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

10th Quarterly Report
2nd Quarter Year 2003

Report prepared for EUROCHLOR

Dr. Jim A. Field and

Dr. Reyes Sierra

Department of Chemical &

Environmental Engineering

University of Arizona

P.O. Box 210011, Tucson

AZ 85721-0011, USA

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ACRONYMS

AHQDS	Anthrahydroquinone-2,6-disulfonic acid
16S rRNA	16S Ribosomal RNA
CB	Chlorobenzene
CBp	Chlorobiphenyl
CDDs	Chlorinated Dibenzo- <i>p</i> -Dioxins
CDFs	Chlorinated Dibenzo- <i>p</i> -Furans
CF	Chloroform
CT	Carbon Tetrachloride
2,4-D	2,4-Dichlorophenoxyacetate
1,2-DCA	1,2-Dichloroethane
DCE	Dichloroethene
DCM	Dichloromethane
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
DNAPL	Dense Non-Aqueous Phase Liquid
E-acceptor	Electron Acceptor
EDB	Ethylene Dibromide or Dibromoethane
E-donor	Electron Donor
FISH	Fluorescence In Situ Hybridization
GST	Glutathione S-transferase (GST)
HCBD	Hexachlorobutadiene
HCH	Hexachlorohexane
PCBs	Polychlorinated Biphenyls
PCP	Pentachlorophenol
PCE	Tetrachloroethylene
PCR	Polymerase Chain Reaction
PeCDD	Penta-Chlorinated Dibenzo- <i>p</i> -Dioxins
MRF smectite	Microbially reduced ferruginous smectite
sMMO	Soluble Methane Monooxygenase
rDNA	Ribosomal DNA
TCA	Trichloroacetic acid
TCE	Trichlorethylene
TOM	Toluene <i>o</i> -monooxygenase (TOM)
VC	Vinyl Chloride

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

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Department of Chemical and Environmental Engineering, University of Arizona
P.O. Box 210011, Tucson, AZ 85721-0011

1. INTRODUCTION

This report presents a review of scientific literature published during the second quarter of 2003 (covering May to July 2003) 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 finding in this quarter for microbial dechlorination is the isolation and characterization of *Dehalococcoides* strain BAV1 which is capable of linking growth to the use of either VC, 1,1-DCE, *cis*-DCE, *trans*-DCE or 1,2-DCA as electron acceptors (e-acceptor) (22). The organism can only use H₂ as the electron donating substrate and requires acetate as a carbon source. In the previous quarter (Eurochlor 9), we reported the same highlight with the initial publication of the isolate and its ability to use VC (21). The newer publication in the

journal *Nature* has better information regarding the identity of the isolate and all possible alternative electron acceptors as well as better kinetic information.

The second most important finding is the demonstration that reductive dechlorination of polychlorinated benzenes can be carried out *in vitro* with cell free extract of the isolate *Dehalococcoides* sp. strain CBDB1 (24). The isolate was previously reported to use trichlorobenzene as a terminal e-acceptor in a respiratory process. The cell free extracts can dechlorinate hexa-, penta-, tetra-, and tri-chlorobenzenes with methylviologen as electron donor (e-donor). Selective inhibitors were used to demonstrate the involvement of vitamin B12 in the *in vitro* dehalogenase activity. Ultracentrifugation experiments and treatments with detergent also demonstrated that the dehalogenase is located on the periphery of the cell membrane.

2.b. Microbial Chlorination

The most important highlight for microbial chlorination is the publication in *Chemosphere* of the proceedings of the second conference on *Naturally Produced Organohalogenes* organized in Heidelberg, Germany, between September 30th and October 4th, 2001 (48). The proceedings contain 26 research publications on natural organohalogenes and their production; four of the publications are review articles.

A second highlight is a report estimating the global quantity of organically bound chlorine present in humus in peatlands and wetlands (27). Globally humification in peatlands has led to the accumulation of approximately 280-1000 million tons of organically bound chlorine during the postglacial period.

3. MICROBIAL DECHLORINATION

3.a. General Reviews

In this quarter, three review articles on biological dechlorination were published. The first reviews the biology of methylotrophic bacteria capable of degrading halomethanes (57). The capacity of aerobic methylotrophic bacteria of utilizing toxic halogenated methane derivatives as sources of carbon and energy are reviewed. Emphasis is placed on the taxonomic, physiological, and biochemical diversity of mono- and dihalomethane-degrading methylbacteria and the enzymatic and genetic aspects of their primary metabolism. The initial

steps of chloromethane dehalogenation to formate and HCl through a methylated corrinoid and methyltetrahydrofolate are catalyzed by inducible cobalamin methyl transferase, made up of two proteins (CmuA and CmuB) encoded by the *cmuA* and *cmuB* genes. At the same time, the primary dehalogenation of dichloromethane to formaldehyde and HCl is catalyzed by cytosolic glutathione transferase with S-chloromethylglutathione as an intermediate. The latter enzyme is encoded by the structural *dcmA* gene and is under the negative control of the regulatory *dcmR* gene. Research is still needed to gain a quantitative evaluation of the ecophysiological role of aerobic methylobacteria in the mineralization of halomethanes and the protection of the biosphere from these toxic pollutants.

The second review article covers recent developments in the reductive dechlorination of C-2 to C-4 chloroalkanes (13). Lower chlorinated members of the group, containing 1-3 chlorine atoms, are generally observed to accumulate in environments where reductive conditions prevail. Their half-lives under these conditions often exceed several decades. To date, success in rapid and complete in situ reductive dechlorination has not been attained for chloroalkanes (although it has with chloroethenes). Except for one 1,2-dichloroethane-dehalorespiring bacterium and a recent isolate which partly dechlorinates some polychloroethanes, all bacterial reductive conversions of C-2 to C-4 chloroalkanes are based on slow, cometabolic dechlorinations. However, energetic consideration of chloroalkane dechlorination suggests that anaerobes may exist which can grow as result of chloroalkane respiration. The authors suggest that the discovery of these microorganisms is needed in order to develop cost-efficient in situ remediation technologies.

A third review article from this quarter is really primarily concerned with the remediation of dense non-aqueous phase liquids (DNAPL) of chlorinated solvents forming source zones at contaminated sites (53). The publication provides the main conclusions of a workshop on chlorinated solvent source zone remediation. Among the possible strategies, several options involving bioaugmentation and biostimulation to promote bioremediation of DNAPL's are reviewed.

3.b. Microbial Dechlorination

Vinyl chloride and Other Chlorinated Ethenes

As indicated in each quarterly report, a large number of studies involve research evaluating the degradation of the higher chlorinated ethenes, perchloroethylene (PCE) and trichloroethene

(TCE) because these are major groundwater contaminants. Thus information regarding the degradation of lower chlorinated ethenes, vinyl chloride (VC) and dichloroethenes (DCE), are found in these studies. Below the studies are categorized based on parent compound investigated, either lower chloroethenes (VC or DCEs) or higher chloroethenes (PCE or TCE).

Vinyl Chloride (VC) and Dichloroethenes (DCE). In this quarter, three studies directly investigated the biodegradation of lower chlorinated ethenes. Two studies were concerned with the anaerobic degradation of VC and DCE whereas one publication evaluated aerobic degradation of VC. The first study is a publication in the journal *Nature* which reports on the successful isolation of a bacterium *Dehalococcoides* strain BAV1 that is able to link growth to the respiration of VC (22). The bacterium utilizes only H₂ as an e-donor, requires acetate as a carbon source, and can utilize either VC, *cis*-DCE, *trans*-DCE, 1,1-DCE, vinyl bromide or 1,2-dichloroethane (1,2-DCA) as e-acceptors. The bacterium is the first isolate known to be able to utilize all three isomers of DCE. The authors showed that the bacterium links its growth to the dechlorination of VC by demonstrating production of biological macromolecules (DNA and protein). The specific activity of VC dechlorination was observed to be 134 nmol min⁻¹ mg⁻¹ protein, the growth yield was 239 mg protein mol⁻¹ VC, and the doubling time was 2.2 days. In the Eurochlor 9 report, another publication by the same research group concerning the same isolate was also summarized (21).

The second publication concerning anaerobic biodegradation of lower chlorinated ethenes is a report on enrichment cultures obtained from landfill leachate impacted sites (8). Among 80 environmental samples, 19 had the ability to anaerobically degrade *cis*-DCE. The best sample (94% *cis*-DCE depletion in one month) was used for sub-culturing. Upon repeated sub-culturing, *cis*-DCE degradation ability decreased. However *cis*-DCE degrading ability could be maintained if Fe(III) was included in the medium, even though it was shown that *cis*-DCE degradation was not linked to dissimilatory iron reduction. The enrichment culture obtained could anaerobically degrade both *cis*-DCE as well as VC. It should be noted that VC was not an intermediate of *cis*-DCE degradation suggesting that other mechanisms aside from reductive dechlorination may have been at play.

This quarter VC degradation was also reported under aerobic conditions. Two microbial cultures obtained from aerobic activated sludge by enrichment were able to utilize VC as a sole source of carbon and energy (3). The specific growth rates of the cultures were slow, ranging from 0.19 to 0.21 d⁻¹ (doubling time 3.3-3.6 d). However, the affinity of the isolates for VC was high with Monod half-saturation constants as low as 0.7 to 1.6 mg L⁻¹. The cultures were

sensitive to VC-feed interruptions and could tolerate shock loads of VC. Ethene could be used to maintain the cultures without loss of VC-degrading ability.

Perchloroethylene (PCE) and Trichloroethene (TCE). In this quarter, there were 11 research reports on the biodegradation of higher chlorinated ethenes. Seven studies evaluated anaerobic degradation; three studies focused on aerobic TCE degradation; and two studies considered the degradation of PCE and TCE under combined anaerobic-aerobic conditions. One of the anaerobic articles (64) and one of the aerobic articles (41), are discussed later in section “3.d. New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds”. Additionally another anaerobic article (53) reports on the conclusions of a workshop on chlorinated solvent source zone remediation and was treated earlier in the section on “3a. General Reviews”.

Five articles on anaerobic dechlorination of higher chlorinated ethenes will be dealt with in this section. The first publication reports on the isolation of the bacterium *Desulfuromonas michiganensis* which has the unique property of utilizing acetate as an e-donor to support the halo-respiration of PCE to *cis*-DCE (54). The bacterium was isolated from pristine river sediments as well as from a contaminated aquifer. Unlike most other halo-respiring bacteria, *D. michiganensis* cannot use H₂ as an e-donor. In addition to acetate, *D. michiganensis* can also utilize lactate, pyruvate, succinate, malate and fumarate as e-donors. Electron acceptors that supported growth were fumarate, malate, ferric iron, sulfur, PCE or TCE. Alternative e-acceptors did not inhibit the use of PCE but were concomitantly utilized with PCE. *D. michiganensis* tolerated high concentrations of PCE and could dechlorinate PCE in the presence of DNAPL's of PCE.

The second anaerobic article describes the isolation of a new halo-respiring bacterium, *Sulfurospirillum halo-respirans* that dechlorinates PCE to *cis*-DCE during halo-respiration (31). The bacterium was isolated from soil polluted with chloroaliphatic compounds. The authors also reclassified another halo-respiring bacterium *Dehalospirillum multivornas* to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans*.

The third anaerobic article evaluated the bioaugmentation of a PCE containing source zone DNAPL's to initiate its remediation (1). An experimental set up utilizing a near field-scale simulated aquifer was established with a known mass of PCE and the groundwater was depleted of oxygen using acetate and lactate prior to culture addition. An active and stable dechlorinating culture was used as an inoculum, and dechlorination activity was observed within 2 weeks following culture transfer. PCE reduction to TCE and *cis*-DCE was accelerated

by the addition of a long-term source of hydrogen (Hydrogen Releasing Compound). *Cis*-DCE was the predominant chlorinated ethene present in the effluent after 225 days of operation, and production of VC and ethene lagged the formation of TCE and *cis*-DCE. However, dechlorination extent continued to improve over time, and VC eventually became a major product. The detection of *Dehalococcoides* species in the source culture and in the simulated aquifer post-inoculation indicated that the metabolic capability to dechlorinate beyond *cis*-DCE was present. The results of this research indicated that adding dechlorinating cultures maybe useful in the application of source zone bioremediation.

The fourth anaerobic article evaluated the use of hollow-fiber membranes to deliver H₂ to engineered PCE bioremediation systems (33). Two glass columns were packed with contaminated soil and were fed a minimal medium spiked with PCE (7 μM). The columns were operated in parallel, with one column receiving H₂ via polyethylene hollow-fiber membranes and a control column receiving no H₂. PCE was initially dechlorinated at a similar rate and to a similar extent in both columns due to the presence of soil organic matter. After 265 days of operation, dechlorination performance declined in the control column and the benefits of membrane-supplied H₂ became evident. Although the membrane supplied H₂ effectively stimulated PCE dechlorination at the end of the experiment (days 359-391), the system was inefficient in that only 5% of the supplied H₂ was used for dechlorination. Most of the remainder was used to support methanogenesis (94%). Despite the dominance of methanogens, nearly complete dechlorination of PCE to ethene was observed in the H₂-fed column.

The final anaerobic article from this quarter, reports on the natural attenuation of TCE in fractured shale bedrock (29). The study was carried out adjacent to a former waste burial site at the Oak Ridge Reservation, Oak Ridge, Tennessee (USA). A contaminant plume containing TCE and its daughter products was detected down-gradient from buried waste pits. Sampling wells indicated the presence of overlapping plumes of TCE, *cis*-DCE, VC, ethylene, ethane, and methane, with the daughter products extending further down-gradient than the parent (TCE). This type of distribution suggests anaerobic biodegradation. The 16S ribosomal DNA (rDNA) sequences from DNA extracted from two wells were similar to sequences of organisms previously implicated in the anaerobic biodegradation of chlorinated solvents. The combined data strongly suggest that intrinsic anaerobic biodegradation of the highly chlorinated compounds was occurring at the site.

Two articles on TCE cooxidation will be dealt with in this section. The first aerobic article describes the operation of a biotrickle reactor used to treat waste gases from a microelectronics

industry, containing TCE, acetone and toluene (14). A microbial consortium acclimatized to the degradation of the organic mixture was used to inoculate the reactor with activated carbon support medium. *Pseudomonas* and *Sphingomonas* strains appeared to be involved in TCE degradation. The steady-state hydrocarbon removal efficiency of the biotrickle column was 95%. Overall mass transfer rate and biokinetic constants were determined. The velocity of TCE degradation was $0.06 \text{ g h}^{-1} \text{ kg}^{-1}$ dry particles (activated carbon and biomass) and the half-saturation constant was 78 ppm TCE in the gas phase.

The other aerobic article evaluated the kinetics of TCE cooxidation by the methanotroph, *Methylosinus trichosporium* OB3b PP35, immobilized in a fibrous-bed bioreactor (28). *M. trichosporium* OB3b PP358 is a strain that expresses the enzyme, soluble methane monooxygenase (sMMO), constitutively. The enzyme sMMO is considered to have a high activity towards TCE cooxidation. Cells were grown on methane as the substrate with aeration, and then used to degrade TCE through the cometabolism with sMMO in the absence of methane. Complete biodegradation of TCE was verified with the TCE and chloride ion mass balance. TCE transformation was inhibited in the presence of methane or methanol. However, without the energy source cells gradually lost most of their capability in degrading TCE, which was attributed to reduced sMMO enzyme activity due to lack of NADH and cell death caused by TCE toxicity. The reactor was able to recover its TCE degradation ability after rejuvenating and re-growing cells with methane and air. Compared to free cell and other immobilized cell systems, the cells immobilized in the fibrous-bed bioreactor not only showed a much higher TCE degradation rate (up to $85 \text{ mg l}^{-1} \text{ d}^{-1}$ or - 32 times higher compared to free cells), but also had a better tolerance to TCE (22.6 mg l^{-1} or 11 times higher than free cells). With periodical rejuvenation, the bioreactor could be used for long-term treatment of TCE-contaminated water.

Finally two articles considered the coupled anaerobic-aerobic degradation of PCE or TCE in a single bioreactor. The first of these evaluated the effect of elemental oxygen exposure on the survival of reductive dechlorination ability of biomass engaged in the anaerobic reduction of PCE and TCE (35). Results from this research showed that both methanogenic and sulfidogenic reductive dechlorination could resume after transient exposures to both oxygen and hydrogen peroxide (H_2O_2). In fact, for cycles as frequent as 10 days between aerobic treatment cycles, reductive dechlorination was observed to resume at rates at least as rapid as microcosms not exposed to aerobic treatments.

The second article evaluated the degradation of TCE in a coupled methanogenic-methanotrophic bioreactor with four aeration and four organic loading rate (OLR) schedules (32). Ethanol was used as the e-donating substrate. Microcosm and PCR studies demonstrated

that methanotrophs capable of mineralizing TCE and methanogens were present in the biomass throughout the study. The gene for the particulate form of methane monooxygenase (pMMO) was detected by PCR, but not that for the soluble form (sMMO). Low TCE concentrations were measured in effluent and off-gas samples in all cases. *Cis*-DCE was also observed. Volatilization losses were 0-5%. Changes in the aeration rate had no effect on TCE removal, but did influence DCE degradation. At low and no-aeration conditions, *cis*-DCE accumulation occurred due to slow DCE degradation. At the higher aeration rates, low *cis*-DCE levels were observed which indicated that conditions in these reactors were amenable to the aerobic co-metabolism of TCE and *cis*-DCE. The OLR did have an effect on TCE removal. TCE and *cis*-DCE removal were negatively affected when the OLR was increased. An OLR of 0.3 g COD l⁻¹ reactor d⁻¹ or lower with an aeration rate of 3 l O₂ l⁻¹ reactor d⁻¹ and higher is the recommended operating condition of a coupled reactor for removal of TCE.

Carbon Tetrachloride (CT) and Chloroform (CF)

This quarter there was one article on the biodegradation of carbon tetrachloride. Complete removal of carbon tetrachloride (CT) by *Pseudomonas cepacia* was observed in batch assays and in continuous fixed-biofilm reactor studies (25). Optimum biodegradation of CT by was observed for culture redox potentials between -100 and -200 mv. These results were part of a broader study aimed at developing a protocol to assess the biological treatability of toxic pollutants.

Chloromethane (CM) and Dichloromethane (DCM)

This quarter there was only one report on the dechlorination of dichloromethane. As discussed above in heading “3.a. Microbial dechlorination: General reviews”, a review on the degradation of halomethanes by methylotrophic bacteria was published (57).

Fractionation of chlorine isotopes during dichloromethane (DCM) metabolism by the *Methylobacterium dichloromethanicum* grown on was reported (65). The aerobic methylotrophic bacterium was found to utilize DCM as the source of C and energy.

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

Three studies tested the microbial degradation of chlorinated ethanes this quarter. In addition, one study reports on the reduction of hexachloroethane by natural organic matter (26). Furthermore, as discussed above in heading “3.a. Microbial dechlorination: General reviews”, a publication presents a review on the microbial reductive dechlorination of C-2 to C-4 chloroalkanes (13). The first of the articles on microbial degradation chlorinated ethanes dealt with the anaerobic halorespiration of 1,2-DCA (22) and was already discussed above in the section on “*Vinyl Chloride (VC) and Dichloroethenes (DCE)*”. The second article concerns further studies on substrate-enzyme interactions between 1,2-DCA and haloalkane dehalogenase from *Xanthobacter Autotrophicus* (Dh1A) (52). The article is discussed in detail in section: “3.c. *In Vitro Degradation of Chlorinated Compounds*”. The last article on microbial degradation chlorinated ethanes reports the dehydrochlorination of 1,1,1-trichloroethane (1,1,1-TCA) and pentachloroethane (PCA) by microbially reduced ferruginous (MRF) smectite (7). Reduction of structural Fe(III) in smectite clay minerals by two microbial cultures, *Shewanella oneidensis* strain MR-1 and an enrichment culture from rice paddy soils, was attained in aqueous suspension under anoxic conditions. 1,1,1-TCA was dechlorinated in the presence of MRF smectite to 1,1-DCE with up to 60% conversion in 3 h. In contrast, no formation of 1,1-DCE was observed after incubation of 1,1,1-TCA with chemically reduced ferruginous smectite for 24 h. PCA was dehydrochlorinated by MRF smectite to PCE. PCE yields in 3 h varied from 15 to 80% depending on the microbial culture. These results indicate that ferric iron in clay minerals has the potential to be an important reductant controlling the fate of organic chemicals in contaminated sediments.

Electrochemically reduced soil humus and anthrahydroquinone-2,6-disulfonic acid (AHQDS), a model compound for hydroquinone moieties in humic acid, were shown to reduce hexachloroethane at appreciable rates (26). The initial reaction followed pseudo-first-order reaction kinetics, and PCE was the only halogenated product. These results suggest that reduced humic acids could play a significant role as reductants in the reductive transformation of subsurface contaminants. Reduced humic acid can be formed by a variety of microbiological and chemical processes.

Chlorobenzenes

One publication was found regarding the microbial degradation of chlorobenzenes (CB). An additional study reports on the reductive dehalogenation of tri-, tetra-, penta-, and

hexachlorobenzenes in cell extracts of *Dehalococcoides* sp strain CBDB1 (24). The latter report is discussed below in heading “3.c. *In Vitro Degradation of Chlorinated Compounds*”.

Invertebrate and microbial communities were characterized to assess the condition of the hyporheic zone of a river (Sebasticook River, Maine, USA) subject to 80 years of contamination by chlorobenzenes (63). Study of river bed sediments at three contaminated and two reference sites showed that variance in the faunal and microbial data was mainly attributable to redox potential, ammonium levels, and downwelling, rather than to CB contamination. Genetic fingerprinting revealed a unique microbial community at the site most heavily contaminated with CB, but a high degree of similarity among the other two mill sites and the reference sites. The reference sites were found to be contaminated with ketones and methyl chloride while the study was in progress.

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

In this quarter, one study reports on the microbial degradation of chlorinated dibenzo-*p*-dioxins (CDDs). In addition, biodegradation of the model dioxin compound, 2,7-dichlorodibenzo-*p*-dioxin (2,7-DiCDD), was shown to be enhanced by the addition of culture filtrates of several strains of fungi (46). The latter study will be discussed below in heading “3.c. *In Vitro Degradation of Chlorinated Compounds*”.

Partial degradation of 2,3-dichlorodibenzo-*p*-dioxin (2,3-DiCDD) and polychlorinated biphenyl (KC-300) by several marine bacterial strains, which were enriched by growth on dibenzofuran or biphenyl, was demonstrated (18). The marine microorganisms were not able to degrade 2,7-DiCDD and 2,4,8-trichlorodibenzofuran. Analysis of 16S rDNA indicated that the strains were more closely related to *Alteromonas macleodii* (96.3% identity) and *Cycloclasticus pugetii* (97.3% identity).

Hexachlorobutadiene and Octachlorostyrene

Only one publication on the microbial dechlorination of hexachlorobutadiene (HCBd) was found in the second quarter of 2003. HCBd was among the pollutants used as a model in a study focusing on the development of a protocol to evaluate the treatability of toxic pollutants generated by chemical industries (25). HCBd was observed to be effectively degraded under oxidizing environmental conditions in the presence of an acclimated mixed bacterial culture.

Polychlorinated Biphenyls (PCBs)

In this quarter, nine publications reported on the microbial degradation of PCBs. Two of these publications address anaerobic degradation of PCBs ((17) (5)), and six examine PCB degradation by aerobic bacteria, in the absence ((18)), (15, 39, 55) or presence ((16, 45)) of compatible plants. An additional study reports on PCB biodegradation in marine sponges (40).

Microbial reductive dechlorination of highly chlorinated PCBs in a contaminated marine sediment (PCB concentration approx. 1 mg kg^{-1}) into tri- and di-chlorinated, *ortho*-substituted biphenyls was observed in pasteurized and non-pasteurized anaerobic slurry microcosms after 11 weeks of incubation (17). Dechlorination was accompanied by significant consumption of sulfate and no methane production. In contrast, no significant PCB transformation was observed in microcosms supplemented with molybdate, eubacteria-inhibiting antibiotics or exogenous carbon sources. When exogenous 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-PCB) was added, preferential dechlorination of this compound at the *meta*- and *para*-positions was observed. Addition of 2,3,4,5,6-PCB did not alter the dechlorination rate. The authors conclude that reductive dechlorination of PCBs in the marine sediment appears to be mediated by spore-forming, sulfate-reducing bacteria.

Microbial reductive dechlorination of river sediments (Housatonic River, Lenox, MA, USA) contaminated with Aroclor 1260 (commercial mixture composed of mainly hexa- and heptachlorobiphenyls) was shown earlier to proceed by three different dechlorination processes (Target chlorine: flanked *meta* (process N); flanked *para* (process P); flanked & unflanked *para* (process LP)), which differ in congener selectivity, position of the chlorine removed and terminal products (Deweerd & Bedard, 1999, *Env. Sci. Technol.* 33(12):2057-2063). In a recent publication, the same authors investigated strategies for the selective enrichment of micro-organisms carrying out the reductive dechlorination of PCBs by processes for each distinct dechlorination activity (5). Pasteurization completely inhibited dechlorination processes N, P and LP, but molybdate had little or no effect. The antibiotics, penicillin G and vancomycin, did not inhibit dechlorination processes N or P, but streptomycin completely inhibited both. Based on these results, the authors conclude that bacteria responsible for PCB-dechlorination processes N, P, and LP are not spore-formers, and not sulfate reducers. Furthermore, they indicated that antibiotics (penicillin G, vancomycin) and molybdate can be used to selectively eliminate non-essential microorganism and develop highly enriched microcosms for each PCB dechlorination process.

Partial degradation of polychlorinated biphenyl (KC-300) by two marine bacterial strains closely resembling *Alteromonas macleodii* (96.3% identity) and *Cycloclasticus pugetii* (97.3% identity) was reported (18).

The aerobic *Sinorhizobium meliloti* electrotransportant carrying the oxygenolytic *ortho*-dechlorination (*ohb*) gene was shown to display enhanced PCB dechlorination (55). The recombinant strain grew on up to 100 mg l⁻¹ of 2',3,4-PCB without any adverse effect on its growth and nodule formation ability with the alfalfa plant. The wild type *S. meliloti* depleted 15% PCB whereas its electransportant dechlorinated 100%.

Microbial degradation of PCBs (Aroclor 1242) in a contaminated sandy sediment by a mixed culture of acclimatized aerobic bacteria in slurry phase reactors was reported (34). Aroclor 1242 elimination averaged 61% after 4 months of treatment. The rate of degradation decreased with increasing PCB chlorination degree, as indicated by the removal of dichlorinated- (100% removal), trichlorinated- (92%) and tetrachlorinated biphenyls (24%). Addition of biphenyl as a cosubstrate was shown to result in a significant enhancement of PCB biodegradation.

Two different studies consider the aerobic biodegradation of PCBs in vegetated soil (45) (16). In the first study, the removal of PCB in a contaminated soil was shown to be affected by the cultivated plant species (tobacco, black nightshade and alfalfa) (45). The highest decrease of PCB concentration was measured in soil vegetated with tobacco (34% comparing to bulk soil). Following isolation, characterization of degradative capabilities, and phylogenetic analysis of PCB-degraders in the contaminated soil, *Pseudomonas pseudoalcaligenes* was identified as an efficient PCB degrader. In the second paper, indigenous mixed soil bacteria in presence of a compatible plant, the leguminous alfalfa (*Medicago sativa*), were shown more efficient in PCB degradation than known (*i.e.*, PCB co-metabolizing bacteria such as *Comamonas testosteroni* and *Rhodococcus* sp.,) and unknown single cultures (16). In this study, the congener 2',3,4-PCB was used as known standard to spike the mixture of clean soil and vermiculite.

In this quarter, two different studies addressed the role of plant terpenes as possible inducers of PCB-degrading bacteria (39) (15). In one of the publications, plant terpenes were shown to enhance the survivability of the PCB-degrading *Pseudomonas pseudoalcaligenes* strain KF707 in microcosms contaminated with PCB (39). *P. pseudoalcaligenes* KF707 was genetically tagged using a transposon with *gfp* (green fluorescent protein) as a reporter gene in order to facilitate examination of the population dynamics. The study found about 10-100-fold increase in the population of *P. pseudoalcaligenes* KF707 when terpene was added, compared

with control (non-terpenes samples and biphenyl added samples). A previous study by the same research group showed that plant terpenes (*p*-cymene, (S)-(-)-limonene, α -pynene, and α -terpinene) were utilized by a PCB degrader and induce the biphenyl dioxygenase gene in pure culture. In a related study, two terpenes, carvone and limonene, were investigated as possible inducers of biodegradation of PCBs (15). None of the terpenes could serve as a sole carbon source. Carvone was shown to enhance the degradation of a commercial mixture of PCBs, DELOR 103, when xylose was used as the carbon source. Removal of individual PCB congeners ranged 7-37% and 30-70%, respectively, in the absence and presence of carvone.

The last publication examined the use of a marine sponge, *Spongia officinalis*, as biomonitor of PCB contamination (40). Analysis of twenty-four PCB congeners along a pollution gradient both in sponges and seawater indicated PCB metabolism in the sponges. The ratio of two persistent PCB compounds (CB138/CB153) in the sponge samples varied from 1 at the most polluted site to 0.3 at the reference site. These values are lower compared to the CB138/CB153 ratio characteristic of commercial PCB mixtures as well as seawater (1.2), indicating degradation of CB138 in sponges. Further work is needed to elucidate the processes responsible for PCB degradation. Biodegradation of PCBs by microorganisms known to live in symbiotic association with sponges can not be excluded.

Miscellaneous Chlorinated Compounds

The search query used is specifically designed to review literature on the target compounds listed in the *Introduction* section. Interesting publications concerned with compounds outside of the range list which are found in the search process are briefly discussed below. This quarter our search retrieved fifteen reports on the biodegradation of miscellaneous chlorinated pollutants, including, six publications on chlorophenol (2, 9, 10, 23, 42, 44), three publications on trichloroacetic acid (TCAA) (6, 47, 49), and one report on the degradation of each of the following compounds, 2,4-dichlorophenoxyacetic acid (2,4-D) (11), chloronaphthalene (36), pentachloronitrobenzene (50), 3,4-dichloroaniline (56), and DDT (59).

Chlorophenols. Six articles reported on chlorophenol degradation. One article reports on the anaerobic bacterium *Anaeromyxobacter dehalogenans* that is known to for its ability to halorespire 2-chlorophenol. The article demonstrates that *A. dehalogenans* can also utilize Fe(III) as an alternative e-acceptor (23). The second article describes the biodegradation of 2,4,6-trichlorophenol in a coupled anaerobic-aerobic bioreactor (9). The four remaining

articles are concerned with the aerobic degradation of chlorophenols. One article reports on the degradation of pentachlorophenol in wood chips mixed with soil (42). Another article reports on 2-chlorophenol and 2,4,6-trichlorophenol degradation is a laboratory simulated aerobic sandy aquifer (2). A third article examined the impact of ozonation pretreatments of 2,4-dichlorophenol on subsequent measurements of its biological oxygen demand (10). In the last article, the aerobic degradation of 2,4-dichlorophenol was evaluated in an airlift reactor with a ceramic honeycomb matrix for biofilm support (44).

Trichloroacetic acid. A brief review on the microbial degradation of TCAA is presented within a review article reporting on the fluxes of TCAA between atmosphere, biota, soil, and groundwater (47). TCAA was shown to be biodegraded in a Norway spruce/soil system (49). TCAA biodegradation in soil depended on TCAA concentration, soil humidity and other factors. CO₂ was the only TCAA metabolite detected. Complete degradation of TCAA in soil (approx. 50 ng (g of soil)⁻¹) was observed within 26 days of application, suggesting relatively rapid removal of TCA in soil (6).

2,4-D. A new method for the determination of 2,4-D and its major transformation product, 2,4-dichlorophenol, in soil samples by liquid chromatographic analysis was described (11). Neither 2,4-D nor its degradation product were detected in soil samples 30 days after the application of the herbicide to eucalyptus crops.

Chloronaphthalenes. Biodegradation of chloronaphthalenes (1-chloronaphthalene and 2-chloronaphthalene) by the white-rot fungus *Phlebia lindtneri* was reported (36). 2-chloronaphthalene was metabolized to 3-chloro-2-naphtol, 6-chloro-1-naphtol and two other chloronaphtols, chloronaphthalene-dihydrodiols and chloronaphthalene-diols. Significant inhibition of the degradation of these substrates was observed when they were incubated with cytochrome P-450 monooxygenase inhibitors, suggesting that *P. lindtneri* initially oxidizes these substrates by a P-450 monooxygenase.

Chloronitrobenzenes. Isolation and identification of a degrading bacterium, *Alcaligenes xylosoxidans* PCNB-2, from agricultural soil at is able to utilize and grow on pentachloronitrobenzene (100 mg l⁻¹) as a sole carbon source was reported (50).

Chloroanilines. The chlorinated aniline, 3,4-dichloroaniline, was shown to be susceptible to biodegradation by the aerobic bacterium *Pseudomonas fluorescens* strain 26-K (56).

Lindane. Two *Pseudomonas* strains isolated from an agricultural soil were found to possess γ -HCH degrading ability when the isolates were grown in a mineral salt medium containing γ -HCH as the sole source of carbon (38).

DDT. Arsenic co-contamination in soil was shown to inhibit breakdown of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) (59).

3.c. In Vitro Degradation of Chlorinated Compounds

In this quarter, four articles reported on the *in vitro* degradation of halogenated compounds by enzymes or their cofactors, and/or were devoted to the characterization of dehalogenating enzymes.

Enzymatic reductive dehalogenation of tri-, tetra-, penta-, and hexachlorobenzenes (TCB, TeCB, PCB, HCB) was demonstrated in cell extracts from the chlorobenzene-respiring anaerobe *Dehalococcoides* sp. strain CBDB1 (24). The highest specific activities were observed with 1,2,3,4-TeCB and PCB. 1,2,3-TCB dehalogenase activity was studied further and found to be associated with the membrane fraction. Light-reversible inhibition by alkyl iodides indicated the presence of a corrinoid cofactor. Treatments with low concentrations of detergents solubilized 1,2,3-TCB dehalogenase suggesting its peripheral localization, which is in keeping with its role in halorespiration.

Enhancement of biodegradation of a model dioxin compound, 2,7-dichlorodibenzo-*p*-dioxin (2,7-DiCDD), by addition of culture filtrate derived from the white-rot basidiomycetous fungus *Phanerochaete sordida* YK-624 was demonstrated (46). Removal of 2,7-DCDD after 3 weeks incubation in a YK-624 culture containing these filtrates was greater (30%) than that in the culture of YK-624 alone (15%).

A *bphK* gene encoding glutathione S-transferase (GST) activity is located in the *bph* operon in *Burkholderia* sp. strain LB400. The enzyme BphK in strain LB400 was shown to display dechlorination activity against 4-chlorobenzoate, an end product of *bph*-promoted degradation of PCBs (19). The author hypothesized that the enzyme may have a role in protection of other *bph* enzymes against certain chlorinated metabolites of PCB degradation.

The halogenated ethane, 1,2-dichloroethane (1,2-DCA), is a substrate of haloalkane dehalogenase from *Xanthobacter Autotrophicus* (Dh1A), an enzyme that catalyzes the conversion of 1,2-DCA to 2-chloroethanol. In a recent paper, the nature of substrate-enzyme interactions were investigated in relation to the electric field created by the environment in which the enzymatic reaction is taking place (52).

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

Characterization of Microbial Populations

Two specific 16S rRNA-targeted oligonucleotide probes have been developed for the detection of *Dehalococcoides* species by Fluorescence In Situ Hybridization (FISH) (64). In situ hybridization of probe Dhe1259t with *D. ethenogenes* strain 195 and two enrichment cultures demonstrated the applicability of the probe for monitoring the abundance of active *Dehalococcoides* species in these samples. *Dehalococcoides ethenogenes* is the only known cultivated organism capable of complete dehalogenation of PCE to ethane.

Proteomics

Proteomics was utilized to characterize the proteins in a recombinant *Escherichia coli* engineered for enhanced TCE cooxidation with the enzyme toluene *o*-monooxygenase (TOM) (41). Comparison of 2-dimensional electrophoresis gels for *E coli* cells with and without the ability to synthesize TOM revealed 31 new proteins in TOM-containing cells as well as nine proteins not detected in those cells but present in the plasmid control strain. Exposure of TOM-containing cells to TCE led to the synthesis of four new proteins and the loss of only one protein. Thus, this example of metabolic engineering has a substantial and complex impact on the physiology of these cells that was clearly revealed using a proteomic approach.

4. MICROBIAL CHLORINATION

4.a. General Reviews

A special issue on naturally produced organohalogens was published this quarter in the journal *Chemosphere* (48). The issue collects 26 papers from a conference on the topic that took place in Heidelberg, Germany, in the Fall of the year 2001. The main objectives of the conference were as follows: 1) to review sources, fluxes and transformations of organohalogens in different environmental compartments; 2) to review mechanisms of halogenation and dehalogenation by abiotic and biotic processes; 3) to assess the significance of a natural contribution to the global organohalogen burden of the environment; 4) to identify and discuss scientific controversies (48). The papers most relevant for this report are discussed below; among them are four review papers.

The first publication provides a review on the biochemical basis for important biological dehalogenation and halogenation reactions (58). The second reports on the diversity and sources of naturally produced organohalogens (20). Presently more than 3800 naturally occurring organohalogen compounds are known. As of February 2002, the breakdown of these compounds is approximately: organochlorine (2200); organobromine (1950); organoiodine (95); organofluorine (100) (Gribble, Unpublished). In the concluding remarks, the authors emphasize the scientific and societal significance of emerging evidence that chlorinated dioxins and dibenzofurans have several natural sources—both abiogenic and biogenic. Recent studies indicate that dioxins are formed in peat and forest soil, presumably via the enzymatic oxidative dimerization of natural chlorophenols (Silk *et al.*, 1997, *Chemosphere* 35, 2865-2880; Hoekstra *et al.*, 1999, *Environ. Sci. Technol.* 33, 2543-2549). The third review deals with the patterns and sources of naturally produced organohalogen compounds in the marine environment, with emphasis on the reaction pathways of natural organohalogens and on the chemistry of haloperoxidases (4). The last paper presents a review on the fluxes of trichloroacetic acid (TCAA) between atmosphere, biota, soil, and groundwater (47). Based on mass balances, the study estimates the yearly formation of TCA in soil at $2.16 \text{ kg ha}^{-1} \text{ year}^{-1}$. The authors warn about that the uncertainties of the latter estimate are enormous due to the large standard deviation of about 80%.

4.b. Microbial Chlorination in the Environment

Chloromethanes and other Chlorinated Compounds

In addition to the aforementioned review articles, there were several reports on the microbial formation of organohalogenes this quarter.

Production of methyl bromide (CH_3Br) in a temperate forest soil was reported by Varner *et al.* (60). The authors hypothesized that fungi could be responsible for the production of methyl bromide. Silk *et al.* (51) investigated the biosynthesis of chloroarylpropane diols by the basidiomycete *Bjerkandera adusta*. The study concluded that the aromatic aldehydes benzaldehyde, 4-hydroxybenzaldehyde and 4-methoxybenzaldehyde are likely C7-unit precursors in the carboligation reaction(s) that leads to 1-arylpropane-1,2-diol biosynthesis.

Chlorinated Natural Organic Matter

A recent study showed that organically bound chlorine is a major natural constituent of peat that can account for up to 0.2% of dry mass (27). The authors report that the latter value exceeds the German threshold value for sewage sludge by a factor of four. Humification of plant material is believed to be the initial process for the formation of organic halogens in peatlands. It is estimated that this process has led to the accumulation of approximately 280-1000 million tons of organically bound chlorine in peatlands during the postglacial period. Based on these estimates, peatlands are a major sink of chlorine in the terrestrial environment.

4.c. Chlorination by Marine and Freshwater Organisms

Chloromethanes

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

Other Chlorinated Compounds

Several studies report on the identification and characterization of new halogenated metabolites from marine organisms, including: alga (43), sponges (12, 30), and unidentified natural marine sources (62, 63).

Seasonal variation in the concentrations of organobromine compounds were detected in an eutrophic lake, with concentrations up to five times higher in late summer as compared to the rest of the year (43). Batch experiments with lake water showed that formation of organobromine compounds required light and the presence of algae, suggesting that it is biological in nature. New chlorinated acetylenes from the San Diego sponge *Haliclona lunisimilis* were isolated and characterized (12). Brominated polyacetylenes were characterized from the Philippines sponge *Diplastrella* sp. (30). Vetter *et al.* (62) reported new spectroscopic and analytical data on the natural product Q1, 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole. Q1 is a non-polar halogenated compound that bioaccumulates in marine samples. Based on the newly resolved structure the highest concentration of Q1 observed in the environment so far is the blubber of marine mammals from Australia is 14 mg/kg lipid. A number of possible lower chlorinated congeners is proposed. The naturally occurring compound, 4,6-dibromo-2-(2',4'-dibromo)phenoxyanisole (BC-2), and several other natural brominated compounds have been detected in different marine animals and even in the milk of Eskimo women who consume whale blubber (61). The latter study is the first report on the bioaccumulation of natural organohalogens in humans.

4.d. Chlorinating Enzymes

Except for the aforementioned review article (4), there was only one article on the enzymatic formation of organohalogens this quarter. The biosynthesis and enzymology of fluorometabolite production by the bacterium *Streptomyces cattleya* was investigated (37). Fluoroacetaldehyde is known to be a common intermediate in the biosynthesis of the fluorinated compounds, fluoroacetate and 4-fluorothreonine, by *S. cattleya*. In the current study, two enzymes, an aldehyde dehydrogenase and a threonine transaldolase, were identified that are involved in the biotransformation of fluoroacetaldehyde to fluoroacetate and 4-fluorothreonine.

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6. ANNEX

Adamson, D. T., J. M. McDade, et al. (2003). "Inoculation of DNAPL source zone to initiate reductive dechlorination of PCE." *Environmental Science & Technology* 37(11): 2525-2533.

The ability to inoculate a PCE-NAPL source zone with no prior dechlorinating activity was examined using a near field-scale simulated aquifer. A known mass of PCE was added to establish a source zone, and the groundwater was depleted of oxygen using acetate and lactate prior to culture addition. An active and stable dechlorinating culture was used as an inoculum, and dechlorination activity was observed within 2 weeks following culture transfer. PCE reduction to TCE and cis-DCE was observed initially, and the formation of these compounds was accelerated by the addition of a long-term source of hydrogen (Hydrogen Releasing Compound). cis-DCE was the predominant chlorinated ethene present in the effluent after 225 days of operation, and production of VC and ethene lagged the formation of TCE and cis-DCE. However, dechlorination extent continued to improve over time, and VC eventually became a major product, suggesting that reinoculation was unnecessary. The detection of *Dehalococcoides* species in the source culture and in the simulated aquifer postinoculation indicated that the metabolic capability to dechlorinate beyond cis-DCE (t = 86 days and t = 245 days) was present. Elevated levels of TCE and cis-DCE were present in the source zone, but neither VC nor ethene were detected in the vicinity of NAPL. The results of this research indicated that adding dechlorinating cultures may be useful in the application of source zone bioremediation but that dechlorination beyond cis-DCE may be limited to regions downgradient of the source zone.

Ahn, Y. B., S. K. Rhee, et al. (2003). "Reductive dehalogenation of brominated phenolic compounds by microorganisms associated with the marine sponge *Aplysina aerophoba*." *Applied and Environmental Microbiology* 69(7): 4159-4166.

Marine sponges are natural sources of brominated organic compounds, including bromoindoles, bromophenols, and bromopyrroles, that may comprise up to 12% of the sponge dry weight. *Aplysina aerophoba* sponges harbor large numbers of bacteria that can amount to 40% of the biomass of the animal. We postulated that there might be mechanisms for microbially mediated degradation of these halogenated chemicals within the sponges. The capability of anaerobic microorganisms associated with the marine sponge to transform haloaromatic compounds was tested under different electron-accepting conditions (i.e., denitrifying, sulfidogenic, and methanogenic). We observed dehalogenation activity of sponge-associated microorganisms with various haloaromatics. 2-Bromo-, 3-bromo-, 4-bromo-, 2,6- dibromo-, and 2,4,6-tribromophenol, and 3,5-dibromo-4-hydroxybenzoate were reductively debrominated under methanogenic and sulfidogenic conditions with no activity observed in the presence of nitrate. Monochlorinated phenols were not transformed over a period of 1 year. Debromination of 2,4,6-tribromophenol, and 2,6-dibromophenol to 2-bromophenol was more rapid than the debromination of the monobrominated phenols. Ampicillin and chloramphenicol inhibited activity, suggesting that dehalogenation was mediated by bacteria. Characterization of the debrominating methanogenic consortia by using terminal restriction fragment length polymorphism (TRFLP) and denaturing gradient gel electrophoresis analysis indicated that different 16S ribosomal DNA (rDNA) phylotypes were enriched on the different halogenated substrates. Sponge-associated microorganisms enriched on organobromine compounds had distinct 16S rDNA TRFLP patterns and were most closely related to the delta subgroup of the proteobacteria. The presence of homologous reductive dehalogenase gene motifs in the sponge-associated microorganisms suggested that reductive dehalogenation might be coupled to dehalorespiration.

Antizar-Ladislao, B. and N. I. Galil (2003). "Kinetics of biodegradation during remediation of consecutive accidental spills of chlorophenols in a sandy aquifer." *Water Science and Technology* 47(9): 157-164.

Kinetics of biodegradation of chlorophenols were studied in six sandy aquifer columns (0.06 m I.D.; 1.00 m L). Remediation of chlorophenols was enhanced by using a "closed-loop" configuration system, where local groundwater was recirculated through the polluted site in a controlled, manner. Consecutive accidental spills of phenol, 2-monochlorophenol (2-MCP) and 2,4,6-trichlorophenol(2,4,6-TCP) as single pollutants were removed following first order kinetics. The removal of chlorophenols increased by one order of magnitude following consecutive accidental spills demonstrating adaptation of the resident micro flora. The biodegradation rate constants in this study were in the same range and agreed with those reported in the literature for biodegradation in aerobic,aquifers. Following the fate of the resident micro flora (enhanced by adding NH₄Cl and KH₂PO₄ at a ratio C/N/P equal to 120:10:1), biomass growth was observed in the sandy aquifer columns and particle size analyses of the aqueous phase recirculated through the polluted site experimentally proved aggregation of cells. Aggregation of cells has been hypothesized as one of the causes for low biodegradation rates found in the field compared to those calculated using biodegradation rate constants determined in batch-culture.

Aulenta, F., M. Majone, et al. (2003). "Enrichment from activated sludges of aerobic mixed cultures capable to degrade vinyl chloride (VC) as the sole carbon source." *Annali Di Chimica* 93(4): 337-346.

Two microbial cultures able to degrade high concentrations of VC as the sole carbon source have been obtained by enrichment from activated sludge. The cultures began consuming VC (0.02 mmol l⁻¹) only after a long initial acclimation period (1-2 months). After then the concentration of VC was gradually increased (from 0.02 to 0.8 mmol l⁻¹) and the cultures were able to maintain VC degrading ability for long time (over 500 days). VC-degrading biomass in the two cultures was characterized by low specific maximum growth rates (0.19-0.21 d⁻¹) compared to heterotrophic organisms typically present in activated sludge processes. Monod half-saturation constant was rather low (0.7-1.6 mgVC l⁻¹) indicating that it is possible to effectively remove VC to low residual concentrations. The cultures were highly sensitive to even short periods of VC lack (with quick decrease of VC degradation rates) whereas they were not to sudden load increases (up to 3.4 mmol l⁻¹). After being cultured with only ethene as the sole carbon and energy source, the cultures kept the ability of degrading VC. Possibility of maintaining the mixed cultures on non-toxic ethene, without loosing VC degradation ability, is very promising for bioaugmentation treatments.

Ballschmiter, K. (2003). "Pattern and sources of naturally produced organohalogenes in the marine environment: biogenic formation of organohalogenes." *Chemosphere* 52(2): 313-324.

The pattern of organohalogenes found in the marine environment is complex and includes compounds, only assignable to natural (chloromethane) or anthropogenic (hexachlorobenzene, PCBs) sources as well as compounds of a mixed origin (trichloromethane, halogenated methyl phenyl ether).The chemistry of the formation of natural organohalogenes is summarized. The focus is put on volatile compounds carrying the halogens Cl, Br, and I, respectively. Though marine natural organohalogenes are quite numerous as defined components, they are mostly not produced as major compounds. The most relevant in terms of global annual production is chloromethane (methyl chloride). The global atmospheric mixing ratio requires an annual production of 3.5-5 million tons per year. The chemistry of the group of haloperoxidases is discussed. Incubation experiments reveal that a wide spectrum of unknown compounds is formed in side reactions by haloperoxidases in pathways not yet understood.

Bedard, D. L., G. V. Jerzak, et al. (2003). "Strategies for the selective enrichment of micro-organisms carrying out reductive dechlorination of polychlorinated biphenyls in freshwater sediments." *Fresenius Environmental Bulletin* 12(3): 276-285.

The sediments of the Housatonic River (Lenox, Massachusetts, USA) are contaminated with Aroclor 1260, a commercial polychlorinated biphenyl (PCB) mixture composed of mainly hexa- and heptachlorobiphenyls. We previously identified specific halogenated biphenyls that selectively stimulate indigenous microorganisms in

these sediments to dechlorinate the PCBs by three different dechlorination processes (Processes N, P, and LP), which differ in congener selectivity, position of the chlorine removed, and terminal products. Here we summarize, further characterize, and compare dechlorination processes N, P, and LP. We also develop strategies to selectively enrich the microbial consortia responsible for each distinct dechlorination activity and eliminate many nonessential microorganisms. Pasteurization completely inhibited - Dechlorination processes N and LP, but molybdate, which inhibits sulfate reducers, had little or no effect. Penicillin G and vancomycin did not inhibit dechlorination processes N or P, but streptomycin completely inhibited both. These and other data indicate that the PCB-dechlorinating bacteria responsible for dechlorination processes N, P, and LP are not spore-formers, and not sulfate reducers. We conclude that penicillin G, vancomycin, and molybdate can be used to selectively eliminate non-essential microorganisms, while the PCB-dechlorinating population is simultaneously enriched by repeated transfer using selective primers, temperature, and pH. Our findings lay the basis for developing highly enriched microcosms for each PCB dechlorination process. This research may lead to effective and environmentally compatible ways to accelerate the detoxification and degradation of PCBs in situ.

Cape, J. N., N. M. Reeves, et al. (2003). "Long-term exposure of Sitka spruce seedlings to trichloroacetic acid." *Environmental Science & Technology* 37(13): 2953-2957.

Trichloroacetic acid (TCA) has been implicated as an airborne pollutant responsible for adverse effects on forest health. There is considerable debate as to whether TCA observed in trees and forest soils is derived from atmospheric deposition or from in situ production. This experiment reports the results from treating 4-year-old Sitka spruce (*Picea sitchensis* (Bong.) Carr) plants in a greenhouse over a growing season with TCA supplied either to the soil or to the foliage at concentrations of 10 and 100 ng mL⁻¹. Similar uptake of TCA by needles was observed for both modes of treatment, with significant accumulation of TCA (300 ng g⁻¹ dry wt) at the higher concentration. Larger concentrations in stem tissue were seen for the foliar-applied TCA (280 ng g⁻¹) than for the soil-applied TCA (70 ng g⁻¹), suggesting that direct stem uptake may be important. Six months after treatments stopped, TCA concentrations in the needles of plants exposed to 100 ng mL⁻¹ TCA were still enhanced, showing that biological degradation of TCA in needles was slow over the winter. By contrast, no significant enhancement of TCA in soil could be detected in the directly treated soils even during the experiment. The protein content of needles treated with the higher concentration of TCA by either route was significantly smaller than for the controls, but there was no effect of TCA on the conjugation of 1-chloro-2,4-dinitrobenzene in roots nor on the conjugation of 1,2-dichloro-4-nitrobenzene in needles.

Cervini-Silva, J., J. E. Kostka, et al. (2003). "Dehydrochlorination of 1,1,1-trichloroethane and pentachloroethane by microbially reduced ferruginous smectite." *Environmental Toxicology and Chemistry* 22(5): 1046-1050.

Reduction of structural Fe(III) in smectite clay minerals has been identified as a means to promote dechlorination of polychlorinated ethanes, but its environmental significance has yet to be fully assessed because Fe reduction has normally been achieved by agents uncommon in the environment (e.g., dithionite). This study reports the dehydrochlorination of pentachloroethane and 1,1,1-trichloroethane in the presence of ferruginous smectite reduced by two cultures of microorganisms, *Shewanella oneidensis* strain MR-1 (MR-R) and an enrichment culture from rice paddy soils (PS-R), in aqueous suspension under anoxic conditions. Microbially reduced ferruginous smectite facilitated dehydrochlorination of 1,1,1-trichloroethane to 1,1-dichloroethene with up to 60% conversion within 3 h of incubation time. In contrast, no formation of 1,1-dichloroethene was observed after incubation of 1,1,1-trichloroethane with chemically reduced ferruginous smectite for 24 h. Microbially reduced ferruginous smectite by MR-R and PS-R promoted the dehydrochlorination of pentachloroethane to tetrachloroethene by 80 and 15%, respectively, after 3 h of incubation time. The conversion of pentachloroethane to tetrachloroethene in the presence of chemically reduced ferruginous smectite after 24 h was 65%. These results indicate that structural Fe(II) in clay minerals has the potential to be an important reductant controlling the fate of organic chemicals in contaminated sediments.

Chang, Y. C., K. Jung, et al. (2003). "Anaerobic degradation of cis-1,2-dichloroethylene by cultures enriched from a landfill leachate sediment." *Journal of Microbiology and Biotechnology* 13(3): 366-372.

The production of microbiologically enriched cultures that degrade cis-1,2-dichloroethylene (DCE) under anaerobic conditions was investigated. Among 80 environmental samples, 19 displayed significant degradation of 10 µM cis-DCE during 1 month of anaerobic incubation, and one sediment sample collected at a landfill area (Nanji-do, Seoul, Korea) showed the greatest degradation (94%). When this sediment culture was subcultured repeatedly, the ability to degrade cis-DCE gradually decreased. However, under Fe(III)-reducing conditions, cis-DCE degradation by the subculture was found to be maintained effectively. In the Fe(III)-reducing subculture, vinyl chloride (VC) was also degraded at the same extent as cis-DCE. No accumulation of VC during the cis-DCE degradation was observed. Thus, Fe(III)-reducing microbes might be involved in the anaerobic degradation of the chlorinated ethenes. However, the subcultures established with Fe(III) could function even in the absence of Fe(III), showing that the degradation of cis-DCE and VC was not directly coupled with the Fe(III) reduction. Consequently, the two series of enrichment cultures could not be obtained that degrade both cis-DCE and VC in the presence or absence of Fe(III). Considering the lack of VC accumulation, both cultures reported herein may involve interesting mechanism(s) for the microbial remediation of environments contaminated with chlorinated ethenes. A number of fermentative reducers (microbes) which are known to reduce Fe(III) during their anaerobic growth are potential candidates involved in cis-DCE degradation in the presence and absence of Fe(III).

Chen, Y. C., H. Y. Zhan, et al. (2003). "Coupled anaerobic/aerobic biodegradation of 2,4,6 trichlorophenol." *Journal of Environmental Sciences-China* 15(4): 469-474.

Degradation of 2, 4, 6-trichlorophenol(TCP) with co- immobilizing anaerobic granular sludge and isolated aerobic bacterial species was studied in coupled anaerobic/aerobic integrated reactors. The synergism of aerobes and anaerobes within co-immobilized granule might facilitate degrading the TCP and exchange of anaerobic metabolites 4-CP, which promoted system organic removal efficiency and recovered from organic shock-loads more quickly. The biomass specific activities experiment further confirmed that strict anaerobes be not affected over the course of this experiment by the presence of an oxic environment, aerobic activity predominated in the outer co-immobilized granule layers, while the interior was characterized by anaerobic activity. The co-immobilized granule could thus enable both aerobic and anaerobic microbes function in the same reactor and thereby integrate the oxidative and reductive catabolism.

Contreras, S., M. Rodriguez, et al. (2003). "Contribution of the ozonation pre-treatment to the biodegradation of aqueous solutions of 2,4-dichlorophenol." *Water Research* 37(13): 3164-3171.

The effect of ozonation on the biodegradability of 100-ppm aqueous solutions of 2,4-dichlorophenol has been investigated. BOD at 5, 10 and 21 days, BOD/COD and BOD/TOC ratios and the average oxidation state are presented. Biodegradability measured as BOD5/COD ratio was increased from 0 of the original solution to 0.25 at the moment of removing all the initial compound (corresponding to an ozone dose of 0.12 g L⁻¹, 0.48 for BOD21/COD ratio). To test the effect of this pre-treatment, the biological oxidation of these pre-ozonated solutions was performed in two semi-continuous stirred tank reactors, one with non-acclimated sludge and one with acclimated-to-phenol sludge. The study showed that the TOC content of the pre-treated solution could be removed up to 68% by an aerobic biological treatment as well as co-digested with municipal wastewater (TOC removal up to 82%), with similar operating retention times to a municipal wastewater plant (12-24 h). Kinetic studies based on Monod model have also been carried out. Pseudo-first-order kinetic constants were found to be in the range of 0.5-0.8 L g TVSS⁻¹ h⁻¹. (C) 2003 Elsevier Science Ltd. All rights reserved.

de Amarante, O. P., N. M. Brito, et al. (2003). "Determination of 2,4-dichlorophenoxyacetic acid and its major transformation product in soil samples by liquid chromatographic analysis." *Talanta* 60(1): 115-121.

The 2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most applied herbicides around the world to control broad leaf herbs in many crops: In this study, a method was developed for simultaneous extraction and determination of 2,4D and its major transformation product, i.e., the 2,4-dichlorophenol (2,4-DCP), in soil samples. The herbicide and its degradation product were extracted twice from soil samples, after acidification, by dichloromethane on ultrasound system for 1 h. Both extracts were combined and filtrated in qualitative filter paper and Celitee. The total extract was concentrated in rotatory evaporator, dried under N₂ and finally dissolved in 1 ml of methanol. High Performance Liquid Chromatography with UV detection at 230 nm was used for analysis. Recoveries were obtained from soil samples fortified at 0.1, 1.0, 2.0, 3.0 and 4.0 mg kg⁻¹ levels and the results varied from 85 to 111% (for 2,4-D) and from 95 to 98% (for 2,4-DCP). For both compounds, the limits of quantification were 0.1 mg kg⁻¹, which were the loss level at which the accuracy and the precision were studied. Nevertheless, the limits of detection, calculated by considering the blank standard deviation and the minimum concentration level, were 0.03 and 0.02 mg kg for 2,4-D and 2,4-DCP, respectively. This proposed method was applied to soil samples of eucalyptus crops, which was previously treated with the herbicide. Despite that, neither 2,4-D nor its degradation product were detected 30 days after the herbicide application. (C) 2003 Elsevier Science B.V. All rights reserved.

de Jesus, R. P. and D. J. Faulkner (2003). "Chlorinated Acetylenes from the San Diego Sponge *Haliclona lunisimilis*." J. Nat. Prod.; (Technical Note); 2003; ASAP Article.

De Wildeman, S. and W. Verstraete (2003). "The quest for microbial reductive dechlorination of C-2 to C-4 chloroalkanes is warranted." Applied Microbiology and Biotechnology 61(2): 94-102.

C-2 to C-4 chloroalkanes have been used for a wide range of industrial applications. Consequently, numerous leaks to the environment have occurred. It is generally observed that the lower chlorinated members of the group, containing 1-3 chlorine atoms, accumulate in environments where reductive conditions prevail. Their half-lives under these conditions often exceed several decades. To date, successes in rapid and complete in situ reductive dechlorination have only been obtained with tetrachloroethene (PCE) and trichloroethene (TCE), but not with chloroalkanes. Since the key-player PCE- and TCE-dechlorinating bacteria involved have been studied, these organisms could be used as very efficient tools for low-cost in situ bioremediation. Except for one 1,2-dichloroethane- dehalorespiring bacterium with limited application possibilities and a recent isolate which partly dechlorinates some polychloroethanes, all bacterial reductive conversions of C-2 to C-4 chloroalkanes are based on slow, mostly incomplete and poorly controllable cometabolic dechlorinations. Furthermore, metals such as Fe(0) cannot dechlorinate most lower-chlorinated C-2 to C-4 alkanes. Hence, pump and treat, or aerobic degradation are the applied technologies, although they are expensive and time-intensive. However, energetic consideration of chloroalkane dechlorination suggests that metabolizing anaerobes may exist. Isolation and characterization of these organisms is warranted in order to develop cost-efficient, controlled, fast and complete in situ remediation technologies.

Den, W., C. P. Huang, et al. (2003). "Biotrickling filtration for control of volatile organic compounds from microelectronics industry." Journal of Environmental Engineering-Asce 129(7): 610-619.

This study investigated the transient and steady-state performance of a bench-scale biotrickling filter for the removal of an organic mixture (acetone, toluene, and trichloroethylene) typically emitted by the microelectronics industry. The, microbial consortium consisting of seven bacterial strains that were fully acclimated prior to inoculation onto activated carbon media. Among the seven strains, the *Pseudomonas* and *Sphingomonas* strains appeared to be the major groups degrading toluene (> 25 ppmv/h (.) 10⁸ cell) and trichloroethylene (>2.3 ppmv/h. 10⁸.) cell), while *Mycobacteria* and *Acetobacteriaceae* strains were the primary decomposers of acetone (> 90 ppmv/h (.) 10⁸ cell). The column performance was evaluated by examining its responses to the fluctuating influent total hydrocarbon concentrations, which varied from 850 to 2,400 ppmv. Excellent steady-state removal efficiencies greater than 95% were consistently observed, and system recovery was

typically within two days after a significant increase, in the inlet loading was experienced. The overall mass-transfer rate and the biokinetic constants were determined for each organic component. Mathematical simulations based on these parameters demonstrated that the removal of acetone was kinetically limiting, whereas the removals of toluene and trichloroethylene were, at least partially mass-transfer limiting.

Dercova, K., R. Tandlich, et al. (2003). "Application of terpenes as possible inducers of biodegradation of PCBs." *Fresenius Environmental Bulletin* 12(3): 286-290.

The study evaluates the effect of two terpenes, carvone and limonene, as potential inducers of the PCB-degrading pathway of a commercial mixture of PCBs, DELOR 103. The purpose was to find out whether addition of terpenes stimulates biodegradation and how this effect depends on terpene concentrations. The addition of the studied terpenes, which could not serve as a sole carbon source, exerted an enhancing effect on PCB degradation when xylose was used as the carbon source and carvone as the possible inducer. In this case, already 7-37% of the individual PCB congeners were degraded without carvone addition, while 30-70% of the congeners were degraded after carvone addition. Good water solubility of carvone, its low costs and effectiveness make it a potential inducer for practical cleanup of DELOR-contaminated soil.

Dutta, S. K., A. Adam, et al. (2003). "Indigenous mixed soil bacteria in presence of compatible plants are more efficient in PCB degradation." *Fresenius Environmental Bulletin* 12(3): 314-319.

This paper presents data on comparative studies of unknown mixed cultures of indigenous microorganisms versus single cultures isolated from PCB-contaminated soil samples containing varying concentrations of PCBs (polychlorinated biphenyls). Remediation of PCBs was studied using isolated indigenous mixed cultures, by indigenous single colony cultures, in presence or absence of the leguminous alfalfa (*Medicago sativa*) plant. The congener 2', 3, 4-PCB was used as known standard to spike the mixture of clean soil and vermiculite. Analytical methods such as HPLC (high performance liquid chromatography) and GC (gas chromatography) were used for PCB degradation quantification. The plant experiments were carried out in a controlled growth chamber environment with a 16-hr fluorescent and incandescent light period which mimicked day light. Results obtained suggest that higher the PCB concentration in soil, the more efficient the indigenous soil bacteria particularly in presence of plants. Mixed indigenous cultures were 2-3 times more efficient than single cultures. When compared to known PCB co-metabolizing bacteria such as *Comamonas testosteroni* and *Rhodococcus* sp., indigenous bacteria showed not only higher amount of PCB degradation, but also significantly better growth as evidenced by the CFU (colony forming units) counts. When the alfalfa plant was present, the indigenous mixed cultures were even more effective than known and unknown single cultures. These findings suggest that a combination of endemic microorganisms utilized with alfalfa plant is a promising approach for bioremediation of PCB-contaminated soils.

Fahimi, I. J., F. Keppler, et al. (2003). "Formation of chloroacetic acids from soil, humic acid and phenolic moieties." *Chemosphere* 52(2): 513-520.

The mechanism of formation of chloroacetates, which are important toxic environmental substances, has been controversial. Whereas the anthropogenic production has been well established, a natural formation has also been suggested. In this study the natural formation of chloroacetic acids from soil, as well as from humic material which is present in soil and from phenolic model substances has been investigated. It is shown that chloroacetates are formed from humic material with a linear relationship between the amount of humic acid used and chloroacetates found. More dichloroacetate (DCA) than trichloroacetate (TCA) is produced. The addition of Fe²⁺, Fe³⁺ and H₂O₂ leads to an increased yield. NaCl was added as a source of chloride. We further examined the relationship between the structure and reactivity of phenolic substances, which can be considered as monomeric units of humic acids. Ethoxyphenol with built-in ethyl groups forms large amounts of DCA and TCA. The experiments with phenoxyacetic acid yielded large amounts of monochloroacetate (MCA). With other phenolic substances a ring cleavage was observed. Our investigations indicate that chloroacetates are formed abiotically from humic material and soils in addition to their known biotic mode of formation.

Fava, F., G. Zanaroli, et al. (2003). "Microbial reductive dechlorination of pre-existing PCBs and spiked 2,3,4,5,6-pentachlorobiphenyl in anaerobic slurries of a contaminated sediment of Venice Lagoon (Italy)." *FEMS Microbiology Ecology* 44(3): 309-318.

Reductive dechlorination of polychlorinated biphenyls (PCBs) pre-existing (at [ap]1 mg kg⁻¹) in a marine sediment of Porto Marghera (Venice Lagoon, Italy) was investigated in anaerobic slurries developed in water of the same contaminated site. Some microcosms were pasteurized whereas others were amended with 2-bromoethanesulfonic acid, molybdate or eubacteria-inhibiting antibiotics (without and in the presence of exogenous carbon sources) to preliminarily characterize the microbial populations involved in the process. Bioconversion of highly chlorinated PCBs into tri- and di-chlorinated, ortho-substituted biphenyls was detected from the 11th week of incubation both in the non-amended and in the pasteurized microcosms, where a significant consumption of sulfate and no methane production were observed. Conversely, no significant PCB transformation was detected in the microcosms with molybdate, where no sulfate consumption and a significant methane evolution occurred. Neither was PCB transformation observed in the microcosms supplemented with antibiotics and exogenous carbon sources, where a strong methane evolution and no sulfate consumption were recorded until the 11th week. The addition of exogenous 2,3,4,5,6-pentachlorobiphenyl showed preferential dechlorination at the meta and para positions, and did not significantly influence the onset of pre-existing PCB dechlorination. These results indicate that endogenous PCBs pre-existing in the marine sediment underwent reductive dechlorination. They also suggest that the process was not 'primed' upon 2,3,4,5,6-pentachlorobiphenyl addition, and was likely to be mediated by sulfate-reducing, spore-forming bacteria.

Felsot, A. S., K. D. Racke, et al. (2003). Disposal and degradation of pesticide waste. *Reviews of Environmental Contamination and Toxicology*, Vol 177. 177: 123-200.

Fuse, H., O. Takimura, et al. (2003). "Degradation of chlorinated biphenyl, dibenzofuran, and dibenzo-p-dioxin by marine bacteria that degrade biphenyl, carbazole, or dibenzofuran." *Bioscience Biotechnology and Biochemistry* 67(5): 1121-1125.

Marine bacterial strains (BP-PH, CAR-SF, and DBF-MAK) were isolated using biphenyl, carbazole (CAR), or dibenzofuran (DF) respectively as substrates for growth. Their 16S ribosomal DNA sequences showed that the species closest to strain BP-PH, strain CAR-SF, and strain DBF-MAK are *Alteromonas macleodii* (96.3% identity), *Neptunomonas naphthovorans* (93.1% identity), and *Cycloclasticus pugetii* (97.3% identity), respectively. The metabolites produced suggested that strain CAR-SF degrades CAR via dioxygenation in the angular position and by the meta- cleavage pathway, and that strain DBF-MAK degrades DF via both lateral and angular dioxygenation. Polychlorinated biphenyl (KC-300) and 2,3-dichlorodibenzo-p-dioxin were partially degraded by strain BP-PH and strain DBF-MAK, while 2,7- dichlorodibenzo-p-dioxin and 2,4,8-trichlorodibenzofuran remained virtually unchanged.

Gilmartin, N., D. Ryan, et al. (2003). "BphK shows dechlorination activity against 4-chlorobenzoate, an end product of bph-promoted degradation of PCBs." *Fems Microbiology Letters* 222(2): 251-255.

A bphK gene encoding glutathione S-transferase (GST) activity is located in the bph operon in *Burkholderia* sp. strain LB400 but its role in polychlorinated biphenyl (PCB) metabolism is unknown. This gene was over-expressed in *Escherichia coli* and an in vivo assay based on growth of *E. coli* containing GST activity was used to identify potential novel substrates for this enzyme. Using this assay, 4-chlorobenzoate (4-CBA) was identified as a substrate for the BphK enzyme. High pressure liquid chromatography analysis and chloride ion detection showed removal of 4-CBA and an equivalent increase of chloride in cell extracts when incubated with this enzyme. These results would indicate that this BphK enzyme has dechlorination activity in relation to 4-CBA and may have a role in protection of other Bph enzymes against certain chlorinated metabolites of PCB

degradation. (C) 2003 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Gribble, G. W. (2003). "The diversity of naturally produced organohalogens." *Chemosphere* 52(2): 289-297.

More than 3800 organohalogen compounds, mainly containing chlorine or bromine but a few with iodine and fluorine, are produced by living organisms or are formed during natural abiogenic processes, such as volcanoes, forest fires, and other geothermal processes. The oceans are the single largest source of biogenic organohalogens, which are biosynthesized by myriad seaweeds, sponges, corals, tunicates, bacteria, and other marine life. Terrestrial plants, fungi, lichen, bacteria, insects, some higher animals, and even humans also account for a diverse collection of organohalogens.

He, J. Z., K. M. Ritalahti, et al. (2003). "Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a *Dehalococcoides* species." *Applied and Environmental Microbiology* 69(2): 996-1003.

A major obstacle in the implementation of the reductive dechlorination process at chloroethene-contaminated sites is the accumulation of the intermediate vinyl chloride (VC), a proven human carcinogen. To shed light on the microbiology involved in the final critical dechlorination step, a sediment-free, nonmethanogenic, VC-dechlorinating enrichment culture was derived from tetrachloroethene (PCE)-to-ethene-dechlorinating microcosms established with material from the chloroethene-contaminated Bachman Road site aquifer in Oscoda, Mich. After 40 consecutive transfers in defined, reduced mineral salts medium amended with VC, the culture lost the ability to use PCE and trichloroethene (TCE) as metabolic electron acceptors. PCE and TCE dechlorination occurred in the presence of VC, presumably in a cometabolic process. Enrichment cultures supplied with lactate or pyruvate as electron donor dechlorinated VC to ethene at rates up to 54 $\mu\text{mol liter}^{-1} \text{day}^{-1}$, and dichloroethenes (DCEs) were dechlorinated at about 50% of this rate. The half-saturation constant (K_s) for VC was 5.8 μM , which was about one-third lower than the concentrations determined for *cis*-DCE and *trans*-DCE. Similar VC dechlorination rates were observed at temperatures between 22 and 30°C, and negligible dechlorination occurred at 4 and 35°C. Reductive dechlorination in medium amended with ampicillin was strictly dependent on H₂ as electron donor. VC-dechlorinating cultures consumed H₂ to threshold concentrations of 0.12 ppm by volume. 16S rRNA gene-based tools identified a *Dehalococcoides* population, and *Dehalococcoides*-targeted quantitative real-time PCR confirmed VC-dependent growth of this population. These findings demonstrate that *Dehalococcoides* populations exist that use DCEs and VC but not PCE or TCE as metabolic electron acceptors.

He, J. Z., K. M. Ritalahti, et al. (2003). "Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium." *Nature* 424(6944): 62-65.

Tetrachloroethene (PCE) and trichloroethene (TCE) are ideal solvents for numerous applications, and their widespread use makes them prominent groundwater pollutants. Even more troubling, natural biotic and abiotic processes acting on these solvents lead to the accumulation of toxic intermediates (such as dichloroethenes) and carcinogenic intermediates (such as vinyl chloride)(1-4). Vinyl chloride was found in at least 496 of the 1,430 National Priorities List sites identified by the US Environmental Protection Agency, and its precursors PCE and TCE are present in at least 771 and 852 of these sites, respectively(5). Here we describe an unusual, strictly anaerobic bacterium that destroys dichloroethenes and vinyl chloride as part of its energy metabolism, generating environmentally benign products (biomass, ethene and inorganic chloride). This organism might be useful for cleaning contaminated subsurface environments and restoring drinking- water reservoirs.

He, Q. and R. A. Sanford (2003). "Characterization of Fe(III) Reduction by Chlororespiring Anaeromyxobacter dehalogenans." *Applied and Environmental Microbiology* 69(5): 2712-2718.

Anaeromyxobacter dehalogenans strain 2CP-C has been shown to grow by coupling the oxidation of acetate to the reduction of ortho-substituted halophenols, oxygen, nitrate, nitrite, or fumarate. In this study, strain 2CP-C was also found to grow by coupling Fe(III) reduction to the oxidation of acetate, making it one of the few isolates capable of growth by both metal reduction and chlororespiration. Doubling times for growth of 9.2 and 10.2 h were determined for Fe(III) and 2-chlorophenol reduction, respectively. These were determined by using the rate of [¹⁴C]acetate uptake into biomass. Fe(III) compounds used by strain 2CP-C include ferric citrate, ferric pyrophosphate, and amorphous ferric oxyhydroxide. The addition of the humic acid analog anthraquinone 2,6-disulfonate (AQDS) increased the reduction rate of amorphous ferric iron oxide, suggesting AQDS was used as an electron shuttle by strain 2CP-C. The addition of chloramphenicol to fumarate-grown cells did not inhibit Fe(III) reduction, indicating that the latter activity is constitutive. In contrast, the addition of chloramphenicol inhibited dechlorination activity, indicating that chlororespiration is inducible. The presence of insoluble Fe(III) oxyhydroxide did not significantly affect dechlorination, whereas the presence of soluble ferric pyrophosphate inhibited dechlorination. With its ability to respire chlorinated organic compounds and metals such as Fe(III), strain 2CP-C is a promising model organism for the study of the interaction of these potentially competing processes in contaminated environments.

Hoekstra, E. J. (2003). "Review of concentrations and chemistry of trichloroacetate in the environment." *Chemosphere* 52(2): 355-369.

This paper reviews the concentrations of trichloroacetate (TCA) in the atmosphere-plant-soil system. Data originate mainly from Europe. The median TCA concentration in rainwater and canopy drip decreased until 1995. From then the median TCA concentration in rainwater remains rather constant while for canopy drip later data are not available. The same seems to hold for concentrations in air although a very limited data set is available. The median concentrations in coniferous needles and groundwater are constant for the period observed. The median TCA concentrations in soil decreased until 1992 and then remained constant. The TCA formation from chlorinated solvents in the atmosphere may explain a substantial percentage of the TCA amount in the atmosphere. The TCA concentrations in rainwater and canopy drip indicate that there will be other sources contributing to 10-50%. Waste incineration, biomass burning and natural formation in the marine boundary layer are potential candidate sources of TCA, but nothing can be said as yet on their TCA emission rates. Anthropogenic emissions of chlorine could also be a source. TCA can be formed from chlorinated solvents by biota. However, for coniferous trees the uptake of TCA from soil may be the predominant route. Biotic and abiotic reactions can cause to formation of TCA in soil, but also formation of TCA from chlorinated solvents by biota that excrete TCA, may contribute. Mass balance calculations of the bioactive soil top layer show that the production rate of TCA in certain soil types could be substantial. The mass balance calculations could not distinguish between natural and anthropogenic sources in soil.

Holscher, T., H. Gorisch, et al. (2003). "Reductive dehalogenation of chlorobenzene congeners in cell extracts of Dehalococcoides sp strain CBDB1." *Applied and Environmental Microbiology* 69(5): 2999-3001.

Enzymatic reductive dehalogenation of tri-, tetra-, penta-, and hexachlorobenzenes was demonstrated in cell extracts with low protein concentration (0.5 to 1 µg of protein/ml) derived from the chlorobenzene-respiring anaerobe Dehalococcoides sp. strain CBDB1. 1,2,3-trichlorobenzene dehalogenase activity was associated with the membrane fraction. Light-reversible inhibition by alkyl iodides indicated the presence of a corrinoid cofactor.

Jin, G., A. J. Englande, et al. (2003). "An integrated treatability protocol for biotreatment/bioremediation of toxic pollutants generated by chemical industries." *Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances & Environmental Engineering* 38(4): 597-607.

To optimize the efficiency of bioremediation, treatability studies are needed to understand the fate of pollutants and environmental conditions under which microorganism growth is promoted and efficient degradation of these pollutants result. This article presents a recommended procedure which may achieve these goals. Results and treatability comparisons for, candidate compounds including carbon, tetrachloride (CT), methyl-tert-butyl-ether (MTBE) and hexachlorobutadiene (HCBT) are presented and discussed. Culture redox potential (ORP) which is an indicator of free electron activity of a system appeared to have a significant impact on CT biodegradation. Optimum biodegradation of CT by *Pseudomonas cepacia* was observed between -100 and -200 mv. Under the optimum environmental conditions established during the batch-scale biotransformation study, 98 to 99.9% of CT and 70% of MTBE introduced into the continuous fixed-biofilm reactor were degraded. The biphasic model simulating biodegradation of CT and MTBE provided an excellent correlation in the fixed-biofilm study and was simple to apply as compared with other models.

Kappler, A. and S. B. Haderlein (2003). "Natural organic matter as reductant for chlorinated aliphatic pollutants." *Environmental Science & Technology* 37(12): 2714-2719.

Humic acids (HA) are ubiquitous redox-active compounds of natural aquatic and soil systems. Here we studied the potential of HA as reductants for chlorinated aliphatic pollutants. To avoid artifacts potentially involved when studying chemically reduced HA, we prepared electrochemically reduced soil, aquatic and synthetic HA, and anthrahydroquinone-2,6-disulfonic acid (AHQDS), a model compound for hydroquinone moieties in HA. Both reduced HA and AHQDS reduced hexachloroethane (HCE) at appreciable rates. Some reduction of HCE by HA, however, occurred even before electrochemical reduction of the humic acids. This indicates that a small fraction of reduced moieties in HA persists at oxic conditions for some time. The initial reaction followed pseudo-first-order reaction kinetics, and tetra chloroethylene was the only halogenated product. The relatively small variations in carbon-normalized rate constants, $k(\text{DOC})$, found indicate that despite inherent variations in concentration, accessibility, and reactivity of redox-active groups in HA of various origins their overall dechlorination activity is fairly constant. However, HCE transformation rate constants and reducing capacities of different HA did not correlate. Rate constants normalized to both carbon content and reducing capacity of HA clearly indicate that reduced functional groups in different HA exhibit different reactivities. Our results together with the fact that reduced HA can be formed by a variety of microbiological and chemical processes suggest that HA could play a significant role as reductants in the reductive transformation of subsurface contaminants and that such a process could potentially be enhanced at contaminated sites by addition of reducible natural organic matter.

Keppler, F. and H. Biester (2003). "Peatlands: a major sink of naturally formed organic chlorine." *Chemosphere* 52(2): 451-453.

It is a little known fact that many chlorinated organic compounds occur naturally and that some are also indispensable to life on earth. Here, we show that chlorination of organic compounds during humification processes in peat is widespread in nature. Globally this process has led to the accumulation of approximately 280-1000 million tons of organically bound chlorine in peatlands during the postglacial period.

Keppler, F., R. Borchers, et al. (2003). "Formation of volatile iodinated alkanes in soil: results from laboratory studies." *Chemosphere* 52(2): 477-483.

Volatile iodinated organic compounds play an important role in the tropospheric photochemical system, but the current knowledge of the known sources and sinks of these alkyl iodides is still incomplete. This paper describes a new source of alkyl iodides from the pedosphere. Different organic-rich soils and humic acid were investigated for their release of volatile organoiodides. Six volatile organoiodides, iodomethane, iodoethane, 1-iodopropane, 2-iodopropane, 1-iodobutane and 2-iodobutane were identified and their release rates were determined. We assume an abiotic reaction mechanism induced by the oxidation of organic matter by iron(III). The influence of iron(III), iodide and pH on the formation of alkyl iodides was investigated. Additionally, different organic substances regarded as monomeric constituents of humus were examined for the production of alkyl

iodides. Two possible reaction pathways for the chemical formation of alkyl iodides are discussed. As humic acids and iron(III) are widespread in the terrestrial environment, and the concentration of iodide in soil is strongly enriched (compared to seawater), this soil source of naturally occurring organoiodides is suggested to contribute significantly to the input of iodine into the troposphere.

Kneidel, A. L. and S. T. Yang (2003). "Kinetic study of trichloroethylene biodegradation by *Methylosinus trichosporium* OB3b PP358 immobilized in a fibrous-bed bioreactor." *Journal of the Chinese Institute of Chemical Engineers* 34(1): 65-73.

Biodegradation of trichloroethylene (TCE) by resting cells of methanotrophic *Methylosinus trichosporium* OB3b PP358, which constitutively expresses soluble methane monooxygenase (sMMO), was studied in a fibrous-bed bioreactor operated in the recycle batch mode. Cells were grown on methane as the substrate with aeration, and then used to degrade TCE through the cometabolism with sMMO in the absence of methane. Complete biodegradation of TCE was verified with the TCE and chloride ion mass balance. In general, TCE biodegradation was faster in the absence of nitrate and with a higher initial TCE concentration of up to similar to 12 mg/l, and followed the first-order reaction kinetics. TCE transformation was inhibited in the presence of methane or methanol. Without the energy source and after being exposed to TCE for an extended period, cells gradually lost most of their capability in degrading TCE, which was attributed to reduced sMMO enzyme activity due to lack of NADH and cell death caused by TCE toxicity and oxygen starvation. However, the reactor was able to recover its TCE degradation ability after rejuvenating and regrowing cells with methane and air. Compared to free cell and other immobilized cell systems, the cells immobilized in the fibrous-bed bioreactor not only showed a much higher TCE degradation rate (up to 84.77 mg/(1 day) or - 32 times of that from free cells), but also had a better tolerance to TCE (22.6 mg/l or similar to 11 times higher than that with free cells). With periodical rejuvenation, the bioreactor could be used for long-term treatment of TCE-contaminated water.

Lenczewski, M., P. Jardine, et al. (2003). "Natural attenuation of trichloroethylene in fractured shale bedrock." *Journal of Contaminant Hydrology* 64(3-4): 151-168.

This paper describes one of the first well-documented field examples of natural attenuation of trichloroethylene (TCE) in groundwater in a fractured shale bedrock. The study was carried out adjacent to a former waste burial site in Waste Area Grouping 5 (WAG5) on the Oak Ridge Reservation, Oak Ridge, TN. A contaminant plume containing TCE and its daughter products were detected downgradient from the buried waste pits, with most of the contamination occurring in the upper 6 in of the bedrock. The monitoring well array consists of a 35-m-long transect of multilevel sampling wells, situated along a line between the waste pits and a seep which discharges into a small stream. Concentrations of volatile organic carbons (VOCs) were highest in the waste trenches and decreased with distance downgradient towards the seep. Sampling wells indicated the presence of overlapping plumes of TCE, cis-dichloroethylene (cDCE), vinyl chloride (VC), ethylene, ethane, and methane, with the daughter products extending further downgradient than the parent (TCE). This type of distribution suggests anaerobic biodegradation. Measurements of redox potential at the site indicated that iron-reduction, sulfate reduction, and potentially methanogenesis were occurring and are conducive to dechlorination of TCE. Bacteria enrichment of groundwater samples revealed the presence of methanotrophs, methanogens, iron-reducing bacteria and sulfate-reducing bacteria, all of which have previously been implicated in anaerobic biodegradation of TCE. 16S rDNA sequence from DNA extracted from two wells were similar to sequences of organisms previously implicated in the anaerobic biodegradation of chlorinated solvents. The combined data strongly suggest that anaerobic biodegradation of the highly chlorinated compounds is occurring. Aerobic biodegradation may also be occurring in oxygenated zones, including near a seep where groundwater exits the site, or in the upper bedrock during seasonal fluctuations in water table elevation and oxygen levels. (C) 2003 Elsevier Science B.V All rights reserved.

Lerch, M. L., M. K. Harper, et al. (2003). "Brominated Polyacetylenes from the Philippines Sponge *Diplastrella* sp." *J. Nat. Prod.*

Luijten, M., J. de Weert, et al. (2003). "Description of *Sulfurospirillum halorespirans* sp nov., an anaerobic, tetrachloroethene-respiring bacterium, and transfer of *Dehalospirillum multivorans* to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. nov." *International Journal of Systematic and Evolutionary Microbiology* 53: 787-793.

An anaerobic, halorespiring bacterium (strain PCE-M2(T) = DSM 13726(T) = ATCC BAA-583(T)) able to reduce tetrachloroethene to cis-dichloroethene was isolated from an anaerobic soil polluted with chlorinated aliphatic compounds. The isolate is assigned to the genus *Sulfurospirillum* as a novel species, *Sulfurospirillum halorespirans* sp. nov. Furthermore, on the basis of all available data, a related organism, *Dehalospirillum multivorans* DSM 12446(T), is reclassified to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. nov.

Lyew, D. and S. Guiot (2003). "Effects of aeration and organic loading rates on degradation of trichloroethylene in a methanogenic-methanotrophic coupled reactor." *Applied Microbiology and Biotechnology* 61(3): 206-213.

The effects of four aeration and four organic loading (OLR) rates on trichloroethylene (TCE) degradation in methanogenic- methanotrophic coupled reactors were studied using ethanol as the carbon source for the methanogens. Microcosm and PCR studies demonstrated that methanotrophs capable of mineralizing TCE and methanogens were present in the biomass throughout the study. The gene for the particulate form of methane monooxygenase (pMMO) was detected by PCR, but not that for the soluble form (sMMO). TCE mineralization by methanotrophs was therefore due primarily to pMMO activity. Low TCE concentrations were measured in effluent and off-gas samples in all cases. Volatilization losses were 0-5%. Dichloroethylene (DCE) was also observed, but vinyl chloride and ethylene were never detected. Changes in the aeration rate had no effect on TCE removal, but did influence DCE degradation. Reductive dechlorination of TCE to DCE was favored at low and no-aeration conditions, and DCE accumulation occurred due to slow DCE degradation. Low DCE levels were observed at the higher aeration rates, which indicated that conditions in these reactors were amenable to the aerobic co-metabolism of TCE and DCE. The OLR did have an effect on TCE removal. TCE and DCE removal were negatively affected when the OLR was increased. An OLR of 0.3 g COD $1(\text{rx})(-1)\text{day}(-1)$ or lower with an aeration rate of 3 $1(02) 1(\text{rx})(-1)\text{day}(-1)$ and higher is the recommended operating condition of a coupled reactor for removal of TCE.

Ma, X., P. J. Novak, et al. (2003). "Evaluation of polyethylene hollow-fiber membranes for hydrogen delivery to support reductive dechlorination in a soil column." *Water Research* 37(12): 2905-2918.

Engineered systems are often needed to supply an electron donor, such as hydrogen (H₂), to the subsurface to stimulate the biological dehalogenation of perchloroethene (PCE) to ethene. A column study was performed to evaluate the ability of gas permeable hollow-fiber membranes to supply H₂ directly to PCE-contaminated groundwater to facilitate bioremediation. Two glass columns were packed with soil obtained from a trichloroethene-contaminated site at Cape Canaveral, Florida, and were fed a minimal medium spiked with PCE (7 μM) for 391 days. The columns were operated in parallel, with one column receiving H₂ via polyethylene hollow-fiber membranes (lumen H₂ pressure of approximately 1 atm) and a control column receiving no H₂. PCE was initially dechlorinated at a similar rate and to a similar extent in both columns, likely due to the presence of soil organic matter that was able to support dechlorination. After 265 days of operation, dechlorination performance declined in the control column and the benefits of membrane-supplied H₂ became evident. Although the membrane-supplied H₂ effectively stimulated PCE dechlorination at the end of the experiment (days 359-391), the system was inefficient in that only 5% of the supplied H₂ was used for dechlorination. Most of the remainder was used to support methanogenesis (94%). Despite the dominance of methanogens, nearly complete dechlorination of PCE to ethene was observed in the H₂-fed column. In addition to the inefficient use of H₂,

operational problems included excessive foulant accumulation on the outside of the membrane fibers and water condensation inside the fibers. Use of alternative membrane materials and changes to the operating approach (e.g. pulsing or supplying H₂ at low partial pressures) may help to overcome these problems so that this technology can provide effective and stable remediation of aquifers contaminated with chlorinated ethenes. (C) 2003 Elsevier Science Ltd. All rights reserved.

Manzano, M. A., J. A. Perales, et al. (2003). "Microbial degradation and chemical oxidation of sandy sediment contaminated with polychlorinated biphenyl." *Environmental Engineering Science* 20(2): 91-101.

This paper reports the results of various biodegradation experiments on polychlorinated biphenyl (PCB)-contaminated sandy sediment employing a mixed culture of acclimatized bacteria. Following the optimization of different variables, the elimination rate achieved of Aroclor 1242 in slurry phase reactors was 61% after 4 months of treatment. The presence of biphenyl as a cosubstrate was the most important factor affecting PCB biodegradation. The biodegradation occurred as a first-order process, and proved most effective with respect to dichlorinated (100% removal), followed by trichlorinated (92%) and tetrachlorinated biphenyls (24%). The results of the treatment of polychlorinated biphenyl (PCB) contaminated sandy sediment with the Fenton advanced oxidation process (AOP) confirm that the oxidation process occurs on the PCBs adsorbed to particles, producing 98% elimination of the original PCB structure after 72 h. The degree of elimination was found to be dependent on the level of congener chlorination, and the process follows pseudo first-order kinetics. In addition, the Fenton chemical oxidation process may be complemented by subsequent aerobic biologic degradation which, after 15 days, produces 70% mineralization of the products generated during the chemical oxidation process.

McCue, T., S. Hoxworth, et al. (2003). "Degradation of halogenated aliphatic compounds utilizing sequential anaerobic/aerobic treatments." *Water Science and Technology* 47(10): 79-84.

The objective of this research was to determine if either methanogenic or sulfidogenic reductive dechlorination could survive an alternating anaerobic/aerobic sequence to biologically transform halogenated aliphatic hydrocarbons (HACs), specifically tetrachloroethylene (PCE), trichloroethylene (TCE), cis-1,2 dichloroethylene (cDCE), trans-1,2 dichloroethylene (tDCE), 1,1-dichloroethylene (1,1DCE) and vinyl chloride (VC). This ability was considered to be a necessary prerequisite for complete-anaerobic/aerobic mineralization of halogenated aliphatic hydrocarbons by a single microbial consortia. Chlorinated solvents, which are among the most common groundwater contaminants, have been partially dechlorinated using single-stage anaerobic environmental treatment strategies. Various types of bacteria typically reductively dechlorinate PCE and TCE to cDCE and VC in an anaerobic environment, including methanogens, sulfidogens, and homoacetogens. The problem lies in the fact that reductive dechlorination typically leads to an accumulation of daughter compounds (cDCE, VC) which are more toxic than their parent compounds (PCE, TCE). Furthermore, PCE and (to a lesser extent) TCE, are resistant to dechlorination in aerobic environments. In contrast, VC and cDCE are readily oxidized co-metabolically in an aerobic environment by methanotrophic bacteria, and others using oxygenases (e.g. toluene oxidizers). Results from this research showed that both methanogenic and sulfidogenic reductive dechlorination could resume after transient exposures to both oxygen and hydrogen peroxide (H₂O₂). In fact; for cycles as frequent as 10 days between aerobic treatment cycles, reductive dechlorination was observed to resume at rates at least as rapid as microcosms not exposed to aerobic treatments:

Mori, T., S. Kitano, et al. (2003). "Biodegradation of chloronaphthalenes and polycyclic aromatic hydrocarbons by the white-rot fungus *Phlebia lindtneri*." *Applied Microbiology and Biotechnology* 61(4): 380-383.

The biodegradation of chloronaphthalene (CN) and polycyclic aromatic hydrocarbons by the white-rot fungus *Phlebia lindtneri*, which can degrade dichlorinated dioxins and non-chlorinated dioxin-like compounds, was investigated. Naphthalene, phenanthrene, 1-chloronaphthalene (1-CN) and 2-chloronaphthalene (2-CN) were metabolized by the fungus to form several oxidized products. Naphthalene and phenanthrene were metabolized to

the corresponding hydroxylated and dihydrodihydroxylated metabolites. 2-CN was metabolized to 3-chloro-2-naphthol, 6-chloro-1-naphthol and two other chloronaphthols, CN-dihydrodiols and CN-diols. Significant inhibition of the degradation of these substrates was observed when they were incubated with the cytochrome P-450 monooxygenase inhibitors 1-aminobenzotriazole and piperonyl butoxide. These results suggest that *P. lindtneri* initially oxidizes these substrates by a cytochrome P-450 monooxygenase.

Murphy, C. D., C. Schaffrath, et al. (2003). "Fluorinated natural products: the biosynthesis of fluoroacetate and 4-fluorothreonine in *Streptomyces cattleya*." *Chemosphere* 52(2): 455-461.

Organofluorine compounds are rare in Nature, with only a handful known to be produced by some species of plant and two microorganisms. Consequently, the mechanism of enzymatic carbon-fluorine bond formation is poorly understood. The bacterium *Streptomyces cattleya* biosynthesizes fluoroacetate and 4-fluorothreonine as secondary metabolites and is a convenient system to study the biosynthesis and enzymology of fluorometabolite production. Using stable-isotope labelled precursors it has been shown that there is a common intermediate in the biosynthesis of the fluorometabolites, which has recently been identified as fluoroacetaldehyde. Studies with cell-free extracts of *S. cattleya* have identified two enzymes, an aldehyde dehydrogenase and a threonine transaldolase, that are involved in the biotransformation of fluoroacetaldehyde to fluoroacetate and 4-fluorothreonine.

Nawab, A., A. Aleem, et al. (2003). "Determination of organochlorine pesticides in agricultural soil with special reference to [gamma]-HCH degradation by *Pseudomonas* strains." *Bioresource Technology* 88(1): 41-46.

Soil samples were taken from different agricultural fields and analyzed for organochlorine pesticide residues by gas chromatography. The analysis indicated that the soil samples contained some common organochlorine pesticides DDT, DDD, DDE, HCH and Aldrin. [gamma]-HCH was detected as 47.35 ppb whereas the concentrations of [alpha]-HCH, [beta]-HCH, p,p'-DDE, o,p'-DDT were 38.81, 1.79, 7.10 and 13.30 ppb, respectively, in the same soil. Two *Pseudomonas* strains isolated from agricultural soil were found to possess [gamma]-hexachlorocyclohexane degrading ability when the isolates were grown in a mineral salt medium containing [gamma]-HCH as the sole source of carbon and a number of metabolites were produced and detected by the gas chromatography. These bacterial isolates were further tested for carbohydrate and amino acid utilization as well as for their susceptibility against 10 commonly used antibiotics namely amoxicillin, chloramphenicol, cloxacillin, doxycycline, methicillin, nalidixic acid, neomycin, nitrofurantoin, streptomycin and tetracycline. Both the isolates were also screened for plasmid DNA and found to harbour a single plasmid.

Oh, E. T., S. C. Koh, et al. (2003). "Plant terpenes enhance survivability of polychlorinated biphenyl (PCB) degrading *Pseudomonas pseudoalcaligenes* KF707 labeled with *gfp* in microcosms contaminated with PCB." *Journal of Microbiology and Biotechnology* 13(3): 463-468.

Polychlorinated biphenyls are toxic pollutants and their degradation is quite slow in the environment. Recently, interest in bioremediation using PCB-degrading bacteria has increased. In a previous report, plant terpenes (p-cymene, (S)-(-)-limonene, alpha-pyrene, and alpha-terpinene) have been found to be utilized by a PCB degrader and to induce the biphenyl dioxygenase gene in pure culture. In this study, *Pseudomonas pseudoalcaligenes* KF707, a PCB-degrading Gram-negative soil bacterium, was used to determine whether the terpene stimulation of PCB degrader occurred in the natural environment. First, *P. pseudoalcaligenes* KF707 was genetically tagged using a transposon with *gfp* (green fluorescent protein) as a reporter gene. The population dynamics of *P. pseudoalcaligenes* KF707 harboring *gfp* gene in a PCB-contaminated environment was examined with or without terpenoids added to the microcosm. About 10-100-fold increase was found in the population of PCB degraders when terpene was added, compared with control (non-terpenes samples and biphenyl added samples). It was proposed that the *gfp*-monitoring system is very useful and terpenes enhance the survivability of PCB degraders in PCB-contaminated environments.

Perez, T., E. Wafo, et al. (2003). "Marine sponges as biomonitor of polychlorobiphenyl contamination: Concentration and fate of 24 congeners." *Environmental Science & Technology* 37(10): 2152-2158.

The aim of this study was first to assess the relevance of a marine sponge, *Spongia officinalis*, as a biomonitor of PCB. Twenty-four chlorobiphenyl congeners have been measured along a pollution gradient both in sponges and seawater. *S. officinalis* displays a capacity to accumulate all types of congeners. The highest concentration factors were found for hexa- and heptachlorobiphenyls. Concentrations recorded in sponges agreed quite well with the PCB concentrations of study sites. The prevalence of CB138 and CB153 definitely demonstrated the urban origin of the PCB detected, despite the ban on their production and the existence of a wastewater treatment plant since 1987. The CB138/CB153 ratio is similar to 1.2 in commercial mixtures as well as in seawater. In sponges, this ratio varies strongly in space and time, from 1 in sponges at the most polluted site to 0.3 at the reference site. This change in the ratio of these two very persistent congeners, which is not observed in seawater, indicates a metabolism of CB138 in sponges. As it was recently demonstrated for nonpersistent organic contaminants, sponges might well be able to degrade PCB, but further work is needed to identify the processes involved.

Pferdeort, V. A., T. K. Wood, et al. (2003). "Proteomic changes in Escherichia coli TG1 after metabolic engineering for enhanced trichloroethene biodegradation." *Proteomics* 3(6): 1066-1069.

Through metabolic engineering, new enzymatic pathways can be introduced into cells to enable or enhance production or biotransformation of chemicals. However, these changes have physiological consequences that can be important but are not well understood. Here we describe the use of two-dimensional gel electrophoresis (2-DE) to detect changes in the proteome of *Escherichia coli* cells that have been engineered to transform the pollutant trichloroethene (TCE) with the enzyme toluene *o*-monooxygenase (TOM). Comparison of 2-DE gels (isoelectric point range 4-7) for *E. coli* cells with and without the ability to synthesize TOM revealed 31 new proteins in TOM-containing cells as well as nine proteins not detected in those cells but present in the plasmid control strain. Exposure of TOM-containing cells to TCE led to the synthesis of four new proteins and the loss of only one protein. Thus, this example of metabolic engineering has a substantial and complex impact on the physiology of these cells that was clearly revealed using a proteomic approach.

Prewitt, M. L., H. Borazjani, et al. (2003). "Soil-enhanced microbial degradation of pentachlorophenol-treated wood." *Forest Products Journal* 53(6): 44-50.

This research examined the addition of soil to enhance the microbial depletion of two pentachlorophenol (PCP) concentrations in wood flakes from a treated utility pole. Treatments in the first study consisted of wood flakes containing 1540 µg/g PCP mixed with and without soil or microorganisms. By the end of the study, the PCP concentration was reduced by approximately 40 percent in treatments containing wood or wood plus bacterium and reduced by 68 percent in wood inoculated with fungus. PCP reduction was 40, 88, and 95 percent in treatments containing wood plus autoclaved soil and wood plus autoclaved soil plus a bacterium or fungus, respectively. In non-autoclaved soil treatments, PCP concentration was reduced by 88 percent or greater with or without added bacterium or fungus. The addition of non-autoclaved soil significantly enhanced the degradation of PCP compared to autoclaved soil. Degradation was initially faster in fungal- than bacterial-inoculated soil treatments but was equal at the end of the study. The reduction in PCP concentration also corresponded to a reduction in toxicity. Treatments for the second study consisted of PCP-treated wood flakes (15,000 µg/g), wood flakes plus non-autoclaved soil, and wood flakes plus non-autoclaved soil plus added bacterium or fungus. The PCP concentration in the wood flakes was reduced by 32 percent in treatments containing soil plus bacterium and 45 percent in treatments containing soil plus fungus. Results from both studies indicated that non-autoclaved soil addition significantly enhanced microbial degradation of PCP in wood.

Putschew, A., M. Mania, et al. (2003). "Occurrence and source of brominated organic compounds in surface waters." *Chemosphere* 52(2): 399-407.

Monitoring of organic halogen compounds, measured as adsorbable organically bound bromine (AOBr), in an eutrophic lake, which is influenced by treated waste water, revealed repeatedly high concentrations of

organobromine compounds in late summer, whereas five times lower values were measured during the rest of the year. It was possible to reproduce the in situ observed AOB_r increase in the laboratory. Batch experiments were performed with lake water from two different lakes and an algae culture. It could be shown that the AOB_r production is not limited to strong waste water influenced lakes. Furthermore, the AOB_r formation requires light and the presence of algae, and thus is most probably biotic in nature. A low content of nutrients favours the formation of organic bromine compounds. To our knowledge this is the first report about the seasonally occurrence of naturally produced organic bromine compounds in lakes/surface waters.

Quan, X. C., H. C. Shi, et al. (2003). "Biodegradation of 2,4-dichlorophenol in an air-lift honeycomb-like ceramic reactor." *Process Biochemistry* 38(11): 1545-1551.

A novel air-lift bioreactor, with a honeycomb-like ceramic column packed in the inner draft tube as the carrier for immobilization of microbial cells, was developed in this laboratory. A microorganism, identified as *Achromobacter* sp. and capable of degrading 2,4-dichlorophenol (2,4-DCP), was immobilized in the ceramic carrier and used for biodegradation of 2,4-DCP. Semi-continuous biodegradation of 2,4-DCP as a single substrate and in the presence of phenol as co-substrate was investigated. The results showed that when 2,4-DCP occurs alone, its biodegradation rate increased gradually from Run 1 to Run 6 and the degradation process could be described with zero-order kinetics model. When phenol was used as co-substrate, the existence of phenol could inhibit the biodegradation of 2,4-DCP and the biodegradation rate of 2,4-DCP decreased gradually. However, the biodegradation of phenol increased with the increase of run number of the batch experiments. In addition, continuous degradation of 2,4-DCP was also investigated. The results indicated that 2,4-DCP at the concentration ranged from 6.86 to 102.38 mg l⁻¹ could be degraded at a dilution rate of 0.16 h⁻¹ and the removal percentage ranged between 84 and 100%. The effect of interruption of 2,4-DCP supply to the bioreactor on the degradation ability of microbial cells was investigated by replacing 2,4-DCP with sodium acetate as the sole carbon source for 12 days. Intermission of 2,4-DCP supply did not cause the loss of chlorophenol-degrading ability. (C) 2003 Elsevier Science Ltd. All rights reserved.

Ryslava, E., Z. Krejci, et al. (2003). "Study of PCB degradation in real contaminated soil." *Fresenius Environmental Bulletin* 12(3): 296-301.

In this study three different plant species (tobacco, black nightshade and alfalfa) were cultivated in a real soil from an industrial site, long-term contaminated by PCBs. After 6 months the decrease of PCB content in soil and PCBs accumulation in plant tissue were analysed. The highest decrease of PCB concentration was measured in soil vegetated with tobacco (34% comparing to bulk soil). Bacteria, which potentially participate in biodegradation of PCBs were isolated from polluted soil and tested for the presence of known genes of biphenyl operon by PCR amplification of *bphA1* and *bphC* genes. The degradative capabilities were confirmed in presence of biphenyl as co-substrate in liquid minimal medium at laboratory conditions. Bacterial strains showing the best growth and degradative properties were taxonomically identified by determination of nucleotide sequence of 16S ribosomal DNA. Strain JAB1 efficiently degrading PCBs (56% of original concentration) was identified as *Pseudomonas pseudoalcaligenes*.

Sato, A., T. Watanabe, et al. (2003). "Enhancement of biodegradation of 2,7-dichlorodibenzo-p-dioxin by addition of fungal culture filtrate." *World Journal of Microbiology & Biotechnology* 19(4): 439-441.

The hydrophilicity of 2,7-dichlorodibenzo-p-dioxin (2,7-DCDD), a model dioxin compound, increased when incubated with the culture filtrates of several strains of fungi. The possibility that the addition of these filtrates could enhance the biodegradation of 2,7-DCDD by the white-rot basidiomycetous fungus *Phanerochaete sordida* YK-624 was examined. The decrease of 2,7-DCDD after 3 weeks incubation in a YK-624 culture containing these filtrates was greater (30%) than that in the culture of YK-624 alone (15%). This is the first report describing the enhancement of dioxin decrease by the addition of a fungal filtrate.

Scholer, H. F., F. Keppler, et al. (2003). "Fluxes of trichloroacetic acid between atmosphere, biota, soil, and groundwater." *Chemosphere* 52(2): 339-354.

Trichloroacetic acid (TCA), in former times used as a herbicide in agriculture, is now ubiquitous and almost evenly distributed in precipitations of the Northern and Southern Hemisphere, despite larger emissions of the possible precursors tetrachloroethene and 1,1,1-trichloroethane in the Northern Hemisphere. The permanent input of a herbicidal compound into most vulnerable ecosystems might lead to adverse effects to biota (plants, microorganisms, etc.). TCA soil levels of coniferous forests in mountainous regions of Central Europe are significantly elevated. Mass balance calculations show that precipitation as sole source of TCA in soil seems to be of minor importance and provide evidence for a natural formation of TCA within soil itself. In addition, the isolation of a chlorinating enzyme in soil and laboratory experiments with humic acid, iron and halide point to an omnipresent chlorinating capability of nature producing polyhalogenated organic compounds such as TCA. In this paper we present an overview of TCA levels in the environment and provide a new estimate about the extent of a natural TCA formation, especially in soil.

Scholer, H. F., K. H. van Pee, et al. (2003). "Special Issue on Naturally Produced Organohalogenes." *Chemosphere* 52(2): 287-287.

Schroder, P., M. Matucha, et al. (2003). "Uptake, translocation and fate of trichloroacetic acid in a Norway spruce/soil system." *Chemosphere* 52(2): 437-442.

Trichloroacetic acid (TCA) is a secondary atmospheric pollutant formed by photooxidation of chlorinated solvents in the troposphere--it has, however, recently been ranked among natural organohalogenes. Its herbicidal properties might be one of the factors adversely affecting forest health. TCA accumulates rapidly in conifer needles and influences the detoxification capacity in the trees. The aim of the investigations--a survey of which is briefly given here--was to elucidate the uptake, distribution and fate of TCA in Norway spruce. For this purpose young nursery-grown plants of Norway spruce (*Picea abies* (L.) Karst.) were exposed to [^{14}C]TCA and the fate of the compound was followed in needles, wood, roots, soil and air with appropriate radio-indicator methods. As shown by radioactivity monitoring, the uptake of TCA from soil by roots proceeded most rapidly into current needles at the beginning of the TCA treatment and was redistributed at later dates so that TCA content in older needles increased. The only product of TCA metabolism/biodegradation found in the plant/soil-system was CO_2 (and corresponding assimilates). TCA biodegradation in soil depends on TCA concentration, soil humidity and other factors.

Schuth, C., H. Taubald, et al. (2003). "Carbon and hydrogen isotope effects during sorption of organic contaminants on carbonaceous materials." *Journal of Contaminant Hydrology* 64(3-4): 269-281.

Stable carbon and hydrogen isotopes can be an efficient means to validate biodegradation of organic contaminants in groundwater since it results in an isotopic fractionation. A prerequisite in applying this method in the field is the proof that other processes decreasing the contaminant concentration are conservative with respect to isotope effects. In this paper we show for carbon isotopes of halogenated hydrocarbon compounds [trichloroethene (TCE), cis-dichloroethene (c-DCE), vinylchloride (VC)] and carbon and hydrogen isotopes of BTEX compounds (benzene, toluene, p-xylene) that no significant fractionation occurs during equilibrium sorption onto activated carbon, lignite coke and lignite. In general, effects were in the range of the reproducibility limit of the analytical instrument (0.5 parts per thousand for $\delta^{13}\text{C}$, and 8 parts per thousand for $\delta^2\text{H}$). This observation was made for fractions sorbed of less than 5% to more than 95%. Also for rate-limited sorption of TCE onto activated carbon, no significant fractionation in carbon isotopes could be observed. These findings support the assumption that for these classes of compounds, sorption processes in aquifer systems are conservative with respect to isotope effects. (C) 2003 Elsevier Science B.V. All rights reserved.

Shin, S. K., J. E. Kim, et al. (2003). "Isolation and identification of a pentachloronitrobenzene (PCNB) degrading bacterium *Alcaligenes xylosoxidans* PCNB-2 from agricultural soil." *Journal of Microbiology* 41(2): 165-168.

We report a new PCNB-degrading strain (PCNB-2) that is able to utilize and grow on PCNB (100 ppm) as a sole carbon source. This strain was identified as *Alcaligenes xylosoxidans* based upon 16S rDNA sequence analysis, API 20 NE tests and cell membrane lipid analysis. The new PCNB degrader *Alcaligenes xylosoxidans* PCNB-2 could find use in bioremediation of PCNB, which is environmentally persistent.

Silk, P. J. and J. B. Macaulay (2003). "Stereoselective biosynthesis of chloroarylpropane diols by the basidiomycete *Bjerkandera adusta*." *Chemosphere* 52(2): 503-512.

Previously we have shown that 1-arylpropane-1,2-diols are catabolic products of *B. adusta* that are stereoselectively biosynthesized from a C7-unit (ring & benzylic carbon) and a C2-unit as predominantly erythro 1R, 2S enantiomers. In order to probe the mechanism of 1-arylpropane-1,2-diol formation, the products of the incubation of isotopically labelled aromatic aldehydes as substrates with *Bjerkandera adusta* (DAOM 215869) have been characterized. The aromatic aldehydes were benzaldehyde (ring D5) and 4-methoxy- and 4-hydroxybenzaldehydes (ring 13C6). These aldehydes were all stereoselectively incorporated into the corresponding 1-arylpropane-1,2-diols, including the chloro analogues, as well as into the corresponding [alpha]-ketols (phenyl acetyl carbinols (PAC's) and 2-hydroxy propiophenones (2-HPP's)) the presumed precursors of the diols. Benzoic acid (ring D5) was likewise incorporated into the diols, chlorodiols and [alpha]-ketols. These results lead us to conclude that the aromatic aldehydes benzaldehyde, 4-hydroxybenzaldehyde and 4-methoxybenzaldehyde are likely C7-unit precursors in the carboligation reaction(s) that leads to 1-arylpropane-1,2-diol biosynthesis. The metabolic role of the diols remains to be elucidated but they may be important intermediates in CAM (chlorinated anisyl metabolite) aldehyde-alcohol cycling and also act as substrates for the chlorination/hydroxylation enzymes yet to be identified in white rot fungi.

Soriano, A., E. Silla, et al. (2003). "Internal rotation of 1,2-dichloroethane in haloalkane dehalogenase. A test case for analyzing electrostatic effects in enzymes." *Journal of Physical Chemistry B* 107(25): 6234-6238.

1,2-Dichloroethane (DCE) is a prototypical molecule for studying electrostatic solvent effects on molecular conformation as far as rotation around the carbon-carbon bond notably changes the electric properties of the molecule and especially the dipole moment. While the apolar trans conformation is the absolute free energy minimum in the gas phase, solvents of increased polarity relatively favor the population of the gauche conformers. DCE is also a substrate of haloalkane dehalogenase from *Xanthobacter Autotrophicus* (Dh1A), an enzyme that catalyzes the conversion of DCE to 2-chloroethanol. We here investigate the nature of substrate-enzyme interactions, obtaining the free energy profiles of rotation around the C-C bond in the gas phase, in aqueous solution, and in the enzymic environment. In the enzyme only the gauche conformers are free energy minima, the trans conformer being a free energy maximum. Differences between the aqueous solution and enzyme energy profiles are rationalized taking into account the different magnitudes and orientations of the electric field created by the environment in both cases. In aqueous solution DCE feels a reaction field several times lower in modulus than in the enzyme active site. Consequences on enzyme catalysis are also discussed.

Stroo, H. F., M. Unger, et al. (2003). "Remediating chlorinated solvent source zones." *Environmental Science & Technology* 37(11): 224A-230A.

Sung, Y., K. M. Ritalahti, et al. (2003). "Characterization of Two Tetrachloroethene-Reducing, Acetate-Oxidizing Anaerobic Bacteria and Their Description as *Desulfuromonas michiganensis* sp. nov." *Applied and Environmental Microbiology* 69: 2964-2974.

Two tetrachloroethene (PCE)-dechlorinating populations, designated strains BB1 and BRS1, were isolated from pristine river sediment and chloroethene-contaminated aquifer material, respectively. PCE-to-cis-1,2-dichloroethene-dechlorinating activity could be transferred in defined basal salts medium with acetate as the electron donor and PCE as the electron acceptor. Taxonomic analysis based on 16S rRNA gene sequencing placed both isolates within the *Desulfuromonas* cluster in the subdivision of the Proteobacteria. PCE was dechlorinated at rates of at least 139 nmol min⁻¹ mg of protein⁻¹ at pH values between 7.0 and 7.5 and temperatures between 25 and 30°C. Dechlorination also occurred at 10°C. The electron donors that supported dechlorination included acetate, lactate, pyruvate, succinate, malate, and fumarate but not hydrogen, formate, ethanol, propionate, or sulfide. Growth occurred with malate or fumarate alone, whereas oxidation of the other electron donors depended strictly on the presence of fumarate, malate, ferric iron, sulfur, PCE, or TCE as an electron acceptor. Nitrate, sulfate, sulfite, thiosulfate, and other chlorinated compounds were not used as electron acceptors. Sulfite had a strong inhibitory effect on growth and dechlorination. Alternate electron acceptors (e.g., fumarate or ferric iron) did not inhibit PCE dechlorination and were consumed concomitantly. The putative fumarate, PCE, and ferric iron reductases were induced by their respective substrates and were not constitutively present. Sulfide was required for growth. Both strains tolerated high concentrations of PCE, and dechlorination occurred in the presence of free-phase PCE (dense non-aqueous-phase liquids). Repeated growth with acetate and fumarate as substrates yielded a BB1 variant that had lost the ability to dechlorinate PCE. Due to the 16S rRNA gene sequence differences with the closest relatives and the unique phenotypic characteristics, we propose that the new isolates are members of a new species, *Desulfuromonas michiganensis*, within the *Desulfuromonas* cluster of the Geobacteraceae.

Toure, O., Y. Q. Chen, et al. (2003). "Sinorhizobium meliloti electrotransportant containing ortho-dechlorination gene shows enhanced PCB dechlorination." *Fresenius Environmental Bulletin* 12(3): 320-322.

This paper reports that we have successfully electro-transformed competent *S. meliloti* strains using the plasmid pPE43, carrying the oxygenolytic ortho-dechlorination (ohb) gene. The resulting recombinant variant grew on up to 100 ppm of 2', 3, 4-PCB without any adverse effect on its growth and nodule formation ability with the alfalfa plant. Quantification of PCBs was performed using colorimetric methods for chlorine release and BPLC (High Performance Liquid Chromatography). The CFU (colony forming unit) counts allowed us to measure viable count of microorganisms and estimate effect of PCB toxicity on the growth of the *S. meliloti* electrotransformants. The wild type *S. meliloti* depleted 15% PCB whereas its electrotransportant dechlorinated 100%. Nitrogen "fixing" by genetically modified *S. meliloti* not only provides nitrogen to the plants they nodulate, but also leaves behind excess nitrogen in the soil, potentially reducing the need for nitrogen fertilizers in the next growing season.

Travkin, V. M. and L. A. Golovleva (2003). "The degradation of 3,4-dichloroaniline by *Pseudomonas fluorescens* strain 26-K." *Microbiology* 72(2): 240-243.

Trotsenko, Y. A. and N. V. Doronina (2003). "The biology of methylobacteria capable of degrading halomethanes." *Microbiology* 72(2): 121-131.

Recent data on the biology of aerobic methylotrophic bacteria capable of utilizing toxic halogenated methane derivatives as sources of carbon and energy are reviewed, with particular emphasis on the taxonomic, physiological, and biochemical diversity of mono- and dihalomethane-degrading methylobacteria and the enzymatic and genetic aspects of their primary metabolism. The initial steps of chloromethane dehalogenation to formate and HCl through a methylated corrinoid and methyltetrahydrofolate are catalyzed by inducible cobalamin methyl transferase, made up of two proteins (CmuA and CmuB) encoded by the *cmuA* and *cmuB* genes. At the

same time, the primary dehalogenation of dichloromethane to formaldehyde and HCl is catalyzed by cytosolic glutathione transferase with S-chloromethylglutathione as an intermediate. The latter enzyme is encoded by the structural *dcmA* gene and is under the negative control of the regulatory *dcmR* gene. In spite of considerable progress in the study of halomethane dehalogenation, some aspects concerning the structural and functional organization of this process and its regulation remain unknown, including the mechanisms of halomethane transport, the release of toxic dehalogenation products (S-chloromethylglutathione, CH₂O, and HCl) from cells, and the maintenance of intracellular pH. Of particular interest is a quantitative evaluation of the ecophysiological role of aerobic methylotrophs in the mineralization of halomethanes and the protection of the biosphere from these toxic pollutants.

van Pee, K.-H. and S. Unversucht (2003). "Biological dehalogenation and halogenation reactions." *Chemosphere* 52(2): 299-312.

A large number of halogenated compounds is produced by chemical synthesis. Some of these compounds are very toxic and cause enormous problems to human health and to the environment. Investigations on the degradation of halocompounds by microorganisms have led to the detection of various dehalogenating enzymes catalyzing the removal of halogen atoms under aerobic and anaerobic conditions involving different mechanisms. On the other hand, more than 3500 halocompounds are known to be produced biologically, some of them in great amounts. Until 1997, only haloperoxidases were thought to be responsible for incorporation of halogen atoms into organic compounds. However, recent investigations into the biosynthesis of halogenated metabolites by bacteria have shown that a novel type of halogenating enzymes, FADH₂-dependent halogenases, are involved in biosyntheses of halogenated metabolites. In every gene cluster coding for the biosynthesis of a halogenated metabolite, isolated so far, one or several genes for FADH₂-dependent halogenases have been identified.

Van Zwieten, L., M. R. Ayres, et al. (2003). "Influence of arsenic co-contamination on DDT breakdown and microbial activity." *Environmental Pollution* 124(2): 331-339.

The impacts of arsenic co-contamination on the natural breakdown of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) in soil are investigated in a study of 12 former cattle dip sites located in northeastern NSW, Australia. This study examines the relationship between the intrinsic breakdown of DDT to 1, 1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD) and 1, 1-dichloro-2,2-bis(4-chlorophenyl)ethylene (DDE), and the impacts of arsenic co-contamination on this breakdown. Between-site analysis demonstrated that arsenic at 2000 mg/kg gave a 50% reduction in the concentration of DDD compared to background arsenic of 5 mg/kg. Within-site analysis also showed the ratio of DDT:DDD increased in soils as arsenic concentrations increased. This within-site trend was also apparent with the DDT:DDE ratio, suggesting inhibition of DDT breakdown by arsenic co-contamination. Microbial activity was inhibited as residues of total DDTs and arsenic increased. Hence arsenic co-contamination and high concentrations of DDT in soil may result in an increased persistence of DDT in the environment studied. Crown Copyright (C) 2003 Published by Elsevier Science Ltd. All rights reserved.

Varner, R. K., M. L. White, et al. (2003). "Production of methyl bromide in a temperate forest soil." *Geophysical Research Letters* 30(10): art. no.-1521.

[1] Field enclosure measurements of a temperate forest soil show net uptake of ambient methyl bromide (CH₃Br), an important trace gas in both tropospheric and stratospheric ozone cycling. The net flux for 1999 was estimated to be -168 ± 72 μg CH₃Br m⁻² (negative indicates loss from the atmosphere). Individual enclosure flux measurements ranged from 4.0 to +3.3 μg CH₃Br m⁻² d⁻¹. Soil consumption of CH₃Br was estimated from laboratory soil incubations. Production of CH₃Br was calculated as the difference between net flux and predicted consumption. Fungi could be responsible for the production of CH₃Br in this temperate forest soil.

Vetter, W. and W. Jun (2003). "Non-polar halogenated natural products bioaccumulated in marine samples. II. Brominated and mixed halogenated compounds." *Chemosphere* 52(2): 423-431.

Several identified and potential natural brominated bioaccumulative compounds were studied in this work. 4,6-dibromo-2-(2',4'-dibromophenoxy)anisole (BC-2) previously detected in Australian marine mammals and isolated from sponges, was synthesized. Two byproducts (a tetrabromo isomer and a tribromo congener) were investigated as well. The byproducts of the synthesis were not identified in the environmental samples investigated. Previously described natural brominated compounds (BC-1, BC-2, BC-3, BC-10, BC-11, MHC-1) and anthropogenic brominated diphenyl ethers (BDE-47, BDE-99, BDE-100, BDE-154) were detected in a sample of human milk. The sample was from a woman from the Faeroe Islands who frequently consumed fish as well as whale blubber and meat. The most abundant compound originated from the natural tetrabromo phenoxyanisole BC-3 which may have a 3:1 distribution of bromine on the two phenyl units. This sample also accumulated a dibromochloroanisole, as well as a previously unknown mixed halogenated compound (MHC-X) and an unknown, most likely aromatic brominated compound. Co-elutions on a DB-5 column were found for BDE-99 and BC-11 as well as BDE-154 and the unknown brominated compound. This suggests that quantification of these two compounds has to be carried out carefully. Two samples of lower trophic level, namely Baltic cod liver and Mexican mussel tissue, were investigated as well. The cod liver samples contained BDE congeners but also abundant signals for the natural 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole Q1 and tribromoanisole (TBA). The mussel sample contained Q1, TBA, another halogenated anisole, BC-1, BC-2, and BC-3, as well as additional, potential natural brominated compounds in the elution range of tribromophenoxyanisoles.

Vetter, W., W. Jun, et al. (2003). "Non-polar halogenated natural products bioaccumulated in marine samples. I. 2,3,3',4,4',5,5'-Heptachloro-1'-methyl-1,2'-bipyrrole (Q1)." *Chemosphere* 52(2): 415-422.

This presentation adds new spectroscopic and analytical data on the natural product Q1 that was recently identified by synthesis as 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole. Solid state magic angle spinning ¹³C NMR data of Q1 is presented as an option for structural proof. Furthermore, the UV spectrum of neat Q1 (absorption maximum at 223 nm) was recorded and, with NMR spectroscopic data, confirmed a twisted bipyrrole ring system. A quantitative standard of Q1 was prepared which allowed to correct previous concentration estimates relative to the electron capture detector response factor of trans-nonachlor. As a result, the actual Q1 response was only 0.65 ± 15% of the response factor of trans-nonachlor. Therefore, actual Q1 levels are about 50% higher than the previous estimates. With this result the highest (corrected) Q1 concentration determined to date in the blubber of marine mammals from Australia is 14 mg/kg lipid. Analysis of Q1 and trans-nonachlor in specimens from the German North Sea coast suggests that harbor seals are more able to metabolize Q1 than harbor porpoises. Finally, we calculated that 79 congeners of Q1 (i.e. lower chlorinated 1'-methyl-1,2'-bipyrroles) are theoretically possible and present their structures.

Weissflog, L., G. Kruger, et al. (2003). "Input of trichloroacetic acid into the vegetation of various climate zones--measurements on several continents." *Chemosphere* 52(2): 443-449.

Trichloroacetic acid (TCA, CCl₃COOH) is a phytotoxic chemical. Although TCA salts and derivatives were once used as herbicides to combat perennial grasses and weeds, they have since been banned because of their indiscriminate herbicidal effects on woody plant species. However, TCA can also be formed in the atmosphere. For instance, the high-volatile C₂-chlorohydrocarbons tetrachloroethene (TECE, C₂Cl₄) and 1,1,1-trichloroethane (TCE, CCl₃CH₃) can react under oxidative conditions in the atmosphere to form TCA and other substances. The ongoing industrialisation of Southeast Asia, South Africa and South America means that use of TECE as solvents in the metal and textile industries of these regions in the southern hemisphere can be expected to rise. The increasing emissions of this substance--together with the rise in the atmospheric oxidation potential caused by urban activities, slash and burn agriculture and forest fires in the southern hemisphere--could lead to a greater input/formation of TCA in the vegetation located in the lee of these emission sources. By means of biomonitoring studies, the input/formation of TCA in vegetation was detected at various locations in South America, North America, Africa, and Europe.

Williams, D. D. and R. R. Fulthorpe (2003). "Using invertebrate and microbial communities to assess the condition of the hyporheic zone of a river subject to 80 years of contamination by chlorobenzenes." *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 81(5): 789-802.

For over 80 years, chlorobenzenes were discharged into the Sebasticook River, Maine, from a woollen mill. Environmental conditions were assessed using invertebrate and bacterial techniques that were applied to river bed sediments at three contaminated and two reference sites. Invertebrate densities and species richness did not differ markedly among the impacted sites, one reference site, and data in the literature from clean waters. Paradoxically, the highest diversity and densities of invertebrates and their eggs occurred at the most contaminated site. Insect representation was low compared with other hyporheic zones. Although chlorobenzene concentrations were much greater than published limits for freshwater life, certain species (e.g., mayflies, caddisflies, and midges) were associated with high concentrations. The majority of variance in the faunal and microbial data was attributable to redox potential, ammonium levels, and downwelling, rather than to chlorobenzene. Genetic fingerprinting revealed a unique microbial community at the site most heavily contaminated with chlorobenzenes, but a high degree of similarity among the other two mill sites and the reference sites (although the latter proved subsequently to be contaminated with ketones and methyl chloride). There were no differences in taxonomic richness among sites.

Yang, Y. and J. Zeyer (2003). "Specific Detection of Dehalococcoides Species by Fluorescence In Situ Hybridization with 16S rRNA-Targeted Oligonucleotide Probes." *Applied and Environmental Microbiology* 69: 2879-2883.

Dehalococcoides ethenogenes is the only known cultivated organism capable of complete dehalogenation of tetrachloroethene (PCE) to ethene. The prevalence of Dehalococcoides species in the environment and their association with complete dehalogenation of chloroethenes suggest that they play an important role in natural attenuation of chloroethenes and are promising candidates for engineered bioremediation of these contaminants. Both natural attenuation and bioremediation require reliable and sensitive methods to monitor the presence, distribution, and fate of the organisms of interest. Here we report the development of 16S rRNA-targeted oligonucleotide probes for Dehalococcoides species. The two designed probes together encompass 28 sequences of 16S rRNA genes retrieved from the public database. Except D. ethenogenes and CBDB1, all the others are environmental clones obtained from sites contaminated with chlorinated ethenes. They are all closely related and form a unique cluster of Dehalococcoides species. In situ hybridization of probe Dhe1259t with D. ethenogenes strain 195 and two enrichment cultures demonstrated the applicability of the probe to monitoring the abundance of active Dehalococcoides species in these enrichment samples.

Zyakun, A. M., N. V. Doronina, et al. (2003). "The fractionation of chlorine isotopes by the aerobic methylotrophic bacterium Methylobacterium dichloromethanicum grown on dichloromethane." *Microbiology* 72(3): 347-351.

Methylobacterium dichloromethanicum was found to be able to utilize dichloromethane (DCM) as the source of carbon and energy with the production of biomass, CO₂, and HCl. A comparative analysis of the abundances of the major DCM isotopomers; Cl-35(2) (CH₂)-C-12-H-1, (ClCICH₂)-Cl-35-Cl-37-C-12-H-1, and (37)Cl(2)(12)CH(2)1H(2) made it possible to estimate the fractionation of chlorine isotopes during the bacterial metabolism of DCM. The kinetic chlorine isotope effects for (ClCICH₂)-Cl-35-Cl-37-C-12-H-1 (m/z, 86) and (Cl₂CH₂)-Cl-37-C-12-H-1 (m/z 88) relative to (Cl₂CH₂)-Cl-35-C-12-H-1 (m/z 84) were characterized by $\alpha(86/84) = 1.006 \pm 0.002$ and $\alpha(88/84) = 1.023 \pm 0.003$, respectively. The inference is made that the growth of M. dichloromethanicum on DCM is accompanied by the mass-independent fractionation of the DCM isotopomers.