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

8th Quarterly Report
4th Quarter Year 2002

Report prepared for EUROCHLOR

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TABLE OF CONTENTS

| | |
|---|----|
| Acronyms | 3 |
| 1. Introduction | 4 |
| 2. Summary of most important developments | 4 |
| 2.a. Microbial dechlorination | 4 |
| 2.b. Microbial chlorination | 5 |
| 3. Microbial dechlorination | 6 |
| 3.a. General reviews | 6 |
| 3.b. Microbial dechlorination | 7 |
| Vinyl chloride (VC) and other chlorinated ethenes | 7 |
| Carbon tetrachloride (CT) and chloroform (CF) | 11 |
| Chloromethane (CM) and dichloromethane (DCM) | 11 |
| 1,2-Dichloroethane (1,2-DCA) and other chlorinated ethanes | 11 |
| Chlorobenzenes | 13 |
| Chlorinated dibenzo- <i>p</i> -dioxins and -furans (CDDs/CDFs) | 13 |
| Hexachlorobutadiene and octachlorostyrene | 15 |
| Polychlorinated biphenyls (PCBs) | 15 |
| Miscellaneous | 17 |
| 3.c. In vitro degradation of chlorinated compounds | 18 |
| 3.d. New tools & techniques to assess the biodegradation of chlorinated compounds | 19 |
| Isotope fractionation | 20 |
| Characterization of microbial populations | 20 |
| Metabolic Engineering | 20 |
| 4. Microbial chlorination | 22 |
| 4.a. General reviews | 22 |
| 4.b. Microbial chlorination in the environments | 22 |
| Chloromethanes | 22 |
| Other chlorinated compounds | 22 |
| Chlorinated Natural Organic Matter | 23 |
| 4.c. Chlorination by freshwater and marine organisms | 23 |
| Chloromethanes | 23 |
| Other chlorinated compounds | 23 |
| 4.d. Chlorinating enzymes | 23 |
| 5. References Cited | 24 |
| 6. Annex | 30 |

ACRONYMS

| | |
|--------------------|---|
| AOX | Adsorbable Organic Halogen |
| 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 |
| DCA | Dichloroethane |
| DCE | Dichloroethene |
| DCM | Dichloromethane |
| DD | Dibenzo- <i>p</i> -dioxins |
| DDD | 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane |
| DDT | 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane |
| 3,4'- DiCBP | 3,4'-dichlorobiphenyl |
| DiCDD | Di-chlorinated Dibenzo- <i>p</i> -Dioxins |
| DiCDF | Di-chlorinated Dibenzofuran |
| 3,4-DiCPA | Dichlorophenylacetic acid |
| E-acceptor | Electron Acceptor |
| EDB | Ethylene Dibromide or Dibromoethane |
| E-donor | Electron Donor |
| HAC | Halogenated Aromatic Compounds |
| HCH | Hexachlorohexane |
| HPD | 2-hydroxypenta-2,4-dienoate |
| HRT | Hydraulic Retention Time |
| PCBs | Polychlorinated Biphenyls |
| PCE | Tetrachloroethylene |
| PCR | Polymerase Chain Reaction |
| PeCDD | Penta-Chlorinated Dibenzo- <i>p</i> -Dioxins |
| sMMO | Soluble Methane Monooxygenase |
| TBOS | Tetrabutoxysilane |
| TCA | Trichloroethane |
| TCE | Trichloroethylene |
| TeCDD | Tetra- Chlorinated Dibenzo- <i>p</i> -Dioxins |
| TKEBS | Tetrakis(2-ethylbutoxy)silane |
| TrCDD | Tri-Chlorinated Dibenzo- <i>p</i> -Dioxins |
| TSS | Total Suspended Solids |
| VC | Vinyl Chloride |

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

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4th Quarter– Year 2002**

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1. INTRODUCTION

This report presents a review of scientific literature published during the fourth quarter of 2002 (covering November 2002 to January, 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 findings on microbial dechlorination in this quarter are as follows:

- Two reports on the biodegradation of vinyl chloride (10, 14).
- One report indicating halorespiration on 1,1,1-trichloroethane (50).
- Three reports on the biodegradation of chlorinated dibenzo-*p*-dioxins (6, 30, 31).
- One report of a novel metal-dependent hydrolytic dechlorinating enzyme (45).

The evidence for the biodegradation of vinyl chloride (VC) is further strengthened by two reports this quarter. The first of these examined numerous sites contaminated with this chlorinated solvent to determine if aerobic VC-degrading bacteria could be isolated (10). In 23 out of 37 sites, aerobic VC degradation was observed. Twelve bacterial strains were isolated, mostly from the genus *Mycobacterium*. The second article provides further evidence for anaerobic dechlorination of VC to ethene (14). From a parent culture dechlorinating perchloroethylene (PCE) to ethene, a daughter enrichment culture was developed with VC. The culture maintained its ability to dechlorinate VC but lost its ability to dechlorinate PCE.

This quarter halorespiration on 1,1,1-trichloroethane (111-TCA) is reported for the first time (50). Halorespiration refers to the use of a halogenated compound as a terminal electron acceptor (*e-acceptor*) to support growth (in analogy to O₂ by aerobes). 111-TCA was reduced to 1,1-dichloroethane and then to chloroethane.

This quarter three reports on chlorinated dibenzo-*p*-dioxins (CDDs) biodegradation were found. The first of these studies reports for the first time the reductive dechlorination of polychlorinated dioxins by a pure culture of anaerobic bacteria (6). The culture was shown to dechlorinate the environmentally significant CDD congener, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin. The other two reports consider chlorinated dioxin degradation by white rot fungi. In a screening, *Phlebia lindtneri* was shown to be capable of partial mineralization of radiolabeled 2,7-dichlorodibenzo-*p*-dioxin (2,7-DiCDD) to ¹⁴CO₂ (30). In the sequel article, short term incubations of *Phlebia lindtneri* with 2,7-DiCDD revealed the occurrence of hydroxy-2,7-DiCDD as an intermediate in the degradation process (31).

An atrazine chlorohydrolase was discovered this quarter (45). The enzyme hydrolytically dechlorinates atrazine to produce hydroxy-atrazine and requires metals for activity. The report constitutes the first time a metal-dependent enzyme has been found with hydrolytic dechlorinating activity.

2.b. Microbial Chlorination

The most important findings on biohalogenation in this quarter are as follows:

- Evidence for the formation of organochlorine compounds during wastewater treatment in a sewage treatment plant (33).
- One report indicating that kinetic isotope effects occur during enzymatic chlorination with chloroperoxidase (40).

Measurements of adsorbable organic halogens (AOX) showed the natural formation of organochlorines in a sewage plant (33). The report identified 3,4-dichlorophenylacetate as one of the most important naturally formed organochlorine compounds in the sewage plant.

Chloroperoxidase was shown to chlorinate 1,3,5-trimethylbenzene and 3,5-dimethylphenol with a slight kinetic isotopic preference for ^{35}Cl (40). Based on these findings, the authors propose that chlorine isotope ratios might be used to distinguish between natural and anthropogenic sources of chlorinated compounds.

3. MICROBIAL DECHLORINATION

3.a. General Reviews

In this quarter there was a review article on the biodegradation of dioxins by a novel reaction known as angular dioxygenation carried out by certain aerobic bacteria, which attack biphenylic compounds at carbon positions carrying the ether bond and an adjacent carbon (34). These novel dioxygenases have non-heme iron containing proteins (Rieske proteins). The 2,2',3-trihydroxy-diphenyl ether derivatives formed are degraded through *meta* cleavage. Some dioxin degradation pathways have been studied biochemically and genetically. In addition, feasibility studies have shown that some dioxin-degrading strains can function in actual dioxin-contaminated soil.

Several other review articles have been published in the last quarter of 2002 that are concerned with the certain aspects of bioremediation or xenobiotic biodegradation. These articles do not specifically focus on chlorinated compounds but, occasionally, the biodegradation of chlorinated compounds is discussed as an example. One review article examines recent developments in biodegradation, biotransformation, and biocatalysis of priority pollutants (37). This review covers several chlorinated compounds, including: triclosan, atrazine, mecoprop, 2,4-dichlorophenoxyacetic acid, 3-chlorobenzoate, 2,4,6-trichlorophenol, perchloroethene (PCE), trichloroethene (TCE), and polychlorinated biphenyls (PCB); among others. Another review deals with horizontal transfer of biodegradative genes between bacterial species in the environment (e.g. via plasmids) (51). The third review covers molecular genetic techniques for studying and improving microorganisms involved in bioremediation (53).

One review article that was missed from the second quarter was discovered. This paper reviews the biochemistry and genetics of methyl halide utilization in several aerobic bacteria utilizing methyl halides as a sole source of carbon and energy (29). The bacteria are all members of the alpha-Proteobacteria and were isolated from a variety of polluted and pristine terrestrial environments. An understanding of the genetics of these bacteria identified a unique gene (*cmuA*) involved in the degradation of methyl halides, which codes for a protein (CmuA) with unique methyltransferase and corrinoid (a cobalt containing enzyme cofactor) functions. This unique functional gene is being used to develop molecular ecology techniques to examine the diversity and distribution of methyl halide-utilizing bacteria in the environment.

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 based 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. The first study considered the biodiversity of bacteria capable of degrading VC as a sole source of carbon and energy source (10). Aerobic VC-degradation was observed in 23 out of 37 microcosms and enrichments inoculated with samples from various chlorinated solvent contaminated sites. Twelve different bacteria (11 *Mycobacterium* strains and 1 *Nocardioides* strain) capable of growth on VC as the sole carbon source were isolated. The growth rates of the *Mycobacterium* strains were in the range of 0.17-0.23 d⁻¹, with specific cell yields of 5.4-6.6 g of protein/mol of VC. The maximum specific substrate utilization rates were 9-16 nmol/min/mg of protein. The one *Nocardioides* strain had a higher growth rate (0.71 d⁻¹), and specific cell yield of 10.3 g of protein/mol. The substrate utilization rate was 43 nmol/min/mg of protein. The half-velocity constant (K_s) values for VC were between 0.5 and 3.2 μ M, indicating a high affinity for the substrate. The results taken as a whole suggest that aerobic bacteria responsible for VC

degradation are widely distributed at chlorinated solvent contaminated sites, and most isolates belong to the genus *Mycobacterium*.

The second article evaluated anaerobic enrichment cultures that utilized *cis*-dichloroethene (*cis*-DCE) and VC as electron acceptors, dechlorinating them to ethene (14). The enrichment cultures were derived from a culture capable of dechlorinating PCE to ethene. The enrichments developed on the lower chlorinated ethenes lost their ability to dechlorinate PCE. Fingerprinting with 16S rRNA resulted in gene fragments that showed homology with *Dehalococcoides ethenogens*, the only known bacterium capable of dechlorinating PCE to ethene. Chloroform was found to strongly inhibit the conversion of VC to ethene.

The third article reports on the aerobic degradation of 1,1-dichloroethene (1,1-DCE) by an aerobic mixed culture oxidizing butane (21). The specific activity of the culture on butane and 1,1-DCE was 2.6 and 1.3 $\mu\text{mol}/\text{mg TSS}/\text{h}$, respectively. The half-velocity constant, K_s , for the two substrates were 19 and 1.5 μM , respectively. The culture thus had an outstanding affinity for 1,1-DCE.

Perchloroethylene (PCE) and Trichloroethene (TCE). In this quarter, there were 15 reports on the biodegradation of higher chlorinated ethenes. Eight studies evaluated anaerobic degradation, of which two considered sequential anaerobic-aerobic degradation.

The first anaerobic article reports on a field study conducted at Kelly Air Force Base in which microorganisms were injected into wells (bioaugmentation) to stimulate the complete anaerobic dechlorination of PCE to ethene (26). The site groundwater contained about 1 mg/L of PCE and lower amounts of TCE and *cis*-DCE. Laboratory microcosms inoculated with soil and groundwater from the site exhibited partial dechlorination of TCE to *cis*-DCE when amended with lactate or methanol as electron donors (*e-donors*). Following the addition of a dechlorinating enrichment culture, KB-1, the chlorinated ethenes in the microcosms were completely converted to ethene. The ability of KB-1 to stimulate biodegradation of chlorinated ethenes *in situ* in the field was explored using a closed-loop recirculation cell with a pore volume of approximately 64 m^3 . The groundwater was first amended with methanol and acetate to establish reducing conditions, which permitted dechlorination of PCE to *cis*-DCE. Subsequently, 13 liters of KB-1 enrichment culture were injected into the groundwater. Within 200 days, the concentrations of PCE, TCE, and *cis*-DCE were all below 5 $\mu\text{g}/\text{L}$, and ethene production accounted for the observed mass loss. The maximum rates of dechlorination estimated from field data showed half-lives of a few hours. The groundwater samples were

assayed for the presence of *Dehalococcoides* (only known genus of bacteria capable of converting PCE to ethene) and the bacterium was detected in bioaugmented groundwater.

In the second anaerobic study, a new bacterium was discovered, *Desulfitobacterium metallireducens* that utilizes PCE, TCE and 3-chloro-4-hydroxyphenylacetate, as e-acceptors while utilizing lactate as an e-donor (15). Lactate also supported growth with Fe(III), Mn(IV), humus or elemental sulfur serving as the e-acceptor.

In the third anaerobic study, two types of slow release substrates were considered as e-donors to support the reductive dechlorination of TCE in microcosms obtained from three contaminated sites (55). Both tetrabutoxysilane (TBOS) and tetrakis(2-ethylbutoxy)silane (TKEBS) were considered as the slow-release anaerobic substrate. Poisoned microcosms showed that 1 mol of TBOS slowly and abiotically hydrolyzes to 4 mol of 1-butanol, while the live microcosms showed the 1-butanol ferments to butyrate and/or acetate, producing H₂. The hydrolysis of TBOS and TKEBS was abiotic and was not enhanced by biotic processes under the anaerobic conditions of these tests. Hydrogen consumption was correlated with reductive dehalogenation, indicating that H₂ served as an e-donor for reductive dehalogenation. Electron balances indicated that 14% of the electron equivalents in TBOS or TKEBS were used for dechlorination.

Two additional articles evaluated the role of an enzyme cofactor (vitamin B12) on reductive dechlorination of PCE (22, 46) are discussed in section "3.c. *In vitro degradation of chlorinated compounds*". Another article considers isotopic fractionation of chlorine during the reductive dechlorination of PCE (35) as discussed in section "3.d. *New tools to assess the biodegradation of chlorinated compounds Isotopic fractionation*".

Two studies evaluated a sequential degradation scheme in which PCE is first rapidly converted to DCE under anaerobic conditions. Subsequently, accumulated DCE is further degraded by aerobic cooxidation. The philosophy behind the strategy is that PCE degradation is only feasible under anaerobic conditions; whereas DCE degradation is more rapid under aerobic than anaerobic conditions. The objective of the first study was to develop a sequential anaerobic-aerobic reactive biobarrier utilizing a layer of slow-release organic substrate and a subsequent layer of slow-release oxygen source (19). The organic-releasing material consisted of sludge cakes from a domestic wastewater treatment plant. The oxygen-releasing material contained calcium peroxide, which is able to generate oxygen continuously upon contact with water. The observed release rates of oxygen and organics (measured as chemical oxygen demand) were approximately 0.0368 and 0.0416 mg/d/g of material, respectively. A laboratory-scale column experiment was then conducted to test the feasibility of this proposed

system for the bioremediation of PCE-contaminated groundwater. Simulated PCE-contaminated groundwater with a flow rate of 0.25 L/d was pumped into this system. Effluent samples showed that up to 99% of PCE was removed in this passive system.

In the second anaerobic-aerobic study, a laboratory sequential bioreactor system was set up to degrade PCE which consisted of an anaerobic fixed-film reactor and two aerobic chemostats (25). The aerobic chemostats included a transformation reactor and a growth reactor. The anaerobic fixed-film bioreactor fed 5 to 35 μM PCE showed more than 99% of PCE transformation into *cis*-DCE at hydraulic retention time (HRT) of 48h. A phenol degrading strain, *Alcaligenes* sp. R5, which can efficiently degrade *cis*-DCE by co-metabolic oxidation, was used as inoculum for the aerobic chemostats. Efficient degradation *cis*-DCE in the aerobic system could not be achieved due to oxygen limitation. However, 54% of the maximum *cis*-DCE degradation was obtained when 10 μmol of hydrogen peroxide was supplemented to the transformation reactor as an additional oxygen source.

TCE degradation under aerobic conditions was evaluated in seven studies. One study reports on the feasibility of using biofiltration technology to remediate TCE-contaminated air streams (24). A bench-scale biofilter system containing 42 L of organic packing material was inoculated with a propane-oxidizing microbial consortium. Propane, the primary substrate, and TCE were introduced into the biofilter. The maximum TCE removal efficiency from the air stream achieved was 98%. The high efficiency could only be achieved if propane and TCE were pulsed into the reactor at different times; continuous addition of both substrates resulted in only 25% removal efficiency of TCE (most likely due to competitive inhibition of propane).

In the second article, bacterial isolates were screened for their ability to cooxidize TCE with phenol as the primary substrate (43). The study found that remarkably few isolates in phenol degrading mixed cultures were capable of TCE cooxidation.

In the third article, a toluene degrading TCE-cooxidizing organism, *Ralstonia pickettii*, was investigated (38). The study evaluated the biochemical basis for tolerance displayed by this organism towards TCE toxicity. Two systems were identified, one that is responsible for the tolerance to solvent stress (e.g. disturbance of bacterial membrane by hydrophobic compounds), and the other that tolerates toxic intermediate stress (*i.e.* the ability of the organisms to survive toxic intermediates produced from the oxidation of TCE).

In the fourth article, the degradation of TCE as well as ethylene dibromide (EDB; dibromoethane) by plants during phytoremediation was evaluated (13). The tropical leguminous tree, *Leuceana leticocephala*, was shown to take up and metabolize EDB and TCE. The plants were grown in sterile hydroponic solution without its bacterial symbiont, *Rhizobium*.

EDB and TCE were both metabolized by the plant, as indicated by the formation of bromide ion from EDB, and trichloroethanol from TCE.

Two additional articles report on the cloning and expression methane monooxygenase, implicated in TCE cooxidation, in a heterologous host (32, 54) as described in section “3.d. *New tools to assess the biodegradation of chlorinated compounds. Metabolic engineering*”.

Finally, the last article describes real time PCR techniques for the detection of methane monooxygenase in the field (20) as described in section “3.d. *New tools to assess the biodegradation of chlorinated compounds. Characterization of microbial populations*”.

Carbon Tetrachloride (CT) and Chloroform (CF)

This quarter there was only one article on the biodegradation of carbon tetrachloride. A model is developed to describe the degradation of CT in the field by a denitrifying bacterium, *Pseudomonas stutzeri* strain KC, which has been used to bioaugment CT-contaminated field sites (39). The model focuses on the transport of the bacterium in porous aquifer medium. The resulting model enabled improved understanding of the complex coupled processes and enabled a test of the model for field-scale design and transport studies. Batch studies were used to identify initial degradation and microbial transport processes, and constrained optimization methods were used to estimate a set of reaction rates that best describe data from a soil column experiment. The CT degradation rate in the columns was lower than values obtained from batch studies, and processes in addition to the growth and decay of strain KC cells (due to native flora) are necessary to describe the observed nitrate consumption.

Chloromethane (CM) and Dichloromethane (DCM)

This quarter there are no reports on the biodegradation of chloromethane or dichloromethane. A review on the aerobic degradation of chloromethane as well as other methyl halides (29) is discussed above in section “3.a. *General reviews*”.

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

Only one publication actually tested the biodegradation of 1,2-dichloroethane (1,2-DCA) this quarter. An anaerobic mixed culture enriched for the dechlorination of TCE was tested for its ability to dechlorinate 1,2-DCA but after several months of incubation no dechlorination was observed (14). This observation was in contrast to results obtained elsewhere with other TCE-dechlorinating pure and mixed cultures that have been shown to readily dechlorinate 1,2-DCA.

Another publication reported for the first time on the isolation of a bacterial pure culture (strain TCA1) capable of utilizing 1,1,1-trichloroethane (1,1,1-TCA) as an e-acceptor (halorespiration) with H_2 as a required e-donor (50). 1,1,1-TCA was converted to 1,1-dichloroethane (1,1-DCA) and subsequently to chloroethane (Figure 1). 1,1-DCA was also shown to serve as an e-acceptor for the bacterium. Several other chlorinated solvents were tested and found not to be dechlorinated (PCE, TCE, 1,1,2-TCA and 1,2-chloropropane). Phylogenetic analysis indicated that strain TCA1 is related to gram-positive bacteria whose closest relative is *Dehalobacter restrictus*, an obligate H_2 -oxidizing, chloroethene-respiring bacterium.

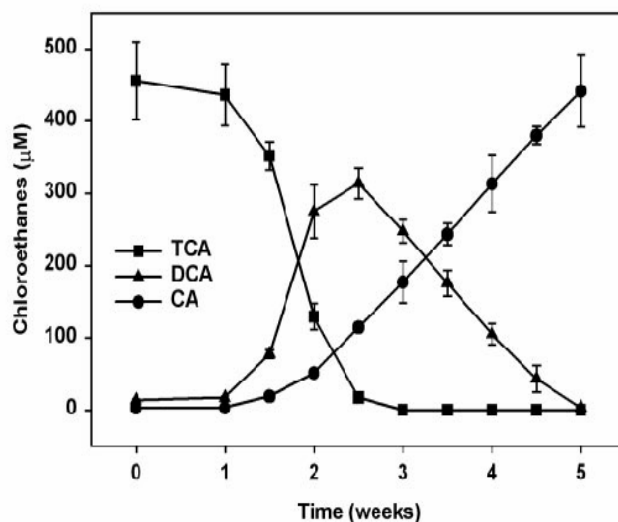


Fig. 1. Stoichiometry of the dechlorination of TCA to DCA and CA by strain TCA1 [50].

A third study evaluated the aerobic cooxidation of the chloroethanes, 1,1,1-TCA and 1,1-DCA, by a butane-oxidizing bacterial culture (21). The specific rate of cooxidation observed was 0.49 and 0.19 $\mu\text{mol}/\text{mg TSS}/\text{h}$ for 1,1-DCA and 1,1,1-TCA, respectively. The half saturation constants (K_s) were 19 and 12 μM for 1,1-DCA and 1,1,1-TCA, respectively. Butane was a strong competitive inhibitor of chloroethane cooxidation.

Chlorobenzenes

Mono-chlorobenzene, Dichlorobenzenes and Trichlorobenzenes: Only one publication was found regarding the microbial degradation of mono-chlorobenzene (5). The study evaluated aerobic chlorobenzene degradation by soil to estimate the fate of the compound in an agricultural soil. The volatilized ^{14}C -compounds and ^{14}C - CO_2 were trapped separately, thus allowing a clear differentiation between volatilization and mineralization. The results show clearly that volatilization is the main loss mechanism of mono-chlorobenzene from soils; whereas, mineralization is of minor importance. Additional nutrition sources could not increase the mineralization process.

Hexachlorobenzene: In the fourth quarter of 2002, no publication was found reporting on the biodegradation of hexachlorobenzene.

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

In this quarter, three studies report on the degradation of chlorinated dibenzo-*p*-dioxins (CDDs). Two studies involved CDD degradation by white-rot fungi (wood-degrading fungi). The first article reports on a screening of 74 strains of white-rot fungi from 66 species for their ability to co-metabolically degrade dibenzo-*p*-dioxins (DD) (30). Evidence for DD degradation was observed in 12 strains, and these were then tested for their ability to degrade ^{14}C -uniformly-labeled 2,7-dichlorodibenzo-*p*-dioxins (2,7-DiCDD). Three of the fungi showed high rates of $^{14}\text{CO}_2$ accumulation. These were an unidentified strain (MZ-227), *Phlebia* sp. MG-60 and *Phlebia lindtneri*. The strains MZ-227, *Phlebia* sp. MG-60, and *P. lindtneri* converted 250 nmol of 2,7-diCDD to 196, 155 and 149 nmol of $^{14}\text{CO}_2$, respectively, during a 30-day incubation period. This corresponds to a mineralization of 2,7-DiCDD ranging from 5.0 to 6.5%.

The second article was a follow-up studying evaluating *P. lindtneri* ability to degrade 2,7-DiCDD and 2,8-dichlorodibenzofuran (2,8-DiCDF) (31). *P. lindtneri* was responsible for removing 2,7-DiCDD by 55% over a 20-day incubation period. Hydroxy-DiCDD and hydroxy-DiCDF were identified as one of the metabolites produced by *P. lindtneri* after 5-day incubation with 2,7-DiCDD or 2,8-DiCDF, respectively.

The third publication on CDD degradation this quarter is very significant. A group of researchers have identified for the first time a pure culture of bacteria capable of reductive dechlorination of several polychlorinated dioxins (6). The strain utilized was *Dehalococcoides* sp. CBDB1, which was previously shown to be responsible for reductive dehalogenation of hexachlorobenzene. Also the bacterium is phylogenetically related to bacteria responsible for

the reductive dechlorination of PCE to ethene. The study also demonstrated the presence of *Dehalococcoides* in CDD-dechlorinating enrichment cultures derived from freshwater sediments. An environmentally significant dioxin, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, was among the CDD congeners dechlorinated by strain CBDB1. The patterns of dechlorination observed in the study with the bacterium *Dehalococcoides* sp. strain CBDB1 are shown in Figure 2.

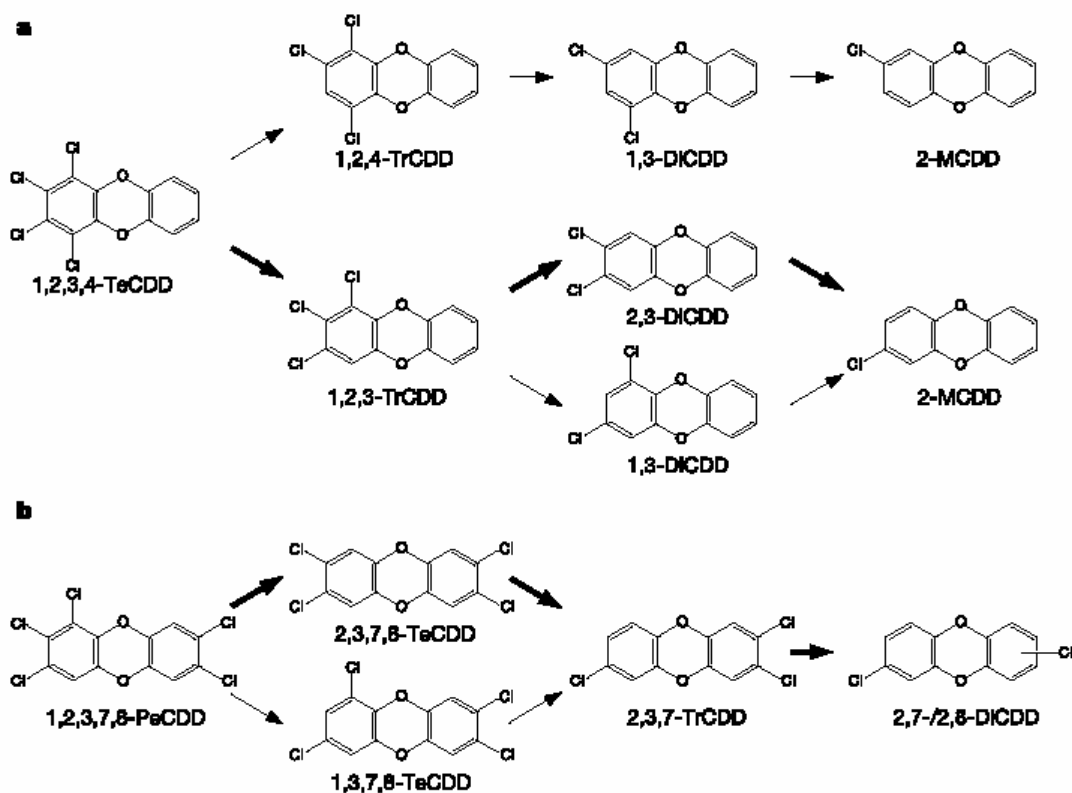


Fig. 2. Proposed pathways of reductive dechlorination of spiked 1,2,3,4-TeCDD (**a**) and 1,2,3,7,8-PeCDD (**b**) by a pure culture of *Dehalococcoides* sp. strain CBDB1. The major routes are marked with bold arrows. The results of reductive dechlorination of spiked 1,2,4-TrCDD, 1,2,3-TrCDD and 2,3-DiCDD are included in (**a**) [6].

Hexachlorobutadiene and Octachlorostyrene

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

Polychlorinated Biphenyls (PCBs)

In this quarter 13 publications reported on the microbial degradation of PCBs. Three of these publications address anaerobic degradation of PCB. The first study examined the reductive dechlorination of 2,2',3,4,4',5'-hexachlorobiphenyl in landfill leachate (41). The effect of biosurfactants on dechlorination rates and congeners patterns was evaluated. Rhamnolipids had little effect on the rates. The biologically active landfill samples averaged 68.6% degradation of 2,2',3,4,4',5' hexachlorobiphenyl over the first 7 days. The majority of this was accounted for in the observed reductive dechlorination products, 2,2',4',5-tetrachlorobiphenyl, 2,2',3,5'-tetrachlorobiphenyl and 2,2',3,4-tetrachlorobiphenyl. All observed breakdown products contained four chlorines and represent the terminal PCB congeners observed in these anaerobic studies. These results indicate that initial reductive dechlorination rates for highly chlorinated PCBs may be rapid in landfill environments.

The second study evaluated congener patterns in deep, dated sediment cores from the Ashtabula River (OH, USA) (17). A factor analysis model with nonnegative constraints was used to investigate the sources and patterns of PCBs. Additionally, a new model, based on a least squares method, was developed to identify possible patterns of anaerobic dechlorination of PCBs in the sediments, and to quantify the relevant dechlorination pathways. The analysis revealed the likelihood of anaerobic dechlorination of PCBs in Ashtabula River sediments.

The third study examined the enhancement of reductive dechlorination of PCBs in sediment-free enrichment cultures by the presence of other halogenated aromatic compounds (HAC) (chlorobenzoates, chlorophenols or chlorobenzenes) (7). In HAC-amended PCB sediments there was a long lag in PCB dechlorination until the HACs were reduced to a plateau level. Despite this lag, once PCB dechlorination started it was faster in the HAC-amended sediments compared to the unamended controls. The overall extent of PCB dechlorination was significantly enhanced by all HACs except pentachlorophenol and pentachlorobenzene, but the extent as well as the pattern of the enhancement varied.

Ten studies considered aerobic degradation of PCBs. The first study evaluated the effect of PCB degradation on the DNA content in cells of the aerobic bacterium, *Comamonas testosteroni* (16). By developing mutants which accumulated PCB-intermediates (hydroxylated-PCBs), it was observed that intermediates caused an increase in the content of

cell DNA. Direct addition of the hydroxy-PCB intermediates to wild type cells caused the same effects. Electron microscopy revealed that cell-cell separation was inhibited in this culture.

The second study, considers the biodegradation of PCBs by a *Pseudomonas* strain in a surfactant solution used to wash PCB-contaminated soil (4). Nonionic surfactants washed more PCBs from the soil (up to 89%) but inhibited their biodegradation. Anionic surfactants washed less PCBs from the soil but were more effective in biodegradation tests, removing up to 67% of total PCBs.

The third study also examined the biodegradation of PCBs in a surfactant solution used to wash PCB-contaminated soil, but this time three strains of white-rot fungi were compared for their ability to metabolize the extracted PCBs (42). The highest PCB transformation (70%) was obtained with *Trametes versicolor* at an initial PCB concentration of 1800 mg/l. *T. versicolor* removed both low- and high-chlorinated congeners.

The fourth study correlated PCB degrading capacity in plant cell cultures with the ability of various cell cultures to oxidize a dye (as a screening tool) (8).

The fifth study evaluated the effect of plant terpenes on gene expression of *bphC* gene encoding 2,3-dihydroxybiphenyl-1,2-dioxygenase (a PCB degradative gene in the bacterium, *Ralstonia eutropha*) (18). The results indicate that terpenes widely distributed in nature could be a potential inducing substrate for effective PCB biodegradation in the soil.

The sixth study elucidates the third step in the aerobic PCB degradation pathway (corresponding to 2,3-dihydroxybiphenyl-1,2-dioxygenase) (11). The study demonstrates that *ortho*-chlorinated PCB metabolites strongly inhibit the dioxygenase, promote its suicide inactivation and interfere with the degradation of other compounds. For example, the kinetics for 2',6'-dichloro-2,3-dihydroxybiphenyl was reduced by a factor of similar to 7,000 relative to 2,3-dihydroxybiphenyl, and the PCB metabolite bound with sufficient affinity to competitively inhibit to 2,3-dihydroxybiphenyl cleavage at nanomolar concentrations. An explanation for these phenomena was elucidated with a crystal structure of the bound enzyme complex.

The seventh study evaluated the ability of several PCB-degrading bacteria for their ability to differentiate between the enantiomers of four atropisomeric PCB congeners (47). Enantioselectivity was shown to vary with respect to strain, congener, and cosubstrate.

Finally the last three studies considered genetic engineering to improve or better understand PCB degradation (28, 36, 44), as is discussed in section “3.d. *New tools to assess the biodegradation of chlorinated compounds: Metabolic engineering*”.

Miscellaneous Chlorinated Compounds

This quarter there were eleven reports on the biodegradation of miscellaneous chlorinated pollutants. These included: atrazine, 2,4-dichlorophenoxy-acetate (2,4-D), chlorophenols and hexachlorocyclohexane (HCH) and DDT.

Three articles report on atrazine. The first study evaluated the ability of the aerobic bacterium *Arthrobacter aureescens* to metabolize various substituted atrazines (49). *A. aureescens* is metabolically diverse and grew on a wider range of s-triazine compounds than any bacterium previously characterized. The 23 s-triazine substrates serving as the sole nitrogen source included the herbicides ametryn, atratone, cyanazine, prometryn, and simazine. Moreover, atrazine substrate analogs containing fluorine, mercaptan, and cyano groups in place of the chlorine substituent were also growth substrates. *A. aureescens* strain TC1 also metabolized compounds containing chlorine plus N-ethyl, N-propyl, N-butyl, N-s-butyl, N-isobutyl, or N-*t*-butyl substituents on the s-triazine ring.

The second study evaluated attenuation processes in a macrocosm (aquarium or outdoor ponds) of three pesticides, atrazine, metolachlor and chlorpyrifos (27). In the outdoor systems, the half-lives were 27 days and 12 days, respectively, for atrazine and metolachlor, for the herbicide-treated (low dosage) pond. Very low levels of two degradation products of atrazine, 6-amino-2-chloro-4-iso-propylamino-s-triazine and 2-chloro-4-ethylamino-6-ethylamino-s-triazine, were observed in the outdoor ponds.

The third article on atrazine reports on the activity of chlorohydrolase involved in atrazine degradation (45), as is discussed in section "3.c. *In vitro* degradation of chlorinated compounds".

This quarter there is one report on 2,4-D degradation. The objective of the study was to evaluate the mineralization of ¹⁴C-labeled 2,4-D in Egyptian soils under aerobic or anaerobic conditions (56). Under aerobic conditions, 10%-14% of applied dose was mineralized during 90 d, irrespective of soil type. The soil-extractable pesticide residues decreased with time and the bound residues gradually increased.

This quarter there is one report on chloroaniline degradation. The study reports on the isolation of a diversity of bacteria from soil capable of 3-chloroaniline and 3,4-dichloroaniline (12). All isolates contained a plasmid involved in chloroaniline degradation.

Two studies reported on chlorophenol degradation. The first study describes the methylation of 2,4,6-trichlorophenol to the corresponding trichloroanisole by various fungal strains, accounting for the cork taint (malodor) of wines (2). The second study examined the

oxidation of chlorophenols by laccase (48), as is discussed in section “3.c. *In vitro* degradation of chlorinated compounds”.

Two studies evaluated the degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT). The first one considered DDT degradation by the bacterium *Alcaligenes denitrificans* under aerobic and anaerobic conditions (1). Aerobic degradation was inhibited by the presence of sodium acetate and sodium succinate, respectively, but remained uninhibited in the presence of glucose. Under anaerobic conditions, DDT is metabolized into 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD). Anaerobic degradation of DDT is further enhanced by the presence of glucose.

The second study examined aerobic degradation of DDT by the bacterium *Serratia marcescens* (3). Complete degradation was observed up to 15 mg DDT/L, followed by inhibitory effects at higher concentrations showing a total loss of degradative ability at 50 mg DDT/L. Degradation was inhibited in the presence of auxiliary carbon sources such as citrate, rice straw hydrolysate. However, the presence of yeast extract, peptone, glycerol and tryptone soya broth (TSB) enabled complete disappearance of DDT.

Two studies examined the degradation of hexachlorocyclohexane (HCH). The first study evaluated the fate of HCH in bay and river sediments by comparing isomers accumulating in these sediments. Sediments were shown to be a sink for γ -HCH. Also concentrations of α - and γ -HCH were negatively correlated with bacterial activity. A second paper describes the heterologous expression of HCH dehydrochlorinase genes from *Sphingomonas pauchmobilis* (23), as is discussed in section “3.d. *New tools to assess the biodegradation of chlorinated compounds: Metabolic engineering*”.

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. Two of these articles concerned PCE. The first article reports on the dehalogenase of *Dehalospirillum multivorans*, a well-known bacterium capable of dechlorinating PCE (46). A strain was found that was not able to dechlorinate PCE, yet it contained normal genes for the dehalogenase enzyme responsible for PCE dechlorination. Analysis of the corrinoids (a cobalt containing cofactor) revealed the absence of the specific corrinoid, which is the cofactor of the PCE dehalogenase. The results indicate that the inability of the unusual strain to dechlorinate is due to the absence of the corrinoid cofactor.

The second article examined direct *in vitro* dechlorination of PCE and TCE by the corrinoid cofactor, vitamin B12 (22). The study evaluated zero valent zinc and zero valent iron as electron donors to the direct dechlorination process. Zero valent zinc was a more potent reductant of vitamin B12, reducing it to the oxidation state I, which enabled complete dechlorination of PCE and TCE. On the other hand, zero valent iron only reduced vitamin B12 to oxidation state II, and thus only caused partial dechlorination.

One article examined an atrazine dechlorinating enzyme, chlorohydrolase, from a *Pseudomonas* (45). Atrazine chlorohydrolase (AtzA) catalyzes a hydrolytic dechlorination reaction to produce hydroxyatrazine. Sequence analysis revealed AtzA to be homologous to metalloenzymes within the amidohydrolase protein superfamily. Loss of activity obtained by incubating AtzA with the chelator 1,10-phenanthroline or oxalic acid was reversible upon addition of Fe(II), Mn(II), or Co(II) salts. This study is the first report of a metal-dependent dechlorinating enzyme that proceeds via a hydrolytic mechanism.

Finally, the *in vitro* oxidation of chlorophenolic compounds by laccase from *Lentinula edodes* was tested (48). The reaction rate and removal efficiency were affected by the nature and position of substituents on the aromatic ring. Chlorinated phenols and/or anilines bearing an additional substituent with a lone electron pair were preferentially oxidized by the enzyme. In contrast, chlorinated substrates bearing an electron-withdrawing substituent, such as the nitro group, were not oxidized at all.

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

Isotope fractionation

One study evaluated the fractionation of chlorine isotopes during the reductive dechlorination of PCE and TCE to *cis*-DCE (35). The changes in the $\text{Cl}^{37}/\text{Cl}^{35}$ ratio observed during the one-step reaction (TCE to *cis*-DCE) can be explained by the regioselective elimination of chlorine accompanied by the Rayleigh fractionation. The fractionation factors (α) of the TCE dechlorination by three kinds of anaerobic cultures were approximately 0.994-0.995 at 30 °C. The enrichment of Cl^{37} in the organic chlorine during the two-step reaction (PCE to *cis*-DCE) can be explained by the random elimination of one chlorine atom in the PCE molecule followed by the regioselective elimination of one chlorine atom in the TCE molecule.

Characterization of Microbial Populations

One publication used real time polymerase chain reaction (PCR) to monitor for the specific and rapid enumeration of an aerobic TCE-degrading methanotroph, *Methylocystis* sp. M in groundwater (20). The primer set was designed for a specific sequence of the gene encoding soluble methane monooxygenase (sMMO). The presence of other methanotrophs in samples did not affect the reliability of enumeration; and recovery of the cells with a membrane filter enabled the quantification of the *Methylocystis* cells in groundwater.

Metabolic Engineering

This quarter six articles report on the application of metabolic engineering to improve or better understand the degradation of chlorinated compounds. Two of these articles involve studies on the aerobic cooxidation of TCE. The first study reports on active expression of soluble methane monooxygenase from *Methylosinus trichosporium* in *Pseudomonas putida* (54). The pollutant TCE was degraded at 5 nmol/min/mg protein using whole cells, and stoichiometric amounts of chloride were generated to show active expression of this large enzyme. *P. putida* lacking the recombinant *sMMO* gene was unable to degrade TCE when grown with glucose in the absence of toluene, conditions that do not allow for expression of toluene dioxygenase; thus, the ability to degrade TCE was from the expression of active sMMO in this strain. Wild type *P. putida* is unable to degrade chloroform even under conditions that express its toluene dioxygenase, but when the recombinant genes were expressed in this host chloroform was degraded as a result of active expression of sMMO. A main advantage of the recombinant expression is that regulation of *sMMO* by copper can be bypassed. The sMMO's are the most active forms of methane monooxygenases but are typically down-regulated in the presence of copper.

The second paper summarizes previous works in which the recombinant sMMO from *Methylosinus trichosporium* has been successfully expressed in either homologous or heterologous hosts (32). Homologous expression yielded higher sMMO activities than in systems with a heterologous host.

Three articles concern the degradation of PCB. The first article reports on a new genetic approach to improving biodegradative capacities applied to the *bph* operon (a cluster of biphenyl degrading genes with a common regulatory sequence) of *Pseudomonas* sp. strain KKS102 to enhance its biphenyl- PCB-degrading activity (36). A native promoter of the *bph* operon, which was under control, was replaced through homologous recombination by a series of promoters that had constitutive activity. This strategy removes rate-limitations associated

with transcription and has the potential to improve the degradation activity of a wide variety of microorganisms.

The second article on metabolic engineering of PCB-degrading organisms, reports on PCB degradation by the aerobic bacterium *Rhodococcus* sp. (44). The bacterium metabolizes PCB via 2-hydroxypenta-2,4-dienoate (HPD) and benzoate metabolic pathways. The HPD metabolic pathway genes were cloned from the *Rhodococcus* strain. The paper describes the homology of different genes in the HPD pathway with that of other bacteria metabolizing PCB, phenol or toluene. Also the promoter region of the genes were characterized. The insertional inactivation of the several HPD pathway genes resulted in the loss of the corresponding enzyme activities and severe growth interference was observed during growth on biphenyl. The growth defects were partially restored by the introduction of plasmids containing the respective intact genes.

The third article on metabolic engineering of PCB-degrading organisms reports on the bacterium *Pseudomonas acidovorans* strain M3GY, a novel recombinant strain with the ability to utilize 3,4'-dichlorobiphenyl (3,4'- DiCBP) as a growth substrate. The strain presumably produced chlorobenzoates while degrading 3,4'-DiCBP; however, the chlorobenzoates were never detected. In this study a mutant strain was made which was unable to grow on 3-chlorobenzoate. It accumulated 3-chlorocatechol from 3,4'-DiCBP when grown on biphenyl. Thus, M3GY attacks both rings, and the failure to isolate 3-chlorobenzoate or 3-chlorocatechol is due to rapid turnover by the enzymes of the *ortho*-chlorocatechol pathway in the wild-type strain.

The last article describes the cloning and characterization of lindane degradative (*lin*) genes in *Sphingomonas paucimobilis* (23). Two nonidentical copies of the *linA* gene encoding HCH dehydrochlorinase, which were designated *linA1* and *linA2*, were found in *S. paucimobilis* B90. The *linA1* and *linA2* genes could be heterologously expressed in *Escherichia coli*, leading to dehydrochlorination of α -, γ -, and δ -HCH but not of β -HCH (the most problematic isomer), suggesting another pathway for the initial steps of β -HCH degradation.

4. MICROBIAL CHLORINATION

4.a. General Reviews

In the fourth quarter of 2002, no review articles on biohalogenation were found.

4.b. Microbial Chlorination in the Environment

Chloromethanes

In the fourth quarter of 2002, no articles on natural chloromethane formation were found.

Other Chlorinated Compounds

An interesting article was found reporting on *de novo* formation of adsorbable organic halogen (AOX) and 3,4-dichlorophenylacetic acid (3,4-DiCPA) during wastewater treatment in a sewage plant (33). The amount of AOX increased 15-fold inside the sewage treatment plant, accounting for the production of more than 6 kg of AOX/d. 3,4-DiCPA was the compound of no known anthropogenic origin found at the highest concentration. In one case, more than 1 g/kg of this compound was detected. A slaughterhouse that emits phenylacetic acid is probably the origin of 3,4-DiCPA formation. Experiments examining the chlorination of phenylacetic acid with hypochlorite (HOCl) did not produce 3,4-DiCPA. Thus a mechanism involving hypochlorite can be excluded.

Four new chlorinated anthraquinone metabolites were reported from a lichen (9). The structures of these new chloroanthraquinones are shown in Figure 3.

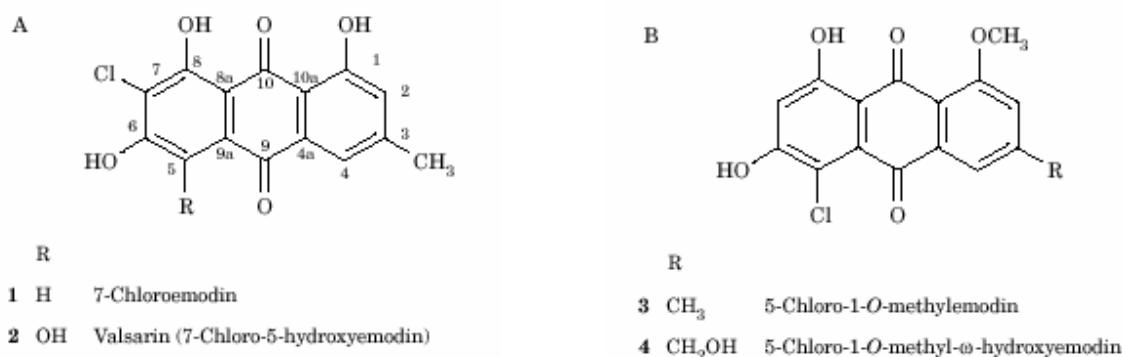


Fig. 3. Structure of novel A) 7-chloro- and B) 5-chloro-anthraquinone metabolites from the lichen *Lasallia papulosa* [9].

Chlorinated Natural Organic Matter

Except for the aforementioned article (33), there were no further reports on natural AOX formation this quarter.

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

A new dibromotyrosine metabolite was isolated from a marine organisms, a sponge (52). The structure of the new metabolite is shown in Figure 4.

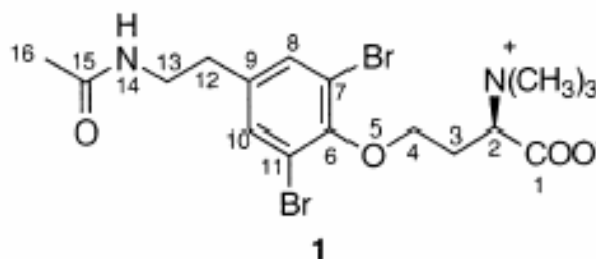


Fig. 4. A novel bromotyrosine metabolite isolated from a Verongid sponge [52].

4.d. Chlorinating Enzymes

The kinetic isotope effects of chloroperoxidase from the fungus *Caldariomyces fumago* were examined (40). Two aromatic substrates, 1,3,5-trimethylbenzene and 3,5-dimethylphenol, were treated with the chloroperoxidase. A kinetic isotope effect $^{35}\text{Cl}/^{37}\text{Cl}$ was calculated to be 1.012 for 1,3,5-trimethylbenzene and 1.011 for 3,5-dimethylphenol. The chemical reaction with hypochlorite yielded a much smaller kinetic isotope effect. These results indicate that a substantial kinetic isotope effect exists for the enzymatic process, suggesting possible use of chlorine isotope ratios to distinguish between anthropogenic and natural sources of chlorinated compounds.

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7. ANNEX

Ahuja, R. and A. Kumar (2003). "Metabolism of DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] by *Alcaligenes denitrificans* ITRC-4 under aerobic and anaerobic conditions." *Current Microbiology* 46(1): 65-69.

An isolated bacterium, *Alcaligenes denitrificans* ITRC-4, metabolizes 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) under both aerobic and anaerobic conditions. The aerobic metabolism is inhibited by 38% and 47% in the presence of 1.0 g L⁻¹ of sodium acetate and sodium succinate, respectively, but remains uninhibited in the presence of 1.0 g L⁻¹ of glucose. Also, the metabolism is inhibited completely in the presence of biphenyl vapors, as well as 0.8 g L⁻¹ of 2,2'-bipyridyl. Under anaerobic conditions, DDT is metabolized into 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD), which is further enhanced by 50% in the presence of 1.0 g L⁻¹ of glucose. Besides, the bacterium also metabolizes 4-chlorobenzoate, which is accompanied by the release of chloride ions.

Alvarez-Rodriguez, M. L., L. Lopez-Ocana, et al. (2002). "Cork taint of wines: Role of the filamentous fungi isolated from cork in the formation of 2,4,6-trichloroanisole by O methylation of 2,4,6-trichlorophenol." *Applied and Environmental Microbiology* 68(12): 5860-5869.

Cork taint is a musty or moldy off-odor in wine mainly caused by 2,4,6-trichloroanisole (2,4,6-TCA). We examined the role of 14 fungal strains isolated from cork samples in the production of 2,4,6-TCA by O methylation of 2,4,6-trichlorophenol (2,4,6-TCP). The fungal strains isolated belong to the genera *Penicillium* (four isolates); *Trichoderma* (two isolates); and *Acremonium*, *Chrysonilia*, *Cladosporium*, *Fusarium*, *Mortierella*, *Mucor*, *Paecilomyces*, and *Verticillium* (one isolate each). Eleven of these strains could produce 2,4,6-TCA when they were grown directly on cork in the presence of 2,4,6-TCP. The highest levels of bioconversion were carried out by the *Trichoderma* and *Fusarium* strains. One strain of *Trichoderma longibrachiatum* could also efficiently produce 2,4,6-TCA in liquid medium. However, no detectable levels of 2,4,6-TCA production by this strain could be detected on cork when putative precursors other than 2,4,6-TCP, including several anisoles, dichlorophenols, trichlorophenols, or other highly chlorinated compounds, were tested. Time course expression studies with liquid cultures showed that the formation of 2,4,6-TCA was not affected by a high concentration of glucose (2% or 111 mM) or by ammonium salts at concentrations up to 60 mM. In *T. longibrachiatum* the O methylation of 2,4,6-TCP was catalyzed by a mycelium-associated S-adenosyl-L-methionine (SAM)-dependent methyltransferase that was strongly induced by 2,4,6-TCP. The reaction was inhibited by S-adenosyl-L-homocysteine, an inhibitor of SAM-dependent methylation, suggesting that SAM is the natural methyl donor. These findings increase our understanding of the mechanism underlying the origin of 2,4,6-TCA on cork, which is poorly understood despite its great economic importance for the wine industry, and they could also help us improve our knowledge about the biodegradation and detoxification processes associated with chlorinated phenols.

Bidlan, R. and H. K. Manonmani (2002). "Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by *Serratia marcescens* DT-1P." *Process Biochemistry* 38(1): 49-56.

Microbial degradation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT)-residues is one of the mechanisms for the removal of this compound from the environment. A DDT-degrading consortium was isolated by long term enrichment of soil samples collected from DDT-contaminated fields. This consortium was acclimated by repeated passages through a mineral salt medium containing increasing concentrations of DDT. This acclimated consortium could degrade 25 ppm of DDT in 144 h. The consortium consisted of four bacteria. Of these, *Serratia marcescens* DT-1P was used for further studies. Various factors such as inoculum size, concentration of DDT, pH, temperature, presence of co-substrates, the type of carbon source used influenced the degradation of DDT in shake flasks. Complete degradation was observed up to 15 ppm DDT, followed by inhibitory effects at higher concentrations showing a total loss of degradative ability at 50 ppm DDT. Effective degradation of DDT was obtained with the inoculum pre-exposed to DDT for 72 h. Degradation was inhibited in the presence of auxiliary carbon sources such as citrate, rice straw hydrolysate. However, the presence of yeast extract, peptone, glycerol and tryptone soya broth (TSB) showed complete disappearance of DDT. Mesophilic

temperatures (26-30 degreesC) and near neutral pH (6.0-8.0) were most favourable for degradation. This microbial culture holds the potential for use in bioremediation of DDT-contaminated soils, waste deposits and water bodies. (C) 2002 Elsevier Science Ltd. All rights reserved.

Billingsley, K. A., S. M. Backus, et al. (2002). "Remediation of PCBs in soil by surfactant washing and biodegradation in the wash by *Pseudomonas* sp LB400." *Biotechnology Letters* 24(21): 1827-1832.

Solutions from the washing of polychlorinated biphenyl (PCB)- contaminated soil with a variety of commercial nonionic or anionic surfactants were incubated with *Pseudomonas* sp. LB400 in an attempt to remediate the soil and destroy the PCBs. Nonionic surfactants washed more PCBs from the soil (up to 89%) but inhibited their biodegradation. Anionic surfactants washed less PCBs from the soil but were more effective in biodegradation tests, removing up to 67% of total PCBs.

Brahushi, F., U. Dorfler, et al. (2002). "Environmental behavior of monochlorobenzene in an arable soil." *Fresenius Environmental Bulletin* 11(9A): 599-604.

In a closed aerated laboratory system C-14-monochlorobenzene (C-14-MCB) was incubated with sieved soil, amended with different substrates, to investigate the behavior of this compound in an arable soil. The volatilized C-14-compounds and (CO₂)-C-14 were trapped separately thus allowing a clear differentiation between volatilization and mineralization of MCB. At the end of the experiment extractable and non- extractable residues were determined and a C-14-mass balance was established. The results show clearly, that volatilization is the main loss mechanism of MCB from soils whereas mineralization is of minor importance. Additional nutrition sources could not increase the mineralization process.

Bunge, M., L. Adrian, et al. (2003). "Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium." *Nature* 421(6921): 357-360.

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) are among the most notorious environmental pollutants. Some congeners, particularly those with lateral chlorine substitutions at positions 2, 3, 7 and 8, are extremely toxic and carcinogenic to humans(1). One particularly promising mechanism for the detoxification of PCDDs and PCDFs is microbial reductive dechlorination. So far only a limited number of phylogenetically diverse anaerobic bacteria have been found that couple the reductive dehalogenation of chlorinated compounds-the substitution of a chlorine for a hydrogen atom-to energy conservation and growth in a process called dehalorespiration(2). Microbial dechlorination of PCDDs occurs in sediments and anaerobic mixed cultures from sediments, but the responsible organisms have not yet been identified or isolated. Here we show the presence of a *Dehalococcoides* species in four dioxin-dechlorinating enrichment cultures from a freshwater sediment highly contaminated with PCDDs and PCDFs. We also show that the previously described chlorobenzene- dehalorespiring bacterium *Dehalococcoides* sp. strain CBDB1 (ref. 3) is able to reductively dechlorinate selected dioxin congeners. Reductive dechlorination of 1, 2,3,7,8- pentachlorodibenzo-p-dioxin (PeCDD) demonstrates that environmentally significant dioxins are attacked by this bacterium.

Cho, Y. C., E. B. Ostrofsky, et al. (2002). "Enhancement of microbial PCB dechlorination by chlorobenzoates, chlorophenols and chlorobenzenes." *Fems Microbiology Ecology* 42(1): 51-58.

We investigated the effects of chlorobenzoates (3-, 2,3-, 2,4-, 2,5-, 2,3,5- and 2,4,6-chlorobenzoate), chlorophenols (2,3-, 3,4-, 2,5-, 2,3,6- and penta-chlorophenol), and chlorobenzenes (1,2-, 1,2,3-, 1,2,4- and penta-chlorobenzene) on polychlorinated biphenyl (PCB) dechlorination and on the enrichment of PCB-dechlorinating microorganisms. When the natural microbial populations eluted from St. Lawrence River sediments were enriched with each of the 15 haloaromatic compounds (HACs) in PCB-free sediments, PCB-dechlorinating microorganisms were found in all but pentachlorophenol-amended sediments. Similarly, dechlorinating microorganisms were also found in PCB-spiked sediments amended with all HACs, except for those with pentachlorophenol. In HAC-amended PCB sediments there was a long lag in PCB dechlorination until the HACs were reduced to a plateau level. Despite this lag, once PCB dechlorination started it was faster in the HAC-amended sediments compared to

the unamended controls. The overall extent of PCB dechlorination was significantly enhanced by all HACs except pentachlorophenol and pentachlorobenzene, but the extent as well as the pattern of the enhancement varied. Of the 13 effective HACs, six (2,3-, 2,4- and 2,4,6-chlorobenzoates; 3,4- and 2,3,6-chlorophenols; and 1,2,3-chlorobenzene) enhanced only meta-dechlorination, whereas five (3-chlorobenzoate; 2,3- and 2,5-chlorophenols; and 1,2- and 1,2,4-chlorobenzenes) increased both meta- and para-dechlorination, and two (2,5- and 2,3,5-chlorobenzoates) promoted overall, substitution non-specific dechlorination. When the maximum extent of dechlorination was plotted against the highest number of PCB-dechlorinating microorganisms for each HAC, there was a linear relationship ($P < 0.01$), suggesting that dechlorination enhancement was related to the increase in their population size. However, there was also evidence to suggest that different dechlorinating microorganisms were selected. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

Chroma, L., T. Macek, et al. (2002). "Decolorization of RBBR by plant cells and correlation with the transformation of PCBs." *Chemosphere* 49(7): 739-748.

An extracellular H₂O₂-requiring Remazol Brilliant Blue R (RBBR) decolorizing enzyme activity was detected after cultivation of cells of various plant species both in liquid medium and when growing on agar plates containing RBBR. Level of the enzyme activity was compared with the ability to metabolize polychlorinated biphenyls (PCBs). The ability to decolorize RBBR was tested in the presence and absence of PCBs. The cultures with high PCB-transforming activity proved to exhibit RBBR oxidase much more resistant towards the influence of PCBs. In addition low activities of lignin peroxidase (LiP) and manganese dependent peroxidase (MnP) were detected in medium and in plant cells. No correlation of MnP and LiP activities with PCB degradation could be found. The RBBR decolorization could be used as a rough screening method for plant cultures able to metabolize PCBs. (C) 2002 Elsevier Science Ltd.

Cohen, P. A. (2002). "Halogenated anthraquinones from the rare southern Illinois lichen *Lasallia papulosa*." *Lichenologist* 34: 521-525.

Four anthraquinones were isolated from the foliose lichen, *Lasallia papulosa* (Ach.) Llano. Two of the anthraquinones are known compounds, previously isolated from *Lasallia papulosa*, while the other two were reported previously as secondary metabolites from laboratory-cultured *Nephroma laevigatum*, and are isolated here for the first time from lichens in their natural habitat. All compounds were characterized by UV spectrophotometry, mass spectrometry, H-1 NMR and C-13 NMR. The products were identified as 7-chloroemodin, valsarin (7-chloro-5-hydroxyemodin), 5-chloro-1-O-methylemodin and 5-chloro-1-O-methyl-omega-hydroxyemodin. (C) 2002 The British Lichen Society. Published by Elsevier Science Ltd.

Coleman, N. V., T. E. Mattes, et al. (2002). "Phylogenetic and kinetic diversity of aerobic vinyl chloride-assimilating bacteria from contaminated sites." *Applied and Environmental Microbiology* 68(12): 6162-6171.

Aerobic bacteria that grow on vinyl chloride (VC) have been isolated previously, but their diversity and distribution are largely unknown. It is also unclear whether such bacteria contribute to the natural attenuation of VC at chlorinated-ethene-contaminated sites. We detected aerobic VC biodegradation in 23 of 37 microcosms and enrichments inoculated with samples from various sites. Twelve different bacteria (11 *Mycobacterium* strains and 1 *Nocardioideis* strain) capable of growth on VC as the sole carbon source were isolated, and 5 representative strains were examined further. All the isolates grew on ethene in addition to VC and contained VC-inducible ethene monooxygenase activity. The *Mycobacterium* strains (JS60, JS61, JS616, and JS617) all had similar growth yields (5.4 to 6.6 g of protein/mol), maximum specific growth rates (0.17 to 0.23 day⁻¹), and maximum specific substrate utilization rates (9 to 16 nmol/min/mg of protein) with VC. The *Nocardioideis* strain (JS614) had a higher growth yield (10.3 g of protein/mol), growth rate (0.71 day⁻¹), and substrate utilization rate (43 nmol/min/mg of protein) with VC but was much more sensitive to VC starvation. Half-velocity constant (K_s) values for VC were between 0.5 and 3.2 μM, while K_s values for oxygen ranged from 0.03 to 0.3 mg/liter. Our results indicate that aerobic VC-degrading microorganisms (predominantly *Mycobacterium* strains) are widely distributed at sites contaminated with chlorinated solvents and are likely to be responsible for the natural attenuation of VC.

Dai, S. D., F. H. Vaillancourt, et al. (2002). "Identification and analysis of a bottleneck in PCB biodegradation." *Nature Structural Biology* 9(12): 934-939.

The microbial degradation of polychlorinated biphenyls (PCBs) provides the potential to destroy these widespread, toxic and persistent environmental pollutants. For example, the four-step upper bph pathway transforms some of the more than 100 different PCBs found in commercial mixtures and is being engineered for more effective PCB degradation. In the critical third step of this pathway, 2,3-dihydroxybiphenyl (DHB) 1,2-dioxygenase (DHBD; EC 1.13.11.39) catalyzes aromatic ring cleavage. Here we demonstrate that ortho-chlorinated PCB metabolites strongly inhibit DHBD, promote its suicide inactivation and interfere with the degradation of other compounds. For example, k_{cat}/K_m for 2',6'-diCl DHB was reduced by a factor of similar to 7,000 relative to DHB, and it bound with sufficient affinity to competitively inhibit DHB cleavage at nanomolar concentrations. Crystal structures of two complexes of DHBD with ortho-chlorinated metabolites at 1.7 Å resolution reveal an explanation for these phenomena, which have important implications for bioremediation strategies.

Dejonghe, W., J. Goris, et al. (2002). "Diversity of 3-chloroaniline and 3,4-dichloroaniline degrading bacteria isolated from three different soils and involvement of their plasmids in chloroaniline degradation." *Fems Microbiology Ecology* 42(2): 315-325.

Attempts were made to isolate 3-chloroaniline (3-CA) and 3,4-dichloroaniline (3,4-DCA) degrading bacteria from the A and 13-horizon of three different soils. A variety of 3-CA degrading bacteria was obtained from all soils, whereas 3,4-DCA degrading strains were only isolated from one soil. Amongst the 3-CA and 3,4-DCA degraders, two belong to the gamma-Proteobacteria and seven to the beta-Proteobacteria. Of the latter group, five are members of the family of the Comamonadaceae. Interestingly, all isolates contained an IncP-1beta plasmid. These plasmids could be divided into four major groups based on restriction digest patterns. While all plasmids that were detected in the isolates, except one, encode total degradation of 3-CA, no indigenous plasmid that codes for total degradation of 3,4-DCA was found. This is the first study that reports the presence of diverse transferable plasmids that encode mineralisation of 3-CA in different 3-CA degrading species. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V.. All rights reserved.

Doty, S. L., T. Q. Shang, et al. (2003). "Metabolism of the soil and groundwater contaminants, ethylene dibromide and trichloroethylene, by the tropical leguminous tree, *Leuceana leucocephala*." *Water Research* 37(2): 441-449.

Ethylene dibromide (EDB; dibromoethane) and trichloroethylene (TCE) are hazardous environmental pollutants. The use of plants to treat polluted sites and groundwater, termed phytoremediation, requires plants that can both effectively remove the pollutant as well as grow in the climatic region of the site. In this paper, we report that the tropical leguminous tree, *Leuceana leucocephala* var. K636, is able to take up and metabolize EDB and TCE. The plants were grown in sterile hydroponic solution without its symbiont, *Rhizobium*. EDB and TCE were both metabolized by the plant, as indicated by the formation of bromide ion from EDB and trichloroethanol from TCE. Each plant organ was independently capable of debromination of EDB. *L. leucocephala* is being used to treat perched groundwater as part of a remedial alternative to address an accidental EDB spill in Hawaii. Bromide levels of plant tissues from the trees grown in the phytoremediation treatment cells at the Hawaii Site were elevated, indicating uptake and degradation of brominated compounds in the trees. This report is the first evidence of a tropical tree effectively metabolizing these common organic pollutants. (C) 2002 Elsevier Science Ltd.

Duhamel, M., S. D. Wehr, et al. (2002). "Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis-dichloroethene and vinyl chloride." *Water Research* 36(17): 4193-4202.

An anaerobic mixed microbial culture was enriched from soil and groundwater taken from a site contaminated with trichloroethene (TCE). This enrichment culture was divided into four subcultures amended separately with either perchloroethene (PCE), TCE, cis-dichloroethene (cDCE) or vinyl chloride (VC). In each of

the four subcultures, the chlorinated ethenes were rapidly, consistently, and completely converted to ethene at rates of 30-50 $\mu\text{mol/l}$ of culture per day, or an average 160 μe^- equivalents/l of culture per day. These cultures were capable of sustained and rapid dechlorination of VC, and could not dechlorinate 1,2-dichloroethane, differentiating them from *Dehalococcoides* ethenogenes, the only known isolate capable of complete dechlorination of PCE to ethene. Chloroform (CF) and 1,1,1-trichloroethane, frequent groundwater co-contaminants with TCE and PCE, inhibited chlorinated ethene dechlorination. Most strongly inhibited was the final conversion of VC to ethene, with complete inhibition occurring at an aqueous CIF concentration of 2.5 μM . Differences in rates and community composition developed between the different subcultures, including the loss of the VC enrichment culture's ability to dechlorinate PCE. Denaturing gradient gel electrophoresis of amplified bacterial 16S rRNA gene fragments identified three different DNA sequences in the enrichment cultures, all phylogenetically related to *D. ethenogenes*. Based on the PCR-DGGE results and substrate utilization patterns, it is apparent that significant mechanistic differences exist between each step of dechlorination from TCE to ethene, especially for the last important dechlorination step from VC to ethene. (C) 2002 Elsevier Science Ltd.

Duhamel, M., S. D. Wehr, et al. (2002). "Comparison of anaerobic dechlorinating enrichment cultures maintained on tetrachloroethene, trichloroethene, cis- dichloroethene and vinyl chloride." *Water Research* 36(17): 4193-4202.

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Finneran, K. T., H. M. Forbush, et al. (2002). "Desulfitobacterium metallireducens sp nov., an anaerobic bacterium that couples growth to the reduction of metals and humic acids as well as chlorinated compounds." *International Journal of Systematic and Evolutionary Microbiology* 52: 1929-1935.

Strain 853-15A(T) was enriched and isolated from uranium- contaminated aquifer sediment by its ability to grow under anaerobic conditions via the oxidation of lactate coupled to the reduction of anthraquinone-2,6-disulfonate (AQDS) to anthrahydroquinone-2,6-disulfonate (AHQDS). Lactate was oxidized incompletely to acetate and carbon dioxide according to the reaction $\text{CH}_3\text{CHOHCOO}^- + 2\text{AQDS} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + 2\text{AHQDS} + \text{CO}_2$. Additional electron donors utilized included formate, ethanol, butanol, butyrate, malate and pyruvate. Lactate also supported growth with Fe(III) citrate, Mn(IV) oxide, humic substances, elemental sulfur, 3-chloro-4-hydroxyphenylacetate, trichloroethylene or tetrachloroethylene serving as the electron acceptor. Growth was not observed with sulfate, sulfite, nitrate or fumarate as the terminal electron acceptor. The temperature optimum for growth was 30 degreesC, but growth was also observed at 20 and 37 degreesC. The pH optimum was approximately 7.0. The 16S rDNA sequence of strain 853-15A(T) suggested that it was most closely related to *Desulfitobacterium dehalogenans* and closely related to *Desulfitobacterium chlororespirans* and *Desulfitobacterium frappieri*. The phylogenetic and physiological properties exhibited by strain 853-15A(T) (= ATCC BAA-636(T)) place it within the genus *Desulfitobacterium* as the type strain of a novel species, *Desulfitobacterium metallireducens* sp. nov.

Gander, J. W., G. F. Parkin, et al. (2002). "Kinetics of 1,1,1-trichloroethane transformation by iron sulfide and a methanogenic consortium." *Environmental Science & Technology* 36(21): 4540-4546.

To evaluate the effect of potential interactions between methanogenic bacteria and iron sulfide minerals during transformation of 1,1,1-trichloroethane (1,1,1-TCA), we measured the kinetics of 1,1,1-TCA transformation by mackinawite (FeS(1-x), but abbreviated as FeS) and a methanogenic consortium enriched on lactate (termed LEC). Results from batch kinetic experiments show that 1,1,1-TCA transformation by FeS and resting LEC can be described by second-order rate expressions, with rates depending on 1,1,1-TCA concentration (M), FeS surface area concentration (m² L⁻¹), and LEC concentration (as measured by mg L⁻¹ volatile suspended solids (VSS)). In reactors containing FeS alone, 1,1,1-dichloroethane (1,1-DCA) and 2-butyne were identified as products, but only accounted for 6% of the 1,1,1-TCA transformed. In reactors containing LEC alone, the only identified product was 1,1-DCA, which accounted for 46 +/- 8% of the 1,1,1-TCA transformed. Supernatant from LEC-alone reactors also transformed 1,1,1-TCA, suggesting that 1,1,1-TCA may be transformed by some non-cell component (such as an extracellular compound excreted by the organisms) that either reacts directly with 1,1,1-TCA or with the abiotic media to form a reactive species. Comparison of 1,1,1-TCA transformation rates from experiments with combinations of FeS (varying surface area concentrations) and LEC (varying VSS concentrations) to those with just FeS alone or LEC alone suggests some synergism occurs between the two reactive species. Observed enhancements took the form of faster 1,1,1-TCA transformation and faster 1,1,1-DCA appearance but less production of 1,1-DCA per unit of 1,1,1-TCA transformed. These observations suggest that the faster 1,1,1-TCA transformation in the combined systems (compared to the FeS-alone and LEC-alone experiments) is due to increased reactivity of both FeS and LEC, possibly due to production of soluble microbial products that make the FeS more reactive or less inhibition of LEC by 1,1,1-TCA due to FeS transformation of 1,1,1-TCA.

Hiraoka, Y., T. Yamada, et al. (2002). "Flow cytometry analysis of changes in the DNA content of the polychlorinated biphenyl degrader *Comamonas testosteroni* TK102: Effect of metabolites on cell-cell separation." *Applied and Environmental Microbiology* 68(10): 5104-5112.

Flow cytometry was used to monitor changes in the DNA content of the polychlorinated biphenyl (PCB)degrading bacterium *Comamonas testosteroni* TK102 during growth in the presence or absence of PCBs. In culture medium without PCBs, the majority of stationary-phase cells contained a single chromosome. In the presence of PCBs, the percentage of cells containing two chromosomes increased from 12% to approximately 50%. In contrast, addition of PCBs did not change the DNA contents of three species that are unable to degrade PCBs. In addition, highly chlorinated PCBs that are not degraded by TK102 did not result in a change in the DNA content. These results suggest that PCBs did not affect the DNA content of the cells directly; rather, the intermediate metabolites resulting from the degradation of PCBs caused the increase in DNA content. To study the effect of intermediate metabolites on the DNA content of the cells, four bph genes, bphA1, bphB, bphC, and bphD, were disrupted by gene replacement. The resulting mutant strains accumulated intermediate metabolites when they were grown in the presence of PCBs or biphenyl (BP). When the bphB gene was disrupted, the percentage of cells containing two chromosomes increased in cultures grown with PCBs or BP. When grown with BP, cultures of this mutant accumulated two intermediate metabolites, 2-hydroxybiphenyl (2-OHBP) and 3-OHBP. Addition of 2- or 3-OHBP to a wild-type TK102 and non-PCB-degrading species culture also resulted in an increase in the percentage of cells containing two chromosomes. Electron microscopy revealed that cell-cell separation was inhibited in this culture. This is the first report that hydroxy-BPs can inhibit bacterial cell separation while allowing continued DNA replication.

Imamoglu, I., K. Li, et al. (2002). "Modeling polychlorinated biphenyl congener patterns and dechlorination in dated sediments from the Ashtabula River, Ohio, USA." *Environmental Toxicology and Chemistry* 21(11): 2283-2291.

Polychlorinated biphenyl (PCB) congeners were analyzed in four deep, dated sediment cores from the Ashtabula River (OH, USA), for the purpose of identifying relevant PCB sources and congener patterns. The time span for three of the cores is from the mid 1960s to 1998, whereas the fourth has a time span of six years. The total

PCB concentrations are in the range of 0.4 to 6.8 µg/g dry weight, with the highest concentrations observed in samples from the 1970s. A factor analysis (FA) model with nonnegative constraints was used to investigate the sources and patterns of PCBs. Additionally, a new model, based on a least squares method, was developed to identify possible patterns of anaerobic dechlorination of PCBs in the sediments, and to quantify the relevant dechlorination pathways. Both models were validated successfully either by artificially created data sets (FA model) or by using laboratory data from the literature (dechlorination model). The FA model revealed two significant sources. The first was identified as a slightly altered Aroclor 1248. The second did not resemble any Aroclor closely, but was very similar to the overall average congener profile of all samples. Simulation of anaerobic dechlorination on an Aroclor 1248 profile from the literature, according to dechlorination activities H/H', as defined in the literature, yielded a congener profile very similar to that of the second pattern. This indicates the likelihood of anaerobic dechlorination of PCBs in Ashtabula River sediments.

Jung, K. J., B. H. Kim, et al. (2002). "Monitoring expression of bphC gene from *Ralstonia eutropha* H850 induced by plant terpenes in soil." *Journal of Microbiology* 40(4): 340-343.

A PCB degrader, *Ralstonia eutropha* 14850 was shown to induce bphC gene encoding 2,3-dihydroxybiphenyl-1,2-dioxygenase in a carvone-amended pure culture in our previous study (Park et al., 1999). The present study was carried out to examine how plant terpenes, as natural substrates, would cause an expression of a PCB degradative gene in soil that was amended with terpenes. The population of *Ralstonia eutropha* 14850 was maintained at least around 10⁸ (CFU/g fresh soil) in the soil amended with carvone or limonene in the presence of succinate as a growth substrate at 50 th day. The gene expression was monitored by RT-PCR using total RNA directly extracted from each soil and bphC gene primers. The bphC gene expression of the seeded strain 14850 was observed in the soil amended with biphenyl (4 days) but not with succinate, carvone and limonene. These results indicate that terpenes widely distributed in nature could be a potential inducing substrate for effective PCB biodegradation in the soil but their bioavailability and specific induction behavior should be taken into account before PCB bioremediation implementation.

Kao, C. M., S. C. Chen, et al. (2003). "Remediation of PCE-contaminated aquifer by an in situ two-layer biobarrier: laboratory batch and column studies." *Water Research* 37(1): 27-38.

The industrial solvent tetrachloroethylene (PCE) is among the most ubiquitous chlorinated compounds found in groundwater contamination. The objective of this study was to develop an in situ two-layer biobarrier system consisting of an organic-releasing material layer followed by an oxygen-releasing material layer. The organic-releasing material, which contained sludge cakes from a domestic wastewater treatment plant, is able to release biodegradable organics continuously. The oxygen-releasing material, which contained calcium peroxide, is able to release oxygen continuously upon contact with water. The first organic-releasing material layer was to supply organics (primary substrates) to reductively dechlorinate PCE in situ. The second oxygen-releasing material layer was to release oxygen to aerobic biodegrade or cometabolize PCE degradation byproducts from the first anaerobic layer. Batch experiments were conducted to design and identify the components of the organic and oxygen-releasing materials, and evaluate the organic substrate (presented as chemical oxygen demand (COD) equivalent) and oxygen release rates from the organic-releasing material and oxygen-releasing materials, respectively. The observed oxygen and COD release rates were approximately 0.0368 and 0.0416 mg/d/g of material, respectively. A laboratory-scale column experiment was then conducted to evaluate the feasibility of this proposed system for the bioremediation of PCE-contaminated groundwater. This system was performed using a series of continuous-flow glass columns including a soil column, an organic-releasing material column, two consecutive soil columns, and an oxygen-releasing material column, followed by two other consecutive soil columns. Anaerobic acclimated sludges were inoculated in the first four columns, and aerobic acclimated sludges were inoculated in the last three columns to provide microbial consortia for contaminant biodegradation. Simulated PCE-contaminated groundwater with a flow rate of 0.25 L/d was pumped into this system. Effluent samples from each column were analyzed for PCE and its degradation byproducts. Results show that up to 99% of PCE removal efficiency was obtained in this passive system. Thus, the biobarrier treatment scheme has the potential to be developed into an environmentally and economically acceptable remediation technology for the in situ treatment of PCE-contaminated aquifer. (C) 2002 Elsevier Science Ltd. All rights reserved.

Kikuchi, T., K. Iwasaki, et al. (2002). "Quantitative and rapid detection of the trichloroethylene- degrading bacterium *Methylocystis* sp M in groundwater by real- time PCR." *Applied Microbiology and Biotechnology* 59(6): 731-736.

We developed a method based on real-time PCR for the specific and rapid enumeration of a trichloroethylene-degrading methanotroph, *Methylocystis* sp. M, with the aim of monitoring the strain in groundwater. A primer set designed from the nucleotide sequence of the *mmoC* gene of a soluble methane monooxygenase (sMMO) gene cluster from *Methylocystis* sp. M was specific to amplify the DNA region from the strain and no PCR products were amplified with the sMMO gene clusters from six other methanotroph strains. The real-time PCR reliably quantified *Methylocystis* sp. M over at least five orders of magnitude (5×10^6 to 5×10^2) cells/PCR tube, or 2×10^8 to 2×10^4 cells/ml). Five cells of *Methylocystis* sp. M per PCR tube (2×10^2) cells/ml) were detectable when the cells were suspended in distilled water. The concomitant presence of other methanotrophs in samples did not affect the reliability of enumeration; and recovery of the cells with a membrane filter enabled us to quantify cells of the strain in groundwater. This quantification procedure was completed within 3 h, including preparation time of environmental samples. We conclude that real-time PCR using the *mmoC* primer set can be used practically to analyze the behavior of *Methylocystis* sp. M at bioremediation sites.

Kim, Y., D. J. Arp, et al. (2002). "Kinetic and inhibition studies for the aerobic cometabolism of 1,1,1-trichloroethane, 1,1-dichloroethylene, and 1,1- dichloroethane by a butane-grown mixed culture." *Biotechnology and Bioengineering* 80(5): 498-508.

Batch kinetic and inhibition studies were performed for the aerobic cometabolism of 1,1,1-trichloroethane (1,1,1-TCA), 1,1- dichloroethylene (1,1-DCE), and 1,1-dichloroethane (1,1-DCA) by a butane-grown mixed culture. These chlorinated aliphatic hydrocarbons (CAHs) are often found together as cocontaminants in groundwater. The maximum degradation rates ($k(\max)$) and half-saturation coefficients (K_s) were determined in single compound kinetic tests. The highest $k(\max)$ was obtained for butane (2.6 $\mu\text{mol}/\text{mg TSS}/\text{h}$) followed by 1,1-DCE (1.3 $\mu\text{mol}/\text{mg TSS}/\text{h}$), 1,1-DCA (0.49 $\mu\text{mol}/\text{mg TSS}/\text{h}$), and 1,1,1-TCA (0.19 $\mu\text{mol}/\text{mg TSS}/\text{h}$), while the order of K_s from the highest to lowest was 1,1-DCA (19 μM), butane (19 μM), 1,1,1-TCA (12 μM) and 1,1-DCE (1.5 μM). The inhibition types were determined using direct linear plots, while inhibition coefficients (K_{ic} and K_{iu}) were estimated by nonlinear least squares regression (NLSR) fits to the kinetic model of the identified inhibition type. Two different inhibition types were observed among the compounds. Competitive inhibition among CAHs was indicated from direct linear plots, and the CAHs also competitively inhibited butane utilization. 1,1-DCE was a stronger inhibitor than the other CAHs. Mixed inhibition of 1,1,1-TCA, 1,1-DCA, and 1,1-DCE transformations by butane was observed. Thus, both competitive and mixed inhibitions are important in cometabolism of CAHs by this butane culture. For competitive inhibition between CAHs, the ratio of the K_s values was a reasonable indicator of competitive inhibition observed. Butane was a strong inhibitor of CAH transformation, having a much lower inhibition coefficient than the K_s value of butane, while the CAHs were weak inhibitors of butane utilization. Model simulations of reactor systems where both the growth substrate and the CAHs are present indicate that reactor performance is significantly affected by inhibition type and inhibition coefficients. Thus, determining inhibition type and measuring inhibition coefficients is important in designing CAH treatment systems. (C) 2002 Wiley Periodicals, Inc.

Kim, Y., D. J. Arp, et al. (2002). "Kinetic and inhibition studies for the aerobic cometabolism of 1,1,1-trichloroethane, 1,1-dichloroethylene, and 1,1- dichloroethane by a butane-grown mixed culture." *Biotechnology and Bioengineering* 80(5): 498-508.

Batch kinetic and inhibition studies were performed for the aerobic cometabolism of 1,1,1-trichloroethane (1,1,1-TCA), 1,1- dichloroethylene (1,1-DCE), and 1,1-dichloroethane (1,1-DCA) by a butane-grown mixed culture. These chlorinated aliphatic hydrocarbons (CAHs) are often found together as cocontaminants in groundwater. The maximum degradation rates ($k(\max)$) and half-saturation coefficients (K_s) were determined in single compound kinetic tests. The highest $k(\max)$ was obtained for butane (2.6 $\mu\text{mol}/\text{mg TSS}/\text{h}$) followed by 1,1-DCE (1.3 $\mu\text{mol}/\text{mg TSS}/\text{h}$), 1,1-DCA (0.49 $\mu\text{mol}/\text{mg TSS}/\text{h}$), and 1,1,1-TCA (0.19 $\mu\text{mol}/\text{mg TSS}/\text{h}$), while the order of K_s from the highest to lowest was 1,1-DCA (19 μM), butane (19 μM), 1,1,1-TCA (12

muM) and 1,1-DCE (1.5 muM). The inhibition types were determined using direct linear plots, while inhibition coefficients (K_{ic} and K_{iu}) were estimated by nonlinear least squares regression (NLSR) fits to the kinetic model of the identified inhibition type. Two different inhibition types were observed among the compounds. Competitive inhibition among CAHs was indicated from direct linear plots, and the CAHs also competitively inhibited butane utilization. 1,1-DCE was a stronger inhibitor than the other CAHs. Mixed inhibition of 1,1,1-TCA, 1,1-DCA, and 1,1-DCE transformations by butane was observed. Thus, both competitive and mixed inhibitions are important in cometabolism of CAHs by this butane culture. For competitive inhibition between CAHs, the ratio of the K_s values was a reasonable indicator of competitive inhibition observed. Butane was a strong inhibitor of CAH transformation, having a much lower inhibition coefficient than the K_s value of butane, while the CAHs were weak inhibitors of butane utilization. Model simulations of reactor systems where both the growth substrate and the CAHs are present indicate that reactor performance is significantly affected by inhibition type and inhibition coefficients. Thus, determining inhibition type and measuring inhibition coefficients is important in designing CAH treatment systems. (C) 2002 Wiley Periodicals, Inc.

Kim, Y. H. and E. R. Carraway (2002). "Reductive dechlorination of PCE and TCE by vitamin B-12 and ZVMs." *Environmental Technology* 23(10): 1135-1145.

The reductive dechlorination of tetrachloroethene (PCE) and trichloroethene (TCE) catalyzed by vitamin B-12 was examined when zero valent metals (ZVMs) were used as bulk electron donors in batch reactors. UV-visible spectra showed that zinc reduces vitamin B-12 Co(III) to vitamin B-12 Co(I) through B-12 Co(II) and iron reduces vitamin B-12 Co(III) to vitamin B-12 Co(II). Thus iron, the most popular ZVM reductant, does not have enough reduction potential to reduce vitamin B-12 to the super-reduced vitamin B-12(I), which has been shown to be an active species in reductive dechlorination. Dechlorination of PCE and TCE by iron and zinc in the presence of vitamin B-12 showed that the zinc and vitamin B-12 combination greatly enhances the reaction rates for both PCE and TCE, but iron and vitamin B-12 result in an increase in reactivity only for PCE degradation, not for TCE degradation in comparison with metals only. This result indicates vitamin B-12(I) is active towards both PCE and TCE degradation while vitamin B-12(II) is active only towards PCE. Calculated activation energies for the dechlorination of PCE in the presence of Vitamin B-12 showed that vitamin B-12 lowered the activation energy about 40-60 kJ mol⁻¹ for both metals.

Kumari, R., S. Subudhi, et al. (2002). "Cloning and characterization of lin genes responsible for the degradation of hexachlorocyclohexane isomers by *Sphingomonas paucimobilis* strain B90." *Applied and Environmental Microbiology* 68(12): 6021-6028.

Hexachlorocyclohexane (HCH) has been used extensively against agricultural pests and in public health programs for the control of mosquitoes. Commercial formulations of HCH consist of a mixture of four isomers, alpha, beta, gamma, and delta. While all these isomers pose serious environmental problems, beta-HCH is more problematic due to its longer persistence in the environment. We have studied the degradation of HCH isomers by *Sphingomonas paucimobilis* strain B90 and characterized the lin genes encoding enzymes from strain B90 responsible for the degradation of HCH isomers. Two nonidentical copies of the linA gene encoding HCH dehydrochlorinase, which were designated linA1 and linA2, were found in *S. paucimobilis* B90. The linA1 and linA2 genes could be expressed in *Escherichia coli*, leading to dehydrochlorination of alpha-, gamma-, and delta-HCH but not of beta-HCH, suggesting that *S. paucimobilis* B90 contains another pathway for the initial steps of beta-HCH degradation. The cloning and characterization of the halohydrolyase (linB), dehydrogenase (linC and linX), and reductive dechlorinase (linD) genes from *S. paucimobilis* B90 revealed that they share similar to 96 to 99% identical nucleotides with the corresponding genes of *S. paucimobilis* UT26. No evidence was found for the presence of a linE-like gene, coding for a ring cleavage dioxygenase, in strain B90. The gene structures around the linA1 and linA2 genes of strain B90, compared to those in strain UT26, are suggestive of a recombination between linA1 and linA2, which formed linA4 of strain UT26.

Lackey, L. W., J. R. Gamble, et al. (2002). "Bench-scale evaluation of a biofiltration system used to mitigate trichloroethylene contaminated air streams." *Advances in Environmental Research* 7(1): 97-104.

Improper disposal practices of the once widely used short- chained chlorinated aliphatics have made them a major component of groundwater contamination. Historically, pump and treat technology has been implemented to remediate and contain such aquifer water. After being pumped to the surface, the water is frequently treated with stripper technology. As a result, there is a high volume, low concentration contaminated air stream to either be directly emitted or treated prior to release in an effort to meet regulatory compliance. This work studied the feasibility of using biofiltration technology to remediate trichloroethylene (TCE) contaminated air streams. Furthermore, work focused on operational schemes that influenced the TCE degradation potential within the system. The bench-scale biofilter system contained 42 l of organic packing material and was inoculated with a propane-oxidizing microbial consortium. Propane, the primary substrate, and TCE were introduced into the biofilter in two distinct modes. Initially, TCE and propane were both continuously added to the biofilter system. The maximum TCE degradation observed under this continuous feeding scheme was 25%. Secondly, the TCE and propane were alternately pulsed or cycled in a step-wise fashion into the biofilter system. Under this operating environment, greater than 98% removal of TCE from the air stream was achieved. (C) 2002 Elsevier Science Ltd.

Lee, T. H., M. Ike, et al. (2002). "A reactor system combining reductive dechlorination with cometabolic oxidation for complete degradation of tetrachloroethylene." *Journal of Environmental Sciences-China* 14(4): 445-450.

A laboratory sequential anaerobic-aerobic bioreactor system, which consisted of an anaerobic fixed film reactor and two aerobic chemostats, was set up to degrade tetrachloroethylene (PCE) without accumulating highly toxic degradation intermediates. A soil enrichment culture, which could reductively dechlorinate 900 µM (ca. 150 mg/L) of PCE stoichiometrically into cis-1,2-dichloroethylene (cis-DCE), was attached to ceramic media in the anaerobic fixed film reactor. A phenol degrading strain, *Alcaligenes* sp. R5, which can efficiently degrade cis-DCE by co-metabolic oxidation, was used as inoculum for the aerobic chemostats consisted of a transformation reactor and a growth reactor. The anaerobic fixed film bioreactor showed more than 99% of PCE transformation into cis-DCE in the range of influent PCE concentration from 5 µM to 35 µM at hydraulic retention time of 48h. On the other hand, efficient degradation of the resultant cis-DCE by strain R5 in the following aerobic system could not be achieved due to oxygen limitation. However, 54% of the maximum cis-DCE degradation was obtained when 10 µmol of hydrogen peroxide (H₂O₂) was supplemented to the transformation reactor as an additional oxygen source. Further studies are needed to achieve more efficient co-metabolic degradation of cis-DCE in the aerobic reactor.

Major, D. W., M. L. McMaster, et al. (2002). "Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene." *Environmental Science & Technology* 36(23): 5106-5116.

A laboratory microcosm study and a pilot scale field test were conducted to evaluate biostimulation and bioaugmentation to dechlorinate tetrachloroethene (PCE) to ethene at Kelly Air Force Base. The site groundwater contained about 1 mg/L of PCE and lower amounts of trichloroethene (TCE) and cis-1,2- dichloroethene (cDCE). Laboratory microcosms inoculated with soil and groundwater from the site exhibited partial dechlorination of TCE to cDCE when amended with lactate or methanol. Following the addition of a dechlorinating enrichment culture, KB-1, the chlorinated ethenes in the microcosms were completely converted to ethene. The KB-1 culture is a natural dechlorinating microbial consortium that contains phylogenetic relatives of *Dehalococcoides* ethenogenes. The ability of KB-1 to stimulate biodegradation of chlorinated ethenes in situ was explored using a closed loop recirculation cell with a pore volume of approximately 64 000 L. The pilot test area (PTA) groundwater was first amended with methanol and acetate to establish reducing conditions. Under these conditions, dechlorination of PCE to cDCE was observed. Thirteen liters of the KB-1 culture were then injected into the subsurface. Within 200 days, the concentrations of PCE, TCE, and cis-1,2-DCE within the PTA were all below 5 µg/L, and ethene production accounted for the observed mass loss. The maximum rates of dechlorination estimated from field data were rapid (half-lives of a few hours). Throughout the pilot test period, groundwater samples were assayed for the presence of *Dehalococcoides* using both a *Dehalococcoides*-specific PCR assay and 16S rDNA sequence information. The sequences detected in the PTA after bioaugmentation were specific to the *Dehalococcoides*

species in the KB-1 culture. These sequences were observed to progressively increase in abundance and spread downgradient within the PTA. populated the PTA aquifer and contributed to the stimulation of dechlorination beyond cDCE to ethene.

Mazanti, L., C. Rice, et al. (2003). "Aqueous-phase disappearance of atrazine, metolachlor, and chlorpyrifos in laboratory aquaria and outdoor macrocosms." *Archives of Environmental Contamination and Toxicology* 44(1): 67-76.

Dissipation processes are described for a combination of commonly used pesticides-atrazine (6-chloro-4-ethylamino-6- isopropylamino-s-triazine), metolachlor (2-chloro-N-[2-ethyl-6- methyl-phenyl]-N-[2-methoxy-1-methylethyl] acetamide), and chlorpyrifos (O-O diethyl O-[3,5,6-trichloro-2-pyridinyl] phosphorothioate)-in a laboratory and outdoor pond systems. Dosing rates and timing were designed to duplicate those common in the n-mid-Atlantic Coastal Plain, USA. Treatments ranged from 2 and 2.5 mg/L to 0.2 and 0.25 mg/L respectively for atrazine and metolachlor, and chlorpyrifos was added at 1.0 and 0.1 mg/L in the aquaria and at 0.1 mg/L in the outdoor macrocosms. Chlorpyrifos disappearance was rapid in all of the systems and followed a two-phase sequence. Initial half-lives varied from 0.16 day to 0.38 day and showed similar rates in the aquaria and the outdoor systems. The second phase of the chlorpyrifos loss pattern was slower (18-20 days) in all the treatments except for the low herbicide treatment in the outdoor test, where it was 3.4 days. Compared to the outdoor system, herbicide losses were much slower in the aquaria, e.g., 150 days for atrazine and 55 days for metolachlor, and no appreciable loss of herbicide was apparent in the high-treated aquaria. In the outdoor systems, the half-lives for the low herbicide treatment were 27 days and 12 days, respectively, for atrazine and metolachlor, and 48 and 20 days, respectively for the high herbicide-treated pond. Very low levels of CIAT (6- amino-2-chloro-4-iso-propylamino-s-triazine) and CEAT (2- chloro-4-ethylamino-6-ethylamino-s-triazine), degradation products of atrazine, were observed in the outdoor studies.

McCullar, M. V., S. C. Koh, et al. (2002). "The use of mutants to discern the degradation pathway of 3,4'-dichlorobiphenyl in *Pseudomonas acidovorans* M3GY." *Fems Microbiology Ecology* 42(1): 81-87.

Pseudomonas acidovorans strain M3GY is a recombinant bacterium with the novel ability to utilize 3,4'-dichlorobiphenyl (3,4'- DCBP) as a growth substrate. This strain was previously shown to oxidize the 3'-ring and produce 4-chlorobenzoate (4-CBa) through the standard biphenyl pathway. Although 4-CBa was metabolized through the meta-fission pathway, the genes encoding the ortho-chlorocatechol pathway were retained. Nevertheless, neither 3-CBa nor 3-chlorocatechol (3-CC) were detected as intermediates during metabolism of 3,4'-DCBP, nor was 4-CBa utilized as a sole carbon source, by this strain. Two mutant strains were produced to resolve these anomalies. Mutant strain M3GY-9 was obtained by Tn5 insertion and selection for growth on biphenyl, and was unable to grow on 3-CBa. It accumulated 3-CC from 3,4'-DCBP when grown on biphenyl. Thus, M3GY attacks both rings, and the failure to isolate 3-CBa or 3- CC is due to rapid turnover by the enzymes of the ortho- chlorocatechol pathway in the wild-type strain. Mutant strain M3GY-I grew on 4-CBa, unlike the wild-type strain. Washed cell suspensions of mutant strain MEGY-1 consumed 4-fluorobenzoate, 4-bromobenzoate, and, to a lesser extent 4-iodobenzoate. The mutation that resulted in the ability of mutant strain M3GY-I to effectively utilize 4-CBa as a sole carbon source was associated with a transport mechanism. (C) 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Microbiological Societies.

McDonald, I. R., K. L. Warner, et al. (2002). "A review of bacterial methyl halide degradation: biochemistry, genetics and molecular ecology." *Environmental Microbiology* 4(4): 193-203.

Methyl halide-degrading bacteria are a diverse group of organisms that are found in both terrestrial and marine environments. They potentially play an important role in mitigating ozone depletion resulting from methyl chloride and methyl bromide emissions. The first step in the pathway(s) of methyl halide degradation involves a methyltransferase and, recently, the presence of this pathway has been studied in a number of bacteria. This paper reviews the biochemistry and genetics of methyl halide utilization in the aerobic bacteria *Methylobacterium chloromethanicum* CM4(T) , *Hyphomicrobium chloromethanicum* CM2(T) , *Aminobacter* strain IMB-1 and *Aminobacter* strain CC495. These bacteria are able to use methyl halides as a sole source of carbon and energy,

are all members of the alpha-Proteobacteria and were isolated from a variety of polluted and pristine terrestrial environments. An understanding of the genetics of these bacteria identified a unique gene (*cmuA*) involved in the degradation of methyl halides, which codes for a protein (CmuA) with unique methyltransferase and corrinoid functions. This unique functional gene, *cmuA*, is being used to develop molecular ecology techniques to examine the diversity and distribution of methyl halide-utilizing bacteria in the environment and hopefully to understand their role in methyl halide degradation in different environments. These techniques will also enable the detection of potentially novel methyl halide-degrading bacteria.

Mori, T. and R. Kondo (2002). "Degradation of 2,7-dichlorodibenzo-p-dioxin by wood-rotting fungi, screened by dioxin degrading ability." *Fems Microbiology Letters* 213(1): 127-131.

One hundred thirty-six strains of wood-rot fungi (74 strains, 66 species of 19 genera, and 62 unidentified strains) were screened for the dibenzo-p-dioxin (DD) decreasing activity. It was observed that 20% of additional DD (1 pmol) disappeared in the cultures of eight strains belonging to four genera (*Aleurodiscus* (one strain), *Ceriporia* (one strain), *Phanerochaete* (one strain), *Phlebia* (five strains)) and four unidentified strains. These 12 fungal strains were used for the examination of the degradation of [¹⁴C-14]2,7-dichlorodibenzo-p-dioxin (2,7-diCDD). The fungi unidentified strain MZ-227, *Phlebia* sp. MG-60 and *Phlebia lindtneri* showed higher cumulative (CO₂)-C-14 evolution rates than the other nine fungi. MZ-227, *Phlebia* sp. MG-60, and *P. lindtneri* converted 250 nmol of 2,7-diCDD to 196, 155 and 149 nmol of (CO₂)-C-14, respectively, during a 30-day incubation period. (C) 2002 Federation of Eur. Microbiological Societies. Published by Elsevier Science B.V.

Mori, T. and R. Kondo (2002). "Oxidation of chlorinated dibenzo-p-dioxin and dibenzofuran by white-rot fungus, *Phlebia lindtneri*." *Fems Microbiology Letters* 216(2): 223-227.

The actions of a white-rot fungus on two chlorinated aromatic compounds, known to be persistent environmental contaminants, were studied. Two models, both-ring chlorinated dioxin, 2,7-dichlorodibenzo-p-dioxin (2,7-diCDD) and 2,8-dichlorodibenzofuran (2,8-diCDF), were metabolized by the white-rot fungus *Phlebia lindtneri*. 2,7-DiCDD disappeared linearly in the culture of *P. lindtneri*; over a 20-day incubation period, with only 45% remaining in the culture. One of the metabolites produced by *P. lindtneri* from a 5-day incubated culture with 2,7-diCDD or 2,8-diCDF was identified by gas chromatography-mass spectrometry. *P. lindtneri* was shown to metabolize 2,7-diCDD and 2,8-diCDF to hydroxy-diCDD and hydroxy-diCDF, respectively. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Murrell, J. C. (2002). "Expression of soluble methane monooxygenase genes." *Microbiology-Sgm* 148: 3329-3330.

Niedan, V. W., F. Keppler, et al. (2003). "De novo formation of organochlorines in a sewage treatment plant." *Biogeochemistry* 62(3): 277-287.

The de novo formation of organochlorines was observed in a municipal sewage treatment plant. Due to this formation, the amount of organically bound halogens (AOX) increased 15-fold inside the sewage treatment plant. Per day, more than 6 kg of organically-bound chlorine were produced. This formation is not based on a metabolism of present organochlorines, it is a de novo formation out of inorganic chloride and organic substrates. The AOX trigger concentration in sewage sludge in Germany is 500 mg kg⁻¹ and was sometimes exceeded by a factor of 10. No known anthropogenic organohalogenes were found which could explain the elevated AOX concentrations. Instead many chlorinated compounds could be identified which were not known to be of anthropogenic origin. The compound with the highest concentration was the 3,4-dichlorophenylacetic acid (3,4-CPAc). In one case, more than 1 g kg⁻¹ of this compound was detected. A slaughterhouse that emits phenylacetic acid is probably the origin of that formation. In model experiments phenylacetic acid was chlorinated with HOCl but chlorinated phenylacetic acids other than 3,4-CPAc were found. Therefore it can be excluded that the chlorination in the sewage treatment plant takes place by an abiotic reaction with hypochlorite that might have been introduced there. We assume that the occurring microorganisms are responsible for the de novo formation in

the sewage treatment plant. The obtained knowledge could also be useful to understand natural chlorination processes.

Nojiri, H. and T. Omori (2002). "Molecular bases of aerobic bacterial degradation of dioxins: Involvement of angular dioxygenation." *Bioscience Biotechnology and Biochemistry* 66(10): 2001-2016.

In the last decade, extensive investigation has been done on the bacterial degradation of dioxins and its related compounds, because this class of chemicals is highly toxic and has been widely distributed in the environment. These studies have revealed the primary importance of a novel dioxygenation reaction, called angular dioxygenation, in the aerobic bacterial degradation pathway of dioxin. Accompanied by the electron transport proteins, Rieske nonheme iron oxygenase catalyzes the incorporation of oxygen atoms to the ether bond-carrying carbon (the angular position) and an adjacent carbon, resulting in the irreversible cleavage of the recalcitrant aryl ether bond. The 2,21,3-trihydroxybiphenyl or 2,2',3'-trihydroxydiphenyl ether derivatives formed are degraded through meta cleavage. In addition to the degradation system of dibenzofuran and dibenzo-p-dioxin (the nonchlorinated model compounds of dioxin), those of fluorene and carbazole were shown to function in dioxin degradation. Some dioxin degradation pathways have been studied biochemically and genetically. In addition, feasibility studies have shown that some dioxin-degrading strains can function in actual dioxin-contaminated soil. These studies provide useful information for the establishment of a bioremediation method for dioxin contamination. This review summarizes recent progress on molecular and biochemical bases of the bacterial aerobic degradation of dioxin and related compounds.

Numata, M., N. Nakamura, et al. (2002). "Chlorine isotope fractionation during reductive dechlorination of chlorinated ethenes by anaerobic bacteria." *Environmental Science & Technology* 36(20): 4389-4394.

Chlorine isotope fractionation during reductive dechlorination of trichloroethene (TCE) and tetrachloroethene (PCE) to cis-1,2-dichloroethene (cDCE) by anaerobic bacteria was investigated. The changes in the (Cl)-C-37/(Cl)-C-35 ratio observed during the one-step reaction (TCE to cDCE) can be explained by the regioselective elimination of chlorine accompanied by the Rayleigh fractionation. The fractionation factors (α) of the TCE dechlorination by three kinds of anaerobic cultures were approximately 0.994-0.995 at 30 degreesC. The enrichment of (Cl)-C-37 in the organic chlorine during the two-step reaction (PCE to cDCE) can be explained by the random elimination of one chlorine atom in the PCE molecule followed by the regioselective elimination of one chlorine atom in the TCE molecule. The fractionation factors for: the first step of the PCE dechlorination with three kinds of anaerobic cultures were estimated to be 0.987-0.991 at 30 degreesC using a mathematical model. Isotope fractionation during the first step would be the primary factor for the chlorine isotope fractionation during the PCE dechlorination to cDCE. The developed models can be utilized to evaluate the fractionation factors of regioselective and multistep reactions.

Ohtsubo, Y., M. Shimura, et al. (2003). "Novel approach to the improvement of biphenyl and polychlorinated biphenyl degradation activity: Promoter implantation by homologous recombination." *Applied and Environmental Microbiology* 69(1): 146-153.

To improve the capabilities of microorganisms relevant for biodegradation, we developed a new genetic approach and applied it to the bph operon (bphEGF[orf4]A1A2A3CD[orf1]A4R) of *Pseudomonas* sp. strain KKS102 to enhance its biphenyl- and polychlorinated biphenyl (PCB)-degrading activity. A native promoter of the bph operon, which was under control, was replaced through homologous recombination by a series of promoters that had constitutive activity. By testing a series of promoters with various strengths, we were able to obtain strains that have enhanced degradation activity for biphenyl and PCBs. This strategy removes the rate-limiting factor associated with transcription and has the potential to improve the degradation activity of a wide variety of microorganisms involved in biodegradation.

Padma, T. V. and R. M. Dickhut (2002). "Spatial and temporal variation in hexachlorocyclohexane isomers in a temperate estuary." *Marine Pollution Bulletin* 44(12): 1345-1353.

Hexachlorocyclohexanes (HCHs) are pesticides that persist in air and water of the Northern hemisphere. To understand the spatial and temporal variability in HCH levels in estuarine surface waters we measured concentrations of two HCH isomers (alpha-HCH and gamma-HCH) at six sites in the York River estuary at bimonthly intervals for a year. Bacterial abundance and activity were also monitored using acridine orange direct counts and uptake of tritiated substrates, respectively. alpha-HCH was consistently observed to be significantly higher in marine water compared to river water entering the estuary, suggesting that the Chesapeake Bay or Atlantic Ocean is a larger source of this compound to the York River estuary compared to riverine input. Moreover, following periods of high freshwater flow into the estuary during spring and early summer, both alpha- and gamma-HCH mixing curves indicated an additional source of these pollutants to the estuary such as land-derived runoff or groundwater discharge. In contrast, during low freshwater flow (late summer and fall) the estuary was a sink for HCHs, with gamma-HCH more rapidly removed from the estuary than alpha-HCH. During the period of low freshwater flow, concentrations of both alpha- and gamma-HCH were negatively correlated with bacterial activity. Bacterial activity as opposed to abundance appears to control HCH degradation in estuarine surface waters. (C) 2002 Published by Elsevier Science Ltd.

Parales, R. E., N. C. Bruce, et al. (2002). "Biodegradation, biotransformation, and biocatalysis (B3)." *Applied and Environmental Microbiology* 68(10): 4699-4709.

As John Archibald Wheeler said, "We live on an island of knowledge surrounded by a sea of ignorance. As our island of knowledge grows, so does the shore of our ignorance." So as the B3 conference added to our collective store of knowledge, it also helped point out more of our areas of ignorance. As we ponder the future, an important question to ask is the following: How broad is natural biocatalysis? This question is also important in the broader context of genomic biology, whereby genome annotators seek to match up sequences with functions. Since most microbial genes encode enzymes that catalyze reactions, we desperately need to know whether or not we are knowledgeable about most of the reaction types catalyzed by microbes. The answer to this question has important implications for genome annotation and biotechnological advancement. There are some indicators suggesting that, regarding reaction types, our shore of ignorance is large. A significant proportion of the B3 conference was concerned with the action of oxygenases, which are critically important in aerobic biodegradation and for commercial biotransformations. However, we are still ignorant about the metabolism of several dozen chemical functional groups found in biologically produced compounds (94). This is despite the fact that many new reactions have been discovered recently; for example, biological equivalents of the following named organic reactions have been discovered: the Diels-Alder reaction, the Bamberger rearrangement, the Beckman rearrangement, and the Kolbe-Schmidt reaction. The B3 conference discussed novel biocatalysis and new ways of using well-known enzymes, but clearly more novel metabolism will be discovered before the next B3 conference convenes. Such discovery will increase the toolkit of enzymes that are usable in biotechnology. The expanded toolkit will provide new routes to synthesize a broad array of commodity chemicals from renewable resources. The use of renewable resources for clean and sustainable industry is clearly one of the major outcomes of B3.

Park, J., J. J. Kukor, et al. (2002). "Characterization of the adaptive response to trichloroethylene-mediated stresses in *Ralstonia pickettii* PKO1." *Applied and Environmental Microbiology* 68(11): 5231-5240.

In *Ralstonia pickettii* PKO1, a denitrifying toluene oxidizer that carries a toluene-3-monooxygenase (T3MO) pathway, the biodegradation of toluene and trichloroethylene (TCE) by the organism is induced by TCE at high concentrations. In this study, the effect of TCE preexposure was studied in the context of bacterial protective response to TCE-mediated toxicity in this organism. The results of TCE degradation experiments showed that cells induced by TCE at 110 mg/liter were more tolerant to TCE-mediated stress than were those induced by TCE at lower concentrations, indicating an ability of PKO1 to adapt to TCE-mediated stress. To characterize the bacterial protective response to TCE-mediated stress, the effect of TCE itself (solvent stress) was isolated from TCE degradation-dependent stress (toxic intermediate stress) in the subsequent chlorinated ethylene toxicity assays with both nondegradable tetrachloroethylene and degradable TCE. The results of the toxicity assays

showed that TCE preexposure led to an increase in tolerance to TCE degradation-dependent stress rather than to solvent stress. The possibility that such tolerance was selected by TCE degradation-dependent stress during TCE preexposure was ruled out because a similar extent of tolerance was observed in cells that were induced by toluene, whose metabolism does not produce any toxic products. These findings suggest that the adaptation of TCE-induced cells to TCE degradation-dependent stress was caused by the combined effects of solvent stress response and T3MO pathway expression.

Phanikumar, M. S., D. W. Hyndman, et al. (2002). "Simulation of microbial transport and carbon tetrachloride biodegradation in intermittently-fed aquifer columns." *Water Resources Research* 38(4): art. no.-1033.

[1] This paper evaluates the microbial transport and degradation processes associated with carbon tetrachloride (CT) biodegradation in laboratory aquifer columns operated with a pulsed microbial feeding strategy. A seven component reactive transport model based on modified saturation kinetics and on a two-site sorption model was developed to describe the linked physical, chemical, and biological processes involved in CT degradation by *Pseudomonas stutzeri* KC, a denitrifying bacterium that cometabolically converts CT to harmless end products. After evaluating several expressions for attachment and detachment, we selected a dynamic partitioning model in which strain KC detachment decreases at low substrate concentrations. The resulting model enabled improved understanding of the complex coupled processes operative within our system and enabled us to test a model for field-scale design and transport studies. Batch studies were used to identify initial degradation and microbial transport processes, and constrained optimization methods were used to estimate a set of reaction rates that best describe the column experiment data. The optimal set of parameters for one column provided a reasonable prediction of solute and microbial concentrations in a second column operated under different conditions, providing an initial test of the model. This modeling strategy improved our understanding of biodegradation processes and rates. The CT degradation rate in the columns was lower than values obtained from batch studies, and processes in addition to the growth and decay of strain KC cells (due to native flora) are necessary to describe the observed nitrate consumption.

Reddy, C. M., L. Xu, et al. (2002). "A chlorine isotope effect for enzyme-catalyzed chlorination." *Journal of the American Chemical Society* 124(49): 14526-14527.

Several chlorinated organic compounds (COCs) that have been detected in a wide range of human, animal, and environmental samples may be derived from natural or anthropogenic sources. To determine whether the Cl isotope ratios of these compounds could be used to differentiate sources, we investigated the chlorine isotope effect for enzyme-catalyzed chlorination. Two aromatic substrates, 1,3,5-trimethylbenzene (TMB) and 3,5-dimethylphenol (DMP), were treated with a chloroperoxidase isolated from the fungus *Caldariomyces fumago*. A kinetic isotope effect (KIE) (in terms of k_{35}/k_{37}) was calculated to be 1.012 for TMB and 1.011 for DMP. A similar reaction, but not catalyzed, with hypochlorite yielded a much smaller KIE. These results indicate that a substantial KIE exists for this process. Furthermore, natural COCs synthesized by this enzymatic pathway may have Cl isotope ratios that will be easily distinguished from anthropogenic COCs.

Royal, C. L., D. R. Preston, et al. (2003). "Reductive dechlorination of polychlorinated biphenyls in landfill leachate." *International Biodeterioration & Biodegradation* 51(1): 61-66.

Sequential anaerobic reductive dechlorination of polychlorinated biphenyl (PCB) 2,2',3,4,4',5' chlorobiphenyl (138-CB) was studied in landfill leachate from a Subtitle D designed landfill for type 11, or municipal, wastes. The effect of biosurfactant on congener product profiles and rates was also examined. Rhamnolipid biosurfactant amendment had little effect, perhaps due to a lack of weathering of the spiked PCBs, or due to the presence of endogenous chemical surfactants in the leachate. The redox of the leachate averaged -405 ± 19.6 mV with a pH of 7.8 ± 0.07 . The biologically active landfill samples averaged 68.6% degradation of 2,2', 3,4,4', 5' chlorobiphenyl (138-CB) over the first 7 days. The majority of this was accounted for in the observed reductive dechlorination products; 2, 2', 4', 5 tetrachlorobiphenyl (48-CB), 2,2',3,5' tetrachlorobiphenyl (44-C), and 2,2',3,4 tetrachlorobiphenyl (42-CB). 2,2',3,5' tetrachlorobiphenyl (44-CB) was the major product accounting for

36.4% of the observed breakdown products with biosurfactant amended leachate, 66.1% of those observed in unamended leachate, and 65.2% of the breakdown products observed in the negative control. All observed breakdown products contain four chlorines and represent the terminal PCB congeners observed in these anaerobic studies. These results indicate that initial reductive dechlorination rates for highly chlorinated PCBs may be rapid in landfill environments, however, certain congener dechlorination products, such as those observed here, are expected to exhibit greater longevity in landfills. (C) 2002 Published by Elsevier Science Ltd.

Ruiz-Aguilar, G. M. L., J. M. Fernandez-Sanchez, et al. (2002). "Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil." *Advances in Environmental Research* 6(4): 559-568.

White-rot fungi are known to degrade a wide variety of recalcitrant pollutants. In this work, three white-rot fungi were used to degrade a mixture of PCBs at high initial concentrations from 600 to 3000 mg/l, in the presence of a non-ionic surfactant (Tween 80). The PCBs were extracted from a historically PCB-contaminated soil. Preliminary experiments showed that Tween 80 exhibited the highest emulsification index of the three surfactants tested (Tergitol NP-10, Triton X-100 and Tween 80). Tween 80 had no inhibitory effect on fungal radial growth, whereas the other surfactants inhibited the growth rate by 75-95%. Three initial PCB concentrations (600, 1800 and 3000 mg/l) were assayed with three fungi for the PCB degradation tests. The extent of PCB modification was found to depend on PCB concentration ($P < 0.001$) and fungal species ($P < 0.001$). PCB degradation ranged from 29 to 70%, 34 to 73% and 0 to 33% for *Trametes versicolor*, *Phanerochaete chrysosporium* and *Lentinus edodes*, respectively, in 10-day incubation tests. The highest PCB transformation (70%) was obtained with *T. versicolor* at an initial PCB concentration of 1800 mg/l, whereas *P. chrysosporium* could modify 73% at 600 mg/l. Interestingly, *P. chrysosporium* was the most effective for PCB metabolization at an initial concentration of 3000 mg/l, and it reduced up to 34% of the PCB mixture. As an overall effect, an increase in the initial PCB concentration led to a decrease in the pollutant degradation, from 57% to 21%. *P. chrysosporium* and *L. edodes* accumulated low chlorinated congeners. In contrast, *T. versicolor* removed both low and high-chlorinated congeners of PCBs. (C) 2002 Elsevier Science Ltd. All rights reserved.

Ruzicka, J., J. Muller, et al. (2002). "Biotransformation of trichloroethene by pure bacterial cultures." *Folia Microbiologica* 47(5): 467-472.

From natural samples 11 isolates able to remove trichloroethene (CCl_2CHCl) from an aqueous environment were obtained which were capable of cometabolic degradation of CCl_2CHCl by an enzyme system for phenol degradation. At an initial CCl_2CHCl concentration of 1 mg/L, the resting cells of particular cultures degraded 33-94 % CCl_2CHCl during 1 d and their transformation capacity ranged from 0.3 to 3.1 Mg CCl_2CHCl per g organic fraction. An analysis of a mixed phenol-fed culture with an excellent trichloroethene-degrading ability found a markedly minority isolate represented in the consortium to be responsible for this property. This culture degraded CCl_2CHCl even at a low inoculum concentration and attained a transformation capacity of 14.7 mg CCl_2CHCl per g. The increase in chloride concentration after degradation was quantitative when compared with the decrease in organically bound chlorine. The degree of CCl_2CHCl degradation was affected by Me2S2; this substance can significantly reduce the degrading ability of some tested cultures (> 60 %); however, it does not cause this inhibition with others.

Sakai, M., K. Miyauchi, et al. (2003). "2-hydroxypenta-2,4-dienoate metabolic pathway genes in a strong polychlorinated biphenyl degrader, *Rhodococcus* sp strain RHA1." *Applied and Environmental Microbiology* 69(1): 427-433.

A gram-positive polychlorinated biphenyl (PCB) degrader, *Rhodococcus* sp. strain RRA1, metabolizes biphenyl through the 2-hydroxypenta-2,4-dienoate (HPD) and benzoate metabolic pathways. The HPD metabolic pathway genes, the HPD hydratase (*bphE1*), 4-hydroxy-2-oxovalerate aldolase (*bphF1*), and acetaldehyde dehydrogenase (acylating) (*bphG*) genes, were cloned from RHA1. The deduced amino acid sequences of *bphGF1E1* have 30 to 58% identity with those of the HPD metabolic pathway genes of gram-negative bacteria. The order of these genes, *bphG-bphF1-bphE1*, differs from that of the HPD metabolic pathway genes, *bphE-*

bphG-bphF, in gram-negative degraders of PCB, phenol, and toluene. Reverse transcription-PCR experiments indicated that the bphGF1E1 genes are inducibly cotranscribed in cells grown on biphenyl and ethylbenzene. Primer extension analysis revealed that the transcriptional initiation site exists within the bphR gene located adjacent to and upstream of bphG, which is deduced to code a transcriptional regulator. The respective enzyme activities of bphGF1E1 gene products were detected in *Rhodococcus erythropolis* IkM1399 carrying a bphGF1E1 plasmid. The insertional inactivation of the bphE1, bphF1, and bphG genes resulted in the loss of the corresponding enzyme activities and diminished growth on both biphenyl and ethylbenzene. Severe growth interference was observed during growth on biphenyl. The growth defects were partially restored by the introduction of plasmids containing the respective intact genes. These results indicated that the cloned bphGF1E1 genes are not only responsible for the primary metabolism of HPD during growth on both biphenyl and ethylbenzene but are also involved in preventing the accumulation of unexpected toxic metabolites, which interfere with the growth of RHA1.

Seffernick, J. L., H. McTavish, et al. (2002). "Atrazine chlorohydrolase from *Pseudomonas* sp strain ADP is a metalloenzyme." *Biochemistry* 41(48): 14430-14437.

Atrazine chlorohydrolase (AtzA) from *Pseudomonas* sp. ADP initiates the metabolism of tile herbicide atrazine by catalyzing a hydrolytic dechlorination reaction to produce hydroxyatrazine. Sequence analysis revealed AtzA to be homologous to metalloenzymes within the amidohydrolase protein superfamily. AtzA activity was experimentally shown to depend on an enzyme-bound, divalent transition-metal ion. Loss of activity obtained by incubating AtzA with the chelator 1.10-phenanthroline or oxalic acid was reversible upon addition of Fe(II), Mn(II), or Co(II) salts. Experimental evidence suggests a 1: 1 metal to subunit stoichiometry, with the native metal being Fe(II). Our data show that the inhibitory effects of metals such as Zn(II) and Cu(II) are not the result of displacing the active site metal. Taken together, these data indicate that AtzA is a functional metalloenzyme, making this the first report, to our knowledge, of a metal-dependent dechlorinating enzyme that proceeds via a hydrolytic mechanism.

Siebert, A., A. Neumann, et al. (2002). "A non-dechlorinating strain of *Dehalospirillum multivorans*: evidence for a key role of the corrinoid cofactor in the synthesis of an active tetrachloroethene dehalogenase." *Archives of Microbiology* 178(6): 443-449.

A strain of *Dehalospirillum multivorans*, designated strain N, was isolated from the same source as the formerly described tetrachloroethene (PCE)-dechlorinating *D. multivorans*, herein after referred to as strain K. Neither growing cells nor cell extracts of strain N were able to dechlorinate PCE. The *pceA* and *pceB* genes encoding for the PCE-reductive dehalogenase were detected in cells of strain N; and they were 100% homologous to the corresponding genes of strain K. Since the PCE dehalogenase of *D. multivorans* strain K contains a corrinoid cofactor, the corrinoids of strain N cells were extracted. Analysis of the corrinoids revealed the absence of the specific corrinoid, which is the cofactor of the PCE dehalogenase of strain K cells. RT-PCR of mRNA indicated that the *pceA* gene was transcribed in strain N cells to a far lower extent than the *pceA* gene of strain K under the same experimental conditions. Western blot analysis of crude extracts of strain N showed that, if at all, an insignificant amount of the apoprotein of the PCE dehalogenase was present. The results indicate that the inability of strain N to dechlorinate is due to the absence of the corrinoid cofactor of the enzyme mediating PCE dechlorination.

Singer, A. C., C. S. Wong, et al. (2002). "Differential enantioselective transformation of atropisomeric polychlorinated biphenyls by multiple bacterial strains with different inducing compounds." *Applied and Environmental Microbiology* 68(11): 5756-5759.

Five polychlorinated biphenyl (PCB)-degrading bacteria were tested for the ability to differentiate between the enantiomers of four atropisomeric PCB congeners (2,2',3,6-tetra-CB; 2,2',3,3',6-penta-CB; 2,2',3,4',6-penta-CB; and 2,2',3,5',6-penta-CB) after growth in the presence of tryptone-soyone, biphenyl, carvone, or cymene. Enantioselectivity was shown to vary with respect to strain, congener, and cosubstrate.

Stazi, S. R., A. D'Annibale, et al. (2002). "Kinetic behaviour and degradative capability of Lentinula edodes laccase isoenzymes with differently substituted chlorinated substrates." *Fresenius Environmental Bulletin* 11(9A): 583-588.

Partially purified *Lentinula edodes* laccase was tested for its ability to oxidize several putative mono-, di- and tri- chlorinated substrates. Both the initial reaction rate and removal efficiency were remarkably affected by the nature of substituents and their position on the aromatic ring. Chlorinated phenols and/or anilines bearing an additional substituent with at least one lone pair electrons adjacent to the benzene ring were preferentially oxidized by the enzyme. In contrast, chlorinated substrates bearing an electron- withdrawing substituent, such as the nitro group, were not oxidized at all. The extent of removal of the most reactive chlorinated compounds after 1 h incubation with laccase was in the following order: chlorohydroquinone > 4,6- dichlororesorcinol > 2-amino-4-chlorophenol > 2,6- dichlorophenol > 2,4-dichlorophenol > 2,4,6-trichlorophenol > 2-chlorophenol > 4-chlorophenol.

Strong, L. C., C. Rosendahl, et al. (2002). "Arthrobacter aurescens TC1 metabolizes diverse s-triazine ring compounds." *Applied and Environmental Microbiology* 68(12): 5973-5980.

Arthrobacter aurescens strain TC1 was isolated without enrichment by plating atrazine-contaminated soil directly onto atrazine-clearing plates. *A. aurescens* TC1 grew in liquid medium with atrazine as the sole source of nitrogen, carbon, and energy, consuming up to 3,000 mg of atrazine per liter. *A. aurescens* TC1 is metabolically diverse and grew on a wider range of s-triazine compounds than any bacterium previously characterized. The 23 s-triazine substrates serving as the sole nitrogen source included the herbicides ametryn, atratone, cyanazine, prometryn, and simazine. Moreover, atrazine substrate analogs containing fluorine, mercaptan, and cyano groups in place of the chlorine substituent were also growth substrates. Analogues containing hydrogen, azido, and amino functionalities in place of chlorine were not growth substrates. *A. aurescens* TC1 also metabolized compounds containing chlorine plus N-ethyl, N-propyl, N-butyl, N-s-butyl, N-isobutyl, or N-t-butyl substituents on the s-triazine ring. Atrazine was metabolized to alkylamines and cyanuric acid, the latter accumulating stoichiometrically. Ethylamine and isopropylamine each served as the source of carbon and nitrogen for growth. PCR experiments identified genes with high sequence identity to *atzB* and *atzC*, but not to *atzA*, from *Pseudomonas* sp. strain ADP.

Sun, B. L., B. M. Griffin, et al. (2002). "Microbial dehalorespiration with 1,1,1-trichloroethane." *Science* 298(5595): 1023-1025.

1,1,1-Trichloroethane (TCA) is a ubiquitous environmental pollutant because of its widespread use as an industrial solvent, its improper disposal, and its substantial emission to the atmosphere. We report the isolation of an anaerobic bacterium, strain TCA1, that reductively dechlorinates TCA to 1,1-dichloroethane and chloroethane. Strain TCA1 required H₂ as an electron donor and TCA as an electron acceptor for growth, indicating that dechlorination is a respiratory process. Phylogenetic analysis indicated that strain TCA1 is related to gram-positive bacteria with low DNA G + C content and that its closest relative is *Dehalobacter restrictus*, an obligate H₂-oxidizing, chloroethene-respiring bacterium.

Top, E. M., D. Springael, et al. (2002). "Catabolic mobile genetic elements and their potential use in bioaugmentation of polluted soils and waters." *Fems Microbiology Ecology* 42(2): 199-208.

Genes that encode the degradation of both naturally occurring and xenobiotic organic compounds are often located on plasmids, transposons or other mobile and/or integrative elements. The list of published reports of such mobile genetic elements (MGEs) keeps growing as researchers continue to isolate and characterize new degrading bacteria and their corresponding degradative genes. There is also growing evidence that horizontal exchange of catabolic (degradative) genes among bacteria in microbial communities plays an important role in the evolution of catabolic pathways. Around 10 years ago the hypothesis was raised that we might be able to accelerate this natural gene exchange and pathway construction by introducing and subsequently spreading degradative genes, located on MGEs, into well established, competitive indigenous microbial populations as a means of bioaugmentation of polluted soils and waters. During the last decade, only a few reports on successful

MGE- mediated bioaugmentation have been published. After summarizing the diversity of degradative MGEs, this review presents an overview of studies that have monitored the transfer of degradative genes in soil microcosms and in activated sludge and other wastewater treatment reactors, with emphasis on those that have clearly shown a direct effect of gene transfer on accelerated biodegradation. A few successful cases suggest that the strategy could indeed work under specific conditions, such as when the in situ degradation potential is absent and the pollutant degrading transconjugants can grow and become numerically dominant populations in the bacterial community. Further studies in this area are obviously needed to improve our current knowledge on the efficiency of gene dissemination as a tool in bioremediation. (C) 2002 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Tsuda, M., T. Endo, et al. (2002). "Nakirodin A, a bromotyrosine alkaloid from a verongid sponge." *Journal of Natural Products* 65(11): 1670-1671.

A new bromotyrosine alkaloid, nakirodin A (1), has been isolated from an Okinawan marine Verongid sponge. The structure was elucidated on the basis of spectroscopic data.

Widada, J., H. Nojiri, et al. (2002). "Recent developments in molecular techniques for identification and monitoring of xenobiotic-degrading bacteria and their catabolic genes in bioremediation." *Applied Microbiology and Biotechnology* 60(1-2): 45-59.

The pollution of soil and water with xenobiotics is widespread in the environment and is creating major health problems. The utilization of microorganisms to clean up xenobiotics from a polluted environment represents a potential solution to such environmental problems. Recent developments in molecular- biology-based techniques have led to rapid and accurate strategies for monitoring, discovery and identification of novel bacteria and their catabolic genes involved in the degradation of xenobiotics. Application of these techniques to bioremediation has also improved our understanding of the composition, phylogeny, and physiology of metabolically active members of the microbial community in the environment. This review provides an overview of recent developments in molecular-biology-based techniques and their application in bioremediation of xenobiotics.

Williams, J. B. (2002). "Phytoremediation in wetland ecosystems: Progress, problems, and potential." *Critical Reviews in Plant Sciences* 21(6): 607-635.

Assessing the phytoremediation potential of wetlands is complex due to variable conditions of hydrology, soil/sediment types, plant species diversity, growing season, and water chemistry. Conclusions about long-term phytoremediation potential are further complicated by the process of ecological succession in wetlands. This review of wetlands phytoremediation addresses the role of wetland plants in reducing contaminant loads in water and sediments, including metals; volatile organic compounds (VOC), pesticides, and other organohalogenes; TNT and other explosives; and petroleum hydrocarbons and additives. The review focuses on natural wetland conditions and does not attempt to review constructed wetland technologies. Physico- chemical properties of wetlands provide many positive attributes for remediating contaminants. The expansive rhizosphere of wetland herbaceous shrub and tree species provides an enriched culture zone for microbes involved in degradation. Redox conditions in most wetland soil/sediment zones enhance degradation pathways requiring reducing conditions. However, heterogeneity complicates generalizations within and between systems. Wetland phytoremediation studies have mainly involved laboratory microcosm and mesocosm technologies, with the exception of planted poplar communities. Fewer large-scale field studies have addressed remediation actions by natural wetland communities. Laboratory findings are encouraging with regards to phytoextraction and degradation by rhizosphere and plant tissue enzymes. However, the next phase in advancing the acceptance of phytoremediation as a regulatory alternative must demonstrate sustained contaminant removal by intact natural wetland ecosystems.

Wood, T. K. (2002). "Active expression of soluble methane monooxygenase from *Methylosinus trichosporium* OB3b in heterologous hosts." *Microbiology-Sgm* 148: 3328-3329.

Yu, S. H. and L. Semprini (2002). "Comparison of trichloroethylene reductive dehalogenation by microbial communities stimulated on silicon-based organic compounds as slow-release anaerobic substrates." *Water Research* 36(20): 4985-4996.

Microcosm studies were conducted to demonstrate the effectiveness of tetrabutoxysilane (TBOS) as a slow-release anaerobic substrate to promote reductive dehalogenation of trichloroethylene (TCE). The abiotic hydrolysis of TBOS and tetrakis(2-ethylbutoxy)silane (TKEBS), and the biotic transformations of the hydrolysis products from both were also investigated. Comparison of TCE reductive dehalogenation was performed with microbial communities stimulated from three different sites: Site 300 Lawrence Livermore National Laboratory (LLNL), CA, Point Mugu Naval Weapons Facility, CA, and the Evanite site in Corvallis, OR. Poisoned microcosms showed that 1 mol of TBOS slowly and abiotically hydrolyzes to 4 mol of 1-butanol, while the live microcosms showed the 1-butanol ferments to butyrate and/or acetate, producing H₂. The hydrolysis of TBOS and TKEBS was abiotic and not enhanced by biotic processes under the anaerobic conditions of these tests. Hydrogen consumption was correlated with reductive dehalogenation, indicating it served as an electron donor for reductive dehalogenation. TBOS was found to be a slow-release anaerobic substrate to support long-term dechlorination of TCE to ethylene in Point Mugu microcosms, and in the LLNL microcosm bioaugmented with the Evanite culture. Electron mass balances showed most of the electron flow went into the creation of organic acids, especially acetate, and the production of methane. Electron efficiencies for reductive dechlorination were as high as 14% based on the electrons used for, dechlorination to the total electrons associated with the mass of TBOS and TKEBS hydrolyzed. Rates of TBOS hydrolysis increased with greater TBOS concentrations as a light nonaqueous-phase liquids (LNAPL). These results indicate that TBOS has promise as an effective anaerobic substrate for remediating a wide range of CAH concentrations at different CAH contaminated sites. (C) 2002 Elsevier Science Ltd. All rights reserved.

Zayed, S., M. Farghaly, et al. (2002). "Mineralization of C-14-ring labelled 2,4-D in Egyptian soils under aerobic and anaerobic conditions." *Biomedical and Environmental Sciences* 15(4): 306-314.

Objectives To study the mineralization of 2,4-D in clay and clay loam Egyptian soils under subtropical conditions over a period of 90 d. **Methods** Using C-14-ring labelled pesticide, laboratory studies under aerobic and anaerobic conditions were conducted. C-14-activity in solutions was directly determined by liquid scintillation counting. Unextractable soil residues were determined by combustion. The nature of methanolic C-14- residues was determined by thin layer and high performance. liquid chromatographic analysis. **Results** Under aerobic conditions 10%-14% of applied dose was mineralized during 90 d irrespective of soil type. The soil extractable pesticide residues decreased with time and the bound residues gradually increased. The highest binding capacity of about 26%-29% was observed in clay soil under aerobic conditions after 90 d. A good balance sheet was obtained and the percentage recovery was generally between 91% and 100%. **Conclusion** The mineralization of 2,4-D in clay soil was higher than that in clay loam soil under anaerobic conditions. Under aerobic conditions, the soil type had no influence on mineralization capacity of 2,4-D during 90 d. The soil binding increased with time whereby the extractable C-14-residues simultaneously decreased. Chromatographic analysis of the methanol extractable C-14-residues of soils revealed the presence of 2,4-D as a main product together with 2,4-dichlorophenol.