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

**14<sup>th</sup> Quarterly Report  
2<sup>nd</sup> Quarter Year 2004**

**Report prepared for Euro Chlor**

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September 15, 2004

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**ACRONYMS**

<b>16S rRNA</b>	16S Ribosomal RNA
<b>CB</b>	Chlorobenzene
<b>CBA</b>	Chlorobenzoic acid
<b>CBc</b>	Chlorobenzoate
<b>CBp</b>	Chlorobiphenyl
<b>CDDs</b>	Chlorinated dibenzo- <i>p</i> -dioxins
<b>CDFs</b>	Chlorinated dibenzo- <i>p</i> -furans
<b>CF</b>	Chloroform
<b>CM</b>	Chloromethane
<b>CP</b>	Chlorophenol
<b>CPO</b>	Chloroperoxidase
<b>CT</b>	Carbon tetrachloride
<b>2,4-D</b>	2,4-dichlorophenoxyacetate
<b>1,2-DCA</b>	1,2-dichloroethane
<b>DCB</b>	Dichlorobenzene
<b>DCE</b>	Dichloroethene
<b>DCM</b>	Dichloromethane
<b>DBP</b>	4,4'-dichlorobenzophenone
<b>DDD</b>	1,1,1-trichloro-2,2-bis ( <i>p</i> -chlorophenyl) ethane
<b>DDE</b>	1,1,1-trichloro-2,2-bis ( <i>p</i> -chlorophenyl) ethane
<b>DDT</b>	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane
<b>DNAPLs</b>	Dense non-aqueous phase liquids
<b>Dw</b>	Dry weight
<b>E-acceptor</b>	Electron acceptor
<b>E-donor</b>	Electron donor
<b>ETH</b>	Ethylene, ethene
<b>HCB</b>	Hexachlorobenzene
<b>HCH</b>	Hexachlorohexane
<b>MCB</b>	Monochlorobenzene
<b>MeBr</b>	Methyl bromide
<b>MPN</b>	Most probable number
<b>PCA</b>	Pentachloroethane

**ACRONYMS** (*Continued*)

<b>PCBs</b>	Polychlorinated biphenyls
<b>PCP</b>	Pentachlorophenol
<b>PCE</b>	Tetrachloroethylene
<b>PCR</b>	Polymerase chain reaction
<b>QSARs</b>	Quantitative structure-activity relationships
<b>TCA</b>	Trichloroacetic acid
<b>TCB</b>	Trichlorobenzene
<b>TCDF</b>	Trichlorodibenzofuran
<b>TCE</b>	Trichlorethylene
<b>VC</b>	Vinyl chloride

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

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## **1. INTRODUCTION**

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

## **2. SUMMARY OF MOST IMPORTANT DEVELOPMENTS**

### **2.a. Microbial Dechlorination**

The most important findings in this quarter for microbial dechlorination are firstly three articles which have detected isotopic fraction of carbon during the biodegradation of chlorinated pollutants. These include studies involving the aerobic utilization as well as cooxidation of vinyl chloride (14), the aerobic utilization of chloromethane (40) and the reductive dehalogenation of 1,2,4- and 1,2,3-trichlorobenzene (24). Isotopic fractionation is important because it provides a forensic tool for determining if changes occurring in the field are due to biodegradation. Using <sup>13</sup>C-labelled chloromethane, populations assimilating chloromethane could be detected by

evaluating  $^{13}\text{C}$ -DNA (24). This type of analysis conducted with known genes for aerobic chloromethane degradation as well as 16S-rRNA genes indicated that there are a large number of chloromethane degraders in the environment utilizing novel genes for chloromethane degradation. The results suggest a high level of biodiversity towards aerobic chloromethane degradation.

Another highlight is the discovery that bacteria which are typically inactivated during the cooxidation of chlorinated solvents such as trichloroethene (TCE) can have their activity restored if the primary substrate is fed again after the inactivation (42). The authors devised a refeeding schedule to maintain TCE oxidizing cells bacterium *Pseudomonas putida* F1 active over 6 cycles of refeeding. This is an important breakthrough since previously it was assumed that bacteria cooxidizing chlorinated solvents were inactivated permanently.

## **2.b. Microbial Chlorination**

The most important highlights for microbial chlorination are two articles concerned with organohalogen formation measured in field studies. In the first of these, terrestrial sources and sinks of halomethanes near Cape Grim, Tasmania were investigated over one year period (17). Chloromethane production was evident at coastal wetlands with a flux of  $300 \text{ ng m}^{-2} \text{ h}^{-1}$ . All field sites resulted in chloroform production with fluxes ranging up to  $5000 \text{ ng m}^{-2} \text{ h}^{-1}$  at certain locations. In the second field study, Concentrations of two halomethanes (chloroform and tetrachloromethane) and several other important chlorinated compounds (1,1,1-trichloroethane, trichloroethene (TCE), tetrachloroethene (PCE) and trichloroacetic acid (TCA)) were monitored in water from lakes, rivers and springs with differing levels of salinity of the Kalmykian Steppe (Southern Russia) (71). The measurements indicate that in particular water from salt lakes located in semiarid/ and areas of the study region must be considered as new types of natural emittents of PER and other chlorinated hydrocarbons as well as trichloroacetic acid.

### 3. MICROBIAL DECHLORINATION

#### 3.a. General Reviews

In this quarter, three review articles were published that discuss various aspects of biological dechlorination (46, 50, 55, 68). A copy of the article entitled “*Halometabolites and cellular dehalogenase systems: An evolutionary perspective*” by Valverde et al. (2004) (68) could not be obtained in time. Thus, the latter article will be discussed in the upcoming quarterly report.

One of the papers provides an overview of the most recent advances in the knowledge of metabolic pathways and enzymes for aerobic and anaerobic degradation (55). The paper emphasizes the significance of genomic studies for gaining new insights into the metabolic potential and activity of microorganisms. Several sections of the paper focus on the microbial degradation of organohalogenes, including a revision on new chloroaromatic metabolic enzymes and pathways and discussion of the substrate diversity and microorganisms involved in dehalorespiration. Parales & Haddock (2004) (50) reviewed the role of microbial reactions in biocatalysis and biodegradation. A brief review on the microbial degradation of chlorinated aliphatic and aromatic compounds is included as part of this publication. Similarly to Pieper *et al.* (55), the authors discuss how the increasing availability of genome sequences is contributing to new insights on the metabolic capabilities of microorganisms and their potential for use in bioremediation applications. Finally, a review on the phytodegradation of organic compounds, that focuses on chlorinated solvents, PCBs and chlorinated pesticides, among other contaminants, was also recently published (46).

#### 3.b. Microbial Dechlorination

##### Vinyl chloride and Other Chlorinated Ethenes

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

studies are categorized based on parent compound investigated, either lower chlorinated ethenes (VC or DCEs) or higher chlorinated ethenes (PCE or TCE).

**Vinyl Chloride (VC) and Dichloroethenes (DCE).** In this quarter, there were only three studies which directly investigated the biodegradation of lower chlorinated ethenes as parent compounds. One study reviewed the work by a Japanese research group on the anaerobe *Clostridium* sp strain DC1 which is capable of degrading 1,2-*cis*-DCE (*cis*-DCE) and VC when supplied with other primary substrates (25). VC and *cis*-DCE were shown to be dechlorinated based on stoichiometric release of chloride; however, expected intermediates of reductive dechlorination (e.g., VC from DCE and ethane from either VC or DCE) were not detected. It is not clear what products are formed instead. However, the anaerobic degradation of chloroethenes without accumulation of harmful products such as VC is implicated and thus marks an interesting development. A second study evaluated carbon-isotope fractionation during the aerobic degradation of VC and *cis*-DCE (14). TCE was also evaluated. The largest fractionation was observed during VC degradation. Significant fractionation was observed for a culture utilizing VC as the sole carbon and energy source under aerobic conditions (*Mycobacterium aurum* L1) as well as for cometabolic (co-oxidative) degradation with methane as the primary growth substrate (*Methylosinus trichosporium* OB3b). *M. aurum* L1 caused the greatest fractionation (the enrichment factor,  $\epsilon = -5.7$ ) while for the cometabolic cultures,  $\epsilon$  values ranged from -3.2 to -4.8. No isotope fractionation was observed during aerobic degradation of *cis*-DCE and only very low fractionation was observed during TCE degradation. The results of the study suggest that isotopic shifts could be used to distinguish between aerobic and anaerobic degradation of VC. The only other article this quarter focused on lower chlorinated ethenes is about a model developed to predict gas migration through the soil surrounding the landfill (1). The model was applied to predict methane and VC concentrations at different distances from the landfill.

**Perchloroethylene (PCE) and Trichloroethene (TCE).** In this quarter, there were 11 publications reporting on either PCE or TCE microbial degradation. One of the citations is an abstract that describes the role of cobalt containing vitamin B12 on PCE and TCE degradation (23) however the abstract provides no details. One of the articles discusses the development of a new decision making model for applied to TCE bioremediation (63) and will be discussed in Section “3.d. New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds”. Of the remaining 9 articles 6 are concerned with anaerobic degradation. Of the 6

anaerobic articles, 4 are about PCE and 2 are about TCE. The first of the anaerobic articles addresses the topic of H<sub>2</sub> threshold values (37). Halorespiring microorganisms have a high affinity for H<sub>2</sub>. The role of H<sub>2</sub>-threshold concentrations in pure halorespiring cultures was evaluated and compared with mixed cultures and field data. H<sub>2</sub>-threshold values between 0.05 and 0.08 nM for *Sulfurospirillum halorespirans*, *S. multivorans* and *Dehalobacter restrictus* under PCE-reducing and nitrate-reducing conditions were observed. The reduction of PCE and TCE can proceed at H<sub>2</sub> concentrations of below 1 nM at a polluted site. However, a higher H<sub>2</sub> concentration is required for the reduction of lower chlorinated ethenes. The measured H<sub>2</sub> concentration in situ can therefore be an indicator of the extent of anaerobic reductive dechlorination. The second anaerobic article considers the use of co-solvents as electron donors (e-donor) to support reductive dechlorination of PCE by halorespiring mixed culture (29). Co-solvents are utilized to flush dense non-aqueous phase liquids (DNAPLs) in the source area of chlorinated solvent plumes. Thus the question has arisen if residual co-solvents can be utilized to support reductive dechlorination of residual PCE. To investigate this possibility ethyl-lactate (a so called “green solvent”) was examined for two functions, DNAPL flushing and e-donor to support bioremediation. Biomass growth and PCE dechlorination were observed by protein and chloride production, respectively, in the culture; with a specific dechlorination rate of 50-150 μg mg<sup>-1</sup> cell d<sup>-1</sup>. Ethyl lactate abiotically breaks down to ethanol and lactate, the latter being a rich source of hydrogen for reductive dechlorination. The results demonstrate that ethyl lactate may be promising for in situ bioremediation following NAPL extraction. The third anaerobic article looks into the factors that are controlling bioenhancement of DNAPL dissolution by halorespiring microorganisms (15). A novel two-dimensional numerical model was used to fully resolve the evolution of biomass profiles and the distributions of compounds within source zones. The extent of bioenhanced dissolution was found to be influenced by dehalogenation kinetics, e-donor levels, NAPL configurations, and e-donor competition among microorganisms. The model predicts enhance of DNAPL dissolution rates by several fold in the presence of biological activity. The fourth anaerobic article compares degradation of PCE with adsorption of PCE and TCE in a zero valent iron column (19). Degradation and sorption were evaluated in the presence of compounds common to contaminated groundwater with varying physicochemical properties. The potential competitors were chlorinated ethenes, monocyclic aromatic hydrocarbons, and humic acids. TCE sorption and degradation decreased by 33% and 30%, respectively, while TCE (10 mg L<sup>-1</sup>) decreased PCE degradation by 30%. In the presence of nonreactive hydrophobic hydrocarbons (BTEX at 100 mg L<sup>-1</sup>), TCE and PCE sorption decreased by 73% and 55%, respectively. The presence of the hydrocarbons had no effect on TCE

degradation and increased PCE reduction rates by 50%, suggesting that the displacement of the chloroethenes from the sorption sites by the aromatic hydrocarbons enhanced the degradation rates. Humic acids did not interfere significantly with chloroethene sorption or with TCE degradation but lowered PCE degradation kinetics by 36% when present at high concentrations ( $100 \text{ mg L}^{-1}$ ).

The two remaining anaerobic articles considered the bioremediation and natural attenuation of TCE. The first of these evaluated the bioremediation of TCE in laboratory columns with a core of weathered fractured bedrock material collected from Oak Ridge, Tennessee (36). Natural, uncontaminated ground water from the site, which was degassed and spiked with dissolved phase TCE, was continuously pumped through one column containing the natural microbial communities (the biotic column). In a second column, the microorganisms were inhibited and the dissolved phase TCE was added under aerobic conditions (dissolved oxygen conditions  $> 2 \text{ mg L}^{-1}$ ). In effluent from the biotic column, reducing conditions rapidly developed and evidence of anaerobic biodegradation of TCE, by the production of 1,2-cDCE, first appeared approximately 31 days after addition of TCE. Reductive dechlorination of TCE occurred after iron-reducing conditions were established and about the same time that sulfate reduction began. Natural organic matter that occurs in the weathered bedrock and ground water at the site is the most likely primary e-donor, supporting reductive dechlorination of TCE. By comparison there were no indicators of TCE degradation in the abiotic column. The second article considering the anaerobic biodegradation of TCE, evaluated the natural attenuation of the chlorinated solvent in lake sediments from Lake Michigan (2). Samples from the contaminated sediment revealed methanogenic conditions and reductive dechlorination products of TCE, namely isomers of DCE, VC and ethane. Loss rates were then calculated from data collected along plume transects using an analytical solution of a two-dimensional advective-dispersive-reactive transport equation. Net apparent rate constants for TCE, DCE and VC ranged from 0.3-1.7, 0.26-3.3 and  $0.15\text{-}2.6 \text{ y}^{-1}$ , respectively. This study provides field evidence for natural attenuation of TCE.

Three articles this quarter report on the degradation of TCE by aerobic bacteria. The first of these reports on the diversity of methane oxidizing bacteria found in a TCE polluted plume containing methane (45). Methane oxidizers are implicated in the co-oxidation of TCE. Primers that specifically target methanotroph 16S rRNA genes or genes that code for subunits of soluble or particulate methane monooxygenase, *mmoX* and *pmoA*, respectively, were used to characterize the indigenous methanotrophs. The majority of clone sequences in type II methanotroph 16S rRNA, *pmoA*, and *mmoX* gene libraries grouped closely with sequences in the *Methylocystis* genus. A subset of the type II methanotroph clones from the aquifer had sequences

that aligned most closely to *Methylosinus trichosporium* OB3b and *Methylocystis* spp., which are known TCE-co-metabolizing methanotrophs. A second study reports on utilizing groundwater from a gas field containing an active population of methane oxidizers to inoculate a TCE contaminated aquifer (65). The concentration of TCE at a monitoring well 2 m down-gradient of the injection pit decreased from 128  $\mu\text{g L}^{-1}$  before the injection to less than the lower detection limit of 12.5  $\mu\text{g L}^{-1}$  after the injection. A control injection of clean water only resulted in a slight decrease to 86  $\mu\text{g L}^{-1}$  at the down-gradient well. Finally the third article on the aerobic degradation of TCE evaluated the restoration of TCE degrading activity in resting cells of the bacterium *Pseudomonas putida* F1 after inactivation during co-oxidation of TCE by toluene dioxygenase (TDO) (42). A novel finding is that subsequent addition of the primary substrate, toluene, resulted in an immediate toluene-degrading activity, which was followed by TCE degradation after toluene was consumed. TCE was degraded simultaneously if cumene was added instead of toluene and the TCE degradation was more extensive. This cycle of pseudo-inactivation and restoration of TCE degradation was observed repeatedly without a significant decrease in the number of viable cells, even after six additions of toluene spread over 30 h. The results obtained in this study demonstrate a new type of restoration of TCE degradation.

### **Carbon Tetrachloride (CT) and Chloroform (CF)**

This quarter three articles report on the biodegradation of higher chlorinated methanes, chloroform (CF) and carbon tetrachloride (CT). The first of these reports on the use of cytochrome P450 from *Sulfolobus solfataricus* CYP119, an extremophilic archaeobacteria found in sulfurous volcanic hot springs (9). The cytochrome from this isolate has an unusually high denaturation point of 90°C. This cytochrome immobilized on an electrode was shown to transfer electrochemical current to support the dehalogenation of CT at high temperatures. Catalysis at the higher temperatures increased the production of  $\text{CH}_4$  from CT by 35-fold compared to room temperature. The second study evaluated CT dechlorination by zero valent iron and discussed problems of metal hydroxide precipitation on the zero valent iron, which lowered rates of CT degradation (35). The article however was restricted to abiotic reactions of the metallic iron. Finally mutations of the monooxygenase of *Burkholderia cepacia* G4 were directed at improving CF co-oxidation (59). A mutant, A106F variant, had 2.8-times-better CF degradation activity based on gas chromatography ( $V_{\text{max}}$  of 2.61 versus 0.95  $\text{nmol min}^{-1} \text{mg}^{-1}$  and unchanged affinity constant,  $K_m$ ).

### **Chloromethane (CM) and Dichloromethane (DCM)**

This quarter also three articles examined the biodegradation of lower chlorinated methanes, dichloromethane (DCM) and chloromethane (CM). In the first article, stable C isotope fractionation and stable isotope probing was used to identify reactions and the responsible microorganisms for CM and methyl bromide (MeBr) degradation in soil (40). Bacterial oxidation of CM and MeBr resulted in significant isotopic fractionation of C. Stable isotope probing revealed that different populations of soil bacteria assimilated added C<sup>13</sup>-labeled MeBr and CM. The identity of the active MeBr and CM degrading bacteria in soil was determined by analysis of 16S rRNA gene sequences amplified from C<sup>13</sup>-DNA fractions, which identified a number of sequences from organisms not previously thought to be involved in methyl halide degradation. These included *Burkholderia*, the major clone type in the C-13-MeBr fraction, and *Rhodobacter*, *Lysobacter* and *Nocardioides* the major clone types in the C-13-MeCl fraction. Functional gene clone types closely related to *Aminobacter* spp. were identified in libraries containing the sequences for the *cmuA* gene, which codes for the enzyme known to catalyze the initial step in the oxidation of MeBr and CM. The *cmuA* gene was limited to members of the  $\alpha$ -Proteobacteria whereas the greater diversity demonstrated by the 16S rRNA gene may indicate that other enzymes catalyze methyl halide oxidation in different groups of bacteria. In the second article, mutagenesis of the CM-utilizing bacterium *Hyphomicrobium chloromethanicum* CM2 was utilized to discover new genes involved in chloromethane utilization (10). The mutational and transcriptional analysis data indicated that, in *H. chloromethanicum*, CM is metabolized via a corrinoid-specific (*cmuA*) and tetrahydrofolate-dependent (*metF*, *purU*, *fold*) methyltransfer system. The last article evaluated the role of vitamin B12 on DNA biosynthesis during aerobic growth of *Methylobacterium dichloromethanicum* on DCM (18). During growth on DCM, 10  $\mu$ g of corrinoids were produced per g dry weight (dw) of biomass.

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

One study this quarter evaluated the bioremediation of 1,2-DCA (47). The study evaluated the mitigation of a 1,2-DCA groundwater plume by use of a physical barrier (cement-bentonite diaphragm wall) and 12 extraction wells. Field investigations provided evidence that 1,2-DCA was naturally biodegraded into VC and ethene under the natural anaerobic-reducing conditions at the site. Source control measures were implemented to accelerate the overall remediation

process. A pilot test is planned to evaluate the enhancement of these processes through the use of a biosparging system. This test will be implemented near the source to achieve sequential aerobic-anaerobic treatment zones.

Another study evaluated the biodegradation of pentachloroethane (PCA) by a genetically engineered microorganisms, biphenyl-degrading *Alcaligenes* sp. strain KF711 (28). The strain expresses a modified camphor monooxygenase and a hybrid dioxygenase consisting of TodC1 (a large subunit of toluene dioxygenase of *Pseudomonas putida* F1) and BphA2-BphA3-pbhA4 (a small subunit, ferredoxin and ferredoxin reductase of biphenyl dioxygenase, respectively, in strain KF707). Modified camphor monooxygenase genes (*camCAB*) were supplied as a plasmid and the *todC1* gene was integrated within the chromosomal *bph* gene cluster by a single crossover recombination. The resultant strain KF711S-3cam dechlorinated PCA to TCE by the action of the modified camphor monooxygenase under anaerobic conditions. The same strain subsequently degraded TCE oxidatively by the action of the Tol-Bph hybrid dioxygenase under aerobic conditions. Thus sequential anaerobic and aerobic treatments of the KF711S-3cam resting cells resulted in efficient and total degradation of PCA

### **Chlorobenzenes (CB)**

Five reports were found regarding the microbial degradation of chlorobenzene compounds (5, 60, 75) (5, 24, 66).

The effect of a non-ionic surfactant (Tween 60) on the phase distribution of hexachlorobenzene (HCB) in an azide-inactivated, methanogenic culture capable of HCB dechlorination was assessed and subsequently used as the basis for the development of a model that describes the bioavailability and reductive dechlorination of HCB in a surfactant/biomass system (75).

The performance of a biotrickling filter treating a waste gas containing a mixture of chlorobenzene and 1,2-dichlorobenzene was investigated (60). Removal efficiencies exceeding 95% were obtained at mass loading rates of  $1,800 \text{ g m}^{-3} \text{ day}^{-1}$ . Dimensionless concentration profiles showed that the chlorobenzenes were simultaneously degraded. Complete mineralization of the chlorobenzene contaminants was confirmed by the low dissolved organic carbon and stoichiometric recovery of chloride in the effluent.

Carbon isotope fractionation was evaluated during aerobic and anaerobic transformation of trichlorobenzenes (TCB) (24). Mineralization of 1,2,4-TCB by the aerobic strain *Pseudomonas* sp. P51 was not accompanied by a significant isotope fractionation. In contrast, reductive

dehalogenation of 1,2,4-TCB and 1,2,3-TCB by the anaerobic strain *Dehalococcoides* sp. strain CBDB1 revealed significant isotope fractionation. These results suggest that carbon isotope fractionation could be applied for evaluating *in situ* biodegradation of halogenated benzenes in anoxic environments.

Biodegradation of mono-chlorobenzene (MCB, 500 mg L<sup>-1</sup>) under consecutive aerobic-anaerobic conditions was evaluated in laboratory microcosms inoculated with the indigenous microbial community from a polluted site (5). Under aerobic conditions, MCB was degraded via the modified *ortho*-pathway. Respiratory chain quinones and SSCP analysis suggested dominance of the genera *Acidovorax* and *Pseudomonas*. Under anoxic conditions, MCB was biotransformed but not dechlorinated. A shift to anoxic conditions resulted in MCB biotransformation but no dechlorination. However, anoxic metabolites could not be detected neither by HPLC (polar compounds) nor GC (volatile, less polar compounds).

Tebes-Stevens *et al.* (2004) (66) reported on the estimation of microbial reductive transformation rates for 25 chlorinated benzenes using quantitative structure-activity relationships (QSARs). The final QSAR included four descriptors: the logarithm of the octanol-water partition coefficient ( $K_{ow}$ ), the summation of the Hammett sigma constants, and the sigma induction constants in the *ortho* and *meta* positions relative to the transformation reaction center.

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

No reports were found this quarter on the microbial degradation of CDDs/CDFs. In a related study, seventeen different xenobiotic degrading *Sphingomonas* strains were screened for the presence of conjugative degradative plasmids (6). Most of the strains were shown to contain 2-5 plasmids with sizes of about 50 to 500 kb. Hybridization experiments with labeled gene probes suggested that large plasmids are involved in the degradation of dibenzo-*p*-dioxin, dibenzofuran, and naphthalenesulfonates in *S. wittichii* RW1, *Sphingomonas* sp. HH69, and *S. xenophaga* BN6, respectively. Conjugative transfer of plasmids between different *Sphingomonas* strain was observed. In contrast, no indications for the transfer of a *Sphingomonas* plasmid to bacteria outside of the *Sphingomonadaceae* were obtained.

## Hexachlorobutadiene and Octachlorostyrene

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

## Polychlorinated Biphenyls (PCBs)

In this quarter, four publications reported on the microbial degradation of polychlorinated biphenyls (PCBs) by aerobic bacteria, namely, *Escherichia coli* (11), *Pseudomonas* sp. strain B4 (12), *Rhodococcus* sp RHA1 (64) and unidentified microorganisms in conventional activated sludge treatment of sewage (31). One publication addressed PCB removal by phytoremediation (13). An additional report concerned with the degradation of PCBs by fungal laccase is discussed in Section 3.c “*In Vitro Degradation of Chlorinated Compounds*”. Finally, a new PCR-based method for the detection of genetically modified microorganisms (26) is discussed in Section 3.d “*New Tools and Techniques to Assess the Biodegradation of Chlorinated Compounds*”. This study utilized a PCB-degrading bacterium, *Pseudomonas fluorescens* F113rifPCB.

The toxicity of diverse PCBs and their biotransformation into the first two metabolic intermediates of the biphenyl pathway, were determined in studies with various recombinant *E. coli* strains expressing different subsets of bph genes of the well-know PCB degrader, *Burkholderia* sp. strain LB400 (11). The recombinant *E. coli* strains accumulated metabolic intermediates from (chloro)biphenyls. Dihydrodiols and dihydroxybiphenyls (eg. 2,3-dihydroxybiphenyl) were shown to be very toxic for bacteria at concentrations of 2mM even after short incubation times, affecting the cell viability much more than (chloro)biphenyls.

Growth of PCB-degrading bacteria in the presence of biphenyl and PCB was shown to generate oxidative stress and massive accumulation of inorganic polyphosphate in studies with *Pseudomonas* sp. strain B4 (12). Large polyphosphate deposits were observed in strain B4 using transmission electron microscopy-dispersive X-ray analysis and electron energy loss spectroscopy following a shift from glucose to biphenyl or chlorobiphenyls. Concomitant increases in the levels of the general stress protein GroEI and reactive oxygen species were also observed in the PCB-grown cells.

The occurrence and removal of PCB contaminants (PCB-52, PCB-110, PCB-180 and heptachlor-exo-epoxide) in sewage during conventional activated sludge treatment was

determined in a plant of Thessaloniki, northern Greece (31). Removal percentages throughout the whole treatment process ranged from 65% to 81% for individual PCB species. Based on the strong correlation between PCB removal efficiency and PCB hydrophobicity (*i.e.*, log  $K_{ow}$ ), the authors hypothesize that sorption to sludge particles was the dominant mechanism contributing to PCB removal. The total concentration of PCB in sewage sludge ranged between 185 and 765  $\text{ng g}^{-1}$  dw.

Significant biodegradation of the PCB Arochlor 1248 ( $100 \text{ mg kg}^{-1}$  of soil) in contaminated soil was observed during experiments comparing the effectiveness of alfalfa, flatpea, sericea lespedeza, deertongue, reed canarygrass, switchgrass, and tall fescue for PCB phytoremediation (13). 38% or less of the initial PCB was recovered from planted pots, compared to more than 82% from the unplanted control soils. The presence of plants did also increase significantly the biological activity as monitored by microbial counts and enzyme activity assays.

Five transcriptional promoters of biphenyl-degradation genes in the PCB-degrading bacterium, *Rhodococcus* sp. RHA1, were characterized (64).

## Miscellaneous Chlorinated Compounds

The search query used is specifically designed to review literature on the target compounds listed in the *Introduction* section. Interesting publications concerned with compounds outside of the range list which are found in the search process are briefly discussed below.

**2,4-D.** Two articles report on 2,4-dichlorophenoxyacetate (2,4-D) biodegradation. One compares rates of 2,4-D sorption and mineralization in five soils (53). Biodegradation half lives ranged from 3 to 22 days. The other article evaluates the spatial distribution of 2,4D-degraders in soil (49).

**3,3'-dichlorobenzidine** . One study evaluated the biodegradation of 3,3'-dichlorobenzidine in laboratory freshwater lake sediment microcosms (48).

**Acetochlor.** The persistence of the pesticide, acetochlor [2'-ethyl-6'-methyl-N-(ethoxymethyl)-2-chloroacetylanilide], in two New Zealand field soils was measured over two years (48). The half-lives of acetochlor loss were approximately 9 and 18 d at low and high pesticide application rates, respectively.

**Atrazine.** Two studies evaluated atrazine degradation. In the first of these, experiments were conducted to determine the degradation of atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) and metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-

(methoxyprop-2-yl)acetamide) in surface water, and to evaluate the contribution of sediment to their dissipation (57). Sediment significantly reduced concentrations of atrazine and metolachlor in the surface water as a result of greater degradation and the partitioning of the herbicide in the sediment. Half-lives of atrazine and metolachlor loss were 42 and 8 d in surface water-sediment incubation systems. In the second study, the soil fungus, strain INBI 2-26(-), was shown to degrade atrazine (33).

**TCAA.** Microbial degradation of trichloroacetic acid (TCA) was evaluated in two studies. One study demonstrated that TCA dissipation in Norway spruce leaf canopies was due to biodegradation based on controls in which antibiotics were added or with the use of axenic plants (22). The other study stressed the importance of halting biological activity in soil samples to obtain good measurements of TCA by preventing losses due to TCA biodegradation (38).

**Chloroaniline.** One study described how the transfer of a 3-chloroaniline degrading plasmid to bacteria in activated sludge enabled the activated sludge to degraded 3-chloroaniline (7).

**Chlorobenzoates.** Two studies covered chlorobenzoate (CBc) degradation. One study described 2-CBc degradation by the genetically engineered *Burkholderia cepacia*, which contains an hemoglobin to support CBc degradation at low oxygen levels (67). The other articles describes the genes of a meta-cleavage pathway involved in 4-CBc degradation by the bacterium, *Pseudomonas* sp. S-47 (51).

**Chlorothalonil.** *Ochrobactrum anthropi* SH35B is bacterial strain known to degraded the fungicide, chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile). A gene responsible for the chlorothalonil biotransformation was cloned from the chromosomal DNA of *Ochrobactrum anthropi* SH35B and was shown to efficiently degrade the chlorothalonil (34).

**Chlorophenols.** Twelve reports were found this quarter with information on chlorophenol (CP) biodegradation. Three of these were concerned with the aerobic degradation of 4-CP by bacteria (4, 27, 41). A new aerobic 4-CP degrader was isolated (27). Three studies evaluated 2,4-dichlorophenol (2,4-DCP) degradation in different biological systems: conventional aerobic activated sludge (56); an upward-flow anaerobic sludge bed reactor (3); and oxidation of 2,4-DCP by manganese peroxidase in organic medium with redox mediators (39). *Citrobacter* strain HPC255 was isolated from sludge which could metabolize several chlorophenols (44). An interesting article was the report of a genetically modified cotton plant which expressed recombinant laccase from its roots, the secreted laccase oxidized 2,4,6-trichlorophenol (70). The crystal structure of the 4-chlorocatechol 1,2-dioxygenase from the Gram-positive bacterium *Rhodococcus opacus* was resolved (21). This report constitutes the first structure elucidation of

an intradiol dioxygenases that specifically catalyze the cleavage of chlorocatechols, key intermediates in the aerobic catabolism of toxic chloroaromatics. Finally, three studies evaluated pentachlorophenol degradation in three distinct biological systems: new white rot fungal strains (69); direct oxidation by PCP 4-monooxygenase in a study evaluating site directed mutagenesis (43); and anaerobic degradation in an upward-flow anaerobic sludge bed reactor, with removal rates up to  $150 \text{ mg PCP l}_{\text{reactor}}^{-1} \text{ d}^{-1}$  (74).

**Dichloropropane.** To shed light on the populations involved in the anaerobic degradation of 1,2-dichloropropane, a comprehensive 16S rRNA gene-based bacterial community analysis of two enrichment cultures derived from geographically distinct locations was performed (58). The results indicated that the well known halo-respiring bacterium *Dehalococcoides* was involved in 1,2-dichloropropane dechlorination in both enrichment cultures. The findings expand the spectrum of chloroorganic compounds used by *Dehalococcoides* species as growth-supporting electron acceptors (e-acceptor).

**DDT.** One study evaluated the degradation of 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDT) in soil with sea weed as electron donating cosubstrate (30). Low levels of sea weed stimulated DDT degradation; whereas high levels lowered degradation. During soil incubation 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDD) was the major metabolite found with small amounts of 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane (DDE) and 4,4'-dichlorobenzophenone (DBP) produced.

**HCH.** Two studies evaluated the bioremediation of hexachlorocyclohexane (HCH) (8, 52). Extensive mineralization of radiolabelled  $\gamma$ -HCH occurred in aerobic soil microcosms (52). Bioremediation was shown to lower the phytotoxicity of technical grade HCH applied to soil (8).

**Trichlorotoluene.** A sequential series of bioreactors operated in methanogenic, denitrifying and aerobic modes were tested for their ability to biodegrade trichlorotoluene (62). TCT was transformed to toluene and dichlorotoluene under anaerobic and anoxic conditions.

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

Degradation of hydroxy polychlorinated biphenyls (hydroxy PCBs) catalyzed by laccase from the white rot fungi *Trametes versicolor* and *Pleurotus ostreatus* was reported (32). 3-hydroxy biphenyl was more resistant to laccase degradation than 2- or 4-hydroxy analogues. Degradation rate constants decreased with increase of chlorination and no degradation was observed with tetra-, penta- and hexa-chloro hydroxy PCBs in non-mediated reactions. However, the tetra- to

hexa-chloro hydroxy PCBs were degraded by laccase from *T versicolor* in the presence of the mediator 2,2,6,6-tetramethylpiperidine-N-oxyl radical. The other mediators, 4-ethyl-2-methoxyphenol, 2,2'-azino-bis(3-ethylbenzthiazoline sulfonic acid) diammonium salt and 1-hydroxybenzotriazole and humic acid, also enhanced degradation of all the hydroxy PCBs except 4-hydroxy-2',3,3',4',5,5'-hexachlorobiphenyl. Significant linear-correlations were found between the ionization potentials and the removal rate constants of hydroxy PCBs.

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

#### **Expert System**

A generalized methodology that enables the translation of expert knowledge about any complex process involved in a remedial decision into easy-to-use decision tools was developed. The methodology is applied to evaluate reductive dechlorination as a remedial possibility at sites contaminated with TCE, building on an existing protocol/scoring system put forth by the US Air Force and the US EPA (63). An alternate scoring system is proposed, which has two major advantages. First it attributes relative weights to findings based on expert beliefs. Second it systematically includes negative weights for negative findings. The ability of the proposed scoring system to assess the bioattenuation potential of TCE is demonstrated using data from extensively studied sites.

#### **Detection of Microbial Populations**

A PCB-degrading strain (*Pseudomonas fluorescens* F113rifPCB) was genetically modified by introducing the *bph*<sub>LB400</sub> operon (for biphenyl and PCB co-metabolism) (26). The donor of the *bph*<sub>LB400</sub> operon was the PCB-degrading microorganisms *Burkholderia* sp. strain LB400. The unique DNA sequence at the insertion site allowed the development of a specific PCR detection system that can detect the genetically modified microorganism from soil in less than 90 min and at levels below the detection limits of standard PCR or cultivable counts on selective media. The developed method utilized real time PCR using fluorescence resonance energy transfer probes.

## 4. MICROBIAL CHLORINATION

### 4.a. General Reviews

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

### 4.b. Microbial Chlorination in the Environment

#### Chloromethanes

Terrestrial sources and sinks of halomethanes near Cape Grim, Tasmania were investigated over one year (17). The sites studied include soil/plant litter under melaleuca scrub and eucalypt forest canopies, native tussock grass/soil, improved pasture grass/soil and coastal wetland. The seasonal variation, the interspecies correlations and ratios for the net flux data were investigated and the ratios compared to the in situ data. All sites were significant sources of  $\text{CHCl}_3$ . The largest  $\text{CHCl}_3$  net flux observed was from the eucalypt soil site and this is likely to be due to the high chlorine content of eucalyptus leaves. The  $\text{CHCl}_3$  emissions observed at the coastal wetland site were 1–2 orders of magnitude smaller than the other sites. The coastal wetland site was also a source methyl bromide ( $\text{CH}_3\text{Br}$ ) and methyl iodide ( $\text{CH}_3\text{I}$ ). Natural formation of methyl bromide was also detected in the grassland sites (pasture and tussock). A seasonal variation in the net flux was observed at the coastal wetland site for  $\text{CH}_3\text{Cl}$  and  $\text{CH}_3\text{Br}$ . Table 1 summarizes the mean net fluxes determined for the various halomethanes and sites. Molar emission ratios for natural halomethanes were studied for the various sites. Molar emissions of  $\text{CH}_3\text{Br}$  and  $\text{CH}_3\text{Cl}$  were strongly correlated suggesting a common production mechanism for these methyl halides at some of these sites.

Concentrations of two halomethanes (chloroform and tetrachloromethane) and several other important chlorinated compounds (1,1,1-trichloroethane, trichloroethene (TCE), tetrachloroethene (PCE) and trichloroacetic acid (TCA)) were monitored in water from lakes, rivers and springs with differing levels of salinity of the Kalmykian Steppe (Southern Russia) (71). The study indicated that salt lakes located in semiarid areas of the study region are sources of natural chlorinated hydrocarbons. Formation of the detected chlorohydrocarbons was attributed to abiotic and biotic reactions in saline soils, on the surfaces of salt crystals recrystallised in the water body and in the sediments of salt lakes, respectively. Furthermore, the

authors hypothesize a link between the occurrence of these toxic substances and the desertification observed in this area since the mid-20th century.

## Other Chlorinated Compounds

One publication was found during this quarter on the formation of organohalogenes, other than methyl halides, in the terrestrial environment. Natural emissions of TCA, 1,1,1-trichloroethane, TCE and PCE were measured for salt lakes (71) as was described above.

## Chlorinated Natural Organic Matter

This quarter reports were not found on the natural formation of organic matter in the terrestrial environment.

**Table 1.** Net fluxes ( $\text{ng m}^{-2} \text{h}^{-1}$ ) of several halomethanes, *i.e.*, methyl chloride, methyl bromide, methyl iodide, chloroform, and dichloromethane, determined in various sites in the vicinity of Cape Grim (17))

The site description, dominant vegetation type, number of experiments, the mean (standard deviation: average of the individual standard deviations for each experiment calculated from the curve fit and instrumental precision) net fluxes ( $\text{ng m}^{-2} \text{h}^{-1}$ ) and their ranges for  $\text{CH}_3\text{Cl}$ ,  $\text{CH}_3\text{Br}$ ,  $\text{CH}_3\text{I}$ ,  $\text{CHCl}_3$  and  $\text{CH}_2\text{Cl}_2$  (positive flux = source, negative = sink)

Site description (location)	Dominant vegetation	<i>n</i>	$\text{CH}_3\text{Cl}$	$\text{CH}_3\text{Br}$	$\text{CH}_3\text{I}$	$\text{CHCl}_3$	$\text{CH}_2\text{Cl}_2$
1. Tussock grass (Cape Grim)	<i>Poa poiformis</i>	10	-510 (570) -1700 to 310	34 (26) -7.1 to 210	73 (52) 6.4 to 260	1800 (1600) 46 to 4800	-6.8 (12) -49 to 27
2. Soil/litter: sub-Melaleuca canopy (Cape Grim)	<i>Melaleuca squarrosa</i>	10	-1000 (1100) -1700 to -120	-34 (19) -83 to -11	-13 (3.7) -23 to -7.0	320 (300) 160 to 950	-37 (11) -120 to -18
3. Pasture (Cape Grim)	<i>Lolium perenne</i>	10	-510 (420) -1100 to -13	31 (23) -5.5 to 110	41 (30) 2.1 to 150	1200 (1100) 72 to 2100	-12 (8.0) -30 to 15
4. Soil/litter: sub-Eucalypt canopy (Welcome Forest; 10 km E of Cape Grim)	<i>Eucalyptus Ovata</i>	8	-180 (650) -1100 to 1300	-22 (11) -30 to -10	-6.0 (3.6) -15 to 5.4	3000 (2900) 1600 to 5000	-6.6 (10) -29 to 29
5. Coastal wetland (Robbins Crossing; 20 km E of Cape Grim)	<i>Pachycornia arbuscula</i>	10	300 (320) -34 to 760	190 (130) 27 to 470	250 (180) 34 to 840	73 (46) 15 to 270	16 (22) -10 to 90

## .c. Chlorination by Marine Organisms

### Chloromethanes

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

### Other Chlorinated Compounds

Several publications reported on the detection and characterization of halogenated metabolites from bacteria (54) and from marine microorganisms, including cyanobacteria (72); sponges (20), red alga (61, 76) and brown alga (73). In addition, new cytotoxic polychlorinated sulfolipids were characterized from contaminated Adriatic mussels, although the producing (micro)organism was unknown (16).

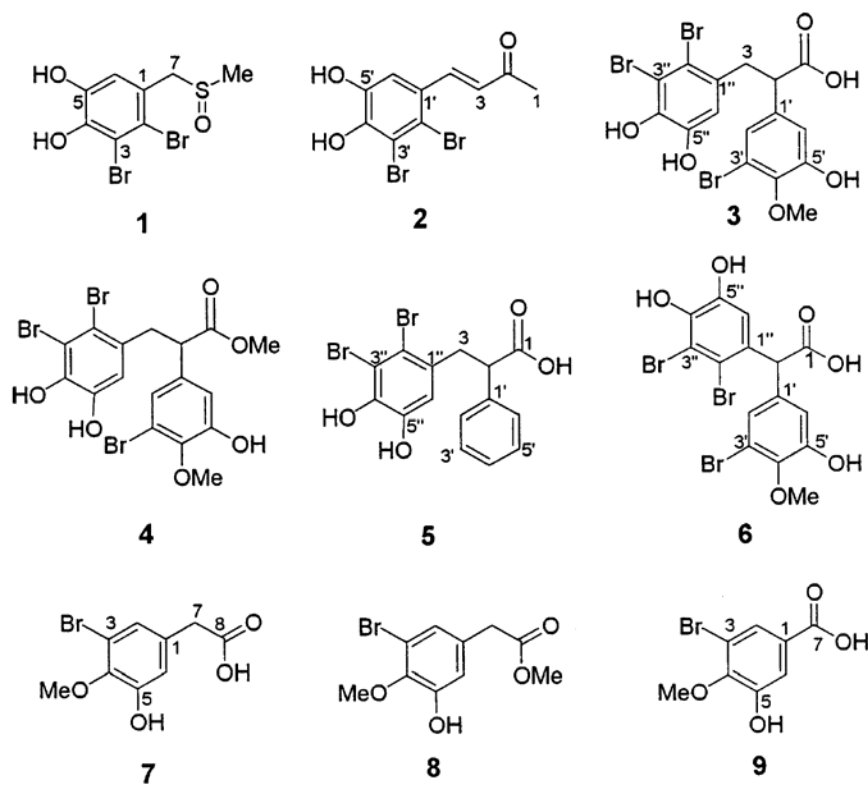
A recently published review paper describes natural products that are shown or suspected to be synthesized by symbiotic bacteria (54). These metabolites include numerous chlorinated and brominated compounds. The review includes 349 references and covers the literature in this field through 2003. Eleven novel chlorinated lipids (taveuniamides) were isolated from the cyanobacteria *Lyngbya majuscula* and *Schizothrix* sp. (72). The structure of these novel metabolites was elucidated using a NMR and mass spectrometry techniques. Several taveuniamides were shown to be a potent brine shrimp toxins with LC50s between 1.7–1.9 mg mL<sup>-1</sup>.

A new cytotoxic polychlorinated sulfolipid from contaminated Adriatic mussels was characterized (16). The compound showed to possess cytotoxic activity against two different human cancer cell lines. The producing organism is as yet unknown. Eight new dimeric bromopyrrole alkaloids (nagelamides A-H) were isolated from the Okinawan marine sponge *Agelas* spp., and the structures were elucidated from spectroscopic data (20). All the nagelamides exhibited antibacterial activity against Gram-positive bacteria. Eight new bromophenol derivatives from the red alga *Rhodomela confervoides* were characterized (76). Four of the isolated compounds were tested for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* and showed no growth inhibition at 100 µg mL<sup>-1</sup>. Likewise, the compounds were found inactive against several human cancer cell lines at 10 µg mL<sup>-1</sup>. Six novel dibenzyl bromophenols with different dimerization patterns and two propyl bromophenol derivatives, together with 11 known bromophenol derivatives, were isolated from the ethanolic extract of the brown alga *Leathesia nana* (73). Some compounds showed in vitro

selective cytotoxicity against several human cancer cell lines. This is the first brown alga to be reported containing bromophenols. Four novel bromophenols were also isolated from another red alga, *Polysiphonia lanossa*, in studies conducted by Shoeib *et al.* (61). Some of these compounds displayed cytotoxic activity in assays with human cell lines were synthesized. The compound, 2,5-dibromo-3,4-dihydroxybenzyl *n*-propyl ether, was the most active compound with IC50 values lower than 2  $\mu$ M.

#### 4.d. Chlorinating Enzymes

No reports were found on the involvement of chlorinating enzymes on the natural formation of organochlorine compounds.



**Figure 1.** Structure of the novel bromophenol derivatives from the red alga *Rhodomela confervoides* [Zhao, 2004 #207].

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## 6. ANNEX

**Abdul-Wahab, S. A. (2004). "Modelling methane and vinyl chloride in soil surrounding landfills." *International Journal of Environment and Pollution* 21(4): 339-349.**

Sanitary landfilling is used in many countries as a preferred method for disposal of household wastes for reasons of simplicity and economics. Immediately following its deposition within a landfill, most of the organic fraction of waste will begin to undergo degradation through chemical and bacterial action. Landfill gas (LFG) is a product of biodegradation and consists of primarily methane (explosive) and carbon dioxide, with trace amounts of other volatiles that are often toxic gases (for example, vinyl chloride). LFG can migrate through the soil away from the landfill site and appear at the surface away from where it started. Since methane presents a fire or explosive threat, LFG must be controlled to protect property and public safety. To aid this, consideration must be given to models. Therefore, this study was undertaken to develop a simple numerical model by using a finite difference method in order to predict gas migration through the soil surrounding the landfill. The model construction was described as well as the landfill and its surrounding soil. The model was applied to predict methane and vinyl chloride concentrations at different distances from the landfill. Comparison between the predicted and measured values was calculated to evaluate the validity of the model. The agreement between measured and predicted concentrations was found, and this agreement is sufficiently good.

**An, Y. J., D. H. Kampbell, et al. (2004). "Natural attenuation of trichloroethene and its degradation products at a lake-shore site." *Environmental Pollution* 130(3): 325-335.**

Subsurface contamination by trichloroethene (TCE) was detected at a Michigan National Priorities List (NPL) site in 1982. The TCE plume resulted from the disposal of spent solvent and other chemicals at an industrial facility located in the eastern shore of Lake Michigan. TCE degradation products of three dichloroethene (DCE) isomers, vinyl chloride (VC) and ethene were present. The plume was depleted of oxygen and methanogenic at certain depths. Transects of the plume were sampled by slotted auger borings the year after the TCE plume was first discovered. Water samples were also taken from lake sediments to a depth of 12 m about 100 m offshore. Later samples were taken along the shoreline of the lake with a hand-driven probe. Later in 1998 water was taken from sediments about 3-m from the shoreline. The average concentration of each chemical and net apparent base coefficient between appropriate pairs of transects between the lower site and lakeshore were calculated. Loss rates were then calculated from an analytical solution of the two-dimensional advective-dispersive-reactive transport equation. Net apparent rate coefficients and a set of coupled reaction rate equations were used to extract the apparent loss coefficients. This study showed the field evidence for natural attenuation of TCE. (C) 2004 Elsevier Ltd. All rights reserved.

**Anderson, W. B., P. G. Board, et al. (2004). "Glutathione transferase zeta-catalyzed bioactivation of dichloroacetic acid: Reaction of glyoxylate with amino acid nucleophiles." *Chemical Research in Toxicology* 17(5): 650-662.**

Dichloroacetic acid (DCA) is a drinking water contaminant, a therapeutic agent, and a rodent carcinogen. Glutathione transferase zeta (GSTZ1-1) catalyzes the biotransformation of a range of alpha-haloalkanoates and the cis-trans isomerization of maleylacetoacetate. GSTZ1-1 catalyzes the bioactivation of fluorine-lacking dihaloacetates to S-(alpha-halocarboxymethyl)glutathione, a reactive intermediate that covalently modifies and inactivates the enzyme or is hydrolyzed to glyoxylate. The purpose of this study was to examine the GSTZ1-1-catalyzed bioactivation of DCA, including the reaction of DCA-derived glyoxylate with amino acid nucleophiles and the characterization of the structures and kinetics of adduct formation by LC/MS. The binding of [1-C-14]DCA-derived label to bovine serum albumin required both GSTZ1-1 and GSH, whereas the binding to dialyzed rat liver cytosolic protein was increased in the presence of GSH. Studies with model peptides (antiflammin-2 and IL-8 inhibitor) indicated that glyoxylate, rather than S-(alpha-chlorocarboxymethyl)glutathione, was the reactive species that modified amino acid nucleophiles. Both addition (Da) and addition-elimination (Da) adducts of glyoxylic acid were observed. Addition adducts (Da) could not be characterized completely by mass spectrometry, whereas

addition-elimination adducts (Da) were characterized as 2-carboxy-4-imidazolidinones. 2-Carboxy-4-imidazolidinones were formed by the rapid equilibrium reaction of glyoxylate with the N-terminal amino group of antinflammin-2 to give an intermediate carbinolamine ( $K_{eq} = 0.63 \text{ mM}^{-1}$ ), which slowly eliminated water to give an intermediate imine ( $k_2 = 0.067 \text{ hour}^{-1}$ ), which rapidly cyclized to give the 2-carboxy-4-imidazolidinone. Glucose 6-phosphate dehydrogenase was inactivated partially by glyoxylate when reactants were reduced with sodium borodeuteride, which may indicate that glyoxylate reacts with selective lysine epsilon-amino groups. The results of the present study demonstrate that GSTZ1-1 catalyzes the bioactivation of DCA to the reactive metabolite glyoxylate. The reaction of glyoxylate with cellular macromolecules may be associated with the multiorgan toxicity of DCA.

**Atuanya, E. I. and T. Chakrabarti (2004). "Kinetics of biotransformation of 2,4-dichlorophenol using UASB-reactor." *Environmental Monitoring and Assessment* 96(1-3): 129-141.**

Chlorophenol compounds are environmental pollutants that are both anthropogenic and xenobiotics. Some of these chemicals are carcinogens and are both toxic to a number biochemical processes. Biotransformation of 2,4-dichlorophenol (2,4-DCP) was studied in the presence of glucose on an upflow anaerobic sludge blanket reactor (UASB) using mixed culture. A continuously operated UASB reactor was employed using mixed synthetic wastewater. Results obtained from the 1.8 L volume capacity UASB reactor were subjected to kinetic evaluation constants. Results indicate that the degradation of 2,4-DCP in the presence of glucose was strongly influenced by the concentration of the compound. High degradation levels were observed when the concentration of 2,4-DCP was in the range of 50-150 mg L<sup>-1</sup>. Concentrations of 2,4-DCP above 160 mg L<sup>-1</sup> were toxic to microbes even in the presence of glucose. The maximum degradation of 2,4-DCP was found to be 70.4% when initial concentration of 2,4-DCP was 124 mg L<sup>-1</sup> and glucose concentration of 500 mg L<sup>-1</sup> at hydraulic retention time of 13.2 hr. The biodegradation followed first order reaction kinetics with a rate constant (K) of 0.67, V-max of 0.244 kg m<sup>(-3)</sup> day<sup>(-1)</sup>, K-s of 0.117 kg m<sup>(-3)</sup> day<sup>(-1)</sup> and correlation coefficient of 0.766.

**Backman, A., N. Maraha, et al. (2004). "Impact of temperature on the physiological status of a potential bioremediation inoculant, *Arthrobacter chlorophenolicus* A6." *Applied and Environmental Microbiology* 70(5): 2952-2958.**

*Arthrobacter chlorophenolicus* A6 (A6) can degrade large amounts of 4-chlorophenol in soil at 5 and 28degreesC. In this study, we investigated the effects of temperature on the physiological status of this bacterium in pure culture and in soil. A derivative of A6 tagged with the *gfp* gene (encoding green fluorescent protein [GFP]) was used to specifically quantify A6 cells in soil. In addition, cyano-ditolyl-tetrazoliumchloride was used to stain GFP-fluorescent cells with an active electron transfer system ("viable cellis") whereas propidium iodide (PI) was used to stain cells with damaged membranes ("dead cells"). Another derivative of the strain (tagged with the firefly luciferase gene [*luc*]) was used to monitor the metabolic activity of the cell population, since the bioluminescence phenotype is dependent on cellular energy reserves. When the cells were incubated in soil at 28degreesC, the majority were stained with PI, indicating that they had lost their cell integrity. In addition, there was a corresponding decline in metabolic activity and in the ability to be grown in cultures on agar plates after incubation in soil at 28degreesC, indicating that the cells were dying under those conditions. When the cells were incubated in soil at 5degreesC, by contrast, the majority of the cells remained intact and a large fraction of the population remained metabolically active. A similar trend towards better cell survival at lower temperatures was found in pure-culture experiments. These results make *A. chlorophenolicus* A6 a good candidate for the treatment of chlorophenol-contaminated soil in cold climates.

**Balcke, G. U., L. P. Turunen, et al. (2004). "Chlorobenzene biodegradation under consecutive aerobic-anaerobic conditions." *Fems Microbiology Ecology* 49(1): 109-120.**

The biodegradation of monochlorobenzene, the main contaminant in a quaternary aquifer at Bitterfeld, Central Germany, was studied in microcosm experiments employing either original groundwater or defined mineral media together with the indigenous microbial community from the polluted site. The impact of consecutive aerobic-anaerobic aerobic incubations on monochlorobenzene biodegradation, microbial diversity, and pH development was

examined. The related changes in microbial community composition were analyzed by 16S rRNA gene-based single-strand conformation polymorphism (SSCP) fingerprints and sequencing of dominant bands and by quantitative analysis of bacterial respiratory chain quinones as biomarkers. Under aerobic conditions, the indigenous microbial community of the groundwater degraded monochlorobenzene mainly via the modified ortho-pathway. Respiratory chain quinones and SSCP analysis suggested dominance of the genera *Acidovorax* and *Pseudomonas*. A shift to anoxic conditions resulted in monochlorobenzene biotransformation but no dechlorination. The ability to degrade monochlorobenzene aerobically remained preserved throughout a fortnightly anoxic period at sufficiently high buffer capacity. Acidification, caused by monochlorobenzene biodegradation, was alkalinity-controlled. At low initial alkalinity a substantial decrease in pH, monochlorobenzene degradation, and total counts of live cells, accompanied by a change of the microbial community composition, was observed. (C) 2004 Federation of European Microbiological Societies. Published by Elsevier B.V. All rights reserved.

**Basta, T., A. Keck, et al. (2004). "Detection and characterization of conjugative degradative plasmids in xenobiotic-degrading *Sphingomonas* strains." *Journal of Bacteriology* 186(12): 3862-3872.**

A systematic survey for the presence of plasmids in 17 different xenobiotic-degrading *Sphingomonas* strains was performed. In almost all analyzed strains, two to five plasmids with sizes of about 50 to 500 kb were detected by using pulsed-field gel electrophoresis. A comparison of plasmid preparations untreated or treated with SI nuclease suggested that, in general, *Sphingomonas* plasmids are circular. Hybridization experiments with labeled gene probes suggested that large plasmids are involved in the degradation of dibenzo-p-dioxin, dibenzofuran, and naphthalenesulfonates in *S. wittichii* RW1, *Sphingomonas* sp. HH69, and *S. xenophaga* BN6, respectively. The plasmids which are responsible for the degradation of naphthalene, biphenyl, and toluene by *S. aromaticivorans* F199 (pNL1) and of naphthalenesulfonates by *S. xenophaga* BN6 (pBN6) were site-specifically labeled with a kanamycin resistance cassette. The conjugative transfer of these labeled plasmids was attempted with various bacterial strains as putative recipient strains. Thus, a conjugative transfer of plasmid pBN6 from *S. xenophaga* BN6 to a cured mutant of strain BN6 and to *Sphingomonas* sp. SS3 was observed. The conjugation experiments with plasmid pNL1 suggested a broader host range of this plasmid, because it was transferred without any obvious structural changes to *S. yanoikuyae* B1, *Sphingomonas* sp. SS3, and *S. herbicidovorans*. In contrast, major plasmid rearrangements were observed in the transconjugants after the transfer of plasmid pNL1 to *Sphingomonas* sp. HH69 and of pBN6 to *Sphingomonas* sp. SS3. No indications for the transfer of a *Sphingomonas* plasmid to bacteria outside of the Sphingomonadaceae were obtained.

**Bathe, S. (2004). "Conjugal transfer of plasmid pNB2 to activated sludge bacteria leads to 3-chloroaniline degradation in enrichment cultures." *Letters in Applied Microbiology* 38(6): 527-531.**

**Aims:** The involvement of the aniline-degradative plasmid pNB2 in degradation of 3-chloroaniline (3-CA) was investigated. **Methods and Results:** Plate matings of a *Pseudomonas putida* strain containing pNB2 with a mixed bacterial culture derived from activated sludge was carried out. After inoculation of the mating mixtures into batch cultures containing 3-CA, degradation of the compound was observed. A total of five different transconjugant strains could be isolated from one of the batch cultures and two of them were able to degrade 3-CA. These two isolates were identified as *Comamonas testosteroni* by partial 16S rDNA sequencing. **Conclusions:** It can be assumed that pNB2 carries a part of the genes involved in the catabolism of 3-CA, but that completion of the pathway must be provided by chromosomal genes in the host strain. **Significance and Impact of the Study:** pNB2 is a candidate plasmid which can be used in plasmid-mediated bioaugmentation of wastewater bacteria involved in degradation of chlorinated anilines.

**Bidlan, R., M. Afsar, et al. (2004). "Bioremediation of HCH-contaminated soil: elimination of inhibitory effects of the insecticide on radish and green gram seed germination." *Chemosphere* 56(8): 803-811.**

The effects of technical grade hexachlorocyclohexane (tech-HCH) on the germination of different seeds were tested. Two types of seeds, radish and green gram showed marked reduction in germination percentage and seeding vigour index. The abnormalities and reduction in germination increased with increasing concentration of tech-HCH. At 100 mug HCH level the germination of radish and green gram seeds was inhibited almost completely

on moist filter paper and soil. Protease and amylase activities were reduced in seeds grown in soil spiked with tech-HCH. Bioremediation of HCH-spiked soils with a HCH-degrading microbial consortium helped in eliminating the toxic effects of tech-HCH towards seed germination. The degradation of 25 µg tech-HCH g(-1) soil was complete by 120 It. The seed germination and the activities of the assayed enzymes, amylase and protease, were same as before or better in bioremediated soils. (C) 2004 Elsevier Ltd. All rights reserved.

**Blair, E., J. Greaves, et al. (2004). "High-temperature electrocatalysis using thermophilic P450 CYP119: Dehalogenation of CCl<sub>4</sub> to CH<sub>4</sub>." *Journal of the American Chemical Society* 126(28): 8632-8633.**

**Borodina, E., I. R. McDonald, et al. (2004). "Chloromethane-dependent expression of the *cmu* gene cluster of *Hyphomicrobium chloromethanicum*." *Applied and Environmental Microbiology* 70(7): 4177-4186.**

The methylotrophic bacterium *Hyphomicrobium chloromethanicum* CM2 can utilize chloromethane (CH<sub>3</sub>Cl) as the sole carbon and energy source. Previously genes *cmuB*, *cmuC*, *cmuA*, and *fold* were shown to be essential for the growth of *Methylobacterium chloromethanicum* on CH<sub>3</sub>Cl. These CH<sub>3</sub>Cl-specific genes were subsequently detected in *H. chloromethanicum*. Transposon and marker exchange mutagenesis studies were carried out to identify the genes essential for CH<sub>3</sub>Cl metabolism in *H. chloromethanicum*. New developments in genetic manipulation of *Hyphomicrobium* are presented in this study. An electroporation protocol has been optimized and successfully applied for transformation of mutagenesis plasmids into *H. chloromethanicum* to generate stable CH<sub>3</sub>Cl-negative mutants. Both transposon and marker exchange mutageneses were highly applicable for genetic analysis of *Hyphomicrobium*. A reliable and reproducible selection procedure for screening of CH<sub>3</sub>Cl utilization-negative mutants has also been developed. Mutational inactivation of *cmuB*, *cmuC*, or *hutI* resulted in strains that were unable to utilize CH<sub>3</sub>Cl or to express the CH<sub>3</sub>Cl-dependent polypeptide *CmuA*. Reverse transcription-PCR analysis indicated that *cmuB*, *cmuC*, *cmuA*, *fmdB*, *paaE*, *hutI*, and *metF* formed a single *cmuBCA-metF* operon and were coregulated and coexpressed in *H. chloromethanicum*. This finding led to the conclusion that, in *cmuB* and *cmuC* mutants, impaired expression of *cmuA* was likely to be due to a polar effect of the defective gene (*cmuB* or *cmuC*) located upstream (5') of *cmuA*. The detrimental effect of mutation in *hutI* on the upstream (5')-located *cmuA* is not clear but indicated that all the genes located within the *cmuBCA-metF* operon are coordinately expressed. Expression of the *cmuBCA-metF* transcript was also shown to be strictly CH<sub>3</sub>Cl inducible and was not repressed by the alternative C-1 substrate methanol. Sequence analysis of a transposon mutant (D20) led to the discovery of the previously undetected *hutI* and *metF* genes located 3' of the *paaE* gene in *H. chloromethanicum*. *MetF*, a putative methylene-tetrahydrofolate reductase, had 27% identity to *MetF* from *M. chloromethanicum*. Mutational and transcriptional analysis data indicated that, in *H. chloromethanicum*, CH<sub>3</sub>Cl is metabolized via a corrinoid-specific (*cmuA*) and tetrahydrofolate-dependent (*metF*, *purU*, *fold*) methyltransfer system.

**Camara, B., C. Herrera, et al. (2004). "From PCBs to highly toxic metabolites by the biphenyl pathway." *Environmental Microbiology* 6(8): 842-850.**

The degradation of polychlorobiphenyls (PCBs) by diverse bacteria, including *Burkholderia* sp. LB400, is incomplete with a concomitant accumulation of metabolic intermediates. In this study, the toxicity of diverse (chloro)biphenyls and of their biotransformation into the first two metabolic intermediates of the biphenyl pathway, were determined for the model bacterium *Escherichia coli*. Recombinant *E. coli* strains expressing different subsets of *bph* genes of strain LB400 accumulated metabolic intermediates from (chloro)biphenyls. During biotransformation of these compounds into metabolic intermediates, the viability and metabolic kinetics were determined. The toxicity of biotransformation of (chloro)biphenyls into different metabolic intermediates of (chloro)biphenyls varied. Dihydrodiols and dihydroxybiphenyls are very toxic metabolites for bacteria even after short incubation times, affecting the cell viability much more than (chloro)biphenyls. When bacteria transformed 2-CB into dihydrodiol or dihydroxybiphenyl, a great decrease of intact cells and abundant cell lysis was observed by transmission electronic microscopy. Cell viability of *Burkholderia* sp. LB400 and of *E. coli* exposed directly to 2,3-dihydroxybiphenyl decreased also drastically. The toxicity of metabolites generated during oxidation of PCBs may partly explain the recalcitrance to biodegradation of these pollutants. Conversion of less toxic compounds into products with increased toxicity resembles the bioactivation of xenobiotics in higher organisms.

**Chavez, F. P., H. Lunsdorf, et al. (2004). "Growth of polychlorinated-biphenyl-degrading bacteria in the presence of biphenyl and chlorobiphenyls generates oxidative stress and massive accumulation of inorganic polyphosphate." *Applied and Environmental Microbiology* 70(5): 3064-3072.**

Inorganic polyphosphate (polyP) plays a significant role in increasing bacterial cell resistance to unfavorable environmental conditions and in regulating different biochemical processes. Using transmission electron microscopy of the polychlorinated biphenyl (PCB)-degrading bacterium *Pseudomonas* sp. strain B4 grown in defined medium with biphenyl as the sole carbon source, we observed large and abundant electron-dense granules at all stages of growth and following a shift from glucose to biphenyl or chlorobiphenyls. Using energy dispersive X-ray analysis and electron energy loss spectroscopy, with an integrated energy-filtered transmission electron microscope, we demonstrated that these granules were mainly composed of phosphate. Using sensitive enzymatic methods to quantify cellular polyP, we confirmed that this polymer accumulates in PCB-degrading bacteria when they grow in the presence of biphenyl and chlorobiphenyls. Concomitant increases in the levels of the general stress protein GroEI and reactive oxygen species were also observed in chlorobiphenyl-grown cells, indicating that these bacteria adjust their physiology with a stress response when they are confronted with compounds that serve as carbon and energy sources and at the same time are chemical stressors.

**Chekol, T., L. R. Vough, et al. (2004). "Phytoremediation of polychlorinated biphenyl-contaminated soils: the rhizosphere effect." *Environment International* 30(6): 799-804.**

The objective in the first phase of this study was to screen alfalfa, flatpea, sericea lespedeza, deertongue, reed canarygrass, switchgrass, and tall fescue for phytoremediation of polychlorinated biphenyl (PCB)-contaminated soil. During the second phase, the focus was rhizosphere characterization to optimize PCB phytoremediation. Aroclor 1248 (PCB) was added to soil at 100 mg kg<sup>-1</sup> of soil. In the first phase, all of the plant species treatments showed significantly greater PCB biodegradation compared to the unplanted controls and the two most effective species were selected for further study. During the rhizosphere characterization study, soil irradiation did not affect PCB biodegradation, but planting significantly increased PCB biodegradation; 38% or less of the initial PCB was recovered from planted pots, compared to more than 82% from the unplanted control soils. Presence of plants significantly increased the biological activity (microbial counts and enzyme activity) of both irradiated and unirradiated soils. Greater bacterial counts and soil enzyme activity were closely related to higher levels of PCB biodegradation. The data showed that Aroclor 1248 biodegradation in soil seem to be positively influenced by the presence of plants and plant-bacteria interactions. Our results suggested that phytoremediation could be an environmentally friendly alternative for PCB -contaminated soils. (C) 2004 Elsevier Ltd. All rights reserved.

**Chu, K. H., S. Mahendra, et al. (2004). "Stable carbon isotope fractionation during aerobic biodegradation of chlorinated ethenes." *Environmental Science & Technology* 38(11): 3126-3130.**

Stable isotope analysis is recognized as a powerful tool for monitoring, assessing, and validating in-situ bioremediation processes. In this study, kinetic carbon isotope fractionation factors (epsilon) associated with the aerobic biodegradation of vinyl chloride (VC), cis-1,2-dichloroethylene (cDCE), and trichloroethylene (TCE) were examined. Of the three solvents, the largest fractionation effects were observed for biodegradation of VC. Both metabolic and cometabolic VC degradation were studied using *Mycobacterium aurum* L1 (grown on VC), *Methylosinus trichosporium* OB3b (grown on methane), *Mycobacterium vaccae* JOB5 (grown on propane), and two VC enrichment cultures seeded from contaminated soils of Alameda Point and Travis Air Force Base, CA. *M. aurum* L1 caused the greatest fractionation (epsilon = -5.7) while for the cometabolic cultures, epsilon values ranged from -3.2 to -4.8. VC fractionation patterns for the enrichment cultures were within the range of those observed for the metabolic and cometabolic cultures (epsilon = -4.5 to -5.5). The fractionation for cometabolic degradation of TCE by *Me. trichosporium* OB3b was low (epsilon = -1.1), while no quantifiable carbon isotopic fractionation was observed during the cometabolic degradation of cDCE. For all three of the tested chlorinated ethenes, isotopic fractionation measured during aerobic degradation was significantly smaller than that reported for anaerobic reductive dechlorination. This study suggests that analysis of compound-specific isotopic fractionation could assist in determining whether aerobic or anaerobic degradation of VC and cDCE predominates in field applications of in-

situ bioremediation. In contrast, isotopic fractionation effects associated with metabolic and cometabolic reactions are not sufficiently dissimilar to distinguish these processes in the field.

**Chu, M., P. K. Kitanidis, et al. (2004). "Possible factors controlling the effectiveness of bioenhanced dissolution of non-aqueous phase tetrachloroethene." *Advances in Water Resources* 27(6): 601-615.**

Tetrachloroethene (PCE) is a widespread subsurface contaminant. When PCE exists in the form of non-aqueous phase liquid (NAPL), it becomes a long term threat to groundwater quality. It has been recognized that treating the resulting plumes from PCE source zones alone is not effective because the long life of NAPL source zones may dictate the overall remediation time. Recent studies have shown the possibility of using microbial reductive dehalogenation to shorten the life of NAPL source zones by accelerating the dissolution process. To better understand the underlying mechanisms and the possible controlling factors of this technology, we used a novel two-dimensional numerical model to fully resolve the evolution of biomass profiles and the distributions of compounds within source zones. It was found that dehalogenation kinetics, electron donor (ED) levels, NAPL configurations, and ED competition among microorganisms all affect the extent of bio-enhanced dissolution. The simulation results point out the need to better characterize dehalogenation kinetics at high concentrations of PCE and its metabolic products. (C) 2004 Elsevier Ltd. All rights reserved.

**Ciminiello, P., C. Dell'Aversano, et al. (2004). "A new cytotoxic polychlorinated sulfolipid from contaminated Adriatic mussels." *Tetrahedron* 60(33): 7093-7098.**

A detailed analysis of toxic shellfish collected in the Adriatic sea in October 2000 allowed us to isolate a new cytotoxic chlorosulfolipid (3). Its gross structure has been elucidated through an extensive NMR analysis including various 2D techniques; the relative stereochemistry has been solved by applying the Murata's method. Compound 3 showed to possess cytotoxic activity against WEHI 164 and J774 cells. The presence of chlorosulfolipids in toxic mussels from the northern Adriatic sea has not to be considered incidental as we have been detecting these cytotoxic compounds since 1998. Their simultaneous and constant presence together with typical marine biotoxins represents a further risk both to consumers' health and aquacultures economic proceeds. (C) 2004 Elsevier Ltd. All rights reserved.

**Cox, M. L., P. J. Fraser, et al. (2004). "Terrestrial sources and sinks of halomethanes near Cape Grim, Tasmania." *Atmospheric Environment* 38(23): 3839-3852.**

The terrestrial sources and sinks of halomethanes, at and nearby the Cape Grim Baseline Air Pollution Station, Tasmania (41 degreesS, 145 degreesE), have been investigated over 12 months (July 2000-June 2001) using a flux chamber technique. The sites studied are representative of the soils and vegetation within 20 km of Cape Grim and include soil/plant litter under melaleuca scrub and eucalypt forest canopies, native tussock grass/soil, improved pasture grass/soil and coastal wetland. On average, the soil, tussock and pasture sites were found to be sinks for methyl chloride (CH<sub>3</sub>Cl), whereas the coastal wetland site was a source for CH<sub>3</sub>Cl, methyl bromide (CH<sub>3</sub>Br) and methyl iodide (CH<sub>3</sub>I). The grassland sites (pasture and tussock) were a small source for CH<sub>3</sub>Br and the soil sites (melaleuca and eucalypt) a sink. All sites were significant sources of chloroform (CHCl<sub>3</sub>). The interspecies ratios for significantly correlated halomethane net fluxes at some terrestrial sites were compared with the ratios found for AGAGE Cape Grim in situ data characterising north and west coast Tasmania. Flux ratios for CH<sub>3</sub>Br/CH<sub>3</sub>I at the coastal wetland and pasture sites were found to be similar to ratios found for the Cape Grim in situ data. Common production mechanisms were indicated for CH<sub>3</sub>Br and CH<sub>3</sub>Cl which were significantly correlated at the coastal wetland, eucalypt and melaleuca sites and for CH<sub>3</sub>Br and CH<sub>3</sub>I at the pasture and coastal wetland sites. A seasonal variation in the net flux was observed at the coastal wetland site for CH<sub>3</sub>Cl and CH<sub>3</sub>Br. Crown Copyright (C) 2004 Published by Elsevier Ltd. All rights reserved.

**Danilova, I. V., N. V. Doronina, et al. (2004). "The aeration-dependent effect of vitamin B-12 on DNA biosynthesis in *Methylobacterium dichloromethanicum*." *Microbiology* 73(2): 134-138.**

The effect of vitamin B-12 (cobalamin) on DNA biosynthesis in *Methylobacterium dichloromethanicum* was studied. When cultivated in media with methanol or dichloromethane, the bacterium produced approximately 10 mug corrinoids per gram dry biomass, compared to about 7 mug/g when cultivated on ethanol or succinate. Exogenous adenosylcobalamin (AdoCbl) stimulated DNA biosynthesis in *M. dichloromethanicum* cells grown under poor aeration, the effect being mediated by AdoCbl-dependent ribonucleotide reductase. In vitro studies showed that *M. dichloromethanicum* also has AdoCbl-independent ribonucleotide reductase. Under good aeration, exogenous AdoCbl had no effect on DNA biosynthesis, while hydroxyurea suppressed it. These data suggest that AdoCbl-independent ribonucleotide reductase, which is likely to be activated by oxygen, plays an important part in DNA biosynthesis when *M. dichloromethanicum* is cultured with good aeration, whereas AdoCbl-dependent ribonucleotide reductase is active under conditions of poor aeration.

**Dong, C. J., A. Kotsch, et al. (2004). "Crystallization and X-ray diffraction of a halogenating enzyme, tryptophan 7-halogenase, from *Pseudomonas fluorescens*." *Acta Crystallographica Section D-Biological Crystallography* 60: 1438-1440.**

Chlorination of natural products is often required for their biological activity; notable examples include vancomycin, the last-ditch antibiotic. It is now known that many chlorinated natural products are made not by haloperoxidases, but by FADH(2)-dependent halogenases. The mechanism of the flavin-containing enzymes is obscure and there are no structural data. Here, crystals of PrnA ( tryptophan 7-halogenase), an enzyme that regioselectively chlorinates tryptophan, cocrystallized with tryptophan and FAD are reported. The crystals belong to the tetragonal space group P4(3)2(1)2 or P4(1)2(1)2, with unit-cell parameters  $a = b = 67.8$ ,  $c = 276.9$  Angstrom. A data set to 1.8 Angstrom with 93% completeness and an R-merge of 7.1% has been collected from a single flash-cooled crystal. A method for incorporating selenomethionine in a *Pseudomonas fluorescens* expression system also is reported.

**Dries, J., L. Bastiaens, et al. (2004). "Competition for sorption and degradation of chlorinated ethenes in batch zero-valent iron systems." *Environmental Science & Technology* 38(10): 2879-2884.**

The sorption and degradation of the chlorinated ethenes tetrachloroethene (PCE, 5 mg L<sup>-1</sup>) and trichloroethene (TCE, 10 mg L<sup>-1</sup>) were investigated in zero-valent iron systems (ZVI, 100 g L<sup>-1</sup>) in the presence of compounds common to contaminated groundwater with varying physicochemical properties. The potential competitors were chlorinated ethenes, monocyclic aromatic hydrocarbons, and humic acids. The effect of a complex matrix was tested with landfill contaminated groundwater. Nonlinear Freundlich isotherms adequately described chloroethene sorption to ZVI. In the presence of the more hydrophobic PCE (5 mg L<sup>-1</sup>), TCE sorption and degradation decreased by 33% and 30%, respectively, while TCE (10 mg L<sup>-1</sup>) decreased PCE degradation by 30%. In the presence of nonreactive hydrophobic hydrocarbons (i.e., benzene, toluene, and m-xylene at 100 mg L<sup>-1</sup>), TCE and PCE sorption decreased by 73% and 55%, respectively. The presence of the hydrocarbons had no effect on TCE degradation and increased PCE reduction rates by 50%, suggesting that the displacement of the chloroethenes from the sorption sites by the aromatic hydrocarbons enhanced the degradation rates. Humic acids did not interfere significantly with chloroethene sorption or with TCE degradation but lowered PCE degradation kinetics by 36% when present at high concentrations (100 mg L<sup>-1</sup>). The landfill groundwater with an organic carbon content of 109 mg L<sup>-1</sup> C had no effect on chloroethene sorption but inhibited TCE and PCE degradation by 60% and 70%, respectively.

**Endo, T., M. Tsuda, et al. (2004). "Nagelamides A-H, New Dimeric Bromopyrrole Alkaloids from Marine Sponge *Agelas* Species." *Journal of Natural Products* 67(8): 1262 -1267.**

Eight new dimeric bromopyrrole alkaloids, nagelamides A-H (1-8) and a monomeric one, 9,10-dihydrokeramadine (9), have been isolated from the Okinawan marine sponge *Agelas* sp., and the structures were elucidated from spectroscopic data. Nagelamides A-H (1-8) exhibited antibacterial activity against Gram-positive bacteria. Nagelamide G (7) inhibited protein phosphatase 2A activity.

**Ferraroni, M., I. P. Solyanikova, et al. (2004). "Crystal structure of 4-chlorocatechol 1,2-dioxygenase from the chlorophenol-utilizing gram-positive *Rhodococcus opacus* 1CP." *Journal of Biological Chemistry* 279(26): 27646-27655.**

The crystal structure of the 4-chlorocatechol 1,2-dioxygenase from the Gram-positive bacterium *Rhodococcus opacus* (erythropolis) 1CP, a Fe(III) ion-containing enzyme involved in the aerobic biodegradation of chloroaromatic compounds, has been solved by multiple wavelength anomalous dispersion using the weak anomalous signal of the two catalytic irons (1 Fe/257 amino acids) and refined at a 2.5 Angstrom resolution (R-free 28.7%; R factor 21.4%). The analysis of the structure and its comparison with the structure of catechol 1,2-dioxygenase from *Acinetobacter calcoaceticus* ADP1 (Ac 1,2-CTD) highlight significant differences between these enzymes. The general topology of the present enzyme comprises two catalytic domains (one for each subunit) related by a noncrystallographic 2-fold axis and separated by a common alpha-helical zipper motif consisting of five N-terminal helices from each subunit; furthermore the C-terminal tail is shortened significantly with respect to the known Ac 1,2-CTD. The presence of two phospholipids binding in a hydrophobic tunnel along the dimer axis is shown here to be a common feature for this class of enzyme. The active site cavity presents several dissimilarities with respect to the known catechol-cleaving enzyme. The catalytic nonheme iron(III) ion is bound to the side chains of Tyr-134, Tyr-169, His-194, and His-196, and a cocrystallized benzoate ion, bound to the metal center, reveals details on a novel mode of binding of bidentate inhibitors and a distinctive hydrogen bond network with the surrounding ligands. Among the amino acid residues expected to interact with substrates, several are different from the corresponding analogs of Ac 1,2-CTD: Asp-52, Ala-53, Gly-76, Phe-78, and Cys-224; in addition, regions of largely conserved amino acid residues in the catalytic cleft show different shapes resulting from several substantial backbone and side chain shifts. The present structure is the first of intradiol dioxygenases that specifically catalyze the cleavage of chlorocatechols, key intermediates in the aerobic catabolism of toxic chloroaromatics.

**Forczek, S. T., H. Uhlirova, et al. (2004). "Trichloroacetic acid in Norway spruce/soil-system. II. Distribution and degradation in the plant." *Chemosphere* 56(4): 327-333.**

Independently from its origin, trichloroacetic acid (TCA) as a phytotoxic substance affects coniferous trees. Its uptake, distribution and degradation were thus investigated in the Norway spruce/soil-system using C-14 labeling. TCA is distributed in the tree mainly by the transpiration stream. As in soil, TCA seems to be degraded microbially, presumably by phyllosphere microorganisms in spruce needles. Indication of TCA biodegradation in trees is shown using both antibiotics and axenic plants. (C) 2004 Elsevier Ltd. All rights reserved.

**Fritsch, J. M., J. J. Klappa, et al. (2003). "Reductive dehalogenation of perchloroethylene and trichloroethylene with vitamin B-12 mimics." *Abstracts of Papers of the American Chemical Society* 225: U803-U803.**

**Griebler, C., L. Adrian, et al. (2004). "Stable carbon isotope fractionation during aerobic and anaerobic transformation of trichlorobenzene." *Fems Microbiology Ecology* 48(3): 313-321.**

Fractionation of stable carbon isotopes upon degradation of trichlorobenzenes was studied under aerobic and anaerobic conditions. Mineralization of 1,2,4-trichlorobenzene by the aerobic strain *Pseudomonas* sp. P51 which uses a dioxygenase for the initial enzymatic reaction was not accompanied by a significant isotope fractionation. In contrast, reductive dehalogenation by the anaerobic strain *Dehalococcoides* sp. strain CBDB1 revealed average isotope enrichment factors (epsilon) between -3.1 and -3.7 for 1,2,3- and 1,2,4-trichlorobenzene, respectively. The significant isotope fractionation during reductive dehalogenation would allow tracing the in situ biodegradation of halogenated benzenes in contaminated anoxic aquifers, whereas the lack of isotope fractionation during aerobic transformation limits the use of this approach in oxic environments. (C) 2004 Published by Elsevier B.V.

**Hata, J., N. Miyata, et al. (2004). "Anaerobic degradation of cis-1,2-dichloroethylene and vinyl chloride by Clostridium sp strain DC1 isolated from landfill leachate sediment." Journal of Bioscience and Bioengineering 97(3): 196-201.**

The bacterial community structure of anaerobic enrichment cultures that are capable of degrading both cis-1,2-dichloroethylene (cis-DCE) and vinyl chloride (VC) and isolation of the organism responsible for the degradation were investigated. Denaturing gradient gel electrophoresis (DGGE) of a PCR-amplified 16S rRNA gene from the cultures showed the possible predominance of Clostridium species. One isolate, designated strain DC1, was closely related to members of Clostridiaceae, based on 16S rRNA gene analysis, and the highest sequence similarity (98.9%) was obtained for Clostridium saccharobutylicum. In culture experiments, strain DC1 was shown to degrade cis-DCE and VC during the stationary phase of growth without accumulation of VC and/or ethene. The bacterial growth was not linked to the degradation of cis-DCE and VC. Stoichiometric analysis revealed that two moles of chloride ions as released from one mole of cis-DCE during the incubation period, indicating that cis-DCE was fully dechlorinated. The results appear consistent with the presence of a mechanism of oxidative dechlorination rather than respiratory reductive dechlorination; the latter is accompanied by transient formation of dechlorinated ethenes from cis-DCE and VC.

**Hogan, J., O. Sherlock, et al. (2004). "Fluorescence Resonance Energy Transfer (FRET) based molecular detection of a genetically modified PCB degrader in soil." Fems Microbiology Letters 236(2): 349-357.**

Genetic analysis of the location of a mini-Tn5 promoted insertion of the LB400 bph operon in the rhizosphere coloniser Pseudomonas fluorescens F113rifPCB, allowed the development of a specific PCR detection system based on the unique DNA sequence at this insertion site. Real time PCR using both SYBR green chemistry and Fluorescence Resonance Energy Transfer probes allowed the precise identification of the recombinant strain and its quantitative detection in soil microcosms over a (bacteria/g) range of five orders of magnitude. This new assay can detect the genetically modified microorganism from soil in less than 90 min and at levels below the detection limits of standard PCR or cultivable counts on selective media. (C) 2004 Federation of European Microbiological Societies.

**Im, W. T., H. S. Bae, et al. (2004). "Herbaspirillum chlorophenicum sp nov., a 4-chlorophenol-degrading bacterium." International Journal of Systematic and Evolutionary Microbiology 54: 851-855.**

A 4-chlorophenol-degrading bacterial strain, formerly designated as a strain of Comamonas testosteroni, was reclassified as a member of the genus Herbaspirillum based on its phenotypic and chemotaxonomic characteristics, as well as phylogenetic analysis using 16S rDNA sequences. Phylogenetic inference based on 16S rDNA sequences showed that strain CPW301(T) clusters in a phylogenetic branch that contains Herbaspirillum species. 16S rDNA sequence similarity of strain CPW301(T) to species of the genus Herbaspirillum with validly published names is in the range 98.7-98.9%. Despite the considerably high 16S rDNA sequence similarity, strain CPW301(T) could be distinguished clearly from type strains of Herbaspirillum species with validly published names by DNA-DNA relatedness values, which were < 15.7%. The genomic DNA G C content of strain CPW301(T) is 61.3 mol%. The predominant ubiquinone is Q-8 and the major cellular fatty acids are C-16:0 and Cyclo-C-17:0. The strain does not fix nitrogen and is not plant-associated. It is an aerobic rod with one unipolar flagellum. On the basis of these characteristics, a novel Herbaspirillum species, Herbaspirillum chlorophenicum sp. nov., is proposed. The type strain of the novel species is strain CPW301(T) (= KCTC 12096(T) = IAM 15024(T)).

**Iwakiri, R., K. Yoshihira, et al. (2004). "Total degradation of pentachloroethane by an engineered Alcaligenes strain expressing a modified camphor monooxygenase and a hybrid dioxygenase." Bioscience Biotechnology and Biochemistry 68(6): 1353-1356.**

We engineered biphenyl-degrading Alcaligenes sp. strain KF711 for total degradation of pentachloroethane (PCA), which expresses a modified camphor monooxygenase and a hybrid dioxygenase consisting of TodC1 (a large subunit of toluene dioxygenase of Pseudomonas putida F1) and BphA2-BphA3-pbhA4 (a small subunit, ferredoxin and ferredoxin reductase of biphenyl dioxygenase, respectively, in strain KF707). Modified camphor monooxygenase genes (camCAB) were supplied as a plasmid and the todC1 gene was integrated within the

chromosomal bph gene cluster by a single crossover recombination. The resultant strain KF711S-3cam dechlorinated PCA to trichloroethene by the action of the modified camphor monooxygenase under anaerobic conditions. The same strain subsequently degraded trichloroethene formed oxidatively by the action of the Tol-Bph hybrid dioxygenase under aerobic conditions. Thus sequential anaerobic and aerobic treatments of the KF711S-3cam resting cells resulted in efficient and total degradation of PCA.

**Jayaraj, J., K. J. Rockne, et al. (2004). "Reductive dechlorination of tetrachloroethene by a mixed bacterial culture growing on ethyl lactate." *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering* 39(6): 1399-1414.**

Chloroethenes like tetrachloroethene (PCE) are the most prevalent groundwater contaminants in the USA. Their presence as nonaqueous phase liquids (NAPLs) makes remediation difficult. Among options for NAPL cleanup, co-solvent injection has demonstrated success. However, the process has the potential to leave considerable residue of the co-solvent as well as residual chloroethene. Our rationale in this study was to examine whether this residual solvent could be a potential electron donor for the remediation of the residual chloroethene. We hypothesized that ethyl lactate, a "green" solvent, could serve both as a NAPL extraction solvent and an electron donor for reductive dechlorination of residual chloroethene. We examined whether a mixed culture known to degrade PCE with lactate could also grow on ethyl lactate and whether it could stimulate PCE dechlorination. Biomass growth and PCE dechlorination were observed by protein and chloride production, respectively, in the culture; with a specific dechlorination rate of 50-150 mug (mg cell d)<sup>-1</sup>. Ethyl lactate abiotically breaks down to ethanol and lactate, the latter being a rich source of hydrogen for reductive dechlorination. The results demonstrate that ethyl lactate may be promising for in situ bioremediation following NAPL extraction.

**Kantachote, D., R. Naidu, et al. (2004). "Bioremediation of DDT-contaminated soil: Enhancement by seaweed addition." *Journal of Chemical Technology and Biotechnology* 79(6): 632-638.**

DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] is a major environmental pollutant and economical methods to remove DDT from the environment are required. In this work we used seaweed (dried and ground) to enhance DDT transformation in waterlogged soils. Initial daily rates of DDT biodegradation increased in the following order relating to the percentage by weight of added seaweed to soil 0.5 > 1 > 3 > 5 > 13 (w/w). The actual percentages of DDT biodegradation occurring within 6 weeks were 80, 64, 60, 50, 40 and 34 respectively. During soil incubation DDD [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] was the major metabolite found with small amounts of DDE [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] produced. The maximum amount of 4,4'-dichlorobenzophenone (DBP) (2.5%) produced was found in soil amended with 0.5% (w/w) seaweed, indicating that further degradation of DDD occurred. High levels of dissolved organic carbon (DOC), between 309 and 509 mg kg<sup>-1</sup> soil, were present in soil amended with 3-13% (w/w) seaweed immediately after seaweed addition. It is possible that the high levels of DOC in soils amended with larger amounts of seaweed significantly retarded DDT biodegradation, possibly due to binding of DDT to DOC and subsequently decreasing the bioavailability of DDT to soil microbes. (C) 2004 Society of Chemical Industry.

**Katsoyiannis, A. and C. Samara (2004). "Persistent organic pollutants (POPs) in the sewage treatment plant of Thessaloniki, northern Greece: occurrence and removal." *Water Research* 38(11): 2685-2698.**

The occurrence and the removal of persistent organic pollutants (POPs) during the conventional activated sludge treatment process were investigated in the wastewater treatment plant of the city of Thessaloniki, northern Greece. POPs of interest were seven polychlorinated biphenyls (PCBs) and 19 organochlorine pesticides. Target compounds were determined at six different points across the treatment system. Most abundant compounds in raw wastewater at all treatment stages were PCB-52, PCB-110, PCB-180 and Heptachlor-exo-epoxide. Quintozene occurred frequently but in relatively low concentrations. Hexachlorocyclohexanes, DDT and its metabolites (DDE, DDD) and Aldrin, Dieldrin, Endrin, Isodrin ("Drins") were found at medium or low frequencies and in concentrations close to their detection limits. Removal percentages throughout the whole treatment process ranged from 65% to 91% for individual POP species. Significant linear relationship was observed between removal efficiency and log K<sub>ow</sub> for PCBs suggesting that compounds with a strong hydrophobic character are principally

removed through sorption to sludge particles and transfer to the sludge processing systems. Total PCBs' concentrations in sewage sludge ranged between 185 and 765 ng g(-1) dw being below the EU limit for use of sludge in agriculture. (C) 2004 Elsevier Ltd. All rights reserved.

**Keum, Y. S. and Q. X. Li (2004). "Fungal laccase-catalyzed degradation of hydroxy polychlorinated biphenyls." *Chemosphere* 56(1): 23-30.**

Hydroxy polychlorinated biphenyls (hydroxy PCBs) are toxic metabolites of PCBs. Their toxicity such as strong endocrine disruption demands effective remediation methods. Laccases from *Trametes versicolor* and *Pleurotus ostreatus* were tested to degrade hydroxy PCBs. Optimum pHs for both enzymes were around 4.0. Laccase from *T. versicolor* degrades hydroxy PCBs more rapidly than that from *P. ostreatus*. The enzymatic activities remained little changes in up to 10% organic solvents, but decreased rapidly in more than 10% acetone, acetonitrile or DMSO. Degradation rate constants decreased with increase of chlorination and no degradation was observed with tetra-, penta- and hexa-chloro hydroxy PCBs in non-mediated reactions. However, the tetra- to hexa-chloro hydroxy PCBs were degraded by laccase from *T. versicolor* in the presence of the mediator 2,2,6,6-tetramethylpiperidine-N-oxyl radical. The other mediators, 4-ethyl-2-methoxyphenol, 2,2'-azino-bis(3-ethylbenzthiazoline sulfonic acid) diammonium salt and 1-hydroxybenzotriazole and humic acid, also enhanced degradation of all the hydroxy PCBs except 4-hydroxy-2',3,3',4',5,5'-hexachlorobiphenyl. The results showed that 3-hydroxy biphenyl was more resistant to laccase degradation than 2- or 4-hydroxy analogues. Significant linear-correlations (coefficient of determination,  $r^2 = 0.9097$  and  $0.8186$  for laccases from *P. ostreatus* and *T. versicolor*, respectively) were found between the ionization potentials and the removal rate constants of hydroxy PCBs. (C) 2004 Elsevier Ltd. All rights reserved.

**Khromonygina, V. V., A. I. Saltykova, et al. (2004). "Degradation of the herbicide atrazine by the soil mycelial fungus INBI 2-26(-), a producer of cellobiose dehydrogenase." *Applied Biochemistry and Microbiology* 40(3): 285-290.**

Nonsporulating mycelial fungi producing cellobiose dehydrogenase (CDH) and isolated from soils of South Vietnam with a high residual content of dioxins are capable of growing on a solid medium in the presence of high atrazine concentrations (to 500 mg/l). At 20 and 50 mg/l atrazine, the area of fungal colonies was 1.5-1.2-fold larger, respectively, than the control colonies of the same age, whereas development of the colonies at 500 mg/l atrazine was delayed by 5 days, compared with controls grown in the absence of atrazine. Surface cultivation of the fungus on a minimal medium with glucose as a sole source of carbon and energy decreased the initial concentration of atrazine (20 mg/l) 50 times in 40 days; in addition, no pronounced sorption of atrazine by mycelium was detected. This was paralleled by an accumulation in the culture medium of extracellular CDH; atrazine increased the synthesis of this enzyme two- to threefold. Accumulation of beta-glucosidase (a mycelium-associated enzyme) and cellulases preceded the formation of CDH.

**Kim, Y. M., K. Park, et al. (2004). "Glutathione-dependent biotransformation of the fungicide chlorothalonil." *Journal of Agricultural and Food Chemistry* 52(13): 4192-4196.**

A gene responsible for the chlorothalonil biotransformation was cloned from the chromosomal DNA of *Ochrobactrum anthropi* SH35B, capable of efficiently dissipating the chlorothalonil. The gene encoding glutathione S-transferase (GST) of *O. anthropi* SH35B was expressed in *Escherichia coli*, and the GST was subsequently purified by affinity chromatography. The fungicide chlorothalonil was rapidly transformed by the GST in the presence of glutathione. LC-MS analysis supported the formation of mono-, di-, and triglutathione conjugates of chlorothalonil by the GST. The monoglutathione conjugate was observed as an intermediate in the enzymatic reaction. The triglutathione conjugate has not been previously reported and seems to be the final metabolite in the biotransformation of chlorothalonil. The glutathione-dependent biotransformation of chlorothalonil catalyzed by the bacterial GST is reported.

**Lee, G., S. Rho, et al. (2004). "Design considerations for groundwater remediation using reduced metals." Korean Journal of Chemical Engineering 21(3): 621-628.**

Use of reduced metals has attracted much attention since it possesses a great potential for eliminating reducible contaminants in groundwater such as heavy metals and chlorinated compounds. However, products of metal-mediated reactions for many chlorinated hydrocarbons have not clearly been identified. In addition, consumption of the metals, generation and release of metal ions, formation of insoluble metal oxides and hydroxides on the clean metal surface, and rise of pH inevitably accompany the reactions. Due to these properties of metal-mediated reactions, the reaction rate could decrease as the reaction proceeds, and effluent quality could decay. It was shown in this study using chlorine mass balance and GC analysis that chloroform is formed from carbon tetrachloride by reduced iron. It is also well-known that nitrate is reduced mostly to ammonia by metals, which indicates that the metal process is inappropriate for denitrification of nitrate-contaminated aquifers. These results indicate that groundwater remediation using metal process requires careful consideration for the safety of reaction products. It was also shown that mixing rate strongly affects reaction rate since metal-mediated reaction occurs on the surface of metals. In addition, reaction rate was decreased due to metal hydroxide deposition on the surface of metal granules that was seen by scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis. Generation of iron ions (consumption of reduced iron) released from reduction of zero-valent iron was also shown by using an ion chromatograph (IC). In this study, some methods were suggested to solve the above-mentioned problems. Acid washing appeared effective for removing corrosion products on the surface of metal granules, by which a reduction rate could be maintained high for an extended time of reaction. Use of iron sulfide decreased an extent of pH rise during metal-mediated reaction; thereby precipitation of insoluble metal (hydr)oxides is expectedly decreased. It was also shown that inexpensive iron scrap instead of fine metal powders can be used for metal processes.

**Lenczewski, M. E., L. D. McKay, et al. (2004). "Anaerobic biodegradation of TCE in laboratory columns of fractured saprolite." Ground Water 42(4): 534-541.**

An experiment was conducted to determine if biodegradation of trichloroethylene (TCE) can occur in previously uncontaminated ground water in saturated fractured saprolite (highly weathered material derived from sedimentary rocks). Two undisturbed columns (0.23 m diameter by 0.25 m long) of fractured saprolite were collected from -2 m depth at an uncontaminated site on the Oak Ridge Reservation, Oak Ridge, Tennessee. Natural, uncontaminated ground water from the site, which was degassed and spiked with dissolved phase TCE, was continuously pumped through one column containing the natural microbial communities (the biotic column). In a second column, the microorganisms were inhibited and the dissolved phase TCE was added under aerobic conditions (dissolved oxygen conditions > 2 ppm). In effluent from the biotic column, reducing conditions rapidly developed and evidence of anaerobic biodegradation of TCE, by the production of cDCE, first appeared similar to 31 days after addition of TCE. Reductive dechlorination of TCE occurred after iron-reducing conditions were established and about the same time that sulfate reduction began. There was no evidence of methanogenesis. Analyses using polymerase chain reaction with specific primers sets detected the bacteria *Geothrix*, *Geobacter*, and *Desulfococcus-Desulfonema-Desulfosarcina* in the effluent of the biotic column, but no methanogens. The presence of these bacteria is consistent with iron- and sulfate-reducing conditions. In the inhibited column, there were no indicators of TCE degradation. Natural organic matter that occurs in the saprolite and ground water at the site is the most likely primary electron donor for supporting reductive dechlorination of TCE. The relatively rapid appearance of indicators of TCE dechlorination suggests that these processes may occur even in settings where low oxygen conditions occur seasonally due to changes in the water table.

**Luijten, M., W. Roelofsen, et al. (2004). "Hydrogen threshold concentrations in pure cultures of halo-respiring bacteria and at a site polluted with chlorinated ethenes." Environmental Microbiology 6(6): 646-650.**

Halo-respiring microorganisms are not only able to oxidize organic electron donors such as formate, acetate, pyruvate and lactate, but also H<sub>2</sub>. Because these microorganisms have a high affinity for H<sub>2</sub>, this may be the most important electron donor for halo-respiration in the environment. We have studied the role of H<sub>2</sub>-threshold concentrations in pure halo-respiring cultures and compared them with mixed cultures and field data. We have found H<sub>2</sub>-threshold values between 0.05 and 0.08 nM for *Sulfurospirillum* halo-respirans, *S. multivorans* and *Dehalobacter*

restrictus under PCE-reducing and nitrate-reducing conditions. The reduction of PCE and TCE can proceed at H-2 concentrations of below 1 nM at a polluted site. However, for the reduction of lower chlorinated ethenes a higher H-2 concentration is required. This indicates that the measured H-2 concentration in situ can be an indicator of the extent of anaerobic reductive dechlorination.

**Ma, Q. L., A. Rahman, et al. (2004). "Field dissipation of acetochlor in two New Zealand soils at two application rates." *Journal of Environmental Quality* 33(3): 930-938.**

The persistence of pesticides in soils has both economic and environmental significance and is often used as a key parameter in pesticide risk assessment. Persistence of acetochlor [2'-ethyl-6'-methyl-N-(ethoxymethyl)-2-chloroacetylanilide] in two New Zealand field soils was measured over two years and the data were used to identify models that adequately describe acetochlor persistence in the field. Acetochlor was sprayed onto six fallow plots (3 X 9 m each) at each site at the recommended rate (2.5 kg a.i. ha<sup>-1</sup>) and at twice that rate. Acetochlor concentrations were measured in soil cores. Simple first-order kinetics (Model 1) adequately described acetochlor persistence in Hamilton clay loam soil (Humic Hapludoll, Illuvial Spadic) at the high application rate, but overestimated it at the low application rate. A quadratic model (Model 2), a first-order double-exponential model (Model 3), a first-order biphasic model (Model 4), or a two-compartment model (Model 5) better described acetochlor persistence at the low application rate. The time for 50% (DT50) and 90% (DT90) of initial acetochlor loss was approximately 9 and 56 d, and 18 and 63 d at low and high application rates, respectively. The more complex Models 2 through 5 also better described the biphasic dissipation of acetochlor in Horotiu sandy loam soil (Typic Orthic Allophanic) than Model 1, with Model 1 significantly underestimating acetochlor concentrations on the day of application at both application rates. The DT50 and DT90 values were 5 and 29 d and 7 and 31 d at low and high application rates, respectively. Overall, application rate significantly affected the DT50 and DT90 values in the Hamilton soil, but not in the Horotiu soil. Faster acetochlor loss in the Horotiu soil possibly resulted from the higher soil organic carbon content that retained more acetochlor near the soil surface where higher temperature and photolysis accelerated the loss.

**Matucha, M., M. Gryndler, et al. (2004). "Microbiological aspects of determination of trichloroacetic acid in soil." *Folia Microbiologica* 49(2): 117-122.**

Soils have been shown to possess a strong microbial trichloroacetic acid (TCA)-degrading activity. High TCA-degradation rate was also observed during soil extraction with water. For correct measurements of TCA levels in soil all TCA-degrading activities have to be inhibited immediately after sampling before analysis. We used rapid freezing of soil samples (optimally in liquid nitrogen) with subsequent storage and slow thawing before analysis as an efficient technique for suppressing the degradation. Frozen soil samples stored overnight at -20 degreesC and then thawed slowly exhibited very low residual TCA-degrading activity for several hours. Omitting the above procedure could lead to the confusing differences between the TCA levels previously reported in the literature.

**Michizoe, J., Y. Uchimura, et al. (2004). "Activation of manganese peroxidase in an organic medium using a mediator." *Biochemical Engineering Journal* 19(1): 43-46.**

We found that both unsaturated fatty acids (UFAs) and 1-hydroxybenzotriazole (HBT) enhance the enzymatic activity of manganese peroxidase in an organic medium. The effects of hydrophobic unsaturated fatty acids directly dissolved in an organic medium and hydrophilic HBT encapsulated in reverse micelles on the oxidation activity of a surfactant-manganese peroxidase (MnP) complex were investigated. The addition of UFAs or HBT (mediator) using reverse micelles improved the oxidation of 2,4-dichlorophenol in toluene up to 3-fold the oxidation without each mediator. This study presents, for the first time, the possibility of MnP-catalyzed oxidation coupled with mediators in organic media. The capability of the latter system using HBT was further assessed by the oxidative conversion of environmental pollutants, i.e. 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and bisphenol A (100, 84, 91, and 100% conversions at 12 h of reaction, respectively). (C) 2003 Elsevier B.V. All rights reserved.

**Miller, L. G., K. L. Warner, et al. (2004). "Degradation of methyl bromide and methyl chloride in soil microcosms: Use of stable C isotope fractionation and stable isotope probing to identify reactions and the responsible microorganisms." *Geochimica Et Cosmochimica Acta* 68(15): 3271-3283.**

Bacteria in soil microcosm experiments oxidized elevated levels of methyl chloride (MeCl) and methyl bromide (MeBr), the former compound more rapidly than the latter. MeBr was also removed by chemical reactions while MeCl was not. Chemical degradation dominated the early removal of MeBr and accounted for more than half of its total loss. Fractionation of stable carbon isotopes during chemical degradation of MeBr resulted in a kinetic isotope effect (KIE) of 59 ‰ parts per thousand. Soil bacterial oxidation dominated the later removal of MeBr and MeCl and was characterized by different KIEs for each compound. The KIE for MeBr oxidation was 69 ‰ parts per thousand and the KIE for MeCl oxidation was 49 ‰ parts per thousand. Stable isotope probing revealed that different populations of soil bacteria assimilated added C-13-labeled MeBr and MeCl. The identity of the active MeBr and MeCl degrading bacteria in soil was determined by analysis of 16S rRNA gene sequences amplified from C-13-DNA fractions, which identified a number of sequences from organisms not previously thought to be involved in methyl halide degradation. These included Burkholderia, the major clone type in the C-13-MeBr fraction, and Rhodobacter, Lysobacter and Nocardioideis the major clone types in the C-13-MeCl fraction. None of the 16S rRNA gene sequences for methyl halide oxidizing bacteria currently in culture (including Aminobacter strain IMB-1 isolated from fumigated soil) were identified. Functional gene clone types closely related to Aminobacter spp. were identified in libraries containing the sequences for the *cmuA* gene, which codes for the enzyme known to catalyze the initial step in the oxidation of MeBr and MeCl. The *cmuA* gene was limited to members of the alpha-Proteobacteria whereas the greater diversity demonstrated by the 16S rRNA gene may indicate that other enzymes catalyze methyl halide oxidation in different groups of bacteria. Copyright (C) 2004 Elsevier Ltd.

**Moreno, G. and G. Buitron (2004). "Influence of the origin of the inoculum and the acclimation strategy on the degradation of 4-chlorophenol." *Bioresource Technology* 94(2): 215-218.**

The influence of the inoculum source and the acclimation strategy on the 4-chlorophenol (4CP) degradation in a sequencing batch reactor (SBR) was studied. Three different sources of inocula were obtained from the aeration tank of domestic, municipal and industrial wastewater treatment plants. The acclimation was performed using two strategies, the first one fixing the reaction time, independent of the removal efficiency (fixed time) and the second one fixing a removal efficiency of 90% as 4CP (variable time). The degradative activity was followed for each condition. Bacterial identification was carried out at the beginning and at the end of the experiments. Variable time strategy produced a microbial community with higher specific activity compared with those obtained for the fixed time strategy. The microbial activity was dependent of the origin of the inoculum. Each inoculum presented different specific activity to 4CP degradation. It was observed that the use of the fixed time strategy for the acclimation reduced the bacterial community diversity. (C) 2004 Elsevier Ltd. All rights reserved.

**Morono, Y., H. Unno, et al. (2004). "Addition of aromatic substrates restores trichloroethylene degradation activity in *Pseudomonas putida* F1." *Applied and Environmental Microbiology* 70(5): 2830-2835.**

The rate of trichloroethylene (TCE) degradation by toluene dioxygenase (TDO) in resting cells of *Pseudomonas putida* F1 gradually decreased and eventually stopped within 1.5 h, as in previous reports. However, the subsequent addition of toluene, which is the principal substrate of TDO, resulted in its immediate degradation without a lag phase. After the consumption of toluene, degradation of TCE restarted at a rate similar to its initial degradation, suggesting that this degradation was mediated by TDO molecules that were present before the cessation of TCE degradation. The addition of benzene and cumene, which are also substrates of TDO, also caused restoration of TCE degradation activity: TCE was degraded simultaneously with cumene, and a larger amount of TCE was degraded after cumene was added than after toluene or benzene was added. But substrates that were expected to supply the cells with NADH or energy did not restore TCE degradation activity. This cycle of pseudoinactivation and restoration of TCE degradation was observed repeatedly without a significant decrease in the number of viable cells, even after six additions of toluene spread over 30 h. The results obtained in this study demonstrate a new type of restoration of TCE degradation that has not been previously reported.

**Nakamura, T., T. Motoyama, et al. (2004). "Identification, characterization, and site-directed mutagenesis of recombinant pentachlorophenol 4-monoxygenase." *Biochimica Et Biophysica Acta-Proteins and Proteomics* 1700(2): 151-159.**

In a previous study, we constructed a three-dimensional (3D) structure of pentachlorophenol 4-monoxygenase (PcpB). In this study, further analyses are performed to examine the important amino acid residues in the catalytic reaction by identification of the proteins with mass spectrometry, circular dichroism (CD) and UV spectrometry, and determination of kinetic parameters. Recombinant histidine-tagged PcpB protein was produced and shown to have a similar activity to the native protein. Mutant proteins of PcpB were then produced (F85A, Y216A, Y216F, R235A, R235E, R235K, Y397A and Y397F) on the basis of the proposed 3D structure. The CD spectra of the proteins showed that there were no major changes in the structures of the mutant proteins, with the exception of R235E. Steady-state kinetics showed a 20-fold reduction in  $k(\text{cat})/K_m$  and a ninefold increase in  $K_m$  for Y216F and a threefold reduction in  $k(\text{cat})/k(m)$  and a sixfold increase in  $K_m$  for Y397F compared to the wild type. On the other hand, the value of  $k(\text{cat})/K_m$  of R235K mutant was the same as that of wild type. As a result, it was confirmed that Y216 and Y397 play an important role with respect to the recognition of the substrate. (C) 2004 Elsevier B.V All rights reserved.

**Narde, G. K., A. Kapley, et al. (2004). "Isolation and characterization of *Citrobacter* strain HPC255 for broad-range substrate specificity for chlorophenols." *Current Microbiology* 48(6): 419-423.**

This study aims at development of an approach for selection of strain, which has capability for oxidation of broad-range of chloro-substitute phenols. A multiplex PCR was optimized targeting loci involved in phenol and chlorophenol degradation, which was used to select activated sludge samples and also to assess the degradative genotype of isolates. The isolated strains were screened on the basis of RAPD analysis. In parallel, physiological experiments were carried out with activated sludge samples and isolated bacteria by respirometric analysis. Based on cluster analysis of RAPD pattern and respirometric data, the isolate G20 was selected and identified by using 16S rDNA sequence analysis as *Citrobacter freundii* strain HPC255. The strain could oxidize different substituted chlorophenol molecules. Such strains could provide the pool of intermediates, which can further be degraded by the associated population, thus helping in maintaining the synergistic association of catabolic activity in activated sludge.

**Newby, D. T., D. W. Reed, et al. (2004). "Diversity of methanotroph communities in a basalt aquifer." *Fems Microbiology Ecology* 48(3): 333-344.**

Methanotrophic bacteria play an important role in global cycling of carbon and co-metabolism of contaminants. Methanotrophs from pristine regions of the Snake River Plain Aquifer (SRPA; Idaho, USA) were studied in order to gain insight into the native, groundwater communities genetic potential to carry out TCE co-metabolism. Wells were selected that were proximal to a TCE plume believed to be undergoing natural attenuation. Methane concentrations ranged from 1 to >1000 nM. Carbon isotope ratios and diversity data together suggest that the SRPA contains active communities of methanotrophs that oxidize microbially produced methane. Microorganisms removed from groundwater by filtration were used as inocula for enrichments or frozen immediately and DNA was subsequently extracted for molecular characterization. Primers that specifically target methanotroph 16S rRNA genes or genes that code for subunits of soluble or particulate methane monoxygenase, *mmoX* and *pmoA*, respectively, were used to characterize the indigenous methanotrophs via PCR, cloning, RFLP analysis, and sequencing. Type I methanotroph clones aligned with *Methylomonas*, *Methylocaldum*, and *Methylobacter* sequences and a distinct 16S rRNA phylogenetic lineage grouped near *Methylobacter*. The majority of clone sequences in type 11 methanotroph 16S rRNA, *pmoA*, and *mmoX* gene libraries grouped closely with sequences in the *Methylocystis* genus. A subset of the type 11 methanotroph clones from the aquifer had sequences that aligned most closely to *Methylosinus trichosporium* OB3b and *Methylocystis* spp., known TCE-co-metabolizing methanotrophs. (C) 2004 Federation of European Microbiological Societies.

**Nobre, R. C. M. and M. M. M. Nobre (2004). "Natural attenuation of chlorinated organics in a shallow sand aquifer." *Journal of Hazardous Materials* 110(1-3): 129-137.**

This work presents the second phase of a groundwater remediation program for the migration control of a 1,2-dichloroethane (1,2-DCA) contaminated plume which includes natural attenuation at a distance downgradient from the source area. The conceived system for the plume migration control, implemented just after a major accidental release of 1,2-DCA in the soil, included a 300 m long physical barrier (cement-bentonite diaphragm wall) and 12 extraction wells. Results of field investigations have provided evidence that 1,2-DCA was naturally biodegrading into vinyl chloride as well as ethene under the natural anaerobic-reducing conditions at the site. In that case, source control measures were implemented to accelerate the overall remediation process. Although the results are favorable, the natural degradation of the 1,2-DCA does not guarantee acceptable levels of concentrations. Therefore, a pilot test to evaluate the enhancement of these processes is being carried out through the use of a biosparging system. This test is being implemented near the source to achieve sequential aerobic-anaerobic treatment zones. (C) 2004 Elsevier B.V. All rights reserved.

**Nyman, M. C., J. Harden, et al. (2004). "Biodegradation of 3,3'-dichlorobenzidine in freshwater lake sediments." *Journal of Environmental Engineering and Science* 3(2): 89-95.**

3,3'-Dichlorobenzidine (DCB) and its degradation products, 3-chlorobenzidine (MCB) and benzidine, are of environmental concern due to their toxic and carcinogenic nature. Laboratory experiments have been conducted to elucidate the biodegradation behavior of DCB in Lake Macatawa (Holland, Michigan) sediment-water systems at 4, 24, and 30 degreesC incubation temperatures. Sediment samples varied in pH (6.45-7.41), total organic carbon (OC) content (1.4-17.1), and particle size distribution (silty-clay to sandy). Biodegradation of DCB was observed in non-autoclaved samples that were incubated at 4, 24, and 30 degreesC for approximately 12 months. The dechlorination rate as a function of OC content was essentially found constant at 4 degreesC and 24 degreesC, but demonstrated a roughly linear increase at 30 degreesC, thereby suggesting that OC content might be correlated to dechlorination rate at 30 degreesC incubation temperature. The rate as a function of particle size (e.g., <74 µm) did not demonstrate a clearly defined relationship.

**Pallud, C., A. Dechesne, et al. (2004). "Modification of spatial distribution of 2,4-dichloro-phenoxyacetic acid degrader microhabitats during growth in soil columns." *Applied and Environmental Microbiology* 70(5): 2709-2716.**

Bacterial processes in soil, including biodegradation, require contact between bacteria and substrates. Knowledge of the three-dimensional spatial distribution of bacteria at the microscale is necessary to understand and predict such processes. Using a soil microsampling strategy combined with a mathematical spatial analysis, we studied the spatial distribution of 2,4-dichlorophenoxyacetic acid (2,4-D) degrader microhabitats as a function of 2,4-D degrader abundance. Soil columns that allowed natural flow were percolated with 2,4-D to increase the 2,4-D degrader abundance. Hundreds of soil microsamples (minimum diameter, 125 µm) were collected and transferred to culture medium to check for the presence of 2,4-D degraders. Spatial distributions of bacterial microhabitats were characterized by determining the average size of colonized soil patches and the average number of patches per gram of soil. The spatial distribution of 2,4-D degrader microhabitats was not affected by water flow, but there was an overall increase in colonized patch sizes after 2,4-D amendment; colonized microsamples were dispersed in the soil at low 2,4-D degrader densities and clustered in patches that were more than 0.5 mm in diameter at higher densities. During growth, spreading of 2,4-D degraders within the soil and an increase in 2,4-D degradation were observed. We hypothesized that spreading of the bacteria increased the probability of encounters with 2,4-D and resulted in better interception of the degradable substrate. This work showed that characterization of bacterial microscale spatial distribution is relevant to microbial ecology studies. It improved quantitative bacterial microhabitat description and suggested that sporadic movement of cells occurs. Furthermore, it offered perspectives for linking microbial function to the soil physicochemical environment.

**Park, D. W., K. Lee, et al. (2004). "Genetic structure of xyl gene cluster responsible for complete degradation of (4-chloro)benzoate from Pseudomonas sp S-47." *Journal of Microbiology and Biotechnology* 14(3): 483-489.**

*Pseudomonas* sp. S-47 is a bacterium capable of degrading benzoate as well as 4-chlorobenzoate (4CBA). Benzoate and 4CBA are known to be degraded via a meta-cleavage pathway characterized by a series of enzymes encoded by xyl genes. The meta-cleavage pathway operon in *Pseudomonas* sp. S-47 encodes a set of enzymes which transform benzoate and 4CBA into TCA cycle intermediates via the meta-cleavage of (4-chloro)catechol to produce C pyruvate and acetyl-CoA. In the current Study, the meta-pathway gene cluster was cloned from the chromosomal DNA of S-47 strain to obtain pCS1, which included the degradation activities for 4CBA and catechol. The genetic organization of the operon was then examined by cloning the meta-pathway genes into a pBluescript SKII(+) vector. As such, the meta-pathway operon from *Pseudomonas* sp. S-47 was found to contain 13 genes in the order of xylXYZLTEGFJQKIH. The two regulatory genes, xyls and xylR, that control the expression of the meta-pathway operon, were located adjacently downstream of the meta-pathway operon. The xyl genes from strain S-47 exhibited a high nucleoside sequence homology to those from *Pseudomonas putida* mt-2, except for the xylJQK genes, which were more homologous to the corresponding three genes from *P. stutzeri* AN10. One open reading frame was found between the xylH and xylS genes, which may play a role of a transposase. Accordingly, the current results suggest that the xyl gene cluster in *Pseudomonas* sp. S-47 responsible for the complete degradation of benzoate was recombined with the corresponding genes from *P. putida* mt-2 and *P. stutzeri* AN10.

**Phillips, T. M., H. Lee, et al. (2004). "Mineralization of hexachlorocyclohexane in soil during solid-phase bioremediation." *Journal of Industrial Microbiology & Biotechnology* 31(5): 216-222.**

with C-14-gamma-HCH and then subjected to bioremediation in bench-scale microcosms to determine the rate and extent of mineralization of the C-14-labeled HCH to (CO<sub>2</sub>)-C-14. The soil was treated using two different DARAMEND amendments, D6386 and D6390. The amendments were previously found to enhance natural HCH bioremediation as determined by measuring the disappearance of parent compounds under either strictly oxic conditions (D6386), or cycled anoxic/oxic conditions (D6390). Within 80 days of the initiation of treatment, mineralization was observed in all of the strictly oxic microcosms. However, mineralization was negligible in the cycled anoxic/oxic microcosms throughout the 275-day study, even after cycling was ceased at 84 days and although significant removal (up to 51%) of indigenous gamma-HCH (146 mg/kg) was detected by GC with electron capture detector. Of the amended, strictly oxic treatments, only one, in which 47% of the spiked C-14-HCH was recovered as (CO<sub>2</sub>)-C-14, enhanced mineralization compared with an unamended treatment (in which 34% recovery was measured). Other oxic treatments involving higher amendment application rates or auxiliary carbon sources were inhibitory to mineralization. Thus, although HCH degradation occurs during the application of either oxic or cycled anoxic/oxic DARAMEND treatments, mineralization of gamma-HCH may be inhibited depending on the amendment and treatment protocol.

**Picton, P. and A. Farenhorst (2004). "Factors influencing 2,4-D sorption and mineralization in soil." *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes* 39(3): 367-379.**

This study quantified 2,4-D [(2,4-dichlorophenoxy)acetic acid] sorption and mineralization rates in five soils as influenced by soil characteristics and nutrient contents. Results indicated that 2,4-D was weakly sorbed by soil, with Freundlich distribution coefficients ranging from 0.81 to 2.89  $\mu\text{g}(1-1/n) \text{ g}(-1) \text{ mL}(1/n)$ . First-order mineralization rate constants varied from 0.03 to 0.26, corresponding to calculated mineralization half-lives of 3 and 22 days, respectively. Herbicide sorption generally increased with increasing soil organic carbon content, but the extent of 2,4-D sorption per unit organic carbon varied among the soils due to differences in soil pH, clay content and/or organic matter quality. Herbicide mineralization rates were greater in soils that sorbed more 2,4-D per unit organic carbon, and that had greater soil nitrogen contents. We conclude that the effect of sorption on herbicide degradation cannot be generalized without a better understanding of the effects of soil characteristics and nutrient content on herbicide behavior in soil.

**Piel, J. (2004). "Metabolites from symbiotic bacteria." *Natural Product Reports* 512-538(4).**

Covering: the literature through 2003. This review describes natural products that are shown or suspected to be synthesized by symbiotic bacteria. It includes 349 references and covers the literature in this field through 2003.

**Pieper, D. H., V. dos Santos, et al. (2004). "Genomic and mechanistic insights into the biodegradation of organic pollutants." *Current Opinion in Biotechnology* 15(3): 215-224.**

Several new methodologies have enabled recent studies on the microbial biodegradation mechanisms of organic pollutants. Culture-independent techniques for analysis of the genetic and metabolic potential of natural and model microbial communities that degrade organic pollutants have identified new metabolic pathways and enzymes for aerobic and anaerobic degradation. Furthermore, structural studies of the enzymes involved have revealed the specificities and activities of key catabolic enzymes, such as dioxygenases. Genome sequencing of several biodegradation-relevant microorganisms have provided the first whole-genome insights into the genetic background of the metabolic capability and biodegradation versatility of these organisms. Systems biology approaches are still in their infancy, but are becoming increasingly helpful to unravel, predict and quantify metabolic abilities within particular organisms or microbial consortia.

**Quan, X. C., H. C. Shi, et al. (2004). "Removal of 2,4-dichlorophenol in a conventional activated sludge system through bioaugmentation." *Process Biochemistry* 39(11): 1701-1707.**

For the removal of toxic and recalcitrant organic substances intermittently appearing in wastewater, bioaugmentation with bacteria having specific degradation ability could be a powerful tool to improve the treatment process. 2,4-Dichlorophenol (2,4-DCP) was chosen as the target recalcitrant substance and a 2,4-DCP degrading special mixed culture was used as bioaugmentation microorganisms. The feasibility and strategies to combine bioaugmentation into a conventional activated sludge (CAS) system in terms of enhancing its efficiency and reliability was investigated. Results showed that for domestic wastewater with multiple chlorophenols, bioaugmentation with a 2,4-DCP degrading culture in a CAS system not only enhanced the removal of 2,4-DCP effectively, but also improved the removal of other chlorophenols such as 4-monochlorophenol (4-MCP) and 2,4,5-trichlorophenol (2,4,5-TCP). A separate bioaugmented bioreactor was combined into the original CAS system at different locations and the effects of the bioaugmentation location on the performance of the combined biotreatment process were studied. Results indicated that the CAS-Bioaug system, in which the bioaugmented bioreactor was set at a location after the original CAS reactor, performed better than the Bioaug-CAS system, in which the bioaugmented bioreactor was placed before the original CAS reactor. Bioaugmentation could be used as an effective and efficient method to improve a CAS process facing sudden toxic pollutant shock loading. (C) 2003 Elsevier Ltd.

**Rice, P. J., T. A. Anderson, et al. (2004). "Effect of sediment on the fate of metolachlor and atrazine in surface water." *Environmental Toxicology and Chemistry* 23(5): 1145-1155.**

In aquatic environments, pesticides can partition between the dissolved phase and particulate phase depending on the type of suspended sediment present and the physical and chemical properties of the pesticides and water. Particulate matter and sediment can alter the bioavailability of contaminants to organisms and therefore influence their toxicity and availability for microbial degradation. Experiments were conducted to determine the degradation of atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) and metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(methoxyprop-2-yl)acetamide) in surface water, and to evaluate the contribution of sediment to their dissipation. Sediment significantly reduced concentrations of atrazine and metolachlor in the Surface water as a result of greater degradation, evident by increased quantities of degradates in file Surface water, and the partitioning of the herbicide or herbicide degradates in the sediment. First-order 50% dissipation time (DT50) Values for atrazine and metolachlor were 42 and 8 d in the surface water-sediment incubation systems, which were almost four times less than the DT50s Calculated for the sediment-free systems. The results of this research illustrate the importance of sediment in the fate of pesticides ill surface water. Greater comprehension of

the role of sediment to sequester or influence degradation of agrichemicals in aquatic systems will provide a better understanding of the bioavailability and potential toxicity of these contaminants to aquatic organisms.

**Ritalahti, K. M. and F. E. Loffler (2004). "Populations implicated in anaerobic reductive dechlorination of 1,2-dichloropropane in highly enriched bacterial communities." *Applied and Environmental Microbiology* 70(7): 4088-4095.**

1,2-Dichloropropane (1,2-D), a widespread groundwater contaminant, can be reductively dechlorinated to propene by anaerobic bacteria. To shed light on the populations involved in the detoxification process, a comprehensive 16S rRNA gene-based bacterial community analysis of two enrichment cultures derived from geographically distinct locations was performed. Analysis of terminal restriction fragments, amplicons obtained with dechlorinator-specific PCR primers, and enumeration with quantitative real-time PCR as well as screening clone libraries all implied that Dehalococcoides populations were involved in 1,2-D dechlorination in both enrichment cultures. Physiological traits (e.g., dechlorination in the presence of ampicillin and a requirement for hydrogen as the electron donor) supported the involvement of Dehalococcoides populations in the dechlorination process. These findings expand the spectrum of chloroorganic compounds used by Dehalococcoides species as growth-supporting electron acceptors. The combined molecular approach allowed a comparison between different 16S rRNA gene-based approaches for the detection of Dehalococcoides populations.

**Rui, L. Y., Y. M. Kwon, et al. (2004). "Saturation mutagenesis of toluene ortho-monooxygenase of Burkholderia cepacia G4 for enhanced 1-naphthol synthesis and chloroform degradation." *Applied and Environmental Microbiology* 70(6): 3246-3252.**

Directed evolution of toluene ortho-monooxygenase (TOM) of Burkholderia cepacia G4 previously created the hydroxylase alpha-subunit (TomA3) V106A variant (TOM-Green) with increased activity for both trichloroethylene degradation (twofold enhancement) and naphthalene oxidation (six-times-higher activity). In the present study, saturation mutagenesis was performed at position A106 with Escherichia coli TG1/pBS(Kan)TOMV106A to improve TOM activity for both chloroform degradation and naphthalene oxidation. Whole cells expressing the A106E variant had two times better naphthalene-to-1-naphthol activity than the wild-type cells (V-max of 9.3 versus 4.5 nmol (.) min<sup>-1</sup> (.) mg of protein<sup>-1</sup>) and unchanged K<sub>m</sub>, and the regiospecificity of the A106E variant was unchanged, with 98% 1-naphthol formed, as was confirmed with high-pressure liquid chromatography. The A106E variant degrades its natural substrate toluene 63% faster than wild-type TOM does (2.12 ± 0.07 versus 1.30 ± 0.06 nmol (.) min<sup>-1</sup> (.) mg of protein<sup>-1</sup> [mean ± standard deviation]) at 91 μM and has a substantial decrease in regiospecificity, since o-cresol (50%), m-cresol (25%), and p-cresol (25%) are formed, in contrast to the 98% o-cresol formed by wild-type TOM. The A106E variant also has an elevated expression level compared to that of wild-type TOM, as evidenced by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Another variant, the A106F variant, has 2.8-times-better chloroform degradation activity based on gas chromatography (V-max of 2.61 versus 0.95 nmol (.) min<sup>-1</sup> (.) mg of protein<sup>-1</sup>) and unchanged K<sub>m</sub> and chloride release (0.034 ± 0.002 versus 0.012 ± 0.001 nmol (.) min<sup>-1</sup> (.) mg of protein<sup>-1</sup>). The A106F variant also was expressed at levels similar to those of wild-type TOM and 62%-better toluene oxidation activity than wild-type TOM (2.11 ± 0.3 versus 1.30 ± 0.06 nmol (.) min<sup>-1</sup> (.) mg of protein<sup>-1</sup>). A shift in regiospecificity of toluene hydroxylation was also observed for the A106F variant, with o-cresol (28%), m-cresol (18%), and p-cresol (54%) being formed. Statistical analysis was used to estimate that 292 colonies must be screened for a 99% probability that all 64 codons were sampled during saturation mutagenesis.

**Seigneur, C., N. Adler, et al. (2004). "Steady-state and transient-state performance of a biotrickling filter treating chlorobenzene-containing waste gas." *Applied Microbiology and Biotechnology* 65(1): 33-37.**

Biotrickling filter (BTF) technology was applied for the treatment of waste gas containing a mixture of chlorobenzene and 1,2-dichlorobenzene. An adapted microbial community was immobilised on a structured packing material. The strategy followed was to reach high removal efficiencies at initially low mass loading rates followed by an increase of the latter. This procedure was successful and resulted in a short start-up period of only 2 weeks. A 3-month operation under steady-state conditions showed good performance, with >95% removal efficiency at a mass

loading rate of 1,800 g m<sup>-3</sup> day<sup>-1</sup>). Dimensionless concentration profiles showed that the chlorobenzenes were simultaneously degraded. Low dissolved organic carbon of 15 mg l<sup>-1</sup> and stoichiometric chloride concentrations in the trickling liquid indicated complete mineralisation of the pollutant. Transient-state experiments with five times higher mass loading rates caused a decrease in the removal efficiency that recovered rapidly once the mass loading rate returned to its original steady-state level. A progressive increase of the mass loading rate in a long-term performance experiment showed that the removal efficiency could be kept stable between 95 and 99% at loads of up to 5,200 g m<sup>-3</sup> day<sup>-1</sup> over several days. Above this mass loading rate, the elimination capacity did not increase any further. These results demonstrated that with a well-adapted inoculum and optimal operation parameters, a BTF system with excellent performance and stability that efficiently removes a mixture of chlorobenzene vapours from air can be obtained.

**Shoeib, N. A., M. C. Bibby, et al. (2004). "In-vitro Cytotoxic Activities of the Major Bromophenols of the Red Alga *Polysiphonia lanosa* and Some Novel Synthetic Isomers." *Journal of Natural Products Web Release* Date: 09-Jul-2004;**

Bioassay-guided fractionation was applied to the cytotoxic chloroform fraction of the red alga *Polysiphonia lanosa*. The major compounds of the most active fraction were identified using GLC-MS analysis as lanosol (1), methyl, ethyl, and n-propyl ethers of lanosol (1a, 1b, and 1c, respectively), and aldehyde of lanosol (2), although 1b appears to be an artifact arising during the fractionation procedure. These compounds and other known bromophenols were synthesized in addition to four novel isomers (3, 3a-c). The cytotoxic activities of all the synthetic compounds were determined against DLD-1 cells using the MTT assay. Compounds with IC<sub>50</sub> < 20 μmol were also tested against HCT-116 cells. Compound 3c (2,5-dibromo-3,4-dihydroxybenzyl n-propyl ether) was the most active compound against both cell lines (IC<sub>50</sub> ) 1.72 and 0.80 μmol, respectively), and its effect on the cell cycle was studied using flow cytometry.

**Solyanikova, I. P. and L. A. Golovleva (2004). "Bacterial degradation of chlorophenols: Pathways, biochemica, and genetic aspects." *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes* 39(3): 333-351.**

Chlorophenols belong to the group of toxic and persistent to microbial attack xenobiotics. Nevertheless, due to the adaptation microorganisms acquire the ability to use chlorophenols as the sole source of carbon and energy. The present review describes the diversity of aerobic pathways for the utilization of halogenated phenols by bacteria with the emphasis on the main reactions and intermediates formed, enzymes responsible for these reactions and their genetic basis. Taking into account (i) the fact that enzymes degrading chlorophenols are similar to the ones involved in the conversion of other (chloro)aromatic compounds and (ii) that present numerous publications describing the properties of separated enzymes or encoding their genes are published, this review was planned as the attempt to present both, the most general and specific aspects in chlorophenols degradation with the emphasis on the literature of the last ten years.

**Sponza, D. T. and H. Atalay (2004). "Simultaneous toxicity and nutrient removals in simulated DEPHANOX (anaerobic/anoxic/oxic sequentials) process treating dinitrotoluene and trichlorotoluene." *Water Science and Technology* 49(5-6): 237-244.**

A modified DEPHANOX process including two upflow sludge blanket reactors (USB) (anaerobic-upflow sludge blanket -UASB and anoxic-upflow anoxic sludge blanket -UA(N)SB) and one completely stirred tank reactor (CSTR) system was simulated in order to detect the simultaneous removal of dinitrotoluene (DNT), trichlorotoluene (TCT), and nutrients. The phosphorus uptake and nitrification was excessively determined in aerobic CSTR reactor. Influent DNT was transformed to toluene, NH<sub>4</sub>-N and total aromatic amines (TAA) while TCT was transformed to toluene and dichlorotoluene (DCT) under anaerobic and anoxic conditions. Increasing the volumetric loading rate of DNT and TCT from 18 mg/L.day and 0.35 g/L.day to 60 mg/l.day and 1.2 g/L.day, respectively, resulted in higher COD conversion (70-80%) rates and methane productions (250-300 ml/day) in anaerobic reactor. 90% NO<sub>3</sub>-N and 87% PO<sub>4</sub>-P were achieved in anoxic and aerobic reactors at DNT and TCT loading rates as high as 40-60 mg/L.day and 0.8-1.2 g/L.day, respectively. The TAA produced under anaerobic and anoxic conditions were ultimately

removed under the aerobic stage. The UASB and anoxic UASB reactor effluents were less toxic relative to the influent when analyzed by anaerobic toxicity tests and specific methanogenic activity tests, indicating that such anaerobic/anoxic aerobic sequential treatments could be able to reduce toxic organics together with nutrient removal.

**Stiber, N. A., M. Pantazidou, et al. (2004). "Embedding expert knowledge in a decision model: evaluating natural attenuation at TCE sites." *Journal of Hazardous Materials* 110(1-3): 151-160.**

This paper describes a generalized methodology that enables the translation of expert knowledge about any complex process involved in a remedial decision into easy-to-use decision tools. The methodology is applied to evaluate reductive dechlorination as a remedial possibility at sites contaminated with trichloroethene (TCE), building on an existing protocol/scoring system put forth by the US Air Force and the US EPA. An alternate scoring system is proposed, which has two major advantages, namely that it: (i) attributes relative weights to findings based on expert beliefs; and (ii) systematically includes negative weights for negative findings. The ability of the proposed scoring system to assess the bioattenuation potential of TCE is demonstrated using data from extensively studied sites. (C) 2004 Elsevier B.V.

**Takeda, H., N. Hara, et al. (2004). "Biphenyl-inducible promoters in a polychlorinated biphenyl-degrading bacterium, *Rhodococcus* sp RHA1." *Bioscience Biotechnology and Biochemistry* 68(6): 1249-1258.**

Five transcriptional promoters of biphenyl-degradation genes in *Rhodococcus* sp. RHA1 were characterized. We newly identified the *etbA4* promoter region, which was located adjacent upstream from a ferredoxin reductase gene, *etbA4* and a dihydrodiol dehydrogenase gene, *bphB2*. The *etbA4* promoter activity was determined in RHA1 using a promoter probe vector with a *luxAB* luciferase reporter gene, and was induced by a variety of aromatic compounds as well as the *bphA1*, *ebdA1*, *etbA1*, and *etbD1* promoters. All these promoters were induced by aromatic compounds in a closely related heterologous host, *R. erythropolis* IAM1399 in the presence of RHA1 *bphST* genes, suggesting that these five promoters are under the control of *bphST*-coding two-component regulatory system. Sequence comparison of the *bphA1* promoter with the *ebdA1* and *etbA1* promoters, whose transcription starts were determined by primer extension analysis, revealed a consensus sequence centering 42-bp upstream from the transcription start. This consensus was also conserved in the *etbA4* and *etbD1* promoters, and deletions of the *bphA1* promoter affecting the consensus impaired inducible promoter activity. These results suggest that this consensus plays a role in transcription induction and/or the promotion of biphenyl degradation genes in RHA1.

**Takeuchi, M., K. Nanba, et al. (2004). "Natural groundwater of a gas field utilizable for a bioremediation of trichloroethylene-contamination." *Environmental Geology* 45(7): 891-898.**

Groundwater from a shallow aquifer in Mobarra, a city in a natural gas field in Chiba Prefecture, Japan, was found to contain a significant amount of dissolved methane (<3.1 mM) along with nitrate, phosphate and methane-oxidizing bacteria (methanotrophs, <9.9x10(6) MPN ml(-1)) which can degrade trichloroethylene (TCE). This water exhibited high methanotroph growth activity and rapid degradation of TCE. This water was introduced into a TCE-contaminated aquifer. The concentration of TCE at the monitoring well 2 m down-gradient of the injection pit decreased from 128 mug L-1 before the injection to less than the lower detection limit of 12.5 mug L-1 after the injection, while it decreased only slightly (to 86 mug L-1) when control water was injected. These results demonstrate the feasibility of utilizing a natural groundwater resource containing methane and methanotrophs without any additives for bioremediation of a TCE-contaminated site.

**Tebes-Stevens, C. L. and W. J. Jones (2004). "Estimation of microbial reductive transformation rates for chlorinated benzenes and phenols using a quantitative structure-activity relationship approach." *Environmental Toxicology & Chemistry* 23(7): 1600-1609.**

A set of literature data was used to derive several quantitative structure-activity relationships (QSARs) to predict the rate constants for the microbial reductive dehalogenation of chlorinated aromatics. Dechlorination rate

constants for 25 chloroaromatics were corrected for the effects of hydrophobic partitioning and adjusted for the observed distribution of product species. A number of physicochemical properties and molecular parameters were considered for inclusion in the QSARs. Multivariate statistical analyses were used to select the optimal set of descriptors to minimize multicollinearity between the descriptors, as well as to minimize the p-value of the regression coefficients. The final QSAR included four descriptors: The logarithm of the octanol-water partition coefficient ( $K_{ow}$ ), the summation of the Hammett sigma constants, and the sigma induction constants in the ortho and meta positions relative to the transformation reaction center. The predictive ability of this QSAR was evaluated using 24 site-specific rate constants that were measured in five separate studies and were not used to derive the expression. The peer-reviewed literature was screened carefully to ensure that all rate constant data were representative of environmentally relevant conditions.

**Urgun-Demirtas, M., K. R. Pagilla, et al. (2004). "Enhanced kinetics of genetically engineered Burkholderia cepacia: Role of vgb in the hypoxic cometabolism of 2-CBA." *Biotechnology and Bioengineering* 87(1): 110-118.**

Application of Vitreoscilla hemoglobin (VHb) technology to 2-CBA degradation by *Burkholderia cepacia* strain DNT under hypoxic conditions was studied in continuous culture chemostats. Dechlorination abilities of both recombinant (VHb gene (vgb) containing) and untransformed cells were investigated at various dilution rates to ensure complete degradation of 2-CBA. As the dilution rate increased from 0.025 to 0.25 h<sup>-1</sup>, the ratios of chloride release to degraded 2-CBA concentration decreased from 0.95 to 0.72 and from 0.89 to 0.39 for recombinant and untransformed cells, respectively. A nonstoichiometric relationship between chloride release and 2-CBA degradation was more pronounced for untransformed cells. Recombinant cell densities were 0.1-0.2 g L<sup>-1</sup> greater than untransformed cell densities for a range of dilution rates. As the dilution rate increased, the oxygen uptake rate (OUR) and the substrate utilization rate (SUR) decreased for both strains. The OUR/SUR ratio increased as the dilution rate increased for both strains but was much higher for the recombinant strain compared to untransformed cells. The specific 2-CBA degradation rate of recombinant cells was greater than that of untransformed cells (1.17 vs. 0.46 mg CBA (mg)<sup>-1</sup> day<sup>-1</sup>), and half-saturation constants for recombinant cells were lower than those of untransformed cells (0.18 and 0.32 mg CBA L<sup>-1</sup>, respectively). The pseudo-first-order degradation constants,  $k(1CBA)$  and  $k(1ACE)$ , were higher for recombinant cells (6.5 L (mg cells)<sup>-1</sup> day<sup>-1</sup> and 95.6 L (mg cells)<sup>-1</sup> day<sup>-1</sup>, respectively) than those of untransformed cells (1.44 L (mg cells)<sup>-1</sup> day<sup>-1</sup> and 73.7 L (mg cells)<sup>-1</sup> day<sup>-1</sup>, respectively). (C) 2004 Wiley Periodicals, Inc.

**Valverde-R, C., A. Orozco, et al. (2004). Halometabolites and cellular dehalogenase systems: An evolutionary perspective. *International Review of Cytology - a Survey of Cell Biology*, Vol. 234. 234: 143-199.**

**Walter, M., L. Boul, et al. (2004). "Growth substrate selection and biodegradation of PCP by New Zealand white-rot fungi." *Journal of Environmental Management* 71(4): 361-369.**

Nine New Zealand native white-rot fungi were studied for their ability to grow and survive on different substrates formulated from bark, wheat straw, sawdust, apple pomace and maize products in order to identify their pentachlorophenol (PCP) biodegradation potential and to select a fungal carrier for bioaugmentation of polluted soils. Isolates were also evaluated to mineralize C-14-PCP in liquid culture and in soil. The American fungus *Phanerochaete chrysosporium* outgrew the native fungi on the substrates tested, but the high colonisation did not result in superior PCP dechlorination as measured by chloride release. Whilst *Trametes versicolor* inocula produced on wheat straw and SCS (sawdust-corn meal-starch-mix) gave the highest chloride release, colonization of these two substrates as measured by biological potential was lower compared to the pomace and pomace - sawdust-mix. Neither lignin peroxidase nor manganese peroxidase production were measured for New Zealand white-rot fungi during the experiments. Laccase was the only enzyme detected. In liquid culture, the mineralisation rate was higher for *T. versicolor* isolates compared to *P. chrysosporium*. Very little to no pentachloroanisole (PCA) was captured in the volatile fraction of *T. versicolor* isolates, whereas 75% of the volatile fraction of *P. chrysosporium* consisted of PCA. The soil microcosms studies, using contaminated soil from a timber treatment site, clearly showed that the

New Zealand *T. versicolor* isolates mineralized PCP. Degradation of PCP in non-sterile soil was higher in the presence of white-rot fungi than in soil without white-rot fungus. This demonstrates that viable white-rot fungus is necessary for significant PCP degradation and that *T. versicolor* isolates showed PCP remediation potential. Wheat straw and SCS could be suitable carriers for New Zealand native *T. versicolor* isolates for bioremediation of PCP polluted soil sites. (C) 2004 Elsevier Ltd. All rights reserved.

**Wang, G. D., Q. J. Li, et al. (2004). "Ex planta phytoremediation of trichlorophenol and phenolic allelochemicals via an engineered secretory laccase." *Nature Biotechnology* 22(7): 893-897.**

Plant roots release a range of enzymes capable of degrading chemical compounds in their immediate vicinity(1-2). We present a system of phytoremediation ex planta based on the overexpression of one such enzyme, a secretory laccase. Laccases catalyze the oxidation of a broad range of phenolic compounds(3), including polychlorinated phenols such as 2,4,6-trichlorophenol (TCP), that are among the most hazardous and recalcitrant pollutants in the environment(4). We isolated a secretory laccase cDNA of LAC1, which is specifically expressed in the roots of *Gossypium arboreum* (cotton). Transgenic *Arabidopsis thaliana* plants overexpressing LAC1 exhibited enhanced resistance to several phenolic allelochemicals and TCP. The secretory laccase activity in these plants was responsible for the conversion of sinapic acid into a mono-lactone type dimer and for the transformation of TCP.

**Weissflog, L., N. Elansky, et al. (2004). "Trichloroacetic acid in the vegetation of polluted and remote areas of both hemispheres - Part II: salt lakes as novel sources of natural chlorohydrocarbons." *Atmospheric Environment* 38(25): 4197-4204.**

One of the issues provided for by the 1993 existing substances regulation (793/93/EEC) is the assessment of the environmental risk emanating from waste materials. One such material is the highly volatile substance perchloroethene (PER; TECE). PER is produced in large quantities all over the world by the chemical industry. There are many industrial processes in which PER escapes into the environment, especially the atmosphere. It has since been proven that after entering plants via the air/leaf pathway, airborne PER can be metabolised into the phytotoxic substance trichloroacetic acid. However our own studies detected relatively high levels of TCA in environmental compartments in regions far away from industry which cannot be explained by the anthropogenic input of airborne substances into the relevant ecosystems. This indicates that natural PER emittents also exist and must be identified, in order to find out more about the global spread of PER. This paper reports on the findings of related fieldwork in the Kalmykian Steppe. This area of steppe in southern Russia spans an area extending west-to-east from the Black Sea and the Caspian Sea and north-to-south between the Greater Caucasus and Volgograd. The main aim of the experiments in the Kalmykian Steppe was to study water from lakes, rivers and springs with differing levels of salinity. The concentrations of the chlorinated hydrocarbons (VCHCs) chloroform (CHCl<sub>3</sub>), tetrachloromethane (CCl<sub>4</sub>), 1,1,1-trichloroethane (1,1,1-C<sub>2</sub>H<sub>3</sub>Cl<sub>3</sub>), trichloroethene (TRI; C<sub>2</sub>HCl<sub>3</sub>), tetrachloroethene (PER; C<sub>2</sub>Cl<sub>4</sub>) and TCA in these waters were measured, along with the levels of cations and anions and the pH-value of the waters. The measurements indicate that in particular water from salt lakes located in semiarid/ and areas of the study region must be considered as new types of natural emittents of PER and other chlorinated hydrocarbons as well as trichloroacetic acid. Furthermore, attention is drawn to ecological impacts resulting from the occurrence of these substances in connection with the desertification observed in this area since the mid-20th century. Possible global associations between TCA phytotoxicity, the consumption of water by contaminated plants and the resulting impact on the regional water cycle are discussed. (C) 2004 Elsevier Ltd. All rights reserved.

**Williamson, R. T., I. P. Singh, et al. (2004). "Taveuniamides: new chlorinated toxins from a mixed assemblage of marine cyanobacteria." *Tetrahedron* 60: 7025-7033.**

Abstract—Brine shrimp toxicity guided fractionation of the extracts from two mixed Fijian collections of the cyanobacteria *Lyngbya majuscula* and *Schizothrix* sp. led to the isolation of eleven novel chlorinated lipids. All of these metabolites show an intriguing constellation of unsaturation (olefinic and acetylenic bonds) and chlorination at the two termini of a 15-carbon chain. The central carbon atom of the chain (C-8) is substituted in each case with an N-acetate function. Taveuniamides A–E have an adjacent carbomethoxy group at C-9 to form a protected β-amino

acid while taveuniamides F–K have a methylene group at this position. A standard assortment of 2D NMR techniques in concert with mass spectrometry and other analytical techniques were used to define the structures of these novel metabolites. Taveuniamides F, G and K were the most potent brine shrimp toxins with LD50s between 1.7–1.9 mg/mL.

**Xu, X., F. Song, et al. (2004). "Dibenzyl Bromophenols with Diverse Dimerization Patterns from the Brown Alga *Leathesia nana*." *Journal of Natural Products* Web Release Date: 01-Sep-2004;.**

Six novel dibenzyl bromophenols (1-6) with different dimerization patterns and two propyl bromophenol derivatives (7 and 8), together with 11 known bromophenol derivatives, were isolated from the ethanolic extract of the brown alga *Leathesia nana*. On the basis of spectroscopic methods the structures of the new compounds were determined as 5,6-*diethyloxymethyl-3,4,2-tribromo-2,3,4-trihydroxydiphenyl ether* (1), 2-(2,3-dibromo-4,5-dihydroxybenzyl)-3,5-dihydroxy-4-methoxybenzyl alcohol (2), 6-(2,3-dibromo-4,5-dihydroxybenzyl)-2,3-dibromo-4,5-dihydroxy benzyl methyl ether (3), 9,10-dihydro-9,10-dimethoxy-3,4,7,8-tetrabromo-1,2,5,6-tetrahydroanthracene (4), (E)-3-(2,3-dibromo-4,5-dihydroxyphenyl)-4-bromo-5,6-dihydroxy-1,3-dihydroisobenzofuran (5), rel-(4aS\*,10aR\*)-(1)-6,7-dibromo-4a-hydroxy-3,8-dihydroxymethyl-10a-methoxy-1,4,4a,10a-tetrahydrodibenzo[b,e][1,4]dioxin-1-one (6), (E)-2-methyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)propenal (7), and 2-methyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)-1-propanol (8). Some compounds including 3 showed *in vitro* selective cytotoxicity against several human cancer cell lines. This is the first brown alga to be reported containing bromophenols.

**Ye, F. X., D. S. Shen, et al. (2004). "Anaerobic granule development for removal of pentachlorophenol in an upflow anaerobic sludge blanket (UASB) reactor." *Process Biochemistry* 39(10): 1249-1256.**

The granulation process using synthetic wastewater containing pentachlorophenol (PCP) in four 1.11 laboratory scale upflow anaerobic sludge blanket (UASB) reactors was studied, and the anaerobic biotransformation of PCP during the granulation process investigated. After 110 days granular sludge was developed and up to 160 and 180 mg/l of PCP was added into the reactors R1 and R2, respectively, when they were inoculated with acclimated anaerobic sludge from an anaerobic digester of a citric acid plant. The inoculum was predominately composed of bacilli and filamentous bacteria. Granulation did not occur in reactors R3 and R4 which were inoculated with acclimated anaerobic sludge from aerobic sludge of the municipal sewage treatment plant which consisted mainly of cocci. Despite similar bacilli in the granule, the filamentous bacteria from reactor R1 were thicker than those of reactor R2. The granular sludge had a maximum diameter of 2.5 and 2.2 mm, and SMA of 1.44 and 1.32 gCOD/gTVS per day for reactors R1 and R2, respectively. Over 98% chemical oxygen demand (COD) removal rate and 99% of PCP removal rate were achieved when reactors R1 and R2 were operated at PCP and COD loading rates of 150 and 7.5 g/l per day, respectively. H<sub>2</sub>-producing acetogens were the dominant anaerobes in the granular sludge. (C) 2003 Elsevier Ltd. All rights reserved.

**Yeh, D. H. and S. G. Pavlostathis (2004). "Phase distribution of hexachlorobenzene in a suspended-growth culture amended with a polysorbate surfactant." *Water Environment Research* 76(2): 137-148.**

The effect of a non-ionic surfactant on the phase distribution of hexachlorobenzene (HCB) in a suspended-growth culture system was assessed. Tween 60, a polyoxyethylene sorbitan stearate ester surfactant, and an azide-inactivated, mixed, methanogenic, HCB-dechlorinating culture were used in this work. The sorption of HCB on the biomass as well as sorption and aggregation/precipitation of Tween 60 were experimentally quantified. The values of the HCB and Tween 60 distribution parameters were determined and the phase distribution of HCB in the presence of surfactant and biomass was described quantitatively. Both the HCB and surfactant distribution are highly dependent on the total amount of surfactant present in the system. At low initial surfactant concentrations, most of the HCB is associated with the biomass. As the surfactant concentration increases, the effect of surfactant sorption and precipitation diminishes and the higher pool of surfactant micelles shifts the distribution of HCB from the solid phase to the solution phase. The HCB phase distribution in the presence of the surfactant may have a significant effect on HCB bioavailability for reductive dechlorination. The quantitative description of the HCB

phase distribution presented here was subsequently used as the basis for the development of a model that describes the bioavailability and reductive dechlorination of HCB in a surfactant/biomass system.

**Zhao, J., X. Fan, et al. (2004). "Bromophenol Derivatives from the Red Alga *Rhodomela confervoides*." *J. Natural Products* 67(6): 1032 - 1035.**

Eight new bromophenol derivatives, 2,3-dibromo-4,5-dihydroxybenzyl methyl sulfoxide (1), 4-(2,3-dibromo-4,5-dihydroxyphenyl)-3-butene-2-one (2), 2-(3-bromo-5-hydroxy-4-methoxyphenyl)-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid (3), 2-(3-bromo-5-hydroxy-4-methoxyphenyl)-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid methyl ester (4), 2-phenyl-3-(2,3-dibromo-4,5-dihydroxyphenyl)propionic acid (5), 4-methoxy-2,3,3,3-tribromo-4,5,5-trihydroxydiphenylacetic acid (6), and 3-bromo-5-hydroxy-4-methoxyphenylacetic acid (7) and its methyl ester (8), together with a known bromophenol, 3-bromo-5-hydroxy-4-methoxybenzoic acid (9), were isolated from the red alga *Rhodomela confervoides*. Their structures were elucidated by spectroscopic methods including IR, EIMS, FABMS, ESIMS, HRFABMS, HRESIMS, 1D and 2D NMR, and single-crystal X-ray structure analysis. Compounds 1-4, 8, and 9 were found inactive against several human cancer cell lines and microorganisms.