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

5th Quarterly Report

1st Quarter Year 2002

Report prepared for EUROCHLOR

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ACRONYMS

BTEX	Benzene, Toluene, Ethyl Benzene and Xylene
CAHs	Chlorinated Aliphatic Hydrocarbons
CB	Chorobenzene
4-CBA-CoA	4-Chlorobenzoyl Coenzyme A
CBp	Chlorobiphenyl
CDDs	Chlorinated dibenzo- <i>p</i> -dioxins
CDFs	Chlorinated Dibenzo- <i>p</i> -furans
CF	Chloroform
CT	Carbon tetrachloride
CPO	Chloroperoxidase
1,2-DCA	1,2-Dichloroethane
DCB	Dichlorobenzene
<i>Cis</i>-DCE	<i>Cis</i> -1,2-dichloroethene
1,2-DCP	1,2-Dichloropropane
DCM	Dichloromethane
DDD	1,1-dichloro-2,2- bis(<i>p</i> -chlorophenyl)-ethane
DDT	1,1,1-trichloro-2,2-bis(<i>p</i> -chlorophenyl)-ethane
DGGE	Denaturing Gradient Gel Electrophoresis
E-acceptor	Electron Acceptor
E-donor	Electron Donor
ETH	Ethene
FISH	Fluorescent In Situ Hybridization
HCB	Hexachlorobenzene
HCH	Hexachlorohexane
PCBs	Polychlorinated Biphenyls
PCE	Tetrachloroethylene
PCR	Polymerase Chain Reaction
PDTC	Pyridine-2,6-bis(thiocarboxylate)
RAMEB	Randomly Methylated Beta Cyclodextrins
TCA	1,1,1-Trichloroethane
TCE	Trichlorethylene
TCB	Trichlorobenzene
TIMS	Thermal Ionization Mass Spectrometry
TOM	Toluene <i>Ortho</i> -Monooxygenase
UASB	Upflow Anaerobic Sludge Blanket reactor
VC	Vinyl Chloride

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1. INTRODUCTION

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

2. SUMMARY OF MOST IMPORTANT DEVELOPMENTS

2.a. Microbial Dechlorination

The most important findings on microbial dechlorination in the first quarter of 2002 are:

- The correspondence of bacteria from the genus *Dehalococcoides* with chloroethene contaminated sites with the anaerobic degradation of chloroethenes to ethene; without accumulation of vinyl chloride and *cis*-dichloroethene intermediates (27). The results indicate that a simple gene probe based on 16S ribosomal DNA specific for *Dehalococcoides* will be predictive in estimating if a given chloroethene-contaminated

(including vinyl chloride) site can be bioremediated under anaerobic conditions to environmentally-benign products.

- Identification of an anaerobic bacterium that specifically catalyzes the reductive dechlorination of PCBs with doubly flanked chlorines was reported for the first time (55).
- The complete biodegradation of yperite (bis(2-chloroethyl) sulfide), a chemical warfare agent, is reported for the first time (54). White rot fungi were shown to be responsible for yperite degradation.

2.b. Microbial Chlorination

The most important highlights from this quarter concerning biological halogenation are as follows:

- Chlorination of tyrosine residues in bacterial proteins by hydrochlorous acid was shown to be a mechanism contributing to phagocytosis of bacteria such as *Staphylococcus aureus* by human white blood cells (13).
- The structure of an important unidentified bioaccumulating natural organohalogen metabolite recovered from fish fat, known as metabolite Q1 ($C_9H_3Cl_7N_2$) has been elucidated further (51). The newly obtained mass spectra indicate that Q1 has a bipyrrrole backbone.

3. MICROBIAL DECHLORINATION

3.a. General Reviews

In this quarter there were 5 review articles. These include: one minireview on anaerobic polychlorinated benzene degradation (3); one review on the reasons for the resistance of xenobiotics to bacterial degradation (40); one review on the family of bacterial glutathione S-transferases (53) and two reviews on phytoremediation of chlorinated hydrocarbons (10, 14).

The publication on the anaerobic degradation polychlorinated benzenes (PCBs) reviews examples of microbial consortia in anaerobic sludges and sediments, which have been found to

reductively dechlorinate PCBs (3). The review reports on a recent strain, which has been found that can couple growth to the use of PCBs as electron acceptors (e-acceptors); whereby the chlorobenzenes are dechlorinated. The results reviewed in the article suggest that additional anaerobic bacteria, utilizing chlorobenzenes or chlorobiphenylic compounds to support respiration (halorespiration), can be isolated. The review also presents pathways for the anaerobic degradation of chlorinated benzenes.

The review on the resistance of xenobiotics to bacterial degradation is mostly concerned with pollutants with multiple electron-withdrawing substituents (azo, chloro and nitro) which are poorly biodegradable under anaerobic conditions but are readily reductively biotransformed under anaerobic conditions (40). The reductively biotransformed products in turn are readily degraded under aerobic conditions. The article reviews several examples of sequenced anaerobic-aerobic degradation to obtain the full degradation of the recalcitrant pollutants. The article also discusses interdisciplinary approaches to the design of environmentally benign substances but the examples given are mostly for non-halogenated compounds in the textile industry.

One review article reports on the screening of glutathione S-transferases, which constitutes a large family of enzymes that catalyze the addition of glutathione to endogenous or xenobiotic, often toxic electrophilic chemicals (53). Dichloromethane (DCM), dichloroethane oxide and tetrachloro-1,4-hydroquinone are examples of chlorinated compounds degraded by this class of enzymes. The enzymes involved such as dichloromethane dehalogenase, 1,2-dichloroepoxyethane epoxidase and tetrachlorohydroquinone reductase, are catabolic enzymes with an essential role for growth on the recalcitrant chemicals. Information from bacterial genome sequencing projects now suggests that glutathione S-transferases are present in large numbers in proteobacteria. The genomes of three *Pseudomonas* species each include at least ten different glutathione S-transferase genes. Several of the corresponding proteins define new classes of the glutathione S-transferase family and may also have novel functions that remain to be elucidated.

The first review of phytoremediation deals with benzene, toluene, ethyl benzene and xylene (BTEX) and trichloroethene (TCE) (14). The environmental problems arising from the use of chlorinated solvents and BTEX compounds are described, and an overview about active management strategies for remediation with special emphasis on phytoremediation are presented to achieve a reduction of the total mass of chlorinated solvents and BTEX. The feasibility of phytoremediation is discussed with a focus on the uptake and metabolism of the compounds by plants. The second review of phytoremediation considers the literature on PCBs and dioxins (10). This review focuses on particular problems encountered in biodegradation and

bioavailability of PCBs and polychlorinated dioxins/dibenzofurans. It highlights the potential and limitations of plants and microorganisms as bioremediation agents and summarizes how plants can be used to augment bacterial activity. Phytoremediation is shown to provide some new possibilities in reducing risks associated with dioxins and PCBs.

3.b. Microbial Dechlorination

Vinyl chloride and Other Chlorinated Ethenes

As indicated in previous reports, relatively few reports are directly concerned with vinyl chloride. A larger number of reports involve research evaluating the degradation of the higher chlorinated ethenes, perchloroethylene (PCE) and trichloroethene (TCE) because these are major groundwater contaminants. Thus information regarding the degradation of chlorinated ethenes will include both vinyl chloride and the higher chlorinated ethenes.

Anaerobic Degradation of PCE and TCE. Four publications in this quarter considered the anaerobic degradation of PCE. The first publication concerns the distribution of the halorespiring organisms *Dehalococcoides* at various sites throughout America and Europe (27). The genus *Dehalococcoides* is associated with chloroethene contaminated sites and this genus is considered to contain the only bacterial strains responsible for the complete reduction of chloroethenes to ethene including lower chlorinated ethenes such as dichloroethene (DCE) and VC. The presence of the organism was tested with a 16S RNA gene probe specific for *Dehalococcoides*. A good association was found between chloroethene contaminated sites displaying complete degradation (to ethene) and the presence of *Dehalococcoides*. Also the absence of detection of *Dehalococcoides* was associated with sites where PCE and TCE were only partially degraded, stopping at the level *cis*-1,2-dichloroethene. Taken as a whole the results indicate that a gene probe for *Dehalococcoides* will be very predictive in determining if at any given site, PCE or TCE will be completely anaerobically degraded to ethene or just partially degraded to DCE and/or VC.

The second publication reports on the effect of carbon tetrachloride (CT) and PCE mixtures on the anaerobic degradation of each chlorinated solvent (2). The presence of CT was found to slow down the rate of PCE degradation but did not prevent PCE degradation. Also CT was degraded in the presence of PCE.

The third publication reports on the synergy between a sulfate reducing bacteria (*Desulfovibrio fructosivorans*) and a halorespiring bacterium (*Desulfotobacterium frappieri*),

responsible for the dehalogenation of PCE to *cis*-DCE utilizing fructose as the electron-donating substrate (20). Fructose is a substrate of the sulfate reducer, but is not a substrate for the halorespiring bacterium. At high sulfate levels all of the substrate consumption was utilized to reduce sulfate, and the bacterial counts of *D. frappieri* were low. At low or no sulfate, a large portion of the substrate was utilized to reduce PCE to *cis*-DCE and the bacterial counts of *D. frappieri* were high. The results indicate that there is electron transfer between the sulfate reducing bacterium and the halorespiring bacterium. H₂ was demonstrated to be the interspecies form of electron transfer.

The fourth publication reports on the use of Tenax as an adsorbent to deliver PCE in biodegradation assays (8). Tenax is proposed as an alternative to the more commonly used hexadecane. The use of Tenax as an adsorbent buffers the toxicity of PCE and its reduction products; enabling the study of chloroethene biodegradation without toxicity side effects to organisms responsible for the degradation.

Aerobic Degradation of TCE. Seven publications evaluated aerobic co-oxidation of TCE this quarter. The first publication considers the degradation of ¹⁴C labeled TCE in wetland microcosms with and without plants (5). In the various microcosms, the most important fate of TCE was volatilization accounting for >50% of the label. In microcosms without plants, the conversion of TCE to CO₂ ranged from 3.2 to 15.6% in organic and sandy soils, respectively. In microcosms with plants the conversion to CO₂ increased to 5.3% in organic soils.

The second publication, considers the aerobic cooxidation of TCE and 1,1,1-trichloroethane (TCA) by a *Mycobacterium* strain grown on ethane (26). The V_{max} and Km for TCE was 9.8 nmol/min/mg cells and 61.9 μM. The V_{max} and Km for TCA was 0.11 nmol/min/mg cells and 3.1 μM. The intermediates of the cooxidation were 2,2,2-trichloroethanol, trichloroacetic acid, chloral and dichloroacetic acid. The chlorinated acetic acids were not metabolized further.

The third publication describes the application of directed evolution to improve the toluene *ortho* monooxygenase (TOM) gene for the cooxidation of TCE (11). Directed evolution refers to the use of Polymerase chain Reaction (PCR) under sub-optimal conditions to provoke minor mutations of the gene, which can be screened for the search of improved gene function. One TOM mutant denoted TOM-green was found to produce an enzyme which could oxidize TCE twice as fast as the wild type enzyme, demonstrating the success of the directed evolution approach.

The fourth publication reports on the bioaugmentation of a TCE co-oxidizing bacterium, *Ralstonia eutrophia* into a field site well together with toluene to promote TCE bioremediation

(50). The study evaluated the survival of the bioaugmented cells by two methods, a gene probe based on the phenol hydroxylase gene and fluorescent in situ hybridization (FISH) targeting 16S rRNA.

The fifth publication examined the isotope fractionation of TCE during its co-oxidation by *Burkholderia cepacia* (6). The study concludes that carbon isotopic fractionation occurs during aerobic cooxidation and its extent is greater than that occurring during anaerobic degradation.

The sixth publication describes the elimination of volatile organic carbon in a peat packed biofilter treating off-gases including chlorinated components such as TCE and chloroform (56). At an empty bed retention time of 1.5 min, the removal efficiency was highest for isoprene (93%), and lowest for chloroform (84%).

The seventh publication reports on a new method to measure soluble methane monooxygenase, an enzyme implicated in the aerobic co-oxidation of TCE, utilizing fluorescence produced with the substrate coumarin (34). The method was tested with *Methylosinus trichosporium*, which expresses soluble methane monooxygenases. The fluorescent product of coumarin was identified as 7-hydroxycoumarin.

Carbon Tetrachloride (CT) and Chloroform (CF)

In the first quarter of 2002, one publication reported on the anaerobic degradation of chloroform (CF) (38). Furthermore, numerous reports focusing on the microbial dechlorination of carbon tetrachloride were found in this period. Two publications focused on the anaerobic degradation of carbon tetrachloride (CT) (38, 46), another one presented data regarding gene regulation during CT degradation by an aerobic bacterial strain (43), and two additional publications dealt with CT degradation by cofactors or metabolites produced by aerobic (47) and anaerobic microorganisms (4). The last two studies are discussed later in the report under the heading “3.c. *In Vitro Degradation of Chlorinated Compounds*”.

The influence of redox potential on the degradation of the halogenated methanes, chloroform (CHCl_3), carbon tetrachloride (CCl_4), and trichlorofluoromethane (CFCl_3), was investigated (38). The extent of dehalogenation caused by live sludge, heat-killed sludge, and abiotic controls (without sludge) was examined. The highest rates for the dehalogenation of the three compounds were observed for the heat-killed sludge treatments at -348 mV, that was the lowest redox potential tested (range tested: +534 to -348 mV). Dechlorination rates by the heat-resistant catalysts was approximately two-fold higher than the live treatments. Enhanced degradation rates by heat-killed sludge was attributed to the absence of physical barriers such as cell wall and cell membranes. CT and CF were dechlorinated abiotically (in the absence of

sludge) by Ti (III) citrate, but CFCl_3 was not. This study reports the Gibbs free energy and the redox potential for the dehalogenation reactions utilizing Ti (III) citrate and acetate as e-donors.

Simultaneous granulation, biomass retainment and carbon tetrachloride (CT) removal in an upflow anaerobic sludge blanket (UASB) reactor was reported (46). The information presented in this manuscript overlaps to a great extent a different publication by the same author (45) that has already been discussed in the 4th Quarterly Eurochlor Report of the year 2001).

The characteristics and role of different genes in the synthesis and activity of pyridine-2,6-bis(thiocarboxylate) (PDTC), the secreted cofactor responsible for carbon tetrachloride dechlorination by *Pseudomonas stutzeri* strain KC, was investigated (43). Additional results from this study indicate that *P. stutzeri* strain KC may possess a distinct biosynthetic pathway for PDTC production compared to other pseudomonads. Screening of a selection of *Pseudomonas* strains, including a strain known to produce PDTC, for the presence of the genes characterized in this study showed that none of the strains tested positive.

Chloromethane and Dichloromethane

No publications regarding the microbial degradation of the aliphatic chlorinated compounds, chloromethane and dichloromethane, were found in this quarter. Only brief mention of dichloromethane was made in the review article on glutathione S-transferases enzymes as is discussed in section 3.a. under the heading, "General Reviews".

Dichloroethane (1,2-DCA)

Only one publication was found in this quarter that is concerned with the dechlorination of 1,2-DCA. The study that describes the degradation of 1,2-DCA and other chlorinated compounds by the enzyme haloalkane dehalogenase from a *Sphingomonas paucimobilis* strain (37), it is discussed in the heading "3.c. In Vitro Degradation of Chlorinated Compounds".

A method suitable to determine inhibition type, kinetic parameters and inhibition coefficients for the aerobic cometabolism of chlorinated aliphatic compounds (CAHs) was described (28). The method was validated by applying it to data obtained from batch kinetics of the aerobic cometabolism of a compound structurally related to 1,2-DCA, 1,1,1-trichloroethane (TCA), by a butane-grown mixed culture. TCA, which is one of the major contaminants in groundwater, is difficult to treat through aerobic cometabolism. However, in a previous study by the same authors showed that a butane-grown mixed culture could transform TCA effectively (Kim *et al. J. Environ. Eng.* 2000, 126:934-942). The results of this study indicated that two different inhibition types occurred during TCA cometabolism: competitive inhibition of TCA on butane degradation, and mixed inhibition of TCA transformation by butane.

Competitive inhibition can be an important process in the aerobic metabolism of CAHs because there is competition between CAH and growth substrates for enzyme active sites due to lack of specificity. The types of inhibition mechanisms observed may differ with different microorganisms, growth substrates, and CAHs. Based on the overall results from this study, the authors conclude that use of the direct linear plot method (*i.e.*, a method different from well-know linear plot methods as Lineweaver-Burk and Hanse plots) to identify the inhibition type, coupled with initial guesses from linearized plots for nonlinear least squares regression analysis, results in an accurate method for determining inhibition types and coefficients.

The kinetic constants of TCA degradation by ethane grown cells of the bacterium *Mycobacterium* were discussed previously (26), under the section "*Aerobic TCE degradation*"

Chlorobenzenes

A mini-review on the microbial transformation of chlorinated benzenes under anaerobic conditions (3) is discussed above in heading "*3.a. General Reviews*".

Mono-chlorobenzene, Dichlorobenzenes and Trichlorobenzenes: Two publications regarding the microbial degradation of mono-, di- and/or tri-chlorobenzenes were found in the first quarter of the year 2002. The first study reports on the aerobic degradation of chlorobenzene (CB) and *o*-dichlorobenzene (DCB) in a bench-scale (40 L) biotrickling filter reactor filled with inert packing material (42). The chloroaromatics were supplied as a mixture in a mass ratio of 2:1 (CB:DCB) at total concentrations ranging from 1 to 2.9 mg/l. The total load of the CB and DCB mixture was increased from 0.43 to 1.50 kg C/m³/day in order to establish the performance of the biotrickling filter. The highest CB and DCB elimination capacity was obtained at a total load of 1.10 kg C/m³/day, and an empty bed retention time of 1.7 min. The maximum removal efficiency was 62 and 72% for CB and DCB, respectively.

In the second publication, the biodegradation of chlorobenzenes in a contaminated aquifer was investigated in laboratory reactors and in on-site column experiments under aerobic conditions (17). The groundwater used was highly contaminated and contained chiefly monochlorobenzene (CB), at an average concentration of 25 mg/l, and concentrations of 1,4-dichlorobenzene (1,4-DCB), 1,2-DCB and benzene at least two orders of magnitude lower. The authors point out that the ratio of aromatic concentrations in the groundwater suggests that an anaerobic degradation process takes place in the aquifer with CB as the preliminary end product. Furthermore, the hypothesized that the natural anaerobic dechlorination of highly chlorinated aromatics, followed by the aerobic in-situ degradation of CB, may be a successful remediation strategy. Initial on-site column experiments showed moderate reductive dechlorination of 1,4-DCB under anaerobic conditions (25% elimination) but no elimination of

CB and benzene. Results from laboratory experiments and on-site column tests performed under aerobic conditions were found to be consistent. Aerobic biodegradation of CB was shown to be only limited by a lack of oxygen, and CB concentrations as high as 100 mg/l were shown to be mineralized by indigenous microorganisms. On the other hand, the aerobic biodegradability of DCBs was found to depend on the arrangement of the chlorine substituents. 1,4-DCB was mineralized by the indigenous microorganisms. In contrast, the degradation rates for the other two isomeric dichlorobenzenes (1,2-DCB, 1,3-DCB) under aerobic conditions were significantly lower. Similarly, very low degradation rates were detected for 1,2,4-trichlorobenzene (1,2,4-TCB) in laboratory experiments using groundwater spiked with this chloroaromatic. The higher susceptibility of 1,2-DCB to biodegradation is attributed to the maximum distance of the chlorine substituents in this compound. The authors found indications that once the oxygen was depleted, Fe(III) species served as alternative electron acceptors. These results confirm the well-known facts that the resistance of CBs to aerobic attack increases with increasing chlorine substitution and that the susceptibility of chlorobenzenes to degradation depends on the position of the chlorine atoms.

Hexachlorobenzene (HCB): No publications regarding the microbial degradation of hexachlorobenzene, other than the review article discussed in heading “3.a. General Reviews”.

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

No publications concerned with the microbial dechlorination of CDD/CDFs hexachlorobutadiene and octachlorostyrene were found in the first quarter of 2002 other than the review article on phytoremediation in heading, "3.a. General Reviews".

Polychlorinated Biphenyls (PCBs)

Seven publications concerned with the microbial dechlorination of PCBs were found this quarter. The first study reported on PCB dechlorination by anaerobic microorganisms (55), the second focused on PCB biodegradation under anaerobic-aerobic conditions (32), two publications presented results on the effect of humus and exogenous additions on bioavailability and aerobic degradation of PCBs (21, 22), two additional reports were concerned with plant-microbe or plant-microbe-soil interactions on the biotransformation of PCBs (30, 33), and the last study was concerned with the new methods for monitoring the stereoselectivity of biodegradation of chiral PCBs (23), and it is discussed in section “3.d. New tools to assess the biodegradation of chlorinated compounds”.

Identification of an anaerobic bacterium that specifically catalyzes the reductive dechlorination of PCBs with doubly flanked chlorines was reported for the first time (55). The culture used (DF culture) is a non-methanogenic microbial community that can *meta* dechlorinate 2,3,4-chlorobiphenyl (2,3,4-CBp) and 2,3,4,6-CBp and *para* dechlorinate 3,4,5-CBp and 2,3,4,5-CBp, but it does not dechlorinate congeners lacking doubly flanked chlorines, including 3-CBp, 4-CBp, 2,3-CBp, 2,4-CBp, 2,5-CBp, 3,4-CBp, 3,5-CBp, 2,3,5-CBp, 2,3,6-CBp, 2,4,6-CBp, 2,3,5,6-CBp, and 2,4,5-2,4,5-CBp. The species in the DF culture that catalyze reductive PCB dechlorination have not been identified previously. The present study established that the DF enrichment culture is a co-culture of a sulfate-reducing vibrato and bacterium DF-1. Identification was accomplished by analysis of community 16S ribosomal DNA (DNA) sequences from a culture enriched in the presence of 2,3,4,5-tetrachlorobiphenyl (2,3,4,5-CBp). The 16S DNA sequence of the PCB dechlorinators, designated bacterium DF-1, was found most similar to that of bacterium O-17 (89% similarity), an uncultured *ortho*-PCB-dechlorinating bacterium. DF-1 reductively dechlorinates congeners with doubly flanked chlorines when it is supplied with formate or H₂-CO₂ (80:20). Preliminary research by the same researchers (unpublished results) suggests that bacterium DF-1 or very similar microorganisms may be widespread.

Sequential anaerobic-aerobic treatment of soil contaminated with weathered Aroclor 1260 was investigated in laboratory-scale experiments (32). Aroclor 1260 consists of a mixture of with highly chlorinated PCB congeners, chiefly substituted with 6-8 chlorine atoms. The anaerobic stage was inoculated with an anaerobic enrichment culture and the aerobic step used *Burkholderia* sp. strain. LB400. Anaerobic treatment led to extensive dechlorination of Aroclor 1260 components after 3 months. PCB homologue classes with 5-8 chlorine substituents were completely removed. The major products of reductive dechlorination were 24-24-tetra-CBp and 24-26-tetra-CBp. Other dechlorination products detected at lower concentrations were 23-2-tri-CBp or 26-4-tri-CBp and 235-2-tetra-CBp or 24-25-tetra-CBp. The molar concentration of PCBs did not decrease during the anaerobic treatment, indicating that the anaerobic process results in PCB dechlorination but in little or no degradation of the biphenyl molecule. All of the major products formed during the anaerobic treatment were degraded in the subsequent aerobic treatment. Overall, after 4 months of anaerobic treatment followed by 28 days of aerobic treatment, the concentration of PCBs was decreased by 67% (from 59 µg/g of soil to 20µg/g of soil), the number of moles PCBs was decreased by 65%, and the average number of chlorine atoms per PCB molecule decreased from 6.4 to 5. PCB degradation was not detected in soil bioaugmented with LB400 without prior anaerobic treatment, a result in agreement with the recalcitrance of highly chlorinated PCB congeners (> 6 chlorine substituent atoms) to

degradation by *Burkholderia* LB400. These results confirm the potential for biological treatment to attain extensive removal of PCBs from contaminated soil.

It is well known that the bioavailability of the highly hydrophobic PCB compounds and thereby their susceptibility to biodegradation, can be significantly enhanced by soil treatment with surface-active agents. In this quarter, two studies report the effectiveness of two different surfactants, randomly methylated-beta-cyclodextrins (RAMEB) (21) and the naturally occurring humic substances (22), to enhance significantly the bioavailability and aerobic biodegradation of PCBs in soil. Selection of RAMEB was based on their environmental advantages as compared to synthetic surfactants (*i.e.*, lack of toxicity, partial biodegradability) and lower costs than pure cyclodextrins. RAMEB supplementation at 1-5% (w/w) was found to enhance the water solubility and aerobic biodegradation of PCBs in loamy, humic and sandy soils, artificially contaminated under laboratory-scale conditions. On the other hand, supplementation of humic substances at the 1.5% rate of addition resulted in significant enhancement of the mobility and biodegradability of PCBs in slurry-phase microcosms of a spiked soil. The positive role of humic substances was attributed to solubilization of apolar PCBs in the hydrophobic domains present in the humic structure and the increased accessibility of PCBs to PCB-degrading bacteria through the proxy hydrophilic domains of HS. Humic substances are non-toxic and considerably less costly than products commonly used to enhance PCBs biodegradation in contaminated soil. The authors cautioned that the results above were obtained on artificially contaminated soils under well-defined laboratory conditions, and therefore they should be verified by testing RAMEB or HM on real PCB-contaminated soils.

Root turnover was reported to be an important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants (30). The authors established that, upon death, the fine roots (<1 mm diameter) of mulberry (*Morus* sp.) can serve as a source of phenolic substrate for PCB-degrading bacteria. The dead fine roots were shown to accumulate phenolic compounds, consisting chiefly of three flavones (morusin, morusinol; and kuwanon C), that reached a maximum value of 38 mg/g dry weight. These flavones were shown to support the growth of the bacterium *Burkholderia* sp. LB400, a degrader of PCBs. The identified flavones were degraded at the same rate as biphenyl, a substrate often used to promote the growth of PCB-degrading bacteria. It is interesting to note that several *Burkholderia* strains are known to be plant-growth promoting rhizobacteria. In addition to providing substrates to degradative bacteria, root turnover also provides oxygen essential for the activity of dioxygenase and monooxygenase enzymes that catalyze the first step in aerobic degradation of aromatic contaminants. These results establish for the first time that the chemical content and turnover rate of fine roots should be considered an important aspect of rhizosphere remediation.

The effects of plant-microbe-soil interactions on the biotransformation of PCBs in a rhizosphere soil were investigated by Mehmannaavaz *et al.* ((33)). Laboratory experiments were performed in containers packed with a soil contaminated with Aroclor 1242, 1248, 1254 and 1260 that were seeded with alfalfa (*Medicago sativa* L.) and augmented with its symbiotic N₂-fixing host rhizobium (*Sinorhizobium meliloti* strain A-025). The experimental design was based on a factorial combination including treatments in the presence/absence of alfalfa and with/without rhizobium-inoculation of the soil. Up to 44 days after planting, when the alfalfa was not fully developed, alfalfa and *S. meliloti* together were the most effective in PCB transformation/depletion, whereas alfalfa only was the least effective. However, by the last day of the experimental period (Day 270), when alfalfa growth was robust and full, alfalfa alone was the most effective, whereas *S. meliloti* alone was the least. The author's conclude that depletion, loss or change in PCB levels may be attributed to either direct or indirect biotransformation, biotranslocation and adsorption of PCBs due to the presence of alfalfa and/or rhizobial inoculation. However, detailed studies of the mechanisms underlying PCB biotransformation and removal were not performed. The results suggest the possibility of using plant-rhizobacterial associations to phytoremediate soils contaminated with PCBs.

Miscellaneous

Six recent reports of microbial dechlorination are discussed here, three dealing with hexachlorocyclohexane (HCH) (1, 7, 35), one with the herbicides atrazine and metolachlor (44), one with the fire retardant tetra-Cl-bis-phenol A (52), and the last one with the chlorinated warfare agent, yperite (54). Data on the microbial dechlorination of the pesticide 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT) are also presented in the publication of Abou-Arab (1). None of these chlorinated compounds are included in our priority list. Nonetheless, these publications are briefly discussed because of their relevance to this review.

The effect of meat starter on the degradation of *p,p'*-DDT and γ -HCH (also called lindane) was investigated (1). Degradation of the pesticides by two different microorganisms known to be present in meat starter, *Lactobacillus plantarum* and *Micrococcus varians*, was evaluated. DDT and lindane were both recalcitrant to degradation by *L. plantarum*. In contrast, both pesticides were degraded by *M. varians* (46-48% elimination after 15 days). DDT was metabolized mainly to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDD). The main degradation products of lindane included 2,4-, 2,5-, 2,6- and 3,4-dichlorophenol; 2,3,4- and 2,3,5-trichlorophenol; hexachlorobenzene; and pentachlorophenol. These results suggest that the activity of meat starter can result in reduction of pesticide residues during the fermentation process in meat products.

LinD and LinE activities are known to be responsible for the degradation of γ -HCH by *Sphingomonas paucimobilis* UT26. These enzymes are inducibly expressed in the presence of their substrates, 2,5-dichlorohydroquinone and chlorohydroquinone. Cloning and characterization of the newly described *linR* gene, was reported by Miyauchi and coworkers (35). The *linR* gene was shown to be directly involved in the inducible expression of the *linD* and *linE* genes.

A non-steady-state multi-compartmental mass balance model, the so-called POPCYCLING-Baltic model, was applied to estimate the behaviour of two hexachlorocyclohexanes (α -HCH and γ -HCH) in the Baltic Sea environment from 1970 to 2000 (7). The model has previously been shown to accurately describe many aspects of the historical behavior of α - and γ -HCH within the Baltic Sea region (Breivik and Wania, *Environ. Sci. Technol.* 2002, 36, 1014-1023). Comparison of model results with measured concentration data in air, seawater, sediments, and foliage indicated a general agreement within a factor of 2 and the replication of the observed spatial and temporal variability of these two HCH isomers. In the present study, the model predicts that the bulk of the α - and γ -HCHs is degraded in the soils receiving the pesticide application and that the remaining fraction of HCHs is transferred to the atmosphere by evaporation. For example, the model estimates that 17% of the total amount of α -HCH used within the Baltic Sea environment has been emitted to the agricultural soils of the Gulf of Riga region, and about the same amount has been lost by degradation in these soils. Pesticide degradation in the soils of application was also found by the authors to be very important when modeling the fate of α -HCH globally (Wania and Mackay, *Environ. Toxicol. Chem.* 1999, 18, 1400-1407). Furthermore, the model predicts that the atmosphere will effectively distribute the HCHs within the Baltic Sea environment and beyond, resulting in relatively uniform concentrations in environmental compartments that do not directly receive emissions. The authors conclude that model can provide valuable insights in the fate and distribution of HCHs, and that in spite of the fact that the simulation is subject to significant uncertainties (e.g., due to the incomplete knowledge about the emissions of HCH within the area of study, large spatial and temporal variability of environmental characteristics affecting HCH fate, difficulties involved in the validation of some model results, etc.).

Anaerobic biotransformation of the flame-retardants tetrachlorobisphenol A and tetrabromobisphenol A was examined in anoxic estuarine sediments (52). Tetrabromobisphenol A and tetrachlorobisphenol A were completely dehalogenated to bisphenol A and to an isomer of bisphenol A, respectively, under both methanogenic and sulfate-reducing conditions. Additional experiments indicated that bisphenol A was highly persistent and that it was not

degraded within 162 days under conditions promoting either methanogenesis, sulfate-reduction, Fe(III)-reduction, or nitrate-reduction.

Degradation of atrazine (6-chloro-N-2-ethyl-N-4-isopropyl-1,3,5-triazine-2,4-diamine) and metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide] were determined in anaerobic wetland soil microcosms (44). In anaerobic soil, the half-life was 38 d for atrazine and 62 d for metolachlor. In the aqueous phase above the soil, the half-life was 86 d for atrazine and 40 d for metolachlor. Metabolites detected in the anaerobic soil were hydroxyatrazine and deethylatrazine for atrazine, and relatively small amounts of ethanesulfonic acid and oxanilic acid for metolachlor. Metabolites detected in the aqueous phase above the soil were hydroxyatrazine, deethylatrazine, and deisopropylatrazine for atrazine, and ethanesulfonic acid and oxanilic acid for metolachlor. The results indicate that atrazine and metolachlor can be degraded under the strongly reducing conditions found in wetland soils.

Complete degradation of yperite (bis(2-chloroethyl) sulfide), a chemical warfare agent, by two basidiomycetous strains (*Coriulus versicolor* IFO 30340 and *Tyromyces palustris* IFO 30339) was reported (54). Two distinct metabolic pathways were detected in each fungus during degradation of Yperite. The major path involved a non-enzymatic hydrolysis to generate thiodiglycol.

3.c. In Vitro Degradation of Chlorinated Compounds

In this quarter, four articles reported on the *in vitro* degradation of chlorinated compounds by enzymes.

The first publication evaluates the kinetics of haloalkane dehalogenase from the bacterium, from *Sphingomonas paucimobilis* UT26 (37). Haloalkane dehalogenases catalyze the hydrolysis of haloalkanes to their corresponding alcohols and inorganic halides. This study reports on the kinetic and structural analysis of dehalogenase (LinB) in complex mixtures of 1,2-DCA and 1,2-dichloropropane (1,2-DCP) and the reaction product of 1-chlorobutane. LinB had weak but measurable activities with 1,2-DCA and 1,2-DCP with V_{\max} of 0.012 and 0.027 nmol/s/mg of enzyme, respectively. The activity of LinB on 1-chlorobutane is much higher, 68.2 nmol/s/mg of enzyme. Inhibition analysis reveals that both 1,2-DCA and 1,2-DCP act as simple competitive inhibitors of the substrate 1-chlorobutane.

The second and third publication report on halohydrin dehalogenase from the bacterium *Agrobacterium radiobacter* (16, 49). Halohydrin dehalogenases are involved in the degradation of several important environmental pollutants, such as 1,3-dichloro-2-propanol and

epichlorohydrin. These enzymes catalyze the intra-molecular nucleophilic displacement of a halogen by a hydroxyl group in halohydrins, producing the corresponding epoxides, and also catalyze the reverse reaction. For some halohydrins this reaction proceeds with high enantioselectivity, which makes halohydrin dehalogenases valuable tools for biocatalysis. Halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 is a homo-tetrameric protein containing three cysteines per 28 kDa subunit. Under oxidizing conditions the enzyme was found to be susceptible to inactivation which could be prevented by the addition of beta-mercapto-ethanol or glycerol. Studies indicate that inactivation coincided with intramolecular disulfide bond formation. To confirm the involvement of cysteine residues in the inactivation, a set of cysteine mutant enzymes was constructed (49). Some of the mutants displayed higher stability compared to wild type enzyme indicating cysteine involvement in activation and suggesting a strategy to improve the enzyme for biocatalysis. The importance of the improved stability of the mutant enzymes was demonstrated by performing kinetic resolution experiments with racemic 2-chloro-1-phenylethanol, which resulted in higher enantiomeric excess values of the remaining halohydrin compared to conversions catalyzed by wild-type enzyme. The halohydrin dehalogenase was crystallized and a preliminary X-ray analysis was performed (16).

The fourth article studies the chlorine kinetic isotopic effect of 4-chlorobenzoyl-Coenzyme-A dehalogenase (4-CBA-CoA) catalyzed by *Pseudomonas* sp. strain 4-CBA-CoA (31). This enzyme participates in the 4-chlorobenzoate degradation pathway operational in bacteria adapted to the use of this soil pollutant as an energy source. The 4-CBA-CoA dehalogenase employs covalent catalysis. Following substrate binding to the enzyme, nucleophilic displacement of chloride ion by attack of an active-site carboxylate residue on C₄ of the benzoyl ring occurs. The measured value of $(37)k = 1.0090$ is larger than the chlorine kinetic isotopic effects recently measured for haloalkane and fluoroacetate dehalogenase. This indicates that the transition-state for dissociation of chloride ion from the Meisenheimer intermediate is sensitive to the chlorine isotopic substitution.

Two articles in this quarter reported on the *in vitro* degradation of chlorinated compounds by microbial metabolites or biogenic products. The first article characterizes a metal binding metabolite produced by *Pseudomonas* spp., pyridine -2,6-bis(monothiocarboxylic acid) (PDTC) (47). The metabolite is known to promote the biodegradation of carbon tetrachloride (CT). The characterization identified the first three protonation constants (pK) of the metabolite which were 5.48, 2.58 and 1.3. The study also measured the stability (affinity) constants (log K) for iron(III), nickel(II), and cobalt(III) by potentiometric or spectrophotometric titration; which were 33.36, 33.28 and 33.93; respectively. The results shows that over a full range of physiological pH the PDTC-Fe(III) complex is one of the most stable biological iron-chelators.

The dechlorination of CT by biogenic products of sulfate- and iron-reducing bacteria, Fe(II), S²⁻ and FeS was examined with and without the presence of vitamin B12, a cofactor known to catalyze CT reduction (4). Homogenous phase Fe(II) or S²⁻ reaction with CT resulted in formation of dichloromethane (DCM) and chloroform (CF). Dechlorination products were also observed in heterogeneous systems where FeS (75 to 200 mM) acted as the bulk reductant and the addition of vitamin B12 resulted in an enhancement of the dechlorination reaction. When 4 mM of vitamin B-12 were added to 200 mM FeS, CT was removed continuously and the amount of CF and DCM formed increased significantly over time, yielding a mass recovery of 40% and higher after 1 h and a pseudo-first-order rate constant of 1.91 h⁻¹. The reductive dechlorination of CT in the absence of vitamin B12, resulted in a slower disappearance of CT and the formation of smaller amounts of CF and DCM accounting for only 1% of the mass loss.

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

Characterization of Microbial Populations

A molecular method to assess the diversity of *Burkholderia* species in environmental samples was described (41). The PCR-denaturing gradient gel electrophoresis (DGGE) method developed was capable of distinguish the majority of the 14 *Burkholderia* species tested. Analysis of the *Burkholderia* communities in two grassland plots revealed differences in diversity mainly between bulk and rhizosphere soil samples; the communities in the latter samples produced more complex patterns. The PCR-DGGE method developed could be a useful tool to monitor the role of *Burkholderia* spp in the reduction of organochlorine compounds from contaminated soil by natural attenuation or bioremediation efforts. The genus *Burkholderia* is an important component of the soil microbial community, and several *Burkholderia* strains have been shown to degrade organochlorine compounds.

Isotopic analysis

The ratios of two stable Cl isotopes (³⁷Cl/ ³⁵Cl) can be a useful indicators for tracing the sources of pollutants and their reactions in the environment. An improved method for chlorine stable isotope determination of chlorinated aliphatic hydrocarbons by thermal ionization mass spectrometry (TIMS) of C₂Cl⁺ has been developed (36). The study presents δ ³⁷Cl values (parts per mil deviations from the standard seawater (ratio ³⁷Cl/ ³⁵Cl) for 10 commercial chlorinated aliphatic hydrocarbons (three chloromethanes, two chloroethanes and five chloroethenes), provided by six suppliers.

Monitoring of Biodegradation Stereoselectivity

Biphenyls with chlorine atoms in *ortho* positions of the phenyl rings can be defined as molecules with axial chirality, in which rotation about the axis is hindered by steric congestion. The enantiomers of chiral compounds may have different biological and toxicological effects. Electrokinetic chromatography with cyclodextrins was used as a method to monitor the stereoselectivity of biodegradation of chiral PCBs (23). The biodegradation of several chiral polychlorinated biphenyls (PCBs, IUPAC numbers 45, 88, 91, 95, 136, 144, 149, and 176) by a naturally occurring soil bacterium (identified as *Jonibacter* sp.) as well as the stereoselectivity of such process was investigated using electrokinetic chromatography. To date, the technique most widely employed to analyse achiral or chiral PCBs has been gas chromatography. The results showed a high degree of biodegradation (from 61% to 94%) for the eight PCBs studied after 262 hours of incubation. The highest degradation rates were observed for the least chlorinated congeners (PCB45 and PCB88). Secondly, measurement of the atropisomeric ratio of the PCB atropisomers during the biodegradation process revealed no significant variation of the atropisomeric ratios with the degradation time, indicating a non-stereoselective degradation of PCBs by the microorganism.

4. MICROBIAL CHLORINATION

4.a. General Reviews

Review articles on microbial dehalogenation were not available in the first quarter of the year 2002. Some attention is given to heme-containing chloroperoxidases in a recent review on the molecular aspects (*i.e.* structural features, molecular genetics) and the potential biotechnological applications of fungal peroxidases (15). Similarly to other haloperoxidases, chloroperoxidase catalyses oxidative dehydrogenation reactions, oxygen transfer reactions, H₂O₂ disproportionations and oxidative chlorinations in which Cl is transferred to an organic substrate.

4.b. Microbial Chlorination in Soils

Chloromethanes

No reports concerning the formation of chloromethanes by soil microorganisms were found in the first quarter of 2002.

Other Chlorinated Compounds

Two new chlorinated diphenyl ethers (compounds **5** and **6** in Fig. 1) have been isolated from the culture broth of an *Aspergillus* species obtained from leaf litter (25).

A structurally related (compound **7**) mono-chloro derivative has been isolated previously from *Penicillium citrinum*. Chlorinated diphenyl ethers are common constituents of lichens and marine sponges, but are relatively rare as fungal metabolites. In fungi, two main types are observed. Chlorination in the A-ring is found in metabolites from *Penicillium* and *Aspergillus* spp., whereas B-ring chlorination occurs in metabolites from *Xylaria* and *Pestalotiopsis* spp.

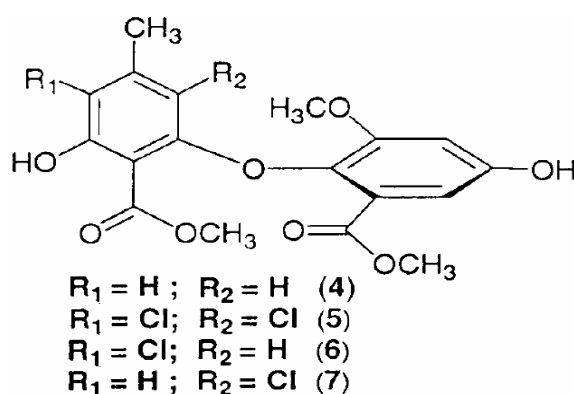


Figure 1. Chlorinated diphenyl ether metabolites isolated from the culture medium of an *Aspergillus* species (25).

Chemical synthesis of two chlorinated fungal metabolites found in the natural environment, (1R,2S)-1-(3'-chloro-4'-methoxyphenyl)-1,2-propanediol (Trametol) and (1R,2S)-1-(3',5'-dichloro-4'-methoxyphenyl)-1,2-propanediol, was described by Kousaka *et al.* (2002). The former compound was isolated earlier in cultures of the basidiomycete fungi, *Trametes* sp. IVP-F640 and *Bjerkandera* sp. BOS55. The latter compound is another metabolite of *Bjerkandera* sp. BOS55

4.c. Chlorination by Marine and Freshwater Organisms

Chloromethanes

No reports concerning the formation of chloromethanes by marine and freshwater microorganisms were found in the first quarter of 2002.

Other Chlorinated Compounds

Chlorination of tyrosine residues in bacterial proteins by hydrochlorous acid was shown to be a mechanism contributing to phagocytosis of bacteria such as *Staphylococcus aureus* by white blood cells (13). Furthermore, in the study, myeloperoxidase is proposed to play a central role in bacterial killing by generating hydrochlorous acid within neutrophil phagosomes (a type of inflammatory cells). Myeloperoxidase is an enzyme known to catalyze the formation of hypochlorous acid from hydrogen peroxide and chloride. Surprisingly, the majority of hypochlorous acid produced during phagocytosis was found to react with neutrophil components rather than the ingested bacteria. Only 6% of the chlorinated tyrosines were present in bacterial proteins. These results provide evidence to support the hypothesis that neutrophils use the myeloperoxidase/hydrogen peroxide/chloride system to generate hypochlorous acid for antimicrobial defense.

Earlier studies (cited by the authors) had already shown that hypohalous acids are formed when neutrophils undergo phagocytosis. Similarly, several studies indicate that myeloperoxidase is required for the majority of oxygen-dependent killing of certain bacteria such as *Staphylococcus aureus*. However, this is the first study that demonstrates that hypochlorous acid is produced in phagosomes when neutrophils engulf bacteria and that this oxidant reacts with bacteria.

Five publications report on the isolation and structural characterization of several new halogenated metabolites from marine organisms. Three of these studies are concerned with red algae, one with a green alga, and one with a sponge. An additional publication presents bioaccumulation data of a natural organohalogen of unknown origin, the so-called Q1

compound, in several marine animals. A bromo-sesquiterpene, bromocyclococanol 1, containing fused cyclopropane-cyclopentane rings leading a novel carbon skeleton has been isolated from the red alga *Laurencia obtusa* (9).

Three new minor linear polyhalohydroxylated marine monoterpenes, plocamenols A-C, have been isolated from the red alga *Plocamium cartilagineum* collected in Chile (18). The molecular formula of the plocamenol compounds contains two bromine atoms and one chlorine atom (compounds **1-3** in Fig. 2). Two of these compounds contain a terminal bromohydrin, and the other compound is the corresponding keto derivative. Marine monoterpene compounds with these functionalities are unusual. It is well known from the literature that most of the polyhalogenated metabolites within the marine monoterpene family (both cyclic and acyclic) are characterized by possessing a terminal 1-chloro- or 1-bromovinyl systems or the corresponding dehalo terminal double bond.

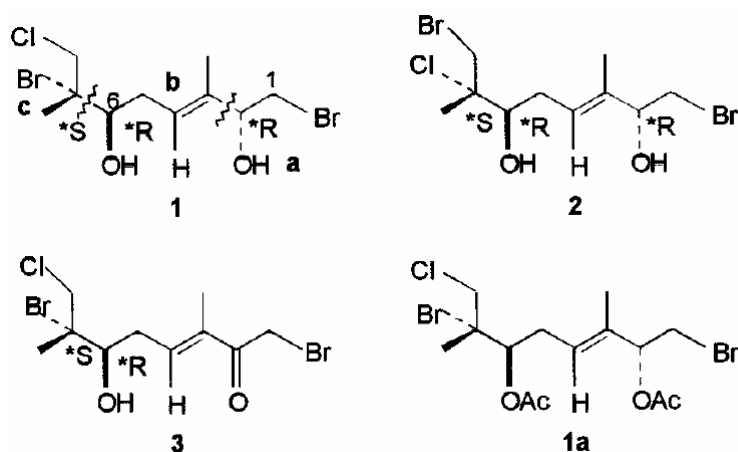


Figure 2. Chemical structure of the polyhalogenated plocamenols isolated from the red alga *Plocamium cartilagineum* (18).

A novel brominated diterpene, named neoirietetraol, has been isolated along with a halogenated C15 acetogenin, (3Z)-laurenyne, from a new Okinawan red alga, *Laurencia yonaguniensis* (48). Neoirietetraol was toxic in a bioassay using shrimp and also showed weak antibacterial activities against two marine bacteria. Six new prenylated bromohydroquinones and a related 6-hydroxy derivative of cymopochromenol have been isolated from the green marine alga *Cymopolia barbata* (19). The structures of these metabolites were determined by spectral methods. One study reports on the isolation of new bromoindoles in the Southern

Australian marine sponge *Hymeniacidon* sp. (12). The structure of these compounds was assigned on the basis of detailed spectroscopic analysis and total synthesis. The newly described compounds displayed nematocidal activity.

Environmental occurrence of Q1, a natural heptachloro compound with the molecular formula $C_9H_3Cl_7N_2$, in samples from the Northern Hemisphere was investigated by Vetter et al. (2002) (51). The structure of this compound it is still to be elucidated, but the authors report that mass spectra suggest that Q1 has a bipyrrole backbone (compounds **1** or **4**, Fig. 3). A related natural organohalogen, the hexabromobipyrrole **2** (Fig. 3), was isolated in the 70's from marine bacteria (*Chromobacterium* sp.). This study as well as earlier reports by the same authors (e.g., see discussion of Vetter *et al.* 2001 in 4th Quaterly report – Year 2001) show that Q1 is a persistent and a bioaccumulative contaminant. The highest Q1 concentrations were found in the Southern Hemisphere, but the compound was also detected in environmental samples from the Northern Hemisphere. In addition to marine mammals and birds, Q1 was also detected in fish from the Mediterranean Sea and the Antarctic. Traces were also detected in SRM 1588 certified cod liver oil, but Q1 was not detected in fish from Hong Kong and Lake Baikal.

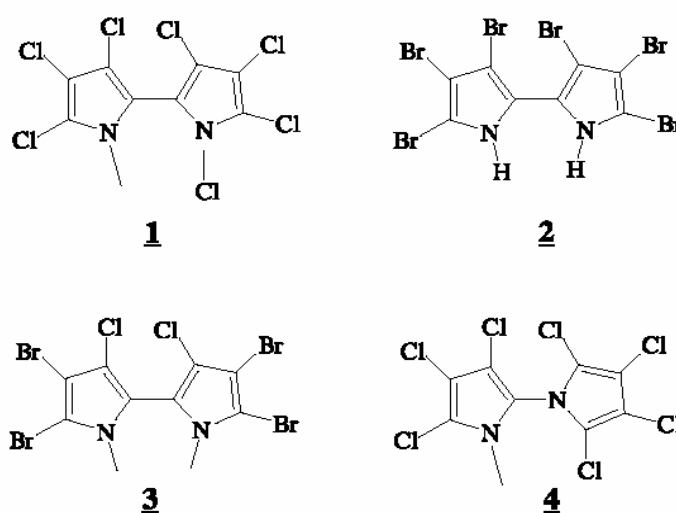


Figure 3. Structures of halogenated bipyrroles detected in the environment: (**1**) suggested structure for Q1 (1',3,3',4,4',5,5'-heptachloro-1-methyl-2,2'-bipyrrole), (**2**) 3,3',4,4',5,5'-hexa-bromo-2,2'-bipyrrole, (**3**) 5,5'-dichloro-1,1'-dimethyl-3,3',4,4'-tetrabromo-2,2'-bipyrrole, (**4**) 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (alternative structure suggested for Q1) (51).

4.d. Chlorinating Enzymes

No articles were found this quarter on the formation of the target chlorinated compounds by halogenating enzymes. One publication is discussed in this section that reports on the function of a halogenase and a haloperoxidase/perhydrolase in the biosynthesis of glycopeptides by the actinomycete *Amycolatopsis mediterranei* DSM5908 (39). Glycopeptides are important antibiotics consisting of a glycosylated and chlorinated heptapeptide backbone. The best known example is vancomycin, an antibiotic of last resort used against multi-resistant gram-positive bacteria. Interest in the development of new glycopeptides, and as a consequence in understanding the biosynthesis of these compounds, is increasing with recent reports of the occurrence of vancomycin-resistant bacteria. Puk *et al.* (2002) used the antibiotic balhimycin as a model system to investigate gene function on the halogenation of glycopeptides. The balhimycin biosynthetic gene cluster is known to contain a gene (*bhaA*) putatively coding for an FADH₂-dependent halogenase. In this study, the balhimycin biosynthetic gene cluster was also shown to contain a gene (*bhp*) that putatively codes for a perhydrolase. However, only the halogenase BhaA was found to be required for chlorination of balhimycin.

Another publication reports on the immobilization of a chloroperoxidase (CPO) on from the fungus *Caldariomyces fumago* (24). The CPO was immobilized onto a mesoporous silicate material, mesocellular foam. The immobilized CPO was shown to retain its activity. The optimal pH at which the maximum amount of enzyme is immobilized was determined to be pH 3.4. A weak ionic interaction between the enzyme and the surface of the inorganic substrate is thought to be critical in maintaining the activity of the immobilized enzyme. The loading capacity of MCF is 122 mg protein per g of mesocellular foam.

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6. ANNEX

Abou-Arab, A. A. K. (2002). "Degradation of organochlorine pesticides by meat starter in liquid media and fermented sausage." *Food and Chemical Toxicology* 40(1): 33-41.

The effect of meat starter on the degradation of DDT and lindane was investigated. The insignificant role of *Lactobacillus plantarum* in degrading p,p'-DDT and lindane presented in tryptone soya broth (TSB) and mineral salt medium (MSM) with or without 120 ppm nitrite was observed. The degradation of DDT and lindane by *Micrococcus varians* in TSB and MSM with or without nitrite were studied. The results indicated that DDT or lindane were degraded during the incubation period. The reduction in DDT at the end of the incubation period (15 days) was about 24.1 and 32.5% in TSB and MSM without nitrite, respectively. Corresponding values in the same media with nitrite were 37.5 and 46.4%. Regarding the reduction in lindane, it was recorded as 27.9 and 40.0% in TSB and MSM without nitrite, respectively and 38.4 and 48.4% in the same media with nitrite. The results indicated that culture media *M. varians* metabolized DDT mainly to DDD and lindane mainly to 2,4-, 2,5-, 2,6- and 3,4-dichlorophenol; 2,3,4- and 2,3,5-trichlorophenol; hexachlorobenzene; and pentachlorophenol. The effect of pesticides on the growth rate of meat starter was also investigated. The addition of DDT or lindane resulted in a slight decrease in counts of the strains during the initial incubation in TSB or MSM. Then the microorganisms recovered and began to grow logarithmically, but not as well as in a normal situation. The effect of fermentation stage by meat starter on DDT and lindane in fermented sausage was recorded. The results indicated that during the 72 h of fermentation, the reduction was 10 and 18% of DDT and lindane, respectively. These results confirmed that the fermentation process in meat products reduced pesticide residues and these reductions were due to the activity of meat starter. (C) 2001 Elsevier Science Ltd.

Adamson, D. T. and G. F. Parkin (2001). "Product distribution during transformation of multiple contaminants by a high-rate, tetrachlorethene-dechlorinating enrichment culture." *Biodegradation* 12(5): 337-348.

Radiolabeled tetrachloroethene (PCE) and carbon tetrachloride (CT) were added to batch systems containing a lactate- enrichment culture displaying apparent dehalorespiration abilities to analyze the influence of mixtures on product distribution. Both CT and PCE were readily dechlorinated, although significant carbon disulfide (CS₂) formation was observed during CT transformation. Calculated 1,2-C-14-PCE recoveries for biotic treatments were between 91 and 104%, but an inability to recover products such as CS₂ led to lower recoveries of C-14-CT (55 to 62%). While the majority of activity in C-14-CT-spiked treatments was recovered in the volatile fraction, (CO₂)-C-14 increased significantly over time. 1,2-C-14-PCE was primarily recovered in volatile and non-strippable fractions, but a significant increase in (CO₂)-C-14 relative to cell-free controls suggested that the presence of a non-specific dechlorination pathway complementing dehalorespiration. The addition of both CT and PCE inhibited the transformation of the individual compounds and reduced the percentages recovered as (CO₂)-C-14. However, the magnitude of these reductions was not severe and appeared to be the result of slower overall transformation rather than a complete inhibition of mineralization pathways.

Adrian, L. and H. Gorisch (2002). "Microbial transformation of chlorinated benzenes under anaerobic conditions." *Research in Microbiology* 153(3): 131-137.

Chlorobenzenes are reductively dechlorinated by anaerobic bacterial cultures obtained from sediments and sludge. Recently a strain was isolated that couples reductive dechlorination of chlorobenzenes with energy conservation. The results reviewed in this article suggest that additional anaerobic bacteria, thriving by dehalogenation of chlorobenzenes or chlorobiphenylic compounds, can be isolated. (C) 2002 Elsevier SAS.

Assaf-Anid, N. and K. Y. Lin (2002). "Carbon tetrachloride reduction by Fe²⁺, S²⁻, and FeS with vitamin B-12 as organic amendment." *Journal of Environmental Engineering-Asce* 128(1): 94-99.

The reductive dechlorination of carbon tetrachloride (CT) was examined in the presence of free iron ions (Fe²⁺), sulfide ions (S²⁻), and freshly precipitated ferrous sulfide (FeS) as reducing agents, and vitamin B-12 as organic amendment. The reductive dechlorination of CT by the reducing ions Fe²⁺ and S²⁻ in homogeneous phase resulted in the formation of variable amounts of the mono- and di-dechlorination products chloroform (CF) and

dichloromethane (DCM). In the ferrous chloride (FeCl_2) solutions (200 mM) where Fe^{2+} was the only electron source, 76% of the original CT was depleted within 1/2 h and about 28% and 8% went to CF and DCM, respectively, at a pH of 3.1 and with no buffer present. These same dechlorination products were observed in unbuffered sodium sulfide (Na_2S) solutions (110 and 200 mM) with S^{2-} as the electron source at pH 13.4. Dechlorination products were also observed in heterogeneous systems where FeS (75-200 mM) acted as the bulk reductant and the addition of vitamin B-12 (0-4 mM) resulted in an enhancement of the dechlorination reaction. When 4 mM of vitamin B-12 were added to 200 mM FeS , CT was removed continuously and the amount of CF and DCM formed increased significantly over time, yielding a mass recovery of 40% and higher after 1 h and a pseudofirst-order rate constant of 1.91 h^{-1} . The reductive dechlorination of CT in the absence of vitamin B12, resulted in a slower disappearance of CT and the formation of smaller amounts of CF and DCM accounting for only 1% of the mass loss in the 75 mM FeS suspensions to 15% in the 200 mM FeS suspensions after 2 h.

Bankston, J. L., D. L. Sola, et al. (2002). "Degradation of trichloroethylene in wetland microcosms containing broad-leaved cattail and eastern cottonwood." *Water Research* 36(6): 1539-1546.

Remediation of aquifers containing trichloroethylene (TCE) relies primarily on physical extraction of contaminated groundwater and soil. Unfortunately, this is typically expensive and does not always attain the desired treatment goals. In situ bioremediation via natural attenuation is an alternative treatment process in which TCE is transformed by indigenous microorganisms and plants. In this study, TCE was observed in a surficial aquifer that discharges into a wetland. Experiments were undertaken to determine whether natural attenuation of TCE in the wetland was possible. Microcosms were constructed using sandy soil +/- eastern cottonwoods (*Populus deltoides*) from the wetland's edge and organic soil +/- broad-leaved cattails (*Typha latifolia*) from the wetland's interior. (C-14) TCE was added to each microcosm (1.27 μCi). Overtime, C-14 was recovered from four microcosm compartments: (1) as C-14 bound to soil and water, (2) as volatilized (C-14) TCE, (3) as (C-14) CO_2 produced by mineralization of (C-14) TCE, and (4) as C-14 incorporated into the plants. Total recoveries of the C-14-label ranged from 73.6% to 95.8%. Volatilized (C-14) TCE accounted for the majority (> 50%) of the recovered label. In microcosms without plants, (C-14) CO_2 represented 3.2% (organic soil) to 15.6% (sandy soil) of the recovered ^{14}C , indicating that TCE was mineralized by indigenous microorganisms. The presence of the broad-leaved cattail resulted in increased production of (C-14) CO_2 to 5.3% in the organic soil. The data thus suggest that natural attenuation is a potential bioremediative strategy for TCE-contaminated wetlands.

Barth, J. A. C., G. Slater, et al. (2002). "Carbon Isotope Fractionation during Aerobic Biodegradation of Trichloroethene by *Burkholderia cepacia* G4: a Tool To Map Degradation Mechanisms." *Appl. Environ. Microbiol.* 68(4): 1728-1734.

The strain *Burkholderia cepacia* G4 aerobically mineralized trichloroethene (TCE) to CO_2 over a time period of [~] 20 h. Three biodegradation experiments were conducted with different bacterial optical densities at 540 nm (OD_{540s}) in order to test whether isotope fractionation was consistent. The resulting TCE degradation was 93, 83.8, and 57.2% (i.e., 7.0, 16.2, and 42.8% TCE remaining) at OD_{540s} of 2.0, 1.1, and 0.6, respectively. ODs also correlated linearly with zero-order degradation rates (1.99, 1.11, and 0.64 $\mu\text{mol h}^{-1}$). While initial nonequilibrium mass losses of TCE produced only minor carbon isotope shifts (expressed in per mille $\delta^{13}\text{C}$ VPDB), they were 57.2, 39.6, and 17.0 {per thousand} between the initial and final TCE levels for the three experiments, in decreasing order of their OD_{540s}. Despite these strong isotope shifts, we found a largely uniform isotope fractionation. The latter is expressed with a Rayleigh enrichment factor, ϵ , and was -18.2 when all experiments were grouped to a common point of 42.8% TCE remaining. Although, decreases of ϵ to -20.7 were observed near complete degradation, our enrichment factors were significantly more negative than those reported for anaerobic dehalogenation of TCE. This indicates typical isotope fractionation for specific enzymatic mechanisms that can help to differentiate between degradation pathways.

Brevik, K. and F. Wania (2002). "Mass budgets, pathways, and equilibrium states of two hexachlorocyclohexanes in the Baltic Sea environment." *Environ Sci Technol* 36(5): 1024-32.

The POPCYCLING-Baltic model, a non-steady-state multicompartamental mass balance model of long-term chemical fate in the Baltic Sea environment, is used to derive a quantitative understanding of the behavior of

alpha- and gamma-hexachlorocyclohexane (HCH) from 1970 to 2000. The atmosphere is found to effectively distribute the HCHs within the Baltic Sea environment and beyond, resulting in relatively uniform concentrations in environmental compartments that do not directly receive emissions. This uniformity is the result of a large-scale redistribution of a relatively small fraction of the emitted HCHs from the agricultural systems in source areas to all other environmental compartments throughout the Baltic Sea region. The major fraction of the HCHs is degraded in the soils receiving the pesticide application. In areas where HCH-containing pesticides are used, HCHs evaporate from soils and water bodies and are advected away in the atmosphere. They are deposited to forests and water bodies when they reach remote regions. This redistribution is driven by the inclination of the HCHs to equalize their chemical potential within the environment, which is illustrated through the use of fugacity fractions. The model is believed to provide useful insight into the complex set of interactions that determine the overall fate of an environmental contaminant but which are inaccessible to measurements.

Brennan, R. A. and R. A. Sanford (2002). "Continuous steady-state method using tenax for delivering tetrachloroethene to chloro-respiring bacteria." *Applied and Environmental Microbiology* 68(3): 1464-1467.

Tenax-TA, a solid-phase sorbent, was used as an alternative to hexadecane for continuous delivery of tetrachloroethene (PCE) to *Desulfuromonas* strain BB1, a chloro-respiring microorganism. In both batch and bioreactor configurations, Tenax not only maintained low, steady-state concentrations of PCE in an active culture for several months but also adsorbed the product of dechlorination, cis-1,2-dichloroethene, before it approached toxic levels.

Brito, I., M. Cueto, et al. (2002). "Bromocyclococanol, a halogenated sesquiterpene with a novel carbon skeleton from the red alga *Laurencia obtusa*." *Tetrahedron Letters* 43(14): 2551-2553.

A bromo sesquiterpene, bromocyclococanol 1, containing fused cyclopropane-cyclopentane rings leading a novel carbon skeleton has been isolated from the red alga *Laurencia obtusa*. The structure and stereochemistry were established by spectroscopic evidence and biogenetic considerations. A biogenetic route for this compound has also been proposed.

Campanella, B. F., C. Bock, et al. (2002). "Phytoremediation to increase the degradation of PCBs and PCDD/Fs. Potential and limitations." *Environ Sci Pollut Res Int* 9(1): 73-85.

Phytoremediation is already regarded as an efficient technique to remove or degrade various pollutants in soils, water and sediments. However, hydrophobic organic molecules such as PAHs, PCBs and PCDD/Fs are much less responsive to bioremediation strategies than, for example, BTEX or LAS. PCDD/Fs and PCBs represent 3 prominent groups of persistent organic pollutants that share common chemical, toxicological and environmental properties. Their widespread presence in the environment may be explained by their chemical and biological stability. This review considers their fate and dissipation mechanisms. It is then possible to identify major sinks and to understand biological activities useful for remediation. Public health and economic priorities lead to the conclusion that alternative techniques to physical treatments are required. This review focuses on particular problems encountered in biodegradation and bioavailability of PCDD/Fs and PCBs. It highlights the potential and limitations of plants and micro-organisms as bioremediation agents and summarises how plants can be used to augment bacterial activity. Phytoremediation is shown to provide some new possibilities in reducing risks associated with dioxins and PCBs.

Canada, K. A., S. Iwashita, et al. (2002). "Directed evolution of toluene ortho-monooxygenase for enhanced 1-naphthol synthesis and chlorinated ethene degradation." *Journal of Bacteriology* 184(2): 344-349.

Trichloroethylene (TCE) is the most frequently detected groundwater contaminant, and 1-naphthol is an important chemical manufacturing intermediate. Directed evolution was used to increase the activity of toluene ortho-monooxygenase (TOM) of *Burkholderia cepacia* G4 for both chlorinated ethenes and naphthalene oxidation. When expressed in *Escherichia coli*, the variant TOM-Green degraded TCE (2.5 +/- 0.3 versus 1.39 +/- 0.05 nmol/min/mg of protein), 1,1-dichloroethylene, and trans-dichloroethylene more rapidly. Whole cells expressing TOM-Green synthesized 1-naphthol at a rate that was six times faster than that mediated by the wild-type enzyme

at a concentration of 0.1 mM (0.19 +/- 0.03 versus 0.029 +/- 0.004 nmol/min/mg of protein), whereas at 5 mM, the mutant enzyme was active (0.07 +/- 0.03 nmol/min/mg of protein) in contrast to the wild-type enzyme, which had no detectable activity. The regiospecificity of TOM-Green was unchanged, with greater than 97% 1-naphthol formed. The beneficial mutation of TOM-Green is the substitution of valine to alanine in position 106 of the alpha-subunit of the hydroxylase, which appears to act as a smaller "gate" to the diiron active center. This hypothesis was supported by the ability of *E. coli* expressing TOM-Green to oxidize the three-ring compounds, phenanthrene, fluorene, and anthracene faster than the wild-type enzyme. These results show clearly that random, in vitro protein engineering can be used to improve a large multisubunit protein for multiple functions, including environmental restoration and green chemistry.

Capon, R. J., C. Skene, et al. (2002). "Equilibrating isomers: bromoindoles and a seco-xanthine encountered during a study of nematocides from the Southern Australian marine sponge *Hymeniacion* sp." *Journal of Natural Products* 65(3): 368-370.

Bioassay-directed fractionation of a *Hymeniacion* sp. yielded as nematocidal agents the equilibrating E/Z bromoindole ethyl esters 1 and 2 and corresponding methyl esters 3 and 4. Also isolated for the first time as a natural product was an equilibrating mixture of seco-xanthine formamides, attributed the trivial name hymeniacionin (5). The structure for 5 was assigned on the basis of detailed spectroscopic analysis and total synthesis.

Carvalho, M. F., C. C. T. Alves, et al. (2002). "Isolation and initial characterization of a bacterial consortium able to mineralize fluorobenzene." *Appl. Environ. Microbiol.* 68(1): 102-105.

Fluorinated compounds are known to be more resistant to microbial degradation than other halogenated chemicals. A microbial consortium capable of aerobic biodegradation of fluorobenzene (FB) as the sole source of carbon and energy was isolated by selective enrichment from sediments collected in a drain near an industrial site. A combination of three microbial strains recovered from the enriched consortium was shown to be necessary for complete FB mineralization. Two of the strains (F1 and F3) were classified by 16S rRNA analysis as belonging to the *Sphingobacterium*/*Flavobacterium* group, while the third (F4) falls in the β -Proteobacteria group, clustering with *Alcaligenes* species. Strain F4 was consistently found in the liquid cultures in a much greater proportion than strains F1 and F3 (86:8:6 for F4, F1, and F3, respectively). Stoichiometric release of fluoride ions was measured in batch and fed-batch cultures. In batch cultures, the consortium was able to use FB up to concentrations of 400 mg liter⁻¹ and was able to utilize a range of other organic compounds, including 4-fluorophenol and 4-fluorobenzoate. To our knowledge this is the first time biodegradation of FB as a sole carbon source has been reported.

Chapman, A. L. P., M. B. Hampton, et al. (2002). "Chlorination of bacterial and neutrophil proteins during phagocytosis and killing of *Staphylococcus aureus*." *Journal of Biological Chemistry* 277(12): 9757-9762.

Myeloperoxidase is proposed to play a central role in bacterial killing by generating hypochlorous acid within neutrophil phagosomes. However, it has yet to be demonstrated that these inflammatory cells target hypochlorous acid against bacteria inside phagosomes. In this investigation, we treated *Staphylococcus aureus* with varying concentrations of reagent hypochlorous acid and found that even at sublethal doses, it converted some tyrosine residues in their proteins to 3-chlorotyrosine and 3,5-dichlorotyrosine. To determine whether or not ingested bacteria were exposed to hypochlorous acid in neutrophil phagosomes, we labeled their proteins with [¹³C-13(6)]tyrosine and used gas chromatography with mass spectrometry to identify the corresponding chlorinated isotopes after the bacteria had been phagocytosed. Chlorinated tyrosines were detected in bacterial proteins 5 min after phagocytosis and reached levels of approximately 2.5/1000 mol of tyrosine at 60 min. Inhibitor studies revealed that chlorination was dependent on myeloperoxidase. Chlorinated neutrophil proteins were also detected and accounted for 94% of total chlorinated tyrosine residues formed during phagocytosis. We conclude that hypochlorous acid is a major intracellular product of the respiratory burst. Although some reacts with the bacteria, most reacts with neutrophil components.

Collins, C., F. Laturus, et al. (2002). Remediation of BTEX and trichloroethene. Current knowledge with special emphasis on phytoremediation. *Environ Sci Pollut Res Int.* 9: 86-94.

The widespread use of industrial chemicals in our highly industrialized society has often caused contamination of large terrestrial and marine areas due to the deliberate and accidental release of organic pollutants into the soil and groundwater. In this review, environmental problems arising from the use of chlorinated solvents and BTEX compounds are described, and an overview about active management strategies for remediation with special emphasis on phytoremediation are presented to achieve a reduction of the total mass of chlorinated solvents and BTEX compounds in contaminated areas. Phytoremediation has been proposed as an efficient, low-cost remediation technique to restore areas contaminated with chlorinated solvents and BTEX compounds. The feasibility of phytoremediation as a remediation tool for these compounds is discussed with particular reference to the uptake and metabolism of these compounds, and a future perspective on the use of phytoremediation for the removal of chlorinated solvents and BTEX compounds is given.

Conesa, A., P. J. Punt, et al. (2002). Fungal peroxidases: molecular aspects and applications. *Journal of Biotechnology.* 93: 143-158.

Peroxidases are oxidoreductases that utilize hydrogen peroxide to catalyze oxidative reactions. A large number of peroxidases have been identified in fungal species and are being characterized at the molecular level. In this manuscript we review the current knowledge on the molecular aspects of this type of enzymes. We present an overview of the research efforts undertaken in deciphering the structural basis of the catalytic properties of fungal peroxidases and discuss molecular genetics and protein homology aspects of this enzyme class. Finally, we summarize the potential biotechnological applications of these enzymes and evaluate recent advances on their expression in heterologous systems for production purposes. (C) 2002 Elsevier Science B.V.

de Jong, R. M., H. J. Rozeboom, et al. (2002). "Crystallization and preliminary X-ray analysis of an enantioselective halohydrin dehalogenase from *Agrobacterium radiobacter* AD1." *Acta Crystallographica Section D-Biological Crystallography* 58: 176-178.

Halohydrin dehalogenases are key enzymes in the bacterial degradation of vicinal halopropanols and structurally related nematocides. Crystals of the enantioselective halohydrin dehalogenase HheC from *Agrobacterium radiobacter* AD1 have been obtained at room temperature from hanging-drop vapour-diffusion experiments against 50-70% saturated ammonium sulfate solution at pH 6.5-7.3. The crystals belong to space group P4(1)2(1)2 or P4(3)2(1)2, with unit-cell parameters $a = b = 104.5$, $c = 121.4$ Angstrom, and contain two monomers in the asymmetric unit. The crystals diffract to 3.0 Angstrom resolution with X-rays from a Cu K alpha rotating-anode generator.

Dermietzel, J. and A. Vieth (2002). "Chloroaromatics in groundwater: chances of bioremediation." *Environmental Geology* 41(6): 683-689.

The potential for biodegrading of mono-, di- and trichlorobenzenes in a contaminated aquifer in Bitterfeld (Saxony-Anhalt) was tested both in the laboratory and using on-site column experiments. Under the prevailing anaerobic conditions, the reductive dechlorination of 1,4-dichlorobenzene (1,4-DCB) takes place very slowly. Under aerobic conditions the indigenous micro-organisms are able to mineralize monochlorobenzene (MCB) and 1,4-DCB. The degradation rates for the other two isomeric dichlorobenzenes and for 1,2,4-trichlorobenzene (1,2,4-TCB) under aerobic conditions are significantly lower. Indications were found that once the oxygen has been consumed, Fe(III) species can be used as alternative electron acceptors.

Díaz-Marrero, A. R., J. Rovirosa, et al. (2002). "Plocamenols A-C, novel linear polyhalohydroxylated monoterpenes from *Plocamium cartilagineum*." *Journal of Natural Products* 65(4): 585-588.

Three new minor linear polyhalohydroxylated marine monoterpenes, plocamenols A-C (1-3), have been isolated from the red alga *Plocamium cartilagineum*. The structure and relative stereochemistry of these compounds were determined on the basis of spectroscopic evidence.

Dorta, E., J. Darias, et al. (2002). "New Prenylated Bromoquinols from the Green Alga *Cymopolia barbata*." *Journal of Natural Products* 65(3): 329-3333.

Six new prenylated bromohydroquinones, 3-*o*-methoxy-7-hydroxycymopol (1), 3-hydroxycymopolone (2), 3,7-dihydroxycymopolone (3), 7-hydroxycymopochromanone (4), 7-hydroxycymopochromenol (5), and a related 6-hydroxy derivative of cymopochromenol (6), have been isolated from the green marine alga *Cymopolia barbata*. The structures of these cymopol-related metabolites were determined by spectral methods.

Drzyzga, O. and J. C. Gottschal (2002). "Tetrachloroethene dehalorespiration and growth of *Desulfitobacterium frappieri* TCE1 in strict dependence on the activity of *Desulfovibrio fructosivorans*." *Appl. Environ. Microbiol.* 68(2): 642-649.

Tetrachloroethene (PCE) dehalorespiration was investigated in a continuous coculture of the sulfate-reducing bacterium *Desulfovibrio fructosivorans* and the dehalorespiring *Desulfitobacterium frappieri* TCE1 at different sulfate concentrations and in the absence of sulfate. Fructose (2.5 mM) was the single electron donor, which could be used only by the sulfate reducer. With 2.5 mM sulfate, the dehalogenating strain was outnumbered by the sulfate-reducing bacterium, sulfate reduction was the dominating process, and only trace amounts of PCE were dehalogenated by strain TCE1. With 1 mM sulfate in the medium, complete sulfate reduction and complete PCE dehalogenation to *cis*-dichloroethene (*cis*-DCE) occurred. In the absence of sulfate, PCE was also completely dehalogenated to *cis*-DCE, and the population size of strain TCE1 increased significantly. The results presented here describe for the first time dehalogenation of PCE by a dehalorespiring anaerobe in strict dependence on the activity of a sulfate-reducing bacterium with a substrate that is exclusively used by the sulfate reducer. This interaction was studied under strictly controlled and quantifiable conditions in continuous culture and shown to depend on interspecies hydrogen transfer under sulfate-depleted conditions. Interspecies hydrogen transfer was demonstrated by direct H₂ measurements of the gas phase and by the production of methane after the addition of a third organism, *Methanobacterium formicicum*.

Fava, F. and V. F. Ciccotosto (2002). "Effects of randomly methylated-beta-cyclodextrins (RAMEB) on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in three pristine soils spiked with a transformer oil." *Applied Microbiology and Biotechnology* 58(3): 393-399.

The low bioavailability of polychlorinated biphenyls (PCBs) in soils often results in their slow and partial aerobic biodegradation. The process can be enhanced by supplementing soils with cyclodextrins. However, pure cyclodextrins are expensive and we have therefore explored the use of a less costly technical-grade mixture of randomly methylated-beta-cyclodextrins (RAMEB). RAMEB was tested at 0, 1, 3 and 5% (w/w) in the aerobic bioremediation and detoxification of a loamy-, a humic- and a sandy-soil, each artificially contaminated with a PCB-containing transformer oil (added PCBs: about 450 or 700 mg/kg), inoculated with an exogenous aerobic PCB-biodegrading bacterial co-culture and treated in slurry- and solid-phase laboratory conditions. Significant depletions of the spiked PCBs were observed in all microcosms of the three soils after 90 days of treatment; however, interesting yields of PCB dechlorination and detectable decreases of the original soil ecotoxicity were observed in the slurry-phase microcosms. RAMEB generally enhanced PCB-metabolism with effects which were dependent on the concentration at which it was applied, the physical-chemical nature of the amended soil, and the soil treatment conditions employed. RAMEB, which was slowly metabolized by soil microorganisms, enhanced the presence of PCBs and PCB-cometabolizing bacteria in the soil-water phase, suggesting that RAMEB enhances aerobic biodegradation of PCBs by increasing pollutant bioavailability in soil microcosms.

Fava, F. and A. Piccolo (2002). "Effects of humic substances on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in a model soil." *Biotechnology and Bioengineering* 77(2): 204-211.

The very high hydrophobicity of polychlorinated biphenyls (PCBs) strongly reduces their bioavailability in aged contaminated soils, thus limiting their bioremediation. The biodegradability of PCBs in heavily contaminated soils can be significantly enhanced by soil treatment with surface-active agents. In this work, the effects of naturally occurring surfactants such as humic substances (HS) on the aerobic biodegradation of PCBs in a model soil were studied. The soil was amended with biphenyl (4 g/kg), Fenclor 42 (1,000 mg/kg), the aerobic

PCB-biodegrading bacterial co-culture ECO3 (inoculum: 10(8) CFU/mL), and treated in aerobic batch slurry-phase conditions (17.5% w/v) with and without the addition of HS at the rates of 1.5 and 3.0% (w/w). Low PCBs biodegradation and dechlorination yields were observed in the HS-free microcosms, probably as a result of the rapid disappearance of inoculated bacteria. The presence of HS influenced significantly the activity of the specialized biomass and the biodegradation of PCBs in the microcosms. The microcosms that received HS at the 1.5% rate showed a higher persistence of the specialized bacteria and yields of PCB biodegradation and dechlorination about 150 and 100%, respectively, larger than those found for the HS-free microcosms. Lower stimulating effects were observed in the microcosms added with the HS at 3.0% rate. These effects were attributed to an increased solubilization of PCBs in the hydrophobic domains of the humic supramolecular associations and to a different accessibility of PCBs by the specialized bacteria at the different rates of HS addition. Although the slurry-phase treatment generally showed a decrease of the original soil ecotoxicity, the addition of the originally non-toxic HS decreased soil ecotoxicity for the *Collembola* animal biomarker and increased that towards the *Lepidium sativum* vegetal biomarker. (C) 2002 John Wiley Sons, Inc.

Garcia-Ruiz, C., R. Andres, et al. (2002). "Monitoring the stereoselectivity of biodegradation of chiral polychlorinated biphenyls using electrokinetic chromatography." *Journal of Separation Science* 25(1-2): 17-22.

A study of the biodegradation of several chiral polychlorinated biphenyls (PCBs, IUPAC numbers 45, 88, 91, 95, 136, 144, 149, and 176) by a naturally occurring soil bacterium as well as the stereoselectivity of such process has been performed using Electrokinetic Chromatography with cyclodextrins (CD-EKC). In order to achieve the analysis of chiral PCBs, a CD-EKC system based on 50 mM 2-morpholinoethanesulfonic acid (MES) buffer (pH 6.5) containing 2 M urea, 20 mM carboxymethylated gamma-cyclodextrin (CM-gamma-CD), and 10 mM beta-cyclodextrin (beta-CD) was used. First, the percentage degradation of PCBs by the microorganism used in this work was measured for different incubation times (22, 46, 71, 93, 165, 213, and 262 hours). The results showed a high degree of biodegradation (from 61% to 94%) for the eight PCBs studied after 262 hours of incubation with the microorganism, degradation being favoured for the least chlorinated congeners. Secondly, measurement of the atropisomeric ratio of the PCB atropisomers during the biodegradation process revealed no significant variation of the atropisomeric ratios with the degradation time, indicating a non-stereoselective degradation of PCBs by the microorganism.

Han, Y. J., J. T. Watson, et al. (2002). "Catalytic activity of mesoporous silicate-immobilized chloroperoxidase." *Journal of Molecular Catalysis B-Enzymatic* 17(1): 1-8.

A versatile enzyme, FeHeme chloroperoxidase (CPO) from *Caldariomyces fumago*, is immobilized in the mesoporous silicate material, mesocellular foam (MCF). MCF is a promising material for immobilizing enzymes, due to its large pore structure and high loading capacity compared to other mesoporous materials, such as MCM-48, SBA-16 and SBA-15. The immobilized CPO in MCF retains its activity. The optimal pH at which the maximum amount of enzyme is immobilized was determined to be pH 3.4, slightly below the isoelectric point of the enzyme. A weak ionic interaction between the enzyme and the surface of the inorganic substrate is thought to be critical in maintaining the activity of the immobilized enzyme. The loading capacity of MCF is 122 mg protein per 1 g of MCF. We demonstrate the advantage of MCF as an inorganic substrate for immobilization of enzymes. (C) 2002 Elsevier Science B.V.

Hargreaves, J., J.-O. Park, et al. (2002). "New chlorinated diphenyl ethers from an *Aspergillus* species." *Journal of Natural Products* 65(1): 7-10.

Two new chlorinated diphenyl ethers (5, 6) have been isolated from the culture broth of an *Aspergillus* species obtained from leaf litter, together with the known benzophenone sulochrin (1), the grisandiene geodin (2), and the diphenyl ether astringic acid (3). The structure of another metabolite, methyl astringate (4), was confirmed by single-crystal X-ray structure analysis.

Hashimoto, A., K. Iwasaki, et al. (2002). "Degradation pathways of trichloro ethylene and 1,1,1-trichloroethane by *Mycobacterium* sp TA27." *Bioscience Biotechnology and Biochemistry* 66(2): 385-390.

We analyzed the kinetics and metabolic pathways of trichloroethylene and 1,1,1-trichloroethane degradation by the ethane-utilizing *Mycobacterium* sp. TA27. The apparent V_{max} and K_m of trichloroethylene were $9.8 \text{ nmol min}^{-1} \text{ mg of cells}^{-1}$ and $61.9 \text{ } \mu\text{mol}$, respectively. The apparent V_{max} and K_m of 1,1,1-trichloroethane were $0.11 \text{ nmol min}^{-1} \text{ mg of cells}^{-1}$ and $3.1 \text{ } \mu\text{mol}$, respectively. 2,2,2-trichloroethanol, trichloroacetic acid, chloral, and dichloroacetic acid were detected as metabolites of trichloroethylene. 2,2,2-trichloroethanol, trichloroacetic acid, and dichloroacetic acid were also detected as metabolites of 1,1,1-trichloroethane. The amounts of 2,2,2-trichloroethanol, trichloroacetic acid, chloral, and dichloroacetic acid derived from the degradation of $3.60 \text{ } \mu\text{mol}$ trichloroethylene were $0.16 \text{ } \mu\text{mol}$ (4.4%), $0.11 \text{ } \mu\text{mol}$ (3.1%), $0.02 \text{ } \mu\text{mol}$ (0.6%), and $0.02 \text{ } \mu\text{mol}$ (0.6%), respectively. The amounts of 2,2,2-trichloroethanol, trichloroacetic acid and dichloroacetic acid derived from the degradation of $1.73 \text{ } \mu\text{mol}$ 1,1,1-trichloroethane were $1.48 \text{ } \mu\text{mol}$ (85.5%), $0.22 \text{ } \mu\text{mol}$ (12.7%), and $0.02 \text{ } \mu\text{mol}$ (1.2%), respectively. More than 90% of theoretical total chloride was released in trichloroethylene degradation. Chloral and 2,2,2-trichloroethanol were transformed into each other, and were finally converted to trichloroacetic acid, and dichloroacetic acid. Trichloroacetic acid and dichloroacetic acid were not degraded by strain TA27.

Hendrickson, E. R., J. A. Payne, et al. (2002). "Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe." *Appl Environ Microbiol* 68(2): 485-95.

The environmental distribution of *Dehalococcoides* group organisms and their association with chloroethene-contaminated sites were examined. Samples from 24 chloroethene-dechlorinating sites scattered throughout North America and Europe were tested for the presence of members of the *Dehalococcoides* group by using a PCR assay developed to detect *Dehalococcoides* 16S rRNA gene (rDNA) sequences. Sequences identified by sequence analysis as sequences of members of the *Dehalococcoides* group were detected at 21 sites. Full dechlorination of chloroethenes to ethene occurred at these sites. *Dehalococcoides* sequences were not detected in samples from three sites at which partial dechlorination of chloroethenes occurred, where dechlorination appeared to stop at 1,2-cis-dichloroethene. Phylogenetic analysis of the 16S rDNA amplicons confirmed that *Dehalococcoides* sequences formed a unique 16S rDNA group. These 16S rDNA sequences were divided into three subgroups based on specific base substitution patterns in variable regions 2 and 6 of the *Dehalococcoides* 16S rDNA sequence. Analyses also demonstrated that specific base substitution patterns were signature patterns. The specific base substitutions distinguished the three sequence subgroups phylogenetically. These results demonstrated that members of the *Dehalococcoides* group are widely distributed in nature and can be found in a variety of geological formations and in different climatic zones. Furthermore, the association of these organisms with full dechlorination of chloroethenes suggests that they are promising candidates for engineered bioremediation and may be important contributors to natural attenuation of chloroethenes.

Kim, Y., D. J. Arp, et al. (2002). "A combined method for determining inhibition type, kinetic parameters, and inhibition coefficients for aerobic cometabolism of 1,1,1-trichloroethane by a butane-grown mixed culture." *Biotechnology and Bioengineering* 77(5): 564-576.

A combined method for determining inhibition type, kinetic parameters, and inhibition coefficients is developed and presented. The method was validated by applying it to data obtained from batch kinetics of the aerobic cometabolism of 1,1,1-trichloroethane (1,1,1-TCA) by a butane-grown mixed culture. The maximum degradation rates (k_{max}) and half-saturation coefficients (K_s) were independently determined in single compound tests, and compared with those obtained from inhibition tests. The inhibition type was determined using direct linear plots at various substrate and inhibitor concentrations. Kinetic parameters (k_{max} and K_s) and inhibition coefficients (K_{ic} and K_{iu}) were determined by nonlinear least squares regression (NLSR) fits of the inhibition model determined from the direct linear plots. Initial guesses of the kinetic parameters for NLSR were determined from linearized inhibition equations that were derived from the correlations between apparent maximum degradation rates ($k_{max}(app)$) and/or the apparent half-saturation coefficient ($K_s(app)$) and the k_{max} , K_s , and inhibitor concentration ($I-L$) for each inhibition equation. Two different inhibition types were indicated from the direct linear plots: competitive inhibition of 1,1,1-TCA on butane degradation, and mixed inhibition of 1,1,1-TCA transformation by butane. Good agreement was achieved between independently measured k_{max} , and K_s values and those obtained from both NLSR and the linearized inhibition equations. The

initial guesses of all the kinetic parameters determined from linear plots were in the range of the values estimated from NLSR analysis. Overall the results show that use of the direct linear plot method to identify the inhibition type, coupled with initial guesses from linearized plots for NLSR analysis, results in an accurate method for determining inhibition types and coefficients. Detailed studies with pure cultures and purified enzymes are needed to further demonstrate the utility of this method. (C) 2002 John Wiley Sons, Inc.

Kousaka, T. and K. Mori (2002). "Synthesis of (1R,2S)-1-(3'-Chloro-4'-methoxyphenyl)-1,2- propanediol (Trametes) and (1R,2S)-1-(3',5'-dichloro-4'- methoxyphenyl)-1,2-propanediol, chlorinated fungal metabolites in the natural environment." *Bioscience Biotechnology and Biochemistry* 66(3): 697-701.

(1 R,2 S)-1-(3'-Chloro-4'-methoxyphenyl)-1,2-propanediol (Trametes, 3), a metabolite of the fungus *Trametes* sp. IVP-F640 and *Bjerkandera* sp. BOS55, was synthesized by employing Sharpless asymmetric dihydroxylation as the key step. Similarly, the (1R,2S) isomer of 1-(3',5'-dichloro-4'- methoxyphenyl)-1,2-propanediol (4), another metabolite of *Bjerkandera* sp. BOS55, was synthesized by asymmetric dihydroxylation.

Leigh, M. B., J. S. Fletcher, et al. (2002). "Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants." *Environmental Science & Technology* 36(7): 1579-1583.

The growth dynamics and phenolic content of mulberry (*Morus* sp.) fine roots (<1 mm diameter) were determined and examined in relationship to rhizosphere remediation of recalcitrant soil contaminants. Root turnover measurements of rhizotron-grown plants showed that 58% of the fine roots produced during a 6- month growing season (June-November) died at the end of the season. The concentration of phenolic compounds in fine roots increased approximately 2-fold during the later stages of the season, and the total phenolic content of dead fine roots reached a maximum value of 38 mg/g dry weight. The late-season increase in total phenolics was primarily due to accumulation of three different flavones (morusin, morusinol; and kuwanon C). These three flavones were shown to support the growth of the bacterium *Burkholderia* sp. LB400, a degrader of polychlorinated biphenyls (PCBs). Thus, it has been established that, upon death, the fine roots of mulberry can serve as a source of substrate for PCB-degrading bacteria. These results establish for the first time that the chemical content and turnover rate of fine roots should be considered an important aspect of rhizosphere remediation.

Lewandowicz, A., J. Rudzinski, et al. (2002). "Determination of the chlorine kinetic isotope effect, on the 4-chlorobenzoyl-CoA dehalogenase-catalyzed nucleophilic aromatic substitution." *Archives of Biochemistry and Biophysics* 398(2): 249-252.

The chlorine kinetic isotope effect (KIE) on the de- halogenation of 4-chlorobenzoyl-CoA catalyzed by 4-chlorobenzoyl-CoA dehalogenase has been measured at room temperature and optimal pH. The measured value of $k = 1.0090 \pm 0.0006$ is larger than the KIEs recently measured for haloalkane and fluoroacetate dehalogenase. This indicates that the transition state for dissociation of chloride ion from the Meisenheimer intermediate is sensitive to the chlorine isotopic substitution. Simple modeling suggests that this sensitivity originates in the high isotopic sensitivity of the C-Cl bond bending modes. (C) 2002 Elsevier Science (USA).

Master, E. R., V. W.-M. Lai, et al. (2002). "Sequential anaerobic-aerobic treatment of soil contaminated with weathered Aroclor 1260." *Environmental Science & Technology* 36(1): 100-103.

Soil contaminated with weathered Aroclor 1260 was bioremediated by sequential anaerobic and aerobic laboratory-scale treatment. The initial concentration was 59 g of PCBs/g of soil. Following 4 months of anaerobic treatment with an enrichment culture, all of the major components in Aroclor 1260 were completely or partially transformed to less chlorinated PCB congeners. The major products of reductive dechlorination were 2,4-dichlorobiphenyl and 2,4,6-trichlorobiphenyl, and the average chlorine substituents per PCB molecule decreased from 6.4 to 5.2. The molar concentration of PCBs did not decrease during the anaerobic treatment. All of the major products formed during the anaerobic treatment were degraded in the subsequent aerobic treatment using *Burkholderia* sp. strain LB400. After 28 days of the aerobic treatment, the concentration of PCBs was reduced to 20 g/g of soil. PCBs were not significantly removed in aerobic treatments unless they were

bioaugmented with LB400. Also, PCB degradation was not detected in soil bioaugmented with LB400 without prior anaerobic treatment. These results confirm the potential for extensive biological destruction of highly chlorinated, weathered PCB congeners in soil.

Mehmannavaz, R., S. O. Prasher, et al. (2002). "Rhizospheric effects of alfalfa on biotransformation of polychlorinated biphenyls in a contaminated soil augmented with *Sinorhizobium meliloti*." *Process Biochemistry* 37(9): 955-963.

The effects of plant-microbe-soil interactions on the biotransformation of polychlorinated biphenyls (PCBs) in a rhizosphere soil were investigated. Containers packed with 350 g of a soil contaminated with Aroclor 1242, 1248, 1254 and 1260, were planted with alfalfa (*Medicago sativa* L.) and augmented with its symbiotic N₂-fixing host rhizobium (*Sinorhizobium meliloti*, strain A-025). The four treatment setups comprised a factorial combination of the presence/absence of alfalfa with the non-inoculation/inoculation of soil with *S. meliloti* in a completely randomized design with two replicates. Up to 44 days after planting, when the alfalfa was not fully developed, alfalfa and *S. meliloti* together were the most effective in PCB transformation/depletion, whereas alfalfa only was the least effective. However, by the last day of the experimental period (Day 270), when alfalfa growth was robust and full, alfalfa alone was the most effective, whereas *S. meliloti* alone was the least. In rhizobium-inoculated soil, soil hardness increased, soil moisture contents decreased, and both plant growth and yield were lowered, compared to non-inoculated soil. The depletion, loss or change in PCB levels may be attributed to either direct or indirect biotransformation, biotranslocation and adsorption of PCBs due to the presence of alfalfa and/or rhizobial inoculation. Either possibility underscores the possibility of using plant-rhizobacterial associations to phytoremediate soils contaminated with PCBs. (C) 2002 Elsevier Science Ltd. All rights reserved.

Miller, A. R., W. K. Keener, et al. (2002). A rapid fluorescence-based assay for detecting soluble methane monoxygenase. *Applied Microbiology and Biotechnology*. 58: 183-188.

A fluorescence-based assay was developed to estimate soluble methane monoxygenase (sMMO) activity in solution. Whole cells of *Methylosinus trichosporium* OB3b expressing sMMO were used to oxidize various compounds to screen for fluorescent products. Of the 12 compounds tested, only coumarin yielded a fluorescent product. The UV absorbance spectrum of the product matches that of 7-hydroxycoumarin, and this identification was confirmed by C-13-NMR spectroscopy. The dependence of the fluorescent reaction on sMMO activity was investigated by pre-incubation with acetylene, a known inhibitor of sMMO activity. Apparent kinetic parameters for whole cells were determined to be $K_m(\text{app}) = 262 \mu\text{M}$ and $V_{\text{max}}(\text{app}) = 821 \text{ nmol 7-hydroxycoumarin min}^{-1} \text{ mg protein}^{-1}$. The rate of coumarin oxidation by sMMO correlates well with those of trichloroethylene degradation and naphthalene oxidation. Advantages of the fluorescence-based coumarin oxidation assay over the naphthalene oxidation assay include a more stable product, direct detection of the product without additional reagents, and greater speed and convenience.

Miyauchi, K., H. S. Lee, et al. (2002). "Cloning and characterization of *linR*, involved in regulation of the downstream pathway for gamma-hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26." *Appl. Environ. Microbiol.* 68(4): 1803-1807.

In *Sphingomonas paucimobilis* UT26, *LinD* and *LinE* activities, which are responsible for the degradation of gamma-hexachlorocyclohexane, are inducibly expressed in the presence of their substrates, 2,5-dichlorohydroquinone (2,5-DCHQ) and chlorohydroquinone (CHQ). The nucleotide sequence of the 1-kb upstream region of the *linE* gene was determined, and an open reading frame (ORF) was found in divergent orientation from *linE*. Because the putative protein product of the ORF showed similarity to the LysR-type transcriptional regulator (LTTR) family, we named it *linR*. The fragment containing the putative LTTR recognition sequence (a palindromic TN11A sequence), which exists immediately upstream of *linE*, was ligated with the reporter gene *lacZ* and was inserted into the plasmid expressing *LinR* under the control of the *lac* promoter. When the resultant plasmid was introduced into *Escherichia coli*, the *LacZ* activity rose in the presence of 2,5-DCHQ and CHQ. RNA slot blot analysis for the total RNAs of UT26 and UT102, which has an insertional mutation in *linR*, revealed that the expression of the *linD* and *linE* genes was induced in the presence of 2,5-DCHQ, CHQ, and

hydroquinone in UT26 but not in UT102. These results indicated that the *linR* gene is directly involved in the inducible expression of the *linD* and *linE* genes.

Numata, M., N. Nakamura, et al. (2002). "Chlorine stable isotope measurements of chlorinated aliphatic hydrocarbons by thermal ionization mass spectrometry." *Analytica Chimica Acta* 455(1): 1-9.

An improved method for chlorine isotopic analysis using thermal ionization mass spectrometry (TIMS) of Cs_2Cl^+ has been investigated. The $\delta \text{Cl-37}$ values (parts per mil deviations from the standard seawater $\text{Cl-37}/\text{Cl-35}$ ratio) for 10 commercial chlorinated aliphatic hydrocarbons (CAHs), provided by six suppliers are presented. The CAHs were treated with a sodiumbiphenyl reagent. The liberated chloride ions were converted to CsCl and their isotopic compositions were determined by TIMS. Replicate analysis of the CAHs gave an external precision of 0.1-0.4 parts per thousand (1 σ (S.D.)). As each compound and each supplier has a distinctive $\delta \text{Cl-37}$ value ($\delta \text{Cl-37}$ values: -5.0 to +2.9 parts per thousand), Cl isotope data can be used to trace a specific source of pollutants in the subsurface environment. Trichloroethene (TCE) and tetrachloroethene (PCE) were extracted from aqueous solutions with toluene and their chlorine isotope compositions were analyzed. The solvent extraction method presented here may be applicable to the analysis of actual contaminated water samples. Chlorine atoms were recovered as inorganic chloride from unsaturated chlorinated aliphatic compounds (TCE and PCE) by KMnO_4 oxidation. However, the saturated compounds were resistant to KMnO_4 oxidation. The isotopic compositions of the recovered chloride ion were then determined by TIMS. It is suggested that such compound-specific isotopic analyses may give information about the past record of each contaminant. (C) 2002 Elsevier Science B.V. All rights reserved.

Oakley, A. J., Z. Prokop, et al. (2002). "Exploring the structure and activity of haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26: Evidence for product- and water-mediated inhibition." *Biochemistry* 41(15): 4847-4855.

The hydrolysis of haloalkanes to their corresponding alcohols and inorganic halides is catalyzed by α/β -hydrolases called haloalkane dehalogenases. The study of haloalkane dehalogenases is vital for the development of these enzymes if they are to be utilized for bioremediation of organohalide-contaminated industrial waste. We report the kinetic and structural analysis of the haloalkane dehalogenase from *Sphingomonas paucimobilis* UT26 (*LinB*) in complex with each of 1,2-dichloroethane and 1,2-dichloropropane and the reaction product of 1-chlorobutane turnover. Activity studies showed very weak but detectable activity of *LinB* with 1,2-dichloroethane [0.012 nmol s^{-1} (mg of enzyme) $^{-1}$] and 1,2-dichloropropane [0.027 nmol s^{-1} (mg of enzyme) $^{-1}$]. These activities are much weaker compared, for example, to the activity of *LinB* with 1-chlorobutane [68.2 nmol s^{-1} (mg of enzyme) $^{-1}$]. Inhibition analysis reveals that both 1,2-dichloroethane and 1,2-dichloropropane act as simple competitive inhibitors of the substrate 1-chlorobutane and that 1,2-dichloroethane binds to *LinB* with lower affinity than 1,2-dichloropropane. Docking calculations on the enzyme in the absence of active site water molecules and halide ions confirm that these compounds could bind productively. However, when these moieties were included in the calculations, they bound in a manner similar to that observed in the crystal structure. These data provide an explanation for the low activity of *LinB* with small, chlorinated alkanes and show the importance of active site water molecules and reaction products in molecular docking.

Olivas, Y., J. Dolfing, et al. (2002). "The influence of redox potential on the degradation of halogenated methanes." *Environmental Toxicology and Chemistry* 21(3): 493-499.

To determine the influence of redox potential on the reaction mechanism and to quantify kinetics of the dechlorination by digester sludge, the test compounds trichlorofluoromethane (CFCl_3), carbon tetrachloride (CCl_4), and chloroform (CHCl_3) were incubated in the presence of sludge and variable concentrations of reducing agent. Different sources of dehalogenation were examined, including live sludge and heat-killed sludge, and abiotic mechanisms were quantified in the absence of sludge. Batch incubations were done under redox conditions ranging from +534 to -348 mV. The highest rates for the dehalogenation of the three compounds were observed at -348 mV. The dechlorination rate of all the compounds by the heat-resistant catalysts was approximately twofold higher than the live treatments. It was proposed that the higher degradation rates by heat-killed sludge were due to the absence of physical barriers such as cell wall and cell membranes. There was no abiotic dechlorination of

CFC13, whereas CCl4 and CHCl3 were both reduced in the absence of sludge catalyst by Ti (III) citrate at greater than or equal to 2.5 mM. The degradation pathways of CFC13 and CHCl3 appeared to be only partially reductive since the production of reduced metabolites was low in comparison with the total amount of original halogenated compounds degraded. For CFC13, the partial reductive degradation implied that different intra- and extracellular pathways were concurrent. The Gibbs free energy and the redox potential for the dehalogenation reactions utilizing Ti (III) citrate and acetate as electron donors are reported here for the first time.

Puk, O., P. Huber, et al. (2002). Glycopeptide biosynthesis in *Amycolatopsis mediterranei* DSM5908: Function of a haloenase and a haloperoxidase/perhydrolase. *Chemistry & Biology*. 9: 225-235.

Glycopeptides are important clinical emergency antibiotics consisting of a glycosylated and chlorinated heptapeptide backbone. The understanding of the biosynthesis is crucial for development of new glycopeptides. With balhimycin as a model system, this work focuses on the investigation of the putative halogenase gene (*bhaA*) and the putative haloperoxidase/perhydrolase gene (*bhp*) of the balhimycin biosynthesis gene cluster. An in-frame deletion mutant in the haloperoxidase/perhydrolase gene *bhp* (OP696) did not produce balhimycin. Feeding experiments revealed that *bhp* is involved in the biosynthesis of P-hydroxytyrosine, a precursor of balhimycin. A *bhaA* in-frame deletion mutant (PH4) accumulated glycosylated but nonchlorinated balhimycin variants. The mutants indicated that only the halogenase *BhaA* is required for chlorination of balhimycin. Nonglycosylated and/or nonhalogenated metabolites can serve as starting points for combinatorial approaches for novel glycopeptides.

Rieger, P. G., H. M. Meier, et al. (2002). "Xenobiotics in the environment: present and future strategies to obviate the problem of biological persistence." *Journal of Biotechnology* 94(1): 101-123.

Sustainable chemistry aims at an improved efficiency of using natural resources which are used to meet human needs for chemical products. Chemists in science and industry, have become aware of the importance to design environmentally benign chemicals. One aspect is the biological persistence and the present paper reviews work in this field focussing on the degradation of xenobiotics in the environment. Different structural reasons for chemical and biological persistence are described and strategies to use single bacterial isolates or microbial communities for the elimination of xenobiotic pollutants in the environment are summarized. Perspectives and limitations to evolve and use this catabolic potential are critically discussed with respect to the complexity of mixtures of xenobiotics often found in practice. An interdisciplinary approach for the prospective design of environmentally benign substances is presented and examples for new commodity chemicals that better fit the naturally existing catabolic potential are included. (C) 2002 Elsevier Science B.V. All rights reserved.

Salles, J. F., F. A. De Souza, et al. (2002). "Molecular Method To Assess the Diversity of Burkholderia Species in Environmental Samples." *Appl. Environ. Microbiol.* 68(4): 1595-1603.

In spite of the importance of many members of the genus *Burkholderia* in the soil microbial community, no direct method to assess the diversity of this genus has been developed so far. The aim of this work was the development of soil DNA-based PCR-denaturing gradient gel electrophoresis (DGGE), a powerful tool for studying the diversity of microbial communities, for detection and analysis of the *Burkholderia* diversity in soil samples. Primers specific for the genus *Burkholderia* were developed based on the 16S rRNA gene sequence and were evaluated in PCRs performed with genomic DNAs from *Burkholderia* and non-*Burkholderia* species as the templates. The primer system used exhibited good specificity and sensitivity for the majority of established species of the genus *Burkholderia*. DGGE analyses of the PCR products obtained showed that there were sufficient differences in migration behavior to distinguish the majority of the 14 *Burkholderia* species tested. Sequence analysis of amplicons generated with soil DNA exclusively revealed sequences affiliated with sequences of *Burkholderia* species, demonstrating that the PCR-DGGE method is suitable for studying the diversity of this genus in natural settings. A PCR-DGGE analysis of the *Burkholderia* communities in two grassland plots revealed differences in diversity mainly between bulk and rhizosphere soil samples; the communities in the latter samples produced more complex patterns.

Seigneur, C., A. Atti, et al. (2002). Effect of biotrickling filter operating parameters on chlorobenzenes degradation. *Journal of Environmental Engineering-Asce*. 128: 360-366.

In this paper the effect of operating parameters on biotrickling filter performance degrading chlorobenzene and o- dichlorobenzene mixture were studied. The large laboratory scale biofilter, total volume 40 L, filled with inert packing material was used. The biomass adaptation and cultivation were performed in a batch fermentor and were used to inoculate the biotrickling filter. After a starting period, the influence of the substrate load increase, liquid recirculation flow rate, and empty bed retention time on elimination capacity and removal efficiency were found. The most important recirculation liquid parameters were analyzed every day, that is: concentration of metabolites, dissolved organic carbon, nitrate, chloride, and biomass. A good correlation was found between intermediate concentration and the removal efficiency of the biotrickling filter. The measurements of the absorbance, very easy and rapid, can be used as a control parameter of the biofiltration efficiency.

Sepulveda-Torres, L. D., A. Huang, et al. (2002). "Analysis of regulatory elements and genes required for carbon tetrachloride degradation in *Pseudomonas stutzeri* strain KC." *Journal of Molecular Microbiology and Biotechnology* 4(2): 151-161.

Previously, we described the generation and initial characterization of four Tn5 mutants of *Pseudomonas stutzeri* strain KC with impaired ability to degrade carbon tetrachloride (Sepulveda-Torres et al., 1999). In this study, we show cloning and sequencing of an 8.3 kbp region in which all four transposons were located. This fragment encodes eight potential genes and is located in the central part of the 25 kbp fragment recently identified by Lewis et al. (2000) and shown by them to be sufficient to confer carbon tetrachloride transformation capability upon other pseudomonads. The four transposon insertion mutants mapped in ORF's F and I designated by Lewis et al. (2000). This is consistent with the results by Lewis et al. (2000) that orfF is required for carbon tetrachloride degradation. We further established that orfI is required for CCl₄ degradation since the three mutants in this ORF were unable to degrade carbon tetrachloride. We present our analysis of the gene and protein sequences from the 8.3 kbp region and propose a tentative model for the role of different genes in the synthesis and activity of pyridine-2,6-bis(thiocarboxylate) (PDTC), the secreted factor responsible for carbon tetrachloride dechlorination. We also found a putative promoter that overlaps with a Fur-box-like sequence in the region upstream of mutated genes. To test this putative promoter region and Fur-box, we generated and ligated DNA fragments containing wild-type and mutant Fur-boxes to a lacZ reporter. The wild-type fragment showed promoter activity that is regulated by the concentration of iron in the medium. Finally, we screened a selection of *Pseudomonas* strains, including *P. putida* DSMZ 3601 - a strain known to produce PDTC for the presence of the genes characterized in this study. None of the strains tested positive, suggesting that *Pseudomonas stutzeri* strain KC may possess a distinct biosynthetic pathway for PDTC production.

Seybold, C. A., W. Mersie, et al. (2001). "Anaerobic degradation of atrazine and metolachlor and metabolite formation in wetland soil and water microcosms." *Journal of Environmental Quality* 30(4): 1271-1277.

The half-lives, degradation rates, and metabolite formation patterns of atrazine (6-chloro-N-2-ethyl-N-4-isopropyl-1,3,5- triazine-2,4-diamine) and metolachlor (2-chloro-N-(2-ethyl-6- methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide) were determined in an anaerobic wetland soil incubated at 24degreesC for 112 d. At 0, 7, 14, 28, 42, 56, and 112 d, the soil and water were analyzed for atrazine and metolachlor, and their major metabolites. The soil oxidation-reduction potential reached -200 mV after 14 d. Degradation reaction rates were first-order for atrazine in anaerobic soil and for metolachlor in the aqueous phase. Zero-order reaction rates were best fit for atrazine in the aqueous phase and metolachlor in anaerobic soil. In anaerobic soil, the half-life was 38 d for atrazine and 62 d for metolachlor. In the aqueous phase above the soil, the half-life was 86 d for atrazine and 40 d for metolachlor. Metabolites detected in the anaerobic soil were hydroxyatrazine and deethylatrazine for atrazine, and relatively small amounts of ethanesulfonic acid and oxanilic acid for metolachlor. Metabolites detected in the aqueous phase above the soil were hydroxyatrazine, deethylatrazine, and deisopropylatrazine for atrazine, and ethanesulfonic acid and oxanilic acid for metolachlor. Concentrations of metabolites in the aqueous phase generally peaked within the first 25 d and then declined. Results indicate that atrazine and metolachlor can degrade under strongly reducing conditions found in wetland soils. Metolachlor metabolites, ethanesulfonic acid, and oxanilic acid are not significantly formed under anaerobic conditions.

Sponza, D. T. (2001). "Performance of upflow anaerobic sludge blanket (UASB) reactor treating wastewaters containing carbon tetrachloride." *World Journal of Microbiology & Biotechnology* 17(9): 839-847.

The anaerobic biodegradation of carbon tetrachloride (CT) was investigated during the granulation process by reducing the hydraulic retention time, increasing the chemical oxygen demand (COD) and CT loadings in a 2 l laboratory-scale upflow anaerobic sludge blanket (UASB) reactor. Anaerobic unacclimated sludge and glucose were used as seed and primary substrate, respectively. Granules were developed 4 weeks after start-up, which grew at an accelerated rate for 8 months, and then became fully grown. The effect of operational parameters such as influent CT concentrations, COD, CT loading, food to biomass ratio and specific methanogenic activity (SMA) were also considered during granulation. The granular sludge cultivated had a maximum diameter of 2.1 mm and SMA of 1.6 g COD/g total suspended solid (TSS) day. COD and CT removal efficiencies of 92 and 88% were achieved when the reactor was firstly operating at CT and COD loading rates of 17.5 mg/l day and 12.5 g/l day, respectively. This corresponds to hydraulic retention time of 0.28 day and food to biomass ratio of 0.5 g COD/g TSS day. Kinetic coefficients of maximum specific substrate utilization rate, half velocity coefficient, growth yield coefficient and decay coefficient were determined to be 2.4×10^{-3} mg CT/TSS day⁽⁻¹⁾, 1.37 mg CT/l, 0.69 mg TSS/mg CT and 0.046 day⁽⁻¹⁾, respectively for CT biotransformation during granulation.

Sponza, D. T. (2002). "Simultaneous granulation, biomass retainment and carbon tetrachloride (CT) removal in an upflow anaerobic sludge blanket (UASB) reactor." *Process Biochemistry* 37(10): 1091-1101.

The granulation process was examined during carbon tetrachloride (CT) biodegradation in a laboratory scale upflow anaerobic sludge blanket (UASB) reactor operated at 35 degreesC for 220 days. Anaerobic, unacclimated sludge and glucose were used as seed and primary substrate, respectively. CT degrading granules developed after 48 days of start-up. They grew at an accelerated pace for 6.5 months and had a maximum diameter of 2.2 mm following the pre-granulation and maturation period. Maximum specific methanogenic activity (SMA) was 1.63 g chemical oxygen demand (COD) per g total suspended solid (TSS) per day while, maximum specific organic loading rate to achieve 96% of COD removal efficiency was determined to be 12.5 g COD per 1 per day. About 95% CT removal efficiencies were achieved when the reactor was operating at loading rates as high as 17.5 mg CT per 1 per day after 220 days of continuous operation. The methane content in the total biogas collected was between 50 and 68% depending to shock organic loadings. About 96% of the total COD removed, was converted to methane, 0.024 g of TSS was yielded for each gram of COD removed at the end of 230 days of operation period. Kinetic coefficients of k (maximum specific substrate utilization rate), K , (half velocity coefficient), were determined to be 2.4×10^{-3} mg CT per mg TSS per day and 1.37 mg CT per 1, respectively, for CT biotransformation during granulation. *Methanothrix* (*Methanosaeta*) sp., *Methanosarcina mazei*, *Methanobacterium* sp., *Methanobrevibacter* sp., *Syntrophobacter* sp., *Methanolobus vulcani* and *Acetobacter woodii* were identified in UASB. (C) 2002 Elsevier Science Ltd.

Stolworthy, J. C., A. Paszczynski, et al. (2001). "Metal binding by pyridine-2,6-bis(monothiocarboxylic acid), a biochelator produced by *Pseudomonas stutzeri* and *Pseudomonas putida*." *Biodegradation* 12(6): 411-418.

Pyridine-2,6-bis(monothiocarboxylic acid) (pdtc), a natural metal chelator produced by *Pseudomonas stutzeri* and *Pseudomonas putida* that promotes the degradation of carbon tetrachloride, was synthesized and studied by potentiometric and spectrophotometric techniques. The first two stepwise protonation constants (pK) for successive proton addition to pdtc were found to be 5.48 and 2.58. The third stepwise protonation constant was estimated to be 1.3. The stability (affinity) constants for iron(III), nickel(II), and cobalt(III) were determined by potentiometric or spectrophotometric titration. The results show that pdtc has strong affinity for Fe(III) and comparable affinities for various other metals. The stability constants (log K) are 33.93 for Co(pdtc)(2)(1)-; 33.36 for Fe(pdtc)(2)(1)-; and 33.28 for Ni(pdtc)(2)(2)-. These protonation constants and high affinity constants show that over a physiological pH range the ferric pdtc complex has one of the highest effective stability constants for iron binding among known bacterial chelators.

Takahashi, Y., M. Daitoh, et al. (2002). "Halogenated Metabolites from the New Okinawan Red Alga *Laurencia yonaguniensis*." *Journal of Natural Products* 65(3): 395-398.

A novel brominated diterpene based on the rare neoirieane skeleton, named neoirietetraol (1), has been isolated along with a halogenated C15 acetogenin, (3Z)-laurenyne (2), from a new *Laurencia* species, *L. yonaguniensis* Masuda et Abe, species inedita, collected at Yonaguni Island, Okinawa Prefecture, Japan. The structures of these metabolites were elucidated by spectroscopic data (IR, ¹H NMR, ¹³C NMR, 2D NMR, and MS). Neoirietetraol (1) was toxic to the brine shrimp (*Artemia salina*; LC50, 40.1 μM) and also showed weak antibacterial activities against two marine bacteria, *Alcaligenes aquamarinus* and *Escherichia coli*.

Tang, L. X., J. E. T. van Hylckama, et al. (2002). Improved stability of halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 by replacement of cysteine residues. *Enzyme and Microbial Technology*. 30: 251-258.

Halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 is a homo-tetrameric protein containing three cysteines per 28 kDa subunit. Under oxidizing conditions the enzyme was found to be susceptible to inactivation which could be prevented by the addition of beta-mercaptoethanol or glycerol. Gel filtration experiments and SDS-PAGE analysis revealed that inactivation coincided with monomerization and intramolecular disulfide bond formation. To identify the cysteine residues involved in the inactivation process, a set of cysteine mutant enzymes was constructed. All the purified mutants (C30A, C153S, C229A and C153S/C229A) showed a similar activity as wild-type enzyme, indicating that no cysteine is directly involved in catalysis. The C153S and C30A mutants displayed a higher stability than wild-type enzyme, whereas mutating Cys229 resulted in decreased enzyme stability. SDS-PAGE analysis showed that in wild-type enzyme Cys30-Cys229 and Cys153-Cys229 disulfide bonds were readily formed while almost no formation of the Cys30-Cys153 disulfide bond could be observed. From this, it was concluded that all three cysteine residues are involved in the enzyme inactivation process. The importance of the improved stability of the C153S and C30A mutant enzymes was demonstrated by performing kinetic resolution experiments with racemic 2-chloro-1-phenylethanol, which resulted in higher enantiomeric excess values of the remaining halohydrin when compared to conversions catalyzed by wild-type enzyme. (C) 2002 Elsevier Science Inc. All rights

Tani, K., M. Muneta, et al. (2002). "Monitoring of *Ralstonia eutropha* KT1 in groundwater in an experimental bioaugmentation field by in situ PCR." *Applied and Environmental Microbiology* 68(1): 412-416.

Ralstonia eutropha KT1, which degrades trichloroethylene, was injected into the aquifer after activation with toluene, and then the number of bacteria was monitored by in situ PCR targeting the phenol hydroxylase gene and by fluorescent in situ hybridization (FISH) targeting 16S rRNA. Before injection of the bacterial suspension, the total concentration of bacteria in the groundwater was approximately 3×10^5 cells/ml and the amount of *Ralstonia* and bacteria carrying the phenol hydroxylase gene as a percentage of total bacterial cells was less than 0.1%. The concentration of bacteria carrying the phenol hydroxylase gene detected by in situ PCR was approximately 3×10^7 cells/ml 1 h after injection, and the concentration of *Ralstonia* detected by FISH was similar. The number of bacteria detected by in situ PCR was similar to that detected by FISH 4 days after the start of the extraction of groundwater. On and after day 7, however, the number of bacterial cells detected by FISH was less than that detected by in situ PCR.

Vetter, W. (2002). "Environmental occurrence of Q1, a C₉H₃Cl₇N₂ compound, that has been identified as a natural bioaccumulative organochlorine." *Chemosphere* 46(9-10): 1477-1483.

Environmental appearance of Q1, a natural heptachloro compound with the molecular formula C₉H₃Cl₇N₂, was studied in samples from different sites all over the world. Q1 was expected to have a bipyrrrole backbone, similar to other compounds ascribed to natural sources. A method for isolation of Q1 was developed by combination of adsorption chromatography on silica and normal phase HPLC with an amino phase. UV-detection of Q1 supports the aromatic character of the compound. The high levels detected in samples of marine mammals and birds suggested that Q1 is both a persistent and a bioaccumulative contaminant. This was underscored by calculated log K_{OW} in the range of other lipophilic organo-halogens. In accordance with earlier studies, highest Q1 concentrations were found in the Southern Hemisphere, but with a highly selective GC/ECNI-MS-SIM method, detection of Q1 was also achieved in many samples from the Northern Hemisphere. In addition to marine mammals and birds, Q1 was also detected in fish from the Mediterranean Sea and the Antarctic. Traces were also

detected in SRM 1588 certified cod liver oil, but Q1 was not detected in fish from Hong Kong and Lake Baikal. (C) 2002 Elsevier Science Ltd. All rights reserved.

Voordeckers, J.W., D.E. Fennell, et al. (2002). "Anaerobic biotransformation of tetrabromobisphenol A, tetrachlorobisphenol A, and bisphenol A in estuarine sediments." *Environmental Science & Technology* 36(4): 696-701.

Biotransformation of the flame retardants tetrabromobisphenol A and tetrachlorobisphenol A, and their ultimate biodehalogenation product, bisphenol A, was examined in anoxic estuarine sediments. Dehalogenation of tetrabromobisphenol A and tetrachlorobisphenol A was examined under conditions promoting either methanogenesis or sulfate reduction as the primary terminal electron-accepting process. Complete dehalogenation of tetrabromobisphenol A to bisphenol A with no further degradation of bisphenol A, was observed under both methanogenic and sulfate-reducing conditions. Dehalogenation of tetra chlorobisphenol A under both methanogenic and sulfate-reducing conditions resulted in the accumulation of a persistent dichlorinated bisphenol A isomer, while no bisphenol A was formed. Co-amendment of sediment enrichments with either 2,6-dibromo- or 2,6-dichlorophenol did not affect the extent of dehalogenation as compared to sediments that were amended only with the flame retardants. Sediment cultures pre-acclimated on 2-bromophenol dehalogenated the flame retardants in a manner similar to that of fresh sediments. No loss of bisphenol A was observed in separate incubations within 162 days under conditions promoting either methanogenesis, sulfate-reduction, iron(III)-reduction, or nitrate-reduction. Furthermore, identical enrichments that readily degraded 4-hydroxybenzoate, a structural analogue of bisphenol A did not exhibit bisphenol A degradation. The dehalogenation of tetrabromo- and tetrachlorobisphenol A and the potential for accumulation of bisphenol A in anoxic sediments is significant given the widespread use of these chemicals.

Vuilleumier, S. and M. Pagni (2002). "The elusive roles of bacterial glutathione S-transferases: new lessons from genomes." *Applied Microbiology and Biotechnology* 58(2): 138-146.

Glutathione S-transferases constitute a large family of enzymes which catalyze the addition of glutathione to endogenous or xenobiotic, often toxic electrophilic chemicals. Eukaryotic glutathione S-transferases usually promote the inactivation, degradation or excretion of a wide range of compounds by formation of the corresponding glutathione conjugates. In bacteria, by contrast, the few glutathione S-transferases for which substrates are known, such as dichloromethane dehalogenase, 1,2-dichloroepoxyethane epoxidase and tetrachlorohydroquinone reductase, are catabolic enzymes with an essential role for growth on recalcitrant chemicals. Glutathione S-transferase genes have also been found in bacterial operons and gene clusters involved in the degradation of aromatic compounds. Information from bacterial genome sequencing projects now suggests that glutathione S-transferases are present in large numbers in proteobacteria. In particular, the genomes of three *Pseudomonas* species each include at least ten different glutathione S-transferase genes. Several of the corresponding proteins define new classes of the glutathione S-transferase family and may also have novel functions that remain to be elucidated.

Wariishi, H., N. Itoh, et al. (2002). "Complete degradation of Yperite, a chemical warfare agent, by basidiomycetes." *Biotechnology Letters* 24(6): 501-505.

The complete degradation of Yperite (bis(2-chloroethyl) sulfide), a chemical warfare agent, was achieved by two basidiomycetous cultures. Two distinct metabolic pathways were detected in each fungus during degradation of Yperite. The major path involved a non-enzymatic hydrolysis to generate thiodiglycol. In the minor path, the sulfide bond was cleaved prior to the hydrolytic dechlorination reaction, yielding chloroethanol and chloromercaptoethane, both of which were then metabolized completely.

Wu, Q. Z., J.E.M. Watts, et al. (2002). "Identification of a Bacterium That Specifically Catalyzes the Reductive Dechlorination of Polychlorinated Biphenyls with Doubly Flanked Chlorines." *Appl. Environ. Microbiol.* 68(2): 807-812.

A microorganism whose growth is linked to the dechlorination of polychlorinated biphenyls (PCBs) with doubly flanked chlorines was identified. Identification was made by reductive analysis of community 16S ribosomal DNA (rDNA) sequences from a culture enriched in the presence of 2,3,4,5-tetrachlorobiphenyl (2,3,4,5-CB), which was dechlorinated at the para position. Denaturing gradient gel electrophoresis (DGGE) analysis of total 16S rDNA extracted from the culture led to identification of three operational taxonomic units (OTUs 1, 2, and 3). OTU 1 was always detected when 2,3,4,5-CB or other congeners with doubly flanked chlorines were present and dechlorinated. Only OTUs 2 and 3 were detected in the absence of PCBs and when other PCBs (i.e., PCBs lacking doubly flanked chlorines) were not dechlorinated. Partial sequences of OTUs 2 and 3 exhibited 98.2% similarity to the sequence of "Desulfovibrio caledoniensis" (accession no. DCU53465). A sulfate-reducing vibrio isolated from the culture generated OTUs 2 and 3. This organism could not dechlorinate 2,3,4,5-CB. From these results we concluded that OTU 1 represents the dechlorinating bacterium growing in a coculture with a Desulfovibrio sp. The 16S rDNA sequence of OTU 1 is most similar to the 16S rDNA sequence of bacterium o-17 (89% similarity), an ortho-PCB-dechlorinating bacterium. The PCB dechlorinator, designated bacterium DF-1, reductively dechlorinates congeners with doubly flanked chlorines when it is supplied with formate or H₂-CO₂ (80:20).

Yoon, I. K. and C. H. Park (2002). "Effects of gas flow rate, inlet concentration and temperature on biofiltration of volatile organic compounds in a peat-packed biofilter." *Journal of Bioscience and Bioengineering* 93(2): 165-169.

The effects of incoming gas concentration, empty bed residence time (EBRT), and column temperature on the removal efficiency of volatile organic compounds (isoprene, dimethyl sulfide, chloroform, benzene, trichloroethylene, toluene, m-xylene, o-xylene and styrene) were studied for 101 d in a biofilter comprising two glass columns (I.D. 5.0 cm x height 62 cm) packed with peat. At an EBRT of 3 min the removal efficiency increased up to 90% 34 d after start up at both 25degreesC and 45degreesC when the incoming gas concentration was raised stepwise to 65 g . m⁽⁻³⁾. When the incoming gas concentration increased to 83 g . m⁽⁻³⁾, the removal efficiency was 93% at 25degreesC, but dropped to 74% at 45degreesC. At an incoming gas concentration of 92 g . m⁽⁻³⁾ and an EBRT of 1.5 min, the removal efficiencies were 91% and 94% at 25degreesC and 32degreesC, respectively. However, at 1 min of EBRT, the removal efficiencies decreased to 68% and 81% at 25degreesC and 32degreesC, respectively. The removal rate per unit time and per unit volume of the biofilter was proportional to the incoming gas rate up to 3483 g VOC . m⁽⁻³⁾ . h⁽⁻¹⁾. Further increase of the incoming gas rate lowered the removal rate as compared to that predicted by the proportionality. The maximum removal rate was 3977 g . m⁽⁻³⁾ . h⁽⁻¹⁾ at 32degreesC. At an EBRT of 1.5 min, the removal efficiency was highest for isoprene (93%), and lowest for chloroform (84%). Aromatic compounds (benzene, toluene, and xylene) were removed by 93- 94%. The cell concentration increased 100-fold from the initial value, and reached 1.12x10⁽⁸⁾ cells - (g of dry peat)⁽⁻¹⁾. At 32degreesC, 67% of the incoming VOC was removed in the first quarter of the column.