

---

---

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

4<sup>th</sup> Quarterly Report

4<sup>th</sup> Quarter Year 2001

Report prepared for EUROCHLOR

**Dr. Jim A. Field**

Department of Chemical &

Environmental Engineering

University of Arizona

P.O. Box 210011, Tucson

AZ 85721-0011, USA

February 2002

---

---

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

4<sup>th</sup> Quarterly Report  
4<sup>th</sup> Quarter Year 2001

Report prepared for EUROCHLOR

**Dr. Jim A. Field**

Department of Chemical &  
Environmental Engineering  
University of Arizona

February 2002

## TABLE OF CONTENTS

	<b>Acronyms</b>	3
1.	<b>Introduction</b>	4
2.	<b>Summary of most important developments</b>	4
	2.a. Microbial dechlorination	4
	2.b. Microbial chlorination	5
3.	<b>Microbial dechlorination</b>	7
	3.a. General reviews	7
	3.b. Microbial dechlorination	7
	Vinyl chloride (VC) and other chlorinated ethenes	7
	Carbon tetrachloride (CT)	10
	Chloromethane, dichloromethane, chloroform	11
	1,2-Dichloroethane (1,2-DCA)	11
	Chlorobenzenes	11
	Chlorinated dibenzo- <i>p</i> -dioxins and -furans (CDDs/CDFs)	12
	Hexachlorobutadiene and octachlorostyrene	13
	Polychlorinated biphenyls (PCBs)	13
	Miscellaneous	14
	3.c. In vitro degradation of chlorinated compounds	14
	3.d. New tools to assess the biodegradation of chlorinated compounds	16
	Gene cloning	16
	Characterization of microbial populations	16
4.	<b>Microbial chlorination</b>	18
	4.a. General reviews	18
	4.b. Microbial chlorination in soils	19
	Chloromethanes	19
	Other chlorinated compounds	19
	4.c. Chlorination by freshwater and marine organisms	20
	Chloromethanes	20
	Other chlorinated compounds	20
	4.d. Chlorinating enzymes	21
5.	<b>References Cited</b>	23
6.	<b>Annex</b>	28

---

**ACRONYMS**

<b>ARDRA</b>	Amplified rDNA Restriction Analysis
<b>BPO</b>	Bromoperoxidase
<b>CB</b>	Chlorinated Benzene
<b>CDDs</b>	Chlorinated dibenzo- <i>p</i> -dioxins
<b>CDFs</b>	Chlorinated Dibenzo- <i>p</i> -furans
<b>COD</b>	Chemical Oxygen Demand
<b>CPO</b>	Chloroperoxidase
<b>CT</b>	Carbon tetrachloride
<b>DBF</b>	Dibenzofurane
<b>1,2-DCA</b>	1,2-Dichloroethane
<b>2,3-DCBp</b>	2,3-Dichlorobiphenyl
<b><i>Cis</i>-DCE</b>	<i>Cis</i> -1,2-dichloroethene
<b>DDD</b>	1,1-dichloro-2,2- bis( <i>p</i> -chlorophenyl)-ethane
<b>DDE</b>	1,1-dichloro-2,2-bis( <i>p</i> - chlorophenyl)-ethylene
<b>DDT</b>	1,1,1-trichloro-2,2-bis( <i>p</i> -chlorophenyl)-ethane
<b>DGGE</b>	Denaturing Gradient Gel Electrophoresis
<b>D.w.</b>	Dry Weight
<b>E-acceptor</b>	Electron Acceptor
<b>E-donor</b>	Electron Donor
<b>ETH</b>	Ethene
<b>GAC</b>	Granulated Activated Carbon
<b>HCB</b>	Hexachlorobenzene
<b>HCH</b>	Hexachlorohexane
<b>HRT</b>	Hydraulic Retention Time
<b>PCBs</b>	Polychlorinated Biphenyls
<b>PCE</b>	Tetrachloroethylene
<b>pMMO</b>	Particulate Methane Monooxygenase
<b>QCB</b>	Pentachlorobenzene
<b>sMMO</b>	Soluble Methane Monooxygenase
<b>TCA</b>	1,1,1-Trichloroethane
<b>TCE</b>	Trichloroethylene
<b>TCB</b>	Trichlorobenzene
<b>TeCB</b>	Tetrachlorobenzene
<b>UASB</b>	Upflow Anaerobic Sludge Blanket reactor
<b>VC</b>	Vinyl Chloride

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

## **4<sup>th</sup> Quarterly Report – Year 2001**

Dr. Jim A. Field

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

### **1. INTRODUCTION**

This report presents a review of scientific literature published during the last quarter of 2001 on the microbial halogenation and dehalogenation of the following compounds: vinyl chloride, dichloroethane, chloroform, dichloromethane, hexachlorobenzene, chlorobenzene, trichlorobenzenes (1,2,4-TCB, 1,2,3-TCB, 1,3,5-TCB), hexachlorobutadiene, octachloro-styrene, 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 fourth quarter of 2001 are:

- 1) The first identification of anaerobic microorganisms that catalyse the dechlorination of polychlorinated biphenyl (PCB) compounds was attained by two different research groups. Identification of an anaerobic bacterium that links its growth to the reductive dechlorination of 2,3,5,6-chlorobiphenyl was performed through denaturing gradient gel electrophoresis (DGGE) of 16S rDNA from a highly enriched dechlorinating culture (6). Preliminary identification of anaerobic microorganisms that selectively dechlorinated double-flanked chlorines in a PCB compound (*i.e.*, congener 2,3,4,5 chlorinated biphenyl) was attained by

three different molecular techniques, namely amplified rDNA restriction analysis (ARDRA) of a clone library, denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (TRFLP), in combination with selective microbial enrichment (45). Numerous, as yet unsuccessful, attempts have been made to isolate and identify anaerobic bacteria that dechlorinate PCBs.

- \
- 2) Additional reports of dioxin degradation [10,37,42] were also published in this period. Extensive anaerobic degradation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was demonstrated under anaerobic conditions [37]. Selected aerobic bacteria were also observed to utilize mono and di-chlorodibenzo-*p*-dioxins as sole carbon source and, could degrade tri- to tetrachlorodibenzo-*p*-dioxin cometabolically [10]. [29, 33-36].
  - 3) The rapid evolution of bacterial catabolic enzymes is the topic of a recent publication by Seffernick *et al.* (2001) (31). The authors present a review of the evolution of new metabolism and how enzymes can acquire distinctly new biological functions by changes in their amino acid sequence. In the publication, atrazine chlorohydrolase is used as a case study to illustrate enzyme plasticity and how enzymes evolve in response to selective evolutionary pressure. Atrazine chlorohydrolase is shown to have evolved in recent decades for the function of catabolizing atrazine. The amino acid sequence of atrazine chlorohydrolase is shown to be 98% identical with that of melamine deaminase, an enzyme that catalyzes deamination reactions. Similarly to atrazine chlorohydrolase, many other enzymes may have evolved or will evolve novel catabolic activities in response to the introduction of xenobiotic compounds in the environment. Table 1 lists newly discovered reactions involved in the transformation of synthetic compounds, including several chlorinated organics once considered to be recalcitrant to microbial attack.
  - 4) Additional evidence was found in this period for a DNA-repair mechanism in bacteria that co-oxidize chlorinated solvents (Yeager *et al.* 2001) (46). This finding is important in understanding how bacteria tolerate transient toxic intermediates that are produced during co-oxidation of chlorinated solvents.

## 2.b. Microbial Chlorination

There is an interesting highlight from this quarter concerning microbial halogenation. The occurrence of several unknown organohalogen compounds (*i.e.*, containing bromo, or bromo

and chlorine substitutions) in the environment, probably of natural origin, was demonstrated by Vetter *et al.* (2001) (41). A major organohalogen detected in all samples analyzed (the so-called Q1 compound) was identified as a bioaccumulative, persistent natural organochlorine that is most likely a heptachlorobipyrrole. In contrast with the widely accepted idea that environmental concentrations of organohalogens are associated with anthropogenic compounds such as PCBs, DDT, and other chloropesticides, the yet unidentified organohalogens reached higher concentrations in the adipose tissues of the tested marine mammals than well-known halogenated xenobiotics. The significance of unidentified natural organohalogens compounds such as those detected in this study is unclear since these compounds are not included in monitoring programs and thus have routinely been ignored.

**Table 1:** Enzymes that catalyze newly discovered reactions with industrial chemicals as substrates (41).

Enzyme	PFam designation <sup>a</sup>	Family or homologue (%identity) <sup>b</sup>
Tetrachloroethene reductive dehalogenase	PF00037: 4Fe-4S	hypothetical proteins (20-28%)
Tetrachlorohydroquinone Reductive dehalogenase	PF00043: glutathione S-transferase	glutathione S-transferase (19-30%)
Cyanamide hydrolase	None	hypothetical proteins (17-33%)
Pentaerythritol tetranitrate reductase	PF00724: NADH:flavin oxidoreductase/NADH oxidase	87.7% N-ethylmaleimide reductase (P77258) (30-50%) reductases
Phosphotriesterase	PF02126: phosphotriesterase family	amidohydrolase superfamily (19-70%)
Styrene epoxide isomerase	None	40.5% p-cymene methyl hydrolase (O84920)

<sup>a</sup> University of Minnesota Biocatalysis and Biodegradation Database enzyme number.

<sup>b</sup> Pfam (Protein family database of alignments).

### 3. MICROBIAL DECHLORINATION

#### 3.a. General Reviews

General reviews on the microbial dechlorination of organohalogen compounds were not found for the fourth quarter of 2001.

#### 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.

**Vinyl Chloride (VC).** The aerobic biodegradation of VC by *Pseudomonas aeruginosa* strain DL1 was studied (39). This strain was isolated in experiments utilizing ethene as the source of energy and carbon. Ethene is known to accumulate at some anaerobic PCE- or TCE-contaminated sites as the final product of chlorinated ethene degradation. When ethene-grown cells of *P. aeruginosa* strain DL1 were spiked with VC, they were able to consume VC, indicating a cometabolic process. After an extended period of incubation (> 40 days), a transition occurred that allowed *P. aeruginosa* strain DL1 to begin using VC as a primary growth substrate. The authors measured kinetic data of *P. aeruginosa* strain DL1 growth on VC. The observed, maximum specific growth rate was  $0.046 \text{ d}^{-1}$ , and the Monod half saturation coefficient was  $1.17 \mu\text{M VC}$  at  $22^\circ\text{C}$ . The cell yield was 21% (dry weight (d.w.) cells:VC consumed). Inhibition studies with acetylene, indicate involvement of a monooxygenase in the initial steps of degradation. A kinetic model was developed to describe substrate interactions between ethene and VC.

Aerobic VC degradation at a TCE and 1,1,1-trichloroethane (TCA) contaminated Superfund site was investigated (13). The anaerobic zone of the site produced expected degradation products: VC, *cis*-dichloroethene (*cis*-DCE) along with ethene and methane. The anaerobic plume transitions into an aerobic plume down-gradient. The paper considers the substrate interactions affecting VC and *cis*-DCE degradation. When methane and ethene were compared as primary substrates to promote aerobic cometabolism of VC, ethene was found to

be a better substrate. Methane inhibited cometabolism of VC by ethene. The results taken as a whole suggest that ethene plays an important role in stimulating aerobic VC degradation at chlorinated ethene contaminated sites. Enrichments were also established that could aerobically degraded VC as a sole carbon and energy source, but initially a lag phase of 75 days passed before degradation occurred. The enrichment was also able to degraded *cis*-DCE.

**Anaerobic Degradation of PCE and TCE.** Six publications in this quarter considered the anaerobic degradation of PCE and TCE

The halorespiring bacterium *Desulfitobacterium* sp. strain Y-51 was used in combination with zero valent iron (ZVI) (as an electron donor, *e-donor*) to promote the reductive dechlorination of PCE (24). The bacterium alone resulted in the conversion of PCE to *cis*-DCE; whereas in combination with ZVI, PCE was converted to ethene and ethane. ZVI was shown to cause the chemical reduction of *cis*-DCE to ethene and ethane. Furthermore, anaerobic corrosion of ZVI was found to stimulate the biological reductive dechlorination of PCE by keeping proper levels of pH and oxidation-reduction potential and, by producing cathodic hydrogen, which might be used as an e-donor for respiratory PCE dechlorination.

Peat as a biobarrier material for the reductive dechlorination of PCE plumes was evaluated in a study reported in this quarter (21). The study was similar to another publication by the same research group, in the third-quarterly report. Peat is used as a slow release electron-donating substrate; whereas anaerobic sludge was used as a source of dechlorinating microorganisms. A simulated PCE-contaminated groundwater was pumped through the inoculated peat, up to 98% removal-efficiency of PCE was observed. TCE, DCE, VC and ethene were observed as products of PCE degradation.

Hydrogen (H<sub>2</sub>) is ultimately the species used during anaerobic degradation as an e-donor for reductive dechlorination of PCE. The optimal form of supplementing H<sub>2</sub> is a continuous supply at low concentrations. One study evaluated an improved method of supplying H<sub>2</sub> (47). Iron wire was used as electrodes in combination with an anaerobic bacteria enrichment for PCE reduction. Experiments were conducted by switching the polarities of electrodes periodically and supplying electrical power in an intermittent way. The results showed that an electrochemical bioreactor that was switched once every 10 min and operated only 8 h/day was able to produce more hydrogen than that operated 24 h/day at 0.4 V without polarity switching. The H<sub>2</sub> produced was available for microbial growth and PCE dechlorination.

The steady state H<sub>2</sub> concentrations that developed during the reductive dehalogenation of various chlorinated ethenes were measured (25). Each chlorinated ethene (PCE, *cis*-DCE and

VC) was incubated under different electron-accepting conditions (nitrate, manganese oxide, ferrous iron, sulfate, CO<sub>2</sub>), and volatile fatty acids were used as e-donor. The results showed that redox processes with nitrate, manganese oxide and ferric iron as the electron acceptors (*e-acceptors*) exhibited hydrogen threshold values close to PCE/TCE dechlorination, whereas *cis*-DCE and VC dechlorinations exhibited H<sub>2</sub> threshold values in the range of sulfate reduction and methanogenesis, respectively. The characteristic steady-state H<sub>2</sub> levels (in nM) were: denitrification, 0.1-0.4; manganese reduction, 0.1-2.0; iron reduction, 0.1-0.4; sulfate reduction, 1.5-4.5; methanogenesis, 2.5-24; PCE/TCE dechlorination, 0.6-0.9; *cis*-DCE dechlorination, 0.1-2.5; and VC dechlorination, 2-24.

In a similar study, the steady state H<sub>2</sub> concentration was determined for sulfate reducing sediments undergoing PCE dechlorination (27). PCE spiked sediments which were actively reducing PCE to TCE had a steady state H<sub>2</sub> concentration of 0.5 nM which was significantly lower than the unspiked sulfate reducing sediments 0.8 nM, suggesting that halorespirers in this sediment would win the competition for H<sub>2</sub>.

The occurrence of reductive dehalogenation of TCE in a fractured dolomite rock contaminated by the chlorinated solvent was the topic of another paper (17). A survey of geochemical parameters indicated that TCE dechlorination was occurring and that electrons were being donated from an unidentified pool of organic matter. The dissolved inorganic carbon at the site had a light isotopic ratio (low in <sup>13</sup>C). Hydrocarbons in the rock also had a light isotopic ratio, suggesting that they were being utilized as e-donor to support dehalogenation. This hypothesis was confirmed by demonstrating that the hydrocarbons extracted from the rock were indeed being utilized as an e-donor. Also pulverized rock, but not combusted pulverized rock, stimulated reductive dehalogenation. The actual hydrocarbons responsible were not yet identified, only it was proven that hexadecane was not responsible. Molecular characterization of the population in the site indicated that microorganisms related to *Dehalococcoides ethenogens*, a halorespiring bacteria that can convert PCE/TCE to ethene, were present at the site.

**Aerobic Degradation of TCE.** Six publications evaluated aerobic co-oxidation of TCE this quarter. Two publications screened for microorganisms based on gene probes for methane monooxygenase (see section “3.d. Characterization of Microbial Populations”), and one study evaluated the purified enzyme, biphenyl dioxygenase, for its TCE oxidizing activity (see section “3.c. In Vitro Degradation of Chlorinated Compounds”). The other three articles will be discussed here.

Cells of the aerobic methane oxidizing bacterium *Methylosinus trichosporium* OB3b were grown in mineral medium free of copper (a condition which promotes soluble methane monooxygenase production, the most potent TCE-oxidizing monooxygenase) with methane. The role of low concentrations of methane or methanol on stimulating TCE oxidation rates by the harvested cells was evaluated (20). Stimulation was observed up to 70  $\mu\text{M}$  methane and up to 400  $\mu\text{M}$  methanol stimulated TCE oxidation, higher concentrations were inhibitory.

The distribution of methanotrophs was examined in shallow aquifers contaminated with TCE in the Southern Kanto gas field, Japan (33). The aquifer was characterized by the distribution of the total population of methanotrophs and soluble methane monooxygenase-producing-methanotrophs. The large population of methanotrophs in certain strata of the aquifer was suggested to play a role for in situ bioremediation of the site.

Genes, which are possibly responsible for DNA-repair in the TCE co-oxidizing *Burkholderia cepacia* G4 strain were researched (46). The hypothesis is that wild type strains have a DNA repair system to tolerate toxicity of TCE oxidation products. Mutants were generated which were sensitive to TCE degradation. The most sensitive mutant to TCE oxidation was also found to be sensitive to UV and  $\text{H}_2\text{O}_2$ . The data from this study establishes that there is a link between DNA repair and the ability of *B. cepacia* G4 cells to survive following TCE transformation.

### **Carbon Tetrachloride (CT)**

Laboratory studies indicate that upflow anaerobic sludge blanket (UASB) bioreactor technology is applicable to treating wastewaters containing carbon tetrachloride (CT) (32). Anaerobic unacclimated sludge and glucose were used as seed and primary substrate, respectively. CT was shown to be highly inhibitory to non-adapted anaerobic bacteria (50% inhibition at 2.2 mg CT/l) in batch bioassays. Nonetheless, upon biomass acclimation, high CT and chemical oxygen demand (COD) removal efficiencies (over 88%) were observed when the continuous UASB reactor was operated with a synthetic wastewater containing 5.8 mg CT/l at a hydraulic retention time (HRT) of 0.28 day, with CT loading rates of up to 17.5 mg/(l\*day). At high loading rates, the COD and CT removal efficiencies dropped sharply, but they rapidly recovered upon reducing the loading rates. Formation of stable anaerobic biomass granules (*i.e.*, highly active, dense methanogenic biofilm aggregates that enable efficient operation of UASB bioreactors under conditions of high organic loading and low HRT) with high dechlorinating activity was observed starting 4 weeks after start-up. Kinetic coefficients of maximum specific substrate utilization rate, half velocity coefficient, growth yield coefficient

and decay coefficient were determined to be  $2.4 \times 10^{-3}$  mg CT/(mg TSS\*day), 1.37 mg CT/l, 0.69 mg TSS/mg CT and  $0.046 \text{ day}^{-1}$ , respectively for CT biotransformation during granulation.

### **Chloromethane, Dichloromethane and Chloroform**

No publications regarding the microbial degradation of the aliphatic chlorinated compounds, chloromethane, dichloromethane and chloroform, were found in this quarter.

### **Dichloroethane (1,2-DCA)**

The potential of the UASB bioreactor technology for the decontamination of waters containing 1,2-dichloroethane (1,2-DCA) was demonstrated in laboratory-scale experiments (8). This report investigated the dechlorination capacity of granular methanogenic biomass in the presence of high amounts of 1,2-DCA (up to 100 mg/l) and ethanol as the main carbon source. Two types of UASB reactors were studied, the first consisting of a sludge blanket and the second containing an additional layer of granulated activated carbon (GAC). Concentrations of 1,2-DCA ranging 20-100 mg/l were dechlorinated to the non-toxic ethene (65-80% recovery) and ethane (<1%). No chlorinated intermediates or residues were found. Reductive dechlorination of 1,2-DCA was shown to be catalyzed by the anaerobic microbial community in the bioreactor. The best configuration (GAC-supplemented bioreactor) degraded the toxic compound for 82% at 88 mg/(l\*day). The degradation rate in the reactor without GAC was approximately half of that of the GAC-supplemented reactor, suggesting that the addition of activated carbon primarily had a stabilizing effect, buffering the anaerobic biomass against variable inputs of 1,2-DCA. The reactor effluents were less toxic relative to the influent when analyzed by Nitrox tests, suggesting that anaerobic treatment of DCA-containing wastewaters can protect a subsequent aerobic nitrifying system.

### **Chlorobenzenes**

#### **Mono-chlorobenzene, Dichlorobenzenes and Trichlorobenzenes**

No publications regarding the microbial degradation of mono-, di- and tri-chlorobenzenes were found in the last quarter of the year 2001.

#### **Hexachlorobenzene (HCB):**

Anaerobic mixed cultures collected from tropical sediment were found to possess the ability to dechlorinate pentachlorobenzene (QCB) and hexachlorobenzene (HCB) after a long-term acclimation in HCB (5). The primary product for HCB and QCB was 1,3,5- trichlorobenzene. A tetrachlorobenzene (1,2,3,5-TeCB) was the main intermediate in the dechlorination of

pentachlorobenzene. In contrast, penta- and tetra-CB were not detected during the HCB dechlorination.

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

Three publications concerned with the microbial dechlorination of CDDs were found this quarter, two utilizing aerobic microorganisms, and the other using anaerobic mixed cultures.

Aerobic dechlorination of polychlorinated dibenzo-*p*-dioxins was studied with new bacterial strains isolated from soil and aerobic sediments contaminated by dioxins (10). Mono- and diCDDs could be utilized as a sole carbon source and degraded by the isolated bacterial strains, but tri-CDDs were poorly degraded. Co-metabolic degradation of *o*-dichlorobenzene enhanced the biodegradability of triCDD and tetraCDDs by an isolated *Pseudomonas* sp. strain. In this case, triCDD (1.2 mg/l) was degraded by 33.1% in 3 weeks and the degradation rate enhanced more than 2 fold; also tetraCDD (0.1 mg/l) biodegraded by 37.8% in 3 weeks. More highly chlorinated dioxins (*i.e.*, penta- through octaCDDs) tested were recalcitrant to biodegradation and accumulated in bacterial cells.

*Sphingomonas* sp. RW1 is an aerobic bacterial strain capable of dioxin degradation. The degradation pathway for dibenzofuran (DBF) in the RW1 strain proceeds through salicylate and gentisate into intermediate metabolism. The glutathione S-transferase (GST) OrfE3 of this strain was recently shown to display maleyl-pyruvate isomerase activity (42). However, data obtained with mutants of strain RW1 suggest that the *orfE3* GST gene (*i.e.*, gene of unknown function detected earlier in one of the gene clusters responsible for DBF degradation) is not essential for growth of strain RW1 with dibenzofuran, salicylate or gentisate. Many GSTs of bacteria have been described in recent years. Bacterial GSTs whose function has been elucidated (*e.g.* dichloromethane dehalogenase, tetrahydrochloroquinone reductase and epoxide ring opening enzymes) are involved in the catabolism and the subsequent mineralization of toxic or xenobiotic chemicals.

The anaerobic biotransformation of a model dioxin compound, 1,2,3,4-tetraCDD, under sulfate-reducing, methanogenic, and iron-reducing conditions was examined with enrichment cultures obtained from estuarine sediments (37). In addition, the effect of prior enrichment on 2-bromophenol or a mixture of 2-, 3-, and 4-bromophenol on dioxin dechlorination was examined. Reductive dechlorination of 1,2,3,4-tetraCDD (initial conc. 1 mg/l) was initially observed only under methanogenic conditions in the cultures enriched on all three bromophenol isomers. The tetraCDD compound was dechlorinated in the lateral position to 1,2,4-triCDD. Dechlorination of the tetraCDD compound to 1,2,4-triCDD was observed after 2 months, with further dechlorination to 1,3-diCDD within 17 months. These results indicate that relatively

---

harmless brominated aromatic compounds could be utilized to enhance PCDD dechlorination. Brominated aromatics are readily degraded under anaerobic conditions. These compounds contribute to enrich a microbial community capable of dechlorinating polyCDDs, and also provide an e-donor that may stimulate dehalogenation activity.

### **Hexachlorobutadiene and Octachlorostyrene**

No publications regarding the microbial hexachlorobutadiene and octachlorostyrene were found in the last quarter of 2001.

### **Polychlorinated Biphenyls (PCBs)**

Five publications concerned with the microbial dechlorination of PCBs were found this quarter. The first study reported on PCB biodegradation by an anaerobic bacteria (6); the second presented results from the remediation of PCB-contaminated soil by composting (28); the third focused on the characterization of a reductive dehalogenase from an anaerobic bacteria capable of PCB dechlorination (Krasotkina *et al.*, 2001 (23), discussed in section “3.c. *In Vitro Degradation of Chlorinated Compounds*”); and the last two publications are concerned with the analysis of PCB-dechlorinating communities by different molecular techniques (Watts *et al.*, 2001 (45) and Ahn *et al.* 2001 (1), both discussed in section “3.d. *New tools to assess the biodegradation of chlorinated compounds: Characterization of microbial populations*”).

An anaerobic bacterium that links cell growth to the reductive dechlorination of 2,3,5,6-chlorobiphenyl was identified (6). Identification was performed through denaturing gradient gel electrophoresis (DGGE) of 16S rDNA from a highly enriched *ortho*-PCB dechlorinating culture. The organism has high sequence similarity with the green non-sulphur bacteria and with a group that includes *Dehalococcoides ethenogenes*, and it required acetate for dechlorination and growth. No dechlorination was observed when H<sub>2</sub>/CO<sub>2</sub> was used. This is the first identification of a microorganism that catalyses the reductive dechlorination of a PCB. It is interesting to note that the identified organism is closely related to a bacterium that dechlorinates PCE.

Bioremediation of a PCB-contaminated soil via composting was investigated by Michel *et al.* (2001) (28). A PCB-contaminated soil from a former paper mill (16 ± 1 mg PCB/kg d.w., 4 Cl/biphenyl on average) was mixed with a yard trimmings amendment (14% to 82% by weight) and composted in field scale piles (25 m<sup>3</sup>) to determine the effect of soil to amendment ratio on PCB degradation. The authors report up to a 40% loss of PCBs with amendment levels of 60% or higher. PCB loss observed during the composting of the contaminated soil appeared to be largely due to biodegradation and not volatilization (less than 1% PCB volatilization

during compost incubation with forced aeration at 55°C). Less chlorinated PCB congeners (1-3 Cl/biphenyl) were preferentially degraded, suggesting that additional remediation processes will be required to eliminate PCB congeners with greater than 3 chlorines.

### Miscellaneous

Two recent reports of microbial dechlorination are discussed here, one dealing with hexachlorocyclohexane (HCH) and the other with the pesticide 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT).

Aerobic degradation of  $\alpha$ -,  $\delta$ - and  $\gamma$  isomers of HCH by two strains of *Alcaligenes faecalis* isolated from a contaminated site was demonstrated in a recent study (15). Strain 1 (S-1) and 2 (S-2) degraded effectively the latter HCH isomers in liquid cultures spiked with 1-5  $\mu\text{g}$  HCH/ml (over 80% elimination in 8 days). Degradation of the  $\beta$ -HCH isomer containing (conc. 1-5  $\mu\text{g}$  HCH/ml) by S-2 was considerably slower (only 25-32% elimination in 28 days), and this isomer was resistant to degradation by strain S-2.  $\beta$ -HCH is known to be most stable isomer of HCH.

The effectiveness of supplementing a nonionic surfactant for enhancing the bioavailability as well as the anaerobic biodegradability of DDT and its metabolites -1,1-dichloro-2,2- bis(*p*-chlorophenyl)-ethane (DDD) and 1,1-dichloro-2,2-bis(*p*- chlorophenyl)-ethylene (DDE)- in contaminated soil was evaluated (43). Dosing of 12 mg surfactant/g soil was required to attain pesticide concentrations greater than the critical micelle concentration in the liquid phase in soil microcosms. At greater doses, solubilization of the various chlorinated compounds increased linearly with the surfactant dose. Significantly greater rates and extents of DDT degradation were observed in anaerobic microcosms amended with surfactant or fed a cellulose substrate relative to controls. The surfactant substantially increased the rate of DDT degradation during the first 9 weeks, although there were no significant differences between cellulose-fed microcosms and surfactant-amended microcosms after 31 weeks. In addition, DDD accumulated at less than stoichiometric amounts in surfactant-amended microcosms, whereas DDD accumulated nearly stoichiometrically with DDT loss in all other microcosms. Concentrations of DDE were unchanged throughout the course of the experiment.

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

Biphenyl dioxygenase is a multi-component enzyme consisting of an iron-sulfur protein that is composed of alpha (BphA1) and beta (BphA2) subunits, a ferredoxin (FDBphA3), and a

ferredoxin reductase (FDRBphA4). A hybrid enzyme in which BphA1 is replaced with TodC1 (alpha sub-unit of toluene dioxygenase) shows very high activity for TCE oxidation. A study in this quarter characterized the role of the components of the hybrid enzymes for TCE oxidation (26). The molecular activity ( $k_0$ ) of the hybrid dioxygenase for TCE was  $4.1 \text{ min}^{-1}$ , which is comparable to that of toluene dioxygenase. The  $K_m$  value of the hybrid dioxygenase for TCE was  $130 \mu\text{M}$ , which was lower than  $250 \mu\text{M}$  for toluene dioxygenase. These results suggest that the alpha sub-unit of the iron-sulfur protein is crucial for the determination of substrate specificity.

Kahng *et al.* (2001) (19) reported on the cloning and characterization of a new gene cluster encoding multi-component monooxygenases that allow *Burkholderia* sp. strain JS150 to carry out the initial oxidation of toluene and a variety of related alkyl- and chloro-substituted hydrocarbons. The *tbc* genes (for toluene, benzene, and chlorobenzene utilization) were shown to be organized into two divergently transcribed operons, *tbc1* and *tbc2*, that present significant homology to multi-component cresol and phenol hydroxylases, and to toluene and benzene monooxygenases, respectively. The results of this study provide further evidence for the existence of multiple, functionally redundant alkyl- and chloroaromatic monooxygenases in strain JS150.

Three additional publications report on the characterization of specific dehalogenating enzymes: *i.e.*, 1) a reductive dehalogenase from a chlororespiring bacterium (23); 2) a 2-haloacid dehalogenase (or halidohydrolase) from a *Burkholderia* sp. strain (30), and 3) an immobilized, commercial haloalkane dehalogenase (9).

The corrinoid and iron-sulfur dependent reductive dehalogenase (CprA) from *Desulfitobacterium chlororespirans* was characterized by Krasotkina *et al.* (2001) (23). *D. chlororespirans* is a microorganism that can utilize chlorinated phenols as terminal e-acceptors in an energy-generating process recently described known as dehalorespiration. In this process, a reductive dehalogenase couples the oxidation of an e-donor to the reductive elimination of chloride. The reductive dehalogenase of *D. chlororespirans* was found to require a hydroxyl group *ortho* to the halide, as well as another functional group *meta* to the chlorine to be eliminated (carboxyl > acetyl > chloro) for substrate binding. The studies represent the first description of a purified dehalogenase that can catalyze the dechlorination of an hydroxylated PCB (3,3',5,5'-tetra-chloro-4,4'-biphenyldiol), albeit at a rate about 100-fold slower of that with the most reactive substrate tested so far (*i.e.*, 3-chloro-4-hydroxybenzoate).

Mutagenic analysis of the conserved residues in dehalogenase IVa (*DehIVa*) of *Burkholderia cepacia* MBA4 suggested that the majority of the protein is indispensable for dehalogenation (30). This dehalogenase catalyzes the hydrolytic dehalogenation of halogen-

carbon bond(s) in L-2-halogenated aliphatic acids, with inversion of the C2 configuration producing D-2-hydroxyacids.

Immobilization of a commercial haloalkane dehalogenase from *Rhodococcus rhodochrous* onto an inorganic  $\gamma$ -alumina carrier, and haloalkane hydrolysis by the immobilized enzyme was investigated by Dravis *et al.* (2001) (9). Haloalkane dehalogenase from *R. rhodochrous* is an enzyme that can cleave, via hydrolysis, a single carbon-halogen bond of primary substituted haloalkanes (*i.e.*, various mono-, di-, and tri-substituted halogenated (Cl, Br, I) aliphatic hydrocarbons (C<sub>3</sub> to C<sub>9</sub>)) with formation of the corresponding primary alcohol. Kinetic characterization of the dehalogenating enzyme showed that immobilization did not cause significant internal and external mass transfer limitations. Furthermore, the enzyme retained greater than 40% of its original activity, maintained its pH dependence, and exhibited a greatly enhanced thermostability and resistance towards inactivation by organic solvents after immobilization.

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

#### Gene Cloning

A whole-cell bacterial sensor system for short-chain halogenated organic acids was constructed, using 2-chloropropionic acid as a model pollutant (36). The final construct, an *E. coli* strain, contained DNA elements from two additional bacteria: the sensing element, a dehalogenase promoter, from a *Pseudomonas* sp., and the reporting element, bioluminescence (*lux*) genes from *Photobacterium luminescens*. The construct responded to the presence of 2-chloropropionic acid by dose-dependent light emission, in a highly specific albeit a very insensitive manner. The authors conclude that further genetic work is needed in order to make such a construct practical for environmental monitoring purposes.

#### Characterization of Microbial Populations

In this quarter, two studies utilized gene probes of methane monooxygenase to characterize methane-oxidizing bacteria in contaminated responsible for aerobic TCE co-oxidation. At one site, gene probes were based on particulate methane monooxygenase (pMMO) (3). The results indicated a high biodiversity of pMMO's in the biostimulated zone of the aquifer. A predominance of type I methanotrophs (closely related to *Methylomonas* sp) over type II methanotrophs (closely related to *Methylocystis* sp.) was found in all biostimulation trials. Gene

---

probes based on soluble methane monooxygenase (sMMO) revealed a lower biodiversity of sMMO's at the site.

A rapid and specific enumeration method for a TCE-degrading methanotroph, *Methylocystis* sp. strain M (type II), based on a most probable number-polymerase chain reaction (MPN-PCR) method was developed for monitoring the bacterium at bioremediation sites (22). The primers were based on a sMMO specific to strain M. The detection limit for strain M in effluent of a soil column fed TCE-contaminated groundwater by the MPN-PCR method was 4 to 8 x 10<sup>2</sup> cells/ml.

Two additional studies were found that characterized PCB-degrading microbial communities, one study utilized transformed strains with a green fluorescent protein gene (1), and the other compared three molecular screening techniques commonly used in environmental microbiology (45).

Labeling of two PCB-degrading psychrotolerant *Pseudomonas* spp with a green fluorescent protein gene (*gfp*) combined with examination by epifluorescent microscopy was used as an approach to follow the survival of these strains in soil microcosms at 4° and 22°C (1). The *gfp* was inserted into the chromosome of each psychrotolerant strain and was stable with no apparent adverse effects on the metabolism and growth of these organisms. Parent and transformed strains degraded biphenyl and 2,3-dichlorobiphenyl (2,3-DCBp) at nearly the same rate and they exhibited similar BphC enzymatic activity (*i.e.*, activity of the enzyme 2,3-dihydroxybiphenyl 1,2-dioxygenase). The *gfp*-transformed strains survived in high numbers (5.6-8.0 log cfu/g) when incubated for 16-18 weeks at 4° and 22°C into non-sterilized soil spiked with 2,3-DCBp. Soil amendment with both strains enhanced the biodegradation of 2,3-DCBp (T=4°C: from 5% 2,3-DCBp elimination in uninoculated soil microcosms to 59-69% elimination in 18 weeks; T=22°C: from 30% to 64-75% elimination in 16 weeks).

The efficacy of different microbial community analysis techniques for monitoring the microbial community associated with PCB-dechlorination was compared in a recent study (45). Three techniques, *i.e.*, amplified rDNA restriction analysis of a clone library, denaturing gradient gel electrophoresis (DGGE), and terminal restriction fragment length polymorphism (TRFLP), were applied to enrichment cultures that selectively dechlorinated double-flanked chlorines in the PCB congener 2,3,4,5 chlorinated biphenyl. DGGE analysis of the highly enriched communities after growth with different e-donors and antibiotic treatments proved to be the most rapid and effective tool for the monitoring of microorganisms within the enrichment. However, each method identified the same organisms, namely, a low G + C Gram-positive eubacterium, an organism most similar to the green non-sulphur bacteria, an *Aminobacterium* sp. and a *Desulfovibrio* sp. The authors conclude that these methods,

particularly DGGE and TRPLP, in combination with selective enrichment are highly effective for identifying major components from microbial communities without isolation. The molecular screening techniques are a valuable alternative to traditional methods that require isolation for identifying species. Attempts to isolate and culture species capable of PCB dechlorination have been as yet unsuccessful.

## 4. MICROBIAL CHLORINATION

### 4.a. General Reviews

Review articles on microbial dehalogenation were not available in the fourth quarter of the year 2001. In this period, however, 3 different publications reviewed the metabolites formed by marine organisms (11) and endophyte microorganisms (34), and new naturally occurring sesquiterpenoids (12). Numerous chlorinated and other halogenated compounds (chiefly brominated compounds) are included among the natural compounds discussed in the latter reports.

Faulkner's report (11) is an extensive review of the marine natural products literature for the year 2000. The report is organized phylogenetically, with sections on marine microorganisms and phytoplankton, green algae, brown algae, red algae, sponges, coelenterates, bryozoans, molluscs, tunicates, echinoderms and miscellaneous marine organisms. Several new chlorinated metabolites were described in different marine organisms, including marine bacteria, sponges and brown algae. Production of metabolites that contained either a 7,7-dichloro-2,2-dimethyl-3-hydroxyoctanoic acid moiety (cited refs. 86–88) or a chloromethylene moiety (cited refs. 81, 83) has been reported in different specimens of the marine cyanobacterium (*Lyngbya majuscula*). Furthermore, a culture of *Pseudoalteromonas luteoviolacea* isolated from the surface of a Hawaiian produced 2,4-dibromo-6-chlorophenol (cited ref. 37). Herbacinic acid, a 5,5,5-trichlorinated leucine derivative, was isolated from a specimen of the sponge *Dysidea herbacea* (cited ref. 214). Furthermore, two polyhalogenated monoterpenes containing chloro and bromo substituents were isolated from a brown alga (*Plocamium cartilagineum*) (cited ref. 142). It is well known that many marine microorganisms produce a variety of brominated metabolites. Numerous studies were reviewed that describe the isolation of new brominated terpenoids by bacteria isolated from marine sediments (cited refs. 33-35); several brominated anisoles and cresols (cited ref. 131), brominated benzyl derivatives (cited ref. 132) and brominated diterpenes (cited refs. 147-148) in red algae; brominated aromatic pigments (rubrolides) in a tunicate species (ascidians) (cited ref. 523); and

bromoindole alkaloids (cited refs. 269, 272, 276), bromotyrosine derivative (cited refs. 295, 299, 301), polybrominated diphenyl ethers (cited refs. 303 and 379), and a brominated anisole (cited ref. 304) in different sponge species.

Tan and Zou (2001) (34) reviewed studies concerned with the functional metabolites of endophytes in literature prior to the year 2000. An endophyte is a bacterial or fungal microorganism, which colonizes the tissue of a host plant for a period encompassing part or the whole life cycle of the microbe. Three chlorinated metabolites from endophytic fungi were reported including the sesquiterpenes, heptelidic acid chlorohydrin and (-)-mycorrhizin A, and (+)-cryptosporiopsin. These compounds were isolated from cultures of balsam fir needle endophyte *Phyllosticta* sp. strain (cited ref. 69), tree endophytes *Pezicula* sp. and *P. livida* strain 1156 (cited ref. 87), respectively.

Fraga's review (12) of the isolation and structures of new naturally occurring sesquiterpenoids, cites one report concerning the formation of a new chlorinated sesquiterpenoid, sesquiterpene 6-chloro-10a-hydroxymelliolide, by the basidiomyceteous fungus *Armillaria novae-zelandiae* (cited ref. 188).

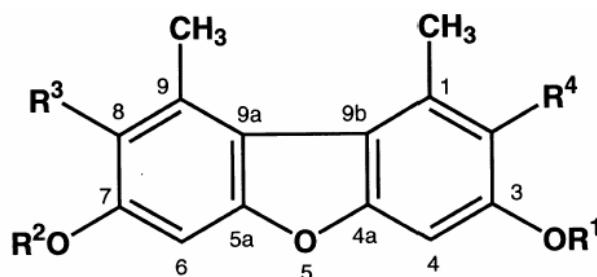
#### **4.b. Microbial Chlorination in Soils**

##### **Chloromethanes**

No reports concerning the formation of chloromethanes by soil microorganisms were found in the fourth quarter of 2001.

##### **Other Chlorinated Compounds**

Five dibenzofurans (DBF), four of which are new, were isolated from the cultures of the spore-derived mycobionts of the lichen *Lecanora cinereocarnea*, namely 3,7-dihydroxy-1,9-dimethyldibenzofuran, 2-chloro-3,7-dihydroxy-1,9-dimethyl-DBF, 2,8-dichloro-3,7-dihydroxy-1,9-dimethyl-DBF, 3-hydroxy-7-methoxy-1,9-dimethyl-DBF, and 2-chloro-7-hydroxy-3-methoxy-1,9-dimethyl-DBF (Fig. 1) (35). Their structures were determined by spectroscopic methods. The known organochlorine metabolites ascochlorin and 6-chlorocollectorin B have been isolated from cultures of the coprophilous fungus *Nigrosabulum globosum* (4).



- 1:  $R^1=R^2=R^3=R^4=H$   
 2:  $R^1=R^2=R^3=H, R^4=Cl$   
 3:  $R^1=R^2=H, R^3=R^4=Cl$   
 4:  $R^1=R^3=R^4=H, R^2=CH_3$   
 5:  $R^1=CH_3, R^2=R^3=H, R^4=Cl$

**Figure 1.** Chlorinated dibenzofurans (compounds 2, 3 and 4) isolated from cultures of the spore-derived mycobionts of the lichen *Lecanora cinereocarnea* (4).

#### 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 last quarter of 2001. One study focusing on the physiological function of vanadium bromoperoxidase and bromoform in marine algae is discussed below in section “4.d. Chlorinating Enzymes” (29).

##### Other Chlorinated Compounds

Three new polyhalogenated furanoid monoterpenes with an unusual chlorobromo vinyl functional group, have been isolated from the red alga *Plocamium cartilagineum* (7). The structure and relative stereochemistry of these compounds, named furoplocamioids A-C, was determined based on spectroscopic data and molecular mechanics calculations. Five new halogenated prostanoids were isolated from the soft coral *Clavularia viridis* and their structure was elucidated (44).

Several studies report on the isolation of chlorinated and other halogenated compounds in sponges. Novel polychlorinated metabolites, dysideaprolines A-F and barbaleucamides A-B,

were isolated from a marine sponge (*Dysidea* sp.) collected in the Philippines (16). Dysideaprolines A-F are proline-derived analogues of dysidenin. Barbaleucamides A-B are structural analogues of barbamide, a metabolite originally isolated from the cyanobacterium *Lyngbya majuscula*. The authors indicate that the isolated barbaleucamide compounds are probably derived from a symbiotic cyanobacterium found in close association with the sponge. Two known bromopyrrole metabolites and a new bromopyrrole alkaloid compound, N-methyldibromoisophakellin, were isolated and identified from the Caribbean sponge *Stylissa caribica* (2). A new dibromo-seco-isophakellin metabolite, ugibohlin, was isolated from a marine sponge (*Axinella carteri*) in the Philippines (14). The known cyclic derivative dibromoisophakellin was also detected in the sponge extract. The structure of the isolated metabolites was elucidated by spectroscopic techniques.

The non-anthropogenic compound Q1, along with a range of man-made organohalogen compounds were detected in the blubber of four bottlenose dolphins (*Tursiops truncatus*), one common dolphin (*Delphinus delphis*), and seven dugongs (*Dugong dugon*), as well as in adipose tissue of a green turtle (*Chelonia mydas*) and a python (*Morelia spilota*) from northeast Queensland (Australia) (41). Q1 was identified as a bioaccumulative, persistent natural organochlorine with molecular formula  $C_9H_3Cl_7N_2$  that is most likely a heptachlorobipyrrole (40). Q1 was a major organohalogen detected in all samples analyzed, ranging from 450-9,100  $\mu\text{g}/\text{kg}$  lipids. The highest levels of the non-anthropogenic compound Q1 were found in dugongs (160  $\text{mg}/\text{kg}$  lipids) a species that is exclusively herbivorous. Recent studies cited by the authors demonstrate that this natural organohalogen compound is widely distributed in the Southern Hemisphere. Furthermore, a series of unknown brominated compounds were observed, probably including a tetrabromo-methoxy-diphenylether. Brominated methoxy-diphenyl ethers are known to occur naturally in both the green and marine sponges. In conclusion, this study demonstrates the occurrence of several unknown organohalogen compounds in the environment that may reach higher concentrations than the well-known PCBs, DDT, and other chloropesticides. Several studies are cited which also report the existence of a major unidentified source(s) of specific organohalogen compounds, probably of natural origin, in the Southern Hemisphere.

#### 4.d. Chlorinating Enzymes

Only one article was found on the formation of the target chlorinated compounds by halogenating enzymes (38). Two additional articles are discussed in this section that report on the production of brominated compounds in red algae by bromoperoxidases (18, 29).

Biocatalytic chlorination of aromatic hydrocarbons by chloroperoxidase (CPO) of the imperfect fungus *Caldariomyces fumago* was investigated by Vazquez-Duhalt *et al.* (2001) (38). CPO is one of a variety of halogenase enzymes that can be found in nature; other enzymes such as vanadium and non-heme halogenases are also potentially able to chlorinate organic pollutants. CPO is a peroxide-dependent enzyme in the hemoprotein family that exhibits a broad spectrum of chemical reactivities. The compounds tested included: 9-methylanthracene, azulene, anthracene, 2-methylanthracene, 7,12-dimethylbenzanthracene, benzo[a]pyrene, 7-methylbenzo[a]pyrene, acenaphthene, pyrene, benzo[ghi]perylene, perylene, biphenylene, phenanthrene, fluoranthene, fluorene, triphenylene, naphthalene, biphenyl, dibenzofuran and anthrone. The CPO of *C. fumago* was found able to chlorinate 17 of 20 assayed aromatic hydrocarbons at reaction rates varying from 0.6 min<sup>-1</sup> for naphthalene to 758 min<sup>-1</sup> for 9-methylanthracene. Only biphenyl, and the oxygen-containing dibenzofuran and anthrone, were not substrates for CPO under the applied reaction conditions. Mono-, di- and tri-chlorinated compounds were obtained from the CPO-mediated reaction on aromatic compounds, and no oxygenated products could be detected. Dichloroacenaphthene, trichloroacenaphthene, 9,10-dichloroanthracene, chloropyrene, dichloropyrene, dichlorobiphenylene and trichlorobiphenylene were identified as products from acenaphthene, anthracene, pyrene and biophenylene respectively. Polycyclic aromatic hydrocarbons (PAHs) with 5 and 6 aromatic rings were also substrates for the CPO reaction. These results show that the transformation of aromatic pollutants into chlorinated derivatives by microbial enzymes may occur in polluted sites.

Ohsawa *et al.* (2001) (29) demonstrated that the main physiological function of bromoperoxidase in the red marine alga *Corallina pilulifera* is production of bromoform as an allelochemical and, the simultaneous elimination of hydrogen peroxide. Bromoform produced by *C. pilulifera* was shown to play an important role in eliminating epiphytic organisms, especially microalgae on its surface. *C. pilulifera* and other coralline alga (*Lithophyllum yessoense*, a major inhabitant of the deforested marine environment) were shown to be capable of utilizing exogenous H<sub>2</sub>O<sub>2</sub> to produce bromoform, the supply of H<sub>2</sub>O<sub>2</sub> to the BPO reaction being the rate-determining step of bromoform formation. On the other hand, the seasonal change of BPO activity in *C. pilulifera* in vivo was in proportion to superoxide dismutase activity and in inverse proportion to catalase activity. These results imply indicate that BPO could be a potential substitute for catalase, because the enzyme catalyzes an efficient Br-dependent catalase reaction.

The red algae *Laurencia* spp. produces eight-membered cyclic bromo-ethers (18). These compounds are divided into laurenan and lauthisan groups based on the type of ring

formation. Biosynthesis of both series of laurenan and lauthisan compounds directly from laurediols (linear C15 compounds) by the two-electron oxidation of bromide ion with BPO and hydrogen peroxide was demonstrated recently (18).

## 5. REFERENCES CITED

1. **Ahn, Y. B., L. A. Beaudette, H. Lee, and J. T. Trevors.** 2001. Survival of a GFP-labeled polychlorinated biphenyl degrading psychrotolerant *Pseudomonas* spp. in 4 and 22 degrees C soil microcosms. *Microbial Ecology* **42**:614-623.
2. **Assmann, M., R. W. M. van Soest, and M. Kock.** 2001. New antifeedant bromopyrrole alkaloid from the Caribbean sponge *Stylissa caribica*. *Journal of Natural Products* **64**:1345-1347.
3. **Baker, P. W., H. Futamata, S. Harayama, and K. Watanabe.** 2001. Molecular diversity of pMMO and sMMO in a TCE-contaminated aquifer during bioremediation. *Fems Microbiology Ecology* **38**:161-167.
4. **Che, Y., D. C. Swenson, J. B. Gloer, B. Koster, and D. Malloch.** 2001. Pseudodestruxins A and B: New cyclic depsipeptides from the coprophilous fungus *Nigrosabulum globosum*. *Journal of Natural Products* **64**:555-558.
5. **Chen, I. M., F. C. Chang, B. V. Chang, and Y. S. Wang.** 2000. Specificity of microbial activities in the reductive dechlorination of chlorinated benzenes. *Water Environment Research* **72**:675-679.
6. **Cutter, L. A., J. E. M. Watts, K. R. Sowers, and H. D. May.** 2001. Identification of a microorganism that links its growth to the reductive dechlorination of 2,3,5,6-chlorobiphenyl. *Environmental Microbiol.* **3**:699-709.
7. **Darias, J., J. Rovirosa, A. San Martin, A. R. Diaz, E. Dorta, and M. Cueto.** 2001. Furoplocamioids A-C, novel polyhalogenated furanoid monoterpenes from *Plocamium cartilagineum*. *Journal of Natural Products* **64**:1383-1387.
8. **De Wildeman, S., H. Nollet, H. Van Langenhove, and W. Verstraete.** 2001. Reductive biodegradation of 1,2-dichloroethane by methanogenic granular sludge in lab-scale UASB reactors. *Advances in Environmental Research* **6**:17-27.

- 
9. **Dravis, B. C., P. E. Swanson, and A. J. Russell.** 2001. Haloalkane hydrolysis with an immobilized haloalkane dehalogenase. *Biotechnology and Bioengineering* **75**:416-423.
  10. **Du, X. Y., N. K. Zhu, X. J. Xia, Z. C. Bao, and X. B. Xu.** 2001. Enhancement of biodegradability of polychlorinated dibenzo-*p*-dioxins. *J. Environ. Sci. & Health Part A- Toxic/Hazardous Substances & Environ. Eng.* **36**:1589-1595.
  11. **Faulkner, D. J.** 2001. Marine natural products. *Natural Product Rep.* **18**:1-49.
  12. **Fraga, B. N.** 2001. Natural sesquiterpenoids. *Natural Product Rep.* **18**:650-673.
  13. **Freedman, D. L., A. S. Danko, and M. F. Verce.** 2001. Substrate interactions during aerobic biodegradation of methane, ethene, vinyl chloride and 1,2-dichloroethenes. *Water-Science-and-Technology* **43**:333-340.
  14. **Goetz, G. H., G. G. Harrigan, and J. Likos.** 2001. Ugibohlin: A new dibromo-seco-isophakellin from *Axinella carteri*. *J. of Natural Products* **64**:1581-1582.
  15. **Gupta, A., C. P. Kaushik, and A. Kaushik.** 2001. Degradation of hexachlorocyclohexane isomers by two strains of *Alcaligenes faecalis* isolated from a contaminated site. *Bull. Environ. Contamination & Toxicol.* **66**:794-800.
  16. **Harrigan, G. G., G. H. Goetz, H. Luesch, S. T. Yang, and J. Likos.** 2001. Dysideaprolines A-F and barbaleucamides A-B, novel polychlorinated compounds from a *Dysidea* species. *Journal of Natural Products* **64**:1133-1138.
  17. **Hohnstock-Ashe, A. M., S. M. Plummer, R. M. Yager, et al.** 2001. Further biogeochemical characterization of a trichloroethene- contaminated fractured dolomite aquifer: Electron source and microbial communities involved in reductive dechlorination. *Env. Sci. Technol.* **35**:4449-4456.
  18. **Ishihara, J., and A. Murai.** 2001. Biosynthesis of cyclic bromo-ethers, derived from red algae. *Journal of Synthetic Organic Chemistry Japan* **59**:1181-1189.
  19. **Kahng, H.-Y., J. C. Malinverni, M. M. Majko, and J. J. Kukor.** 2001. Genetic and functional analysis of the tbc operons for catabolism of alkyl- and chloroaromatic compounds in *Burkholderia* sp. Strain JS150. *Applied and Environmental Microbiology* **67**:4805-4816.
  20. **Kang, J. M., E. Y. Lee, and S. Park.** 2001. Co-metabolic biodegradation of trichloroethylene by *Methylosinus trichosporium* is stimulated by low concentrations methane or methanol. *Biotechnology Letters* **23**:1877-1882.

- 
21. **Kao, C. M., S. C. Chen, and J. K. Liu.** 2001. Development of a biobarrier for the remediation of PCE-contaminated aquifer. *Chemosphere* **43**:1071-1078.
  22. **Kikuchi, T., K. Iwasaki, H. Nishihara, Y. Takamura, and O. Yagi.** 2001. Quantitative and specific detection of a trichloroethylene- degrading methanotroph, *Methylocystis* sp strain M, by a most probable number-polymerase chain reaction method. *Bioscience Biotechnology & Biochemistry* **65**:2673-2681.
  23. **Krasotkina, J., T. Walters, K. A. Maruya, and S. W. Ragsdale.** 2001. Characterization of the B-12- and iron-sulfur-containing reductive dehalogenase from *Desulfitobacterium chlororespirans*. *J. Biological Chem.* **276**:40991-40997.
  24. **Lee, T., T. Tokunaga, A. Suyama, and K. Furukawa.** 2001. Efficient dechlorination of tetrachloroethylene in soil slurry by combined use of an anaerobic *Desulfitobacterium* sp strain Y- 51 and zero-valent iron. *Journal of Bioscience and Bioengineering* **92**:453-458.
  25. **Lu, X. X., S. Tao, T. Bosma, and J. Gerritse.** 2001. Characteristic hydrogen concentrations for various redox processes in batch study. *J. Environ. Sci. & Health Part A- Toxic/Hazardous Substances & Environ. Eng.* **36**:1725-1734.
  26. **Maeda, T., Y. Takahashi, H. Suenaga, A. Suyama, M. Goto, and K. Furukawa.** 2001. Functional analyses of Bph-Tod hybrid dioxygenase, which exhibits high degradation activity toward trichloroethylene. *Journal of Biological Chemistry* **276**:29833-29838.
  27. **Mazur, C. S., and W. J. Jones.** 2001. Hydrogen concentrations in sulfate-reducing estuarine sediments during PCE dehalogenation. *Environmental Science & Technology* **35**:4783-4788.
  28. **Michel, F. C., J. Quensen, and C. A. Reddy.** 2001. Bioremediation of a PCB-contaminated soil via composting. *Compost Science & Utilization* **9**:274-284.
  29. **Ohsawa, N., Y. Ogata, N. Okada, and N. Itoh.** 2001. Physiological function of bromoperoxidase in the red marine alga, *Corallina pilulifera*: production of bromoform as an allelochemical and the simultaneous elimination of hydrogen peroxide. *Phytochemistry* **58**:683-692.
  30. **Pang, B. C. M., and J. S. H. Tsang.** 2001. Mutagenic analysis of the conserved residues in dehalogenase IVa of *Burkholderia cepacia* MBA4. *Fems Microbiology Letters* **204**:135-140.

- 
31. **Seffernick, J. L., and L. P. Wackett.** 2001. Rapid evolution of bacterial catabolic enzymes: A case study with atrazine chlorohydrolase. *Biochemistry* **40**:12747-12753.
  32. **Sponza, D. T.** 2001. Performance of upflow anaerobic sludge blanket (UASB) reactor treating wastewaters containing carbon tetrachloride. *World Journal of Microbiology & Biotechnology* **17**:839-847.
  33. **Takeuchi, M., K. Nanba, H. Iwamoto, H. Nirei, T. Kusuda, O. Kazaoka, and K. Furuya.** 2001. Distribution of methanotrophs in trichloroethylene-contaminated aquifers in a natural gas field. *Geomicrobiology Journal* **18**:387-399.
  34. **Tan, R. X., and W. X. Zou.** 2001. Endophytes: a rich source of functional metabolites. *Natural Product Reports* **18**:448-459.
  35. **Tanahashi, T., Y. Takenaka, N. Nagakura, and N. Hamada.** 2001. Dibenzofurans from the cultured lichen mycobionts of *Lecanora cinereocarnea*. *Phytochemistry* **58**:1129-1134.
  36. **Tauber, M., R. Rosen, and S. Belkin.** 2001. Whole-cell biodetection of halogenated organic acids. *Talanta* **55**:959-964.
  37. **Vargas, C., D. E. Fennell, and M. M. Haggblom.** 2001. Anaerobic reductive dechlorination of chlorinated dioxins in estuarine sediments. *Applied Microbiology and Biotechnology* **57**:786-790.
  38. **Vazquez-Duhalt, R., M. Ayala, and F. J. Marquez-Rocha.** 2001. Biocatalytic chlorination of aromatic hydrocarbons by chloroperoxidase of *Caldariomyces fumago*. *Phytochemistry* **58**:929-933.
  39. **Verce, M. F., R. L. Ulrich, and D. L. Freedman.** 2001. Transition from cometabolic to growth-linked biodegradation of vinyl chloride by a *Pseudomonas* sp isolated on ethene. *Environmental Science & Technology* **35**:4242-4251.
  40. **Vetter, W., L. Alder, R. Kallenborn, and M. Schlabach.** 2000. Determination of Q1, an unknown organochlorine contaminant, in human milk, Antarctic air and further environmental samples. *Environmental Pollution* **110**:401-409.
  41. **Vetter, W., E. Scholz, C. Gaus, J. F. Mueller, and D. Haynes.** 2001. Anthropogenic and natural organohalogen compounds in blubber of dolphins and

- 
- dugongs (*Dugong dugon*) from northeastern Australia. Archives of Environmental Contamination and Toxicology **41**:221-231.
42. **Vuilleumier, S., Z. Ucurum, S. Oelhafen, T. Leisinger, J. Armengaud, R. M. Wittich, and K. N. Timmis.** 2001. The glutathione S-transferase OrfE3 of the dioxin-degrading bacterium *Sphingomonas* sp. RW1 displays maleylpyruvate isomerase activity. Chemico Biological Interactions **133**:265-267.
43. **Walters, G. W., and M. D. Aitken.** 2001. Surfactant-enhanced solubilization and anaerobic biodegradation of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (DDT) in contaminated soil. Water Environment Research **73**:15-23.
44. **Watanabe, M., M. Sekine, H. Takahashi, and K. Iguchi.** 2001. New halogenated marine prostanoids with cytotoxic activity from the Okinawan soft coral *Clavularia viridis*. Journal of Natural Products **64**:1421-1425.
45. **Watts, J. E. M., Q. Z. Wu, S. B. Schreier, H. D. May, and K. R. Sowers.** 2001. Comparative analysis of polychlorinated biphenyl-dechlorinating communities in enrichment cultures using three different molecular screening techniques. Environmental Microbiology **3**:710-719.
46. **Yeager, C. M., P. J. Bottomley, and D. J. Arp.** 2001. Requirement of DNA repair mechanisms for survival of *Burkholderia cepacia* G4 upon degradation of trichloroethylene. Appl. Environ. Microbiol. **67**:5384-5391.
47. **Zhang, X. H., G. W. Sewell, and S. Y. Cui.** 2001. An improved method of hydrogen production as electron donor for anaerobic bioremediation. Journal of Environmental Science and Health Part A- Toxic/Hazardous Substances & Environmental Engineering **36**:1661-1670.

## 6.ANNEX

**Ahn, Y. B., L. A. Beaudette, et al. (2001).** "Survival of a GFP-labeled polychlorinated biphenyl degrading psychrotolerant *Pseudomonas* spp. in 4 and 22 degrees C soil microcosms." *Microbial Ecology* 42(4): 614-623.

The green fluorescent protein gene (*gfp*) was inserted into the chromosome of *Pseudomonas* spp. Cam-1 and Sag-50G, two psychrotolerant polychlorinated biphenyl (PCB)-degrading bacteria. The *gfp*-transformed microorganisms, designated Cam-1-*gfp*1, Cam-1-*gfp*2, Sag-50G-*gfp*1, and Sag-50G-*gfp*2, exhibited green fluorescence under an epifluorescent microscope. The *gfp* was inserted into the chromosome of each psychrotolerant strain and was stable with no apparent adverse effects on the metabolism and growth of each organism. Activity of *gfp*-transformed microorganisms against biphenyl and 2,3-dichlorobiphenyl was determined by assaying for BphC activity and by resting cell assays. The patterns of BphC activity at two different growth temperatures in batch cultures were similar for each of the *gfp*-transformed microorganisms. Resting cell assays of both the parent strains (Cam-1, Sag-50G) and the *gfp*-transformed strains (Cam-1-*gfp*1, Cam-1-*gfp*2, Sag-50G-*gfp*1, Sag-50G-*gfp*2), grown on glycerol or glucose, exhibited BphC activity to a lesser extent and at a slower rate than those observed for biphenyl grown cells. In addition, all *gfp*-transformed microorganisms degraded 2,3-dichlorobiphenyl (2,3-DCB) in broth to the same extent as the parent strains. When Cam-1-*gfp*1 and Sag-50G-*gfp*1 were used as a bioremediation amendment in soil microcosms spiked with 2,3-DCB, both strains survived in high numbers (5.6 to 7.9 log cfu g<sup>-1</sup>) and (5.6 to 8.0 log cfu g<sup>-1</sup>) when inoculated into nonsterilized soil over 16 weeks at 22 degrees C and 18 weeks at 4 degrees C, respectively. Biodegradation of 2,3-DCB was enhanced with the microbial amendment; however, the addition of sunflower oil did not help the PCB degrading bacteria and may have enhanced the growth of the indigenous population, thereby decreasing the amended PCB-degrading population.

**Assmann, M., R. W. M. van Soest, et al. (2001).** "New antifeedant bromopyrrole alkaloid from the Caribbean sponge *Stylissa caribica*." *Journal of Natural Products* 64(10): 1345-1347.

In this first report on the chemistry of the sponge *Stylissa caribica*, two known bromopyrrole metabolites and a new compound, N-methyldibromoisophakellin (1), were isolated and identified. The structure of 1 was determined using spectroscopic methods and the computer program COCON. N-Methyldibromoisophakellin (1) was shown to be the only secondary metabolite in *Stylissa caribica* that, at its natural concentration, is active as a feeding deterrent against a common omnivorous reef fish.

**Baker, P. W., H. Futamata, et al. (2001).** "Molecular diversity of pMMO and sMMO in a TCE-contaminated aquifer during bioremediation." *Fems Microbiology Ecology* 38(2-3): 161-167.

The particulate methane monooxygenase genes (pMMO) were amplified from methane-biostimulated aquifer samples using two sets of primers, A 189 plus A682 and pmof2 plus pmor, and the products were cloned and sequenced. The analysis of the sequences revealed a high diversity of pMMO genes in the aquifers. Some of the pMMO gene sequences (LP20 and LP21) isolated in this study were unique and unrelated to methanotrophs previously isolated from this site. Throughout all the samples collected from the different methane biostimulation trials, competitive PCR amplification using specific pMMO primers revealed that type I methanotrophs predominated over type 11 methanotrophs. The partial soluble methane monooxygenase genes were also amplified from the aquifer samples using specially designed primers but sequencing revealed a lower diversity. (C) 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Chen, I. M., F. C. Chang, et al. (2000).** "Specificity of microbial activities in the reductive dechlorination of chlorinated benzenes." *Water Environment Research* 72(6): 675-679.

Anaerobic mixed cultures collected from tropical sediment were found to possess the ability to dechlorinate pentachlorobenzene (QCB) and hexachlorobenzene (HCB) after a long-term acclimation in HCB. The primary product for HCB and QCB was 1,3,5-trichlorobenzene (1,3,5-TCB). During QCB dechlorination, 1,2,3,5-tetrachlorobenzene (1,2,3,5-TeCB) is accumulated as the intermediate, but during the HCB dechlorination, QCB and 1,2,3,5-TeCB are not found. When treated with chloramphenicol, the microorganisms precultured in HCB were found to dechlorinate HCB for only 1 day and dechlorinate QCB for several days. However, microorganisms precultured in QCB could not dechlorinate HCB at all. Results also indicate that HCB is not directly dechlorinated

to 1,3,5-TCB by a single microbial activity but that various microbial systems are responsible for each step of dechlorination from HCB to 1,3,5-TCB.

**Cutter, L. A., J. E. M. Watts, et al.** (2001). "Identification of a microorganism that links its growth to the reductive dechlorination of 2,3,5,6-chlorobiphenyl." *Environmental Microbiology* 3(11): 699-709.

Anaerobic bacteria reductively dechlorinate polychlorinated biphenyls (PCBs) in aquatic sediments, but these microorganisms remain uncultured and, until now, unidentified. Through denaturing gradient gel electrophoresis (DGGE) of 16S rDNA from a highly enriched ortho-PCB dechlorinating culture, the growth of a single microorganism was shown to be dependent upon the presence and dechlorination of 2,3,5,6-tetrachlorobiphenyl. This is the first identification of a microorganism that catalyses the reductive dechlorination of a PCB. The organism, bacterium o-17, has high sequence similarity with the green non-sulphur bacteria and with a group that includes *Dehalococcoides ethenogenes*. Bacterium o-17 required acetate for dechlorination and growth. H<sub>2</sub>:CO<sub>2</sub> (80:20 at 101 kPa) did not support dechlorination or growth of the dechlorinator. Archaeal 16S rDNA was not detected in actively dechlorinating bromoethanesulphonate-treated non-methanogenic cultures, which indicated that methanogenic Archaea were not required for dechlorination. The consistent association with dechlorinating activity combined with high similarity to other known dechlorinating microorganisms indicates that bacterium o-17 catalyses the reductive ortho-dechlorination of 2,3,5,6-tetrachlorobiphenyl.

**Darias, J., J. Roviroso, et al.** (2001). "Furoplacamioids A-C, novel polyhalogenated furanoid monoterpenes from *Plocamium cartilagineum*." *Journal of Natural Products* 64(11): 1383-1387.

Three new tetrahydrofuran derivatives, marine monoterpenes, with an unusual chlorobromo vinyl functional group, 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 and molecular mechanics (MM2) calculations. These compounds are related to pantofuranoids isolated from the antarctic *Pantoneura plocamioides*, which strongly suggests a close relationship between these species.

**De Wildeman, S., H. Nollet, et al.** (2001). "Reductive biodegradation of 1,2-dichloroethane by methanogenic granular sludge in lab-scale UASB reactors." *Advances in Environmental Research* 6(1): 17-27.

Dechlorination of 1,2-dichloroethane (1,2-DCA) dosed to a model wastewater in lab-scale upflow anaerobic sludge blanket (UASB) reactors was examined. Anaerobic granular sludge was used as a biocatalyst. Ethanol served as the main methanogenic substrate. For 3 months, two types of UASB reactors were studied, the first type consisting of a sludge blanket and the second type containing an additional layer of activated carbon. When subjected to 1,2-DCA at an average volumetric loading rate of 87.6 mg l<sup>-1</sup> day<sup>-1</sup>, the latter type obtained an average removal efficiency of 82%. Increasing the volumetric loading rate of ethanol from 5 to 15 g COD l<sup>-1</sup> day<sup>-1</sup> resulted in higher 1,2-DCA conversion rates. No chlorinated intermediates or residues were found. 1,2-DCA was converted mainly to ethene (65-80%) and ethane (<1%). Both autoclaved sludge and cell extracts were not able to degrade 1,2-DCA, which indicates the need for metabolic activity. The reactor effluents were less toxic relative to the influent when analyzed by Nitrox tests, indicating that such UASB treatments can protect a subsequent aerobic nitrifying system. The 1,2-DCA removal rates achieved, and the safe nature of the endproducts, warrant the combination of granular sludge and UASB technology for practical decontamination of waters containing such types of organochlorines. (C) 2001 Elsevier Science Ltd. All rights reserved.

**Dravis, B. C., P. E. Swanson, et al.** (2001). "Haloalkane hydrolysis with an immobilized haloalkane dehalogenase." *Biotechnology and Bioengineering* 75(4): 416-423.

Haloalkane dehalogenase from *Rhodococcus rhodochrous* was covalently immobilized onto a polyethyleneimine impregnated gamma-alumina support. The dehalogenating enzyme was found to retain greater than 40% of its original activity after immobilization, displaying an optimal loading (max. activity/supported protein) of 70 to 75 mg/g with an apparent maximum (max. protein/support) of 156 mg/g. The substrate, 1,2,3-triichloropropane, was found to favorably partition (adsorb) onto the inorganic alumina carrier (10 to 20 mg/g), thereby increasing the local reactant concentration with respect to the catalyst's environment, whereas the product, 2,3-dichloropropan-1-ol, demonstrated no affinity. Additionally, the inorganic alumina support exhibited no adverse effects because of solvent/component incompatibilities or deterioration due to pH variance (pH 7.0 to 10.5). As a result of the large surface area to volume ratio of the support matrix and the accessibility of the bound protein, the immobilized biocatalyst was not subject to internal mass transfer limitations. External diffusional

restrictions could be eliminated with simple agitation (mixing speed: 50 rpm; flux: 4.22 cm/min). The pH-dependence of the immobilized dehalogenase was essentially the same as that for the native enzyme. Finally, both the thermostability and resistance toward inactivation by organic solvent were improved by more than an order of magnitude after immobilization, (C) 2001 John Wiley & Sons, Inc.

**Du, X. Y., N. K. Zhu, et al.** (2001). "Enhancement of biodegradability of polychlorinated dibenzo-p-dioxins." *Journal of Environmental Science and Health Part a- Toxic/Hazardous Substances & Environmental Engineering* 36(9): 1589-1595.

Enhancement of biodegradability of PCDDs was studied with new isolated bacterial strains from soil and oxic-sediments contaminated by PCDDs. The results indicated that mono- and di-chlorinated dibenzo-p-dioxins could be utilized as a sole carbon source and degraded by isolated bacterial strains, but tri-chlorinated dibenzo-p-dioxin (TrCDD) was hardly degraded. The biodegradability of TrCDD and tetra-chlorinated dibenzo-p-dioxin (TCDD) by the strain *Pseudomonas* sp. EE41, a new isolated one, could be enhanced through primary nutrient of co-metabolism of *o*-dichlorobenzene (*o*-DCB). In this case, TrCDD (1.2 mg/l for 3 weeks) was degraded by 33.1% and the degradation rate enhanced more than 2 fold; also TCDD (0.1 mg/l for 3 weeks) biodegraded by 37.8%. Most highly chlorinated, Penta-, Hexa-, Hepta-, and Octa-chlorinated, dibenzo-p-dioxins (P-CDD, H-6-, H-7-CDD and OCDD) tested in this study could not be degraded while accumulated in bacterial cells.

**Faulkner, D. J.** (2001). "Marine natural products." *Natural Product Reports* 18(1): 1-49.

This review covers the marine natural products literature for the year 2000 and is organized phylogenetically, with sections on marine microorganisms and phytoplankton, green algae, brown algae, red algae, sponges, coelenterates, bryozoans, molluscs, tunicates, echinoderms and miscellaneous marine organisms. There is an emphasis on new structures, stressing their biological activities, source organisms and countries of origin, and also syntheses that confirm the structures of known compounds. The review contains 869 structures and 592 references, of which 434 appeared between January and December 2000.

**Fraga, B. N.** (2001). "Natural sesquiterpenoids." *Natural Product Reports* 18(6): 650-673.

This report follows the model of its predecessors describing both the isolation and structures of new naturally occurring sesquiterpenoids, and, in a concise form, partial and total syntheses in the area.

**Freedman, D. L., A. S. Danko, et al.** (2001). "Substrate interactions during aerobic biodegradation of methane, ethene, vinyl chloride and 1,2-dichloroethenes." *Water-Science-and-Technology* 43(5): 333-340.

Intrinsic biodegradation of trichloroethene and 1,1,1-trichloroethane in groundwater at a Superfund site in California, USA was observed. An anaerobic zone exists in the area closest to the source location, yielding the expected complement of reductive dechlorination daughter products, including *cis*-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC). Significant levels of methane and ethene were also generated in the anaerobic zone. The groundwater returns to aerobic conditions downgradient of the source, with methane, ethene, VC, and several other compounds still present. Attenuation of VC in the aerobic zone suggests that it is being biodegraded. In this study, microcosms were used to evaluate the role of methane and ethene as primary substrates for aerobic biodegradation of VC. Biodegradation of VC was fastest in the bottles containing ethene, with 40  $\mu$ mol of VC consumed over a 150 day period, compared to approximately 15-20  $\mu$ mol with methane or a mixture of methane and ethene. VC did not noticeably inhibit ethene biodegradation but did slow the rate of methane use. Methane inhibited ethene metabolism, which apparently caused a reduction in VC biodegradation when methane was present with ethene. These results suggest that ethene plays an important role during *in situ* natural attenuation of VC under aerobic conditions. Microcosms were also set up with VC alone. Following a 75 day lag period, VC consumption began and subsequent additions were consumed without a lag, suggesting the presence of organisms capable of using VC as a growth substrate. After providing VC alone for nearly 400 days, aliquots of the enrichment culture were used to evaluate its ability to biodegrade *cis*- and *trans*-DCE. Both compounds were readily consumed, although addition of VC as the primary substrate was needed to sustain biodegradation of repeated additions. This result suggests that organisms capable of using VC as a sole substrate may play an active role in aerobic natural attenuation of DCEs.

**Goetz, G. H., G. G. Harrigan, et al.** (2001). "Ugibohlin: A new dibromo-seco-isophakellin from *Axinella carteri*." *Journal of Natural Products* 64(12): 1581-1582.

Chemical investigation of a marine sponge, *Axinella carteri*, collected on a reed slope of Talakanen Island, Phillipines, has afforded the new metabolite ugibohlin (1), along with its known cyclic derivative dibromoisophakellin (2). Structure elucidation of the isolated metabolites involved high-field 2D NMR spectroscopy including H-1-H-1 COSY, HSQC, and HMBC. Revised chemical shift assignments are provided for 2.

**Gupta, A., C. P. Kaushik, et al.** (2001). "Degradation of hexachlorocyclohexane isomers by two strains of *Alcaligenes faecalis* isolated from a contaminated site." *Bulletin of Environmental Contamination and Toxicology* 66(6): 794-800. Abstract not available online

**Harrigan, G. G., G. Goetz, et al.** (2001). "Dysideaprolines and barbaleucamides: Novel polychlorinated peptides from a *Dysidea* species." *Abstracts of Papers of the American Chemical Society* 222: 136-MEDI.

**Harrigan, G. G., G. H. Goetz, et al.** (2001). "Dysideaprolines A-F and barbaleucamides A-B, novel polychlorinated compounds from a *Dysidea* species." *Journal of Natural Products* 64(9): 1133-1138.

Chemical investigation of a marine sponge, *Dysidea* sp., collected at Bararin Island, Philippines, has afforded the novel metabolites 1-6, proline-derived analogues of dysidenin (7). We have termed compounds 1-6 dysideaprolines A-F, respectively. Also isolated were compounds 8 and 9, structural analogues of barbamide (10), a metabolite originally isolated from the cyanobacterium *Lyngbya majuscula*. We have termed these novel compounds barbaleucamides A (8) and B (9). It is most probable that the compounds presented here are actually derived from a symbiotic cyanobacterium found in close association with the *Dysidea* sp. Structure elucidation of the isolated metabolites involved high-field 2D NMR spectroscopy including H-1-H-1 COSY, HSQC, and HMBC.

**Hohnstock-Ashe, A. M., S. M. Plummer, et al.** (2001). "Further biogeochemical characterization of a trichloroethene- contaminated fractured dolomite aquifer: Electron source and microbial communities involved in reductive dechlorination." *Environmental Science & Technology* 35(22): 4449-4456.

A recent article presented geochemical and microbial evidence establishing metabolic adaptation to and in-situ reductive dechlorination of trichloroethene (TICE) in a fractured dolomite aquifer. This study was designed to further explore site conditions and microbial populations and to explain previously reported enhancement of reductive dechlorination by the addition of pulverized dolomite to laboratory microcosms. A survey of groundwater geochemical parameters (chlorinated ethenes, ethene, H<sub>2</sub>, CH<sub>4</sub>, DIC, DOC, and delta C-13 values for CH<sub>4</sub>, DIC, and DOC) indicated that in situ reductive dechlorination was ongoing and that an unidentified pool of organic carbon was contributing, likely via microbial respiration, to the large and relatively light onsite DIC pool. Petroleum hydrocarbons associated with the dolomite rock were analyzed by GC/MS and featured a characteristically low delta C-13 value. Straight chain hydrocarbons were extracted from the dolomite previously found to stimulate reductive dechlorination; these were particularly depleted in hexadecane (HD). Thus, we hypothesized that HD and related hydrocarbons might be anaerobically respired and serve both as the source of onsite DIC and support reductive dechlorination of TCE. Microcosms amended with pulverized dolomite demonstrated reductive dechlorination, whereas a combusted dolomite amendment did not. HD-amended microcosms were also inactive. Therefore, the stimulatory factor in the pulverized dolomite was heat labile, but that component was not HD. Amplified Ribosomal DNA Restriction Analysis (ARDRA) of the microbial populations in well waters indicated that a relatively low diversity, sulfur-transforming community outside the plume was shifted toward a high diversity community including *Dehalococcoides ethenogenes*-type microorganisms inside the zone of contamination. These observations illustrate biogeochemical intricacies of in situ reductive dechlorination reactions.

**Ishihara, J. and A. Murai** (2001). "Biosynthesis of cyclic bromo-ethers, derived from red algae." *Journal of Synthetic Organic Chemistry Japan* 59(12): 1181-1189.

Various halogenated organic compounds, originated from marine livings, have been well-known over five decades. Particularly, cyclic bromo-ether have attracted many chemists to study their existence, biogenetic syntheses and artificial syntheses, owing to their unique structures. *Laurencia* species produce eight-membered ethers as major products, although their syntheses by chemical procedures are generally difficult. These compounds are divided into laurenan and lauthisan groups based on the type of ring formation. The laurenan compounds are characteristic in respect of C 6-S and C 7-S configurations, while the lauthisan series have R and R

at the requisite positions. It has been proposed without any proof that the bromine atoms in the marine compounds could be introduced via bromo cationic species, generated by the two-electron oxidation of bromide ion with bromoperoxidase (BPO) and hydrogen peroxide. We studied on the enzymatic formation of these bromo-ethers, and found that both series of laurenan and lauthisan compounds could be biosynthesized directly from linear C 15 compounds, laurediols. Herein, we mention our study of these natural products, and the recent studies around this field.

**Kahng, H.-Y., J. C. Malinverni, et al.** (2001). "Genetic and Functional Analysis of the *tbc* Operons for Catabolism of Alkyl- and Chloroaromatic Compounds in *Burkholderia* sp. Strain JS150." *Applied and Environmental Microbiology* 67(10): 4805-4816.

*Burkholderia* sp. strain JS150 is able to metabolize a wide range of alkyl- and chloroaromatic hydrocarbons through multiple, apparently redundant catabolic pathways. Previous research has shown that strain JS150 is able to synthesize enzymes for multiple upper pathways as well as multiple lower pathways to accommodate variously substituted catechols that result from degradation of complex mixtures of monoaromatic compounds. We report here the genetic organization and functional characterization of a gene cluster, designated *tbc* (for toluene, benzene, and chlorobenzene utilization), which has been cloned as a 14.3-kb DNA fragment from strain JS150 into vector pRO1727. The cloned DNA fragment expressed in *Pseudomonas aeruginosa* PAO1c allowed the recombinant to grow on toluene or benzene and to transform chlorobenzene, trichloroethylene, phenol, and cresols. The *tbc* genes are organized into two divergently transcribed operons, *tbc1* and *tbc2*, each comprised of six open reading frames. Similarity searches of databases revealed that the *tbc1* and *tbc2* genes showed significant homology to multicomponent cresol and phenol hydroxylases and to toluene and benzene monooxygenases, respectively. Deletion mutagenesis and product analysis were used to demonstrate that *tbc2* plays a role in the initial catabolism of the unactivated alkyl- or chloroaromatic substrate and that the *tbc1* gene products play a role in the catabolism of the first metabolite that results from transformation of the initial substrate. Phylogenetic analysis was used to compare individual components of these *tbc* monooxygenases with similar sequences in the databases. These results provide further evidence for the existence of multiple, functionally redundant alkyl- and chloroaromatic monooxygenases in strain JS150.

**Kang, J. M., E. Y. Lee, et al.** (2001). "Co-metabolic biodegradation of trichloroethylene by *Methylosinus trichosporium* is stimulated by low concentrations methane or methanol." *Biotechnology Letters* 23(22): 1877-1882.

Co-metabolic biodegradation of trichloroethylene by *Methylosinus trichosporium* OB3b was stimulated by low concentrations of methane (up to 70  $\mu$ M) or methanol (up to 0.4 mM) but inhibited at higher concentrations of them. A kinetic equation describing the dual effects of methane or methanol is proposed and the relevant kinetic constants have been determined.

**Kao, C. M., S. C. Chen, et al.** (2001). "Development of a biobarrier for the remediation of PCE-contaminated aquifer." *Chemosphere* 43(8): 1071-1078. Abstract not available on line

**Kikuchi, T., K. Iwasaki, et al.** (2001). "Quantitative and specific detection of a trichloroethylene-degrading methanotroph, *Methylocystis* sp strain M, by a most probable number-polymerase chain reaction method." *Bioscience Biotechnology and Biochemistry* 65(12): 2673-2681.

We developed a rapid and specific enumeration method for a trichloroethylene-degrading methanotroph, *Methylocystis* sp. strain M, based on a most probable number-polymerase chain reaction method for monitoring the bacterium at bioremediation sites. The primers designed for the *mmoC* gene of the soluble methane monooxygenase gene cluster were specific to strain M. Recovery of the cells with a membrane filter enabled us to detect strain M in trichloroethylene-contaminated groundwater. We used the enumeration method to monitor the number of strain M cells in effluent from soil columns supplied with trichloroethylene-contaminated groundwater. The number of strain M cells in the effluent depended on the amount of the strain M inoculated and the number of cells measured by the most probable number-polymerase chain reaction method was correlated with that measured by a culture method. The detection limit for strain M in effluent detected by MPN-PCR method was 4 to 8 x 10<sup>2</sup> cells/ml.

Krasotkina, J., T. Walters, et al. (2001). "Characterization of the B-12- and iron-sulfur-containing reductive dehalogenase from *Desulfitobacterium chlororespirans*." *Journal of Biological Chemistry* 276(44): 40991-40997.

The United Nations and the U.S. Environmental Protection Agency have identified a variety of chlorinated aromatics that constitute a significant health and environmental risk as "priority organic pollutants," the so-called "dirty dozen." Microbes have evolved the ability to utilize chlorinated aromatics as terminal electron acceptors in an energy-generating process called dehalorespiration. In this process, a reductive dehalogenase (CprA), couples the oxidation of an electron donor to the reductive elimination of chloride. We have characterized the B-12 and iron-sulfur cluster-containing 3-chloro-4-hydroxybenzoate reductive dehalogenase from *Desulfitobacterium chlororespirans*. By defining the substrate and inhibitor specificity for the dehalogenase, the enzyme was found to require a hydroxyl group ortho to the halide. Inhibition studies indicate that the hydroxyl group is required for substrate binding. The carboxyl group can be replaced by other functionalities, e.g. acetyl or halide groups, ortho or meta to the chloride to be eliminated. The purified *D. chlororespirans* enzyme could dechlorinate an hydroxylated PCB (3,3',5,5'-tetra-chloro-4,4'-biphenyldiol) at a rate about 1% of that with 3-chloro-4-hydroxybenzoate. Solvent deuterium isotope effect studies indicate that transfer of a single proton is partially rate-limiting in the dehalogenation reaction.

**Lee, T., T. Tokunaga, et al.** (2001). "Efficient dechlorination of tetrachloroethylene in soil slurry by combined use of an anaerobic *Desulfitobacterium* sp strain Y-51 and zero-valent iron." *Journal of Bioscience and Bioengineering* 92(5): 453-458.

A laboratory test was conducted to examine the combined effect of bioaugmentation of an anaerobic bacterial *Desulfitobacterium* sp. strain Y-51 and addition of zero-valent iron (Fe-0) on the reductive dechlorination of tetrachloroethylene (PCE) in a non-sterile soil slurry. Introduction of a strain Y-51 culture in soil (3 mg vss (volatile suspended solids)/kg soil) containing PCE (at 60  $\mu\text{mol/kg}$  soil) led to complete conversion of PCE to cis-1,2-dichloroethylene (cis-DCE) within 40 d. Treatments of the same soil slurry with Fe-0 (0.1-1.0%) resulted in extended PCE dechlorination to ethylene (ETH) and ethane (ETA). The combined use of a strain Y-51 culture and Fe-0 showed effective dechlorination of PCE than did the, individual use. The cis-DCE produced from biological PCE dechlorination by strain Y-51 was totally converted to non-chlorinated end products by the following chemical reduction by, Fe-0. Furthermore, anaerobic corrosion of Fe-0 was found to stimulate the biological reductive dechlorination of PCE by keeping proper levels of pH and oxidation-reduction potential (ORP) and by producing cathodic hydrogen, which might be used as an electron donor for respiratory PCE dechlorination. These findings suggest that the combined use of bacterial strain Y-51 and Fe-0 is effective for practical treatment of PCE and other chlorinated ethylenes in contaminated sites.

**Lu, X. X., S. Tao, et al.** (2001). "Characteristic hydrogen concentrations for various redox processes in batch study." *Journal of Environmental Science and Health Part A- Toxic/Hazardous Substances & Environmental Engineering* 36(9): 1725-1734.

The dissolved hydrogen concentrations under various redox processes were investigated based on batch experiments. Chloroethenes including tetrachloroethene (PCE), cis-dichloroethene (cis-DCE) and vinylchloride (VC) were respectively used as culture substrates. For each chloroethene, a series of bottles were prepared with the additions of different electron acceptors or donors such as nitrate, manganese oxide, ferrous iron, sulfate, carbondioxide and volatile fatty acids. Hydrogen concentrations as well as redox species were measured over time to ensure the achievements of characteristic hydrogen levels in various enrichment batches. The results showed that redox processes with nitrate, manganese oxide and ferric iron as the electron acceptors exhibited hydrogen threshold values close to PCE/TCE dechlorination, whereas cis-DCE and VC dechlorinations exhibited hydrogen threshold values in the range of sulfate reduction and methanogenesis, respectively. Characteristic hydrogen concentrations for various redox processes were as follows (nM): denitrification, 0.1-0.4; manganese reduction, 0.1-2.0; iron reduction, 0.1-0.4; sulfate reduction, 1.5-4.5; methanogenesis, 2.5-24; PCE/TCE dechlorination, 0.6-0.9; cis-DCE dechlorination, 0.1-2.5; and VC dechlorination, 2-24.

**Maeda, T., Y. Takahashi, et al.** (2001). "Functional analyses of Bph-Tod hybrid dioxygenase, which exhibits high degradation activity toward trichloroethylene." *Journal of Biological Chemistry* 276(32): 29833-29838.

Biphenyl dioxygenase (BphDox) in *Pseudomonas pseudoalcaligenes* KF707 is a multicomponent enzyme consisting of an iron-sulfur protein (ISP) that is composed of alpha (BphA1) and beta (BphA2) subunits, a ferredoxin (FDBphA3), and a ferredoxin reductase (FDRBphA4). A recombinant *Escherichia coli* strain

expressing hybrid Dox that had replaced BphA1 with TodC1 (alpha subunit of toluene dioxygenase (TolDox) of *Pseudomonas putida*) exhibited high activity toward trichloroethylene (TCE) (Furukawa, K., Hirose, J., Hayashida, S., and Nakamura, K. (1994) *J. Bacteriol.* 176, 2121-2123). In this study, ISP, FD, and FDR were purified and characterized. Reconstitution of the dioxygenase components consisting of purified ISPTodC1BphA2, FDBphA3, and FDRBphA4 exhibited oxygenation activities toward biphenyl, toluene, and TCE. Native polyacrylamide gel electrophoresis followed by the Ferguson plot analyses demonstrated that ISPTodC1BphA2 and ISPBphA1A2 were present as heterohexamers, whereas ISPTodC1C2 was present as a heterotetramer. The molecular activity ( $k_0$ ) of the hybrid Dox for TCE was 4.1 min<sup>-1</sup>, which is comparable to that of TolDox. The  $K_m$  value of the hybrid Dox for TCE was 130  $\mu$ M, which was lower than 250  $\mu$ M for TolDox. These results suggest that the alpha subunit of ISP is crucial for the determination of substrate specificity and that the change in the alpha subunit conformation of ISP from alpha2beta2 to alpha3beta3 results in the acquisition of higher affinity to TCE, which may lead to high TCE degradation activity.

**Mazur, C. S. and W. J. Jones** (2001). "Hydrogen concentrations in sulfate-reducing estuarine sediments during PCE dehalogenation." *Environmental Science & Technology* 35(24): 4783-4788.

Despite recent progress made evaluating the role of hydrogen (H<sub>2</sub>) as a key electron donor in the anaerobic remediation of chloroethenes, few studies have focused on the evaluation of hydrogen thresholds relative to reductive dehalogenation in sulfidogenic environments. Competition for hydrogen exists among microbial populations in anaerobic sediments, and direct evidence indicates that lower hydrogen thresholds are observed with more energetically favorable electron-accepting processes. This study examined aqueous hydrogen concentrations associated with sulfate reduction and perchloroethylene (PCE) dehalogenation in anoxic estuarine sediment slurry microcosms and evaluated the competition for H<sub>2</sub>-reducing equivalents within these systems. After an initial lag period of 13 days, PCE was reductively transformed to trichloroethylene (TCE). During the time of continuous PCE dehalogenation, a significantly ( $P < 0.05$ ) lower hydrogen concentration (0.5 nM) was observed in the sediment slurries amended with PCE as compared to slurries without PCE (0.8 nM). Sulfate reduction to sulfide was observed in all sediment slurries, but in microcosms actively dechlorinating PCE, the amount of reducing equivalents directed to sulfate reduction was approximately half the amount in sediment slurries without PCE. These findings provide evidence that a lower hydrogen threshold exists in anoxic estuarine sediment slurries with PCE as a terminal electron acceptor as compared to sediment slurries in which sulfate reduction was the predominant electron-accepting process. Furthermore, our results utilizing the inhibitor molybdate indicated that H<sub>2</sub>-utilizing methanogens may have the potential to effectively compete with dechlorinators for hydrogen when sulfate reduction is initially inhibited.

**Michel, F. C., J. Quensen, et al.** (2001). "Bioremediation of a PCB-contaminated soil via composting." *Compost Science & Utilization* 9(4): 274-284.

Polychlorinated biphenyls (PCBs) were widely used in the past and now contaminate many industrial and natural areas. In this study, a PCB-contaminated soil from a former paper mill was mixed with a yard trimmings amendment and composted in field scale piles to determine the effect of soil to amendment ratio on PCB degradation. Temperature, oxygen concentrations, and a number of other environmental parameters that influence microbial activity during composting were monitored. The PCBs in contaminated soil had a concentration of 16 +/- 1 mg/kg dw and an average of 4 chlorines per biphenyl. The soil was composted with five levels of yard trimmings amendment (14% to 82% by weight) in pilot scale compost piles (25 m<sup>3</sup>) turned once per month. Results showed up to a 40% loss of PCBs with amendment levels of 60%, and 82%. Congener specific PCB analysis indicated that less chlorinated PCB congeners (1-3 chlorines per biphenyl) were preferentially degraded. Bench-scale studies indicated that less than 1% of the PCBs in the contaminated soil were volatilized from composts during incubation with forced aeration at 55 degreesC. In conclusion, PCB loss observed during the composting of the PCB-contaminated soil appeared to be largely due to biodegradation and not volatilization. Effective bioremediation of aged PCB-contaminated soils may require coupling of composting with additional remediation technologies to reduce levels of PCB congeners with greater than 4 chlorines.

**Ohsawa, N., Y. Ogata, et al.** (2001). "Physiological function of bromoperoxidase in the red marine alga, *Corallina pilulifera*: production of bromoform as an allelochemical and the simultaneous elimination of hydrogen peroxide." *Phytochemistry* 58(5): 683-692.

The physiological function of vanadium-bromoperoxidase (BPO) in the marine red alga, *Corallina pilulifera*, has been characterized from the viewpoint of allelochemical formation. The algae emit bromoform (CHBr<sub>3</sub>) depending on the enzyme activity level in vivo (Itoh, N., Shinya, M., 1994. Seasonal evolution of bromomethanes from coralline algae and its effect on atmospheric ozone. *Marine Chemistry* 45, 95-103). We demonstrated that bromoform produced by *C. pilulifera* played an important role in eliminating epiphytic organisms, especially microalgae on the surface. Such data suggest a strong relationship between the coralline algae and the coralline flat (deforested area in the marine environment: called isoyake in Japanese). *Lithophyllum yessoense*, the main inhabitant of coralline flats in Japan, produced a lower level of CHBr<sub>3</sub> than *C. pilulifera*, and showed BPO activity. On the other hand, the seasonal change of BPO activity in *C. pilulifera* in vivo was in proportion to superoxide dismutase (SOD) activity and in inverse proportion to catalase activity. The phenomenon implies that BPO could be a potential substitute for catalase, because the enzyme catalyzes an efficient Br-dependent catalase reaction.

**Pang, B. C. M. and J. S. H. Tsang (2001).** "Mutagenic analysis of the conserved residues in dehalogenase IVa of *Burkholderia cepacia* MBA4." *Fems Microbiology Letters* 204(1): 135-140.

Amino and carboxyl terminal deletion derivatives of dehalogenase IVa (DehIVa) of *Burkholderia cepacia* MBA4 were constructed and analyzed for enzyme activity and for protein integrity. The results suggested that the majority of the protein is indispensable. Point mutations on 29 conserved charged and/or polar residues were generated and characterized. Derivatives D11E, D11N, D11S and D181N were totally inactive while mutant N178D was defective in catalysis. Mutations of other conserved residues displayed varying effects. Mutation that enhances DehIVa activity has been shown to be inhibitory in other dehalogenase and essential conserved residues in DehIVa have been shown to be dispensable in others. This suggests there is no general rule for the importance of these conserved residues. (C) 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Seffernick, J. L. and L. P. Wackett (2001).** "Rapid evolution of bacterial catabolic enzymes: A case study with atrazine chlorohydrolase." *Biochemistry* 40(43): 12747-12753. Abstract not available online

**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  $2.4 \times 10^{-3}$  mg CT/TSS day<sup>-1</sup>, 1.37 mg CT/l, 0.69 mg TSS/mg CT and 0.046 day<sup>-1</sup>, respectively for CT biotransformation during granulation.

**Takeuchi, M., K. Nanba, et al. (2001).** "Distribution of methanotrophs in trichloroethylene-contaminated aquifers in a natural gas field." *Geomicrobiology Journal* 18(4): 387-399.

The distribution of methanotrophs was examined in shallow aquifers contaminated with trichloroethylene in the southern Kanto gas field, Chiba, Japan. The total populations of methanotrophs and the numbers of methanotrophs producing soluble methane monooxygenase (sMMO) were determined separately. Hydrostratigraphic units of a Pleistocene to Holocene stratum consisted of three aquifers separated by nontransmissive silt layers. Dissolved methane concentrations increased with depth and were highest in the third aquifer. The number of methanotrophs was higher in the second aquifer than in the first aquifer. A clear relationship was observed between microbial populations and lithofacies. The greatest abundance of methanotrophs was observed in the coarse sand layers of the second aquifer, with the lowest abundance observed

in silt layers. The high abundance of methanotrophs in the coarse sand in the second aquifer implied that this part of the stratum plays an important role in in situ bioremediation.

**Tan, R. X. and W. X. Zou (2001).** "Endophytes: a rich source of functional metabolites." *Natural Product Reports* 18(4): 448-459.

An endophyte is a bacterial (including actinomycete) or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant (Fig. 1), typically causing no apparent symptoms of disease.<sup>1,2</sup> The endophytic population of a given species varies from several to a few hundreds of bacterial and fungal strains. Endophytes can be isolated from mildly surface-sterilized plant tissues and cultivated on nutrient agar (Fig. 2). The relationship between the endophyte and its host plant may range from latent phytopathogenesis to mutualistic symbiosis. Presumably owing to their specialised niches, no substantial body of work has accumulated since the first discovery of endophytic fungus in darnel in 1904.<sup>4</sup> However, much renewed attention is now being paid to the chemistry and bioactivity of endophyte metabolites, and to endophytic biodiversity and related ecological functions. This review, beginning with a brief survey of the biological aspects of endophytes, describes endophyte metabolites characterized before 2000.

**Tanahashi, T., Y. Takenaka, et al. (2001).** "Dibenzofurans from the cultured lichen mycobionts of *Lecanora cinereocarnea*." *Phytochemistry* 58(7): 1129-1134.

From the cultures of the spore-derived mycobionts of the lichen *Lecanora cinereocarnea*, five dibenzofurans, 3,7-dihydroxy-1,9-dimethyldibenzofuran, 2-chloro-3,7-dihydroxy-1,9-dimethyldibenzofuran, 2,8-dichloro-3,7-dihydroxy-1,9-dimethyldibenzofuran, 3-hydroxy-7-methoxy-1,9-dimethyldibenzofuran, and 2-chloro-7-hydroxy-3-methoxy-1,9-dimethyldibenzofuran, were isolated.

**Tauber, M., R. Rosen, et al. (2001).** Whole-cell biodetection of halogenated organic acids." *Talanta* 55(5): 959-964.

A whole-cell bacterial sensor system for short-chain halo-organic acids was constructed, using 2-chloropropionic acid (2-CPA) as a model pollutant. An *Escherichia coli* host was transformed with a moderate-copy plasmid containing a fusion of two foreign genetic elements: (a) a promoter-containing segment of the *Pseudomonas* DL-DEX (DL-2-haloacid dehalogenase) encoding gene and (b) bioluminescence (*luxCDABE*) genes of *Photobacterium luminescens*. The resulting construct, named MT1, responded to the presence of 2-CPA by dose-dependent light emission, in a highly specific albeit a very insensitive manner. Thus, while the desired concept was successfully demonstrated, further genetic work is needed in order to make such a construct practical for environmental monitoring purposes. (C) 2001 Elsevier Science B.V. All rights reserved.

**Vargas, C., D. E. Fennell, et al. (2001).** "Anaerobic reductive dechlorination of chlorinated dioxins in estuarine sediments." *Applied Microbiology and Biotechnology* 57(5-6): 786-790.

The biotransformation of 1,2,3,4-tetrachlorodibenzo-p-dioxin (1,2,3,4-tetraCDD) under anaerobic sulfate-reducing, methanogenic, and iron-reducing conditions was examined with anaerobic enrichment cultures established with sediment from an estuarine intertidal strait in the New York/New Jersey harbor. In addition, the effect of prior enrichment on 2-bromophenol or a mixture of 2-, 3-, and 4-bromophenol on dioxin dechlorination was examined. All enrichments were spiked with 1 ppm 1,2,3,4-tetraCDD and monitored by gas chromatography-mass spectrometry for up to a 3-year period. Reductive dechlorination was initially observed only under methanogenic conditions in the cultures enriched on all three bromophenol isomers. 1,2,3,4-TetraCDD was dechlorinated in the lateral position to 1,2,4-triCDD. The initial appearance of 1,2,4-triCDD was observed after 2 months, with further dechlorination to 1,3-diCDD within 17 months.

**Vazquez-Duhalt, R., M. Ayala, et al. (2001).** "Biocatalytic chlorination of aromatic hydrocarbons by chloroperoxidase of *Caldariomyces fumago*." *Phytochemistry* 58(6): 929-933.

Chloroperoxidase from *Caldariomyces fumago* was able to chlorinate 17 of 20 aromatic hydrocarbons assayed in the presence of hydrogen peroxide and chloride ions. Reaction rates varied from 0.6 min<sup>-1</sup> for naphthalene to 758 min<sup>-1</sup> for 9-methylanthracene. Mono-, di- and tri-chlorinated compounds were obtained from the chloroperoxidase-mediated reaction on aromatic compounds. Dichloroacenaphthene, trichloroacenaphthene, 9,10-dichloroanthracene, chloropyrene, dichloropyrene, dichlorobiphenylene and trichlorobiphenylene were identified by mass spectral analyses as products from acenaphthene, anthracene, pyrene and biophenylene

respectively. Polycyclic aromatic hydrocarbons with 5 and 6 aromatic rings were also substrates for the chloroperoxidase reaction. The importance of the microbial chlorination of aromatic pollutants and its potential environmental impact are discussed.

**Verce, M. F., R. L. Ulrich, et al.** (2001). "Transition from cometabolic to growth-linked biodegradation of vinyl chloride by a *Pseudomonas* sp isolated on ethene." *Environmental Science & Technology* 35(21): 4242-4251.

*Pseudomonas aeruginosa* strain DL1 was isolated on ethene as a sole carbon and energy source. When ethene-grown DL1 was first exposed to vinyl chloride (VC), the rate of VC consumption was very rapid and then declined sharply, indicative of a cometabolic process. A lack of growth and significant release of soluble products during this interval also indicates that the initial activity on VC was cometabolic. Following the rapid initial rate of VC cometabolism, a slow rate of VC utilization continued. After an extended period of incubation (> 40 days), a transition occurred that allowed DL1 to begin using VC as a primary growth substrate, with an observed yield, maximum growth rate, and Monod half saturation coefficient of 0.21 mg of total suspended solids/mg VC, 0.046 d<sup>-1</sup>, and 1.17 μM VC, respectively, at 22 degreesC. Acetylene inhibits consumption of ethene and VC by ethene-grown cells, suggesting a monooxygenase is responsible for initiating metabolism of these alkenes. Resting cells grown on ethene cometabolized VC with an observed transformation capacity of 9.1 μmol VC/mg total suspended solids and a transformation yield of 0.22 mol VC/mol ethene. The presence of 40 μM ethene increased the rate and amount of VC cometabolized. However, consumption of higher concentrations of ethene decreased the total amount of VC consumed, and VC inhibited ethene utilization. A kinetic model was developed that describes substrate interactions during batch depletion of ethene and VC for a range of initial concentrations. The results suggest that ethene may stimulate in situ biodegradation of VC either by functioning as a primary substrate to support cometabolism of VC or by selecting for organisms that can utilize VC as a primary substrate.

**Vetter, W., E. Scholz, et al.** (2001). "Anthropogenic and natural organohalogen compounds in blubber of dolphins and dugongs (*Dugong dugon*) from northeastern Australia." *Archives of Environmental Contamination and Toxicology* 41(2): 221-231.

A range of organohalogen compounds (10 polychlorinated biphenyl (PCB) congeners, DDT and metabolites, chlordane-related compounds, the potential natural organochlorine compound Q1, toxaphene, hexachlorobenzene, hexachlorocyclohexanes, dieldrin, and several yet unidentified brominated compounds) were detected in the blubber of four bottlenose dolphins (*Tursiops truncatus*), one common dolphin (*Delphinus delphis*), and seven dugongs (*Dugong dugon*), as well as in adipose tissue of a green turtle (*Chelonia mydas*) and a python (*Morelia spilota*) from northeast Queensland (Australia). The green turtle and dugongs accumulated lower organohalogen levels than the dolphins. Lower levels in dugongs were expected because this species is exclusively herbivorous. Highest PCB and DDT levels recorded in dugongs were 209 and 173 μg/kg lipids, respectively. Levels of the nonanthropogenic heptachlorinated compound Q1 (highest level in dugongs was 160 μg/kg lipids) were estimated using the ECD response factor of trans-nonachlor. Highest organohalogen levels were found in blubber of dolphins for sumDDT (575-52,500 μg/kg) and PCBs (600-25,500 μg/kg lipids). Furthermore, Q1 was a major organohalogen detected in all samples analyzed, ranging from 450-9,100 μg/kg lipids. The highest concentration of Q1 determined in this study represents the highest concentration reported to date in an environmental sample. Levels of chlordane-related compounds were also high (280-7,700 μg/kg, mainly derived from trans-nonachlor), but concentrations of hexachlorobenzene, hexachlorocyclohexanes, dieldrin, and toxaphene were relatively low and contributed little to the overall organohalogen contamination. Furthermore, a series of three major (BC-1, BC-2, and BC-3) and six minor (BC-4 through BC-9) unknown brominated compounds were observable by extracting m/z 79 and m/z 81 from the GC/ECNI-MS full scan run. Structural proposals were made for the two major recalcitrant compounds (referred to as BC-1 and BC-2). BC-2 appears to be a tetrabromo-methoxy-diphenylether (512 u) and BC-1 has 14 u (corresponding with an additional CH<sub>2</sub> group) more relative to BC-1. In general, the organohalogen pattern observed in blubber of dolphins was different compared to similar samples from other locations in the world, which is apparent from the fact that the four major abundant signals in the GC/ECD chromatogram of *D. delphis* originated from the four unknown compounds Q1, BC-1, BC-2, and BC-3.

**Vetter, W., L. Alder, R. Kallenborn, and M. Schlabach.** 2000. Determination of Q1, an unknown organochlorine contaminant, in human milk, Antarctic air and further environmental samples. *Environmental Pollution* 110:401-409.

**Walters, G. W. and M. D. Aitken** (2001). "Surfactant-enhanced solubilization and anaerobic biodegradation of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (DDT) in contaminated soil." *Water Environment Research* 73(1): 15-23. No abstract available

**Watanabe, M., M. Sekine, et al.** (2001). "New halogenated marine prostanoids with cytotoxic activity from the Okinawan soft coral *Clavularia viridis*." *Journal of Natural Products* 64(11): 1421-1425.

Five new halogenated prostanoids 1-4 and 6 were isolated from the Okinawan soft coral *Clavularia viridis*. The gross structure of I was elucidated mainly on the basis of NMR spectral data. The relative and absolute configurations were determined by analysis of NOESY and CD data, chemical conversion, and the modified Mosher's method. The structures of 2-4 and 6 were deduced by comparison of their spectral data with those of 1. Compound 1 demonstrated cytotoxic activity.

**Watts, J. E. M., Q. Z. Wu, et al.** (2001). "Comparative analysis of polychlorinated biphenyl-dechlorinating communities in enrichment cultures using three different molecular screening techniques." *Environmental Microbiology* 3(11): 710-719.

The catalysts for many microbially mediated environmental processes such as the dechlorination of polychlorinated biphenyls (PCBs) have been difficult to identify by traditional isolation techniques. Numerous, as yet unsuccessful, attempts have been made to isolate and culture the dechlorinating species. To overcome this limitation, amplified rDNA restriction analysis (ARDRA) of a clone library, denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (TRFLP) were used concurrently to compare their effectiveness for characterizing an enriched microbial community. These methods were applied to enrichment cultures that selectively dechlorinated double-flanked chlorines in the PCB congener 2,3,4,5 chlorinated biphenyl. The methods have different biases, which were apparent from discrepancies in the relative clone frequencies (ARDRA), band intensities (DGGE) or peak heights (TRFLP) from the same enrichment culture. However, each method was effectively qualitative and identified the same organisms: a low G + C Gram-positive eubacterium, an organism most similar to the green non-sulphur bacteria, an *Aminobacterium* sp. and a *Desulfovibrio* sp. Overall, in community fingerprinting and preliminary identification, DGGE proved to be the most rapid and effective tool for the monitoring of microorganisms within a highly enriched culture. TRFLP results corroborated DGGE fingerprint analysis; however, identification required the additional step of creating a clone library. ARDRA provided an in-depth analysis of the community and this technique detected slight intraspecies sequence variation in 16S rDNA. These molecular methods are common in environmental microbiology, but rarely are they compared with the same sample site or culture. In general, all three methods detected similar community profiles, but inherent biases resulted in different detection limits for individual OTUs (operational taxonomic units).

**Yeager, C. M., P. J. Bottomley, et al.** (2001). "Requirement of DNA Repair Mechanisms for Survival of *Burkholderia cepacia* G4 upon Degradation of Trichloroethylene." *Appl. Environ. Microbiol.* 67: 5384-5391.

A Tn5-based mutagenesis strategy was used to generate a collection of trichloroethylene (TCE)-sensitive (TCS) mutants in order to identify repair systems or protective mechanisms that shield *Burkholderia cepacia* G4 from the toxic effects associated with TCE oxidation. Single Tn5 insertion sites were mapped within open reading frames putatively encoding enzymes involved in DNA repair (UvrB, RuvB, RecA, and RecG) in 7 of the 11 TCS strains obtained (4 of the TCS strains had a single Tn5 insertion within a *uvrB* homolog). The data revealed that the *uvrB*-disrupted strains were exceptionally susceptible to killing by TCE oxidation, followed by the *recA* strain, while the *ruvB* and *recG* strains were just slightly more sensitive to TCE than the wild type. The *uvrB* and *recA* strains were also extremely sensitive to UV light and, to a lesser extent, to exposure to mitomycin C and H<sub>2</sub>O<sub>2</sub>. The data from this study establishes that there is a link between DNA repair and the ability of *B. cepacia* G4 cells to survive following TCE transformation. A possible role for nucleotide excision repair and recombination repair activities in TCE-damaged cells is discussed.

**Zhang, X. H., G. W. Sewell, et al.** (2001). "An improved method of hydrogen production as electron donor for anaerobic bioremediation." *Journal of Environmental Science and Health Part a Toxic/Hazardous Substances & Environmental Engineering* 36(9): 1661-1670.

This paper investigated an improved electrochemical approach that is able to provide hydrogen for anaerobic bioremediation of chloroethenes in subsurface. Hydrogen is the ultimate electron donor of biodechlorination processes. In experiments, iron wire was used as electrodes, an anaerobic bacteria enrichment collected from a site contaminated with chloroethenes as test microbes, perchloroethylene (PCE) as model chloroethene. Experiments were conducted by switching the polarities of electrodes periodically and supplying electrical power in an intermittent way. The results showed that an electrochemical bioreactor that was switched 1 time/10 min and operated only 8 li a day was able to produce more hydrogen than that operated 24 h a day at 0.4 V without polarity switching, stimulating microbial growth more effectively. The intermittent operation also resulted in periodical release of overpotentials that built up on electrode surfaces, thus prevented charged ions and particles from attaching on electrodes. The hydrogen produced was available for microbial growth and PCE dechlorination. It is suggested that the improved electrochemical process developed in this study has significant implications to anaerobic bioremediation.