MICROBIAL REDUCTIVE DECHLORINATION OF WEATHERED POLYCHLORINATED DIBENZO-*P*-DIOXINS AND DIBENZOFURANS IN CONTAMINATED SEDIMENTS

By

HUI LIU

A dissertation submitted to the Graduate School-New Brunswick

Rutgers, The State University of New Jersey

in partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Environmental Sciences

Written under the direction of

Dr. Max Häggblom

and approved by

New Brunswick, New Jersey

OCTOBER, 2010

ABSTRACT OF THE DISSERTATION

Microbial Reductive Dechlorination of Weathered Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Contaminated Sediments

By HUI LIU

Dissertation Director:

Dr. Max Häggblom

Sediments contaminated with weathered polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are problematic around the world. River Kymijoki is highly contaminated with PCDFs mainly originating from the production of the wood preservative Ky-5 until the 1980s. Limited information is available on the *in situ* microbial reductive dechlorination of weathered PCDD/Fs. The overall objectives of my work were to assess the potential for anaerobic microbial dechlorination of weathered PCDFs and to gain information on the application in bioremediation and detoxification of contaminated sediment using Kymijoki River sediments as a case model. Experiments in mesocosms (30 L), microcosms (200 mL) and enrichment cultures (40 mL) demonstrated the potential for dechlorination of weathered PCDFs as well as spiked PCDD/Fs by the indigenous microorganisms of Kymijoki River sediment. The relative decrease of highly chlorinated dibenzofurans (CDFs) was accompanied with an increase of tetra- and penta-CDFs over a 7-year period in mesocosms. Amendment with halogenated co-substrates,

tetrachlorobenzene (TeCB) and pentachloronitrobenzene (PCNB), and bioaugmentation with *Dehalococcoides ethenogenes* strain 195 selectively stimulated dechlorination of weathered PCDFs after 18 months in microcosms. Dechlorinating enrichment cultures were established from the microcosms and spiked with 1,2,3,4-tetra-CDD/F and octaCDF with PCNB as a co-substrate. 1,2,3,4-tetra-CDD was dechlorinated mainly via 1,2,3-triCDD to 2,3-di-CDD and 2-mono-CDD over 13 months incubation. Dechlorination of 1,2,3,4-tetra-CDF was slow and less extensive compared to that of 1,2,3,4-tetra-CDD, with 2,4-di-CDF as the most abundant dechlorination product accumulating after 13 months. More extensive dechlorination of 1,2,3,4-tetra-CDD was observed in the presence of PCNB, which demonstrated that PCNB was capable of enhancing the dechlorinating potential of the indigenous microorganisms in Kymijoki sediments. Dechlorination of spiked octa-CDF was observed with the production of hepta-, hexa-, penta- and tetra-CDFs over 5.5 months incubation. DGGE analysis revealed diverse *Chloroflexi* community in mesocosms and a highly selected *Chloroflexi* community in the enrichment cultures. One of the stimulated *Chloroflexi* populations clustered closely with the Pinellas subgroup of *Dehalococcoides*. Our results suggest that dechlorination of weathered PCDD/Fs contaminants may be mediated by indigenous microbial populations as a means for *in situ* bioremediation of PCDD/F contaminated sediments.

Acknowledgement

It evokes my feelings to acknowledge lots of people who made this thesis possible.

I would like to thank my Ph.D. supervisor, Dr. Max Häggblom for his enormous patience and great support during the past 5 years. His enthusiasm, his inspiration, his intelligence, and his great efforts in explaining things clearly to me, lead me through my Ph.D. life with fun and exciting discoveries. His supervision tailored my mind scientifically and socially. During the thesis-writing period, Max provided a lot of encouragement, a lot of patience, a lot of good advice, a lot of instruction, and a lot of good ideas. Max is my best company during my Ph.D. period. I will never overstate my gratitude to him.

I would like to thank Dr. Joong-wook Park for his instructions on microbial molecular work that have been done in this thesis and also his many ideas and suggestions of solving problems met in science, academia and also daily life.

I would like to thank Dr. Lisa Rodenburg for her short online essay about women in science. Her essay gave me strong determinations and courage to conquer all the difficulties that I met in writing this thesis and eventually succeed in my Ph.D. life.

I would like to thank Dr. John Reinfelder not only because he provided essential administrative information and instructions towards the completion of my Ph.D. study, but also for his fantastic course instruction and help with my first course paper in English after I came to USA. His help and instruction allowed me to know what, how and why I should write a paper.

I also want to thank many other professors that have taught me all the courses at Rutgers: Dr. Donna Fennell, Dr. Lee Kerkhof, Dr. Elisabetta Bini, Dr. Christopher Uchrin, Dr. Jerry Kukor, Dr. Peter Strom, Dr. Peter Morin, and Dr. Tamar Barkay. They are

intelligent and knowledgeable. They gave me best instructions that will accompany me forever. I also would like to thank Dr. Donna Fennell, Dr. Lee Kerkhof, Dr. Elisabetta Bini and Dr. Yuan Ren for their serving my thesis committee.

I am grateful to the administrative staffs who helped the department run smoothly and for assisting me in many different ways: Ms. Marsha Rajaei, Ms. Helene Press, Ms. Down Skouboe and Mr. Michael Ferner from Department of Environmental Science; Ms. Kathy Maguire, Ms. Eileen Glick, Ms. Arleen Nebel, Ms. Jessie Maguire, and Mr. Peter Anderson from the Department of Biochemistry and Microbiology.

I would like to thank many of my student and postdoc colleagues for providing a stimulating, caring and fun environment to learn and grow. I am especially grateful to Isabella Gray, Ines Rauschenbach, Jessica McCormick, Laura Youngster, Nora Lopez, Young-Boem Ahn, Val Krumins, Priya Narasingarao, Beth Ravit, Joanne Ahn, Nathan Giren, Suman Rawat, Jennifer McConnell and Tong Liu.

I wish to thank many of my friends for their support and company during the past 5 years: Yi Wang, Na Li, Yan Xu, Yan Liu, Songyan Du, Riqing Yu, Yi Tan, Wenyi Zhu, Fang Liu, Yun Li, Chaochao Gao, Chuching Lin, Huajun Zhen, Wen Liu, Jia Guo, Haibing Li, Liang Chen, and Lucy (Yun) Hsu. Their company made my life at USA much easier and full of happy memories.

I wish to thank my entire extended family for providing a supporting and lovely environment for me. I am grateful for my parents and my in-laws helping with my two kids during the past 3 years. My father (Herui Liu) passed away unfortunately on May 25th 2010, and I wish this thesis could be a late present to him and give him a peace mind and forever company up in the heaven. I am grateful to my sister, Min Liu, for sharing a

lot of my feelings up and down and her taking care of my mom during the most difficult time of our family.

Lastly, but importantly, I would like to thank my husband, Zhidong Lu, for his enormous support, encouragement, understanding and love during the past few years. I will also thank my two kids: Alexander Lu and Audrey Lu, who have been giving us a lot of funs and challenges that allow us to learn and grow and start to understand parenthood.

This research was supported by the Strategic Environmental Research and Development Program of the U.S. Department of Defense, USA. The author greatly appreciates Dr. Matti Vertra (Finnish Environment Institute) for kindly providing Kymijoki River sediments and Dr. Katri Mattila for assistance with mesocosm maintenance. The author was supported in part by a graduate fellowship from the graduate program in Environmental Sciences and teaching assistantship from the Department of Biochemistry and Microbiology.

Table of Contents

List of tables

List of figures

Chapter 1

Current Knowledge of Microbial Reductive Dechlorination of Freshly Spiked and Weathered Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans in Contaminated Sediments

1.1. Abstract

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are a group of hetocyclic organohalogen compounds that have extremely low water solubility, low vapor pressure and high octanol-water partition coefficients. Therefore, they tend to associate with organic particles. Sediments and soils thus become their important and ultimate sinks in the environment. They are mainly produced as un-wanted byproducts during chlorine involved industrial processes and incinerations of organic waste. Currently, PCDD/F contamination is found everywhere with several highly contaminated sites that arouse great concern due to their toxicity, resistance and consequently continuous impact to the health of human beings. The Kymijoki River in Finland is one of the most contaminated sites in the world with PCDD/Fs up to 193,000 μg/kg dry weight (d.w.) of sediment. Microbial reductive dechlorination of PCDD/Fs is one of few known reactions of highly chlorinated congeners, in which chlorines are substituted by hydrogen in a step by step sequence and generating less chlorinated congeners. These are then more susceptible to subsequent reactions including attack by mono- and dioxygenases. Much effort has been made to enrich, isolate and identify the microorganisms responsible for PCDD/F dechlorination. *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* spp. CBDB1 are the two known isolates that are capable of dechlorinating select PCDD/F congeners. Another highly enriched culture, *Dehalococcoides* DCMB5, was also capable of dechlorinating certain triCDDs. These strains all belong to the phylum of *Chloroflexi*. In addition, microbial molecular techniques as well as phylogenetic analysis of various microbial consortia all indicated that bacteria in the phylum of *Chloroflexi* are involved in the dechlorination of PCDD/Fs.

Dechlorination of PCDD/Fs is extremely slow with time frames of months or years. Efforts to stimulate this process include the addition of PCDD/F structural analogs, additional electron donors, and bioaugmentation using known PCDD/F dechlorinating bacteria such as *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. strain CBDB1. Successful stimulation of PCDD/F dechlorination has been reported for spiked congeners of various enrichment cultures. Several studies showed that the profiles of weathered PCDDs changed in present sediment layers compared to archived sediment layers samples. However, the knowledge on dechlorination potentials of indigenous microorganisms in contaminated sediment on weathered PCDFs is very limited. It should be noted that certain environmental factors will affect the dechlorination process. These factors include temperature, pH value, salinity, alternative electron donors and acceptors in the environment, and physical chemical properties of sediments or soils. Successful strategies to remediate PCDD/F contaminated sediments are limited by various aspects, including inadequate knowledge of dechlorination pathway of PCDD/Fs, and limited bioavailability of PCDD/Fs in sediment or soils, limited knowledge of responsible dechlorinating bacteria and the overall dechlorinating community.

1.2. Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) contamination in sediments and soils

1.2.1. Sources, sinks, properties, toxicity and environmental behavior of PCDD/Fs

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are a group of heterocyclic ring-structured organohalogen compounds that are made up of carbon, oxygen, hydrogen and chlorines (Fig. 1-1). PCDDs consist of two benzene rings connected to each other by two oxygen bridges with eight substituents of either hydrogen or chlorine (Fig. 1-1a). For PCDFs, two benzene rings are connected via one oxygen bridge and one carbon-carbon bond, with the remaining eight substitutions on the benzene rings being hydrogen or chlorine (Fig. 1-1b). There are 75 PCDD congeners and 135 PCDF congeners that differ in the number and position of chlorine substitution. The general nomenclature dioxins will be adopted in this paper, which includes all 210 PCDD/Fs and 12 coplanar polychlorinated PCBs (PCB-77, -81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189), which usually exist as a mixture of various congeners in the environment and arouse great social and scientific concerns due to the severe environmental and health problems coming from their environmental persistence, reaction recalcitrance and biological toxicity.

1.2.1.1. Sources of PCDD/Fs

Unlike other halogenated compounds, such as PCBs, chlorinated solvents or pesticides, PCDD/Fs have never been produced intentionally and in large amounts. They are discharged into the environment mainly as impurities or unwanted by-products from a

variety of processes and in varying quantities depending upon the source. Anthropogenic sources are the principal routes for PCDD/Fs entering the environment (Fiedler et al., 1990, 1996; Esposito et al., 1980; Hutzinger & Fiedler, 1988a, 1988b; Rappe, 1993; and see review by Kulkarni et al., 2008). Three major sources have been identified: industrial processes, thermal or combustion processes, and environmental reservoirs.

The industrial sources discharge PCDD/Fs as unwanted byproducts and impurities into the environment, including the manufacture of chlorinated compounds such as chlorophenols, chlorophenolic pesticides, chlorinated benzenes and polychlorinated biphenyls (PCBs) (Hagenmaier and Brunner, 1987; Masunaga et al., 2001), chlorine bleaching by pulp and paper industry (LaFleur et al., 1990), dry cleaning distillation residues, and metal refining industries (Wagrowski et al., 2000; Anderson and Fisher, 2002). Chlorophenols are known as one of the most important combustion precursors of PCDD/Fs in the gas phase at temperatures between 400 and 800 °C. The major mechanisms for PCDD/F formation include self-condensation of chlorophenols and chlorophenoxy acids as well as cyclization of intermediates (see review by Altarawneh et al., 2009). Insufficient oxygen supply and limited transit time will increase the emission of PCDDs in the gas phase during combustion of chlorophenol formulations (Jansson et al., 1978). During chlorine involved bleaching and dry cleaning processes, PCDD/Fs can be formed through chlorination of naturally occurring phenolic compounds like those present in wood pulp (Rappe et al., 1987). Metal refining industries that need a high temperature operation, such as steel production and metal recovery furnaces are typical sources of PCDD/F emissions into the environment (Anderson and Fisher, 2002).

PCDD/Fs from combustion sources enter the environment through three major ways: 1) emission into the atmosphere (Buser et al., 1978; Andersson et al., 1998; Shen et al., 2009; Liu 2009; Nakai et al., 2007); 2) fly ash disposal in the landfills, then through water leachate back to the environment (Koester and Hites, 1992b; Cheung et al., 2007; Lundin and Marklund, 2007); and 3) wastewater effluent (Hoffman et al., 1995). Thermal or combustion sources include large stationary plants, such as incinerators for municipal solid waste and hazardous waste, sewage sludge, sintering plants and many types of recycling plants. Some individual sources like automobile exhaust, home heating activity, cigarette smoke, and combustion of landfill gas also contribute to the emission of PCDD/F to the environment (Hart et al., 1991; Hoffman et al., 1995; Schettler et al., 1999; Im et al., 2002). In addition to volcanic eruptions, PCDD/Fs are also generated by various fire accidents, e.g. PCB fires, building fires, and forest fires (Hoffman et al., 1995; Ingersoll et al., 1997). After being emitted from these combustion sources, lightly chlorinated dioxins (relative higher vapor pressure) can travel atmospherically, some dioxins can also react in the atmosphere, and some dioxins (lower vapor pressure, highly chlorinated dioxins) will deposit to Earth through wet or dry deposition, and some eventually accumulate in biota (Tysklind et al., 1993; Lohmann, 1998; Nessel et al., 1991; Koester and Hites, 1992a).

Once PCDD/Fs are associated with organic matter, deposited in soils, sediments, landfill sites, sewage sludge or compost, they can be redistributed and circulated in the environment via water leachate, sediment or dust re-suspension and transport, or directly

as components when these “reservoirs” are applied onto agricultural and horticultural soils (Hutzinger, 1985a, 1985b; Kjeller and Rappe, 1995; Rotard et al., 1994).

Dioxins are also generated through a variety of geogenic and biogenic processes. Recent studies suggest that biomass burning and subsequent deposition (Gaus et al., 2001; Green et al., 2001) contribute to the PCDD/Fs in sediments and clays too. Enzymatic oxidative dimerization of natural chlorophenols has been reported to produce PCDD/Fs in peat and forest soils (Hoekstra, 1999; Silk, 1997). The photochemical synthesis of octa-CDD and partially hepta-CDDs from pentachlorophenol (PCP) in atmospheric condensed water has been proposed as the most significant source of octa-CDD and hepta-CDDs into the environment (Baker et al., 2000).

Historical events associated with the production and application of the herbicide Agent Orange during the Vietnam War (Alastair, 1978) and the Seveso industrial accident (Bertazzi et al., 1998; Alastair, 1977) have discharged large amounts of highly toxic 2,3,7,8-tetra-CDD into the environment. About 3 kg of 2,3,7,8-tetra-CDD was released to the local environment of Seveso, Italy (Meharg and Osborn 1995), and around 221 to 336 kg of 2,3,7,8-tetra-CDD were released to local soils and sediments as impurities of the Agent Orange in Vietnam during the war (Stellman et al., 2003). Impurities of 2,3,7,8-tetra-CDD was mainly produced during the process of manufacturing 2,4,5-trichlorophenol (TrCP), which is the precursor of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Fig. 1-2). Agent Orange is made up of 2,4-D and 2,4,5-T at the ratio of 1:1. Therefore, 2,3,7,8-tetraCDD in Agent Orange mainly comes from 2,4,5-T (Hites, 2010).

1.2.1.2. Physical properties determine the toxicity, sink and behavior of PCDD/Fs in environment

Table 1-1 summarizes the physical properties of different PCDD/F congeners selected by Mackay et al. (1992). The key properties that affect the environmental behavior of PCDD/Fs include water solubility, C^s, octanol-water partition coefficient, K_{ow}, vapor pressure, P, and Henry's law constant, H. As chlorination increases, both vapor pressure and water solubility decrease, while the octanol-water partition coefficient increases. Although the water solubility of mono-chlorinated and octa-chlorinated congeners varies greatly, they are all highly hydrophobic. As a result of their hydrophobicity, chlorinated dioxins are expected to associate with organic particles and ultimately deposit into soil or sediment as their environmental sink. The different properties of lesser chlorinated products and higher chlorinated parent congeners (increasing solubility and vapor pressure accompanied with decreasing octanol-water partition coefficient and increasing Henry's law constants as the number of chlorine substitution decreased) affect their bioavailability and thus the ultimate fate of these compounds in the environment.

PCDD/Fs have extremely low water solubility, low vapor pressure, and high octanol-water partition coefficients (Table 1-1). Consequently, biotic exposure to PCDD/F toxicity is chronic and widespread as a result of their hydrophobicity and recalcitrance in the environment. The toxicity of PCDD/Fs is primarily associated with a specific substitution pattern of chlorines on the two benzene rings. Among all the 210 congeners of PCDD/Fs, 2,3,7,8-chlorine substituted tetra-CDD/Fs are the most toxic congeners and

can be highly accumulated in fatty tissues of animals and humans due to their high octanol-water coefficients (K_{ow}) (Harding and Pomeroy, 1990; Koistinen, 1990; Cooper et al., 1992). Seven of the 75 possible PCDD congeners and ten of the 135 PCDF congeners contain chlorine substitutions at 2-, 3-, 7-, and 8- lateral positions (Table 1-2). 2,3,7,8-tetra-CDD is the most toxic congener among all the PCDD/Fs (Van der Berg et al., 1998, 2006). It and other 2,3,7,8-substituted congeners are toxic to biota through a common mechanism, which is binding to the cellular aryl hydrocarbon (Ah) Receptor (Poland and Knutson, 1982). The toxicity of these 2,3,7,8-substituted congeners varies with altered potency determined by their relative agonism to the Ah Receptor (Haws et al., 2006).

PCDD/F contamination in the environment is generally found as mixtures containing different kinds of congeners and different homolog groups as well as other dioxin-like compounds including certain PCBs. To measure the total toxicity of such a mixture as a single value and make toxicities comparable, the concept of "International Toxic Equivalents" (TEQ) has been developed (Van den Berg et al., 2006). In this toxicity weighing system, each of the less toxic compounds has been attributed a specific "Toxic Equivalency Factor" (TEF), representing its toxicity relative to 2,3,7,8-tetra-CDD, which has been assigned a reference value of 1. When reporting results, the concentration of each congener is multiplied by the corresponding TEF, as shown in equation 1-1:

$$TEQ = \sum_{i=1}^{n} TEF_i \times M_i \qquad (1\text{-}1)$$

where TEF_i: TEF value of certain congener i in one sample, M_i: mass of certain congener i in gram in one sample, n: the total number of congeners detected in each sample.

These values are then summed to give a total toxic equivalency (TEQ). It should be mentioned that the TEQ scheme refers only to adverse effects resulting from the interactions with the cellular aryl hydrocarbon (Ah) receptors (Boening, 1998; Kerkvliet, 2002). Other toxic effects of PCDD/Fs and similar compounds are not quantified by this method. TEF values are different for different animal species. Currently there are two common schemes for calculating TEQs: International TEQ and TEF (I-TEQ, I-TEF), and the World Health Organization TEQ and TEF (WHO-TEQ, WHO-TEF). The I-TEF scheme is proposed by the North Atlantic Treaty Organization, Committee on Challenges to Modern Society (NATO/CCMS) (Kutz et al., 1990). The TEF and TEQ approach assigns each of the 17 toxic 2,3,7,8- chlorine substituted PCDD/Fs a TEF. The remaining non 2,3,7,8-substituted congeners are considered biologically inactive with regard to the Ah receptor (Boening, 1998; Kerkvliet, 2002), and a TEF of zero has been assigned to them. In 1998, WHO revised and expanded the I-TEF scheme to provide TEF values for humans and wildlife (Van den Berg et al., 1998) and further updated them for human beings in 2005 (Table 1-3) (Van den Berg et al., 2005). The two schemes are different from each other by assigning 1,2,3,7,8-PeCDD WHO-TEF of 1, while I-TEF is 0.5; and WHO-TEF for octa-CDD/F is 0.0001, while I-TEF is 0.001.

1.2.2. PCDD/F contamination in sediments and soils

Sediments and soils are the most important environmental sinks for PCDD/Fs. Table 1-4 summarizes some of the current known PCDD/F contamination in sediments and soils around the world. Most of the contamination listed in Table 1-4 occurred between

1950s~1970s as a combined result of combustion and industrial processes. Studies found that the PCDD/F levels near industrial areas in the United States were very low until about the 1920s and reached their environmental peak amount between the 1970s and 1980s (Alcock and Jones, 1996; Czuczwa et al., 1984). Among worldwide PCDD/F contamination data, three major hotspots can be identified: 1) Kymijoki River, Finland; 2) Newark Bay, Passaic River, NJ, USA; and 3) Bitterfeld area, Germany.

The Kymijoki River in Finland was heavily contaminated with PCDD/Fs as impurities and by-products of chlorinated phenols from 1940s to 1980s due to the manufacture of chlorophenolic fungicide Ky-5 in the city of Kuusankoski (Malve et al., 2003). Large amounts of PCDD/Fs were discharged into the river sediment as impurities of Ky-5 during this time. Concentrations of PCDD/Fs as high as 120-193,000 μg/kg (d.w.) in the river sediment were reported (Salo et al., 2008). Although the production of Ky-5 was banned in the 1980s, the river sediment itself still remains a major source of PCDD/Fs to the Gulf of Finland (Salo et al., 2008).

In the USA, many areas have been identified with PCDD contamination due to the production, transportation and storage of Agent Orange, including Newark Bay (NJ) (Bopp, 1991), and the Passaic River (NJ) (Wenning et al., 1993). Passaic River sediment was reported to contain PCDDs in the range of 415-23,300 ng/kg d.w. and PCDFs 37-8,400 ng/kg d.w. (Wenning et al., 1993). In addition to these Agent Orange associated hotspots, many other places in USA were also identified as having contamination by PCDD/Fs. Love Canal in the state of NY was contaminated by PCDD/Fs as a result of

the dumping place of municipal and chemical wastes during 1940-1953 (Smith et al., 1983). Times Beach (MO) soils were contaminated with dioxins due to the application of hexachlorophene industrial waste as road pavement in 1972 to 1976 (Hites, 2010). Houston ship channel sediments contain PCDD/Fs up to 140 ng WHO-TEQ/kg d.w. as consequence of petrochemical transports as well as municipal and industrial discharges that happened in the 1960s (Suarez et al., 2006).

Similarly, the application of Agent Orange during the Vietnam War in 1970s caused PCDD/F contamination in many parts of Vietnam. High concentrations of PCDD/Fs in the sediment and soil of Vietnam have been reported (Schecter et al., 2001). The total concentration of PCDD/Fs in southern Vietnam soil was 11-1,800 μg/kg d.w. as a consequence of spraying with the herbicide Agent Orange, and Bien Hoa soil was found to contain PCDD/Fs around 12-1,792 μg/kg d.w. where used to be the storage place of Agent Orange, the Bien Hung lake sediment contained PCDD/Fs 208-2,104 ng/kg d.w. (Schecter et al., 2001).

The Bitterfeld area of Germany has been heavily contaminated with PCDD/Fs from the 1950s to the 1990s mainly due to the metal refining industry. Rastatt soil contained PCDD/Fs at 4-7,926 ng/kg d.w. (She et al., 1996). Spittelwasser River sediments contained PCDD/Fs up to 120,000 pg I-TEQ/g d.w. (Bunge et al., 2001, 2003). Pevestorf sediments contain PCDD/Fs up to 500 ng/g d.w. (Götz et al., 2007). Grosser Arbersee sediment contained around 1,000-2,300 ng/kg d.w. PCDD/Fs (Bruckmeier et al., 1997).

In addition to the above hotspots, PCDD/F contamination has been reported for many other places in the world mainly due to combustion and / or industrial activities (Table 1-4). The Seveso industrial accident in 1976 discharged 1.5-580 μg/m^2 of 2,3,7,8-tetra-CDD, the most toxic congener, into the nearby soil (Bertazzi et al., 1998). A number of lake and river sediments in China (Liu et al., 2009; Terauchi et al., 2009, Ren et al., 2009; Zhang et al., 2008) have been reported with PCDD/F contamination at different concentrations. Many other places are also been reported for PCDD/F contamination, such as Korea (Koh et al., 2004), Canada (Shen et al., 2008; 2009), UK (Rose, 1996), Japan (Nakai et al., 2007). In summary, PCDD/Fs contamination is ubiquitous in the world with sediment and soils being their most important sinks and consequently attracts social and scientific concerns on their burden in such places.

1.3. Microbial reductive dechlorination of PCDD/Fs and PCBs

1.3.1. Microbial reductive dechlorination of spiked and weathered PCDD/Fs and PCBs

Because of their persistence and recalcitrance in the environment, the natural attenuation rate of PCDD/Fs is very slow and with limited extent. This process is highly restricted to a few environmental conditions and varies among different homologs (for reviews see Wittich, 1998a, b; Chang, 2008; Field and Sierra-Alvarez 2008; Hiraishi et al., 2008; Bunge and Lechner, 2009).

Aerobic degradation of PCDD/Fs by bacteria is restricted to chlorinated congeners with less than 4 chlorine atoms. Complete mineralization can occur to the non-chlorinated aryl

ring of congeners with one or no chlorine substituents, in which aerobic bacteria gain carbon and energy for growth (Wittich, 1998a, b; Hong et al., 2004). Co-metabolism is the common mechanism for aerobic bacteria attacking di- to tetra-chlorinated congeners while utilizing another growth substrate (Habe et al., 2001; Hong et al., 2002). In this case, PCDD/Fs are metabolized to chlorinated salicylic acid or chlorocatechols.

White-rot fungi are capable of co-metabolizing a number of PCDD/F congeners via the activity of extracellular lignin and manganese peroxidases and cytochrom P450 monooxygenases (Takada et al., 1996; Valli et al., 1992; Manji and Ishihara, 2004: Mori and Konda 2002).

Under anaerobic conditions, bacteria mediate the step-wise reductive dechlorination of PCDD/F congeners. This is one of the very few known mechanisms that transform highly chlorinated congeners in environment. In this process, PCDD/Fs may serve as an electron acceptor and anaerobic bacteria may gain energy for growth. This process is also known as (de)halorespiration, in which electron transfer was coupled with proton gradient across the membrane, thus driving ATP formation (Holliger et al., 1999; Smidt et al., 2000). The environmental importance of microbial reductive dechlorination lies in the initial attack of highly chlorinated congeners that are otherwise recalcitrant to microbial activities. The resulting less chlorinated congeners are more susceptible for subsequent aerobic degradation.

1.3.1.1. Microbial reductive dechlorination of spiked PCDD/Fs and PCBs

Knowledge of microbial reductive dechlorination of PCDD/Fs has mostly been obtained from the study of a few selected single spiked PCDD congeners using different enrichment cultures or sediment microcosms derived from freshwater, estuarine and marine sediments (Table 1-5).

Adriaens and Grbic-Galic (1994 and 1995) reported the first evidence of biologically mediated reductive dechlorination of PCDD/Fs. They observed lesser chlorinated congeners of CDD/Fs, which accounted for up to 30% of the initially spiked penta-, hexa- and hepta-CDD/Fs in active anaerobic microcosms using Hudson River sediment as inoculum incubated for more than 2 years. Beurskens et al. (1995) reported the first microbial reductive dechlorination of spiked 1,2,3,4-tetra-CDD using a sediment-free enrichment culture derived from hexachlorobenzene dechlorinating microcosms from Rhine River sediments over a time course of 20 days. In their experiment, 1,2,3,4-tetra-CDD was reductively dechlorinated to both 1,2,3- and 1,2,4-tri-CDDs, which were further dechlorinated to 1,3- and 2,3-di-CDDs at a ratio of 5:2. Trace amount of 2-mono-CDD was also observed. Later work by Ballerstedt et al. (1997) using Saale River (Germany) sediments found that dechlorination of 1,2,3,4-tetra-CDD produced 1,3-di-CDD as the main product with minor amounts of 1,2,4- and 1,2,3-tri-CDDs. The authors analyzed the dechlorination of 1,2,3- and 1,2,4-tri-CDDs using a subculture of the original sediment consortium (Ballerstedt et al. 1997). They found that 1,2,4-tri-CDD yielded only 1,3-di-CDD, while 1,2,3-tri-CDD was slowly dechlorinated to equal amounts of 1,3- and 2,3-di-CDD. They concluded that the dechlorination pathway of

1,2,3,4-tetra-CDD to 1,3-di-CDD was primarily via the lateral removal of chlorine atom via 1,2,4-tri-CDD. Bunge et al. (2001) reported a similar dechlorination pathway for spiked 1,2,3,4-tetra-CDD using an anaerobic microbial consortium enriched from Spittelwasser sediments (Germany). They proposed the concept of "regiospecific dechlorination" pathway of spiked 1,2,3,4-tetra-CDD, and 1,2,3- and 1,2,4-tri-CDDs. In their explanation, the Spittelwasser microorganisms were capable of simultaneous dechlorination of *peri* and lateral chlorines, and / or restricted to dechlorination at positions flanked by chlorine atoms on both sides. In addition to the positive dechlorination results of sediment microcosms and enrichment cultures derived from above mentioned sites for 1,2,3,4-tetra-CDD, Arthur Kill sediments (NJ, USA) (Vargas et al., 2001), Graving Dock and Paleta Creek sediment (SD, USA) as well as pristine sediment from Tuckerton (NJ, USA) (Ahn et al., 2005) have shown similar dechlorination of spiked 1,2,3,4-tetra-CDD. In addition to different dechlorination pathways, dechlorination of 1,2,3,4-tetra-CDD has been observed with carbon isotope fraction by a mixed culture containing *Dehalococcoides ethenogenes* strain 195 very recently (Liu et al., 2010). The 1,2,4-triCDD dechlorination product was enriched in ^{13}C relative to 1,2,3,4-tetra-CDD in cultures with and without perchloroethene (PCE) as a co-substrate. The further dechlorination product 1,3-di-CDD was depleted in ^{13}C relative to 1,2,4-tri-CDD. However, no carbon isotope fractionation factors could be determined for 1,2,3,4-tetra-CDD in their study. Similar carbon isotope fractionation during sequential reductive dechlorination of 1,2,4- and 1,2,3-tri-CDDs was also reported by Ewald and co-workers (2007). Their study showed that the intermediate 1,3- and 2,3-di-CDDs were enriched, and the final product 2-mono-CDD significantly depleted in ^{13}C.

Only two studies have been conducted on dechlorination of 1,2,3,4-tetra-CDF, including one enrichment cultures derived from Paleta Creek sediment (USA) (Ahn et al., 2005) and one pure isolate of *Dehalococcoides ethenogenes* strain 195 (Fennell et al., 2004). Tri-, di- and mono-CDFs were detected in both studies as the dechlorination products, but due to the lack of commercially available standards, no conclusive dechlorination pathway of 1,2,3,4-tetra-CDF is available currently.

Fu and co-workers (2005) studied the dechlorination pathway of 1,2,3,4,6,7,8-hepta-CDD using Passaic River sediment (NJ, USA) in the presence of acetate, butyrate, and benzoate. They found that the relative yields of dechlorination product formation did not exceed 30% of the mother congener. The dechlorination of spiked octa-CDD, 1,2,3,4,6,7,8-hepta-CDD, 1,2,3,4,7,8-, 1,2,4,6,8,9-, 1,2,4,6,7,9-hexa-CDDs, 1,2,3,4,6,7,8-hepta-CDF, 1,2,4,6,8-penta-CDF, and 2,3,7,8-tetra-CDD was also tested using different sediment sources (Adriaens and Grbic-Galic, 1994, 1995; Barkovskii and Adriaens, 1996; Kao et al., 2001). They demonstrated the dechlorination activity and identified several dechlorination products, but there was no conclusive pathway being identified yet.

Microbial reductive dechlorination of spiked single PCB congeners or PCB mixtures has been observed in enrichment cultures and sediment microcosms derived from many places over the world (Table 1-6). Compared to PCDD/Fs, dechlorination knowledge of PCB single congener and mixtures are better understood in numbers of PCB congeners

and mixtures being studied and dechlorination pathways being demonstrated (Brown et al., 1984; Quensen et al., 1988, 1990; Van Dort and Bedard, 1991; Hartkamp-Commandeur et al., 1996; Kuo et al., 1999; Williams, 1997; Chen et al., 2001; Zanaroli et al., 2010; Baba et al., 2007; Krumins et al., 2009; for reviews see Bedard and Quensen, 1995; Bdeard, 2003; 2008).

1.3.1.2. Dechlorination of weathered PCDD/Fs and PCBs in contaminated sediments

Evidence for the microbial reductive dechlorination of weathered PCDD/Fs and PCBs in the environment is available from a few studies conducted with soil or sediment (Table 1-7 and 1-8). Dated sediment cores from aquatic depositional systems have the potential to provide knowledge on the chronologies of pollutant input as well as provide information on the possible fates of these pollutants. To date, only a few studies have been performed in this category. Beurskens et al. (1993) observed significant disappearance of four higher chlorinated dioxin (PCDDs) congeners when compared with archived sediment samples in Lake Ketelmeer, Rhine River sedimentation area, the Netherlands. Gaus et al. (2002) reported that sediment age was correlated with increasing proportions of lower chlorinated PCDDs and decreasing proportions of octa-CDD for Queensland sediment (Australia). These limited studies conducted directly to the sediments in the field provided very important evidence of microbial reductive dechlorination of weathered PCDD/Fs *in situ*, yet with inadequate knowledge about the dechlorination process over time and the response of microbial community in the sediment.

In addition to the knowledge obtained by comparing profiles of weathered PCDD/Fs vs. sediments at different age, dechlorination of weathered PCDD/Fs were also observed in microcosms or enrichment cultures derived from the contaminated sites.

Historical contamination of PCDD in Passaic River sediments, hepta- and penta-CDDs in particular, decreased in anaerobic microcosms over two months after spiking high concentration (ppm) of similar highly oxidized compounds to sediments (Barkovskii et al., 1994). Aged 2,3,7,8-tetra-CDD was stoichiometrically converted to tri- and mono-CDDs in a microbial consortium derived from dioxin-contaminated Passaic River sediments (Barkovskii and Adriaens, 1996). Using the same sediment source (Passaic River, USA), Albrecht et al. (1999) demonstrated the dechlorination capability of indigenous microorganisms. Sediment-associated 2,3,7,8-substituted PCDD/F residues in general and 2,3,7,8-tetra-CDD in particular, may be dechlorination products of octa-CDD and hepta-CDDs, which may be further dechlorinated to 2-mono-CDD. The addition of organic acids, 2-monobromodibenzo-*p*-dioxin and hydrogen stimulated the reactions.

By employing polytopic vector analysis (PVA) as a statistical pattern recognition technique, Barabás et al. (2004) estimated the dechlorination of dioxins in the field for the first time. They identified a dechlorination fingerprint in the sediment of Passaic River, New Jersey, with a highly positive 2,3,7,8-tetra-CDD component and a highly negative hepta-CDD component. About 2~4% of their data variance was contributed by dechlorination, which equaled an average of 1.2 μg/kg of 2,3,7,8-TCDD per sample at

the expense of hepta-CDD. This postulation was further validated by the ratio of 2,3,7,8-tetra-CDD/ total 2,3,7,8-PCDD, which indicated dechlorination in the laboratory incubations. Their work combines laboratory and field scale analysis, which provide strong support on the occurrence of natural PCDD/F dechlorination.

Yoshida et al. (2005) incubated polluted river sediment (Japan) in anaerobic microcosms for more than 1 year with periodic supplementation of organic medium. They found that the total concentration and TEQ level of aged PCDD/Fs in the sediment decreased without significant accumulation of 1-3CDD/Fs congeners as intermediates or end products during the overall period of incubation after a lag time of a few weeks. They also observed an increasing amount of catechol and salicylic acid in the aqueous phase.

Compared to weathered PCDD/Fs, the knowledge of dechlorination of weathered PCBs were obtained by comparing PCB profiles at different layers of sediments and different locations as well as to the congener profiles of commercial PCB mixtures (Aroclor products). The first changing PCB distribution pattern was reported for the lower layers of Hudson River sediment when compared to commercial Aroclor 1242 (Brown et al., 1984). Alder et al. (1993) found dechlorination of both spiked Aroclor 1242 and historical (pre-existing) PCBs in cultures established with New Bedford Harbor sediment resulting in accumulation of di-, tri- and tetra-chlorobiphenyls. Later, Adriaens et al. (1995) detected more mono-, di- and tri-PCBs with decreased mol% of tetra-, penta-, and hexa-, hepta-PCBs in anaerobic microcosm inoculated with Aroclor 1242 contaminated Hudson River sediment after 400 days than that of starting time point as well as Aroclor

1242 standard. Different locations of Woods Pond sediment (MA, USA) contained characteristic congener distribution of Aroclor 1260 with decreased percentages of key hexa-, and hepta-CBs as well as increased percentages of tri-, tetra- and penta-CBs as compared to Aroclor 1260 (Bedard and May, 1996). Margar et al. (2005) reported sediment depth associated preferential loss of *meta*- and *para*- chlorines of PCBs and accumulation of lower chlorinated congeners dominated by *ortho*- chlorine substitutions in Lake Hartwell (SC, USA).

1.3.1.3. Dechlorination patterns of PCDD/Fs and PCBs

Chlorine substitutions on PCDD/F congeners can be categorized into two groups: (1) *peri* chlorines at positions of -1, -4, -6, and -9; (2) lateral chlorines at positions of -2, -3, -7, and -8. As indicated above from the different dechlorination pathways of PCDDs, these two groups of chlorines have different tendencies of being substituted by hydrogen during dechlorination. Two different dechlorination pathways have been shown with 1,2,3,4-tetra-CDD, 1,2,4- and 1,2,3-tri-CDDs by many researchers using river, estuarine and marine sediments (Beurskens et al., 1995; Ballerstedt et al., 1997; Vargas et al., 2001; Bunge, 2001; Ahn et al., 2005). Dechlorination of these congeners can occur on either *peri* or lateral chlorines sequentially or simultaneously depending on the source of inoculum. Different reaction rates of *peri* dechlorination vs. lateral dechlorination will result in different dominant dechlorination pathways. Theoretical analysis by Gibbs free energy, molecular orbital calculation as well as density-functional calculations indicated that reductive dechlorination of octa-CDD would tend to occur at the lateral chlorines, which is thus not likely to result in dominant formation of laterally substituted toxic

congeners (Huang et al., 1996; Wehrmeier et al, 1998; Lynam et al., 1998; Fueno et al., 2002). However, dechlorination of PCDD/Fs mediated by microorganisms is very complicated and affected by environmental conditions. Consequently, the ultimate dechlorination pathway of PCDD/Fs will be determined not only by the number and positions of chlorines, but also by the microbial populations (Wiegel and Wu, 2000) available in the contaminated sediments.

Chlorine substitutions of PCB congeners are categorized into three groups based on their relative positions to the carbon bridge: (1) *ortho* chlorines at position of -2, -2', -6, and -6'; (2) *meta* chlorines at positions of -3, -3', -5, and -5'; (3) *para* chlorines at positions of -4 and -4'. Microbial reductive dechlorination of PCBs has been observed for all three positions with varied preferences in microcosms or enrichment cultures derived from different sites (for reviews see Bedard, 2003, 2008; Bedard and Quensen, 1995). For PCBs, generally, higher chlorinated biphenyls are preferentially dechlorinated over lower chlorinated congeners, resulting in the accumulation of mono-, di-, and trichlorobiphenyls (Quensen and Tiedje, 1997). The removal of chlorine from a PCB congener is not completely determined by the position (*ortho*-, *meta*- or *para*-) of the chlorine relative to the opposite phenyl ring; the surrounding chlorine configuration, the chlorine configuration on the opposite ring, and the total number of chlorines on both rings (Brown et al., 1987; Bedard and Quensen, 1995) all affect specificity of dechlorination. This similar mechanism holds true for PCDD/F dechlorination too. The regioselectivity of PCDD/F dechlorination not only depends on the number of chlorines, but also the position of the chlorine substitution, the chlorine configuration on the both

benzene rings. Dechlorination products of 1,2,4-tri-CDD have been reported to be mainly 1,3-di-CDD (Ballerstedt et al., 1997; Bunge et al., 2001; Vargas et al., 2001; Ahn et al., 2005). In comparison, dechlorination products of 1,2,3-tri-CDD included 1,3- and 2,3-di-CDDs as combined results of *peri* and lateral dechlorination (Ballerstedt et al., 1997; Bunge et al., 2001).

1.3.2. Reductive dechlorination properties of pure culture and highly enriched mixed/sediment-free cultures

1.3.2.1. PCDD/F dechlorination by pure and highly enriched cultures

The pseudo-first-order transformation rates of aged PCBs in contaminated sediments tend to be orders of magnitude lower than those of freshly spiked PCBs (concentration ranged from 20-500 mg/kg) (Brown et al., 1984; Quensen et al., 1988, 1990; Anid et al., 1991; Bedard et al., 1992). Abramowicz and co-workers (1993) reported that the dechlorination rate of aged PCB in Hudson River sediments was 80% of that of the freshly spiked PCBs. These observations could provide some references for understanding the very limited dechlorination of weathered PCDD/Fs and PCBs *in situ*. Obviously, the extreme hydrophobicity, poor bioavailability and limit transportability of PCDD/Fs are important factors that affect the dechlorination rates of PCDD/Fs under natural conditions. But there are almost certainly others, including abiotic factors such as presence of humic acid, which also affect dechlorination of PCDD/Fs *in situ* (Barkovskii and Adriaens, 1998). Dechlorination of PCDDs mediated by model humic constituents (MHCs) including resorcinol, 3,4-dihydroxybenzoic acid (3,4-DHBA), and catechol, ceased at tetra-CDDs in microbial consortium derived from Passaic River sediment,

microbial acitivity caused the further dechlorination to produce tri-, di- and mono-CDDs (Barkovskii and Adriaens, 1998). The key to determine the role of these other factors on dechlorination of PCDD/Fs will be to identify microorganisms involved in the dechlorination process, and to elucidate how they deal with the stresses posed by PCDD/Fs and how their activity may be enhanced.

In recent years, much research focus has been given to the development of methods to unequivocally demonstrate ongoing reductive dechlorination in anaerobic environments, on approaches to stimulate such reactions, and on characterization of the microbial communities responsible for anaerobic dechlorination of PCDD/Fs, with the goal of developing successful and comprehensive strategies for the bioremediation of PCDD/Fs contamination in the world. Currently, there are only two known pure cultures and one highly enriched culture from the genus *Dehalococcoides* that are capable of dechlorinating various PCDD/Fs congeners (Table 1-9) (Bunge et al., 2003, 2008; Fennell et al., 2004; Liu and Fennell, 2008; Liu et al., 2010).

Dehalococcoides ethenogenes strain 195 was initially identified and isolated as a perchloroethene (PCE) dechlorinator (Maymó-Gatell et al., 1997). In the presence of butyric acid and PCE, a mixed culture containing strain 195 was capable of dechlorinating 1,2,3,4-tetra-CDD, with 1,2,4-tri-CDD as the first intermediate before 1,3-di-CDD in 250 days (Fennell et al., 2004). TriCDFs were the only detectable dechlorination products of 1,2,3,4-tetra-CDF in the same period for strain 195 (Fennell et al., 2004). Strain 195 also dechlorinated lateral chlorines of 1,2,3,4,7,8-hexa-CDF to

generate non-2,3,7,8 substituted penta and tetra-CDFs in the presence of butyric acid (Liu and Fennell, 2008). However, it is not known if these processes are growth supporting. Strain 195 was also capable of dechlorinating 2,3,4,5,6-PCB to 2,3,4,6- and 2,3,5,6-PCB then further to 2,4,6-PCB in presence of butyric acid and PCE (Fennell et al., 2004). Dechlorination of 1,2,3,4-tetra-CDD was also coupled with carbon isotope fractionation with ^{13}C enriched in 1,2,4-tri-CDD compared to 1,2,3,4-tetra-CDD and ^{13}C depleted in 1,3-di-CDD compared to 1,2,4-tri-CDD. However, no carbon isotope fractionation factors could be determined for 1,2,3,4-tetra-CDD (Liu et al., 2010). He et al. (2006) reported the debromination activities of strain 195 on octa- brominated diphenyl ethers (octa-BDE) producing hepta- through penta-BDEs within six months. More extensive debromination of tetra- and di-BDEs was observed with an enriched culture containing strain 195 during 3 months incubation.

Dehalococcoides sp. CBDB1 was initially known for its ability to dechlorinate chlorinated benzenes (Adrian et al., 2000). Bunge et al. (2003) investigated its capability of dechlorinating spiked 1,2,3,4-tetra-CDD, 1,2,3-/1,2,4-tri-CDD, and 1,2,3,7,8-PeCDD. Their study showed a preferred removal of *peri*-chlorines followed by subsequent lateral removal, with 2-mono-CDD as a final product for 1,2,3,4-tetra-CDD. The dechlorination of 1,2,3,4-tetra-CDD was coupled to the growth of strain CBDB1. The dechlorination rate of 1,2,3,7,8-PeCDD was relatively slower than that of 1,2,3,4-tetra-CDD, producing 2,3,7,8- and 1,3,7,8-tetra-CDD. 2,3,7,8-tetra-CDD could be further dechlorinated to 2,3,7-tri-CDD and then to 2,7- and 2,8-di-CDD. Strain CBDB1 was also reported to carry out *para* and *meta* dechlorination of Aroclor 1260 and 1248 (Adrian et al., 2009). Strain

CBDB1 was also capable of dechlorinating 2,3-chlorophenol (Adrian et al., 2007). The electron donor and carbon sources for the above dechlorination reaction are H_2 and acetate.

Dehalococcoides sp. DCMB5 was recently enriched from Spittlewasser creek sediment by Bunge and co-workers (2008). It was initially enriched with spiked 1,2,4-tri-CDD (Ewald et al. 2007) and finally isolated in two-liquid phase cultures with 1,2,3-trichlorobenzene in presence of mixed organic acid solution (Bunge et al. 2008). Both 1,2,4-triCDD and 1,2,3-trichlorobenzene are growth supporting electron acceptors for strain DCMB5. This highly enriched culture can mediate the sequential *peri*-dechlorination of 1,2,4-tri-CDD to produce 2-mono-CDD as an end product (Bunge et al., 2008).

In addition to *Dehalococcoides ethenogenes* strain 195, *Dehalococcoides* sp. CBDB1, and highly enriched *Dehalococcoides* sp. DCMB5, one sediment-free triCDDs dechlorinating mixed culture was enriched from the sediment of River Saale, Germany (Ballerstedt et al., 2004). This sediment-free tri-CDD dechlorinating mixed culture was enriched from River Saale sediment (Germany) using pyruvate, lactate, and fumarate as electron donors (Ballerstedt et al., 2004). It is capable of mediating *peri*-dechlorination of 1,2,4-tri-CDD to produce 1,3-di-CDD and then to 2-mono-CDD; *peri* and lateral dechlorination of 1,2,3-tri-CDD to produce 1,3- and 2,3-di-CDDs and then to 2-mono-CDD.

1.3.2.2. PCB dechlorination by pure and highly enriched cultures

The knowledge of PCB dechlorinators is relatively well established and helps to understand the process of PCDD/Fs dechlorination. Several enrichment cultures and different bacteria phylo-types have been reported for their PCB dechlorinating activities (Table 1-10).

Chloroflexi o-17 is the first PCB dechlorinator in pure culture reported in the literature (Cutter et al., 2001). It mediates the doubly flanked *meta*- and flanked *ortho*-dechlorination of 2,3,5,6-PCB (Cutter et al., 2001). This process was demonstrated to support growth of the bacterium. *Dehalococcoides* sp. DF-1 initially co-existed with a *Desulfovibrio* sp., and mediates the *para*-dechlorination of 2,3,4,5-PCB to support its growth (Wu et al., 2002). May et al. (2008) succeeded in maintaining its dechlorination capability in pure culture. Phylotypes DEH10, SF1 and SF2 were isolated from sediment of Baltimore Harbor, co-existing as a mixed culture and were consistently detected via 16S rRNA gene analysis (Fagervold et al., 2005, 2007). This group of phylotypes is capable of mediating dechlorination of various PCB congeners through different routes to gain energy for growth (Fagervold et al., 2005, 2007). *Dehalococcoides* sp. JN is a sediment free culture enriched from the sediment of Housatonic River, USA, which is capable of carrying out process N dechlorination (flanked *para* dechlorination) of commercial Aroclor 1260 to support its growth (Bedard et al., 2007).

PCDD/F dechlorination is ubiquitously observed in various anoxic habitats (Beurskens et al. 1995; Adriaens and Grbic-Galic, 1994, 1995; Barkovskii and Adriaens, 1996;

Ballerstedt et al., 1997; Kao et al., 2001; Vargas et al., 2001; Bunge, 2001; Ahn et al., 2005; Fu et al., 2005). Anaerobic microbial communities usually exhibit strong interactions among their members including dependence between non-dechlorinating and dechlorinating species (Wu et al. 2002), which is most likely the case for PCDD/Fs-dechlorinating communities. Dechlorinating species may be dependent on non-dechlorinating species producing vital growth factors, or maintaining low level of inhibitory factors for dechlorinating species. There might be important syntrophic relationship between the different members of the community. Unlike PCB dechlorinating enrichments (soil/sediment free culture; Hartcamp-Commandeur et al., 1996; Cutter et al., 1998), there is only one sediment-free enrichment capable of dechlorinating tri-CDDs (Ballerstedt et al., 2004) and one *Dehalococcoides* sp. strain DCMB5 enriched with 1,2,4-tri-CDD and isolated on 1,2,3-trichlorobenzene (Bunge et al. 2008). The other two *Dehalococcoides* strain 195 and CBDB1 were initially isolated as PCE or chlorobenzene dechlorinators and their capability of dechlorinating PCDD/Fs were later demonstrated (Fennell et al. 2004; Liu and Fennell 2008, Bunge et al. 2000, 2003). The limited cultivation and isolation of PCDD/F dechlorinating microorganisms are most likely due to the limited bioavailability of the extreme hydrophobicity and stable steiochemical structure of PCDD/F congeners (Shiu et al., 1988)

Despite of all the difficulties, it is still of special interest to enrich and to characterize indigenous microbial communities responsible for the dechlorination processes in various sediments. This might provide information on the actual potential of sediments to undergo *in situ* natural remediation. Additional isolates will give more information about

the dynamics of microbial transformation of environmentally significant PCDD/F congeners both in culture and in the natural environment. Isolation and characterization of the bacteria or consortia responsible for microbial reductive dechlorination of PCDD/Fs would allow for a better understanding of the mechanisms of this activity in the environment. This would provide a better prediction of both the dechlorination potential and products of PCDD/Fs.

1.3.2.3. Phylogeny of dechlorinating communities

Known PCDD/F as well as PCB dechlorinating bacteria all belong to the phylum of *Chloroflexi*, which is a deep-branching phylogenetic group dominated by uncultured microorganisms and most closely related to the green non-sulfur bacteria. The detection of 16S rRNA gene sequences affiliated with the phylum *Chloroflexi*, including *Dehalococcoides* and its relatives, in contaminated sediments as well as dechlorinating enrichment cultures indicated their widespread existence in the environment (Dennis et al., 2003; Duhamel et al., 2004; Fennell et al., 2001; Gu et al., 2004; He et al., 2003; Hendrickson et al., 2002; Kassenga et al., 2004; Löffler et al. 2000; Major et al. 2002; Richardson et al., 2002; Smits et al. 2004) and their importance in dechlorination of PCDD/Fs and PCBs (Holoman et al., 1999; Yoshida et al., 2005; Hiraishi et al., 2005; Ahn et al., 2007, 2008, Krumins et al., 2009). Multiple nonidentical reductive-dehalogenase-homologues (*rdh*) genes are commonly detected in *Dehalococcoides* (Hölscher et al., 2004) on the base of whole genome analysis. *Dehalococcoides ethenogense* Strain 195 and CBDB1 are reported to contain 17 (Seshadri et al., 2005) and 32 (Kube et al., 2005) reductive dehalogenase (*rdh*) genes, respectively, although the

overall functions of these genes are still unclear. This information suggests that members of *Chloroflexi* are habitating a wide range of contaminated environments and play important roles in the transformation of PCDD/Fs and other chlorinated contaminants.

In addition to the evidence for *Dehalococcoides* spp. as mediators of PCDD/F and PCB dechlorination, also other bacteria are capable of carrying out microbial reductive dechlorination (see Häggblom and Barrot, 2003). Ye et al. (1992) reported that anaerobic sporeformers (survived heat and ethanol) in upper Hudson River sediment may have been responsible for *meta* dechlorination of Aroclor 1242, while the heat and ethanol sensitive microorganisms were responsible for *para* dechlorination of Aroclor 1242. Hiraishi et al. (2001) reported that the quinone profiles of the microbial population in PCDD/F heavily polluted soil were distinctly separate from that of non-polluted soils, and the size of *in situ* microbial population was reversely related to the level of PCDD/F pollution. PCDD/F conamination exhibited a significant effect on microbial populations in soil or sediment in terms of quantity and activity. By using a most-probable-number technique, Kim and Rhee (1997) found that the time course of Aroclor 1248 dechlorination was reversely related to the growth of dechlorinators. Dechlorination ensued after dechlorinator populations increased by 2 orders of magnitude, and once the dechlorination activity reached a plateau, the number of dechlorinators started to decrease.

In summary, the process of microbial mediated reductive dechlorination of PCDD/Fs consists of the sequential reduction of highly chlorinated, toxic and bioaccumulable

PCDD/Fs into lower chlorinated congeners that are normally less toxic and more susceptible to subsequent mineralization by aerobic bacteria (see reviews Field and Sierra-Alvarez 2008; Chang, 2008). Respiratory microbial reductive dechlorination (also known as dechlororespiration) is a process by which dechlorinating microorganisms derive energy for growth via mediating the removal of chlorine by hydrogen and employing the organohalides as ultimate electron acceptors. If occurring *in situ*, it has a good potential of mediating a detoxification of the contaminated sites when hydrogen substitution occurs to the lateral chlorines (2,3,7,8-) of PCDD/Fs. With this prospect, microbial reductive dechlorination could ultimately become a competitive alternative method to the expensive and invasive traditional sediment remediation methods such as dredging or capping.

1.3.3. Environmental Factors that Affect the dechlorination of PCDD/Fs

The successful dechlorination under laboratory conditions provides us possible strategies to remediate PCDD/F contamination in sediments and soils. However, for successful application in the field, we have to find strategies to explore the full capacity of PCDD/F dechlorinating microbes. One aspect is to investigate the relationship between different dechlorinators in terms of interspecies transfer of halogenated intermediates of PCDD/Fs, which contribute to the combined turnover of PCDD/F congeners. The other aspect is to investigate the relationship between dechlorinators vs. non-dechlorinators in the environment. Dechlorinators may depend on non-dechlorinators to provide growth factors such as vitamin B12, or may depend on non-dechlorinators to limit growth

inhibitors (Kim and Rhee, 1999, review by Hiraishi, 2008). As for microorganisms in general, environmental factors including temperature, pH value, salinity, available electron donors, alternate electron acceptors and the physical and chemical properties of sediments or soils, also affect the dechlorination of PCDDD/Fs and PCBs.

The only reported optimal temperature for remarkable reduction of PCDD/F in microcosm inoculated with polluted river sediment in Japan was 25-37 °C, compared to much lower reduction yield at higher or lower temperatures (Yoshida et al., 2005). The PCDD/F dechlorination of mixed culture derived from river Saale (Germany) was inhibited by pasteurization, but not remarkably sensitive to inhibitors of methanogens, sulfate-reducing bacteria or gram-positive bacteria. Other available information about effect of temperature on dechlorination was obtained by PCB dechlorinators. Ye et al. (1992) observed that sporeformers from Hudson River sediment do not dechlorinate Aroclor 1242 when incubated above 37 °C. Wu et al. (1997) reported the different temperature ranges for PCB dechlorination processes N, P, and LP. Process N occurs at 8-30 °C, process P occurs at 2-34 °C, process LP occurs at 18-30°C. In most cases, these dechlorinations occur below 40 °C, this might be due to their existence in the sediment and soil is seldom above 40 °C.

To date, no study has been reported on the effect of pH on dechlorination of PCDD/Fs. However, several similar studies have been done with PCB dechlorinators. Rysavy et al. (2005) reported the inhibition of pH between 8 and 8.5 for dechlorination of 3,4,5-PCB. Yan et al. (2006) reported the profound effects of different concentrations of sodium

bicarbonate on the dechlorination of 2,3,4,5-PCB in sediment cultures containing *Dehalococcoides* like organisms as putative dechlorinators. This can be an indirect effect of pH on dechlorination of PCB, since sodium bicarbonate is one of the determine factors of pH value in the medium.

Fu et al. (2001) evaluated the transformation of PCDD/Fs in river sediments under different geochemical conditions including freshwater, estuarine and marine environments. The yield of dechlorinated products increased with salinity in general and the production of 2,3,7,8-tetra-CDD increased with decreased salinity and the amendment of dissolved organic matter on the contrary. Barkovskii and co-workers reported that lateral dechlorination patterns of PCDD/Fs were dominant under freshwater conditions (Barkovskii & Adriaens, 1996; Fu et al, 2001).

The effect of electron donors on dechlorination has been studied by several groups of sciencetists. The major electron donors that have been studied include H_2, acetate, lactate, pyruvate, propionate, and butyrate. H_2 is one of the most important electron donors for dehalorespiring microorganisms under anaerobic conditions. During dechlorination process, the substitution of chlorine atom by hydrogen atom is coupled with electron transfer across the cell membrane and thus drives ATP formation to support growth or metabolic activity of microorganisms (Holliger et al., 1999; Smidt et al., 2000). And this process is It is reported that 1.0 atm H_2 in the headspace completely inhibit dechlorination of 3,4,5-PCB in microcosm but not in the pre-enriched sediment cultures;

0.001 atm H_2, on the contrary, enriched the PCB dechlorinators, decreasing the lag period prior to dechlorination (Rysavy et al., 2005).

The effect of organic substrates on dechlorination was studied as early as 1990, when Nies and Vogel (1990) found that the relative rates and extents of dechlorination of Aroclor 1242 was greatest for methanol-, glucose-, and acetone-fed anaerobic cultures and least for acetate-fed cultures, although significant dechlorination over time was observed in all treatments. Pulliam Holoman et al. (1998) showed that changing the mixed carbon source of butyrate, propionate and acetate with acetate only as electron donor resulted in a dramatic shift in the microbial community. The dechlorination rate of 2,3,5,6-tetrachlorobiphenyl with acetate alone was greater than in the enrichment with a mixture of fatty acids.

In addition to various possible electron donors, alternate electron acceptors in the environment also affect dechlorination of PCDD/Fs and PCBs. The presence of alternate electron donors provided different anaerobic conditions, including methongenesis, sulfidogenesis, iron-reducing conditions.

Reductive dechlorination of PCDD/Fs and PCBs has been observed mainly under methanogenesis conditions. However, some exception was reported (Alder et al., 1993; Barkovskii et al., 1994; Cutter et al., 1998; Bunge et al., 2003, Ahn et al., 2005, 2008). Ye et al. (1992) found that methanogenesis was not required for the *meta*- dechlorination

of Aroclor 1242 by sporeforming dechlorinators in upper Hudson river sediment, however, responsible for *para* dechlorination of Aroclor 1242 (Ye et al., 1995).

Addition of sulfate inhibited or extended the lag phase of PCB dechlorination (Alder et al., 1993; Berkaw et al., 1996; Brown et al., 1990; Suflita et al., 1988; Hartkamp-Commandeur, 1996), including microcosms enriched with strain DF-1 (Wu et al., 2002a). However, ferrous sulfate ($FeSO_4$) was reported to stimulate extensive anaerobic PCB dechlorination in Hudson River sediment cultures (Zwiernik et al., 1998). Sulfate was explained to stimulate growth of sulfate reducing bacteria, which were responsible for PCB dechlorination, while Fe^{2+} restricted sulfide bioavailability and toxicity by forming the insoluble precipitate FeS. However, this has not been confirmed in other studies. Fu et al. (2005) reported that sulfidogenic conditions resulted in a lower 2,3,7,8-tetra-CDD formation from spiked hepta-CDD using estuarine sediment of Passaic River. Ahn et al. (2005) reported that dechlorination of 1,2,3,4-tetra-CDD occurred to a greater extent under methanogenic than under sulfogenic conditions.

Under iron-reducing conditions, Rysavy et al. (2005) reported the inhibition of Fe^{2+} at pH of 8.5 for dechlorination of 3,4,5-trichlorobiphenyl. Efficient 2,3,7,8-tetra-CDD removal (up to 86%) was observed under anaerobic reductive dechlorination conditions, whereas, no significant tetra-CDD removal in the microcosm prepared under aerobic or iron reduction conditions (Kao et al. 2001). Vargas et al. (2001) found very limited dechlorination of 1,2,3,4-tetra-CDD under anaerobic sulfate reducing or iron-reducing conditions using Arthur Kill estuarine sediment over 31 months.

Physical and chemical characteristics of contaminated sediment also affect the dechlorination of PCDD/Fs and PCBs. In a study on the impact of organic and inorganic electron transfer molecules on the dechlorination of PCDD/Fs in reduced environments, Adriaens et al. (1996) found that resorcinol, catechol, 3,4-dichlorobenzoate, Vitamin B12 and zerovalent zinc all mediated the dechlorination of octa-CDD/F and/or 1,2,3,7,8-penta-CDD to lesser chlorinated congeners. Their later study (Barkovskii and Adriaens, 1998) showed that *peri*-dechlorination of PCDD was stimulated by model humic constitutents (MHCs) with production of hepta-, hexa- and tetra-CDDs, whereas, microbial activity mediated the further dechlorination to tri-, di- and mono-CDDs. Fu et al. (1999) investigated the dissolved organic carbon DOC-mediated dechlorination of dioxin by using 1,2,3,4,6,7,9-hepta-CDD, Aldrich humic acid (AHA) and polymaleic acid (PMA) as model compounds. Hexa- and penta-CDDs were dominant in all incubations with comparable yields observed in the presence of humic constituents of catechol and resorcinol when normalized to phenolic acidity. The end points of microbial reductive dechlorination were mono-CDDs. Dechlorination of PCBs was found to be consistent with the total organic carbon content in the sediment (Kjellerup et al., 2008). Finally, the sediment type and particle size were found to have minimal influence on the dechlorination reactions (Kjellerup et al., 2008, Kim and Rhee, 2001). Kim and Rhee (2001) also showed that the physical sediment characteristics did not influence the dechlorination pattern in sediment spiked with Aroclor 1248.

In addition to the above mentioned environmental factors, PCDD/F concentrations will also affect the dechlorination. Hiraishi et al. (2005) reported a statistically well-fit first order kinetic relationship between the concentrations of PCDD/Fs and incubation time (r^2 ranged 0.9196~0.9766) in semi-anaerobic sediment microcosms. Concentrations of PCDD/Fs in these microcosm ranged from 18 ~2,900 pmol/g d.w.. In another study, the rate of one chlorine removal of 1,2,4-tri-CDD increased with the increasing concentration of 1,2,4-tri-CDD (Ballerstedt et al., 2004). Laboratory PCB dechlorination was reported to be dependent on the concentration of total PCB concentration (Abramowicz et al., 1993; Fish 1996; Rhee et al., 1993; and Sokol et al., 1995), which may be a result of different biomass of PCB dechlorinators or different affinity of dechlorinating enzymes. Thus the knowledge of the size of the dechlorinating populations in the contaminated sites will indicate the potential for further dechlorination of dioxins *in situ*. In this regard, it is very important to find any relationship between the PCDD/F concentration and the biomass of the dechlorinators. Additionally, PCDD/F concentrations vary from site to site, so we need to know the impact of PCDD/F concentrations on dechlorination, the bioavailability under different PCDD/F concentration levels.

In addition to the effect of PCDD/F concentration, the co-existence of other halogenated compounds will also affect the dechlorination of PCDD/Fs. May and co-workers (2006) tested the stimulatory and inhibitory effects of organohalides (26 PCBs, 12 chlorobenzenes, and 6 chloroethenes) on the dechlorinating activities of PCB-dechlorinating baceterium *Chloroflexi* o-17. They found that Aroclor 1260 and 246-PCB were not dechlorinated by the culture, but inhibited the dechlorination of 2,3,5,6-PCB.

The dechlorination of 2,3,4- and 2,3,5-PCBs, and 1,2,3,5- and 1,2,4,5-tetrachlorobenzene occurred only when a more extensively chlorinated parent compound was present for *Chloroflexi* o-17. Ahn and co-worker's work showed the stimulating effect of several halogenated structure analogs of PCDD/Fs on the dechlorination of spiked 1,2,3,4-tetra-CDD/Fs using enrichment cultures derived from various sediments (Ahn et al., 2005, 2007, 2008).

1.4. Strategies to enhance microbial reductive dechlorination of PCDD/Fs

Microbial reductive dechlorination of highly chlorinated PCDD/Fs by indigenous microorganisms is a slow process and happens only to limited extent, which is especially true under field conditions (Barkovskii and Adriaens, 1996; Beurskens et al., 1993; Albrecht et al., 1999; Barabás et al., 2004). Hence, the stimulation of PCDD/F dechlorination has been attempted by adding a wide range of halogenated compounds as co-substrates for the purpose of "priming" the dechlorination reactions. These halogenated co-substrates are known as "haloprimers" (Bedard et al., 1998). Many PCDD/F structural analogs have been tested for such purpose, including 2-monobromodibenzo-p-dioxin, brominated phenols, chlorinated benzenes and chlorinated anisoles (Table 1-11). These "haloprimers" are relatively easier to be dechlorinated and can be used to initially stimulate the growth of dechlorinators or induce the expression of responsible dechlorinating enzymes.

The first successful enhancement of PCDD/F dechlorination was observed for the aged PCDD/Fs in Passaic River sediment (USA) when amended with 2-monobromodibenzo-*p*-dioxin and H_2 in presence of acetate, butyrate, and benzoate (Albrecht et al., 1999). After this, enhancement of dechlorination has been investigated mostly with spiked 1,2,3,4-tetra-CDD (Vargas et al., 2001; Ahn et al., 2005, 2008). Ahn et al. (2005) tested the priming effects of 1,2,3,4-tetrachlorobenzene (TeCB), 2,3,4,5-tetrachloroanisole (TeCA), 2,3,4,5-tetrachlorophenol (TeCP), 2,3,5,6-tetrachlorobenzoate (TeCBA), 2',3',4'-trichloroacetophenone (TrCAP), phenol, 2-chlorophenol (CP), 3-CP, 4-CP, 2-bromophenol (BP), 3-BP, 4-BP on the dechlorination of 1,2,3,4-tetra-CDD/F using estuarine sediments from San Diego Bay (CA, USA) and Tuckerton (NJ, USA). Their work showed that TeCB, TeCA, TeCP and TrCAP plus lactate and propionate displayed the most effective stimulation on the dechlorination of tetra-CDD/Fs compared to no dechlorination without these amendments and less dechlorination and long lag phase for the other priming compounds. TeCB was dechlorinated to 1,3- and 1,2-DiCB within 3 months. No other information was available for the fate of priming compounds. Later the authors characterized the response of microbial community accompanied with the enhanced dechlorination activity (Ahn et al. 2007, 2008). Distinct microbial populations were enriched with each priming compound, and *Chloroflexi*-like rRNA genes were detected and associated with the dechlorination reactions. The authors postulate that specific *Chloroflexi* related to *Dehalococcoides* are involved in the reductive dechlorination of 1,2,3,4-tetra-CDD (Ahn et al., 2007).

In addition to 1,2,3,4-tetra-CDD, dechlorination of 1,2,3,4,6,7,8-hepta-CDD was enhanced by 2-bromodibenzo-*p*-dioxin (2-BrDD) in estuarine sediments of the Passaic River (NJ, USA) (Fu et al., 2005). Their results showed that 2-BrDD not only increased the dechlorination yields, but also decreased the formation of 2,3,7,8-tetra-CDD through the enhanced lateral dechlorination over *peri* chlorines. Dechlorination of 1,2,3,4,7,8-hexa-CDF by *Dehalococcoides ethenogenes* strain 195 can also be enhanced by adding 1,2,3,4-TeCB (Liu and Fennell, 2008). Besides adding priming co-substrates to initiate dechlorination reactions in the system, Ahn and co-workers (2008) also used the mixed culture of *D. ethenogenes* strain 195 to bioaugment this reaction.

Compared to PCDD/Fs, the successful enhancement of PCB dechlorination has been reported for not only single spiked congeners but also PCB mixtures. One of the early enhancements of PCB dechlorination was reported for spiked 2,2',4,4',5,5'- and 2,2',4,4',6,6'-PCBs by adding 2,3,6-trichlorobenzoate using Rhine River sediment (the Netherlands) (Hartkamp-Comandeur et al., 1996). 2,3,6-Trichlorobenzoate (2,3,6-TrBT) was dechlorinated to 2,5-dichlorobenzoate (2,5-DiBT) during this process. In addition to these two congeners, dechlorination of 3,3',4,4'- and 3,4,4',5-PCBs was enhanced by toluene or 3-chlorobenzoate-adapted sediments (Taiwan) (Kuo et al., 1999). Adding 0.1g Fe^0/g sediment was found to enhance the dechlorination of 3,4,5-trichlorobiphenyl (3,4,5-PCB) and 2,2',3,4,4',5,5'-heptachlorobiphenyl (22'344'55'-PCB) in Baltimore Harbor sediment (Rysavy et al. 2005). While the stimulating effect of $FeSO_4$ on the dechlorination of Aroclor 1242 was observed by Zwiernik and co-workers for the sediment of Hudson River (1998). In both cases, Fe^0 and Fe^{2+} behaved as the electron

donor and also inhibitor of S^{2-}. Sulfate was used to stimulate the growth of sulfate reducing bacteria that were able to dechlorinate PCBs.

In addition to an effect on single PCB congeners, enhanced dechlorination was observed for PCB mixtures like Aroclor 1242, 1260 by adding brominated biphenyls (Bedard et al., 1998; Wu et al., 1999) and bromo- or iodo-benzoates (Deweerd and Bedard, 1999). Adding certain inhibitors such as sodium molybdate has been demonstrated to enhance the dechlorination of PCB mixtures using Japanese burnt soil as inoculum (Baba and Katayama, 2007). Different priming compounds are known to prime different dechlorination patterns (N, P, LP) for PCB (Bedard et al., 1998). Bedard et al. (1998) reported that the concentration of 2,6-polybrominated biphenyl (2,6-PBB) as a priming compound affected the rate and final extent of PCB dechlorination; the optimal range was 200-500 μM. Dechlorination of historical PCBs has been enhanced by adding a mixed culture containing *Dehalococcoides ethenogenes* strain 195 and pentachloronitrobenzene (PCNB) (Krumins et al., 2009).

Much effort has been made to enhance the reductive dechlorination of microorganisms, including the stimulation effects of several haloprimers as co-substrates. However, the question that still remains to be answered is to what extent the structure analog co-substrates will be able to enhance PCDD/F dechlorination. One major concern or enhancing microbial reductive dechlorination is the accumulation of secondary products, including volatile fatty acid (acetate, propionate), metal (iron, magnesium), and various dechlorination intermediates either from dioxin or from haloprimers, which may lead to

an increased toxicity or generate secondary problematic environmental pollutants. In order to resolve these issues, defined system need to be employed to monitor the possible side reactions of electron donors besides dechlorination, ecotoxicity experiments need to be done to test the toxic or inhibit impact on the indigenous microbes.

1.5. Hypothesis and objectives

Although dechlorination of PCDD/Fs have been shown with several spiked congeners by pure isolates or enrichment cultures derived from various contaminated sediments, limited knowledge is available for dechlorination of weathered PCDD/Fs, in particular for weathered PCDFs associated with sediments for many years. There is still a lack of information on the strategies to stimulate such activities *in* situ. Amendment with structural analog co-substrates ("haloprimer") or bioaugmentation with known dechlorinating bacteria were shown to enhance dechlorination of spiked PCDD/Fs, yet their effect on the dechlorination of weathered PCDD/Fs in contaminated sediments is unknown. The consistent detection of *Chloroflexi* bacteria in PCDD/F dechlorinating community indicated their important role in the dechlorination of PCDD/Fs. However, only two isolates and one highly enriched culture of *Dehalococcoides* belonging to *Chloroflexi* are capable of dechlorinating certain spiked PCDD/F congeners.

In order to resolve the issues mentioned above, Kymijoki River sediment in Finland was used as a case model to assess the potential for anaerobic microbial reductive dechlorination of weathered PCDFs and to gain information on the application in bioremediation and detoxification of contaminated sediments. This river has been heavily

contaminated with weathered PCDFs due to the production of chlorophenolic fungicide Ky-5 from 1940s to 1980s (Malve et al., 2003), with high concentration of PCDD/Fs from 120-193,000 μg/kg (d.w.) in the river sediment (Salo et al., 2008). In view of the current knowledge of microbial reductive dechlorination of spiked and weathered PCDD/Fs in contaminated sediments, it was hypothesized that long-term (> 50 years) contamination of weathered PCDD/Fs has enriched for a group of dechlorinating microorganisms. Three objectives were proposed to demonstrate the hypothesis: 1) find evidence of dechlorination of weathered PCDFs in Kymijoki sediment; 2) investigate the feasibility of stimulating dechlorination of weathered PCDFs by different amendments; 3) enrich and identify the dechlorinating microorganisms responsible for dechlorination. Experiments in mesocosms (30 L), microcosms (200 mL) and enrichment cultures (40 mL) were set up to determine each objective, as described detail in Chapters 2, 3 and 4.

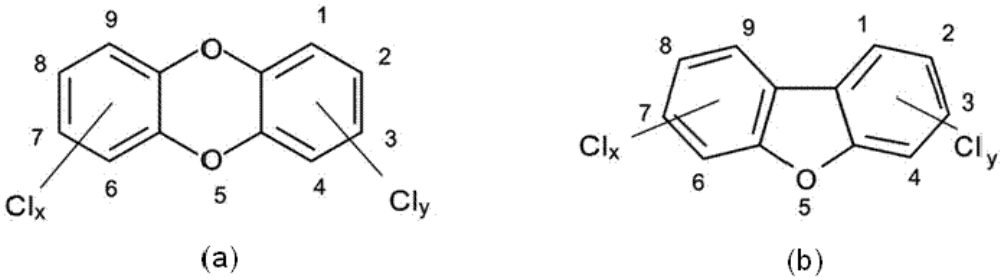

Figure 1-1. Molecular structure of polychlorinated dibenzo-*p*-dioxins (PCDDs) (a) and dibenzofurans (PCDFs) (b). x and y indicate the number of chlorine substitutions.

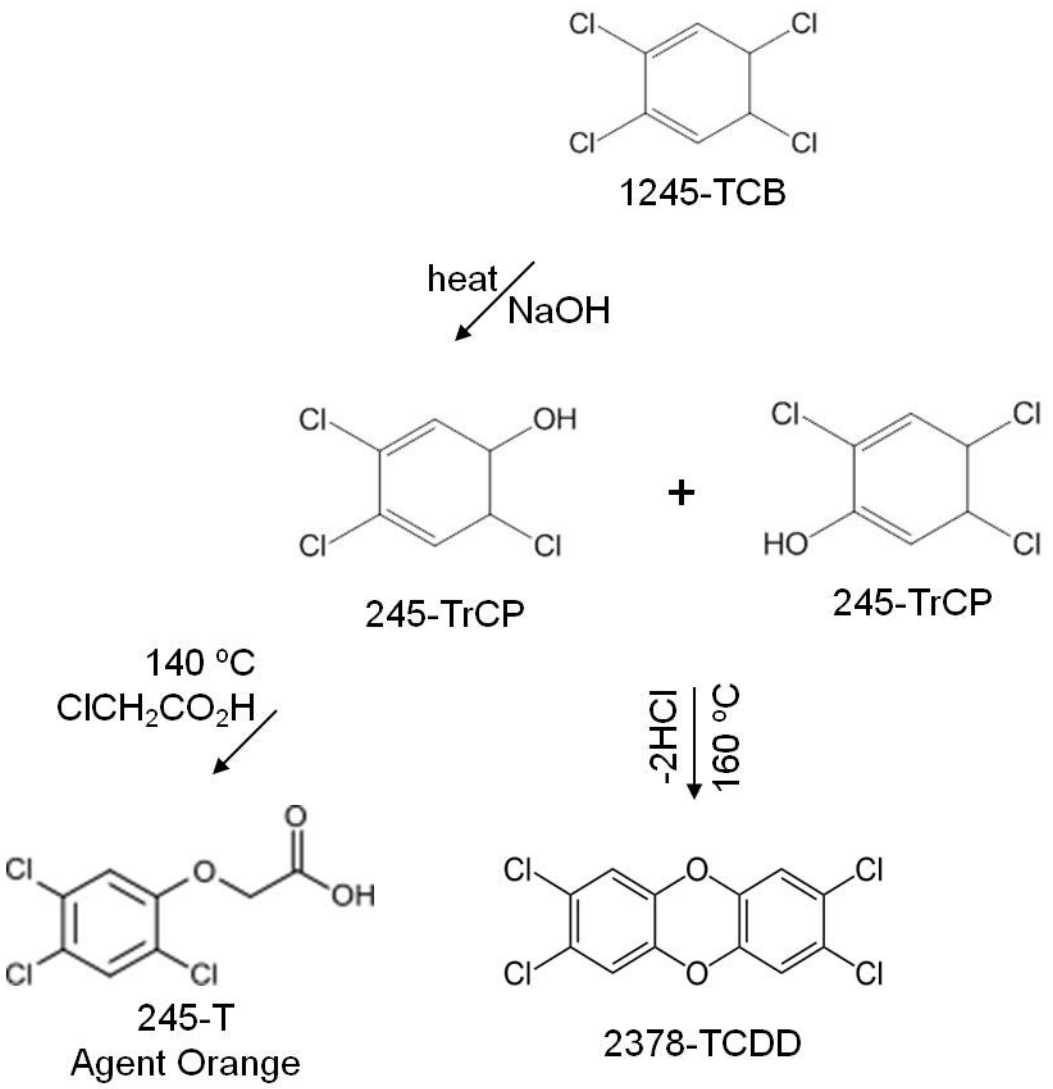

Figure 1-2. Production of 2,3,7,8-tetra-CDD during the manufacture processes of 2,4,5-trichlorophenol (245-TrCP) from 1,2,4,5-tetrachlorobenzene (1245-TCB) and 2,4,5-trichlorophenoxyacetic acid (245-T) from 245-TrCP. Agent Orange is made up of 2,4-dichlorophenoxyacetic acid (24-D) and 245-T at 1:1 ratio (Adapted from Hites, 2010).

Table 1-1. Key properties of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (Adapted from Mackay et al., 1992).

Congener	Water Solubility C^s (mg/m^3)	Vapor Pressure P ($Pa \times 10^{-5}$)	Henry's Law Constant H ($Pa\ m^3/mol$)	Log K_{ow}
1-mono-CDD	417	1200	8.38	4.2-5.47
2-mono-CDD	257-1260	1600-1700	14.82	4.2-5.71
23-di-CDD	14.9-34.1	39-40	6.61	4.7-6.23
27-di-CDD	3.75-311	12~13	8.11	4.7-6.72
28-di-CDD	16.7	13~14	2.13	4.7-5.6
124-tri-CDD	8.41-8.5	10	3.84	5.1-7.77
1234-tetra-CDD	0.47-25.6	0.63-10.44	3.77	5.5-8.97
1237-tetra-CDD	0.042-0.48	0.1-0.07	0.77	5.5-8.81
1368-tetra-CDD	0.168-26.4	0.07-5.8	0.71	5.5-9.43
2378-tetra-CDD	0.0072-19.4	0.00933-6	0.0021-7.93	5.38-7.70
12347-penta-CDD	0.0855-5.78	0.0088-0.1	0.264	6.60-10.05
123478-hexa-CDD	0.004-0.044	0.00051-0.396	4.52	7.3-10.89
1234678-hepta-CDD	0.00203-0.561	0.000075-0.193	0.133	7.92~11.98
octa-CDD	0.00007-0.18	0.000011-2.4	0.683	7.53-13.08
28-di-CDF	14.5	39	6.377[a]	4.91-6.16
2378-tetra-CDF	0.419-3.51	0.2-12.28	1.5	5.82~7.70
23478-penta-CDF	0.236-0.515	0.035-2.17	0.505[a]	6.92~7.82
123478-hexa-CDF	0.00825	0.0032-0.8093	1.454[a]	7.7
1234678-hepta-CDF	0.00135-0.0108	0.00047-1.93	1.425[a]	7.92~9.25
1234789-hepta-CDF	0.00004	0.00062-0.1011	---	6.9
octa-CDF	0.00116	0.0005-0.026	0.1	7.6~13.93

All properties were chosen to be representative of those that occur at 25 °C.
a: calculated constant (Mackay, 1992)

Table 1-2. Total numbers of isomers and 2,3,7,8-substituted congeners in each homolog group of the PCDD/Fs.

Abbreviation	**# of possible congeners**	**# of possible 2378-substituted congeners**
mono-CDD	2	0
di-CDD	10	0
tri-CDD	14	0
tetra-CDD	22	1
penta-CDD	14	1
hexa-CDD	10	3
hepta-CDD	2	1
octa-CDD	1	1
mono-CDF	4	0
di-CDF	16	0
tri-CDF	28	0
tetra-CDF	38	1
penta-CDF	28	2
hexa-CDF	16	4
hepta-CDF	4	2
octa-CDF	1	1

Table 1-3. WHO-TEF (2005) values of 2,3,7,8- substituted PCDD/Fs and dioxin-like PCBs for humans and mammals (Adapted from van den Berg, 2006).

Congeners	**WHO 2005 TEF**	**Congeners**	**WHO 2005 TEF**
Dioxins		Biphenyls	
2378-tetra-CDD	1	33’44’-TeCB (77)	0.0001
12378-penta-CDD	1	344’5-TeCB (81)	0.0003
123478-hexa-CDD	0.1	233’44’-PnCB (105)	0.00003
123678-hexa-CDD	0.1	2344’5-PnCB (114)	0.00003
123789-hexa-CDD	0.1	23’44’5-PnCB (118)	0.00003
1234678-hepta-CDD	0.01	2’344’5-PnCB (123)	0.00003
octa-CDD	0.0003	33’44’5-PnCB (126)	0.1
Furans		233’44’5-HxCB (156)	0.00003
2378-tetra-CDF	0.1	233’44’5’-HxCB (157)	0.00003
12378-penta-CDF	0.03	23’44’55’-HxCB (167)	0.00003
23478-penta-CDF	0.3	33’44’55’-HxCB (169)	0.03
123478-hexa-CDF	0.1	233’44’55’-HpCB (189)	0.00003
123678-hexa-CDF	0.1		
234678-hexa-CDF	0.1		
123789-hexa-CDF	0.1		
1234678-hepta-CDF	0.01		
1234789-hepta-CDF	0.01		
octa-CDF	0.0003		

Table 1-4. Global PCDD/F contamination in soils and sediments as well as some major problematic sites.

Location	Source	PCDD/Fs (µg/kg d.w.)	Period	Reference
Kymijoki River sediment, Finland	Manufacture of Fungicide Ky5	120-193,000	1940s-1980s	Salo et al., 2008
Gulf of Finland sediment, Finland	Manufacture of Fungicide Ky-5	1.03-52.9		
Brentella Canal sediment, Italy	Municipal and industrial activities	3-64[a]	1920s-1970s	Frignani et al. 2001
Seveso soil, Italy	Industrial accident of chlorophenol manufacture	1.5-580[b]	1976	Bertazzi et al. 1998
Southern Vietnam soil, Vietnam	Herbicide Agent orange spray	11-1800	1970s	Schecter et al. 2001
Bien Hoa soil, Vietnam	Agent orange storage	12-1792	1970s	Schecter et al. 2001

Location	Source	PCDD/Fs (µg/kg d.w.)	Period	Reference
Newark Bay, USA	Diamond Shamrock-Occidental, AO manufacture	4-8[c]	---	Bopp 1991
Passaic River sediment, USA	Manufacture of chlorinated compounds	0.5-32	1970s	Wenning et al. 1993
Love Canal, USA	Dumping of municipal and chemical waste	---	1940-1953	Smith et al. 1983
Times Beach, USA	Road pavement of hexachlorophene contaminated clay	---	1972-1976	Hites 2010
Rastatt soil, Germany	Dust from thermal metal recycling processes	8	1985	She et al. 1996
Spittelwasser River sediment, Germany	Magnesium and chlorine industry	Up to 120[a]	---	Bunge et al. 2001, 2003
Pevestorf sediments, Germany	Metallurgical processes	Up to 507	1950s	Götz et al. 2007

Location	Source	PCDD/Fs (μg/kg d.w.)	Period	Reference
Grosser Arbersee sediment, Germany	Gaseous emission from mining and smelting or iron-ores and glass production	2.3	1960s-1990s	Bruckmeier et al. 1997
Lake sediment of Erie and Ontario, Canada	Industrial discharge	10	1970s	Shen et al. 2008
Lake Superior sediment, Canada	Atmospheric deposition	18	---	Shen et al. 2009
Lake Huron sediments, Canada	Atmospheric deposition	27	---	Shen et al. 2009
Taihu sediment, China	---	1.3	---	Zhang et al. 2005
Eastern city soil, China	Combustion	1.1	---	Liu, 2009
Eastern city sediment, China	Combustion	1.5	---	Liu, 2009
Hongkong marine sediment, China	---	2.5-6.3	---	Terauchi et al. 2009

Location	Source	PCDD/Fs (µg/kg d.w.)	Period	Reference
East River sediment, China	Copper industry	2.1-4.8	---	Ren 2009
Daliao River sediment, China	Organochlorine production	3.0	1980s-present	Zhang et al. 2008
Daliao Soil, China	Organochlorine productions and metal industry	8.7	1980s - present	Zhang et al. 2008
Lake sediments, UK	Atmospheric deposition and local municipal acitivities	4.5	---	Rose 1996
Nose soil, Japan	Municipal waste incinerator	559	---	Nakai et al. 2007
Hachiouji soil, Japan	Municipal waste incinerator	68.4	---	Nakai et al., 2007
Akita Paddy soil, Japan	Herbicide application	18-540	---	Kiguchi et al. 2007

Location	Source	PCDD/Fs (µg/kg d.w.)	Period	Reference
Coastal area sediments, Caribbean	---	3.3	---	Mohammed et al. 2009
Hyeongsan River sediment, Korea	---	1.6	---	Koh et al. 2004
Port Jackson sediments, Australia	industrial	4.4[d]	---	Birch et al. 2007

a: µg I-TEQ/kg d.w,
b: 2378-tetra-CDD, µg/m^2;
c: 2378-tetra-CDD, kg;
d: µg WHO-TEQ/kg d.w,

Table 1-5. Microbial reductive dechlorination of spiked PCDD/Fs in enrichment cultures and sediment microcosms.

Substrate	Conc. (µM)	Products	Inoculum	Electron Donors	Reference
123478-hexa-CDD 124689-hexa-CDD 124679-hexa-CDD 1234678-hepta-CDD 12468-penta-CDF 1234678-hepta-CDF	145 ± 15*	*Peri*-preferred dechlorination with higher removal rate than autoclaved control	Hudson River sediment, USA	---	Adriaens and Grbic-Galic, 1994, 1995
1234-tetra-CDD	0.5	123-, 124-→13-, 23-→2-	Sediment-free, HxCB pre-enriched, Rhine River sediment, the Netherlands, 20 days.	Lactate	Beurskens et al., 1995
octa-CDD	11.4 ± 0.5	Hp-, hexa-, penta-, tetra-, tri-, di- and mono-CDDs	Passaic River sediment, USA, 7 months	Acetate, butyrate, benzoate	Barkovskii and Adriaens, 1996

Substrate	Conc. (μM)	Products	Inoculum	Electron Donors	Reference
1234-tetra-CDD	50	124-, 123-→13 (main)	Saale River sediment, Germany,	Pyruvate, acetate, benzoate, fumarate,	Ballerstedt et al., 1997
123-, 124-tri-CDD	10	124-→13 123-→13,23			
2378-tetra-CDD	~0.78	Up to 86% removal of 2378-tetra-CDD	2378-tetra-CDD contaminated sediments or anaerobic sludge, Taiwan,	Acetate, sludge cake	Kao et al., 2001
1234-tetra-CDD	2	124-→13	Arthur Kill Estuary sediment, USA, 3 yrs	---	Vargas et al., 2001
1234-tetra-CDD	50	124-, 123-	Spittelwasser River sediment, Bitterfeld, Germany	Fumarate, Pyruvate, acetate and benzoate	Bunge et al., 2001
123-tri-CDD	25	13-, 23-			
124-tri-CDD	25	13-			

Substrate	Conc. (μM)	Products	Inoculum	Electron Donors	Reference
1234-tetra-CDD	31	124-→13-→2-	Graving dock and Paleta Creek, San Diego, USA	Lactate and propionate	Ahn et al., 2005
		124-→13-	Shelter dock, San Diego; Tuckerton sediment, NJ; USA		
1234-tetra-CDF	49	Tri-, di-CDFs	Paleta Creek sediment, USA		
1234678-hepta-CDD	0.47	Hx-, penta-, tetra-, tri-, di- and/or mono-CDDs	Passaic River Estuary sediment, NJ, USA	Acetate, butyrate, and benzoate	Fu et al., 2005

*: concentration in ng/g

Table 1-6. Microbial reductive dechlorination of spiked PCBs in enrichment cultures and sediment microcosms.

Substrate	Conc. (μM)	Products	Inoculum	Electron Donors	Reference
2356-PCB	350	236-, 25-, 26-, *ortho* and *meta* dechlorination	Wood pond sediment, MA, USA	---	Van Dort and Bedard, 1991
Aroclor 1254	---	*Meta* and *para* chlorines were preferentially removed, resulting in accumulation of *ortho*-substutited mono-, di-, and tri-chlorobiphenyls	Hudson River sediment, NY, USA	Fatty acid mixture	Alder et al., 1993
			New Bedford Harbor sediment, MA, USA		
Aroclor 1260	---	Dechlorination of tri- and tetra-chlorobiphenyls	Silver Lake sediment, MA, USA		
22'33'44'-hxCBp	1.4	Mainly *ortho* dechlorination to penta- and tetra-CBps	Chemie Harbour of Rhine river sediment, Estuarine, the Netherlands	---	Hartkamp-Commandeur et al., 1996
22'33'66'-hxCBp					

Substrate	Conc. (µM)	Products	Inoculum	Electron Donors	Reference
22'44'55'-hxCBp	1.9	*Para* dechlorination products of penta-, tetra-, and tri-CBps	Biesbosch of Rhine River, Estuarine, The Netherlands	---	Hartkamp-Commandeur et al., 1996
22'44'66'-hxCBp	1.73				
Aroclor 1242	---	---	Hudson River sediment, USA	---	Williams, 1997
23'4'6-PCB	---	---	Tansui and Erjen River sediment, estuarine, Taiwan	---	Kuo et al., 1999
33'44'-PCB					
344'5-PCB					
33'44'5-PCB					
234-trCBp	---	---	Ho-Tsin river sediment, Taiwan	---	Chen et al., 2001
345-trCBp					
2345-PCB					

Substrate	Conc. (μM)	Products	Inoculum	Electron Donors	Reference
2345-PCB	100	235-, 245-	Baltimore Harbour estuarine sediment, USA,	Fe(0)/acetate, propionate, butyrate	Yan et al., 2006
PCB mixture	---	---	Burnt Soil culture, Japan	---	Baba, 2007
23456-PCB	---	Decreasing mol% of 23456-PCB	Anacostia River sediment, USA	---	Krumins et al., 2009
33’44’-PCB 33’44’5-PCB 23’44’5-PCB 33’44’55’-PCB 23’44’5-PCB	---	---	Brentell Canal, marine sediment, Italy		Zanaroli et al., 2010

Table 1-7. Dechlorination of weathered PCDD/Fs in sediments.

Location	Result	Characteristics	Reference
Lake Ketelmeer, Rhine River sediment, the Nethelands	Significant disappearance of 4 higher chlorinated dioxin congeners	Compared to archived sediment samples	Beurskens et al., 1993
Passaic River sediment, NJ, USA	After spiking high concentrations (ppm) of similar highly oxidized compounds to the sediment, previous contamination of PCDD decreased in anaerobic microcosms over two months, in particular hepta- and penta-CDDs, no measurement of lesser (tri- and di-) congeners yet. Pn-CDD was observed in the presence of solvent and electron shuttles catechol and resorcinol	Microcosm containing contaminated sediments, compared to the changes of spiked octa-CDD at time 0 and after 2 months	Barkovskii et al., 1994

Location	Result	Characteristics	Reference
Passaic River sediment, NJ, USA	Aged 2378-tetra-CDD was stoichiometrically converted to tri- and mono-CDDs	Results of anaerobic microcosm derived from site	Barkovskii and Adriaens, 1996
Passaic River sediment, NJ, USA	Production and dechlorination of 2378-tetra-CDD	Anaerobic microcosm with site sediment as inoculates	Albrecht et al., 1999
Queensland sediment, Australia	Sediment age was correlated with increasing proportions of lower chlorinated PCDDs that was corresponding to decreasing proportions of octa-CDD	Compared to archived sediment samples	Gaus et al., 2002
Passaic River Sediment, NJ, USA	About 2~4% of data variance was contributed by dechlorination with fingerprint of a highly positive 2378-TCDD and a highly negative hepta-CDD.	Employing polytopic vector analysis and validated by laboratory incubations	Barabas et al., 2004
PCDD/F contaminated river sediments, Japan	All PCDD/F congeners detected were equally reduced without accumulation of less-chlorinated congeners as intermediate or end products. Large amount of catechol and salicylic acid were produced in the upper aqueous phase.	Anaerobic microcosm amended with organic media inoculated with contaminated site sediments	Yoshida et al., 2005

Table 1-8. Dechlorination of weathered PCBs in sediments.

Location	Result	Characteristics	Reference
Hudson River sediment, NY, USA	Different PCB distribution pattern in the lower layers of sediment	Compared to commercial Aroclor 1242	Brown et al., 1984
Hudson River sediment, NY, USA	Mo-, di-, and tri-CBps increased with decrease of higher CBps	Compared to commercial Aroclor 1242, as well as time 0	Adriaens et al., 1995
Woods Pond sediment, Lenox, MA, USA	Characteristic congener distribution of Aroclor 1260. But key hexa-, hepta- decreased (max. 45%) relative to Aroclor 1260, with increase of tri-, tetra- and penta-CBps	Different locations of pond sediment	Bedard and May, 1996
Lake Hartwell sediment, Pickens County, SC, USA	Preferential loss of *meta* and *para* chlorines of PCBs with sediment depth, accompanied with the accumulation of lower chlorinated congeners dominated by *ortho* chlorine substituents.	Over sediment depth	Magar et al., 2005

Table 1-9. PCDD/Fs dechlorination by pure culture or highly enriched mixed cultures.

Isolate/Culture	Electron donor/priming reagent	Electron Acceptor (PCDD/Fs)	Growth Support	source	reference
Dehalococcoides ethenogenes strain 195	Butyric acid and PCE	1234-tetra-CDD→124-→13-1234-tetra-CDF→tri-CDFs	---	---	Fennell et al. 2004
	Butyric acid and PCE	23456-PCB→2346-/2356-→246-	---	---	
	---	Hexa- ~ non-BDE → penta, hexa, and hepta-BDEs	---	---	He, J. 2006
	butyric acid	123478-hxCDF→13478-, 12478-→1378-, 1248-CDFs	---	---	Liu and Fennell 2008

Isolate/Culture	Electron donor/priming reagent	Electron Acceptor (PCDD/Fs)	Growth Support	source	reference
Dehalococcoides sp. CBDB1	H_2, ED acetate for carbon source	12378-penta-CDD→2378-1378-tetra-CDD, 2378-tetra-CDD→237-→27-, 28	---	---	Bunge et al. 2003
	H_2, ED acetate for carbon source	1234-tetra-CDD→123, 124 (m)→23-(m), 24-, 13-→2-mono-CDD	Yes	---	
	---	23-chlorophenol	---	---	Adrian et al. 2007
	H_2, ED acetate for carbon source	Aroclor 1260 and 1248, *para* and *meta* dechlorination	---	---	Adrian et al. 2009

Isolate/Culture	**Electron donor/priming reagent**	**Electron Acceptor (PCDD/Fs)**	**Growth Support**	**source**	**reference**
Dehalococcoides sp. DCMB5	Acetate (C), formate, fumarate, and Pyruvate	124→13→2-CDD	Yes	Spittlewasser, Creek/river	Bunge et al. 2008
TriCDD- dechlorinating sediment-free mixed culture	Pyruvate, lactate, and fumarate	124→13→2; 123→13, 23→2	---	River Saale sediment, Germany	Ballerstedt et al. 2004

Table 1-10. PCB dechlorination by pure culture or highly enriched mixed cultures and highly enriched PBDE debrominators.

Isolate /Culture	**Electron donor/priming reagent**	**Electron Acceptor (PCB)**	**Growth Support**	**source**	**reference**
Chloroflexi o-17	acetate	2356-PCB doubly flanked *meta*- and flanked *ortho* dechlorination	Yes	Estuarine	Cutter et al. 2001
Chloroflexi DF-1 and *Desulfovibrio* sp. co-culture	H_2:CO_2=80:20 (v/v) and formate	2345-PCB *Para*-dechlorination doubly flanked chlorine	Yes	Estuarine	Wu, Q. et al. 2002
Chloroflexi DEH10, phylotype	Fatty acid mixture (acetate, propionate, and butyrate)	Remove doubly flanked chlorine in 234-substituted PCBs with a preference for *para*-flanked *meta*-chlorines when no double-flanked chlorines were available	Yes	Baltimore Harbor sediment	Fagervold et al., 2005, 2007

Isolate /Culture	**Electron donor/priming reagent**	**Electron Acceptor (PCB)**	**Growth Support**	**source**	**reference**
Chloroflexi SF1 phylotype More close to o-17 and DF-1	Fatty acid mixture (acetate, propionate, and butyrate)	Dechlorinate all 2345-substituted PCBs of double-flanked *meta* positions and 2356-, 236- and 235- congeners in *ortho*-flanked *meta* positions	Yes	Baltimore Harbor sediment	Fagervold et al. 2005, 2007
Chloroflexi SF2 phylotype, More close to o-17	Fatty acid mixture (acetate, propionate, and butyrate)	2346-substutited chlorophenyl ring (PCB 183) in double-flanked *meta* position and 2356-substituted chlorophenyl ring of PCB 151 in *ortho* position	Yes	Baltimore Harbor sediment	Fagervold et al. 2007
Sediment free *Dehalococcoides* JN culture	Acetate and hydrogen	Commercial Aroclor 1260, Process N dechlorination	Yes	Housatonic River sediment	Bedard et al. 2007

Isolate /Culture	**Electron donor/priming reagent**	**Electron Acceptor (PCB)**	**Growth Support**	**source**	**reference**
Chloroflexi o-17& DF-1	---	Aroclor 1260	---	---	Bedard et al. 2008
Chloroflexi DF-1 pure culture	H_2:CO_2=80:20 (v/v) and formate	Doubly flanked PCBs, Aroclor 1260 and weathered Aroclors	Yes, need *Desulfovibrio* spp. co-culture or cell extract	Estuarine	May et al., 2008
Dehalococcoides species	---	*ortho* debromination of PBDE	---	---	Robrock et al., 2008 Lee et al., 2010

Table 1-11. Approaches to enhance dechlorination of PCDD/Fs and PCBs.

Source	Country	Substrate	Enhancement	Reference
Passaic River sediment	USA	Aged PCDD/Fs in the sediment	Acetate, butyrate, benzoate, 2-MonobromoDD and H_2	Albrecht et al., 1999
Arthur Kill estuarine sediment	USA	1234-tetra-CDD	2-, 3-, 4-bromophenol	Vargas et al., 2001
Passaic River sediment	USA	1234678-hepta-CDD	2-BrDD	Fu et al., 2005
San Diego Bay sediment, Tuckerton sediment	USA	1234-tetra-CDD/F	1234-TeCB, 2345-tetrachloroanisole, 2345-tetrachlorophenol, 2'3'4'-trichloroacetophenone	Ahn et al., 2005, 2007
Kymijoki sediment,	Finland	1234-tetra-CDD	1234-tetrachlorobenzene	Ahn et al., 2008
Gulf Island Pond sediment,	USA	1234-tetra-CDD	1234-tetrachlorobenzene	Ahn et al., 2008

Source	Country	Substrate	Enhancement	Reference
D. ethenogenes 195		123478-hexa-	1234-tetrachlorobenzene	Liu and Fennell,
mixed culture		CDF		2008
River Rhine sediment	the	22'44'55'-,	236-trichlorobenzoate→25-	Hartkamp-
	Netherlands	22'44'66'-PCB		Commandeur et al.,
				1996
Hudson river	USA	Aroclor 1242	246-PCB, *para* dechechlorination	Williams et al., 1997
			236-PCB, *meta* dechechlorination	
Hudson River sediment	USA	Aroclor 1242	$FeSO_4$	Zwiernik et al., 1998
Housatonic River	USA	Aroclor 1260	Brominated biphenyls	Bedard et al., 1998
Woods Pond sediment	USA	Aroclor 1260	26-Dibromobiphenyl	Wu et al., 1999
Housatonic River	USA	Aroclor 1260	4-bromobenzoate,	Deweerd and
			4-iodobenzoate,	Bedard, 1999
			25-, 26-dibromobenzoate	

Source	Country	Substrate	Enhancement	Reference
Erjen river sediment,	Taiwan	33'44'-, 344'5-PCBs,	Toluene or 3-chlorobenzoate-adapted sediments	Kuo et al. 1999
Baltimore Harbor estuarine sediment,	USA	345-PCB, 22'344'55'-PCB	0.1g Fe^0/g sediment	Rysavy et al., 2005
Burnt Soil,	Japan	PCBs Kanechlor-300 & -400	Sodium molybdate	Baba et al., 2007
Uncontaminated paddy soil	Japan	Kanechlor 300, 400	Burnt soil	Baba et al., 2007b
Anacostia River sediment	USA	Historical PCBs	*Dehalococcoides* Strain 195 mixed culture, pentachloronitrobenzene	Krumins et al., 2009

Chapter 2

Microbial Reductive Dechlorination of Weathered Polychlorinated Dibenzofurans in Kymijoki Sediment Mesocosms

ABSTRACT

Little is known about the potential for indigenous microorganisms to reductively dechlorinate weathered polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in contaminated aquatic sediments. The sediments of River Kymijoki in Finland were heavily contaminated by PCDFs resulting from pulp and paper production and the manufacture of the chlorophenol-based wood preservative Ky-5 from the 1940's to the 1980's. In order to investigate naturally occurring microbial reductive dechlorination of weathered PCDFs and to determine the feasibility of stimulating such activities, we incubated River Kymijoki sediments in 30-L mesocosms at 18-21 °C. Treatments in attempt to stimulate dechlorination consisted of the addition of electron donors and a halogenated co-substrate (tetrachlorobenzene, TeCB), and bioaugmentation with a mixed culture containing *Dehalococcoides ethenogenes* strain 195. Following 7 years of incubation in electron donor plus TeCB treatments, we observed that the average mol% ratio of 1,2,3,4,6,7,8- vs. 1,2,3,4,6,8,9-hepta-CDF increased initially and then decreased, which was unlike the constantly increasing mol% ratio of these two hepta-CDFs in no addition controls and bioaugmented only treatments. Compared to no addition controls, these electron donor plus TeCB amended sediments showed a decrease in the mol% of octa-CDF and 1,2,3,4,6,8,9-hepta-CDF after 7 years that was accompanied by an increase in the mol% of 1,2,3,4,6,7,8-hepta-CDF and certain penta-CDFs and tetra-CDFs. These observations suggested that there was a mixed removal of *peri*-lateral-chlorines for hepta-CDFs in the presence of electron donor and TeCB, as compared to a general initial removal of *peri*-chlorines under other situations. For penta-CDFs an increasing mol% ratio of *peri* vs. total chlorines and decreasing mol% ratio of

lateral vs. total chlorines was observed in electron donor plus TeCB amended mesocosms over 7 years, suggesting the selective enhancement of *peri*-dechlorination for penta-CDFs under these conditions. PCR-DGGE analysis of 16S rRNA genes revealed a diverse *Chloroflexi* community in Kymijoki sediments. The overall observations allows us to conclude that dechlorination of weathered PCDFs in Kymijoki sediments by indigenous microorganism was occurring to different extents for different congeners, and amendement with TeCB plus electron donors may selectively stimulate dechlorination. Further characterization of the microbial communities mediating these transformations and future research on the dechlorination pathways of PCDFs may aid in the assessment of PCDD/Fs contamination and their natural attenuation processes. These accomplishments will eventually help developing effective bioremediation technologies.

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are generated mainly as unwanted byproducts from chlorine involved industries as well as thermal and combustion processes (Esposito et al., 1980; Fiedler et al., 1990; Rappe 1993; Kulkarni et al., 2008). Because of their extremely low water solubilities, low vapor pressures, and strong absorption to particles and surfaces (high K_{ow}) (Shiu et al., 1988), aquatic sediments and soils constitute typical sinks for PCDD/Fs in the environment. The burden of PCDD/Fs in various environmental compartments has been well documented (Wenning et al., 1992a; Clarke et al., 1996; Alcock et al., 1996; Thomas et al., 1996) with several hot spots that are heavily contaminated with PCDD/Fs (Wenning et al., 1993; Bertazzi et al., 1998; Bunge et al., 2001; Schecter et al., 2001; Kiguchi et al., 2007), including sediments of the Kymijoki River in Finland, which contains PCDD/Fs up to 120-193,000 µg/kg d.w. (Salo et al., 2008).

Dechlorination of spiked as well as weathered PCDDs under different redox conditions has been observed in microcosms and enrichment cultures derived from various sources (Adriaens and Grbic-Galic, 1994, 1995; Beurskens et al., 1995; Barkovskii and Adriaens, 1996; Ballerstedt et al., 1997; Bunge et al., 2001, 2003; Vargas et al., 2001; Ahn et al., 2005, 2008). Reductive dechlorination under anaerobic conditions is the only biological process observed so far capable of transforming highly chlorinated CDDs. This process can be stimulated by addition of PCDD structural analogs, also known as “haloprimers”, such as tetrachlorobenzene (TeCB) (Ahn et al., 2005, 2007, 2008).

Bacteria of the phylum *Chloroflexi,* with genus *Dehalococcoides* in particular, contain the first known and only isolated bacteria that are able to transform highly chlorinated dibenozo-*p*-dioxins. The chlorobenzene-dehalorespiring bacterium *Dehalococcoides* sp. strain CBDB1 (Adrian et al., 2000) and chloroethene-dehalorespiring *Dehalococcoides ethenogenes* strain 195 are the only isolated strains, so far, shown to be capable of reductively dechlorinating select PCDD congeners (Bunge et al., 2003; Fennell et al. 2004). Another highly enriched culture, "*Dehalococcoides* strain DCMB5", was enriched initially with 1,2,4-tri-CDD (Ewald et al., 2007) and finally isolated in two-phase liquid culture by 1,2,3-trichlorobenzene (Bunge et al., 2008). Strain CBDB1 and DCMB5 derive energy for growth during dechlorination of PCDDs. Members of *Dehalococcoides*-related *Chloroflexi* were reported to be putative dechlorinators in anaerobic enrichment cultures that dechlorinate various chlorinated compounds including PCBs (Cutter et al., 2001, Wu et al., 2002; Yan et. al., 2006), chlorobenzenes (Wu et al., 2002) and tri-CDDs (Ballerstedt et al., 2004). The detection of 16S rRNA gene sequences affiliated with *Dehalococcoides* spp. and its relatives in contaminated environments and dechlorinating enrichment cultures suggests a wide distribution of these bacteria in nature and their major roles in transforming chlorinated pollutants in the environment (Holoman et al., 1999; Löffler et al., 2000; Fennell et al., 2001; Hendrickson et al., 2002; He et al., 2003; Gu et al., 2004; Smits et al., 2004).

Unlike microcosms and enrichment cultures under laboratory conditions, the potential of indigenous microorganisms to dechlorinate weathered PCDD/Fs in contaminated sediments under field conditions has been less investigated and limited information is

available on the effectiveness of stimulating strategies for such processes. Knowledge of the dechlorination of weathered PCDDs in sediments was obtained by comparing present and archived sediments from Lake Ketelmeer, the Netherlands (Beurskens et al., 1993). Loss of higher chlorinated PCDD congeners was observed in the recently collected sediments when compared with archived sediment cores. Increasing proportions of lower chlorinated PCDDs corresponded to decreasing proportions of octa-CDD were also correlated with sediment ages in Queensland sediment, Australia (Gaus et al., 2002). Later Barabás et al. (2004) applied polytopic vector analysis (PVC) to estimate dechlorination of PCDDs in the field using Passaic River sediment, New Jersey. Their work identified a dechlorination fingerprint with a highly positive 2,3,7,8-tetra-CDD component and a highly negative hepta-CDD component. About 2~4% of data variance was contribuated by dechlorination, which corresponds to 1.2 μg/kg 2,3,7,8-TCDD per sample in expense of hepta-CDD according to PVA. This postulation was validated by the laboratory value for the ratio of 2,3,7,8-TCDD/2,3,7,8-PCDD (Barkovskii and Adriaens, 1996). Barabás and co-workders (2004) provide important links between laboratory dechlorination results with field scale analysis, which supported the occurrence of natural PCDD dechlorination *in situ*. However, these observations lack any detailed knowledge of the trend of difference over time and the corresponding responses of the microbial community. Unlike PCDDs, knowledge of the potential biotransformation of weathered PCDFs in contaminated sediments is limited. Currently, the biotransformation knowledge of PCDFs is mainly based on a few spiked single congeners studies using either soil or sediment derived microcosms containing pure or mixed cultures (Adriaens and Grbic-Galic, 1994; Fennell et al., 2004; Ahn et al., 2005;

Liu et al., 2008). Consequently, there is an inadequate understanding of natural attenuation of weathered PCDFs as a result of reductive dechlorination mediated by the indigenous microorganisms.

In this study we incubated historically PCDF-contaminated sediment from Kymijoki, Finland in 30-L mesocosms at 18~21ºC for seven years with different amendments provided at the start of the experiment, including 1,2,3,4-tetrachlorobenzene (TeCB) as a "haloprimer", bioaugmentation with a mixed culture containing *Dehalococcoides ethenogenes* strain 195, and lactate and propionate as electron donors. The purpose of this study was to find evidence of microbial reductive dechlorination of weathered PCDFs in Kymijoki sediments and to investigate the feasibility of stimulating such activities *in situ*. Sediment samples were taken after over the 7 years of incubation for the analysis of PCDFs and the microbial community.

MATERIALS AND METHODS

Treatment/Incubations of Contaminated Sediments. The Kymijoki River was heavily contaminated with PCDD/Fs from the 1940's to the 1980's due to chlorine bleaching in paper industry and the production and use of a chlorophenol fungicide in the saw mill industry along the river (Salo et al., 2008; Verkasalo 2004; Paasivirta 1996; Verta et al. 1999). Contaminated sediments were collected in 2002 by Dr. Martti Verta (Finnish Environment Institute, Helsinki, Finland) by dredging from downstream of Myllykoski in River Kymijoki, Finland, where the production of the chlorophenol fungicide Ky-5 constituted the main source of extensive PCDD/Fs contamination. The major chlorinated

compounds are polychlorinated phenols (PCPs), PCDDs, and PCDFs; among them 1,2,3,4,5,6,7,8- and 1,2,3,4,5,6,8,9-heptaCDFs and octa-CDF are the dominant congeners (Verta et al., 1999; Koistinen et al., 1995). It is estimated that the total amount of PCDD/Fs in the sediments range from 4,000 to 5,000 kg in the contaminated area of River Kymijoki (Verta et al., 1999), and the surface sediment remains a significant source of PCDD/Fs downstream (Isosaari et al., 2002; Malve et al., 2003). The dredged sediments were mixed and aliquoted in 30 L plastic fermentation buckets sealed and fitted with an air lock, then spiked at the very beginning with 25 μM 1,2,3,4-tetrachlorobenzene (TeCB) as haloprimer or amended with 200 ml of a mixed culture containing *Dehalococcoides ethenogenes* strain 195 with 1 mM lactate and propionate as electron donors. Mesocosm controls amended with electron donors only or no additions were set up at the same time. All mesocosm treatments were conducted in triplicate 30 L plastic fermentation buckets sealed and incubated at room temperature (18~21 °C) to sustain the dechlorination capacity since 2002. At select time points (after 0, 2, 3, 5 and 7 years of the incubation) the fermentation buckets was opened, the sediments mixed with a rod and a 30 to 50 mL samples collected in glass jars. The sediment samples were stored at -20 °C for chemical analysis of PCDFs and at -70 °C for microbial community analysis.

Analysis of PCDFs. Liquid/solid extraction was performed to quantify PCDFs and transformation products as follows: 3 g (wet weight) of sediments was extracted with 4 ml toluene and acetone (2:1) with 625 μg (each) PCB 14 (3,5-DiCB), PCB 23 (2,3,5-TrCB), PCB 65 (2,3,5,6-TeCB) and PCB 166 (2,3,4,4',5,6-HxCB) added as extraction

efficiency surrogates. Extraction tubes were capped with Telflon-lined screw caps, mixed vigorously for 1 min, and sonicated in an ultrasound bath for 30 minutes before shaking overnight on a wrist-action shaker. The extraction mixtures were centrifuged at 2500 rpm for 3 min before transferring the solvent phase to a clean 7 ml extraction vial. The extraction procedure was repeated 3 times, the upper solvent phase was pooled and finally evaporated down to a volume of 1 ml, which was then loaded on to toluene pre-rinsed Florisil (Sigma-Aldrich, St. Louis, MO) pipette columns and eluted with 6 ml toluene. The florisil-cleaned sample was concentrated to 1 ml with a stream of N_2. PCDFs parent and daughter compounds were analyzed on an Agilent 6890 series gas chromatograph (GC) equipped with an Agilent 5973 series Mass Selective Detector (MSD) (GC-MS, Agilent Technologies, Inc., Santa Clara, CA) equipped with a HP-5MS capillary column (60 m ×0.25 mm, 0.25 μm film thickness, J&W Scientific, Folsom, CA). The temperature cycle program was: 60 °C, increased by 10 °C/min to 170 °C, 2 °C/min to 200 °C, 5 °C/min to 220 °C, and hold at 220 °C for 16 minutes, and increased by 5 °C/min to 235 °C, hold at 235 °C for 7 minutes, and increased by 5 °C/min to 250 °C, and finally 1 °C/min to 280 °C, and a 5 min 300 °C hold. Helium was used as the carrier gas at a constant flow rate of 1.5 ml/min. Mono-, di-, tri-, tetra-, penta-, hexa-, hepta- and octa-chlorinated dibenzofuran congeners are abbreviated as mono-CDF, di-CDF, tri-CDF, tetra-CDF, penta-CDF, hexa-CDF, hepta-CDF and octa-CDF, respectively. PCDFs denote penta- to octa-chlorinated dibenzofuran congeners, whereas 1-4CDFs stands for all congeners of mono-CDFs, di-CDFs, tri-CDFs and tetra-CDFs.

Detection, Identification and Quantification of PCDFs by PCB Surrogates and Internal Standards. PCDFs were detected and identified based on the retention times of standards and their most abundant molecular ions, and a qualifying ion was monitored to assure the correct identification (Table 2-1). PCB 14, PCB 23, PCB 65 and PCB 166 were used as surrogates to estimate the extraction efficiencies of PCDFs from the historically polluted sediments. PCB 30 and 204 were used as internal standards to calculate response factors (RF) for the quantification of the parent and daughter PCDFs in the sediments based on the recovery of PCB surrogates throughout the extraction, cleanup and concentration procedures. RFs were calculated by equation (2-1):

$$RF = \frac{C_s}{C_{is}} \times \frac{A_{is}}{A_s} \qquad (2\text{-}1)$$

where, RF: response factors of PCB surrogates (PCB 14, 23, 65, 166) versus PCB internal standards (PCB 30, 204) respectively; C_s: PCB surrogate concentration; C_{is}: PCB internal standards concentration; A_s: integrated chromatographic area of PCB surrogate; A_{is}: integrated chromatographic area of PCB internal standards.

Weathered PCDFs were quantified by equation (2-2):

$$C_p = \frac{A_p}{A_{is}} \times C_{is} \times RF \qquad (2\text{-}2)$$

where, C_p: concentration of weathered PCDFs or PCB surrogate; A_p: integrated chromatographic area of PCDF congener or PCB surrogate; A_{is}: integrated chromatographic area of PCB internal standards; C_{is}: PCB internal standards concentration; RF: response factors of PCB surrogates (PCB 14, 23, 65, 166) versus PCB internal standards (PCB 30, 204) respectively.

According to their retention time (rt) ranges, PCB 30 (rt, 22.02 min) was used in quantification of PCB 14 (rt, 21.11 min), PCB 23 (rt, 24.76 min), PCB 65 (rt, 28.76 min), PCB 166 (rt, 47.49) and weathered PCDFs with less than three chlorines. However, due to their extremely low concentrations in Kymijoki sediment, no weathered PCDFs with less than three chlorines were detected in our experiment. PCB 204 (rt, 52.52 min) was used in quantification of weathered PCDFs (tetra: 40-50 min, penta: 50-60 min, hexa: 60-70 min, octa-CDF: 85 min). The average extraction efficiency of PCB surrogates ranged from 80~105% for PCB 14, 23, 65 and 166 (n=60, triplicates samples from 4 treatments at 5 time points). The detection limits for PCDFs were approximately 0.5 ng/g (d.w). Since dechlorination reactions result in the loss of mass but not in total molar concentration, the congener concentrations are calculated and reported in molar percentages (and molar fractions). Each congener was calculated in mol/g d.w., and its concentration was calculated in following equation (2-3):

$$Mol\% = 100\% \times \frac{C_i}{\sum_{i=1}^{n} C_i} \qquad (2\text{-}3)$$

where, C_i: concentration of certain congener in mol/g d.w, ΣC_i: sum concentrations of all congeners detected in same sample in mol/g d.w, n: the number of congeners detected in each sample for each time point.

PCR-DGGE. Bulk DNA was extracted from mesocosm sediments by using the PowerSoil DNA purification kit (MoBio, CA) and following the manufacturer's instructions. The resulted DNA solution was used for polymerase chain reaction (PCR)

and denaturing gradient gel electrophoresis (DGGE). Nested PCR were performed to specifically amplify the *Chloroflexi* community following the method of Park et al. (2010). The 1st PCR was amplified by forward primer 338F and reverse primer Dchl1101R; and the 2nd PCR were amplified with 341F plus a GC clamp on the 5' terminus and reverse primer 534R (Table 2-2). PCR reagents were purchased from GenScript (Piscataway, NJ). The reaction contained 2 µl of DNA as template and 5 µl of 10 X reaction buffer, 100 µM of Dntp, 0.01 U of Taq DNA polymerase, 0.2 µM of forward primer and 0.2 µM of reverse primer in each 50 µl PCR reaction mixture. PCR was performed by preheating the mixture at 94 ºC for 5 minutes, followed by 35 cycles of denaturation at 94 ºC for 20 seconds, annealing at 55 ºC for 45 seconds, and extension at 72 ºC for 45 seconds, and a final post extension step at 72 ºC for 7 min. DGGE was performed according to the standard protocol (Muyzer et al., 1993) using the Bio-Rad Dcode system (Bio-Rad laboritaries, Hercules, CA). The denaturing gradient gel was prepared to contain 0.5 X TAE buffer (20 Mm Tris, 10 Mm acetate, 0.5 Mm Na_2-EDTA, Ph 8.0), 6% acrylamide-bis-acrylamide, and 50 to 70% denaturant (100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was performed at 40 V for 17 hours in 0.5 X TAE buffer at 60 ºC. PCR products separated on the gel were stained with EtBr for 20 min and then photographed under illumination by UV light. Major DGGE bands were cut from the gel, eluted in PCR water overnight and PCR amplified with 2nd set of primers (341F-gc/534R) under the same conditions as described above.

Sequencing and Phylogenetic Analysis. Purified single bands from DGGE gels were re-amplified with primers of 341F and 534R under the same PCR conditions as above. The

resulting PCR products were separated by agarose gel electrophoresis, purified and pre-added 341F primer before sending to GeneWiz for sequencing (GeneWiz, South Plainfield, NJ). Homologous sequence searches were performed using BLAST (Altschul et al., 1997) and selected sequences aligned using CLUSTAL X (Thompson et al., 1997). Phylogenetic analyses by the Neighbor-joining method were conducted using MEGA version 4 (Tamura et al., 2007). The partial 16S rRNA gene sequences are available in appendix 3.

RESULTS

Detected and identified congeners of weathered PCDFs in Kymijoki sediment mesocosms. Kymijoki sediments contain a mixture of PCDF congeners. Altogether 20 peaks corresponding to PCDF congeners with four or more chlorines were detected, and among them, 14 peaks were identified. Two peaks identified as hexa-CDFs and 4 peaks identified as tetra-CDFs were unknown congeners (Table 2-3). Data for one triplicate mesocosm amended with ED and TeCB is shown as an example (Fig. 2-1). In addition to the most abundant peaks of octa-CDF (peak 20), 1,2,3,4,6,8,9-hepta-CDF (peak 19) and 1,2,3,4,6,7,8-hepta-CDF (peak 18) (Fig. 2-1a & b), altogether eight hexa-CDF peaks were detected (Fig. 2-1c & d), including 6 known congeners identified by comparing their elution time and sequence based on Humppi and Heinola (1985) and two unknown isomers, peaks 14 and 16. Five peaks were detected and identified as penta-CDFs (Fig. 2-1e & f) (Humppi and Heinola, 1985). Four peaks were detected as tetra-CDFs congeners (peaks 01 to 04) (Fig. 2-1g & h) but without identification due to the unavailability of these tetra-CDF standards. Neither 1,2,3,4- nor 2,3,7,8-tetra-CDF were detected in

Kymijoki sediment mesocosms. The identified congeners have different combinations of *peri* and lateral chlorine substitutions (Table 2-3).

Evidence for dechlorination of weathered PCDFs in Kymijoki sediment. Evidence for dechlorination was found by comparing the Selected Ion Chromatograms for all the congeners at time 0 vs. 7 years, which showed changes in the intensity of the different peaks (congeners) over time, suggestive of reductive dechlorination. In the case of one Kymijoki mesocosm amended with electron donors plus TeCB at time 0 vs. after 7 years (Fig. 2-1), the relative abundance of 1,2,3,4,6,8,9-hepta-CDF (peak 19) was initially greater than that of 1,2,3,4,6,7,8-hepta-CDF (peak 18). After 7 years of incubation, the abundance of the 1,2,3,4,6,8,9-hepta-CDF (peak 19) was decreased relative to that of 1,2,3,4,6,7,8-hepta-CDF (peak 18). The relative abundance of 1,2,4,6,8,9-hexa-CDF (peak 12) decreased and 1,2,3,6,8,9-hexa-CDF (peak 13) increased over time. The abundance of 2,3,4,7,8- (peak 09), 1,2,3,7,8,- (peak 07) and 1,2,4,6,8- (peak 05) penta-CDFs increased after 7 years. The abundance of tetra-CDF-3 (peak 03) and -1 (peak 01) increased after 7 years.

Evidence for dechlorination of weathered PCDFs was further supported by comparing the molar fraction of 1,2,3,4,6,7,8-hepta-CDF vs. 1,2,3,4,6,8,9-hepta-CDF over 7 years incubation in each treatment (Fig. 2-2). The average molar fraction of 1,2,3,4,6,7,8-hepta-CDF vs. 1,2,3,4,6,8,9-hepta-CDF increased from 0.7 ± 0.1 at time 0 to 1.0 ± 0.2 after 7 years in the no addition control. A similar trend was also observed in the treatment amended with electron donor plus *D. ethenogenes* strain 195. The average molar fraction

of 1,2,3,4,6,7,8-hepta-CDF vs. 1,2,3,4,6,8,9-hepta-CDF increased from 0.9 ± 0.1 at time 0 to 1.2 ± 0.3 after 7 years. For the meosocosms amended with electron donors plus TeCB, in comparison, the average molar fraction of 1,2,3,4,6,7,8-hepta-CDF vs. 1,2,3,4,6,8,9-hepta-CDF increased 83% from 0.7 ± 0.2 at time 0 to 1.3 ± 0.2 after 2 years and then this molar fraction remained stable above 1, with a slight decreasing trend till after 7 years. In the electron donor only mesocosm control, similar changes were observed over the 7-year incubation period.

Effect of electron donors and TeCB on the dechlorination of weathered PCDFs. All the PCDF chlorines can be classified into two groups: lateral chlorines for 2,3,7,8-substitutions or *peri* chlorines for 1,4,6,9-substitutions. Therefore, dechlorination will cause changes in their respective mol% among the total chlorines for the congeners of each individual homolog (Fig. 2-3). A trend of decreasing average mol% of *peri* chlorines and increasing average mol% of lateral chlorines for hepta-CDFs was observed in the no addition control over 7 years (Fig. 2-3a). A trend of decreasing average mol% of *peri* chlorines and increasing average mol% of lateral chlorines for penta-CDFs was observed in the electron donor and TeCB treatment over 7 years (Fig. 2-3c). These observations suggest a *peri* preferred dechlorination for weathered hepta-CDFs in the no addition control and for weathered penta-CDFs in the electron donor plus TeCB treatment over 7 years. No such trends could be determined for hexa-CDFs and tetra-CDFs (due to unavailable congener information) in the other treatments over 7 years. No such trend of penta-CDFs was observed in no addition control and mesocosms amended with electron donor with or without *D. ethenogenes* strain 195 over 7 years (Fig. 2-3).

Comparison of the average mol% changes of weathered PCDFs in mesocosms amended with electron donors plus TeCB vs. no addition control after 7 years (Fig. 2-4a & b) showed that congeners decreasing over time included octa-CDF (peak 20), 1,2,3,4,6,8,9-hepta-CDF (peak 19), 1,2,4,6,8,9-hexa-CDF (peak 12), 1,2,4,6,7,8-hexa-CDF (peak 11), 1,2,3,4,6,8-hexa-CDF (peak 10), 1,2,3,7,8-penta-CDF (peak 07), 1,2,4,6,8-penta-CDF (peak 05), tetra-CDF-4 (peak 04) and congeners increasing over time included 1,2,3,4,6,7,8-hepta-CDF (peak 18), 1,2,3,6,8,9-hexa-CDF (peak 13), 2,3,4,7,8-penta-CDF (peak 09), 1,2,4,7,8-penta-CDF (peak 06), tetra-CDF-3 (peak 03), tetra-CDF-1 (peak 01) (Fig. 2-4c).

Analysis of Putative Dechlorinating *Chloroflexi* Community. In order to identify the responsible microbial populations for dechlorination of weathered PCDFs in Kymijoki sediment mesocosms, the community structure of *Chloroflexi* in each treatment was characterized. The microbial succession in the mesocosms monitored by PCR-DGGE profiling of the 16S rRNA gene using *Chloroflexi*-specific primers (Fig. 2-5) showed a diverse *Chloroflexi* or *Dehalococcoides*-like community in Kymijoki sediment over 7 years of treatment. Six DGGE single bands highlighted in Figure 5 were purified and sequenced for phylogenetic analysis (Fig. 2-6). The sequences of six different DGGE bands clustered with *Dehalococcoides* spp. and various uncultured *Chloroflexi*, found previously in analysis of dechlorinating microbial consortia or methanogenic environments containing benzenes (Winderi et al., 2008; Hori et al., 2007; Kunapuli et

al., 2007). Among them, Band 6 is most closely related to *Dehalococcoides ethenogenes* strain 195.

DISCUSSION

This study demonstrates the dechlorination of weathered PCDFs in Kymijoki sediment mesocosms during 7 years of incubation. Compared to the no addition control, the addition of electron donors plus TeCB enhanced dechlorination and stimulating dechlorination of weathered octa-CDF to produce 1,2,3,4,6,7,8-hepta-CDF, stimulating dechlorination of weathered 1,2,3,4,6,8,9-hepta-CDF by stimulating *peri*-preferred dechlorination of weathered penta-CDFs during this period. The increase in the average mol% ratio of 1,2,3,4,6,7,8-hepta-CDFs vs. 1,2,3,4,6,8,9-hepta-CDF from less than 1 to more than 1 in all mesocosms over 7 years of incubations (Fig. 2-2) provides evidence for dechlorination of weathered PCDFs in Kymijoki sediments. In addition, the average mol% ratio of *peri* chlorines vs. total chlorines for hepta-CDFs decreased and the average mol% ratio of lateral chlorines vs. total chlorines for hepta-CDFs increased in all mesocosms over 7 years of incubation (Fig. 2-3a). Evidence that addition of electron donor and TeCB enhanced dechlorination of weathered PCDFs is presented in three ways. First, comparing the selected ion chromatograms of hepta-CDFs, penta-CDFs and tetra-CDFs at time 0 and after 7 year, we found that the relative abundance ratio of 1,2,3,4,6,7,8-hepta-CDF and 1,2,3,4,6,8,9-hepta-CDF changed from less than 1 to more than 1 (Fig. 2-1a & b) and the relative abundance of four penta-CDFs (Fig. 2-1e & f) together with two tetra-CDFs (Fig. 2-1g & h) increased after 7 years incubation. Second, the average mol% ratio of *peri* chlorines vs. total chlorines for penta-CDFs decreased and

the average mol% ratio of lateral chlorines vs. total chlorines for penta-CDFs increased in mesocosms amended with electron donors and TeCB (Fig. 2-3c). This observation indicated a *peri*-perferred dechlorination for penta-CDFs. Third, comparing the average mol% of each congener of weathered PCDFs in mesocosms amended with electron donors and TeCB to the no addition control during 7 years incubation, we found that congeners with a decreasing average mol% after 7 years included octa-CDF (peak 20), 1,2,3,4,6,8,9-hepta-CDF (peak 19), 1,2,4,6,8,9-hexa-CDF (peak 12), 1,2,4,6,7,8-hexa-CDF (peak 11), 1,2,3,4,6,8-hexa-CDF (peak 10), 1,2,3,7,8-penta-CDF (peak 07), 1,2,4,6,8-penta-CDF (peak 05), tetra-CDF-4 (peak 04). In contrast, congeners with an increasing mol% after 7 years included 1,2,3,4,6,7,8-hepta-CDF (peak 18), 1,2,3,6,8,9-hexa-CDF (peak 13), 2,3,4,7,8-penta-CDF (peak 09), 1,2,4,7,8-penta-CDF (peak 06), tetra-CDF-3 (peak 03), tetra-CDF-1 (peak 01) (Fig. 2-4b). In addition to the results of chemical analysis, 16S rRNA gene microbial community analysis by DGGE showed a diverse *Chloroflexi* community (Fig. 2-5) and sequences of selected DGGE bands clustered with bacteria found in areas with similar contamination or in dechlorinating enrichment cultures (Fig. 2-6). It should be noted that since here we specifically targeted only *Chloroflexi* community, there exist possibility that non-*Chloroflexi* bacteria contributed to dechlorination but were missed by this method. These non-*Chloroflexi* bacteria may be involved in dechlorination of weathered PCDFs directly or indirectly by providing vital growth factors for dechlorinating bacteria in the sediments.

Based on the identification of certain increasing and decreasing PCDF congeners in mesocosms amended with electron donrs and TeCB, we proposed a possible

dechlorination pathway for weathered PCDF in Kymijoki sediment (Fig. 2-7). In most cases, *peri*-dechlorination could explain the decrease of highly chlorinated congeners and the corresponding increase of less chlorinated congeners, namely *peri*-dechlorination of octa-CDF to 1,2,3,4,6,7,8-hepta-CDF, 1,2,3,4,6,8,9-hepta-CDF to 1,2,3,6,8,9-hexa-CDF, and 1,2,4,6,7,8-hexa-CDF to 1,2,4,7,8-penta-CDF. The increase in 2,3,4,7,8-penta-CDF may be the result of a two-step *peri*-dechlorination product produced from 1,2,3,4,6,7,8-hepta-CDF via 2,3,4,6,7,8-hexa-CDF. Considering the *peri*-preferred dechlorination reaction for hepta-CDFs in no addition controls and mesocosms amended with electron donors and strain 195 over 7 years as well as for penta-CDFs in mesocosms amended with electron donors and TeCB, it is reasonable to postulate that *peri*-dechlorination might be a preferred dechlorination route for weathered PCDFs in Kymijoki sediment, and amendment with electron donors and TeCB selectively enhanced this reaction. In this scenario Kymijoki sediment contained a *peri*-preferrential dechlorinating microbial community, and amendment with electron donors and TeCB either stimulated the growth of *peri*-dechlorinating microbial community or induced the expression of *peri*-dechlorinating enzymes. This possible *peri*-preferred dechlorination may have the risk of generating toxic intermediates, such as 2,3,7,8-tetra-CDF. However, whether this should be a concern for Kymijoki sediment needs further studies.

In contrast to the theoretical dechlorination pattern proposed for PCDDs (Fueno et al., 2002; Lynam et al., 1998), which implied that lateral chlorines were more amenable for dechlorination due to the positive charges of lateral carbons and marginal preference of chlorines on them, dechlorination mediated by microorganisms in the sediment has been

shown to occur preferentially at the *peri* chlorines. Barkovskii and Adriaens (1996) indicated that *peri*-dechlorination of 2,3,7,8-substituted hepta to penta-CDDs produced 2,3,7,8-tetra-CDD; and *peri*-lateral dechlorination was occurring to non-2,3,7,8-subsituted congeners in the microbial consortium eluted from dioxin-contaminated Passaic River sediments. These observations are in agreement with what we have reported here for the dechlorination of weathered PCDFs in Kymijoki sediment mediated by the indigenous microorganisms. Similarly, dechlorination study of spiked 1,2,3,4-/ 2,3,7,8-tetra-CDDs and 1,2,3,7,8-penta-CDD by *Dehalococcoides* sp. strain CBDB1 showed *peri*-lateral dechlorination sequence (Bunge et al., 2003). However, a mixed culture containing *Dehalococcoides ethenogenes* strain 195 mediated a lateral-*peri* – dechlorination sequence for spiked 1,2,3,4,7,8-hexa-CDF and 1,2,3,4-tetra-CDD instead (Liu and Fennell, 2008; Fennell et al., 2004). These results from already known dechlorinators further supported the strong dechlorinating potentials of microorganisms.

A diverse *Chloroflexi* community in the Kymijoki sediment microcosms was detected by PCR-DGGE analysis, further confirming the importance of this group of bacteria in dechlorinating PCDD/Fs and other polychlorinated pollutants. Members of *Chloroflexi* bacteria were reported to be putative dechlorinators in anaerobic enrichment cultures capable of dechlorinating various chlorinated compounds, including 2,3,5,6- and 2,3,4,5-tetrachlorinated biphenyls (Cutter et al., 2001, Wu et al., 2002; Yan et. al, 2006), tri- to hexa-chlorobenzenes (Wu et al., 2002) and 1,2,4- and 1,2,3- tri-CDDs (Ballerstedt et al., 2004). In addition to one highly enriched culture of *Dehalococcoides* sp. DCMB5, *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. CBDB1 are two

known isolates that are capable of dechlorinating selected PCDD/F congeners (Fennell et al., 2004; Bunge et al., 2003, 2008). The presence of *Dehalococcoides* spp. has also been confirmed in Anacostia River (Washington DC) sediment microcosms showing enhanced dechlorination of low concentration historical PCBs (Krumins et al., 2009; Park et al., 2010). The detection of 16S rRNA gene sequences affiliated with "*Dehalococcoides*" and its relatives in contaminated environments and dechlorinating enrichment cultures suggests the wide distribution of these bacteria in nature and their major roles in transforming chlorinated pollutants in the environment (Dennis et al., 2003; Duhamel et al. 2004; Fennell et al., 2001; Löffler et al., 2000; Major et al., 2002; Gu et al., 2004; He et al., 2003; Hendrickson et al., 2002; Richardson et al., 2002; Holoman et al., 1999; Kassenga et al., 2004; Smits et al., 2004; Ahn et al., 2007, 2008).

The available information on the dechlorination potential of weathered PCDD/Fs congeners was mainly postulated from two major sources. One source is the dechlorination of certain spiked PCDD/F congeners in microcosms or enrichment cultures derived from contaminated sediments and soils (Beurskens et al., 1995; Adriaens and Grbic-Galic, 1994, 1995; Ballerstedt et al., 1997; Vargas et al., 2001; Bunge et al., 2001). The other one source is by comparing sediment cores at different age and depth (Beurskens et al., 1993; Gaus et al., 2002) or by assessing the dechlorination potential of microbial consortia derived from historically contaminated sediments (Barkovskii et al., 1994; Barkovskii and Adriaens, 1996; Albrecht et al., 1999). In this study, we have provided direct evidence of dechlorination and the corresponding time course of weathered PCDFs in Kymijoki sediment mesocosms over 7 years incubation. The

stimulating effect of TeCB on dechlorination was previously shown for spiked PCDD/F congeners in similar microcosms and enrichment cultures, including in Kymijoki sediments (Ahn et al., 2007, 2008).

The mol% of *peri* and lateral chlorines among total chlorines for hexa-CDFs remained constant over 7 years in Kymijoki sediment mesocosms under all conditions including the no-amendment controls. The average mol% of *peri* and lateral chlorines among total chlorines for hexa-CDF was approximately 58% and 38% (since we are still missing the identification of several detected HxCDFs in the sediment, Table 2-3) under all conditions during 7 years of incubation. It is unlikely that the lack of bioavailability specifically of hexa-CDFs was the explanation for this. Consequently, a possible explanation is that weathered hexa-CDF congeners in Kymijoki sediment were actually in a state of flux, which means that dechlorination occurred to certain selected hexa-CDFs congeners that would balance hexa-CDFs produced by dechlorination of hepta-CDFs over time. It may not necessarily be the same hexa-CDF isomer that was produced from hepta-CDFs dechlorination to be further dechlorinated to less chlorinated congeners, since we here only consider chlorines removals at *peri* or lateral positions regardless of individual isomers. Similarly to what we have proposed for weathered hexa-CDFs in Kymijoki sediment, weathered 2,3,7,8-substituted PCDDs in general and 2,3,7,8-tetra-CDD in particular, were shown to be in a state of flux in Passaic River sediment (Albrecht et al., 1999). In addition, dechlorination of spiked 1,2,3,4,6,7,8-hepta-CDF inoculated with Hudson River sediment (Adriaens and Grbic-Galic, 1994) and dechlorination of spiked 1,2,3,4,7,8-hexa-CDF by a mixed culture containing

Dehalococcoides ethenogenes strain 195 (Liu and Fennell, 2008) all supported this postulation. To confirm this explanation, however, further work is needed to clarify the exact dechlorination pathway of 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-hepta-CDFs and all the hexa-CDFs detected in Kymijoki sediment.

This study also showed a diverse *Chloroflexi* community in Kymijoki sediment mesocosms (Fig. 2-5). The sequences of selected DGGE bands clusted with *Chloroflexi* sequences found at other sites with similar contaminations (Winderi et al., 2008; Hori et al., 2007; Kunapuli et al., 2007). The various treatments were introduced once to the mesocosm only at the very beginning, it is likely that any major changes would subside over time, or that the treatments did not induce changes in the *Chloroflexi* community that we could detect using current method. This can be supported by the fact that the dechlorination observed in Kymijoki sediment mesocosms was very limited compared to enrichment cultures for the dechlorination of spiked PCDD/F congeners. Just like their widespread existence in the environment (Dennis et al., 2003; Duhamel et al., 2004; Fennell et al., 2001; Gu et al., 2004; He et al., 2003; Hendrickson et al., 2002; Kassenga et al., 2004; Löffler et al., 2000; Major et al., 2002; Richardson et al., 2002; Smits et al., 2004) and their importance in dechlorination of PCDD/Fs (Holoman et al., 1999; Yoshida et al., 2005; Hiraishi et al., 2005; Ahn et al., 2007, 2008, Krumins et al., 2009), this diverse *Chloroflexi* community in Kymijoki mesocosm (Fig. 2-5) suggest their important roles in mediating dechlorination of weathered PCDFs *in situ*.

In view of these collective data above, Kymijoki sediments appear to contain active indigenous dechlorinating bacteria that may be stimulated with proper amendments to reductively dechlorinate weathered PCDFs. Knowledge of the microbial community members in the contaminated sediments, especially those whose presence correlates with activity and dechlorination, can potentially be valuable in detecting, stimulating and cultivating the relevant dechlorinating populations. The question of whether abiotic reductive dechlorination of PCDD/Fs (Adriaens et al., 1996; Lee and Batchelor, 2004) is involved here should also be clarified by further study. Understanding of the microbial attenuation of weathered PCDFs in sediments like Kymijoki will be of crucial importance for risk assessment and development of strategies for bioremediation of contaminated sites.

Conclusions

In conclusion, following 7 years of incubation, dechlorination of weathered PCDFs in Kymijoki sediment was observed. An increase in the mol% ratio of 1,2,3,4,6,7,8- vs. 1,2,3,4,6,8,9-hepta-CDF was observed in all mesocosms. Compared to no addition control after 7 years, decreasing mol% of octa-CDF and 1,2,3,4,6,8,9-hepta-CDF as well as increasing mol% of 1,2,3,4,6,7,8-hepta-CDF were observed in addition to mol% loss of penta-CDFs as well as tetra-CDFs in mesocoms amended with electron donors and TeCB. This suggests that *peri*-chlorines of hepta-CDFs were removed in general, while for penta-CDFs, *peri*-chlorines were removed preferentially only with amendment of electron donors and TeCB. No dechlorination preference was observed for weathered hexa-CDFs in Kymijoki sediments. However, the data is insufficient to conclude whether

this *peri* dechlorination may result in toxic 2,3,7,8-substituted intermediates. PCR-DGGE analysis revealed a diverse *Chloroflexi* community in Kymijoki sediment. Phylogenetic analysis of DGGE bands indicated that community members were closely related to *Chloroflexi* found in other environments polluted by chlorinated compounds. The mesocosm studies implied that natural attenuation of weathered PCDFs was occurring in Kymijoki sediment. However, the possible dechlorination pathways and reactions were complicated considering the integrated role of abiotic and biotic dechlorination potentials that may co-exist in the sediment. The very limited extent of dechlorination observed also complicates the analysis. The poor survival of *D. ethenogenes* strain 195 may restrict its application to enhance dechlorination of weathered PCDFs in Kymijoki sediment. Characterization of the microbial communities mediating these transformations as well as further research in the fate and pathways of PCDD/Fs dechlorination will aid in the assessment of weathered PCDD/Fs contamination and natural attenuation processes, which will help the development of bioremediation technologies for PCDD/Fs pollution.

Table 2-1. The most abundant molecular ions and qualifying molecular ions of different PCDF and PCB congeners.

Congener	Most abundant Molecular Ion (*m/z*)	Qualifying Molecular Ion (*m/z*)
octa-CDF	442	444
hepta-CDF	408	410
hexa-CDF	374	376
penta-CDF	340	342
tetra-CDF	306	304
TriCDF	272	270
di-CDF	238	236
mono-CDF	204	202
PCB 14	222	224
PCB 30 & 23	256	258
PCB 65	292	290
PCB 166	360	362
PCB 204	430	428

Table 2-2. Primers of nested-PCR DGGE reaction.

Primers	**Sequence**
338F	5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CC CTC CTA CGG GAG GCA GCA G-3'
Dch1101R	5’-CTC GCK AGA AMA TKT AAC TAG CAA C-3’
341F-GC	5’-GCC CGC CGC GCG CGG CGG GCG GGG CGG GGG CAC GGG GGG, CCT ACG GGA GGC AGC AG-3’
534R	5’-CCA GCA GCC GCG GTA AT-3’

Table 2-3. Detected and identified congeners of weathered PCDFs in Kymijoki sediments at time 0.

Homolog	Peak #	Congener Name	Retention Time (min)	Conc. (ng/g d.w.)	Mol% (avg±SD)	# of *peri* Cl	# of lateral Cl
tetra-CDFs	01	tetra-CDF-1	43.84	49.0±13.3	0.53±0.12	---	---
	02	tetra-CDF-2	46.98	13.9±3.50	0.15±0.03	---	---
	03	tetra-CDF-3	47.66	35.9±7.10	0.40±0.05	---	---
	04	tetra-CDF-4	48.23	25.4±5.59	0.29±0.07	---	---
penta-CDFs	05	1,2,4,6,8-penta-CDF	52.69	32.6±15.1	0.30±0.09	3	2
	06	1,2,4,7,8-penta-CDF	54.80	104±30.9	0.99±0.28	2	3
	07	1,2,3,7,8-penta-CDF	55.49	15.3±2.09	0.16±0.04	1	4
	08	2,3,4,6,8-penta-CDF	57.20	16.2±3.17	0.18±0.06	2	3
	09	2,3,4,7,8-penta-CDF	60.57	47.7±30.5	0.48±0.22	1	4
hexa-CDFs	10	1,2,3,4,6,8-hexa-CDF	60.97	127±38.5	1.07±0.10	3	3
	11	1,2,4,6,7,8-hexa-CDF	61.37	350±109	2.82±0.27	3	3
	12	1,2,4,6,8,9-hexa-CDF	62.62	1561±345	12.4±1.82	4	2
	13	1,2,3,6,8,9-hexa-CDF	63.14	68.0±25.9	0.58±0.20	3	3
	14	hexa-CDF-5	63.65	21.1±4.51	0.21±0.03	---	---
	15	1,2,3,4,7,8-hexa-CDF*	64.05	13.1±4.61	0.12±0.05	2	4
	16	2,3,4,6,7,8-hexa-CDF	65.31	24.6±5.25	0.22±0.05	2	4
	17	hexa-CDF-8	67.71	30.3±11.9	0.27±0.11	---	---

Homolog	Peak #	Congener Name	Retention Time (min)	Conc. (ng/g d.w.)	Mol% (avg±SD)	# of *peri* Cl	# of lateral Cl
hepta-CDFs	18	1,2,3,4,6,7,8-hepta-CDF	72.73	3281±1009	26.2±2.55	3	4
	19	1,2,3,4,6,8,9-hepta-CDF	73.93	4443±696	34.9±1.81	4	3
octa-CDF	20	octa-CDF	86.27	2491±758	17.8±2.08	4	4

For tetra-CDFs being detected, no 1,2,3,4- or 2,3,7,8-tetra-CDFs were identfied. All congeners being identified bases on Humppi and Heinola (1985) except 1,2,3,4,7,8-hexa-CDF (*), which was identified, based on the information of chemical standard. Mol% and concentration in ng/g d.w. of each congener was in average (avg) ±SD of 12 mesocosm samples.

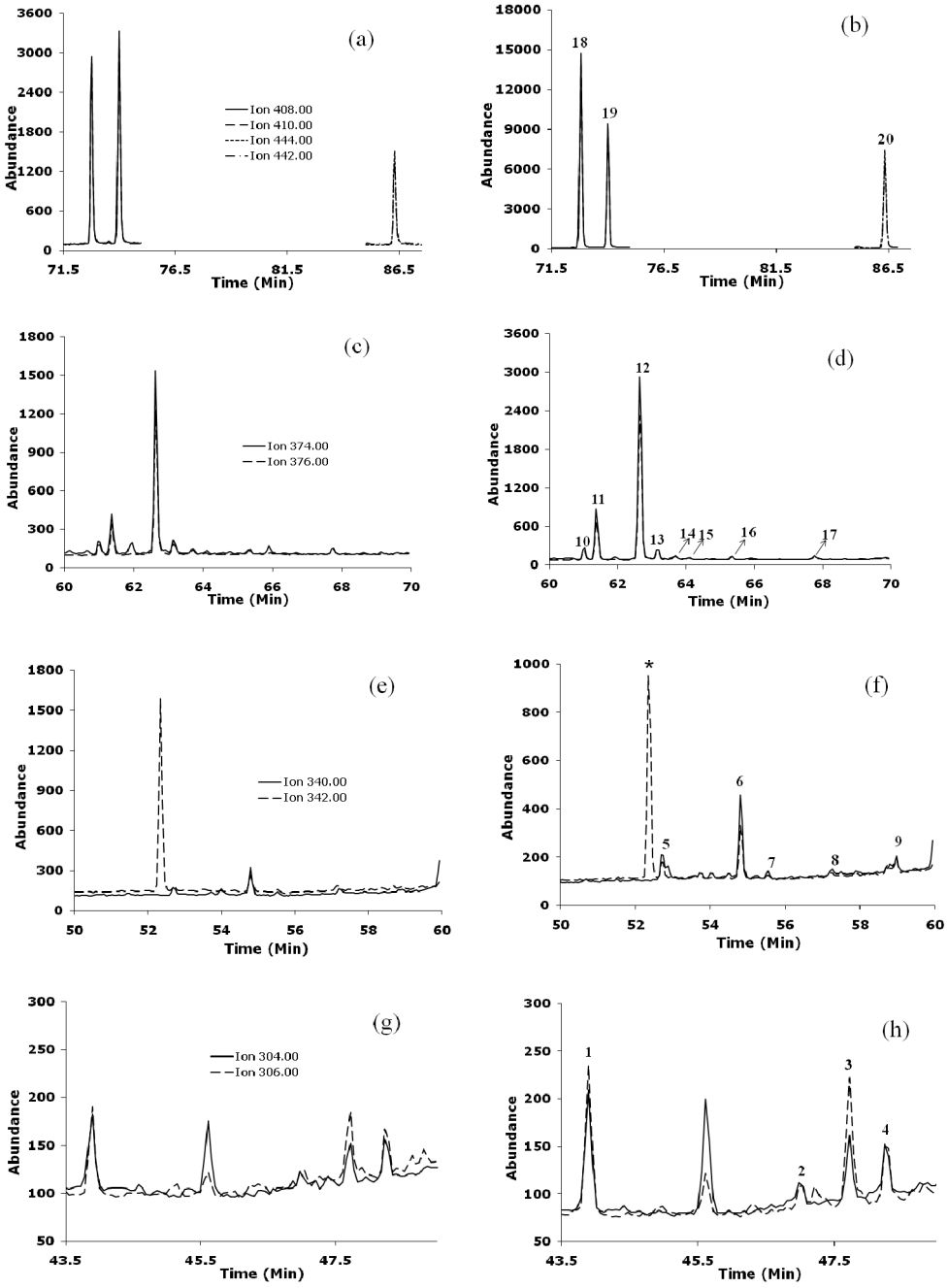

Figure 2-1. Selected ion chromatograms for hepta-CDFs and octa-CDF at year-0 (a) and year-7 (b), hexa-CDFs at year-0 (c) and year-7 (d), penta-CDFs at year-0 (e) and year-7 (f), tetra-CDFs at year-0 (g) and year-7 (h) in the same Kymijoki mesocosm amended with electron donor plus TeCB. The peak labeled with a * is not a PnCDF congener since it does not contain the ions of 340 and 342 in the correct ratio (f).

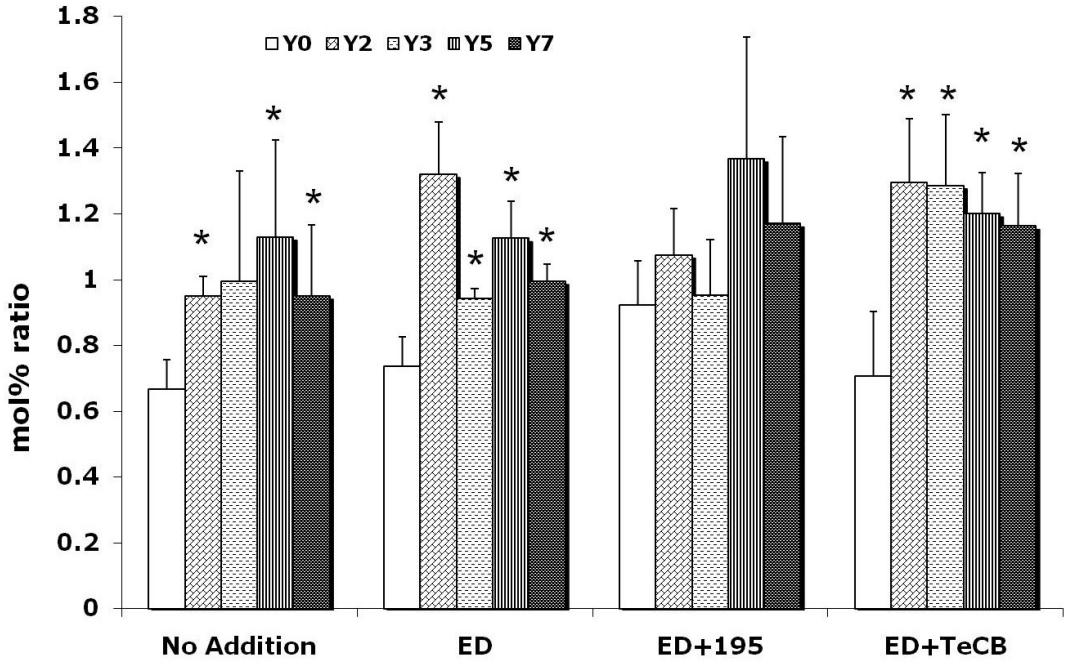

Figure 2-2. Average mol% ratio of 1,2,3,4,6,7,8-hepta-CDF (peak 18) vs. 1,2,3,4,6,8,9-hepta-CDF (peak 19) over 7 years incubation in different Kymijoki mesocosm treatments. * Indicates that the mol% ratio that is significantly different from that of year 0 by student T-test ($\alpha<0.05$).

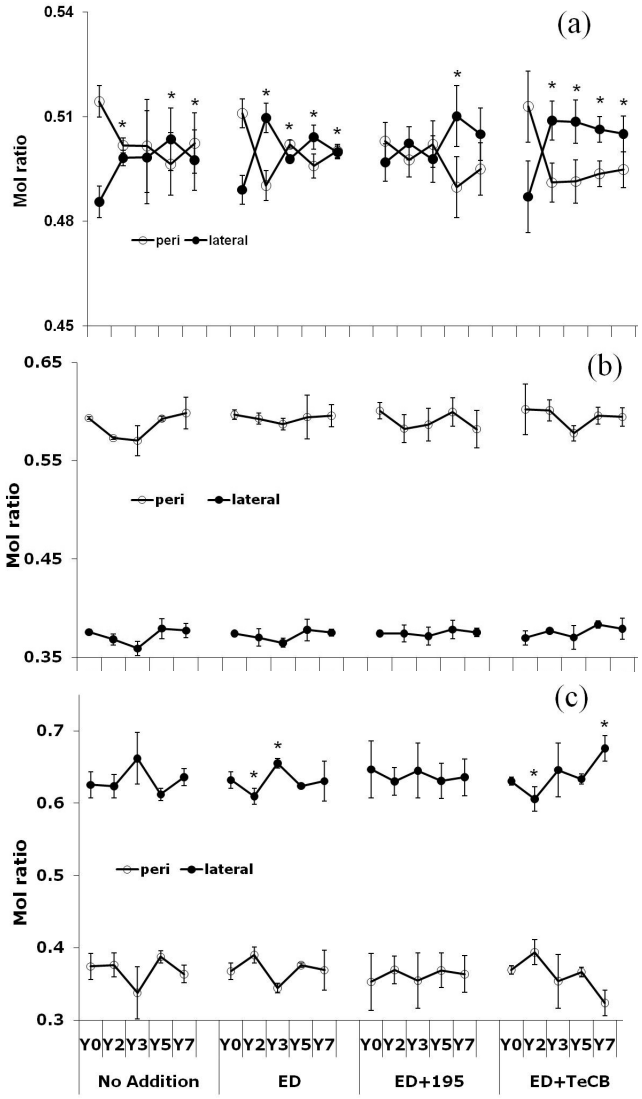

Figure 2-3. Average mol ratios of *peri* and lateral chlorines vs. total chlorines per dibenzofuran molecule over time for hepta-CDFs (a), hexa-CDFs (b) and penta-CDFs (c) identified in Kymijoki mesocosms amended with no addition, electron donor only (ED), and electron donor plus *D. ethenogenes* strain 195 (ED + 195) or plus 1,2,3,4-tetrachlorobenzene (ED + TeCB). Y0, Y2, Y3, Y5, Y7 stands for year 0, 2, 3, 5, and 7. Value labled with a * Indicated that was significantly different from the value at time 0 by student t-test ($\alpha<0.05$). Both *peri* and lateral Cl ratio shared the same t-test value, only the t-test result of *peri* Cl was showed.

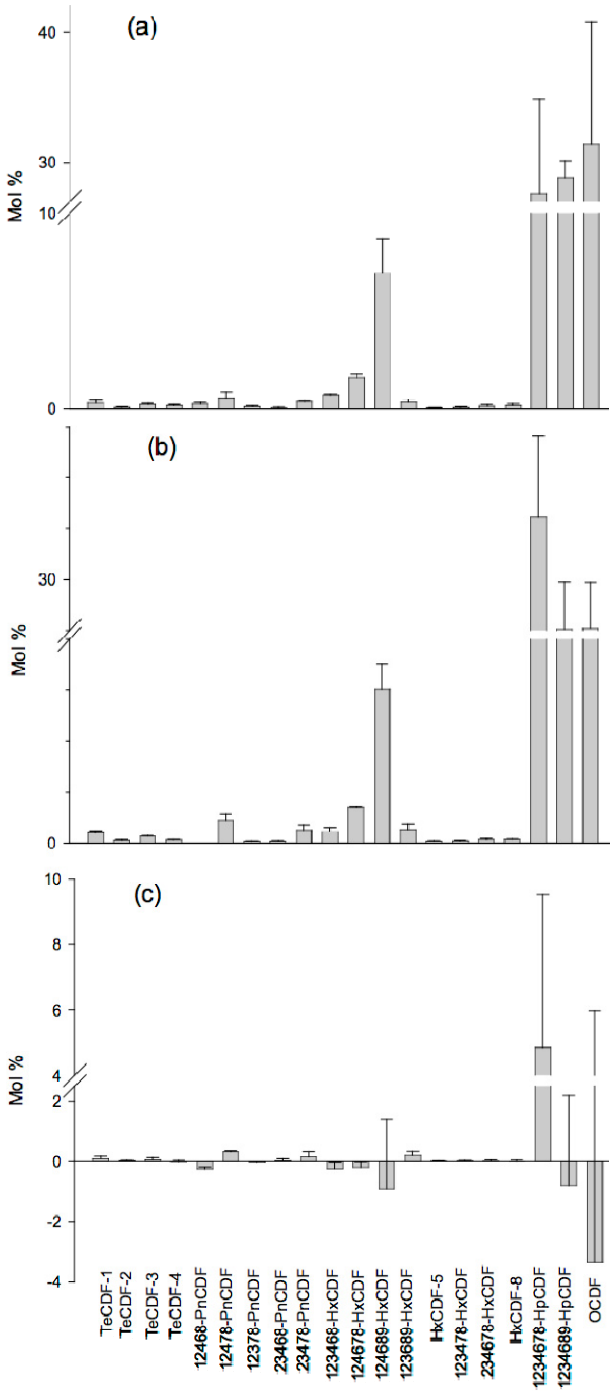

Figure 2-4. Average mol% of weathered PCDF congeners in no addition control (a), electron donor plus TeCB amendment (b), and difference of electron donor plus TeCB vs. no addition after 7 years (c). Bars are the average of triplicates ± SD. * Indicated statistically significant difference by student t-test ($\alpha<0.05$).

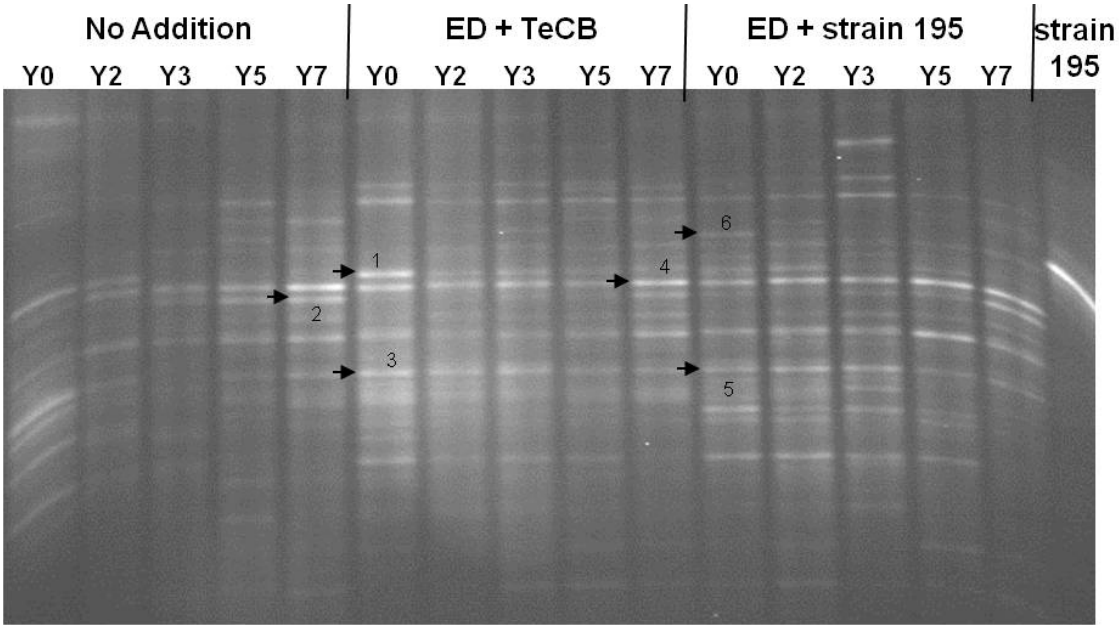

Figure 2-5. Nested PCR-DGGE analysis of putative *Chloroflexi* 16S rRNA genes in Kymijoki mesocosms over 7 years of treatment. A diverse *Chloroflexi* community was observed with different amendments over time. 6 DGGE single bands as highlighted above were purified and sequenced for phylogenetic analysis.

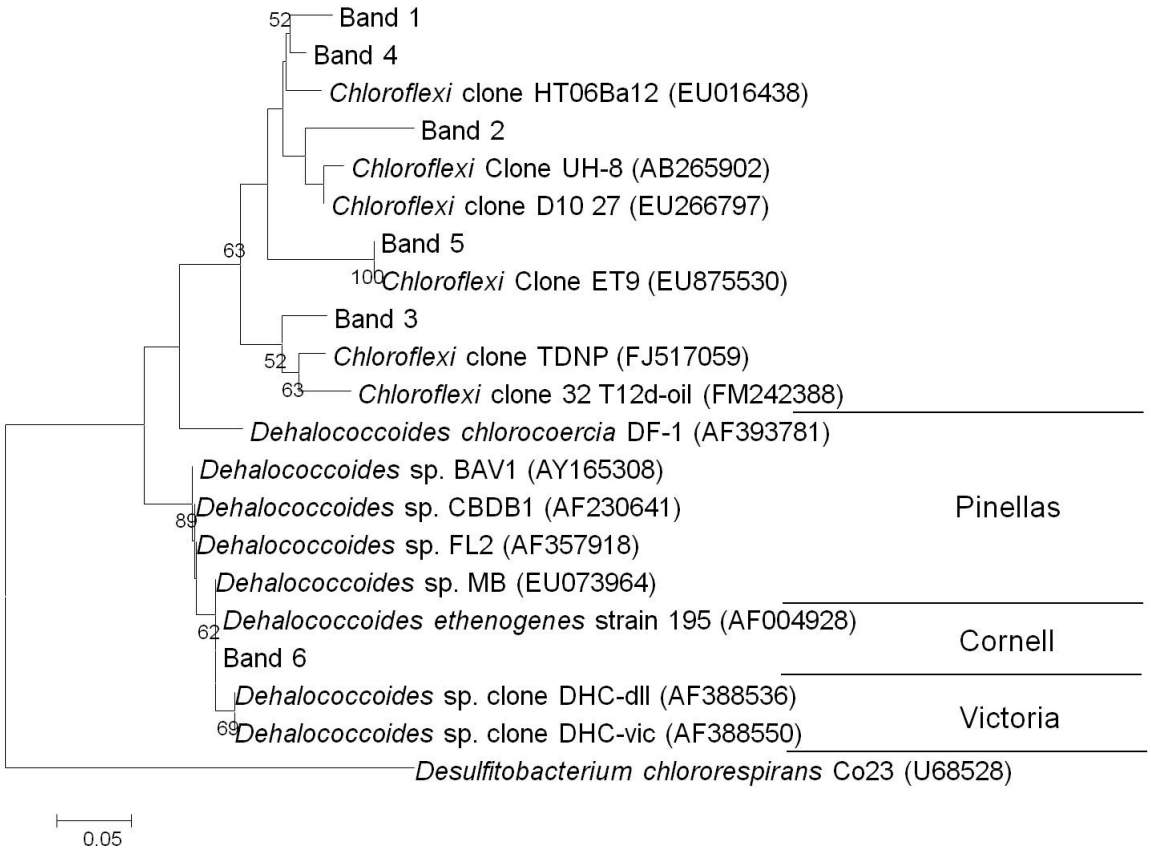

Figure 2-6. Neighbor-joining tree of DGGE bands in Kymijoki mesocosm and related *Chloroflexi* 16S rRNA genes from published sequences. Bootstrap values are indicated at the branch points. The tree was derived from variable region 3 of 16S rRNA genes, which was able to distinguish three subgroups of *Dehalococcoides* species.

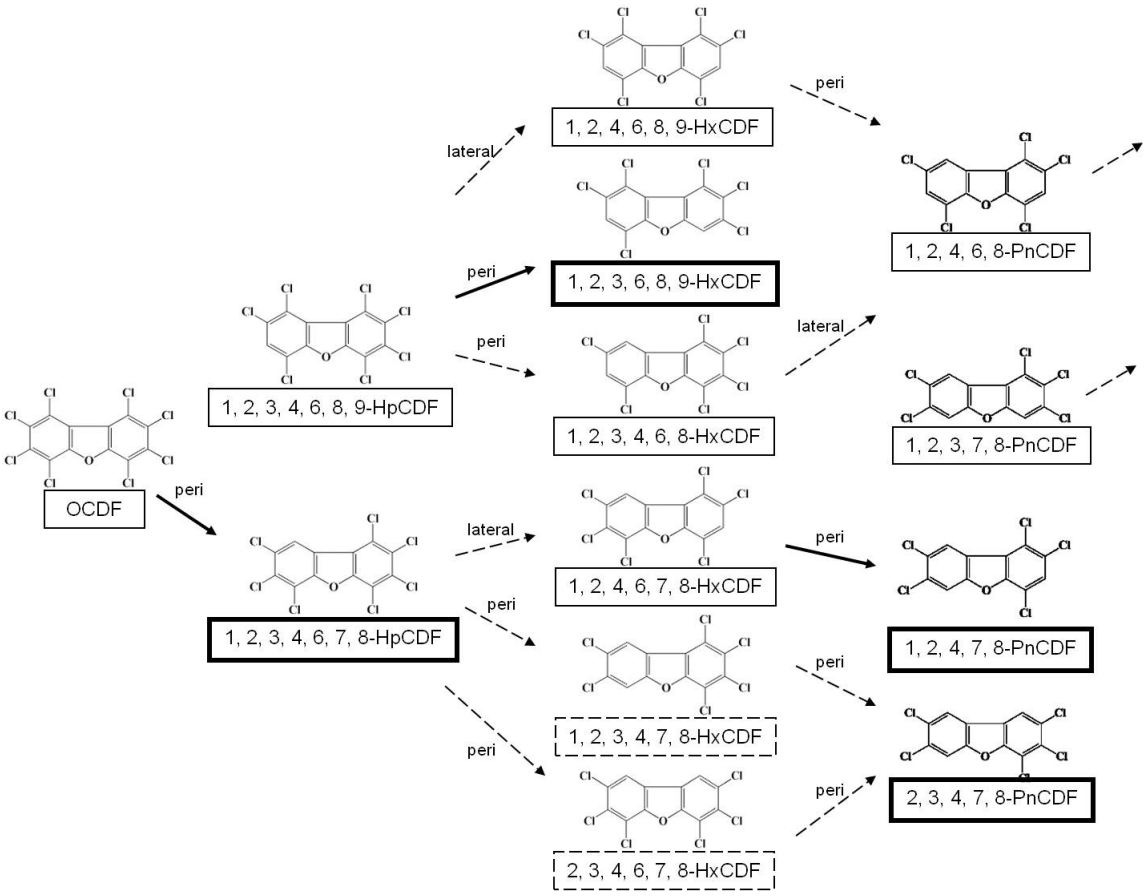

Figure 2-7. Proposed dechlorination pathway for weathered PCDFs in Kymijoki sediment mesocosms amended with electron donor plus TeCB. Bold box highlights increasing congeners in electron donor plus TeCB vs. no addition control after 7 years, light box highlights decreasing congeners in electron donor plus TeCB vs. no addition control after 7 years, dashed box highlights possible intermediates. Dashed arrows represent possible dechlorination pathways. Solid arrows represent dechlorination pathways that were observed in this study.

Chapter 3

Stimulating Microbial Reductive Dechlorination of Weathered Polychlorinated Dibenzofurans in Contaminated Kymijoki Sediment, Finland

Abstract

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are unwanted byproducts from various industrial processes. These compounds are of major concern due to their extreme toxicity and high resistance to microbial degradation. Anaerobic bacteria are known to dechlorinate PCDD/Fs, but their activity is low. To test whether amendments can stimulate the reductive dechlorination of weathered PCDFs we established 200 mL microcosms with contaminated sediment from Kymijoki River, Finland. The effects of spiked 1,2,3,4-tetrachlorodibenzofuran (1,2,3,4-tetra-CDF), 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB) and 2,3,4,5,6-pentachloronitrobenzene (PCNB) as "haloprimers" in the presence of combined electron donors were examined. Vegetable oil was tested for the effect on enhancing dechlorination of weathered PCDF congeners in the sediments with and without bioaugmentation of *Dehalococcoides ethenogenes* strain 195. Dechlorination of octa-CDF, 1,2,3,4,6,8,9-hepta-CDF, and hexa-CDFs was accompanied by production of 1,2,3,4,6,7,8-hepta-CDF and non-2,3,7,8-tetra-CDFs in bioaugmented and electron donor amended microcosms after 18 months, suggesting a *peri*-preferred dechlorination pathway. Dechlorination via a different pathway was observed for bioaugmentation with vegetable oil amended microcosms. In this case, dechlorination of octa-CDF, 1,2,3,4,6,7,8-hepta-CDF was accompanied by production of 1,2,3,4,6,8,9-hepta-CDF and hexa-CDFs after 18 months. In one of these triplicate microcosms, dechlorination of 1,2,3,4,6,7,8-hepta-CDF was accompanied with production of 1,2,3,4,6,8-hexa-CDF. The overall observations for bioaugmentation and vegetable oil amended microcosms suggest that lateral dechlorination might be the preferred dechlorination pattern under this condition. Dechlorination of hexa-CDFs was

accompanied by production of non-2,3,7,8 substituted tetra-CDFs in electron donor and TeCB or PCNB amended microcosms. Very limited dechlorination was observed for microcosms amended with tetra-CDF and electron donors, controls of electron donors only, or controls of vegetable oil only. These results suggest that bioaugmentation of strain 195 with electron donor or vegetable oil amendment can stimulate microbial reductive dechlorination of weathered PCDFs in Kymijoki sediment. Further studies to identify the responsible microorganisms or reductive dehalogenase genes of potential dechlorinating microbes in Kymijoki sediment could provide more information on developing successful strategies for *in situ* bioremediation of PCDD/F contaminated sediments.

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are mainly generated as un-wanted byproducts or impurities from various industrial and thermal combustion processes (for review Kulkarni et al., 2008; Hites, 2010). They have extremely low water solubility, low vapor pressure and high octanol-water partition coefficients (Shiu et al., 1988); sediments are consequently one of their most important sinks in the environment. Because of their toxicity and recalcitrance, remediation of sediments contaminated by PCDD/Fs has become one of the most difficult and challenging issues in the field of environmental science and technology. Microbial reductive dechlorination is one of the very few known reactions that initiate the transformation of highly chlorinated congeners in sediments, with production of less chlorinated congeners that are more susceptible to subsequent aerobic degradation (see review of Bunge and Lechner, 2009). However, due to their environmental recalcitrance and chemical inertness, the natural attenuation rate of PCDD/Fs in the sediment is extremely slow and occurs only to very limited extent (Beurskens et al., 1993; Barkovskii et al., 1994; Adriaens and Grbic-Galic 1994, 1995; Barkovskii and Adriaens, 1996; Albrecht et al., 1999; Gaus et al., 2002).

There has been much interest in stimulating the rate and extent of microbial reductive dechlorination of either freshly spiked or historic weathered PCDDs by adding structurally analogous co-substrates (Albrecht et al., 1999; Vargas et al., 2001; Fu et al., 2005; Ahn et al., 2005, 2007, 2008). The amended co-substrate is also known as a "haloprimer", which is thought to initiate the dechlorination reaction of indigenous

microorganisms either by promoting their growth or inducing the functional enzymes (Bedard et al., 1998). Previously, the dechlorination of weathered PCDDs in Passaic River sediment (NJ, USA) was enhanced by adding 2-monobromodibenzo-*p*-dioxin (2-BrDD) and H_2 in the presence of acetate, butyrate and benzoate (Albrecht et al., 1999). 2-BrDD was also used to stimulate the dechlorination of spiked 1,2,3,4,6,7,8-hepta-CDD using sediment from the same site as inoculum (Fu et al., 2005). 1,2,3,4-TeCB, 2,3,4,5-tetrachlorinated anisole, 2,3,4,5-tetrachlorianted phenol, and 2',3',4'-trichloroacetophenone as well as monobromophenol were all shown to enhance the dechlorination of spiked 1,2,3,4-tetra-CDD/F using estuarine and river sediment from different sites (Vargas et al., 2001; Ahn et al., 2005, 2007, 2008). Liu and Fennell (2008) later used 1,2,3,4-TeCB to stimulate the dechlorination of spiked 1,2,3,4,7,8-hexa-CDF by a mixed culture containing *Dehalococcoides ethenogenes* strain 195. Ahn and co-workers (2008) showed the enhancement effect of bioaugmentation by adding a mixed culture containing *Dehalococcoides ethenogenes* strain 195 to microcosms spiked with 1,2,3,4-tetra-CDD. Compared to PCDDs, the current knowledge on the stimulation potentials of sediment-associated PCDFs is inadequate. In addition, the above-mentioned PCDD/F structurally analogous "haloprimers" are also environmental pollutants; they have the potential of being re-introduced as pollutants in the environment while being used to stimulate PCDD/F dechlorination. Thus it is necessary to find a safe and efficient strategy to enhance the dechlorination of weathered PCDD/Fs in contaminated sediments.

The objectives of this study were to 1) assess the stimulating effects of different co-substrates (TeCB, PCNB and tetra-CDF) on the dechlorination of weathered PCDFs in

contaminated sediments; 2) examine the effectiveness of bioaugmentation by a mixed culture containing *Dehalococcoides ethenogenes* strain 195 for enhancing dechlorination of weathered PCDFs in the sediment; and 3) investigate the effectiveness of vegetable oil or solvents on stimulating dechlorination of weathered PCDFs in the sediment.

Materials and Methods

Chemicals. PCDD/Fs standards were purchased from Ultra Scientific (North Kingstown, RI). Polychlorinated biphenyl (PCB) 14 (3,5-dichlorobiphenyl (DiCB)), PCB 23 (2,3,5-trichlorobiphenyl (TrCB)), PCB 65 (2,3,5,6-tetrachlorobiphenyl (TeCB)), PCB 166 (2,3,4,4',5,6-hexachlorobiphenyl (HxCB)), PCB 30 (2,4,6-trichlorobiphenyl (TrCB)) and 204 (octachlorobiphenyl (OcCB)) were purchased from AccuStandard, Inc. (New Haven, CT). The 1,2,3,4-tetrachlorobenzene (TeCB); 2,3,4,5,6-pentachloronitrobenzene (PCNB) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

Contaminated Sediment Treatment/Incubations. Contaminated sediments were collected by dredging from downstream of Myllykoski along River Kymijoki, Finland, where the production of chlorophenol fungicide Ky-5 constitutes the main source of heavy PCDFs contamination between the 1940s and the 1980s (see Chapter 2). The major chlorinated compounds in Kymijoki sediment are polychlorinated phenols (PCPs), PCDDs, and PCDFs of 1,2,3,4,5,6,7,8- and 1,2,3,4,5,6,8,9-heptaCDFs and octa-CDF are the dominant congeners (Verta et al., 1999; Koistinen et al., 1995). It is estimated that the total amount of combined PCDD/Fs in the sediments range from 4000 to 5000 kg in the contaminated area of River Kymijoki (Verta et al., 1999). The experimental setup matrix

is listed in Table 3-1. Sediment microcosms (200 mL) were fed at the very beginning with co-substrates of 1,2,3,4-tetrachlorobenzene (TeCB, 25 μM) (EDTeCB), or 2,3,4,5,6-pentachloronitrobenzene (PCNB, 25 μM) (EDPCNB), or 1,2,3,4-tetrachlorodibenzofuran (tetra-CDF, 5 μM) (EDTCDF), respectively and a mixed electron donor solution (lactate, propionate and butyrate, 50 μM each) to study the effect of priming compounds on the dechlorination of weathered PCDFs in Kymijoki sediment. Twenty mL of a mixed culture containing *Dehalococcoides ethenogenes* strain 195 (Fennell et al., 1998) was introduced to yield a cell density of approximately 2~3 × 10^6 cells mL^{-1} of the microcosm (Fennell et al., 2004; Krumins et al., 2009) in the presence of mixed electron donor solution (EDB) or 4 ml vegetable oil (VOB). Sediment controls of only mixed electron donors (ED), solvent (sol) or vegetable oil (VO) were set up simultaneously in addition to no-amendment background controls (NA) and killed controls (K). All sediment microcosms (200 mL sediment with a thin layer of site water on top) were incubated at room temperature (18~21 °C) to sustain the dechlorination capacity. All batch biotransformation assays were conducted in triplicate 250-mL glass jars that were flushed with N_2 above the sediment surface and sealed tightly with a rubber stopper. 2 mL (approximately, 3g of wet weight) of sediment samples were collected in glass vials in the presence of N_2 stream at time 0 and after 18 months during the incubation period for purpose of PCDFs analysis.

Analysis of PCDFs. The liquid/solid extraction to quantify PCDFs and transformation products was performed as described in Chapter 2. PCDFs, PCB surrogates and internal standards were detected, identified and quantified as shown in Chapter 2.

According to their relative retention time (rt), PCB 30 (rt, 22.02 min) was used to determine extraction efficiencies of PCB 14 (rt, 21.106 min), 23 (rt, 24.761 min), 65 (rt, 28.76 min) and PCB 204 (rt, 52.52 min) was used to determine extraction efficiencies of weathered PCDFs (tetra: 39-50min, penta: 50-60min, hexa: 60-70min, octa-CDF: 85min). The average extraction efficiency of PCB surrogates ranged from 80~105% for PCB 14, 23 and 65. The detection limit for PCDFs was approximately 0.5 ng/g (d.w).

Results

Detection and identification of weathered PCDFs in Kymijoki sediment microcosms. Kymijoki sediments contain a mixture of PCDF congeners. Altogether 18 peaks corresponding to PCDF congeners with four or more chlorines were detected, and among them, 11 peaks were identified, 1 peak of hexa-CDF and 6 peaks of tetra-CDFs were unknown congeners (Table 3-2). Selected Ion Chromatograms of hexa-CDFs, hepta-CDFs and octa-CDF after 0 and 18 months for one triplicate microcosm with no addition controls, amended with electron donors (ED), amended with vegetable oil (VO), amended with vegetable oil and bioaugmentation (VOB) with strain 195 are shown as examples (Fig. 3-1 & 3-2). In addition to the most abundant congeners of octa-CDF (peak 18), 1,2,3,4,6,8,9-hepta-CDF (peak 17) and 1,2,3,4,6,7,8-hepta-CDF (peak 16) (Fig. 3-1), altogether six hexa-CDF congeners were detected (Fig. 3-2), including 5 known isomers (Humppi and Heinola, 1985) and one unknown isomer (peak 14). Three peaks were detected and identified as penta-CDFs (data not shown) (Humppi and Heinola, 1985). Six peaks were detected as tetra-CDFs isomers (data not shown) but without identification

due to the unavailability of tetra-CDF standards. Neither 1,2,3,4- nor 2,3,7,8-tetra-CDFs were detected in Kymijoki sediment microcosms.

For background control microcosms of (no addition controls), the relative abundance of the 1,2,3,4,6,7,8-hepta-CDF (peak 16) and 1,2,3,4,6,8,9-hepta-CDF (peak 17) did not change substantially after 18 months incubation (Fig. 3-1a vs. b). However, in microcosms amended with electron donors (ED), vegetable oil (VO), vegetable oil and bioaugmentation with strain 195 (VOB), the relative abundance of the two hepta-CDFs changed substantially after 18 months of incubation, with an increase of 1,2,3,4,6,8,9-hepta-CDF relative to that of 1,2,3,4,6,7,8-hepta-CDFs (Fig. 3-1c~h). Selected ion chromatograms of major hexa-CDFs isomers in these microcosms indicated that 1,2,3,4,6,8-hexa-CDF (peak 10) increased remarkably in one microcosm of vegetable oil and bioaugmentation of strain 195 amendment (VOB) after 18 months (Fig. 3-2g vs. h). No such observations were made for the microcosms amended with electron donors and tetra-CDF, PCNB, or TeCB, killed controls and solvent (Sol) controls (data not shown).

Impact of bioaugmentation on the dechlorination of weathered PCDFs in Kymijoki sediment microcosms. In microcosms amended with electron donors and bioaugmented with strain 195 (EDB), decreasing mol% of octa-CDF, 1,2,3,4,6,8,9-hepta-CDF and hexa-CDFs was accompanied by increasing mol% of 1,2,3,4,6,7,8-hepta-CDF and tetra-CDFs after 18 months (Fig. 3-3a). Similar trends were also observed when comparing average mol% of different PCDF congeners in these microcosms to background, killed control, and electron donors only control microcosms (ED) after 18 months (Fig. 3-3a).

Compared to microcosm controls of electron donors only (ED), the decreasing mol% of 1,2,3,4,6,8,9-hepta-CDF and hexa-CDF was accompanied by increasing mol% of tetra-CDFs after 18 months, indicating the additional dechlorination activities stimulated by bioaugmentation of *Dehalococcoides ethenogenes* strain 195 in addition to the effect of electron donors (Fig. 3-3a).

For microcosms amended with vegetable oil and bioaugmented with strain 195 (VOB), a different dechlorination pattern was enhanced in comparison to microcosms amended with electron donors and bioagumentation (EDB), unlike the decreasing mol% of hexa-CDFs, 1,2,3,4,6,8,9-hepta-CDF, and increasing mol% of 1,2,3,4,6,7,8-hepta-CDF in microcosms amended with electron donor and bioaugmentation (EDB) (Fig. 3-3a); increasing mol% of hexa-CDFs, 1,2,3,4,6,8,9-hepta-CDF and decreasing mol% of 1,2,3,4,6,7,8-hepta-CDF were observed in addition to the decreasing mol% of octa-CDF in microcosms amended with vegetable oil and bioagumentation (VOB) (Fig. 3-3b). Similar changes were also observed in microcrosms of VOB when compared to background level, killed controls (Fig. 3-3b). Compared to microcosms of vegetable oil only (VO) after 18 months, mol% of octa-CDF, 1,2,3,4,6,8,9-hepta-CDF, and 1,2,3,4,6,7,8-hepta-CDF decreased and more hexa-CDFs were produced, suggesting enhanced dechlorination of hepta-CDFs and octa-CDF with bioaugmentation of strain 195 and vegetable oil amendment.

Impact of "haloprimers" on the dechlorination of weathered PCDFs in Kymijoki sediment microcosms. Increasing mol% of tetra-CDFs and decreasing mol% of hexa-CDFs were observed after 18 months in microcosms amended with TeCB and electron donors (EDTeCB) (Fig. 3-4a). The average mol% of octa-CDF, 1,2,3,4,6,8,9-hepta-CDF, 1,2,3,4,6,7,8-hepta-CDF and penta-CDFs remained constant over time. Compared to background and electron donors only (ED) microcosm controls, the decreasing mol% of hexa-CDFs was accompanied by increasing mol% of tetra-CDFs and penta-CDFs. This suggests that TeCB might enhance the dechlorination of hexa-CDFs to penta-CDFs and tetra-CDFs over time. The relative higher mol% of hexa-CDFs in microcosms amended with TeCB and electron donors (EDTeCB) than that in killed control or solvent control suggested a possible but very limited dechlorination of hepta-CDFs and octa-CDFs. However, since the mol% of hepta-CDFs and octa-CDF remained constant, no conclusive observations can be made. For sediment microcosms amended with PCNB and electron donors, the average mol% of tetra-CDFs and penta-CDFs remained constant, while the average mol% of hexa-CDFs decreased after 18 months (Fig. 3-4b). Compared to background level, decreasing mol% of hexa-CDFs was accompanied by increasing mol% of tetra-CDFs. No such observations were made for microcosms amended with tetra-CDF and electron donors (EDtetra-CDF) over time (data not shown), and no accumulation of lesser chlorinated, tri-, di- and mono-CDF, congeners were observed with any of the amendments (data not shown).

Discussion

Dechlorination of weathered PCDFs, particularly the higher chlorinated congeners, namely octa-CDF, 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-hepta-CDFs, in Kymijoki sediment

microcosms was enhanced to different extent by different amendments. Amendment with electron donor mixtures, vegetable oil, or combined with bioaugmentation of *D. ethenogenes* strain 195 all stimulated a change in the relative abundance of 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-hepta-CDFs after 18 months. A decrease of octa-CDF mol% was observed for microcosms bioagumented with strain 195 combined with electron donor (EDB) or vegetable oil (VOB) amendments. However, the impact of EDB and VOB amendments on the dechlorination of weathered PCDFs in Kymijoki sediment microcosms is different as being shown by the mol% difference of individual congeners or homologs after 18 months (Fig. 3-3). The extent of octa-CDF dechlorination was similar in both situations as shown by the limited difference of average mol% of octa-CDF in both situations after 18 months (Fig. 3-3a vs. b). However, the dechlorination pattern of octa-CDF was affected differently when adding electron donors (ED) or vegetable oil (VO). For microcosms bioaugmented with strain 195 and amended with electron donors, decreasing mol% of 1,2,3,4,6,8,9-hepta-CDF and hexa-CDFs was accompanied by increasing mol% of 1,2,3,4,6,7,8-hepta-CDF after 18 months. This observation suggested that the dechlorination product of octa-CDF was 1,2,3,4,6,7,8-hepta-CDF, which could be produced by removing *peri* chlorine at position 9 of octa-CDF. Dechlorination of 1,2,3,4,6,8,9-hepta-CDF and accumulation of certain hexa-CDFs were also detected, however due to the incomplete information of hexa-CDFs congeners, no dechlorination pathway can be proposed at this point. For microcosms bioaugmented with strain 195 and amended with vegetable oil (VOB), decreasing mol% of octa-CDF and 1,2,3,4,6,7,8-hepta-CDF was accompanied by increasing mol% of 1,2,3,4,6,8,9-hepta-CDF and hexa-CDFs after 18 months. This suggestes that dechlorination product of

octa-CDF in VOB was 1,2,3,4,6,8,9-hepta-CDF, which could be produced by removing the lateral chlorine at position 7 of octa-CDF. Dechlorination of 1,2,3,4,6,7,8-hepta-CDF produced certain hexa-CDFs. However, no dechlorination pathway can be derived due to the incomplete information of hexa-CDFs congeners being detected in Kymijoki sediment. These combined results suggest that: 1) there were multiple dechlorinating populations or multiple dechlorination functional enzymes in Kymijoki sediments which could dechlorinate both lateral and *peri* chlorines; 2) by adding different amendments, e.g. EDB or VOB, these different dechlorination activities can be enhanced differently.

Previously, dechlorination of aged PCDDs in Passaic River sediment (NJ, USA) was enhanced by addition of acetate, butyrate, benzoate, 2-monobromodibenzodioxin and H_2 (Albrecht et al., 1999). Also, bioaugmentation with a mixed culture of *Dehalococcoides ethenogenes* strain 195 stimulated dechlorination of aged PCBs in Anacostia River sediment (Baltimore, USA) (Krumins et al., 2009). Dechlorination of spiked 1,2,3,4-tetra-CDD in enrichment cultures inoculated with Kymijoki River sediment (Finland) was also enhanced by bioagumentaion of strain 195 (Ahn et al., 2008). The different primary dechlorination products indicated that the dechlorination pathway of 1,2,3,4-tetra-CDD was also affected by amendement of TeCB and bioaugmentation (Ahn et al., 2008).

Mixed cultures containing *Dehalococcoides ethengoenes* strain 195 did not dechlorinate spiked deca-brominated dibenzoether (BDE) and octa-CDD (Liu and Fennell, 2008). The enhanced dechlorination of weathered OCDF observed in this study may be mediated by

the indigenous dechlorinating microorganisms in Kymijoki sediment. The addition of strain 195 may provide important growth support for the indigenous dechlorinating microorganisms in Kymijoki sediment. This is another important role of bioaugmentation to remediate PCDD/F contaminated sediments.

Adriaens and Grbic-Galic (1994, 1995) reported that *peri* dechlorination was the preferred reaction for certain PCDD/F congeners including 1,2,3,4,6,7,8-hepta-CDF when using Hudson River sediment (USA) as inoculum. *Peri* dechlorination of weathered PCDFs in Kymijoki sediment was also observed in our mesocosom studies (Chapter 2). However, whether this preferred reaction was a result of the specific dechlorination microbial populations or individual congeners needs further investigation.

Structural analogs of tetra-CDD such as TeCB and PCNB have been reported for their stimulating effects on dechlorination of spiked 1,2,3,4-tetra-CDD in sediments from Kymijoki (Finland) and Gulf Island Pond (USA) (Ahn et al., 2008), spiked 1,2,3,4,7,8-hexa-CDF in mixed culture of *Dehalococcoides ethenogenes* strain 195 (Liu and Fennell, 2008), as well as aged PCBs in Anacostia River sediment (USA) (Krumins et al., 2009). Unlike these previous reports, the priming effects of TeCB and PCNB was not observed for the dechlorination of weathered octa-CDF and hepta-CDFs in Kymijoki sediment microcosms (Fig. 3-3 a & b). A decrease in the mol% of hexa-CDFs and increase in the mol% of tetra-CDFs was observed in microcosms amended with TeCB or PCNB after 18 months which suggested that dechlorination under these conditions might occur to hexa-CDFs and lower chlorinated congeners. The limited dechlorination of spiked 1,2,3,4-

tetra-CDF might also be a result of limited accessibility to the dechlorinating microbes in Kymijoki sediment microcosms.

Future studies should monitor the side reactions of amended electron donors, haloprimers (TeCB, PCNB, tetra-CDF) and vegetable oil for the purpose of a better understanding on the environmental behavior of co-substrates beside their stimulating effects on dechlorination of PCDD/Fs *in situ*. It is also necessary to know the response and interactions of both dechlorinating microorganisms and non-dechlorinating species in the sediment under different stimulating selections. In order to utilizing mixed culture of *D. ethenogenes* strain 195 for bioaugmentation to enhance the dechlorination of PCDD/Fs, it is important to have knowledge of its survival, growth, movement and competition with the indigenous microbial community and possible PCDD/F-dechlorinating populations habitating the contaminated sediments. It is equally important to know the biological toxicity and environmental impact of all kinds of intermediates generated from dechlorination of PCDD/Fs as well as co-substrates. The accomplishment of all the above research aspects will allow a better-controlled application of stimulating strategies on dechlorination of the weathered PCDD/Fs in the sediment.

Table 3-1. Sediment microcosm experiment set up

Microcosms	**Amendment**
Killed control	120 °C autoclaved 20 min for 3 consecutive days
No addition control	none
ED	Mixed lactate, propionate and butyrate solution, 50 μM for each
ED+tetra-CDF	Mixed lactate, propionate and butyrate solution, 50 μM for each; 5 μM tetra-CDF
ED+TeCB	Mixed lactate, propionate and butyrate solution, 50 μM for each; 25 μM TeCB
ED+PCNB	Mixed lactate, propionate and butyrate solution, 50 μM for each; 25 μM PCNB
Sol	Methanol + acetone + butanol (1:1:1), 4 ml in total
VO	4 ml vegetable oil
ED+B	Mixed lactate, propionate and butyrate solution, 50 μM for each; 20 ml of strain 195 culture
VO+B	4 ml vegetable oil; 20 ml of strain 195 culture

ED: electron donors;
Sol: solvent,
VO: vegetable oil,
B: bioaugmentation.

Table 3-2. Detected and identified congeners of weathered PCDFs in Kymijoki sediments microcosms.

Homologs	Peak #	Congener Name	Retention Time (Min)	Conc. (ng/g d.w.)	Mol% (avg±SD)
tetra-CDFs	01~06	---	39~49	374 ± 105	2.79 ± 2.4
penta-CDFs	07	1,2,4,6,8-penta-CDF	52.17	108 ± 47	0.36 ± 0.1
	08	1,2,4,7,8-penta-CDF	54.29	263 ± 114	0.90 ± 0.3
	09	1,2,3,7,8-penta-CDF	55.03	53 ± 23	0.21 ± 0.1
hexa-CDFs	10	1,2,3,4,6,8-hexa-CDF	60.28	318 ± 375	1.08 ± 0.5
	11	1,2,4,6,7,8-hexa-CDF	60.68	1178 ± 884	3.09 ± 0.9
	12	1,2,4,6,8,9-hexa-CDF	61.94	4455 ± 3439	11.2 ± 2.8
	13	1,2,3,6,8,9-hexa-CDF	62.57	317 ± 150	0.84 ± 0.2
	14	Hexa-CDF-5	63.01	0	0
	15	2,3,4,6,7,8-hexa-CDF	64.74	95.3 ± 90	0.24 ± 0.3
hepta-CDFs	16	1,2,3,4,6,7,8-hepta-CDF	72.73	16092 ± 9099	34.8 ± 3.6
	17	1,2,3,4,6,8,9-hepta-CDF	73.93	11605 ± 6386	26.0 ± 1.9
octa-CDF	18	octa-CDF	86.27	8979 ± 4176	17.9 ± 4.3

No 1,2,3,4- or 2,3,7,8-tetra-CDF was detected. All congeners being identified bases on elution order and retention time ranges of hexa-CDFs isomers in Humppi and Heinola (1985). Mol% of each congener was in average (avg) ± SD of 29 sediment microcosm samples at time 0.

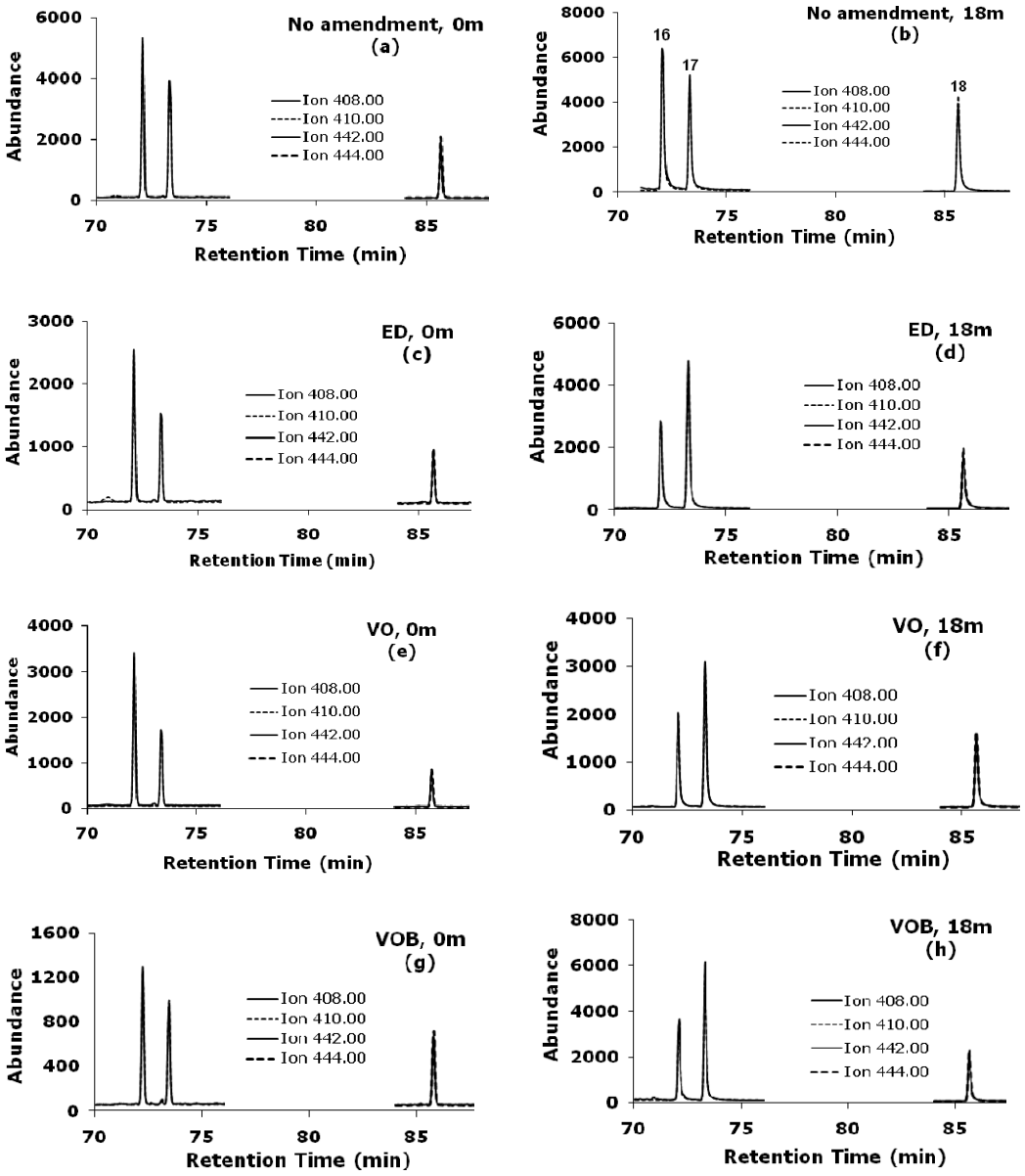

Figure 3-1. Selected ion chromatograms of hepta-CDFs (408, 410) and octa-CDF (442, 444) in Kymijoki sediment microbosms of background control after 0 month (a) and 18 months (b), amended with electron donors (ED) after 0 month (c) and 18 months (d), amended with vegetable oil (VO) after 0 month (e) and 18 months (f), amended with vegetable oil and bioaugmentation with a mixed culture containing *Dehalococcoides ethenogenes* strain 195 (VOB) after 0 month (g) and 18 months (h). Data from one representative triplicate microcosm is shown for each condition.

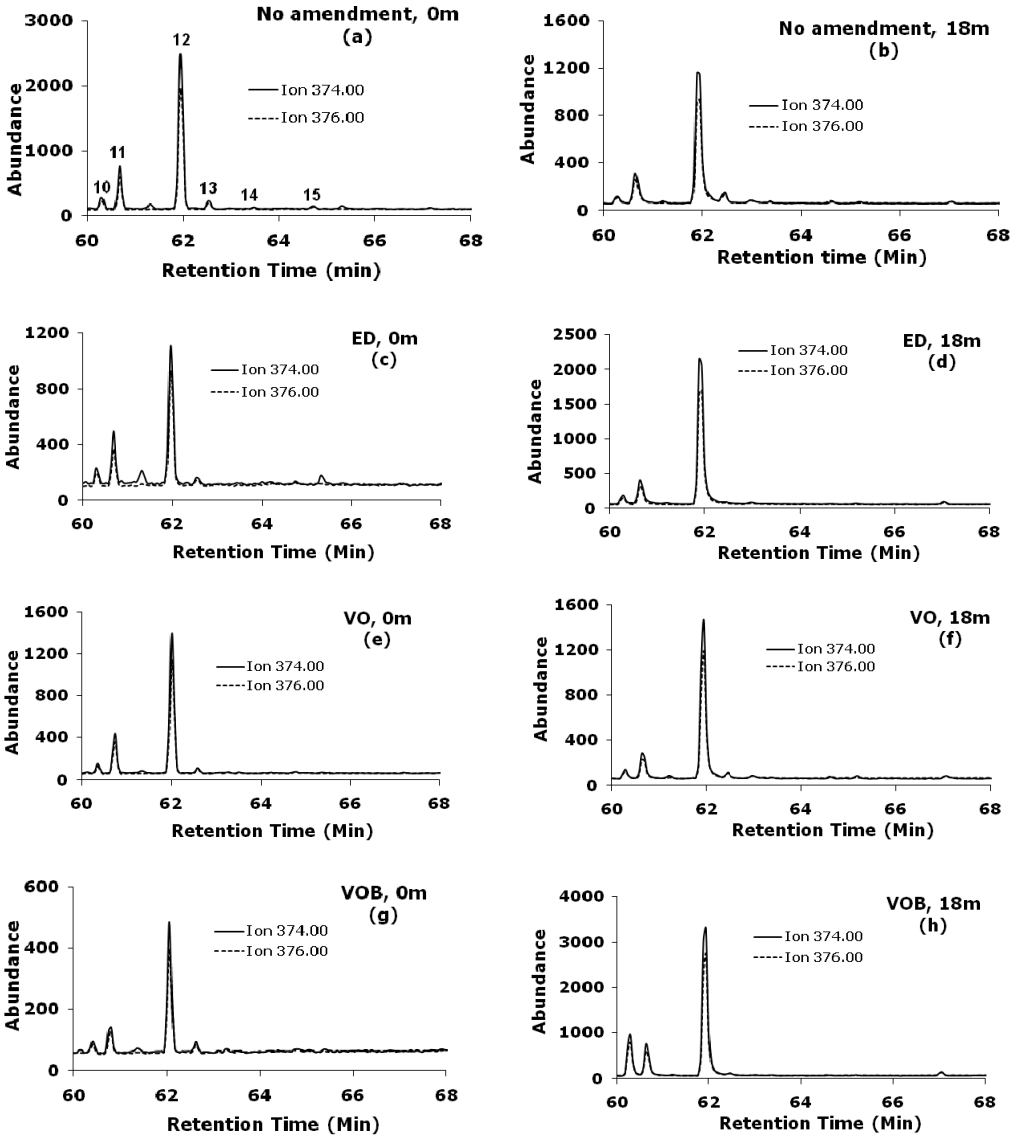

Figure 3-2. Selected ion chromatograms of hexa-CDFs (374, 376) in Kymijoki sediment microcosm with no addition control after 0 month (a) and 18 months (b), amended with electron donors (ED) after 0 month (c) and 18 months (d), amended with vegetable oil (VO) after 0 month (e) and 18 months (f), amended with vegetable oil and bioaugmentation with a mixed culture containing *Dehalococcoides ethenogenes* strain 195 (VOB) after 0 month (g) and 18 months (h). Data from one representative triplicate microcosm is shown for each condition.

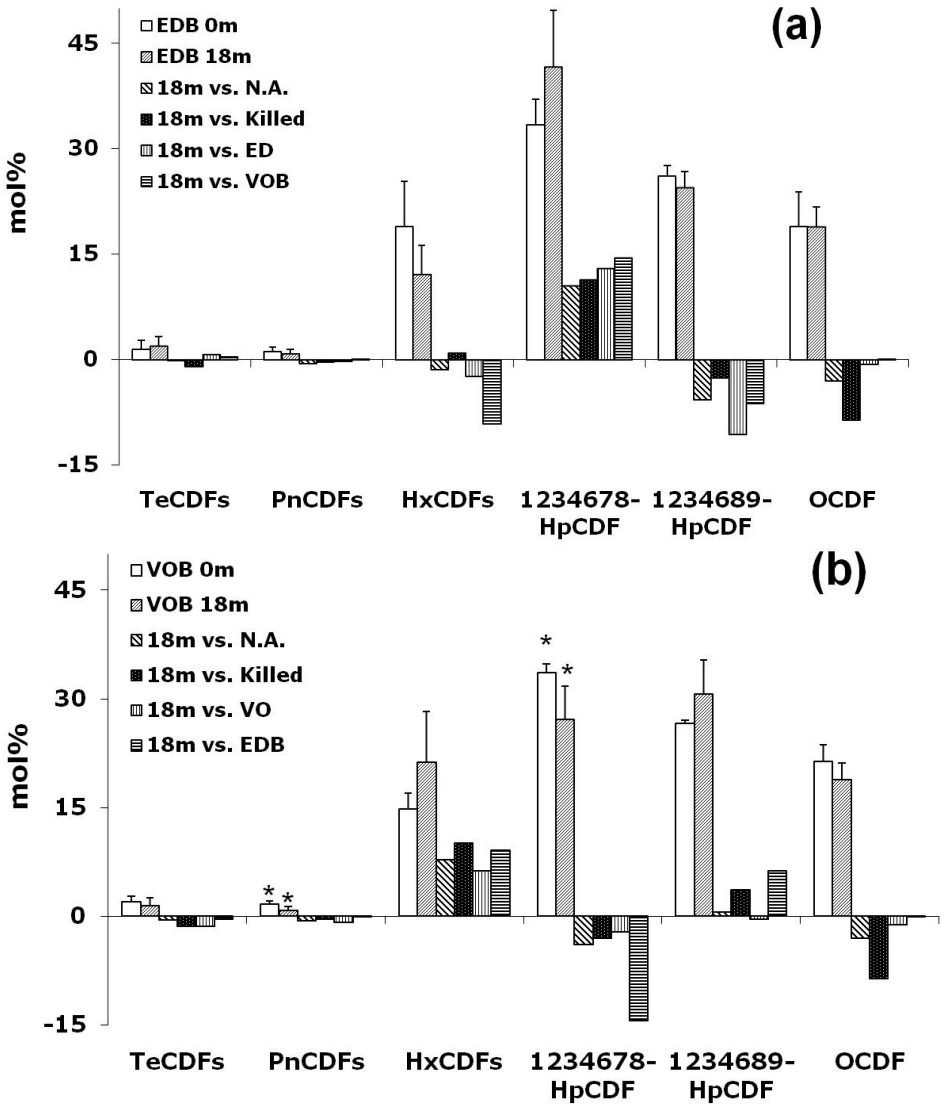

Figure 3-3. Molar percentages of octa-CDF, 1,2,3,4,6,7,8-hepta-CDF, 1,2,3,4,6,8,9-hepta-CDF, hexa-CDFs, penta-CDFs, and tetra-CDFs in Kymijoki sediment microcosm amended with electron donors and bioaugmentation (EDB) (a), vegetable oil and bioaugmentation (VOB) (b) at time 0, after 18 months, and their corresponding mol% difference vs. no addition control (N.A.), killed control, sediment microcosms amended with electron donors (ED), vegetable oil only (VO) after 18 months. * Indicated congeners with mol% after 18 months statistically significant different from that of time 0 by student t-test ($\alpha<0.05$).

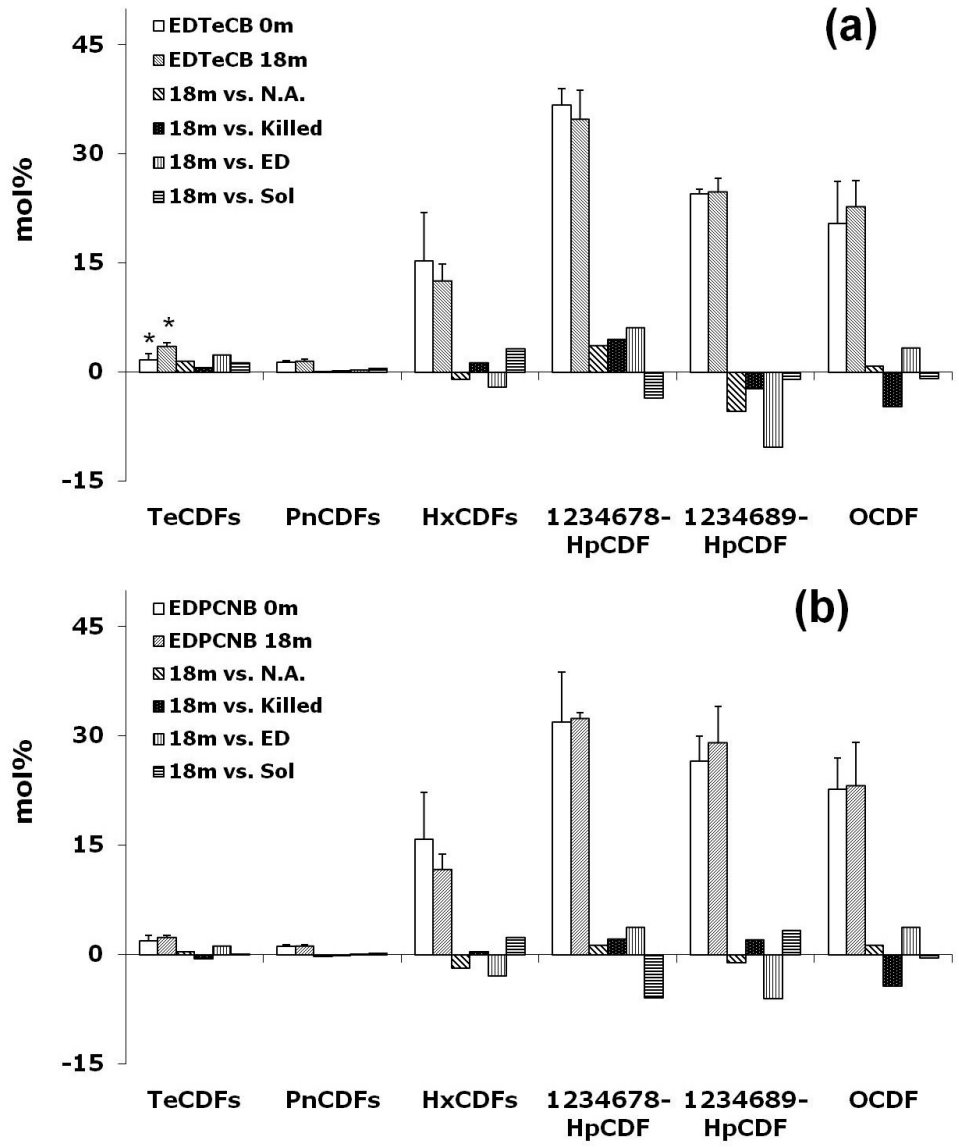

Figure 3-4. Molar percentages of octa-CDF, 1,2,3,4,6,7,8-hepta-CDF, 1,2,3,4,6,8,9-hepta-CDF, hexa-CDFs, penta-CDFs, and tetra-CDFs in Kymijoki sediment microcosm amended with electron donors plus TeCB (EDTeCB) (a), electron donors plus PCNB (EDPCNB) (b), at time 0, after 18 months, and their corresponding mol% difference vs. no addition control (N.A.), killed control, sediment microcosms amended with electron donors (ED) and solvent (Sol) after 18 months. * Indicated congeners with mol% after 18 months statistically significant different from that of time 0 by student t-test ($\alpha<0.05$).

Chapter 4

Microbial Reductive Dechlorination of 1,2,3,4-Tetrachlorinated Dibenzo-*p*-dioxin and Dibenzofuran and Octachlorinated Dibenzofuran

Abstract

Anaerobic enrichment cultures derived from historically contaminated Kymijoki River sediment (Finland) were able to dechlorinate 1,2,3,4-tetrachloro-dibenzo-*p*-dioxin (1,2,3,4-tetra-CDD), 1,2,3,4-tetrachlorodibenzofuran (1,2,3,4-tetra-CDF) and octachlorodibenzofuran (octa-CDF). The rate and extent of 1,2,3,4-tetra-CDD dechlorination was enhanced by the addition of pentacholornitrobenzene (PCNB) as a cosubstrate. 1,2,3,4-tetra-CDD was dechlorinated via 1,2,3-tri-CDD and 1,2,4-tri-CDD as short-life intermediates mainly to 23,-di-CDD and 2-mono-CDD over a 13-month incubation period. Dechlorination of 1,2,3,4-tetra-CDF was slower than that of 1,2,3,4-tetra-CDD, with 2,4-di-CDF as the most abundant dechlorination product after 13 months. PCNB enhanced *peri* dechlorination of 1,2,3,4-tetra-CDD, while it had no effect on lateral dechlorination during 13 months incubation. Dechlorination of spiked octa-CDF was observed with the production of hepta-, hexa-, penta- and tetra-chlorinated dibenzofurans (CDFs) over 5.5 months incubation. Analysis of *Chloroflexi* specific 16S rRNA genes from the tetra-CDD- and tetra-CDF-dechlorinating enrichment cultures by denaturing gradient gel electrophoresis (DGGE) showed two major bands that were enhanced over the incubation period. Phylogenetic analysis indicated that one population (band) identical to the Pinellas subgroup of *Dehalococcoides* was enhanced. Analysis of 12 *rdh* genes in the community showed an increase in *rdh* gene abundance with 1,2,3,4-tetra-CDD/F+PCNB amendment compared to controls amended with electron donors only. These findings provided additional knowledge on the fate and interaction of 1,2,3,4-tetra-CDD/F, octa-CDF, PCNB and PCA in the sediment system and their impact on the indigenous microbial community.

Introduction

Sediments are important environmental sinks for polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) once they are emitted from combustion sources or discharged from various industrial processes. Microbial reductive dechlorination under anaerobic conditions, typical of aquatic sediments, is the most important biological process that may transform PCDD/F and potentially decrease their toxicity through the removal of lateral chlorines (Ballerstedt et al., 1997; Vargas et al., 2001; Gruden et al., 2003; Häggblom et al., 2003; Fennell et al., 2004; Ahn 2005, 2007; Yoshida et al., 2005; Liu et al., 2008). Consequently, less-chlorinated congeners are usually more susceptible to subsequent aerobic degradation (Wittich, 1998). However, the dechlorination of PCDD/Fs is notoriously slow, which may be attributed to their very limited bioavailability in the environment and to their overall chemical stability. Different dechlorination rates in presence of different amendments have been observed in various enrichment and microcosms inoculated with sediments from a number of sites. Previous studies have demonstrated that the addition of alternate halogenated co-substrates enhanced the reductive dechlorination of PCDD/Fs. (Beurskens et al., 1995; Albrecht et al., 1999; Vargas et al., 2001; Ahn et al., 2005; 2007; 2008). This suggested that the diversity of PCDD/F dechlorinating microorganisms could be stimulated or selected by certain compounds (Ahn et al., 2005, 2007).

Different dechlorination routes of 1,2,3,4-tetra-CDD via both *peri*- and lateral- removal of chlorine have been observed for various sediment enrichments from different sites (Beurskens et al., 1995; Ballerstedt et al., 1997; Vargas et al., 2001; Bunge et al., 2001).

These different dechlorination pathways might be the result of the diverse microbial community and / or diverse metabolic activities in response to the stresses of specific environmental contaminants or adaptation of individual enrichment conditions by priming co-substrates (Vargas et al., 2001; Ahn et al., 2008). Only two known isolates: *Dehalococcoides* sp. strain CBDB1 and *Dehalococcoides ethenogenes* strain 195, have been reported for their capability to dechlorinate CDD/Fs (Bunge et al., 2003; Fennell et al., 2004; Liu & Fennell, 2008; Liu et al., 2010). Both of these strains as well as other *Dehalococcoides* spp., contain multiple genes predicted to encode enzymes mediating putative reductive dehalogenation (*rdh* genes) (Hölscher et al., 2004; Seshadri et al., 2005). Therefore *rdh* genes can be good tools to assess microbial molecular changes in response to dechlorination activities. The detection of *Dehalococcoides*-like species in CDD/F-dechlorinating enrichment cultures (Ballerstedt et al., 2004; Yoshida et al., 2005; Ahn et al., 2007) or PCB-dechlorinating enrichment cultures (Yan et al., 2006; Bedard et al., 2007; Fagervold et al., 2007; Krumins et al. 2009) suggests their importance in the environmental biotransformation of these compounds. However, the fate of priming compounds involved in the dechlorination reactions is not well monitored. The cause-effect relationship between the influence of priming compounds on the microbial community structure and dechlorination activity endpoints is not fully understood yet. The impact of priming compounds on the kinetics of dechlorination reactions is not known.

In addition to a number of selected CDD congeners, dechlorination reactions of PCDFs have only been shown for 1,2,3,4-TCDF (Ahn et al., 2005), 1,2,4,6,8-penta-CDF, and

1,2,3,4,6,7,8-hepta-CDF (Adriaens and Grbic-Galic 1994, 1995) with unknown dechlorination routes or unspecified products. octa-CDF, 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-hepta-CDFs are the most abundant congeners in contaminated Kymijoki sediment. Therefore, knowledge about possible dechlorination reactions for these congeners is important to assess the potential for sediment remediation.

The objectives of the research reported here were to assess (a) the dechlorination of 1,2,3,4-tetra-CDD/F and octa-CDF by dechlorinating enrichment cultures derived from historically contaminated Kymijoki sediment; (b) the priming effect of PCNB on the dechlorination; (c) the fate of PCNB as a priming compound for dechlorination; and (d) the microbial community response to different treatments.

Materials and Methods

Chemicals. Mono-, di-, tri-, tetra- chlorinated dibenzo-*p*-dioxin congeners are abbreviated as mono-CDD, di-CDD, tri-CDD, and tetra-CDD, respectively. Mono-, di-, tri-, tetra-, penta-, hexa-, hepta- and octa-chlorinated dibenzofuran congeners are abbreviated as mono-CDF, di-CDF, tri-CDF, tetra-CDF, penta-CDF, hexa-CDF, hepta-CDF and octa-CDF, respectively. PCDFs denote penta- to octa-chlorinated congeners, whereas 1-4CDD/Fs stands for all congeners of mono-CDD/Fs, di-CDD/Fs, tri-CDD/Fs and tetra-CDD/Fs. Specially, TCDD stands for 1,2,3,4-tetra-CDD, TCDF stands for 1,2,3,4-tetra-CDF in this paper. Mono-, di-, tri-, tetra- and penta-chlorinated anilines (CA) are abbreviated as MoCA, DiCA, TrCA, TeCA and PCA, which were all denoted by 1-5CAs. TCDD, 1,2,3- and 1,2,4-tri-CDDs, 1,3- and 2,3-di-CDDs, 2- and 4-mono-

CDDs, TCDF, 1,2,3-tri-CDF, 24-di-CDF, octa-CDF, 2,2',5-PCB and PCNB were purchased from AccuStandard Inc. (New Haven, CT). Standards of expected PCNB dechlorination products, including penta-chloroaniline (PCA), 2,3,4,5- and 2,3,5,6-TeCA, 2,3,4-, 2,4,5- and 2,4,6-TrCA, 2,4/2,5-DiCA, 4- and 2-MoCA, were purchased from UltraScientific (North Kingstown, RI).

Enrichment Culture Setup. The enrichment cultures were developed from contaminated sediment obtained from downstream of Myllykoski in Kymijoki River, Finland, which was heavily contaminated with chlorinated phenols from 1940's to 1980's from the manufacture of chlorophenolic fungicide Ky-5 in the city of Kuusankoski (Malve et al., 2003). Large amounts of PCDD/Fs were discharged into the river sediment as impurities of Ky-5 during this period. The most abundant polychlorinated dibenzofuran (PCDF) congeners are 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-hepta-CDFs and octa-CDF. Sediments were initially incubated in 30-L drums at room temperature for 7 years (see Chapter 2) and subsamples used for enrichment cultures were packed into glass jars, sealed, and stored at 4 ˚C until used. Methanogenic enrichment cultures were established as described previously (Vargas et al., 2001; Ahn et al., 2005). Briefly, 1 g of dry sterile sediment was added to each culture flask and spiked with a stock solution of TCDD/F and/or PCNB in toluene. Toluene was allowed to evaporate under a sterile N_2 purge, leaving a coating of TCDD, TCDF and/or PCNB on the dry sediment. Enrichment cultures were prepared by dispensing 40 ml volume of well-mixed 20% (v/v) sediment slurry to each TCDD/F and PCNB-spiked 50 ml serum bottle under 70%/30% N_2/CO_2. The anaerobic medium used for enrichment cultures was prepared as described previously (Fennell et al., 2004).

Cultures were capped with poly (tetrafluoroethene) (PTFE)-coated butyl rubber septa and crimped with aluminum caps. The resulting nominal concentrations of TCDD/F and PCNB (assuming no partitioning and uniform distribution throughout the enrichment culture volume) was 50 μM. Sodium lactate, sodium acetate and sodium propionate were added as electron donors to the designated treatments with a final concentration of 500 μM each. Killed controls were prepared by autoclaving at 121 ˚C for 30 min on 3 consecutive days. Enrichment cultures were incubated quiescently at room temperature. Enrichment cultures were sampled over a 13 month period for both chemical analysis and microbial community analysis.

Parallel methanogenic enrichment cultures were prepared in the same way as described above to study octa-CDF dechlorination in the presence of PCA and TCDD or TCDF. Sodium lactate, sodium acetate and sodium propionate were added as electron donors to the designated treatments to a final concentration of 500 μM each, and re-spiked every 3 months. The resulting nominal concentrations of octa-CDF, PCA and TCDD/F (assuming no partitioning and uniform distribution throughout the microcosm volume) were 20 μM, 20 μM, 10 μM, and 10 μM, respectively. Killed controls were prepared by autoclaving at 121 ˚C for 30 min on 3 consecutive days. Enrichment cultures were incubated quiescently at room temperature. Enrichment cultures were sampled after 0, 3 and 5.5 months for chemical analysis.

Analytical Methods. For sampling, the cultures were shaken thoroughly and 2 ml of slurry were withdrawn with a sterile syringe flushed with oxygen-free N_2. Sediment samples were extracted with toluene and acetone (1:1, v/v) for TCDD/F, PCNB, PCA and

PCDFs analysis. The CDD/Fs analysis was performed as described by Vargas et al. (2001). Briefly, samples were separated into aqueous and solid phases by centrifugation. Water was removed from the solid phase by an acetone rinse. 2,2',5-trichlorobiphenyl (2,2',5-PCB) was spiked to the solid phase as an internal standard (1 mg in total) and then the solid phase was extracted with toluene and acetone (1:1, v/v) overnight for 3 times. The toluene-acetone extracts were pooled. Acetone was removed by reverse partition into water, and the toluene extract was gently concentrated under a stream of N_2. Samples were analyzed by gas chromatography-mass spectrometry using an Agilent 6890 series gas chromatograph equipped with a 5973 series Mass Selective Detector (MSD) and a DB-5MS fused silica column (30 m, 0.25 mm i.d., film thickness 0.2 μm, J&W Scientific, Folsom, CA). The temperature program for 1-4CDD/Fs was as follows: 70˚C initial temperature, increased by 20˚C/min to 230˚C, 10˚C/min to 250 ˚C, and finally 15˚C/min to 300˚C. The temperature program for PCNB and the daughter products was as follows: 60˚C initial temperature, increased by 12 ˚C/min to 180 ˚C, and then by 15˚C/min to 250 ˚C. The temperature program for PCDFs was: 60°C, increased by 10°C/min to 170°C, 2°C/min to 200°C, 5°C/min to 220°C, and hold 220 °C for 16 minutes, and increased by 5°C/min to 235°C, hold 235 °C for 7 minutes, and increased by 5°C/min to 250°C, and finally 1°C/min to 280°C, and a 5 min 300 °C hold.

Spiked TCDD/F, octa-CDF, PCNB, PCA and their dechlorination products, as well as the historic PCDFs were detected and identified based on the retention times of standards and their most abundant molecular ions, qualifying ions were monitored to assure the correct identification (m/z: 256, 258, 22'5-PCB; 442, 444, octa-CDF; 408, 410, hepta-

CDF; 374, 376, hexa-CDF; 340, 342, penta-CDF; 322, 320, tetra-CDD; 306, 304, tetra-CDF; 286, 288, tri-CDD; 270, 272, tri-CDF; 252, 254, di-CDD; 236, 238, di-CDF; 218, 220, mono-CDD; 202, 204, mono-CDF; 297, 295, PCNB; 267, 265, PCA; 231, 229, TeCA; 195, 197, TrCA; 161, 163, DiCA; 127, 129, MoCA). Tetra-CDF and octa-CDF dechlorination products were identified by the number and position of chlorine substituents for commercially available and by the number of chlorines substituents only for unavailable congeners. Of the PCNB/PCA dechlorination products, 24- and 25-DiCA co-eluted and could not be separated by GC-MS analysis.

Quantification of 1-4CDD/F, PCDFs, PCNB and 1-5CAs was performed against comparison to a 5-point curve (concentration range). For unknown historical PCDFs as well as unknown TCDF dechlorination daughter products, quantification was based on the response factors of available octa-, hepta-, hexa-, penta-, tetra-, tri-, di-, and mono-CDFs. The results for 1-4CDD/Fs, PCDF, PCNB and 1-5CAs are presented by expressing each compound as a mole percent (mol%) of the total concentration of the congeners detected at each sampling point. Data are presented as the average of triplicate data points ± standard deviation (SD). The total amount of TCDD/F and PCDFs recovered from each sample varied because of slight differences of aqueous-phase/sediment-phase volumes sampled at each time point and because of differences in the solubility of dechlorination products. The extraction efficiency for TCDD, TCDF and octa-CDF spiked to sediment slurries by the method used here was approximately 80 ~ 90 % and the detection limit was approximately 10 ppt (~0.03 nM) for TCDD/F and 2 ppt (~4.5 nM) for octa-CDF. The method of data presentation assumes that no anaerobic

degradation of dibenzo-*p*-dioxin, dibenzofuran and benzene structure occurred and that the CDD/Fs and PCA underwent no significant reactions other than dechlorination.

PCR-DGGE Analysis. Bulk DNA was extracted by using the PowerSoil DNA isolation kit (MO BIO, Solana Beach, CA) following the standard protocol. Nested PCR amplification was performed to analyze the *Chloroflexi* community. The first PCR product was amplified by forward primer 338F and reverse primer Dchl1101R; and the 2nd PCR were amplified with 341F plus a GC clamp on the 5' terminus and reverse primer 534R as Chapter 2 (Krumins et al., 2009; Park et al., 2010). PCR reagents were purchased from GenScript (Piscataway, NJ). The PCR reaction contained 2 µl of DNA extraction as template and 5 µl of 10X reaction buffer, 100 µM of dNTP, 0.01U of Taq DNA polymerase, 0.2 µM of forward primer and 0.2 µM of reverse primer in each 50 µl PCR reaction mixture. PCR was performed by preheating the mixture at 94 ºC for 5 minutes, followed by 35 cycles of denaturation at 94 ºC for 20 seconds, annealing at 55 ºC for 45 seconds, and extension at 72 ºC for 45 seconds, and the final extension step at 72 ºC for 7 min. DGGE was performed according to the standard protocol (Muyzer et al., 1993) using the Bio-Rad Dcode system (Bio-Rad laboritaries, Hercules, CA). The denaturing gradient gel was prepared to contain 0.5 X TAE buffer (20 mM Tris, 10 mM acetate, 0.5 mM Na_2-EDTA, pH 8.0), 6% acrylamide-bis-acrylamide, and 50 to 70% denaturant (100% denaturant contains 7 M urea and 40% formamide). Electrophoresis was performed at 40 V for 17 hours in 1 X TAE buffer at 60ºC. PCR products separated on the gel were stained with EtBr for 20 min and then photographed under illumination by UV light. Major DGGE bands were cut from the gel, eluted in PCR water overnight

and PCR re-amplified with the 2nd set of primers (341F-gc/534R) under the same conditions as described above until confirmed for band purity.

Sequencing and Phylogenetic Analysis. Confirmed single bands from DGGE gels were amplified again with 341F and 534R under the same PCR conditions as above. The PCR products were separated by agarose gel electrophoresis, purified and pre-added 341F primer before sending to GeneWiz for sequencing analysis (GeneWiz, South Plainfield, NJ). Sequence similarity searches and alignments were performed using BLAST (Altschul et al., 2007). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura et al., 2007).

PCR and intensity analysis of reductive dehalogenase genes

Twelve sets of reductive dehalogenase gene were amplified and quantified based on Park et al. (2010). Briefly, the temperature profile for all 12 sets was 94 °C for 5 min followed by 35 cycles of 94 °C for 20 s, 52 °C for 60 s, and 72 °C for 60 s. A final extension step was carried out for 7 min at 72 °C, after which the DNA was stored at 4 °C. PCR products were loaded in one 1.5% agarose gel with same well size for electrophoresis and a resulting gel image was used to measure relative band intensities by using the ImageJ quantification software (ver. 1.33u, National Institutes of Health, USA), according to the manufacturer's protocol (http://rsbweb.nih.gov/ij/).

Results

Dechlorination of TCDF and TCDD. Dechlorination of TCDF started after 9 months, with the detection of three tri-CDFs, including 1,2,3-tri-CDF, and three Di-CDFs, including 2,4-di-CDF as dechlorination products (Fig. 4-1a). 4-mono-CDF was detected by the end of 11 months. After 13 months, approximately 50 mol% of TCDF remained, with 4-mono-CDF accounting for approximately 12 mol% among all the CDFs. In the TCDD enrichment cultures without PCNB, dechlorination of TCDD started after 8 months (Fig. 4-1b) with the detection of 1,2,3-tri-CDD and 1,3- and 2,3-di-CDDs. Approximately 98 mol% TCDD remained in the culture at this time point. 2,3-di-CDD reached its peak amount of 14 ± 5.5 mol% after 11 months, followed by depletion to 4.5 ± 0.6 mol% by the end of 13 months. 2-mono-CDD was observed after 9 months, and became the dominant daughter congener later on in the culture, at approximately 58 mol% among all the CDD congeners in the culture after 13 months. By the end of 13 months, the remaining TCDD in the culture was approximately 34 mol%. In the TCDD enrichment cultures amended with PCNB, dechlorination of TCDD started after 5 months (Fig. 4-1c). 1,3- and 2,3-di-CDDs (14 mol% in total) and 2-mono-CDD (15 mol%) were detected after 7 months, with approximately 68 mol% of TCDD remaining in the culture at this time. 2,3-di-CDD reached its peak amount of 56 ± 8.6 mol% after 8 months, followed by depletion to 11 ± 2.8 mol% by the end of 13 months. By the end of 13 months, the remaining TCDD was only 9 mol%, and 2-mono-CDD was more than 77 mol% in the culture. No dechlorination of TCDD was observed in killed controls over 13-month incubation in the presence of PCNB (data not shown).

Dechlorinatino of PCNB. PCNB was transformed to pentachloroaniline (PCA) in the first month (Fig. 4-2a). PCA was dechlorinated to 2,3,5,6- and 2,3,4,5-TeCAs after 2 months, which were further dechlorinated to 2,4,5- and 2,4,6-TrCAs by around 5 months incubation. Accumulation of 2,4,6-TrCA was observed over time, and exceeded more than 60 mol% in the system after 11 months. Co-eluting 2,4- and 2,5-DiCAs were detected in the culture after 7 months and increased to around 18 mol% by the end of 11 months. 4-MoCA was constantly detected since 7th month. Over 90 mol% of PCA was dechlorinated by the end of 11 months, with 2,4,6-TrCA accumulating to more than 60 mol%. PCNB was also transformed to pentachloroaniline (PCA) in killed controls after 1 month with slight amount of (around 2 mol%) 2,3,4,5-TeCA, 2,4,6-TrCA and 4-MoCA being detected occasionally in killed control over 11 months (data not shown).

Dechlorination of octa-CDF. In the octa-CDF amended enrichment cultures, two additional hepta-CDFs (peaks 1 & 2) were detected at time 0 as impurities of octa-CDF, in addition to the historical 1,2,3,4,6,8,9- and 1,2,3,4,6,7,8-hepta-CDFs, which were the only peaks that were detected in background controls over time (Fig. 4-3). Two new hexa-CDFs (peaks 3 & 4) were detected after 3 months and increased after 5.5 months in treatments amended with PCNB and TCDD or tetra-CDF (Fig. 4-4). Three new penta-CDFs (peaks 5, 6, & 7) were detected after 5.5 months in both treatments (Fig. 4-5). One new tetra-CDF (peak 8) was first detected after 3 months and increased by 5.5 months, and another new tetra-CDF (peak 9) was first detected after 5.5 months (Fig. 4-6) in treatments amended with PCNB and TCDD. Dechlorination of spiked octa-CDF thus resulted in accumulation of one unknown hepta-CDF, two new unknown hexa-CDFs,

three new unknown penta-CDFs, and two new unknown tetra-CDFs. No such new peaks were detected in killed or background controls over time. Comparing average mol% of each PCDF congeners in both treatments vs. killed control after 0, 3 and 5.5 months showed that the average mol% of the new CDF peaks kept increasing, including 1 hepta-CDF (peak 2), 2 new hexa-CDFs (peaks 3 & 4), 3 penta-CDFs (peaks 5, 6, & 7), and 2 new tetra-CDFs (peaks 8 & 9) (Fig. 4-7). Spiked TCDD/F and PCA all quickly underwent dechlorinations to produce different daughter products as shown above.

Response of *Chloroflexi* community in Kymijoki sediment enrichment cultures to dechlorination of TCDD/F and PCNB. DGGE analysis of *Chloroflexi* specific 16S rRNA genes in the enrichment cultures revealed different band patterns that were closely related to the dechlorination activities observed in each treatment over time (Fig. 4-8). The band patterns of the microbial community in all enrichment cultures after 5 months were similar to controls amended with only electron donors after 11 months. This observation was in correspondence to the lack of dechlorination of TCDD/F by 5 months, but just the beginning of PCNB dechlorination. The microbial community greatly shifted after the onset of dechlorination, with 2 major groups of bands enhanced to different extent corresponding to different dechlorination activities in enrichment cultures amended with electron donors and TCDD/F. In enrichment cultures amended with electron donors and TCDD as well as PCNB, only bands in upper position were enhanced after 7 and 11 months. Phylogenetic analysis of the 11 purified DGGE bands showed that DGGE bands in upper postion including (ED+TCDD)-1, -3, (ED+TCDD+PCNB)-1, -2 and (ED+TCDF)-2 were 100% identical to the sequence of *Dehalococcoides* sp. strain

CBDB1; and DGGE bands in group II including (ED+TCDD)-2, (ED+TCDF)-1 and -3 are closely related to a group of *Chloroflexi* clones that have been only enhanced with 1,2,3,4-tetra-CDD/F amendment (Fig. 4-9). *Chloroflexi* clone D15 27 (EU266866) was toluene degrader found in the redox zone of a tar oil contaminant plume (Winderl et al., 2008). *Cloroflexi* HalAS B2 (AM998347) was found in anoxic sediment of Marmara Sea (unpublished data); *Chloroflexi* GA46 (EF613767) was found in paddy soil (Kim et al., 2008).

***rdh* gene analysis in the microcosm community.** 12 *rdh* genes were analyzed for samples after 7 months in all treatments and controls (Fig. 4-10). The abundance of all 12 *rdh* genes were increased in the presence of TCDD, TCDF and PCNB compared to electron donor only control. For *rdh*09, adding PNCB exclusively stimulated its abundance as compared to treatment adding TCDD or TCDF only or electron donors only. It should be noted that the relative abundance of different *rdh* genes are not directly comparable since the primers for each gene has different reaction efficiency.

Discussion

This study investigated the process of microbial reductive dechlorination of TCDD, TCDF and octa-CDF and the stimulating effect of PCNB in enrichment cultures derived from historically contaminated Kymijoki sediment. Three main aspects can be concluded from the results. First, TCDF is more resistant to microbial reductive dechlorination than TCDD (Fig. 4-1a & b). Second, dechlorination of octa-CDF produced new peaks corresponding to one hepta-CDF, two hexa-CDFs, three penta-CDFs and two tetra-CDFs

(Figs. 4-3 to 4-7). Third, PCNB enhanced the dechlorination rate and extent of TCDD. More resistant dechlorination of TCDF than that of TCDD was indicated by the later onset of dechlorination and lesser extent of dechlorination by the end of 13 months (remaining TCDF of 51 vs. remaining TCDD of 34 mol%) (Fig. 4-1a & b). The maximum rate of TCDD decrease (0.27 ± 0.08 mol% • $month^{-1}$) was 1.7 times higher than that of TCDF (0.16 ± 0.08 mol% • $month^{-1}$) in Kymijoki enrichment cultures (Table 4-1). Consequently, the estimated half-life of TCDF and TCDD were approximately 15 vs. 11 months, respectively, under same conditions (Table 4-1). A slower dechlorination rate of TCDF compared to TCDD was also observed for a mixed culture containing *D. ethenogenes* 195 (Fennell et al., 2004). Similar observations were also made for the more limited extent of dechlorination of spiked PCDFs than that of PCDDs using freshwater sediments (Adriaens et al., 1995). The stimulating effect of PCNB on the dechlorination of TCDD was exhibited in a shorter lag phase of TCDD dechlorination (5 months vs. 8 months) and greater extent of dechlorination (remaining TCDD of 10 mol% with PCNB vs. 34 mol% without PCNB) (Fig. 4-1b & c and Table 4-1). The maximum rate of TCDD decrease with PCNB (0.85 ± 0.18 mol% • $month^{-1}$) was 3.2 times higher than that of TCDD without PCNB (0.27 ± 0.08 mol% • $month^{-1}$) (Table 4-1). Consequently, the estimated half-life of TCDD was 11 months vs. 7 months with the addition of PCNB (Table 4-1). In addition to PCNB, also TeCB has been shown to function as priming co-substrate stimulating the dechlorination of TCDD (Ahn et al., 2005, 2008). The maximum rate constant of TCDD decrease in the presence of TeCB was 0.18 mol%·$month^{-1}$ (R^2=0.9425) before the second re-amendment and 0.24 mol%·$month^{-1}$ (R^2=0.9987) after the second re-amendment for methanogenic consortium using Paleta

Creek sediment (San Diego Bay, CA, USA) (Ahn et al., 2005). However, the exact stimulating effects on the dechlorination kinetics were not determined. Compared to the rate constants in our experiment, they were approximately 4 folds less than that of adding PCNB (0.85 mol%·month^{-1}). However, since there was no lag phase for the dechlorination of TCDD, the half-life time of TCDD was 5.8 ~ 4.3 months in their study (Ahn et al., 2005).

The priming compound PCNB was reductively dechlorinated by indigenous microorganisms in the enrichment cultures. PCNB was transformed to PCA, which was sequentially dechlorinated to 2,3,4,6- and 2,3,4,5-TeCA to 2,4,5-, 2,4,6-TrCAs to 2,4/2,5-DiCAs to 2- and 4-MoCA after 5 months and 2,4,6-TrCA was the dominant dechlorination product after 11 months (64 ± 2.6 mol%) (Fig. 4-2a). Dechlorination of PCA occurred prior to that of TCDD (Fig. 4-1c vs. Fig. 4-2a). A slightly different reductive dechlorination route of PCA has been observed in a first generation mixed methanogenic culture developed from contaminated estuarine sediment (Tas and Pavlostathis 2005). Instead of 2,3,4,6-TeCA and 2,4,5-TrCA as shown here, they observed 2,3,5,6-TeCA and 2,3,5-TrCA in addition to 2,3,4,5-TeCA and 2,4,6-TrCA. 3- and 4-MoCA were the end dechlorination products (Tas and Pavlostathis 2005) compared to 2- and 4-MoCAs in the current study. These differences suggest that the responsible dechlorinating microorganisms for PCNB reductive transformation are different.

According to the identified dechlorination products of TCDD over time, we propose a dominant *peri*-lateral dechlorination route of TCDD via 1,2,3-tri-CDD to 2,3-di-CDD

and 2-mono-CDD in the Kymijoki enrichment cultures stimulated by PCNB (Fig. 4-11). *Peri*-chlorines at position 1 and 4 were more readily removed than lateral chlorines at position 2 and 3. 1,2,3-TriCDD was produced as a short-life intermediate (Fig. 4-1c). 2,3-Di-CDD reached its peak value of 56 ± 9 mol% among all congeners when *peri* dechlorination of TCDD reached its reaction plateau. This was implied by the stable mol% of TCDD after 8 months in the system (Fig. 4-1c). Depletion of 2,3-di-CDD started simultaneously as a result of subsided *peri* dechlorination but still active lateral dechlorination. 2-Mono-CDD accumulated in the enrichments, indicating that lateral dechlorination of 2,3-di-CDD was occurring as soon as 2,3-di-CDD was produced in the enrichment culture. The accumulation 2,3-di-CDD before 8 months was due to the slower reaction rate of lateral dechlorination (2 or 3) than that of *peri* dechlorination (1 ad 4). Consequently, the reaction occurred to the lateral chlorine is the rate-limiting step for complete dechlorination of TCDD. However, unlike the equal dechlorination chance of *peri* chlorines at position 1 and 4, lateral chlorines at position 2 and 3 may not have equal opportunity for dechlorination since only 2-mono-CDD was detected as the final dechlorination product but no non-chlorinated DD. (4) 1,2,4-tri-CDD and 1,3-di-CDD were detected in minor amounts in the enrichment culture (Fig. 4-1c). In summary, *peri* dechlorination was reacting more easily than lateral dechlorination for TCDD in Kymijoki enrichment culture. It should be noted that both *peri* and lateral dechlorination reached their reaction plateaus when TCDD and 2,3-di-CDD were about 10 mol% among all congeners in the system (Fig. 4-1c), which might be the reaction thresholds for dechlorination at *peri* and lateral positions. The different removal rate of *peri* and lateral

chlorines of TCDD may be due to different microorganisms or different reductive dehalogenase genes (*rdh*) catalyzing these reactions.

The dechlorination pathway of TCDD in Kymijoki enrichment cultures was similar with or without PCNB, including initial accumulation and later depletion of 2,3-di-CDD as well as 1,2,3- and 1,2,4-tri-CDDs being short-life intermediates in the system (Fig. 4-1b). The near identical accumulation rates of 2-mono-CDD with and without PCNB were 11.9±0.5 vs. 13.0±0.4 mol%•month^{-1}. From this it can be postulated that PCNB enhanced *peri* dechlorination of TCDD to 1,2,3-tri-CDD then to 2,3-di-CDD, but did not affect the rate of lateral dechlorination of 2,3-di-CDD to produce 2-mono-CDD. Since most congeners of tri-, di-CDFs are not commercially available; no dechlorination pathway for TCDF could be defined. However, similar dechlorination products of TCDF were also reported for microcosms using Paleta Creek sediment (San Diego, CA, USA) (Ahn et al., 2005).

Dechlorination of spiked TCDD via both 1,2,3- and 1,2,4-tri-CDD to a mixture of 1,3- and 2,3-di-CDD was also observed in a sediment-free culture enriched with hexachlorobenzene (HCB) from Lake Ketelmeer sediment (Beurskens et al., 1995) as well as different primary enrichment cultures from the highly PCDD/F-contaminated sediment of River Spittelwasser (Bunge et al., 2001). Similar dechlorination pathways of 1,2,4-tri-CDD to 1,3-di-CDD to 2-mono-CDD and 1,2,3-tri-CDD to 1,3- and 2,3-di-CDDs to 2-mono-CDD were reported for enrichments of different sites (River Rhine, Spittelwasser and Saale) (Ballerstedt et al., 2004; Toussaint et al., 1998). These two

dechlorination pathways suggest the existence of different dechlorinating microbial populations that catalyze chlorine removal from distinct positions. This diversity may also be due to the diversity of reductive dehalogenase genes (*rdh*) that can be induced to different extent in the environment.

After understanding the stimulation effect of PCNB on *peri*-dechlorinations, according to the increasing and decreasing peaks of PCDFs, there are several postulations can be made in terms of octa-CDF dechlorination pathway despite the commercial unavailability of some PCDF standards. Firstly, the dominant dechlorination product of octa-CDF should be 1,2,3,4,7,8,9-hepta-CDF (peak 2) considering PCNB's stimulation on *peri* dechlorination, and the known 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-hepta-CDF. Peak 1 is postulated to be 1,2,3,4,6,7,9-hepta-CDF. Secondly, if we assume the occurring two hexa-CDFs resulted from 1,2,3,4,6,7,8-hepta-CDF dechlorination (as shown by its decreasing average mol% during 5.5 months incubation) in treatments amended with TCDD, PCNB and octa-CDF (Fig. 4-7), considering the already known peaks of hexa-CDFs in the sediment, the occurring two hexa-CDFs (peaks 3 and 4) would be 1,2,3,6,7,8-hexa-CDF and 1,2,3,4,7,8-hexa-CDF. This still needs to be confirmed with authentic standards. There was not enough information available currently to predict the congener structures of the new penta-CDFs and tetra-CDFs.

Different dechlorination rates on chlorines at different positions have been observed in other studies, as well. Accumulation and depletion of 2,3,6-trichlorobiphenyl as dechlorination intermediate of 2,3,5,6-tetrachlorobiphenyl (2,3,5,6-PCB) was observed

by Van Dort and Bedard (1991). Depletion of 2,3,6-trichlorobiphenyl (2,3,6-TrCBp) was accompanied by an increase of 2,6-dichlorobiphenyl (2,6-DiCBp). They proposed that two different dechlorinating populations were responsible for the observed dechlorination of 2,3,5,6-PCB. One population was capable of dechlorinating 2,3,5,6-PCB to 2,3,5-PCB, the other population was capable of dechlorinating 2,3,6-TrCBp to 2,6-DiCBp, but no activity on 2,3,5,6- or 2,3,5-CBp. The reductive dechlorination of TCDD by a methaonogenic consortium enriched on bromophenols also displayed a similar pattern (Vargas et al., 2001). Depletion of 1,2,4-tri-CDD followed the initial accumulation due to the reductive dechlorination of TCDD, and then accompanied by a level-off phase of TCDD. 1,3-Di-CDD kept increasing after 1,2,4-tri-CDD reached the peak amount between 250-300 days.

Ahn et al. (2005) reported the greater stimulating effects of 1,2,3,4-TeCB, 2,3,4,5-tetrachloroanisole (TeCA) and 2',3,'4'-trichloroacetophenone (TrCAP) on the dechlorination of TCDD using Paleta Creek sediment (San Diego Bay, CA, USA) in methanogenic media. In all cases, TCDD was dechlorinated to 1,2,4-tri-CDD, which was subsequently dechlorinated to 1,3-di-CDD and then 2-mono-CDD after 15 months. Ballerstedt et al. (1997) did not observe the dechlorination of carry-over TCDD in the second transfer when amended with 1,2,4- or 1,2,3-tri-CDDs. They explained this non-dechlorinated TCDD as the non-available fraction of TCDD in the sediment. Kim and Rhee (1997) reported that once dechlorination reached a plateau, the number of dechlorinators began to decrease, which implied that PCB concentrations may have to exceed a certain threshold to maintain the growth of PCB dechlorinators. Jayachandran et

al. (2003) reported different 1,2,3-trichlorobenzene (1,2,3-TrCB), pentachlorobenzene (PnCB), and hexachlorobenzene (HxCB) dechlorination rates for strain CBDB1 with cells pregrown on different chlorobenzene congeners as electron acceptors, suggesting that dehalogenases are induced by their respective substrates (electron acceptors).

Removal of a chlorine atom at lateral position of CDDs was reported as a preferential reaction based on the Gibbs free energy of formation (Huang et al., 1996) and by the semiempirical molecular orbital calculations (Wehrmeier et al., 1998). However in this study, *peri* chlorines at 1 or 4 positions were more amenable for hydrogen substitution. Similar regioselectivity of microbial reductive dechlorination was also reported by Barkovskii and Adriaens (Barkovskii and Adriaens, 1996). Later, a theoretical study by Fueno (2002) for octa-CDD showed very small activation energy difference between 1- and 2 chlorine (only 0.1 kcal/mol), and the authors concluded that the hydrogen dechlorination of octa-CDD will not lead to a dominant formation of the toxic congener with lateral chlorine atoms.

DGGE analysis of putative 16S rRNA genes of *Chloroflexi* in the enrichment cultures revealed a shift in microbial community pattern corresponding to active dechlorination of TCDD/F and PCNB over time compared to controls (Fig. 4-8). The enriched microbial population closely clustered with the Pinellas subgroup of *Chloroflexi*, which includes *Dehalococcoides* strain CBDB1, which actually has the same dechlorination route of TCDD as shown in this study. A second enriched microbial population closely clustered with another division of the *Chloroflexi*, which has also been found in anaerobic sludge

or sediment with aromatic contamination (Fig. 4-9). It remains to be determined whether these bacteria participate directly in the dechlorination process, or just played a role in co-substrate utilization. DGGE enables the monitoring of temporal changes in the microbial community structure of enrichment, and provides the information of the dominant microbial species. However, since only 93 bp fragment of the 16S rRNA gene was used here, it may lack the specificity required for phylogenetic identification of some organisms (Gilbride et al., 2006). Although the known *Dehalococcoides* isolates only contain one copy of the 16S rRNA gene (Seshadri et al., 2005; Kube et al., 2005), heterogeneous 16S rRNA gene may exist in potential uncultured dechlorinating microorganisms, which may produce multiple bands on gels (Nubel et al., 1997). Band intensity may not truly reflect the abundance of the microbial population, but merely more gene copy number. The activities of non-dechlorinating bacteria on degrading organic acids in the mixed consortia is most likely important for the overall dechlorinating process by providing potential electron donors, carbon sources and other nutrients.

Conclusions

In summary, microbial reductive dechlorination of TCDD/F was investigated using historically PCDF contaminated Kymijoki sediments in methanogenic enrichment cultures. PCNB amendment stimulated the rate and extent of TCDD dechlorination over time. TCDF was more resistant to microbial reductive dechlorination than TCDD. The dominant dechlorination pathway of TCDD in Kymijoki enrichment cultures was TCDD to 1,2,3-tri-CDD to 2,3-di-CDD to 2-mono-CDD. PCNB specifically stimulated *peri*-

dechlorination, while it had minimum effect on lateral dechlorination of TCDD in Kymijoki enrichment cultures. Analysis of *Chloroflexi* specific 16S rRNA genes from the enrichment culture by DGGE revealed a shift in the microbial community composition in response to dechlorination of TCDD/F and PCNB. Phylogenetic analysis of the purified DGGE bands showed that at least one population belonging to the Pinellas subgroup of *Dehalococcoides*, which contains strain CBDB1, is responsible for the observed dechlorination activities. Another population (band) observed only in the presence of TCDD/F is related to *Chloroflexi* clones that were found in anaerobic sludge or sediment with aromatic contamination (Winderl et al., 2008; Kim et al., 2008). These results indicated that PCNB is able to stimulate the growth of dechlorinators in the sediment or induce certain reductive dehalogenase genes (*rdh*s) that were active in the dechlorination of PCDD/Fs.

Parallel enrichment cultures amended with PCNB and octa-CDF with TCDD or TCDF demonstrated dechlorination of octa-CDF to hepta-CDFs and further to hexa-CDFs, penta-CDFs and tetra-CDFs. Although the exact dechlorination pathways are not clear, it still provides strong evidence of dechlorination of PCDFs in Kymijoki sediment. This work may assist future attempts to enrich and isolate PCDD/F-dechlorinating bacteria in mixed culture or at contaminated sites. Further characterization of the selective and synergistic activities of PCDD/F dechlorinating microorganisms with different dioxin mixtures is essential for generating models to predict the dechlorination potentials at specific dioxin- impacted sites and design effective *in situ* treatment strategies.

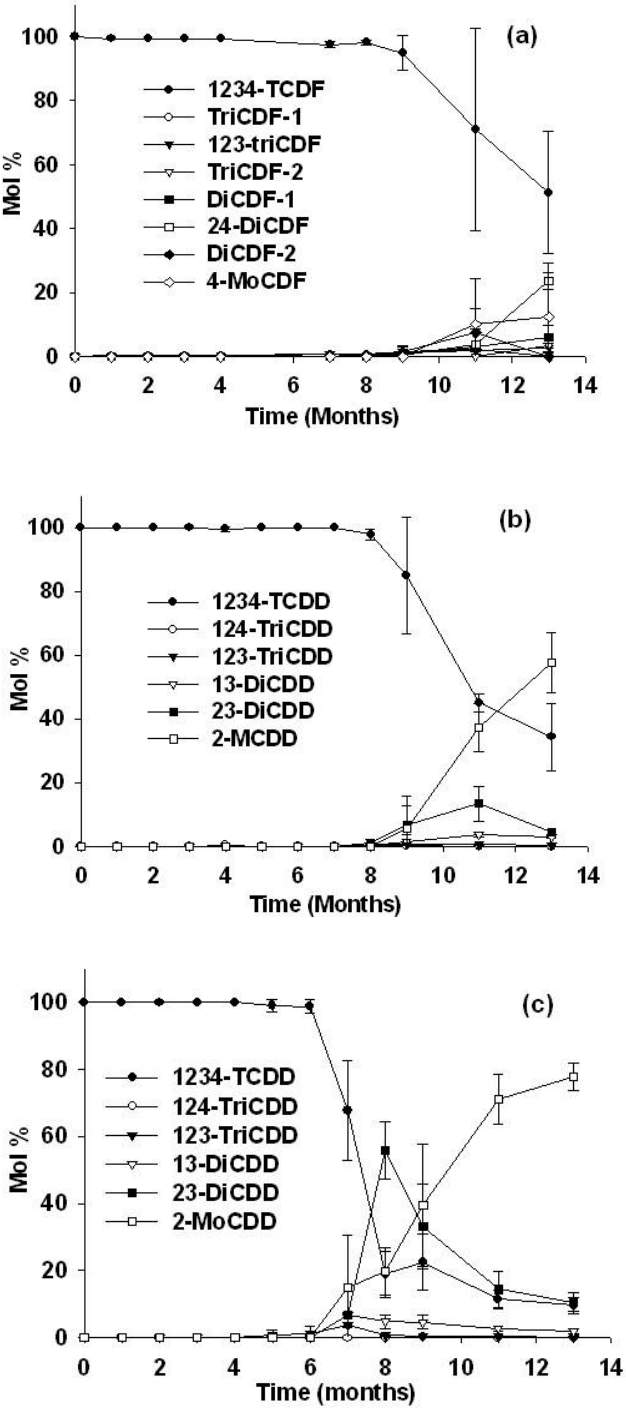

Figure 4-1. Dechlorination of 1,2,3,4-TCDF (a), dechlorination of 1,2,3,4-TCDD without PCNB (b) and with PCNB as co-substrate (c). All cultures were supplemented with an electron donor mixture. Fresh anaerobic medium was added up to 40 ml after 6 months to replenish the whole volume.

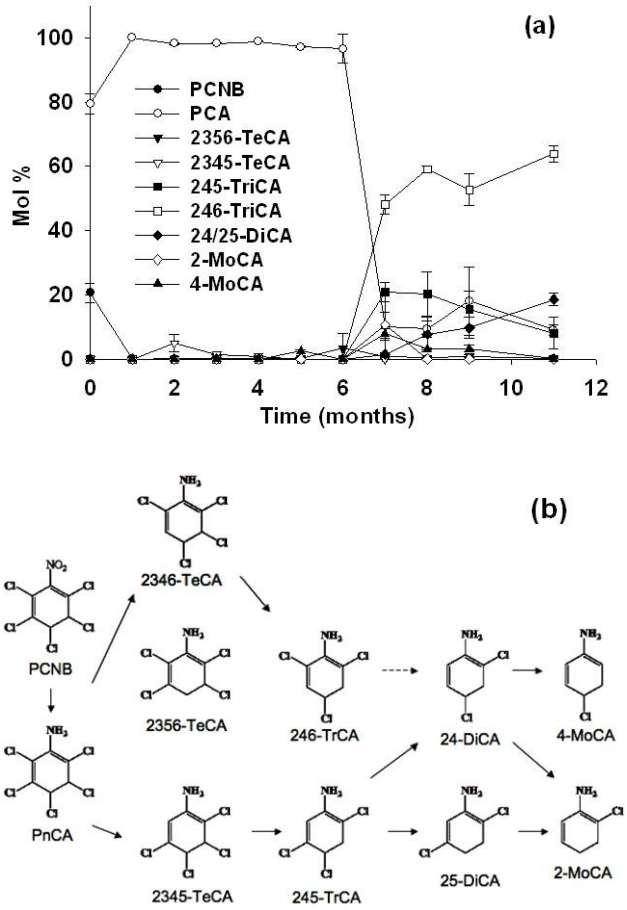

Figure 4-2. Dechlorination of PCNB in cultures spiked with TCDD and PCNB (a) and the proposed dechlorination pathway based on the dechlorination products identified (b). 2356-TeCA is the commercially available standard. 2346-TeCA is the intermediate that have been postulated based on the identified subsequent TrCAs intermediates and the co-elution fact of 2356-tetrachlorophenol and 2346-tetrachlorophenol (Häggblom et al., 1988).

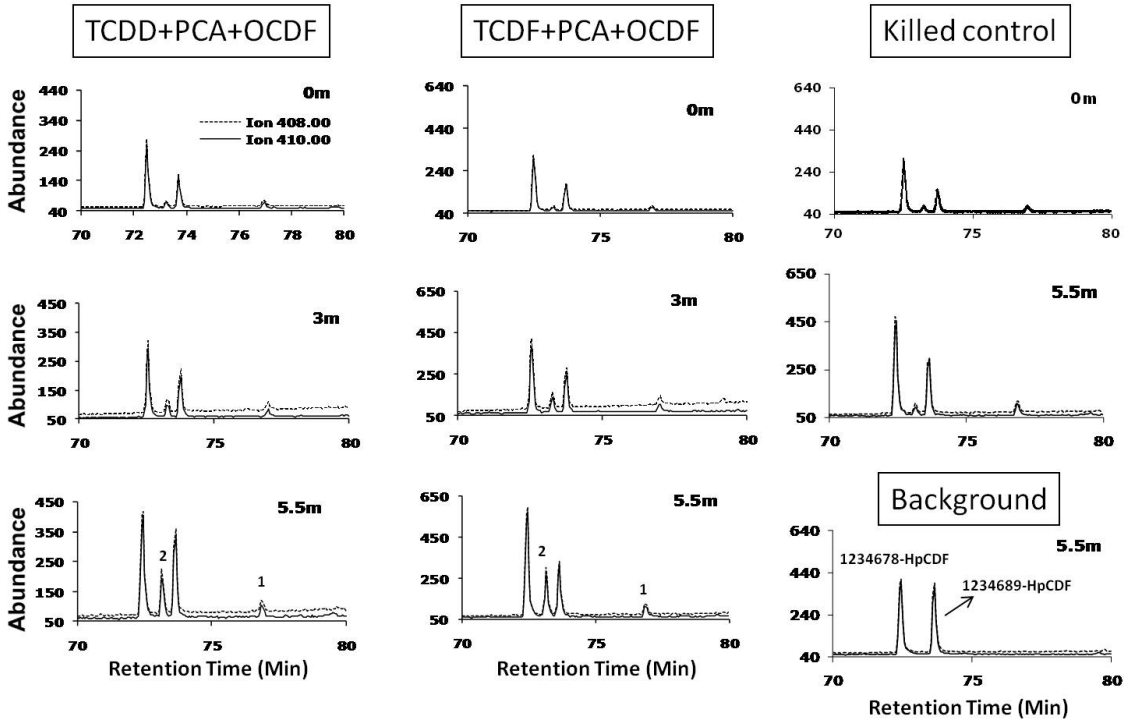

Figure 4-3. Selected ion chromatograms of hepta-CDFs in enrichment cultures amended with octa-CDF and TCDD + PCNB (a), octa-CDF and TCDF + PCNB (b) after 0, 3, and 5.5 months, and background and octa-CDF-amended killed controls after 5.5 months (c).

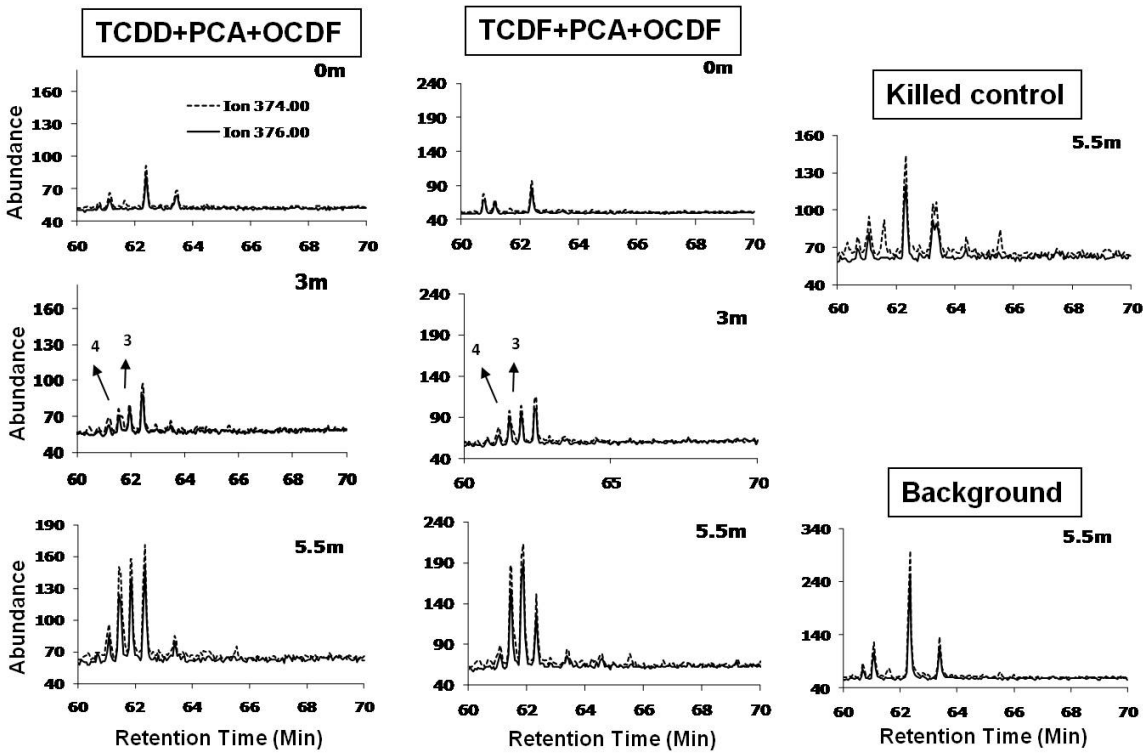

Figure 4-4. Selected ion chromatogram of hexa-CDFs in enrichment cultures amended with TCDD+PCNB+octa-CDF after 0, 3, and 5.5 months (m); TCDF+PCNB+octa-CDF after 0, 3, and 5.5 months (m); Killed control after 3 and 5.5 month (m); No addition control after 5.5 months (m).

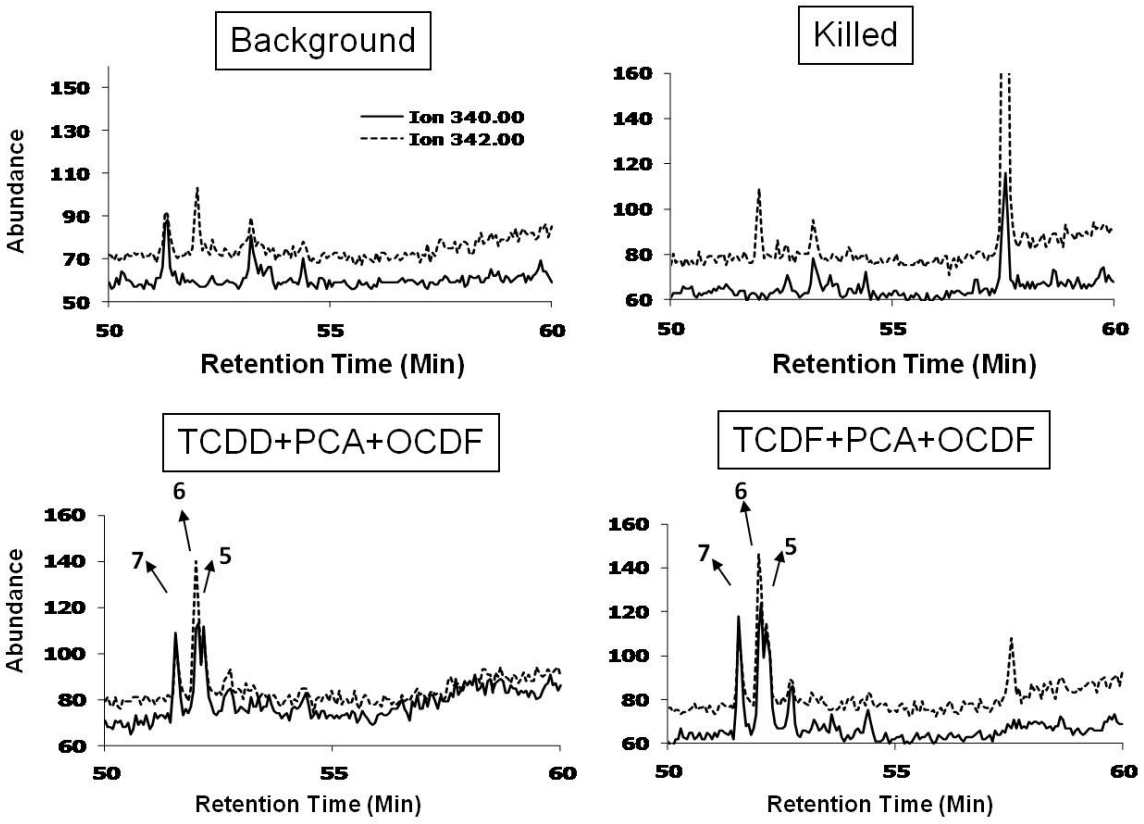

Figure 4-5. Selected ion chromatograms of penta-CDFs in enrichment culture of no addition control, killed control, treatment amended with TCDD+PCNB+octa-CDF and TCDF+PCNB+octa-CDF after 5.5 months. Two new penta-CDFs were detected after 5.5 months in enrichment cultures amended with TCDD/F+PCNB+octa-CDF, but not after 0 or 3 months.

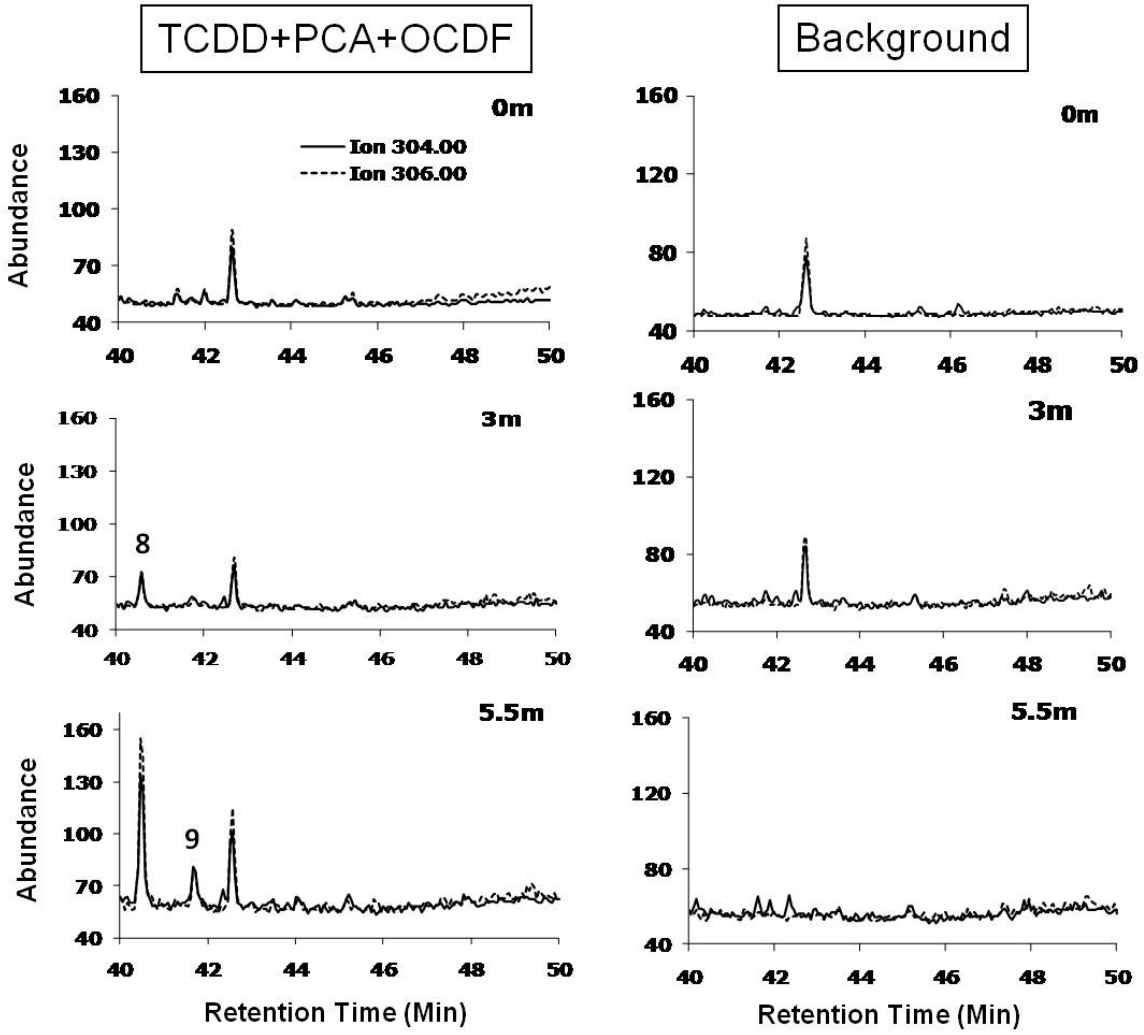

Figure 4-6. Selected ion chromatograms of tetra-CDFs (m/z 304 and 306) in enrichment cultures amending TCDD+octa-CDF+PCNB after 0, 3, and 5.5 months; no addition control after 0, 3, and 5.5 months. No data shown here for enrichment cultures amended with TCDF+PCNB+octa-CDF and Killed control. TCDD: 1234-TCDD, TCDF: 1234-TCDF.

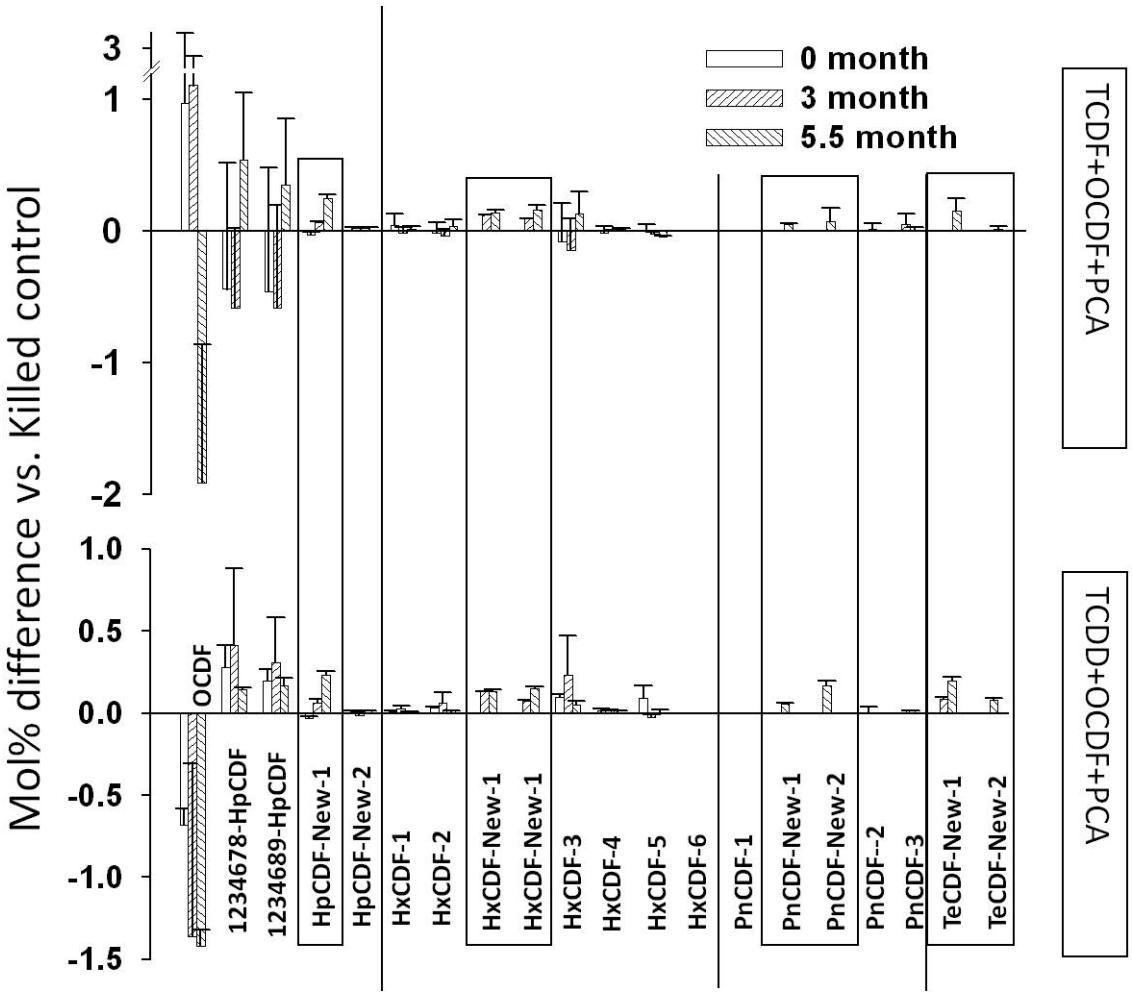

Figure 4-7. Average mol% difference of CDD congeners of triplicate enrichment cultures amdended with octa-CDF + TCDD + PNCB vs. killed control (a) octa-CDF + TCDF + PCNB vs. killed control (b) after 0, 3, and 5.5 months.

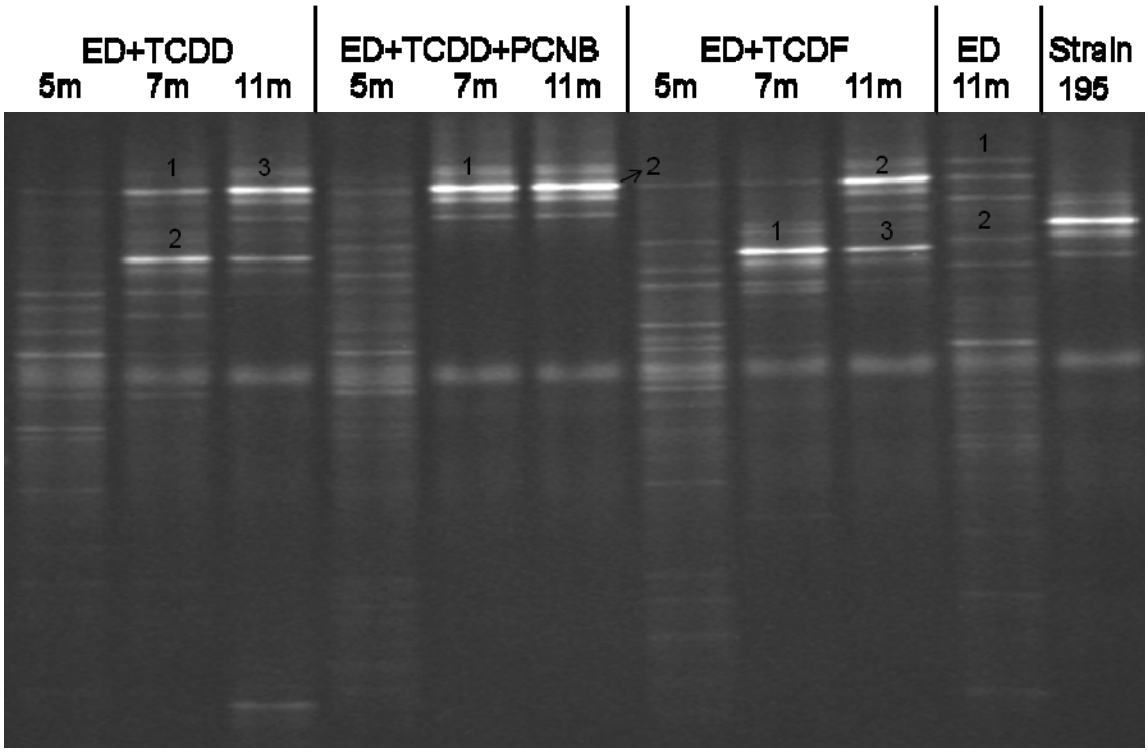

Figure 4-8. DGGE profiles of microbial community in cultures spiked 1234-TCDD, 1234-TCDD and PCNB, and 1234-TCDF after 5 months, 7 months and 11 months incubation (all cultures received electron donor) compared to electron donor only control after 11 months.

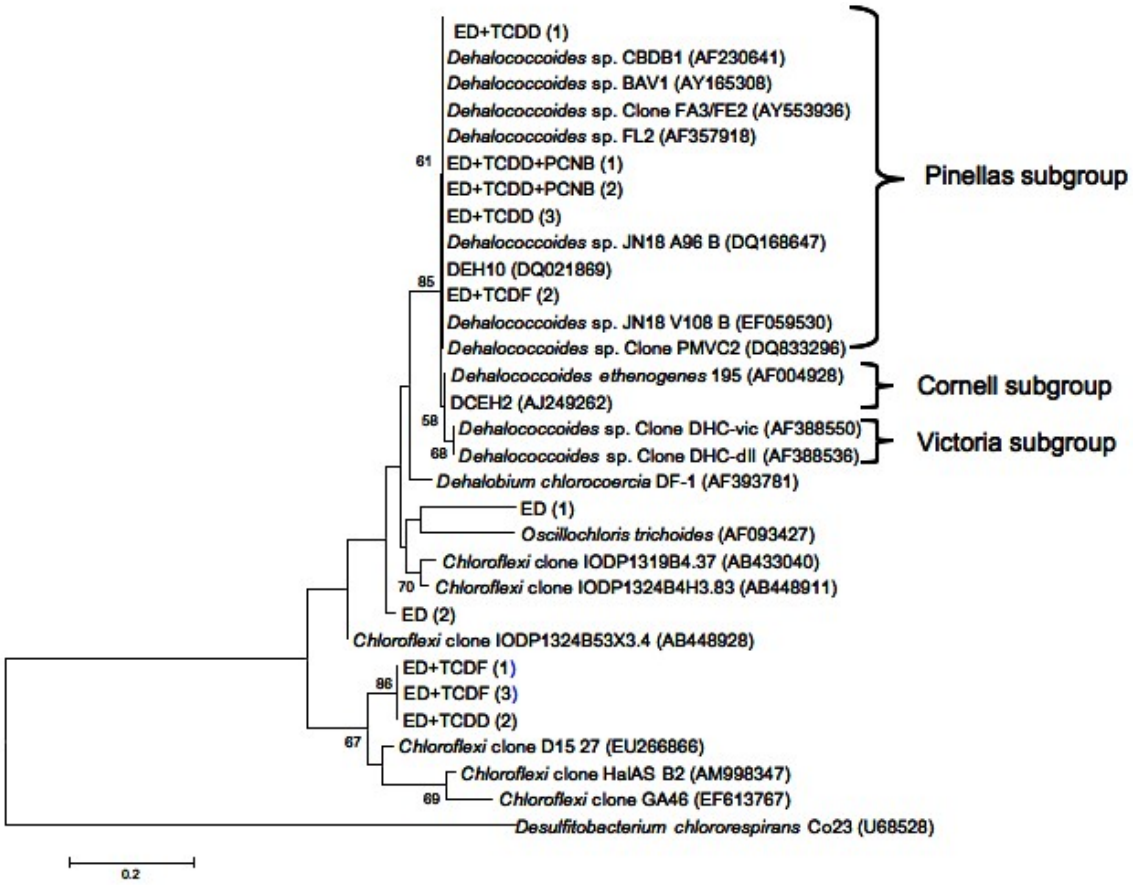

Figure 4-9. Phylogenetic analysis of 16S rRNA gene DGGE band sequences compared closely related reference sequences. Single alignment and construction of the phylogenetic tree with the minimum evolution method with 1000 bootstrap re-samplings were performed using the MEGA version 4 program. Bootstrap values at nodes are the percentages of 1000 iterations. The reference bar indicates 20 nucleotide exchanges per 100 nucleotides.

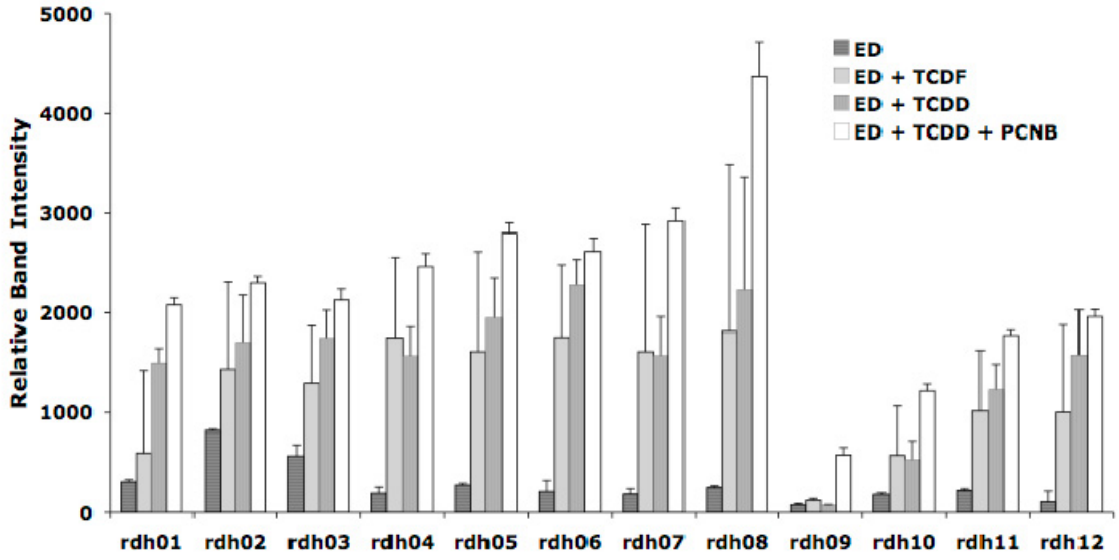

Figure 4-10. Quantification of 12 putative *rdh* genes in enrichment cultures derived from Kymijoki River sediment after 7 months of incubation. Putative *rdh* gene amplicons correspond to the following *D. ethenogenes* strain 195 and *Dehalococcoides* sp. CBDB1 genes: *rdh* 01 (DET0180 and cbdb_A187), *rdh* 02 (DET0235 and cbdb_A243), *rdh* 03 (DET0302 and cbdb_A237), *rdh* 04 (DET0306 and cbdb_A1495), *rdh* 05 (DET0311 and cbdb_A88), *rdh* 06 (DET0318 and cbdb_A1588), *rdh* 07 (DET1171 and cbdb_A1092), *rdh* 08 (DET1519 and cbdb_A1575), *rdh* 09 (DET1522 and cbdb_A1570), *rdh* 10 (DET1535 and cbdb_A1595), *rdh* 11 (DET1538 and cbdb_A1627), and *rdh* 12 (DET1545 and cbdb_A1638). ED: electron donor, TCDD: 1,2,3,4-tetrachlorodibenzo-*p*-dioxin, PCNB: pentachloronitrobenzene, TCDF: 1,2,3,4-tetrachlorodibenzofuran.

Table 4-1. Comparison of dechlorinating kinetics of TCDD/F in Kymijoki sediment enrichment cultures amdended with TCDD+PCNB, TCDD only and TCDF. All cultures received the electron donor mixture.

Treatment	ED+TCDD+PCNB	ED+TCDD	ED+TCDF
Target Compound	TCDD	TCDD	TCDF
Lag Phase (months)	5	8	9
Dominant product	2-mono-CDD	2-mono-CDD	24-di-CDF
Dominant product mol%	77.7±4.2	57.6±9.5	23.7±2.6
Dechlorination rate* (mol%•month^{-1})	0.85±0.18	0.27±0.08	0.16±0.08
Estimated half life (month)	6.9±0.2	10.7±0.8	13.9±2.4

*: First order rate regression was calculated for decreasing molar percentages of TCDD/F in the enrichments vs. time in the period with fastest depletion rate: for 1,2,3,4-TCDD in ED+TCDD+PCNB, month-6, -7, and -8; for 1234-TCDD in ED + TCDD, month-8, -9, and -11; for 1,2,3,4-TCDF in ED + TCDF, month-9, -11, and -13. Values are provided with average +/- SD of triplicate cultures.

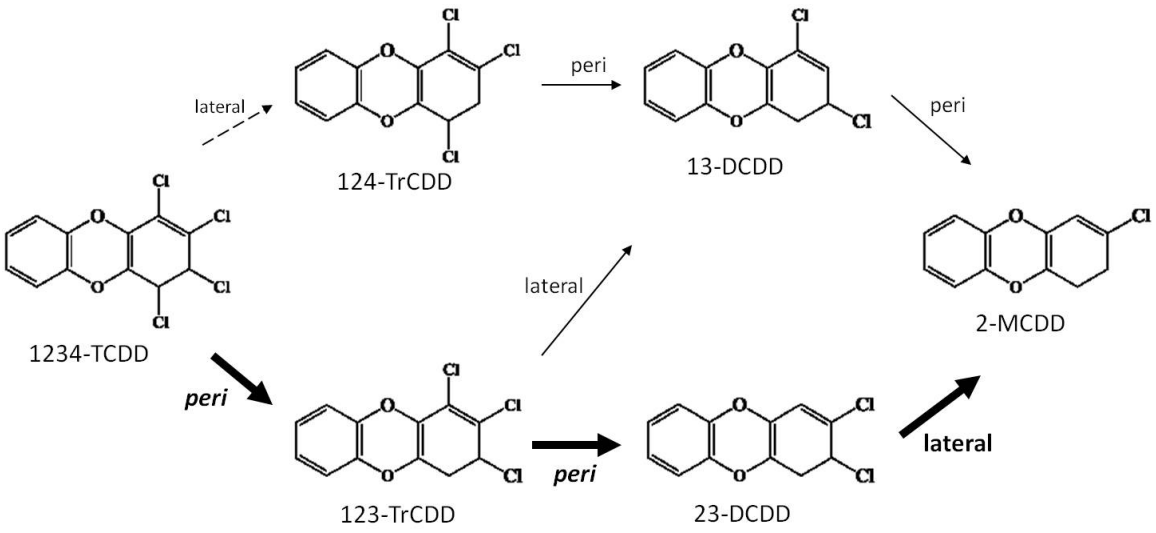

Figure 4-11. Proposed dechlorination pathway of 1,2,3,4-TCDD (TCDD) in Kymijoki enrichment cultures amended with PCNB as co-substrate. TrCDD: tri-CDD, DCDD: di-CDD, MCDD: mono-CDD. The principle dechlorination pathway of 1234-TCDD dechlorination observed in Kymijoki enrichment cultures amended with PCNB as co-substrate was highlighted in heavy arrow.

Chapter 5

Discussion and Conclusions

The combined work in this thesis shows that Kymijoki sediment has a dechlorinating community that actively dechlorinates spiked PCDD/Fs. Even more importantly also weathered PCDFs in Kymijoki sediment can be dechlorinated, although the activity is very slow.

Dechlorination of weathered PCDFs in Kymijoki mesocosms was demonstrated in two ways: 1) the increasing average mol% ratio of 1,2,3,4,6,7,8-hepta-CDF vs. 1,2,3,4,6,8,9-hepta-CDF from less than 1 to more than 1 in all mesocosms over 7 years of incubation (Chapter 2; Fig. 2-2); 2) the decreasing average mol ratio of *peri* chlorines vs. total chlorines of hepta-CDFs and increasing average mol ratio of lateral chlorines vs. total chlorines of hepta-CDFs in all mesocosms over 7 years of incubation (Fig. 2-3a). In addition to the above general trend of dechlorination of weathered PCDFs, for mesocosms amended with electron donors and TeCB, *peri*-dechlorination of penta-CDFs was selectively enhanced over 7 years which was demonstrated by the decreasing mol ratio of *peri* chlorines vs. total chlorines and increasing mol ratio of lateral chlorines vs. total chlorines (Fig. 2-3c).

The stimulating effects of amendments on dechlorination of weathered PCDFs were further studied in sediment microcosms (Chapter 3). Bioaugmentation with a mixed culture containing *Dehalococcoides ethenogenes* strain 195 and amendment with electron donors stimulated *peri* dechlorination of octa-CDF to produce 1,2,3,4,6,7,8-hepta-CDF in addition to the enhanced dechlorination of 1,2,3,4,6,8,9-hepta-CDF and hexa-CDFs and production of non-2,3,7,8-tetra-CDFs (Fig. 3-3c). Bioaugmentation with *D. ethenogenes*

strain 195 and amendment with vegetable oil stimulated lateral dechlorination of octa-CDF to produce 1,2,3,4,6,8,9-hepta-CDF in addition to the enhanced dechlorination of 1,2,3,4,6,7,8-hepta-CDF (Fig. 3-3d). Amendment of electron donors and "haloprimers", such as TeCB or PCNB, may stimulate the dechlorination of hexa-CDFs and production of non-2,3,7,8-tetra-CDFs after 18 months incubation in these microcosms as indicated by the mol% change of the detected congeners over time as well as compared to controls (Fig. 3-4a & b).

Further dechlorinating potential of indigenous microorganisms in Kymijoki sediment was confirmed in the enrichment cultures (Chapter 4) derived from the sediment microcosms (Chapter 3). 1,2,3,4-tetra-CDF was more resistant to dechlorination than 1,2,3,4-tetra-CDD (Fig. 4-1a vs. 4-1b); and dechlorination of octa-CDF produced new peaks corresponding to one hepta-CDF, two hexa-CDFs, three penta-CDFs and two tetra-CDFs (Fig. 4-3 to 4-7). Dechlorination of 1,2,3,4-tetra-CDF was slower than that of 1,2,3,4-tetra-CDD, with 2,4-di-CDF as the most abundant dechlorination product after 13 months (Table 4-1). 1,2,3,4-tetra-CDD was dechlorinated via 1,2,3-tri-CDD and 1,2,4-tri-CDD as short-life intermediates mainly to 2,3-di-CDD and 2-mono-CDD over 13 months incubation (Fig. 4-11). In addition, the stimulating effect of PCNB on dechlorination was also confirmed by the enhanced dechlorination rate and extent of 1,2,3,4-tetra-CDD (Fig. 4-1b vs. c). PCNB did not affect the dominant dechlorination pathway of 1,2,3,4-tetra-CDD (Fig. 4-11). PCNB stimulated dechlorination by enhancing removal of *peri* chlorines on 1,2,3,4-tetra-CDD, while it had minimum effect on the removal of lateral chlorines (Fig. 4-1b vs. c and table 4-1). During the process of stimulating 1,2,3,4-tetra-

CDD dechlorination, PCNB was reductively transformed to PCA (pentachloroaniline), which then underwent sequential dechlorination to 2,3,4,5- and 2,3,4,6-tetra-CAs to 2,4,5- and 2,4,6-tri-CAs to 2,4-/2,5-di-CAs to 2- and 4-mono-CAs (Fig. 4-2). A second transfer of these enrichment showed shortened lag phase and similar dechlorination reactions of 1,2,3,4-tetra-CDD/F (data not shown).

This study has provided direct evidence of dechlorination of weathered PCDFs in Kymijoki sediment mesocosms over 7 years incubation (Chapter 2). Previously, dechlorination of aged PCDDs in Passaic River sediment (NJ, USA) was enhanced by addition of acetate, butyrate, benzoate, 2-monobromodibenzodioxin and H_2 (Albrecht et al., 1999). Also, bioaugmentation with a mixed culture containing *Dehalococcoides ethenogenes* strain 195 stimulated dechlorination of aged PCBs in Anacostia River sediment (Baltimore, USA) (Krumins et al., 2009). Dechlorination of spiked 1,2,3,4-TCDD in enrichment cultures inoculated with Kymijoki River sediment (Finland) was also enhanced by bioagumentaion of strain 195 (Ahn et al., 2008), which resulted in different primary dechlorination products (Ahn et al., 2008). In our study, we showed that different amendments including "haloprimers" like TeCB and PCNB as well as bioaugmentation of strain 195 with electron donors or vegetable oil selectively stimulated dechlorination of weathered PCDFs in microcosms (Chapter 3). The priming effect of PCNB on dechlorination of spiked 1,2,3,4-tetra-CDD was further demonstrated to enhance the removal of *peri* chlorines, but had minimal effect on the removal of lateral chlorines (Chapter 4).

Based on the identification of certain increasing and decreasing congeners in mesocosms amended with electron donors and TeCB, possible *peri*-preferred dechlorination pathways for weathered PCDFs were proposed (Chapter 2) (Fig. 2-7). *Peri* dechlorination of octa-CDF to 1,2,3,4,6,7,8-hepta-CDF was also further stimulated in microcosms amended with bioaugmentation of *D. ethenogenes* strain 195 and electron donors. In contrast, lateral dechlorination of octa-CDF to 1,2,3,4,6,8,9-hepta-CDF was stimulated in microcosms bioaugmented with *D. ethenogenes* strain 195 and amended with vegetable oil (Chapter 3). It should be noted that the mixed culture containing *Dehalococcoides ethenogenes* strain 195 did not dechlorinate spiked octa-CDD and deca-chlorobiphenyl (Liu and Fennell, 2008). This information indicated that the indigenous microorganisms in Kymijoki sediment might mediate the dechlorination of weathered octa-CDF observed in this study. Dechlorination of spiked 1,2,3,4-tetra-CDD in the enrichment culture showed a *peri*-lateral sequence (Chapter 4) (Fig. 4-11). The combined evidence showed that the dechlorinating potential of indigenous microorganisms in Kymijoki sediment on *peri* or lateral chlorines can be selectively stimulated with different amendments, but also can happen sequentially. The possible *peri*-preferred dechlorination may have the risk of generating toxic intermediates, such as 2,3,7,8-tetra-CDF. However, whether this should be a concern for Kymijoki sediment needs further studies.

In contrast to the theoretical dechlorination pattern proposed for PCDDs (Fueno et al., 2002; Lynam et al., 1998), which indicated that lateral chlorines were more amenable to dechlorination due to the positive charges of lateral carbons and marginal preference of

chlorines on them, dechlorination mediated by microorganisms in the sediment has been shown to occur preferentially at the *peri* chlorines. Barkovskii and Adriaens (1996) indicated that *peri*-dechlorination of 2,3,7,8-substituted hepta to penta-CDDs produced 2,3,7,8-tetra-CDD; and *peri*-lateral dechlorination was occurring to non-2,3,7,8-subsituted congeners in the microbial consortium eluted from dioxin-contaminated Passaic River sediments. Adriaens and Grbic-Galic (1994, 1995) reported that *peri* dechlorination was preferred reaction for certain PCDD/F congeners, including 1,2,3,4,6,7,8-hepta-CDF, when using Hudson River sediment (USA) as inoculum. These observations are in agreement with what we have reported here for the dechlorination of weathered PCDFs in Kymijoki sediment microcosms and mesocosms, as well as spiked 1,2,3,4-tetra-CDD.

Molecular community analysis (PCR-DGGE) revealed a diverse *Chloroflexi* community in Kymijoki sediments (Fig. 2-5). DGGE analysis revealed a highly selected *Chloroflexi* community in the enrichment culture (Fig. 4-8). One of the stimulated *Chloroflexi* populations clustered within the Pinellas subgroup of *Dehalococcoides*, which includes strain CBDB1, which dechlorinates 1,2,3,4-tetra-CDD to similar products as the Kymijoki sediment enrichments (Fig. 4-9). The second enriched microbial population was closely related to another division of *Chloroflexi*, which have been found in anaerobic sludge or sediment with aromatic contamination (Fig. 4-9). It remains to be determined whether these bacteria participate directly in the dechlorination process, or just played a role in co-substrate utilization. The activities of non-dechlorinating bacteria on degrading organic acids in the mixed consortia is most likely important for the overall

dechlorinating process by providing potential electron donors, carbon sources and other nutrients (Wu et al., 2002). A second transfer of the enrichment culture has a shortened the lag phase and maintained the same dechlorination activity. DGGE analysis showed that same *Chloroflexi* population had been enriched (data not shown). DGGE may lack the specificity required for phylogenetic identification of some organisms (Gilbride et al., 2006) due to the limited length of 16S rRNA genes were used. It may underestimate the microbial community due to the unidentified heterogenous 16rRNA genes for some potential uncultured dechlorinating microorganisms (Nubel et al., 1997). Despite of all these incompleteness, DGGE still provided important information on the dominant *Chlorolfexi* species in Kymijoki enrichment cultures.

In conclusion, following 7 years of incubation, dechlorination of weathered PCDFs in Kymijoki sediment mesocosms was observed. An increase in the mol% ratio of 1,2,3,4,6,7,8- vs. 1,2,3,4,6,8,9-hepta-CDF was observed in all mesocosms. The available information suggested that *peri*-chlorines of hepta-CDFs were removed in general, while for penta-CDFs, *peri*-chlorines were removed preferentially only with the amendment of electron donors and TeCB. Microcosm studies further indicated that dechlorination of weathered PCDFs in Kymijoki sediment was selectively stimulated with different amendments. Bioaugmentation with strain 195 and amendment with electron donors stimulated *peri* dechlorination of weathered octa-CDF to produce 1,2,3,4,6,7,8-hepta-CDF, while bioaugmentation with strain 195 and amendment with vegetable oil stimulated lateral dechlorination of weathered octa-CDF to produce 1,2,3,4,6,8,9-hepta-CDF in Kymijoki sediment microcosms after 18 months. The dechlorinating potential of

indigenous microorganisms in Kymijoki sediment was further confirmed in the enrichment cultures spiked 1,2,3,4-tetra-CDD/F and octa-CDF. 1,2,3,4-tetra-CDD was dechlorinated via 1,2,3-tri-CDD and 1,2,4-tri-CDD as short-life intermediates mainly to 2,3-di-CDD and 2-mono-CDD over a 13 month incubation period. Dechlorination of 1,2,3,4-tetra-CDF was slower than that of 1,2,3,4-tetra-CDD, with 2,4-di-CDF as the most abundant dechlorination product after 13 months. PCNB enhanced *peri* dechlorination of 1,2,3,4-tetra-CDD, while it had minimum effect on lateral dechlorination during 13 months incubation. Dechlorination of spiked octa-CDF was observed with the production of hepta-, hexa-, penta- and tetra-chlorinated dibenzofurans (CDFs) over 5.5 months incubation. Molecular community analysis (PCR-DGGE) revealed a diverse *Chloroflexi* community in Kymijoki sediments, while a highly selected *Chloroflexi* community in the enrichment culture. One of the stimulated *Chloroflexi* populations in enrichment culture clustered closely with the Pinellas subgroup of *Dehalococcoides* including strain CBDB1, which dechlorinates 1,2,3,4-tetra-CDD to similar products as the Kymijoki sediment enrichment.

These results suggest that dechlorination of weathered PCDD/Fs contaminants may be mediated by indigenous microbial populations as a means for *in situ* bioremediation of PCDD/F contaminated sediments. The meso- and microcosm experiments allow us to conclude that dechlorination of weathered PCDFs in Kymijoki sediments by indigenous microorganism was occurring and can be stimulated by bioaugmentation with srain 195 and amendment with electron donor or vegetable oil. Further characterization of the microbial communities mediating these transformations and future research in the

dechlorination pathways of PCDFs will aid in the assessment of PCDD/Fs contamination and their natural attenuation processes, and eventually may help in developing effective bioremediation technologies.

References

Abramowicz DA, Brennan MJ, Van Dort HM, Gallager EL (1993) Factors influencing the rate of polychlorinated biphenyl dechlorination in Hudson River sediments. Environmental Science and Technology 28: 1125-1132

Adriaens P, Grbic-Galic D. (1994) Reductive dechlorination of PCDD/F by anaerobic cultures and sediments. Chemosphere 29: 2253-2259

Adriaens P, Chang PR, Barkovskii AL (1996) Dechlorination of PCDD/F by organic and inorganic electron transfer molecules in reduced environments. Chemosphere 32: 433-441

Adriaens P, Fu Q, Grbic-Galic D (1995) Bioavailability and transformation of highly chlorinated dibenzo-p-dioxins and dibenzofurans in anaerobic soils and sediments. Environmental Science and Technology 29: 2252-2260

Adrian L, Dudková V, Demnerová K, Bedard DL (2009) "*Dehalococcoides*" sp. strain CBDB1 extensively dechlorinates the commercial polychlorinated biphenyl mixture Aroclor 1260. Applied and Environmental Microbiology 75: 4516-4524

Adrian L, Szewzyk U, Wecke J, Görisch H (2000) Bacterial dehalorespiration with chlorinated benzenes. Nature 408: 580-583

Adrian L, Lechner U (2004) Anaerobic transformation of chlorinated dioxins by microorganisms. Organohalogen Compounds 66: 2266-2271

Ahn YB, Häggblom MM, Fennell DE (2005) Co-amendment with halogenated compounds enhances anaerobic microbial dechlorination of 1234-tetrachlorodibenzo-*p*-dioxin and 1234-tetrachlorodibenzofuran in estuarine sediments. Environmental Toxicology and Chemistry 24: 2775-2784

Ahn YB, Häggblom MM, Kerkhof LJ (2007) Comparison of anaerobic microbial communities from estuarine sediments amended with halogenated compounds to enhance dechlorination of 1234-tetrachlorodibenzo-*p*-dioxin. FEMS Microbiological Ecology 61: 362-371

Ahn YB, Liu F, Fennell DE, Häggblom MM (2008) Biostimulation and bioaugmentation to enhance dechlorination of PCDD in contaminated sediments. FEMS Microbial Ecology 66: 271-281

Alastair H (1977) Seveso: dioxin damage. Nature 266:7-8

Alastair H (1978) Vietnam dioxin problem. Nature 271:597-598

Albrecht ID, Barkovskii AL, Adriaens P (1999) Production and dechlorination of 2378-tetrachlorodibenzo-*p*-dioxin in historically-contaminated estuarine sediments. Environmental Science and Technology 33: 737-744

Alcock RE, Jones KC (1996) Dioxins in the environment: a review of trend data. Environmental Science and Technology 30: 3133-3143

Alder AC, Häggblom MM, Oppenheimer SR, Young LY (1993) Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. Environmental Science and Technology 27: 530-538

Alcock RE, Jones KC (1996) Dioxins in the environment: a review of trend data. Environmental Science and Technology 30: 3133-3142

Altarawnech M, Dlugogorski BZ, Kennedy EM, Mackie JC (2009) Mechanisms for formation, chlorination, dechlorination and destruction of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs). Progress in energy and combustion science 35: 245-274

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search program. Nucleic Acids Res 25: 3389-3402

Anderson DR, Fisher R (2002) Sources of dioxins in the United Kingdom: the steel industry and other sources. Chemosphere 46: 371-381

Anderson P, Rappe C, Maaskant O, Unsworth JF, Marklund S (1998) Low temperature catalytic destruction of PCDD/F in flue gas from waste incineration. Organohalogen Compounds 36: 109-112

Anid PJ, Nies L, Vogel TM (1991) In *on-site Bioremediation;* Hinchee, R.E, Olfenbuttel, R.F., Eds: Butteworth-Heinemann; Stoneham, MA.

Aulenta F, Pera A, Rossetti S, Papini MP, Majone M (2007) Relevance of side reactions in anaerobic reductive dechlorination microcosms amended with different electron donors. Water Research 41: 27-38

Baba D, Yoshida N, Katayama A (2007a) Effects of inhibitors on anaerobic microbial consortium with enhanced dechlorination activity in polychlorinated biphenyl mixture. Journal of Bioscience and Bioengineering 104: 268-274

Baba D, Katayama A (2007b) Enhanced anaerobic biodegradation of polychlorinated biphenyls in burnt soil culture. Journal of Bioscience and Bioengineering 104: 62-68

Baker JI, Hites RA (2000) Is combustion the major source of polychlorinated dibenzo-p-dioxins and dibenzofurans to the environment? A mass balance investigation. Environmental Science and Technology 34: 2879-2886

Ballerstedt H, Kraus A, Lechner U (1997) Reductive dechlorination of 1234-tetrachlorodibenzo-*p*-dioxin and its products by anaerobic mixed cultures from Saale River Sediment. Envrionmental Science and Technology 31: 1749-1753

Ballerstedt H, Hantke J, Bunge M, Werner B, Gerritse J, Andreesen JR, Lechner U (2004) Properties of a trichlorodibenzo-p-dioxin-dechlorinating mixed culture with a *Dehalococcoides* as putative dechlorinating species. FEMS Microbiology Ecology 47: 223-234

Barabás N, Gooverts P, Adriaens P (2004) Modified polytopic vector analysis to identify and quantify a dioxin dechlorination signature in sediments. 2. Application to the Passaic River. Environmental Science and Technology 38: 1821-1827

Barkovskii A, Fu Q, Adriaens P (1994) Biological and abiotic dechlorination of highly chlorinated PCDD/PCDFs: issues of bioavailability and pathways. Organohalogen Compounds 21: 469-473

Barkovskii AL, Adriaens P (1996) Microbial dechlorination of historically present and freshly spiked chlorinated dioxins and diversity of dioxin-dechlorinating populations Applied and Environmental Microbiology 62: 4556-4562

Barkovskii AL, Adriaens P (1998) Impact of humic constituents on microbial dechlorination of polychlorinated dioxins. Environmental Toxicology and Chemistry 17: 1013-1020

Bedard DL, May RJ (1996) Characterization of the polychlorinated biphenyls in the sediments of Woods Pond: Evidence for microbial dechlorination of Aroclor 1260 *in situ*. Environmental Science and Technology 30: 237-245

Bedard DL, Van Dort H, Deweerd KA (1998) Brominated biphenyls prime extensive microbial reductive dehalogenation of Aroclor 1260 in Housatonic River sediment. Applied and Environmental Microbiology 64: 1786-1795

Bedard DL, van Dort HM (1992) Abstract of ASM conference Anaerobic Dehalogenation and its Environmental Implications. Athens, GA, August; ASM: Materials Park, OH.

Bedard DL, Ritalahti, KM, Löffler FE (2007) The *Dehalococcoides* population in sediment-free mixed cultures metabolically dechlorinates the commercial polychlorinated biphenyl mixture Aroclor 1260. Applied and Environmental Microbiology 73: 2513-2521

Bedard DL, Quensen JF (1995) Microbial reductive dechlorination of polychlorinated biphenyls, in Microbial Transformation and Degradation of Toxic Organic Chemicals (Edited by L.Y.Young and C.Cerniglia), pp. 127-216. Wiley-Liss Division, John Wiley & Sons, Inc, New York

Bedard DL (2008) A case study for microbial biodegradation: anaerobic bacteria reductive dechlorination of polychlorinated biphenyls-from sediment to defined medium. Annual Review of Microbiology 62: 253-270

Berkaw M, Sowers KR, May HD (1996) Anaerobic *ortho* dechlorination of polychlorinated biphenyls by estuarine sediments from Baltimore Harbor. Applied and Environmental Microbiology 62: 2534-2539

Bertazzi PA, Bernucci I, Brambilla G, Consonni D, Pesatori AC (1998) The Seveso studies on early and long-term effects of dioxin exposure: a review. Environ Health Perspectives 106: 625-943

Beurskens JEM, Mol GAJ, Barreveld HL, van Munster B, Winkels HJ (1993) Geochronology of priority pollutants in a sedimentation area of the Rhine River. Environmental Toxicology and Chemistry 12: 1549-1566

Beurskens JEM, Stortelder PBM (1995) Microbial transformation of PCBs in sediments: What can we learn to solve practical problems? Water Science Technology 31: 99-107

Beurskens JEM, Toussant M, De Wolf J, van der Stee JMD, Slot PC, Commandeur LCM, Parsons JR (1995) Dehalogenation of chlorinated dioxins by an anaerobic microbial consortium from sediment. Environmental Toxicolology and Chemistry 14: 939-943

Birch GF, Harrington C, Symons RK, Hunt JW (2007) The source and distribution of polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofurans in sediments of Port Jackson, Australia. Marine Pollution Bulletin 54: 295-308

Boening DW (1998) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to several ecological receptor groups: a short review. Ecotoxicology Environment Safety 39: 155-163

Bopp RF, Gross ML, Tong H, Simpson HJ, Monson SJ, Deck BL, Moser FC (1991) A major incident of dioxin contamination: sediments of New Jersey estuaries. Environmental Science and Technology 25: 951-956

Brochu C, Moore S, Pelletier E (1995) Polychlorinated dibenzo-p-dioxins and dibenzofurans in sediments and biota of the Saguenay Fjord and the St Lawrence Estuary. Marine Pollution Bulletin 30: 515-523

Brown Jr JF, Wagner RE, Bedard DL, Brennan MJ, Carnahan JC, May RJ (1984) PCB transformations in upper Hudson sediments. Northeastern Environmental Science 3: 166-178

Brown Jr JF, Wagner RE, Feng H, Bedard DL, Brennan MJ, Carnahan JC, May RJ (1987) Environmental dechlorination of PCBs. Environmental Toxicology and Chemistry 6: 579-593

Brown Jr JF, Wagner RE (1990) PCB movement, dechlorination, and deteoxification in the Acushnet estuary. Environmental Toxicology and Chemistry 9: 1215-1233

Bruckmeier BFA, Juttner I, Schramm KW, Winkler R, Steinberg CEW, Kettrup A (1997) PCBs and PCDD/Fs in Lake sediments of Groβer Arbersee, Bavarian Forest, South Germany. Environmental Pollution 95: 19-25

Bunge M, Ballerstedt H, Lechner U (2001) Regiospecific dechlorination of spiked tetra- and trichlorodibenzo-p-dioxins by anaerobic bacteria from PCDD/F-contaminated Spittelwasser sediments. Chemosphere 43: 675-681

Bunge M, Lechner U (2009) Anaerobic reductive dehalogenation of polychlorinated dioxins. Applied Microbiol Biotech 84:429-444

Bunge M, Wagner A, Fischer M, Andreesen JR, Lechner U (2008) Enrichment of a dioixn-dehalogenating *Dehalococcoides* species in two-liquid phase cultures. Environmental Microbiology 10: 2670-2683

Bunge M, Adrian L, Kraus A, Opel M, Lorenz WG, Andreesen JR, Gorisch H, Lechner U (2003) Reductive dehalogenation of chlorinated dioxins by an anerobic bacteriu. Nature 421: 357-360

Buser HR, Bosshardt HP, Rappe C, Lindahl R (1978) Identification of polychlorinated dibenzofuran isomers in fly-ash and PCB pyrolysis. Chemosphere 7: 419-429

Chang YS (2008) Recent developments in microbial biotransformation and biodegradation of dioxins. Journal of Molecular Microbiology and Biotechnology 15: 152-171

Chen IM, Chang FC, Wang YS (2001) Correlation of gas chromatographic properties of chlorobenzenes and polychlorinated biphenyls with the occurrence of reductive dechlorination by untamed microorganisms. Chemosphere 45: 223-229

Cheung WH, Lee VKC, McKay G (2007) Minimizing dioxin emissions from integrated MSW thermal treatment. Environmental Science and Technology 41: 2001-2007

Clarke AN, Wilson DJ, Megehee MM, Lowe DL (1996) A review of 2,3,7,8-tetra chlorinated dibenzodioxins and toxicity equivalencies in fish in the United States and international waterways. Hazardous Waste & Hazardous Material 13:419-443

Cooper KR, Cristini A, Bergqvist P, Rappe C (1992). Bioavailability and bioconcentration of polychlorinated dioxins and furans to organisms inhabitating heavily contaminated estuarine ecosystems. Chemosphere 25: 25-28

Cutter LA, Sowers KR, May HD (1998) Microbial transformation of 2356-tetrachlorobiphenyl under anaerobic conditions in the absence of soil or sediment. Applied Environmental Microbiology 64: 2966-2969

Cutter LA, Watts JEM, Sowers KR, May HD (2001) Identification of a microorganism that links its growth to the reductive dechlorination of 2356-chlorobiphenyl. Environmental Microbiology 3: 699-709

Czuczwa JM, Hites RA (1984) Environmental fate of combustion-generated polychlorinated dioxins and furans. Environmental Science and Technology 18: 444-450

Dannenberger D, Andersson R, Rappe C (1997) Levels and patterns of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in surface sediments from the

western Baltic sea (Arkona Basin) and the Oder River Estuarine System. Marine Pollution Bulletin 34: 1016-1024

Dennis PC, Sleep BE, Fulthorpe RR, Liss SN (2003) Phylogenetic analysis of bacterial populations in an anaerobic microbial consortium capable of degrading saturation concentrations of tetrachloroethylene. Canadian Journal of Microbiology 49: 15-27

Deweerd K, Bedard DL (1999) Use of halogenated benzoates and other halogenated aromatic compounds to stimulate the microbial dechlorination of PCBs. Environmental Science and Technology 33: 2057-2063

Duhamel M, Mo K, Edwards EA (2004) Characterization of a highly enriched *Dehalococcoides*-containing culture that grows on vinyl chloride and trichloroethylene. Applied and Environmental Microbiology 70: 5538-5545

Eljarrat E, Caixach J, Rivera J (2001) Evaluation of dioxin contamination in sewage sludge discharges on coastal sediments from Catalonia, Spain. Water Research 35: 2799-2803

El-Kady AA, Abdel-Wahhab MA, Henkelmann B, Belal MH, Morsi MKS, Galal SM, Schramm K (2007) Polychlorinated biphenyl, Polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran residues in sediments and fish of the River Nile in the Cairo region. Chemsophere 68: 1660-1668

Esposito MP, Tiernan TO, Dryden FE (1980) Dioxins EPA/600/2-80-197

Ewald EM, Wagner A, Nijenhuis I, Richnow HH, Lechner U (2007) Microbial dehalogenation of trichlorinated dibenzo-*p*-dioxins by a *Dehalococcoides*-containing mixed culture is coupled to carbon isotope fractionation. Environmental Science and Technology 41: 7744-7751

Fagervold SK, Watts JEM, May HD, Sowers KR (2005) Sequential reductive dechlorination of *meta*-chlorinated PCB congeners in sediment microcosms by two different phylotypes of *Chloroflexi.* Applied Environmental Microbiology 71: 8085-8090

Fagervold SK, May D, Sowers KR (2007) Microbial reductive dechlorination of Aroclor 1260 in Baltimore Harbor sediment microcosms is catalyzed by three phylotypes within the Phylum *Chloroflexi*. Applied Environmental Microbiology 73: 3009-3018

Fennell DE, Nijenhuis I, Wilson SF, Zinder SH, Häggblom MM (2004) *Dehalococcoides ethenogenes* strain 195 reductively dechlorinate diverse chlorinated aromatic pollutants. Environmental Science and Technology 38: 2075-2081

Fennell DE, Carroll AB, Gossett JM, and Zinder SH (2001) Assessment of indigenous reductive dechlorinating potential at a TCE-contaminated site using microcosms, polymerase chain reaction analysis, and site data. Environmental Science and Technology 35: 1830-1839

Fiedler H (1996) Sources of PCDD/F and impact on the Environment. Chemosphere 32: 55-64

Fiedler H (1993) Formation and sources of PCDD/F. Organohalogen Compounds 11: 221-228

Fiedler H, Timms CW, Hutzinger O (1990) Dioxins: Sources of environmental load and human exposure. Toxicolology Environmental Chemistry 29: 157-234

Field JA, Sierra-Alvarez R (2008) Microbial degradation of chlorinated dioxins. Chemosphere 71: 1005-1018

Fish KM (1996) Influence of Aroclor 1242 concentration on polychlorinated biphenyl biotransformations in Hudson River test tube microcosms. Applied and Environmental Microbiology 62: 3014-3016

Frignani M, Bellucci LG, Carraro C, Favotto M (2001) Accumulation of polychlorinated dibenzo-p-dioxins and dibenzofurans in sediments of the Venice Lagoon and the industrial area of Porto Marghera. Marine Pollution Bulletin 42: 544-553

Fu QS, Barkovskii AL, Adriaens P (1999) Reductive transformation of dioxins: an assessment of the contribution of dissolved organic matter to dechlorination reactions. Environmental Science and Technology 33: 3837-3842

Fu QS, Barkovskii AL, Adriaens P (2001) Dioxin cycling in aquatic sediments: the Passaic River Estuary. Chemosphere 43: 643-648

Fu QS, Barkovskii A, Adriaens P (2005) Microbial dechlorination of dioxins in estuarine enrichment cultures: effects of respiratory conditions and priming compound on community structure and dechlorination patterns. Marine Environmental Research 59: 177-195

Fueno H, Tanaka K, Sugawa S (2002) Theoretical study of dechlorination reaction pathways of octachlorodibenzo-*p*-dioxin. Chemosphere 48: 771-778

Gao LR, Zheng MH, Zhang B, Liu WB, Zhao XR, Zhang QH (2008) Declining polychlorinated dibenzo-p-dioxins and dibenzofurans levels in the sediments from Dongting Lake in China. Chemosphere 73: S176-S179

Gaus C, Päpke O, Dennison N, Haynes D, Shaw GR, Connell DW, Müller JF (2001) Evidence for the presence of a widespread PCDD source in coastal sediments and soils from Queensland, Australia. Chemosphere 43: 549-558

Gevao B, Jaward FM, Uddin S, Al-Ghadban AN (2009) Occurrence and concentrations of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in coastal marine sediments in Kuwait. Marine Pollution Bulletin 58: 424-455

Gilbride KA, Lee DY, Beaudette LA (2006) Molecular techniques in wastewater: understanding microbial communities, detecting pathogens, and real-time process control. Journal of Microbial Methods 66: 1-20

Götz R, Steiner B, Friesel P, Roch K, Walkow F, Maaβ V, Reincke H, Stachel B (1998) Dioxin (PCDD/F) in the river Elbe –investigations of their origin by multivariate statistical methods. Chemosphere 37: 1987-2002

Götz R, Bauer OH, Friesel P, Herrmann T, Jantzen E, Kutzke M, Lauer R, Paepke O, Roch K, Rohweder U, Schwartz R, Sievers S, Stachel B (2007) Vertical profile of PCDD/Fs, dioxin-like PCBs, other PCBs, PAHs, chlorobenzenes, DDX, HCHS, organotin compounds and chlorinated ethers in dated sediment/soil cores from flood-plains of the river Elbe, Germany. Chemosphere 67: 592-603

Green, N.J.L, Jones, J.L, Johnston, A.E, Jones, K.C. Further evidence for the existence of PCDD/Fs in the enironment prior to 1900. Environmental Science and Technology 2001, 35, 1974-1981.

Gruden CL, Fu QS, Barkovskii AL, Albrecht ID, Lynam MM, Adrians P (2003) Dechlorination of sediment dioxins: catalysts, mechanisms, and implications for remedial strategies and dioxin cycling. Dehalogenation: Microbial Processes and Environmental Applications (Häggblom MM & Bossert ID, eds), pp. 347-372, Kluwer Academic Publishers, Boston, MA.

Gu AZ, Hedlund BP, Staley JT, Strand SE, Stensel HD (2004) Analysis and comparison of the microbial community structures of two enrichment cultures capable of reductively dechlorinating TCE and *cis*-DCE. Environmental Microbiology 6: 45-54

Habe H, Chung JS, Lee JH, Kasuga K, Yoshida T, Nojiri H, Omori T (2001) Degradation of chlorinated dibenzofurans and dibenzo-*p*-dioxins by two types of bacteria having angular dioxygenases with different features. Applied and Environmental Microbiology 67: 3610-3617

Häggblom MM, Ahn YB, Fennell DE, Kerkhof LJ, Rhee SK (2003) Anaerobic dehalogenation of organohalide contaminants in the marine environment. Advance Appllied Microbiology 53: 61-84

Häggblom MM, Nohynek LJ, Salkinoja-Salonen MS (1988) Degradation and o-methylation of chlorinated phenolic compounds by *Rhodococcus* and *Mycobacterium* strains. Applied and environmental microbiology 54:3043-3052

Hagenmaier H, Brunner H (1987) Isomer specific analysis of pentachlorophenol and sodium pentachlorophenate for 2378-substituted PCDD and PCDF at sub-ppb levels. Chemosphere 16: 1759-1764

Harding LE, Pomerory WM (1990) Dioxin and furan levels in sediments, fish and invertebrates from fishery closure areas of coastal British Columbia, Environ. Can. Reg. Data Rep. 90-09, North Vancouver, British Columbia

Hartkamp-Cammandeur LCM, Gerritse J, Govers HAJ, Parsons JR (1996) Reductive dehalogenation of polychlorinated biphenyls by anaerobic microorganisms enriched from Dutch sediments. Chemosphere 32 1275-1286

Hart J, Holdren C, Schneider R, Shirley C (1991) Toxics A to Z: A guide to everyday pollution hazards, University of California Press, Berkeley and Los Angeles, CA

Haws LC, Su SH, Harris M, DeVito MJ, Walker NJ, Farland WH, Finley B, Birnbaum LS (2006) Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. Toxicological Science 89: 4-30

He J, Ritalahti KM, Aiello MR, Loffler FE (2003) Complete detoxification of vinyl chloride by an anaerobic enrichment culture and identification of the reductively dechlorinating population as a *Dehalococcoides* species. Applied and Environmental Microbiology 69: 996-1003

He J, Robrock KR, Alvarez-Cohen L (2006) Microbial reductive debromination of polybrominated diphenyl ethers (PBDEs). Environmental Science and Technology 40: 4429-4434

Hendrickson ER, Payne JA, Young RM, Starr MG, Perry MP, Fahnestock S, Ellis DE, Ebersole RC (2002) Molecular analysis of *Dehalococcoides* 16S ribosomal DNA from chloroethene-contaminated sites throughout North America and Europe. Applied and Environmental Microbiology 68: 485-495

Hiraishi A, Kaiya S, Miyakoda H, Futamata H (2005) Biotransformation of polychlorinated dioxins and microbial community dynamics in sediment microcosms at different contamination levels. Microbes and Environments 20, 227-242

Hiraishi A, Miyakoda H, Lim BR, Hu HY, Fujie K, Suzuki J (2001) Toward the bioremediation of dioxin-polluted soil: structural and functional analyses of *in situ* microbial populations by quinone profiling and culture-dependent methods. Applied Microbiology and Biotechnology 57: 248-256

Hites RA (2010) Dioxin: an overview and history. Environmental Science and Technology in press

Hoekstra EJ, Weerd H, Leer EWB, Brinkman UA (1999) Natural formation of chlorinated phenols, dibenzo-p-dioxins, and dibenzofurans in soil of a Douglas fir forest. Environmental Science and Technology 33: 2543-2549

Hoffman DJ, Rattner BA, Burton GA, Cairnes J (1995) Handbook of ecotoxicology, CRC Press, Boca Raton, FL

Holliger C, Wohlfahrt G, Diekert G (1999) Reductive dechlorination in the energy metabolism of anaerobic bacteria. FEMS Microbiology Review 22: 383-398

Holoman TR, Elberson MA, Cutter LA, May HD, Sowers KR (1999) Charaterization of a defined 2356-tetrachlorobiphenyl-*ortho*-dechlorinating microbial community by comparative sequence analysis of genes coding for 16S rRNA. Applied and Enviromental Microbiology 64: 3359-3367

Hölscher T, Krajmalnik-Brown R, Ritalahti K, Von Wintzingerode F, Görisch H, Löffler F, Adrian L (2004) Multiple nonidentical reductive-dehalogenase-homologous genes are common in *Dehalococcoides*. Applied Environmental Microbiology 70: 5290-5297

Hong HB, Chang YS, Nam IH, Fortnagel P, Schmidt S (2002) Biotransformation of 2,7-dichloro- and 1234-tetrachlorodibenzo-*p*-dioxin by *Sphingomonas wittichi* RW1. Applied and Environmental Microbiology 68: 2584-2588

Hong HB, Nam IH, Murugesan K, Kim YM, Chang YS (2004) Biodegradation of dibenzo-*p*-dioxins by *Pseudomonas veronii* PH-03. Biodegradation 15: 303-313

Hori T, Noll M, Igarashi Y, Friedrich MW, Conrad R (2007) Identification of acetate-assimilating microorganisms under methanogenic conditions in anoxic rice field soil by comparative stable probing of RNA. Applied Environmental Microbiology 73: 101-109

Howard PH (1991) Handbook of Environmental Fate and Exposure Data for Organic Chemicals. Volume III. Pesticides; Lewis Publisher: Chelsea, MI., pp 549-558

Huang CL, Harrison BK, Madura J, Dolfing J (1996) Gibbs free energies of formation of PCDDs, evaluation of estimation methods and application for predicting dehalogenation pathways. Environmental Toxicology and Chemistry 15, 824-836

Hutzinger O, Fiedler H (1988a) Formation of dioxins and related compounds in combustion processes, Pilot Study on international information exchange on dioxins and related compounds, report number 172, August, North Atlantic Treaty Organization, Committee on Challenges of Modern Society, Brusseis, Belgium;

Hutzinger O, Fiedler H (1988b), Formation of dioxins and related compounds in industrial processes, Pilot study on international information exchange on dioxins and related compounds, report number 173, August, North Atlantic Treaty Organization, Committee on challenges of modern society, Brussels, Belgium;

Im SH, Kannan K, Matsuda M, Giesy JP, Wakimoto T (2002) Sources and distribution of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in sediments from Masan Bay, Korea. Environmental Toxicology and Chemistry 21: 245-252

Ingersoll CG, Dillon T, Biddinger GR (1997) Ecological risk assessment of contaminated sediments, SETAC press, Pensacola, FL

Jansson B, Sundström G, Ahling B (1978) Formation of polychlorinated dibenzo-*p*-dioxins during combustion of chlorophenol formulations. Science of the total environment 10: 209-217

Jayachandran G, Görisch H, Adrian L (2003) Dehalorespiration with penta- and hexachlorobenzene by *Dehalococcoides* sp. strain CBDB1. Archive of Microbiology, 180: 411-416

Kao CM, Chen SC, Liu JK, Wu MJ (2001) Evaluation of tetra-CDD bioavailability under different redox conditions. Chemosphere 44: 1447-1454

Kassenga G, Pardue JH, Moe WM, Bowman KS (2004) Hydrogen thresholds as indicators of dehalorespiration in constructed treatment wetlands. Environmental Science and Technology 38: 1024-1030

Kerkvliet NI (2002) Recent advances in understanding the mechanisms of tetra-CDD immunotoxicity. International Journal of Immunopharmacology 2: 277-291

Kim J, Rhee GY (1997) Population dynamics of polychlorinated biphenyl-dechlorinating microorganisms in contaminated sediments. Applied and Environmental Microbiology 63: 1771-1776

Kim J, Rhee GY (1999) Reductive dechlorination of polychlorinated biphenyls: interactions of dechlorinating microorganisms with methanogens and sulfate reducers. Enviromental Toxicology and Chemistry 18: 696-2702

Kiguchi O, Kobayashi T, Wada Y, Saitoh K, Ogawa, N (2007) Polychlorinated dibenzo-p-dioxins and dibenzofurans in paddy soils and river sediments in Akita, Japan. Chemosphere 67: 557-573

Kjeller LO, Rappe C (1995) Time trends in levels, patterns, and profiles for polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in a sediment core from the Baltic Proper. Environmental Science and Technology 29: 346-355

Kjellerup BV, Sun X, Ghosh U, May HD, Sowers KR (2008) Site specific microbial communities in three PCB-impacted sediments are associated with different *in situ* dechlorinating activities. Environmental Microbiology 10: 1296-1309

Koester JC, Hites RA (1992a) Wet and dry deposition of chlorinated dioxins and furans. Environmental Science and Technology 26: 1375-1382

Koester JC, Hites RA (1992b) Photodegradation of polychlorinated dioxins and dibenzofurans absorbed to fly ash. Environemntal Science and Technology 26: 502-507

Koh CH, Khim JS, Kannan K, Villeneuve DL, Senthilkuman K, Giesy JP (2004) Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs) and polychlorinated aromatic hydrocarbons (PAHs) in sediment from the Hyeongsan River, Korea. Environmental Pollution 132: 489-501

Koistinen L (1990) Residues of planar polychloroaromatic compounds in Baltic fish and seal. Chemosphere 20: 1043-1048

Koistinen J, Paasivirta J, Suonpera M, Hyvarinen H (1995) Contamination of pike and sediment from the Kymijoki River by PCDEs, PCDDs and PCDFs: Contents and patterns compared to pike and sediment from the Bothnian Bay and seals from Lake Saimaa. Environmental Science and Technology 29: 2541-2547

Kube M, Beck A, Zinder S, Kuhl H, Reinhardt R, Adrian L (2005) Genome sequence of the chlorinated compound-respiring bacterium *Dehalococcoides* species strain CBDB1. Nature Biotechnology 23: 1269-1273

Kulkarni PS, Crespo JG, Afonso CAM (2008) Dioxins sources and current remediation technologies---A review. Environmental International 34: 139-153

Kunapuli U, Lueders T, Meckenstock RU (2007) The use of stable isotope probing to identify key iron-reducing microorganisms involved in anaerobic benzene degradation. ISME J 1: 643-653

Kuo C, Liu S, Liu C (1999) Biodegradation of coplanar polychlorinated biphenyls by anaerobic microorganisms from estuarine sediments. Chemosphere 39: 1445-1458

Krumins V, Park JW, Son EK, Rodenburg LA, Kerkhof LJ, Häggblom MM, Fennell DE (2009) PCB dechlorination enhancement in Anacostia River sediment microcosms. Water Research 43: 4549-4558

LaFleur L, Brunck B, McDonough T, Ramage K, Gillespie W, Malcolm E (1990) Studies on the mechanism of PCDD/PCDF formation during the bleaching of pulp. Chemosphere 20: 1731-1738

Lee LP, He J (2010) Reductive debromination of polybrominated diphenyl ethers by anaerobic bacteria from soils and sediments. Applied and Environmental Microbiology 76: 794-802

Lièvremont D, Murandi FS, Guyod JL (1996) Effects of culture parameters on pentachloronitrobenzene removal by *Sporothrix cyanescens*. Chemosphere 32: 361-375

Liu F, Cichocka D, Nijenhuis I, Richnow H, Fennell DE (2010) Carbon isotope fractionation during dechlorination of 1234-tetrachlorodibenzo-*p*-dioxin by a *Dehalococcoides*-containing culture. Chemosphere in press

Liu F, Fennell DE (2008) Dechlorination and detoxification of 123478-hexa-CDF by a mixed culture containing *Dehalococcoides ethenogenes* strain 195. Environmental Science and Technology 42: 602-607

Liu J, Liu W (2009) Distribution of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dioxin-like PCBs) in the soil in a typical area of eastern China. Journal of Hazardous Materials 163: 959-966

Löffler FE, Sun Q, Li J, Tiedje, JM (2000) 16S rRNA gene-based detection of tetrachloroethene-dechlorinating *Desulfuromonas* and *Dehalococcoides* species. Applied and Environmental Microbiology 66: 1369-1374

Lohmann R, Jones KC (1998) Dioxins and furans in air and deposition: A review of levels, behavior and processes. Science of the total Environment 219: 53-81

Lopes TJ, Furlong ET (2001) Occurrence and potential adverse effects of semivolatile organic compounds in streambed sediment, United States, 1992-1995. Environmental Toxicology and Chemistry 20: 727-737

Lynam MM, Kuty M, Damborsky J, Koca J, Adriaens P (1998) Molecular orbital calculations to describe microbial reductive dechlorination of polychlorinated dioxins. Environmental Toxicology and Chemistry 17: 988-997

Lundin L, Marklund S (2007) Thermal degradation of PCDD/F, PCB and HCB in municipal solid waste ash. Chemosphere 67: 474-481

Mackay D, Shiu WY, Ma KC (1992) Illustrated Handbook of physical-chemical properties and environmental fate for organic chemicals Monoaromatic hydrocarbons, chlorobenzenes and PCBs Vols 1 and 2. Lewis Publishers, Chelsea, MI.

Magar VS, Brenner RC, Johnson GW, Quensen III JF (2005) Long-term recovery of PCB-contaminated sediments at the lake Hartwell Superfund site: PCB

dechlorination. 2. Rates and Extent. Environmental Science and Technology 39: 3548-3554

Maji S, Ishihara A (2004) Screening of tetrachlorodibenzo-*p*-dioxin-degrading fungi capable of producing extracellular peroxidases under various conditions. Applied and Microbiological Biotechnology 63: 438-444

Major DW, McMaster ML, Cox EE, Edwards EA, Dworatzek SM, Hendrickson ER, Starr MG, Payne JA, Buonamici LW (2002) Field emonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene. Environmental Science and Technology 36: 5106-5116

Malve O, Salo S, Verta M, Forsius J (2003) Modeling the transport of PCDD/F compounds in a contaminated river and the possible influence of restoration dredging on calculated fluxes. Environmental Science and Technology 37: 3413-3421

Masunaga S, Takasuga T, Nakanishi J (2001) Dioxin and dioxin-like PCB impurities in some Japanese agrochemical formulations. Chemosphere 44: 873-885

Maymó-Gatell X, Chien Y, Gossett JM, Zinder SH (1997) Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethane. Science 276: 1568-1571

May HD, Miller GS, Kjellerup BV, Sowers KR (2008) Dehalorespiration with polychlorinated biphenyls by an anaerobic ultramicrobacterium. Applied and Environmental Microbiology 74: 2089-2094

May HD, Cutter LA, Miller GS, Milliken CE, Watts JEM, Sowers KR (2006) Stimulatory and inhibitory effects of organohalides on the dehalogenating activities of PCB-dechlorinating Bacterium *o*-17. Environmental Science and Technology 40: 5704-5709

Meharg AA, Osborn D (1995) Dioxins released from chemical accidents. Nature 375: 353-354

Mohammed A, Orazio C, Peterman P, Echols K, Feltz K, Manoo A, Maraj D, Agard J (2009) Polychlorinated dibenzo-p-dioxin (PCDDs) and polychlorinated dibenzofurans (PCDFs) in Harbor sediments from Sea Lots, Port-of-Spain, Trinidad and Tobago. Marine Pollution Bulletin 58: 928-946

Mori T, Kondo R (2002) Oxidation of dibenzo-*p*-dioxin, dibenzofuran, biphenyl, and diphenyl ether by the white-rot fungus *Phlebia lindtneri*. Applied and Microbiological Biotechnology 60: 200-205

Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Enviornmental Micorbiology 59: 695-700

Nakai S, Kishita S, Nomura Y, Hosomin M (2007) Polychlorinated dibenzothiophenes in Japanese environmental samples and their photodegradability and dioxin-like endocrine-disruption potential. Chemosphere 67: 1852-1857

Nessel CS, Butler JP, Post GB, Held JL, Gochfeld M, Gallo MA (1991) Evaluation of the relative contribution of exposure routes in a health assessment of dioxin emissions from a municipal waste incinerator. Journal of exposure analysis and environmental epidemiology 1: 283-307

Nie L, Vogel TM (1990) Effects of organic substrates on dechlorination of Aroclor 1242 in anaerobic sediments. Applied and Environmental Microbiology 56: 2612-2617

Nubel U, Garcia-Pichel F, Muyzer G (1997) PCR primers to amplify 16S rRNA genes from cyanobacteria. Applied Environmental Microbiology 63: 3327-3332

Okutman Tas D, Pavlostathis SG (2005) Microbial reductive transformation of pentachloronitrobenzene under methanogenic conditions. Environmental Science and Technology 39: 8264-8272

Pan J, Yang Y, Geng C, Yeung LWY, Cao X, Dai T (2010) Polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins and dibenzofurans in marine and lacustrine sediments from the Shandong Peninsula, China. Journal of Hazardous Materials 176: 274-279

Poland A, Knutson JC (1982) 2378-Tetrachlorodibenzo-para-dioxin and related halogenated aromatic-hydrocarbons-examination of the mechanism of toxicity. Annual Review of Pharmacology and Toxicology 22: 517-554

Quensen III JF, Boyd SA, Tiedje JM (1988) Redeuctive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. Science 242: 752-754

Quensen III JF, Boyd SA, Tiedje JM (1990) Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. Applied and Environmental Microbiology, 56: 2360-2369

Quensen III JF, Tiedje JM (1997) Evaluation of PCB dechlorination in sediments. In *Methods in Biotechnology: Bioremediation Protocols;* Sheehan, D., Ed, Humana Press: Totowa, NJ, pp 257-273

Rappe C (1993) Sources of exposure, environmental concentrations and exposure assessment of PCDD and PCDFs. Chemosphere 27: 211-225

Ren M, Peng P, Chen D, Chen P, Li X (2009) Patterns and sources of PCDD/Fs and dioxin-like PCBs in surface sediments from the East River, China. Journal of Hazardous Materials 170: 473-478

Rhee GY, Bush B, Bethoney CM, DeNucci A, Oh HM, Sokol RC (1993) Reductive dechlorination of Aroclor 1242 in anaerobic sediments: pattern, rate and concentration dependence. Environmental Toxicology and Chemistry 12: 1025-1032

Richardson RE, Bhupathiraju VK, Song DL, Goulet TA, Alvarez-Cohen L (2002) Phylogenetic characterization of microbial communities that reductively dechlorinate TCE based upon a combination of molecular techniques. Environmental Science and Technology 36: 2652-2662

Robrock KR, Korytár P, Alvarez-Cohen L (2008) Pathways for the anaerobic microbial debromination of polybrominated diphenyl ethers. Environmental Science and Technology 42: 2845-2852

Rotard W, Christmann W, Knoth W (1994) Background levels of PCDD/F in soils of Germany. Chemosphere 29: 2193-2200

Rose CL, McKay WA (1996) PCDDs (Dioxins) and PCDFs (furans) in selected UK lake and reservoir sites-concentrations and TEQs in sediment and fish samples. The Science of the Total Environment 177; 43-56

Rysavy JP, Yan T, Novak PJ (2005) Enrichment of anaerobic polychlorinated biphenyl dechlorinators from sediment with iron as a hydrogen sources. Water Research 39: 569-578

Schettler T, Soloman G, Valenti M, Huddle A (1999) Generations at Risk, MIT Press, Cambridge, Massachusetts

Schecter A, Dai LC, Papke O, Prange J, Constable JD, Matsuda M, Thao VD, Piskac AL (2001) Recent Dioxin contamination from Agent Orange in residents of a Southern Vietnam City. Journal of Occupational and Environmental Medicine 43: 435-443

Seshadri R, Adrian L, Fouts DE, Eisen JA, Philippy AM, Methe BA, Ward NL, Nelson WC, Deboy RT, Khouri HM, Kolonay JF, Dodson RJ, Daugherty SC, Brinkac LM, Sullivan SA, Madupu R, Nelson KE, Kang KH, Impraim M, Tran K, Robinson JM, Forberger HA, Fraser CM, Zinder SH, Heidelberg JF (2005) Genome sequence of the PCE-dechlorinating bacterium *Dehalococcoides ethengogenes*. Science 307: 105-108

She J, Hagenmaier H (1996) Levels and fate of PCDD/Fs in soil around Rastatt in Southwest Germany. Organohalogen Compounds 28: 31-34

Shen L, Gewurtz SB, Reiner EJ, MacPherson KA, Kolic TM, Helm PA, Brindle ID, Marvin CH (2008) Patterns and sources of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in surficial sediments of Lakes Erie and Ontario. Environmental Pollution 156: 515-525

Shen L, Gewurtz SB, Reiner EJ, MacPherson KA, Kolic TM, Khurana V, Helm PA, Howell ET, Bruniston DA, Brindle ID, Marvin CH (2009) Occurrence and sources of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls in surficial sediments of Lake Superior and Huron. Environmental Pollution 157: 1210-1218

Shiu WY, Doucette W, Gobas FAPC, Andren A, Mackay D (1988) Physical-chemical properties of chlorinated dibenzo-p-dioxins. Environmental Science and Technology 22: 651-658

Silk PJ, Lonergan GC, Arsenault TL, Boyle CD (1997) Evidence of natural organochlorine formation in peat bogs. Chemosphere 35: 2865-2880

Smidt H, Akkermans ADL, van der Oost J, de Vos WM (2000) Halorespiring bacteria-molecular characterization and detection. Enzyme Microbiology Technology 27: 812-820

Smits TH, Devenoges C, Szynalski K, Maillard J, Holliger C (2004) Development of a real-time PCR method for quantification of the three genera *Dehalobacter, Dehalococcoides,* and *Desulfitobacterium* in microbial communities. Journal of Microbiological Methods 57: 369-378

Sokol RC, Bethoney CM, Rhee GY (1995) Effect of PCB concentration on reductive dechlorination and dechlorination potential in natural sediments. Water Research 29: 45-48

Stapleton RD, Ripp S, Jimenez L, Cheol-Koh S, Fleming JT, Gregory IR, Sayler GS (1998) Nucleic acid analytical approaches in bioremediation: site assessment and characterization. Journal of Microbiological Methods 32, 165-178

Stellman JM, Stellman SD, Christian R, Weber T, Tomasallo C (2003) The extent and patterns of usage of Agent Orange and other herbicides in Vietnam. Nature 422: 681-687

Suarez MP, Rifai HS, Palachek R, Dean K, Koenig L (2006) Distribution of polychlorinated dibenzo-p-dioxins and dibenzofurans in suspended sediments, dissolved phase and bottom sediment in the Houston Ship Channel. Chemosphere 62: 417-429

Suflita JM, Gibson SA, Beeman RE (1988) Anaerobic biotransformations of pollutant chemicals in aquifers. Journal Industry Micriobiology 3: 179-194

Susarla S, Masunaga S, Yonezawa Y (1996) Transformations of chloronitrobenzenes in anaerobic sediment. Chemosphere 32: 967-977

Tamura K; Hasegawa Y, Kudo T, Yamaguchi I (1995) Isolation and characterization of PCNB degrading bacterium, *Pseudomonas aeruginosa* strain I-41. Journal of Pesticide Science 20: 145-151

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary generic analysis (MEGA) software version 4.0. Molecular Biolology Evolution 24: 1596-1599

Takada S, Nakamura M, Matsueda T, Kondo R, Sakai K (1996) Degradation of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans by the white rot fungus *Phanerochaete sordida* YK-624. Applied and Environmental Microbiology 62: 4323-4328

Terauchi H, Takahashi S, Lam PKS, Min BY, Tanabe S (2009) Polybrominated, polychlorinated and monbromo-polychlorinated dibenzo-p-dioxins/dibenzofurans and dioxin-like polychlorinated biphenyls in marine surface sediments from Hongkong and Korea. Environmental Pollution 157: 724-730

Thomas VM, Spiro TG (1996) The U.S. dioxin inventory: Are there missing sources? Environmental Science and Technology 30: 82A-85A

Toussaint M, van Herwijnen R, Parsons JR (1998) Anaerobic bacterial dehalogenation of PCDD/F. In: Biodegradation of dioxins and furans (Wittich, R., -M., Ed.) Springer, Berlin, pp. 29-60

Tysklind M, Fängmark I, Marklund S, Lindskog A, Thaning L, Rappe C (1993) Atmospheric transport and transformation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Environmental Science and Technology 27: 2190-2197

US Environmental Protection Agency (2003) Waste Minimization Priority Chemicals and Chemical Fact Sheets Website (http://www.epa.gov/epaoswer/hazwaste/minimize/chemlist.htm); office of Solid Waste and Emergency Response: Washinnton, DC.

US Environmental Protection Agency (2006) Reregistration Eligibility Decision for Pentachloronitrobenzene, List A, Case No. 0128; U.S. EPA, Office of Prevention, Pesticides and Toxic Substances: Washington DC.

Vali K, Wariishi H, Gold MH (1992) Degradation of 2,7-dichlorodibenzo-*p*-dioxin by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Journal of Bateriol 174: 2131-2137

Van Dort HM, Bedard DL (1991) Reductive *ortho* and *meta* dechlorination of a polychlorinated biphenyl congener by anaerobic microorganisms. Applied and environmental microbiology 57: 1576-1578

Van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Freely M, Giesy J.P, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FXR, Liem AKD, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environmental Health Persepect 106: 775-792

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE (2006) The 2005 World

Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicological Science 93: 223-241

Vargas C, Fennell DE, Häggblom MM (2001) Anaerobic reductive dechlorination of chlorinated dioxins in estuarine sediments. Applied Microbiology and Biotechnology 57: 786-790

Verta M, Lehtoranta J, Salo S, Korhonen M, Kiviranta H (1999) High concentrations of PCDD and PCDF in river Kymijoki sediments, south-eastern Finland, caused by wood preservative Ky-5. Organohalogen Compounds 43: 261-264

Verkasalo PK, Kokki E, Pukkala E, Vartiainen T, Kiviranta H, Penttinen A, Pekkanen J (2004) Cancer risk near a polluted river in Finland. Environmental Health Perspectives 112: 1026-1031

Wagrowski DM, Hites RA (2000) Insights into the global distribution of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Environmental Science and Technology 34: 2952-2958

Wehrmeier A, Lenoir D, Schramm KW, Zimmermann R, Hahn K, Henkelmann B, Kettrup A (1998) Patterns of isomers of chlorinated dibenzo-*p*-dioxins as tool for elucidation of thermal formation mechanisms. Chemosphere 36: 2775-2801

Wenning RJ, Harris MA, Ungs MJ, Paustenbach DJ, Bedbury H (1992) Chemometric comparisons of polychlorinated dibenzo-p-dioxin and dibenzofuran residues in surficial sediments from Newark Bay, New Jersey and other industrialized waterways. Archives of Environmental Contamination Toxicology 22: 397-413

Wiegel J, Wu Q (2000) Microbial reductive dehalogenation of polychlorinated biphenyls. FEMS Microbiology Ecology 32: 1-15

Williams WA (1997) Stimulation and enrichment of two microbial polychlorinated biphenyl reductive dechlorination activities. Chemosphere 34: 655-669

Winderi C, Anneser B, Griebler G, Meckenstock RU, Lueders T (2008) Depth-resolved quantification of anaerobic toluene degraders and aquifer microbial community patterns in distinct redox zones of a tar oil contaminant plume. Applied Environmental Microbiology 74: 792-801

Wittich RM (1998a) Aerobic degradation by bacteria of dibenzo-*p*-dioxins, dibenzofurans, diphenyl ethers and their halogenated derivatives. In: Wittich RM (ed) Biodegradation of dioxins and furans. Springer, Berlin, pp 1-28

Wittich RM (1988b) Degradation of dioxin-like compounds by microorganisms. Applied and Microbiolgocial Biotechnology 49: 489-499

Witt G, Schramm KW, Henkelmann B (1997) Occurrence and distribution of polychlorinated dibenzo-p-dioxins and dibenzofurans in sediments of the western Baltic Sea. Chemosphere 35: 1465-1473

Wu Q, Sowers KR, May HD (2002a) Identification of a bacterium that specifically catalyzes the reductive dechlorination of polychlorinated biphenyls with doubly flanked chlorines. Applied and Environmental Microbiology 68: 807-812

Wu Q, Milliken CE, Meier GP, Watts JEM, Sowers KR, May HD (2002b) Dechlorination of chlorobenzenes by a culture containing bacterium DF-1, a PCB dechlorinating microorganism. Environmental Science and Technology 36: 3290-3294

Wu Q, Bedard QL, Wiegel J (1997) Temperature determines the pattern of anaerobic microbial reductive dechlorination of Aroclor 1260 primed by 2346-

tetrachlorobiphenyl in Woods Pond sediment. Applied and Environmental Microbiology 63: 4818-4825

Wu Q, Bedard DL, Wiegel J (1999) 2,6-dibromobiphenyl primes extensive dechlorination of Aroclor 1260 in contaminated sediment at 8-30 °C by stimulating growth of PCB-dehalogenating microorganisms. Environmental Science and Technology 33: 595-602

Yan T, LaPara TM, Novak PJ (2006) The effect of varying levels of sodium bicarbonate on polychlorinated biphenyl dechlorination in Hudson River sediment cultures. Environmental Microbiology 8: 1288-1298

Yan T, LaPara TM, Novak PJ (2006) The reductive dechlorination of 2345-tetrachlorobiphenyl in three different sediment cultures: evidence for the involvement of phylogenetically similar *Dehalococcoides*-like bacterial populations. FEMS Microbiology Ecology 55: 248-261

Ye D, Quensen III JF, Tiedje JM, Boyd SA (1992) Anaerobic dechlorination of polychlorinated biphenyls (Aroclor 1242) by pasteurized and ethanol-treated microorganisms from sediment. Applied and environmental Microbiology 58: 1110-1114

Ye D, Quensen III JF, Tiedje JM, Boyd SA (1995) Evidence for *para* dechlorination of polychlorinatedbiphenyls by methanogenic bacteria. Applied and Environmental Microbiology 61: 2166-2171

Persson Y (2007) Chlorinated Organic Pollutants in Soil and Groundwater at Chlorophenol-Contaminated Sawmill Sites, Ph.D. Thesis, Department of Chemistry, Environmental Chemistry Umea University, Umea.

Yoshida N, Takahashi N, Hiraishi A (2005) Phylogenetic characterization of a polychlorinated-dioxin-dechlorinating microbial community by use of microcosm studies. Applied and environmental microbiology 71: 4325-4334

Zanaroli G, Balloi A, Negroni A, Daffonchio D, Young LY, Fava F (2010) Characterization of the microbial community from the marine sediment of the Venice lagoon capable of reductive dechlorination of coplanar polychlorinated biphenyls (PCBs). Journal of Hazardous Materials 2010 in press

Zhang QH, Jiang GB (2005) Polychlorinated dibenzo-p-dioxins/furans and polychlorinated biphenyls in sediments and aquatic organisms from the Taihu Lake, China. Chemosphere 61: 314-322

Zwiernik MJ, Quensen JF, Boyd SA (1998) $FeSO_4$ amendments stimulate extensive anaerobic PCB dechlorination. Environmental Science and Technology 32: 3360-3365

Appendix 1. Raw data of Kymijoki sediment mesocosm. M.W.: molecular weight, R.T.: retention time (min), std: standard, Live: no addition control, ED: electron donors only, ED195: electron donors plus bioaugmentation with strain 195, EDTeCB: amendment with electron donors and TeCB, sed D.W.: sediment dry weight (g).

Table A-1. Raw data of primary ions of major PCDF congeners in Kymijoki sediment mesocosms at year 0.

						Sed D.W. (g)	1.39	1.06	0.99	1.27	1.31	0.94	0.89	1.29	1.22	1.26	1.19	1.01
		PCB S (ppb)	70	35	17.5	PCB S (ng)	70	70	70	70	70	70	70	150	70	70	70	70
		PCB IS (ppb)	12.5	25	50	PCB IS (ng)	25	25	25	25	25	25	25	25	25	25	25	25
M.W.	R.T.	Name	PCB std 1	PCB std 2	PCB std 3	PCB std2 R	Live-1	Live-2	Live-3	ED-1	ED-2	ED-3	ED195-1	ED195-2	ED195-3	EDTeCB-1	EDTeCB-2	EDTeCB-3
222	21.906	PCB 14	7429	3731	1861	4180	16132	19836	22406	21426	21728	20813	16437	20723	13147	20617	29115	20789
256	22.82	PCB 30	1391	2775	5575	3098	4043	3320	4266	3588	3968	3701	3930	4382	3745	3683	4465	4314
256	25.561	PCB 23	9279	4609	2316	5236	17766	21652	24578	22632	24225	23653	18804	23636	15296	23619	34269	24003
292	29.615	PCB 65	4102	2090	1114	2426	9262	12514	11794	11876	13819	12623	9820	14121	8186	13189	20946	14669
360	48.118	PCB 166					8977	10927	12051	11465	12222	11904	9923	12010	8008	11643	16834	12078
430	53.03	PCB 204	559	1120	2255	1538	1843	1610	1984	1689	1850	1706	1864	2032	1734	1662	1963	2032
374	60.97	123468-HxCDF					1987	1874	2282	1350	2351	1120	5267	1162	1878	2731	2193	857
	61.37	124678-HxCDF					4838	4602	5887	2930	6476	3522	15436	4698	3403	9012	5702	2333
	62.62	124689-HxCDF					18400	24324	21296	11986	31270	16852	59871	16115	15426	56319	22108	10218
	63.14	123689-HxCDF					877	2604	750	617	2529	757	1453	709	562	733	641	863
	63.65	HxCDF-5					247	302	291	271	373	298	483	435	497	291	408	387
	64.05	123478-HxCDF					192	332	216	168	452	240	254	147	27	161	182	178
	65.31	234678-HxCDF					326	480	302	223	613	298	528	240	226	226	240	332
	67.71	HxCDF-8					507	959	661	295	874	353	617	267	206	463	260	428
408	72.732	1234678-HpCDF					44045	30821	29438	21532	73197	27984	77689	70541	37003	41162	40964	23612
	73.931	1234689-HpCDF					49239	41399	42348	26244	74842	35821	86431	60869	31462	72612	41773	25349
444	86.267	OCDF					27309	21772	14775	13230	43794	17616	45299	38123	16153	22923	13158	13689
306	43.84	TeCDF-1					699	1786	1066	709	2166	850	1628	744	802	651	929	716
	46.98	TeCDF-2					243	278	367	291	603	206	507	367	230	295	161	188
	47.66	TeCDF-3					548	1018	757	603	1285	641	1333	685	641	524	768	637
	48.23	TeCDF-4					555	668	692	511	932	435	846	336	411	298	634	425
322	45.72	TeCDD-1					391	418	469	346	439	473	730	548	511	469	538	641
340	52.69	12468-PnCDF					415	1240	583	411	1518	206	1501	987	792	353	343	326

M.W.	R.T.	Name	PCB std 1	PCB std 2	PCB std 3	PCB std2 R	Live-1	Live-2	Live-3	ED-1	ED-2	ED-3	ED195-1	ED195-2	ED195-3	EDTeCB-1	EDTeCB-2	EDTeCB-3
340	54.8	12478-PnCDF					1446	4232	2224	1340	5270	1816	3265	1357	1374	1586	1528	1830
	55.49	**12378-PnCDF**					367	336	439	291	583	243	524	250	202	212	493	281
	57.2	23468-PnCDF					284	206	353	295	517	473	473	373	250	312	353	535
	60.57	**23478-PnCDF**					702	1096	812	521	2083	750	1110	3286	1802	569	435	500

Table A-2. Raw data of primary ions of major PCDF congeners in Kymijoki sediment mesocosms at year 2.

						Sed D.W. (g)	1.33	1.32	1.07	1.14	0.98	1.04	1.19	1.18	0.97	1.01	0.89	0.96
						PCB S (ng)	100	100	100	75	100	100	100	100	100	100	100	100
						PCB IS (ng)	25	25	25	25	25	25	25	25	25	25	25	25
		PCB S (ppb)	70	35	17.5	35	200	200	200	150	200	200	200	200	200	200	200	200
		PCB IS (ppb)	12.5	25	50	25	50	50	50	50	50	50	50	50	50	50	50	50
M.W.	R.T.	Name	PCB std 1	PCB std 2	PCB std 3	PCB std2 R	Live-1	Live-2	Live-3	ED-1	ED-2	ED-3	ED195-1	ED195-2	ED195-3	EDTeCB-1	EDTeCB-2	EDTeCB-3
222	21.906	PCB 14	4513	2190	1055	2662	5496	11616	12767	12222	10961	11829	10410	10917	22964	14049	12640	12616
256	22.82	PCB 30	867	1576	3224	2070	2378	2450	2710	2464	2536	2416	2789	2371	2827	2721	2498	2590
256	25.561	PCB 23	5407	2618	1336	3536	5962	13212	14919	14083	12483	13980	12459	13202	27937	16660	15745	15659
292	29.615	PCB 65	2481	1237	641	1665	3002	6716	8796	7700	7274	7696	7483	8357	17893	11441	10146	9759
360	48.118	PCB 166					2995	6668	7713	7021	6538	6795	6346	6730	14607	8830	8484	8165
430	53.03	PCB 204	367	706	1388	1014	1172	1175	1271	1172	1230	1096	1278	1124	1316	1275	1216	1227
374	60.97	123468-HxCDF					353	408	826	596	1107	836	706	483	1196	1014	1566	3262
	61.37	124678-HxCDF					1172	1275	1926	2200	5322	3344	2220	1422	3231	4376	3834	10300
	62.62	124689-HxCDF					4509	5352	7963	10259	21474	16574	11373	5301	11616	15851	13946	41426
	63.14	123689-HxCDF					298	497	685	648	2100	857	713	524	963	833	548	1422
	63.65	HxCDF-5					284	336	387	404	521	415	353	387	380	356	346	370
	64.05	123478-HxCDF					65	58	130	144	394	151	103	82	199	154	110	219
	65.31	234678-HxCDF					96	134	257	216	627	199	219	137	326	216	212	38
	67.71	HxCDF-8					144	188	250	264	397	435	223	134	216	226	278	260
408	72.732	1234678-HpCDF					14457	17006	25061	46395	167521	78374	29718	21899	39309	75942	69163	105087
	73.931	1234689-HpCDF					12682	14713	24250	34591	106383	45991	27844	16173	29280	46032	42348	84196
444	86.267	OCDF					12791	11417	27515	34344	157644	52086	20330	16961	17304	29047	48235	49764
306	43.84	TeCDF-1					336	524	627	545	1792	692	702	439	1676	661	613	1042
	46.98	TeCDF-2					120	243	284	116	497	312	511	192	397	319	425	445
	47.66	TeCDF-3					391	466	528	531	1120	524	610	593	1086	737	610	822
	48.23	TeCDF-4					103	202	432	336	778	250	370	243	970	350	319	425
322	45.72	TeCDD-1					370	308	363	322	373	339	356	377	418	572	377	463
340	52.69	12468-PnCDF					182	377	411	449	1456	648	456	368	1144	836	685	877
	54.8	12478-PnCDF					726	881	1251	1059	4434	1412	1432	911	2748	1330	1312	2505
	55.49	**12378-PnCDF**					96	113	243	247	504	158	312	144	350	202	233	274

M.W.	R.T.	Name	PCB std 1	PCB std 2	PCB std 3	PCB std2 R	Live-1	Live-2	Live-3	ED-1	ED-2	ED-3	ED195-1	ED195-2	ED195-3	EDTeCB-1	EDTeCB-2	EDTeCB-3
340	57.2	23468-PnCDF					178	206	230	332	254	202	305	326	305	346	319	305
	58.8	**23478-PnCDF**					425	391	356	490	997	572	833	740	1014	733	846	360

Table A-3. Raw data of primary ions of major PCDF congeners in Kymijoki sediment mesocosms at year 3.

						Sed D.W. (g)	1.33	1.42	1.39	1.3	1.27	1.41	1.26	1.22	1.11	1.06	1.08	1.31
		PCB S (ppb)	70	35	17.5	35	200	200	200	200	200	200	200	200	200	200	200	200
		PCB IS (ppb)	12.5	25	50	25	50	50	50	50	50	50	50	50	50	50	50	50
M.W.	R.T.	Name	PCB std 1	PCB std 2	PCB std 3	PCB std2 R	Live-1	Live-2	Live-3	ED-1	ED-2	ED-3	ED195-1	ED195-2	ED195-3	EDTeCB-1	EDTeCB-2	EDTeCB-3
222	21.906	PCB 14	7340	3728	1826	4431	20313	18290	13237	14583		17630	14313	19054	16913	17304	17838	16523
256	22.82	PCB 30	1425	2656	5099	3447	3639	3821	3550	3598		3440	3618	3704	4009	3934	3988	3817
256	25.561	PCB 23	9022	4506	2179	5376	21381	20693	15341	17194		20257	17328	22485	20545	20093	22475	20624
292	29.615	PCB 65	4143	2111	1093	3063	13655	12092	7724	9714		10523	10180	14645	13442	14042	15820	14014
360	48.118	PCB 166					11551	10821	8439	8974		10626	9348	12623	11198	11746	12798	11753
430	53.03	PCB 204	569	1206	2313	1789	1967	2005	1915	1905		1768	1847	1768	2001	1861	1960	1881
374	60.97	123468-HxCDF					970	1789	439	1641		1357	3810	1107	3992	1449	939	3502
	61.37	124678-HxCDF					2988	5630	1700	4876		3707	15549	4550	8556	3101	3403	11869
	62.62	124689-HxCDF					11674	33474	6719	22063		17801	66587	18716	30222	14306	12932	45641
	63.14	123689-HxCDF					1117	2220	452	1299		1083	3543	1501	1631	1031	702	5154
	63.65	HxCDF-5					812	798	723	949		1018	939	1275	983	863	987	1350
	64.05	123478-HxCDF					175	350	96	182		171	702	291	350	192	168	918
	65.31	234678-HxCDF					271	480	209	421		312	953	483	507	274	281	1374
	67.71	HxCDF-8					353	1991	219	339		421	1497	689	445	418	329	757
408	72.732	1234678-HpCDF					50722	61592	27207	76936		49663	151306	60539	92971	47022	73119	289574
	73.931	1234689-HpCDF					35009	85704	20672	68417		46254	171011	51015	73807	38781	43935	178255
444	86.267	OCDF					30746	61794	18185	47608		33385	122981	39699	51072	34450	36928	168455
306	43.84	TeCDF-1					1227	1501	596	1357		1223	3522	2087	1538	1093	894	4962
	46.98	TeCDF-2					315	428	236	504		648	1158	593	346	274	737	1244
	47.66	TeCDF-3					1042	1388	565	1158		908	2104	1463	1264	1049	905	1244
	48.23	TeCDF-4					480	634	233	552		565	1295	764	737	500	452	1987
322	45.72	TeCDD-1					1353	2087	1175	1038		1652	997	915	1035	1073	1323	1120
340	52.69	12468-PnCDF					980	1347	326	1100		874	2868	1162	1114	651	2470	4287
	54.8	12478-PnCDF					2073	3200	857	2608		2306	7415	3492	3461	1785	1549	11582
	55.49	12378-PnCDF					223	319	134	487		356	863	394	367	257	302	1165
	58.8	23468-PnCDF					1295	1501	1155	833		600	860	822	908	1001	1031	2193
	60.57	23478-PnCDF					2759	2354	2899	3026		2090	2085	4156	4033	3481	3988	4619

Table A-4. Raw data of primary ions of major PCDF congeners in Kymijoki sediment mesocosms at year 5.

			Sed D.W. (g)	0.85	1.09	0.55	0.81	0.85	0.56	0.75	0.57	0.65	0.62	0.52	0.79
			PCB S (ppb)	35	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	70	62.5	70
			PCB IS (ppb)	25	25	25	25	25	25	25	25	25	25	25	25	25
M.W.	R.T.	Name	PCB std	ED195-1	ED195-2	ED195-3	ED-1	ED-2	ED-3	EDTeCB-1	EDTeCB-2	EDTeCB-3	Live-1	Live-2	Live-3
222	21.049	PCB 14	3608	4684	5924	5438	5393	5143	5678	5088	5287	5184	4016	5081	5273
256	21.963	PCB 30	2052	2289	2429	2865	2618	3022	2950	2628	2779	2913	2827	2645	2632
256	24.704	PCB 23	4506	6719	8135	6894	7203	7065	7833	7141	6980	6911	5215	7031	7206
292	28.701	PCB 65	2464	4708	5414	4879	4879	5136	5393	4729	5064	5092	3046	4554	4112
360	47.319	PCB 166		3865	4406	3910	4033	3964	4335	4006	3903	3875	370	3851	332
430	52.34	PCB 204	1031	1364	1329	1542	1528	1655	1645	1497	1511	1628	1621	1525	1463
374	60.168	123468-HxCDF		1096	853	853	785	774	483	898	620	6795	1467	620	1891
	60.568	124678-HxCDF		2409	4057	3920	1902	1912	1624	1542	1676	8488	7230	2022	3241
	61.825	124689-HxCDF		9659	18071	12719	12431	6795	6092	6284	5476	33978	32857	10351	11109
	62.396	123689-HxCDF		1374	620	500	511	922	702	490	271	942	3999	1172	778
	62.91	HxCDF-5		86	103	58	0	106	86	99	27	154	192	103	58
	63.309	123478-HxCDF		195	106	120	96	127	89	89	72	260	493	140	182
	64.57	234678-HxCDF		264	140	99	96	212	137	99	65	274	709	168	161
	66.964	HxCDF-8		367	373	257	209	240	284	243	113	404	1090	493	257
408	71.99	1234678-HpCDF		41827	73398	102901	48376	21474	27974	20384	22752	93499	66481	48704	33457
	73.189	1234689-HpCDF		30684	55208	49150	38072	17763	19165	16159	14652	67379	64374	28684	27350
444	85.52	OCDF		24948	46498	31726	47491	8813	14371	11935	9382	39796	25692	30284	16410
306	43.15	TeCDF-1		1103	480	394	326	675	545	421	428	946	2426	692	747
	46.234	TeCDF-2		219	226	161	130	233	212	65	110	212	401	188	206
	46.919	TeCDF-3		531	367	298	188	356	421	288	233	562	1114	384	452
	47.49	TeCDF-4		480	199	212	89	278	257	202	151	415	754	233	367
322	43.893	TeCDD-1		120	130	144	130	158	178	130	182	110	140	96	185
340	51.945	12468-PnCDF		730	428	397	212	493	384	260	199	733	2364	562	565
	54.058	12478-PnCDF		2200	1021	706	709	1525	1278	925	545	2039	6562	1693	1569
	54.8	12378-PnCDF		288	113	130	106	240	151	185	106	428	466	151	247
	56.456	23468-PnCDF		103	110	219	195	117	113	182	55	182	247	113	140
	59.83	23478-PnCDF		785	504	980	315	572	507	442	326	843	2190	562	665

Table A-5. Raw data of primary ions of major PCDF congeners in Kymijoki sediment mesocosms at year 7.

					D.W. (g)	0.99	0.68	0.59	0.81	0.73	0.48	0.53	0.5	0.88	0.54	0.61	1.08
		PCB S (ppb)	150	100	50	70	70	70	70	70	70	70	70	70	70	70	70
		PCB IS (ppb)	50	100	150	50	50	50	50	50	50	50	50	50	50	50	50
M.W.	R.T.	Name	PCB std 1	PCB std 2	PCB std 3	Live-1	Live-2	Live-3	ED-1	ED-2	ED-3	ED195-1	ED195-2	ED195-3	EDTeCB-1	EDTeCB-2	EDTeCB-3
222	21.906	PCB 14	38076	15008	9108	6089	7898	8518	6987	6637	5054	6449	6486	7658	8470	6908	6891
256	22.82	PCB 30	9800	13110	24369	5506	6582	5931	5945	5328	6257	5544	5763	6599	6020	6079	6534
256	25.561	PCB 23	31280	12616	7809	7086	9371	9937	8398	8251	6110	8231	8196	9704	10208	8751	8796
292	29.615	PCB 65	21779	9207	5777	3142	4259	5085	4290	4609	3115	4530	4568	5020	5376	4341	4516
360	48.118	PCB 166	21011	8477	4986												
430	53.03	PCB 204	4872	6435	12171	2512	2954	2597	2642	2453	2851	2594	2738	2978	2741	2786	2895
374	60.97	123468-HxCDF				1234	3533	3934	1203	3601	1076	336	1182	1535	1497	3331	1357
	61.37	124678-HxCDF				2618	8152	9608	3755	8751	3361	1096	4263	5808	5551	7429	5239
	62.62	124689-HxCDF				10331	32244	47567	14922	38628	14850	4143	18472	17667	28619	27964	20552
	63.14	123689-HxCDF				747	2207	757	1025	641	1446	377	1336	603	2100	3954	1234
	63.65	HxCDF-5				113	363	370	185	476	195	267	271	223	329	302	353
	64.05	123478-HxCDF				175	442	195	288	188	226	127	257	161	356	579	254
	65.31	234678-HxCDF				257	809	288	349	370	387	171	401	329	480	857	356
	67.71	HxCDF-8				449	744	569	552	586	565	188	589	524	699	785	531
408	72.732	1234678-HpCDF				27772	143032	141066	50753	76589	45538	13579	64751	86698	96853	141297	109248
	73.931	1234689-HpCDF				33837	113706	112979	43732	69555	37256	11486	54228	50414	84892	97429	73896
444	86.267	OCDF				49126	99115	89798	44281	39631	27785	9642	39950	28974	75133	105163	65340
306	43.84	TeCDF-1				559	2529	997	843	1405	1487	267	1319	850	2005	2505	1206
	46.98	TeCDF-2				175	606	288	326		360		346		528	843	298
	47.66	TeCDF-3				394	1679	908	740	1158	970	329	949	822	1319	1494	1093
	48.23	TeCDF-4				271	1360	696	500	850	709	199	538	401	699	771	507
322	45.72	TeCDD-1				264	408	401	514	415	322	452	421	500	569	538	658
340	52.69	12468-PnCDF				483	2162	1288									
	54.8	12478-PnCDF				1264	5493	1607	2005	1765	3080	720	2751	1360	4352	7418	2755
	55.49	12378-PnCDF				223	1072	487		511	353	130	411	192	394	504	236
	57.2	23468-PnCDF					401	514	295	418	332	264	346	425	514	439	298
	60.57	23478-PnCDF				812	2395	2193			905		1090		3625	2549	1843

Appendix 2. Raw data of Kymijoki sediment microcosm. M.W.: molecular weight, R.T.: retention time (min), Live: no addition control, ED: electron donors only, EDTeCDF: electron donors plus TeCDF; EDPCNB: electron donors plus PCNB, EDTeCB: amendment with electron donors and TeCB, Sol: solvents only, VO: vegetable oil only, EDB: electron donors plus bioaugmentation with strain 195, VOB: vegetable oil plus bioaugmentation with strain 195, hexan: control of PCB 14, 23, 65, 166 and 204 only, sed D.W.: sediment dry weight (g).

Table A-6. Raw data of primary ions of major PCDF congeners in Kymijoki sediment microcosms at time 0.

ppb	62.5	25	62.5	62.5	62.5	25								
M.W.	222	256		290	360	428	374							
R.T.	21.106	22.02	24.818	28.816	47.49	52.52	60.283	60.682	61.939	62.567	64.737	72.104	73.303	85.639
Compound	PCB14	PCB30	PCB23	PCB65	PCB166	PCB204	123468-Hx	124678-Hx	124689-Hx	123689-Hx	234678-Hx	1234678-Hp	1234689-Hp	OcCDF
Live-1	4557	2166	4694	2854	5081	1891	308	1371	4136	363		10461	8087	3670
Live-2	11654	3615	15693	6870	8076	1864	630	2087	7288	702	332	17626	12980	5736
Live-3	11468	3396	14693	5952	7579	1710	1216	4149	16238	980		37562	30345	14203
Kill-1	16893	3982	20861	9183	10574	2008	380	1645	5095	360		12661	9125	4890
Kill-2	16478	4849	21419	8405	10890	2470	1189	2748	9796	1025		28834	20395	12503
ED-1	14946	5164	19524	8892	11150	3005	997	3437	12058	1107	466	42269	26925	15961
ED-2	15159	4670	20672	9008	12603	2933	709	2429	6901	449	278	17773	12291	6815
ED-3	5006	3824	5003	3406	4869	2854	1576	4890	18089	857		33765	31983	12202
EDTeCDF-1	3862	3725	3855	2587	3964	2841	1062	4547	15505	2656	785	72919	45795	29605
EDTeCDF-2	3235	3104	3163	2039	3543	2501	881	3241	14210	1703		41673	31983	22738
EDTeCDF-3	3046	2590	3015	2104	3557	2193	839	1672	4962	524	219	14491	11493	6939
EDPCNB-1	3334	2494	3022	2231	4122	2330	10533	11513	53820	2097		99410	104858	53015
EDPCNB-2	2275	1912	2237	1638	3012	1953	1946	6654	30883	1881		54927	53073	40022
EDPCNB-3	1569	1473	1518	1240	2299	1768	798	2885	10153	1234	343	67753	39449	38048
EDTeCB-1	946	911	891	846	1631	1319	949	2474	7333	408		15659	11034	5493
EDTeCB-2	898	843	894	908	1576	1278	408	1525	5407	894	178	27080	17489	15916
EDTeCB-3	740	586	658	750	1234	1004	452	1765	7429	925	401	31476	20463	14628
Solvents-1	469	531	483	418	764	959	726	2745	10005	822	367	38301	26103	14107
Solvents-2	559	387	449	555	644	648	284	668	2100	233	161	6956	4934	2166
Solvents-3	634	511	490	713	1049	953	627	2320	9313	682	418	28807	22289	14570
VO-1	586	548	377	511	750	973	411	1237	4509	291	236	12990	10882	6175

R.T.	21.106	22.02	24.818	28.816	47.49	52.52	60.283	60.682	61.939	62.567	64.737	72.104	73.303	85.639
Compound	PCB14	PCB30	PCB23	PCB65	PCB166	PCB204	123468-Hx	124678-Hx	124689-Hx	123689-Hx	234678-Hx	1234678-Hp	1234689-Hp	OcCDF
VO-2	613	524	391	586	977	1025	1446	3841	16002	1049		44801	30153	11575
VO-3	620	535	329	583	836	1007	555	2467	8549	339		23006	13908	6151
EDB-1	394	473	353	531	607	672	1874	4509	17133	500		26939	22728	9423
EDB-2	350	363	288	524	562	661	274	874	3375	223		12370	8059	6438
EDB-3	305	288	319	528	517	538	240	445	1645	144	89	4197	3677	2375
VOB-1	257	353	257	363	514	637	264	651	2666	332	151	8881	6939	5225
VOB-2	247	370	250	391	500	644	257	778	2707	240	86	7035	5794	3457
VOB-3	336	298	254	360	497	583	408	1045	3783	360	171	13956	10780	7463
Hexane	1172	240	696	432	531	195								

Table A-6 continued. Raw data of primary ions of major PCDF congeners in Kymijoki sediment microcosms at time 0.

R.T.	39.81	41.38	42.293	43.321	45.092	46.405	47.09	52.173	54.286	55.028	
Compound	TeCDF-1	TeCDF-2	1234-TeCDF	TeCDF-3	TeCDF-4	TeCDF-5	TeCDF-6	12468-Pn	12478-Pn	12378-Pn	sed D.W. (g)
Live-1	278	397		408	250		315	93	134	339	1.56
Live-2	384	764		548	1014	308	312	435	1220	206	1.7
Live-3	600	692		1007	1525	435	613	788	2522	384	1.46
Kill-1	555	737		435	987	326	230	257	1066	284	1.55
Kill-2	3615	805		1388	2217	3000		1001	1960	541	1.49
ED-1	682	524		956	1120	565	764	1000	2484	401	1.94
ED-2	555	768		425	1672	380	583	493	1028	332	1.48
ED-3	500	449		747	1449	445	466	836	1816	459	1.29
EDTeCDF-1			245520					1765	5352	572	1.47
EDTeCDF-2			226950					1062	2940	284	1.53
EDTeCDF-3			302231					408	1059	284	1.36
EDPeCN-1	514	350	7052	1251	675	353	637	1326	4119	394	1.73
EDPeCN-2	350	298	1254	1014	651	250	531	1168	3115	456	1.75
EDPeCN-3	284	236	315	589	517	164	312	678	2131	257	1.38
EDTeCB-1	192	178	305	281	236	99	164	326	689	185	1.64
EDTeCB-2	75	209		363	175	195	134	356	1038	182	1.53
EDTeCB-3	137	93		346	79	72	154	569	1220	185	1.28
Solvents-1	130	55		308	120	82	185	425	1038	209	1.47
Solvents-2	103	62		120	93	21	55	106	284	41	1.38
Solvents-3	154	79		264	127	89	93	332	867	247	1.7
VO-1	137	45		137	79	72	55	281	367	168	1.4
VO-2	154	120		247	113	117	127	480	1264	140	1.55
VO-3	120	110		192	134	58	93	336	665	96	1.53
EDB-1	117	69		140	89	86	116	192	415	144	1.34
EDB-2	48	65		134		58	72	161	291	45	1.48
EDB-3	89	62		107	99	48	72	89	284	69	1.69
VOB-1	41	10		127	113	161	82	99	356	96	1.65
VOB-2	123	185		130	117	62	103	171	487	106	1.5
VOB-3	24	55		240	106	151	127	274	696	151	1.44

Table A-7. Raw data of primary ions of major PCDF congeners in Kymijoki sediment microcosms after 18 months.

ppb	35	25	35	35	35	25									
M.W.	222	256	256	290	360	428	374						408		442
R.T.	21.049	21.96	24.76	28.759	45.663	52.402	60.283	60.625	61.939	62.453	62.967	64.623	72.104	73.303	85.582
Compounds	PCB 14	PCB 30	PCB 23	PCB 65	PCB 166	PCB 204	123468-Hx	124678-Hx	124689-Hx	123689-Hx	HxCDF-5	234678-Hx	1234678-Hp	1234689-Hp	OCDF
solv	10708	3869	12462	5167	5990	1501									
Live-1	2752	2933	2793	1066	243	1268	103	490	1939	216	713	65	5510	5030	3060
Live-2	2580	3111	2779	1035	164	1312	394	1096	5397	637	397		11890	15656	6339
Live-3	3433	3461	3694	1381	257	1377	565	2426	11030	843	350		67543	50712	45614
Kill-1	3636	4009	3653	1391	730	1607	120	466	1881	147	202	120	6329	6243	4759
Kill-2	3601	3660	3557	1323	10242	1535	182	627	2323	202	267		9725	7884	7621
ED-1	3543	3868	3560	1432	305	1518	411	1247	5208	641	199	161	10523	14535	5140
ED-2	3906	3721	3995	1511	257	1515	970	3135	19678				30712	45877	20909
ED-3	4088	3810	4084	1542	343	1569	768	4300	17047	1658			83812	76394	47269
EDTeCDF-1	3845	3855	3934	1648	421	1552	353	901	3773	411	202		14658	10657	8943
EDTeCDF-2	4105	4153	4393	1795	1042	1580	260	815	3039	332	257	110	10434	7528	4406
EDTeCDF-3	3797	3557	3865	1720	1813	1525	212	658	2234	216	171		8501	6781	5530
EDPCNB-1	3738	3827	3855	1607	661	1569	329	1066	4167	332	168		19007	14748	14679
EDPCNB-2	4163	4235	5619	1861	483	1621	466	1871	7234	315			22015	24095	10506
EDPCNB-3	3396	3831	4170	1703	606	1538	668	2385	7216	497			24095	19987	13658
EDTeCB-1	3988	4115	3865	1672	257	1604	178	720	2416	130	154		6812	6038	4441
EDTeCB-2	3635	3087	3714	1699	2251	1258	343	1196	4670	360	192		22063	14114	13391
EDTeCB-3	3546	4009	3769	1473	192	1508	158	511	1655	140	223		6692	4338	2861
Solvents-1	3618	3841	3752	1669	3601	1542	356	1014	3903	219	134		18380	13802	16742
Solvents-2	3677	4016	3992	1628	353	1689	288	802	2919	209	146	123	13404	10427	6192
Solvents-3	3379	3913	3488	1535	158	1515	404	2152	7415				49815	26912	15532
VO-1	3409	3869	3303	1559	298	1706	493	1319	4615	370	223		21467	15810	12092
VO-2	3368	3930	3403	1343	819	1497	257	990	3903	308	164		7949	8964	2851
VO-3	3269	3954	3375	1395	336	1610	579	2005	11475	476			18551	27203	16297
EDB-1	3803	3875	3834	1511	278	1501	456	2409	3372	264	151		11753	8618	5983
EDB-2	3618	3793	3581	1391	3211	1576	1251	4629	18435	637			92866	59402	39391
EDB-3	3396	3653	3639	1456	288	1456	401	1247	3591	219	147		26110	11633	6939
VOB-1	4167	3810	4228	1604	1059	1566	6980	5301	27785				33584	54286	22557

R.T.	21.049	21.96	24.76	28.759	45.663	52.402	60.283	60.625	61.939	62.453	62.967	64.623	72.104	73.303	85.582
Compounds	PCB 14	PCB 30	PCB 23	PCB 65	PCB 166	PCB 204	123468-Hx	124678-Hx	124689-Hx	123689-Hx	HxCDF-5	234678-Hx	1234678-Hp	1234689-Hp	OCDF
VOB-2	4040	3821	4218	1679	469	1580	576	1302	4883	356	178		15183	14518	8837
VOB-3	3488	3968	3543	1532	274	1758	4341	7980	26299				35883	33689	18894
hexane	14138	5068	15872	6685	8508	1967									

Table A-7 continued. Raw data of primary ions of major PCDF congeners in Kymijoki sediment microcosms after 18 months.

M.W.	304						340				
R.T.	39.781	41.265	42.236	43.207	44.92	46.291	46.976	52.002	54.172	54.857	
Compounds	TeCDF-1	TeCDF-2	1234-TeCDF	TeCDF-3	TeCDF-4	TeCDF-5	TeCDF-6	12468-Pn	12478-Pn	12378-Pn	sed D.W. (g)
Live-1	100	178		120	127	75	75	113	322	45	0.56
Live-2	151	116		373	161	86	254	278	1014	127	0.62
Live-3	164	123		466	168	161	219	428	1347	116	0.71
Kill-1	223	147		154	278	103	72	75	326	86	0.47
Kill-2	178	137		151	158	34	86	106	284	45	0.52
ED-1	200	168		284	206	82	117	343	977	144	0.79
ED-2	195	188		134	295	48	62	99	243	65	0.48
ED-3	200	212		678	339	147	326	815	2518	236	0.6
EDTeCDF-1			101417					322	816	147	0.69
EDTeCDF-2			75643					206	596	79	0.55
EDTeCDF-3			87764					199	531	96	0.6
EDPCNB-1	171	284	435	271	271	48	151	302	672	144	0.62
EDPCNB-2	300	312		281	535	192	195	202	771	182	0.76
EDPCNB-3	300	305	110	415	757	202	164	288	1045	171	0.68
EDTeCB-1	195	178		147	363	151	72	82	278	62	0.59
EDTeCB-2	250	343	178	469	689	161	158	281	781	116	0.86
EDTeCB-3	134	182	34	123	140		103	82	360	34	0.72
Solvents-1	168	178	72	171	332	75	93	55	449	158	0.64
Solvents-2	289	408	137	295	524	113	171	192	541	206	0.71
Solvents-3	158	120		168	312	38	96	89	257	65	0.79
VO-1	189	209	113	305	576	151	147	271	733	151	0.46
VO-2	147	202	127	367	421	89	147	305	757	154	0.45
VO-3	182	212	51	298	397	161	151	288	874	134	0.35
EDB-1	189	192		236	439	113	144	168	521	96	0.61
EDB-2	190	154	21	432	264	164	202	439	1049	127	0.5
EDB-3	200	147		271	384	120	178	147	606	55	0.48
VOB-1	171	380		243	439	137	120	305	617	65	0.51
VOB-2	200	188		291	586	116	188	182	747	103	0.46
VOB-3	185	140		195	504	34	140	202	507	168	0.41

Appendix 3. Selected DGGE bands sequences from Kymijoki mesocosm (Fig. 2-5 &6) (Chapter 2).

>Band 6
GCTTTCGGGTTGTAAACTcTTTCANCGGGAATAATGACGGTACCTGTGGAATA
AGCTCGGCTAACTACGTGCCAGCAGCCGCGGTAAT

>Band 1
CGGCGAGCCAGCCCAGCCGNTGCCAGCGGGAACTGAAATGAGCGCTTCGCGT
CTGTAAACCTCTTTATCTGAGGGNAGAGATAGGACGGTACCTCAGGAATAAG
TCTCGGCTAACTACGTGCCAGCAGCCGCGGTAAT

>Band 2
AGCCTGGAGCAGCAACGCCGCGTGGGAGTGATGAAGGCCTTCGGGTCTGTAA
ACACTCCTTTTCTGGAGGGACGATAATGGACCGGGTACCCTCTAGGGAANAA
GGTCCCGGGCCTAACCTNCCGGTGTGCCNNCCAGCCCGCGGTAAT--

>Band 4
GCACGCCGCGTGAGGGACGAAAGCCTTCGGGTCGTAAACCTCTTTTCTGGGG
NAAGAGCAAGGACGGTACCTCAGGAATAAGTCTCGGCTAACTACGTGCCAGC
AGCCGCGGTAAT

>Band 5
AGCACGCCGCGTGGNAGGACGAAGGCCTTCGGGTCGTAAACCTCCTTTTNCG
GGGGAAGAGGAAGGACGGTACCCCGAGAATAAGTCACGGCTAACTACGTGC
CAGCAGCCGCGGTAAT

>Band 3
CGGGNGCCAGCGATGCCGACTGATGAATAGCCTTCGGGTCTGTAAAATCCTT
TATCTGAGGGACGATAATGACGGTACCTCAGGAATAAGTCCCGGCTAACTAC
GTGCCAGCAGCCGCGGTAAT

Appendix 4. Selected DGGE bands sequences from Kymijoki enrichment cultures (Fig. 4-9 &8) (Chapter 4).

>ED+TCDD (1)
GGCGAGCCTGACCCAGCAACGCCGCGTGAGGGATGAAGGCTTTCGGGTTGTAAACCTCTT
TTCATAGGGAAGAATAATGACGGTACCTGTGGAATAAGCTTCGGCTAACTACGTG

>ED+TCDD (3)
GCCTGACCCAGCAACGCCGCGTGAGGGATGAAGGCTTTCGGGTTGTAAACCTCTTTTCAT
AGGGAAGAATAATGACGGTACCTGTGGAATAAGCTTCGGCTAACTACGTG

>ED+TCDD+PCNB (1)
AGCCTGACCCAGCAACGCCGCGTGAGGGATGAAGGCTTTCGGGTTGTAAACCTCTTTTCA
TAGGGAAGAATAATGACGGTACCTGTGGAATAAGCTTCGGCTAACTACGTG

>ED+TCDD+PCNB (2)
AGCCTGACCCAGCAACGCCGCGTGAGGGATGAAGGCTTTCGGGTTGTAAACCTCTTTTCA
TAGGGAAGAATAATGACGGTACCTGTGGAATAAGCTTCGGCTAACTACGTG

>ED+TCDF (2)
GGCGAGCCTGACCCAGCAACGCCGCGTGAGGGATGAAGGCTTTCGGGTTGTAAACCTCTT
TTCATAGGGAAGAATAATGACGGTACCTGTGGAATAAGCTTCGGCTAACTACGTG

>ED+TCDD (2)
GACAGCAACGCCGCGTGGGTGAAGAAGGCTTTCGAGTCGTAAAACCCTTTTCTAGGGGAT
GAGTAAGGACGGTACCCTAGGAATAAGTCTCGGCTAACTACGTG

>ED+TCDF (1)
TGGGTGAAGAAAGGCTTTCGAGTCGTAAAACCCTTTTCTAGGGGATGAGTAAGGACGGTA
CCCTAGGAATAAGTCTCGGCTAACTACGTG

>ED+TCDF (3)
TGACGCGGCAGCCAGCRTGGGTGAAGAAAGGCTTTCGAGTCGTAAAACCCTTTTCTAGGG
GATGAGTAAGGACGGTACCCTAGGAATAAGTCTCGGCTAACTACGTG

>ED (1)
GCAGCCAGCATSAASGATAGATAGCCTTCGGGTCATAAACTTCTTTTTTCAGG
GATGAAT
AATGACAGTACCTGGAGAATAAGTCACGGCTAACTACGTG

>ED (2)

ACGGGCAGCCAGCATGMGGGATRATAGGCSTTCCGGTTGTAAACCTCTTTTCT
CANGGAA
GATTAATGACGGTACCTGGGGAATAAGTCTCGGCTAACTACGTG

Curriculum Vitae

HUI LIU

EDUCATION

Oct. 2010	Ph.D. Environmental Science, Rutgers, The State University of New Jersey, U.S.A.
Jul. 2004	M.S., Environmental Microbiology, Nanjing University, Nanjing, P.R.China
Jul. 2001	B.S., Biology, Inner Mongolia University, Huhhot, P.R.China

RESEARCH EXPERIENCE

09/2005-present	Graduate assistant, Rutgers University, New Brunswick, NJ
09/2001-07/2004	Graduate assistant, Nanjing University, Nanjing, P.R.China
09/2004-12/2005	Research fellow, Tongji University, Shanghai, P.R.China

TEACHING EXPERIENCE

09/2007-12/2007	Teaching assistant for Analytical Microbiology, Rutgers University, New Brunswick, NJ

PUBLICATIONS

- Study on terephthalic acid degrading yeast and its activities. CHEN Peng, XIAO Lin, WU Jian, **LIU Hui**, YANG Liuyan, ZHU Jianzhong, China Environmental Science, 2003, 23(4):412-416;
- Studies on Yeast Degrading Phenol and Simultaneously Cultivating *Pityrosporum* sp. as Single Cell Protein. **LIU Hui**, YANG Liuyan, XIAO Lin, CHEN Peng, Journal of Agro-Environment Science, 2004, 23(4):810-813;
- Heavy metal ions' effects on the process of yeast degrading phenol, **LIU Hui**, YANG Liuyan, XIAO Lin, CHEN Peng, Environmental Chemistry, 2004, 23(5):495-500.