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Review

Microbial degradation of chlorinated dioxins

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Abstract

Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) were introduced into the biosphere on a large scale as by-products from the manufacture of chlorinated phenols and the incineration of wastes. Due to their high toxicity they have been the subject of great public and scientific scrutiny. The evidence in the literature suggests that PCDD/F compounds are subject to biodegradation in the environment as part of the natural chlorine cycle. Lower chlorinated dioxins can be degraded by aerobic bacteria from the genera of *Sphingomonas*, *Pseudomonas* and *Burkholderia*. Most studies have evaluated the cometabolism of monochlorinated dioxins with unsubstituted dioxin as the primary substrate. The degradation is usually initiated by unique angular dioxygenases that attack the ring adjacent to the ether oxygen. Chlorinated dioxins can also be attacked cometabolically under aerobic conditions by white-rot fungi that utilize extracellular lignin degrading peroxidases. Recently, bacteria that can grow on monochlorinated dibenzo-*p*-dioxins as a sole source of carbon and energy have also been characterized (*Pseudomonas veronii*). Higher chlorinated dioxins are known to be reductively dechlorinated in anaerobic sediments. Similar to PCB and chlorinated benzenes, halorespiring bacteria from the genus *Dehalococcoides* are implicated in the dechlorination reactions. Anaerobic sediments have been shown to convert tetrachloro- to octachlorodibenzo-*p*-dioxins to lower chlorinated dioxins including monochlorinated congeners. Taken as a whole, these findings indicate that biodegradation is likely to contribute to the natural attenuation processes affecting PCDD/F compounds.

Keywords: Biodegradation; Biotransformation; Dechlorination; Polychlorinated dibenzodioxins; Polychlorinated furans

Contents

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1. Introduction

Chlorinated dioxins refer to two families of tricyclic, planar, aromatic compounds. One of these families is the polychlorinated dibenzo-*p*-dioxins (PCDD) with 75 possible congeners and the other is the polychlorinated dibenzofurans (PCDF) with 135 different congeners. Dioxins were introduced into the biosphere on a large scale as by-products from the manufacture of chlorinated phenols, which started to gain importance in the late 1930s as pesticides (Hutzinger et al., 1985). Dioxins have also been released into the environment by incineration of wastes (McKay, 2002; Tuppurainen et al., 2003). Aside from the anthropogenic input, dioxins are present naturally in the environment as evidenced by low levels detected in archived samples of soils and plant tissue from periods prior to the industrial revolution (Alcock and Jones, 1996; Green et al., 2004). The natural formation of octachloro- and heptachlorodioxin congeners has been demonstrated during composting (Krauss et al., 1994) and during sewage treatment (Klimm et al., 1998). Oxidative enzymes such as peroxidases can catalyze the coupling of chlorophenols into dioxins (Oberg and Rappe, 1992; Wittsiepe et al., 2000), which could account for the natural formation of chlorinated dioxins. Another natural source of chlorinated dioxins is forest fires, 130 pounds of PCDDs are estimated to be produced by Canadian forest fires annually (Gribble, 1994).

PCDD/Fs are stable hydrophobic contaminants which persist in the environment (Hutzinger et al., 1985; Alcock and Jones, 1996). Congeners with lateral chlorine atoms, such as in 2,3,7,8-tetrachlorodibenzo-*p*-dioxins (2378-TeCDD) are highly toxic to mammals (Landers and Bunce, 1991; Pohjanvirta and Tuomisto, 1994) and other organisms (Boening, 1998). Only the isomers with chlorine groups in the 2,3,7,8 positions are considered to be toxic to higher organisms. Thus the number of dioxin congeners of interest from a toxicological standpoint are 17 PCDDs and 10 PCDFs (Srogi, in press). Dioxins have a high tendency to become adsorbed onto soil and sediments as well as bioaccumulate in organisms (Matsumura and Benezet, 1973; Hutzinger et al., 1985). It has long been recognized that dioxins are subject to photodegradation (Crosby and Wong, 1977; Hutzinger et al., 1985; McPeters and Overcash, 1993). On the other hand, only recently has the role of biodegradation been evaluated as a possible environmental fate of dioxins. Biodegradation studies were initiated in the mid-1980s which demonstrated the microbial conversion of PCDD and PCDF by isolated microorganisms. Previously, there has only been one comprehensive review article on the biodegradation of chlorinated dioxins, which was published by Wittich (1998). A review on the aerobic bacterial degradation of dioxins was also provided by Halden and Dwyer (1997). Many biodegradation studies involve dioxin congeners that are not among the 17 congeners of high toxicological risk. This is due to the availability of test compounds used and the limited aerobic biodegradability of the higher chlorinated toxic congeners. Since non-toxic lower chlorinated dioxins are potential biotransformation products of toxic chlorinated congeners, there has been an interest to evaluate their further degradation under aerobic conditions.

The nomenclature to be used for mono-, di-, tri-, tetra-, penta-, hexa-, hepta- and octochloro-dibenzo-*p*-dioxins/dibenzofurans in this paper will be CDD/F, DCDD/F, TCDD/F, TeCDD/F, QCDD/F, HCDD/F, HpCDD/F and OCDD/F, respectively.

2. Biodegradation of PCDD/F compounds

2.1. Degradation in the environment

Evidence for the biodegradation of chlorinated dioxins in the environment is available in a few studies conducted with either soil, surface water or sediments. Dated sediment cores from aquatic depositional environments have the potential to provide chronologies of pollutant input as well as supply information on possible fates such as biodegradation (Alcock and Jones, 1996). A study with dated sediment cores from Lake Ketelmeer (The Netherlands), a sedimentation area of the River Rhine, confirmed significant disappearance of four higher chlorinated congeners of dioxins when compared with archived sediment samples (Beurskens, 1995). The average half-life calculated for these four congeners was 12 years. The results indicated slow biodegradation of these four congeners; however, 13 other dioxin congeners evaluated were persistent. A study of dated estuarine sediment cores collected in Queensland, Australia, indicated that sediment age (since deposition) was correlated with increasing proportions of lower chlorinated PCDDs that corresponded to decreasing proportions of OCDD (Gaus et al., 2002). The lower chlorinated congeners, which accumulated in older sediments, had characteristic substitution patterns expected from the anaerobic microbial dechlorination of OCDD. Lastly, another study of dated sediment cores from the Baltic sea indicated minimum half-lives of PCDD/Fs that ranged from 30 years (for OCDF and HpCDF) to 170 years (for HpCDD) (Kjeller and Rappe, 1995).

Evidence for biodegradation of PCDD and PCDF in anaerobic sediments has been obtained in numerous microcosm studies where sediments collected from rivers, estuaries and bays have been spiked with specific congeners of chlorinated dioxin together with an electron donating substrate (Adriaens and Grbic-Galic, 1994; Adriaens et al., 1995; Beurskens et al., 1995; Ballerstedt et al., 1997; Fu et al., 1999; Bunge et al., 2001). In these studies, the spiked dioxins are converted to lower chlorinated dioxins, representing products of biologically mediated reductive dechlorination. The time scale for the bioconversions in the microcosms ranges from months to a year; whereas in the field many years are implicated. The difference may be due to the many-fold higher dioxin concentrations used in the microcosms, typically added as a fresh spike and

together with an adequate supply of electron donating substrates. In one microcosms study, anaerobic dechlorination was also demonstrated for aged 2378TeCDD pollution in the sediments (Barkovskii and Adriaens, 1996).

Reports on the biodegradation of chlorinated dioxins in soil are conflicting. On the one hand, there are studies which indicate that chlorinated dioxins are persistent. One such study considered chlorinated dioxins that were introduced into soil via land application of sewage sludge (Wilson et al., 1997). Chlorinated dioxin concentrations did not significantly change after 260 d of monitoring. On the other hand, the evidence obtained in other experiments suggests that dioxins are degraded in soil. The concentration of 2378-TeCDD was monitored over 10 years in soil contaminated with agent orange (chlorophenoxy acetates). The chlorophenoxy acetates and the 2378-TeCDD were shown to significantly decrease in concentration (Young, 2006). Biodegradation was also observed in soil microcosms spiked with one to 100 ppm of 2378-TeCDD (Kearney et al., 1972). From 37% to 44% of added 2378-TeCDD was eliminated in one year. In a similar soil microcosm, [^{14}C]-27-DCDD was converted in ten weeks to $^{14}CO_2$ (5% conversion) and an unidentified polar metabolite was identified on a thin-layer chromatography plate (Kearney et al., 1972).

The persistence of dioxins in the environment is mainly due to their poor bioavailability. Dioxins are highly hydrophobic. The aqueous solubility and the logarithm of the octanol–water coefficient ($\log P$) for 2378-TeCDD are 0.019 ppb and 6.5, respectively. Consequently dioxins are prone to be tightly adsorbed by soil and sediments (Kao et al., 2001). The variability in results from different studies with soil may be due to differences in the bioavailability status of the dioxins as well as differences in time allowed for an enrichment of an appropriate microbial population.

The biotransformation of 2378-TeCDD was evaluated in outdoor pond water (Matsumura et al., 1983). The apparent half-life of 2378-TeCDD was approximately one year, based on the measured recoveries of 2378-TCDD of 49.7% and 29.4% after 12 and 25 months, respectively.

2.2. Biodegradation in engineered systems

There are limited research results available on the biodegradation of dioxins in engineered systems. A number of studies have been conducted, evaluating concentrations of chlorinated dioxins during municipal waste treatment (McLachlan et al., 1996; Rogers, 1996; Stevens et al., 2003; Oleszek-Kudlak et al., 2005). Chlorinated dioxins have low volatility and are highly hydrophobic so they tend to adsorb to sludge solids. Municipal digested sludge contains from 10 to 40 ppb of chlorinated dioxins on a dry weight (dwt) basis (Rogers, 1996; Stevens et al., 2001). The predominant isomers detected are the higher chlorinated isomers, especially hepta- and octochloro congeners. Most studies are in agreement that chlorinated dioxins are not significantly degraded during anaerobic sludge digestion (Disse et al., 1995; Stevens et al., 2003; Oleszek-Kudlak et al., 2005). The lack of biodegradation in sludge may be due to higher chlorinated dioxin congeners with limited bioavailability in organic matrices. Microcosm studies with spiked TeCDD provide differing results. In one study, the incubation of 1234TeCDD with anaerobic digester sludge for 13 months resulted in no evidence for its degradation based on monitoring for the formation of dechlorinated daughter products (Ballerstedt et al., 1997). However, another microcosm study evaluating the disappearance of 2378TeCDD in soil mixed with anaerobic sludge found that the compound was removed by 86% in 90 d, while there was no significant removal in poisoned controls (Kao et al., 2001). The success in the latter study may be due to the use of aquifer sediments from historically contaminated sites as inoculum. Significant losses of many chlorinated dioxin congeners were observed during the aerobic digestion of sludge (Disse et al., 1995).

There is limited experience with the bioremediation of dioxins. Most of the studies have considered the impact of adding dioxin-degrading bacterial strains to soils artificially contaminated with defined dioxin congeners. The results, summarized in Table 1, indicate that bacterial strains added to the soils metabolize from 32% to 100% of mono- to trichloro-DD/DF congeners supplied at concentrations ranging from 1 to 10 ppm within a week. In some cases, the addition of dioxin-degrading bacterial strains to actual contaminated soil was evaluated, resulting in 8.3–10% removal of dioxins after 7 d of incubation (Habe et al., 2001b, 2002b). The dioxin-degrading strain *Sphingomonas wittichii* RW1 was used to bioremediate PCDD in a contaminated sample of incinerator fly ash (Nam et al., 2005). After 15 d, 75.5% of the toxic PCDDs were removed. Only 20.2% removal was observed in a control receiving heat-killed inoculum, providing strong evidence for biodegradation.

Another approach towards bioremediation has been the addition of large amounts of organic matter to soil. Autoclaved compost was added as an organic nutrient to a heavily polluted soil, which resulted in the decrease of PCDD and PCDF concentrations by 22% after three months (Nam et al., 2005). The pattern in the time course of the congener removal was interpreted to reflect anaerobic reductive dechlorination. Two studies evaluated anaerobic bioremediation of soil and sediments. In one study, a soil artificially contaminated with 2378-TeCDD (96 ppb) was treated with anaerobic sludge supplied as an inoculum and sludge cake supplied as an electron donor (Kao et al., 2001). After a 90 d incubation period, 86% of the 2378-TeCDD added was removed in the non-sterile microcosms, while only 2% of the compound was eliminated in controls poisoned with a mixture of $HgCl_2$ and NaN_3. A zeroth-order rate constant of 1 ppb d^{-1} was reported. In the other study, addition of organic acids was used as strategy to remediate chlorinated dioxins in polluted river sediments (Yoshida et al., 2005). Up to 32% removal of PCDD/PCDF from the sediments was achieved after 210 d of incubation. A poisoned control (paraformaldehyde) did not result in

Table 1
Bioremediation of soils artificially contaminated with chlorinated dioxins and bioaugmented with bacterial strains

Bacterial strain	Dioxin	Conc. ($\mu g\ l^{-1}$)	Incubation time (d)	Removal (%)	References
Pseudomonas resinovorans CA10	23-DCDD	1000	14	100.0	Widada et al. (2002)
Terrabacter sp. DBF63	28-DCDF	1000	7	90.0	Habe et al. (2002b)
	2-CDF	1000	5	89.0	Habe et al. (2002b)
	2-CDD	1000	5	65.0	Habe et al. (2002b)
	28-DCDF	1000	5	78.0	Habe et al. (2002b)
	23-DCDF	1000	5	32.0	Habe et al. (2002b)
Sphingomonas sp. KA1	2-CDD	1000	7	96.0	Habe et al. (2002a)
	23-DCDD	1000	7	70.0	Habe et al. (2002a)
Pseudomonas sp. CA10	2-CDD	1000	5	97.0	Habe et al. (2001b)
	23-DCDD	1000	5	89.0	Habe et al. (2001b)
	123-TCDD	1000	5	52.0	Habe et al. (2001b)
Pseudomonas sp. CA10	2-CDD	10,000	7	98.5	Halden et al. (1999)

any removal, confirming biodegradation as the main removal mechanism. The best results were obtained in anaerobic microcosms exposed at the surface to air because lower chlorinated congeners formed from reductive dechlorination were subsequently oxidized by aerobic bacteria. The authors demonstrated the formation of catechol and salicylic acid which was indicative of occurrence of aerobic bacteria.

3. Microbiology and biochemistry of PCDD/Fs biodegradation

Chlorinated dioxins are subject to both aerobic and anaerobic metabolism. Under aerobic conditions, lower chlorinated dioxins are susceptible to partial degradation during cometabolic metabolism. In only a few cases have chlorinated dioxins been reported to serve as growth substrates, and these cases are restricted to monochlorinated congeners. Under anaerobic conditions, chlorinated dioxins are subject to reductive dechlorination when suitable electron-donating substrates are available. Recently, several strains of halorespiring bacteria have been reported to utilize polychlorinated dioxins as electron acceptors to support microbial growth.

3.1. Aerobic bacterial cometabolism

Table 2 summarizes literature data on the aerobic degradation of chlorinated dioxins by aerobic bacterial strains. A quick survey of the table reveals that most of the evidence for aerobic biodegradation of chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans has been obtained with monochloro- or dichloro-congeners, which collectively account for 84% of the reported cases of aerobic bacterial chlorinated dioxin degradation. Most of the remaining reports concern tri- and tetrachloro congeners. These findings are in keeping with a general trend that the aerobic biodegradability of chlorinated dioxins increases with a decreasing number of chlorine groups (Parsons and Storms, 1989; Wilkes et al., 1996; Schreiner et al., 1997; Keim et al., 1999; Du et al., 2001). As a general rule, PCDD/F with five chlorine groups or more are not prone to aerobic degradation. One important exception is the recent report that *S. wittichii* strain RW1 is able to slowly transform 123478-HCDD (Nam et al., 2006).

Cometabolism refers to the metabolism of a compound that does not yield growth or energy benefit while another primary substrate is being utilized for energy and/or growth. Aerobic bacterial biodegradation of chlorinated dioxins occurs via cometabolism in the overwhelming majority of the literature reports (91%). The most widely used primary substrate to support chlorinated dioxin cometabolism is the non-halogenated analogue, dibenzofuran (DF) (Table 2). In addition to DF, non-halogenated dibenzo-*p*-dioxin (DD) (Hong et al., 2002), biphenyl (BP) (Parsons et al., 1998), carbazole (CAR) (Habe et al., 2001a), *o*-dichlorobenzene (*o*-DCB) (Du et al., 2001), and benzoic (Bc) or 3-methoxybenzoic acid (3MBc) (Parsons and Storms, 1989) represent common examples of other primary substrates utilized. These primary substrates most likely induce dioxygenases that may be involved in the degradation of the chlorinated dioxins. The most commonly used primary substrates are biphenylic compounds (DF, DD, CAR, and BP) which most likely induce angular dioxygenases implicated in the degradation of DF and DD (Wittich, 1998; Nojiri and Omori, 2002) as well as chlorinated DFs and DDs (Habe et al., 2001a).

The most common biodegradation pathways for the aerobic degradation of chlorinated dioxins are illustrated in Figs. 1 and 2 for 2-CDD and 2-CDF, respectively. The best chlorinated dioxin-degrading bacterial strains initiate the attack of the biphenylic dioxin compounds with angular dioxygenases (Habe et al., 2001a; Nojiri and Omori, 2002). Angular dioxygenases attack a ring adjacent to the ether oxygen bridging the two rings (position 1,10a in dibenzo-*p*-dioxin and position 4,4a in dibenzofuran). Three angular dioxygensases have been described and cloned. These are carbazole 1,9a dioxygenase (CARDO) from *Pseudomonas* sp. strain CA10 (Habe et al., 2001a), dibenzofuran 4,4a dioxygenase (DFDO) from *Terrabacter* sp.

Table 2
Bacterial strains responsible for biodegradation of chlorinated dioxins and identification of biotransformation intermediates/products

Bacterial strain	Compound	Conc. (ppm)	Time (d)	Removal (%)	Products ID[d]	Growth subst.[e]	References
Rhodococcus opacus SAO101	1-CDD	1.0	7	92.0		DF	Kimura and Urushigawa (2001)
Beijerinckia sp. B8/36	1-CDD	500	NS[a]	NS	CDD-dihydrodiols	Succ + BP	Klecka and Gibson (1980)
Pseudomonas veronii PH-03	1-CDD	219	2.5	84.0	3CC	1-CDD	Hong et al. (2004)
Bacillus megaterium AL4V	1-CDD	2.2	0.003	40.0	DH1CDD	NS	Sulistyaningdyah et al. (2004)
Sphingomonas sp. RW1	1-CDD	55	0.7	60.8	3CC, C, CTHDE, 2CMA CHPMA	DF	Wilkes et al. (1996)
Sphingomonas sp. HL7	2-CDD	8.7	0.3	100.0		DF	Fukuda et al. (2002)
Sphingomonas sp. RW1	2-CDD	55	0.7	44.8	4CC, C, CTHDE, 2CMA	DF	Wilkes et al. (1996)
Sphingomonas wittichii RW1	2-CDD	8.7	0.3	100.0		DF	Fukuda et al. (2002)
Pseudomonas sp. CA10	2-CDD	10	0.8	32.5	4CC	CAR	Habe et al. (2001a)
Pseudomonas sp. EE41	2-CDD	2.5	63.0	63.7		*o*-DCB	Du et al. (2001)
Pseudomonas veronii PH-03	2-CDD	219	2.5	90.0	4CC	2-CDD	Hong et al. (2004)
Terrabacter sp. DBF63	2-CDD	10	0.8	75.0	4CC	DF	Habe et al. (2001a)
Burkholderia sp. JB1	2-CDD	0.1	1.0	95.0	4CC, CTHDE	BP	Parsons et al. (1998)
Beijerinckia sp. B8/36	2-CDD	500	NS	NS	CDD-dihydrodiols	Succ + BP	Klecka and Gibson (1980)
Klebsiella sp. HL1	2-CDD	8.7	0.3	45.0		DF	Fukuda et al. (2002)
Alcaligenes sp. JBI	2-CDD	0.3	2.0	97.7	HCDD, DHCDD	Bc or 3MBc	Parsons and Storms (1989)
Coculture[b]	2-CDF	1013	25.0	NS	Chloride,	2-CDF	Wittich et al. (1999)
Sphingomonas sp. HL7	2-CDF	8.1	0.3	100.0		DF	Fukuda et al. (2002)
Sphingomonas wittichii RW1	2-CDF	8.1	0.3	60.0		DF	Fukuda et al. (2002)
Sphingomonas sp. RW1	2-CDF	51	0.7		5CSA, 2MCh	DF	Wilkes et al. (1996)
Sphingomonas sp. RW16	2-CDF	1013	NS	94.4	5CSA	DF	Wittich et al. (1999)
Klebsiella sp. HL1	2-CDF	8.1	0.3	82.5		DF	Fukuda et al. (2002)
Terrabacter sp. DBF63	2-CDF	10	0.8	NS	5CSA	DF	Habe et al. (2001a)
Pseudomonas sp. CA10	2-CDF	10	0.8	100.0	5CSA	CAR	Habe et al. (2001a)
Alcaligenes sp. JBI	2-CDF	NS	NS	NS	CSA	Bc or 3MBc	Parsons et al. (1990)
Burkholderia sp. JB1	2-CDF	0.3	1.0		5CSA, CTHBP	BP	Parsons et al. (1998)
Sphingomonas sp. RW16	3-CDF	1013	NS	NS	4CSA,4CTHBP,4′CTHBP	DF	Wittich et al. (1999)
Sphingomonas sp. RW1	3-CDF	51	0.7	71.6	4CSA	DF	Wilkes et al. (1996)
Coculture (S. RW16 + P. RW10)	3-CDF	1013	25.0	96.0	Chloride, protoanemonin	3-CDF	Wittich et al. (1999)
Pseudomonas sp. HH69	3-CDF	NS	NS	NS	4CSA, SA	DF	Harms et al. (1991)
Pseudomonas sp. HH69/II	3-CDF	NS	NS	NS	4CTHBP,4′CTHBP	Acetate	Harms et al. (1991)
Sphingomonas sp. RW1	4-CDF	51	0.7	100.0	3CSA	DF	Wilkes et al. (1996)
Sphingomonas sp. RW1	4-CDF	1013			3′CTHBP, 3CSA, (2HPA)	4-CDF	Arfmann et al. (1997)
Coculture[c]	4-CDF	1013			Chloride	4-CDF	Arfmann et al. (1997)
Sphingomonas sp. HL7	23-DCDD	10	0.3	100.0	2MCh	DF	Fukuda et al. (2002)
Sphingomonas wittichii RW1	23-DCDD	10	0.3	100.0	2MCh	DF	Fukuda et al. (2002)
Sphingomonas sp. RW1	23-DCDD	63	0.7	28.4	45DCC	DF	Wilkes et al. (1996)
Pseudomonas sp. EE41	23-DCDD	0.3	63.0	70.5		*o*-DCB	Du et al. (2001)
Pseudomonas sp. CA10	23-DCDD	10	0.8	35.0	45DCC	CAR	Habe et al. (2001a)
Rhodococcus opacus SAO101	23-DCDD	1.0	7.0	23.0		DF	Kimura and Urushigawa (2001)
Terrabacter sp. DBF63	23-DCDD	10	0.8	80.0	45DCC	DF	Habe et al. (2001a)
Bacillus megaterium AL4V P450	23-DCDD	2.5	0.042	11.0	H23DCDD	NS	Sulistyaningdyah et al. (2004)
Sphingomonas wittichii RW1	27-DCDD	50	5.0	21.3	4CC	DF or DD	Hong et al. (2002)
Rhodococcus opacus SAO101	27-DCDD	1.0	7.0	16.0		DF	Kimura and Urushigawa (2001)
Pseudomonas sp. CA10	27-DCDD	10	0.8	25.0		CAR	Habe et al. (2001a)
Erwinia sp.	27-DCDD	2.0	1.0	27.6		Succ	Liaw and Srinivasan (1990)

(*continued on next page*)

Table 2 (*continued*)

Bacterial strain	Compound	Conc. (ppm)	Time (d)	Removal (%)	Products ID[d]	Growth subst.[e]	References
Alcaligenes sp. JBI	27-DCDD	0.3	2.8	33.3		Bc or 3MBc	Parsons and Storms (1989)
Rhodococcus opacus SAO101	28-DCDD	1.0	7.0	16.0		DF	Kimura and Urushigawa (2001)
Sphingomonas sp. RW1	23-DCDF	59	0.7	84.8	45DCSA	DF	Wilkes et al. (1996)
Sphingomonas sp. RW1	27-DCDF	NS	NS	NS	6C2MCh, 7C2MCh	DF	Keim et al. (1999)
Sphingomonas sp. HL7	28-DCDF	9.5	0.3	97.5	6C2MCh	DF	Fukuda et al. (2002)
Sphingomonas wittichii RW1	28-DCDF	9.5	0.3	100.0	6C2MCh	DF	Fukuda et al. (2002)
Terrabacter sp. DBF63	28-DCDF	10	0.8	85.0	5CSA	DF	Habe et al. (2001a)
Sphingomonas sp. RW1	28-DCDF	59	0.7	67.2	6C2MCh	DF	Wilkes et al. (1996)
Pseudomonas sp. EE41	237-TCDD	0.3	63.0	39.7		*o*-DCB	Du et al. (2001)
Sphingomonas wittichii RW1	237-TCDD	50	5.0	23.1	345TCC,TCTHDE	DF	Nam et al. (2006)
Bacillus megaterium AL4V P450	237-TCDD	2.9	0.042	0.8	H237TCDD	NS	Sulistyaningdyah et al. (2004)
Sphingomonas sp. RW1	248-TCDF	NS	NS	NS	68DC2MCh	DF	Keim et al. (1999)
Sphingomonas wittichii RW1	1234-TeCDD	50	5.0	13.2	3456TeCC, 3456TeCG	DF or DD	Hong et al. (2002)
Pseudomonas veronii PH-03	1234-TeCDD	322	5.0	18.0	3456TeCC	DF	Hong et al. (2004)
Pseudomonas testosteroni G1036	2378-TeCDD	0.3	238.0	NS	HTeCDD	NS	Philippi et al. (1982)
Bacillus megaterium	2378-TeCDD	0.005	244.0	61.2	Unidentified	DF	Quensen III and Matsumura (1983)
Sphingomonas wittichii RW1	123478-HCDD	50	5.0	10.2	3456TeCC, 3456TeGC, HCTHDE	DF	Nam et al. (2006)

[a] NS = not stated.
[b] Coculture: *Sphingomonas* sp RW16 and *Pseudomonas* sp. RW10.
[c] Coculture: *Sphingomonas* sp. RW1and *Burkholderia* sp. JWS.
[d] Dibenzofuran (DF), dibenzo-*p*-dioxin (DD); biphenyl (BP); succinate (succ); 1,2-dichlorobenzene (*o*-DCB); carbazole (CAR); benzoic acid (Bc); 3-methylbenzoic acid (3MBc).
[e] 4-chlorocatechol (4CC); 3-chlorocatechol (3CC); 4,5-dichlorocatechol (45DCC); 3,4,5-trichlorocatechol (345TCC); 3,4,5,6-tetrachlorocatechol (3456TeCC); 3,4,5,6-tetrachloroguaiacol (3456TeCG); salicylic acid (SA); 5-chlorosalicylate (5CSA); 4-chlorosalicylate (4CSA), 3-chlorosalicylate (3CSA); 4,5-dichlorosalicylic acid (45DCSA); 2-methyl-4H-chromen-4-one (2MCh); 6-chloro-2-methyl-4H-chroman-4-one (6C2MCh); 7-chloro-2-methyl-4H-chroman-4-one (7C2MCh); 6,8-dichloro-2-methyl-4H-chromen-4-one (68DC2MCh); chloro-2,2′,3-trihydroxybiphenyl (CTHBP); 4- chloro-2,2′,3-trihydroxybiphenyl (4CTHBP); 4′-chloro-2,2′,3-trihydroxybiphenyl (4′CTHBP); 3′-chloro-2,2′,3-trihydroxybiphenyl (3′CTHBP); chlorotrihydroxydiphenyl ether (CTHDE); hexachlorotrihydroxydiphenyl ether (HCTHDE); trichlorotrihydroxydiphenyl ether (TCTHDE); 2-hydroxypenta-2,4-dienoate (2HPA); = dihydroxy-1CDD (DH1CDD); hydroxy-23DCDD (H23DCDD); trihydroxy-23DCDD (H236TCDD); 2-chloromuconic acid (2CMA); chloro-(2-hydroxyphenyl)muconic acids (CHPMA); hydroxyl-CDD (HCDD); dihydroxy-CDD (DHDCC); hydroxy-TeCDD (HTeCDD).

strain DBF63 (Habe et al., 2001a) and a dibenzo-*p*-dioxygenase 1,10a from (Wittich et al., 1992; Armengaud et al., 1998). CARDO and DFDO cloned into and expressed by *Escherichia coli* were able to catalyze the biotransformation of 2-CDF, 28-DCDF, 2-CDD, 23-DCDD, 27- DCDD and 123-TCDD (with the exception of 27DCDD which was transformed by DFDO) (Habe et al., 2001a). The angular dioxygenases catalyze the formation of diols which spontaneously form chlorinated 2,2′,3-trihydroxydiphenyl ethers (THDE) and chlorinated 2,2′,3-trihydroxybiphenyl (THB) in the case of PCDD and PCDF, respectively. THDE and THB are subsequently oxidized by dioxygenases causing ring opening by *meta* cleavage of the dihydroxylated ring. The ring opened products are metabolized further yielding chlorinated catechols or chlorinated salicylates from PCDD or PCDF, respectively (Figs. 1 and 2). In addition to chlorinated salicylates, metabolism of PCDF sometimes yields chlorinated 2-methyl-4H-chroman-4-ones, especially when the PCDF has chloro groups on both rings (Keim et al., 1999; Fukuda et al., 2002) as indicated in Table 2.

Aside from angular dioxygenases, dioxygenase activity has also been found in some bacterial strains, accounting for dioxygenation in the lateral positions of chlorinated dioxins. Examples include dihydrodiols recovered from 2-CDD metabolism by *Beijerinckia* sp. strain B8/36 (Klecka and Gibson, 1980) and dihydroxy-2-CDD metabolites from the incubation of *Alcaligenes* sp. strain JB1 (Parsons and Storms, 1989). Bacterial cytochrome P450 from *Bacillus megaterium* has the ability to cause monooxygenation

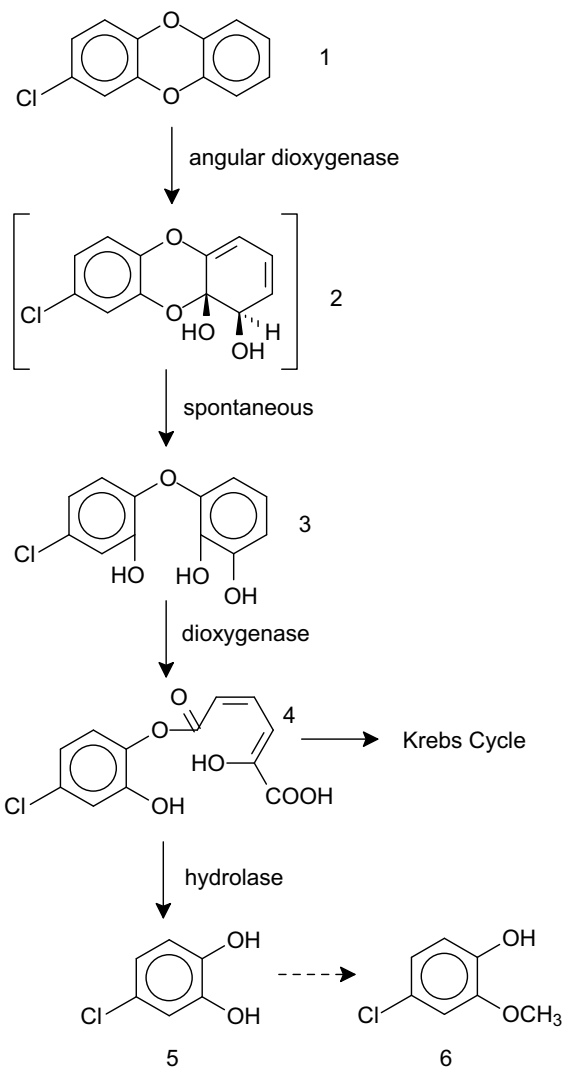

Fig. 1. Pathway of 2-CDD biodegradation by aerobic bacteria. Compound definitions: 2-chlorodibenzo-*p*-dioxin (1); 8-chloro-*cis*-1,10*a*-dihydroxy-1-hydro-dibenzo-*p*-dioxin (2); 4′-chloro-2,2′,3-trihydroxydiphenyl ether (3); 2-hydroxy-6-oxo-6-(4-chloro-2-hydroxyphenoxy)-2,4-hexadienoic acid (4); 4-chlorocatechol (5); 4-chloroguaiacol (6). References: (Wittich, 1998; Habe et al., 2001a; Kimura and Urushigawa, 2001; Nojiri and Omori, 2002; Hong et al., 2004).

of 23-DCDD and 237-TCDD, yielding hydroxylated metabolites of these dioxins (Sulistyaningdyah et al., 2004).

3.2. Aerobic bacterial growth utilizing PCDD/Fs as a sole carbon and energy source

There are surprisingly few well documented examples of chlorinated dioxins serving as a sole source of carbon and energy for pure bacterial strains. *Pseudomonas veronii* PH-03 has been shown to utilize 1-CDD and 2-CDD growing on the aliphatic acids generated from ring cleavage but accumulating 3-chlorocatechol (CC) and 4-CC, respectively, as dead products from the chlorinated ring (Hong et al., 2004). Similarly, *Sphingomonas* sp. strain RW1 can grow on 4-CDF (Arfmann et al., 1997). In this case, the 5-carbon aliphatic acid, 2-hydroxypenta- 2,4-dienoate released from ring cleavage is the substrate that provides carbon and energy; whereas, 3-chlorosalicylic acid (CSA) accumulates as the dead-end product. Complete mineralization of chlorinated dioxins has only been achieved in co-cultures, which combine a CDF-degrader with CSA-degrader. A co-culture of *Sphingomonas* sp. strain RW16 together with *Pseudomonas* sp. strain RW10 completely degraded 2-CDF and 3-CDF (Wittich et al., 1999). *Pseudomonas* sp. RW10 was responsible for mineralizing 5-CSA and 4-CSA that accumulated from 2-CDF and 3-CDF, respectively. About 60% of the chlorine in 3-CDF was recovered as inorganic chloride, indicating an extensive mineralization of 3-CDF by the co-culture. A co-culture constructed from *Sphingomonas* sp. RW1 and *Burkholderia* sp. JWS was used to completely degrade 4-CDF (Arfmann et al., 1997). *Burkholderia* sp. JWS was able to utilize 3-CSA accumulating from 4-CDF degradation for growth. This co-culture released 86% of the chlorine added with 4-CDF.

3.3. Aerobic fungal cometabolism

The only evidence for the degradation of chlorinated dioxins by fungi is limited to wood-, or litter-degrading white-rot fungi. The white-rot fungi constitute the most important group of organisms responsible for the degradation of nature's most complex polymer, lignin. Lignin is formed from the random polymerization of phenyl propanoid units. White-rot fungi use extracellular oxidative enzymes to initiate the attack of lignin. These oxidative enzymes, lignin peroxidase (LiP) and manganese peroxidase (MnP), are also capable of oxidizing a variety of xenobiotic pollutants (Field et al., 1993), including polychlorinated dioxins (Hammel et al., 1986; Valli et al., 1992). In the first study evaluating the potential of white-rot fungi to degrade chlorinated dioxins, *Phanerochaete chrysosporium* was shown to mineralize [^{14}C]-2378-TeCDD by 2.2% to $^{14}CO_2$ in 30 d (Bumpus et al., 1985). The mechanism of DD and 2-CDD oxidation by LiP from *P. chrysosporium* involves successive one-electron oxidations, with the cation radical of DD being the initial intermediate of the process (Hammel et al., 1986). In an elaborate study evaluating the oxidation of 27-DCDD by whole cultures and LiP of *P. chrysosporium*, a pathway of degradation was elucidated (Valli et al., 1992). 27-DCDD supplied at 6.9 mg l^{-1} was degraded by 50% in 27 d by *P. chrysosporium* only under conditions of low-nutrient nitrogen required for the induction of ligninolytic enzymes. Purified LiP catalyzed the conversion of 27-DCDD to 4-chloro-1,2-benzoquinone (2CBQ), 2-hydroxy-1,4-benzoquinone (2HBQ) and chloride. The molar yield of chloride accounted for 19% of the 27-DCDD removed. In whole cultures, the formation of 2CBQ and 1,2,4-trihydroxybenzene (124THB) was demonstrated after short incubation of 48 h with 27-DCDD. These metabolites were added exogenously to the cultures *in vivo* and LiP *in vitro* to elaborate the

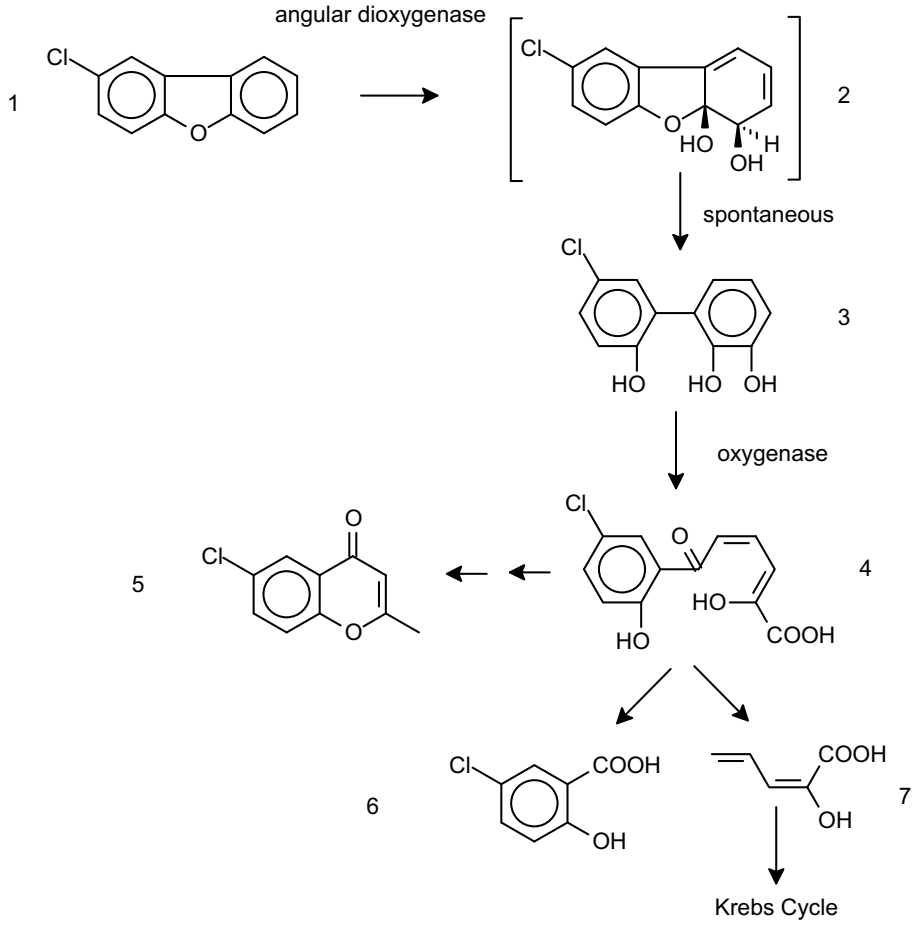

Fig. 2. Pathway of 2-CDF biodegradation by aerobic bacteria. Compound definitions: 2-chlorodibenzofuran (1); 8-chloro-*cis*-4,4*a*-dihydroxy-4-hydrodibenzofuran (2); 5′-chloro-2,2′,3-trihydroxybiphenyl (3); 2-hydroxy-6-oxo-6-(5-chloro-2-hydroxyphenyl)-2,4-hexadienoic acid (4); 6-chloro-2-methyl-4H-chroman-4-one (5); 5-chlorosalicylic acid (6); 2-hydroxypenta-2,4-dienoate (7). References: (Harms et al., 1991; Wilkes et al., 1996; Arfmann et al., 1997; Wittich, 1998; Wittich et al., 1999; Habe et al., 2001a; Nojiri and Omori, 2002).

pathway. The overall pathway shown in Fig. 3 involves cycles of oxidation by LiP and/or MnP forming quinones, followed by reduction to hydroquinones or catechols and subsequent methylations to methoxybenzenes. 124THB is subject to ring cleavage, which could lead to mineralization to CO_2.

The ability of white-rot fungi to degrade chlorinated dioxins is not limited to lower chlorinated congeners. *Phanerochaete sordida* and *P. chrysosporium* were able to remove 34% and 48% of a mixture of higher PCDD/F (penta-, hexa-, hepta-, and octo-PCDD/F, collectively 0.5 $\mu g\,l^{-1}$) after 7 and 14 d of incubation, respectively (Takada et al., 1996). A biological removal mechanism is implicated since the removal is reported in comparison to heat-killed controls and the removal was stimulated by addition of glucose. Additional evidence for a biotransformation reaction was the recovery of 4,5-dichlorocatechol (DCC) and tetrachlorocatechol (TeCC) as metabolites of 2378-TeCDD and OCDD (0.005 $mg\,l^{-1}$) incubations (10 d) with *P. sordida*, respectively (Takada et al., 1996).

Aside from *Phanerochaete*, several other genera of white-rot fungi have been described with outstanding abilities to degrade dioxins. Three strains of fungi, *Phlebia lindtneri*, *Phlebia* sp. MG-60, and an unidentified strain MZ-227, were shown to mineralize [^{14}C]-27-DCDD (7 $mg\,l^{-1}$) to $^{14}CO_2$ by 5.0%, 5.2% and 6.5%, respectively, after 30 d (Mori and Kondo, 2002a). In screening studies that followed, other *Phlebia* strains (BMC3014, BMC9152 and BMC9160) were identified as good 27-DCDD-degraders (Kamei et al., 2005). These strains degraded from 32.4% to 51.1%, 18.4% to 27.8%, 11.9% to 21.1%, 14.2% to 21.5% of 27-DCDD (6.9 $mg\,l^{-1}$), 278-TCDD (7.9 $mg\,l^{-1}$), 1289-TeCDD (8.7 $mg\,l^{-1}$) and 1267-TeCDD (8.7 $mg\,l^{-1}$), respectively after 14 d. *Phlebia brevispora* eliminated 1368-TeCDD by 28% in 28 d and produced 7-methoxy-1368-TeCDD as a metabolite along with traces of hydroxyl-TeCDD, dimethoxy-TeCDD, dimethoxy-TCDD as well as 3,5-DCC (Kamei et al., 2005). *P. lindtneri* was shown to remove 27-DCDD (6.9 $mg\,l^{-1}$) by 55% in 20 d (Mori and Kondo, 2002b). The same strain mineralized [^{14}C]-28-DCDF and [^{14}C]-27-DCDD (6.9 $mg\,l^{-1}$) to $^{14}CO_2$ by 6% and 17%, respectively, in 5 d (Mori and Kondo, 2002b). Additionally, 3-hydroxy-28-DCDF and 3-hydroxy-27-DCDD were identified as metabolites

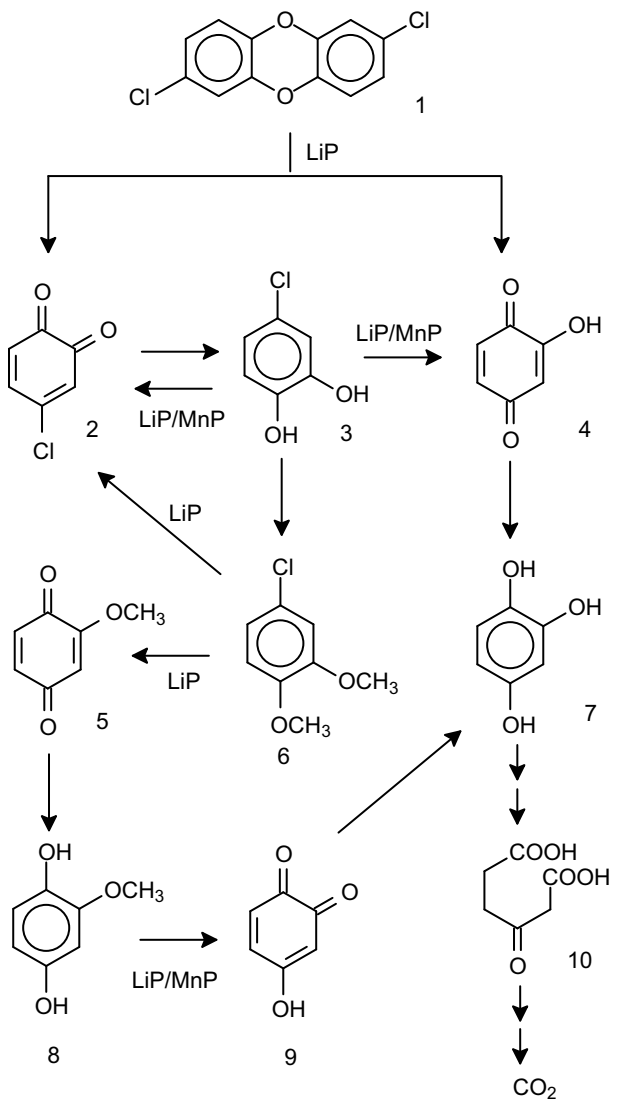

Fig. 3. Proposed pathway of 2,7-dichlorodibenzo-*p*-dioxin (27-DCDD) degradation by the white-rot fungus, *Phanerochaete chrysosporium* (Valli et al., 1992). Legend: 27-DCDD (1); 4-chloro-1,2-benzoquinone (2), 4-chlorocatechol (3); 2-hydroxy-1,4-benzoquinone (4), 2-methoxy-1,4-benzoquinone (5); 4-chloroveratrole (6); 1,2,4-trihydroxybenzene (7); 2-methoxyhydroquinone (8); 4-hydoxy-1,2-benzoquinone (9); β-ketoadipic acid (10); lignin peroxidase (LiP); manganese peroxidase (MnP).

(Mori and Kondo, 2002b). *Panellus stipticus* was identified as another outstanding chlorinated dioxin degrader from another screening study (Sato et al., 2002). *P. stypticus* completely eliminated 27-DCDD (2.8 mg l^{-1}) in 40 d. 4CC was identified as a metabolite of the degradation.

3.4. Anaerobic reductive dechlorination

Chlorinated dioxins undergo reductive dechlorination in anaerobic environments. The first evidence was obtained by spiking anaerobic sediment microcosms with highly chlorinated congeners of dioxins, 1234678-HpCDD, 1234678-HpCDF, 123478-HCDD, and 12468-PeCDF (Adriaens and Grbic-Galic, 1994). These higher chlorinated congeners were shown to be eliminated faster in undisturbed sediments compared to heat-killed sediments, establishing a biological mechanism of removal. The removal rates of PCDD/F in non-autoclaved microcosms were from 19% to 56% higher than in autoclaved controls. Long-term incubation of the congeners up to years revealed the accumulation of lower chlorinated biotransformation products, accounting for up to 30% of the PCDD spiked (Adriaens et al., 1995). In non-autoclaved microcosms, the early studies demonstrated that 1234678-HpCDD and 123478-HCDD were dechlorinated to TeCDD congeners. Likewise, 12468-PCDF was converted to TeCDF congeners. Dechlorination of the PCDD/F was also observed in the heat-killed control microcosms, however, the extent of dechlorination was less and the dechlorination was limited to removal of only one to two chlorines (Adriaens et al., 1995; Barkovskii and Adriaens, 1998). Further studies revealed that model humic compounds and vitamin B_{12} served as redox mediating compounds catalyzing the abiotic reduction of the highly chlorinated (octo to penta-) PCDD/F with either sulfide (present in reduced anaerobic medium) or added Ti(III)citrate (Adriaens et al., 1996; Barkovskii and Adriaens, 1998). In abiotic reactions, the humic model compounds catalyzed the slow removal of one or two chlorine atoms; whereas OCDD was converted in low yields (<10%) to TeCDD by vitamin B_{12}. The results clearly indicate that chemical reduction mechanisms are implicated in some of the initial dechlorination reactions of highly chlorinated.

More extensive dechlorination of the higher chlorinated dioxins requires the activity of microorganisms in sediments. In anaerobic sediment microcosms incubated with 5.3 mg l^{-1} OCDD for 7 months, tri-, di- and mono-chlorinated dioxin congeners were identified, albeit in low molar yields (0.4%) (Barkovskii and Adriaens, 1996, 1998). A number of additional studies have evaluated the bioconversion of penta-, tetra-, tri- and di-chlorinated dioxins added to anaerobic sediments, enrichment cultures and pure cultures of halorespiring bacteria. The results from these studies are summarized in Table 3. Several trends can be recognized. Firstly, that PeCDD and TeCDD have been consistently dechlorinated by two or more chlorine groups to mono-tri- CDD in numerous studies. This confirms the potential of microorganisms to extensively dechlorinate PCDDs. Secondly, previous enrichment of anaerobic cultures derived from sediments with a halogenated compound serving as an electron acceptor (bromophenols, chlorinated benzenes, etc.) is associated with a greater extent of test compound removal and a higher degree of dechlorination. This observation would suggest that the alternative halogenated compounds contribute to the growth of halorespiring bacteria that can utilize PCDD/F as an electron acceptor. Finally, two studies have shown that pure cultures of known halorespiring bacteria from the genus, *Dehaloccocoides*, can effectively dechlorinate

Table 3
Anaerobic biotransformation of chlorinated dioxin congeners spiked into microbial cultures

Microbial culture	Compound	Conc. (ppm)	Time (d)	Removal (%)	Products ID	Growth EA[a]	Electron donor	References
Dehalococcoides sp. CBDB1	23-DCDD	2.5	84	53.0	2-CDD		H_2, Acetate	Bunge et al. (2003)
Anaerobic enrichment	123-TCDD	7.2	55	95.8	13-DCDD		Org. acid mixture	Bunge et al. (2001)
Anaerobic enrichment	123-TCDD	2.9	148	61.9	23-DCDD, 13-DCDD, 2CDD		Org. acid mixture	Ballerstedt et al. (1997)
Dehalococcoides sp. CBDB1	123-TCDD	7.2	84	59.0	2-CDD, 23-DCDD, 13-DCDD[t]		H_2, Acetate	Bunge et al. (2003)
Anaerobic enrichment	124-TCDD	7.2	55	91.9	13-DCDD	1234-TeCDD	Org. acid mixture	Bunge et al. (2001)
Anaerobic enrichment	124-TCDD	2.9	148	86.6	13-DCDD		Org. acid mixture	Ballerstedt et al. (1997)
Dehalococcoides sp. CBDB1	124-TCDD	17.3	84	55.0	2-CDD, 13-DCDD		H_2, Acetate	Bunge et al. (2003)
Anaerobic (sediment)	1234-TeCDD	16.1	244	89.7	124-TCDD, 13-DCDD, 23-DCDD		Org. acid mixture	Bunge et al. (2001)
Anaerobic (sediment)	1234-TeCDD	16.1	396	30.0	13-DCDD		Org. acid mixture	Ballerstedt et al. (1997)
Anaerobic enrichment	1234-TeCDD	0.2	18	42.3	13-DCDD, 23-DCDD, 124-TCDD, 123-TCDD	HCB	Lactate	Beurskens et al. (1995)
Anaerobic enrichment	1234-TeCDD	10.0	225	85.0	124-TCDD, 13-DCDD	PCE	Butyrate + YE	Fennell et al. (2004)
Anaerobic enrichment	1234-TeCDD	10.0	91		13-DCDD, 2CDD	1234-TeCB	Lactate/ propionate	Ahn et al. (2005)
Anaerobic enrichment	1234-TeCDF	13.4	91		TCDF, DCDF	1234-TeCB	Lactate/ propionate	Ahn et al. (2005)
Methanogenic (sediment)	1234-TeCDD	0.6	944	26.9	124-TCDD, 13-DCDD		NS[b]	Vargas et al. (2001)
Methanogenic enrichment	1234-TeCDD	0.6	515	76.6	124-TCDD, 13-DCDD	BrP	NS	Vargas et al. (2001)
Sulfate reducing (sediment)	1234-TeCDD	0.6	944	3.7	124-TCDD		NS	Vargas et al. (2001)
D. ethenogenes 195[d]	1234-TeCDD	10.0	250	40.0	124-TCDD, 13-DCDD[c]	PCE	H_2	Fennell et al. (2004)
Dehalococcoides sp. BDB1	1234-TeCDD	14.8	84	22.0	2-CDD, 23-DCDD		H_2, Acetate	Bunge et al. (2003)
Dehalococcoides sp. CBDB1	12378-PeCDD	1.1	104	2.8	2378-TeCDD, DCDD, 237-TCDD		H_2, Acetate	Bunge et al. (2003)

[a] EA = electron acceptor used for growth during enrichment; BrP = bromophenols; HCB = hexachlorobenzene; TeCB = 1234-TeCB; PCE = perchloroethene.
[b] NS = Not stated.
[c] Trace.
[d] *Dehalococcoides ethenogenes* 195.

various PCDD congeners (Bunge et al., 2003; Fennell et al., 2004). *Dehaloccocoides* sp. strain CBDB1 known for its ability to dehalogenate chlorinated benzenes caused extensive dechlorination of 1234-TeCDD as well as intermediates to 2-CDD (Bunge et al., 2003). *Dehaloccocoides* sp. strain CBDB1 can be sustained for several transfers with 1234-TeCDD as the sole electron acceptor, suggesting that this strain uses 1234-TeCDD as the primary electron acceptor for growth. On the other hand, *Dehalococcoides ethenogenes* strain 195, known for its ability to dehalogenate perchloroethene (PCE), only catalyzed the efficient removal of one chlorine atom from 1234-TeCDD to form 124-TCDD as the main product (Fennell et al., 2004). The ability of *D. ethenogenes* strain 195 to utilize 1234-TeCDD as the primary electron acceptor for growth was not demonstrated since PCE was added during the assay to support the growth of the strain.

Several pathways of PCDD dechlorination have been observed. The most commonly studied model compound for evaluating reductive dehalogenation has been 1234-TeCDD (Table 3). A summary of the pathways observed is provided in Fig. 4. Initial dechlorination is either observed in a lateral position such as with *D. ethenogenes* strain 195 (Fennell et al., 2004), forming 124-TCDD (labeled "195"), or in the *peri* position such as with *Dehaloccocoides* sp. strain CBDB1 (Bunge et al., 2003) (labeled

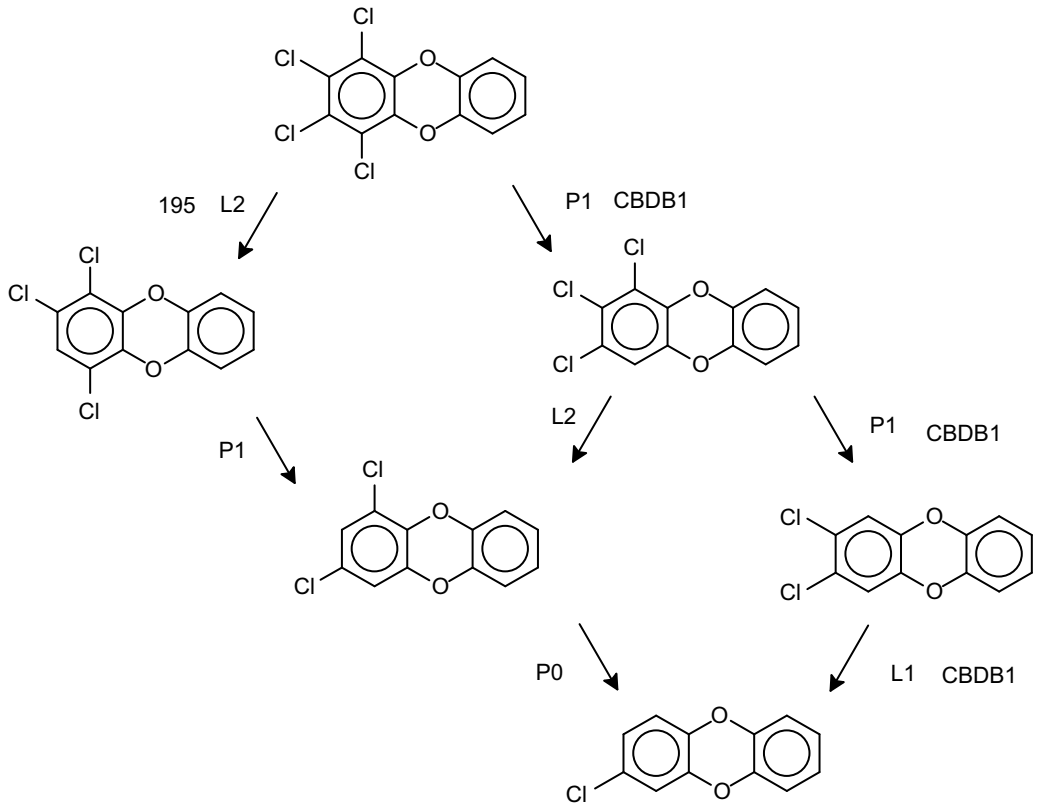

Fig. 4. Anaerobic biotransformation pattern of 1234-TeCDD in anaerobic sediments, enrichment cultures and pure cultures of *Dehalococcoides* spp. (Beurskens et al., 1995; Ballerstedt et al., 1997; Bunge et al., 2001, 2003; Vargas et al., 2001; Fennell et al., 2004; Ahn et al., 2005). Legend: lateral dechlorination surrounded by 2 vicinal chlorines (L2); lateral dechlorination adjacent with 1 vicinal chlorine (L1); *peri*-dechlorination adjacent with one vicinal chlorine (P1); *peri*-dechlorination with no vicinal chlorine (P0); predominant dechlorination reactions carried out by *Dehalococcoides* sp. CBDB1 (CDDB1) (Bunge et al., 2003); predominant dechlorination reactions carried out by *Dehalococcoides ethenogenes* strain 195 (Fennell et al., 2004).

"CBDB1"), forming 123-TCDD. Whereas 124T-CDD is not significantly further degraded by strain 195, organisms present in enrichment cultures and sediment microcosms appear to be capable of continuing the dechlorination of 124-TCDD via *peri*-dechlorinations to 13-DCDD and 2-CDD (Vargas et al., 2001; Fennell et al., 2004). Strain CBDB1 can continue dehalogenating its first major intermediate, 123-TCB, via a second *peri*-dechlorinations to 23-DCDD and a subsequent lateral dechlorination to 2-CDD (Bunge et al., 2003).

Several sediment microcosms and enrichment cultures display both types of activities (195 and CBDB1) (Beurskens et al., 1995; Bunge et al., 2001). One pattern involves organisms solely capable of catalyzing lateral dechlorinations; whereas, the other involves organisms that can catalyze both *peri*- and lateral dechlorinations. Distinct dehalogenation patterns were also observed during the reductive dechlorination of OCDD in a microbial consortium derived from anaerobic sediments (Barkovskii and Adriaens, 1996). Non-spore forming cells were responsible for the *peri*-dechlorination of higher PCDD, resulting in the formation of the toxic 2378-TeCDD intermediate. Spore-forming cells (cells that survive a pasteurization treatment), on the other hand, performed lateral dechlorinations leading to non-2378-TeCDD intermediates; however, the spore-formers were unable to dehalogenate beyond TCDD congeners. The results suggest that there is a diversity of microorganisms that catalyze reductive dehalogenations of chlorinated dioxins.

4. Kinetics of PCDD/F biodegradation

The only reliable data on the microbial kinetics of chlorinated dioxin degradation are those obtained with lower chlorinated congeners under aerobic conditions (Table 4). Most aerobic bacterial strains have been tested under cometabolic conditions. Specific activities of substrate consumption range from an incredibly rapid rate of 42 750 mg g^{-1} dwt cells d^{-1} for 3-CDF metabolism by dibenzofuran grown cells of *Pseudomonas* sp. HH69 (Harms et al., 1991) to moderate rates ranging from several hundred to several thousand mg g^{-1} dwt cells d^{-1} for other monochlorinated dioxin congeners. As the chlorine number increases for either the PCDD or PCDF series, the specific substrate consumption activities as well as the specific O_2-uptake activities decrease by orders of magnitude (Table 4). The results indicate that the oxygenolytic attack

Table 4
Kinetics of aerobic degradation of chlorinated dioxins

Substrate	Role[a]	Culture	Growth rate (d^{-1})	Activity (mg g^{-1} dwt d^{-1})	O_2 uptake rate (mg O_2 g^{-1} dwt d^{-1})	References
1-CDD	Growth/ED	*Pseudomonas veronii* PH-03	2.72			Hong et al. (2004)
1-CDD	Comet (DF)	*Pseudomonas veronii* PH-03			682	Hong et al. (2004)
1-CDD	Comet (Succ)	*Beijerinckia* sp.			1210	Klecka and Gibson (1980)
1-CDD	Comet (DF)	*Sphingomonas* sp. RW1		493		Wilkes et al. (1996)
2-CDD	Growth/ED	*Pseudomonas veronii* PH-03	1.97			Hong et al. (2004)
2-CDD	Comet (DF)	*Pseudomonas veronii* PH-03			597	Hong et al. (2004)
2-CDD	Comet (Succ)	*Beijerinckia* sp.			1212	Klecka and Gibson (1980)
2-CDD	Comet (DF)	*Sphingomonas* sp. RW1		165		Wilkes et al. (1996)
23-DCDD	Comet (Succ)	*Beijerinckia* sp.			258	Klecka and Gibson (1980)
23-DCDD	Comet (DF)	*Sphingomonas* sp. RW1		115		Wilkes et al. (1996)
27-DCDD	Comet (Succ)	*Beijerinckia* sp.			196	Klecka and Gibson (1980)
27-DCDD	Comet (DF)	*Sphingomonas* sp. RW1		18		Wilkes et al. (1996)
28-DCDD	Comet (Succ)	*Beijerinckia* sp.			196	Klecka and Gibson (1980)
124-TCDD	Comet (Succ)	*Beijerinckia* sp.			150	Klecka and Gibson (1980)
124-TCDD	Comet (DF)	*Sphingomonas* sp. RW1		28		Wilkes et al. (1996)
1234-TeCDD	Comet (DF)	*Pseudomonas veronii* PH-03			256	Hong et al. (2004)
2-CDF	Comet (DF)	*Sphingomonas* sp. RW16			9193	Wittich et al. (1999)
2-CDF	Comet (DF)	*Sphingomonas* sp. RW1			2938	Keim et al. (1999)
2-CDF	Comet (DF)	*Sphingomonas* sp. RW1		846		Wilkes et al. (1996)
3-CDF	Comet (DF)	*Sphingomonas* sp. RW16			10 529	Wittich et al. (1999)
3-CDF	Comet (DF)	*Sphingomonas* sp. HH19k		4,377		Harms and Zehnder (1994)
3-CDF	Comet (DF)	*Sphingomonas* sp. RW1			315	Keim et al. (1999)
3-CDF	Comet (DF)	*Sphingomonas* sp. RW1		666		Wilkes et al. (1996)
3-CDF	Comet (DF)	*Pseudomonas* sp. HH69		42 749		Harms et al. (1991)
4-CDF	Growth/ED	*Sphingomonas* sp. RW1	0.87			Arfmann et al. (1997)
4-CDF	Comet (DF)	*Sphingomonas* sp. RW1		856		Wilkes et al. (1996)
4-CDF	Comet (DF)	*Sphingomonas* sp. RW1			5947	Arfmann et al. (1997)
23-DCDF	Comet (DF)	*Sphingomonas* sp. RW1		469		Wilkes et al. (1996)
27-DCDF	Comet (DF)	*Sphingomonas* sp. RW1			518	Keim et al. (1999)
28-DCDF	Comet (DF)	*Sphingomonas* sp. RW1		236		Wilkes et al. (1996)
37-DCDF	Comet (DF)	*Sphingomonas* sp. RW1		117		Wilkes et al. (1996)
248-TCDF	Comet (DF)	*Sphingomonas* sp. RW1			115	Keim et al. (1999)
248-TCDF	Comet (DF)	*Sphingomonas* sp. RW1		16		Wilkes et al. (1996)

[a] Growth/ED = growth on substrate as sole electron donor and carbon source; Comet = Cometabolism (growth substrate in parenthesis); DF = Dibenzofuran; Succ = Succinate.

of dioxins by dioxygenases is greatly impeded by the electron withdrawing properties of multiple chlorine groups.

Microbial kinetic data is lacking for the anaerobic dechlorination of higher chlorinated congeners. One study reported zero-order rate constants in mixed cultures for the bioconversion of 1234-TeCDD of 6.0×10^{-7} and 1.1×10^{-6} mg g^{-1} dwt d^{-1} in anaerobic sediments and digester sludge, respectively (Kao et al., 2001). The remaining kinetic data are limited to half-lives in sediments or culture fluids. Half-lives in anaerobic river sediment microcosms are reported to be 4.1, 2.0, 2.1 and 1.0 y for HeCDD, HCDD, HeCDF and PeCDF, respectively; and in anaerobic aquifer sediments are reported to be 2.9, 2.9, 2.5 and 3.5 y for HeCDD, HCDD, HeCDF and PeCDF, respectively (Adriaens and Grbic-Galic, 1994). An anaerobic enrichment culture derived from lake sediments degraded 1234-TeCDD with a half-life of 15.5 d (Beurskens, 1995).

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