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Reductive dechlorination of 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin and Aroclor 1260, 1254 and 1242 by a mixed culture containing *Dehalococcoides mccartyi* strain 195

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ABSTRACT

A mixed culture containing *Dehalococcoides mccartyi* strain 195 dechlorinated 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD) and selected polychlorinated biphenyl (PCB) congeners in Aroclors 1260, 1254 and 1242. 1,2,3,7,8-PeCDD was dechlorinated to 1,3,7-trichlorodibenzo-*p*-dioxin (1,3,7-TrCDD) and/or 1,3,8-TrCDD via 1,3,7,8-tetrachlorodibenzo-*p*-dioxin (1,3,7,8-TeCDD), a pathway that excludes the production of the toxic congener 2,3,7,8-TeCDD. Dechlorination rate and extent was greatly enhanced by the addition of 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB) as an alternate halogenated substrate and/or incubation temperature increase from 25 °C to 35 °C. The most extensive dechlorination of PCBs occurred for Aroclor 1260 with 13 major congeners making up 44.1 mol% of the original PCBs dechlorinated by 42% over 250 days at 25 °C. When 1,2,3,4-TeCB was amended as co-substrate, the extent of dechlorination increased to 82%, over 250 days. The mixed culture primarily dechlorinated the doubly-flanked chlorines on 2,3,4-, 2,3,4,6-, and 2,3,4,5,6-substituted chlorophenyl rings, whereas it primarily removed the doubly-flanked *para* chlorine from the 2,3,4,5-substituted chlorophenyl ring. Experiments using a 20% dilution of culture with 31.8 μg/mL 1,2,3,4-TeCDD or 2,3,4,4′,5-pentachlorobiphenyl (PCB 114) as sole halogenated substrate exhibited less than 0.1 mol % dechlorination over 120 days. Further, dechlorination of PCBs and PCDDs by the fully grown culture in the absence of 1,2,3,4-TeCB eventually stopped or greatly slowed over the incubation period. Since *Dehalococcoides* spp. only gain energy for growth from organohalide respiration, absence of reductive dechlorination upon transfer and dilution or cessation of dechlorination after long incubation times suggest that it is unlikely that strain 195 can grow using the PCDDs or PCBs utilized in this study.

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1. Introduction

Pollution of the environment by hydrophobic, toxic polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), poses a threat to human and ecosystem health (van den Berg et al., 2006). Many PCB- and PCDD/F-contaminated sites date to improper disposal and accidental spillage events that occurred decades ago (Bedard and May 1995, Hutzinger and Verrkamp, 1981; Meharg and Osborn, 1995; Stellman et al., 2003). The cleanup of those sites remains a challenge because of a lack of economical and effective remediation methods. Previous studies showed that PCBs and PCDD/Fs could be utilized by dechlorinating bacteria as terminal electron acceptors, and thus undergo reductive dechlorination under anaerobic conditions (Beurskens et al., 1995; Brown et al., 1987). The enhancement of this naturally occurring process has been considered as a promising strategy to restore contaminated environments.

To date, *Dehalococcoides* spp. and *Dehalobium* sp. within the Chloroflexi are known as the only bacteria that can reductively dechlorinate PCBs (Adrian et al., 2009; Bedard et al., 2007; Fagervold et al., 2005; Fennell et al., 2004; May et al., 2008) or PCDD/Fs (Bunge et al., 2003, 2008; Fennell et al., 2004). In light of successful field application of bioaugmentation for remediation of chloroethene-contaminated aquifers (Lendvay et al., 2003), the dehalorespiring bacteria have also been considered good candidates for *in situ* bioremediation of PCB- and PCDD/F-contaminated environments. One specific advantage of using *Dehalococcoides* spp. is that these organisms often have a diverse dehalogenating capability, and thus can be grown on relatively soluble substrates such as chloroethenes (Marco-Urrea et al., 2011; Maymó-Gatell et al., 1997; Miller et al., 2005) or chlorobenzenes (Adrian et al., 2000; Fennell et al., 2004; Wu et al., 2002) which would allow production of culture for use in bioaugmentation. Further, the volatile daughter products of dechlorination of these compounds could be removed from the cultures via volatilization prior to amendment to the environment.

Dehalococcoides mccartyi strain 195 (previously known as *Dehalococcoides ethenogenes* strain 195) (Löffler et al., 2013) was first known for its ability to completely dechlorinate tetrachloroethene (PCE) to the environmentally benign product ethene, although the last step from vinyl chloride (VC) to ethene is a co-metabolism process (Maymó-Gatell et al., 1997). Strain 195 has 17 putative reductive dehalogenase genes (Seshadri et al., 2005). The strain respires chlorophenols (Adrian et al., 2007) and could be transferred multiple times with chlorobenzenes as the sole electron acceptor, a likely organohalide respiration process (Fennell et al., 2004). Additionally, pure culture strain 195 debrominated commercial octa-brominated diphenyl ether (BDE) mixtures (He et al., 2006), 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) and 1,2,3,4-tetrachlorodibenzofuran (1,2,3,4-TeCDF), but not 2,3,7,8-TeCDD (Fennell et al., 2004). A mixed culture containing strain 195 also dechlorinated 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF), to non-2,3,7,8-substituted daughter products (Liu and Fennell, 2008). Currently only limited information is available for PCBs: one single PCB congener, 2,3,4,5,6-pentachlorobiphenyl was dechlorinated at doubly-flanked positions (Fennell et al., 2004).

A better understanding of the ability of *Dehalococcoides* spp. for beneficial biotransformation of halogenated pollutants will allow their further exploitation in environmental restoration applications. Further, documenting the ability of these organisms to grow on PCBs or PCDD/Fs would justify their use as bioaugmentation agents that could theoretically be amended at a single time point to accomplish remedial goals. Bunge et al. (2003) reported that *Dehalococcoides mccartyi* strain CBDB1, originally cultivated on trichlorobenzene, dehalogenates and could be transferred on selected chlorinated dibenzo-*p*-dioxins. *Dehalococcoides* spp. in a mixed culture exhibited growth by organohalide respiration with 1,2,4- and 1,2,3-trichlorinated dibenzo-*p*-dioxins (TrCDD) (Ewald et al., 2007). *Dehalococcoides* spp. in culture JN1 also grew on PCB congeners in Aroclor 1260 (Bedard et al., 2007). *Dehalobium chlorocoercia* DF1 respires 2,3,4,5-tetrachlorobiphenyl (May et al., 2008).

We previously reported that rates of dechlorination of weathered PCBs in Anacostia River, Washington, DC microcosms were greater in those microcosms amended with pentachloronitrobenzene (PCNB), bioaugmentation with the mixed culture containing *D. mccartyi* strain 195, or combined PCNB plus bioaugmentation, relative to other treatments (Krumins et al., 2009). Strain 195 was not sustained in the sediment however; and it is still not known whether strain 195 can respire PCBs or PCDD/Fs. Further the pathway of dechlorination of PCB congers with chlorines on both rings (such as those found in Aroclors) or environmentally relevant PCDD congers which have chlorines on both rings is not known for strain 195.

The goal of this study was to determine if the mixed culture containing *D. mccartyi* strain 195 could dechlorinate other environmentally relevant PCDDs and PCBs including 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD), the dioxin-like PCB 2,3,4,4′,5-pentachlorobiphenyl (PCB 114) and commercial PCB mixtures Aroclors 1242, 1254, and 1260. Dechlorination was examined at 25 °C and 35 °C and in the presence or absence of co-amended 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB), a likely growth substrate of strain 195 (Fennell et al., 2004) which has been shown to enhance dechlorination of 1,2,3,4,7,8-HxCDF (Liu and Fennell, 2008). Dilutions of the culture were established on 1,2,3,4-TeCDD and PCB 114 as sole substrates to evaluate growth on these compounds.

2. Materials and methods

The sources for chemicals used and full details of analytical methods are shown in the Supporting Information.

2.1. Dechlorination assessment with undiluted culture

A mixed culture containing *D. mccartyi* strain 195 was grown at 25 °C on PCE and butyric acid, and monitored for chloroethene dechlorination as described previously (Fennell et al., 2004; Liu and Fennell, 2008). The mixed culture containing strain 195 was first described in Fennell et al. (1997). The culture was obtained from Professor James M. Gossett (Cornell University)