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PCB dechlorination enhancement in Anacostia River sediment microcosms

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ABSTRACT

In situ treatment of PCB contaminated sediments via microbial dechlorination is a promising alternative to dredging, which may be reserved for only the most contaminated areas. Reductive dechlorination of low levels of weathered PCB mixtures typical of urban environments may occur at slow rates. Here, we report that biostimulation and bioaugmentation enhanced dechlorination of low concentration (2.1 mg PCBs/kg dry weight) historical PCBs in microcosms prepared with Anacostia River, Washington, DC, sediment. Treatments included electron donors butyrate, lactate, propionate and acetate (1 mM each); alternate halogenated electron acceptors (haloprimers) tetrachlorobenzene (TeCB, 25 μM), pentachloronitrobenzene (PCNB, 25 μM), or 2,3,4,5,6-PCB (PCB116, 2.0 μM); and/or bioaugmentation with a culture containing *Dehalococcoides ethenogenes* strain 195 (3×10^6 cells/mL). Dechlorination rates were enhanced in microcosms receiving bioaugmentation, PCNB and PCNB plus bioaugmentation, compared to other treatments. Microcosm subcultures generated after 415 days and spiked with PCB116 showed sustained capacity for dechlorination of PCB116 in PCNB, PCNB plus bioaugmentation, and TeCB treatments, relative to other treatments. Analysis of *Chloroflexi* 16S rRNA genes showed that TeCB and PCNB increased native *Dehalococcoides* spp. from the Pinellas subgroup; however this increase was correlated to enhanced dechlorination of low concentration weathered PCBs only in PCNB-amended microcosms. *D. ethenogenes* strain 195 was detected only in bioaugmented microcosms and decreased over 281 days. Bioaugmentation with *D. ethenogenes* strain 195 increased PCB dechlorination rates initially, but enhanced capacity for dechlorination of a model congener, PCB116, after 415 days occurred only in microcosms with enhanced native *Dehalococcoides* spp.

1. Introduction

Polychlorinated biphenyls (PCBs) are hydrophobic, persistent toxic organic pollutants that accumulate in sediments and biota. Effective, economical methods for remediation of PCB contaminated sediments are lacking, however, progress has been made in understanding potential for biotransformation of PCBs in recent years. Discovery that anaerobic bacteria of

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the phylum *Chloroflexi*, including the genus *Dehalococcoides* and more distantly-related taxons, can dechlorinate PCBs may offer a promising avenue for bioremediation (Wu et al., 2000; Cutter et al., 2001; Fennell et al., 2004; Watts et al., 2005; Fagervold et al., 2005; Yan et al., 2006; Fagervold et al., 2007; Bedard et al., 2007; May et al., 2008; Kjellerup et al., 2008; and see review by Bedard, 2008). In this study we evaluated whether stimulating native *Dehalococcoides* populations or bioaugmentation could increase dechlorination of low concentrations of weathered PCBs, and whether the treatment effects persist over a relatively long time frame (>1 yr).

Recently, Bedard et al. (2007) reported growth of *Dehalococcoides* spp. coupled to Aroclor 1260 dechlorination and May et al. (2008) showed growth of strain DF-1 a dechlorinating *Chloroflexi* during dechlorination of 2,3,4,5-PCB. Fagervold et al. (2007) reported that three different phylotypes within the *Chloroflexi* increased in response to Aroclor 1260 in Baltimore Harbor sediment, suggesting that a consortium of dechlorinating bacteria with various specificities may result in more extensive dechlorination of mixed PCBs. In addition to the above studies, the tetrachloroethene dehalorespirer, *Dehalococcoides ethenogenes* strain 195, dechlorinated 2,3,4,5,6-PCB (Fennell et al., 2004), although growth was not tested.

One effective strategy for stimulating PCB dechlorination by sediment microorganisms is addition of alternate halogenated electron acceptors/co-substrates (haloprimers), such as 2,3,4,5,6-PCB (PCB116) (Van Dort et al., 1997), 2,6-dibromobiphenyl (2,6-DBB) (Bedard et al., 1998), halobenzoates (Deweerd and Bedard, 1999) and chlorobenzenes and chlorophenols (Cho et al., 2002). Wu et al. (1999) used a most probable number method to show that halopriming with 2,6-DBB increased the number of PCB- and PBB-dehalogenators, suggesting that halopriming works by stimulating growth of dehalorespirers.

Dechlorination rates may depend on PCB concentration (Fish, 1996). In sediment microcosms from the Saint Lawrence River spiked with Aroclor 1242, Cho et al. (2003) reported threshold concentrations of 35–45 mg/kg PCB, below which PCB dechlorination ceased. Fish (1996) found that the maximum dechlorination rate of spiked Aroclor 1242 in sediment microcosms over a range of concentrations from 10 to 250 mg PCBs/kg increased with increasing initial concentration. The data of Fish (1996) generally follow a first order trend, though it should be noted that the rates measured at higher initial concentrations were higher than would be expected from a perfectly linear relationship. Thus, it is not known whether biological treatment of sediments with low PCB concentrations could be effective.

We examined biostimulation and bioaugmentation for enhancing dechlorination of low concentration historical PCBs in microcosms developed using sediments from the Anacostia River, Washington DC. The Anacostia River is a freshwater tidal system within the Potomac River Drainage Basin, which empties into the Chesapeake Bay. The Anacostia River is classified as a warm-water stream with mean temperatures ranging from 3 °C in January to 26 °C in August, and summer temperatures of 18–32 °C (SRC and NOAA, 2000). The lower Anacostia River, downstream of the Washington Naval Yard, is the site of a validation study of active sediment capping technologies (Reible et al., 2006). The area sampled for this study was downstream from a combined sewer overflow (CSO), and sediments are contaminated with PCBs, polycyclic aromatic hydrocarbons, chlorinated pesticides, and heavy metals (Horne Engineering Services, 2003). PCB concentrations in the sediment ranged from 0.4 to 9.1 mg/kg, and the congener profiles are most similar to a mixture of Aroclors 1248, 1254, and 1260 (Horne Engineering Services, 2003). Thus, our study of the Anacostia River site addressed stimulation of PCB dechlorination under conditions of relatively low PCB concentrations arising from a weathered mixture of urban and industrial sources. We used electron donors, haloprimers, and/or bioaugmentation with *D. ethenogenes* strain 195 to stimulate dechlorination of historical PCBs in Anacostia River sediment microcosms. We compared dechlorination rates induced by the treatments, evaluated the persistence of the stimulation after 415 days, and tracked the dechlorinating bacterial population.

2. Experimental methods

2.1. Microcosm preparation

Microcosms were constructed using homogenized sediment recovered from the Anacostia River capping site control plot (Horne Engineering Services and Sevenson Environmental Services, 2004) on 7 July 2006, using a Van Veen dredge. The sediment contained 42–48% total solids. The organic matter content (7%), and textural analysis (41% sand:39% silt:20% clay) were determined by the Rutgers University Soils Testing Laboratory. Microcosms were prepared using 200 mL site sediment in 250 mL stock bottles fitted with rubber stoppers. In order to maintain pore water concentrations as similar as possible to *in situ* conditions, the microcosms were constructed using sediment only – no media or additional site water were added.

Eight treatments were run in triplicate: unamended and killed (autoclaving for 40 min at 121 °C on three successive days) controls; a mixture of electron donors; alternate halogenated electron acceptors (haloprimers) tetrachlorobenzene (TeCB), pentachloronitrobenzene (PCNB), or PCB116; bioaugmentation with a mixed culture containing *D. ethenogenes* strain 195; and PCNB plus bioaugmentation. The electron donor mix containing lactate, propionate, acetate, and butyrate was added to all microcosms to a concentration of 1 mM each, except in the live and killed controls. TeCB and PCNB (both 99%, Sigma–Aldrich, Inc., St. Louis, MO) were added in 100 μL of a 50 mM solution in acetone (B&J Brand, 99.9%, VWR, International, Inc., Pittsburgh, PA). PCB116 (AccuStandard, Inc., New Haven, CT) was added in 100 μL of acetone. The final TeCB and PCNB concentrations were 25 μM; and the final PCB116 concentration was 2 μM (1.5 mg/kg dry weight). Acetone (100 μL, without haloprimer) was added to the electron donor and bioaugmentation microcosms as well, resulting in 6.8 mM acetone in all microcosms except the controls.

The mixed culture containing *D. ethenogenes* strain 195 was grown at 25 °C on PCE and butyric acid using methods described previously (Fennell, 1998). The culture contained 2×10^8 gene copies per mL, based on quantitative PCR targeting the *Dehalococcoides* 16S rRNA gene (see Supporting information). Four mL of the mixed culture was aseptically