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Enhanced reductive dechlorination of polychlorinated biphenyl impacted sediment by bioaugmentation with a dehalorespiring bacterium

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Abstract

Anaerobic reductive dehalogenation of commercial PCBs such as Aroclor 1260 has a critical role of transforming highly chlorinated congeners to less chlorinated congeners that are then susceptible to aerobic degradation. The efficacy of bioaugmentation with the dehalorespiring bacterium “*Dehalobium chlorocoercia*” DF1 was tested in 2-liter laboratory mesocosms containing sediment contaminated with weathered Aroclor 1260 (1.3 ppm) from Baltimore Harbor, MD. Total penta- and higher chlorinated PCBs decreased by approximately 56% (by mass) in bioaugmented mesocosms after 120 days compared with no activity observed in unamended controls. Bioaugmentation with DF-1 enhanced the dechlorination of doubly flanked chlorines and stimulated the dechlorination of single flanked chlorines as a result of an apparent synergistic effect on the indigenous population. Addition of granulated activated carbon had a slight stimulatory effect indicating that anaerobic reductive dechlorination of PCBs at low concentrations was not inhibited by a high background of inorganic carbon that could affect bioavailability. The total number of dehalorespiring bacteria was reduced by approximately half after 60 days. However, a steady state level was maintained that was greater than the indigenous population of putative dehalorespiring bacteria in untreated sediments and DF1 was maintained within the indigenous population after 120 days. The results of this study demonstrate that bioaugmentation with dehalorespiring bacteria has a stimulatory effect on the dechlorination of weathered PCBs and supports the feasibility of using *in situ* bioaugmentation as an environmentally less invasive and lower cost alternate to dredging for treatment of PCB impacted sediments.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that are globally dispersed as a result of cycling between air, water, and soil ^{1–3}. PCBs are hydrophobic and bioaccumulate throughout the food chain by absorption in the fatty tissue of animals and humans where they have been reported to act as endocrine disruptors ⁴ and possible carcinogens ⁵. The most widely applied technologies for treating PCB-impacted sites are

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SUPPORTING INFORMATION AVAILABLE

Image of laboratory mesocosms, phylogenetic tree of Chloroflexi identified in mesocosms, congeners detected in mesocosms, inferred pathways of PCB dechlorination in bioaugmented mesocosm and identification of phylotypes in mesocosms. This information is available free of charge via the Internet at <http://pubs.acs.org/>.

removal by dredging and subsequent transfer to hazardous waste landfills or *in situ* containment in subaqueous sites by capping⁶. In addition to being cost prohibitive for treating large areas of contamination in rivers, lakes and coastal sediments, these technologies are disruptive to environmentally sensitive areas such as marshes and wetlands. Development of an *in situ* treatment system that utilizes microbial activity, bioaugmentation, could potentially reduce high costs and negative environmental impacts associated with dredging and capping.

There have been reports on the potential of aerobic bioaugmentation with bacteria, fungi and plants, but these processes have limited capacity to attack highly chlorinated congeners often found in PCB impacted sites⁷. Microbial reductive dechlorination of higher chlorinated PCB congeners has the potential to complement these processes, but there have been very few studies to date describing the use of anaerobic bioaugmentation to stimulate *in situ* treatment of PCB impacted sediments. Wu and Weigel⁸ and Bedard *et al.*⁹ observed only slight or no stimulation of weathered Aroclor 1260 in Housatonic River sediments bioaugmented with PCB enriched soil and sediment slurries. Natarajan *et al.*¹⁰ reported dechlorination of weathered Aroclor 1242 and 1248 in microcosms inoculated with microbial granules from a UASB digester. However, the possibility could not be ruled out that the observed activity resulted from stimulation of indigenous PCB dechlorinating bacteria by hydrogen generated from fermentation of wood powder added an electron donor rather than the result of bioaugmentation with dehalorespiring bacteria. More recently *Dehalococcoides* spp. and related species within the Chloroflexi have been identified that are capable of reductively dechlorinating PCBs by utilizing PCBs as terminal electron acceptors, a process termed dehalorespiration, and several strains with specific dechlorinating capabilities have been reported^{11–14}. The commercial PCB mixture Aroclor 1260 was reported to be significantly dechlorinated by a consortium consisting of one or more phylotypes enriched from sediment microcosms¹⁵, sediment-free microcosms¹¹ and by an individual species, “*Dehalococcoides*” sp. CBDB1¹⁶. A recent report showed that “*Dehalobium chlorocoercia*” DF-1, bacterium o-17, phylotypes SF1 and DEH10, each with different PCB congener specificities for dechlorination, would alter the overall pathway for dechlorination of spiked Aroclor 1260 differently depending on which microorganisms were added to sediment microcosms¹⁷. Bedard *et al.*¹¹ observed in a sediment-free enrichment culture that a critical mass of cells was required before reductive dechlorination of spiked Aroclor 1260 was detected and proposed that low indigenous numbers of dehalorespiring bacteria might explain why substantial attenuation of PCBs is rarely observed in the environment.

Although these combined reports suggest that bioaugmentation could be a potential strategy for treatment of PCB-impacted sediments, several questions need to be addressed to determine if *in situ* treatment of weathered PCBs by bioaugmentation is possible. Bedard⁹ demonstrated that bioaugmentation with sediment slurries enriched for PCB dechlorinating bacteria combined with PCB 116 as a primer stimulated dechlorination of relatively high levels (50 ppm) of weathered Aroclor 1260 from Housatonic River sediment. However, the effectiveness of bioaugmentation for dechlorinating low levels of PCBs most commonly associated with weathered PCB contaminated sites where both kinetic and availability issues might affect the effectiveness of *in situ* PCB transformation is currently unknown^{18–19}. A recent report by May *et al.*²⁰ showed that bioaugmentation with DF-1 stimulated the reductive dechlorination of weathered Aroclor 1260 (4.6 ppm) in contaminated soil microcosms, and Krumins,²¹ found that the addition of “*D. ethenogenes*” and pentachloronitrobenzene stimulated the dechlorination of weathered Aroclors 1248, 1254 and 1260 (2.1 ppm) in sediment microcosms. Combined, these results suggest that using bioaugmentation for low levels of weathered PCBs is feasible. Another unknown is whether dehalorespiring microorganisms used for *in situ* bioaugmentation can successfully compete

with indigenous populations and be sufficiently sustainable to have a significant impact on reduction of higher chlorinated PCB congeners.

In this report we tested the efficacy of using bioaugmentation with the dehalorespiring bacterium *D. chlorocoercia* DF-1 to stimulate the reductive dechlorination of weathered Aroclor 1260 in sediment mesocosms that simulate *in situ* conditions. Specifically we examined the effects of bioaugmentation on Aroclor transformation over the course of 120 days in the presence of activated carbon to assess any potential effects of bioavailability and monitored the microbial population to assess the sustainability of DF-1 within the background of the indigenous bacterial community.

MATERIALS AND METHODS

Media and growth conditions

“*Dehalobium chlorocoercia*” DF1 was grown anaerobically in estuarine mineral medium (ECI) as described previously^{20, 22}. Sodium formate (10 mM) was added as the electron donor and PCB 61 (2,3,4,5-PCB) was added in acetone (0.1% v/v) at a final concentration of 173 μ M. *Desulfovibrio* sp. extract (1% v/v) was added as a growth factor and titanium(III) nitrilotriacetate (0.5 mM) was added as a chemical reductant^{20, 23}. *D. chlorocoercia* was routinely grown in 50 ml of medium in 160-ml serum bottles sealed with 20-mm Teflon-coated butyl stoppers (West Pharmaceutical, Inc.). All cultures were incubated statically at 30°C in the dark. Growth was monitored by gas chromatographic analysis of PCB 61 dechlorination to PCB 23 (2,3,5-PCB) and by quantitative RT-PCR of 16S rRNA gene copies (described below).

Mesocosm experiments

Mesocosms were prepared in glass 2.2 liter TLC tanks (Fisher Scientific). PCB impacted sediments were sampled on 14 May 2009 from the Northwest Branch of Baltimore Harbor (BH) with a petite Ponar grab sampler at 39°16.8_N, 76°36.2_W and stored in the dark under nitrogen for 19 days at 4 °C prior to use. Sediment was pooled and homogenized anaerobically by stirring in an anaerobic glove bag. Two liters of sediment were added to each mesocosm tank with 2 cm of indigenous water above the sediment surface. A glass plate covered each mesocosm to minimize evaporation with a 1 cm gap on one end for air exchange. Water lost due to evaporation was periodically replenished with deionized water to maintain the osmolarity of the harbor water.

The bioaugmentation inoculum was prepared using ten 50 ml cultures of DF1 grown until 50% of PCB 61 was dechlorinated. The cultures were transferred into 250 ml Oak Ridge bottles in an anaerobic glove box and sealed under nitrogen-carbon dioxide (4:1). The bottles were centrifuged at $22,000 \times g$ for 30 min, decanted, and the pellets were pooled in 50 ml of sterile ECI medium. The concentration of pooled DF1 was approximately 5×10^7 16S rRNA gene copies per ml. Spent medium supernatant was prepared by passing DF-1 culture supernatant through a 0.22 micron filter (Millipore, www.millipore.com) to remove residual cells. Mesocosms were amended with one of four treatments in an anaerobic glove box: (1) 20 ml of concentrated DF1 (5×10^5 cells gram^{-1} sediment); (2) 20 ml of spent cell-free growth medium; (3) 20 ml of concentrated DF1 adsorbed to 25 g granulated activated carbon for 1 hour; or (4) 20 ml of spent cell-free growth medium adsorbed to 25 g activated carbon (CAS# 7440-44-0, type TOG-NDS 80 $\times$ 325, Calgon Carbon Corp.) for 1 hour. The total GAC added in an individual mesocosm (4.4 % sediment dry weight) was similar to concentrations (3–5%) used *in situ* to sequester PCBs from benthic organisms²⁴. No exogenous electron donor was added. Mesocosms were homogenized after addition of the amendments by stirring with a Teflon spoon, then removed from the anaerobic glove box

and incubated at 23°C in the dark. Mesocosms were sampled by taking six cm deep cores using a five ml syringe barrel with the end cut off. Triplicate cores were sampled for each timepoint using a random sampling grid and homogenized prior to analysis for PCBs and DNA as described below.

DNA extraction

DNA was extracted by adding 0.25 g of sediment from each sample core to a PowerBead microfuge tube of a Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc.). The PowerBead tubes were mixed by hand prior to 30 s of bead beating at speed “4.5” using a FastPrep120 (Q-Biogene, 8 CA). Total DNA was then isolated from the PowerBead tubes according to the manufacturer’s directions. DNA was eluted in 100 µl of TE buffer and quantified with a NanoDrop 1000 Spectrophotometer (ThermoScientific). Extracted DNA samples had an A260/280 ratio of ≥ 1.6 and an A260/230 ratio of ≥ 2.0 . All DNA samples were diluted to 2 ng/µl in TE buffer.

Enumeration of PCB dehalorespiring bacteria by quantitative PCR

The quantification of putative dechlorinating Chloroflexi in each subcore was performed by quantitative PCR (qPCR) using iQ SYBR green supermix (Bio-Rad Laboratories) and primers specific for the 16S rRNA gene of a deep branching, putative dechlorinating clade within the Chloroflexi (348F/884R)¹³. Each 25-µl reaction volume contained 1× iQ SYBR green supermix, 500 nM forward and reverse primers and 1 µl of sample DNA. PCR amplification and detection were conducted in an iCycler (Bio-Rad Laboratories). qPCR conditions were as follows: initial denaturation for 15 min at 95°C followed by 35 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 61 °C, elongation for 30 s at 72 °C, with a final extension step of 5 min at 72 °C. One copy of the gene per genome was assumed based on the genomes of *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp, strain CBDB1^{25–26}. qPCR data were analyzed with MJ Opticon Monitor Analysis Software v3.1 and compared to a standard curve of purified DF1 348F/884R 16S rRNA gene product. The standard curve consisted of duplicate dilutions over 6 orders of magnitude. The specificity of qPCR amplification was verified by melting curve analysis followed by gel electrophoresis. Amplification efficiencies of dilutions of gel purified DF1 16S rRNA gene PCR product used as standards were $87\% \pm 7\%$ ($r^2 = 0.99$), and amplification efficiencies of DF1 cells and environmental samples were almost identical to those of the standard curve ($86 \pm 7\%$). The observed departure of amplification efficiencies from 100% was possibly due to the consensus nature of primer 884R, which has 1 mismatch with the DF-1 16S rRNA gene combined with the stringency of the PCR reaction conditions.

Community analysis of PCB dechlorinating bacteria by denaturing HPLC

Denaturing HPLC (DHPLC) analyses were performed using a WAVE 3500 HT system (Transgenomic, Inc.) as described previously²⁷ except that the instrument was equipped with a fluorescence detector (excitation 490 nm, emission 520 nm). The primer set 348F/884R was used for PCR amplification of 16S rRNA genes from bacteria within the Chloroflexi¹³. DHPLC fractions were sequenced as described previously²⁷. A phylogenetic tree was created with Tree Builder (<http://rdp.cme.msu.edu/index.jsp>).

PCB extraction

Sediment samples were extracted using an Accelerated Solvent Extractor (Dionex) following EPA method 3545. Approximately 5 grams wet weight sediment was dried with pelletized diatomaceous earth (Dionex) at room temperature in a desiccator containing $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. The dried sediment (1 g) was transferred to an 11 ml stainless steel extraction cell containing 0.6 g Cu and 2.4 g fluorosil between two cellulose filters on the bottom of

the cell and the remaining cell volume was filled with anhydrous Na₂SO₄. To correct for extraction efficiency, 10 µl of a 400 µg l⁻¹ solution of PCB 166 in hexane was pipetted on top of the Na₂SO₄. The sample containing the surrogate was extracted with approximately 20 ml of pesticide grade hexane (Acros Organics) at 100°C and purged with 13.8 MPa nitrogen. The sample was evaporated to a final volume of 1 ml at 30°C under nitrogen using a N-EVAP 111 nitrogen evaporator (Organomation Associates). Before PCB analysis, 10 µl of PCB 30 and PCB 204 (400 µg l⁻¹ each in acetone) was added to the sample as internal standards.

PCB analysis

PCB congeners were analyzed using a Hewlett-Packard 6890 series II gas chromatograph (GC) with a DB-1 capillary column (60 m by 0.25 mm by 0.25 µm; JW Scientific) and a ⁶³Ni electron capture detector by a modified method of EPA 8082. PCB congeners in a mixture containing 250 µg l⁻¹ Aroclor 1232, 180 µg l⁻¹ Aroclor 1248 and 180 µg l⁻¹ Aroclor 1262 were quantified with a 10-point calibration curve using PCB 30 and PCB 204 as internal standards. Individual congeners and respective concentrations were obtained from Mullins et al.²⁸. Fifty-five additional congeners not present in the Aroclor mixture that were potential dechlorination products were added to the calibration table containing the Aroclor congeners. The additional congeners were quantified with 10-point calibration curves at concentrations of 2, 5, 10, 20, and 40 µg l⁻¹ (in duplicate) for the low range calibration and 40, 100, 200, 400, and 800 µg l⁻¹ (in duplicate) for the high range calibration. Using this protocol 173 congeners were resolved in 130 individual peaks (excluding internal standards PCB 30 and PCB 204 and surrogate PCB166) (Table S1). The final concentration of individual congeners in samples was corrected for the recovery efficiency of the surrogate (82 ± 6%), which was reduced below 100% by the high content of carbon black in Baltimore Harbor sediments³⁰ and the activated carbon amendment. Co-eluting peaks were indicated as multiple congeners. Total chlorines per biphenyl was calculated as the product of the average number of chlorines and molar concentration of each congener divided by the sum of the total molar concentration of all congeners²⁹. When co-eluting peaks were different homologs, equal amounts of each homolog were assumed²⁹. All PCBs (99% purity) were purchased from AccuStandard.

RESULTS

Characteristics of mesocosm sediment

Sediment used in mesocosms was black in color with a slightly gelatinous texture. Relatively high amounts of PAHs, trans-nonachlor and heavy metals have been reported near this site as recently as 1997³¹. Total PCB concentration in the pooled sediment samples was 1.3 ± 0.15 ppm with a mean of 4.76 chlorines per biphenyl. The congener profile was consistent with the first reported survey of PCBs in BH by Morgan and Sommer³² showing that Aroclor 1260 occurred predominantly with smaller amounts of Aroclor 1254 (Table S1). However, less chlorinated congeners were detected that are potential weathered products of Aroclor 1260 (Table S1).

After bioaugmentation the sediments appeared to be anaerobic 1–2 cm below the surface based on black coloration and the distinct odor of sulfide. In mesocosms without GAC the sediment surface developed a grey coloration; with GAC the sediment surface developed a distinct orange-brown coloration. The surface of the mesocosm was disrupted within 1 week by holes (about 0.2 cm in diameter) caused by the activity of benthic worms but the activity was less apparent by day 60 (Figure S1).

Effects of treatments on reductive dechlorination of weathered PCBs

BH sediment mesocosms were bioaugmented with dehalorespiring *D. chlorocoercia* DF-1 to a final concentration of approximately 5×10^5 cells g^{-1} sediment and PCBs were monitored over 120 days. In mesocosms bioaugmented directly with DF1, there was a net decrease in the total amounts of septa- and octa-PCBs (Figure 1C). In mesocosms bioaugmented with DF-1 with GAC, which reduces the bioavailability of PCBs²⁴, there was a net decrease in the total amounts of penta- through octa- PCBs and a net increase in the total amount of tetra- and tri-PCBs over the course of 120 days (Figure 1D). In contrast, there was no obvious change in homolog distribution in non-bioaugmented mesocosms treated with spent cell-free medium with or without GAC. Bioaugmentation both directly and with GAC as an adsorption substrate resulted in 0.6 to 0.7 mol Cl per biphenyl dechlorinated, respectively, after 120 days (Figure 2). A greater rate of dechlorination (0.0067 Cl/biphenyl/day) was observed after bioaugmentation with GAC compared with direct injection (0.0041 Cl/biphenyl/day). A t-Test (two-sample assuming unequal variances, $\alpha=0.05$) showed a significant difference in dechlorination rates between the bioaugmentation treatments with a P-value of 0.041 (df = 4).

Single congener analysis of the mesocosm bioaugmented with DF1 adsorbed to GAC resulted in a significant decrease in higher chlorinated congeners and corresponding increase in lesser chlorinated congeners (Figure 3 and Table S1). The total sum of predicted substrates and products (Figure 3) was $1.26 \pm 0.307 \times 10^{-9}$ mol per gram sediment at day 0 and $1.02 \pm 0.639 \times 10^{-9}$ mol g^{-1} sediment at day 120. Predicted substrate congeners decreased from 1.00 ± 0.228 to $0.291 \pm 0.282 \times 10^{-9}$ mol per gram sediment and product congeners increased from 0.256 ± 0.0786 to $0.729 \pm 0.356 \times 10^{-9}$ mol per gram sediment. The inability to obtain a zero mass balance might have resulted from the absence of possible dechlorination products in the GC method (e.g., PCBs 187, 109, 92, 96, and 84 among others, Table S1 and Table S2), volatilization of less chlorinated congeners or aerobic degradation by indigenous microorganisms. Strikingly, PCB 194 decreased from about 23 to 8 ppb and PCB 133, the predicted product of sequential double flanked *para* reductive dechlorination of PCB 194 (via PCB 172) was found to increase from about 1 to 12 ppb over 120 days (Figure 3 and Table S2). Potential dechlorination products of double flanked reductive dechlorination of PCB 208 (PCB 179 or PCB 136), PCB 195 (PCB 187), PCB 170 (PCB 87), PCB 190 (PCB 163), PCB 180 (PCB153 or PCB 141) and PCB 174 (PCB135 or PCB 149) were detected after 120 days; however, these products did not accumulate to substantial amounts. Furthermore, the less chlorinated congeners that did accumulate were not products resulting from dechlorination of double flanked chlorines (PCB 51, PCB 53, PCB 52, PCB 49, PCB 32, and PCB 57, Table S2). Since DF-1 is reported to reductively dechlorinate only doubly flanked chlorines²⁰ the dechlorination products likely resulted from enhanced activity by the indigenous population of dehalorespiring bacteria. Addition of cell-free medium used to grow DF-1 did not stimulate PCB dechlorination, indicating that the enhanced activity by indigenous microorganisms did not result from “priming” by residual PCBs or biostimulation by the medium.

Sustainability of *D. chlorocoercia* DF1 after bioaugmentation

To determine whether DF-1 was sustainable in the presence of relatively low PCB concentrations and the indigenous microbial community, putative dehalorespiring microorganisms were enumerated during the experiment based on the number of 16S rRNA gene copies g^{-1} dry sediment. In both bioaugmented mesocosms the numbers of putative dehalorespiring bacteria was initially about 2-fold higher than in untreated mesocosms (1.3×10^6 compared to about 6.0×10^5 copies per gram, respectively) indicating that added DF-1 accounted for approximately half the total population of putative dehalorespiring bacteria in those mesocosms. A Student's t-Test ($\alpha=0.05$) showed a significant difference

between initial 16S rRNA gene copy numbers between treatments with a two-tail P-value of 0.01 (df=3). The total 16S rRNA copy numbers in treated mesocosms decreased by 60 days before reaching an apparent steady state for the 120 day incubation period (Figure 4). However, for bioaugmentation treatments by both direct injection and on GAC substrate, the total number of putative dehalorespiring microorganisms remained nearly 2-fold higher in the bioaugmented mesocosms compared with the untreated mesocosms (8.0×10^5 compared to about 4.5×10^5 copies per gram at day 120, respectively). Detection of putative dechlorinating bacteria in untreated inactive mesocosms suggests that only a small proportion of the indigenous bacteria within this clade of the Chloroflexi were capable of dechlorinating PCBs. The results indicate that greater numbers of dehalorespiring microorganisms were maintained in bioaugmented mesocosms during active dechlorination of the weathered Aroclor compared with non-bioaugmented controls.

Seven predominant phylotypes were detected by DHPLC in the BH sediment mesocosms. The community phylotypes were generally similar between mesocosms over 120 days, with the exception of DF1, which was only detected in bioaugmented mesocosms (Figure 5). The putative DF1 fraction was collected, sequenced, and found to be 100% identical to DF1 (Figure S2 and Table S3.). One phylotype present at time zero (BH 4) was 100% identical to phylotype DEH10, a PCB dechlorinating bacterium previously detected BH sediment microcosms^{13, 15}, but no other previously reported BH phylotypes were detected.

DISCUSSION

There have been several reports on aerobic bioremediation by microbes and plants, but these approaches are only effective for treatment of less chlorinated congeners⁷. Although highly chlorinated congeners commonly associated with commercial Aroclors such as Aroclor 1254, 1258, 1260 and 1262 can be reductively dechlorinated to less chlorinated congeners that serve as substrates for complete degradation by aerobic bacteria, concerns over several factors have called into question whether bioaugmentation by dehalorespiring bacteria is an effective strategy for *in situ* treatment of PCB impacted sediments.

Bioavailability

Previous studies on the kinetics of reductive dechlorination in sediment microcosms spiked with Aroclor 1242¹⁸ or 1248¹⁹ reported that activity was limited at concentrations below 37–40 ppm. However, Magar *et al.*³³ reported evidence of reductive dechlorination at PCB concentrations as low as 1–2 ppm in Lake Hartwell over a period of 11 years, which suggested that dehalorespiring bacteria slowly dechlorinated low levels of weathered Aroclor over time. In the current report we show unequivocally that bioaugmentation with the dehalorespiring bacterium DF-1 successfully stimulates the reductive dechlorination of sediment containing 1.3 ppm of weathered Aroclor 1260. Furthermore, addition of GAC, which increases the partition coefficient of PCBs between the sediment matrix and aqueous phase, had no inhibitory effect on dechlorination activity in bioaugmented mesocosms. GAC has been reported to reduce the bioavailability of PCBs to macroorganisms that ingest the particles³⁴, however, the slight stimulatory effect of GAC observed in the bioaugmented mesocosm suggests that PCB dehalorespiring bacteria have mechanisms that enable them to utilize weathered PCBs sequestered in the inorganic carbon fraction. The true threshold of bioavailability for PCB dechlorinating bacteria is not currently known, however, the results clearly demonstrate that bioaugmentation can stimulate reductive dechlorination of low levels of weathered PCBs in the 1–2 ppm range even with activated carbon present to sequester PCBs from any rapid desorption pool of sediment-associated PCB. Although the less chlorinated PCBs generated by bioaugmentation are more bioavailable to benthic organisms, they are generally less toxic because they are non-coplanar or have at least two

adjacent unsubstituted carbon atoms that would make them subject to rapid metabolic degradation³⁵.

Sustainability

One factor that is critical for successful bioaugmentation of weathered PCB-impacted sediments is the ability of the inoculated biocatalyst to compete with the indigenous community of non-dehalorespiring bacteria for electron donors and nutrients. In the current study DF-1 was detected in bioaugmented sediment mesocosms after 120 days indicating that non-native DF1 could coexist with the indigenous sediment community. The total numbers of putative dehalorespiring bacteria decreased by approximately half after 90 days to a steady state of $7-8 \times 10^5$ cells per gram of sediment, but community analysis showed that DF-1 was sustained as a predominant member of the putative dehalorespiring community. Although the total numbers of putative dehalorespiring phylotypes was 1–2 orders of magnitude lower than observed in prior reports of Aroclor 1260 dechlorinating microcosms^{11, 15} neither exogenous electron donors nor electron acceptors were added in our study; therefore the lower steady state numbers likely reflect lower concentrations of indigenous electron donors or acceptors available in the sediment. The observation that dehalorespiring bacteria were sustained in the sediment mesocosms without an exogenous electron donor is consistent with the ability of dehalorespiring bacteria to outcompete hydrogenotrophic sulfate reducers, acetogens, and methanogens in the presence of limited hydrogen concentration^{36–37}. Interestingly, the indigenous phylotypes of putative dehalorespiring bacteria, with the exception of phylotype DEH10 had not been reported previously in Baltimore Harbor sediment Aroclor 1260 enrichment microcosms¹⁵. One possible explanation is that a different population of dehalorespiring bacteria was enriched with high concentrations of spiked Aroclor 1260 in the prior studies and the population in the current study is more relevant at *in situ* concentrations. The results show that DF-1 was able to coexist successfully with the indigenous microbial population even with the lower background concentrations of electron donor.

Another significant observation was the detection of PCB congeners that did not result from dechlorination of doubly flanked chlorines. These dechlorination reactions were not observed in control mesocosms with spent cell-free medium indicating that the activity observed was neither due to a “priming” effect from residual PCB 61 or 23 in the inoculum nor a nutrient effect from carryover of formate or cell byproducts in the medium. This phenomenon has been reported previously. May *et al.*²⁰ observed enhanced dechlorination of congeners without flanked chlorines in Aroclor 1260 impacted soil microcosms inoculated with DF-1. Krumins *et al.*²¹ observed in PCB-impacted Anacostia River sediments bioaugmented with *D. ethenogenes* strain 195 stimulated PCB dechlorination and although this species was not sustained enhanced activity continued, which was attributed to the indigenous dehalorespiring community. One possible explanation for this effect is “priming” by the accumulation of dechlorination products from the initial activity of high numbers of DF-1 inoculated into the sediments. Addition of PCB congeners and analogs is known to stimulate the reductive dechlorination of PCBs in lab studies^{21, 38–39} and in field tests⁴⁰. Overall the results indicate that in addition to initiating and directly dechlorinating weathered PCBs, bioaugmentation with DF-1 had a synergistic effect on the indigenous dehalorespiring community by an as yet unknown mechanism that contributed further to the dechlorination process.

Bioaugmentation

There have been recent attempts to test the effects of bioaugmentation with pure cultures of dehalorespiring bacteria. Krumins *et al.*²¹ reported enhanced reductive dechlorination of weathered PCBs (*ca.* 2 ppm) in sediment microcosms after bioaugmentation with *D.*

ethenogenes strain 195 and May *et al.*²⁰ reported enhanced dechlorination of Aroclor impacted soil (4.6 ppm) after bioaugmentation with DF-1. In contrast to the prior studies where bioaugmentation stimulated the reductive dechlorination of PCB by 0.2 Cl/biphenyl after 415 days and 0.35 Cl/biphenyl after 145 days, respectively, bioaugmentation in the current study stimulated the reductive dechlorination by 0.7 Cl/biphenyl after only 120 days. Furthermore, bioaugmentation results in the current study stimulated 56% by mass reduction of penta- through nona-chlorobiphenyls to lesser-chlorinated congeners (primarily tetrachlorobiphenyls), which are susceptible to aerobic degradation, with no detectable activity in untreated controls. The discrepancies in the rates and extent of dechlorination could possibly occur due to a number of factors including available nutrients, presence of inhibitory co-contaminants, the dehalorespiring strain used and the growth state and numbers of cells used for bioaugmentation. Distribution of cells in the current study was effective either by direct injection or on GAC particles. The ability to use a solid substrate such as GAC for inoculation of cells offers a possible solution for dispersing cells in the field.

The results of this study support the potential feasibility of using bioaugmentation for treatment of PCB impacted sediments. We showed that bioavailability does not prevent bioaugmentation from treating low levels of weathered PCBs in sediment mesocosms and that GAC actually enhanced the overall process. Furthermore, DF-1, a non-indigenous species, was sustained throughout the dechlorination process and had a positive synergistic effect on the indigenous dehalorespiring population that contributed to the process. Although DF-1 was used successfully to bioaugment reductive dechlorination of weathered Aroclor 1260 in Baltimore Harbor sediments, discrepancies between the current and prior studies highlight the importance of testing inoculum with sediments from each site. All PCB bioaugmentation studies including the current study have been conducted on a laboratory scale and field studies will be required ultimately to validate the approach for *in situ* treatment of contaminated sites. However, the overall results of this mesocosm-based study provide compelling evidence to support further testing and development of bioaugmentation with dehalorespiring bacteria as an environmentally less invasive and lower cost alternative for *in situ* treatment of PCB impacted sediments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

PCB analysis by homolog at day 0 (*white bars*) and day 120 (*black bars*) after treatment with filter sterilized spent growth medium (A), sterilized spent growth medium and GAC (B), concentrated DF1 in growth medium inoculated directly into the sediment (C) and concentrated DF1 adsorbed onto GAC (D). Each bar represents the mean and standard deviation of three replicates samples.

Figure 2.

Changes in Cl per biphenyl in mesocosms over time after treatment with sterilized spent growth medium (○), sterilized spent growth medium and GAC (□), DF1 inoculated directly into sediment (●), and DF1 adsorbed onto GAC inoculated into sediment (■). Each datum point represents the mean and standard deviation of three replicates samples.

Figure 3.

Congeners showing greatest change in mesocosms receiving both GAC and DF1 at day 0 (□) and day 120 (■). An asterisk (*) indicates congeners that could be dechlorination substrates of DF1, or products from double flanked dechlorination by DF-1. Each bar represents the mean and standard deviation of three replicates samples.

Figure 4. Enumeration of putative dechlorinating *Chloroflexi* normalized to 16S rRNA gene copies/gram sediment at day 0 (□), day 60 (▨) and day 120 (■). Treatments included spent medium only, spent medium and GAC, amendment with DF-1 by direct injection, and amendment with DF-1 adsorbed to GAC. Each bar represents the mean and standard deviation of three replicates samples.

Figure 5.

DHPLC community analysis of putative dechlorinating *Chloroflexi* 16S rRNA genes in mesocosms at day 0 (*bottom trace*) and day 120 (*top trace*). A, addition of spent growth media alone; B, addition of spent growth media and GAC; C, addition of DF1; D, addition of DF1 and GAC. Peak eluting at about 6.3 minutes (labeled 8/DF1 with *arrow*) in C and D confirmed DF1 16S rRNA gene by sequencing. Other phylotypes of putative dechlorinating *Chloroflexi* labeled 1, 2, 3, 4, 5, 6, or 7 are described in Table S2.