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A Pilot-Scale Field Study: In Situ Treatment of PCB-Impacted Sediments with Bioamended Activated Carbon

Rayford B. Payne,[†] Upal Ghosh,[‡] Harold D. May,[§] Christopher W. Marshall,[∥,⊥] and Kevin R. Sowers*[,†]

[†]Department of Marine Biotechnology, Institute of Marine and Environmental Technology, University of Maryland Baltimore County, Baltimore Maryland 21202, United States

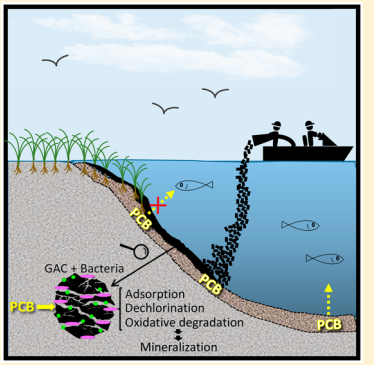
[‡]Department of Chemical, Biochemical, and Environmental Engineering, University of Maryland Baltimore County, Baltimore Maryland 21250, United States

[§]Hollings Marine Laboratory, Department of Microbiology and Immunology, Medical University of South Carolina, Charleston South Carolina 29412, United States

[∥]Biosciences Division, Argonne National Laboratory, Argonne, Illinois 60439, United States

Supporting Information

ABSTRACT: A combined approach involving microbial bioaugmentation and enhanced sorption was demonstrated to be effective for in situ treatment of polychlorinated biphenyls (PCBs). A pilot study was conducted for 409 days on PCB impacted sediments in four 400 m^2 plots located in a watershed drainage pond in Quantico, VA. Treatments with activated carbon (AC) agglomerate bioamended with PCB dechlorinating and oxidizing bacteria decreased the PCB concentration in the top 7.5 cm by up to 52% and the aqueous concentrations of tri- to nonachlorobiphenyl PCB congeners by as much as 95%. Coplanar congeners decreased by up to 80% in sediment and were undetectable in the porewater. There was no significant decrease in PCB concentrations in non-bioamended plots with or without AC. All homologue groups decreased in bioamended sediment and porewater, indicating that both anaerobic dechlorination and aerobic degradation occurred concurrently. The titer of the bioamendments based on quantitative PCR of functional marker genes decreased but were still detectable after 409 days, whereas indigenous microbial diversity was not significantly different between sites, time points, or depths, indicating that bioaugmentation and the addition of activated carbon did not significantly alter total microbial diversity. In situ treatment of PCBs using an AC agglomerate as a delivery system for bioamendments is particularly well-suited for environmentally sensitive sites where there is a need to reduce exposure of the aquatic food web to sediment-bound PCBs with minimal disruption to the environment.



INTRODUCTION

Polychlorinated biphenyls (PCBs) are frequently reported contaminants worldwide[1] that dominate the ecological and human health risk associated with contaminated sediments in the United States, ranking second after mercury as the basis for fish consumption advisories.[2] Hydrophobic pollutants such as PCBs have accumulated in sediments under waterbodies and continue to serve as an ongoing source of the bioaccumulative pollutants to the aquatic food web.[3]

The most widely applied technologies for remediation of contaminated sediments are dredging and disposal or capping with inert or active materials. A recent study by the National Research Council found that of the 26 sediment Superfund Megasites (remediation expense > $50M) that underwent dredging operations, about half did not achieve the set goals of PCB contaminant removal.[4] In addition to being cost prohibitive and potentially ineffective for large areas of contamination in rivers, lakes, and coastal sediments, these technologies are disruptive to environmentally sensitive areas such as marshes and wetlands. In situ studies have demonstrated the feasibility of PCB bioavailability reduction using activated carbon as an amendment.[5] Although in situ amendment studies are effective at reducing PCB bioavailability in sediments, a more desirable goal is to ultimately reduce the inventory of legacy PCBs in sediments while also reducing bioavailability to the food chain.

A large volume of work published in the last two to three decades has demonstrated PCB microbial dechlorination and aerobic degradation in the laboratory (reviewed in refs 6 and 7). However, a lack of understanding of the rate-limiting step in the process has limited translation to sediments in the field. Work by Lombard et al.[8] demonstrated that the low abundance of organohalide respiring bacteria rather than

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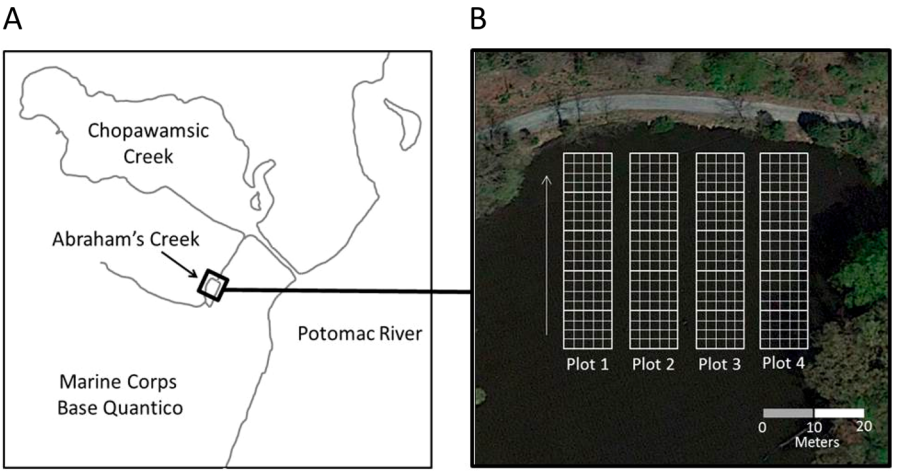

Figure 1. Schematic of (A) Abraham's Creek in Marine Corps Base Quantico and (B) positions of the treatment plots 1 (no treatment), 2 (GAC only), 3 and 4 (bioamended GAC). Pre- and post-treatment sediment samples were taken from random positions from each of five areas (bold grid lines) within the treatment plots. Arrow shows direction of water flow toward corrugated steel culverts that pass under a land bridge and drain into the northernmost portion of the creek. Map data copyright 2018, Google Imagery copyright 2018, Commonwealth of Virginia, U.S. Geological Survey.

bioavailability accounts for the low rates of dechlorination typically observed in sediments. This is consistent with a laboratory mesocosm study that showed bioaugmentation of PCB-contaminated sediment with the organohalide respiring bacterium *Candidatus Dehalobium chlorocoercia* DF1 reductively dechlorinated penta- to nona-chlorobiphenyls to lesser chlorinated PCB congeners (56% mass reduction of chlorines).[9] A subsequent laboratory study showed that concurrent addition of DF1 and aerobic PCB degrader *Paraburkholderia xenovorans* LB400 decreased the total mass of PCBs by 80% after 120 days as a result of oxidation and dechlorination of the less chlorinated PCB congeners produced by DF1.[10] The study also demonstrated the efficacy of using activated carbon (AC) as a medium to deliver microorganisms into sediment. More recently, a laboratory-scale treatability study was conducted with sediments from Abraham's Creek, a watershed drainage pond in Quantico, VA, to determine the optimal bioamendment titer and AC loading rates for field application.[11] Results of that study indicated that a titer of 5×10^5 DF1 and LB400 cells g^{-1} sediment with 1.5% AC as a delivery medium resulted in 78% reduction of total PCBs and 97% reduction of PCBs in the aqueous phase after 375 days.

Based on the optimal cell titer and carbon loading rates determined from the latter study, we conducted the first pilot-scale field application of bioamended AC in Abraham's Creek with the following objectives: (1) demonstrate the scalability of growing PCB respiring microorganisms for field application, (2) develop and test the application of PCB dechlorinating and oxidizing bacteria using pelleted AC as a delivery system, (3) assess the benefits of bioamended AC treatment on concentrations of PCBs in sediments and porewater, (4) assess the fate of the bioamendment over time, and (5) evaluate the impact of treatment on the indigenous microbial populations.

MATERIALS AND METHODS

Pilot Study Location and Design. The field location for this study was a 31600 m^2 watershed drainage pond in Abraham's Creek, a tributary of the Potomac River adjacent to Chopawamsic Creek located 38°29′49″N, 77°18′57″W (Figure 1). The average water depth of the test area varied seasonally and spatially between 120 and 180 cm. Total PCB concentration was 3.4 ± 0.5 mg kg^{-1} distributed among higher homologues (subcategories of PCB congeners that have equal numbers of chlorine substituents) dominated by 18% hexa- and heptachlorobiphenyls typically found in the commercial PCB mixture Aroclor 1260 (Monsanto Co.) and 42% of a second homologue group dominated by tri- and tetrachlorobiphenyls, which are typical dechlorination products.[11] This site is currently in the Remedial Investigation/Feasibility Study phase under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), and bench-scale treatability studies and treatment options are being evaluated.[11,12]

Preparation of Bioamended Activated Carbon. A pelletized activated carbon (AC) agglomerate, SediMite (Sediment Solutions), was manufactured with the addition of 0.1% by weight of cellulose as an electron donor and a residual moisture content of 5−10%.

Growth and scale up of the anaerobic organohalide respiring bacterium *Candidatus D. chlorocoercia* DF1[14] (DF1) and the aerobic PCB oxidizing bacterium *Paraburkholderia xenovorans* LB400[13] (LB400) is described in the SI (Text S1).

Cell suspensions were applied to SediMite pellets at the site by combining DF1 and LB400 to final concentrations of 1.3×10^8 and 1.7×10^8 cells mL^{-1}, respectively, in M9 medium in 4L batches immediately before application.[11] A cell viability test showed the combined strains can be sprayed onto SediMite in the presence of air and passed through a water column without significant loss of cell numbers or viability (Text S2 and Table S1). A measured volume of cell suspension was applied with a 23 L electric sprayer (R&K Pump & Equipment) onto measured aliquots of SediMite in a 0.1 m^3 cement mixer to provide an even distribution on the pellets (Table S2). The concentration for DF1 and LB400 cells was $1.4 \pm 0.9 \times 10^7$ and $1.6 \pm 1.1 \times 10^7$ cells g^{-1} of SediMite, respectively.

Application of Treatments. Four 400 m^2 plots were treated as follows: (1) control plot with no treatment, (2) SediMite containing cellulose as an electron donor, and (3 and

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4) replicate plots treated with bioamended SediMite containing cellulose (Figure 1). The 10 × 40 m treatment plots were mapped by using GPS coordinates and marked with 2.5 cm PVC tubing posts. Treatments in Abrahams Creek were delivered from a flat bottom boat using a 965 kPa 15 cm Venturi Horn Induction (VHI) system (Model TX6-AM, Texas Pneumatic Tools) modified with a 0.5 m length of 10 cm flexible duct (Figure S1) that drew pellets from a hopper. The VHI was attached to a 10.5 m^3 min^{-1} compressor located on shore, and the rate of application and distance of throw was controlled by the operator via a control valve. Unamended or amended SediMite (2 × 500 kg bulk bags) was applied at the dosing rate indicated in Table S2 to achieve approximately 1 × 10^5 cells each of DF1 and LB400 inocula and 3% SediMite.

Sediment Sampling. To address spatial variability of PCB concentration in sediment, five samples were randomly collected from each 400 m^2 treatment plot (Figure 1 and Figure S2) by inserting a 50 × 5 cm push core (Wildco) into the top 30 cm of sediment. Samples were taken prior to treatment (day 0) and 140 and 409 days after treatment (day 140 and day 409). The core device was maintained in a vertical position when advancing and removing the device to minimize disturbance to the sediment. The polycarbonate liner was removed from the core body while maintaining a vertical position, and the liner was sealed with caps. Liners were transported to the lab in a vertical position within an insulated cooler and stored at 4 °C. In the lab, measured depths of 0−7.5 and 7.5−15 cm were extruded from the sediment cores and transferred into 250 or 500 mL I-CHEM Certified borosilicate jars (Thermo Scientific). Each 0−7.5 and 7.5−15 cm depth core sample was homogenized by hand mixing with a Teflon spatula prior to analyses.

Porewater Sampling. Freely dissolved PCBs in sediment porewater and overlying water immediately above the sediment were measured by passive sampling following method described in ref 15. The polyethylene (PE) passive samplers (77 μm 15 cm × 15 cm) were encased in a stainless steel mesh and frame. Triplicate passive samplers were randomly positioned in three locations within each plot (Figure S3) using a 3 m long insertion device that had a 15 × 15 cm metal platform perpendicular to the mesh frame to prevent the top of the frame from being pushed beyond the sediment surface. The samplers were attached to a nylon rope and floatation buoy at the surface for retrieval. A second passive sampler was attached to the retrieval line approximately 1 m below the surface to measure PCBs in the water column. After equilibrating *in situ* for at least 30 days the samplers were retrieved, rinsed with deionized water, and sealed in I-CHEM Certified borosilicate jars. After retrieval, the PE was sectioned into 0−7.5 and 7.5−15 cm depth intervals and transported on ice to the lab. Samplers were stored at 4 °C until they were processed.

Chemical Analyses. Sediment total organic carbon (TOC) analysis was performed as described by Grossman and Ghosh[16] using a Shimadzu TOC analyzer with a solids sample module (TOC-5000A and SSM-5000A) and non-dispersive infrared gas analyzer as recommended by the manufacturer. Activated carbon (AC) falls under the class of black carbon (BC) and was measured in sediments using a BC assay method tailored to include AC in sediments.[16,17] In this method, the natural organic carbon in sediments is first removed by chemical oxidation followed by TOC analysis of the residual carbon that includes any native BC and AC. TOC, BC, and AC values are reported as weight percent of dry sediments.

PCBs were extracted from 5g wet weight aliquots dried with pelletized diatomaceous earth (Dionex, Sunnyvale, CA) in a desiccator at room temperature. The dried sediment was extracted with an Accelerated Solvent Extractor (Dionex) following EPA method 3545 as described previously[9] with PCB 166 (10 μL stock of 400 μg L^{-1} hexane) as a surrogate to correct for extraction efficiency and PCBs 30 and 204 (400 μg L^{-1} each in 10 μL acetone) as internal standards.

PE passive samplers used to measure PCB concentrations in porewater were analyzed as described by Sanders et al.[18] PCB concentrations in PE were converted to estimated PCB concentrations in the porewater phase based on equilibrium partitioning constants (K_{PE}) described by Ghosh et al.[19] PCBs 29, 69, 103, 155, and 192 were included in the PE samplers as performance reference compounds to correct for nonequilibrium conditions and assess porewater concentration using the first order nonequilibrium correction method described in Oen et al.[20]

Gas chromatographic analysis of PCBs and chloroethenes is described in the SI (Text S3).

Biological Analyses. DNA was extracted from 0.25 g sediment (wet wt) aliquots with a Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, CA) as previously described.[9] Extracted DNA samples had an A260/280 ratio of ≥1.8 and an A260/230 ratio of ≥2.0. Microorganisms were enumerated by real-time quantitative PCR (qPCR) using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) with primers CIOP0/CIOP1 specific for the *bph*A gene operon of LB400 and SKFPat9F/SKFPat9R specific for a putative reductive dehalogenase gene of DF-1 (BankIt2186043 Seq1MK423975) using conditions previously described.[9,10,21] Standards (*bph*A or putative reductive dehalogenase) were amplified from LB400 or DF1 genomic DNA using PCR with their respective primers, gel purified, confirmed by sequencing, and then resuspended at 10 ng/μL in TE buffer. Amplification efficiencies of standards and samples were 92 ± 8.0% with R^2 = 0.98. The linear range was 0.1 to 1 × 10^{-6} ng and the y-intercept was 1.2 (CIOP0/CIOP1), and 1.3 (SKFPat9F/SKFPat9R).

DNA for 16S rRNA gene amplicon sequencing analysis was amplified using 12.5 μL of AccuStart II pCR ToughMix (QuantaBio, Beverly, MA), 1 μL of Golay barcode tagged 515 forward primer,[22] 1 μL of 806 reverse primer, 9.5 μL of PCR water, and 1 μL of template DNA. PCR conditions were: 94 °C for 3 min, 35 cycles at 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; with a final extension of 10 min at 72 °C. Amplicons were quantified using PicoGreen (Invitrogen) and pooled to equimolar amounts. A pooled final concentration of 6.75 pM was used with a 10% PhiX spike for sequencing on the Illumina MiSeq Amplicons were sequenced on a 2 × 150 bp paired end MiSeq run using customized sequencing primers and procedures.[22]

Sequences were processed with Qiime2 version 2018.2.[23] Quality filtering and denoising was done with the Qiime2 Dada2 plugin,[24] and taxonomic classification was based on the SILVA 132 release using the 99% operational taxonomic unit (OTU) classifier.[25] α and β diversity, ordination plots, and bar plots were created with Phyloseq v1.22.3,[26] vegan v2.5.1,[27] and FSA v0.8.20.[28] For α and β diversity, samples were rarefied to 5750 sequences per sample with set.seed (04242018) in order to ensure even sampling depth and remove samples with low

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Table 1. Effect of Treatments on the Decrease of Total PCB Levels after 409 Days Showing Mean and Standard Deviation for Five Replicate Sediment Samples at Each Time Point

	day 0		day 140		day 409	
	pretreatment		post-treatment			
treatment (core depth)	mg kg^{-1} $\bar{x}$ (SD)	% decrease[a]	mg kg^{-1} $\bar{x}$ (SD)	% decrease[a]	mg kg^{-1} $\bar{x}$ (SD)	% decrease[a]
1 (0–7.5 cm)	2.6 (0.9)		3.1 (0.6)		2.4 (0.5)	8
2 (0–7.5 cm)	2.5 (0.5)		2.9 (0.6)		2.6 (0.8)	
3 (0–7.5 cm)	2.3 (0.5)		1.9 (0.8)	17	1.6 (0.3)	30[b]
4 (0–7.5 cm)	2.5 (0.3)		1.8 (0.7)	28	1.2 (0.3)	52[b]
1 (7.5–15 cm)	2.9 (1.1)		3.3 (1.0)		3.7 ± 0.8	
2 (7.5–15 cm)	3.0 (0.5)		3.6 (0.7)		3.3 ± 0.8	
3 (7.5–15 cm)	2.7 (0.8)		4.2 (1.2)		3.5 ± 0.6	
4 (7.5–15 cm)	2.5 (0.6)		3.4 (1.0)		3.0 ± 1.1	

[a]Percent decrease of mean value compared to day 0. [b]Significant decrease ($p < 0.05$) compared with day 0.

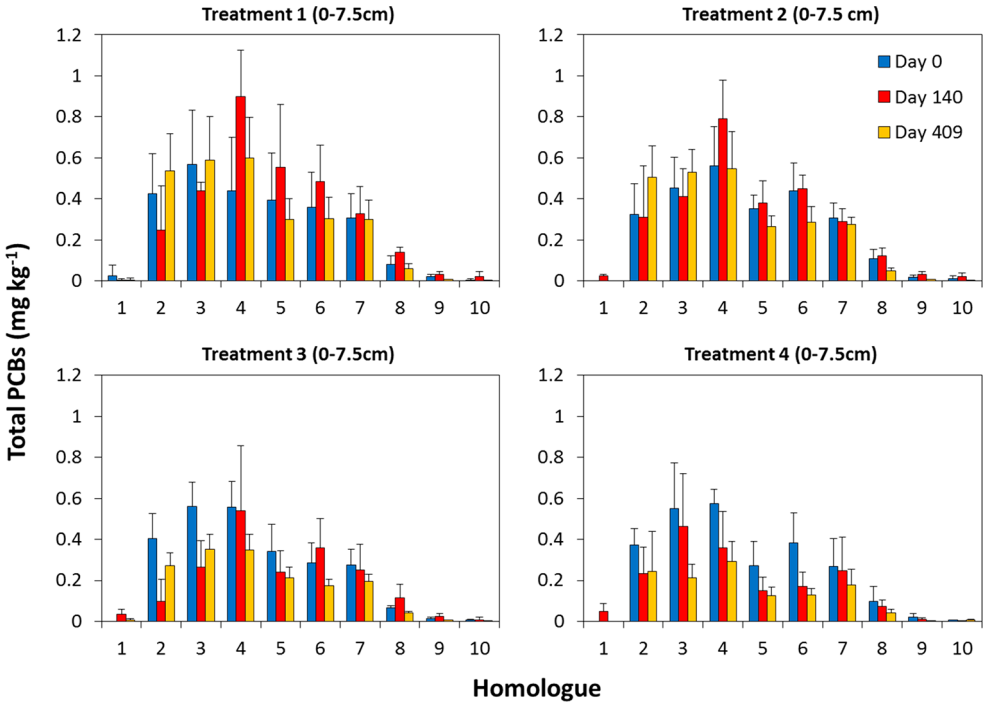

Figure 2. Effect of treatments on PCB homologue (chlorine atoms per biphenyl) concentrations in upper (0–7.5 cm) sediment profile of treatment plots 1 (untreated), 2 (nonamended GAC + cellulose), and 3 and 4 (bioamended GAC + cellulose). Each bar represents mean and standard deviation for five replicate sediment samples.

sequence output (8 samples fell below this threshold and were excluded from these analyses). Command line and R workflow for sequence processing are available here: https://github.com/sirmicrobe/qiime2_workflow_quantico.

Data Availability. Sequences were submitted to NCBI under BioProject PRJNA355587 and accession nos. SAMN10092436–SAMN10092561.

RESULTS AND DISCUSSION

Total Organic and Activated Carbon Measurement. The mean TOC ranged from 2.2 to 3.2% native carbon in all plots prior to treatments and increased in treated plots 2, 3, and 4 as a result of AC addition (Table S3). Background levels of BC ranged from 0.2 to 0.4% in all four plots, and as expected, higher levels of BC were detected in plots 2, 3, and 4 after treatment with unamended and amended AC. However, the mean values of TOC and BC observed in plot 4 were greater than those observed in plot 3, which indicates that although the same amount of measured amendment was applied to each plot (1000 kg), the bioamended AC was not evenly distributed within each plot. The mean concentrations of BC in sample cores taken 0.6, 1.2, and 1.8 m downstream of plot 3 were at background levels, which indicates that bioamended BC remained in place throughout the 409 day post-treatment period.

Effect of Treatments on Total PCBs in Sediments. Table 1 and Figure S4 show the effects of treatments on total PCB concentrations in the sediments. The only plots with a significant decrease in PCB concentration were the top 7.5 cm of bioamended treatments 3 and 4 ($p = 0.028$ and $p = 0.0001$, respectively). The mean total PCB reduction in concentration and apparent rate of degradation for treatments 3 and 4 was 30% at 1.7 ($r^2 = 0.0.29$) μg kg^{-1} day^{-1} and 52% at 3.2 ($r^2 = 0.0.54$) μg kg^{-1} day^{-1}, respectively (Figure S5). The estimated rate in the field is lower than that observed in mesocosm treatability study for Abraham's Creek reported previously,[11]

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where 65% of the PCBs were degraded in the first 30 days at a rate of 73 $\mu g\ kg^{-1}\ day^{-1}$. However, unlike the mesocosm study, the field treatments were not artificially mixed and the site was subject to seasonal temperature changes over the course of the 409 day incubation period rather than a constant 20 °C. There was no detectable decrease in PCB concentration below 7.5 cm, but this is likely below the benthic zone where natural mixing of the bioamendment by bioturbation is expected to occur.

Although treatment of the two bioamended plots 3 and 4 was identical, plot 3 showed less reduction of PCB concentration than plot 4. In order to explain the range of PCB degradation levels within and between bioamended plots 3 and 4, the amounts of bioamended AC in the individual sample cores were compared with the reduction of PCB levels. As shown in Figure S7, there is a very weak correlation in plot 2 between AC dose and reduction in PCB concentration after 409 days. Thus, AC alone is not influencing the mass of PCB remaining in sediment, whereas in bioamended plots 3 and 4, PCB concentrations were significantly lower compared to plot 2 for any AC dose. Four lines of evidence indicate that the decrease in PCB was due to microbial activity and not the AC. First, there was no significant difference in PCB levels between the untreated control and plot 2 treated with unamended AC. Second, a significant decrease in total PCB levels was only observed in bioamended plots 3 and 4. Third, extraction efficiency using methods described in this study is not affected significantly (p = 0.33) by different amounts of black carbon (Figure S6). Fourth, excluding the two outliers in plot 4 (Figure S7) that had exceptionally high concentrations of black carbon (15.3 and 16.6%), the decrease in total PCB concentration was 43% instead of 52% compared with day 0 and remained statistically significant (p = 0.0023). Furthermore, reduction of PCB levels in Abraham's Creek sediment was already confirmed to occur by bioaugmentation in a prior mesocosm study using the same dosage of cells g^{-1} sediment.[11] The results suggest that increasing the dosage of bioamended AC combined with more even application would achieve greater degradation and homogeneity of PCB degradation throughout the treatment area.

An examination of the congener homologues after 409 days (Figure 2) confirms net reduction in the concentration of most mono- to nonachlorobiphenyls, indicating both anaerobic organohalide respiration of highly chlorinated congeners and aerobic degradation of less chlorinated congeners occurred in the bioamended plots (plots 3 and 4). By contrast, there is very little change in the PCB homologues after 409 days in the nonbioamended plots (plots 1 and 2). This uniform decrease suggests that most congener products resulting from anaerobic dechlorination were subject to aerobic degradation, thereby preventing their accumulation. Payne et al. showed previously that the dechlorination pattern in sediments bioamended with DF1 is influenced by the indigenous community.[9,10] DF1 is only capable of attacking chlorines that are flanked by two other chlorines, however, in sediments bioamended with DF1 additional dechlorination patterns are observed, including dechlorination of singly flanked chlorine positions. This pattern shift is possibly the result of indigenous PCB respiring bacteria that are stimulated by an unidentified growth factor provided by the nonorganohalide respiring *Desulfovibrio* sp. grown in coculture with DF1.[14] No significant changes in homologue concentrations were observed in the nonbioamended plot, which indicate that the stimulation was not the result of electron donor added with the GAC.

The effect of treatments on the reduction in toxicity by coplanar PCBs was also examined. Only three coplanar PCBs (114, 156 and 157) were detected in sediment samples from Abraham's Creek. As shown in Table 2, significant reduction (p = 0.026) was only detected in the top 0−7.5 cm of plot 4, which resulted in an 80% reduction in the total toxic equivalency TEQ.[29]

Table 2. Effect of Treatments on Coplanar PCB 114, 156, and 157 Levels Showing Mean and Standard Deviation for Five Replicate Sediment Samples (0−7.5 cm) at Each Time Point

	plot 1	plot 2	plot 3	plot 4
day	$\mu g\ kg^{-1}\ \bar{x}$ (SD)	$\mu g\ kg^{-1}\ \bar{x}$ (SD)	$\mu g\ kg^{-1}\ \bar{x}$ (SD)	$\mu g\ kg^{-1}\ \bar{x}$ (SD)
0	66.2 (45.5)	105.8 (83.8)	45.8 (36.0)	64.5 (41.3)
140	70.8 (13.0)	60.8 (37.0)	58.0 (17.7)	29.5 (21.3)
409	52.4 (31.6)	74.6 (17.6)	46.4 (12.3)	13.1 (10.8)

Effect of Treatments on PCBs in Porewater. The total freely dissolved PCB concentrations in the overlying water ranged from 4 to 8 ng L^{-1}, and there was no significant change with time (Figure S8). The relatively constant PCB concentrations in the water column within and between plots was not unexpected since the nonamended and bioamended treatment plots represented only 1.2% of the total 31600 m^2 area in Abraham's Creek, which was also subject to varying intensities of water flow from precipitation and wind-associated disturbances.

The total freely dissolved PCB concentrations in the bioactive sediment zone (0−7.5 cm) are shown in Figure 3a and Table S4. The average concentration of total PCBs in untreated plot 1 remained close to 130 ng/L over the first 140 days and decreased to 70 ng/L after 409 days. However, most of this decrease was due to a decrease in the more soluble dichlorobiphenyls and was not statistically significant. For the untreated site, tri- to nonachlorobiphenyl congeners averaged 36 ng/L before and after 409 days (Figure 3b). Although there is an apparent increase on day 140, the difference is not significant (p = 0.50). PCB congeners with three or more chlorines are a good representation of the PCBs that bioaccumulate in fish and has been used for assessing dissolved PCB concentrations in the Hudson River remedial investigations.[30]

The initial mean total PCB concentrations in the porewater of the three treatment plots 2, 3, and 4 were slightly higher than plot 1 at about 160 ng/L prior to treatment, although this difference was not significant (p = 0.73, p = 0.705, and p = 0.720, respectively). The dissolved concentrations in porewater were more than an order of magnitude higher than the concentrations observed in the overlying water. Thus, there appears to be a strong gradient for PCB transport from the sediments into the overlying water at this site. The treatment effect appears to manifest into reduction of porewater PCB concentrations over time. Decrease of porewater PCBs were observed in the two bioamended plots on day 140. At day 409, the mean percent reductions in plots 3 and 4 were 73% and 76%, respectively, for total PCBs including dichlorobiphenyl congeners. For the tri- to nonachlorobiphenyl congeners the reductions in bioamended plots 3 and 4 after 409 days were 84% and 95%, respectively, compared to 64% reduction in plot

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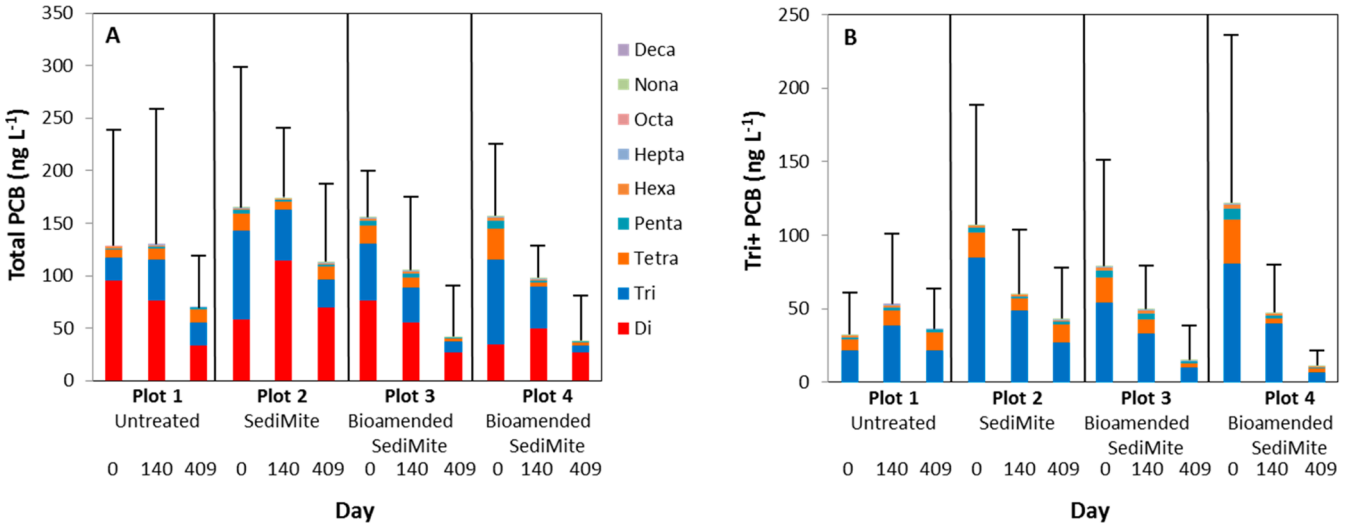

Figure 3. Freely dissolved concentration of total PCBs in sediment porewater in the 0−7.5 cm surface sediments for mono- to decachlorobiphenyls (A) and tri- to decachlorobiphenyls (B). Each bar represents mean and standard deviation for three replicate porewater samples.

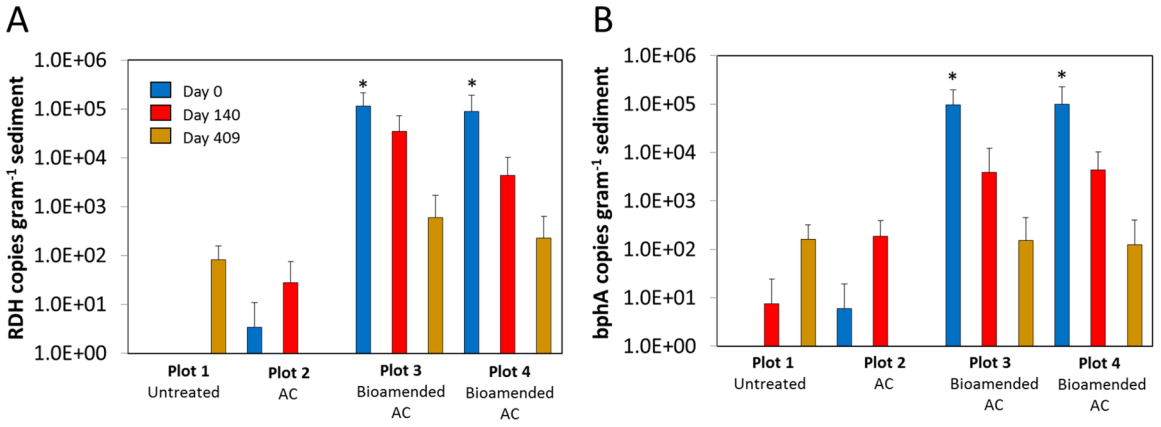

Figure 4. Estimated titer of bioamendments LB400 and DF1 in test plots based on quantitative PCR enumeration of genes encoding a putative reductive dehalogenase in *D. chlorocoercia* DF-1 (A) and biphenyl dioxygenase (*bph*A1) in B. xenovorans LB400 (B). Asterisks indicate estimated cell titer based on amount of bioamended SediMite deployed into plot. Each bar represents mean and standard deviation for five replicate sediment samples.

2 treated with nonbioamended AC. Plots 1 and 2 show a mean reduction of porewater total PCB concentration, but the change was not significant (p = 0.464 and p = 0.587, respectively). The target dose of AC for this demonstration was kept at only 1.5% AC (as percent dry sediment) so as to not overwhelm the treatment with the effect of the AC. There is some spatial variability in measurements of the porewater concentration across the plots and over time. As a result, some of the observed differences in mean concentrations were not statistically significant especially when the dichlorobiphenyls are included. However, the reductions are statistically significant for plots 3 and 4 after 409 days when looking at either all congeners or tri- to nona-chlorobiphenyls. These observed reductions in tri- to nonachlorobiphenyl congeners in the porewater in conjunction with observed reductions of these congeners in the sediment phase confirms the effectiveness of the treatments in reducing both the mass and bioavailability of the tri- to nonachlorobiphenyls at the site.

The concentration of dissolved PCBs in the deeper sediments (7.5−15 cm) did not show a significant change with treatment or over time (data not shown), perhaps because of slow penetration of the amendments to the deeper zone of sediments. However, the bioamended upper layer would effectively serve as a barrier for movement of PCBs toward the sediment surface as a result of diffusion or avective processes. Sites requiring degradation of PCBs below the benthic zone could potentially be treated using mechanical mixing, which has been used successfully in the field for application of activated carbon amendments.[31]

Fate of Bioamendments. Bioamendments *D. chlorocoercia* DF1 and *P. xenovorans* LB400 were monitored after deployment into the test plots (Figure 4). The combined titer of the bioamendments was 2.4 ± 1.9 × 10^7 cells g^{-1} AC with 88% distributed onto AC at the target titer of >1 × 10^7 cells g^{-1} based on random sampling of 16 inoculated AC pellets. Bioamendments were estimated as 3.4 × 10^5 cells g^{-1} dw sediment based on the mean titer of the sampled pellets and distribution of AC in plots 3 and 4. The titer of the organohalide respiring strain (DF1) and aerobic degrader (LB400) decreased by 2 to 3 orders of magnitude after 409 days, which is similar to the decrease in cell numbers observed in the mesocosm treatability study after a similar period of time.[11] One explanation for the observed decrease in DF1 the population is the concentration of dissolved PCBs after

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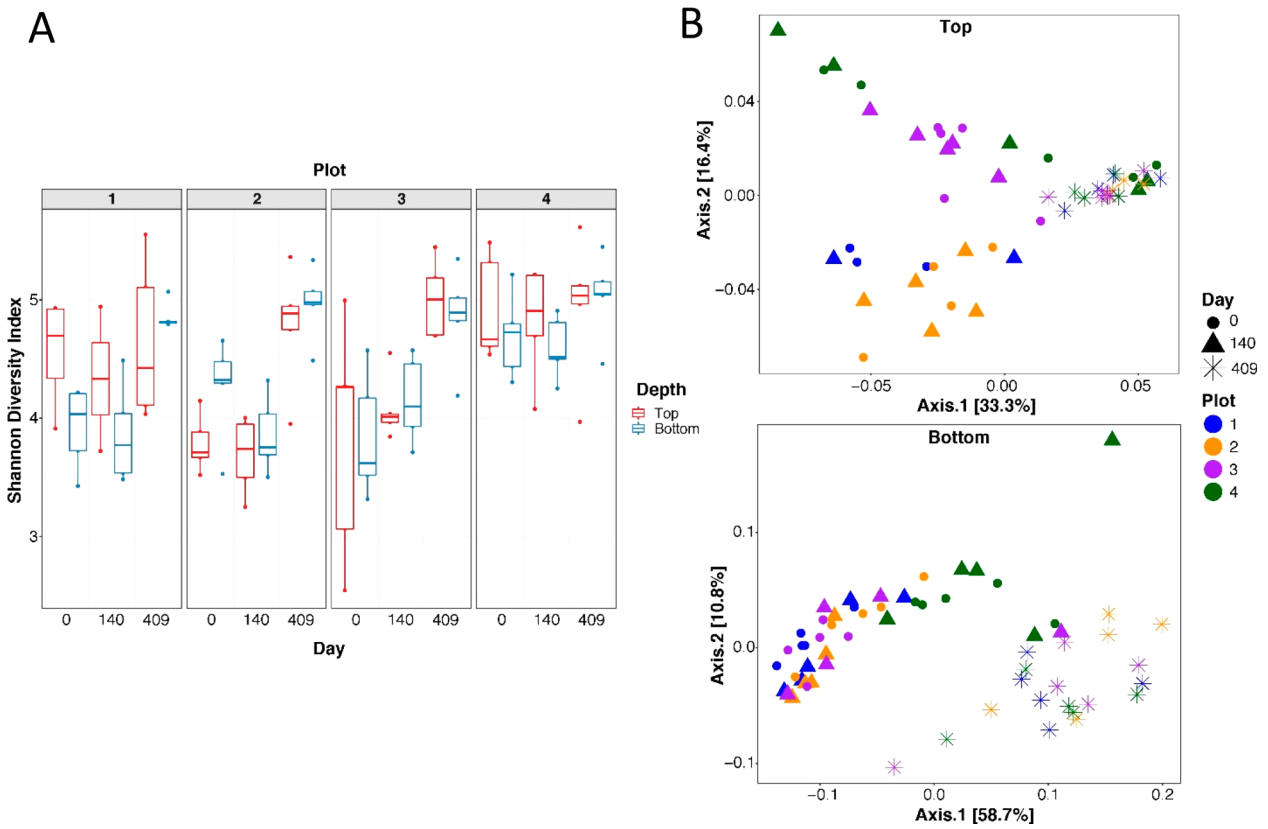

Figure 5. Figure 5. (A) Box and whisker plots of the Shannon alpha diversity measure for top 0−7.5 cm (red) and bottom 7.5−15 cm (blue) sediment depth in each plot at day 0, 140, and 409 days after treatment; (B) principal coordinate analysis using the weighted unifrac dissimilarity measure for the top 0−7.5 cm (top) and bottom 7.5−15 cm (bottom) to determine the relatedness between microbial communities in each sample. Shapes represent samples taken on different days and colors represent samples from different plots.

microbial degradation was below threshold to support the large population of PCB transforming bacteria added as bioamendment.[8] The observed decrease in the population of LB400 is not surprising since metabolism of PCBs by this strain is cometabolic and requires a nonchlorinated substrate for growth.[34] It is possible that naturally occurring substrates present at the site would support growth of LB400, but the decline in LB400 titer over time suggests that they were either not present or present at concentrations that would not support LB400 at the high titer used to amend the sediment. Any remaining dissolved PCBs would be less bioavailable due to adsorption to AC and naturally occurring organic carbon. Some background signal was detected in plots 1 and 2 at low levels between 10^1 and 10^2 cells g^{-1} dw sediment. However, the bioamendments were not detected previously among indigenous microorganisms in sediment from this site,[11] which suggests some cross contamination occurred between plots. Overall, the results indicate that the bioamendment titer decreased but was still retained in sediment after 409 days, which suggests that dechlorination and degradation activity potentially could continue after 409 days.

Impact of Treatments on the Indigenous Microbial Community. In addition to monitoring the bioamendments, the overall microbial diversity was examined before treatment and 140 and 409 days after treatment in each test plot. The Shannon diversity metric was significantly higher in plot 4 overall, but no difference in α diversity was observed between plots 1, 2, and 3 (Figure 5a). Even though plot 4 was significantly more diverse overall, there were no significant differences in Shannon diversity between the plots at day 409. This indicates that the treatments did not significantly reduce overall microbial diversity, a potential concern with any introduction of non-native species. Bioaugmentation with DF1 and LB400 strains, however, did appear to alter the compositions of the microbial communities in the treatment plots 3 and 4 (Figure 5b). The principal coordinate analysis using the weighted unifrac distance describing the dissimilarity between samples based on operational taxonomic unit[32] presence, absence, abundance, and phylogenetic relatedness indicated that plot 2 was significantly different from plots 3 and 4 (PERMANOVA, p = 0.041, 999 permutations, Table S5); however, these differences disappeared by day 409, where no significant difference in any plot was observed in the top 7.5 cm samples. No differences were observed between the plots in the bottom 7.5 cm.

The predominant OTUs present across all plots were members of the Gallionellaceae, Helicobacteraceae (*Sulfuricurvum*), and Pseudomonadaceae families (Figure S9). These families consist of metabolically diverse taxa that include iron and sulfur oxidizing bacteria, and common nitrogen fixing soil and sediment bacteria. In addition to these abundant OTUs, we looked at the composition of the taxonomic families that comprised the bioaugmentation strains. Burkholderiaceae were only observed in the 16S rRNA gene amplicon sequencing analysis in plots 3 and 4 on days 0 and 140 (Figure S10). This observation is consistent with the high 16S rRNA gene copy numbers observed in those at times 0 and 140 and a reduced titer at time 409 (Figure 4). We were unable to detect and/or

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properly classify DF-1 with the universal 16S rRNA gene-targeted primers in the bioamended sediments.

Overall Performance and Future Improvements. This pilot-scale field study shows the feasibility of using bioamended AC for in situ treatment of PCBs in contaminated sediments. The study successfully demonstrated (1) scale up of PCB respiring and oxidizing bioamendments for field-scale treatment, (2) inoculation and deployment of bioamendments on pelleted AC, (3) significant reduction of the total mass of PCBs and the porewater concentration of PCBs compared to untreated sediment and treatment with AC alone, (4) sustainability of bioamendments above background levels in sediments after 1 year, and (5) no significant impact of the treatment on the indigenous microbial community.

The effectiveness of bioamended AC was directly affected by the homogeneity of the application and for full-scale treatment more consistent application would be required to achieve maximum degradation and homogeneity of PCB degradation throughout the site. The VHI used here is advantageous for application in water margins, wetlands, and difficult to access areas such as below piers and under overhanging trees, whereas large open water areas will require methods that ensure even distribution such as a boat-mounted belt spreader or land-based telebelt. In addition to homogeneity of the application, there was a direct relationship between the extent of degradation and the amount of bioamended AC detected in an individual sediment sample (Figure 2). The results suggest that increasing the target dose of bioamended AC would further enhance the effectiveness of the treatment in a full-scale application.

Prior to this study, the only report of in situ bioremediation of PCBs did not show significant reduction of PCBs after repeated inoculation with an aerobic PCB degrader.[33] The total mass reduction of PCBs in the current field study was significant at 52% in the first 409 days, a major advance in this first successful demonstration of in situ bioremediation of PCBs. Lab mesocosm experiments using sediment from the same site indicate that mass reduction could be as high as 78% with further optimization. Part of the discrepancy between lab and field can be attributed to slower natural mixing by bioturbation, uneven distribution of the bioamendment, and seasonal fluctuations in temperature. As the current study was limited to two post-assessments 140 and 409 days after treatment, a multiyear post-treatment assessments would be necessary to fully validate the long-term effectiveness of the bioamended AC to decrease PCB levels in sediments and porewater. In addition, we observed a gradual reduction in the abundance of the bioamendments over time in the field. Future work will explore the feasibility of a second application to further accelerate degradation of PCBs at sites that require a shorter timeline for site closure.

The current study demonstrates the successful treatment of PCB impacted sediments using a combination of in situ treatment with both anaerobic dechlorinating and aerobic oxidizing microbes and AC. The AC serves as substrate for co-colonization by both the anaerobe and aerobe possibly by providing a redox gradient within microniches of its porous structure or within biofilms. Application of bioamended AC to sediments reduces risk by both biological degradation of the total mass and reduction of freely dissolved concentrations of PCBs in sediments. As with any in situ technology, bioamended AC will be most effective in sites that are subject to minimal erosion and with no ongoing upstream sources of PCBs. However, AC amendment provides several advantages over traditional remediation methods such as dredging and capping, including less disruption to benthic habitats in sensitive rivers and wetlands, amenability to shallow or constricted locations, and a smaller carbon footprint. This pilot study shows the promise of bioremediation as a new strategy to help address the widespread need to reduce contamination of the aquatic food web from exposure to sediment-bound PCBs either alone or in combination with other remedies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b05019.

Additional information on scale up of microorganisms, analysis of organochlorines, microbial viability, loading rates, sediment characteristics, sediment, and dissolved PCBs, the VHI, sampling locations, PCB extraction efficiencies, and microbial community analyses (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: (410) 234-8878. Fax: (410) 234-8896. E-mail: sowers@umbc.edu.

ORCID

Upal Ghosh: 0000-0003-2112-1728

Christopher W. Marshall: 0000-0001-6669-3231

Kevin R. Sowers: 0000-0002-8920-9278

Present Address

⊥(C.W.M.) Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, PA 15219.

Notes

The authors declare the following competing financial interest(s): Several authors are co-inventors of patents related to the technology described in this paper for which they are entitled to receive royalties. K.R.S. and H.D.M. are co-inventors of U.S. Patent Nos. 6,946,248 and 7,462,480 B2 issued to the University of Maryland Baltimore County (UMBC) and Medical University of So. Carolina. K.R.S. ad U.G. are co-inventors of U.S. Patent No. 8,945,906 issued to UMBC. U.G. is a co-inventor of US Patent No. 7,101,115 B2 issued to Stanford University and U.S. Patent No. 7,824,129 issued to UMBC. In addition, U.G. and K.R.S. are partners in a startup company (RemBac Environmental) that has licensed the three former technologies; U.G. is a partner in a startup company (Sediment Solutions) that has licensed the latter technology.

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REFERENCES

(1) Kalantzi, O. I.; Alcock, R. E.; Johnston, P. A.; Santillo, D.; Stringer, R. L.; Thomas, G. O.; Jones, K. C. Global distribution of PCBs and organochlorine pesticides in butter. *Environ. Sci. Technol.* **2001**, *35*, 1013−1018.

(2) USEPA. *National Listing of Fish Advisories*; EPA-820-F-13-058; EPA, 2013.

(3) USEPA. *Contaminated Sediment Remediation Guidance for Hazardous Waste Sites*; EPA-540-R-05−012 Office of Solid Waste and Emergency Response OSWER 9355.0-85; EPA, 2005.

(4) Council, N. R. *Sediment Dredging at Superfund Megasites: Assessing the Effectiveness*; The National Academies Press: Washington, DC, 2007.

(5) Ghosh, U.; Luthy, R. G.; Cornelissen, G.; Werner, D.; Menzie, C. A. *In-situ* sorbent Amendments: a new direction in contaminated sediment management. *Environ. Sci. Technol.* **2011**, *45* (4), 1163−1168.

(6) He, J.; Bedard, D. L., The microbiology of anaerobic PCB dechlorination. In *Organohalide Respiring Bacteria*; Adrian, L., Loffler, F. E., Ed.; Springer-Verlag: Berlin, 2016; pp 541−562.

(7) Pieper, D. H.; Seeger, M. Bacterial metabolism of polychlorinated biphenyls. *J. Mol. Microbiol. Biotechnol.* **2008**, *15*, 121−138.

(8) Lombard, N. J.; Ghosh, U.; Kjellerup, B. V.; Sowers, K. R. Kinetics and threshold level of 2,3,4,5-tetrachlorobiphenyl dechlorination by an organohalide respiring bacterium. *Environ. Sci. Technol.* **2014**, *48* (8), 4353−4360.

(9) Payne, R. B.; May, H. D.; Sowers, K. R. Enhanced reductive dechlorination of polychlorinated biphenyl impacted sediment by bioaugmentation with a dehalorespiring bacterium. *Environ. Sci. Technol.* **2011**, *45*, 8772−8779.

(10) Payne, R. B.; Fagervold, S. K.; May, H. D.; Sowers, K. R. Remediation of polychlorinated biphenyl impacted sediment by concurrent bioaugmentation with anaerobic halorespiring and aerobic degrading bacteria. *Environ. Sci. Technol.* **2013**, *47*, 3807−3815.

(11) Payne, R. B.; Ghosh, U.; May, H. D.; Marshall, C. W.; Sowers, K. R. Mesocosm studies on the efficacy of bioamended activated carbon for treating PCB-impacted sediment. *Environ. Sci. Technol.* **2017**, *51* (18), 10691−10699.

(12) *Battelle Feasibility Study for Site 100 Chopawamsic Creek Including Abraham's Creek MCB Quantico VA*, 2008 (https://www.navfac.navy.mil/niris/WASHINGTON/QUANTICO_MCB/BASEWIDE/ADMIN%20RECORD/M00264_001381.pdf).

(13) Bedard, D. L.; Unterman, R.; Bopp, L. H.; Brennan, M. J.; Haberl, M. L.; Johnson, C. Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. *Appl. Environ. Microbiol.* **1986**, *51* (4), 761−8.

(14) May, H. D.; Miller, G. S.; Kjellerup, B. V.; Sowers, K. R. Dehalorespiration with polychlorinated biphenyls by an anaerobic ultramicrobacterium. *Appl. Environ. Microbiol.* **2008**, *74* (7), 2089−2094.

(15) EPA/SERDP/ESTCP. *U. S. Laboratory, Field, and Analytical Procedures for Using Passive Sampling in the Evaluation of Contaminated Sediments: User's Manual*; Office of Research and Development: Washington DC, 2017.

(16) Grossman, A.; Ghosh, U. Measurement of activated carbon and other black carbons in sediments. *Chemosphere* **2009**, *75* (4), 469−475.

(17) Brändli, R. C.; Bergsli, A.; Ghosh, U.; Hartnik, T.; Breedveld, G. D.; Cornelissen, G. Quantification of activated carbon contents in soils and sediments using chemothermal and wet oxidation methods. *Environ. Pollut.* **2009**, *157* (12), 3465−3470.

(18) Sanders, J. P.; Andrade, N. A.; Menzie, C. A.; Amos, B. A.; Gilmour, C. C.; Henry, E. A.; Brown, S. S.; Ghosh, U. Persistent reductions in the bioavailability of PCBs at a tidally inundated Phragmites australis marsh amended with activated carbon. *Environ. Toxicol. Chem.* **2018**, *37* (9), 2496−2505.

(19) Ghosh, U.; Kane Driscoll, S.; Burgess, R.; Gobas, F.; Maruya, K.; Jonker, C.; Gala, W.; Choi, Y.; Beegan, C.; Apitz, S.; Mortimer, M.; Reible, D. Passive sampling methods for contaminated sediments: practical guidance for selection, calibration and implementation. *Integr. Environ. Assess. Manage.* **2014**, *10*, 210−223.

(20) Oen, A. M. P.; Janssen, E. M. L.; Cornelissen, G.; Breedveld, G. D.; Eek, E.; Luthy, R. G. *In situ* measurement of PCB pore water concentration profiles in activated carbon-amended sediment using passive samplers. *Environ. Sci. Technol.* **2011**, *45*, 4053−4059.

(21) Beltrametti, F.; Reniero, D.; Backhaus, S.; Hofer, B. Analysis of transcription of the bph locus of *Burkholderia* sp. strain LB400 and evidence that the ORF0 gene product acts as a regulator of the *bph*A1 promoter. *Microbiology* **2001**, *147*, 2169−2182.

(22) Caporaso, J.; Lauber, C.; Walters, W.; Berg-Lyons, D.; Huntley, J.; Fierer, N.; Owens, S.; Betley, J.; Fraser, L.; Bauer, M.; Gormley, N.; Gilbert, J.; Smith, G.; Knight, R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **2012**, *6* (8), 1621−1624.

(23) Caporaso, J. G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.; Costello, E.; Fierer, N.; Peña, A.; Goodrich, J.; Gordon, J.; Huttley, G.; Kelley, S.; Knights, D.; Koenig, J.; Ley, R.; Lozupone, C.; McDonald, D.; Muegge, B.; Pirrung, M.; Reeder, J.; Sevinsky, J.; Turnbaugh, P.; Walters, W.; Widmann, J.; Yatsunenko, T.; Zaneveld, J.; Knight, R. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335−336.

(24) Callahan, B.; McMurdie, P.; Rosen, M.; Han, A.; Johnson, A.; Holmes, S. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13* (7), 581−583.

(25) Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F. O. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **2012**, *41* (D1), D590−D596.

(26) McMurdie, P. J.; Holmes, S. An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **2013**, *8* (4), No. e61217.

(27) Oksanen, J.; Blanchet, F. G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P. R.; O'Hara, R. B.; Simpson, G. L.; Solymos, P.; Stevens, M. H. H.; Szoecs, E.; Wagner, H. *Vegan: Community Ecology Package*, R package version 2.5-1; 2018.

(28) Ogle, D. *Fisheries Stock Analysis. R Package*, 0.8.20; 2018.

(29) van den Berg, M.; Birnbaum, L. S.; Denison, M.; De Vito, M.; Farland, W.; Feeley, M.; Fiedler, H.; Hakansson, H.; Hanberg, A.; Haws, L.; Rose, M.; Safe, S.; Schrenk, D.; Tohyama, C.; Tritscher, A.; Tuomisto, J.; Tysklind, M.; Walker, N.; Peterson, R. E. The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. *Toxicol. Sci.* **2006**, *93* (2), 223−241.

(30) USEPA. *EPA proposes comprehensive plan to clean up Hudson River PCBs*; USEPA, 2000.

(31) Cho, Y. M.; Ghosh, U.; Kennedy, A. J.; Grossman, A.; Ray, G.; Tomaszewski, J. E.; Smithenry, D. W.; Bridges, T. S.; Luthy, R. G. Field application of activated carbon amendment for in-situ stabilization of polychlorinated biphenyls in marine sediment. *Environ. Sci. Technol.* **2009**, *43* (10), 3815−3823.

(32) Lozupone, C.; Lladser, M. E.; Knights, D.; Stombaugh, J.; Knight, R. UniFrac: an effective distance metric for microbial community comparison. *ISME J.* **2011**, *5* (2), 169−172.

(33) Harkness, M. R.; McDermott, J. B.; Abramowicz, D. A.; Salvo, J. J.; Flanagan, W. P.; Stephens, M. L.; Mondello, F. J.; May, R.; Lobos, J. H.; Carroll, K. M.; Brennan, M. J.; Bracco, A. A.; Fish, K. M.; Warner, G. L.; Wilson, P. R.; Dietrich, D. K.; Lin, D. T.; Morgan, C. B.; Gately, W. L. *In situ* stimulation of aerobic PCB biodegradation in Hudson River sediments. *Science* **1993**, *259*, 503−507.

(34) Goris, J.; De Vos, P.; Caballero-Mellado, J.; Park, J.; Falsen, E.; Quensen, J. F., III; Tiedje, J. M.; Vandamme, P. Classification of the biphenyl- and polychlorinated biphenyl-degrading strain LB400T and relatives as *Burkholderia xenovorans* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 1677−1681.

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX