Microbial-Catalyzed Reductive Dechlorination of Polychlorinated Biphenyls in Hudson and Grasse River Sediment Microcosms: Determination of Dechlorination Preferences and Identification of Rare Ortho Removal Pathways

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Supporting Information

ABSTRACT: Biodegradation of polychlorinated biphenyls (PCBs) is an important transformation and detoxification route in the environment. To better understand the influence of PCB congener compositions on dechlorination, sediments from two rivers, Hudson and Grasse, and two PCB mixtures (PCB 5/12, 64/71, 105/114, and 149/153/170 in Mixture 1 and PCB 5/12, 64/71, 82/97/99, and 144/170 in Mixture 2) were used for this microcosm study. The Grasse River sediment microcosms exhibited more extensive dechlorination than the Hudson River sediment microcosms. The extent of dechlorination was predominantly controlled by sediment itself, not by the PCB compositions. Rare ortho dechlorination, targeting mono-ortho PCB congeners was observed in Grasse sediment, indicating a potential for full dechlorination of some PCBs in this sediment. The identified ortho dechlorination pathways were PCB 28 (24−4-CB) to PCB 15 (4−4-CB) and PCB 25 (24−3-CB) to PCB 13(3−4-CB). The relative abundances of Dehalococcoides were much higher in both sediments spiked with PCBs. An apparent increase of Dehalococcoides 16S rRNA genes coincided with the commencement of dechlorination. The dechlorination preferences were identified using a modified data analysis approach focusing on chlorine neighboring conditions. In both sediments, the overall dechlorination preferred meta > para > ortho. Specially, ortho-/double-flanked meta-chlorines were primarily targeted followed by single-/double-flanked para-chlorines.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs). They contain 209 molecules called congeners and are composed of two linked benzene rings with 1–10 chlorine atoms substituted at the 10 available sites. The sites can be classified as ortho, meta, and para positions. Historically, PCBs were widely used as dielectric fluids and stabilizing additives from 1929 through the mid 1970s due to their chemical stability, heat-resistance, nonflammability, and low water solubility.1,2 However, PCB biological toxicity and adverse effect on the environment were of increasing concern after 1960.3,4 More than 650 000 tons of PCBs were produced in the US, mostly as commercial mixtures called Aroclors, which contained between 63 and 97 of the 209 congeners, and one-third of the total PCBs produced in the US were directly discharged into the environment.5–7 In the Hudson River, approximately 590 tons (1.3 million pounds) of PCBs were released at NPDES-permitted discharge points by the capacitor manufacturing plants of the General Electric Company (GE) from 1947 to 1977.8 Similarly, in the Grasse River, PCBs were discharged by the Aluminum Company of America (Alcoa) when operating an aluminum smelting and fabricating facility in that area. Due to the hydrophobic nature of most of the 209 structurally distinct congeners that make up commercial PCB mixtures, PCBs released to the aquatic environment are predominantly sorbed to organic matter in sediment, where they generally persist. PCBs are toxic. In addition to carcinogenic effects,5 recently, PCBs were found to be associated with autism spectrum disorder and intellectual disability.9,10 Removal of PCBs in sediment is crucial to reduce the risk to the environment and human health. Sediment dredging has been conducted in the Hudson River.9 However, dredging is costly and can cause sediment resuspension that leads to higher PCB concentrations in water and fish.9,11 Therefore, a low cost and environmental friendly remediation alternative based on PCB dechlorination is of great interest.

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Although most PCB congeners are resistant to aerobic biodegradation, microbially catalyzed reductive dechlorination has been observed in many systems and subsequently studied in the laboratory. Different sediments and sediment conditions exhibit distinct dechlorination preferred patterns, often called “Processes”. The processes consist predominately of stepwise dechlorination that removes para- and meta-chlorines and leaves predominantly lightly chlorinated ortho compounds as a result. Rare ortho dechlorination had been reported in Woods Pond and Baltimore Harbor sediments; the Baltimore Harbor estuarine sediment contains a dechlorinator named ortho-17(o-17) which is capable of targeted ortho dechlorination. In addition to o-17, *Dehalobium chlorococcia* strain DF-1 and *Dehalococcoides mccartyi* (formerly *ethenogenes*) strain 195 were found to target double-flanked chlorines on PCB congeners that contain that structure. Further, *Dehalococcoides mccartyi* (formerly sp.) strain CBDB1 was isolated and is capable of dechlorinating 43 congeners in Aroclor 1248 and Aroclor 1260 by targeting flanked (both single- and double-flanked ones) para-chlorines and double-flanked meta-chlorines. Also, three phylotypes, DEH10 (high sequence similarity to Dehalococcoides *mccartyi*), SF1 (o-17/DF-1 like) and SF2 (identical to o-17) were discovered concurrently in Baltimore Harbor sediment microcosms and responsible for extensive Aroclor 1260 dechlorination, targeting flanked meta-, double-flanked meta- and ortho-flanked meta-, double-flanked meta- and ortho-chlorines, respectively. Most recently, four new strains were identified as PCB dechlorinators, *Dehalococcoides* *mccartyi* CG-1, CG-4, CG-5, and JNA. Specially, strain CG-1 mainly removes double-flanked meta-chlorines; strain CG-4 prefers flanked para-chlorines followed by flanked meta-chlorines; strain CG-5 extensively removes flanked meta-chlorines and flanked para-chlorines; and strain JNA was found to be responsible for the removal of flanked meta-chlorines from Aroclor 1260 matching dechlorination Process N. JNA is the first pure culture carrying out a complex environmental dechlorination process.

All previous laboratory PCB dechlorination studies used either commercial PCB Aroclor mixtures or single PCB congeners. Due to limitations in PCB analytical methods and the complexity of dechlorination pathways, not all pathways can be identified and assigned appropriately when using commercial mixtures as substrates. Similarly, only very limited pathway information can be obtained by analyzing the dechlorination products from single PCB congeners, and examining all 209 congeners one-by-one or in an exhaustive matrix of mixtures has proven prohibitively expensive and has not been undertaken. The study of dechlorination has been further challenged by the many putative controlling factors, including biological populations of degraders, sediment geochemical conditions, and concentration/composition of PCBs. Further, methods to assess dechlorination without measuring all 209 congeners have been problematic.

One method to estimate dechlorination rate and extent mathematically, chlorine per biphenyl (CPB) has been successfully applied in PCB dechlorination studies. CPB is not dependent on the total PCB concentrations but on the PCB compositions, which makes it useful for comparing PCB dechlorination in different studies. Rhee and colleagues proposed a three phases CPB curve consisting of a flat lag phase, a rapid decrease phase, and a flat plateau phase as the experimental dechlorination limit. However, use of CPB precludes assessment of fully dechlorinated PCBs (biphenyl) because CPB, which is a ratio of total chlorines to the total PCBs, will increase rather than decrease when full dechlorination to biphenyl removes PCB mass. This will lead to an under-estimation of dechlorination extent if full dechlorination to biphenyl takes place. In addition, CPB does not identify target chlorine neighboring positions, which may be relevant for PCB degradation extent. However, many researchers report that the removal of chlorine is not substitution-position dependent but rather dechlorination pattern (Process) dependent. Therefore, a new approach to classify chlorines with respect to the target chlorine position together with the dechlorination patterns is necessary to assess dechlorination extent in sediments.

The ultimate goal of this study was to investigate the effects of PCP composition on dechlorination activity in two sediments. Two PCB mixtures designed to enable pathway elucidation were each used as substrate in microcosms created from Hudson River and Grasse River sediments. Over a time course of 357 days (51 weeks), dechlorination extent, pathway preferences, and the populations of putative dechlorinating microorganisms were examined and analyzed with a modified CPB analysis approach. The findings of this study can lead to a better understanding of site-specific PCB dechlorination, as well as provide an approach to compare previous studies on PCB dechlorination over time, regardless of initial mixture compositions and initial PCB concentrations.

### MATERIALS AND METHODS

#### Sediment Collection and Characterization.
Three gallons of surficial sediments (the top 4 in. of sediments) were collected using a petite ponar dredge sampler from the Hudson River (N: 43°14’ ‘55.5506”; W: −073° 35’ 37.4080”, Moreau, NY) in October 2008 and the Grasse River (N: 44° 57’ 35.9577”; W: −074° 48’ 59.8695”, Massena, NY) in June 2009. The sediments were transferred into 3-gal plastic buckets and saturated with river water. The sediments were stored in the dark at 4 °C until used to create the microcosms in September 2009. Heavy metals, acid volatile sulfide, total phosphorus and total residue as percent solids were measured by the Severn Trent Laboratories, Inc. (Pittsburgh, PA) and the Huffman Laboratories, Inc. (Golden, CO), following EPA methods SW-846 6020, AVS, MCAA 365.1, and MCAA 160.3. Inorganic anions and carbon content of the sediments were determined in our laboratory using ion chromatography (IC) following EPA method SW-846 9056A and a solids TOC analyzer (O-I-Analytical, College Station, TX). Sediment background PCBs in the Hudson and Grasse sediments were analyzed in our lab using gas chromatography with electron capture detection (GC-ECD) on a DB-XLB column as described previously.

#### Experimental Setup.
PCB congeners spiked in this study met the following criteria: (1) congeners account for more than 0.5% by mass of at least one of the commercial Aroclors or are of significant health risk concern (dioxin-like); (2) congeners were previously observed to dechlorinate following reported pathways in explicitly reported dechlorination processes or in the classification tree dechlorination process generalizations (CTDPG); (3) congeners belong to tracker pairs that showed transformations in the Hudson sediment samples and were added to the microcosms in tracker pair ratio concentrations; (4) first generation products of dechlorination do not overlap with spiked source congeners, and their subsequent generation products can be theoretically assigned to no more than three possible source congeners; (5) congeners themselves and most of their first generation and subsequent generation products do...
not coelute in the GC-ECD method. Based on these criteria, only 13 individual PCB congeners (10 tracker pairs) were found as candidates. They were grouped into two mixtures to ensure that the mixtures contained same PCB congeners in identical concentrations, same congeners in different concentrations, and different congeners belonging to the same homologue. PCB Mixture 1 contained congeners PCB 5/12, 64/71, 105/114, and 149/153/170. PCB Mixture 2 contained congeners PCB 5/12, 64/71, 82/97/99, and 144/170. The structures of the PCBs in each of the mixtures and the first step pathways for stepwise dechlorination of each spiked congener are shown in Supporting Information Figure S1 and Figure S2, providing a full matrix of PCBs that could be produced during reductive dechlorination of these congeners, as well as the possible dechlorination Processes associated with these pathways.

The PCB-spiked dry sediments were prepared as follows. The Hudson and the Grasse wet sediments were air-dried in a laminar flow hood. The specific amount of air-dried sediment for each sediment and PCB mixture combination was massed. PCB Mixture 1 or 2 solution in pure hexane was poured over the sediment in an organic-free 2 L glass beaker. Sediment was covered by the solution, and the beaker was placed in a laminar flow hood. The spiked sediment was mixed with an autoclaved stainless steel spoon periodically until the hexane fully evaporated. The PCB-spiked sediment was mixed thoroughly and stored at 4 °C in amber glass bottle until used.

Modified reduced anaerobic mineral medium (RAMM) was prepared as described elsewhere, except that 2 mM L-cysteine-HCl was used as the reducing agent and 1% (v/v) of Wolfe’s vitamin solution was added. The pH of the medium was adjusted to 7.0 using 1 M NaOH and autoclaved for 20 min at 121 °C under a nitrogen atmosphere.

Microcosms were set up in an anaerobic glovebox with a gas atmosphere of 0.9% H2 in O2-free N2 gas. Unless stated otherwise, 3 g PCB-spiked dry sediment substrate or dry sediment, 3 g fresh sediment inocula (dry weight basis), and freshly prepared RAMM medium were added to a 50 mL serum bottle to achieve a total weight of 30 g. Generally, four PCB-spiked experiments: Hudson sediment with PCB Mixture 1 (H-1), Hudson sediment with PCB Mixture 2 (H-2), Grasse sediment with PCB Mixture 1 (G-1), Hudson sediment with PCB Mixture 2 (G-2); and two no PCB control experiments, one with sediment with PCB Mixture 2 (H-2), Grasse sediment with PCB Mixture 1 (G-1), Hudson spiked experiments: Hudson sediment with PCB Mixture 1 (H-1) or 2 solution in pure hexane was poured over the sediment in an organic-free 2 L glass beaker. Sediment was covered by the solution, and the beaker was placed in a laminar flow hood. The spiked sediment was mixed with an autoclaved stainless steel spoon periodically until the hexane fully evaporated. The PCB-spiked sediment was mixed thoroughly and stored at 4 °C in amber glass bottle until used.

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**Headspace Analysis.** Prior to each slurry withdrawal, a microcosm headspace sample (200 μL) was analyzed for gas composition (hydrogen, methane, carbon dioxide, and trace oxygen) utilizing a gas chromatograph (Agilent 6850 series II) equipped with a thermal conductivity detector. Gas components were separated by packed column (30 in Haysep D, 30 in × 0.125 in). High purity nitrogen at a flow rate of 20 mL min−1 was used as a carrier gas. Oven and detector temperatures were held at 50 and 155 °C, respectively. Considering the increase of headspace pressure caused by biological gas production, the amount of each gas species was simply adjusted with ideal gas law by assuming a 1.0 atm partial pressure of nitrogen in headspace and a room temperature of 20 °C.

**PCB Extraction and Analysis.** After mixing the sediment slurry thoroughly, microcosm bottles were opened and sampled under a stream of nitrogen. Exactly 2.0 g sediment slurry was taken for PCB extraction. To correct for extraction efficiency, 20 μL of a 50 μg/mL solution of PCB 209 in hexane (1 μg of PCB 209) was injected on top of the 2.0 g sediment slurry with a syringe. The vial was placed in a laminar flow hood until the solvent fully evaporated. PCB extraction procedure was modified from the method described elsewhere.

In brief, 2.0 g sediment slurry was first extracted with 10 mL of acetone, followed by 10 mL of 1:1 acetone/hexane (v/v) twice and 3 mL of hexane. Eight mL of organic-free 2% NaCl solution was added to the pooled 33 mL extract to phase-separate the hexane layer. The hexane phase was concentrated down to 2 mL using a nitrogen evaporator. Sulfur removal was conducted as using tetrabutylammonium (TBA) sulfate following EPA Method 3660B. The TBA pretreated PCB extract was subjected to further cleanup with a 10 mm inner diameter glass column packed with 4 g Florisil and 1.5 g anhydrous sodium sulfate. The column was eluted with 30 mL high purity n-hexane followed by 25 mL 4:1 n-hexane/CH2Cl2 (v/v). The elutants were combined and concentrated down to 4.0 mL using a nitrogen evaporator. Individual PCB congeners were identified and quantified by Hewlett-Packard gas chromatograph (Model 6890) coupled with a microelectron capture detector (μECD) and a 30 m DB-XLB capillary column (0.18 mm diameter and 0.18 μm film thickness; Agilent Technologies, Palo Alto, CA) in splitless mode. All PCB commercially available mixture standards, 209 individual congeners and Aroclors were purchased from AccuStandard (AccuStandard, Inc.; New Haven, CT). The GC setting, external standard curves and sample PCB identification and quantification rules were described previously. Detection limits for most PCB congeners were below 1 ng/mL except for monochlorobiphenyls, which were 4 ng/mL. When necessary, 20 times dilutions for the precleaned 4.0 mL of PCB extracts were performed immediately prior to GC injection. The dilution procedure was to ensure that the PCB congener concentrations fell within the range of the series of external standards. The final individual PCB concentrations were corrected by corresponding surrogate recoveries to achieve a better reproducibility for triplicate microcosms. To ensure the uncertainties introduced by coelution and the varied recoveries of individual PCB congeners were within an acceptable range, a method reliability test was conducted by analyzing 209 individual PCB congeners spiked in sediments (Table S2), Aroclor 1242, 1248, and 1262 standards,
PCB mixtures in stock solution and in dry sediment substrates, respectively (data not shown). To confirm complete dechlorination, biphenyl and PAHs were analyzed in selected microcosms (duplicate bottles in each treatment at week 36) by Energy & Environmental Research Center (EERC), University of North Dakota.

DNA Extraction and Quantitative PCR. A 0.5 mL portion of slurry was withdrawn from the microcosm in the same way as the subsamples used for PCB analysis. The slurry was placed in a sterile 1.5 mL microcentrifuge tube and centrifuged at 16,000 g for 8 min. After pipetting off the supernatant, the sediment pellet was stored at −80 °C until needed. Total genomic DNA of the frozen sediment pellet was extracted using a PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA) following the manufacturer's procedures. Equal volumes of the triplicate sediment microcosm DNA extracts were mixed to represent the microbial communities at each sampling point under each treatment condition.

The quantification of 16S rRNA genes of total Bacteria, *Dehalococcoides* and PCB degrading organism strains *ortho*-17 and *Dehalobium chlorocoercia* strain DF-1 (*o*-17/DF-1) were performed by SYBR Green based quantitative PCR (qPCR) using appropriate group-specific primers. Universal primer set BAC338F/534R targets total Bacteria.59 Primer set DHC1200F/1271R is specific to *Dehalococcoides* spp.50 A universal forward primer BAC908F coupled with a modified *o*-17/DF-1 specific reverse primer Dehal1265R was utilized to target *o*-17/DF-1 and some related PCB dechlorinators.45,51,52 Pure 16S rRNA gene templates extracted from the corresponding microorganism 16S rRNA gene clones were used to construct the qPCR external standard curves. Each reaction was conducted in a total volume of 25 μL containing 1 × Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA), 300 nM of each primer, 0.2–2 μL of template sediment microcosm genomic DNA or pure 16S rRNA gene standards ranged from 10³ to 10⁸ copies per well. PCR amplification and detection were conducted in an ABI (Applied Biosystems) 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA). The thermal cycling parameters and dissociation curve analysis were set following the guide of ABI. The qPCR program started with initial denaturation for 10 min at 95 °C, followed by 40 cycles of denaturation for 1 min at 95 °C, annealing and elongation for 1 min at 60 °C, a final extension for 5 min at 60 °C, and the dissociation program from 60 to 95 °C.

### RESULTS

**Characteristics of Sediments.** The Hudson sediment was brown and sandy, while the Grasse sediment was black and clayey. The physical and geochemical properties of the two sediments were significantly different (Table S3). The Grasse sediment contained much higher levels of metals, especially Al and Fe, than the Hudson sediment. This is not unexpected due to the collection location in the Grasse River, which is near Outfall 001 of the Alcoa facility, Massena, New York. The Hudson River sediment had a relatively low organic carbon content (1.26% of TOC), whereas the Grasse River sediment had a high organic matter content (5.73% of TOC). The Hudson River sediment porewater contained much higher concentrations of inorganic anions than the Grasse River sediment. In contrast, the Hudson and the Grasse River grab sediments had similar levels of background total PCBs (1.59 mg/kg dry wt in Hudson and 1.63 mg/kg dry wt in Grasse). However, the Hudson sediment contained more lower chlorinated PCBs than the Grasse sediment; the CPB values were 2.43 in the Hudson sediment and 3.52 in the Grasse sediment.

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**Figure 1.** Total PCB mass concentrations and molar concentrations over time in H-1, G-1, H-2, G-2. H-1: Hudson River spiked with PCB Mixture 1. G-1: Grasse River spiked with PCB Mixture 1. H-2: Hudson River spiked PCB Mixture 2. G-2: Grasse River spiked with PCB Mixture 2. Data plotted are averages of triplicates. Error bars represent standard deviations. Error bars not visible are smaller than the symbol size.
Headspace Gas. Oxygen was not detected in any of the sediment microcosms. Methane was produced in the Hudson and Grasse sediment microcosms spiked with PCB mixtures and in the no PCB live controls, but not in sterile controls. The methane evolution patterns were different in the two sediments. Generally, microcosms using the Grasse sediment generated more methane than those using the Hudson sediment, regardless of PCB amendments. Methane production was affected by the spiked PCB mixtures, but not as significantly as by the sediment source and carbon content. The detailed methane production results are provided in the Supporting Information. Hydrogen was not detected in the Grasse sediment microcosms spiked with PCBs and in the no PCB live controls (G-1, G-2, and G) during the 51 week incubation, while in the Hudson sediment microcosms, H-1, H-2, and H, hydrogen was only detectable at week 0 with concentrations less than 0.2%.

Shifts of Total PCBs in Sediment Microcosms. Sterile controls were not degraded within the 51 week incubation (Supporting Information Table S4 and S5). Except for the sterile controls, all the microcosms spiked with PCBs showed extensive dechlorination. Assessment of dechlorination is complicated by the transformation of one PCB to another following removal of a single chlorine from a congener that has multiple chlorines. This reduces the mass of PCBs in the system, but not the number of molecules of PCBs (moles). The total PCB mass over time for each experiment is shown in Figure 1. The reduction of total PCB concentration was sediment source dependent. Regardless of the PCB mixture spiked, a similar level of residual total PCBs were observed in each sediment after 51 weeks of incubation. In the Hudson sediment microcosms, the total PCB concentrations declined by 23.8% in H-1 and 24.2% in H-2, whereas in the Grasse sediment microcosms, the total PCB concentrations declined by 34.8% and 33.8% in G-1 and G-2, respectively. Also, the CPB values for the Hudson sediment declined by 31.3% and 28.0%, whereas the CPB values for the Grasse sediment declined by 52.2% and 49.9%. Thus, the Grasse sediment treatments showed more extensive dechlorination than the Hudson sediment treatments. However, the lag time and the trends of total PCB concentration change over time were PCB composition and sediment dependent. The Grasse sediment tended to have reduced lag time (approximately 3 weeks) compared to the Hudson sediment (6−9 weeks), and PCB Mixture 1 slightly increased the dechlorination lag time in the Grasse sediment, but decreased the lag time in the Hudson sediment. Moreover, the declining trends of PCB Mixture 1 were similar in both sediments between 9 and 24 weeks, but much less total PCB loss was observed thereafter in the Hudson sediment. Although the final dechlorination extents of PCB S, 12, 64, and 71 at week 51 were similar in both sediments amended with different PCB mixtures, the reduction processes were influenced by the composition of other PCB congeners (Figure S4). Similar to the effects on methane production, PCB Mixture 1 favored more rapid dechlorination of PCB S, 12, 64, and 71 than PCB Mixture 2 in Hudson sediment microcosms. In contrast, the above PCB congeners dechlorinated relatively faster in G-2 than in G-1. The concentration normalized dechlorination of PCB 170 (originally 3.71 mg/kg in Mixture 1 amended microcosms, and 8.75 mg/kg in Mixture 2 amended microcosms), had the same trend as PCB S, 12, 64, and 71 (data not shown). This result indicated that,
although total PCBs concentrations and chlorine contents were similar, PCB congener composition/structure can affect dechlorination.

Since dechlorination generally transforms one PCB congener to another rather than completely dechlorinating a PCB congener to biphenyl, the reduction of PCB mass concentrations is limited. Excluding the experimental loss of PCBs, the reduction of total PCB moles indicates complete dechlorination to biphenyl. In the present study, the time course of total PCB molar concentrations is illustrated in Figure 1. Compared to sterile controls, the reduction of total PCB molar concentrations was significant ($p < 0.001$) in all the four treatments H-1, G-1 and H-2, G-2. Also, biphenyls were detected in the four treatments but not in the no PCB controls H and G (see GC-MS chromatogram in Supporting Information Figure S5). PAH analysis results showed that in the Hudson and the Grasse sediments sister PAHs were too low to account for the biphenyl detected in the microcosms. Therefore, the source of biphenyl was believed to be PCBs. Although blind duplicate microcosms (no sample information was provided) from each treatment were sent for biphenyl and PAHs analysis, the analytical reports of EERC grouped duplicates correctly and noted that the Grasse sediment microcosms contained relatively higher levels of biphenyl than the Hudson sediment microcosms. Assessment of the PCB congener-specific analysis indicates the decrease of total PCB moles in all the treatments was mainly due to the complete dechlorination of PCB 12 (34-CB), the only non-ortho-PCB congener spiked in the sediment microcosms (see Supporting Information Table S6 and Table S7).

**Classification and Identification of Target Chlorines in Dechlorination.** The classification of chlorines is based on their position and neighboring conditions (Supporting Information Figure S6 and Table S8). In general, ortho-chlorines are classified into two categories, unflanked ortho (UF ortho) and flanked ortho; flanked ortho by default flanked in the meta position. Meta-chlorines have four different neighboring conditions. They are unflanked meta (UF meta), ortho-flanked meta (OF meta), para-flanked meta (PF meta), and double-flanked meta (DF meta) (neighborhood with one ortho and one meta chlorine). Para-chlorines were divided into unflanked para (UF para), single-flanked para (SF para) (so-called meta flanked para), and double-flanked para (DF para) subgroups. Any chlorine on a PCB congener is classified into one of the nine categories described above.

The changes of ortho-chlorine per biphenyl (OCPB) over time are shown in Figure 2. Generally, the number of ortho-chlorine remained high, and a slight increase of the ortho-chlorine number was observed in all treatments within the first 36 weeks of incubation, supporting the congener-specific analysis that indicates no significant ortho dechlorination before 36 weeks. OCPB values slightly increased from 1.57 ± 0.01 to 1.69 ± 0.01 in H-1, 1.57 ± 0.01 to 1.70 ± 0.01 in G-1, 1.65 ± 0.01 to 1.82 ± 0.02 in H-2, and 1.65 ± 0.00 to 1.78 ± 0.01 in G-2. The increase of OCPB mainly resulted from the reduction of total molar concentration due to full dechlorination of congeners that did not contain ortho-chlorines (PCB12, 34-CB). A reduction of ortho-chlorine was observed at week 51 in the Grasse sediment (from 1.70 ± 0.01 to 1.60 ± 0.03; see Figure 2B), where the effect

![Figure 3. Changes of meta-chlorine per biphenyl over time. A: H-1. B: G-1. C: H-2. D: G-2. UF: unflanked. OF: ortho-flanked. PF: para-flanked. DF: doubly flanked. All data points are averaged triplicate microcosms. Error bars represent standard deviation. Error bars not visible are smaller than the symbol size.](image-url)
of ortho dechlorination dominated over the reduction of total PCB molar concentration. In all the four treatments (H-1, G-1, H-2, G-2) shown in Figure 2, unflanked ortho-chlorine continued to increase, whereas flanked ortho-chlorine decreased over time. Due the lack of ortho dechorination in the Hudson and the very limited ortho dechorination in the Grasse after 36 weeks, the removal of ortho-flanked and/or double-flanked meta-chlorines was expected under all the four treatment conditions. Moreover, in the Grasse sediment, at the end of incubation, almost all the remaining ortho-chlorines were unflanked. In contrast, approximately 0.2 and 0.3 flanked OCPB were left in the Hudson sediment microcosms H-1 and H-2, respectively, and the trends of the flanked ortho-chlorine curves suggest that they had reached a plateau, where no additional meta-chlorine removal was expected.

The shifts of meta-chlorines are plotted in Figure 3. Meta-chlorine per biphenyl (MCPB) values decreased over time in both sediments but with different trends and extents. By the end of 51 weeks, the averaged MCPB values were reduced from 1.59 ± 0.00 to 0.62 ± 0.14 in H-1 and from 1.50 ± 0.00 to 0.59 ± 0.05 in H-2. Approximately 60% of meta-chlorines were removed from PCB congeners in the Hudson sediment microcosms H-1 and H-2, respectively, and the trends of the flanked ortho-chlorine curves suggest that they had reached a plateau, where no additional meta-chlorine removal was expected. The declining trend of MCPB in the Grasse sediment microcosms suggests the potential for further meta dechlorination. Interestingly, the meta dechlorination rate was dependent on the spiked PCB compositions. In the sediment microcosms spiked with PCB Mixture 1 (H-1 and G-1 groups), a similar meta dechlorination rate was observed in the rapid phase of H-1 (6–21 weeks) and that of G-1 (3–21 weeks) (0.045 ± 0.02 Cl/week in H-1 and 0.043 ± 0.04 Cl/week in G-1). However, a slightly slower meta dechlorination rate was found in the rapid phase of H-2 than that of G-2 (0.038 ± 0.01 Cl/week in H-2 and 0.043 ± 0.02 Cl/week in G-2). Although the decline of MCPB confirmed the meta dechlorination preference in both the Hudson and the Grasse sediments, the specific dechlorination patterns were not clear until comparison of the change of meta-chlorine neighboring conditions. Seen in Figure 3, unflanked meta-chlorines increased, then followed by a slight decrease (solid triangle). Given the lack of ortho dechlorination before 36 weeks, the accumulation of unflanked meta-chlorines can only result from the dechlorination of para-chlorines with one or two neighboring meta-chlorines and there should be no ortho-chlorine in the target phenyl ring. Thereafter, the reduction of unflanked meta was an indicator of unflanked meta dechlorinating pattern. Looking into the decrease of ortho-flanked meta-chlorines (open square), again almost all the loss of ortho-flanked meta-chlorines was due to meta dechlorination. Moreover, in the rapid phase (6–21 weeks in Hudson and 3–21 weeks in Grasse), the faster reduction of meta-flanked ortho-chlorines (0.026 ± 0.001 Cl/week in H-1, 0.019 ± 0.000 Cl/week in G-1, 0.025 ± 0.002 Cl/week in H-2, and 0.027 ± 0.001 Cl/week in G-2) than

the ortho-flanked meta-chlorines (0.013 ± 0.001 Cl/week in H-1, 0.011 ± 0.000 Cl/week in G-1, 0.016 ± 0.002 Cl/week in H-2, 0.017 ± 0.001 Cl/week in G-2) indicates that meta dechlorination concurrently occurred on ortho-flanked meta- and doubly flanked meta-chlorines (Figures 2 and 3). Additionally, by comparing the declining trend of total meta-chlorines and the reduction trend of each of the sub meta-chlorine groups, overall meta dechlorination appeared to be mainly resulting from the decrease of para-flanked meta-chlorines (solid diamond) rather than ortho-flanked meta- or doubly flanked meta-chlorines. This confirmed prevalent meta dechlorination when meta-chlorines were para-flanked. Generally, meta dechlorination was dominant in this study and it occurred under any of the four possible meta-chlorine neighboring conditions (unflanked, ortho-flanked, para-flanked, and doubly flanked).

Para dechlorination was also commonly found in both the Hudson and Grasse sediments spiked with either PCB Mixture 1 or 2 (Figure 4). The Grasse sediment favored more overall para dechlorination than the Hudson sediment. In the first 18 weeks, para dechlorination was even slower in the Grasse sediment microcosms (0.008 ± 0.000 Cl/week in G-1, 0.010 ± 0.001 Cl/week in G-2), than that in the Hudson sediment microcosms (0.028 ± 0.003 Cl/week in H-1, 0.014 ± 0.001 Cl/week in H-2). Thereafter, para chlorination in the Grasse sediment increased rapidly. For both sediments, the extent of para dechlorination was less than that of meta dechlorination. By the end of 51 weeks, para-chlorines were reduced by around 50% while meta-chlorines were reduced by 60% in the Hudson sediment microcosms. Again, a plateau phase was observed after 30 weeks. In the Grasse sediment microcosms, 75% and 80% of para-chlorines were removed in G-1 and G-2, respectively, by the end of 51 weeks, while approximately 90% of meta-chlorines were removed.

Illustrated in Figure 4, unflanked para-chlorines (solid triangle) initially increased as the result of removing neighboring meta-chlorines, then the dechlorination of unflanked para-chlorines dominated in the microcosms and resulted in a decrease of unflanked para-chlorines. In the first 18 weeks, the reduction rates of single-flanked para-chlorines (open square) (0.040 ± 0.003 Cl/week in H-1, 0.019 ± 0.002 Cl/week in H-2, 0.024 ± 0.001 Cl/week in G-1, 0.021 ± 0.001 Cl/week in G-2) were even faster than the removal rates of total para-chlorines shown above, suggesting that (1) para dechlorination was mainly targeting flanked para-chlorines, (2) meta dechlorination cannot be neglected for PCBs with a single meta- and para-chlorines on the same phenyl ring. Double-flanked para-chlorines decreased over time. Here, para and meta dechlorination were both prevalent. Moreover, after 51 weeks of incubation, para-chlorines left in the Grasse sediment microcosms were almost all unflanked para, which indicates a meta dechlorination preference on PCBs with meta- and para-chlorines on the same phenyl ring in the Grasse sediment.

Ortho Dechlorination in the Sediment Microcosms. Congener specific analysis of the comprehensive microcosm experiments revealed that no ortho dechlorination product was found in the Hudson sediment microcosms (Supporting Information Table S6 and Table S7). However, significant levels of ortho dechlorination products were observed in the Grasse sediment microcosms. Two pathways were found to relate to ortho dechlorination in G-1. They were PCB 28 (4-4-CB) to PCB 15 (4-4-CB) and PCB 25 (24-3-CB) to PCB 13(3-4-CB). Another ortho dechlorination pathway PCB 1(2-CB) to biphenyl was also likely to proceed due to the over 10% decrease of PCB 1 from 36–51 weeks in G-1 but not confirmed. PCB 15
was the dominant ortho dechlorination product (4.9, 4.5, and 2.3 nmol/g slurry in triplicates at week 51).

The shifts of PCB 3 (4-CB) in the microcosms were more challenging to interpret. The concentrations of PCB 3 (4-CB) were 11.3 ± 0.4, 5.0 ± 0.4, and 16.8 ± 1.6 nmol/g at weeks 18, 36, and 51, respectively, while the concentrations of PCB 12 (34-CB), the only non-ortho-spiked PCB parent, were 0.6 ± 0.0, 0.3 ± 0.0, and 0.3 ± 0.0 nmol/g at weeks 18, 36, and 51 in G-1 (Table S6). Therefore, the observed high PCB 3 concentration at week 51 was not attributed to the dechlorination of remaining spiked PCB 12. For the pathways, ortho dechlorination of PCB 7 (24-CB) and PCB 8 (2-4-CB) to PCB 3 (4-CB) was possible; production of PCB 3 via meta/para dechlorination of PCB 13 (3-4-CB) or PCB 15 (4-4-CB) was also possible. Regardless of the intermediate pathways, the original spiked parent PCB congeners were the dioxin-like mono-ortho-PCB 105 (234-34-CB) and PCB 114 (2345-4-CB).

**Quantification of Putative PCB Dechlorinating Bacteria.** The changes of 16S rRNA genes of putative PCB dechlorinating bacteria *Dehalococcoides* are illustrated in Figure 5. Initially, the *Dehalococcoides* 16S rRNA genes in the Grasse sediment were 10- to 20-fold higher than those in the Hudson sediment. This difference may explain the longer lag time observed in Hudson microcosms. In H-1 and H-2 treatments, comparing to time zero, 1 order of magnitude increase of *Dehalococcoides* 16S rRNA genes were detected at weeks 6 and 9, respectively. Weeks 6 and 9 were also the first sampling time point at which dechlorination products were detected. This suggests a link of *Dehalococcoides* growth to PCB reductive dechlorination in the Hudson sediments. As mentioned previously, the PCB-spiked Grasse sediment microcosms dechlorinated more extensively than the PCB-spiked Hudson sediment microcosms. However, after 9 weeks of incubation, the Hudson sediment microcosms (H-1, H-2) showed higher numbers of *Dehalococcoides* 16S rRNA genes than the Grasse sediment microcosms (G-1, G-2).

The changes of 16S rRNA genes of o-17/DF-1 were not as obvious as those of *Dehalococcoides* (Figure S7). In H-1, o-17/DF-1 remained at a similar level at weeks 0, 3, and 6. After PCB dechlorination commenced, o-17/DF-1 even decreased to lower concentrations. Until week 51, o-17/DF-1 retrieved to a concentration similar to that at weeks 0, 3, and 6. In H-2, o-17/DF-1 was less than 1 order of magnitude higher at most sampling points (except for week 18) than that at week 0. This suggests the link of o-17/DF-1 growth to PCB dechlorination in the Hudson sediment was not as strong as that of *Dehalococcoides* growth. In the Grasse sediment microcosms, o-17/DF-1 first increased. After 9 weeks of incubation, o-17/DF-1 went down. In G-1, from week 15 to week 36, o-17/DF-1 remained at a relatively low level comparable to that of week 0, followed by a sharp increase at week 51. In G-2, o-17/DF-1 decreased from 12 to 24 weeks, thereafter, increased sharply at week 30, and remained high until 51 weeks. This indicates the complex shifts of active o-17/DF-1 in the Grasse sediment.

The relative abundances of *Dehalococcoides* and o-17/DF-1 were estimated based on the averaged percentage of *Dehalococcoides* 16S rRNA genes in total *Bacteria* 16S rRNA genes. Compared with the no PCB live controls (H, G), the abundances of *Dehalococcoides* in H-1, G-1, H-2, and G-2 were higher (Figure 6). Notably, the relative abundance of *Dehalococcoides* was much higher in the Hudson sediment microcosms (1.6–28.5% in H-1 and 0.8–7.1% in H-2) than in the Grasse sediment microcosms (0.1–1.0% in G-1 and 0.1–0.9% in G-2) after 9 weeks. This indicates more complex bacterial communities in the Grasse sediments spiked with PCBs, which might play a crucial role in cometabolisms for PCB dechlorination and resulted in more extensive dechlorination in the Grasse sediment. On the other hand, the relative abundance of o-17/DF-1 did not apparently shift in either sediment spiked with PCBs (Figure S8). This result together with the low o-17/DF-1 16S rRNA gene level in the Hudson sediment supports that o-17/DF-1 group was not likely to be responsible for the PCB dechlorination observed in the Hudson sediment. In the Grasse sediment, the observed increase of o-17/DF-1 16S rRNA genes was not faster than the growth of total *Bacteria*. Thus, there was no selective enrichment of o-17/DF-1 even when ortho dechlorination was taking place, suggesting that o-17/DF-1 related organisms might be active at relatively low concentrations or other unknown ortho dechlorinating organisms may be present.

**DISCUSSION**

**Impact of PCB Composition and Indigenous Sediment Properties.** This is the first study to examine the effect of PCB
compositions with identical mass concentrations and similar CPB values on dechlorination pathway and extent. The differences observed in the response of the sediments to the PCB mixture spiked suggests different sediment conditions and/or microbial species control the response of the system to specific PCB congeners.17,18,23,37 The composition of PCBs, even with same total concentrations and chlorine contents, could significantly affect dechlorination activity, which might be explained by cometabolism or the effect of certain congeners on priming dechlorination pathways. Previous studies have mentioned the effects of priming PCBs on dechlorination activity,33,58 which indicates that some PCB congeners are likely to serve as haloresposers of other PCBs in a mixture. Interestingly, no matter how fast the parent PCB congeners existing in both mixtures degraded after the lag period, the observed overall dechlorination extents were very close, which indicates that sediments and their indigenous microbial populations played a crucial role on dechlorination potential. This finding suggests that bioaugmentation by inoculating bacterial cultures would be an effective way to enhance dechlorination. This has been supported by many successful bioaugmentation experiments.36,37,55

Although, the overall para dechlorination extent of the Grasse sediment microcosms was greater than that of the Hudson sediment microcosms, before 18 weeks, para dechlorination of the Hudson microcosms (0.028 ± 0.003 Cl/week in H-1, 0.014 ± 0.001 Cl/week in H-2) was faster than that of the Grasse sediment microcosms (0.008 ± 0.000 Cl/week in G-1, 0.010 ± 0.003 Cl/week in G-2). The Hudson sediment had relatively low organic carbon (1.26%) and contained 40 times higher sulfate (174.5 mg/L in porewater) than that of the Grasse sediment (4.11 mg/L in porewater). Previous studies had found that under sulfate reducing conditions, para dechlorination preferred flanked para-chlorines while meta dechlorination was relatively limited, except for doubly flanked meta dechlorination.56,57 This may explain the relatively faster para dechlorination observed in the Hudson sediment before 18 weeks. Then, carbon source, as well as the lower concentration of available flanked para-chlorines, might become limiting factors leading to slower para dechlorination in the Hudson sediment.

Ortho Dechlorination. This work provides the first observation of ortho dechlorinating activity in the Grasse River sediment. Previously, ortho dechlorination capable of targeting PCB congener with two and more ortho-chlorines has been reported.23,25,27,58 o-17, identified from Baltimore Harbor sediment, preferentially removes meta-flanked ortho-chlorine when the meta-chlorine is not para-flanked (so-called ortho-flanked meta-chlorine).59 Recently, marine sediment collected from Hunters Point California was found to be capable of exhibiting ortho dechlorination of PCB 116 (23456-CB) where meta- and para-chlorines are both present.60 Chemical analysis revealed that the only degradation product in Hunters Point sediment spiked with PCB 116 was PCB 14 (3,5-CB), indicating the stepwise removal of the two ortho-chlorines. Ortho dechlorination reported in freshwater sediments from Woods Pond and Silver Lake removed the unflanked ortho-chlorine from PCB 30 (246-CB).61 Interestingly, the directly observed pathways in the present study included PCB 28 (244-CB) to PCB 15 (4-4-CB) and PCB 25 (243-CB) to PCB 13 (3-4-CB) targeting unflanked ortho-chlorine as well. Similarly to Woods Pond and Silver Lake sediments, ortho-chlorine removal in this study preferred a PCB congener with three or fewer chlorine atoms and took a relatively long time (36–51 weeks) to show the ortho dechlorinating activity. These findings suggest the presence of distinct ortho dechlorinating microorganisms between marine sediments and freshwater sediments. This ortho dechlorination preference is very likely to lead to a complete dechlorination of mono-ortho-PCB congeners, especially dioxin-like congeners, which is crucial to ultimate PCB detoxification in the Grasse River. Additionally, ortho dechlorination in this study preferred to targeting congeners with a single unflanked ortho-chlorine atom and unflanked meta- and/or para-chlorines. This suggests that the selective accumulation of intermediate congeners PCB 28 and PCB 25 might favor the ortho dechlorination.

Putative PCB Dechlorinating Microorganisms. Although enhanced growth of Dehalococcoides spp. was detected in PCB-amended sediment microcosms, the increase of Dehalococcoides 16S rRNA genes in the Grasses sediment microcosms was not as obvious as in the Hudson. There are two possible explanations. First, the existing of high concentration of Dehalococcoides could be using alternative natural electron acceptors to obtain growth energy.62 With the addition of PCBs, the existing Dehalococcoides turned to catalyze PCB dechlorination. Alternatively, microorganisms besides Dehalococcoides might be capable of dechlorinating PCBs. Interestingly, after a long incubation time, Dehalococcoides 16S rRNA genes remained at a high level, which indicated continuous dechlorination activities. Moreover, the Grasse sediment contained higher numbers of Dehalococcoides than the Hudson sediment after 30 weeks of incubation. This may be linked to the extended dechlorination activities, with/without ortho dechlorination activities observed in the Grasses sediment. o-17/DF-1 are believed to catalyze PCB dechlorination targeting ortho- or double-flanked meta/para-chlorines.32,50 As mentioned, acetate-utilizing o-17 isolated from Baltimore Harbor marine sediment preferred an ortho-chlorine neighboring with a single-flanked meta (so-called ortho-flanked meta).24,59 Some unknown ortho dechlorinators in Hunters Point marine sediment were responsible for removing ortho-chlorines with a double-flanked meta neighbor.60 Ortho dechlorinators capable of removing unflanked ortho-chlorine from PCB 30 (246-CB) in Woods Pond and Silver Lake sediments remained unclear.61 Unlike previously reported ortho dechlorination preferences, the observed ortho-chlorine removal in the present study targeted single unflanked ortho-chlorines, which indicates the existence of unidentified ortho dechlorinating organisms in Grasse River sediment.

In conclusion, extensive PCB dechlorination was observed in both Hudson and Grasse River sediment microcosms spiked with two different sets of PCB mixtures. The rate and extent of PCB dechlorination were mainly controlled by the indigenous differences in sediment, but not by the PCB mixture compositions. Generally, the carbon-rich and low-sulfate Grasse River sediment exhibited more extensive dechlorination than the relatively low carbon content and high-sulfate Hudson sediment. The occurrence of rare ortho dechlorination was confirmed in the Grasse sediment microcosms, supporting the potential for complete PCB dechlorination. Moreover, we have successfully applied a modified dechlorination pathway analysis approach based on PCB chlorine neighboring conditions and found ortho-/double-flanked meta dechlorination was primarily observed by single-/double-flanked para dechlorination in both sediments.
ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b03892.

Figures S1–S8 and Tables S1–S8 as mentioned in the text (PDF)

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Notes
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