Potential for Polychlorinated Biphenyl Biodegradation in Sediments from Indiana Harbor and Ship Canal

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Abstract

Polychlorinated biphenyls (PCBs) are carcinogenic, persistent, and bioaccumulative contaminants that pose risks to human and environmental health. In this study, we evaluated the PCB biodegradation of sediments from Indiana Harbor and Ship Canal (IHSC), a PCB-contaminated site (average PCB concentration = 12,570 ng/g d.w.). PCB congener profiles and bacterial community structure in a core sediment sample (4.57 m long) were characterized. Analysis of vertical PCB congener profile patterns in sediment and pore water strongly suggest that in situ dechlorination occurred in sediments. However, 16S rRNA genes from putative PCB-dechlorinating Chloroflexi were relatively more abundant in upper 2 m sediments, as were genes indicative of aerobic biodegradation potential (i.e. biphenyl dioxygenase (bphA)). Characterization of the bacterial community by terminal restriction fragment length polymorphism and comparison of these with sediment and pore water PCB congener profiles with the Mantel test revealed a statistical correlation (p<0.001). Sequences classified as Acinetobacter and Acidovorax were highly abundant in deep sediments. Overall, our results suggest that PCB dechlorination has already occurred, and that IHSC sediments have the potential for further aerobic and anaerobic PCB biodegradation.

Keywords

Indiana Harbor and Ship Canal sediment; PCBs; bacterial communities; bphA; Chloroflexi

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of 209 synthetic chemicals that were widely used in industry during the last century. It is estimated that 1.3 million tons of PCBs were produced worldwide between the 1930s and the mid-1980s, and about 30% has entered the environment by the mid-1980s (Breivik et al., 2002; Meijer et al., 2003). The production and commercial use of PCBs were banned by the EPA in 1979 because they were found to be...
toxic, carcinogenic, and bioaccumulative in the food web (Ross, 2004). Nevertheless, due to their high stability, PCBs still exist in the environment, and pose a potential risk to public health more than 30 years later (Bedard, 2004). Currently EPA has established a maximum contaminant level goal of zero and a maximum contaminant level of 0.5 ppb for PCBs in public drinking water supplies (USEPA, 1991).

Despite their environmental persistence, PCBs can be transformed to less toxic forms or even mineralized by microorganisms. Field and laboratory studies indicate two predominant processes: anaerobic dechlorination and aerobic biphenyl ring cleavage (Borja et al., 2005).

Under anaerobic conditions, PCBs are used as electron acceptors, resulting in the removal of chlorine atoms. The less chlorinated congeners are more amenable to aerobic degradation (Alder et al., 1993). Anaerobic PCB-degraders include *Dehalococcoides* spp., the o-17/DF-1 group, as well as some other members of the Chloroflexi (Adrian et al., 2009; Fagervold et al., 2007; Fagervold et al., 2005; Wiegel and Wu, 2000; Yan et al., 2006). *Dehalobacter* spp. could also participate in anaerobic PCB dechlorination (Yoshida et al., 2009). Reductive dehalogenase (RDase) is considered to be the key enzyme catalyzing the PCB dechlorination process (Hiraishi, 2008; Pieper and Seeger, 2008). Nonidentical RDase genes were found in PCB degrading strains such as *Dehalococcoides mccartyi* strain 195 and CBDB1, but no RDase gene was identified to dechlorinate PCBs (Bedard et al., 2007; Fung et al., 2007; Hölscher et al., 2004; Wagner et al., 2009).

Under aerobic conditions, PCBs can be used as electron donors, or fortuitously oxidized by oxygenase enzymes. A variety of PCB-oxidizing bacteria have been identified, including Gram-negative strains of *Pseudomonas*, *Alcaligenes*, *Burkholderia*, *Acinetobacter*, and *Comamonas* and Gram-positive strains of *Corynebacterium*, *Rhodococcus* and *Bacillus* (Bedard et al., 1986; Furukawa and Fujihara, 2008; Pieper and Seeger, 2008). These microbes harbor biphenyl dioxygenase (Bph), the key enzyme catalyzing the first step of aerobic biphenyl ring cleavage, generating a cis-dihydrodiol intermediate, which is further degraded to a chlorobenzoate (Bedard et al., 1986; Gibson and Parales, 2000; Mackova et al., 2010). PCB-oxidizing microbes usually cannot metabolize the chlorobenzoate, thus a consortium of chlorobenzoate-degraders are required for complete PCB mineralization (Pavlů et al., 1999).

The site of this study is the Indiana Harbor and Ship Canal (IHSC), a heavily industrial area of southern Lake Michigan contaminated with a variety of pollutants, including heavy metals, polycyclic aromatic hydrocarbons (PAHs) and PCBs. It is reportedly a major source of PCBs to Lake Michigan, with PCB concentrations in IHSC surficial sediments ranging from 53 to 35,000 ng g⁻¹ dry weight (Martinez et al., 2010). PCB-contaminated IHSC sediments are currently being dredged and permanently stored in a confined disposal facility (CDF) (USACE, 2013).

The purpose of this study is to evaluate both anaerobic and aerobic PCB biodegradation potential in IHSC sediments. We characterized PCB congener profiles and bacterial communities in a 4.57-m long core sediment sample, which revealed evidence of *in situ* aerobic and anaerobic PCB degradation in upper 1.83 m sediments. We also explored the
correlation between the bacterial communities and PCB congener profiles and found that sediment sections with similar PCB congener profiles tend to have similar bacterial community structure. We conclude that microbial communities in IHSC sediments have the potential for aerobic and anaerobic PCB biodegradation. This suggests that natural attenuation of PCBs could continue in IHSC sediments after they are transferred to the CDF.

2. Materials and Methods

2.1. Site description and sampling

In May 2009, a 4.57-m core sediment sample from IHSC was collected using a submersible vibro-coring system with a PVC tube (length 457 cm, internal diameter 9.5 cm) from aboard the U.S. EPA’s research vessel, *Mudpuppy* (Figure 1). The core was sectioned every 0.305 m, and each section was homogenized, placed in plastic bags and kept on ice during transportation. Sediment samples were stored at 4°C in the lab until analysis. The analytical procedure was shown in Figure 2.

2.2. PCB congener analysis

Preparation, extraction and clean-up steps for measuring PCB sediment concentrations have been described previously (Martinez and Hornbuckle, 2011). Briefly, sediments were extracted using pressurized fluid extraction (Accelerated Solvent Extractor, Dionex ASE-300). The extracts were concentrated and eluted through a multilayer silica gel column. Activated granulate copper was used to remove sulfur in solution. Poly-dimethylsiloxane (PDMS) coated fibers were used as passive samplers to determine the sediment pore water PCB concentration. PCB extraction and quantification procedures were also reported previously (Martinez et al., 2013). PCB identification and quantification were conducted employing a modified US EPA method 1668C (USEPA, 2010). Tandem mass spectrometry GC/MS/MS (Quattro Micro GC, Micromass MS Technologies) in multiple reaction monitoring mode was utilized to quantify all 209 congeners in 161 individual or coeluting congeners peaks. For sediment samples, the limit of quantification was ca. 0.4 ng/g dw for individual congeners, and triplicate of three different sediment sections of bulk sediment concentrations yielded a relative standard deviation of less than 9% as reported previously (Martinez and Hornbuckle, 2011). For pore water samples, PCB congeners found in laboratory blanks (PCB 68, PCBs 85+116+117 and PCB 209) were removed from field samples for further analysis. Triplicates of five different sediment sections of freely-dissolved pore water concentrations generated a relative standard deviation of 19% (Martinez et al., 2013).

The molar dechlorination product ratio (MDPR) was used to examine possible PCB dechlorination in core sediments. When determining the MDPR, it is assumed that exclusively ortho-chlorinated PCB congeners undergo no further dechlorination (USEPA, 1997). In this study, five exclusively ortho-chlorinated PCBs (PCB 1, 4, 10, 19, 54) and PCB 8 were selected as the ultimate dechlorination products. The ratio of the sum of the molar concentrations of selected congener over total PCB molar concentration was calculated and defined as MDPR. PCB 8 was considered as a dechlorination product because the proportions of PCB-8 in core sediments (averaged 2.7% of total PCB) were
much higher than that in other Aroclor commercial mixtures (0.48%, 0%, 0% of Aroclor 1248, 1242, 1016, respectively), which indicates the production of this congener in IHSC sediments.

### 2.3. Quantitative PCR

Total DNA from sediments was isolated with the MoBio Ultraclean Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) and stored at −20°C until analysis. The abundance of total bacteria, aerobic PCB-degrading bacteria, and putative PCB dechlorinators were estimated using qPCR targeting bacterial 16S rRNA gene (primer set 16SU f/r) (Nadkarni et al., 2002), bphA (primer set bphA 463f/674r) (Petrić et al., 2011b), putative dechlorinating Chloroflexi 16S rRNA genes (primer set chl348f/dehal884r) (Fagervold et al., 2005), and Dehalococcoides-like 16S rRNA genes (primer set dhc793f/946r) (Yoshida et al., 2005) (Table S1). PCR conditions were as follows: 10 min at 95°C, 40 cycles of 15s at 95°C and 1 min at 60°C followed by a dissociation step. Each 25 µl reaction contained 12.5 µl Power SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA), 2.5 pmol primers and variable amounts of primers and template (Table S2). Bovine serum albumin (0.5 µg) was added to relieve possible PCR inhibition (Kreader, 1996).

For total bacterial 16S rRNA gene qPCR, the standard DNA template was PCR products amplified from Burkholderia xenovorans strain LB400 with primer set 8F/1492R (Grabowski et al., 2005). For bphA, the standard DNA template was the LB400 bphA (amplified with the 463f/674r primer set) cloned into the pCR 2.1-TOPO vector. For putative dechlorinating Chloroflexi 16S rRNA genes and Dehalococcoides-like 16S rRNA gene, standard curves were prepared from pCR 2.1-TOPO vector containing the target PCR products with primer chl348f/dehal884r and dhc793f/946r, respectively. All qPCRs were performed with an ABI 7000 Sequence Detection System (Applied Biosystem, Grand Island, NY) and fluorescence data was analyzed by ABI 7000 System SDS Software (Applied Biosystems, Grand Island, NY). With each primer set, the target gene was not detected in no template (DI water) controls (Ct value > 35). Additional qPCR information, such as primer concentrations, template concentrations, qPCR linear range, qPCR efficiency range of the standard curves, and Y-intercepts are provided in Table S2, in accordance with MIQE guidelines (Bustin et al., 2009).

### 2.4. qPCR quality assurance

To verify the specificity of qPCR primer sets chl348f/dehal884 and dhc793f/946r, two clone libraries were constructed from the amplification products in DNA extracted from sediments at 0–0.30 m and 0.91–1.22 m. Clone libraries were prepared as described in section 2.6. From the chl348f/dehal884r PCR product clone library, 21 unique sequences were obtained from 22 clones and from the dhc793f/946r PCR product clone library, 10 unique sequences were obtained from 10 clones. All of the sequences were determined to be from Chloroflexi, using RDP classifier (Cole et al., 2007), with 91% classified as Dehalogenimonas (Table S3). The specificity of the SYBR green based qPCR was also validated by dissociation curve analysis. This analysis demonstrated that, for each primer set, the PCR products generated had similar melting temperatures and that primer dimer formation was minimal.
2.5. Terminal-Restriction Fragment Length Polymorphism (T-RFLP) analysis

PCR with fluorescently-labeled 6-FAM 8Fm and 533R was performed with 1 ng DNA (Schütte et al., 2008). PCR conditions were: 4 min at 94°C, 30 cycles of 1 min at 94°C, 2 min at 55°C and 1 min at 72°C followed by a final extension step of 10 min at 72°C. PCR product (12 µl) was digested with the restriction enzyme HaeIII (New England BioLabs, Inc., Ipswich, MA), and then precipitated with glycogen (Fermentas International Inc., Vilnius, Lithuania), sodium acetate (pH 5.2), and ethanol at −20°C for 2h. DNA was recovered by centrifugation at 17,800 x g for 15 min and resuspended in distilled water (Invitrogen Corp., Carlsbad, CA). The digested DNA was sent to the University of Iowa DNA facility for electrophoresis for sizing using an Applied Biosystems 3730 DNA analyzer (Life Technologies Corporation, Carlsbad, CA) with the GeneScan 500 LIZ size standard.

Terminal restriction fragment (T-RF) sizes were estimated with Peak Scanner software (Applied Biosystems, Carlsbad, CA). The TRF size matrix containing 15 samples (rows) and 354 unique 16S rRNA gene TRFs (columns) was generated by T-REX software after filtering background peak noise and rounding fragment sizes to the nearest whole number (Culman et al., 2009). TRF profiles were analyzed by nonmetric multidimensional scaling (NMDS) in R (da C Jesus et al., 2009). The Bray-Curtis dissimilarity index was calculated with a random starting configuration, and a two-dimensional solution was reached after seven runs. The final stress was 7.42.

Correlations between PCB congener profiles and T-RFLP profiles were assessed with the Procrustes test, where PCB congener profiles were analyzed by principal components analysis (PCA) and T-RFLP profiles were ordinated by NMDS. The Mantel test was applied to assess the correlation between T-RFLP profiles and PCB profiles, where a Euclidean dissimilarity index was calculated for PCB congener profiles and the Bray-Curtis dissimilarity index was calculated for T-RFLP profiles. Pearson correlation coefficients between each TRF and molar dechlorination product ratio (MDPR) values were also calculated (González et al., 2000).

2.6. PCR amplification, cloning and sequencing of bacterial 16S rRNA

A clone library was constructed with bacterial 16S rRNA genes amplified from sediment at the depth 3.35 – 3.66 m. Relatively high MDPR values (0.08 for sediment and 0.37 for pore water) were observed at this depth. Bacterial 16S rRNA genes were amplified using primer set 8F/1492R (Grabowski et al., 2005). PCR conditions were as described in section 2.5. PCR products were purified with the Qiaquick PCR purification Kit (Qiagen Inc., Valencia, CA), cloned into the pCR 2.1-TOPO vector using the TOPO TA cloning Kit (Invitrogen Corp., Carlsbad, CA), and transformed into One Shot TOP10 chemically competent E.coli cells (Invitrogen Corp., Carlsbad, CA). The transformation efficiency was checked by plating recombinant E.coli on Luria Broth agar with kanamycin (50 mg/L), and X-gal (0.4 mg/plate) and incubated overnight at 37°C. Clones were Sanger sequenced at HTSeq.org (Seattle, WA) with M13F (5’-TGTTAAAACGACGCTATG-3’) and M13R (5’-CAGGAAACAGCTATGAC-3’) primers. Sequence identification and classification were performed using RDP Classifier (Cole et al., 2007) and Basic Local Alignment Search Tool
3. Results and discussion

3.1. Evidence for PCB dechlorination in IHSC sediment congener profiles

Changes in PCB congener distribution patterns with sediment depth can be explained by changes in production, use and discharge of different commercial mixtures, sediment resuspension and transport, and in situ microbial PCB degradation (Li et al., 2009). Sediment resuspension and PCB desorption would be expected to result in accumulation of highly chlorinated PCBs in deeper sediments. However, anaerobic PCB dechlorination in sediments would lead to decreased abundance of higher chlorinated congeners and increased abundance of less chlorinated ones. With this in mind, we examined PCB congener profiles in sediment samples to search for evidence of PCB dechlorination. Both sediment and pore water PCB profiles indicate accumulation of lesser chlorinated PCBs (1–3 chlorine substituents) in deeper core sediments, along with a decrease in medium chlorinated congener abundance (4–6 chlorine substituents) (Figure 3A, 3B). There was also an increasing trend of tri-chlorinated PCBs and a corresponding decreasing fraction of tetra- and penta-chlorinated congeners with increasing depth (Figure S1).

Anaerobic PCB dechlorination in sediments often preferentially removes chlorines at para- and meta- positions, resulting in the enrichment of ortho-chlorinated congeners (Alder et al., 1993; Bedard et al., 1997; Fava et al., 2003). Therefore, if PCB dechlorination had occurred, we would expect to see an increase in the abundance of PCB congeners with ortho-chlorine substituents. Indeed, analysis of the relative abundance of ortho-, meta-, and para-chlorines against core depth revealed an accumulation of ortho-chlorines in deep sediments, in accordance with a decrease in meta- and para-chlorinated PCBs (Figure 3C). In pore water, the enrichment of ortho-chlorinated congeners was also observed with depth (Figure 3D).

MDPR values were estimated in each core section to further examine potential PCB dechlorination in the core sediment. Accumulation of the six selected ortho-chlorinated congeners in the core sediments was observed, which further supports that PCB dechlorination has occurred (Figure 4). Although sediment PCB profiles in the core reportedly mainly resemble the Aroclor 1248 mixture, the average sediment MDPR value (0.052) was much greater than that of Aroclor 1248 (0.009) (Frame et al., 1996; Martinez and Hornbuckle, 2011), which is also a sign of in situ dechlorination.

MDPR values in pore water were higher than those of corresponding sediment sections (Figure 4). This can be explained by the tendency for ortho-chlorine substituents to increase the aqueous solubility of the PCB molecule (Huang and Hong, 2002). In addition, the six lower chlorinated congeners selected for use in the MDPR calculation are more soluble than most PCB congeners (Van Noort et al., 2010). However, PCBs dissolved in pore water are more bioavailable than those sorbed to sediment particles and thus are more vulnerable to in situ dechlorination (Reid et al., 2000).
3.2. Aerobic and anaerobic PCB biodegradation potential in sediment

With the PCB congener-based evidence for reductive dechlorination in IHSC sediments, we aimed next to characterize the PCB biodegradation potential by estimating the abundance of total 16S rRNA genes, putative dechlorinating *Chloroflexi* 16S rRNA genes and *bph* A, a functional gene associated with aerobic PCB oxidation. The total bacterial 16S gene abundance was relative stable with sediment depth (2.37 × 10^9 ± 2.41 × 10^9 16S rRNA genes per g sediment) (Figure 5). The lowest 16S rRNA gene abundance was from 0–0.30 m sediment, possibly because this section was at the water-sediment surface and had relatively high water content compared with the other sediment samples.

The abundance of *bph* A averaged 7.39 × 10^6 ± 3.96 × 10^6 genes per g sediment (0–1.83 m depth), and 3.20 × 10^5 ± 4.58 × 10^5 genes per g sediment (1.83–4.57 m depth). Interestingly, *bph* A gene abundances were significantly correlated with total sediment PCB concentrations in the upper 1.83 m, which could indicate a microbial response to PCB contamination in the sediments (Pearson’s R, p<0.05) (Table S6).

Although the upper 1.83 m was anaerobic as suggested by negative ORP data (−13 to −200mv; Table S4), micro-aerobic conditions could possibly exist in some portions of the sediment (Martinez et al., 2013). The sharp decrease in *bph* A abundance below 1.83 m likely indicates the presence of a stricter anaerobic environment. A strong positive correlation was observed between the *bph* A abundance and the ORP along depth (Pearson’s R, p<0.01) (Table S5).

Both of the primer sets used to estimate the abundance of putative dechlorinating *Chloroflexi* group in the sediment (chl384f/884r and dhc793f/946r) primarily amplified 16S rRNA gene sequences classified as *Dehalogenimonas sp*. (Table S3). Some *Dehalogenimonas* isolates can reductively dehalogenate polychlorinated alkanes (Maness et al., 2012; Yan et al., 2009). *Dehalogenimonas* sp. are also reported to couple their growth to PCB dechlorination in a sediment free enrichment culture (Wang and He, 2013).

Putative dechlorinating *Chloroflexi* 16S rRNA gene abundances were 2.53 × 10^5 ± 2.45 × 10^5 genes per g sediment (0–1.83 m depth) and 1.53 × 10^4 ± 2.80 × 10^4 genes per g sediment (1.83 – 4.57 m depth) as assessed by chl384f/884r, while the gene abundance was 2.15 × 10^5 ± 2.07 × 10^5 genes per g sediment (0 – 1.83 m depth) and 7.64 × 10^3 ± 1.30 × 10^4 genes per g sediment (1.83 – 4.57 m depth) as assessed by dhc793f/946r. These gene abundances are lower than observed in sediments from other PCB-contaminated sites, which ranged from 10^6–10^8 genes per g sediment, possibly because these other sites contained higher PCB concentrations (Table S4) (Kjellerup et al., 2008; Xu et al., 2012).

The co-occurrence of putative dechlorinating *Chloroflexi* 16S rRNA genes and *bph* A indicate the potential for simultaneous aerobic and anaerobic PCB degradation in the upper 1.83 m of the sediment. However, the pattern of putative dechlorinating *Chloroflexi* abundance seems inconsistent with the congener profile which according to the MDPR values, displayed greater dechlorination degree in sediments deeper than 1.83 m. In a previous study of a PCB-contaminated sediment core from the Grasse River, it was concluded that there is a valid relationship between MDPR values and the abundance of...
putative dehalogenating microorganisms (Xu et al., 2012). In the upper 1.83 m of our core sample, the relatively abundant putative dechlorinat

ing Chloroflexi suggest that they may be involved in PCB dechlorination and related to the increasing trend of MDPR values with increasing depth. A possible explanation for the relatively high MDPR values and low abundance of dechlorinating Chloroflexi in sediments 1.83–4.57 m in depths is that the dechlorination activity ceased at some point in the past and dechlorinator abundance has been declining over time. We caution that using a 16S rRNA gene based approach to estimate dechlorinating Chloroflexi abundance does not directly identify the dechlorinating capability of the microbial community. Quantifying the abundance of a functional gene involved in PCB dechlorination (e.g. a reductive dehalogenase gene) would be a better indicator of microbial dechlorination. However, because no PCB reductive dehalogenase has been specifically identified, qPCR primers targeting PCB reductive dehalogenases are not currently available.

### 3.3. Relationships between microbial community structure and PCB congener profiles

To explore the possibility that other microbial community members could be relevant to PCB biodegradation potential in IHSC sediments, we evaluated microbial community structure with depth using T-RFLP analysis. A significant correlation between the overall sediment T-RFLP profile and PCB congener profile was noted when using the Mantel test (p=0.001 for sediment, p=0.001 for pore water) (Table S7). This correlation was confirmed with the Procrustes test (p=0.002 for sediment, p=0.001 for pore water) (Table S7). This suggests that sediment sections with similar PCB congener profiles tend to have similar bacterial community structures.

NMDS ordination of the T-RFLP profiles revealed that bacterial community structure in the shallow sediment sections (0–1.83 m depth) were distinct from the structure in deeper sediments (1.83–4.57 m depth) (Figure 6A). The disparity between shallow and deep sediments was also found by qPCR, which show much less abundant bphA, putative Chloroflexi 16S rRNA genes, and Dehalococcoides-like 16S rRNA genes in sediments of 1.83–4.57 m deep.

The microbial community composition in sediments at 3.35–3.66 m depth was assessed by sequencing 91 clones from a 16S rRNA gene clone library. This indicated that the community was dominated by Proteobacteria (11.0% α-Proteobacteria, 34.1% β-Proteobacteria and 54.9% γ-Proteobacteria). At the genus level, Acinetobacter sp. and Acidovorax sp. were dominant, comprising 45.1% and 20.9% of the clones, respectively.

A 196 bp T-RF, which was abundant in sediments deeper than 1.22 m (26.9% ± 13.0%) (Figure 6B), was significantly correlated with sediment and pore water MDPR values (Pearson’s R, p<0.001). This T-RF was identified to be from an Acidovorax sp. when compared to virtually digested T-RFs from a 16S rRNA gene clone library (91 clones) from sediments of 3.35–3.66 m. The corresponding T-RFs of Acinetobacter were 198 bp, 199 bp and 252 bp (Table 1). A 199 bp T-RF comprised 20.0% of all T-RFs in 3.35–3.66 m section, but was only 0–2.5% in other sections. Clones identified as Methylotenera sp. and unclassified Comamonadaceae also generated a 199 bp T-RF, but the majority of the 199 bp T-RFs in the clone library was classified as Acinetobacter. Two clones from the clone...
library generated a 198 bp T-RF. This T-RF was not found in T-RFLP profiles of 3.35–3.66 m sediments, but was found in 0–1.52 m sediments (1.97–9.51% relative abundance) and had a relative abundance of 18.74% in sediments at 4.27–4.57 m depth. A 252 bp T-RF was also identified as coming from an *Acinetobacter* sp., and had a relative abundance range of 0.62 – 8.10% along the core. Overall, this analysis revealed that although *Acinetobacter* was an abundant (and thus potentially important) community member in the core sediments. However, there was no correlation between TRFs identified as *Acinetobacter* and sediment or pore water MDPR values.

*Proteobacteria* are often found abundant in soils and sediments (de Cárcer et al., 2007; Petrić et al., 2011a; Spain et al., 2009). However, an increase in β- and α-*Proteobacteria* abundance was observed in PCB-exposed samples in a microcosm study (Correa et al., 2010). Many known PCB-degraders are *Proteobacteria* (e.g. *Pseudomonas*, * Acidovorax*, *Acinetobacter*, *C omamonas* and *Burkholderia* (Bedard et al., 1986; Furukawa and Fujihara, 2008)). Both *Acidovorax* and *Acinetobacter* are known to degrade PCBs and were commonly found in PCB-contaminated sites (de Cárcer et al., 2007; Ionescu et al., 2009; Shuai et al., 2010; Slater et al., 2011). Aerobic PCB biodegradation was likely not active in sediments deeper than 1.83 m, as indicated by negative ORP data and the low abundance of *bpha*. So it is unlikely that *Acinetobacter*, *Acidovorax* or other possible PCB degraders were carrying out aerobic PCB degradation in deep sediments.

Some *Acidovorax* species are anaerobic nitrate respirers, but are not known to be associated with dechlorination (Byrne-Bailey et al., 2010; Hohmann et al., 2010). *Acinetobacter* has been detected in sediments deeper than 100 m (Breuker et al., 2011), and studies have reported that some *Acinetobacter* species are capable of dechlorinating and degrading chlorobenzoate, chlorophenols and chloroanilines (Adriaens and Focht, 1991; Copley and Crooks, 1992; Hong sawat and Vangnai, 2011; Kim and Hao, 1999). *Acinetobacter* has been found in PCB-dechlorinating JN cultures (Bedard et al., 2007), although no evidence yet suggests that *Acinetobacter* can dechlorinate PCBs.

*Chloroflexi* sequences were not detected in the 16S rRNA gene clone library, probably because of their relatively low abundance with respect to the total 16S rRNA gene abundance in the sediment sample used to construct the library. Low *Chloroflexi* abundance was verified by our qPCR results. However, PCB congener profile analysis suggested extensive PCB dechlorination in deep sediments. If the extent of degradation is positively correlated to the relative abundance of dechlorinators in sediments, the relatively low abundance of dechlorinating *Chloroflexi* in the deep sediments suggests that unknown species other than *Chloroflexi* were involved in PCB dechlorination.

### 4. Conclusions

In general, both aerobic and anaerobic PCB degradation potential at IHSC were assessed by combining PCB congener analysis and molecular microbial ecology analysis. PCB congener profiles of the core sediment revealed that *in situ* dechlorination has occurred. The presence of taxa known to be involved in anaerobic reductive dechlorination and a functional gene known to participate in aerobic PCB degradation (*bpha*) in the upper 2-m sediments
suggests that the indigenous microbial communities contained the potential for both aerobic and anaerobic PCB degradation. In deeper sediment, analysis of T-RFLP profiles revealed that the microbial communities were different from those in upper sediments. *Acinetobacter* sp. were highly abundant in deep sediments, suggesting their possible role in PCB dechlorination. Overall our results provide a comprehensive understanding of *in situ* PCB degradation potential in IHSC sediments, and suggest that natural attention of PCBs could continue in IHSC sediments stored at the CDF after dredging activities. Continued monitoring of these sediments after they are placed in the CDF using analytical chemistry and molecular biology techniques, along with the aid of metrics like MDPR, as described here has the potential to reveal evidence of further PCB degradation.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

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• PCB congener analysis suggested in situ PCB dechlorination occurred in sediments.
• Putative dechlorinating Chloroflexi abundance was elevated in shallow sediments.
• Biphenyl dioxygenase gene abundance was also elevated in shallow sediments.
• Acinetobacter and Acidovorax spp. were highly abundant in deep sediments.
• Acidovorax sp. abundance was correlated with molar dechlorination product ratios.
Figure 1.
Aerial view of Indiana Harbor and Ship Canal depicting the location of sediment sample analyzed in this study (Red open circle).
Figure 2.
Flow chart depicting the analytical procedures used in this study.
Figure 3.
Fraction of light, medium and heavy PCBs versus depth in core sediment (A) and pore water (B), and fraction of ortho-, meta-, para-chlorines versus depth in core sediment (C) and pore water (D).
Figure 4.
Sediment and pore water MDPR values against core sediment depth.
Figure 5.
Abundances (expressed as genes per g sediment) of total bacteria (bac), bphA, putative Chloroflexi 16S rRNA genes (chl and dhc) against sediment depth as determined by qPCR analysis.
Figure 6.
NMDS ordination and cluster analysis of T-RFLP profiles of core sediment (0–0.30 m, 0.30–0.61 m…) (A), the abundance of T-RF 196 bp (B) and 198 bp+199 bp + 252 bp (C) along sediment depth.
Table 1

16S rRNA gene clones recovered from sediment at the depth of 3.35–3.66 m.

<table>
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<th>Observed T-RF (bp)</th>
<th>Predicted T-RF (bp)</th>
<th>Closest classified relative (% certainty)</th>
<th>No. of clones</th>
<th>NMDS axis 1</th>
<th>NMDS axis 2</th>
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<td><em>Acidovorax</em> (93–100)</td>
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<td>0.717</td>
<td>-0.052</td>
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