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Short communication

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 dw). 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 suggests 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.

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* (Wiegel and Wu, 2000; Fagervold et al., 2005, 2007; Yan et al., 2006; Adrian et al., 2009). *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 (Hölscher et al., 2004; Bedard et al., 2007; Fung et al., 2007; 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

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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 dw (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* (Fig. 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 is shown in Fig. 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.

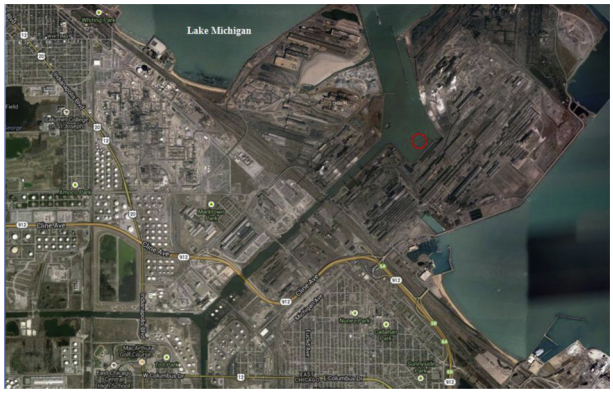

Fig. 1. Aerial view of Indiana Harbor and Ship Canal depicting the location of sediment sample analyzed in this study (red open circle). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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), *bph*A (primer set *bph*A 463f/674r) (Petrić et al., 2011b), and putative dechlorinating *Chloroflexi* 16S rRNA genes (primer set chl348f/dehal884r) (Fagervold et al., 2005) and (primer set dhc793f/946r) (Yoshida et al., 2005) (Table S1). PCR conditions were as follows: 10 min at 95 °C, 40 cycles of 15 s 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 *bph*A, 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, standard curves were prepared from pCR 2.1-TOPO vector containing the target PCR products with primer chl3487f/dehal884r and dhc793f/946r, respectively. All qPCRs were performed with an ABI 7000