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Enhanced anaerobic dechlorination of polychlorinated biphenyl in sediments by bioanode stimulation☆

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ABSTRACT

The application of a low-voltage electric field as an electron donor or acceptor to promote the bioremediation of chlorinated organic compounds represents a promising technology meeting the demand of developing an efficient and cost-effective strategy for *in situ* treatment of PCB-contaminated sediments. Here, we reported that bioanode stimulation with an anodic potential markedly enhanced dechlorination of 2,3,4,5-tetrachlorobiphenyl (PCB 61) contained in the sediment at an electronic waste recycling site of Qingyuan, Guangdong, China. The 110-day incubation of the bioanode with a potential poised at 0.2 V relative to saturated calomel electrode enabled 58% transformation of the total PCB 61 at the initial concentration of 100 μmol kg^{-1}, while only 23% was reduced in the open-circuit reference experiment. The introduction of acetate to the bioelectrochemical reactor (BER) further improved PCB 61 transformation to 82%. Analysis of the bacterial composition showed significant community shifts in response to variations in treatment. At phylum level, the bioanode stimulation resulted in substantially increased abundance of *Actinobacteria*, *Bacteroidetes*, and *Chloroflexi* either capable of PCB dechlorination, or detected in the PCB-contaminated environment. At genus level, the BER contained two types of microorganisms: electrochemically active bacteria (EAB) represented by *Geobacter*, *Ignavibacterium*, and *Dysgonomonas*, and dechlorinating bacteria including *Hydrogenophaga*, *Alcanivorax*, *Sedimentibacter*, *Dehalogenimonas*, *Comamonas* and *Vibrio*. These results suggest that the presence of EAB can promote the population of dechlorinating bacteria which are responsible for PCB 61 transformation.

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of hydrophobic persistent organic pollutants that easily bioaccumulate in animal fatty tissues and can cause immune system damage and carcinogenicity. Despite bans on production in most countries since the late 1970s, the previous usage of PCB-containing products have caused their widespread distribution in the environment, particularly in the bottom sediments of rivers, lakes and harbors all over the world (Brown JR et al., 1987; Wiegel and Wu, 2000). Bottom sediments are the major sinks for PCBs, which are poorly soluble in water. For example, PCBs have been distinctly detected in the aquatic environment of Longtang and Guiyu towns, two major electronic waste recycling sites in Southeast China, since PCBs were widely used as flame-retardant additives to oil insulators, coolants and lubricants in transformers and capacitors in electric and electronic equipment (Leung et al., 2006; Wu et al., 2008; Xing et al., 2009). Many studies (Wiegel and Wu, 2000; Wu et al., 2012; Gomes et al., 2013) have shown the ability of natural anaerobic processes for the microbial transformation of PCBs in the contaminated sediments, with highly chlorinated biphenyls reduced to less chlorinated congeners as a result of replacement of chlorine substituent by hydrogen. Due to the stability and hydrophobicity of PCBs, their anaerobic dechlorination, however, proceeds in the natural environment at a very slow rate. Considerable attempts (DeWeerd and Bedard, 1999; Krumins et al., 2009; Park et al., 2011; Payne et al., 2011) have been made to enhance the PCBs' dechlorination rates by biostimulation using halogenated co-substrates as priming compounds and/or bioaugmentation with dehalorespiring bacteria. These methods turn out to be effective in achieving increased PCB reduction, but could suffer the problems of periodic replenishment of chemicals, bacterial competition for energy sources, cell death and the loss in microbial activity during inoculation.

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A promising *in situ* alternative could consist of application of an electric field to stimulate microbial metabolism for PCB dechlorination. In this approach an electrode system is used, instead of chemicals as a cathodic electron donor or an anodic electron sink. So far, the biocathode stimulation of anaerobic reduction of various chlorinated organic compounds via direct or indirect electron-transfer pathway has been demonstrated (Strycharz et al., 2008, 2010). Frequently studied chlorinated compounds include trichloroethene, pentachloroethene and pentachlorophenol. We have previously attempted to demonstrate the feasibility of biocathode stimulation for anaerobic reduction of PCBs by polarizing the carbon cathode at −0.5 V *vs.* standard calomel electrode (SCE), and observed no effect on PCB dechlorination even after a year of continuous operation of the bioelectrochemical reactor (data unpublished). The result was likely attributed to the low cathode potential applied, which was confirmed recently by Chun et al. They showed that application of a higher potential of 1.5–3.0 V to the titanium electrode significantly enhanced the microbial transformation of PCBs within 88 days of incubation (Chun et al., 2013).

The hypothesis that bioanode stimulation, like biocathode stimulation, is also effective for enhancing the anaerobic reduction of PCBs is proposed here. This approach emerged from a few studies discovering that the presence of electrochemically active bacteria (EAB) grown with easily degradable organics such as acetate, glucose, sucrose or formate can markedly stimulate the anaerobic degradation of toxic and biorefractory polycyclic aromatic hydrocarbons (Wang et al., 2012), azo dyes (Fernando et al., 2012) and pentachlorophenol (Huang et al., 2011). The aim of this study was to demonstrate the concept of bioanode stimulation towards PCB dechlorination. The corresponding microbial community shifts was investigated using Illumina high-throughput sequencing technology that has proven to a feasible method to disclose microbial community fluctuations in the bioelectrochemical reactor (BER) (Liang et al., 2014; Liu et al., 2015b). A BER with a bottom sediment containing 2,3,4,5-tetrachlorobiphenyl (PCB 61) introduced to its anode chamber was operated under the conditions, where no extra organic compounds were added and an anodic potential of 0.2 V *vs.* SCE was applied. The concentrations of PCB 61 and its dechlorination products as a function of time were quantified, and the composition of microbial communities were analyzed.

2. Material and methods

2.1. Chemicals, suppliers, and purities

All PCBs were obtained at >99% purity from AccuStandard (New Haven, CT, USA). High-performance liquid chromatography (HPLC)-grade n-hexane, dichloromethane and acetone were purchased from Sigma–Aldrich (St. Louis, USA), and other chemicals were of reagent grade.

2.2. Configuration and inoculation of BER

The two-chamber BER made of Perspex flames was fabricated as described previously (Feng et al., 2014; Xie et al., 2014). The reactor consists of a bare carbon paper electrode (3 cm × 4 cm × 0.1 cm) horizontally positioned at the bottom of the anode chamber, and a graphite felt electrode (7.0 cm × 4.0 cm × 0.5 cm) placed vertically in the cathode chamber. A titanium wire of 0.6 mm diameter were attached to the carbon paper with conductive adhesive and inserted to the graphite felt providing the external circuit connection. The total void volume of the cathode and anode chambers was 100 mL; the chambers were separated by a cation exchange membrane (Qianqiu Corporation, Zhejiang, P.R.China). Saturated calomel electrode was used as reference electrode. The working and reference electrodes in the anode chamber, and the counter electrode in the cathode chamber, were all connected to a CHI1030 potentiostat (Chenhua Corporation, Shanghai, P.R.China) used to set the anodic potential at the desired value. It should be noted that all the potentials reported throughout this paper were referred to SCE, if not stated otherwise.

2.3. Operation of anode-stimulated BER for PCB dechlorination

The sediment samples were taken from 20 cm below the water-sediment interface in a small river (23°36′ 12.30″ N, 113e°04′ 38.84″ E) near Longtang town (Qingyuan, Guangdong, China), which has a 40-year history of electronic waste recycling. The sediments were black in color, gelatinous in texture, and had a strong petroleum odor. The samples were anaerobically placed to a glass container and stored at ambient temperature in the dark. The total organic carbon (TOC) in the sediment was determined by dry combustion and subsequent measurement of CO_2 with a TOC analyzer for solid samples (Elementar, Germany).

To start the experiments, a stock solution of PCB 61 containing 200 mg/L in hexane was added to the anode chamber of the BER, yielding a final concentration of 100 nmol per 1 g of dry sediment equivalent. The residual volume of anode chamber was filled with phosphate buffered saline (PBS) medium (0.1 M, pH 7.0) containing 5.84 g L^{-1} NaCl, 0.10 g L^{-1} KCl, 0.25 g L^{-1} NH_4Cl, 10 mL $L^{-1}$of vitamin solution and mineral solution. The cathode chamber was also filled with the PBS solution (0.1 M, pH 7.0). To remove the dissolved oxygen, the media added to both chambers were purged with N_2 for 30 min prior to the experiments. The sediment-containing BER was operated at 30 °C with a potential of 0.2 V applied to the anode. The poised anode potential was set at 0.2 V according to many previous studies (Busalmen et al., 2008; Rosenbaum et al., 2011; Feng et al., 2013), which have demonstrated that it was an optimal value for the growth of EAB.

For comparison, four reference experiments were simultaneously conducted, including the BER with the successive replenishment of sodium acetate to the anode chamber, the open-circuit references with and without the addition of sodium acetate, and the killed-cell reference. Duplicate reactors were run for each case. It should be noted that a quarter of the solution in each reactor was replaced with a fresh portion every fifth day. The selection of a five-day cycle was made considering that sodium acetate was consumed in the BER in 5 day as observed in our previous study (Wu et al., 2014). During one cycle, the anodic current immediately increased with the amendment of acetate, reached a maximum value and gradually decayed over time as a result of the consumption of acetate.

2.4. Sampling, extraction and analysis of PCBs

Duplicate enrichments were sampled every 10 days. The reactors were vigorously hand-shaken for 5 min before opening at the aseptic operation table, and approximately 1 mL of the sediment slurry was withdrawn using a sterilized syringe. To quantify absolute concentration of PCBs, sediment slurry samples were exerted to the triple extraction with portions of 25 mL of 1:1 hexane-acetone mixture in a separation funnel shaken for about 6–10 min. Decachlorobiphenyl (PCB 209) in the amount of 1 mL of a 4 mg/L *n*-hexane solution, was added as a surrogate standard. The solvent extracts were collected in a bottle and rinsed with 30 mL of 1:1 hexane-dichloromethane mixture after evaporation of acetone. Then they were purified by passing through chromatography column containing anhydrous Na_2SO_4, acidic silicon powder, neutral silicon powder (80–100 mesh) and glass wool to ensure the removal of residual water and particulate impurities. Subsequently,