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Enhancing tetrabromobisphenol A biodegradation in river sediment microcosms and understanding the corresponding microbial community

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ABSTRACT

In situ remediation of contaminated sediment using microbes is a promising environmental treatment method. This study used bioaugmentation to investigate the biodegradation of tetrabromobisphenol A (TBBPA) in sediment microcosms collected from an electronic-waste recycling site. Treatments included adding possible biodegradation intermediates of TBBPA, including 2,4-dibromophenol (2,4-DBP), 2,4,6-tribromophenol (TBP), and bisphenol A (BPA) as co-substrates. Bioaugmentation was done with *Ochrobactrum* sp. T (TBBPA-degrader) and a mixed culture of *Ochrobactrum* sp. T, *Bacillus* sp. GZT (TBP-degrader) and *Bacillus* sp. GZB (BPA-degrader). Results showed that bioaugmentation with *Ochrobactrum* sp. T significantly improved TBBPA degradation efficiencies in sediment microcosms ($P < 0.01$); aerobic conditions increased the microbes' degradation activities. Co-substrates 2,4-DBP, TBP and BPA inhibited biodegradation of TBBPA. A metagenomic analysis of total 16S rRNA genes from the treated sediment microcosms showed that the following dominant genera: *Ochrobactrum*, *Parasegetibacter*, *Thermithiobacillus*, *Phenylobacterium* and *Sphingomonas*. The genus level of *Ochrobactrum* increased with increased degradation time, within 10-week of incubation. Microbes from genus *Ochrobactrum* are mainly linked to enhance the TBBPA biodegradation.

1. Introduction

Tetrabromobisphenol A (TBBPA) is a widely used brominated flame retardant, and reduces the flammability of final manufactured products (de Wit, 2002). It is released into the environment during the production, use, and disposal of TBBPA-containing products. TBBPA is very persistent and has been detected in a wide range of environmental media, including air, water, soil and sediment (de Wit et al., 2010; Song et al., 2014; Xiong et al., 2014), and even in human tissues and plasma (Fujii et al., 2014a, b). TBBPA persists due to its high lipophilicity (log $K_{ow} = 5.9$), low volatility (7.0×10^{-11} atm m^3 mol^{-1}), low water solubility (4.16 mg L^{-1} at 25 °C in H_2O) and recalcitrance (McCormick et al., 2010). Unfortunately, the evidence increasingly highlights the possible toxic effects of TBBPA (Decherf et al., 2010; An et al., 2011; Koike et al., 2013; Linhartova et al., 2015). Furthermore, like halogenated polychlorinated biphenyls or dioxins, TBBPA may migrate to sediment due to hydrophobicity. When TBBPA enters the environment, photo-oxidation, chemical oxidation, and biodegradation may transform it (Chang et al., 2012b; Liu et al., 2013; Wang et al., 2015).

The in situ remediation of contaminated sediment using microbes is a promising treatment method (Acosta-González et al., 2013; Tischer et al., 2013; Dalvi et al., 2014; Islam et al., 2015). In a previous study, a novel bacterium *Ochrobactrum* sp. T was isolated and tested for its ability to effectively degrade TBBPA in water; the microorganism successfully degraded TBBPA, and used TBBPA as a sole carbon and energy source under aerobic conditions (An et al., 2011). Little is known, however, about the role of *Ochrobactrum* sp. T in enhancing TBBPA degradation and elimination in sediment. As such, further study is needed to support its practical use as in situ remediation strain.

Adding alternative halogenated compounds (referred to as "haloprimers" agents) can enhance the ability of anaerobic

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microorganisms to dechlorinate polychlorinated biphenyls in sediment (Ahn et al., 2005, 2008; Pöritz et al., 2015). A microbial consortium enriched from sediment using a mixture of 2-, 3-, and 4-bromophenol may improve 1,2,3,4-tetrachlorodibenzo-*p*-dioxin dechlorination (Vargas et al., 2001). Halogenated aromatic compounds ("haloprimers") with similar analogous structures to 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (TeCDD)/1,2,3,4-tetrachlorodibenzofuran (TeCDF) could also effectively enhance TeCDD/F dechlorination (Ahn et al., 2005). Using halogenated co-substrates as haloprimers could stimulate anaerobic dechlorination, because certain bacteria can use halogenated co-substrate as a terminal electron acceptor during the respiratory reductive dehalogenation or dehalorespiration process (Hiraishi, 2008). This respiratory activity may play an important role in detoxifying organohalides released into the environment (Park et al., 2011). Based on these past studies, this research explored a number of co-substrates to assess their effect on TBBPA biodegradation.

Understanding the biodegradation enhancement of organics in bioaugmentation experiments also requires to understand bacterial community responses. This is because high densities of aboriginal microorganisms existed in sediment, and sediment-based bacterial communities facilitate organic decomposition (Krumins et al., 2009; Lin et al., 2014). Information about the bacterial community composition in sediment is important to understand TBBPA degradation in sediment ecosystems.

This study had four main elements. First, it investigated TBBPA biodegradation in contaminated sediment undergoing bioaugmentation with *Ochrobactrum* sp. T. Next, it compared the influence of co-substrates, by adding 2,4-dibromophenol (2,4-DBP), 2,4,6-tribromophenol (TBP), or bisphenol A (BPA) as co-substrates for TBBPA biodegradation. Third, it investigated the degree of TBBPA biodegradation under aerobic and anaerobic conditions. Finally, it analyzed bacterial community structure responses to bioaugmentation using high-throughput sequencing.

2. Materials and methods

2.1. Chemicals

TBP and TBBPA, with purities exceeding 99%, were purchased from Sigma–Aldrich (St. Louis, MO, USA). N,O-bis(trimethylsilyl) trifluoroacetamide/trimethychlorosilane (BSTFA:TMCS, 99:1, v/v), 2,4-DBP, and BPA, with purities exceeding 99%, were obtained from Acros Organics (New Jersey, USA). Surrogates, including ^{13}C-TBP, ^{13}C-TBBPA, and ^{13}C-BPA, were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Hexane, methanol, and dichloromethane of high-performance liquid chromatography (HPLC) grade came from CNW technologies (Shanghai ANPEL Scientific Instrument Co., Ltd., Shanghai, China). HPLC grade acetone was from J. T. Baker (Center Valley, PA, USA). All other chemicals were of analytical grade and from Guangzhou Chemical Reagent Co., Inc., China (South of Industry AVE, Guangzhou, China). The LC-Florisil cartridge (1 g, 6 cc) was provided by Sigma–Aldrich (St. Louis, MO, USA); silica sorbent (300–400 mesh) was from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). These were used after Soxhlet extraction, activated and deactivated.

2.2. Sampling and medium

Sediment samples were collected from the Liangjiang River and Beigang River (0–10 cm depth) in South China (23°327′N, 116°342′E). These rivers are heavily contaminated with TBBPA, TBP, BPA, polybrominated diphenyl ethers (PBDEs), and heavy metals (Xiong et al., 2014). Sediment samples were packed into sterile glass jars, sealed, and stored at −20 °C until used.

Preparation of the mineral medium (MM) used in biodegradation and isolation of the TBBPA-degrading bacteria for experiments, and the preparation of the growth medium (GM) were done based on our previously published work (An et al., 2011). The pH value of the basal medium was adjusted to 7.0 before autoclaving at 121 °C for 30 min.

2.3. Microcosm setup

Microcosms were created with homogenized sediment, containing 47% (V/V) sediment, 53% (V/V) MM, and 10 mg L^{-1} of TBBPA. TBBPA was first dissolved in MM at a fixed concentration before being added to the sediment. Each microcosm unit included 150 mL homogenized sediment in a 250 mL serum bottle; bottles with anaerobic microcosms were capped with a silicone stopper.

Eight treatments were created, each with a duplicate: (1) sterile controls (autoclaved at 121 °C for 30 min) under aerobic conditions; (2) unamended controls under aerobic conditions; (3) aerobic conditions plus bioaugmentation with *Ochrobactrum* sp. T; (4) anaerobic conditions plus bioaugmentation with *Ochrobactrum* sp. T; (5) co-substrate TBP plus bioaugmentation with *Ochrobactrum* sp. T under aerobic conditions; (6) co-substrate BPA plus bioaugmentation with *Ochrobactrum* sp. T under aerobic conditions; (7) co-substrate 2,4-DBP plus bioaugmentation with *Ochrobactrum* sp. T under aerobic conditions; and (8) bioaugmentation with a mixed culture containing *Ochrobactrum* sp. T, *Bacillus* sp. GZT and *Bacillus* sp. GZB under aerobic conditions. An equal microbial population was added to each treatment. As such, the microbial population of each bacterium of the mixed culture occupied one-third of the total microbial population.

Previous research demonstrated that *Ochrobactrum* sp. T can degrade and mineralize TBBPA (An et al., 2011); and *Bacillus* sp. GZT can degrade 2,4-DBP and TBP. These latter two substances are also intermediates produced when TBBPA is degraded by *Ochrobactrum* sp. T (Zu et al., 2012); *Bacillus* sp. GZB can degrade BPA, which is also an intermediate produced when TBBPA is degraded by *Ochrobactrum* sp. T (Li et al., 2012).

These bacteria were inoculated in the sterilized GM at 37 °C in a rotary incubator at 200 rpm for 15 h. Then, 30 mL of the incubated GM was centrifuged and rinsed three times with sterilized water to facilitate bacteria collection. The bacteria (0.37 g, wet weight) were then aseptically transferred to the sediment microcosms. All the microcosms were incubated on a horizontal shaker (150 rpm) in the dark at 25 °C. The anaerobic microcosms were obtained by continuously flushing the sealed bottles with N_2 for 1 h.

For the samples to support microbial community analysis, serum bottles were shaken thoroughly and subsamples (5 mL each) were extracted from the main sample with a glass syringe once a week for 10 weeks. At each sampling event, an additional 5 mL sample was collected from the bioaugmentation experiments with a culture containing *Ochrobactrum* sp. T for microbial community analyses.

2.4. Chemical analysis

Sediment samples from microcosms were spiked with 40 ng ^{13}C-TBBPA surrogate standard, freeze-dried, and extracted three times using a 40 kHz ultrasonic processor with 20 mL hexane:acetone (1:1, v/v) for 40 min (Xiong et al., 2014). Elemental sulfur was removed using HCl-activated copper powder. Three extracts were combined and concentrated to 1 mL using ultra-high purity N_2 (99.999%). Cleanup was performed using LC-Florisil cartridges (1 g, 6 cc) (Labadie et al., 2010; Xiong et al., 2014), as follows. First, 0.5 g anhydrous sodium sulfate was packed on the top of the LC-Florisil cartridge, and then sequentially preconditioned with