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ORIGINAL ARTICLE

Microbial communities associated with anaerobic degradation of polybrominated diphenyl ethers in river sediment

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Abstract *Background/purpose:* Polybrominated diphenyl ethers (PBDEs) are extensively used as a class of flame retardants and have become ubiquitous environmental pollutants. We aimed to uncover the changes in microbial community with PBDE anaerobic degradation with and without zero-valent iron in sediment from the Erren River, considered one of the most heavily contaminated rivers in Taiwan.
Methods: PBDE anaerobic degradation in sediment was analyzed by gas chromatography with an electron capture detector. Microbial community composition was analyzed by a pyrosequencing-based metagenomic approach.
Results: The anaerobic degradation rate of BDE-209 was higher than BDE-28 in sediment; the addition of zero-valent iron enhanced the degradation rates of both. In total, 19 known bacterial genera (4 major genera: *Clostridium*, *Lysinibacillus*, *Rummeliibacillus*, and *Brevundimonas*) were considered PBDE degradation-associated bacteria (sequence frequency negatively correlated with PBDE remaining percentage) as were four known archaea genera (*Methanobacterium*, *Methanosarcina*, *Methanocorpusculum*, and *Halalkalicoccus*; sequence frequency positively correlated with PBDE remaining percentage).
Conclusion: The composition of bacteria and that of archaea affected the anaerobic degradation of BDE-28 and BDE-209. The addition of zero-valent iron further decreased the archaea content to undetectable levels.

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Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in various industrial products[1] and their residues are found in a wide variety of environments, with their concentrations increasing exponentially.[2] Increasing evidence shows that PBDEs are bioaccumulated and biomagnified and the compounds have been listed as the new persistent organic pollutants, and their fate and transport in ecosystems have received worldwide attention.

Microbial degradation is believed to be one of the major processes that may be used in remediating PBDE-contaminated sediment. PBDEs can be reductively debrominated by anaerobic microbes.[3] High-brominated PBDE congeners can be transformed to low-brominated PBDEs.[4] To enhance the efficiency of biodegradation, three remedial strategies—natural attenuation, bioaugmentation, and biostimulation—have been proposed.[5] Our previous study showed that BDE-209 can be debrominated successively to BDE-3 by anaerobic microbes from sediment; the addition of brij 30, brij 35, rhamnolipid, surfactin, vitamin B_{12}, zero-valent iron, acetate, lactate, and pyruvate enhanced the anaerobic degradation, with zero-valent iron yielding the highest BDE-209 anaerobic degradation.[6] However, little is known about the microbial communities involved in PBDE anaerobic degradation in sediment.

Molecular-biological methods have allowed for studies of microbial diversity in environmental samples. Metagenomic approaches have revolutionized our ability to explore the microbial world, revealing at higher resolution the structure of complex microbial communities that conventional cloning and sequencing methods have not been able to achieve. The pyrosequencing of 16S ribosomal RNA (rRNA) genes has been developed as a high-throughput metagenomic technology for profiling microbial communities in a resolution at the genus level.[7,8]

We aimed to assess the anaerobic degradation of high- and low-brominated PBDEs with zero-valent iron in sediment from the Erren River, considered one of the most heavily contaminated rivers in Taiwan. We used pyrosequencing to examine the phylogenetic diversity, composition, and structure of the microbial community associated with PBDE degradation. The target PBDEs were BDE-28 and BDE-209.

Methods

Chemicals

BDE-28 and BDE-209 were from Sigma Aldrich (St. Louis, MO, USA). Solvents were from Mallinckrodt, Inc. (Paris, KY, USA). All other chemicals were from Sigma Aldrich.

Sampling and medium

Sediment samples were collected from the Erren River in Taiwan. A detailed description of the sampling site was previously described.[6,9] Anaerobic BDE-adaption was achieved by the addition of 50 μg/g BDE-209 at 14-day intervals under static incubation at 30°C in the dark for 2 years. In this article, such sediment refers to anaerobic BDE-adapted sediment. The anaerobic medium consisted of (in g/L): KH_2PO_4, 0.27; K_2HPO_4, 0.35; NH_4Cl, 1.7; $FeCl_2.4H_2O$, 0.01; $CaCl_2.2H_2O$, 0.1; and $MgCl_2.6H_2O$, 0.1. The pH was adjusted to 7.0 after autoclaving; 0.9mM titanium citrate was added as a reducing reagent.

Experimental design

Experiments involved 12.5-mL serum bottles containing 4.5 mL medium, 0.5 g sediment, 50 μg/g PBDEs (BDE-28 or BDE-209), and zero-valent iron (1 g/L). Anaerobic experiments were conducted in an anaerobic glove box (Forma Scientific, Model 1025S/N, USA (Thermo Fisher Scientific Inc, 81 Wyman StreetWaltham, MA 02451, USA)) filled with N_2 (85%), H_2 (10%), and CO_2 (5%). Bottles were capped with butyl rubber stoppers and crimp seals and wrapped in aluminum foil to prevent photolysis, then incubated without shaking at 30°C in the dark. Each treatment was performed in triplicate. Samples were collected to measure residual PBDEs at 0 days, 30 days and 60 days and to detect bacterial communities at 60 days.

Analysis of PBDEs

PBDEs were extracted twice from whole bottles by use of hexane and acetone (9:1), then extracted again for 20 minutes with use of a Branson 5200 ultrasonic cleaner (Branson Ultrasonics, Americas Headquarters, 41 Eagle Road, Danbury, CT 06810, USA). Extracts were analyzed by use of a gas chromatograph (Hewlett Packard 6890 (Hewlett-Packard Company, 2850 Centerville Road, Wilmington, DE 19808-1610, USA)) equipped with an electron capture detector and Stx-500 capillary column. The initial column temperature was set at 170°C, increased by 10°C/min to 300°C, then increased by 2.5°C/min to 340°C. Injector and detector temperatures were set at 350°C and 370°C, respectively. Nitrogen was used as both a carrier gas (flow rate 4.0 mL/min) and makeup gas (flow rate 16.2 mL/min). The recovery percentages for BDE-28 and BDE-209 were 98.4% and 94.9%, respectively.

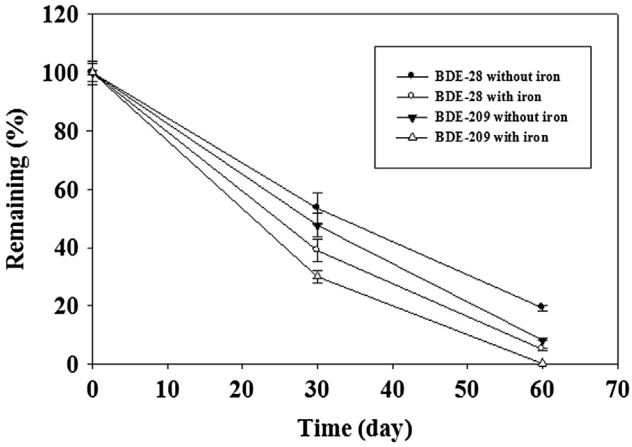

Figure 1. Anaerobic degradation of BDE-28 and BDE-209 with or without zero-valent iron in sediments. BDE = brominated diphenyl ether.