International Biodeterioration & Biodegradation 87 (2014) 60–65

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Reductive debromination of decabromodiphenyl ether by anaerobic microbes from river sediment

Huang-Wen Huang [a], Bea-Ven Chang [a,*], Ching-Chang Lee [b]

[a] Department of Microbiology, Soochow University, Tainan, Taiwan
[b] Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, Tainan, Taiwan

ARTICLE INFO

Article history:
Received 17 February 2013
Received in revised form
13 October 2013
Accepted 14 October 2013
Available online 25 November 2013

Keywords:
Polybrominated diphenyl ethers (PBDEs)
Decabromodiphenyl ether (BDE-209)
Reductive debromination
Sediment

ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are emerging persistent organic pollutants and have consequently drawn much environmental concern. The objective of this study was to evaluate reductive debromination of decabromodiphenyl ether (BDE-209) by anaerobic microbes from river sediment under various conditions. The debromination rates for BDE-209 were enhanced by the addition of brij 30, brij 35, rhamnolipid, surfactin, vitamin B_{12}, zero-valent iron, acetate, lactate, and pyruvate. Zero-valent iron yielded the highest BDE-209 debromination. For the various PBDE congeners, the high-to-low order of debromination rates in sediment was BDE-209 > BDE-99 > BDE-47 > BDE-28 > BDE-15. The intermediate products resulting from the reductive debromination of BDE-209 in sediment were nona-BDE (BDE-207), octa-BDEs (BDE-196, 197), hepta-BDEs (BDE-183, 184, 191), hexa-BDEs (BDE-138, 154), penta-BDEs (BDE-85, 99, 100, 119), tetra-BDEs (BDE-47, 49, 66, 71), tri-BDEs (BDE-17, 28), di-BDEs (BDE-7, 15), and mono-BDE (BDE-3). Our result shows BDE-209 can be debrominated successively to BDE-3 by anaerobic microbes from river sediment. This research offers feasible methods for removal of BDE-209 in river sediment for bioremediation.

1. Introduction

Polybrominated diphenyl ethers (PBDEs), a well-known group of additive brominated flame retardants, have been widely used over the past decades because of their low production costs and highly efficient fire-retardant properties (Hakk and Letcher, 2003). Among the congeners of PBDEs, penta-BDEs and octa-BDEs have been banned from use in both Europe and California; tetra-BDE to hepta-BDE were added to the list of persistent organic chemicals by the Stockholm Convention, and the production of BDE-209 will cease in 2013 (Shih et al., 2012). However, because of long-term use, PBDEs have been dispersed into air, water, sewage sludge, sediment, and human and animal tissue and therefore are of large concern to human health (de Wit, 2002; Schreiber et al., 2010; Chen et al., 2013).

PBDEs can be reductively debrominated by anaerobic microbes (Gerecke et al., 2005). Higher-brominated PBDE congeners (e.g., hepta-BDEs to BDE-209) can be transformed to lower-brominated PBDEs (e.g., tetra- to hexa-BDEs) in various environmental media, including air, water, sediment, and biota (Tomy et al., 2004; Gerecke et al., 2005; Keum and Li, 2005; Stapleton et al., 2006; Tokarz et al., 2008). Less-brominated compounds, such as 4,4′-dibromodiphenyl ether (BDE-15), may be debrominated to 4-bromodiphenyl ether (BDE-3) and diphenyl ether by anaerobic microbial and photochemical degradation pathways (Rayne et al., 2003).

To enhance the efficiency of biodegradation, three remedial strategies, namely, natural attenuation, bioaugmentation, and biostimulation, have been proposed (Yu et al., 2005). Several mixed microbes or pure culture of bacteria strains, including *Sulfurospirillum multivorans*, *Dehalobacter restrictus*, *Dehalococcoides* sp., and *Desulfitobacterium hafniense*, have been reported to carry out PBDE debromination (He et al., 2006; Robrock et al., 2008; Lee and He, 2010). However, higher brominated PBDEs are difficult to be debrominated by these anaerobic strains and a long reaction time is needed (Smidt and deVos, 2004). The addition of brij 30, brij 35, rhamnolipid, and surfactin enhanced biodegradation of dibromodiphenyl ether in river sediment (Huang et al., 2012). Cobalamins, such as coenzyme vitamin B_{12}, are well known to mediate the reductive dehalogenation of hydrophobic halogenated organics in biomimetic laboratory studies (Tokarz et al., 2008). The debromination mechanism of zero-valent iron with different pollutants has been studied (Matheson and Tratnyek, 1994; Ebert et al., 2006). However, little is known about the effects of various factors on the anaerobic debromination of BDE-209 in river sediment.

The climatic characteristics of subtropical regions foster diverse microbial communities. Molecular biological methods enable

* Corresponding author. Tel.: +886 228806628; fax: +886 228831193.
E-mail addresses: bvchang@scu.edu.tw, bvchang@mail.scu.edu.tw (B.-V. Chang).

0964-8305/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ibiod.2013.10.011

studies of microbial diversity in environmental samples. Many studies have used PCR-Denaturing gradient gel electrophoresis (DGGE) to examine the effect of toxic chemicals on microbial communities in the sediment (Castle et al., 2006). Little information is available about the microbial community associated with BDE-209 anaerobic debromination in river sediment. The aim of the present study was to assess the effect of anaerobic debromination of BDE-209 and change in microbial community in river sediment. We also evaluated sequential biotransformation of BDE-209 by anaerobic microbes from river sediment.

2. Materials and methods

2.1. Chemicals

BDE-15, 28, 47, 99, and 209 were purchased from Sigma Aldrich (St. Louis, MO). Solvents were purchased from Mallinckrodt, Inc. (Paris, KY). The biosurfactants used were surfactin and rhamnolipid. The details of surfactin and rhamnolipid production can be found elsewhere by Yeh et al. (2005) and Wei et al. (2005), respectively. All other chemicals were purchased from Sigma Aldrich (St. Louis, MO).

2.2. Sampling and adaptation

Sediment samples were collected from the Erren River, considered one of the most heavily contaminated rivers in Taiwan (TEPA, 1997). Deep sediment (15–30 cm) was collected during low tide by use of a soil core sampler. All sediment samples were collected randomly in triplicate from an area of about 1 m^2 at the center of each selected site. Detailed descriptions of the sampling sites are available by Yuan et al. (2011). The anaerobic medium used in experiments consisted of the following (mg l^{-1}): 0.27 g KH_2PO_4, 0.35 g K_2HPO_4, 1.7 g NH_4Cl, 0.01 g $FeCl_2 \cdot 4H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, and 0.1 g $MgCl_2 \cdot 6H_2O$. The pH was adjusted to 7.0 after autoclaving; 0.9 mM titanium citrate was added as a reducing reagent.

Initial acclimation was achieved by addition of 50 μg g^{-1} BDE-209 to a slurry consisting of sediment to medium ratio of 1:3 at 14-d intervals under static incubation at 30 °C in the dark for 2 years. In this paper, such sediment refers to BDE-adapted sediment. Experiments of the effects of various additives or various BDE-209 concentrations involved 0.5- or 2-year BDE-adapted sediment, respectively.

2.3. Experimental design

Experiments involved 12.5-ml serum bottles containing 4.5 ml medium, 0.5 g sediment and 50 μg g^{-1} BDE-209. The effect of the following factors was measured on BDE-209 debromination in sediment: rhamnolipid, surfactin, brij 30, or brij 35 at a 0.5 critical micelle concentration (CMC; CMC values for brij 30, brij 35, rhamnolipid, and surfactin were determined to be 27.5 μM, 45.5 μM, 75 mg l^{-1}, and 21.5 mg l^{-1}, respectively); vitamin B_{12} (0.025 mg l^{-1}), zero-valent iron (1 g l^{-1}); the electron donors acetate (30 mM), lactate (20 mM), or pyruvate (20 mM); bicarbonate (30 mM) to create methanogenic conditions, sulfate (20 mM) to create sulfate-reducing conditions, or nitrate (20 mM) to create nitrate-reducing conditions; and microbial inhibitors (50 mM (2-bromoethanesulfonate, BESA), 100 mg l^{-1} vancomycin, or 20 mM sodium molybdate-2-hydrate); BDE-15, 28, 47, 99, or 209 (50 μg g^{-1}); and various BDE-209 concentrations (50, 250, 500, and 1000 μg g^{-1}). The concentrations of these factors were chosen based on previous studies (Yuan et al., 2011). Inoculated control samples, considered non-sterile, and BDE-adapted sediment were shaken before incubation at 30 °C and pH 7.0 in the dark. Sterile controls were autoclaved at 121 °C for 30 min on 2 consecutive days.

All experiments were conducted in an anaerobic glove box (Forma Scientific, Model 1025S/N, USA) filled with N_2 (85%), H_2 (10%), and CO_2 (5%) gases. 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 periodically collected to measure residual concentrations of PBDEs and intermediate products. PCR-DGGE was used to analyse the microbial community after the sediment was spiked with various concentrations of BDE-209 as described below.

2.4. Analytical methods

The analytical method used to measure PBDEs concentration in sediment samples was as previously described (Huang et al., 2012). PBDEs were extracted twice from whole bottles by use of hexane and acetone (9:1), then extracted again for 20 min with use of a Branson 5200 ultrasonic cleaner (Branson, USA). Extracts were analysed using a gas chromatograph (Hewlett Packard 6890) equipped with an electron capture detector (GC-ECD) and Stx-500 capillary column (0.25 mm i.d. × 0.1 μm film thickness × 30 m, Restek). The initial column temperature was set at 170 °C, increased by 10 °C min^{-1}–300 °C, then increased by 2.5 °C min^{-1}–340 °C. Injector and detector temperatures were set at 350 and 370 °C, respectively. Nitrogen was used as both a carrier gas (flow rate 4.0 ml min^{-1}) and make-up gas (flow rate 16.2 ml min^{-1}). The recovery percentages for BDE-15, BDE-28, BDE-47, BDE-99, and BDE-209 were 97.1%, 98.4%, 93.3%, 96.3%, 94.9%, respectively. All the concentrations of BDE-15, BDE-28, BDE-47, BDE-99, and BDE-209 were added 50 μg g^{-1}.

Intermediate products of PBDEs in sediment samples were measured as described (Chen et al., 2013). All collected sediment samples were mixed, evaporated to near dryness under a gentle stream of nitrogen, and measured by high-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) (Agilent GC 6890/VG) with a 15-m DB-5HT column (0.25 mm i.d. × 0.1 μm film thickness, J&W Scientific, Folsom, CA).

2.5. DNA extraction and PCR-DGGE analysis

DNA extraction and PCR-DGGE analyses were described previously (Chang et al., 2012). DNA was extracted from sediment samples with use of the PowerSoil DNA kit. FGC968 (*Escherichia coli* position 968–983, 5′-GCCCGGGGCGCGCCCGGGCGGGGCGGGGGCACGGGGGG AACGCGAAGAACCTTAC-3′) and R1401 (*E. coli* position 1401–1385, 5′-CGGTGTGT ACAAGACCC-3′) were used for PCR-DGGE analysis. The PCR cycling parameters were initial denaturation at 94 °C for 10 min; 35 cycles of 45 s at 94 °C, 1 min at 54 °C, and 1 min at 72 °C; and final extension at 72 °C for 10 min. PCR products were analyzed by electrophoresis in 1.2% (wt/vol) nusieve 3:1 agarose gels containing ethidium bromide (1 μg ml^{-1}). DGGE involved a D-gene and D-code system (Bio-Rad Laboratories, CA, USA) with 1-mm gel thickness. Electrophoresis was run in 1× TAE buffer at 60 V and 60 °C for 16 h, then gels were incubated for silver staining. The solutions were 10% (wt vol^{-1}) ethanol plus 0.1% acetic acid for fixation, 0.25 g silver nitrate for staining, freshly prepared developing solution containing 0.01% (wt vol^{-1}) sodium borohydride, 0.4% (vol vol^{-1}) formaldehyde, and 1.2% (wt vol^{-1}) NaOH, and finally, 0.75% sodium carbonate solution to stop development. Gels were dried and documented by use of a video system (Bio Image Products, Ann Arbor, MI).

2.6. Data analysis

The percentage PBDEs remaining in sediment was calculated as the PBDE residue concentration divided by the original PBDE concentration, multiplied by 100. The PBDE debromination data fit well