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

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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

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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 with first-order kinetic equations: $S = S_0 \exp(k_1 t)$, $t_{1/2} = \ln 2/k_1$, where t is the time, S_0 the initial substrate concentration, S the substrate concentration at time t, and k_1 the debromination rate constant. Significant differences were accepted at $p < 0.05$. Principal Component Analysis (PCA) was used to examine the DGGE community structure among different samples that contained multiple variables. Statistics were calculated using the Statistical Package for Social Sciences v10.0 software (SPSS Inc., IL, USA).

3. Results and discussion

3.1. Effect of various factors on the anaerobic debromination of BDE-209 in sediment

The BDE-209 concentrations in sterile controls were first examined at the end of 56-d of incubation. The remaining percentage of BDE-209 in sediment ranged from 91.1% to 96.3%. Therefore, we concluded that the BDE-209 debromination in all treatments was due to microbial activity under different conditions. The effects of the addition of various substrates on anaerobic debromination of BDE-209 with 0.5-year BDE-adapted sediment samples are shown in Table 1. The debromination rates for BDE-209 were enhanced by 27.3%, 45.5%, 50.0%, and 31.8% with addition of rhamnolipid, surfactin, brij 30, and brij 35, respectively. Organic hydrophobic chemicals can be partitioned into a surfactant's micellar phase and are, to some degree, available to be assimilated by microorganism for degradation (Chang et al., 2012).

The debromination rates of BDE-209 were elevated by 36.4% and 86.4% with the addition of vitamin B_{12} and zero-valent iron, respectively. Cobalamins have been identified in cell extracts to have dehalogenating ability as well as dehalogenase enzymes (Stromeyer et al., 1992; Woods and Trobaugh, 1999). Cobalamins from decaying cells have been isolated from environmental samples. Thus, in the presence of low-potential electron donors, free cobalamins may abiotically mediate reductive dehalogenation reactions. The ubiquitous occurrence of vitamin B_{12} in anaerobic microbes, combined with its ability to mediate reductive dehalogenation reactions, provides an excellent experimental model for examining the reductive dehalogenation of PBDE congeners (Gaul et al., 2006; Tokarz et al., 2008). Also, the addition of a zero-valent iron yielded a higher BDE-209 debromination rate than any of the other additives. The addition of zero-valent iron could reduce PBDEs to less-brominated compounds by anaerobic microbes (Kim et al., 2011). As well, sorption could play a role in the BDE-209 debromination process with the addition of zero-valent iron (Shih and Tai, 2010). Therefore, the addition of vitamin B_{12} and zero-valent iron could enhance BDE-209 removal efficiency and debromination ability via abiotic and biotic debromination processes.

Table 1
Effect of various substrates on BDE-209 anaerobic debromination rate constant (k_1) and half-life ($t_{1/2}$) from sediment samples.

Treatment	k_1 (d^{-1})	$t_{1/2}$ (d)	r[a]
Inoculated control[b]	0.022	31.5	0.92
Rhamnolipid (75 mg l^{-1})	0.028	24.8	0.90
Surfactin (21.5 mg l^{-1})	0.032	21.7	0.92
Brij 30 (27.5 μM)	0.033	21.0	0.96
Brij 35 (45.5 μM)	0.029	24.0	0.91
Lactate (20 mM)	0.038	18.2	0.97
Acetate (30 mM)	0.035	19.8	0.96
Pyruvate (20 mM)	0.031	22.4	0.97
Vitamin B_{12} (0.025 mg l^{-1})	0.030	23.1	0.90
Zero-valent iron (1 g l^{-1})	0.041	16.9	0.89
Sulfate (20 mM)	0.030	23.1	0.98
Bicarbonate (30 mM)	0.028	24.8	0.98
Nitrate (20 mM)	0.025	27.7	0.99

Each treatment was significantly different from the inoculated control at $P < 0.05$.

[a] r = correlation coefficient.

[b] Inoculated control: 30 °C, pH 7.0, 50 μg g^{-1} BDE-209.

Table 1 also shows that the addition of electron donors, namely lactate, acetate, or pyruvate increased the debromination rates of BDE-209 by 72.7%, 59.1%, and 40.9%, respectively. Debromination may have been enhanced because of the use of acetate, lactate, or pyruvate as electron donors. Sulfate-reducing bacteria may act on lactate to produce pyruvate plus two electrons, which would cause a higher rate of debromination (Chang et al., 2006). Addition of electron acceptors nitrate, sulfate, or bicarbonate increased the debromination rate of BDE-209 by 13.6%, 36.4%, and 27.3%, respectively. Compared with the inoculated control, the debromination of BDE-209 was enhanced under methanogenic, sulfate-reducing, and nitrate-reducing conditions. The order of rates for BDE-209 debromination in sediments under these conditions was observed to be sulfate-reducing conditions > methanogenic conditions > nitrate-reducing conditions. The data in Table 2 show that addition of molybdate (a selective inhibitor of sulfate-reducing bacteria), BESA (a selective inhibitor of methanogens), or vancomycin (a selective inhibitor of eubacteria) (Arbeli et al., 2006) decreased the degradation rate constants of BDE-209 by 27.3%, 13.6%, and 9.1%, respectively. Sulfate-reducing bacteria belong to eubacteria (domain Bacteria), whereas methanogens are classified within the domain Archaea. The methanogens can only utilize simple substrates such as H_2/CO_2 and acetate, but the sulfate-reducing bacteria can use a wide spectrum of organic compounds for growth in the presence or absence of sulfate. The debromination rate of BDE-209 decreased greatly when the activities of sulfate-reducing bacteria were inhibited. This observation shows that sulfate-reducing bacteria might have played an important role in the debromination of BDE-209.

3.2. Comparison of the anaerobic debromination of various PBDE congeners in sediment

BDE-209 accounts for 83% of all PBDEs used (mass basis), yet the two dominant PBDE congeners detected in biota, including humans, are BDE-47 and BDE-99 (Tokarz et al., 2008). Therefore, we further compared the debromination of various PBDE congeners. The remaining percentages of BDE-15, BDE-28, BDE-47, BDE-99, and BDE-209 after 56 d of incubation were 75.1%, 60.5%, 48.3%, 39.5%, and 20.0%, respectively (Fig. 1). PBDE congeners were extracted by use of whole bottle sediment samples to remove sorption interference, thus microbial debromination of PBDE congeners is a major process. The debromination rates of PBDE congeners were in the order of BDE-209 > BDE-99 > BDE-47 > BDE-28 > BDE-15. PBDEs undergo reductive debromination reactions in sediment at rates corresponding to the number of bromine substitutions, such that the debromination rate of BDE-209 is faster than that of BDE-99, and the debromination rate of BDE-99 is faster than that of BDE-47 (Tokarz et al., 2008). Higher-brominated BDE congeners are debrominated via a reductive process, and the

Table 2
BDE-209 anaerobic debromination rate constants (k_1) and half-lives ($t_{1/2}$) from sediment samples after the addition of 3 microbial inhibitors.

Treatment	k_1 (d^{-1})	$t_{1/2}$ (d)	r[a]
Inoculated control[b]	0.022	31.5	0.92
Vancomycin (100 mg l^{-1})	0.020	34.7	0.99
BESA (50 mM)	0.019	36.5	0.99
Molybdate (20 mM)	0.016	43.3	0.95

Each treatment was significantly different from the inoculated control at $P < 0.05$.

[a] r = correlation coefficient.

[b] Inoculated control: 30 °C, pH 7.0, 50 μg g^{-1} BDE-209.

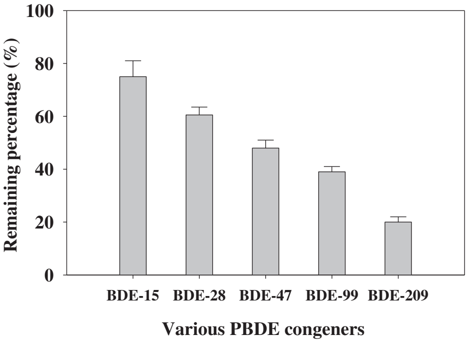

Fig. 1. Comparison of the anaerobic debromination of various polybrominated diphenyl ether (PBDE) congeners from sediment samples.

Table 3
Anaerobic debromination rate constant (k_1) and half-life ($t_{1/2}$) of various BDE-209 concentrations in 2-year BDE-adapted sediment.

Treatment	k_1 (d^{-1})	$t_{1/2}$ (d)	r[a]
50 μg g^{-1}	0.165	4.2	0.94
250 μg g^{-1}	0.062	11.2	0.96
500 μg g^{-1}	0.027	25.7	0.99
1000 μg g^{-1}	0.015	46.2	0.92

Each treatment was significantly different from the inoculated control at $P < 0.05$.
[a] r = correlation coefficient.

degradation rate is higher than with lower-brominated PBDEs (Keum and Li, 2005). We also monitored the products of the PBDE congeners by GC-ECD, but no intermediate products were detectable in these experiments. It may be that the concentration of intermediate products was too low to be detected in this experimental system.

3.3. *Anaerobic debromination of various concentrations of BDE-209 and microbial community analysis*

Two-year BDE-adapted sediment was used to compare the debromination of various concentrations of BDE-209. As shown in Table 3, the higher the concentration of BDE-209, the slower the debromination at 50–1000 μg g^{-1}. This finding may reflect increased toxicity at higher BDE-209 concentrations. Comparison of sediment adapted with BDE-209 for 0.5 or 2 years showed that the adaptation could enhance the capacity of microbial debromination of BDE-209 in the sediment. The adaptation process may increase the BDE-degrading activity of microbes. This finding is consistent with previous study on the effects of adaptation on the anaerobic biotransformation of tetrabromobisphenol-A, tetrachlorobisphenol-A, and bisphenol-A from estuarine sediments (Voordeckers et al., 2002).

Fig. 2 shows the changes in the microbial community in sediment with various treatments after 56 d of incubation. The DGGE profile consisted of at least 6 bands, and the number of bands was changed by the addition of various BDE-209 concentrations. The first principal component (PC1 = 52.6%) and second principal component (PC2 = 38.1%) explained 90.7% of the variation different concentrations. Comparison of 0.5- and 2-year BDE-adapted sediment showed that the microbial community changed significantly. Different microbial communities were present at different BDE-209 concentrations. The microbial community changed more significantly at the higher than lower BDE-209 concentration. High doses (1000 μg g^{-1}) of BDE-209 resulted in greater microbial community change in sediment. This finding is consistent with report by Liu et al. (2011), who reported that high doses of BDE-15 and BDE-209 altered the soil microbial community structure.

3.4. *Identification of debromination products from BDE-209 in sediment*

Biotransformation of BDE-209 was first monitored with 0.5-year BDE-adapted sediment by HRGC/HRMS. The BDE-209 parent starting material was converted by *meta* debromination into nona-BDE (BDE-207). BDE-207 was converted into 2 octa-BDEs (BDE-196, 197). BDE-197 was converted by *meta*-substitution into hepta-BDE (BDE-184), by *ortho*-substitution into penta-BDE (BDE-119), and by *para*-substitution into tetra-BDE (BDE-71). BDE-196 was converted by *ortho*- or *meta*-substitution into hepta-BDE (BDE-191) or hepta-BDE (BDE-183). BDE-183 was converted by *ortho*-substitution into hexa-BDE (BDE-138), by *meta*-substitution into penta-BDE (BDE-85), by *ortho*-substitution into tetra-BDE (BDE-66), and by *meta*-substitution into tri-BDE (BDE-28). BDE-183 was also converted by *meta*-substitution into hexa-BDE (BDE-154), by *meta*-substitution

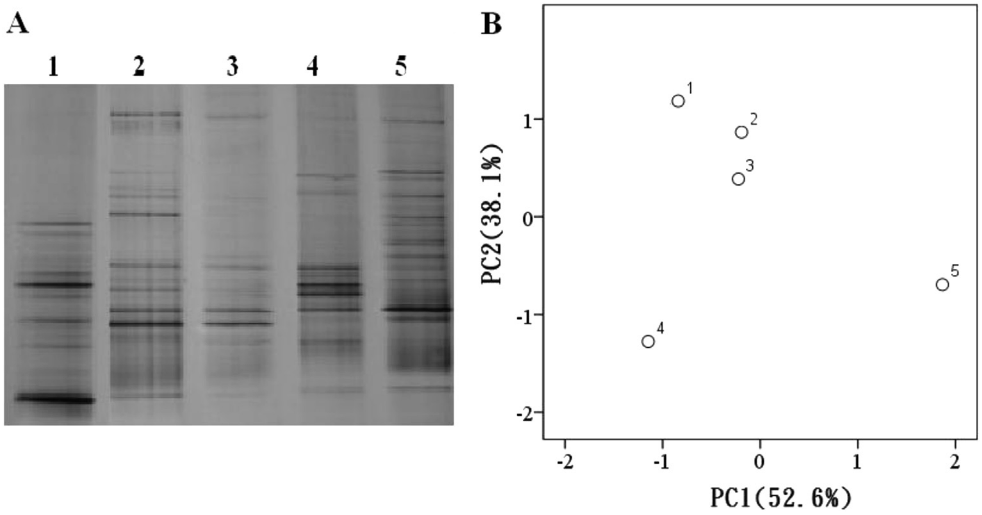

Fig. 2. Changes in denaturing gradient gel electrophoresis (DGGE) band profiles (A) and principal component analysis (PCA) (B) for 16S rDNA fragments, with the addition of various concentrations of decabromodiphenyl ether (BDE-209) for 56 d of incubation. Lane 1 represents 0.5-year BDE-adapted sediment samples spiked with 50 μg g^{-1} BDE-209; lanes 2, 3, 4, and 5 represent 2-year BDE-adapted sediment samples spiked with 50, 250, 500, and 1000 μg g^{-1} BDE-209, respectively.

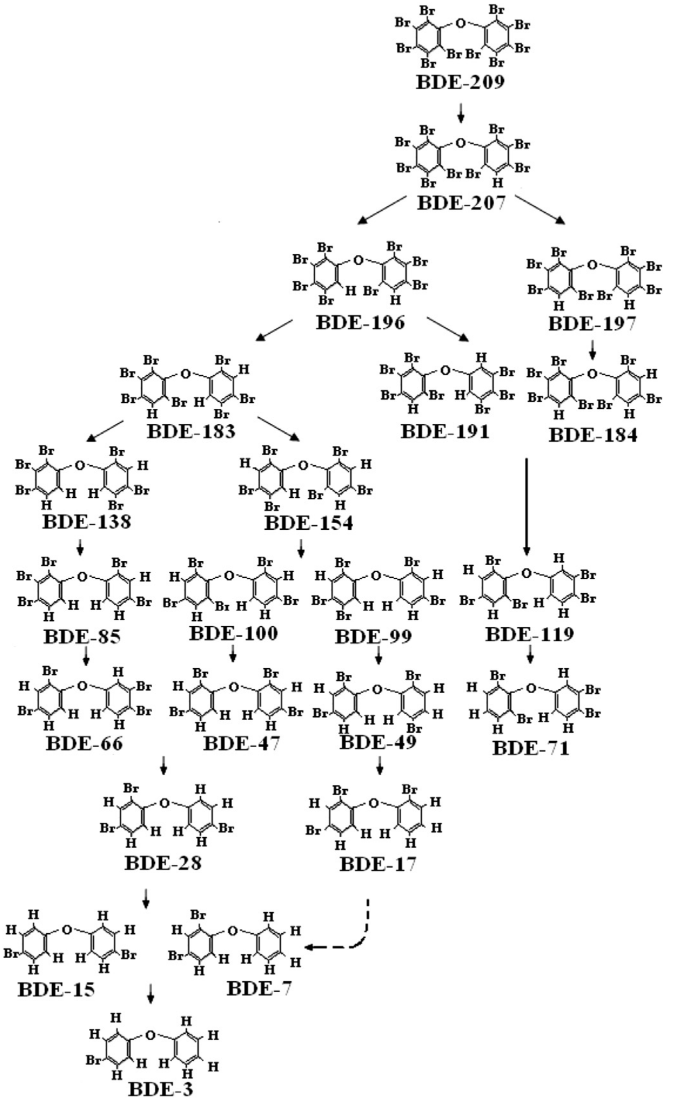

Fig. 3. Proposed debromination pathway for biotransformation of BDE-209 identified by HRGC/HRMS. Solid arrows show debrominations observed and dashed arrows debrominations assumed from sediment samples.

into penta-BDE (BDE-100), by *ortho*-substitution into tetra-BDE (BDE-47), and by *ortho*-substitution into tri-BDE (BDE-28). BDE-154 was also converted by *ortho*-substitution into penta-BDE (BDE-99), by *para*-substitution into tetra-BDE (BDE-49), and by *meta*-substitution into tri-BDE (BDE-17).

Despite the appearance of tri-BDEs from the debromination of BDE-209, another approach was needed to understand the sequential treatment of anaerobic reactions and to determine whether tri-BDE congeners were further debrominated. Thus, we conducted another experiment with tri-BDE (BDE-28) as an initial substrate with 2-year BDE-adapted sediment. BDE-28 was converted by *para*- or *ortho*-substitution into di-BDEs (BDE-7, 15) and by *ortho*- or *para*-substitution into mono-BDE (BDE-3). In addition, tri-BDE (BDE-17) may be converted via debromination to di-BDE (BDE-7). From the two-stage experiments with 0.5- and 2-year BDE-adapted sediment, BDE-209 with *ortho*, *meta*, and *para* specificity dominated debromination in the sediment. BDE-209 could be biotransformed via a debromination process to a 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) in the sediment. The proposed reductive debromination pathway for biotransformation of BDE-209 by BDE-adapted sediment is in Fig. 3.

Keum and Li (2005) revealed that BDE-209 was debrominated to di-BDE (BDE-7, 8, 15) by zero-valent iron treatment. Tokarz et al. (2008) reported that BDE-209 undergoes reductive debromination with vitamin B_{12} to produce various intermediates ranging from nona-BDE to tri-BDEs congeners (BDE-17, 28). Kim et al. (2011) showed reductive debromination of BDE-209 with microscale zero-valent iron produced various intermediates ranging from nona-BDEs to tri-BDEs (BDE-28, 30), and then aerobically treated with *Sphingomonas* sp. PH-07 strain to mono-BDEs.

In conclusion, our study showed successive biotransformation of BDE-209 to mono-BDE (BDE-3) via a reductive debromination process by BDE-adapted sediment. Additionally, we may optimize the reactions for complete debromination of BDE-209 as proposed in Fig. 3. These findings could provide useful information for the bioremediation of PBDE-contaminated sediment. Our next goal is to characterize the diversity of the microbial community involved in the debromination of BDE-209 by anaerobic microbes from river sediment.

Acknowledgments

This research was supported by the National Science Council, Republic of China (no. 98-2627-B-031-001). The authors gratefully acknowledge Dr. Jo-Shu Chang at National Cheng Kung University for generously providing surfactin and rhamnolipid for this study.

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