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Interaction of polybrominated diphenyl ethers (PBDEs) with anaerobic mixed bacterial cultures isolated from river sediment

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ABSTRACT

The degradation of flame retardant polybrominated diphenyl ethers (PBDE), including tetra-brominated diphenyl ether (BDE-47), penta-brominated diphenyl ether (BDE-99 and -100), and hexa-brominated diphenyl ether (BDE-153 and -154), by anaerobic bacterial mixed cultures isolated from river sediment was investigated. The effects of PBDEs on changes of anaerobic bacterial community in sediment culture were also studied. Sediments were collected from Er-Jen River and Nan-Kan River basins, which were both heavily polluted rivers in Taiwan, and bacteria from the sediment samples were enriched before the experiment was conducted. Into the anaerobic bacterial mixed cultures, 0.1 μg/mL of PBDEs was added followed by incubation under 30 °C for 70 days. Residues of PBDE were determined by gas chromatography with electron capture detector (GC-ECD), and the changes of bacterial community were analyzed by denaturing gradient gel electrophoresis (DGGE). Less than 20% of PBDEs were degraded after 70 days of incubation in all samples except for BDE-47 from the Nan-Kan River sediment. In that culture, BDE-47 was found to have notably degraded. In particular, after 42 days of incubation; BDE-47 was degraded, suddenly and sharply, to a negligible level on Day 70, and the result was confirmed by a repeated experiment. An interesting result was that although BDE-47 was degraded fast in the Nan-Kan River sediment, the bacterial communities did not shift significantly as we had speculated at Day 70. From UPGMA dendrograms, PBDEs changed the composition of bacterial communities, and the extents varied with the variety of PBDE congeners. By the amendment with BDE-153 or -154, bacterial communities would be changed immediately and irreversibly throughout the rest of the incubation period. No significant difference in degradation of PBDEs was observed between sediment bacteria from Er-Jen River and Nan-Kan River. However, the results verified the persistence of PBDEs in the environment.

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1. Introduction

Polybrominated diphenyl ethers (PBDE), as a flame retardant, are widely used in commercial products of electronic equipment and building materials such as computer housing, circuit boards in computers and televisions, capacitors and textiles, to reduce the risk of fires.

Owing to the persistent hydrophobic property of PBDEs, accumulation of PBDEs in sediment and biota of aquatic environments was regarded as a serious environmental problem in the world. Among them, a commercial penta-brominated diphenylether (PeBDE) flame retardant mixture, DE-71, has been extensively used for years. BDE-47 and -99 were the major components of DE-71 [1,2]. In the large production volumes of brominated diphenyl, tetra- and penta-brominated congeners predominate in biota; and BDE-47 was the most abundant PBDE congener [3–5]. The toxicity of PBDEs to fishes had been reported, and BDE-47, -49, -99, -100, -153 and -154 were found to be the most abundant congeners in exposed fish (accounting for over 95% of PBDE levels), and increasing with dose [6]. BDE-209 was reported to be the predominant congener in the Shiawassee River and Saginaw River, USA (10.8 and 2.77 ng/g in the river samples; 2.28 and 4.76 ng/g in the sediments, respectively), followed by BDE-47, -99, and -100 in order of decreasing abundance [7]. Biodegradation was considered to be a possible way of removing PBDEs from the environment [8], and could be effectively excluded by microorganisms under anaerobic conditions [9]. Related study on the debromination pathways by three different cultures of anaerobic dehalogenating bacteria was reviewed. It was found that the lesser the bromide in PBDEs, the faster the degradation of PBDEs would be [10]. However, studies on how PBDEs affect the anaerobic bacterial community in sediment are scarce. DGGE technique separates a DNA molecule by single-base difference and provides the knowledge of microbial diversities

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without cultivation [11–13]. Therefore, this study employed such technique to investigate the bacterial community in the sediment.

The fate of PBDEs in sewage sludge after agricultural application has been studied [14]. Concentration of brominated flame retardants of PBDEs including BDE-28, -47, -66, -100, -99, -85, -154, -153, -138 and -209, in floodplain soil samples, surface sediment samples and sediment cores from three rivers were measured [15]. Effects of PBDE-153 on *Daphnia magna* have been assessed with acute and reproductive toxicity tests [16].

Er-Jen River and Nan-Kan River, located at the southern and northern part of Taiwan, suffered marked contamination. Er-Jen River was mainly contaminated by municipal wastewater, industrial effluent, and heavy metals [17], whereas Nan-Kan River was contaminated by municipal wastewater, agricultural wastewater and industrial effluent [18]. The main difference between the two rivers is that there are metal reclamation activities in Er-Jen River, which caused metal pollution in this river, and there exist great differences in locations and contaminants present and experiments were conducted on the sediments of the two rivers.

The present study was designed to investigate the residual percentage of BDE-47 (tetra-brominated), BDE-99 (penta-brominated), BDE-100 (penta-brominated), BDE-153 (hexa-brominated) and BDE-154 (hexa-brominated) in the river sediments. Results thus obtained can shed light on the effects of PBDEs on anaerobic bacterial community in sediment culture, and how the bacterial community changes under 0.1 μg/mL concentration of PBDE.

2. Materials and methods

2.1. Materials

Five PBDEs including tetra-BDE 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), penta-BDEs 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) and 2,2′,4,4′,6′-pentabromodiphenyl ether (BDE-100), and hexa-BDEs 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153) and 2,2′,4,4′,5,6′-hexabromodiphenyl ether (BDE-154) were purchased from AccuStandard Inc., CT, USA. The purity of PBDEs was 100% (by GC–MS) except for BDE-153 (99.3%). Stock standard solution of PBDEs were prepared by adding 1 mL of purchased 50 μg/mL PBDEs into 1 mL of acetone and stored in opaque bottles at −20 °C. Anaerobic sediment samples were collected from river basins of Er-Jen River and Nan-Kan River located at the southern and northern part of Taiwan, respectively. Dates of collection were March 2005 and January 2006, respectively. Both rivers had been reported to be seriously contaminated by halogenated organic pollutants [19,20]. A grab sampler was employed to collect the sediment in the middle of the river at a depth of 0–10 cm. After sampling, the sediment samples were stored in a jar, kept at 4 °C and taken to laboratory for preparation of anaerobic microbe mixed culture. In order to purge oxygen, the jar was immediately taken to the laboratory and stored in an anaerobic modular atmosphere-controlled system (Don Whitley Scientific Co., England).

2.2. Methods

The cultural medium was prepared according to [21] with modifications. The culture medium comprising (in g/L): NH_4Cl (2.7), $MgCl_2{\cdot}6H_2O$ (0.1), $CaCl_2{\cdot}2H_2O$ (0.1), $FeCl_2{\cdot}4H_2O$ (0.02), K_2HPO_4 (0.27), KH_2PO_4 (0.35), yeast extract (1.0) and resazurin (0.001) was neutralized to about 7.0 with NaOH and then sterilized by autoclaving at 121 °C for 30 min. Prior to use, titanium citrate (as a reducing reagent) was added to the cultural medium to achieve a final concentration of 0.9 mM and a redox potential ranging from −280 to −300 mV.

The stock anaerobic sediment mixed culture was prepared by adding slurry sediment (300 mL) and culture medium (500 mL) into a 1-L serum bottle, followed by incubation at 30 °C in anaerobic modular atmosphere-controlled system. The supernatant culture medium was renewed every 2 weeks.

To eliminate the interference of sediment during the experiments, bacteria in anaerobic sediment mixed culture were isolated, as described by [22]. Tween 80 solution (a surfactant) was added into the stock anaerobic sediment mixed culture (200 mL) in a 350-mL serum bottle to achieve the final concentration of 0.1% (v/v) and then sonicated for 5 min. The sediment mixed culture was centrifuged to precipitate the sediment at 500 rpm for 5 min. Then the supernatant was collected and centrifuged again at 3700 rpm for 20 min to precipitate the bacteria. The bacteria thus collected were resuspended in 200 mL of freshly culture medium and incubated at 30 °C for experimental use.

The batch experiment was carried out by shaking 250 mL serum bottles at 120 rpm, with a final concentration of 0.1 μg/mL of PBDE (in acetone), 45 mL of culture medium and 5 mL of anaerobic bacteria mixed culture previously amended. The sterile control was prepared following the same procedure but modified by adding 5 mL of autoclaved (121 °C, 30 min) anaerobic bacterial mixed culture into the serum bottle. During incubation, the serum bottle was sealed with a butyl rubber stopper cap, covered with an aluminum top and then incubated in darkness (to avoid interference of oxygen and photolysis) at 30 °C for 70 days. All experiments were conducted in triplicate. At designated time, 2.0 mL of sample were taken from the serum bottle using sterile syringes. The residues of PBDEs and DNA were extracted and analyzed. PBDEs were analyzed with GC/ECD. DNA was processed by the 16S rDNA polymerase chain reaction (PCR) amplification and denaturing gradient gel electrophoresis (DGGE) was employed to analyze the changes of bacterial community.

2.3. Extraction and analysis of PBDEs

Residues of PBDE in bacterial culture samples were extracted and analyzed. Sample culture (2 mL) was extracted with 2 mL of *n*-hexane for 2 min under shaking. After three extractions, the extracts were combined and treated with anhydrous sodium sulfate and copper to remove H_2O and sulfur, concentrated, and analyzed by GC. A GC-ECD (HP 6890 series GC system, Hewlett Packard Co., USA) equipped with a HP-5 fused silica capillary column (30 m × 0.32 mm ID × 0.25 μm) was employed to identify the PBDE. Nitrogen was used as the carrier gas with a flow rate of 58 cm/s. The programmatic program of column temperature was set at 150 °C for 1 min, increased to 200 °C by 20 °C/min and then increased to 280 °C by 5 °C/min and held for 5 min. The retention times of PBDEs were 10.1, 5.5, 12.6, 17.6 and 15.4 min for BDE-47, -99, -100, -153 and -154, respectively. The recovery test was performed by adding PBDE to 2 mL of anaerobic culture medium to reach a final concentration of 0.1 μg/mL, extracting PBDEs with *n*-hexane and then analyzing with GC-ECD. All experiments were conducted in triplicate. The recoveries were between 87 and 106%.

For quantitative determination, standard curves of PBDEs were prepared by diluting the stock standard solution of PBDEs with *n*-hexane to 0.01, 0.025, 0.075 and 0.125 μg/mL and analyzed with GC-ECD. Linear regression equations with r^2 were obtained by plotting the integration area (y) vs. PBDE concentration (x) for calculating

the analyst as follows:

BDE-47	$y = 68,956x + 131.76$	$r^2 = 0.9998$
BDE-99	$y = 72,155x - 56.834$	$r^2 = 0.9997$
BDE-100	$y = 68,589x + 116.71$	$r^2 = 0.9999$
BDE-153	$y = 61,805x + 333.21$	$r^2 = 0.9984$
BDE-154	$y = 57,568x + 268.59$	$r^2 = 0.9998$

2.4. DNA extraction and purification

Total genomic DNA was extracted from 2 mL of anaerobic microbe mixed culture using an UltraClean Soil DNA kit (MO BIO Laboratories, Inc). Extracted DNA was stored at −20 °C.

2.5. PCR–DGGE techniques

PCR–DGGE analysis was described in our previous research [11]. In brief, PCR was performed with ABgene DNA polymerase and a buffer kit obtained from ABsystem. The PCR mixture (50 μL) containing 0.2 mM of each dNTP, 4.0 mM $MgCl_2$, 1× buffer solution, 1 unit of ABgene DNA polymerase, 0.4 μM of each primer, and 2 μL of template DNA was used. Primers 968f and 1401r were used for the amplification of bacterial 16S rDNA [23]. A GC-clamp was attached to the 5′-end of a 968F [24]. GeneAMP PCR system 9700 (Applied Biosystems, USA) was used in PCR amplification. The 16S rDNA was amplified as follows: 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 1 min, and 72 °C for 1 min, and with a final extension at 72 °C for 10 min. The PCR products were confirmed by using 1% (w/v) agarose gel electrophoresis and ethidium bromide staining. DGGE analysis was performed with a D-Code universal mutation detection system (Bio-Rad, USA). Samples of 20 μL of PCR products were loaded onto 8% (w/v) polyacrylamide gel containing 40–60% denaturing gradient of formamide and urea. The electrophoresis was run at 60 °C in 1× TAE for 12 h at a constant voltage of 75 V. After the electrophoresis, the polyacrylamide gel was stained with SYBR Green I nucleic acid gel stain and visualized on a UV transilluminator. The gel was photographed with a CCD camera.

2.6. Statistical comparison of DGGE pattern

The DGGE profiles were analyzed by unweighted pair-group method using arithmetic averages (UPGMA) and the similarity was calculated by coefficient of DICE, using the Quantity One software (Bio-Rad, USA). The UPGMA method was employed to reveal the change of bacterial community during incubation.

3. Results and discussion

3.1. Degradation of PBDEs in anaerobic bacterial mixed culture

Effects of sterilization on degradation of PBDEs in prepared anaerobic culture medium and in anaerobic bacterial (from sediment) mixed culture were studied. Degradation of BDE-47, -99 and -154, (representing tetra-, penta- and hexa-brominated diphenyl ether, respectively) in sterilized anaerobic culture medium in darkness at 30 °C for 70 days is shown in Fig. 1. Residues (%) in Figs. 1 and 2 indicate the proportion of chemicals determined to the original chemicals amended. As seen in Fig. 1, the degradation of PBDEs in sterilized anaerobic culture medium was limited. On the other hand, degradation of PBDEs by the anaerobic bacterial (from sediment) mixed culture was found to be less than 20% on Day 70 for BDE-47, -99, and -100 except for BDE-47 in the Nan-Kan River sample, and related data have been reported in [25].

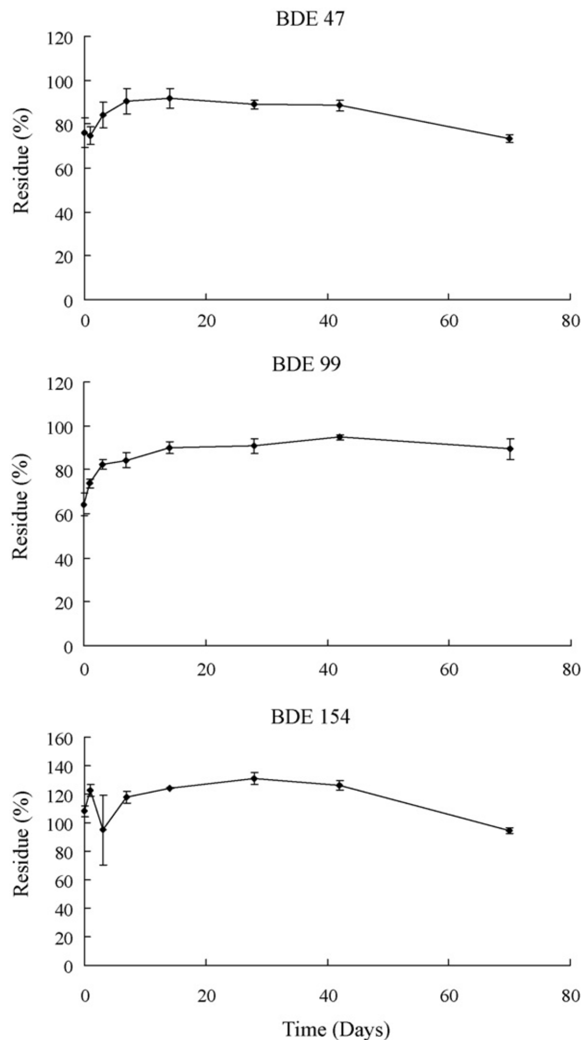

Fig. 1. Degradation of BDE-47, -99, and -154 in sterilized anaerobic culture medium in darkness at 30 °C for 70 days.

Degradation of BDE-153 and -154 (both are hexa-brominated) is shown in Fig. 2. Likewise, the degradation of BDE-47, -99 and -100 has been previously reported [25]. BDE-47 showed a similar degradation rate with BDE-99 and -100 in the bacterial mixed culture in the first 42 days of incubation, but after 42 days, BDE-47 was degraded suddenly and sharply to a negligible level in the sample of bacteria from Nan-Kan River sediment, BDE-47 was degraded to an undetectable level after Day 63 and no other degradation products were found from the GC chromatogram on Day 70. This experiment was repeated and analyzed at Days of 42, 50, 55, 63 and 70, and the results are shown in Table 1. The reason may be that BDE-47 was a lesser brominated congener of PBDE, and could be completely debrominated within weeks [10]. And in Nan-Kan River,

Table 1
Degradation of 0.1 μg/mL concentration of BDE-47 in the bacterial culture from Nan-Kan River sediment at 30 °C for 70 days.

Sampling day	Residues (%) of BDE-47
Before 42nd day	>80
50th day	73
55th day	22.4
63rd day	ND[a]
70th day	ND

[a] ND: not detectable.

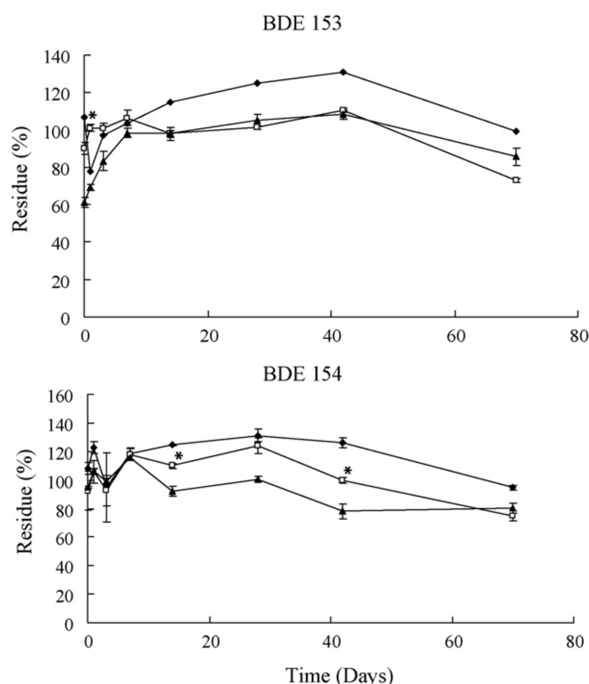

Fig. 2. Degradation of BDE-153 and -154 in sterile (♦), and non-sterile anaerobic bacterial mixed culture from Er-Jen River (▲) and Nan-Kan River (□) sediment at 30 °C for 70 days. *Indicated the significant differences of degradation ($p < 0.05$) between cultures from two rivers.

the metabolites can cause a reduced condition capable of degrading BDE-47, or, some bacteria induced by unidentified factors are favorable for the degradation of BDE-47.

In this study, BDE-153 and -154 were degraded slowly at 30 °C until Day 70, a slight difference in residual amounts was found between sterile and non-sterile treatments (Fig. 2). No significant difference in degradation of PBDEs was observed between sediment from Er-Jen River and Nan-Kan River. Although it is difficult to interpret why the determined values did not show the smooth curves for the first 7 days in the figure, the results verified the persistence of PBDEs in the environment.

3.2. *Structural change of bacterial community*

In parallel with the residual PBDE analysis, DGGE analysis was employed to study the structure of bacterial community in anaerobic bacterial mixed culture amended by PBDEs. BDE-47, -99, -100, -153 or -154 in the anaerobic bacterial mixed culture from Er-Jen River or Nan-Kan River sediment were incubated for 70 days, and 16S rDNA sequence fragments obtained from bacteria were used for DGGE analysis. Cluster analysis of bacterial community structures by UPGMA was also studied.

Fig. 3 shows the DGGE pattern and change in bacterial community structure after incubation with BDE-47 in bacterial culture from the Er-Jen River and Nan-Kan River sediment. The DGGE pattern in bacterial culture from the Er-Jen River sediment (Fig. 3A, upper) showed five bands, B1, B2, B3, B4 and B5, throughout the 70 days of incubation, implying that the bacterial community was not affected by BDE-47. On Day 42, two bands, B6 and B7, were still present, but they were not observed on Day 70, which might be attributed to the lack of nutrition in the culture medium. Two bands B8 and B9 appeared on Day 3 after amending with BDE-47; however, B8 disappeared on Day 28 while B9 showed increase in related band strength in the bacterial community until the end of incubation. The result of cluster analysis in Fig. 3B (upper) showed two clusters in the dendrogram: one was bacteria from samples incubated for 28 and 42 days, and the other was from samples incubated for 1, 3, 7, 14 and 70 days. Vigorous change in bacterial community structure was found by amending with BDE-47, as seen in Fig. 3B (upper), the lower similarity of 53% shown at Days 1 and 3, and of 45% at Days 14 and 28. The lower similarity at first several days indicated that BDE-47 affected the bacterial community structure in bacterial culture from the Er-Jen River sediment.

The DGGE pattern of the bacterial culture of the Nan-Kan River sediment after incubation with BDE-47 (Fig. 3A, beneath) showed five bands, namely B1, B2, B3, B4 and B5. The bands were observed throughout the 70 days of incubation, indicating that they were not affected by BDE-47. In addition, three bands, B6, B7 and B8, appeared after incubation for 28 days, indicating that these bacteria may be the potential bacteria communities contributing to BDE-47 biodegradation. Bands B9 and B10 appeared at Day 70, prior to which, BDE-47 was degraded to a negligible level, BDE-47 decreased sharply from Day 42, and could no longer be detected on Day 63 [23]. It was speculated that the bacterial communities B9 and B10 were first inhibited by BDE-47, and after BDE-47 was degraded by bacterial communities B6, B7 and B8 or by some unidentified reasons, the inhibited bacterial communities B9 and B10 were reactivated. As seen in the cluster analysis results (Fig. 3B, beneath), the changes of bacterial community were not vigorous. It was interesting to note that although BDE-47 was degraded fast in the Nan-Kan River sediment, the bacterial communities did not shift significantly as we had speculated. The communities were also divided into two clusters. The first cluster included three samples of bacterial community incubated for 1, 3 and 7 days, and the second cluster included four samples of bacterial community incubated for 14, 28, 42 and 70 days. The lower similarity (57%) of bacterial community for Days 1 and 3 was attributed to the impact of BDE-47. BDE-47 was degraded in bacterial culture of the Nan-Kan River sediment from Days 42 to 70 (Table 1), so the similarity of bacterial community on Days 42 and 70 was at a higher level of 66%, proving that bacterial communities may recover from the impact of BDE-47.

The DGGE pattern and the dendrogram of the bacterial culture of the Er-Jen River and Nan-Kan River sediment incubated with BDE-99 for 70 days were studied. DGGE pattern of the bacterial culture from the Er-Jen River sediment after incubation (figure not shown) showed five bands throughout the 70 days of incubation, indicating that they were not affected by BDE-99. Two bands were observed at Day 3, another two bands at Day 14, with all bands increasing their band intensity from Day 28. In particular, one of them showed the highest intensity at Day 70, which was observed as a predominant community amongst all species. The results of cluster analysis (Fig. 4A) showed that the change of bacterial community was vigorous. There are two clusters in cluster analysis (Fig. 4A): one was the bacterial community from the sample incubated for 70 days and the other was from six samples incubated for 1, 3, 7, 14, 28 and 42 days. BDE-99 impacted bacterial community structures vigorously; the result showed the similarity of 59% at Days 1 and 3, but decreased to 51% at Days 42 and 70. This change may be attributed to the nutrients being exhausted and secondary metabolites produced in medium with some of the bacterial activities lost.

The DGGE pattern of the bacterial culture of the Nan-Kan River sediment after incubation with BDE-99 (figure not shown) showed five bands maintained in steady state throughout the 70 days of incubation, indicating that they were not impacted by BDE-99. The DGGE pattern also showed one band that was observed until Day 28 but thereafter lost its activity; and two bands that were observed after incubation for 14 days with related intensity increasing as time elapsed. From the result of cluster analysis (Fig. 4B), the bacterial communities were divided into two clusters: one was the bacterial community from samples incubated for 42 and 70 days

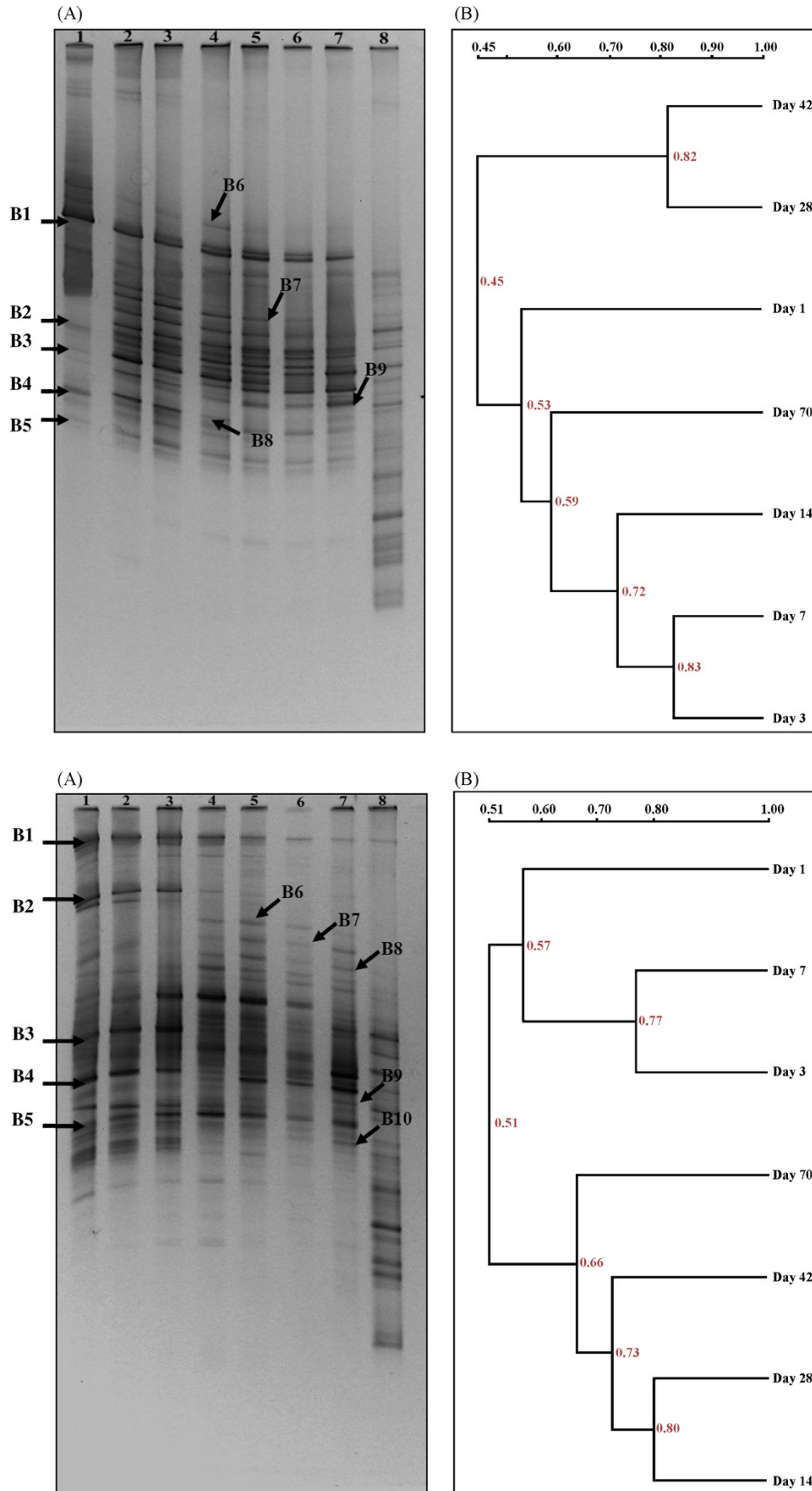

Fig. 3. (A) DGGE pattern of 16S rDNA sequence fragments from anaerobic bacterial mixed culture from Er-Jen River (upper) and Nan-Kan (beneath) sediment incubated with BDE-47 for 70 days and (B) dendrogram of cluster analysis of bacterial community structure by UPGMA. In DGGE pattern, lanes 1–8 represent bacterial community of days 1, 3, 7, 14, 28, 42, 70 and marker, respectively, and in dendrogram, Days 1, 3, 7, 14, 28, 42 and 70 represent the bacterial community in lanes 1–7, respectively.

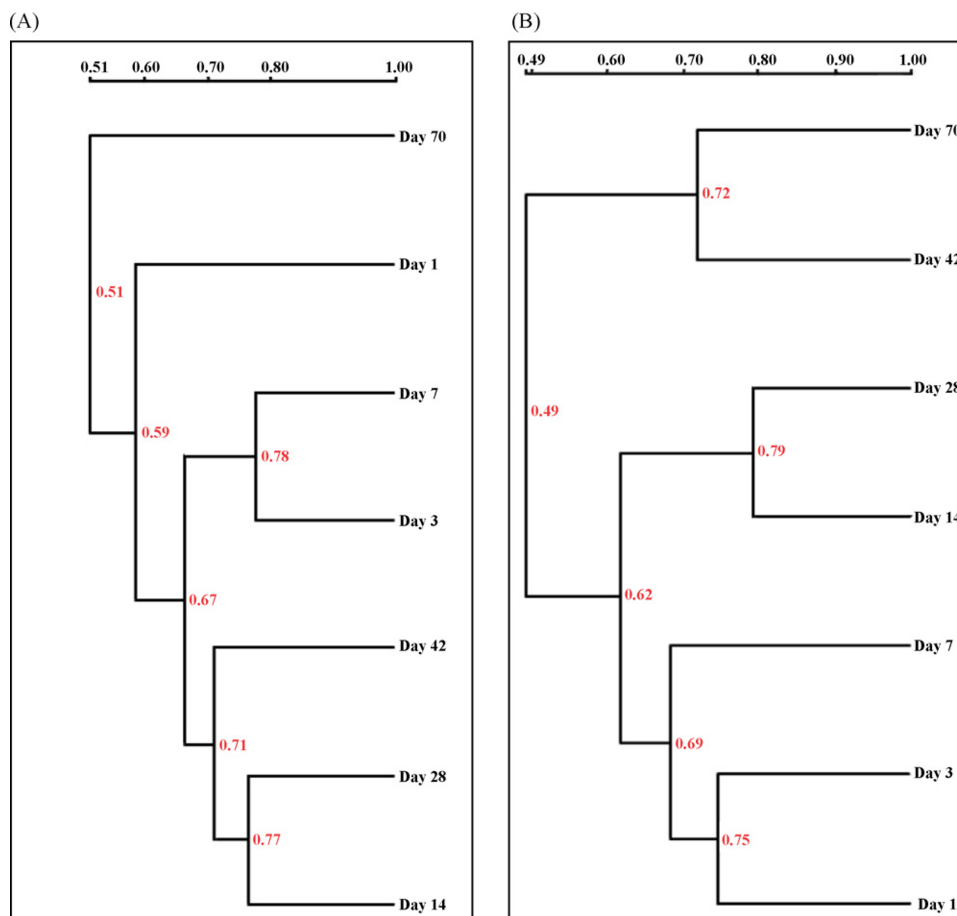

Fig. 4. Cluster analysis by UPGMA of bacterial community structure in the anaerobic bacterial mixed culture from Er-Jen River (A) and Nan-Kan River (B) sediment, incubated with BDE-99 for 70 days. Days 1, 3, 7, 14, 28, 42 and 70 indicate the bacterial community of incubation day.

and the other was from five samples incubated for 1, 3, 7, 14 and 28 days. However, no extreme change of bacterial community was found, similarity of bacterial community was between 79 and 49% throughout 70 days of incubation. The lowest similarity (49%) was found at Days 28 and 42.

Incubating with BDE-47, -99, -100, -153 or -154 in the bacterial culture from the Er-Jen River and Nan-Kan River sediment for 70 days, the band change in DGGE pattern and the cluster analysis of dendrogram are summarized and listed in Tables 2 and 3.

The numbers of bands indicating bacterial populations that steadily existed, and appeared later or disappeared later during 70 days of incubation are shown in Table 2. During 70 days of incubation with different PBDEs, about five bands (not exactly the same band because different PBDEs were used in the amendment) existed from the beginning of incubation in both ER-Jen River and Nan-Kan River sediment cultures. The results also showed that some bacteria increased while some decreased during the 70 days of incubation with PBDEs. There are four bands in the Er-Jen River and six bands in the Nan-Kan River sediment culture that appeared after being incubated with BDE-99 for 70 days. However, no bacterium in the Er-Jen River and only one bacterium in the Nan-Kan River sediment culture disappeared after incubation. This result showed that BDE-99 does not inhibit the bacterial population in either of the river sediments. On the contrary, with BDE-154, no bacterium appeared in either of the river sediment cultures, but four bands in the Er-Jen River and five in the Nan-Kan River sediment culture disappeared after 70 days. All the observations above reveal that BDE-154 showed more inhibition ability to the bacterial

Table 2
Band change in DGGE pattern of incubation with BDE-47, -99, -100, -153 or -154 in bacteria mixture culture from Er-Jen River and Nan-Kan River sediment during 70 days of incubation.

PBDEs	Band change during 70 days					
	Er-Jen River			Nan-Kan River		
	Exist	Appear	Disappear	Exist	Appear	Disappear
BDE-47	5	1	3	5	2	3
BDE-99	5	4	0	5	6	1
BDE-100	4	3	2	5	2	2
BDE-153	5	1	3	5	2	3
BDE-154	5	0	4	4	0	5

Table 3
Cluster analysis of bacterial community after incubation with PBDEs for different days in bacterial culture of Er-Jen River and Nan-Kan River sediment.

PBDE	Cluster analysis			
	Er-Jen River		Nan-Kan River	
	Cluster A	Cluster B	Cluster A	Cluster B
Incubation days, the sample of bacteria community from				
BDE-47	42, 28	1, 3, 7, 14, 70	1, 3, 7	14, 28, 42, 70
BDE-99	70	1, 3, 7, 14, 28, 42	42, 70	1, 3, 7, 14, 28
BDE-100	1, 42, 70	3, 7, 14, 28	1, 3, 7	14, 28, 42, 70
BDE-153	1	3, 7, 14, 28, 42, 70	1	3, 7, 14, 28, 42, 70
BDE-154	1	3, 7, 14, 28, 42, 70	1, 3, 7	14, 28, 42, 70

Dendrogram representation for BDE-47 and -99 are shown in Figs. 3 and 4, respectively.

populations than other PBDEs. The reason was that highly brominated PBDE congeners were slowly debrominated, usually below 10% of nanomolar concentrations of PBDEs transformed after 3 months [10], thus it would be more toxic to the microbes than other PBDEs.

For all the DGGE patterns, cluster analysis divided them into approximately two clusters regardless which PBDE treatment was applied (Table 3). Bacterial communities from 1-day incubation were different from that of 3, 7, 14, 28, 42 and 70 days of incubation with BDE-153 or -154 treatments. This means, by the amendment with BDE-153 or -154, that bacterial communities would be changed immediately and irreversibly throughout the rest of incubation period. Similar trends were observed with the amendment of BDE-47 or -100 in the Nan-Kan River sediment culture, in which the bacterial communities from 1, 3 and 7 days of incubation were different from the other four bacterial communities from 14, 26, 42 and 70 days of incubation. However, cultures amended with BDE-99 showed a diverse result. That was, the bacterial community changed after 70 days in the Er-Jen River and 42 days in the Nan-Kan River sediment culture. However, when amended with BDE-100, the bacterial community from 1, 42 and 70 days of incubation was in the same cluster in that of the Er-Jen River sediment culture, which implies, that the impact of BDE-100 on the ER-Jen River bacterial community was not permanent but recoverable.

4. Conclusions

This study demonstrated that degradation of PBDE compounds in anaerobic bacteria mixed cultures at 0.1 μg/mL concentration was difficult. After 70 days of incubation, degradation of most PBDEs in the Er-Jen River and Nan-Kan River sediment cultures was less than 20%, except for BDE-47 in the Nan-Kan River sediment culture. After 42 days of incubation, BDE-47 was degraded suddenly and sharply to the negligible level. The reason may be that in Nan-Kan River, the microbial metabolites can cause the reduced condition capable of degrading BDE-47, or some bacteria, induced by unidentified factors, could directly utilize BDE-47. Bacterial communities were changed by amendment of PBDEs and the extents differed widely with the variety of PBDEs. In view of the long persistence and bacterial community change of PBDEs in the environment, we have to pay attention to their residual amounts and effects on our environments.

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