

# EFFECTS OF POLYBROMINATED DIPHENYL ETHERS (PBDEs) ON ANAEROBIC MICROORGANISM COMMUNITY OF SEDIMENT

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## Introduction

Polybrominated diphenyl ethers (PBDEs), as a flame retardant, were widely used in the commercial products, such as circuit boards in the computer, computer housing, televisions, capacitors and etc. Due to their persistent and highly hydrophobic property, accumulation of PBDEs in the sediment and biota of aquatic environments had been concerned as a seriously environmental problem in the world. Biodegradation of PBDEs was concerned as the possible way to remove PBDEs from the environments.<sup>1</sup> Biodegradation might be effective to exclude PBDEs by microorganisms under anaerobic conditions.<sup>2</sup> However, the researches about how PBDEs affect the anaerobic microorganism community in sediment still limited. The present study was designed to investigate the effects of PBDEs on anaerobic microorganism community in sediment and to provide a point of view of how the microorganism community changes under 0.01  $\mu$ g/mL concentration of BDE 154 as time elapse.

## Materials and Methods

**Materials:** 2,2',4,4',5,6'-Hexabromodiphenyl ether (BDE-154) with 100 % purities (GC-MS) was purchased from AccuStandard Inc., CT, USA and was dissolved in isooctane. Anaerobic sediment samples were collected from river basin of Er-Jen River and Nan-Kan River located at southern and northern parts of Taiwan, respectively. A grab sampler was used to collect the river sediment in a depth of 0 to 10 cm. After sampling, the samples were stored in a jar, kept in 4°C and taken to laboratory for experiments.

**Methods:** The microorganisms in sediment were first enriched<sup>3</sup> and then separated<sup>4</sup> from sediment following the method of Chiu et al.<sup>3</sup>. In anaerobic conditions, a 5-mL aliquot of the enrichment cultures was transferred into 250-mL sterilized serum bottle containing 45 mL fresh medium, then spiked with 0.01  $\mu$ g/mL BDE-154, sealed and incubated under 30°C for 70 days. Experiments were conducted in triplicate. At designed times, 2.0 mL of sample was taken from serum bottle by using sterilized syringes and residues of BDE-154 and DNA were extracted and analysis. BDE-154 was analyzed with GC/ECD and DNA was proceeded the 16S rDNA PCR (Polymerase Chain Reaction) amplification and then use DGGE (Denature Gradient Gel Electrophoresis) to analyze the change of microorganism community.

**Polybrominated diphenyl ethers (PBDEs) analysis:** Residues of BDE-154 in sediment samples were extracted and analyzed. Two mL of samples were extracted with 2 mL of *n*-hexane for 2 min under shaken. After two times of extraction, the extracts were combined, the sample was further treated with anhydrous sodium sulfate and copper to remove H<sub>2</sub>O and sulfur, 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 x 0.32 mm I.D. x 0.25  $\mu$ m) was used to identify the BDE-154. Nitrogen was used as the carrier gas with a flow rate of 58 cm/sec, split. The programmatic column temperature program was set at 150°C for 1 minute, then increased to 200°C by 20°C/min and then increased to 280°C by 5°C/min and held for 5 minutes.  $\mu$ -ECD was performed at 300°C, retention time was 15.3 min. PCR-DGGE analysis described in previous study.<sup>3</sup>

## Results and Discussion

Anaerobic microbial degradation of BDE-154 in two different river sediments are shown in Figure 1. No significant difference of degradation rate was observed after 70 days of incubation. From the result of PCR-DGGE (Figures 2A and 3A) and dendrogram representation of hierarchical cluster analysis (Figures 2B and 3B), a different responds of microorganism community change was found. The microorganism community change in sediment of Er-Jen River as time elapse can be separated into two clusters (Figure 2B), that is days 3, 7, 14 verses days 28, 42, 70. The DGGE pattern (Figure 2A) of day 1 has only 52% similarity to the others. This result shows that microorganism community in the sediment of Er-Ren River is impacted by the BDE-154. Difference from that, the microorganism community change in the sediment of Nan-Kan River also be separated into two clusters (Figure 3B), the two cluster are days 1, 3, 7 verses days 14, 28, 42, and 70, but BDE-154 doesn't impact the microorganism community, since the Day 1 pattern has 68% similarity to day 3 and day 7 pattern (Figure 3A).

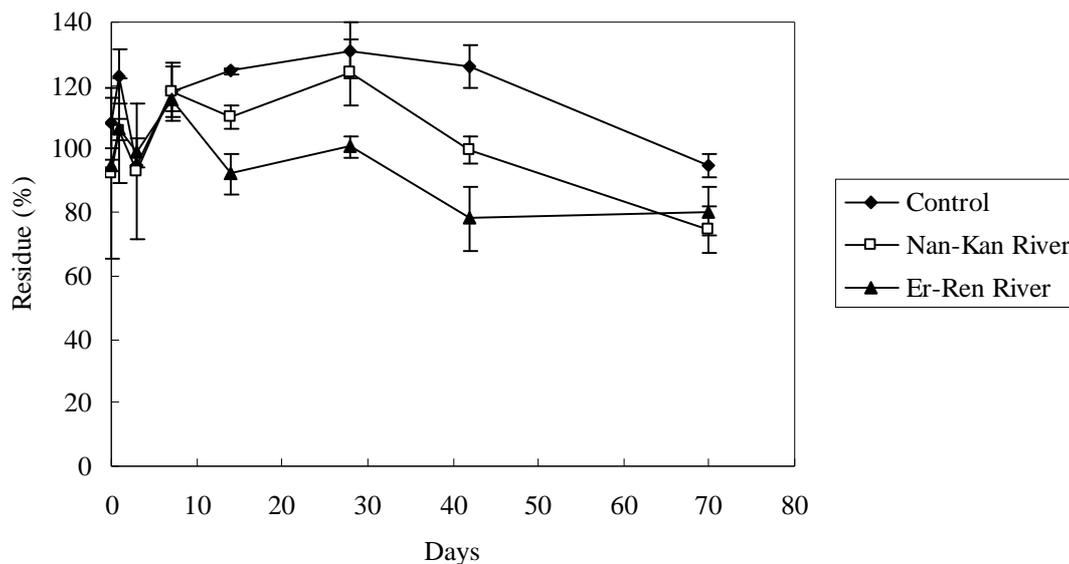


Figure 1. Degradation of BDE-154 in two different river sediments incubated for 70 days..

The result of this study demonstrate that though BDE-154 is difficult to degrade in the anaerobic mixed cultures, it still shows different effect on the different community in anaerobic mixed culture. Effect of other PBDEs on anaerobic mixed culture community is currently in progress.

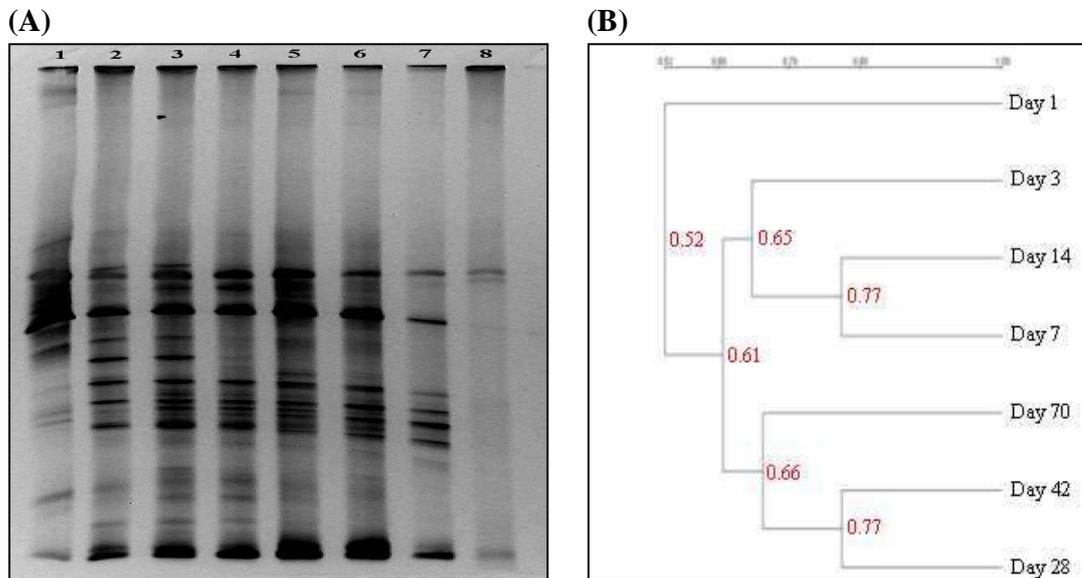


Figure 2. (A) PCR-DGGE analysis of 16S rDNA sequence fragments obtained from the Er-Jen River mixed culture incubated with 0.01  $\mu\text{g/mL}$  BDE-154 during 70 days of incubation periods. Lanes 1 to 7 represent the bacterial community structure in an anaerobic mixed culture for Days 1, 3, 7, 14, 28, 42, and 70, respectively, and lane 8 was used as the marker to compare the relative position to other lanes. (B) Cluster analysis of microbial community structures incubated with BDE-154 by unweighted pairwise grouping method with mathematical averages (UPGMA).

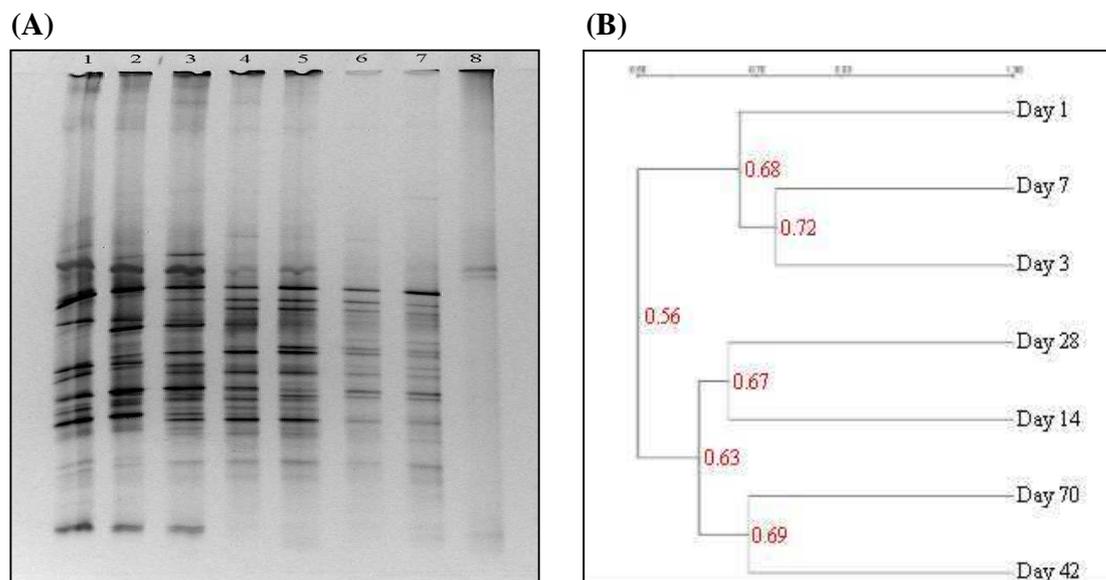


Figure 3. (A) PCR-DGGE analysis of 16S rDNA sequence fragments obtained from the Nan-Kan River mixed culture incubated with 0.01  $\mu\text{g/mL}$  BDE-154 during 70 days of incubation periods. Lanes 1 to 7 represent the bacterial community structure in an anaerobic mixed culture for Days 1, 3, 7, 14, 28, 42, and 70, respectively, and lane 8 was used as the marker to compare the relative position to other lanes. (B) Cluster analysis of microbial community structures incubated with BDE-154 by unweighted pairwise grouping method with mathematical averages (UPGMA).

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