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Short communication

Anaerobic degradation of tetrabromobisphenol-A in river sediment

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ABSTRACT

The contamination of the environment with tetrabromobisphenol-A (TBBPA), an endocrine disruptor, is a concern. We examined anaerobic degradation of TBBPA in sediment samples from the Erren River in southern Taiwan. Anaerobic degradation of TBBPA was enhanced with the addition of humic acid (0.5 g L^{-1}), sodium chloride (1 mass/vol%), zero-valent iron (1 g L^{-1}), vitamin B_{12} (0.025 mg L^{-1}), brij 30 (55 μM), brij 35 (91 μM), rhamnolipid (130 mg L^{-1}), or surfactin (43 mg L^{-1}) but was inhibited by the addition of acetate (30 mM), lactate (20 mM), or pyruvate (20 mM). Sulfate-reducing bacteria, methanogen, and eubacteria are involved in the anaerobic degradation of TBBPA; sulfate-reducing bacteria is a major component of the sediment.

1. Introduction

Tetrabromobisphenol-A (TBBPA) is a flame retardant used in the production of many plastic polymers and electronic circuit boards (de Wit, 2002). It has been found in environmental samples and in human plasma and may have a toxic effect (Darnerud, 2003). Microbial degradation is the primary mechanism for removal of organic toxic compounds in sediment (Yu et al., 2012). Reductive dehalogenation (e.g., substitution of Br or Cl by a hydrogen atom) is an important mechanism (Fetzner, 1998; Davis et al., 2005). Halogenated compounds are electron acceptors in respiratory or cometabolic processes. Environmental factors such as temperature, pH, salinity, plant species selection and the availability of organic carbon and/or inhibiting substances influence the growth and activity of microbes, and the manipulation of some has been investigated (Faulwetter et al., 2009). The addition of sodium chloride, humic acid, zero-valent iron, surfactants, electron donors or electron acceptors influences the anaerobic degradation of organic toxic chemicals in sediment (Chang et al., 2009). However, little is known about the effects of factors on the anaerobic degradation of TBBPA in river sediment.

The climatic characteristics of subtropical regions foster diverse microbial communities (Chang et al., 2009). Several techniques used to study microbial communities in environmental samples include phospholipid fatty acid analysis (e.g., Langer and Rinklebe, 2009, 2011) and molecular-biological methods (Faulwetter et al., 2009). Many studies have used PCR-denaturing gradient gel electrophoresis (PCR-DGGE) to examine the effect of pollutants on microbial communities in sediment (Castle et al., 2006; Chang et al., 2009). Little information is available on the effect of TBBPA anaerobic degradation on the change in bacterial communities in river sediment. We aimed to examine the effect of factors on the anaerobic degradation of TBBPA in the sediment of Erren River, one of the most heavily contaminated rivers in southern Taiwan, and changes in the microbial community in the sediment.

2. Materials and methods

2.1. Chemicals

TBBPA (98.0%) analytical standard was from Aldrich Chemical Co. (Milwaukee, WI). Solvents were from Mallinckrodt, Inc. (Paris, KY). The biosurfactants used in this study were surfactin and rhamnolipid as described by Yeh et al. (2005) and Wei et al. (2005), respectively. All other chemicals were from Sigma Chemical Co. (St. Louis, MO). The log Kow for TBBPA, tribromobisphenol-A, dibromobisphenol-A and monobromobisphenol-A was 4.5, 2.1, 2.1, and 3.7, respectively.

2.2. Sampling and medium

We collected sediment samples from Erren River in July 2008. The three sampling sites, A (22.55°10.98′N, 120.11°3.51′E), B (22.55°14.32′N, 120.11°12.9′E) and C (22.54°51.13′N, 120.13°27.01′E) are well known from previous studies of aquatic pollutants (Yuan et al., 2011). The sediments (>15 cm) were collected by use of a soil core during low tide. Adaptation involved adding 50 μg g^{-1} TBBPA to 500 g sediment at 14-d intervals under

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static incubation at 30 °C without light for 6 months. Here, sediment refers to TBBPA-adapted sediment. For sites A, B, and C, the TBBPA concentration in sediment was 260, 450, and 38.1 ng g^{-1}, respectively. In our previous study of the degradation of TCBPA in sediment from the 3 sampling sites, the anaerobic degradation rate of TCBPA was higher in site B than other sites (Yuan et al., 2011). Therefore, we used the sediment sample from site B in the following experiments.

The experimental medium consisted of (in g L^{-1}) 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, 0.2; and resazurin, 0.001. The pH was adjusted to 7.0 after autoclaving; 0.9 mM titanium citrate was added as a reducing reagent.

2.3. Experimental design

All experiments involved use of 125-mL serum bottles containing 45 mL medium, 5 g river sediment and 50 μg g^{-1} TBBPA. We measured the effect of the following factors on anaerobic degradation in sediment collected from site B: sodium chloride (1 mass/vol%); humic acid (0.5 g L^{-1}); zero-valent iron (1 g L^{-1}); vitamin B_{12} (0.025 mg L^{-1}); surfactants, brij 30, brij 35, rhamnolipid, and surfactin at 1 critical micelle concentration (CMC; the CMC values were 55 μM, 91 μM, 130 mg L^{-1}, and 43 mg L^{-1}, respectively); the electron donor sodium acetate (30 mM), sodium lactate (20 mM) or sodium pyruvate (20 mM); sodium hydrogen carbonate (20 mM), sodium sulfate (20 mM), or sodium nitrate (20 mM) for methanogenic, sulfate-reducing or nitrate-reducing conditions, respectively; and microbial inhibitors (50 mM BESA, 50 mM vancomycin, 50 mM sodium molybdate-2-hydrate). The concentrations of these factors were from previous studies (Yuan et al., 2011). Inoculated control samples (without sodium hydrogen carbonate, sodium sulfate, or sodium nitrate), considered non-sterile 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 3 d.

All experiments were conducted in an anaerobic glove box (Forma Scientific, USA) filled with N_2 (85%), H_2 (10%), and CO_2 (5%). The 125-mL serum bottles were capped with butyl rubber stoppers, wrapped in aluminum foil to prevent photolysis, and incubated without shaking at 30 °C in the dark. Each treatment was applied in triplicate. Samples were collected every 7 d to measure residual TBBPA, then underwent PCR-DGGE. Methane was sampled from the headspace of the serum bottles.

2.4. Analytical methods

TBBPA was extracted twice from sediment samples by use of dichloromethane, then again 20 min at 30 °C with use of a Branson 5200 ultrasonic cleaner. Extracts were analyzed by use of gas chromatography (Hewlett Packard 6890) equipped with an electron capture detector and HP-5 capillary column. The initial column temperature was set at 250 °C, increased by 2 °C min^{-1} to 260 °C, and then increased by 10 °C min^{-1} to 280 °C. Injector and detector temperatures were set at 300 and 320 °C, respectively. The recovery percentage and detection limit for TBBPA was 91.5% and 0.02 mg L^{-1}, respectively. The anaerobic degradation products of TBBPA and methane levels were analyzed as we described previously (Chang et al., 2011).

2.5. DNA extraction and PCR-DGGE analysis

DNA was extracted from sediment samples using the Mo Bio PowerSoil DNA kit (Carlsbad, CA). The primer sequences for DGGE analysis were for FGC968 (*Escherichia coli* position 968–983), 5′-GCCCGGGGCGCGCCCGGGCGGGGCGGGGGCACGGGGG GAACGCGAAGAACCTTAC-3′, and R1401 (*E. coli* position 1401–1385), 5′-CGGTG TGTACAAGACCC-3′ (Chang et al., 2009). DGGE involved use of a D-gene and D-code system (Bio-Rad Laboratories, CA, USA). Electrophoresis involved 1× TAE buffer at voltage 60 V and temperature 60 °C for 16 h. The bands of interest were excised and soaked in elution buffer overnight at 37 °C. The DNA was re-amplified with the primers for FGC968 and R1401. The re-amplified products were again purified and sequenced with use of an ABI-Prism automatic sequencer.

Table 1
Effect of various substrates on TBBPA anaerobic degradation rate constant (k_1) and half-life ($t_{1/2}$) in sediment of Erren River, southern Taiwan.

Treatment	k_1 (d^{-1})	$t_{1/2}$ (d)	r^a
Inoculated control[b]	0.0417	16.6	0.98
Humic acid (0.5 g L^{-1})	0.0491	14.1	0.94
Sodium chloride (1%)	0.0502	13.8	0.95
Zero-valent iron (1 g L^{-1})	0.0541	12.8	0.92
Vitamin B_{12} (0.025 mg L^{-1})	0.0529	13.1	0.89
Rhamnolipid (130 mg L^{-1})	0.0686	10.1	0.94
Surfactin (43 mg L^{-1})	0.0582	11.9	0.92
Brij 30 (55 μM)	0.0510	13.6	0.94
Brij 35 (91 μM)	0.0554	12.5	0.95
Sodium acetate (30 mM)	0.0297	23.3	0.95
Sodium lactate (20 mM)	0.0276	25.1	0.96
Sodium pyruvate (20 mM)	0.0340	20.4	0.97
Sodium hydrogen carbonate (20 mM)	0.0582	11.9	0.89
Sodium sulfate (20 mM)	0.0679	10.2	0.95
Sodium nitrate (20 mM)	0.0525	13.2	0.96

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, TBBPA 50 μg g^{-1}.

2.6. Data analysis

The TBBPA biodegradation data collected for this study fit well with first-order kinetic equations: $S = S_0 \exp(-k_1 t)$, $t_{1/2} = \ln 2/k_1$, where t is time, S_0 is the initial substrate concentration, S is the substrate concentration at time t, and k_1 is the degradation rate constant. Principal component analysis (PCA) was used to examine the DGGE community structure. Statistical analysis involved use of SPSS v10.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Effects of various factors on the anaerobic degradation of TBBPA in the sediment

The TBBPA concentrations in the sterile controls were first examined at the end of the 35-d incubation. The proportion of TBBPA ranged from 92.1% to 95.3%. Therefore, the TBBPA degradation in the following experiments was due to microbial action. The degradation rate and half-life of TBBPA were 0.0417 d^{-1} and 16.6 d, respectively (inoculated control) (Table 1). As compared with the inoculated control, the addition of humic acid, sodium chloride, zero-valent iron, and vitamin B_{12} enhanced the degradation rate of TBBPA by 17.7%, 20.4%, 29.7%, and 26.9%, respectively. Humic acid showed increased showed a higher reducing capacity in deeper layers, probably because of reduction by humic-acid-reducing microorganisms (Kappler et al., 2004). TBBPA degradation was enhanced by the addition of sodium chloride (1%). The types of bacteria colonized in sediment and their biodegradation potential are affected by salinity (Tam et al., 2002). The high salinity of sample sediment may significantly inhibit the degradation rate (Yu et al., 2012). The addition of zero-valent iron enhanced the degradation of TBBPA. Zero-valent iron can be an electron donor and can