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16S ribosomal DNA clone libraries to reveal bacterial diversity in anaerobic reactor-degraded tetrabromobisphenol A

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ABSTRACT

Microorganisms able to rapidly degrade tetrabromobisphenol A (TBBPA) were domesticated in an anaerobic reactor and added to gradually increased concentrations of TBBPA. After 240 days of domestication, the degradation rate reached 96.0% in cultivated batch experiments lasting 20 days. The optimum cultivating temperature and pH were 30 °C and 7.0. The bacterial community's composition and diversity in the reactor was studied by comparative analysis with 16S ribosomal DNA clone libraries. Amplified rDNA restriction analysis of 200 clones from the library indicate that the rDNA richness was high (Coverage *C* 99.5%) and that evenness was not high (Shannon–Weaver index 2.42). Phylogenetic analysis of 63 bacterial sequences from the reactor libraries demonstrated the presence of Betaproteobacteria (33.1%), Gammaproteobacteria (18.7%), Bacteroidetes (13.9%), Firmicutes (11.4%), Chloroflexi (3.6%), Actinobacteria (0.6%), the candidate division TM7 (4.2%) and other unknown, uncultured bacterial groups (14.5%). Comamonas, Achromobacter, Pseudomonas and Flavobacterium were the dominant types.

1. Introduction

Tetrabromobisphenol A (4,4A-isopropylidenebis(2,6-dibromophenol), TBBPA) is one of the 75 different brominated flame retardants (BFRs) and the largest BFR in terms of production (www.bsef.com). TBBPA is an additive that can chemically bind to synthetic matrices such as plastics, textiles, electronic circuitry and other materials to prevent fires (Wit, 2002). It is also used in high tech devices, such as wind turbines and defence systems (www.bsef.com). TBBPA has been detected in a wide range of materials, such as soil, plants, sediments, animals and even in house dust and clothes dryer lint (Stapleton et al., 2005; Li et al, 2011). Although TBBPA is not acutely toxic, some research showed that its release in the environment can cause adverse effects. In recent years, the hazardous effects of TBBPA on the environment and human health have increasingly attracted widespread attention. Boecker et al. (2001) found that TBBPA is toxic to primary hepatocytes, most likely by destroying mitochondria. TBBPA is also highly immunotoxic in cultures, which is demonstrated by its ability specifically to inhibit the expression of CD25 at concentrations as low as 3 μM (Pullen et al., 2003). Kitamura et al. (2005) found that TBBPA exhibited significant thyroid hormonal activity on the rat pituitary cell line GH_3. Tada et al. (2006) showed that TBBPA caused higher serum concentrations of total cholesterol and liver weights in treated dams and offspring than in the control mice. Histological findings in treated dams and offspring showed the increase of focal necrosis of hepatocytes and inflammatory cell infiltration in the liver as well as an increase in the dilation or atrophy of renal tubules and cysts in the kidney. The interpretation of the results depicted an interference of thyroid and vitamin A homeostasis in zebrafish exposed to TBBPA. TBBPA also elicited responses indicating the onset of oxidative stress and general stress responses. Additionally, numerous differentially expressed transcripts could be associated with defence mechanisms or correspond to metabolising enzymes (De Wit et al., 2008). Some studies indicated that TBBPA disrupts thyroid hormone-dependent hypothalamic set-points (Decherf et al., 2010). Therefore, it is important to develop strategies to decrease and eliminate TBBPA residues in the environment.

Microbial degradation involves the use of living microorganisms to detoxify and degrade hazardous materials; it is generally considered to be an effective and safe way to remove contaminants from the environment. It has been widely applied in degrading pollutants such as pesticides (Chanika et al., 2011), plastic (Nakajima et al., 1999), petroleum (Zhang et al., 2010) and surface-active agents (Fuchedzhieva et al., 2008).

Microbes also can degrade TBBPA. TBBPA partially breaks down in both aerobic and anaerobic conditions (WHO, 1995). Ronen and Abeliovich (2000) found that it may be possible to use a sequential anaerobic–aerobic process to completely degrade TBBPA in contaminated soils, reporting an 80% decrease in the TBBPA concentration. Brenner et al. (2006) reported that various organic compounds were used as potential electron donors to enhance the growth of halorespiring bacteria that would debrominate the TBBPA and

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make it available for further aerobic mineralisation. However, little TBBPA biodegradation has been observed under such conditions (2006). George and Häggblom (2008) found that microorganisms can cause *O*-methylation of the flame retardant TBBPA. Nyholm et al. (2010) found that TBBPA is more difficult to degrade than other BRFs in anaerobic soil. In most of the TBBPA degradation studies, the degradation rate and the fate of TBBPA was emphasised. Little attention has been paid to the formation of the microbial population which degrades TBBPA. Appels et al. (2011) indicated that a further development and optimization of anaerobic digestion requires additional fundamental knowledge on the occurring mechanisms on microscale, which should in turn be linked to the macroscale system performance and behavior. Information on the microbial community composition will indeed provide crucial information for the further development and optimization of anaerobic digestion systems.

The aim of this study is to investigate the microorganisms that offer the most efficient microbiological degradation of TBBPA by domestication. The sewage sludge used for enrichment was collected from the anaerobic tanks of wastewater treatment facilities in the Zhongkai industrial park; because the treatment facilities have processed TBBPA in its wastewater for several years, it is possible to hypothesise the presence of microorganisms able to metabolise xenobiotics (Brusa et al., 2001). Laboratory reactor systems based on the conventional sewage sludge process were operated for 240 days to develop a biological process for the degradation of TBBPA. As the domestication time increased, the degradation rate of TBBPA gradually increased. To study the composition of the bacterial community, the 16S ribosomal DNA clone libraries were set up for comparison after the 240-day domestication period. Furthermore, a series of studies was carried out in the present paper, including the transformation of degradation rate in different time groups as well as the influence of pH and temperature on the microorganisms.

2. Methods

2.1. Chemicals and media

The tetrabromobisphenol A (4,4A-isopropylidenebis(2,6-dibromophenol), 99% purity, Mw 543.9 g/mol, CAS number 79-94-7) used in this study was purchased from Sigma Chemical Co. (St Louis, MO, USA). The TBBPA was dissolved in acetone as stock solution (100,000 mg/L), which was sterilised by membrane filtration, and then rationed into the medium to obtain the desired concentrations. All other chemicals and solvents used (acetone and methanol) were of HPLC (high performance liquid chromatography) grade and purchased from Merck, Germany. High quality water was obtained from a Nanopure UV deionisation system, Barnstead/Thermolyne Co. (Dubuque, IA, USA). The sewage sludge was collected from the anaerobic tanks of wastewater treatment facilities in the Zhongkai industrial park in Huizhou, Guangdong province, China.

2.2. Medium composition and enrichment culture

For the start-up of the anaerobic reactor, a synthetic medium was used. The medium contained with the following composition: NH_4Cl 2600 mg/L, $MgCl_2{\cdot}7H_2O$ 1054 mg/L, K_2HPO_4 752 mg/L, $CaCl_2$ 520 mg/L and trace solution 1 mL/L. The carbon sources used in this experiment were the following: $NaHCO_3$ 800 mg/L, $Na_3C_6H_5O_7{\cdot}2H_2O$ 680 mg/L and $C_6H_{12}O_6$ 1000 mg/L. The composition of the trace elements were the following: $NiCl_2{\cdot}7H_2O$ 800 mg/L, $FeCl_3{\cdot}6H_2O$ 1250 mg/L, $ZnCl_2$ 130 mg/L, $CoCl_3{\cdot}6H_2O$ 110 mg/L, $MnCl_2{\cdot}4H_2O$ 220 mg/L, $Na_2BO_3{\cdot}10H_2O$ 44 mg/L, $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$ 80 mg/L and $CuCl_2{\cdot}2H_2O$ 65 mg/L. An initial concentration of 0.1 mg/L of TBBPA was added to the medium, and then the concentration was gradually increased from 0.2 to 0.5 mg/L. The pH of the mixture was adjusted to 7.0 ± 0.2 before seeding.

The anaerobic TBBPA-utilising sludge was enriched in a 3.0 L water-jacketed chemostat reactor at 30 °C. The schematic overview used for the domestication was shown in Fig. 1. The reactor was applied in continuous mode. Hydraulic retention time (HRT) was kept constant for 9 days and the pH was maintained at 6.8–7.2 throughout the study. The reactor was seeded with the anaerobic sludge to treat the medium containing the TBBPA.

2.3. Batch experiments

Three series of batch experiments on the domesticated microorganisms degrading the TBBPA were conducted in 100 mL glass serum vials. The first series was to investigate the degradation rate of TBBPA depending on different periods of domestication. Five batches were run adding 0.5 mg/L of TBBPA after domestication times of 0, 60, 120, 180, 240 d The second series was to investigate the effect of temperature at 25, 30, 35 and 40 °C, respectively. The third series was to investigate the effect of pH value at 5.0, 6.0, 7.0 and 8.0, respectively. The formulation of the nutrient and trace elements for the feed solution followed the formulation used in several previous studies (Jia et al., 1996). The nutrient stock solution was sparged with nitrogen to strip off any dissolved oxygen.

The TBBPA-degrading sludge from the anaerobic reactor was used to seed each batch reactor. The batch reactor contained 60 mL mixed liquor that contained the 100 mg/L VSS. After the addition of TBBPA and adjustment of the pH, each vial was

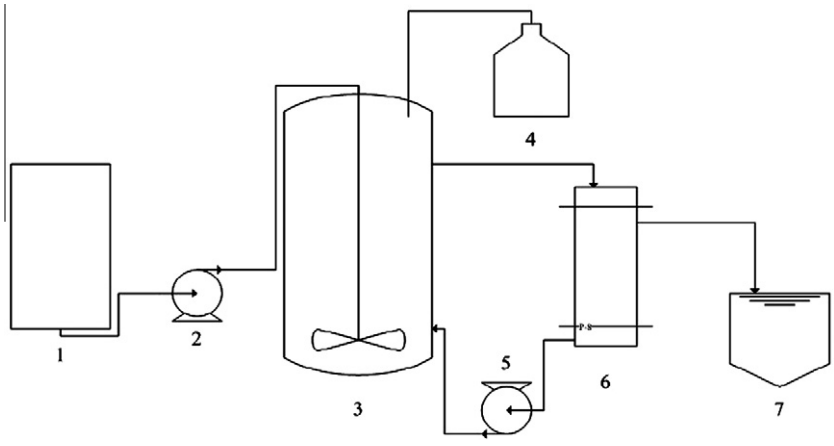

Fig. 1. Schematic diagram of the reactor. 1 container for medium; 2 pump; 3 reactor; 4 Water seal tube; 5 backflow; 6 Sedimentation; 7 wastewater container.