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Concurrent degradation of tetrabromobisphenol A by *Ochrobactrum* sp. T under aerobic condition and estrogenic transition during these processes

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ABSTRACT

The effect of concurrent degradation of tetrabromobisphenol A (TBBPA) by the strain *Ochrobactrum* sp. T under aerobic condition was investigated. The results demonstrated that four extra energy source-addition systems still followed pseudo-first order kinetics. The addition of ethanol or glucose could promote the biodegradation ability of *Ochrobactrum* sp. T to TBBPA, and 90.1 percent and 77.5 percent of TBBPA (5 mg L^{-1}) could be removed with corresponding TBBPA half-lives of 26 and 36 h, respectively, after 96 h reaction. Comparatively, the degradation efficiency of the sole TBBPA system was only 72.9 percent under the same condition. In contrast, two other co-substrates 2,4,6-tribromophenol (TBP) and bisphenol A (BPA) showed a negative effect on the TBBPA biodegradation, and the degradation efficiencies of TBBPA were achieved as 44.7 percent and 67.4 percent, respectively. For the TBBPA+TBP system, the competitive inhibition for the TBBPA debromination was less than the inhibition of the toxicity to the bacterium. While for the TBBPA+BPA system, the degradation of TBBPA could be promoted at the beginning of the reaction, and was then inhibited slightly with further prolonging of reaction time. This is probably due to the substrates being oxidized, and BPA can consume partial oxygen and provide the electrons during the concurrent biodegradation process. In addition, although higher estrogenic activity could be detected for the debrominated intermediates in TBBPA co-degradation process than the original TBBPA, the estrogenicity of the whole system still decreased finally after 96 h degradation.

1. Introduction

Tetrabromobisphenol A (TBBPA) is one of the most widely used brominated flame retardants (BFRs) to effectively reduce the flammability of the final manufactured products around the world (de Wit, 2002). However, it can be emitted and pollute the environment via the use of all sorts of electrical products and the dismantlement of electrical wastes (Li et al., 2008; Ni et al., 2010). What is more, possible toxic effects of TBBPA such as endocrine disruption and acute toxicity to some aquatics have already been reported (Darnerud, 2003; Kuiper et al., 2007; Lilienthal et al., 2008; Kling and Forlin, 2009; Strain et al., 2009). Therefore, the degradation of TBBPA including phototransformation (Eriksson et al., 2004; Horikoshi et al., 2008) and biodegradation (Ronen and Abeliovich, 2000; Voordeckers et al., 2002; Gerecke et al., 2006) was carried out. Previous reports about the phototransformation were mainly focused on debromination, mineralization and identification of the degradation intermediates in aqueous solutions under UV-irradiation. And almost all of the biodegradations were limited by the different conditions between reductive debromination and oxidative mineralization. In our previous study, one bacterial strain *Ochrobactrum* sp. T with the ability to simultaneously debrominate and mineralize TBBPA was isolated (An et al., 2011), which can effectively solve the problem above. Dehalogenation, a process through which dehalogenation bacteria utilize halogenated compounds as election acceptors, can

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be enhanced or inhibited by presented other electron donors and acceptors (de Wit, 2002). So the study of the debromination intermediates which may serve as electron acceptors is also needed.

To the best of our knowledge, additional electron donor and acceptor are essential to the organics metabolism, especially the redox related process (Hong et al., 2007a, 2007b). Nevertheless, most studies on TBBPA biodegradation were mainly focused on the debromination kinetics (Arbeli and Ronen, 2003). Only one work reported the effect of the electron transfer system on the TBBPA anaerobic debromination (Arbeli et al., 2006), which suggested that the substrate TBBPA served as the electron acceptor, and ethanol was utilized as carbon and energy sources. This situation may not be proper for the aerobic degradation of TBBPA. According to our previous study (An et al., 2011), TBBPA aerobic degradation included two simultaneous processes of reduction and oxidation; oxygen is used to oxidize TBBPA first and the other oxidative intermediates consume the oxygen which is supposed to be a competitive electron acceptor of TBBPA, thus providing the reducing power and ensuring that reductive debromination of TBBPA occurs easily. So, to get a complete insight into the aerobic degradation process of TBBPA, the study of concurrent additional energy sources including TBBPA degradation intermediates in the degradation system is necessary.

As a potential toxic compound, the estrogenic activity of TBBPA is still under intensive debate. For instance, some previous studies reported that TBBPA probably possessed the estrogenic activity, showing inhibition of estrogen sulfotransferase in vitro (Hamers et al., 2006) or enhanced proliferation in an estrogen-dependent cell line (Kitamura et al., 2002). While some other studies indicated that the estrogen-like effect of TBBPA was insignificant (Samuelsen et al., 2001; Dorosh et al., 2011), its degradation intermediates might possess higher potential estrogenicity (Samuelsen et al., 2001; Uhnáková et al., 2011). Therefore, it is essential to evaluate the safety of the estrogenic transition during the TBBPA biodegradation process, and the estrogenic activity investigation of TBBPA concurrent biodegradation could provide more information for other brominated analogs of TBBPA.

To compare with the anaerobic debromination process, in this work, four additional energy sources were selected as the electron donors as well as carbon sources to investigate their effect on TBBPA biodegradation kinetics under aerobic condition. Furthermore, 2,4,6-tribromophenol (TBP) and bisphenol A (BPA) were employed as the concurrent substrate to determine the structure analog effect on TBBPA biodegradation, and also to confirm the mechanism of TBBPA aerobic degradation proposed in our previous work. The estrogenic transition was also investigated to measure the toxicity during the TBBPA concurrent biodegradation. As far as we know, this is the first study of TBBPA concurrent degradation carried out under aerobic condition.

2. Materials and methods

2.1. Chemicals and growth medium

TBBPA (purity: 97 percent) and TBP (99 percent) were purchased from Sigma-Aldrich. BPA (97 percent) was offered by Acros Organics (New Jersey, USA). All other chemicals (analytical grade reagents with more than 99 percent purity) used for the preparation of aqueous medium and biochemical experiments were obtained from Guangzhou Chemical Reagent Co., Inc., China. Our previous isolated bacterial strain *Ochrobactrum* sp. T (HM543185) (An et al., 2011) was employed for the concurrent degradation of TBBPA, and the growth medium for cell enrichment and mineral medium (MM) for degradation were all prepared according to Eriksson et al. (2004) and An et al. (2011).

2.2. Concurrent degradation of TBBPA

The strain *Ochrobactrum* sp. T was pre-cultured in the Luria-Bertani (LB) medium for 15 h, collected by centrifugation at 6000*g* for 3 min, and washed with the MM twice. The biodegradation experiments were performed by inoculating 25 mL of harvested cultures into 100 mL MM (in 250 mL shake flasks) containing 5 mg L^{-1} TBBPA at 35 °C, pH 7.0, and 200 rpm for 96 h. The following compounds (g L^{-1}) were used as the carbon sources and electron donors: ethanol 1.0, acetate 1.66, pyruvate 1.0 and glucose 0.5. The pH values of acetate and the pyruvate addition system were adjusted to about 7.0 by 1 mol L^{-1} HCl and 1 mol L^{-1} NaOH, respectively. The growth curve of *Ochrobactrum* sp. T in each energy source was determined by the optical density at 600 nm (OD_{600}) which was measured using a spectrophotometer after 2–3 s vigorous vortexing. For concurrent substrate degradation of TBBPA, TBP and BPA were separately tested at 5.0 mg L^{-1} (Arbeli et al., 2006). All experiments were conducted in triplicate.

2.3. Estrogenic activity assay

The transition of estrogenic activity was detected during the TBBPA biodegradation process as well as during the concurrent degradation process (TBBPA+TBP and TBBPA+BPA, respectively) by measuring the β-galactosidase activity of a recombination yeast cell. The experiments steps and calculation method were all similar to those of our previous study (Li et al., 2012). 17β-estradiol (E2) was still used as a standard control, and ten E2 concentrations 10^{-11}, 2.5×10^{-11}, 5×10^{-11}, 10^{-10}, 2.5×10^{-10}, 5×10^{-10}, 10^{-9}, 10^{-8}, 5×10^{-8}, and 10^{-7} mol L^{-1} were chosen to establish the dose–response curve.

2.4. Analytical methods

Concentrations of TBBPA, BPA and TBP were all measured by high performance liquid chromatography (HPLC) (Agilent 1200 HPLC) equipped with a DAD detector. The detailed detection conditions were similar to those of our previous work (An et al., 2011; Zu et al., 2012). Wavelengths of 230, 280 and 286 nm were used to detect TBBPA, BPA and TBP, respectively. The eluent was a mixed solution of 80 percent methanol, eighteen percent ultra-pure water, and two percent glacial acetic acid at a flow rate of 1 mL min^{-1}. The retention time of TBBPA was 9.644 min, while TBP and BPA showed retention time of 8.394 and 3.530 min, respectively.

3. Results

3.1. Effect of energy sources on TBBPA biodegradation

It is well known that most of the redox power needed for the xenobiotics metabolized by microorganisms is offered through the electron transfer chain. Therefore, four energy sources such as ethanol, acetate, pyruvate and glucose were employed as the additional electron donors as well as carbon sources during the *Ochrobactrum* sp. T degradation of TBBPA process (Fig. 1). Results showed that after 96 h reaction, the highest TBBPA degradation efficiency of 90.1 percent was achieved in the ethanol-addition system, and the value of 77.5 percent in the glucose-addition system. Both of them are higher than the control sample (TBBPA only) value of 72.9 percent. In contrast, lower TBBPA biodegradation efficiencies of 57.9 percent and 48.7 percent were obtained in acetate- and pyruvate-addition systems, respectively. It can also be found that the biodegradation efficiency of TBBPA increased with degradation time in each energy source-addition system within

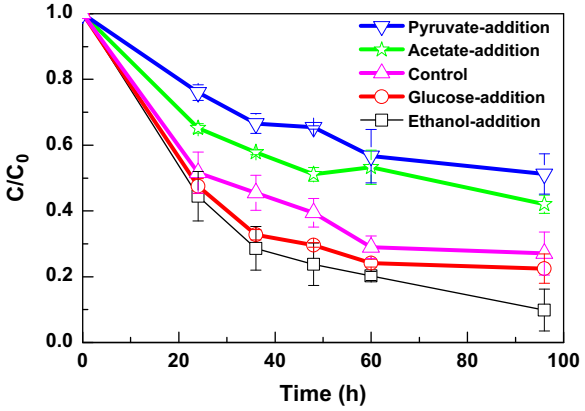

Fig. 1. Biodegradation efficiency of TBBPA associated with different additional energy sources.