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Aerobic biotransformation of decabromodiphenyl ether (PBDE-209) by *Pseudomonas aeruginosa*

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HIGHLIGHTS

- Certain amount of co-metabolic substrates promoted the biodegradation of PBDE-209.
- Degradation was stimulated at low level of Cd^{2+} while inhibited at higher content.
- Br^- was produced during degradation of PBDE-209.
- The lower brominated products of PBDE-209 transformation were presented.
- The mechanism of PBDE-209 degradation by *P. aeruginosa* was put forward.

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ABSTRACT

Aerobic biodegradation of decabromodiphenyl ether (PBDE-209) by *Pseudomonas aeruginosa* under the influence of co-metabolic substrates and heavy metal cadmium ion was studied, The results showed that certain amount of co-metabolic substrates, such as glucose, sucrose, lactose, starch, and beef extract, would promote the biodegradation of PBDE-209, among which glucose most favorably accelerated PBDE-209 degradation by about 36% within 5 d. The highest degradation efficiency was reached at the ratio of PBDE-209 and glucose 1:5 while excessive carbon source would actually hamper the degradation efficiency. Exploration of influences of cadmium ion on PBDE-209 biodegradation indicated that degradation efficiency was stimulated at low concentrations of Cd^{2+} (0.5–2 mg L^{-1}) while inhibited at higher levels (5–10 mg L^{-1}), inferring that the heavy metals of different concentrations possessed mixed reactions on PBDE-209 bioremoval. Bromine ion was produced during the biotransformation process and its concentration had a good negative correlation with the residues of PBDE-209. Two nonabromodiphenyl ethers (PBDE-208, PBDE-207), four octabromodiphenyl ethers (PBDE-203, PBDE-202, PBDE-197, PBDE-196) and one heptabromodiphenyl ethers (PBDE-183) were formed with the decomposition of PBDE-209, demonstrating that the main aerobic transformation mechanism of PBDE-209 was debromination.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of synthetic organic compounds which have been widely used in a variety of industrial products including flame retardants, plastics, electronic appliances, furniture and vehicles (Song et al., 2005; Leung et al., 2007). There are 209 congeners based on the number and position of bromine atoms. In these PBDEs congeners, three major PBDEs, deca-, octa-, and pentabromodiphenyl ethers, are most globally used. Although penta- and octabromodiphenyl ethers have been banned in Europe and the United States since 2006 because of their toxicity, the fully brominated decabromodiphenyl ethers (PBDE-209) is still used in parts of the world and its level in the environment is increasing in recent years.

As a result of massive industrial use, PBDEs have been detected in a wide variety of environmental media such as air (Luo et al., 2009), soil (Leung et al., 2007), sediment and sewage sludge (Song et al., 2005) as well as biological samples including birds (Luo et al., 2009), fish (Hites et al., 2004), human blood serum and breast milk (Miller et al., 2009). PBDEs are found even in the Arctic biosphere (Rotander et al., 2012). In addition to their wide distribution in the environment, PBDEs are also persistent, and can undergo long distance transportation and bioaccumulation in organisms. All of

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these pose detrimental effect on wildlife and human health. Therefore, there is an urgent need to establish effective technological approaches to deal with PBDEs in the environment.

Within the past two decades, a lot of methods have been tried to degrade PBDEs, including photochemical degradation (Mas et al., 2008), hydrothermal treatment (Nose et al., 2007), reduction by zero valent iron (Keum and Li, 2005) and microbial anaerobic or aerobic degradation. Recent work on anaerobic biotransformation has demonstrated that PBDEs were debrominated to less brominated congeners by a variety of anaerobic bacteria. However, this process requires a relatively long period of time, from several months to over a year. Gerecke et al. (2006) reported that degradation of PBDE-209 into lower brominated diphenyl ether congeners in anaerobic mesophilic digester sludge took over 238 d and the degradation rate was only 50%.

Compared with anaerobic biodegradation, aerobic microorganisms may rapidly mineralize a lot of organic compounds. However, very little is known about aerobic biodegradation and biotransformation of PBDEs at present. In the early 1990s, Schmidt et al. (1992) demonstrated the feasibility of aerobic transformation and degradation of PBDEs using two isolated strains *Sphingomonas* sp. SS3 and SS33. *Sphingomonas* sp. SS3 was capable of transforming 4-bromodiphenyl ether and using monobromodiphenyl ethers to grow up, while *Sphingomonas* sp. SS33 had the capability of breaking down 4,4′-dibromodiphenyl ether but was not able to use it as nutrient. In 1999, Hundt et al. (1999) showed that the white-rot fungi possessed the capability of converting 4-bromodiphenyl ether to its hydroxylated analog. Then, Kim et al. (2007) reported that *Sphingomonas* sp. PH-07 could aerobically break down a number of lower- bromodiphenyl ethers congeners, such as mono-, di-, and tribromodiphenyl ethers. In recent years, Robrock et al. (2008, 2009) investigated four strains which could transform mono- through hexabromodiphenyl ethers at ppb level. In these studies, two PCB-degrading strains, *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400 transformed all of the mono- and pentabromodiphenyl ethers and strain LB400 transformed one of the hexabromodiphenyl ethers, while *Rhodococcus* sp. RR1 and ether-degrading strain *Pseudonocardia dioxanivorans* CB1190 were only able to transform a few brominated mono- and dibromodiphenyl ether congeners. In 2011, Deng et al. (2011) isolated an indigenous strain DB-1 from PBDEs polluted sediment which could degrade PBDE-209 to lower-bromodiphenyl ethers using lactate, pyruvate and acetate as carbon sources under aerobic condition.

To our knowledge, studies on the aerobic biodegradation of PBDE-209 are rather few, and the main decomposition mechanism has not been demonstrated yet. In this paper, we described the transformation of PBDE-209 into nona-, octa-, and heptabromodiphenyl ethers by using aerobic bacterium *Pseudomonas aeruginosa*. The release of bromide ions during the process of degradation were determined by utilizing ion chromatography. Taking into account that heavy metals were most abundant pollutants co-existed with PBDEs in environment, we chose cadmium ion, a frequently detected heavy metal in e-waste dismantling site, as model of heavy metal pollutant to examine its influence on the biodegradation of PBDE-209. The results obtained from this investigation are expected to provide valuable information on the aerobic biodegradation and biotransformation of PBDEs, especially in the presence of heavy metals.

2. Materials and methods

2.1. Strain and culture medium

P. aeruginosa, a potential aerobic strain for PBDE-209 biodegradation used in this work, was isolated from an e-waste dismantling area in Guiyu town of Guangdong province, China by our lab members.

Two kinds of culture media were used in this study.

Nutrient medium was used for strain culture, and its composition was as follows (g L^{-1}): glucose 5, peptone 2, and yeast powder 1.

Mineral salt medium (MSM) was used as the degradation medium, and its composition consisted of (g L^{-1}): NH_4NO_3 1, KH_2PO_4 1.5, K_2HPO_4 3, and 2 mL L^{-1} trace elements (g L^{-1}: $MgSO_4$ 4, $CuSO_4$ 1, $MnSO_4$, $FeSO_4{\cdot}7H_2O$ 1, $CaCl_2$ 1), pH was adjusted to 7.5.

All the media were previously sterilized in an autoclave at 121 °C for 30 min.

2.2. Chemicals

PBDE-209 with a purity of >98% was purchased from Sigma–Aldrich (St. Louis, MO, USA) and the standards of heptabromodiphenyl ethers (PBDE-183), octa- bromodiphenyl ethers (PBDE-196, PBDE-197, PBDE-202, PBDE-203), nonabromodiphenyl ethers (PBDE-206, PBDE-207, PBDE-208), PBDE-209 were purchased from Accustandard. Other commercial chemicals were of analytical reagent grade.

2.3. Microbial cultivation

P. aeruginosa was grown in 500 mL Erlenmeyer flask filled with 200 mL of liquid nutrient medium and incubated at 30 °C for 24 h under a rotary shaker at 150 r min^{-1}. Then, the cells were harvested and separated from the medium by centrifugation at 6000 r min^{-1} for 10 min. The separated biomass was washed three times with 0.05 M L^{-1} sterile phosphate buffer (pH 7.3) and suspended in the same buffer, and then it was used for PBDE-209 biodegradation.

2.4. Biodegradation of PBDE-209

All biodegradation experiments were carried out in batch with a 50 mL Erlenmeyer flask containing 20 mL MSM solution with 1 mg L^{-1} PBDE-209. Flasks were inoculated with consortium at an optical density value of 0.45–0.47 at 600 nm (OD_{600}) on a rotary shaker at 30 °C and 150 r min^{-1} in the dark for the desired time. Glucose of 5 mg L^{-1} was supplemented as carbon source. The system without inoculation of *P. aeruginosa* was served as control.

2.5. Effect of additional carbon source on PBDE-209 biodegradation

Glucose, sucrose, lactose, starch, peptone, yeast powder and beef extract at a concentration of 5 mg L^{-1} individually were chosen as co-metabolic substrates for biodegradation of PBDE-209. In addition, to determine the possible influence of concentration of carbon source, glucose was added into several flasks and the final concentrations were set at 0.5, 1, 2, 5, 10 and 20 mg L^{-1}, respectively. *P. aeruginosa* was added to MSM containing 1 mg L^{-1} PBDE-209, and incubated at 30 °C and 150 r min^{-1} in the dark for 5 d. The system without inoculation of *P. aeruginosa* was served as control.

2.6. Effect of Cd^{2+} on Degradation of PBDE-209

In order to investigate the effect of heavy metal on PBDE-209 degradation, $Cd(NO_3)_2$ was added into flasks and the final concentration of cadmium ion was set at 0.1, 0.5, 1, 2, 5 and 10 mg L^{-1}, respectively. *P. aeruginosa* was added to MSM containing 1 mg L^{-1} PBDE-209 and 5 mg L^{-1} of glucose, and incubated at 30 °C and 150 r min^{-1} in the dark for the desired time. The system without cadmium ion was served as control.