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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<sup>2+</sup> while inhibited at higher content.

- Br<sup>-</sup> 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 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. © 2013 Elsevier Ltd. All rights reserved.

# 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 hexabromodiphenvl 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 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<sub>4</sub>NO<sub>3</sub> 1, KH<sub>2</sub>PO<sub>4</sub> 1.5, K<sub>2</sub>HPO<sub>4</sub> 3, and 2 mL L<sup>-1</sup> trace elements  $(g L^{-1})$ : MgSO<sub>4</sub> 4, CuSO<sub>4</sub> 1, MnSO<sub>4</sub>, FeSO<sub>4</sub>·7H<sub>2</sub>O 1, CaCl<sub>2</sub> 1), pH was adjusted to 7.5.

All the media were previously sterilized in an autoclave at 121  $^{\circ}\mathrm{C}$  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<sup>-1</sup>. Then, the cells were harvested and separated from the medium by centrifugation at 6000 r min<sup>-1</sup> for 10 min. The separated biomass was washed three times with 0.05 M L<sup>-1</sup> 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<sup>-1</sup> PBDE-209. Flasks were inoculated with consortium at an optical density value of 0.45-0.47 at 600 nm (OD<sub>600</sub>) on a rotary shaker at 30 °C and 150 r min<sup>-1</sup> in the dark for the desired time. Glucose of 5 mg L<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup>, respectively. *P. aeruginosa* was added to MSM containing 1 mg L<sup>-1</sup> PBDE-209, and incubated at 30 °C and 150 r min<sup>-1</sup> in the dark for 5 d. The system without inoculation of *P. aeruginosa* was served as control.

#### 2.6. Effect of Cd<sup>2+</sup> 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<sup>-1</sup>, respectively. *P. aeruginosa* was added to MSM containing 1 mg L<sup>-1</sup> PBDE-209 and 5 mg L<sup>-1</sup> of glucose, and incubated at 30 °C and 150 r min<sup>-1</sup> in the dark for the desired time. The system without cadmium ion was served as control.

#### 2.7. Determination of Br<sup>-</sup>

The samples were centrifuged at 8000 r min<sup>-1</sup> for 10 min. The bromide ion concentration was measured by ion chromatography ICS 900 equipped with a conductivity detector and an anion-exchange column (IonPac<sup>®</sup> AS14 (4 mm × 250 mm), Dionex USA). The mobile phase was 3.5 mM L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> and 1.0 mM L<sup>-1</sup> NaHCO<sub>3</sub> at 1.2 mL min<sup>-1</sup> flow rate with an injection volume of 20  $\mu$ L. Bromide ion standards were purchased from Inorganic Ventures (USA).

#### 2.8. Analytical methods

*Extraction*: The method of PBDEs extraction came from the literature information (de Boer et al., 2001) coupled with the experience of our study. After designated time of incubation, the whole samples were extracted with an equal volume of n-hexane/dichloromethane for twice with ultrasonic-assistance. Each time the mixture was vigorously shaken for 30 min and allowed to set until phase separation. Two organic parts were collected and treated with anhydrous sodium sulfate to remove water. Then the solution was concentrated by using a rotary evaporator at 40 °C. The resulting residue was dissolved in 10 mL n-hexane for GC–MS analyses. The recovery of extraction was 95–103%.

GC-MS analysis: PBDE-209 was analyzed using GC-MS (7890A-5975C, Agilent Technology, USA) equipped with a type DB-5ms GC column (15 m  $\times$  0.25 mm  $\times$  0.1  $\mu$ m). Helium was used as the carrier gas at a flow rate of 1 mL min<sup>-1</sup>. The column temperature program started at 110 °C, held for 2 min, then the oven was heated to 310 °C at a rate of 15 °C min<sup>-1</sup> and held for 5 min. Splitless injection of 1 µL sample was performed automatically at 280 °C. The temperature of ion source was set at 150 °C, and auxiliary temperature was 290 °C. The identification of degradation products was performed on a same column. The GC oven temperature program was as follows: isothermal at 110 °C for 5 min and 10 °C min<sup>-1</sup> to 310 °C and held at 310 °C for 10 min. The injector, ion source and transfer line temperatures were the same as those when analyzing PBDE-209. The mass spectrometry used negative chemical ionization (NCI) mode with methane as reagent gas and the ionization energy was 70 eV. The samples were both analyzed in full SCAN mode (m/z = 50-1000) and SIM mode (m/z = 79, 81).

#### 2.9. Statistical analysis

All of the experiments were performed in triplicates and the results presented were average values of the three replicates. The standard deviations for all measurements ranged from 1.5% to 8.0%.

## 3. Results and discussion

# 3.1. Degradation of PBDE-209

The degradation of PBDE-209 by aerobic strain *P. aeruginosa* during 1–7 d was conducted and the results were shown in Fig. 1(a). The experiment revealed that the degradation rate of PBDE-209 was 56% after 7 d and the residual amount of PBDE-209 in the system decreased from 0.99 mg L<sup>-1</sup> to 0.44 mg L<sup>-1</sup> within 7 d when using glucose as carbon source. However, the degradation efficiency was only 20% when glucose was absent, which was less than half of that when glucose was present. In the parallel control experiments, no significant change in the residual amount of PBDE-209 occurred over time, implicating that the possible non-biological degradation during the process was negligible.

It was seen from Fig. 1(a) that PBDE-209 residues contained in samples decreased and the degradation rate of PBDE-209 gradually increased with time whether the carbon source was added or not. In the presence of glucose, although the degradation rate of PBDE-209 increased very slowly during the first 2 d, it ascended sharply from the 3rd day on and then tended to stabilize after 5 d. This demonstrated that P. aeruginosa mainly used glucose as nutrient to grow up during the first several days and at the same time the strain gradually adapted to PBDE-209 environment. Then P. aeruginosa started to consume and transform more PBDE-209, resulting in quick increase of degradation efficiency. After 5 d reaction time, the depletion of nutrient slowed down the growth of the strain, leading to the diminishing of the degradation. Compared to PBDE-209 degradation with glucose as carbon source, the PBDE-209 residues decreased slowly without glucose during the process of incubation. It indicated that the accessibility to available carbon sources was an important factor for the biotransformation of PBDE-209.

PBDE-209 cannot be preferentially used by microorganism since it is not the essential nutrient for microorganism growth. Thus, the microorganism requires a period of time to adapt to the PBDE-209 contaminated environment. It has been reported that co-metabolic substrate which microorganisms can easily utilize was sometimes added to the system to deal with refractory compounds, especially persistent organic pollutants (POPs) (Field and Sierra-Alvarez, 2008). Qiang et al. (2009) indicated that the addition of lactose as co-metabolic substrate most favorably accelerated degradation of anthracene by about 37% when using Aspergillus fumigatus to biodegrade anthracene. In order to explore whether the additional co-metabolic substrates influenced the utilization of PBDE-209 by P. aeruginosa, several carbon sources were chosen to test, and it was observed that five carbon sources, including glucose, sucrose, lactose, starch, and beef extract, supported P. aeruginosa growth, among which glucose was proved to be the excellent one, based on the cell growth and the PBDE-209 degradation (Fig. 1(b)).

As illustrated in Fig. 1(c), degradation efficiency increased with increasing concentration of glucose ranged from 0 to 5 mg L<sup>-1</sup>, then the further increase of glucose exerted suppressive effect on PBDE-209 biodegradation. This result suggested that the optimal concentration of glucose was 5 mg L<sup>-1</sup> when dealing with 1 mg L<sup>-1</sup> PBDE-209. When high concentration of additional carbon source existed, the strain would mainly utilize this easily metabolized carbon source, consequently reducing the degradation of the target contaminants. This result was similar to what Ye et al. (2011) reported, in which the highest degradation efficiency was reached at equal concentration of lactose and anthracene when using *A. fumigatus* to biodegrade anthracene, and excessive carbon source would actually inhibit the degradation efficiency.

# 3.2. Effect of $Cd^{2+}$ on degradation of PBDE-209

PBDEs and heavy metals ubiquitously coexist in the environment, especially in e-waste dismantling area. In this experiment, we selected cadmium, a frequently occurred heavy metal in ewaste recycling site, as the model to investigate the effect of heavy metal on degradation of PBDE-209 by *P. aeruginosa*, and the results were shown in Fig. 2.

It was clear that the influence of  $Cd^{2+}$  varied with its concentration. The degradation rate of PBDE-209 at  $Cd^{2+}$  concentration of 0.1 mg L<sup>-1</sup> was similar to that in MSM system. And the removal of PBDE-209 was stimulated at low concentration of  $Cd^{2+}$ , ranging from 0.5 to 2 mg L<sup>-1</sup>, as examined by *t*-tests (p < 0.05). Possible explanations for this result were (i) *P. aeruginosa* in this research was isolated from the electronic wastes surrounded area and was therefore more tolerant of heavy metal. Moreover, some heavy



**Fig. 1.** Degradation efficiency of PBDE-209 (1 mg L<sup>-1</sup>) by *P. aeruginosa* under different conditions. (a) Glucose was added or not over incubation time, (b) addition different carbon source, and (c) addition of different concentration of glucose.

metals, such as  $Cd^{2+}$ , when their concentrations were low, could promote the growth and activity of the bacteria (Hong et al., 2007), thus increasing the utilization rate of PBDE-209 by *P. aeruginosa*. (ii) Functional enzyme for the degradation of PBDE-209 was synthesized at low concentration of  $Cd^{2+}$ . When  $Cd^{2+}$  concentration reached 10 mg L<sup>-1</sup>, however, the degradation efficiency decreased greatly and the maximum degradation rate on 7th day was only 29%, which was approximately half that in MSM system. This was attributed to the toxicity of  $Cd^{2+}$  to *P. aeruginosa* at higher concentrations (5–10 mg L<sup>-1</sup>). In the later study, we found that  $Cd^{2+}$  of higher concentration had strong inhibition effect on activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and ATPase. This suggested that the bacterial cells were damaged and metabolism was negatively affected at higher  $Cd^{2+}$  concentration, thus decreasing the degradation effect of PBDE-209. The similar results were achieved in other studies when exploring the effect of metals on organics degradation by mixed cultures, in which the biodegradation was enhanced at low metal concentrations but inhibited at high levels (Kuo and Genthner, 1996; Roberts et al., 1998). Lin et al. (2007) also obtained a similar result when using *Ochrobactrum cytisi*, a pure culture, to degrade methyl tert-butyl ether that the degradation rate was stimulated at low concentrations of  $Zn^{2+}$  and  $Mn^{2+}$  ( $1-5 \text{ mg L}^{-1}$ ) while inhibited at high concentrations of  $Zn^{2+}$  and  $Mn^{2+}$  ( $20 \text{ mg L}^{-1}$ ).

detected either.



Fig. 2. Degradation of PBDE-209 under different concentrations of  $Cd^{2*}$  (0.1–10 mg  $L^{-1}$ ) over incubation time.



Fig. 3. Concentration of bromide ion released during PBDE-209 degradation.

#### 3.3. Presence and formation of Br-

It was reported that strain RHA1 was able to release large amounts of bromide ions during the transformation of tetrabromodiphenyl ether (PBDE-47) (Robrock et al., 2009). And strain DB-1 debrominated part of PBDE-209 at aerobic incubation using lactate as carbon source (Deng et al., 2011). Considering that the degradation of PBDE-209 by *P. aeruginosa* formed lower brominated congeners in our experiment, we presumed bromide ions would be produced during this process. To confirm this, ion chromatography was used to analyze bromide generation accompanied with PBDE-209 disappearance.

The result showed that none of Br- was detected whether in samples or in controls in the first 2 d of PBDE-209 degradation. From the 3rd day, the concentration of Br<sup>-</sup> increased rapidly and maintained uptrend in the following days (Fig. 3). This trend was similar to that of PBDE-209 degradation. A negative correlation between the concentration of Br<sup>-</sup> and the residues of PBDE-209 (correlation = -0.973) in the sample was revealed by comparing with the experimental data of Br<sup>-</sup> release and PBDE-209 degradation, and conducting correlation analysis between them. The fact that Br<sup>-</sup> was not detected in the first 2 d of experiment despite of the decrease of PBDE-209 amount was due to that the formation of Br<sup>-</sup> was too low to be detected. Further experiments indicated that Br<sup>-</sup> could not be detected when its concentration was below  $5 \,\mu g \, L^{-1}$ . Our theoretical calculation also demonstrated that the concentration of Br<sup>-</sup> was lower than  $5 \,\mu g \, L^{-1}$  during the first 2 d of experiment. In the sterile control experiments, none of bromide ion was observed.

By comparing with the theoretically formed bromide ion based on degradation rate and the measured value over the incubation course, we found that the actual value was less than the theoretical

## 3.4. Presence and formation of lower brominated congeners

PBDE-209 used in this work contained small amounts of three nonabromodiphenyl ethers (about 0.8% PBDE-206, 0.8% PBDE-207, and 0.4% PBDE-208, Fig. 4(a)) which were equivalent to the total of nonabromodiphenyl ethers concentrations in technical PBDE-209 reported in the literature (Tokarz et al., 2008). In this work, two nonabromodiphenyl ethers (PBDE-208 and PBDE-207) were formed in the incubation process with the decrease of PBDE-209, demonstrating that the degradation of PBDE-209 by *P. aeruginosa* was accompanied by debromination.

of bromide ion with the intermediates of PBDE-209 and was not

As shown in Fig. 4(b) and (c), signals for PBDE-208 and PBDE-207 clearly increased relative to the signal of PBDE-206 with increasing incubation time. PBDE-208, formed by para debromination, and PBDE-207, resulted from meta debromination, increased significantly on the 3rd and 7th day, but the formation of PBDE-206 could not be detected conclusively. This result was similar to what Gerecke et al. (2006) reported, in which PBDE-208 and PBDE-207 as well as a number of octabromodiphenyl ether congeners were formed in the anaerobic experiments. It indicated that microbially mediated debromination of PBDE-209 at the ortho po-



**Fig. 4.** GC/MS chromatograms of PBDE-209 and nona-PBDEs (at m/z 486.4 and 488.3). (a) In the sterile control; (b) incubation 3 d; (c) incubation 7 d.



**Fig. 5.** Amount of lower bromodiphenyl ether congeners versus time (a) nonabromodiphenyl ethers; (b) octabromodiphenyl ethers; (c) heptabromodiphenyl ether.



Fig. 6. Amount of loss of PBDE-209 and formation of lower bromodiphenyl ether congeners versus time.

sition was less frequently observed than that at the meta and para positions. The similar results were also reported when dealing with PCBs biodegradation in which the microbial dehalogenation occurred at the same positions (Maltseva et al., 1999; Abraham et al., 2002).

However, it was found that the increased amount of total nonabromodiphenyl ethers was smaller than the loss of PBDE-209 after 3 d degradation, which implied that some other intermediates might have formed during this process. In order to identify as many low bromodiphenyl products in this study as possible, GC retention times and mass spectra of the products were compared with those of standards and previous literatures (Fang et al., 2008). It was confirmed that four octabromodiphenyl ethers (PBDE-203, PBDE-202, PBDE-197, PBDE-196) and one heptabromodiphenyl ether (PBDE-183) were formed during PBDE-209 degradation. Therefore, it could be concluded that the main aerobic biotransformation mechanism of PBDE-209 by P. aeruginosa was debromination. Some studies on PBDEs biodegradation have shown that PBDEs are anaerobically transformed to lower brominated congeners by a variety of bacteria in sediment, digester sludge or other environmental samples (Gerecke et al., 2006; Yen et al., 2009; Lee and He, 2010). But very few works about aerobic degradation of PBDEs, especially PBDE-209, were reported to date. Degradation of PBDEs by aerobic bacteria may act a similar way to that of PCBs. Researches have revealed that the mechanism of aerobic biodegradation of PCBs involved ring opening of PCB congeners when they underwent co-metabolic aerobic oxidation mediated by an enzyme deoxygenases (Vasilyeva and Strijakova, 2007: Furukawa and Fujihara, 2008). However, the ring of fully brominated PBDE-209 is difficult to open directly because of its symmetric molecular structure and enhanced chemical stability by higher bromine constitution. Therefore, the aerobic biodegradation of PBDE-209 was most likely started with debromination, resulting in forming lower- bromodiphenyl ethers, which was easier to be further broken down through ring opening.

Fig. 5 shows the concentration profiles of different metabolic products at different incubation times. The amounts of two nonabromodiphenyl ethers increased in the samples at highest rates. And four octabromodiphenyl ethers and one heptabromodiphenyl ether were observed and their levels ascended 2 and 3 d later, respectively. After 5 d reaction time, the depletion of nutrient slowed down the growth of the strain and a lot of them were inactivated, leading to the stabilization of the amount of lower bromodiphenyl ether products. Similar to the previous findings that nonabromodiphenyl ethers were produced by debromination at the meta and para positions, PBDE-202, formed by para debromination of PBDE-208, and PBDE-197, resulted from meta debromination of PBDE-207, were detected at high level. Contrary to these two congeners. PBDE-203 and PBDE-196 which came from debromination of PBDE-207 on ortho position at different benzene ring were detected at relatively low concentrations in the samples.

The amounts of PBDE-209 disappeared and lower brominated products formed did not match well after 4 d degradation and this difference increased gradually over time (Fig. 6). There may be two reasons for this discrepancy. First, because PBDEs have 209 congeners and not all standards were available, other lower bromodiphenyl ethers that had no standards to compare formed with the decrease of PBDE-209. Second, some unidentified transformation products, such as lower bromo- products through ring opening or hydroxylated/methylated PBDEs were produced. In the sterile controls, neither nonabromodiphenyl ethers nor other lower brominated congeners were formed.

# 4. Conclusions

This work demonstrated that *P. aeruginosa*, isolated from an ewaste dismantling area, exhibited exceptional ability in degrading PBDE-209. When glucose was used as an additional carbon source at concentration of 5 mg L<sup>-1</sup>, *P. aeruginosa* performed best degradation efficiency. Different concentrations of cadmium ions affected the degradation of PBDE-209 in a different way, low cadmium ion concentrations (0.5–2 mg L<sup>-1</sup>) enhanced the degradation of PBDE-209 but high concentrations (5–10 mg L<sup>-1</sup>) inhibited it. Bromine ion was detected during the course of incubation, and the concentration of Br<sup>-</sup> had a good negative correlation with the residues of PBDE-209. The research suggested that the main aerobic biotransformation mechanism of PBDE-209 by *P. aeruginosa* was debromination. 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 during PBDE-209 degradation.

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