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Rapid biodegradation of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) by *Achromobacter xylosoxidans* GYP4

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ABSTRACT

2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47) is a highly toxic polybrominated flame retardant, which causes severe pollution to the environment. In this work, a novel strain of aerobic bacteria *Achromobacter xylosoxidans* (GYP4) was screened out to degrade BDE-47. GYP4 was isolated from polluted paddy soil near an electronic-waste recycling plant. Experimental results showed that GYP4 could degrade BDE-47 as a single carbon source. The degradation rate of GYP4 for 1.0 mg L^{-1} BDE-47 solution was 90.8% within 4 d. GYP4 exhibited the highest degradation efficiency at temperature of 25.0°C–35.0°C and pH of 3.0–7.0. Three hydroxylated polybrominated diphenyl ethers were detected in the biodegradation process. Therefore, hydroxylation reaction was considered to be the main reaction in the metabolism pathway of BDE-47 degraded by GYP4.

Keywords: Aerobic bacteria; Degradation efficiency; Hydroxylation reaction; Metabolism pathway

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of effective brominated flame retardants that are widely used in various commercial products [1,2]. Among the 209 PBDEs congeners, 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47) is a highly toxic and widely used derivative which has been detected in organisms and environment [3]. When electronic products containing PBDEs are scraped, BDE-47 can flow into the water, soil, or get absorbed by organisms. BDE-47 poses serious risks to human health due to its potential biotoxicity [4,5]. It has been reported that BDE-47 induces oxidative stress, DNA damage, and apoptosis in primary rat hippocampal neurons [6]. In humans, BDE-47 can increase lactate dehydrogenase leakage and induce cell apoptosis in neuroblastoma cells [7]. Therefore, it is necessary and urgent to develop the remediation methods to remove BDE-47 from the contaminated environment.

Various methods have been investigated for the remediation of BDE-47 in the environment, such as reduction by zerovalent iron [8,9], photodegradation [10,11], hydrothermal treatment [12], and biodegradation [13,14]. Compared with

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physical and chemical treatment methods, biodegradation is more economical and environmentally friendly. However, studies on microbiological degradation of BDE-47 are rare. In particular, the relevant studies and applications of aerobic biodegradation of BDE-47 are lacking [13,15].

As a typical homologue of polybrominated diphenyl ethers (PBDEs), the research theories and methods developed for PBDEs are also helpful to investigate the remediation of BDE-47 [16,17]. Previous studies have suggested that PBDEs could be degraded into micromolecular brominated congeners by microorganisms under anaerobic or aerobic conditions [18,19]. For example, the anaerobic microbes from sewage sludge and river sediment could degrade deca-BDE through anaerobic reductive debromination [20]. Another study reported that the anaerobic bacterium *Dehalococcoides ethenogenes Strain 195* was able to debrominate octa-BDE to penta-BDE within 6 months [21]. However, the degradation rate of anaerobic biodegradation is slow, which requires long reaction time ranging from several months to a year. On the other hand, aerobic bacteria can metabolize the contaminants more rapidly. Most of the aerobic biodegradation studies until now have focused on BDE-209 while only a few have investigated BDE-47 [22,23]. Schmidt et al. [24]. initially isolated two BDE-degrading bacterial *Sphingomonas* sp. strains SS3 and SS33. Both were verified to have the capability to degrade 4-bromodiphenyl ether and 4,4′-dibromodiphenyl ether, respectively. Afterwards, Kim et al. [25] identified a *Sphingomonas* sp. strain PH-07, which displayed the ability to degrade mono-BDEs to tri-BDEs with biphenyl as the growth substrate. Recently, Robrock et al. [26] investigated the biodegradation of PBDEs by two polychlorinated biphenyl degrading bacteria *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400. Experiments showed that both strains could degrade all of the mono-BDEs to penta-BDEs. However, none of these reported strains could utilize PBDEs as the sole carbon source. Besides, efficient aerobic degradation microorganisms for BDE-47 are still scarce [27,28].

This study aimed to isolate an efficient aerobic degrading bacterium for BDE-47 and investigate the corresponding degradation pathways. First, temperature, pH, substrate concentration, and additional carbon sources were selected as the influencing factors to analyze the effects of environmental conditions on the biodegradation. Then, hydroxylated metabolites of BDE-47 degradation were identified to speculate the mechanism of the biodegradation process. The object of this research is to enrich the aerobic PBDEs degrading bacterium species and provide new options for the remediation of BDE-47 pollution.

2. Experimental

2.1. Materials

2,2′,4,4′-Tetrabromodiphenyl ether (BDE-47), 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE-47), 5-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (5-OH-BDE-47), 4′-hydroxy-2,2′,4-tribromodiphenyl ether (4′-OH-BDE-17), 2,4-dibromophenol (2,4-DBP), 2,3,4,5-tetrachlorobiphenyl (PCB-61), diphenyl ether (DE), and biphenyl were purchased from AccuStandard, Inc, USA. Additional carbon sources of glucose, sucrose, and lactose, as well as yeast extract and other reagents (KH_2PO_4, K_2HPO_4, and $CaCl_2$) were of the highest purity available.

The composition of the mineral salts medium (MSM) was as follows: 5.0 mL phosphate buffer solution (KH_2PO_4 8.5 g L^{-1}, $K_2HPO_4{\cdot}H_2O$ 21.75 g L^{-1}, $Na_2HPO_4{\cdot}12H_2O$ 33.4 g L^{-1}, and NH_4Cl 5.0 g L^{-1}), 3.0 mL $MgSO_4$ solution (22.5 g L^{-1}), 1.0 mL $FeCl_3$ solution (0.25 g L^{-1}), 1.0 mL $CaCl_2$ solution (36.4 g L^{-1}), and 1.0 mL trace element solution ($MnSO_4{\cdot}H_2O$ 39.9 mg L^{-1}, $ZnSO_4{\cdot}H_2O$ 42.8 mg L^{-1}, and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 34.7 mg L^{-1}). The pH value of MSM was adjusted to 7.0–7.2 using 2.0 mol L^{-1} HCl and NaOH solutions. The MSM solution was prepared with distilled water and autoclaved at 121.0°C for 30.0 min. Solid MSM was prepared by adding 2.0% agar into MSM. After autoclaving, a certain amount of BDE-47 stock solution (100.0–600.0 μL and 200.0 mg L^{-1}) was spread on the surface of solid medium. Luria–Bertani (LB) nutrient medium (agar) consisted of beef extract (3.0 g L^{-1}), peptone (10.0 g L^{-1}), NaCl (5.0 g L^{-1}), and 2.0% agar.

2.2. Microbial screening

PBDEs contaminated soil was collected from paddy fields near a dismantling workshop of electronic waste in Guiyu Town, Guangdong Province, China. The collected soil was stored at 4.0°C before use.

For sample analysis, 1.0 g soil was added into 20.0 mL sterile enrichment media, which was supplemented with 50.0 μg L^{-1} BDE-47. The mixture was incubated aerobically at 30°C on a rotary shaker operating at 150.0 rpm. BDE-47 was used as the sole carbon source. 1.0 mL aliquot of the above sample was transferred weekly into fresh culture medium with gradually increasing concentrations of BDE-47. After repeating the above procedure for six times, the final BDE-47 concentration in the culture medium was 5.0 mg L^{-1}. Then, the solution of the final cultivation was streaked in the medium plates with solid mineral salts. The grown colonies on plates were transferred into fresh solid enrichment media plates with 200.0 μL BDE-47 stock solution (200.0 mg L^{-1}). Colonies were transferred into fresh solid enrichment media every 3 d. The final volume of BDE-47 solution on the plates increased to 600.0 μL. The colonies showing better growth were separated on Luria–Bertani (LB) nutrient agar plates. The pure colonies were selected to verify their degradation abilities individually.

2.3. Identification of BDE-47 degrading strain

2.3.1. Colonial morphology

The morphological properties of cells were characterized by observing the colonies on LB solid plates using a scanning electron microscope (SEM, Carl Zeiss Jena Melin). Cell samples were subjected to a series of pretreatment processes before SEM analysis. The cells at exponential phase were first washed by phosphate buffer solution (0.1 M, pH = 7.0). Then, the cells were fixed with 2.50% glutaraldehyde in PBS, washed with PBS, and dehydrated by a graded series of ethanol (30%, 50%, 70%, 80%, 90%, 95%, and 100%). Finally, cells were replaced with tertiary butyl alcohol and freeze dried. Afterwards, samples were coated with gold and observed by SEM.

2.3.2. 16S rDNA

Genomic DNA was extracted with bacterial genomic DNA isolation kit (Sangon Ltd., Shanghai, China). 16S rDNA was amplified by PCR using the genomic DNA as template and the bacterial universal primers: F27 (5′-AGTTTGATCMTGGCTCAG-3′) and R1492 (5′-GGTTA CCTTGTTACGACTT-3′). First, the initial denaturation was performed at 94.0°C for 4.0 min followed by 30.0 cycles of denaturation at 94.0°C for 45.0 s. Then, the sample was annealed at 55.0°C for 45.0 s and elongated at 72.0°C for 1.0 min. Finally, elongation was performed at 72.0°C for 10.0 min. The PCR product was purified by a PCR purification kit (Sangon Ltd., Shanghai, China).

2.3.3. Phylogenetic tree analysis

According to the nucleotide BLAST results of the obtained sequences, the stain shared 100.0% homology with *Achromobacter xylosoxidans*. Therefore, it was identified as *Achromobacter xylosoxidans* and named as GYP4. 16S rDNA sequences of GYP4 were aligned by Clustal X program. The phylogenetic tree of strain GYP4 was structured by the software MEGA 6.0 using the neighbor-joining method according to bootstrap analyses based on 1,000 replicates. The nucleotide sequence of strain GYP4 was preserved in the National Center for Biotechnology Information Genbank under accession number KY697918. The collection number for the GYP4 strain is CCTCC M 2017104 in China Center for Type Culture Collection.

2.4. Biodegradation of BDE-47 and growth curve of strain GYP4

Stock solution of BDE-47 (0.1 mL, with concentration 200.0 mg L^{-1}) was added to 100.0 mL glass vials and evaporated. Strain GYP4 was first harvested in exponential phase on LB nutrient agar plates. It was then resuspended in fresh MSM to obtain a bacterial suspension. Then, 18.0 mL aliquot of MSM and 2.0 mL bacterial suspension were added into each vial to get a cell number of about 10^8 colony forming units (CFU) m L^{-1}. The final concentration of BDE-47 in the biodegradation system was 1.0 mg L^{-1}. Control vials after autoclaving were prepared by introducing the same volume of BDE-47 solution and 20.0 mL MSM, but without adding bacterial suspension. These vials were sealed with sterile sealing membrane to exclude external bacteria and placed in a rotary shaker (30.0°C, 150 rpm) in darkness to avoid photodegradation. Four parallel samples were prepared for each experiment and control set.

At different biodegradation times, all experimental vials and control vials were taken out. One experimental vial and one control vial were used to calculate the microbial population by using dilution-plate method. The remaining vials were used to detect the residual concentration of BDE-47. These remaining samples were extracted with an equal volume (20.0 mL) of *n*-hexane containing PCB-61 (100.0 μg L^{-1}) as an internal standard. The vials were sealed with silicon plug to avoid evaporation of *n*-hexane. Then, the mixture was vigorously shaken to create vortex for 5.0 min. After that, the mixture was ultrasonicated for 30 min. The two phases were allowed to separate. Then, the organic phase was collected and dried over anhydrous sodium sulfate and analyzed by GC–MS.

To identify the possible metabolic products of BDE-47 biodegradation by GYP4, the solution was concentrated for the instrument detection. After incubation with 4 d, all samples were extracted twice by the above-described extraction procedures. The extracted organic phases were collected, combined, and dehydrated with anhydrous sodium sulfate. Then, the solution was concentrated with a rotary evaporator at 40.0°C. The flasks were washed three times with *n*-hexane and the liquid was concentrated using nitrogen. The resulting residue was dissolved in 1.0 mL methanol for UPLC-MS/MS analyses.

2.5. Effects of environmental factors on the biodegradation of BDE-47

In this study, temperature, pH, initial concentration of BDE-47, and the additional carbon sources were varied to investigate the effects of environmental factors on the biodegradation of BDE-47. The detailed description of this part is given in Supplementary Information.

2.6. GC-MS and UPLC-MS/MS analyses

The quantification analysis in this study was performed by GC-MS and UPLC-MS/MS. The detailed description is given in Supplementary Information.

3. Results and discussion

3.1. Identification of strain GYP4

After the incubation, the BDE-47 degrading strain GYP4 with the highest degradation ratio was isolated from PBDE contaminated soil using solid agar plates. On LB nutrient agar plates, colonies of strain GYP4 appeared circular and cream colored with regular margins (shown as Fig. S1). The SEM image is shown in Fig. 1, which presented rod-shaped strain GYP4 with the size of 1.02 × 0.41 μm^2. It suggested that the strain GYP4 was gram-negative. Phylogenetic analysis of 16S rDNA gene sequences demonstrated that the strain GYP4 belonged to the genus of *Achromobacter xylosoxidans*. Its position in the phylogenetic tree is shown in Fig. 2. This is the first report about a strain of *Achromobacter xylosoxidans* which can effectively degrade BDE-47.

3.2. Biodegradation of BDE-47

In the biodegradation experiments, the temperature, pH, and concentration of BDE-47 were set as 30.0°C, 7.0, and 1.0 mg L^{-1}, respectively. BDE-47 served as the sole carbon source in the medium. As shown in Fig. 3, during the first 10.0 h incubation, significant degradation of BDE-47 and rapid growth of strain GYP4 were observed. During incubation for 10.0–31.0 h, the degradation ratio of BDE-47 increased slowly. Correspondingly, the growth of strain GYP4 slowed down during the same period and reached the peak value at 31.0 h. Beyond 31.0 h incubation, the degradation ratio of BDE-47 continued to increase and then stabilized. Beyond 96.0 h, the degradation ratio of

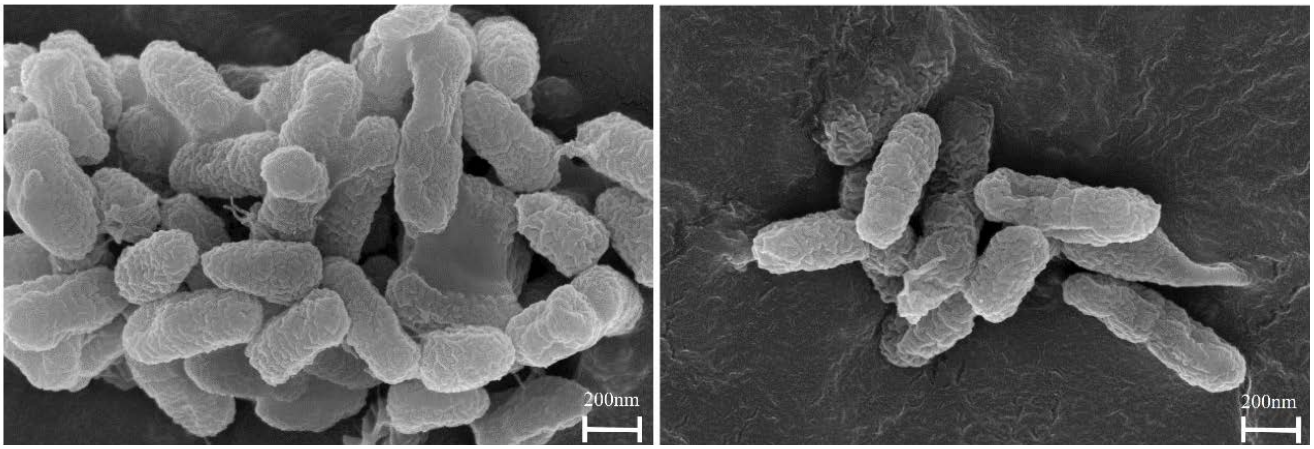

Fig. 1. SEM micrographs of the strain GYP4.

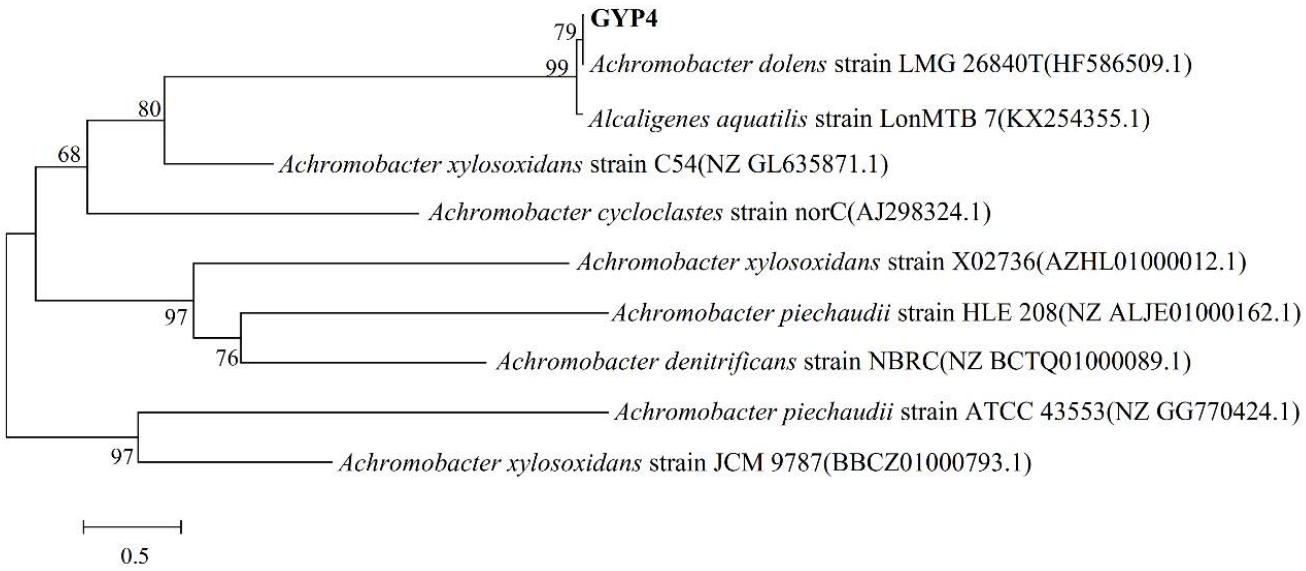

Fig. 2. Phylogenetic analysis of the strain GYP4 and related species by the neighbor-joining approach.

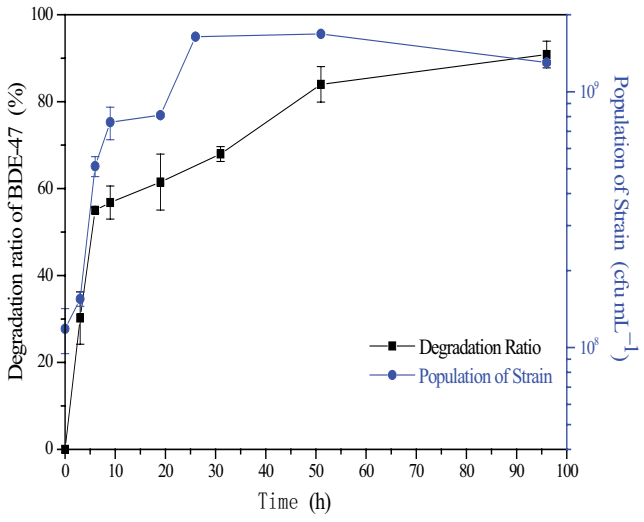

Fig. 3. Growth curve and degradation kinetics of the strain GYP4.

BDE-47 reached 90.8%. It has been reported that a lag phase was observed in biodegradation curve when toxic compounds were used as the sole carbon source in the medium [29]. However, the lag phase was negligible during the degradation of BDE-47. It indicated that the strain GYP4 could adapt to the BDE-47 contaminated environment quickly after initiation in the incubation period. In addition, during the entire biodegradation process of BDE-47, low brominated diphenyl ether and diphenyl ether were not detected. This result indicates that the debromination process was not involved in the degradation of BDE-47. The degradation rate of BDE-47 was calculated by the following equation:

$$\text{Degradation rate}(\%) = \frac{C_0 - C_t}{C_0} \times 100\% \quad (1)$$

where C_0 is the initial concentration of the substrate, mg L^{-1}, and C_t is the substrate concentration at time t, mg L^{-1}.

3.3. Effects of environmental factors

To further investigate the biological mechanism of BDE-47 degradation by GYP4, the effects of temperature, pH, substrate concentration, and additional carbon source were considered in this study. These effects were also used to determine the optimal environment for degradation of BDE-47 by GYP4.

3.3.1. Effects of temperature

The effects of temperature are shown in Fig. 4a. It can be seen that the degradation ratio of BDE-47 varied from 37.2% to 85.0% when the temperature ranged from 15.0°C to 40.0°C during 4 d incubation. When the temperature increased from 15.0°C to 30.0°C, the degradation ratio increased from 54.6% to 85.0% and the maximum degradation ratio was achieved at 30.0°C. When the incubation temperature increased further from 35.0°C to 40.0°C, the degradation ratio of BDE-47 decreased sharply from 83.9% to 37.2%. All the degradation ratios were above 80.0% when the incubation temperature increased from 25.0°C to 35.0°C. These results indicated that GYP4 could maintain excellent degradation capacity over a wide temperature range from 15.0°C to 40.0°C. Moreover, the bioremediation of BDE-47 contaminated environment also takes place at this temperature range, which confirms that the enzyme activities, cell membrane fluidity, and substance solubility are influenced greatly by temperature [30]. In general, at a certain temperature range, enzyme activity, cell membrane fluidity, and substance solubility all increase with the increase in temperature, which ultimately leads to higher degradation ratio of BDE-47. However, beyond this temperature range, cell function might decrease and cause the decrease in degradation ratio of BDE-47.

3.3.2. Effects of pH

As shown in Fig. 4b, the degradation ratios of 1.0 mg L^{-1} BDE-47 after 4 d incubation varied from 62.3% to 93.8%. The pH of the culture media was varied from 3.0 to 9.0, and 4.0 was found to be the optimal pH. When the pH increased from 3.0 to 7.0, the degradation remained at a high rate of more than 84.0%. However, the degradation ratio decreased from 85.0% to 62.3% with further increase in pH from 7.0 to 9.0. The results showed that the strain GYP4 could adapt to a wide range of pH (3.0–9.0) but preferred acidic environment for degradation of BDE-47.

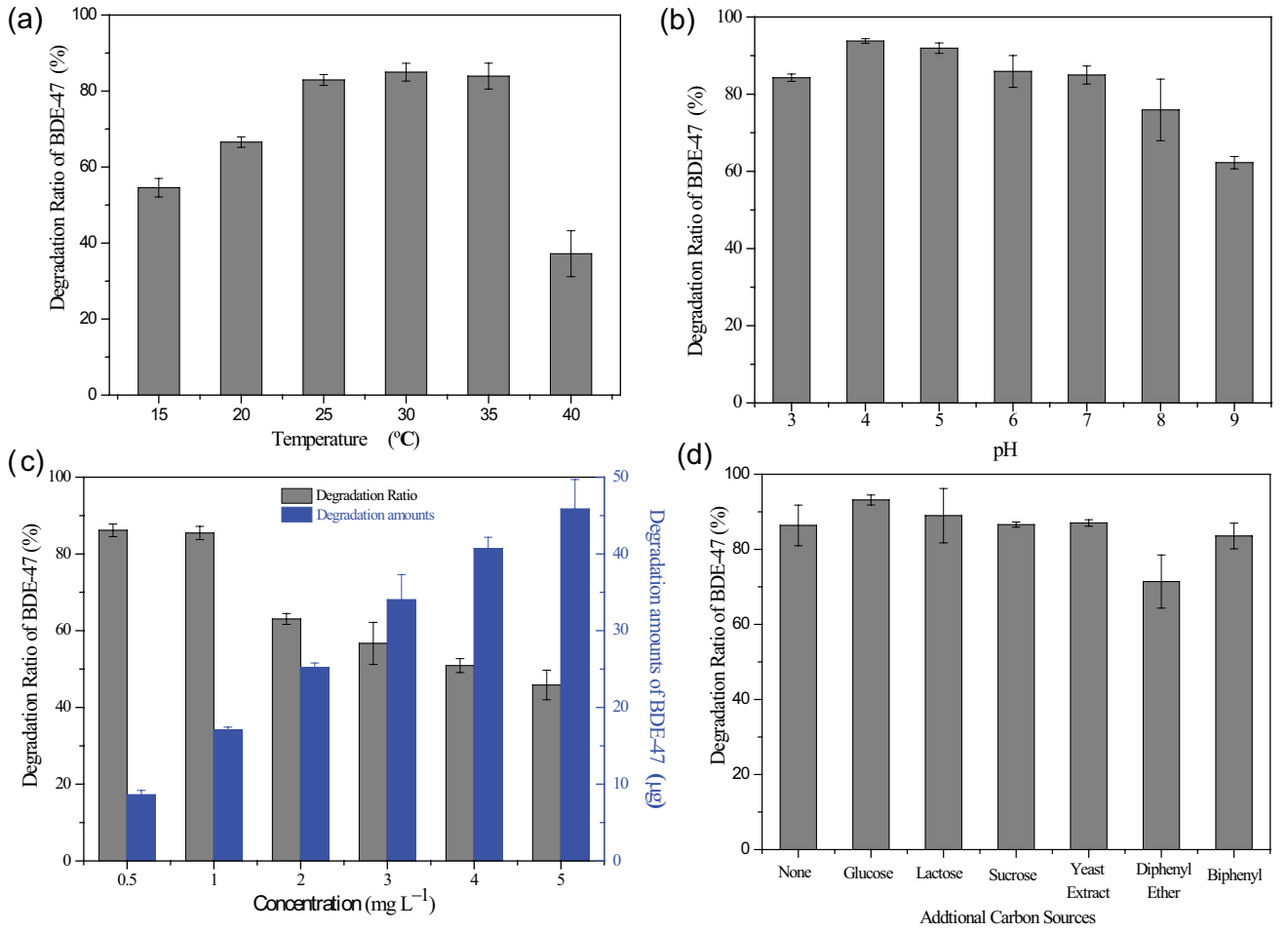

Fig. 4. Degradation ratios of BDE-47 under different conditions: (a) temperature, (b) pH, (c) initial BDE-47 concentration (mg L^{-1}), and (d) additional carbon sources.

Previous studies have indicated that weakly acidic pH can promote the microbial degradation of contaminants. This promotion effect might result from the improved permeability of cell membranes in a weakly acidic media. The higher permeability accelerated the exchange capacity of substances between cells and medium, which made it easy for contaminants to enter the cells [31]. Therefore, the degradation process of contaminants was facilitated by acidic environment.

3.3.3. Effects of initial concentration of BDE-47

The degradation ratios of BDE-47 with different initial concentrations after 4 d incubation are shown in Fig. 4c. The BDE-47 concentrations of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg L^{-1} presented the degradation ratios of 86.2%, 85.5%, 63.1%, 56.7%, 50.9%, and 45.9%, respectively. It can be seen that the degradation ratios of BDE-47 exhibited a declining trend with the increase in initial concentration of BDE-47. However, when the initial concentration of BDE-47 increased from 0.5 to 5.0 mg L^{-1}, the degradation amount of BDE-47 increased from 8.62 to 45.9 μg. This indicated that the strain GYP4 could degrade BDE-47 at high concentrations. Moreover, the toxicity of BDE-47 would not affect the cell function of strain GYP4 when BDE-47 concentration is lower than 5.0 mg L^{-1}.

3.3.4. Effects of additional carbon sources

Previous studies have reported that refractory compounds (such as persistent organic pollutants and co-metabolic substrates) can enhance the biodegradation efficiency of the target pollutants when microorganisms are used for the degradation [32]. As shown in Fig. 4d, glucose, sucrose, lactose, yeast extract, biphenyl, and diphenyl ether were separately used as additional carbon sources. Fig. 4a shows that the degradation ratios of BDE-47 with glucose, sucrose, lactose, yeast extract, biphenyl, and diphenyl ether were 93.1%, 86.6%, 89.0%, 87.0%, 83.6%, and 71.4%, respectively. Compared with the degradation ratio of pure BDE-47 (86.4%), the degradation ratio of BDE-47 was improved by the addition of glucose, sucrose, lactose, and yeast extract; however, the improvement was small. On the contrary, the addition of diphenyl ether significantly inhibited the biodegradation efficiency of BDE-47. It indicated that these additives did not significantly affect the biodegradation except for diphenyl ether.

The negative effect of diphenyl ether on the degradation of BDE-47 was attributed to the following reasons: (a) the additional additives complicated the probable interactions between bacteria and substrates. The mutual inhibition between BDE-47 and additives caused the decrease in removal ratio of individual substrate [33]. The structure of diphenyl ether is similar to that of BDE-47, but the structure of BDE-47 is more complex than that of diphenyl ether. Therefore, the system of BDE-47 mixed with diphenyl ether could be easily metabolized by microorganisms. When diphenyl ether coexisted with BDE-47 in the culture media, the strain GYP4 could utilize diphenyl ether prior to BDE-47 as the carbon source. (b) The biodegradation process of BDE-47 blended with diphenyl ether can produce toxic metabolites. Those toxic metabolites might decrease the cell function or cause other side effects in the biodegradation of BDE-47 [34].

3.4. Speculation of probable metabolism pathways

To further investigate the biodegradation of BDE-47 by GYP4, the probable metabolism pathways were speculated in this study. As shown in Fig. 5, three OH-PBDEs (6-OH-BDE-47, 5-OH-BDE-47, and 4′-OH-BDE-17) and a dibromo phenol of 2,4-DBP were detected in the incubation process. The relevant parameters and concentrations of analytes are given in Supplementary information (Tables S1 and S2). No debrominated metabolites were detected, indicating that hydroxylation was the main biodegradation mechanism for BDE-47. The identification of metabolic products made it possible to deduce the metabolism pathways of BDE-47. Therefore, the biodegradation of BDE-47 could be monitored when applied in actual contaminated environments.

In this study, three probable metabolism pathways of BDE-47 are proposed, as shown in Fig. 6. and discussed below. (a) BDE-47 was hydroxylated to 5-OH-BDE-47 and 6-OH-BDE-47, and then decomposed to 2,4-DBP through the cleavage of the ether bond. (b) BDE-47 was directly decomposed to 2,4-DBP via the diphenyl ether bond cleavage. (c) BDE-47 was hydroxylated to 4′-OH-BDE-17 via the hydroxyl group substituting the bromine atom in the para position of the diphenyl ether oxygen, and then degraded to 2,4-DBP via the ether bond cleavage. Finally, 2,4-DBP was decomposed and metabolized.

Erratico et al. [35] demonstrated that BDE-47 could be hydroxylated to 5-OH-BDE-47 and 6-OH-BDE-47 through in vivo biodegradation. According to previous reports on hydroxylated PBDEs, 6-OH-BDE-47 and 5-OH-BDE-47 are the main metabolites from BDE-47 biodegradation. These metabolites are degraded via the direct insertion of hydroxyl group into the biphenyl ring [36]. Marsh et al. [37] first identified a OH-triBDE derivative with its hydroxyl group in para position to diphenyl ether oxygen in rat feces. They also suggested that the formation of this OH-triBDE was through debromination processes. In this work, OH-triBDE derivative of 4′-OH-BDE-17 was also detected in the biodegradation of BDE-47. During in vivo metabolism, BDE-28 or BDE-17 could be generated from BDE47 via oxidative debromination [38]. However, BDE-17 or BDE-28 were not detected in this biodegradation process of BDE-47. These results suggest that the oxidative debromination could not be caused by GYP4 enzymes. Moreover, BDE-17 or BDE-28 were not the intermediates, that were responsible for the generation of 4′-OH-BDE-17. Thus, this research proposes that 4′-OH-BDE-17 was generated directly through substitution of the bromine atom at the para position of BDE-47 by the hydroxyl group.

3.5. Comparison analysis

In this study, strain GYP4 of *Achromobacter xylosoxidans* was isolated from PBDEs polluted paddy field soil. According to previous studies, this species can denitrify or degrade high-density polyethylene and polycyclic aromatic hydrocarbons [39,40]. However, the research on using *Achromobacter xylosoxidans* to biodegrade PBDEs has not been reported yet. Therefore, this study attempted to investigate

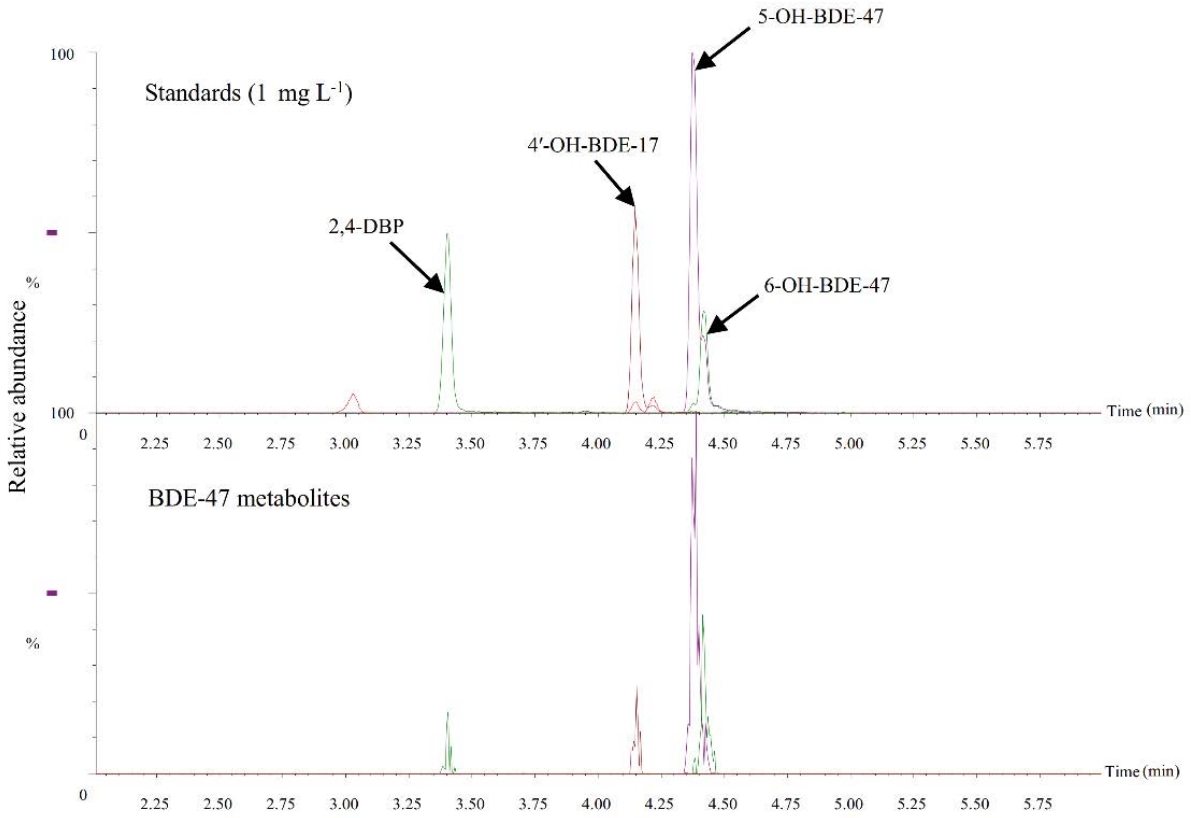

Fig. 5. UPLC-MS/MS chromatograms of biodegradation products of BDE-47.

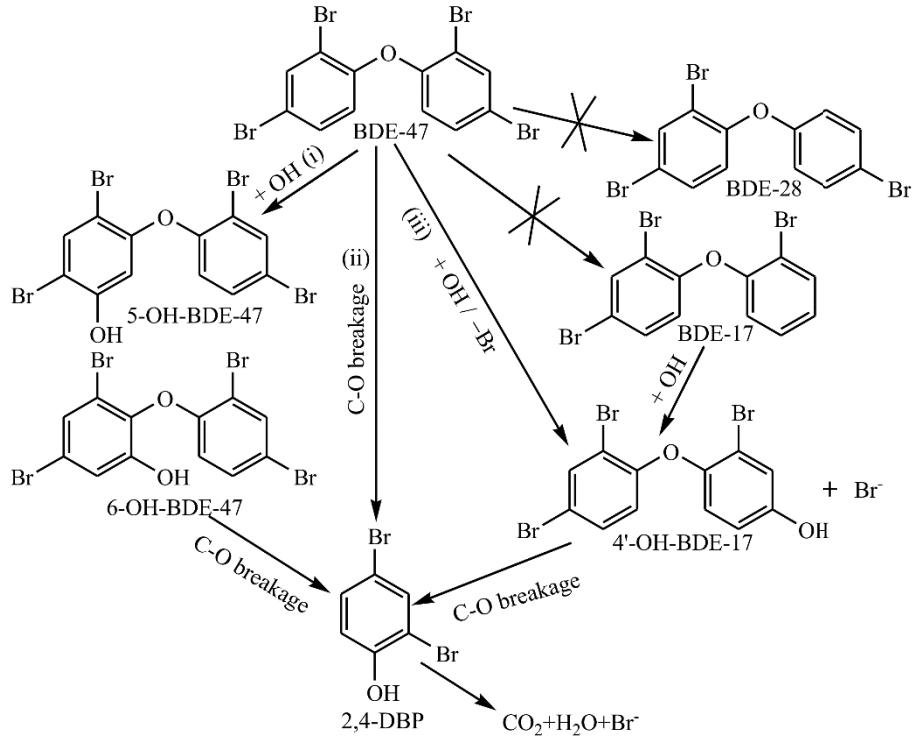

Fig. 6. Probable metabolism pathways of BDE-47 degraded by strain GYP4.

Table 1
Comparison of degradation ratios of GYP4 with other BDE-47 degrading strains

Species	Sources	Initial BDE-47 concentrations	Co-substrates	Degradation ratio	References
Pseudomonas putida sp. TZ-1	Sediment	50 μg L^{-1}	–	50.0%, 7 d	[41]
Pseudomonas stutzeri BFR-01	Soil	20 μg L^{-1}	–	97.9%, 14 d	[42]
Rhodococcus jostii RHA1	–	17 μg L^{-1}	Biphenyl	80.0%, 3 d	[27]
Burkholderia xenovorans LB400	–	17 μg L^{-1}	Biphenyl	50.0%, 3 d	[27]
Phanerochaete chrysosporium	–	0.5 mg L^{-1}	–	64.3%, 5 d	[43]
Achromobacter xylosoxidans GYP4	Soil	1 mg L^{-1}	–	90.8%, 4 d	This study
		5 mg L^{-1}		45.9%, 4 d	

Note: "–" means "no data".

the biodegradation of PBDEs by *Achromobacter xylosoxidans*. To discuss the biodegrading advantages of GYP4, the biodegradation efficiency of GYP4 was compared with other aerobic degrading microorganisms, such as: *Pseudomonas* (*putida* sp. TZ-1, *stutzeri* BFR-01), white-rot fungus (*Phanerochaete chrysosporium*), *Rhodococcus* and *Burkholderia* (*jostii* RHA1, *xenovorans* LB400). All the above microorganisms were able to degrade BDE-47 under aerobic conditions. The comparison results are listed in Table 1. Compared with other strains, GYP4 had a higher degradation ratio for BDE-47 and was able to utilize only BDE-47 as the sole carbon source. Moreover, GYP4 could degrade BDE-47 at a high concentration within a relatively short period.

4. Conclusion

In this study, a novel strain GYP4 of *Achromobacter xylosoxidans* was isolated and demonstrated to have the ability to biodegrade BDE-47. The biodegradation ratio of strain GYP4 was 90.8% for 1.0 mg L^{-1} BDE-47 in 4 d. Moreover, the strain GYP4 exhibited excellent potential to remediate BDE-47 contaminated environments over a wide range of temperature (25.0°C–35.0°C) and pH (3.0~7.0). This strain could also biodegrade BDE-47 at a high concentration. Furthermore, the additional carbon sources were found to have limited effects on the biodegradation ratio of BDE-47, except for diphenyl ether. Finally, the mechanism of BDE-47 biodegradation by GYP4 was speculated to mainly involve hydroxylation. This study will be helpful to solve the pollution remediation of BDE-47 in contaminated environments, especially water.

Conflict of interest

The authors stated that they have read the policy on conflict of interest and confirm that there are no conflicts to declare.

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Supplementary Information

S1. Effects of environmental factors on the biodegradation of BDE-47

Environmental conditions are the major factors, which might affect the biodegradation efficiency when microorganisms are applied to remediate the polluted environment. To study the environmental effects on the biodegradation of BDE-47, the natural environment changes were simulated. Those factors included temperature, pH, initial concentration of BDE-47, and additional carbon sources.

- *Temperature:* The concentration of BDE-47 in the culture media was 1.0 mg L^{-1} and the pH value of the media was adjusted to 7.0. Bacterial suspension (10% v/v) was added into the media and incubated at 15.0°C, 20.0°C, 25.0°C, 30.0°C, 35.0°C, and 40.0°C, respectively.
- *pH:* At the optimal temperature of 30°C, bacterial suspension (10% v/v) was added into the culture media and the concentration of BDE-47 was 1.0 mg L^{-1}. The effects of pH

ranging from 3.0 to 9.0 on the biodegradation of BDE-47 was investigated.
- *Initial concentration of BDE-47:* The pH value of the media was adjusted to 7.0, the volume of bacterial suspension was 10% (v/v), and the temperature was set at 30.0°C. The effects of BDE-47 concentration varied as 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg L^{-1} on the biodegradation of BDE-47 were evaluated.
- *Additional carbon sources:* Glucose, sucrose, lactose, yeast extract, biphenyl, and diphenyl ether at a concentration of 5.0 mg L^{-1} were individually chosen as additional carbon sources to assess their effects on the biodegradation of BDE-47. The initial concentration of BDE-47 was 1.0 mg L^{-1}, pH value of the media was adjusted to 7.0, the temperature was 30.0°C, and the volume of bacterial suspension was 10% (v/v).

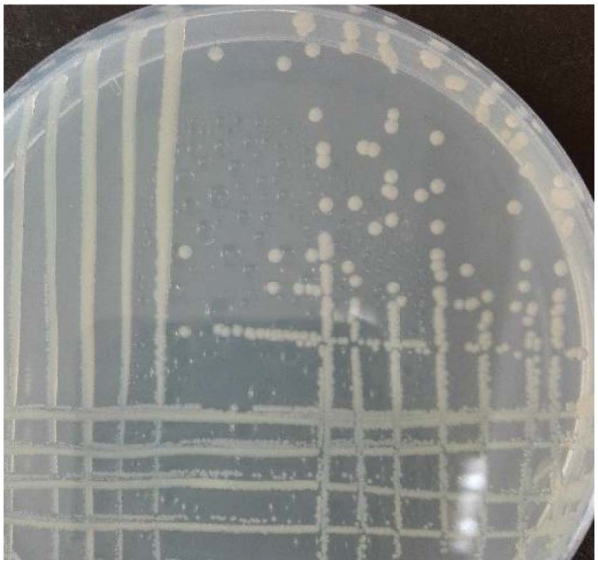

Fig. S1. Photographs of GYP4 colonies on LB nutrient agar plate.

All the experiment samples were incubated on a rotary shaker at 150 r min^{-1}, after 4 d all the vials were withdrawn, extracted, and analyzed.

S2. GC-MS and UPLC-MS/MS analysis

The quantification of BDE-47 was performed by GC-MS coupled with a-Thermo-Trace GC Ultra instrument to a Thermo-DSQ II mass spectrometer (Thermo Electron Corporation, Waltham, USA). The GC column was DB-5MS capillary column (30.0 m × 0.25 mm i.d. × 0.25 μm). Helium was employed as carrier gas at a constant flow of 1.0 mL min^{-1}. 1.0 μL of sample was injected into GC in spitless mode. The injector temperature was 280.0°C. The temperature of oven was held at 100.0°C for 1.0 min and increased to 210.0°C with the heating rate of 20.0°C min^{-1}, finally increased by 4.0°C min^{-1} to 260.0°C hold for 10.0 min. The ionization energy was 70.0 eV. The transfer line, quadrupole, and ion source temperature were set to 290.0°C, 250.0°C, and 280.0°C, respectively.

The BDE-47 metabolic products were identified using the Waters ACQUITY UPLC I-Class System. It was coupled with the Xevo TQ-S triple quadrupole mass spectrometer (UPLC-MS/MS). Instrument analytical procedure was performed based on the method by Kowalczyk et al. [39] but with some modifications. A Phenomenex Kinetex C18 column (100.0 mm × 2.1 mm, 2.6 μm particle size) was selected for chromatographic separation. The injection volume was 10.0 μL and the column temperature was set at 40.0°C. The mobile phase consisted of acetonitrile (component A) and 10.0 mmol L^{-1} ammonium acetate in 18.0 MΩ Milli-Q water (component B). First, it was used with a gradient elution of

Table S1
MRM mass parameters and retention time of analytes

Analytes	Retention time (min)	Parent ion (m/z)	Product ion (m/z)	Collision energy (eV)	Cone voltage (V)
6-OH-BDE-47	4.42	496.8	78.6*	10	28
		498.8	78.6	14	34
		500.7	81.1	10	12
		502.7	80.6	12	38
5-OH-BDE-47	4.37	496.8	78.7	30	68
		498.8	78.7	38	66
		500.7	78.4	26	68
		502.7	80.7*	32	62
4′-OH-BDE-17	4.15	418.8	78.7*	26	66
		420.8	78.7	26	68
		422.8	80.7	32	62
		424.8	80.7	22	62
2,4-DBP	3.41	250.8	78.4	26	6
		252.9	80.7*	18	18

Note: *Quantitative ion.

Table S2
Concentration of analytes after incubation for 4 d.

Analytes	Intensity		Concentration (μg L^{-1})
6-OH-BDE-47	Standard	$3.37e^6$	1,000
	GYP4	$6.35e^3$	0.094
5-OH-BDE-47	Standard	$1.19e^7$	1,000
	GYP4	$1.44e^4$	0.061
4′-OH-BDE-17	Standard	$6.80e^6$	1,000
	GYP4	$3.49e^3$	0.026
2,4-DBP	Standard	$5.94e^6$	1,000
	GYP4	$2.45e^3$	0.021
BDE-28	GYP4	ND	ND
BDE-17	GYP4	ND	ND

Note: ND – not detected.

A:B as 5:95 (v/v) in initial 0.3 min. Then, a linear increase to 50:50 (v/v) from 0.3 to 2 min and increased to 90:10 (v/v) from 2.0 to 3.5 min, continued from 3.5 to 4.0 min. Moreover, it decreased to 5:95 (v/v) from 4.0 to 5.0 min, and 5.0% solvent was lasted from 5.0 to 7.0 min at the flow rate of 0.3 mL min^{-1}. Mass spectrometric detection was performed using ESI source in electrospray negative ionization mode at a capillary voltage of 3,500.0 V. The source and desolation temperature were all set at 350.0°C. The flow rate of desolation gas, collision gas, and cone gas were 750.0 L h^{-1}, 0.15 mL min^{-1}, and 20.0 L h^{-1}, respectively.

All the glass vials used in the experiments were baked at 450.0°C for 4.0 h. Recovery of BDE-47 ranged from 87.1% to 104.6% in water phase. The standard deviations for all measurements ranged from 0.35% to 7.25%.