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One-step process for debromination and aerobic mineralization of tetrabromobisphenol-A by a novel *Ochrobactrum* sp. T isolated from an e-waste recycling site

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ABSTRACT

A novel bacterium, *Ochrobactrum* sp. T, capable of simultaneous debromination and aerobic mineralization of tetrabromobisphenol-A (TBBPA), was isolated from a sludge sample collected from an electronic-waste recycling site. The bacterium exhibited maximal debrominase activity at pH 6.5, 35 °C, and 200 rpm in Luria–Bertani culture medium. Initial TBBPA concentration and pH had more significant effects on degradation efficiency than those of temperature and inoculum size. Degradation and debromination efficiencies of 91.8% and 86.7%, respectively, were achieved within 72 h under optimized conditions of 35 °C, pH 7.0, inoculum volume of 25 mL, and TBBPA concentration of 3 mg L^{-1}. In addition, a 35.6% decrease in total organic carbon was observed after the degradation of 5 mg L^{-1} TBBPA for 120 h. Eight metabolic intermediates were identified during the biodegradation of TBBPA. This study is the first report to propose a one-step process for TBBPA debromination and mineralization by a single bacterial strain.

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1. Introduction

Brominated flame retardants (BFRs) are a group of brominated organic compounds extensively incorporated into many consumer products such as building materials, electronic goods, carpets, upholstery textile, and car panels to reduce the flammability of the final manufactured products (de Wit, 2002). Among of many BFRs, tetrabromobisphenol-A (TBBPA) is one of the most widely used commercially, with the highest global consumption of over 500,000 metric tons annually (Alaee et al., 2003). TBBPA does not leak into the environment because it is covalently bound to polymers. Nevertheless, a growing body of evidence had demonstrated the presence of TBBPA in various environmental samples such as in air (de Wit et al., 2010; Xie et al., 2007), water (de Wit et al., 2010), soil and sediment (de Wit et al., 2010), aquatic animals (de Wit et al., 2010; McHugh et al., 2010), and human tissues (Cariou et al., 2008) due to its persistent, lipophilic, and bioaccumulative properties (Reistad et al., 2005). In animals, TBBPA has been reported to have thyroid hormone-like and estrogen receptor-mediated effects that make estradiol inactive (Stasinakis et al., 2010). It also showed high acute toxicity to algae, mollusks, crustaceans, and fish (Darnerud, 2003). These risks were magnified by TBBPA detection at increasing concentrations in environmental and biological samples worldwide (Zhang et al., 2009).

To date, only a few studies report on the biotic and abiotic transformation of TBBPA in the aquatic environment. Abiotic processes include photodegradation (Eriksson et al., 2004; Horikoshi et al., 2008) and chemical reactions with manganese dioxide (Lin et al., 2009) or singlet oxygen (Han et al., 2008) present in the environment. Even fewer studies focusing on the biodegradation of TBBPA have been published (de Wit, 2002). Thus, deeper knowledge on TBBPA toxicity assessment and fate in water environments, especially the half-lives in water and solid environments, are urgently needed. However, most studies investigated whether TBBPA can be debrominated under anaerobic conditions (Arbeli et al., 2006; Iasur-Kruh et al., 2010) or without further degradation in either under anaerobic or aerobic condition (Brenner et al., 2006). Some studies compared the anaerobic debromination of TBBPA with six other BRFs and found that TBBPA was the most difficult to be degraded (Nyholm et al., 2010). To the best of our knowledge, all previous studies focused on the feasibility of TBBPA debromination under anaerobic conditions rather than mineralization in an aerobic system. A bacterial strain that can directly decontaminate TBBPA has not been isolated up to now, although the anaerobic debromination of TBBPA has been reported previously (Arbeli et al., 2006; Nyholm et al., 2010).

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Bromine substitution may increase the inherent toxicity of a compound (Haggblom and Bossert, 2003). Conversely, debromination is the most crucial process in the detoxification of BFRs and in the reduction of resistance to aerobic biodegradation of toxic intermediates during subsequent metabolic steps (Janssen et al., 2001). Removal of bromine or chlorine atom(s) frequently renders the molecule more susceptible to complete mineralization (Armenante et al., 1999; Li et al., 2008). The complete microbial mineralization of TBBPA was previously accomplished with two steps: reductive debromination of TBBPA under anaerobic conditions with subsequent mineralization of BPA by a Gram-negative aerobic bacterium (Ronen and Abeliovich, 2000). To the best of our knowledge, a one-step process for simultaneous debromination and aerobic mineralization of TBBPA in the same system has never been reported.

In the present study, a novel bacterial strain that can simultaneously debrominate and mineralize TBBPA efficiently in an aerobic system was isolated and identified. The dependence of debrominase activity on growth conditions of the novel bacterial strain was determined. Important parameters, such as substrate concentrations, inoculum volume, initial pH, and temperature, in TBBPA debromination biodegradation and mineralization were optimized. Furthermore, TOC was employed to probe the degree of TBBPA mineralization, whereas gas chromatography–mass spectrometer detector (GC–MSD) was applied to identify debromination and mineralization intermediates. Finally, a TBBPA decontamination mechanism in an aerobic system using the newly-isolated bacterium was also proposed.

2. Methods

2.1. Enrichment and isolation

The bacterial strain was isolated from a sludge sample collected from Guiyu Town in Southeast China, the world's largest electronic-waste recycling site. Detailed descriptions of the chemicals, growth medium, and sampling location are given in Supporting Information. Bacterial strain capable of degrading TBBPA was isolated as follows. About 3 g (wet weight) sludge was added to 100 mL mineral medium (MM) containing 0.5 mg L^{-1} TBBPA. After one week incubation at 35 °C with shaking at 150 rpm, 10% (v/v) of the culture was inoculated into 100 mL MM with the addition of TBBPA ranged from 1.0 to 3.0 mg L^{-1} once every week for two months to enrich the TBBPA-degrading bacteria. Then, 200 μL of the resulting cultures were spread onto Luria–Bertani (LB) agar medium containing 1.0 mg L^{-1} TBBPA, followed by incubation at 30 °C for 3 d. Colonies with a distinct morphological type were picked up and transferred to standard LB agar medium until a pure strain was isolated.

2.2. Bacterial strain identification

Cell morphology of the bacterial strain was observed by a light microscopy and confirmed using a transmission electron microscope (TEM, HITACHI, Japan) to ensure purity of the strain. Physiological and biochemical identification of the strain was performed using standard procedures (Guo, 2007). Characteristics of the bacterial isolate were determined according to Bergey's Manual of Determinative Bacteriology (Buchanan et al., 1984). The identity of the selected bacterial strain was further confirmed by 16S rRNA gene sequence analysis. Experimental details are shown in Supporting Information.

2.3. Determination of growth pattern of the strain

The growth pattern of the bacterial strain was determined by inoculating a loopful over night culture in 75 mL of LB culture medium in 250-mL shake flasks without TBBPA for 24 h. Various growth parameters were optimized afterwards. Unless otherwise specified, all experiments were performed at 35 °C, pH 7.0, and 200 rpm. At the given sampling time, optical density at 600 nm (OD_{600}) of each culture was measured using a spectrophotometer (GE Healthcare) after 2–3 s vigorous vortexing. All experiments were conducted in triplicate.

2.4. Measurement of debromination activity

After incubation, late log growth phase cultures under different growth conditions were harvested by centrifugation at 10,000*g* for 10 min. The cell pellet was re-suspended in sterile distilled water and washed twice, then re-suspended in MM containing 2 mg L^{-1} TBBPA. Finally, the resulting suspensions were incubated at 30 °C and 200 rpm for 12 h. The concentration of free Br^- released from TBBPA during degradation was measured at appropriate intervals according to the standard method (APHA, 1995). Debromination activity of the bacterial strain per gram was expressed and determined as μg mL^{-1} bromine released per hour according to the method reported previously (Olaniran et al., 2001).

2.5. TBBPA biodegradation and decontamination

The bacterial strain was cultured in LB culture medium for 15 h with continuous shaking, after which biodegradation behaviors of the strain under various conditions were investigated. Unless otherwise specified, biodegradation experiments were performed by inoculating 15 mL of late log growth phase cultures in 100 mL of MM and 5 mg L^{-1} TBBPA in 250-mL shake flasks at 30 °C, pH 7.5, and 200 rpm. Since low solubility of TBBPA (Kuramochi et al., 2008) under the experimental conditions used in this study, TBBPA crystals presented in the medium during growth to maintain the concentration of TBBPA near the saturating level. The pH of every sample (each sample point prepared in single flask) was adjusted to about 9.0 to ensure that all TBBPA was dissolved in the solution before analyzed by HPLC. For each batch of experiments, one parameter was varied while others were kept constant. All samples were analyzed in triplicate.

Degradation efficiency of TBBPA was determined every 12 h for 4 d by high performance liquid chromatograph (HPLC) (Agilent 1200 HPLC) equipped with an UV detector set at 230 nm. Separation was achieved on a Kromasil 100-5C18 (AKZONOBEL 250 mm × 4.6 mm id, Sweden) analytical column at ambient temperature. The eluent was a mixed solution of 80% methanol, 18% ultra-pure water, and 2% acetic acid at a flow rate of 1 mL min^{-1}.

CO_2 concentrations in the gas phase of shake flasks were determined by a gas chromatograph coupled with a flame ionization detector (GC–FID) (Shanghai, China) equipped with a methane converter. A TDX-01 column set at 150 °C was used for separation of CO_2. The temperatures of injector and detector were 180 and 230 °C, respectively. MM containing late log growth phase cultures without TBBPA served as a blank sample to deplete other sources of CO_2. Parafilm was used to seal the flask to ensure a closed system. CO_2 was drawn from the closed system by a 500 μL gas-tight locking syringe (Agilent, Australia). Henry's Law was used to calculate CO_2 concentration in the aqueous phase.

2.6. Identification of metabolites

Metabolites in the degradation solution (100 mL) were extracted three times consecutively using 30 mL mixture of dichlormethane/hexane (2:1, v/v) at pH 2.0 and 10.0, respectively, and then at pH 7.0 (1:1, v/v). The mixed extract was concentrated to 1 mL by a rotary evaporator, averaged into two 1.5 mL vials, and then completely dried with a gentle stream of high-purity

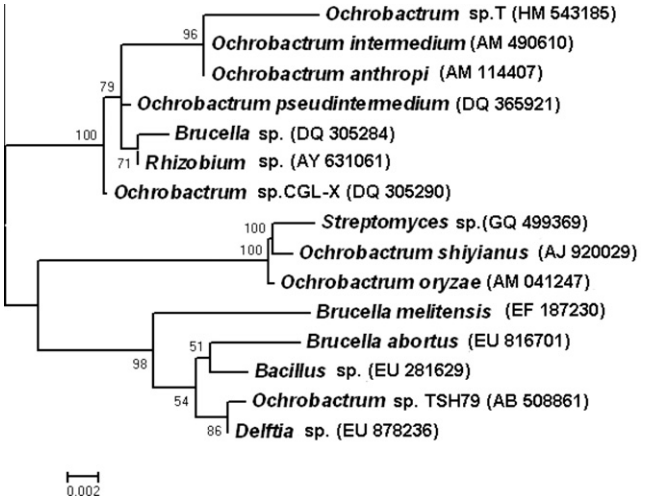

Fig. 1. Phylogenetic tree based on 16S rRNA sequence analysis (1000 bootstrap for the confidence level). The bar represents 0.002 nucleotide substitution per 100 nucleotides.

nitrogen. For direct detection of intermediates, one portion of the sample was re-dissolved in 0.1 mL hexane and then injected into the gas chromatograph (Agilent 7890) coupled with a mass selective detector (Agilent 5975C) (GC–MSD) with a DB-5 column (30 m × 0.25 mm, 0.25 μm film thickness). For indirect identification of polar metabolites, another portion was derived overnight at 25 °C using 50 μL N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) as derivation reagent and 50 μL pyridine as catalyst for GC–MS detection. Column temperature was programed as follows: 60 °C for 1 min, rose to 150 °C at 30 °C min^{-1}, to 300 °C at 5 °C min^{-1}, and then held constant for 5 min. Mass spectra were recorded in electron ionization mode at an ion source temperature of 230 °C and an electron energy of 70 eV.

3. Results and discussion

3.1. Isolation and identification of TBBPA-degrading strain

After two months of acclimatization, three single colonies with different morphological types on agar plate were isolated. A pilot study showed that without any anaerobic treatment, Strain T had the highest TBBPA degradation efficiency, so that it was chosen as the target strain. Purity of Strain T was determined by morphological uniformity of cells from a single colony on solid medium plates via microscopic observation. Strain T was identified by 16S rRNA sequence analysis. Biochemical and physiological characteristics were also investigated. Electrophoresis result of partial 16S rRNA is presented in Fig. S1. The corresponding 1432-bp gene fragment was subjected to sequence analysis. The nucleotide sequence of the 16S rRNA gene of Strain T can be found in GenBank (accession number HM543185). A phylogenetic tree based on the 16S rRNA sequences was constructed using the Neighbor–Joining method after alignment with related sequences from the GenBank database (Fig. 1). Strain T was clustered in the phylogenetic branch of family *Brucellaceae* and in the alpha subdivision of *Proteobacteria*, which comprises of the genera *Brucella*, *Mycoplana*, and *Ochrobactrum*. The closest match of the 16S rRNA sequence of Strain T was that of *Ochrobactrum anthropi* with a homology of 98.1%, followed by that of *Ochrobactrum intermedium* with a homology of 96.2%. Based on these values, the newly-isolated Strain T was classified into the genus *Ochrobactrum*.

The morphological, physiological, and biochemical characteristics of Strain T are presented in Table S1. The bacterium is Gram-negative, rod-shaped, and round-ended (0.4 − 0.8 × 1.6 − 2.2 μm) with peritrichous flagella (Fig. S2). However, it is non-motile because of the exfoliation of flagella. Colonies are round, non-pigmented, and glossy with a diameter of 1–2 mm on agar plate. Based on these findings, Strain T was designated as a novel strain belonging to *Ochrobactrum* and was named as *Ochrobactrum* sp. T.

3.2. Growth and debromination activity of Ochrobactrum sp. T

The growth characteristics of *Ochrobactrum* sp. T are important in TBBPA degradation, since bacterial cells will have different functional metabolism under different growth condition. Therefore, the dependence of debromination activity of this novel bacterial strain on cell growth was investigated. The effect of various parameters such as temperature, pH, and shaking speed on both growth and debromination activity of the bacterial strain were compared. Fig. 2a shows a plot of OD_{600} against culture time in different culture media with pH ranging from 5.0 to 9.0. Results indicate that acidic or alkaline pH inhibits cell growth. At pH 5.0 and 9.0, the lag growth phase of *Ochrobactrum* sp. T was prolonged to 5 h, with OD_{600} of only 0.82 and 0.70, respectively, after 24 h cultivation. These values are much lower than that (i.e. 1.19) at pH 7.5. Fig. 3a illustrates that debromination activity profile of *Ochrobactrum* sp. T changed markedly with changes in pH. As expected, higher values of debromination activity were obtained around pH 7.0. However, the highest debromination value of 0.12 μg mL^{-1} Br^{-1} (g wet biomass)$^{-1}$ h^{-1} was achieved at pH 6.5 instead of the optimal growth pH (i.e. 7.5) of the bacterial strain. Debromination at pH 5.5 was also much higher than that at pH 8.0, although pH 8.0 was favorable to cell's growth. The lowest debromination activities (0.02 μg mL^{-1} Br^{-1} (g wet biomass)$^{-1}$ h^{-1}) were obtained at pH 9.0. Our findings do not consistent with those of other studies, which reported that the dehalogenation process is favored in an alkaline medium (Olaniran et al., 2001). One possible explanation is that dehalogenases are composed of a group of bacterial enzymes from different strains with a diversity of structure and characteristics, so that they have varying responses to different substrates (Sfetsas et al., 2009).

Fig. 2b revealed that the optimum temperature for bacterial growth was 30 °C. OD_{600} of cultures at 40 °C, and especially 20 °C, were notably lower than those grown at other temperatures, for example 25, 30 and 35 °C. Debrominase activity increased from 0.065 to the peak value of 0.125 μg mL^{-1} Br^{-1} (g wet biomass)$^{-1}$ h^{-1} as temperature increased from 20 to 35 °C, and then decreased dramatically to 0.085 μg mL^{-1} Br^{-1} (g wet biomass)$^{-1}$ h^{-1} at 40 °C (Fig. 3b). Interestingly, debromination was much higher at 35 °C than that at 30 °C, although bacterial growth was slightly slower at 35 than that at 30 °C. Our findings are in agreement with previous reports that the optimal temperature for aerobic dechlorination by two other bacterial species was between 30 and 35 °C (Olaniran et al., 2002).

Shaking speed (aeration) can influence dissolved oxygen (DO) in the culture medium and subsequently affect cell growth. Therefore, the effect of shaking speed on strain growth was investigated. Results showed that strain growth increased gradually but not remarkably when shaking speed was increased from 60 to 200 rpm, and then increased much slowly with a further increase of shaking speed to 250 rpm (Fig. 2c). In comparison, debrominase activity was not as profoundly affected (Fig. 3c) and the maximum value was achieved at 200 instead of 250 rpm. This could be explained that debromination is not strictly aerobic, so that a higher DO level might not be favorable for enzyme activity. The results also indicate that cell growth is relatively rapid and debrominase activity is maintained at the highest level at the shaking rate of 200 rpm.

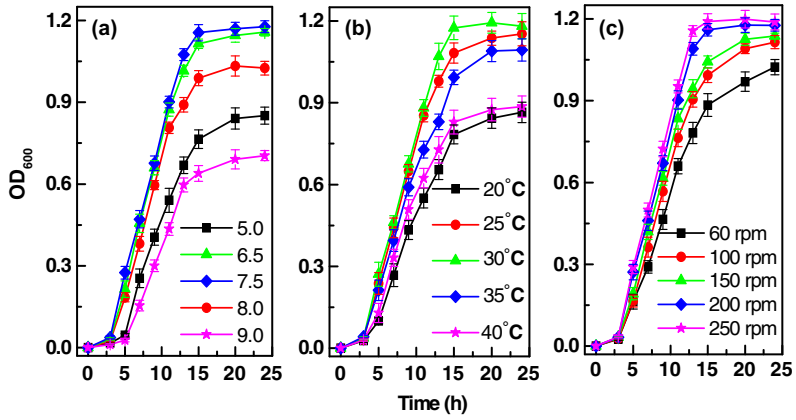

Fig. 2. Effect of pH (a), temperature (b), and rotation speed (c) on cell growth.

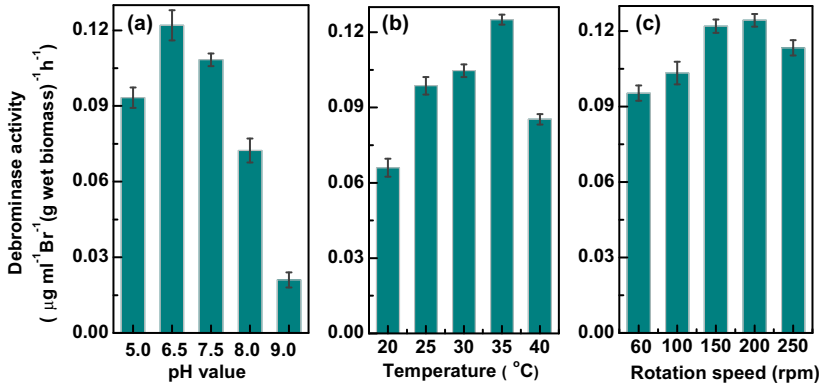

Fig. 3. Effect of pH (a), temperature (b), and rotation speed (c) on debromination activity.

3.3. Biodegradation and debromination of TBBPA

In general, debromination is the first step in the biodegradation of BFRs. Thus, biodegradation and debromination of TBBPA were compared in this study. The effects of various parameters (e.g. initial pH value, initial concentration of TBBPA, temperature, and inoculum volume) on the degradation and debromination of TBBPA were investigated at a fixed shaking speed of 200 rpm. Results showed that TBBPA biodegradation efficiency was affected by the initial concentration of TBBPA with a predominant antagonistic effect and by inoculum volume with a synergistic effect to some extent (Fig. 4a and b). Optimum pH and temperature for TBBPA degradation was 7.0 and 35 °C, respectively (Fig. 4c and d). Biodegradation efficiency decreased steadily from 91.8% to 39.3%, while half-lives increased from 29.8 to 133.2 h when initial concentration of TBBPA was increased from 3 to 25 mg L^{-1} (Table S2). Our results concur with the report that low TBBPA concentration can be easily debrominated with a half-life of 0.59 d (14.2 h) (Gerecke et al., 2006). However, TBBPA biodegradation took more than 100 days (Voordeckers et al., 2002) and up to 430 days (Nyholm et al., 2010) in previous studies. This is because high biodegradation could be achieved at a low substrate concentration (3 mg L^{-1}) with relatively high solubility at optimal condition of a high inoculum volume of 25 mL, a pH of 7.0, and a temperature of 35 °C in the present study.

The effect of various important parameters on debromination efficiency was investigated using procedures similar to those for the study of degradation efficiency (Fig. 4). Under optimized conditions for debromination of pH 7.0, 35 °C, inoculum volume of 25 mL, and TBBPA concentration of 3 mg L^{-1}, a debromination efficiency of up to 86.7% was obtained. Findings indicate that *Ochrobactrum* sp. T has a strong ability to TBBPA degradation and debromination under aerobic condition. This contradicts with the previous reports that microbial debromination occurs under anaerobic condition (Arbeli et al., 2006; Ronen and Abeliovich, 2000; Voordeckers et al., 2002). Results suggest that the bacterial strain isolated in the present study is a novel TBBPA-degrading bacterial strain.

Cell growth was studied during the biodegradation process (Fig. S3). OD_{600} in the biodegradation system was not as high as that in LB, and the log growth phase was prolonged in MM with TBBPA as the degradation substrate. It was found that OD_{600} decreased steadily with increasing substrate concentration, indicating that there may be substrate inhibition that limits TBBPA concentration at a relative small extent. Under the optimized conditions of 35 °C, pH 7.0, 25 mL inoculums, and 3 mg L^{-1} TBBPA, the bacterial strain produced the highest degree of TBBPA biodegradation and cell density reached OD_{600} of 0.387 during log growth phase within 48 h.

3.4. Mineralization of TBBPA

To further test the detoxification of TBBPA by this novel bacterial strain, the complete mineralization product, CO_2, was detected at relatively high TBBPA concentration (Fig. 5). TBBPA mineralization efficiencies increased slightly during the first 60 h, increased rapidly during the next 36 h, and then leveled off with further prolonging of reaction time. About 35.6% of carbon contents of TBBPA (5 mg L^{-1}) were mineralized and completely converted to CO_2. However, only 20.3% of mineralization efficiency could be achieved at a higher TBBPA concentration (25 mg L^{-1}). Results indicate that *Ochrobactrum* sp. T could not only debrominate efficiently, but

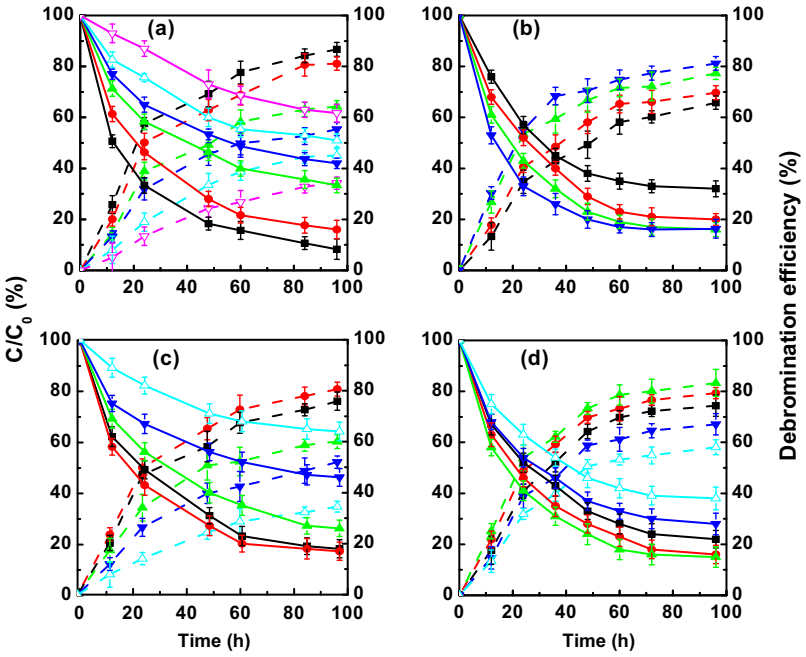

Fig. 4. Effect of (a) TBBPA concentrations (mg L^{-1}): (■) 3; (●) 5; (▲) 10; (▼) 15; (△) 20; (▽) 25; (b) inoculum volume (mL): (■) 10; (●) 15; (▲) 20; (▼) 25; (c) pH value: (■) 6.5; (●) 7.0; (▲) 7.7; (▼) 8.5; (△) 9.0; and (d) temperature: (■) 27 °C; (●) 32 °C; (▲) 35 °C; (▼) 37 °C; (△) 40 °C on TBBPA biodegradation efficiency (solid lines) and debromination efficiency (dashed lines).

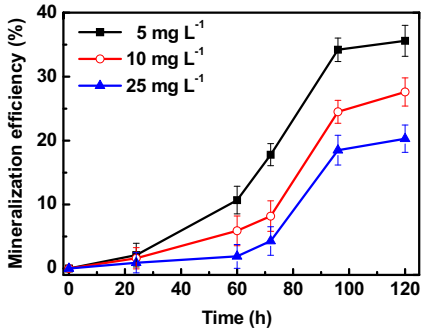

Fig. 5. TBBPA mineralization efficiency by *Ochrobactrum* sp. T.

could also completely mineralize TBBPA if degradation time was sufficiently prolonged. To the best of our knowledge, this study is the first to report that a single bacterial strain can simultaneously debrominate and mineralize TBBPA into CO_2 under aerobic condition.

3.5. Mechanisms of TBBPA aerobic metabolism

To better understand its environmental fate in water environments, the possible biodegradation pathways and mechanism of TBBPA, including its simultaneous debromination and BPA mineralization by *Ochrobactrum* sp. T, were also proposed based on GC–MS results. Two debrominated intermediates, TriBBPA and BPA, and six other ring-opening by-products were detected with and without BSTFA derivatization. Mass spectrum of the eight intermediates (Fig. S4) revealed that two tribrominated intermediates and four dibrominated intermediates could be defined by the isotopic characteristic peak m/z (M − 2):M:(M + 2):(M + 4) = 1:3:3:1 and m/z (M − 2):M:(M + 2) = 1:2:1, respectively. The chemical structure and retention time of the intermediates are summarized in Table S3. The eight degradation intermediates were identified as 2,6-dibromo-4-(propan-2-ylidene)cyclohexa-2,5-dienone (A), 2,6-dibromo-4-(2-hydroxypropan-2-yl)phenol (B), 2,6-dibromo-4-(prop-1-en-2-yl)phenol (C), 2,4,6-tribromophenol (D), 2,6-dibromo-4-(2-methoxypropan-2-yl)phenol (E), TriBBPA (F), BPA (G), and 1-(4-hydroxyphenyl) ethanone (H). Note that intermediate (C) was found only with BSTFA derivatization, while the other seven intermediates were identified both with and without BSTFA derivatization.

Based on the identified intermediates, a possible biodegradation mechanism of TBBPA by the isolated bacterial strain was proposed (Fig. 6). Two distinct pathways might occur simultaneously during biodegradation. First, TBBPA is oxidized to lose an electron. Although the oxidative form of TBBPA was not detected in the GC–MS, its scission product (A) was found in the mass spectra. This step was also established in the oxidation of TBBPA by manganese dioxide (Lin et al., 2009). Note that this step ensures the occurrence of the two subsequent two pathways occurrence. Reductive debromination occurs under anaerobic condition probably because oxygen is a competitive electron acceptor of the substrate TBBPA. This is why most previous studies could achieve debromination products only under anaerobic condition. Analysis of degradation intermediates suggests that in this biodegradation system, oxygen is used to oxidize TBBPA first and the other oxidative intermediates consume the competitive electron acceptor, thus providing the reducing power (i.e. hydrogen) and ensuring that reductive debromination of TBBPA occurs easily. These two pathways could be described as both the oxidation and the reduction routes of TBBPA, respectively. Following Route I (oxidation route), intermediates (A) and (B) are produced directly from the decomposition of oxidative TBBPA, leading to intermediate (C) and 2,6-dibromophenol (not detected) after dehydrogenation and decomposition. Then, intermediate (C) is further converted to 2,4,6-tribromophenol (2,4,6-TBP) (D), which has been reported previously (Barontini et al., 2004). Intermediate (E) also could be produced by the methylation reaction via intermediate (B). The ring cleavage product is further oxidized to CO_2 and Br^{-1} finally. In Route II (reductive debromination), intermediate BPA (G) is produced by the complete reductive debromination of TBBPA *via* TriBBPA (F). Coincidentally, the mechanism of debromination was found to be similar to the biodegradation of 2,4,6-TBP by a strain of *Ochrobactrum* sp. (Yamada et al., 2008). This indicates that *Ochrobactrum* sp. might

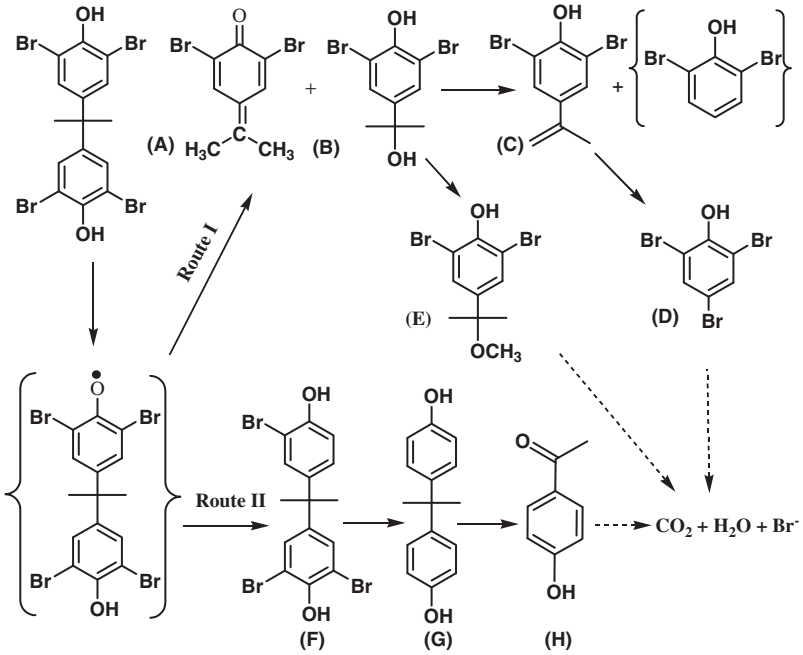

Fig. 6. Proposed aerobic TBBPA biodegradation pathway by *Ochrobactrum* sp. T.

produce an enzyme complex including dehydrogenase and debrominase, thus enabling it to simultaneously reductively debrominate and oxidatively mineralize bromide phenols. With prolonging degradation time, one benzene ring of BPA is further cleaved to produce intermediate (H); this oxidation is also achieved by aerobic degradation of BPA (Lobos et al., 1992) and another two-step process of TBBPA biodegradation by sequential anaerobic-aerobic strains (Ronen and Abeliovich, 2000). Compared with the degradation pathways of TBBPA by manganese dioxide (Lin et al., 2009), photochemical transformation (Eriksson et al., 2004) and other biodegradation ways, we obtained a series of complete debromination cleavage products in this study rather than the brominated phenols reported in previous works (Eriksson et al., 2004; Lin et al., 2009; Ravit et al., 2005; Uhnáková et al., 2009). Note that this one-step decontamination mechanism address the limitation of two-step processes by combining the first step of reductive debromination by an anaerobic system (Ronen and Abeliovich, 2000) and the second step of degradation and mineralization by an aerobic system (Voordeckers et al., 2002).

4. Conclusions

A novel bacterium, *Ochrobactrum* sp. T, was isolated from the world's largest e-waste recycling site in Guiyu, China. The bacterium exhibited excellent TBBPA biodegradation and debromination abilities. Furthermore, the carbon content of TBBPA, as well as those of its metabolites, can be converted into CO_2. Combining the results of TBBPA mineralization and intermediate detection, we proposed a mechanism for a one-step process of debromination and aerobic mineralization of TBBPA by *Ochrobactrum* sp. T.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.06.080.

References

Alaee, M., Arias, P., Sjodin, A., Bergman, A., 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environ. Int. 29, 683–689.
APHA, 1995. Standard Methods for the Examination of Water and Wastewater. 19th ed. United Book Press, Baltimore.
Arbeli, Z., Ronen, Z., Diaz-Baez, M.C., 2006. Reductive dehalogenation of tetrabromobisphenol-A by sediment from a contaminated ephemeral streambed and an enrichment culture. Chemosphere 64, 1472–1478.
Armenante, P.M., Kafkewitz, D., Lewandowski, G.A., Jou, C.J., 1999. Anaerobic–aerobic treatment of halogenated phenolic compounds. Water Res. 33, 681–692.
Barontini, F., Cozzani, V., Marsanich, K., Raffa, V., Petarca, L., 2004. An experimental investigation of tetrabromobisphenol A decomposition pathways. J. Anal. Appl. Pyrolysis 72, 41–53.
Brenner, A., Mukmenev, I., Abeliovich, A., Kushmaro, A., 2006. Biodegradability of tetrabromobisphenol A and tribromophenol by activated sludge. Ecotoxicology 15, 399–402.
Buchanan, P., Schubert, H.W., Holt, J.K., 1984. Bergey's Manual of Determinative Bacteriology. The Williams and Wilklins Company, Baltimore.
Cariou, R., Antignac, J.P., Zalko, D., Berrebi, A., Cravedi, J.P., Maume, D., Marchand, P., Monteau, F., Riu, A., Andre, F., Le bizec, B., 2008. Exposure assessment of French women and their newborns to tetrabromobisphenol-A: occurrence measurements in maternal adipose tissue, serum, breast milk and cord serum. Chemosphere 73, 1036–1041.
Darnerud, P.O., 2003. Toxic effects of brominated flame retardants in man and in wildlife. Environ. Int. 29, 841–853.
de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46, 583–624.
de Wit, C.A., Herzke, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment – Trends and new candidates. Sci. Total Environ. 408, 2885–2918.
Eriksson, J., Rahm, S., Green, N., Bergman, A., Jakobsson, E., 2004. Photochemical transformations of tetrabromobisphenol A and related phenols in water. Chemosphere 54, 117–126.
Gerecke, A.C., Giger, W., Hartmann, P.C., Heeb, N.V., Kohler, H.P.E., Schmid, P., Zennegg, M., Kohler, M., 2006. Anaerobic degradation of brominated flame retardants in sewage sludge. Chemosphere 64, 311–317.
Guo, J.T., 2007. The Identification and Atlas of Microorganism. People's Medical Publishing House, Beijing.
Haggblom, M.M., Bossert, I.D., 2003. Dehalogenation–Microbial processes and environmental applications. Kluwer Academic Publishers, Norwell, Massachusetts.

Han, S.K., Bilski, P., Karriker, B., Sik, R.H., Chignell, C.F., 2008. Oxidation of flame retardant tetrabromobisphenol A by singlet oxygen. Environ. Sci. Technol. 42, 166–172.

Horikoshi, S., Miura, T., Kajitani, M., Horikoshi, N., Serpone, N., 2008. Photodegradation of tetrahalobisphenol-A (X=Cl, Br) flame retardants and delineation of factors affecting the process. Appl. Catal. B: Environ. 84, 797–802.

Iasur-Kruh, L., Ronen, Z., Arbeli, Z., Nejidat, A., 2010. Characterization of an enrichment culture debrominating tetrabromobisphenol A and optimization of its activity under anaerobic conditions. J. Appl. Microbiol. 109, 707–715.

Janssen, D.B., Oppentocht, J.E., Poelarends, G.J., 2001. Microbial dehalogenation. Curr. Opin. Biotechnol. 12, 254–258.

Kuramochi, H., Kawamoto, K., Miyazaki, K., Nagahama, K., Maeda, K., Li, X.-W., Shibata, E., Nakamura, T., Sakai, S.-I., 2008. Determination of physicochemical properties of tetrabromobisphenol A. Environ. Toxicol. Chem. 27, 2413–2418.

Li, M.T., Hao, L.L., Sheng, L.X., Xu, J.B., 2008. Identification and degradation characterization of hexachlorobutadiene degrading strain *Serratia marcescens* HL1. Bioresour. Technol. 99, 6878–6884.

Lin, K., Liu, W., Gan, J., 2009. Reaction of tetrabromobisphenol A (TBBPA) with manganese dioxide: kinetics, products, and pathways. Environ. Sci. Technol. 43, 4480–4486.

Lobos, J.H., Leib, T.K., Su, T.M., 1992. Biodegradation of bisphenol A and other bisphenols by a Gram-negative aerobic bacterium. Appl. Environ. Microbiol. 58, 1823–1831.

McHugh, B., Poole, R., Corcoran, J., Anninou, P., Boyle, B., Joyce, E., Foley, M.B., McGovern, E., 2010. The occurrence of persistent chlorinated and brominated organic contaminants in the European eel (*Anguilla anguilla*) in Irish waters. Chemosphere 79, 305–313.

Nyholm, J.R., Lundberg, C., Andersson, P.L., 2010. Biodegradation kinetics of selected brominated flame retardants in aerobic and anaerobic soil. Environ. Pollut. 58, 2235–2240.

Olaniran, A., Okoh, A., Ajisebutu, S., Golyshin, P., Babalola, G., 2002. The aerobic dechlorination activities of two bacterial species isolated from a refuse dumpsite in Nigeria. Int. Microbiol. 5, 21–24.

Olaniran, A.O., Babalola, G.O., Okoh, A.I., 2001. Aerobic dehalogenation potentials of four bacterial species isolated from soil and sewage sludge. Chemosphere 45, 45–50.

Ravit, B., Ehrenfeld, J.G., Hagblom, M.M., 2005. Salt marsh rhizosphere affects microbial biotransformation of the widespread halogenated contaminant tetrabromobisphenol-A (TBBPA). Soil Biol. Biochem. 37, 1049–1057.

Reistad, T., Mariussen, E., Fonnum, F., 2005. The effect of a brominated flame retardant, tetrabromobisphenol-A, on free radical formation in human neutrophil granulocytes: the involvement of the MAP kinase pathway and protein kinase C. Toxicol. Sci. 56, 95–104.

Ronen, Z., Abeliovich, A., 2000. Anaerobic–aerobic process for microbial degradation of tetrabromobisphenol A. Appl. Environ. Microbiol. 66, 2372–2377.

Sfetsas, C.C., Milios, L., Skopelitou, K., Venieraki, A., Todou, R., Flemetakis, E., Katinakis, P., Labrou, N.E., 2009. Characterization of 1,2-dibromoethane-degrading haloalkane dehalogenase from *Bradyrhizobium japonicum* USDA110. Enzyme Microb. Technol. 45, 397–404.

Stasinakis, A.S., Kordoutis, C.I., Tsiouma, V.C., Gatidou, G., Thomaidis, N.S., 2010. Removal of selected endocrine disrupters in activated sludge systems: effect of sludge retention time on their sorption and biodegradation. Bioresour. Technol. 101, 2090–2095.

Uhnáková, B., Petrícková, A., Biedermann, D., Homolka, L., Vejvoda, V., Bednár, P., Papoušková, B., Šulc, M., Martínková, L., 2009. Biodegradation of brominated aromatics by cultures and laccase of *Trametes versicolor*. Chemosphere 76, 826–832.

Voordeckers, J.W., Fennell, D.E., Jones, K., Haggblom, M.M., 2002. Anaerobic biotransformation of tetrabromobisphenol A, tetrachlorobisphenol A, and bisphenol A in estuarine sediments. Environ. Sci. Technol. 36, 696–701.

Xie, Z., Ebinghaus, R., Lohmann, R., Heemken, O., Caba, A., Pumann, W., 2007. Trace determination of the flame retardant tetrabromobisphenol A in the atmosphere by gas chromatography-mass spectrometry. Anal. Chim. Acta 584, 333–342.

Yamada, T., Takahama, Y., Yamada, Y., 2008. Biodegradation of 2,4,6-Tribromophenol by *Ochrobactrum* sp. Strain TB01. Biosci. Biotechnol. Biochem. 72, 1264–1271.

Zhang, X.L., Luo, X.J., Chen, S.J., Wu, J.P., Mai, B.X., 2009. Spatial distribution and vertical profile of polybrominated diphenyl ethers, tetrabromobisphenol A, and decabromodiphenylethane in river sediment from an industrialized region of South China. Environ. Pollut. 157, 1917–1923.