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BIOTRANSFORMATION OF THE FLAME RETARDANT TETRABROMOBISPHENOL-A (TBBPA) BY FRESHWATER MICROALGAE

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Abstract: Tetrabromobisphenol-A (TBBPA) is the most widely used brominated flame retardant. However, little is known about its biotransformation by algae in aquatic environments. The authors investigated transformations of TBBPA by 6 freshwater green microalgae and identified its transformation products. Transformation experiments were conducted under axenic conditions in a laboratory for 10 d. The results showed that TBBPA could be transformed by the selected microalgae, with nearly complete removal by *Scenedesmus quadricauda* and *Coelastrum sphaericum* following 10-d incubation. Five transformation products were positively identified by mass spectrometry: TBBPA sulfate, TBBPA glucoside, sulfated TBBPA glucoside, TBBPA monomethyl ether, and tribromobisphenol-A. The mechanisms involved in the biotransformation of TBBPA include sulfation, glucosylation, *O*-methylation, and debromination, which could be an important step for its further degradation. This suggests that microalgae can play an important role in the fate of TBBPA in aquatic environments. The present study is the first report on algal transformation of TBBPA, and the proposed transformation products could have significant environmental implications. *Environ Toxicol Chem* 2014;33:1705–1711. © 2014 SETAC

Keywords: Tetrabromobisphenol-A (TBBPA) Algae Degradation Transformation Metabolite

INTRODUCTION

Brominated flame retardants have been widely used in recent decades in various consumer and industrial products such as plastics, textiles, and electronics to improve flame resistance [1]. Tetrabromobisphenol-A (TBBPA) is currently the most widely used brominated flame retardant [2,3]. The annual global consumption of TBBPA was estimated to be more than 170 000 tons [4]. Owing to its similar chemical structure to the thyroid hormone thyroxine, TBBPA has been suspected to be an endocrine-disrupting chemical [5]. It has exhibited some estrogen-like properties in in vitro tests using human cancer cells and in vivo test with mice and multiple hormonal activities in mosquitofish [6–8]. Despite its potential risks to wildlife and human health, TBBPA is still unregulated worldwide.

Tetrabromobisphenol-A can be released into the environment during the production, usage, and disposal of products. It has been detected frequently in various environmental matrices including air, dust, sewage sludge, wastewater, surface water, and sediment [9–15]. As a result of its wide detection, it has become an increasing concern to the general public and the scientific community. Therefore, it is essential to understand its fate in the environment.

The aquatic environment is an important sink for TBBPA resulting from the discharge of various wastewaters [13,16]; and under elevated pH, TBBPA can be quite soluble [17]. Limited laboratory studies have shown potential transformation and/or degradation of TBBPA in the aquatic environment [18–21]. Tetrabromobisphenol-A in water can be photodegraded via dehalogenation and benzene ring cleavage [18]. Feng et al. [19] found that TBBPA could be effectively transformed by the naturally occurring lactase enzyme from *Trametes versicolor*. Tetrabromobisphenol-A is degraded in both anaerobic and aerobic conditions [20–23]. Bacterial degradation studies showed that TBBPA could be reduced under anoxic conditions to dibromobisphenol-A and further to bisphenol-A (BPA) [20,21].

Algae are essential organisms that distribute widely in aquatic environments such as rivers and lakes. It is known that algae can play an important role in the degradation or removal of inorganic and organic contaminants [24,25]. Some algal species have been shown to transform some phenolic compounds such as BPA [26,27] and chlorophenol [28]. Considering its similar chemical structure to BPA, TBBPA may also be transformed or degraded by algae in the aquatic environment. To the best of our knowledge, there is still no report on the metabolism and transformation of TBBPA by algae as well as its metabolites. Thus, the aim of the present study was to investigate transformations of TBBPA by microalgae in the laboratory and to identify potential transformation products. Six freshwater green microalgae species, which are common inhabitants of freshwater lakes and rivers, were selected for algal transformation experiments. The results will improve our understanding of the fate of TBBPA in the aquatic environment and the potential roles of freshwater microalgae in the degradation and transformation of this brominated flame retardant.

MATERIALS AND METHODS

Chemicals and materials

Tetrabromobisphenol-A (CAS number 79-94-7; purity 99%) was purchased from Dr. Ehrenstorfer. A stock solution of TBBPA (1800 μM) was prepared in dimethyl sulfoxide. Mineral salts (analytical grade) used for preparation of algal growth medium were obtained from Tianjin. All the organic solvents used in extraction and analysis (acetonitrile, methanol,

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methylene dichloride, and ethyl acetate) were high-performance liquid chromatography (HPLC)–grade and purchased from Merck or CNW. Ultrapure water was produced by a Milli-Q apparatus from Millipore. Oasis HLB cartridges (500 mg) were obtained from Waters. All glassware was hand-washed with tap water, rinsed with methanol and HPLC-grade water, and baked at 450 °C for more than 4 h before use when necessary.

Microalgae and culture medium

Six algal species (numbered as A1–A6) were used in the present study: *Pseudokirchneriella subcapitata* (A1), *Scenedesmus acuminatus* (A2), *Scenedesmus quadricauda* (A3), *Coelastrum sphaericum* (A4), *Scenedesmus obliquus* (A5), and *Chlorella pyrenoidosa* (A6). These algal species were obtained from the Collection of the Institute of Hydrobiology, Chinese Academy of Sciences and the cultures were unialgal and bacteria-free. The growth medium was BG11 medium, which contains the following chemicals: $NaNO_3$, 1.5 g/L; $K_2HPO_4 \cdot 3H_2O$, 0.04 g/L; $MgSO_4 \cdot 7H_2O$, 0.075 g/L; $CaCl_2 \cdot 2H_2O$, 0.036 g/L; Na_2CO_3, 0.02 g/L; citric acid, 0.006 g/L; ferric ammonium citrate, 0.006 g/L; EDTA, 0.001 g/L; A5 + Co solution (1 mL/L) that consists of H_3BO_3, 2.86 g/L, and $MnCl_2 \cdot 4H_2O$, 1.81 g/L; $ZnSO_4 \cdot 7H_2O$, 0.222 g/L; $CuSO_4 \cdot 5H_2O$, 0.079 g/L; $Na_2MoO_4 \cdot 2H_2O$, 0.390 g/L; and $Co(NO_3)_2 \cdot 6H_2O$, 0.0494 g/L [29].

Algae were cultivated in 150 mL BG11 medium in flasks, which were placed in a shaking incubator with a speed of 150 rpm at 25 °C. Illumination was provided by white fluorescent light at 3000 lux with a light:dark cycle of 12 h:12 h. Before a biotransformation experiment, algal cells were collected by centrifuging the culturing solution at the exponential growth phase, and then the harvested pellets were washed 3 times with Milli-Q water and resuspended in 100 mL Milli-Q water for later use.

Algal growth inhibition tests

Preliminary algal growth inhibition tests (72 h) were performed for the 6 algal species to TBBPA at a concentration of 0.8 μM, which was used in the biotransformation experiment. No significant growth inhibition was found for all 6 species; thus, detailed tests were performed for only 2 species *S. obliquus* (A5) and *C. pyrenoidosa* (A6) with a range of exposure concentrations of TBBPA (in 0.05% dimethyl sulfoxide): 0 μM, 0.3 μM, 0.9 μM, 1.8 μM, 3.7 μM, and 9.2 μM. The end points for the tests were algal cell density (24 h and 72 h) and photosynthetic pigment contents (120 h). Growth inhibition tests were performed in 250-mL Erlenmeyer flasks containing 150 mL with an initial algal density of approximately 10^5 cells/mL, incubated in an orbital shaker at 150 rpm and 25 ± 1 °C. Light was provided by continuous cool white fluorescent lamps at 3000 lux with a light:dark cycle of 12 h:12 h. Constant shaking of 150 rpm was maintained during this test period.

Algal cell density was indicated by optical density (OD) at 650 nm (OD_{650}; *S. obliquus*) or 680 nm (OD_{680}; *C. pyrenoidosa*), which was measured using a microplate reader (BMG Lab Technologies). The relationships between algal density and OD_{650} or OD_{680} are shown in Equations 1 and 2, respectively [25].

$$C.\ pyrenoidosa \text{ cell density } (10^5 \text{ cells/mL}) = 334.74 \times OD_{680} - 6.6694 \ (R = 0.997) \quad (1)$$

$$S.\ obliquus \text{ cell density } (10^5 \text{ cells/mL}) = 78.953 \times OD_{650} - 1.9331 (R = 0.995) \quad (2)$$

To evaluate photosynthetic pigment contents, algal cells (5 mL) were harvested by centrifugation (9169 *g*, 5 min). Algal cells were frozen (–20 °C) for 20 min and thawed (25 °C) for 5 min, and this procedure was repeated 3 times. Then, algal cells were frozen (–20 °C) overnight until the cell walls were broken. Processed algae were extracted by 5 mL of 95% ethyl alcohol at 80 °C for 2 min and an additional 6 h at room temperature before centrifugation (9169 *g*, 5 min) to remove algal cells [25]. Chlorophyll *a*, chlorophyll *b*, and carotenoid contents (C_A, C_B, and C_K) in the supernatant were estimated based on measurement of the absorbance at 665 nm, 649 nm, and 470 nm using a Lambda 850 spectrophotometer (PerkinElmer). Photosynthetic pigment contents were calculated from the absorbance at 665 nm, 649 nm, and 470 nm according to Equations 3, 4, and 5, respectively [30]:

$$C_A = 13.95\, A_{665} - 6.88\, A_{649} \quad (3)$$

$$C_B = 24.96\, A_{649} - 7.32\, A_{665} \quad (4)$$

$$C_K = (1000\, A_{470} - 2.05\, C_A - 114.8\, C_B)/245 \quad (5)$$

Biotransformation experiment

The biotransformation experiment of TBBPA by the 6 algae (A1–A6) was conducted individually under strict axenic conditions. The initial TBBPA concentration (0.8 μM) was achieved by adding certain amounts of its stock solution into the BG11 medium. Algal cells were inoculated at an initial density of approximately 10^5 cells/mL. Culturing was performed in 250-mL Erlenmeyer flasks containing 150 mL culture volume at 25 ± 1 °C. Light was provided by continuous cool white fluorescent lamps at 3000 lux with a dark/light cycle of 12 h/12 h. The experiment was conducted for 240 h, and constant shaking of 150 rpm was maintained during this period. The algae-free control was also included to measure any abiotic loss of TBBPA during the experiment. All treatments were carried out in triplicate. Concentrations of TBBPA in the experimental solutions were monitored at different time intervals (0 h, 24 h, 48 h, 72 h, 120 h, 168 h, and 240 h) during incubation. Separate batches of culture flasks with the same treatments were also set up for the identification of transformation products.

Biotransformation of TBBPA in natural water was also assessed using 2 algal species (A3 and A4) based on the results of the above experiment in BG11 medium. The transformation experiment was conducted by replacing BG11 incubation medium with surface water from the Pearl River at Guangzhou section, South China. The river water had pH 7.72, dissolved organic content 3.6 mg/L, and conductivity 312 μs/cm. Before the experiment, the river water was filtered through glass fiber filters (Whatman GF/F; 0.7-μm effective pore size) to remove particulates and then through a sterile membrane filter to remove microorganisms (0.45-μm pore diameter) with a vacuum filtration apparatus under sterile conditions. Incubation was conducted for 168 h, and other experimental conditions were the same as described in the experiment using BG11 medium. Controls without algal species were also included in this experiment. Concentrations of TBBPA were also monitored at different time intervals (0 h, 72 h, 120 h, and 168 h) during incubation.

Analysis of TBBPA

To monitor the concentrations of TBBPA in biotransformation experiments, exactly 1.5 mL of the culture solution was sampled each time and centrifuged at 9168 *g* for 5 min to remove algal cells, and then 1 mL of the supernatant was extracted with 0.3 mL methylene dichloride 3 times. The extracts were pooled together and reconstituted in the initial mobile phase for instrumental analysis. Tetrabromobisphenol-A in the extracts was analyzed by HPLC coupled with diode-array detection (Agilent 1200 series). Chromatographic separation was performed on a Zorbax Eclipse reversed-phase XDB-C18 column (4.6×150 mm, 5 μm). The column temperature was set at 30 °C. The mobile phase was 70% acetonitrile and 30% water at a flow rate of 1 mL/min. The injection volume was 20 μL, and the ultraviolet wavelength used for detection was 210 nm. The limit of detection for TBBPA was 0.037 μM. The aqueous extraction recoveries for TBBPA were $99 \pm 2\%$. The remaining algal cells from each sample were also collected and extracted for measuring potential sorption. Because the target compound TBBPA was not detected or was detected only at trace levels in algal cells. no further analysis was carried out.

Analysis of transformation products

The experimental medium (150 mL) at different time intervals for transformation product identification was filtered through glass fiber filters (Whatman GF/F; 0.7-μm effective pore size) to remove algal cells. The water phase was collected and extracted by solid phase extraction with an Oasis HLB cartridge. Dichloromethane, ethyl acetate, and methanol were used sequentially as the elution solvents, with a volume of 4 mL individually. The combined extract from each sample was concentrated and dried under a gentle nitrogen stream, and the final extract was reconstituted in methanol for analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

The LC-MS/MS instrument used in the analysis of transformation products was an Agilent 1200 LC-Agilent 6460 QQQ with an electrospray ionization (ESI) source. Chromatographic separation was performed on an Agilent Zorbax SB-C18 column ($100\text{ mm} \times 3\text{ mm}$, 1.8 μm) with a precolumn filter (2.1 mm, 0.2 μm). The mobile phase consisted of methanol and water at a flow rate of 0.3 mL/min. The gradient program used in the analysis was as follows: methanol increased gradually from 30% (0 min) to 80% (25 min) and maintained at 80% for 15 min. The injection volume of each sample was 10 μL. Scan mode was used to search for suspected parent ions in negative ionization mode over the mass to charge ratio (*m/z*) range from 50 to 1200, with the fragmentor voltage at 135 V. Then, a product ion scan was performed based on the selected parent ions to obtain further information of each transformation product, with the fragmentor voltage and collision energy at 200 V and 50 V, respectively.

The identification of transformation products of TBBPA was further confirmed by an Agilent 6540 Q-TOF time-of-flight mass spectrometer (TOF-MS). Accurate molecular weight was obtained for each product compound, which was identified by its isotope abundance score and isotope spacing score with Agilent MassHunter software. The TOF-MS instrument conditions are given in the Supplemental Data.

Statistical analysis

Statistical analysis was carried out using the SPSS 16.0 package. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to check the significance of treatments. The level of significance was set at 5%. Data are presented as mean ± standard deviation unless otherwise stated.

RESULTS

Algal growth inhibition

Preliminary tests showed no significant growth inhibition of the selected 6 algal species to TBBPA at 0.8 μM, which was the concentration used in algal transformation experiments. Further tests for the end point of algal density showed that the growth of both species *S. obliquus* (A5) and *C. pyrenoidosa* (A6) was inhibited by TBBPA only at the highest concentration (9.2 μM) (Supplemental Data, Figure S1). For the end point of photosynthetic pigment contents, the lowest observed inhibition effects were at a TBBPA concentration of 3.7 μM (Supplemental Data, Figure S2). Therefore, no significant algal growth inhibition was expected for TBBPA at concentrations lower than 3.7 μM (ANOVA, $p < 0.05$).

Biotransformation of TBBPA

The biotransformation experiment in BG11 medium demonstrated that TBBPA was transformed or degraded by the selected 6 microalgae after incubation for 240 h (Figure 1). No significant losses were observed for the sterile controls, suggesting that the losses in the nonsterile treatments were the results of algal factors. Within the incubation period, the 6 algae showed different behaviors in the TBBPA transformation process. Significant changes were observed for most treatments after 168 h and 240 h (Figure 1). Almost complete removal of TBBPA in the culture medium after 240 h incubation was found in the treatments by 2 algal species, *S. quadricauda* (A3) and *C. sphaericum* (A4). The other 4 algal species, *P. subcapitata* (A1), *S. acuminatus* (A2), *S. obliquus* (A5), and *C. pyrenoidosa* (A6), removed 60% to 90% of TBBPA after 240-h incubation. Algae showed increasing growth during the incubation period, with OD_{420} values changing from approximately 0.06 at 0 h to 0.3 at 240 h for the selected algal species.

Although *S. quadricauda* (A3) and *C. sphaericum* (A4) grew well in the river water, TBBPA transformation under these

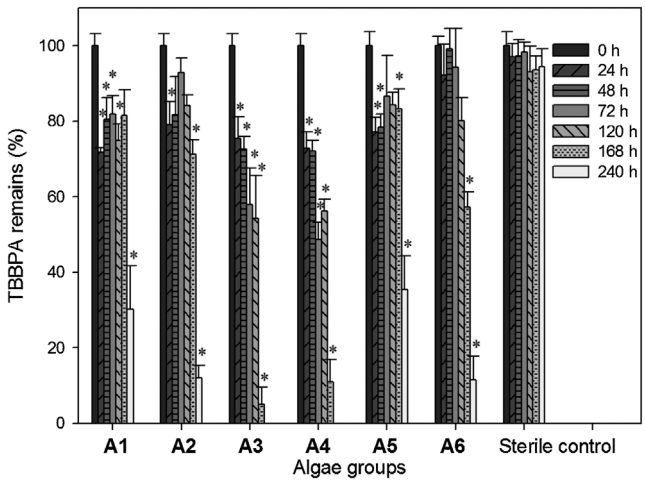

Figure 1. Percentages (mean ± standard deviation, $n = 3$) of tetrabromobisphenol-A (TBBPA) remaining in the medium after incubation for various time intervals. Treatments: 6 algal species, *Pseudokirchneriella subcapitata* (A1), *Scenedesmus acuminatus* (A2), *Scenedesmus quadricauda* (A3), *Coelastrum sphaericum* (A4), *Scenedesmus obliquus* (A5), and *Chlorella pyrenoidosa* (A6), and sterile controls without algae. * Values significantly different ($p < 0.05$) compared with the sterile control at each sampling time.

Table 1. Identification of biotransformation products of TBBPA by liquid chromatography tandem mass spectrometry

Peak	Compound name	Molecular weight	Retention time (min)	Molecular ion	Characteristic product ions
	TBBPA	544	28.35	542.7	447.6, 419.4, 290.5, 80.9
P1	TBBPA sulfate	624	12.97	622.6	541.6, 444.9, 416.7, 290.0, 80.9
P2	TBBPA glucoside	706	22.98	704.7	541.3, 525.5, 444.7
P3	Sulfated TBBPA glucoside	786	7.16	784.7	541.6, 525.0
P4	TBBPA monomethyl ether	558	35.84	556.7	525.2, 80.7
P5	Tribromobisphenol-A	464	26.58	462.8	N/A

TBBPA = tetrabromobisphenol-A; N/A = no product ion scan was performed.

conditions was slower. Compared with the removal of 90% and 85% in the BG11 medium, the 2 algal species removed 88% and 5% of TBBPA in the river water during 168 h of incubation, respectively. Thus, a slower biotransformation is expected for TBBPA in the natural aquatic environment.

Identification of transformation products

Biotransformation products of TBBPA were identified by LC-ESI-MS/MS, based on their LC retention times, molecular weights, isotope patterns, and mass spectra interpretation. The characteristic ions for TBBPA and 5 products (P1–P5) are presented in Table 1. The parent compound TBBPA and products P1 to P4 showed the same characteristic bromine isotope patterns, suggesting that these compounds have 4 bromines in their molecules (Figures 2–4). As the product ion scan showed, products P1 to P4 have fragment ions at *m/z* 541.6 and/or *m/z* 525.0 [541.6-OH], suggesting that the basic chemical structure of TBBPA was kept in the transformation products (Table 1). Higher molecular weights for the 4 products P1 to P4 than that of TBBPA imply addition of a certain functional group(s) in the basic chemical structure of TBBPA as a result of the algal transformation process. Product P1 (MW-H 622.6) was tentatively identified as the sulfated TBBPA, since SO_3H accounts for the additional *m/z* 80 (Figure 3). Product P2 (MW-H 704.7) was tentatively identified as the monoglucosylated derivative of TBBPA because of the addition of a glucosyl group (Figure 3). Product P3 (MW-H 784.7) was identified as the glucosylated derivative of TBBPA sulfate (Figure 3). Product P4 (MW-H 556.7) was found to be the O-methylated derivative of TBBPA (Figure 3). Product P5 (MW-H 462.8) had characteristic bromine isotope patterns showing 3 bromines in its molecule and was identified as tribromobisphenol-A. Accurate molecular weights of TBBPA and its 5 transformation products were obtained by TOF-MS (Supplemental Data, Figure S3), thus further confirming the identities of the products P1 to P5. None of these products were detected in the abiotic control treatment.

According to the LC retention times, TBBPA and its 5 products, P1 to P5, had the following order of decreasing polarity: P3 > P1 > P2 > P5 > TBBPA > P4 (Figures 2 and 4). This polarity order is in accordance with the tentative identification of these products. Clearly, the addition of sulfate and glucose groups to TBBPA in products P1 to P3 would increase their polarity and subsequently shorten their retention time in a reverse-phase chromatogram. As a result of the loss of a bromine atom in the TBBPA molecule, P5 has an increased polarity compared with its parent compound, TBBPA. Product P4 would have a lower polarity and a longer retention time than TBBPA because it was an *O*-methylated derivative of TBBPA.

It should be noted that the 6 algae produced different transformation products. Products P1, P2, and P3 were detected in the treatments with *P. subcapitata* (A1), *S. quadricauda* (A3), and *C. sphaericum* (A4), as well as *S. obliquus* (A5) and *C. pyrenoidosa* (A6); P4 was found only in the treatment with *S. acuminatus* (A2). This experiment demonstrated that TBBPA could be transformed by algae via sulfation, glucosylation, and *O*-methylation (Figure 5). In addition, it should be mentioned that product P5 (tribromobisphenol-A) was detected in all the algae treatments, although it was impossible to quantify tribromobisphenol-A because the peaks were rather small. This suggests that debromination is also a mechanism for algal transformation of TBBPA.

DISCUSSION

The present study found that the main biotransformation mechanisms for TBBPA were via sulfation, glucosylation, and *O*-methylation, as well as debromination. The present study is the first report on the algal biotransformation of TBBPA. Considering its global use, great efforts have been made in recent years to study of TBBPA degradation in the environment. Tetrabromobisphenol-A was previously found to be degraded or transformed by bacteria [21,31,32], by photochemical processes [17,33], and by reaction with manganese oxide [34]. The present study found that among the 6 selected algal species *S. quadricauda* (A3) and *C. sphaericum* (A4) were the most efficient and achieved almost complete TBBPA removal within 10 d, whereas the other 4 species also showed moderate removal (Figure 1). Previous studies reported that algae could remove contaminants by various processes such as adsorption, absorption, biotransformation, and biodegradation [26,27,35]. Sorption should play a certain role in the algal biotransformation of TBBPA. But based on the results from the present study, biotransformation was the dominant process in TBBPA removal with formation of its biotransformation products (P1–P5).

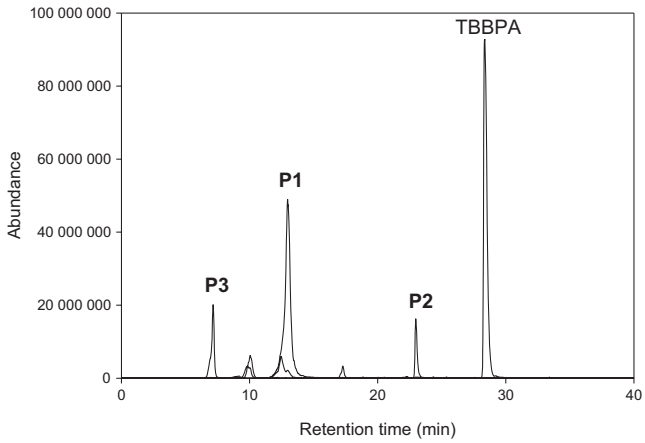

Figure 2. Extracted ion chromatogram of tetrabromobisphenol-A (TBBPA) and its products (P1–P3) by the microalga *Pseudokirchneriella subcapitata* after 10-d incubation.

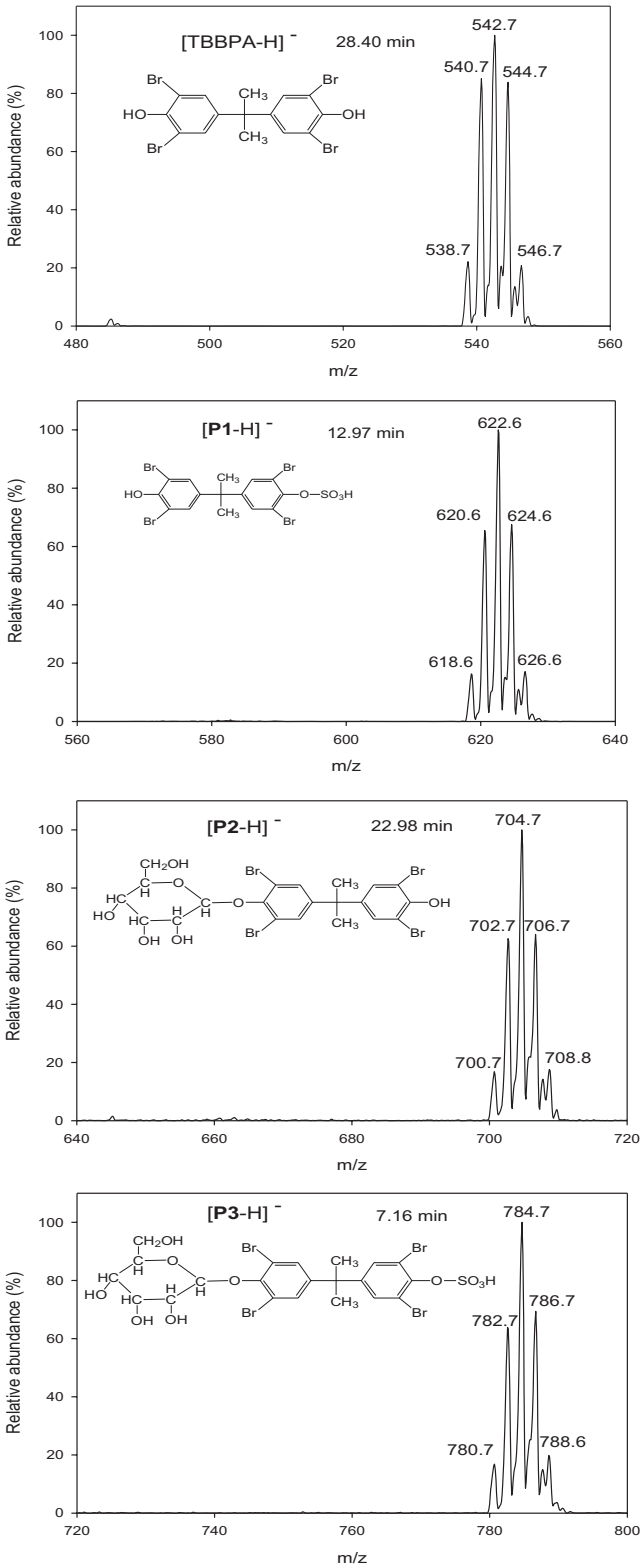

Figure 3. Mass spectra of tetrabromobisphenol-A (TBBPA) and its transformation products (P1–P3) in liquid chromatography tandem mass spectrometric analysis in electrospray ionization mode.

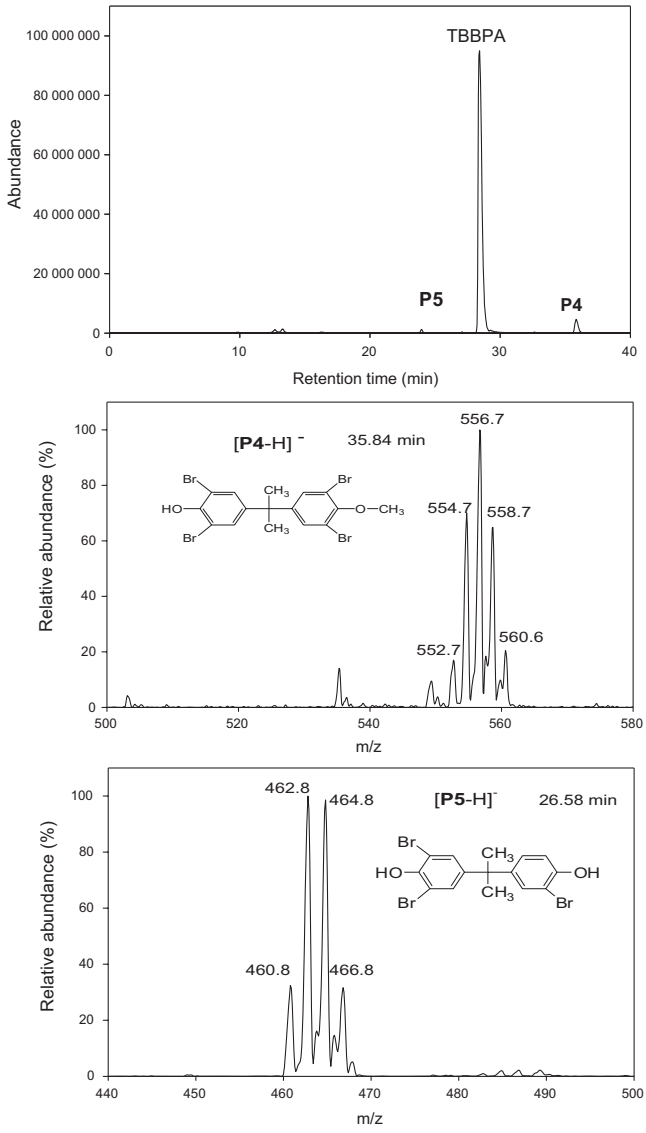

Figure 4. Extracted ion chromatogram of tetrabromobisphenol-A (TBBPA) and its transformation products (P4 and P5) by *Scenedesmus acuminatus* after 10-d incubation and mass spectra of P4 and P5.

The mechanisms involved in the algal biotransformation of TBBPA included sulfation, glucosylation, *O*-methylation, and debromination (Figure 5). As we know, sulfation and glucuronidation are 2 important metabolic pathways in mammals [36]. For example, in toxicokinetic studies of TBBPA in rats and humans, sulfated and glucuronidated conjugates were detected as the main products in blood and urine [36]. In fact, some algae also have sulfating and glycosylating abilities for organic contaminants [27,37]. A previous study demonstrated that the microalgae *Chlorococcum* sp. and *Scenedesmus* sp. could transform α-endosulfan to endosulfan sulfate, which was further degraded in liquid medium [37]. As for glucosylation, Nakajima et al. [27] reported that BPA was metabolized to BPA glycosides by *P. subcapitata*, *Scenedesmus acutus*, *S. quadricauda*, and *Coelastrum reticulatum*; and these metabolites were then released into the culture medium. Thus, it is reasonable to understand the glucosylation of TBBPA because it has a similar chemical structure to BPA. The present study found TBBPA sulfate (P1) and TBBPA glucoside (P2) as well as sulfated TBBPA glucoside (P3) in the medium. Products P1 and P2 were the main degradation products in the treatments with *P. subcapitata* (A1), *S. quadricauda* (A3), and *C. sphaericum* (A4).

Another transformation mechanism proposed in the present study is *O*-methylation of TBBPA by algae. A mono-methyl

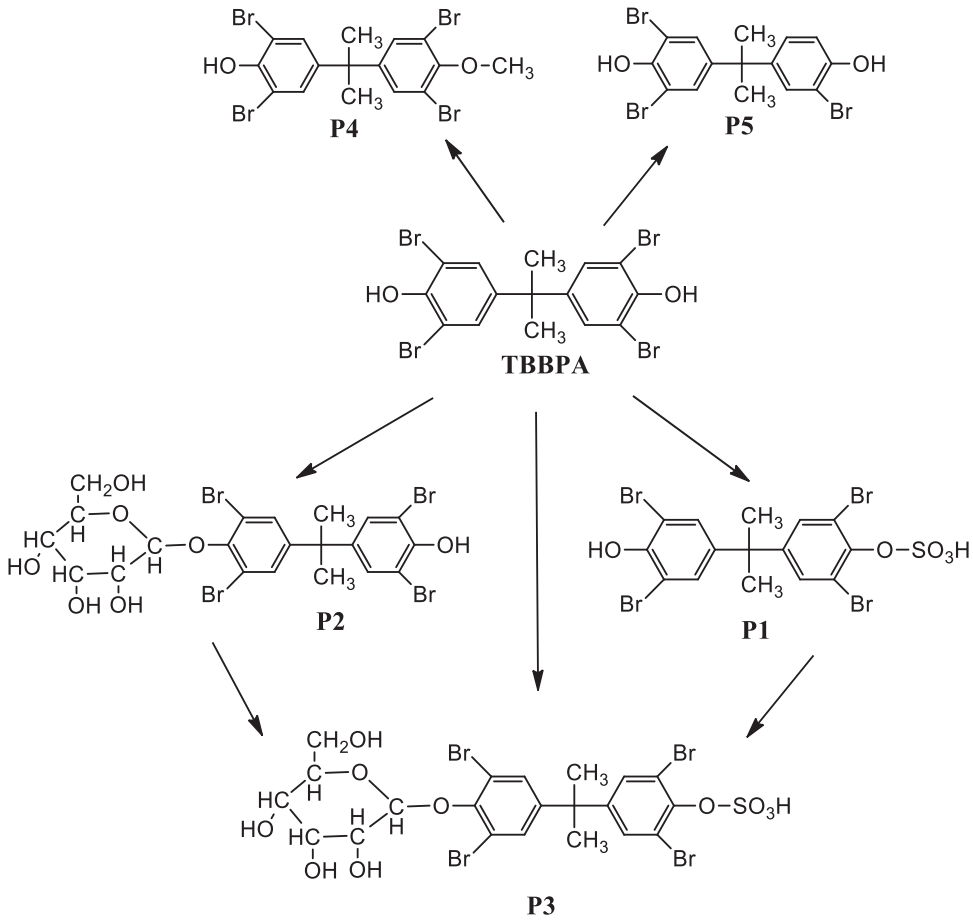

Figure 5. Proposed pathways for tetrabromobisphenol-A (TBBPA) transformation by freshwater microalgae.

ether derivative of TBBPA (P4) was detected in an algal species in the present study. A variety of bacteria and fungi have been demonstrated to be capable of mediating *O*-methylation of halogenated phenols, including TBBPA [38–40]. Under anoxic conditions, TBBPA could be *O*-methylated to its mono- and dimethyl ether derivatives by microorganisms in sediments [39]. Furthermore, *O*-methylated polybrominated diphenyl ethers were also found to be present in the green alga *Cladophora fascicularis* and the red alga *Ceramium tenuicorne* [41,42].

Debromination is usually regarded as an important mechanism of TBBPA removal in the contaminated environment. To date, the reductive debromination of TBBPA by anaerobic bacteria has been documented in anoxic sediments and soils [19,20,32,43]. Debromination of TBBPA is a stepwise process of sequential removal of bromine atoms, with TBBPA being used as an electron acceptor by microorganisms and reductively debrominated to less brominated BPA and finally to BPA under anaerobic conditions [19]. The detection of tribromobisphenol-A (P5) in all algal treatments suggests that debromination of TBBPA could be a common transformation mechanism in freshwater algae. Further debromination of product P5 by algae would be possible if a longer incubation were carried out.

Tetrabromobisphenol-A is one of the main organic contaminants in the aquatic environment of industrialized regions such as the Pearl River Delta region in South China, because of its great consumption [14]. The identified transformation products could have significant environment implications in the environmental fate of TBBPA. Sulfated and glucosylated TBBPA can be easily released and further degraded in the aquatic environment. Debrominated TBBPA would also be important for further degradation by photochemical and bacterial processes. However, *O*-methylated TBBPA would be more lipophilic and bioaccumulative than its parent compound TBBPA, which raises serious environmental concerns. In fact, O-methylated TBBPA derivatives have been detected in river sediments near a Swiss plastic manufacturer [44] and in aquatic organisms such as mussels [45]. Despite the lower TBBPA transformations in river water than in the BG11 medium, we can conclude that microalgae can play an important role in the fate of TBBPA in the aquatic environment. Although we did not find complete degradation of TBBPA by algae, biotransformation, especially debromination, would be an important first step for its subsequent degradation in the environment.

SUPPLEMENTAL DATA

Figures S1–S3. (279 KB DOC).

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