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# Development of methodology for the determination of carbon isotope ratios using gas chromatography/combustion/isotope ratio mass spectrometry and applications in the biodegradation of phenolic brominated flame retardants and their degradation products

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**RATIONALE:** Compound-specific stable carbon isotope analysis of phenolic brominated flame retardants (BFRs) and bisphenol A (BPA) has proven informative to discriminate their source and fate apportionment in the environment. However, because these compounds contain highly polar functional groups and exist as complex mixtures in environment matrices, derivatization is a necessary step, which adds additional non-analyte carbon atoms for analyses and may alter the original stable carbon isotope ratio. It is, therefore, imperative to gain an insight into the relationship between the  $\delta^{13}\text{C}$  values of the BFRs and BPA derivatives and those of underivatized BFRs and BPA.

**METHODS:** The  $\delta^{13}\text{C}$  values of BFRs and BPA *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatives were measured by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS). The  $\delta^{13}\text{C}$  values of the BSTFA reagent and the standard underivatized BFRs and BPA were determined using elemental analyzer/isotope ratio mass spectrometry (EA/IRMS). The experimentally obtained  $\delta^{13}\text{C}$  values for BFRs and BPA derivatives were then compared with the theoretically calculated values.

**RESULTS:** The derivatization process introduces no isotopic fractionation for BFRs and BPA (the average difference between the theoretically calculated and experimentally obtained  $\delta^{13}\text{C}$  values was  $0.06 \pm 0.15\%$ , within the precision limits of the GC/C/IRMS measurements). Therefore, the  $\delta^{13}\text{C}$  values for the original underivatized BFRs and BPA were computed through a mass balance equation.

**CONCLUSIONS:** This work offers a novel tool to research the biotic or abiotic transformation processes of BFRs and BPA in the environment and will offer a perspective for the identification of the environmental source and fate of these organic compounds. Copyright © 2014 John Wiley & Sons, Ltd.

Brominated flame retardants (BFRs) are a group of organic compounds extensively incorporated into a multitude of products such as building materials, electrical and electronic products, textiles and foams to protect materials against ignition.<sup>[1]</sup> Among these BFRs, more than one-third are phenolic BFRs, including tetrabromobisphenol A (TBBPA), 2,4,6-tribromophenol (TBP), 2,4-dibromophenol (2,4-DBP) and 2,6-dibromophenol (2,6-DBP),<sup>[2]</sup> with a total global market that increased from 130 000 tons in 2002 to over 170 000 tons in 2004.<sup>[3]</sup> Bisphenol A (BPA) has also been widely used as an intermediate in the synthesis of polycarbonate plastics, epoxy resins and flame retardants.<sup>[4]</sup> However,

during the production, use or dismantling of these products, a proportion of the BFRs can be released into the environment. Therefore, BFRs and BPA are of great concern because of their ubiquitous presence and recalcitrant persistence in various environmental matrices, including biota, and their potential adverse effects on wildlife and humans.<sup>[5–9]</sup> The biodegradation or photochemical transformation of these substances occurs once they are released into the environment. Among the various environmental transformation processes, microbial degradation is suspected to affect the environmental exposure and the toxicity of the BFRs and BPA,<sup>[10,11]</sup> although there is limited data regarding the extent of their degradative processes in the environment. Our previous work showed that TBP, 2,4-DBP, and 2,6-DBP were the main biodegradation intermediates of TBBPA,<sup>[12]</sup> and BPA was also found as a debromination metabolite of TBBPA.<sup>[13]</sup> However, uncertainty still existed regarding their biodegradation in the real natural environment and the identity of the sources of some of the most abundant bioaccumulating BFRs, and of BPA, in the environment.

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Nevertheless, is reasonable to expect that partial degradation and different synthetic pathways could give rise to discriminatory carbon isotope effects and thus imprint compound-specific  $\delta^{13}\text{C}$  values, which are diagnostic of both the sources and the biodegradation processes of BFRs and BPA.

Although numerous studies on BFRs and BPA have been reported, they were mainly concerned with the degradation of these compounds in the laboratory.<sup>[12–16]</sup> There has, however, been almost no published work on their source and fate in the environment, because BFRs and BPA exist as complex mixtures in environmental matrices.

Compound-specific isotope analysis (CSIA) is one of the effective methods not only for the source identification of the organic compounds, but also for understanding their metabolism in the environment.<sup>[17]</sup> For example, Macko and Estep used compound-specific carbon/nitrogen isotope ratios to study the interaction between microorganisms and organic compounds in order to effectively interpret the diagenesis of organics in sediments,<sup>[18]</sup> and Sakata *et al.* studied the carbon isotopic fractionation associated with lipid biosynthesis by the cyanobacterium in order to interpret biomarker records.<sup>[19]</sup> The isotopic compositions of organic compounds can be measured by gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS).<sup>[20,21]</sup> Although GC/C/IRMS is an ideal tool for carrying out compound-specific  $\delta^{13}\text{C}$  measurements, its application to BFRs and BPA is difficult due to their thermally labile and polarity. Therefore, this technique requires the derivatization of the phenolic hydroxyl groups to form less polar and volatile compounds. To the best of our knowledge, there have been no reports of compound-specific  $\delta^{13}\text{C}$  value measurement for the source and fate identification of BFRs and BPA.

Therefore, we have examined the carbon isotopic effect for BFRs and BPA as their *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) derivatives: the derivatization is a necessary step for compounds such as the BFRs and BPA that contain polar functional groups (e.g., hydroxyl groups).<sup>[22,23]</sup> As the derivatization process adds additional non-analyte carbon atoms, and this could alter the original stable carbon isotope composition,<sup>[21,24]</sup> it is critical that an investigation is conducted into the relationship between the  $\delta^{13}\text{C}$  values of BFRs and BPA derivatives and those of the underivatized BFRs and BPA.

The main aim of this study was to develop a method for the accurate and precise isotopic measurement of BFRs and BPA by derivatization with BSTFA (Fig. 1). Subsequently, this method was implemented in our laboratory for the determination of the  $\delta^{13}\text{C}$  values of BFRs and BPA during biodegradation processes.

## EXPERIMENTAL

### Materials

2,6-DBP, TBP, and TBBPA were purchased from Sigma-Aldrich (St. Louis, MO, USA) with purities exceeding 99%. 2,4-DBP, BPA and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were from Acros Organics (Geel, Belgium) with purities exceeding 99%. HPLC grade dichloromethane was from CNW (ANPEL Scientific Instrument Co., Ltd, Shanghai, China). HPLC grade hexane was from Honeywell Burdick &

Jackson (Muskegon, MI, USA). Water was double distilled. All other chemicals were of analytical grade and from Guangzhou Chemical Reagent Co., Inc. (Guangzhou, China).

The microorganisms used in this experiment were originally isolated and inoculated with BFR-contaminated sludge by our laboratory: *Ochrobactrum* sp. T can degrade and mineralize TBBPA,<sup>[12]</sup> *Bacillus* sp. GZT can degrade and mineralize TBP, 2,4-DBP and 2,6-DBP,<sup>[14]</sup> and *Bacillus* sp. GZB can degrade BPA.<sup>[13]</sup> More detailed information can be found in our previous work.<sup>[10,12–14,25]</sup>

### Derivatization

Prior to isotope ratio measurement, the BFRs and BPA were derivatized with BSTFA: 50% (v/v) of BSTFA was added to the extracts (50  $\mu\text{L}$ ), and the mixtures were stirred for 60 min at 60 °C to replace the hydrogen atom of a hydroxyl group with a trimethylsilyl group.

### Isotope ratio measurement

CSIA was performed using a gas chromatograph (Trace GC Ultra; Thermo Scientific, Bremen, Germany) coupled to a DELTA V PLUS isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany) via a GC Combustion Interface III (Thermo Electron, Bremen, Germany). The GC system was equipped with a DB-5 ms column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ , J&W Scientific, Folsom, CA, USA). For the  $\delta^{13}\text{C}$  measurements of the BFR and BPA BSTFA derivatives, the injector temperature was set at 290 °C, and the column oven temperature was initially held at 60 °C for 1 min, increased to 150 °C at 30 °C  $\text{min}^{-1}$ , and then to 300 °C at 5 °C  $\text{min}^{-1}$ , and held for 10 min. Helium at a flow rate of 1 mL  $\text{min}^{-1}$  was used as the carrier gas. The combustion interface was operated at 1060 °C. The samples were injected in splitless mode, and the injection volume of the samples was 2  $\mu\text{L}$ . The stable isotopic compositions are reported in the delta ( $\delta$ ) notation:

$$\delta = (R_{\text{sample}}/R_{\text{standard}}) - 1 \quad (1)$$

where R is the ratio of the heavy isotope ( $^{13}\text{C}$ ) to light isotope ( $^{12}\text{C}$ ) measured in a sample, relative to that of an international standard. The carbon stable isotope ratios were measured relative to the Vienna Pee Dee Belemnite (VPDB) marine limestone standard. An external  $\text{CO}_2$  reference gas ( $\delta^{13}\text{C} = -21.66\text{‰}$ ) was used to obtain highly accurate  $\delta^{13}\text{C}$  values. The reproducibility and accuracy of carbon isotopic analysis were assessed routinely every day using 10 laboratory isotopic standards ( $\text{C}_{12}$ ,  $\text{C}_{14}$ ,  $\text{C}_{16}$ ,  $\text{C}_{18}$ ,  $\text{C}_{20}$ ,  $\text{C}_{22}$ ,  $\text{C}_{25}$ ,  $\text{C}_{28}$ ,  $\text{C}_{30}$ , and  $\text{C}_{32}$  n-alkanes) with carbon isotope ratio values of  $-31.99\text{‰}$ ,  $-30.69\text{‰}$ ,  $-30.66\text{‰}$ ,  $-31.11\text{‰}$ ,  $-32.35\text{‰}$ ,  $-32.87\text{‰}$ ,  $-28.46\text{‰}$ ,  $-32.21\text{‰}$ ,  $-29.84\text{‰}$ , and  $-29.47\text{‰}$ , respectively.

Elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) measurements were performed as follows. The underivatized BFRs and the BPA standard sample were weighed into clean tin capsules, and the capsules were placed in a FLASH 2000 organic elemental analyzer (Thermo Scientific) and burned at 970 °C in a combustion tube ( $3\text{Cr}_2\text{O}_3$ ,  $2\text{Cu}$ ,  $1\text{CoO}$ ). The combustion gases were swept through a reduction column by a stream of inert helium gas and passed into a gas chromatograph where the  $\text{CO}_2$ , still in the He stream, was separated from other gases. The gas stream then entered a DELTA V PLUS isotope ratio mass spectrometer where the  $\delta^{13}\text{C}$  value of the  $\text{CO}_2$  gas was

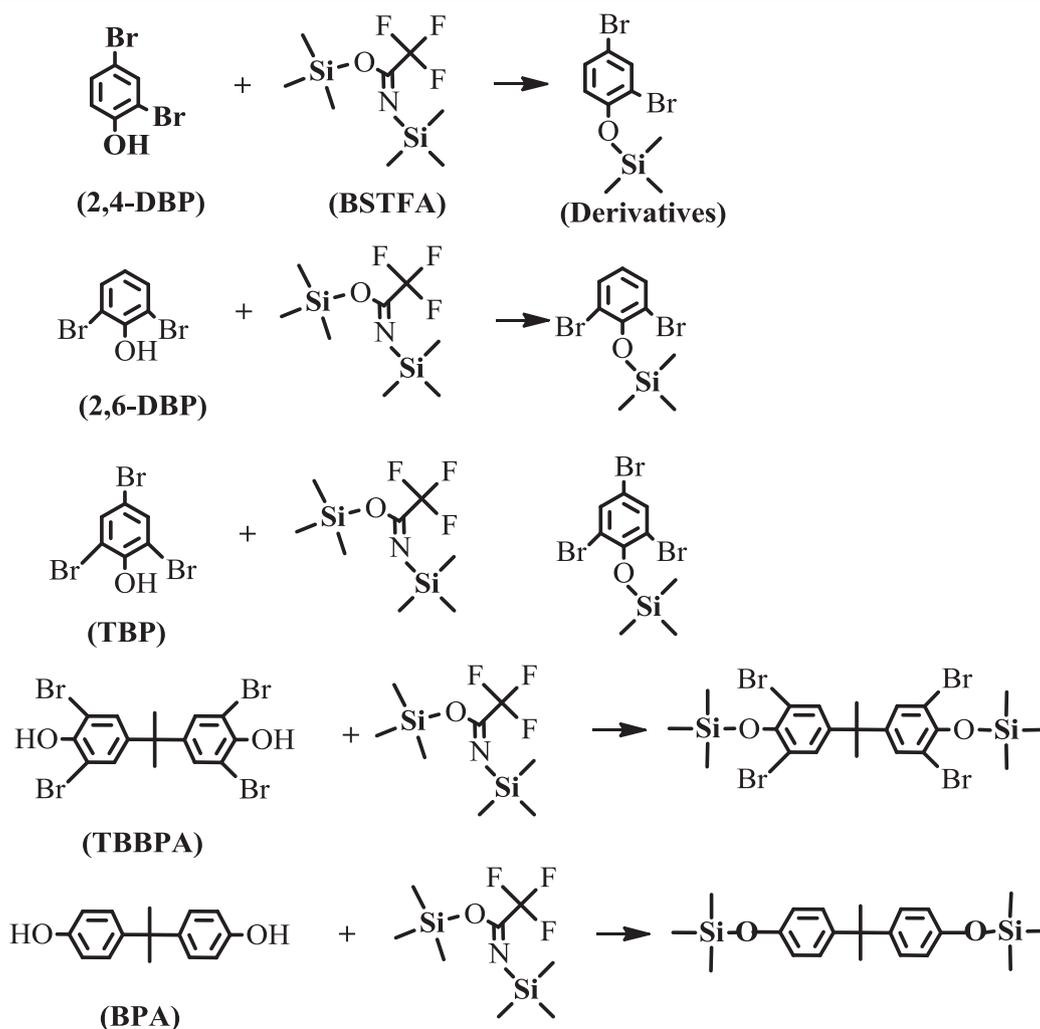


Figure 1. The derivatization scheme of phenolic BFRs and BPA with BSTFA.

determined relative to a reference CO<sub>2</sub> gas of known  $\delta^{13}\text{C}$  value ( $-21.66\%$ ). During every ten samples, a carbon black sample of known  $\delta^{13}\text{C}$  value ( $-22.43\%$ ) was analyzed, and this showed that excellent reproducibility and accuracy were achieved; the standard deviation of the analysis and the deviation between the measured data and pre-determined data were less than 0.2%.

#### Measurement of $\delta^{13}\text{C}$ values of standard materials

The  $\delta^{13}\text{C}$  values of the BSTFA reagent and the underivatized BFRs and BPA used in this study were determined by EA/IRMS. For the BFRs and BPA–BSTFA derivatives, the  $\delta^{13}\text{C}$  values were determined by GC/C/IRMS, with each sample injection being more than 100 ng (linearity range from peak height 1–4 V).

#### Applicability of the method

The method developed herein was tested for the analysis of real biodegradation samples. The microorganisms were inoculated in an autoclaved Luria–Bertani (LB) medium at 37 °C in a rotary incubator-shaker at 200 rpm for 15 h; 30 mL of the incubated LB medium was then centrifuged and rinsed three times with autoclaved water to collect the bacteria. The obtained bacteria were added to 100 mL

autoclaved mineral medium (MM) containing 10 mg L<sup>-1</sup> substrates. The experimental MM consisted of (all concentrations in g L<sup>-1</sup>, Guangzhou Chemical Reagent Co.): K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 2.27; KH<sub>2</sub>PO<sub>4</sub>, 0.68; NH<sub>4</sub>NO<sub>3</sub>, 1.00; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.10; MnSO<sub>4</sub>·H<sub>2</sub>O, 0.03; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.05; and CaCl<sub>2</sub>, 0.02; the pH was adjusted to 7.0–8.0. The vials were incubated at 34 °C in a rotary incubator-shaker at 200 rpm. Biodegradation samples were collected after 0, 24, 36, 48, 72 and 96 h. The detailed methods used to collect the biodegradation samples were described in our previous work.<sup>[12]</sup> The biodegradation samples were extracted, and the extracts were derivatized in the same manner as described previously. The  $\delta^{13}\text{C}$  values of the BFRs and BPA were determined by GC/IRMS. Every sample was analyzed in triplicate and the average was determined.

## RESULTS AND DISCUSSION

#### $\delta^{13}\text{C}$ analysis of BFRs and BPA and their derivatives

The derivatization of the BFRs and BPA with BSTFA may alter their original stable isotope compositions.<sup>[21–23]</sup> Therefore, to correct for any deviation arising from the

introduction of carbon during the derivatization process, it is necessary to evaluate the isotopic reproducibility of the derivatization process. The  $\delta^{13}\text{C}$  values of BFRs and BPA derivatives were determined by GC/C/IRMS, and those of the underivatized BFRs and BPA and BSTFA by EA/IRMS (Table 1). It can be seen that, generally, for the underivatized BFRs and BPA as well as for the BSTFA samples, the reproducibility (standard deviation) of seven replicate analyses was less than 0.2‰ (ranging from 0.06 to 0.16‰). The analytical error of the measurements of the BFR and BPA–BSTFA derivatives ranged from 0.07 to 0.35‰ and averaged  $0.21 \pm 0.07\%$ . Although different molar ratios of BFRs and BPA/BSTFA were used in the derivatization reaction, the analytical error obtained for four GC/C/IRMS analyses of these derivatives still ranged from 0.16 to 0.24‰, with an average of  $0.21 \pm 0.03\%$ . In addition, our achieved reproducibilities compare very well with those obtained in the derivatization of hydroxy groups,<sup>[22]</sup> and are within the uncertainties reported for the GC/C/IRMS determinations of  $\delta^{13}\text{C}$  values.<sup>[26]</sup>

Theoretically, the  $\delta^{13}\text{C}$  values of these derivatized compounds are derived from the contribution of carbon from each component. In principle, if there is no isotope fractionation associated with the BFRs and BPA derivatization reaction, the mass balance equation for BFRs and BPA and their BSTFA derivatives will be:

$$\delta^{13}\text{C}_{\text{derivatives}} = f_{\text{BFRs or BPA}} \delta^{13}\text{C}_{\text{BFRs or BPA}} + f_{\text{BSTFA}} \delta^{13}\text{C}_{\text{BSTFA}} \quad (2)$$

where  $f_{\text{BFRs or BPA}}$  is the mole fractions of carbon, which is the ratio of the carbon of BFRs or BPA to that of the BFRs or BPA–BSTFA derivatives; and  $f_{\text{BSTFA}}$  is the mole fractions of carbon, which is the ratio of the carbon of BSTFA to that of the BFRs or BPA–BSTFA derivatives. In this study,  $f_{\text{BFRs or BPA}}$  has the value of 1/3 for 2,4-DBP, 2,6-DBP and TBP, while the corresponding  $f_{\text{BSTFA}}$  is 2/3; and  $f_{\text{BFRs or BPA}}$  has the value of 5/7 for BPA and TBBPA, while the corresponding value of  $f_{\text{BSTFA}}$  is 2/7. This equation can be used to estimate the carbon isotope composition of the original BFRs or BPA ( $\delta^{13}\text{C}_{\text{BFRs or BPA}}$  values).

Table 1 gives the stable carbon isotopic compositions for the underivatized BFRs and BPA, the BFRs and BPA derivatives, and BSTFA. It can be seen that the experimentally obtained data for the BFRs and BPA derivatives by GC/C/IRMS compared well with the theoretical values calculated by Eqn. (2). Furthermore, the theoretically calculated and experimentally achieved values agreed very well within the precision limits of the GC/C/IRMS system (ranging from –0.23 to 0.33‰). As such, the difference between the theoretically calculated and experimentally obtained values can be ignored.

**Table 1.** The  $\delta^{13}\text{C}$  values of BFRs and BPA as well as their respective BSTFA derivatives versus  $\delta^{13}\text{C}$  values predicted by mass balance equation ( $\delta^{13}\text{C}_{\text{BSTFA}} = -32.00 \pm 0.14\%$ , from 12 analyses determined by EA/IRMS)

	Molar ratios BFRs <sup>a</sup> /BSTFA	$\delta^{13}\text{C}$			$\Delta^g$
		Underivatized BFRs <sup>b,c</sup> (AM $\pm$ SD <sup>d</sup> )	BFR derivatives <sup>b,e</sup> (AM $\pm$ SD <sup>d</sup> )	Predicted BSTFA derivatives <sup>b,f</sup>	
2,4-DBP	1:1	$-28.71 \pm 0.06$ (n = 7)	$-30.07 \pm 0.13$ (n = 4)	–29.80	0.27
	1:10		$-29.88 \pm 0.35$ (n = 4)		0.08
	1:20		$-30.08 \pm 0.16$ (n = 4)		0.28
	1:30		$-29.93 \pm 0.30$ (n = 4)		0.13
2,6-DBP	1:1	$-28.82 \pm 0.14$ (n = 7)	$-29.87 \pm 0.15$ (n = 4)	–29.88	–0.01
	1:10		$-30.03 \pm 0.07$ (n = 4)		0.15
	1:20		$-29.84 \pm 0.18$ (n = 4)		–0.04
	1:30		$-30.00 \pm 0.25$ (n = 4)		0.12
TBP	1:1	$-21.66 \pm 0.16$ (n = 7)	$-24.88 \pm 0.25$ (n = 4)	–25.11	–0.23
	1:10		$-24.98 \pm 0.19$ (n = 4)		–0.13
	1:20		$-25.05 \pm 0.16$ (n = 4)		–0.06
	1:30		$-25.15 \pm 0.31$ (n = 4)		0.04
BPA	1:1	$-41.06 \pm 0.10$ (n = 7)	$-38.80 \pm 0.15$ (n = 4)	–38.47	0.33
	1:10		$-38.70 \pm 0.17$ (n = 4)		0.23
	1:20		$-38.51 \pm 0.19$ (n = 4)		0.04
	1:30		$-38.44 \pm 0.35$ (n = 4)		–0.03
TBBPA	1:1	$-27.44 \pm 0.07$ (n = 7)	$-28.84 \pm 0.23$ (n = 4)	–28.74	–0.10
	1:10		$-28.85 \pm 0.18$ (n = 4)		0.11
	1:20		$-28.71 \pm 0.20$ (n = 4)		–0.03
	1:30		$-28.72 \pm 0.19$ (n = 4)		–0.02

<sup>a</sup>BFRs: BFRs and BPA.

<sup>b</sup>Stable carbon isotopic compositions reported relative to VPDB.

<sup>c</sup> $\delta^{13}\text{C}$  values determined by EA/IRMS.

<sup>d</sup>The arithmetic means and standard deviations.

<sup>e</sup> $\delta^{13}\text{C}$  values determined by GC/C/IRMS.

<sup>f</sup>Based on mass balance relationship (Eqn. (2)) of BFRs and BPA and BSTFA  $\delta^{13}\text{C}$  values determined by EA/IRMS.

<sup>g</sup>Predicted  $\delta^{13}\text{C}$  values – analytical  $\delta^{13}\text{C}$  values.

### Measurement of the $\delta^{13}\text{C}$ values of BFRs and BPA during the biodegradation processes

For the measurement of the  $\delta^{13}\text{C}$  values of TBBPA during the biodegradation process, a *Ochrobactrum* sp. T enrichment culture was employed in a sediment-free system containing TBBPA, which can be degraded by *Ochrobactrum* sp. T.<sup>[12]</sup> Although about 63.5% of the TBBPA was removed after 96 h reaction, the carbon isotope compositions remained constant during this biodegradation process (Fig. 2). The  $\delta^{13}\text{C}$  values at the beginning and after a degradation time of 96 h were  $-29.13 \pm 0.14\text{‰}$  and  $-28.72 \pm 0.10\text{‰}$ , respectively. The maximum fractionation for the carbon isotopes ( $\Delta\delta^{13}\text{C}$ ) during the biodegradation process was 0.41‰, a value which remained virtually constant during the course of the experiment and indicated a very small or no isotope effect.

According to our previous work, TBP, 2,4-DBP, and 2,6-DBP are the main biodegradation intermediates during TBBPA biodegradation by *Ochrobactrum* sp. T,<sup>[12]</sup> and can also be degraded by our isolated *Bacillus* sp. GZT; 2,4-DBP and 2,6-DBP were the debromination metabolites of TBP.<sup>[14]</sup> Therefore, the *Bacillus* sp. GZT enrichment culture was also employed in a sediment-free system containing TBP, 2,4-DBP, and 2,6-DBP to study their carbon isotope fractionation. Similar trends were obtained for the three bromophenols as for TBBPA during the biodegradation process. That is, although 2,4-DBP could be gradually degraded by *Bacillus* sp. GZT with an increase in the degradation time, and about 90.0% of the 2,4-DBP was eliminated after 96 h, this biodegradation process only minimally affected the carbon isotope ratios of the remaining compound (Fig. 3(a)). The  $\delta^{13}\text{C}$  value of 2,4-DBP is also obtained as  $-29.86 \pm 0.30\text{‰}$ . The maximum fractionation for the carbon isotopes ( $\Delta\delta^{13}\text{C}$ ) during the biodegradation process was 0.31‰. This value was even less than the allowed error of the instrument of  $\pm 0.5\text{‰}$ , indicating that there is no carbon isotope fractionation or, at least, no obvious fractionation. Similar results were achieved for 2,6-DBP, which was also gradually degraded by *Bacillus* sp. GZT as time increased, with about 82.0% of the 2,6-DBP being eliminated within 96 h. The fractionation of the carbon isotopes was also not obvious during the biodegradation of this compound (Fig. 3(b)). The

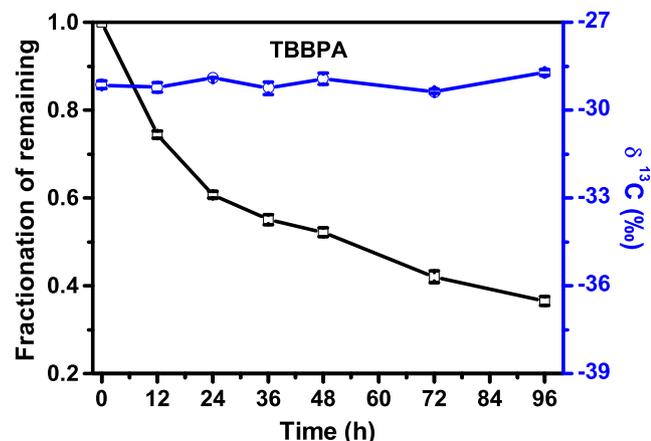


Figure 2. The fraction of TBBPA remaining and the  $\delta^{13}\text{C}$  values of the TBBPA remaining.

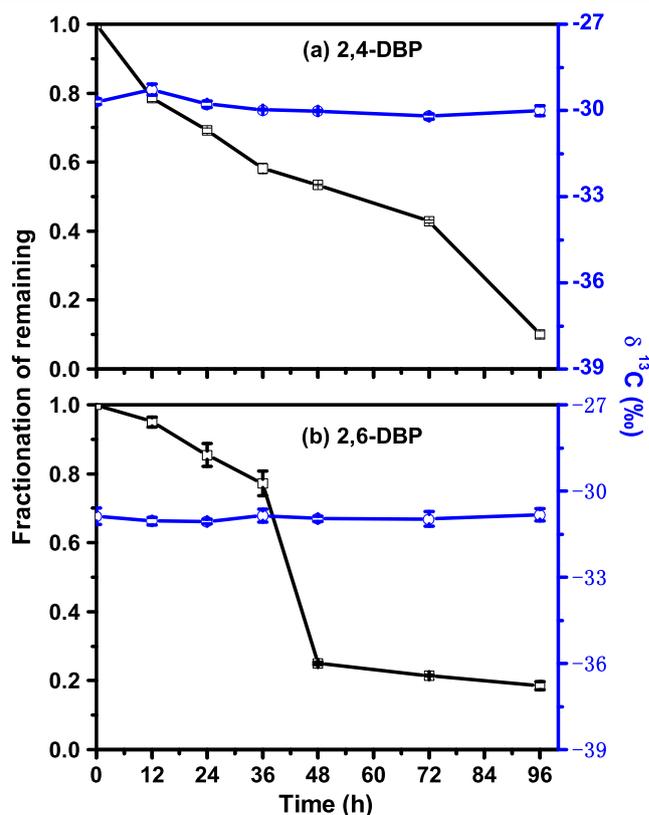


Figure 3. The fraction of substrate remaining and the  $\delta^{13}\text{C}$  values of the two substrates remaining: (a) 2,4-DBP and (b) 2,6-DBP.

$\delta^{13}\text{C}$  value of 2,6-DBP was obtained as  $-30.94 \pm 0.10\text{‰}$ , with a standard deviation of 0.10‰. The maximum fractionation for the carbon isotope ( $\Delta\delta^{13}\text{C}$ ) during the biodegradation process was 0.06‰. Similarly, the carbon isotopic fractionation of TBP was not obvious during the biodegradation process (Fig. 4). The  $\delta^{13}\text{C}$  value of TBP was  $-22.60 \pm 0.12\text{‰}$ , with a standard deviation of 0.12‰. The maximum fractionation for the carbon isotope ( $\Delta\delta^{13}\text{C}$ ) during the biodegradation process was 0.30‰, which was also less than the allowed error of the instrument.

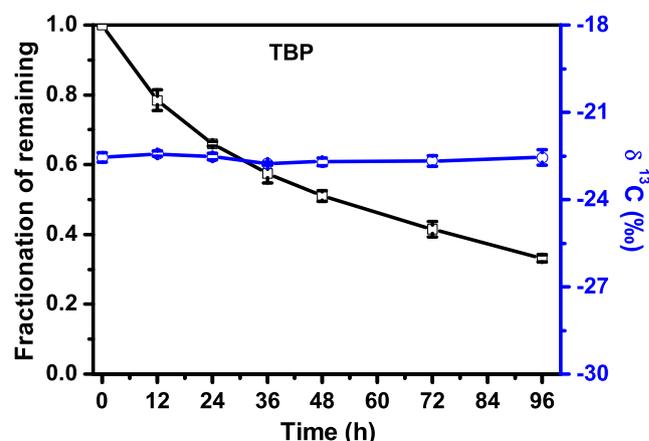


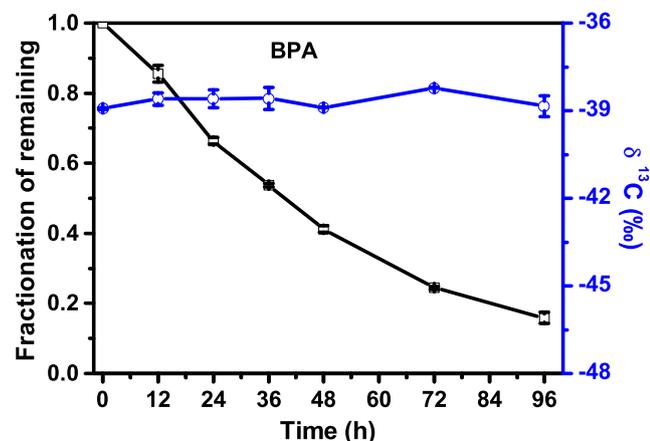
Figure 4. The fraction of TBP remaining and the  $\delta^{13}\text{C}$  values of the TBP remaining.

In addition to the three bromophenols, BPA is also a major biodegradation intermediate during TBBPA biodegradation by various bacteria, as reported by us and other groups.<sup>[12,27,28]</sup> Our previous work proved that BPA can be degraded by *Bacillus* sp. GZB.<sup>[13]</sup> Hence *Bacillus* sp. GZB enrichment culture was employed in a sediment-free system containing BPA to study its isotope fractionation. About 84.1% of the BPA was eliminated within 96 h, but fractionation of the carbon isotopes was not obvious during the biodegradation process (Fig. 5). The  $\delta^{13}\text{C}$  value of BPA was  $-38.66 \pm 0.25\text{‰}$ , with a standard deviation of 0.25‰. The maximum fractionation for the carbon isotopes ( $\Delta\delta^{13}\text{C}$ ) during the biodegradation process was 0.08‰, which was also less than the allowed error of the instrument of  $\pm 0.5\text{‰}$ . These results mean that the carbon isotope fractionation for BFRs and BPA during the biodegradation process was not obvious or there was no fractionation.

The reasons for the absence of a large fractionation may be explained as follows. First, two possible routes exist for the biodegradation of TBBPA by *Ochrobactrum* sp. T<sub>1</sub><sup>[12]</sup> one is the debromination and the other is the cleavage of the molecule, and these may produce competing isotope effects.<sup>[29]</sup> Second, any large fractionation may be diluted by the 15 carbon atoms in the molecule, which are not involved in the reaction. Similar results were observed in other studies: for example, the absence of carbon isotopic fractionation of semi-volatile organic contaminants, such as polycyclic aromatic hydrocarbons<sup>[30]</sup> and polychlorinated biphenyls,<sup>[31]</sup> and the extremely low bromine isotopic fractionation, such as that of brominated phenol,<sup>[32]</sup> during biodegradation. Furthermore, the TBBPA molecule was too large to readily permeate the cell membranes of the microorganism, and the absence of a large fractionation of these brominated phenols and BPA may be for the same reason as for TBBPA.

### Biogeochemical implications and the potential applications

The above results indicate that there is no observable carbon isotopic fractionation during the biodegradation of BFRs and BPA. The absence of an isotope effect may prove equally valuable for examining these biodegradation processes, which do not result in any measurable isotopic fractionation. This, in turn, has advantages for these larger molecules,



**Figure 5.** The fraction of BPA remaining and the  $\delta^{13}\text{C}$  values of the BPA remaining.

meaning that the isotopic fingerprints from these compounds can be used for determining their sources and fate in the various environments, monitoring natural attenuation. Our results showed that the  $\delta^{13}\text{C}$  values of these BFRs and BPA are relatively constant over the course of the biodegradation process. Thus, this study provides the evidence necessary for better understanding the source of these BFRs and BPA pollutants. The  $\delta^{13}\text{C}$  values of these BFRs and BPA are relatively constant over the course of the biodegradation. For example, the  $\delta^{13}\text{C}$  value of TBP is  $-22.60 \pm 0.12\text{‰}$ , while those of 2,4-DBP and 2,6-DBP are  $-29.86 \pm 0.30\text{‰}$  and  $-30.94 \pm 0.10\text{‰}$ , respectively. Because the  $\delta^{13}\text{C}$  values of TBP, 2,4-DBP, and 2,6-DBP are significantly different, there may be different sources of the three BFRs. If the  $\delta^{13}\text{C}$  values of 2,4-DBP and 2,6-DBP are the same as that of TBP, this indicates that the 2,4-DBP and 2,6-DBP may originate from the debromination products of TBP due to the relatively constant  $\delta^{13}\text{C}$  values during the biodegradation process. On the contrary, the 2,4-DBP and 2,6-DBP do not come from the debromination of TBP. The  $\delta^{13}\text{C}$  values of TBBPA and BPA are also relatively constant over the biodegradation course. In addition, different manufacturers use different raw materials and manufacturing processes and thus produce chemicals with distinct isotopic compositions.<sup>[33]</sup> Therefore, for a given site where discrete releases of contaminant from a number of sources are involved, CSIA may also provide a quantifiable means of distinguishing these sources. Thus, the relatively constant isotopic compositions may provide additional evidence for understanding the parent/daughter relationships and the sources of these brominated phenol compounds in the natural environments.

### CONCLUSIONS

A novel methodology for the determination of carbon isotope ratios using GC/C/IRMS for BFRs and BPA was developed in this study. This method relied on the derivatization of the BFRs and BPA with BSTFA. The derivatization process introduced no carbon isotopic fractionation for BFRs and BPA (the average difference between the theoretically calculated and experimentally measured  $\delta^{13}\text{C}$  value was  $0.06 \pm 0.15\text{‰}$ , within the precision limits of the GC/C/IRMS measurement). Therefore, the  $\delta^{13}\text{C}$  values for the original underivatized BFRs and BPA were calculated through a mass balance equation. Using this novel method, the biodegradation of BFRs and BPA was investigated to identify their sources and fate. The results indicated that there is no observable carbon isotopic fractionation during the processes. The absence of carbon isotope effect equally proved to be valuable for examining these biodegradation processes. The developed method proved to be suitable for the analysis of BFRs and BPA and for identifying the environmental source and fate of these organic compounds.

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