Talanta 97 (2012) 445–449

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Compound-specific isotope analysis for aerobic biodegradation of phthalate acid esters

Xuewei Peng [a,b], Xianguo Li [a,*]

[a] Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao 266100, China
[b] College of Chemistry and Chemical Engineering, Weifang University, Weifang 261061, China

ARTICLE INFO

Article history:
Received 16 January 2012
Received in revised form
19 April 2012
Accepted 30 April 2012
Available online 10 May 2012

Keywords:
Compound-specific isotope analysis
Phthalic acid esters
Priority pollutant
Aerobic degradation

ABSTRACT

The degradation of three phthalic acid esters (PAEs) (dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-*n*-butyl phthalate (DBP)) by natural microbial community under aerobic condition and their isotope fractionation were compared by using a laboratory microcosm system with natural marine sediment overlying with natural seawater. The results showed that the degradation of the three tested PAEs followed a first-order kinetics, with rate constants of 0.0541, 0.0352 and 0.00731 day^{-1} for DMP, DBP and DOP, respectively, indicating that the degradation rate of PAEs is a inverse function of the length of the alkyl side chain: the longer the side chain, the slower the rate is. ^{13}C isotope enrichment of the three residual PAEs were evaluated with compound-specific isotope analysis (CSIA). A relatively obvious ^{13}C enrichment, with maximum $\delta^{13}C$ shifts of $\Delta\delta^{13}C_{DMP}=2.05\pm0.21‰$ ($f=0.17$) and $\Delta\delta^{13}C_{DBP}=1.92\pm0.23‰$ ($f=0.08$) in the residual DMP and DBP, respectively, was observed at an advanced stage of biodegradation. No significant ^{13}C enrichment occurred in the residual DOP ($\Delta\delta^{13}C_{DOP}=0.55\pm0.21‰$, $f=0.16$) within the accuracy and reproducibility for GC–C–IRMS ($\pm0.5‰$). The experimental results indicated that the degree of isotopic fractionation in the three residual PAEs appeared to be related to the number of carbon atoms, which is in the order of DMP > DBP > DOP.

1. Introduction

Phthalic acid esters (PAEs) are widely used industrial chemicals serving as additives in plastics manufacturing, paints, adhesives, cardboard, lubricants and fragrances. Owing to their low solubility in water and high octanol/water partition coefficients, PAEs have been identified in diverse environmental samples including groundwater, soil, lake and marine sediments [1]. There is a deep concern about their possible toxicity to human beings and other organisms, since some of them are considered as potential carcinogens, teratogens and mutagens [2]. The United States Environmental Protection Agency (US EPA) and some of its international counterparts have classified the most common PAEs as priority pollutants and as endocrine disruptor compounds [3].

Metabolic breakdown by microorganisms is considered to be one of the major routes of environmental degradation for these compounds. A number of studies have demonstrated the biodegradation of several PAEs in soil, natural water, sediment and wastewater [4–6]. Identification and quantification of degradation processes is important to adjust the remediation strategies of organic pollutants. The traditional methods of monitoring contaminants degradation include detecting changes in concentrations of target compounds, electron acceptors, and identification of microbial metabolites [7], which was done usually using high-performance liquid chromatography [8], gas chromatography [9] and liquid chromatography–mass spectrometry [10]. However, the concentrations of contaminants and electron acceptors might decrease due to physical processes such as volatilization, sorption and dilution. On the other hand, the specific metabolites formed during biotransformation processes were mainly polar materials and degraded more easily than parent compounds, which were difficult to be detected [11]. Thus, the methods mentioned above have some shortcomings, and might inaccurately assess the degradation processes of organic contaminants.

In recent years, stable carbon isotope analysis by CSIA using gas chromatography coupled to isotope ratio mass spectrometry through a combustion interface (GC–C–IRMS) has become a promising tool to assess the degradation processes that control the fates of organic pollutants in environment [12,13]. With CSIA, the relative abundance of the heavy (^{13}C) and light (^{12}C) isotopes in a molecule of a given compound is determined, expressed by the ratio $\delta^{13}C$,

$$\delta^{13}C=\frac{R_{sample}-R_{standard}}{R_{standard}}\times 1000‰ \quad (1)$$

where R_{sample} is the $^{13}C/^{12}C$ ratio in a given sample, and $R_{standard}$ is the $^{13}C/^{12}C$ ratio in a standard reference material, Vienna Pee Dee Belemnite (VPDB).

* Corresponding author. Tel.: +86 532 66782215; fax: +86 532 66782540.
E-mail address: lixg@ouc.edu.cn (X. Li).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.04.060

Owing to the lower activation energies needed to break chemical bonds formed by light compared to heavy isotopes, light isotopes form weaker bonds and thus react faster than the heavy isotopes, leading to an enrichment of heavy isotopes (^{13}C) in the residual fraction. Hence, increases in isotope ratio (a positive isotopic shift) nearly always indicate occurrence of degradation, since degradation is the only known natural attenuation process that may result in significant isotopic fractionation [14]. CSIA has been increasingly used to assess the biodegradation of organic contaminants in the past decade. To date, previous studies have been mainly focused on the investigations of volatile organic compounds, such as chlorinated ethane [15–17], the BTEX (benzene, toluene, ethylbenzene and xylenex) compounds [11,18–20], and methyl *t*-butyl ether [21–24]. As a type of typical priority pollutants and endocrine-disrupting compounds, the study on isotopic fractionation of PAEs in microcosm or field has not been reported.

In the present work, DMP, DBP and DOP, priority pollutants listed by both China National Environmental Monitoring Center and the US EPA, were chosen as the model compounds. The biodegradation of three PAEs was simulated by using these compounds as substrate for marine mixed bacterial. The aim of this work was to investigate the environmental fate of three PAEs under aerobic conditions in marine sediment. Another aim was to investigate the fractionation of stable carbon isotope in the molecules of three PAEs and the relationship between isotopic fractionation and molecular structure during the course of aerobic biodegradation.

2. Materials and methods

2.1. Chemicals

DMP, DBP, and DOP, all with 99.0% analytical grade, were purchased from Shanghai Chemical Reagent Co. (China). Hexamethylbenzene was purchased from Sigma Chemical Co. (St. Louis, MO, USA). HPLC grade hexane, acetone and dichloromethane, purchased from Dima Chemical Co. (China), were used without further purification.

2.2. Sampling

Surface sediment samples were collected using a stainless steel shove from Licun River, an urbanized marine inlet in Qingdao. The top layer (0.5∼1.0 cm) of light brown aerobic sediment was transferred to pre-cleaned 250 mL glass jars. Seawater samples were collected in pre-cleaned glass bottles at the same site. The filled jars were placed in a cooler packed with ice during transportation to laboratory. Sediment and seawater were used for incubations immediately after transporting to laboratory.

2.3. Aerobic degradation

Surface sediments were sieved (0.5 mm mesh) to remove macrofauna and any coarse debris. Then, PAEs (on dry sediment) were spiked into wet sediments and stirred for 2 h to reach a nearly homogeneous distribution. The final concentration of three PAEs in aerobic marine sediment was about 20.0 μg/g dry sediment. Sediments placed in thin polyethylene plugs (2 mm thick, 4.5 cm i.d.) were incubated in a relatively large (∼5 l) water reservoir, which was bubbled with air for 24 h. Thus, solute exchange between sediment and the water reservoir could occur easily. Aerobic conditions were controlled by continually purging the seawater reservoir with CO_2/air (2/98).

Sterile controls were performed in parallel with autoclaved sediment and seawater at 120 °C for 20 min. All incubation experiments were carried out in dark and the temperature was kept constant (18.0 °C).

2.4. Extraction and purification

The procedure of sediment treatment is shown in Fig. 1. The incubated sediment samples were extracted three times at room temperature (ca. 25 °C) in an ultrasonic bath, with 10.0 mL solvent (1:2 acetone–hexane) for 10 min each time. After each extraction, centrifugation at 3000 rpm was conducted for 5 min, and the supernatant was transferred to and combined in a pear-shaped flask. The combined extract was pre-concentrated in a vacuum rotary evaporator to about 0.5 mL at 30 °C and the solvent was then changed (in 3 times) to 1.0 mL of hexane.

The resulted extracts were introduced onto a silica gel chromatographic column to remove interfering compounds prior to instrumental analysis. Silica gel (100∼200 mesh) was activated by heating at 180 °C for 12 h and partially deactivated with Milli-Q water (5% w/w). The glass column (1.0 cm i.d × 18.0 cm length) was packed with glass wool at its base and filled with 7.0 g of deactivated silica gel slurry in hexane under gravity. In order to prevent disturbance by eluting solvent, 1.0 g of anhydrous sodium sulfate was added on the top. After that, 20.0 mL of hexane was added and drained to the top of the sodium sulfate to condition the silica gel column prior to sample loading. Then extracts were quantitatively transferred to column and eluted consecutively with 20.0 mL hexane, 40.0 mL dichloromethane-hexane (3:7 v/v), and 60.0 mL acetone–hexane (2:8 v/v). The third eluate contained PAEs was concentrated and exchanged solvent with hexane.

To remove potentially substances interfering with isotope analysis, the resulted eluate and the three standard PAEs were spotted onto a silica gel plate (60 Å, 500 μm thickness, Liangchen Guiyuan, Anhui) and developed with hexane–acetone (4:1 v/v) mixture (R_f=0.6). The spots developed on the plate were recognized by color with standard under UV analyzer (Jingke, Shanghai), and allowed to dry for 30 min. After scraped off the plate, the silica containing target compound was gently ground to a powder and extracted with hexane in an ultrasonic bath in three times. The combined extracts were concentrated to dry under a gentle stream of high purity nitrogen and spiked with 50.0 μL hexane and 50.0 μL internal standard for analysis.

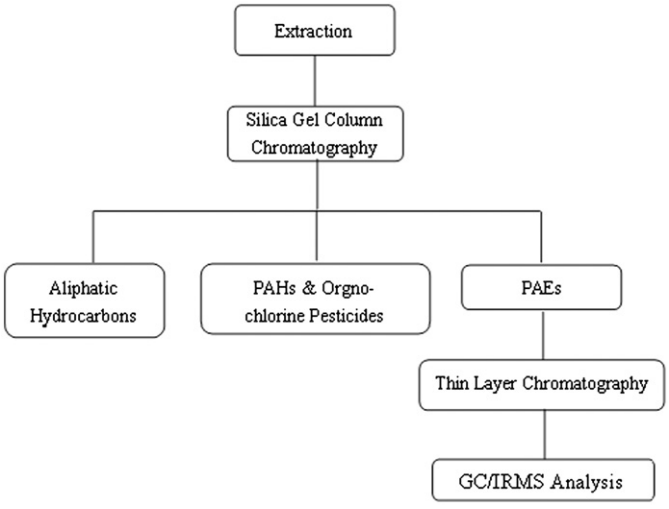

Fig. 1. Analytical procedure for stable carbon isotope ratios of PAEs.

2.5. Analysis methodology

Extracts were analyzed using a gas chromatograph (Shimadzu GC-2010) equipped with flame ionization detector and HP-5 capillary column (film thickness, 0.25 μm; inner diameter, 0.32 mm; length, 30 m). The initial column temperature was set at 130 °C for 1 min, increased by 5 °C/min to 220 °C, then increased by 3 °C/min to 275 °C and maintained for 13 min. Injector and detector temperatures were set at 250 °C and 320 °C, respectively. Nitrogen was used as both a carrier gas (flow rate 0.8 mL/min) and a make-up gas (flow rate 60.0 mL/min).

GC–C–IRMS analyses were performed on a Delta Plus XL mass spectrometer (Thermo Finnigan, Bremen, Germany) equipped with a GC-III combustion interface and linked to a 6980 gas chromatograph (Agilent, USA). Analyses were carried out with a DB-1 fused silica capillary column (film thickness, 0.25 μm; inner diameter, 0.25 mm; length, 60 m). Gas chromatographic conditions were as follows: 3 min isothermal time at 60 °C, heating with 2 °C/min to 310 °C, splitless injector heated at 270 °C and 60 s splitless time. Helium carrier gas flow was 1.5 mL/min. Oxidation of eluting analyses was carried out at 960 °C facilitated by a CuO/NiO/Pt catalyst. Isotopic measurements were performed in duplicate. For calibration of the carbon isotope reference gas (carbon dioxide), a certified reference standard ($\delta^{13}C = -26.65‰$) was used. All data are expressed relative to the VPDB standard.

3. Results and discussion

3.1. Sediment and seawater characterization

The organic matter content, as determined by elemental analyzer (PE 2400 SERIES II, PerkinElmer instruments, USA), was 5.8 ± 0.15% (mean ± SD, $n=3$). The salinity and pH value of the seawater, determined with multi-parameter water analyzer (Hach sension156), were 32.5% and 7.63, respectively.

3.2. Purification of sediment samples and the variation of $\delta^{13}C$ during purification

To accurately determine stable carbon isotope ratio of specific compounds, it is necessary to remove co-extracted substances thoroughly prior to GC–C–IRMS analysis. For this purpose, the purification by silica gel column was carried out first to separate the target pollutants from other substances interfering with GC analysis. Sample purities after purification were verified by GC–FID, and the chromatogram of sample containing DBP after silica gel purification is shown in Fig. 2A (the chromatograms of samples containing DMP and DOP are not shown). As can be clearly seen, the peak resolution and the chromatogram baseline were poor owing to the interference of unresolved complex mixture (UCM). A minor component co-eluting with a target compound can have a significant effect on the isotope ratio if their carbon isotope ratios are significantly different [25]. Thus, further purification for CSIA analysis was necessary. Fig. 2B is the chromatogram of sample containing DBP purified by thin layer chromatography (TLC) after silica gel purification procedure. It is clear that the peaks are well resolved and the chromatogram baseline is low, indicating that most of the background contamination was decreased after TLC purification.

The recoveries of the three PAEs after total purification steps are shown in Table 1. Among the three PAEs, DMP is vulnerable to loss with lower recoveries (75.92%) and higher RSDs, while higher recoveries (88.70%, 92.38%) and lower RSDs were observed for DBP and DOP, which has high-molecular weight. The difference in recovery and RSDs of the three PAEs was mainly due to the evaporative losses including concentration with vacuum rotary evaporator or under gentle N_2 stream, and exposing TLC plate to the air.

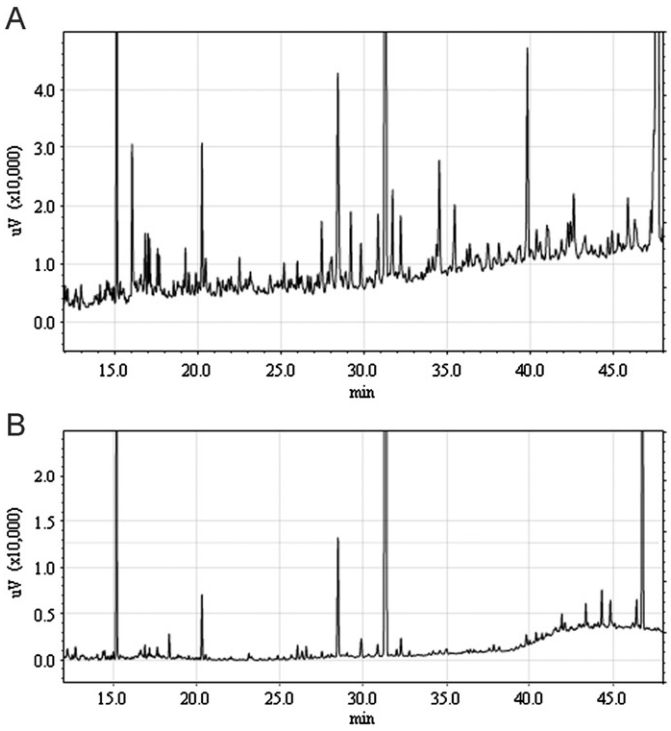

Fig. 2. Chromatograms of sediment sample after purification step. (A) Silica gel column and (B) silica gel column+TLC.

Table 1
Recoveries and RSDs of PAEs for clean-up procedure ($n=4$).

PAEs	Spiking amount/ng	Recovering amount/ng				Average recovery (%)	RSD
		1	2	3	4		
DMP	500	372	325	386	435	75.92	11.9
DBP	500	423	453	437	461	88.70	5.6
DOP	500	474	456	469	448	92.38	2.6

Table 2
$\delta^{13}C$ of PAEs during clean-up procedure ($n=4$).

PAEs	$\delta^{13}C$ (‰)				Mean+SD
	1	2	3	4	
DMP[a]	−29.08	−28.94	−29.14	−28.91	−29.02 ± 0.10
DMP[b]	−29.13	−28.91	−29.02	−29.15	−29.05 ± 0.11
DBP[a]	−27.37	−27.27	−27.25	−27.40	−27.32 ± 0.07
DBP[b]	−27.40	−27.21	−27.32	−27.49	−27.36 ± 0.12
DOP[a]	−26.53	−26.33	−26.30	−26.55	−26.43 ± 0.13
DOP[b]	−26.56	−26.30	−26.52	−26.15	−26.38 ± 0.19

[a] before purification.
[b] after purification.

The stable carbon isotopic signatures of three PAEs before and after purification were evaluated. As can be seen from Table 2, the $\delta^{13}C$ values revealed that no significant alteration in isotopic signature of the three PAEs occurred during the extraction and purification processes [26]. The analytical errors (standard deviation) obtained for GC/C/IRMS analyses of three PAEs during purification were from 0.07‰ to 0.18‰, with an average of

0.13 ± 0.05‰, indicating that the reproducibility was excellent compared with that of the previous studies [17]. Combination analysis of the recoveries and isotope signatures during clean-up indicated that the purification procedure used in this work was available.

3.3. PAEs degradation

During the course of aerobic biodegradation, the non-degraded DMP, DBP and DOP were monitored by GC–FID analysis. Data on the remaining percentages of the three PAEs are presented in Fig. 3. It can be clearly seen that DMP and DBP were degraded quickly, with more than 65% and 50% removed within initial 12 days of incubation, respectively. However, a little degradation of DOP occurred within initial 12 days, which indicated that an apparent lag phase existed during the course of DOP biodegradation in marine sediment. Within 74 days of incubation, DMP and DBP were removed more than 85%. Whereas only 35% of DOP was degraded within 74 days of incubation, indicating that the biodegradation of DOP was slower than that of DMP and DBP.

The high-to-low order of degradation rates, DMP > DBP > DOP, revealed that the biodegradation rate of the three PAEs appeared to be related to the length of the alkyl side chains. Generally, PAEs with shorter alkyl-chains (e.g., DMP and DBP) are more easily degraded than those with longer alkyl-chains (e.g., DOP) [5,27]. The reasons for this might be that PAEs with long alkyl side chains obtain a big three-dimensional structure, which hinders the hydrolytic enzymes from binding to the PAEs and thereby hindering the hydrolyzes [28]. In addition, water solubility is another major factor limiting the degradation of PAEs [29]. PAEs with low water solubility are easily adsorbed to sediment particles, and then the degradation effectiveness of microorganisms may decrease by reducing the bioavailability of PAEs, retarding the degradation process [5].

By fitting the aerobic biodegradation processes of DMP, DBP and DOP with a first order kinetic equation, good linear correlations between the logarithm of the three PAEs concentrations and time were obtained. These results were consistent with those of Yuan et al. [30], who found that degradation of PAEs with mixed microbial population in sediment followed first-order model. The degradation kinetic equations of three PAEs were summarized in Table 3.

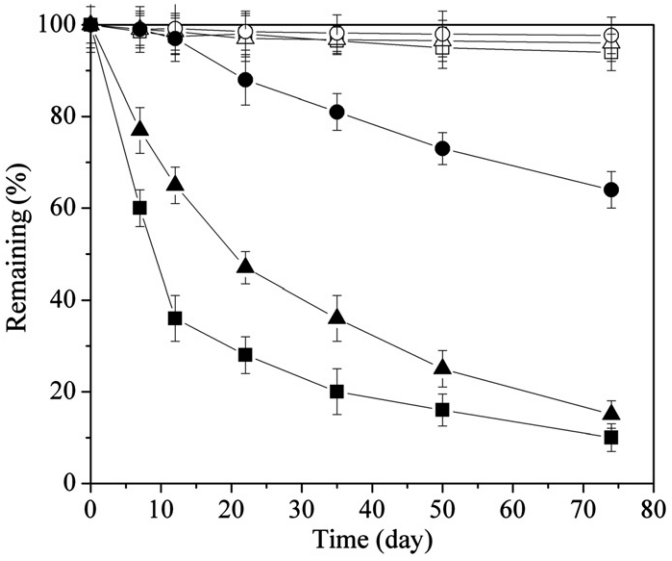

Fig. 3. Biodegradation of DMP, DBP and DOP under aerobic conditions. Symbols: ■, □, DMP; ▲, △, DBP; ●, ○, DOP. Filed symbols, non-sterile sediment; opened symbols, sterile sediment.

Table 3
Kinetic equations of PAEs degradation.

PAEs	Kinetic equation	Rate constant (day^{-1})	Half-life (day)	r^2
DMP	$\ln c = 3.119 - 0.0587t$	0.0587	11.8	0.962
DBP	$\ln c = 3.204 - 0.0472t$	0.0472	14.6	0.985
DOP	$\ln c = 3.265 - 0.00731t$	0.00731	94.8	0.996

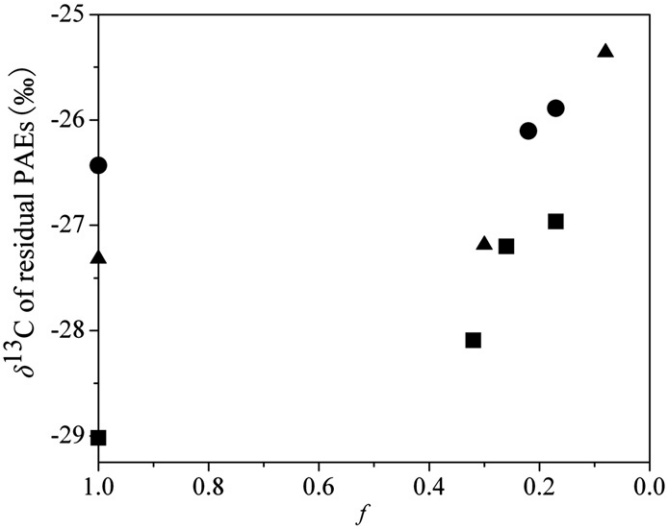

Fig. 4. $\delta^{13}C$ values of residual PAEs versus the fraction of PAEs remaining undegraded in the marine sediments. Initial $\delta^{13}C$ of DMP, DBP and DOP before biodegradation ($f=1.0$) is −29.02‰, −27.32‰ and −26.43‰, respectively. $f = c_t/c_0$, c_0 and c_t were the concentrations of PAEs at $t=0$ and $t=t$. Symbols: ■, DMP; ▲, DBP; ●, DOP.

The degradation half-life of DOP was 94.8 days, which was longer than that of DMP and DBP (11.8 and 14.6 days, respectively). These results indicate that the biodegradation half-life of phthalates homologs do not change significantly with $\log K_{ow}$ value increasing when $\log K_{ow} < 4.5$ [31].

In order to determine whether the losses of DMP, DBP and DOP were the result of microbial action in this study, sterile control experiments were carried out by autoclaving the marine sediment and seawater for 20 min at 120 °C. The results showed that no significant losses in autoclaved controls were observed during the course of biodegradation of three PAEs (Fig. 3). It suggested that abiotic losses of DMP, DBP and DOP could be neglected, and the losses of three PAEs in natural marine sediment were the result of microbial action.

3.4. ^{13}C isotopic fractionation

Previous studies on biodegradation of PAEs have shown that it starts with a hydrolysis of ester bond under both aerobic and anaerobic conditions [4,32,33]. There are two types of bonds (^{12}C–O and ^{13}C–O bonds) in molecule of PAEs based on stable carbon isotopes (^{12}C, ^{13}C). Owing to kinetic isotope effect, ^{13}C–O bonds break slower than ^{12}C–O bonds, which should give rise to ^{13}C enrichment in the residual PAEs. The $\delta^{13}C$ values for DMP, DBP and DBP at different stages of aerobic biodegradation were monitored by GC–C–IRMS (Fig. 4). As can be clearly seen, the $\delta^{13}C$ values of three PAEs were significantly different from each other at $t=0$ ($f=1$), which relates to the raw materials used in production. At an advanced stage of biodegradation, a small but observed ^{13}C enrichment in the residual of DMP and DBP

occurred. The maximum isotopic enrichment observed in the $\delta^{13}C$ value of the residual DMP and DBP were $+2.05 \pm 0.21‰$ ($f=0.17$) and $+1.92 \pm 0.23‰$ ($f=0.08$), respectively. Within accuracy and reproducibility associated with GC–C–IRMS ($\pm 0.5‰$), no obvious isotope enrichment ($\delta^{13}C=0.55 \pm 0.21‰$) occurred after more than 84% of DOP biodegradation. The small isotopic fractionation effect in our experiments is in agreement with the conclusion observed during aerobic [34] and anaerobic [35] biodegradation of toluene and aerobic biodegradation of benzene [36] by mixed microbial consortia, which were none or only a small carbon isotopic fractionation.

The high-to-low order of isotopic fractionation in our experiment, DMP > DBP > DOP, revealed that ^{13}C isotope enrichment also related to the number of carbon atoms in a given molecule. The reason for this was mainly because the dilution effect existed in the intrinsic isotopic fractionation. (The higher the number of respective atoms per molecule, the more diluted becomes the extent of analyzed isotopic fractionation.) Morasch et al. [37] reported that the maximum molecular mass of a compound that could be analyzed with stable isotope fractionation analysis was around 12–14 carbon atoms. For molecules larger than 12 carbon atoms, the expected isotope shifts upon degradation measured by CSIA are not significantly different from the analytical error. In our experiment, an observed ^{13}C enrichment ($\delta^{13}C=1.92 \pm 0.23‰$) occurred in the residual DBP molecule (16 carbon atoms), which was different from the conclusion of Morasch et al. [37]. Moreover, in the molecule of DOP, which has 24 carbon atoms, slight ^{13}C enrichment was observed at an advanced stage of biodegradation. The reason for this may be that stable ^{13}C isotopic effect in a given molecule is influenced by the binding partner, and tends to be greater if the element is bound to heavier atoms. For example, carbon isotope effects are generally larger in cleavage of C–O bonds than that of C–H bonds [38].

The $\delta^{13}C$ values of the controls were measured in order to determine whether the ^{13}C enrichment in the residual DMP, DBP and DOP were the result of microbial action. The results indicated that there was no change from an initial $\delta^{13}C_0$ within accuracy and reproducibility associated with GC–C–IRMS ($\pm 0.5‰$) and therefore not plotted. These results further suggested that the losses of DMP, DBP and DOP in marine sediment under aerobic conditions were the result of microbial action, which were consistent with the conclusion mentioned in Section 3.3.

4. Conclusions

This study investigated the degradation of DMP, DBP and DOP by mixed microbial populations in marine sediment under aerobic conditions. The results indicated that the biodegradation rate of the three PAEs under aerobic conditions was correlated to the number of carbon atoms in a molecule, and the degradation process could be described using a first-order model. CSIA was used to evaluate the biodegradation of DMP, DBP and DOP in microcosm. A small ^{13}C isotope enrichment in the residual DMP and DBP observed at advanced stage of biodegradation provided the direct evidence of their degradation. No significant ^{13}C isotope enrichment occurred at advanced stage of DOP biodegradation.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (Grant nos. 40976041 and 20775074).

References

[1] V.W. Saeger, E.S. Tucker, Appl. Environ. Microbiol. 31 (1976) 29–34.
[2] J.E. Huff, W.M. Kluwe, Prog. Clin. Biol. Res. 141 (1984) 137–154.
[3] N. Barlow, S. Phillips, D. Wallace, M. Sar, K. Gaido, P. Foster, Toxicol. Sci. 73 (2003) 431–441.
[4] J.C. Inman, S.D. Strachan, L.E. Sommers, D.W.J. Nelson, Environ. Sci. Health B 19 (1984) 245–257.
[5] B.V. Chang, C.M. Yang, C.H. Cheng, S.Y. Yuan, Chemosphere 55 (2004) 533–538.
[6] S. Chatterjee, T.K. Dutta, Chemosphere 70 (2008) 933–941.
[7] J.S. Cho, J.T. Wilson, D.C. DiGiulio, Biodegradation 8 (1997) 265–273.
[8] P. Liang, J. Xu, Q. Li, Anal. Chim. Acta 609 (2008) 53–58.
[9] A. Gómez-Hens, M.P. Aguilar-Caballos, TrAC, Trends Anal. Chem. 22 (2003) 847–857.
[10] C. Pérez Feás, M.C. Barciela Alonso, E. Peña-Vázquez, P. Herbello Hermelo, P. Bermejo-Barrera, Talanta 75 (2008) 1184–1189.
[11] S.A. Mancini, G. Lacrampe-Couloume, H. Jonker, B.M. van Breukelen, J. Groen, F. Volkering, B. Sherwood Lolla, Environ. Sci. Technol. 36 (2002) 2464–2470.
[12] D. Hunkeler, R. Aravena, B.J. Butler, Environ. Sci. Technol. 33 (1999) 2733–2738.
[13] T.B. Hofstetter, M. Berg, TrAC, Trends Anal. Chem. 30 (2011) 618–627.
[14] R.U. Meckenstock, B. Morasch, C. Griebler, H.H. Richnow, J. Contam. Hydrol. 75 (2004) 215–255.
[15] I. Nijenhuis, S.H. Zinder, Appl. Environ. Microbiol. 71 (2005) 1664–1667.
[16] C. Courbet, A. Rivière, S. Jeannottat, S. Rinaldi, D. Hunkeler, H. Bendjoudi, G. deMarsily, J. Contam. Hydrol. 126 (2011) 315–329.
[17] L. Zwank, M. Berg, T.C. Schmidt, S.B. Haderlein, Anal. Chem. 75 (2003) 5575–5583.
[18] C. Griebler, M. Safinowski, A. Vieth, H.H. Richnow, R.U. Meckenstock, Environ. Sci. Technol. 38 (2004) 617–631.
[19] B. Morasch, D. Hunkeler, J. Zopfi, B. Temime, P. Höhener, Water Res. 45 (2011) 4459–4469.
[20] C.V. von Eckstaedt, K. Grice, M. Ioppolo-Armanios, G. Chidlow, M. Jones, J. Chromatogr. A 1218 (2011) 6511–6517.
[21] L.E. Lesser, P.C. Johnson, R. Aravean, G.E. Spinnler, C.L. Bruce, J.P. Salanitro, Environ. Sci. Technol. 42 (2008) 6637–6643.
[22] S.F. Thornton, S.H. Bottrell, K.H. Spence, R. Pickup, M.J. Spence, N. Shah, H.E.H. Mallinson, H.H. Richnow, Appl. Geochem. 26 (2011) 828–837.
[23] T.C. Schmidt, TrAC, Trends Anal. Chem. 22 (2003) 776–784.
[24] M. Rosell, S. Lacorte, D. Barceló, TrAC, Trends Anal. Chem. 25 (2006) 1016–1029.
[25] L. Ellis, A.L. Fincannon, Org. Geochem. 29 (1998) 1101–1117.
[26] R.R. Harrington, S.R. Poulson, J.I. Drever, P.J.S. Colberg, E.F. Kelly, Org. Geochem. 30 (1999) 765–775.
[27] H.N. Gavala, F. Alatriste-Mondragon, R. Iranopour, B.K. Ahring, Chemosphere 52 (2003) 673–682.
[28] C.S. Giam, E. Atlas, M.A. Powers, J.E. Leonard, Phthalic acid esters, in: O. Hutzinger (Ed.), Handbook of Environmental Chemistry. Part C, vol. 3, Springer-Verlag, New York, Berlin, 1984, pp. 67–140.
[29] J. Ejlertsson, M. Alnervik, S. Jonsson, B. Svensson, Environ. Sci. Technol. 31 (1997) 2761–2764.
[30] S.Y. Yuan, C. Liu, C.S. Liao, B.V. Chang, Chemosphere 49 (2002) 1295–1299.
[31] P.H. Howard, R.S. Boethling, W.F. Jarvis, W.M. Meylan, E.M. Michalenko, Handbook of Environmental Degradation Rates, Lewis, Chelsea, Mich., 1991.
[32] R. Kurane, T. Suzuki, S. Fukuoka, Appl. Microbiol. Biotechnol. 20 (1984) 378–383.
[33] J. Wang, L. Chen, H. Shi, Y. Qian, Chemosphere 41 (2000) 1245–1248.
[34] B. Sherwood Lollar, G. Slater, J. Ahad, B. Sleep, J. Spivack, M. Brennan, P. MacKenzie, Org. Geochem. 30 (1999) 813–820.
[35] J.M.E. Ahead, B. Sherwood Lollar, E.A. Edwards, G.F. Slater, B.E. Sleep, Environ. Sci. Technol. 34 (2000) 892–896.
[36] L.G. Stehmeier, M.M. Francis, T.R. Jack, E. Diegor, L. Winsor, T.A. Abrajano, Org. Geochem. 30 (1999) 821–833.
[37] B. Morasch, H.H. Richnow, A. Vieth, B. Schink, R.U. Meckenstock, Appl. Environ. Microbiol. 70 (2004) 2935–2940.
[38] W.P. Huskey, in: P.F. Cook (Ed.), Enzyme Mechanism from Isotope Effects, CRC Press, Boca Raton, FL, 1991, pp. 37–72.