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Stable Carbon Isotope Fractionation of Diethyl Phthalate During Oxidic and Anoxic Degradation in Estuarine Sediment

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Diethyl phthalate was degraded by mixed microbial in estuarine sediment under oxic and anoxic conditions in a laboratory-microcosm. The respective degradation rate constants of diethyl phthalate under both conditions were 0.103 and 0.069 day⁻¹, with respective half-lives of 6.7 and 10 days. Stable carbon isotopic fractionation produced by biodegradation of diethyl phthalate was evaluated with compound-specific isotope analysis under both conditions. A small ¹³C-enrichment in residual diethyl phthalate at advanced stages of biodegradation under both conditions was observed, which was 1.8 and 0.7 ‰, respectively. The isotopic enrichment observed in the residue provided the direct evidence of diethyl phthalate biodegradation in estuarine sediment.

Keywords: Diethyl phthalate, Compound-specific isotope analysis, Degradation.

INTRODUCTION

Phthalic acid esters (PAEs), a class of refractory organic compounds widely used as additives in plastics manufacturing, have been identified in diverse environmental samples including groundwater, soil, lake and estuarine sediments¹. Some of them are considered as potential carcinogens, teratogens and mutagens, and have been listed as priority pollutants and endocrine-disrupting compounds by the USEPA and some of its international counterparts².

Metabolic breakdown of phthalic acid esters by micro-organisms is considered to be one of the major routes of environmental degradation for these widespread pollutants. A number of studies have demonstrated the degradation of several phthalic acid esters in soil, natural water, sediment and waste water by bacteria³. The traditional techniques used to verify the occurrence of intrinsic biodegradation include monitoring depletion of target compounds, electron acceptors and the enumeration microbial metabolites. Approaches mentioned above may not provide irrefutable proof of intrinsic biodegradation for physical processes such as volatilization, sorption and dilution also result in decrease of contaminant and electron acceptors concentration.

Carbon isotope analysis by compound-specific isotope analysis (CSIA) has become a promising tool to assess degradation processes that control the fate of organic pollutants in environment⁴. Owing to the kinetic isotope effect (KIE), a certain extent enrichment of heavy isotopes (¹³C) in the residual

fraction of organic contaminant was commonly observed at the advanced stage of degradation⁵. Increases in isotope ratio nearly always indicate occurrence of degradation, since degradation is the only known natural attenuation process that may result in significant isotopic fractionation^{4,5}. Therefore, compound-specific isotope analysis has been increasingly used to assess the biodegradation of organic contaminants. Numerous studies have been mainly focused on chlorinated ethanes⁶, the BTEX (benzene, toluene, ethylbenzene and xylene) compounds⁷ and methyl *t*-butyl ether⁸. To date, no detailed information concerning isotope fractionation in the course of biodegradation of phthalic acid esters under oxic and anoxic conditions is currently available.

In the present work, diethyl phthalate, priority pollutants, listed by both China National Environmental Monitoring Center and the USEPA, was chosen as a model compound. The biodegradation of diethyl phthalate in anoxic and oxic sediments was simulated by using this compound as a substrate for estuarine mixed bacterial. The aim of this study was to investigate environmental fate of diethyl phthalate in estuarine sediment under anoxic and oxic conditions. Another aim was to investigate whether stable carbon isotopic fractionation during the course of oxic and anoxic biodegradation of diethyl phthalate in estuarine sediment.

EXPERIMENTAL

Diethyl phthalate (analytical grade) was purchased from Shanghai Chemical Reagent Co., China. Hexamethylbenzene

was purchased from Sigma (St. Louis). Analytical grade organic solvents [dichloromethane, acetone and hexane (Tianjin, China, purity > 98 %)] and were distilled before use. Diethyl phthalate stock solution was dissolved in hexane at concentrations of 50,000 mg/L and then diluted with hexane to 5000 mg/L before use.

Surface sediment samples were collected using a stainless steel shovel from Jiushui River, an urbanized estuarine inlet in Qingdao. The top layer (0.5-1.0 cm) of light brown oxic sediment and the remaining sulfide-blackened anoxic sediment (2-8 cm) were 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 an incubator packed on ice for transport to the laboratory. Sediments and seawater were used for incubations immediately after transportation to the laboratory.

Oxic degradation: Surface sediments were first sieved (0.5 mm mesh) to remove macrofauna and any coarse debris. Then, diethyl phthalate (on dry sediment) was spiked into wet sediments and stirred for 2 h to reach a nearly homogeneous distribution. The final concentration of diethyl phthalate in oxic estuarine sediment was about 7.2 µg/g dry sediment. The sediment was placed in thin polyethylene plugs (2 mm thick, 4.5 cm i.d.) for use in incubation experiments. The sediment plugs containing diethyl phthalate were incubated in a relatively large (5 liters) water reservoir, which was bubbled with air for 24 h. Thus solute exchange between sediment and the water reservoir could occur easily. Oxic conditions were controlled by continual purging of the seawater reservoir with CO₂/air (2/98).

Anoxic degradation: The treatment of anoxic sediment was carried out in an anoxic glove bag under a nitrogen atmosphere and the program was similar to those of oxic sediment. The final concentration of diethyl phthalate in anoxic sediment was about 3.6 µg/g dry sediment. Strict anoxic conditions were maintained throughout the process. Anoxic condition in biodegradation was controlled by continual purging of the seawater reservoir with CO₂/N₂ (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 the dark and temperature was kept constant (18 °C).

The sediment samples incubated were extracted three times with 30 mL acetone:hexane (1:2 v:v) mixture for 0.5 h at room temperature (25 °C) in an ultrasonic bath. After dried at room temperature under nitrogen and exchanged to 1 mL of hexane, the extracts were quantitatively transferred onto a silica gel chromatographic column and eluted consecutively with 20 mL hexane, 40 mL 3:7 dichloromethane:hexane and 60 mL 2:8 acetone:hexane. The third fraction, containing diethyl phthalate, was concentrated and further purified on a thin layer chromatography (TLC) for isotope analysis. The concentrated sample was spotted onto a silica gel plate (GF254, Liangchen Guiyuan, Anhui) and developed with 4/1 hexane/acetone ($R_f = 0.6$). Silica was then scraped off the plates after the diethyl phthalate spot dried on the plate. The silica was gently ground to a powder and extracted with hexane in an ultra-sonic bath. The combined extracts were concentrated to dry under a gentle stream of high purity nitrogen and then spiked with 50 µL hexane and 50 µL internal standards, respectively.

Quantitative analyses were performed on a gas chromatograph (SHIMADZU GC-2010) equipped with flame ionization detector and HP-5 capillary column (60 m length × 0.32 mm ID × 0.25 µm df). 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 and 320 °C, respectively. Nitrogen was used as both a carrier gas (flow rate 0.8 mL/min) and make-up gas (flow rate 60 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 (60 m length × 0.25 mm ID × 0.25 µm df). Gas chromatographic conditions were as follows: 3 min isothermal time at 80 °C, heating with 5 °C/min to 310 °C, injector heated at 270 °C. 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 was used. All data are expressed relative to the VPDB (Vienna Pee Dee Belemnite) standard.

RESULTS AND DISCUSSION

During the course of biodegradation, the non-degraded residual diethyl phthalate was monitored by GC-FID analysis (Fig. 1). It can be clearly seen that diethyl phthalate was degraded quickly, with more than 65 and 60 % removed within two weeks under anoxic and oxic experiment, respectively. After 4 weeks, the extent of diethyl phthalate degradation was more than 90 %. After that, degradation of diethyl phthalate decreased. Compared the degradation of diethyl phthalate under both conditions, the rate of diethyl phthalate degradation under anoxic is slower than that under the oxic experiment although the concentration of diethyl phthalate under anoxic condition was only half that under oxic condition. This result was in agreement with the conclusions of previous studies that anoxic degradation of phthalates is generally much slower than oxic degradation⁹.

By fitting the biodegradation process of diethyl phthalate under both conditions with the first order kinetic equation, good linear correlation between the logarithm of diethyl phthalate concentration and time was obtained. The results were consistent with those of Yuan *et al.*⁹, who found that degradation of diethyl phthalate with mixed microbial population in sediment followed first-order model. The kinetic equations of diethyl phthalate degradation were summarized in Table-1, which also indicates that degradation rate under oxic (0.1032) is greater than that under anoxic (0.0693).

TABLE-1
KINETIC EQUATIONS OF DIETHYL
PHTHALATE DEGRADATION

Kinetic equation	Rate (day ⁻¹)	Half-life (day)	R ²
$\ln c_{\text{oxic}} = 2.10 - 0.1032t$	0.1032	6.72	0.9748
$\ln c_{\text{anoxic}} = 1.28 - 0.0693t$	0.0693	10	0.9887

Fig. 1. Biodegradation of diethyl phthalate under anoxic and oxic conditions in estuarine sediment ■, □, oxic degradation; ●, ○, anoxic degradation. Blank are the control experiments

Fig. 2. Biodegradation of diethyl phthalate under anoxic and oxic conditions in estuarine sediment □, oxic degradation; ○, anoxic degradation

Previous studies on the degradation of phthalic acid esters have shown that it starts with a hydrolysis of the ester bond under both oxic and anoxic conditions¹⁰. There are two type of bonds (¹³C-O bonds and ¹²C-O bonds) in diethyl phthalate molecule based on carbon stable isotopes (¹²C, ¹³C). Owing to kinetic isotope effect, ¹³C-O bond breaks slower than ¹²C-O bond and this should give rise to ¹³C enrichments in the residual target compound. The ¹³C values of diethyl phthalate before and after biodegradation under both conditions were monitored by GC/C/IRMS and are presented in Fig. 2. It is clear that biodegradation of diethyl phthalate under both oxic and anoxic conditions, using mixed microbial communities in natural estuarine sediment, produced a small ¹³C enrichment in the residual diethyl phthalate. At the advanced stage of oxic (at > 95 % conversion) and anoxic (at > 92 % conversion) biodegradation, the isotopic enrichment observed in the δ¹³C value of

the residual diethyl phthalate was 1.8 % and 0.7 %, respectively. The small isotopic fractionation effect in the experiments may be associated with the mixed microbial populations investigated in the experiment¹¹ and the results were in agreement with the conclusion observed during oxic¹² and anoxic¹¹ biodegradation of toluene and aerobic biodegradation of benzene¹³ by mixed microbial consortia, which was none or only a small carbon isotope fractionation. Also, the δ¹³C of the control was determined in order to confirm whether the ¹³C enrichment in residual diethyl phthalate was the result of microbial action. The result indicated that there was no change from an initial δ¹³C₀ of diethyl phthalate within accuracy and reproducibility associated with GC/C/IRMS (± 0.5 %). These results further dominated that the loss of diethyl phthalate in sediment under oxic and anoxic conditions were the result of microbial action which was consistent with the conclusion mentioned in diethyl phthalate degradation.

Conclusion

Results of this study demonstrated that diethyl phthalate could be rapidly degraded by mixed microbial populations in estuarine sediment under oxic and anoxic conditions. The degradation rate of diethyl phthalate under oxic condition was greater than that under anoxic condition. The kinetics of diethyl phthalate biodegradation under both conditions could be described using a first-order model. Furthermore, CSIA was used to evaluate the biodegradation of diethyl phthalate in microcosm. The results indicated that a small ¹³C enrichment observed in the residual diethyl phthalate at advanced stage of biodegradation provided the direct evidence of diethyl phthalate biodegradation.

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