Biodegradability of four phthalic acid esters under anaerobic condition assessed using natural sediment

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Abstract: Biodegradability of di-n-butyl phthalate (DBP), butylbenzyl phthalate (BBP), di-ethylhexyl phthalate (DEHP), and di-isonyl phthalate (DINP) under an anaerobic condition was evaluated using three natural sediment microcosms obtained from ponds in Osaka, which had not been significantly polluted by the chemicals. The degradabilities of the four phthalic acid esters (PAEs) were analyzed by a first-order kinetic model with a lag phase and ranked as DBP > BBP > DEHP > DINP. The PAEs with shorter alkyl-chains, DBP and BBP, were degraded with quite short lag phases near to zero and short half-lives of a few days. The PAEs with longer alkyl-chains, DEHP and DINP, were degraded with lag phases of 5–30 d and the quite long half-lives of a couple of hundred days. Although no data was available on the anaerobic biodegradability of DINP before this study, it was clarified that DINP can be degraded with slow degradation rates. The fact that all the three intact sediments were capable of biodegradation of the PAEs suggests that potential of anaerobic biodegradation of PAEs is widespread in the aquatic environment.

Keywords: phthalic acid esters; anaerobic biodegradation; sediment; first-order kinetics

Introduction

In recent years phthalic acid esters (PAEs) utilized as plasticizers have been detected throughout the environment and a considerable attention is paid to their toxicity and the biodegradability (Staples et al., 1997). Once PAEs enter into water environment, they are preferentially adsorbed to the suspended particles and sediments due to their low solubility and high hydrophobicity. Actually, some PAEs have been frequently detected in sediments (Fromme et al., 2002; Yuan et al., 2002). Anaerobic biodegradation would play more important roles for PAEs in sediments rather than aerobic biodegradation although the anaerobic degradation rates are usually one order of magnitude lower than the aerobic ones (Staples et al., 1997; Yuan et al., 2002).

A number of studies have been reported on the biodegradation of PAEs, especially under aerobic conditions (Staples et al., 1997). Although several studies on the anaerobic biodegradation of PAEs have been also reported, microorganisms used in those tests were usually well acclimated to the test chemicals or obtained from artificial/semi-artificial environments such as anaerobic digesters (Shelton and Tiedje, 1984; Ziegler et al., 1999; Jianlong et al., 2000; Gavala et al., 2003) and landfill sites (Ejertsson et al., 1997; Jonsson et al., 2003). On the other hand, only a little information exists on the anaerobic biodegradation of PAEs by intact natural sediments/microbes (Johnson and Heitkamp, 1984; Painter and Jones, 1990; Yuan et al., 2002; Chang et al., 2005), which makes it difficult to assess the fates of PAEs under anaerobic conditions in the aquatic environment.

Di-n-butyl phthalate (DBP) and butylbenzyl phthalate (BBP) are suspected to be endocrine disruptors (Jobling et al., 1995; Harris et al., 1997; Beresford et al., 2000), while di-ethylhexyl phthalate (DEHP) and BBP are probable human carcinogens (class B and class C, respectively) (USEPA, 2000). Although di-isonyl phthalate (DINP) is one of the PAEs with the largest production rate as well as DEHP, surprisingly, there is a lack of accurate data on the anaerobic biodegradability. The aim of this study was to evaluate the anaerobic biodegradability of PAEs in the aquatic environment and to estimate their degradation rates. Four commercial PAEs were selected; DBP, BBP, DEHP and DINP to be examined for their biodegradability by three intact pond sediments.

1 Materials and methods

1.1 Chemicals

Analytical grade of BBP (Tokyo Chemical, Tokyo), DBP (Kishida Chemical, Osaka), DEHP (Kishida Chemical, Osaka) and DINP (Wako, Osaka) were utilized in the biodegradation tests. The chemicals were used without any further purification. Analytical grade of acetonitrile (Kishida Chemical, Osaka) was utilized. The chemical composition of mineral salt medium used in the biodegradation test was as follows; K2HPO4, 356 mg; KH2PO4, 272 mg; NH4Cl, 530 mg; MgCl2·6H2O, 100 mg; CaCl2, 75 mg; FeCl2·4H2O, 20 mg; NaHCO3, 1.2 g and 0.1 ml of a trace metal solution in 1 L of deionized water. The trace metal solution consisted of MnCl2·4H2O, 500 mg; ZnCl2, 50 mg; H3BO3, 50 mg; CuCl2, 30 mg; NaMO3·2H2O, 10 mg; CoCl2·6H2O, 500 mg; NiCl2·6H2O, 50 mg and Na2SeO3, 50 mg in 1 L of deionized water.

1.2 Biodegradation test

All experimental procedures followed the general
method for determining anaerobic biodegradation potential from Shelton and Tiedje (1984). The anaerobic microcosm for the primary biodegradation test comprised a 25-ml glass bottle containing a pond sediment sample and a PAE-containing solution. Because of variation in solubility, the PAEs with different initial concentrations was prepared in acetone at 200 g as TOC (total organic carbon) in the bottle and stayed overnight to let acetone evaporated. Sediment samples were collected from the Piano, Ue, and Zuion Ponds in Osaka, Japan in May, 2003 (Table 1). The sediments of the three shallow ponds had not been exposed to strictly-anoxic conditions and no anthropogenic source of PAEs existed near the sampling sites. They were taken as grab samples from 0 to 10 cm depth of the sediment and washed 3 times with a sterile buffer solution (KH2PO4, 2.65 g/L, Na2HPO4, 3.8 g/L, pH 7.0) by centrifugation (4500 g for 10 min). The sediment sample was mixed with 20 ml of the mineral salt medium and dispensed into the bottle. The microcosms made from the sediment of Piano, Ue and Zuion ponds had the original solid content of 106.6, 52.4 and 82.2 g/L dw, respectively. The pH value was adjusted by HCl or NaOH to 7.2. Head space of the bottle was filled with N2 gas for creating the anaerobic condition, and a small amount of Na2S·9H2O was added to the medium as a reducing agent to completely suffocate the test bottle. The bottles were sealed with rubber stoppers and capped with aluminum crimp seals and incubated stationary at 28°C in a dark condition to avoid photolysis. Several replicate bottles were prepared and each sediment sample was reproduced in duplicate for all experiments.

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>Temperature, °C</th>
<th>pH</th>
<th>Solid content, g/L dw</th>
<th>Ignition loss, % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piano Pond</td>
<td>15.7</td>
<td>6.47</td>
<td>106.6</td>
<td>26.9</td>
</tr>
<tr>
<td>Ue Pond</td>
<td>16.3</td>
<td>6.95</td>
<td>52.4</td>
<td>15.7</td>
</tr>
<tr>
<td>Zuion Pond</td>
<td>15.2</td>
<td>6.61</td>
<td>82.2</td>
<td>30.0</td>
</tr>
</tbody>
</table>

1.3 Analytical procedures

Degradation of PAEs was assessed by measuring the disappearance of the parent chemicals by high performance liquid chromatography (HPLC) analysis. One bottle was sacrificed for analysis at each sampling time. Hexane and acetone were used to extract PAEs and evaporated to 2.0 ml before analyzed by HPLC. The HPLC apparatus consisted of a CCPE solvent delivery pump (Tosoh, Japan) with PX-8010 solvent controller (Tosoh, Japan), and UV-8010 spectrophotometric detector (Tosoh, Japan) connected to an advanced computer interface with chromatogram analyses performed by a chromatography work station (AI-450, ver. 3.320J: Dionex, USA). Samples were injected through TSK ODS 80-TM column (Tosoh, Japan) with the mobile phase of acetonitrile and water mixture (90:10 v/v) at a flow rate of 0.5 ml/min. PAEs were detected by UV detector at a wavelength of 254 nm. The detection limit was 100 μg/L for each of PAEs. The errors in the PAEs measurements were less than 5% in all the replicated analyses.

1.4 Kinetic analysis

The PAEs’ biodegradation data obtained in this study were fitted to simple models which have been frequently used, and a modified first-order kinetic model with a lag phase was selected as a best one to properly describe the degradation kinetics:

\[ C = \begin{cases} C_0 & (0 \leq t < t_{lag}) \\ C_0 \exp(-kt) & (t \geq t_{lag}) \end{cases} \]

where \( C \) is the PAE concentration, \( C_0 \) is the initial PAE concentration, \( k \) is the biodegradation rate constant, \( t \) is the time period, \( t_{lag} \) is the lag phase for biodegradation. The half-lives for PAEs degradation were calculated as \( t_{1/2} = \ln 2 / k \). An optimized procedure for determining the parameters was performed using a method that found the minimum of the sum of squared deviations between the observed and model calculations by the 4th-order Runge-Kutta method.

2 Results and discussion

Results of the biodegradation tests of PAEs under the anaerobic condition by the natural sediment microcosms are shown in Fig.1, where the percentage of the remaining parent compounds relative to the initial concentration is plotted against the incubation time. The four PAEs were biodegraded more or less within the experimental period under the anaerobic condition, though the degree and the rate of the degradation between the shorter alkyl-chain PAEs (s-PAEs), BBP and DBP, and the longer alkyl-chain PAEs (l-PAEs), DEHP and DINP were very different. On the other hand, the degradation curves for each PAE were similar independently of the sediment source.

The s-PAEs were efficiently degraded by all the sediment microcosms and their concentration reached below the detection limits within 3 weeks. In contrast, the l-PAEs degradation proceeded very slowly and more than 80% of the initial l-PAEs still remained after 3 months. This tendency coincides with the results from previous studies on the anaerobic degradation of PAEs using digested sewage sludge (Shelton and Tiedje, 1984; Ziogou et al., 1989; Jianlong et al., 2000; Gavala et al., 2003), municipal solid waste under landfilling conditions (Ejlersson et al., 1997; Jonsson et al., 2003), and anaerobic freshwater or salt marsh sediments (Johnson and
Heitkamp, 1984; Pointer and Jones, 1990; Yuan et al., 2002; Chang et al., 2005). To our knowledge, it first reports the biodegradability of DINP under anaerobic degradation of DINP.

We tried to apply several simple kinetic models to describe the anaerobic degradation curves of the PAEs, and selected a modified first-order kinetic model considering a lag phase as the most suitable one. The kinetic analyses for the s-PAEs and l-PAEs degradation by Piano Pond sediment are shown in Fig.2 as an example. The model provided good matches to the experimental data (all the regression resulted in $r^2 > 0.99$ except for DINP degradation by Ue Pond sediment), and the estimated parameters are summarized in Table 2.

The simple first-order kinetic model (without a lag phase) was often applied for describing the biodegradation of s-PAEs such as dimethyl phthalate (DMP) and DBP, and proved to be one of the most suitable model (Jianlong et al., 1995, 1996, 2000; Yang et al., 2002; Chang et al., 2005). In this study, BBP and DBP biodegradation data were compatible with the first-order kinetics with negligible or quite short lag phases (Fig.2a), indicating the simple first-order model was enough to express the anaerobic degradation of s-PAEs as well as the previous studies.

On the other hand, the degradation of the l-PAEs, DEHP and DINP, could not be described well by the simple first-order kinetics, and apparent lag phases were observed at the beginning of the experiments (Fig.2b). The degradation kinetics of l-PAEs, especially under anaerobic conditions, have been rarely discussed until now, however, Madsen et al. (1999) have proposed to use a biphasic model to explain the complex degradation pattern of DEHP in a sludge-amended soil. In their study, the initial degradation phase was described well by the first-order kinetics, whereas the lower degradation activity in the late phase (>40 d) was described better by the fractional power kinetics. Although the anaerobic degradation kinetics of DEHP and DINP were biphasic in our study also, that is initial lag
phases followed by first-order kinetics. The initial lag phases for PAEs biodegradation have never reported before. The long lag phases observed for the degradation of DEHP and DINP in this study suggest that acclimation periods would be necessary for the intact sediment microbes to exhibit the degradation activities toward l-PAEs.

The estimated parameter values for the modified first-order kinetic model (Table 2) quantitatively confirmed the tendency that the s-PAEs were more easily degraded than the l-PAEs. BBP and DBP were degraded with quite short lag phases \((t_{wa})\) of nearly zero and short half-lives \((t_{1/2})\) of a few days, while DEHP and DINP were degraded with long lag phases of 5—30 d and quite long half-lives of a couple of hundred days. According to the parameter values, the degradation rate of BBP was slightly lower than those of DBP, and DINP needed much shorter lag periods but longer half-lives than DEHP in all the microcosms. Considering both \(t_{wa}\) and \(t_{1/2}\) \((t_{wa} + t_{1/2})\) as an index of degradability, the tested PAEs may be ranked by their biodegradability under the anaerobic condition as DBP > BBP > DEHP > DINP.

The fact that all the sediment microcosms, which had never been acclimated to the PAEs, were capable of their biodegradation suggests PAEs-degrading microbes ubiquitously exist in natural sediments, although some reports concluded that DEHP could not be degraded under anaerobic conditions (Shelton et al., 1984; Ejlertsson et al., 1997). Since anaerobic biodegradation of different PAEs should depend on different facilitative and obligate anaerobic microbes (Shelton et al., 1984; Chang et al., 2005), it may be necessary to isolate and characterize the corresponding degrading-microbes for more profoundly understanding and precisely predicting the fate of PAEs under the anaerobic conditions.

3 Conclusions

The ubiquitous existence of anaerobic microcosms responsible for the degradation of PAEs in natural sediments was established. The main factor affecting the biodegradation of PAEs under anaerobic condition was their chemical structure, particularly the alkylation length. The high biodegradability of s-PAEs was demonstrated via the short lag phases and half-lives in first-order kinetic model, whereas, l-PAEs could be induced or triggered to hydrolyze only a minor extent and suspected to persist for months or years.

References:


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