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Effect of Nutrients and Light on Biodegradation of Dibutyl Phthalate and Di-2-ethylexyl Phthalate in Haihe Estuary

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Phthalic acid esters (PAEs) are a class of refractory organic compounds widely used as plasticizers in polyvinyl chloride plastics. They have attracted increasing attention owing to their widespread use, ubiquity in the environment, and endocrine-distrupting activity (Fatoki 1993; Hashizume, 2002). PAEs have been listed as 'priority pollutants' by the US EPA.

Biodegradation is a critical process affecting the environmental fate of PAEs. A number of studies have demonstrated the degradation of several PAEs in soil, natural water, sediment and wastewater by bacteria (Inman, 1984; Jianlong, 1995; Chang, 2005). However, microalgae have been shown to be capable of degrading organic pollutants (Kuritz, 1995; Semple, 1999). In aquatic systems, the proliferation of phytoplankton may therefore enhance the degradation process. Alternatively, competition for resources, or other trophic relationships between phytoplankton and bacteria may inhibit the proliferation of those bacteria in the degradation process. Algae are limited by light and nutrients, and the combined effects of light and nutrients on algae are often more important than their independent effects (Taulbee, 2005). The change of nutrient conditions or illumination may influence the biomass between phytoplankton and bacteria, which also may influence the biodegradation of PAEs. Therefore, it is necessary to examine the effects of nutrient conditions and illumination on biodegradation of PAEs. In this study, we considered two of the most important and abundant PAEs, di-n-butyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP). The biodegradation of DBP and DEHP was studied by a nutrient (N, P) enrichment experiment with and without illumination.

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Materials and Methods

DBP and DEHP, 99.9% analytical standards, were purchased from Sigma Corporation USA. The organic reagents, including hexane and formaldehyde, were of analytical grade and purchased from Tianjin Second Reagent Manufactory. All other chemicals were of analytical-reagent grade and purchased from Tianjin Third Reagent Manufactory.

Water used in this degradation study was sourced from Haihe Estuary and stored in a tank. After one day of equilibration, a series of 250 ml flasks were each filled with 100 ml of the water. Prior to filling, the flasks were sterilized at 160°C for 2 h. The water in the flasks was dosed with PAE-methanol stock solution, yielding final concentrations of 208 to 230 μg/L for DBP and 176 to 216 μg/L for DEHP. Half the flasks were provided with nitrogen (N, as KNO_3) and phosphorus (P, as KH_2PO_4) to enhance the nutrient levels. Then all flasks were kept in a culture room at 25°C. The experiment was divided into four groups and the experimental conditions are given in Table 1. The experiment lasted four days. Water samples were taken at 0, 8, 24, 36, 48, 96 hours. Controls were performed under identical conditions with the addition of formaldehyde (final concentration, 1.3%). PAEs were extracted from 40 ml aliquots of the water samples with 3 ml hexane, three times each for 10 minutes; and the hexane solution was analyzed by gas chromatography. The average recoveries for DBP and DEHP in the range of experimental concentrations were 92.1% and 88.2%, respectively. The method

Table 1 Experimental conditions

No	Light intensity (lux)	PH	TN (mg/L)	TP (mg/L)	N: P	Chlorophyll (mg/L)	SPM (mg/L)
I	4000 ± 100	8.7	12.8	1.05	12	0.051	81
II	—	8.5	12.8	1.05	12	0.051	81
III	4000 ± 100	8.2	4.6	0.02	230	0.014	76
IV	—	8.1	4.6	0.02	230	0.014	76

detection limits for DBP and DEHP were 4 μg/L and 5 μg/L, respectively.

Concentrations of DBP and DEHP in the water samples were determined by an Agilent 6890N gas chromatograph equipped with a flame ionization detector (GC-FID) and HP-5 capillary column (film thickness, 0.25 μm; inner diameter, 0.32mm; length, 30m). Nitrogen (50 mL/min) served as carrier gas; using hydrogen and airflow rates of 37 and 550 mL/min, respectively. The temperature of injector and detector both were set at 250°C. The initial column temperature was set at 120°C for 2 min and then increased by 15°C/min to 250°C, which was held for 3 min.

Chlorophyll, a measure of phytoplankton biomass, was determined using a UV/Vis spectrometry (Crank, 1975). Nutrient determinations were analyzed according to the standard methods (National Environmental Protection Bureau, 1997).

Results and Discussion

Figures 1 and 2 show the biodegradation of DBP and DEHP in the water samples at different ambient N and P concentrations with and without illumination. By fitting the biodegradation process of DBP and DEHP with the first order kinetic equation, good linear correlation between logarithm of PAE concentration and time was obtained (correlation coefficients > 0.9566). From the control tests, the losses of DBP and DEHP under different experimental conditions were in the range of 1.1%–5.6% and 0.9%–9.8%, respectively, over 4 days.

Therefore, it can be considered that the decline of DBP and DEHP concentration in the tested water samples was mainly due to biodegradation.

As shown in Table 2, the first-order biodegradation rate constants of DBP and DEHP under high nutrient conditions are higher than those under low nutrient conditions, regardless of the lighting conditions. Additions of N and P clearly stimulated DBP and DEHP biodegradation. This is partially supported by the increase of suspended particulate matter (SPM) in the water samples, which is related to the biomass of microorganism. Under high nutrient conditions, the increase of SPM in the water samples during the 4-day period was 170 mg/L in the light and 109 mg/L in the dark,

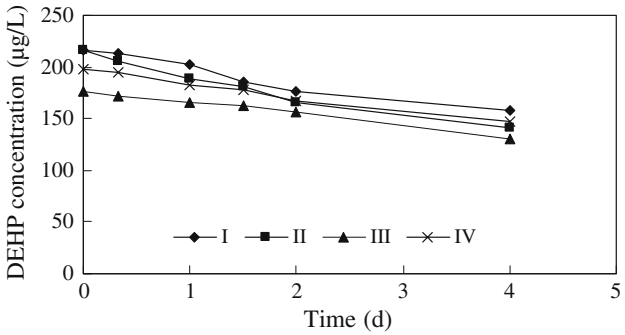

Fig. 1 Biodegradation of DBP under different conditions

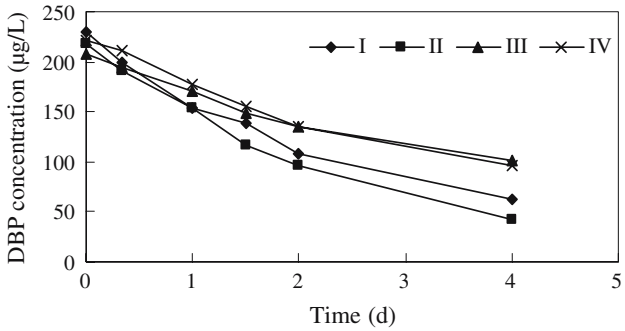

Fig. 2 Biodegradation of DEHP under different conditions

whereas under low nutrient conditions, it was only 44 mg/L in the light and 32 mg/L in the dark. Studies by Rubin et al. (1982) showed that no DEHP mineralization was observed after 60 days in water from an oligotrophic lake. In contrast, mineralization occurred immediately with DEHP in eutrophic lake water. The Knapp et al. (2003) reported alachlor transformation to be strongly associated with aquatic fertility conditions and biotic activity in aquatic mesocosms.

Under the same nutrient and illumination conditions, the first-order biodegradation rate constants of DBP were higher than those of DEHP (Table 2). The increase of the molecular weights could explain the decrease of the biodegradation rates from DBP to DEHP.

In the presence of illumination, biodegradation of DBP and DEHP was retarded under high nutrient conditions, but there was no obvious retardation under low nutrient con-

Table 2 Biodegradation of DBP and DEHP

	I	II	III	IV
Total removal percentage (%)				
DBP	72.4	80.3	51.1	56.4
DEHP	26.7	34.4	26.3	25.9
Removal percentage by biodegradation (%)				
DBP	69.6	77.2	48.3	55.8
DEHP	26.5	32.3	23.7	24.2
Contribution of biotic removal (%)				
DBP	96.1	96.1	94.4	98.9
DEHP	99.1	94.0	90.2	93.4
K (d^{-1})				
DBP	0.32	0.41	0.17	0.20
DEHP	0.082	0.10	0.069	0.072

k, the first-order biodegradation rate constant

ditions (Table 2). Under high nutrient conditions, chlorophyll a, a measure of phytoplankton biomass, increased from 0.051 mg/L to 0.062 mg/L in the experiment with illumination, but slightly decreased in the experiment without illumination over 4 days. Under low nutrient conditions, there was no significant change in chlorophyll a in the experiment with illumination during the 4-day period, implying that illumination does not stimulate the proliferation of phytoplankton. In contrast, there was only a slight decline in chlorophyll a in the experiment without illumination.

Zohary et al. (2005) reported phytoplankton was N and P co-limited in the eastern Mediterranean Sea. Under low nutrient condition, the initial N:P ratio was 230 in this study, indicating a strong deficit in P. Therefore, it can be expected that there was no noticeable phytoplankton response to illumination when phytoplankton were exposed to P deficiency. Our data are consistent with Zohary's results. Under high nutrient conditions, the water samples were initially in a balanced condition with respect to the N: P ratio (N: P = 12). This ratio is adapted to the growth of phytoplankton (Kunikane, 1984; Peng, 1988). Therefore, nutrients were not a factor limiting the proliferation of phytoplankton and light could stimulate the growth of phytoplankton. This was supported by the chlorophyll a increase in the experiment with illumination. In a closed system, the growth of phytoplankton has an effect on bacterial growth because they are often limited by the same resource (mainly inorganic phosphorus) (Currie, 1990; Michael, 1999). Consequently, the dynamics of phytoplankton and bacteria must influence the biodegradation of DBP and DEHP, resulting in the retardation in biodegradation of DBP and DEHP. Mann et al. (2000) found that in the presence of illumination, biodegradation of a nonylphenol ethoxylate was retarded and heterotrophic bacterial proliferation was inhibited. Their studies also showed that algal proliferation in light was concomitant with a reciprocal decrease in heterotrophic bacteria. Our results are consistent with theirs.

Results of this study demonstrate that nutrient level and illumination were significant to cause biodegradation of DBP and DEHP. Biodegradation of DBP and DEHP was influenced by the interactive effects of light and nutrients, and the transformation of DBP and DEHP was primarily biotic. Additions of N and P clearly stimulated DBP and DEHP biodegradation. The N: P ratio had a strong effect on DBP and DEHP biodegradation. In this study, biodegradation of DBP and DEHP was retarded in the presence of illumination under N: P ratio balanced condition (N: P = 12), whereas the retardation in biodegradation was not obvious in a strong P-limited condition (N: P = 230). DBP was biodegraded more easily compared with DEHP.

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