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Activated sludge-mediated biodegradation of dimethyl phthalate under fermentative conditions

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Abstract

The biodegradation of dimethyl phthalate (DMP) was investigated under fermentative conditions in this study. The nature of the intermediate compounds and the extent of mineralization were probed using high-pressure liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) methods. The fermentative bacteria were able to biodegrade the DMP under anaerobic conditions, with the biodegradation rate of 0.36 mg DMP/(L·h). The results demonstrated that the DMP degradation under fermentative conditions followed the modified Gompertz model with the correlation coefficient of 0.99. Monomethyl phthalate (MMP) and phthalic acid (PA) were detected as the intermediates of DMP biodegradation. During the experiment, MMP was rapidly produced and removed; however, PA accumulated as the biodegradation was slower throughout the course of the experiment. The COD_{Cr} concentration decreased from 245.06 to 72.01 mg/L after the experimental operation of 20 d. The volume of methane produced was 3.65 ml over a period of 20 d and the amount of methane recovered corresponded to 40.2% of the stoichiometric value. The COD_{Cr} variation and methane production showed that the DMP could not be completely mineralized under the fermentative conditions, which implied that the fermentative bacteria were not able to biodegrade DMP entirely.

Key words: activated sludge; biodegradation; dimethyl phthalate; fermentation; phthalic acid; mineralization

Introduction

Phthalic acid esters (PAEs) are a class of synthetic organic compounds commonly used as additives of polyvinyl chloride or polyvinyl acetate. Because of the noncovalent nature of the bond between the phthalate plasticizer and polyvinyl chloride or polyvinyl acetate, PAEs can easily migrate to their ambient environment (Nilsson, 1994). The global use of plasticized plastics has made them detectable in every environment in which they have been sought (Giam *et al*., 1984; Johnson *et al*., 1984), with the highest concentrations found adjacent to phthalate production or plastics processing facilities.

PAEs can enter the human food chain, affecting human health, and leading to cancers. In recent times, the behavior of PAEs in the environment has grabbed much attention because they are considered as endocrine-disrupting chemicals (Colon *et al*., 2000; Main *et al*., 2006). They have been listed as priority pollutants and endocrine-disrupting compounds by the United States Environmental Protection Agency and China National Environmental Monitoring Center.

Bioremediation offers a potential solution for the conversion of PAEs into harmless end products such as CO_2 and H_2O. Studies have shown that PAEs are biodegraded by employing different electron acceptors. The biodegradation of PAEs under aerobic conditions has been studied extensively (Wang and Gu, 2006; Vega and Bastide, 2003; Oliver *et al*., 2007; Li *et al*., 2005). On the contrary, few studies exist on the biotransformation of phthalate esters under anoxic (Wang *et al*., 1999; Wu *et al*., 2007) and sulfate reducing conditions (Cheung *et al*., 2007). Moreover, few researches have focused on the biodegradation of dimethyl phthalate (DMP) under fermentative conditions. In the circumstances such as deep-water, soil, and anoxic sediments, fermentation will be the important metabolic pathway because of lack of oxygen (Lamin and Moktar, 2003; Speranza *et al*., 2006). Consequently, a better understanding of the fate of PAEs under anaerobic conditions may provide a deeper insight into their ecotoxicological effects, which is predominantly important for the well being of aquatic ecosystems. Furthermore, information regarding the rates and effectiveness of redox pathways is essential for *in situ* biodegradation of PAEs.

The aim of the present study was to evaluate the biodegradability of PAEs under methanogenic conditions, and to clarify the nature of the intermediate compounds and the extent of mineralization as a result of the biodegradation.

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1 Materials and methods

1.1 Inoculum, culture medium, and enrichment

DMP (> 99.5% purity) was purchased from Guangdong Guanghua Chemical Factory Co. Ltd., China. The anaerobic digested sludge used as inoculum for the present research was collected from the Sibao Wastewater Treatment Plant in Hangzhou, China. Two liter sludge was put into a 4-L glass bottle and mixed with the enrichment medium. The culture was purged with oxygen-free argon gas for 10 min and then incubated at 30°C. The enrichment medium contained 20 mg/L of DMP as source of carbon and energy. The minimal medium was composed of $CaCl_2{\cdot}2H_2O$ (0.3 g/L), $Na_2HPO_4{\cdot}2H_2O$ (0.065 g/L), $MgCl_2{\cdot}6H_2O$ (0.165 g/L), and 1.25 ml/L of trace element solutions I and II, respectively (Qian and Min, 1986). The minimal medium for DMP-degrading cultures was replaced at intervals of approximately 3 d, on the depletion of DMP. During the enrichment, DMP concentration was gradually increased from 20 to 100 mg/L. The DMP-degrading enrichment medium was replaced more than 10 times before being used for biodegradation experiments.

1.2 DMP biodegradation experiment

After the enrichment, the digested sludge was washed thrice, with 0.02 mol/L K_2HPO_4-KH_2PO_4 buffer solution and transferred into 170 ml serum bottles. All the serum bottles contained 135 ml minimal medium containing 15 ml of washed sludge and 100 mg/L DMP. The pH of the medium was adjusted to 7.0 using 1 mol/L NaOH solution. After purging continuously with oxygen-free argon gas for 10 min, the bottles were airtight by applying butyl rubber stoppers and then sealed with paraffin. The volume of methane produced by 170 ml serum bottle cultures was measured daily with the help of a Smith fermentation tube. The Smith fermentation tube contained 40 g/L NaOH solution and carbon dioxide was absorbed. The culture was then incubated at 30°C. The methane production was corrected for background level in a control culture.

The control experiment was also conducted using the same minimal medium (without DMP solution) sterilized with γ-radiation. After sterilization, 2,000 mg/L DMP storage solution, filtered through a 0.22-μm membrane, was added into the sterilized medium and was also incubated at 30°C.

The γ-ray sterilization was carried out at the Zhejiang University Radiation Center, China. The sterilization was manipulated using 18.5×10^{15} Bq source with a round ^{60}Co-γ-ray set. Culture liquid was put at a distance of 0.86 m from the source, sterilization duration was 10 h, and radiation of 20 kGy dosage was used.

Aqueous samples were periodically collected through sterile syringes, and were centrifuged at 6,000 r/min for 5 min. The aqueous solution was filtered through a 0.22-μm membrane and was subsequently analyzed through HPLC.

1.3 Analytical procedures for the detection of DMP and its metabolites

The filtered samples were subsequently analyzed, to determine the amounts of DMP through HPLC (Agilent 1100 Series, USA). The HPLC apparatus was equipped with a Diotron array detector and Zorbax SB C18 (4.6 × 150 mm, 5 μm, Agilent, USA) chromatography column. Methanol-0.5% and acetic acid solution 50:50 (*V/V*) were used as the mobile phase with a flow rate of 1.00 ml/min. The UV detecting wavelength was set at 254 nm, with a 20-nm strip width, as compared with a wavelength of 330 nm having 50 nm strip width.

The DMP biodegradation metabolites were identified using LC-MS (Agilent 1100 SL, USA) analyses. The mass spectrometer was equipped with electrospray ionization (ESI), positive ion scan model, mass scanner with a range of 100–300 amu, fragmentor was with 100 V, drying gas flow was 13.0 L/min, nebulizer pressure was 413 KPa, drying gas temperature was 35°C, and capillary voltage was 4,000 V. The rest of the analytical methods were identical to HPLC.

1.4 Other analytical procedures

Chemical oxygen demand (COD), ammonia, nitrite, nitrate were measured according to the standard methods for examination of water and wastewater (APHA, 1985).

2 Results and discussion

2.1 Variation in the DMP concentration

The variation in the DMP concentrations under anaerobic biodegradation by mixed cultures is presented in Fig.1. The DMP concentration in control (CK) varied in the range of 103.01 to 96.30 mg/L within 16 d; however, it decreased from 103.59 mg/L to an undetectable level within 12 d in the presence of nonsterile inoculum.

Previous researchers used the first-order kinetic model to describe the biodegradation of PAEs under various environmental conditions (Yuan *et al*., 2002; Chang *et al*., 2005; Lertsirisopon *et al*., 2006). However, first-order kinetic model was not suitable to express DMP biodegradation under fermentative conditions in the present research, as the correlation coefficient value was only 0.8954. On the basis of previous studies (Fang *et al*., 2006; Li *et al*.,

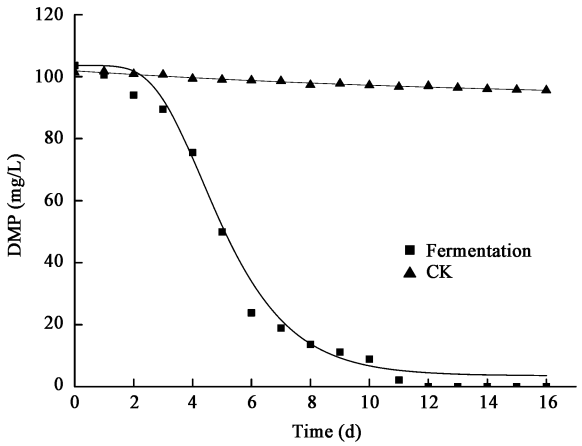

Fig. 1 Variation in dimethyl phthalate (DMP) concentration under fermentative conditions.

2005), a modified Gompertz Model was used to explain the kinetics of phthalic acid degradation. The results in Fig.1 demonstrated that DMP degradation under fermentative conditions followed the modified Gompertz model (Zwietering *et al*., 1990) (the correlation coefficient was 0.9929), which could be expressed as follows:

$$S = S_0 - A \times \exp\left(-\exp\left(\frac{\mu_m \times e}{A} \times (\lambda - t) + 1\right)\right) \quad (1)$$

where, S is the substrate concentration at t time, S_0 is the initial concentration, A indicates the degradation potential, μ_m is the maximum degradation rate, and λ is lag phase.

The kinetic parameters compiled in Table 1 show that DMP degradation under fermentative conditions requires a lag time of 2.5 d and the rate of degradation is 0.896 mg/(L·h).

2.2 Identification of the intermediates of DMP biodegradation

The HPLC chromatogram of DMP biodegradation, under fermentative conditions is shown in Fig.2. Monomethyl phthalate (MMP) and phthalic acid (PA) were detected as intermediates of DMP biodegradation. The results were comparable with the previous studies on the biodegradation of PAEs under aerobic and anaerobic conditions (Chang *et al*., 2005; Gu and Yao, 2006; Wang and Gu, 2006). On the basis of the standard sample, the variations in the MMP and PA concentrations are shown in Fig.3. During the experiment, MMP was rapidly produced and removed; however, PA accumulated and biodegraded more slowly throughout the course of the experiment. Numerous studies had focused on the biodegradation pathways of PAEs. It was recommended that biodegradation of phthalic acid 3,5- or 4,5-dihydrophthalate pathway to procatechuate occurred under aerobic conditions (Eatons and Ribbons, 1987; Nomura *et al*., 1989). Nevertheless, little was known about the pathways of anaerobic catabolism. Benzoate was shown as an intermediate during anaerobic biodegradation of PAEs (Liang *et al.*, 2007; Xia, 2002).

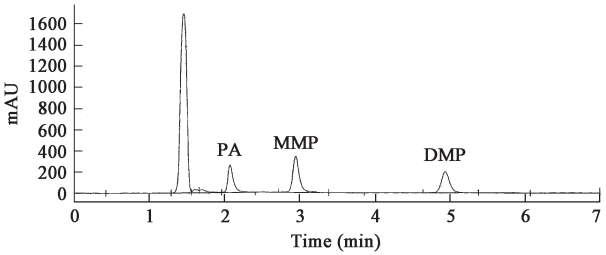

Fig. 2 HPLC chromatogram of DMP degradation.

Table 1 Kinetic parameters of DMP biodegradation under fermentative conditions

Initial concentration (mg/L)	λ (d)	μ_m (mg DMP/(L·d))	A (mg/L)	R^2
104	2.5	21.5	103	0.9929

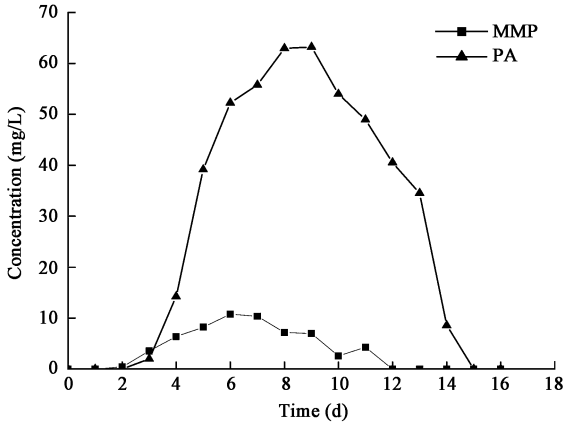

Fig. 3 Variations of monomethyl phthalate (MMP) and phthalic acid (PA) under fermentation condition.

In a chemical reaction, the Gibb's free energy is always used to denote the feasibility of the reaction. In this experiment, to get an insight of the accumulation of PA through the reaction, free energy is used to describe the variation of DMP, MMP, and PA throughout the reaction. The free energy changes during biodegradation, benzoate and their mineralization are shown in Table 2.

It is evident that the free energy of DMP mineralization is –398.07 kJ/mol, which suggests that the reaction can proceed spontaneously (Table 2). The free energy of DMP hydrolysis to benzoate is –189.83 kJ/mol and its value for PA conversion to benzoate is –48.31 kJ/mol. It implies that the free energy for DMP biodegradation to PA is greater than PA degradation to benzoate, hence, PA formation is easier than the formation of benzoate, and that is the reason why PA accumulation throughout the reaction was observed in present work .

2.3 Variation in the COD_{Cr} concentration, pH, and methanogenesis

Chemical oxygen demand is used to describe the biodegradability of PAEs (Wang, 2004; Banat *et al*., 1999; Liang *et al*., 2007). The variations of COD_{Cr} are presented in Fig.4. It is evident that the COD_{Cr} concentration decreases from 245.06 to 72.01 mg/L within 20 d. A previous investigation has achieved 93% removal efficiency (Liang *et al*., 2007), which is much higher than the present study.

At the same time, the authors considered the methane production during the DMP fermentation biodegradation. Under fermentative condition, the DMP was subsequently converted to CH_4 and CO_2 by biodegradation. Therefore, methane production could be an indicator of biodegra-

Table 2 Redox reactions involved in DMP biodegradation, associated free energy change

Biodegradation procedure	Redox pathways for DMP degradation	ΔG (kJ/mol)
DMP⟶PA	$C_{10}H_{10}O_4 + H_2O = C_8H_6O_4 + 1.5CH_4 + 0.5CO_2$	–189.83
PA⟶benzoate	$C_8H_6O_4 = C_7H_6O_2 + CO_2$	–48.31
DMP mineralization	$C_{10}H_{10}O_4 + 5.5H_2O = 4.75CO_2 + 5.25CH_4$	–398.07

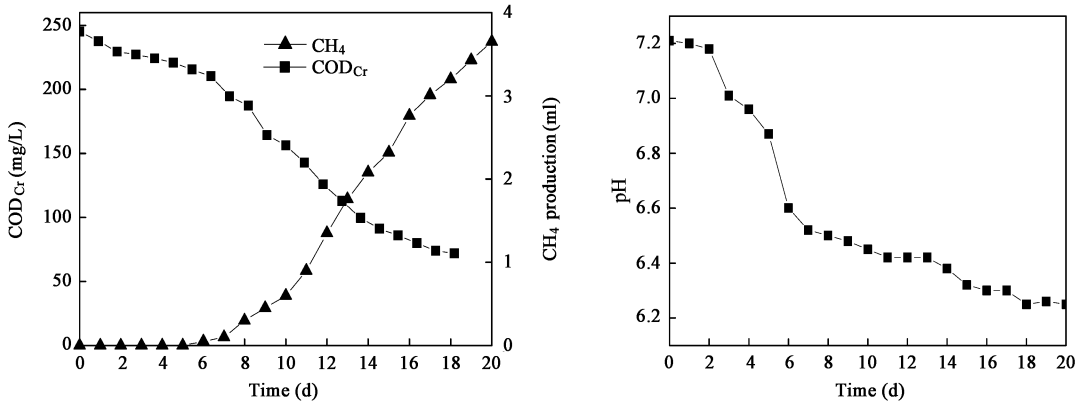

Fig. 4 Variation of COD_{Cr} concentration, methane production, and pH value under fermentative condition.

dation status. The stoichiometry of the methanogenic conversion of DMP is illustrated in Table 1. The stoichiometric analysis indicated that theoretically 9.09 ml methane could be produced from biodegradation of each mol of DMP under anaerobic conditions. In the present experiment, the amount of methane produced is shown in Fig.3.

The volume of methane produced was 3.65 ml over a period of 20 d and the amount of methane recovered from biodegradation of DMP corresponded to 40.2% of the stoichiometric value. Healy and Young (1979) demonstrated that the amount of methane produced was closer to the theoretical value (99% to 100%) when aromatic compounds were mineralized under strictly anaerobic conditions. The present results of the COD_{Cr} variation and methane production showed that the DMP could not be completely mineralized under fermentative conditions, which implied that fermentative bacteria were not able to biodegrade DMP entirely.

The variations in pH of the culture are shown in Fig.4. The pH of the culture medium decreased from 7.21 to 6.25. Compared with the previous research (Chang *et al*., 2005), the medium pH may rise when the PAEs are mineralized by mixed bacterial cultures. The variation in pH value may be owing to the fact that DMP could not be mineralized completely under fermentative conditions, in the present investigation. As DMP and their metabolites cannot be degraded completely and persistently, lack of oxygen under fermentative conditions may become a critical determinant factor in the incomplete biodegradation of DMP. Because of the endocrine disrupting nature of DMP and its metabolites, and also its deleterious effects on human health, better understanding of the intermediates and additional methods to remove DMP under fermentative conditions may be an appropriate approach to cope with these toxic pollutants.

3 Conclusions

The biodegradability of PAEs under methanogenic conditions was evaluated, also the nature of the intermediate compounds and the extent of mineralization were investigated in this study. The DMP concentration was decreased from 103.59 mg/L to an undetectable level in the presence of nonsterile inoculum within 12 d. The DMP degradation under fermentative conditions followed the modified Gompertz Model with a high correlation coefficient. The HPLC chromatograms of DMP biodegradation, under fermentative conditions showed that MMP and PA were intermediates of DMP biodegradation. The present results of the COD_{Cr} variation and methane production showed that the DMP could not be completely mineralized under the fermentative conditions, implying that the fermentative bacteria were not able to biodegrade DMP entirely. It is recommended that the supplementary of electron acceptor could be an efficient approach for the complete removal of DMP under fermentative conditions.

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