Short Communication

Increase in biodegradation of dimethyl phthalate by
Closterium lunula using inorganic carbonHai Yan^{a,*}, Gang Pan^{a,b}^a State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-environmental Sciences,
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Abstract

The effect and mechanism of inorganic carbon (IC) on the biodegradation of dimethyl phthalate (DMP) by a green microalga *Closterium lunula* was investigated. The growth of this microalga and the biodegradation of DMP were significantly enhanced when the initial IC was increased. An intermediate product of DMP biodegradation was identified as phthalic acid (PA) that was accumulated and caused a sharp decrease in pH of microalgal culture medium, which inhibited both the growth of microalga and the biodegradation of DMP. A suggested second-order kinetic equation of organic pollutant biodegradation by microalgae ($-dC/dt = kNr$) fitted well with the experimental data. The increase of IC caused a decline in biodegradation rate constant for organic carbon (k) and an increase in growth (N) by supplying a favorite carbon source and mitigating the decrease of pH. As the net effect, the overall biodegradation rate of DMP was promoted as IC increased, which was dominated by the increase of microalgal growth.

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1. Introduction

Dimethyl phthalate (DMP) is broadly used in many industrial processes including construction, automobile and chemical engineering and is increasingly released into the environment all over the world. Because of the low biodegradability and its toxic effect on aquatic animals (Adams et al., 1995; Rhodes et al., 1995), DMP is listed as a priority pollutant in many countries.

Although bacteria play a key role in the biodegradation of organic pollutant, recent studies indicated that, in addition to providing oxygen for aerobic bacterial biodegradation, microalgae can also biode-

grade organic pollutants directly (Semple et al., 1999). It was reported that more than 30 azo compounds were biodegraded and decolorized by *Chlorella pyrenoidosa*, *Chlorella vulgaris* and *Oscillatoria tenuis*, in which azo dyes were decomposed into simpler aromatic amine (Liu and Liu, 1992). Klekner and Kosaric (1992) found that 2,4-dinitrophenol was quickly biodegraded and converted to an isomer of dimethyl benzenediol by *Chlorella*. In the study of biodegradation of phenol by *Ochromonas danica*, it was found that [U - ^{14}C] phenol could be completely mineralized (Semple and Cain, 1996; Semple, 1998). Furthermore biodegradations of DDT, linear alkylbenzene sulfonate, and tributyltin by microalgae were also studied (Yan et al., 1998; Tsang et al., 1999; Megharaj et al., 2000). However, little information was found on the control of the biodegradation activity of organic pollutants by microalgae.

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Here the biodegradation of DMP by *Closterium lunula* was studied. Phthalic acid (PA) was found to be an intermediate product of DMP biodegradation, which could be further biodegraded by this microalga. The biodegradation rate of DMP increased with the increase of initial concentration of inorganic carbon (IC). The addition of IC caused a decline in degradation rate constant and an increase in microalgal growth.

2. Materials and methods

2.1. Chemicals and microalga

All chemicals used were analytical grade. *C. lunula* was bought from the Institute of Hydrobiology, Chinese Academy of Sciences.

2.2. Culture medium

The culture medium consists of 0.20 g of $(\text{NH}_4)_2\text{SO}_4$, 0.03 g of superphosphate, 0.08 g of MgSO_4 , 0.03 g of KCl, 0.01 g of NaCl, 1.0 ml soil extract, 0.15 ml of 1% (w/v) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution and 1000 ml of distilled water. Different initial IC concentrations in the medium were prepared using NaHCO_3 .

2.3. Experimental and analytical procedures

The toxicity (96-h EC_{50}) of DMP on the inhibition of the growth of *C. lunula* was evaluated to be 331 mg l^{-1} using standard bioassay method (APHA et al., 1985) before the biodegradation experiment. So the initial concentration of DMP was set to 100 mg l^{-1} and initial microalgal cell density was prepared as $0.15 \times 10^5 \text{ ml}^{-1}$ for the biodegradation experiment. *C. lunula* grew in 100 ml-flasks containing 30 ml inoculums in a Climatic Chamber at 24°C with the light intensity of 3000 lux 12-h light-dark circle. A hemocytometer was used to count cell density under a microscope. Concentrations of DMP, PA and IC as well as pH were measured once a day for a period of 6 days. Data presented in the figures are the average values of each sample measured three times with standard deviation.

The culture solution filtered through a $0.22 \mu\text{m}$ membrane was measured for DMP and PA concentrations on a HPLC (Shimadzu-LC10A) at 228 nm using a C_{18} column. The mobile phase was 75% (v/v) methanol–water solution and the flow-rate was 1.0 ml min^{-1} . The injection amount was 20 μl , and the peak areas were used to calculate the concentrations of DMP and PA with the calibrate curves established. pH of culture solution was measure by a pH meter (Orion model 868) and IC in filtered sample was also determined by a TOC meter (Tekmer Dohrmann-Apollo 9000).

2.4. Controls

Controls consist of 100 mg l^{-1} DMP in the culture medium without the alga. Experimental conditions were the same as above. The change of DMP concentration in the control was less than 5% in the period of 6 days. Thus, the rapid decline of DMP concentration in the medium was mainly due to the biodegradation of DMP by *C. lunula*.

3. Results and discussion

3.1. The growth of alga and the biodegradation of DMP

The growth of *C. lunula* was significantly increased as the initial IC increased (Fig. 1a). Concurrently, DMP decreased with the increase of IC with the most rapid decrease happened in day 2 to 3 (Fig. 1b). About 35% of the initial DMP was biodegraded when initial IC was 0.5 mg l^{-1} , but all DMP added was decomposed when initial IC was increased to 21.5 mg l^{-1} during the period of 6 days (Fig. 1b).

Fig. 1. Effect of IC on the growth (a) and the biodegradation of DMP (b) by *C. lunula*.

Fig. 2. Consumption of IC during the growth of the alga and the biodegradation of DMP.

IC was also rapidly consumed during the lag and exponential growth phases of *C. lunula* (Fig. 2). Both IC and organic carbon of DMP could be used as carbon sources to support the growth of microalga. At initial IC of 0.5 mg l^{-1} , IC reduced to nil at day 1 (Fig. 2) and organic carbon from DMP was therefore the sole carbon source for the growth of the alga after day 1 under this condition. Although DMP could be used as the sole carbon source to support a slow heterotrophic growth of *C. lunula*, the growth was apparently increased when more initial IC was added. Compared with the organic carbon of DMP, IC was a more favorite carbon source for the growth of *C. lunula* (Fig. 1a) and the biodegradation of DMP was promoted as the increase in IC (Fig. 1b).

3.2. The production of phthalic acid (PA) during the biodegradation of DMP

Fig. 3a is the profiles of DMP and PA on HPLC, which show that the peaks of standard PA of 50 mg l^{-1} and DMP of 100 mg l^{-1} appear at 1.5 and 2.6 min, respectively. Fig. 3b, c and d are the HPLC profiles in the biodegradation experiment of day 6 at different initial IC, which indicate that the peaks of an intermediate product A of DMP biodegradation by *C. lunula* and DMP also appear at 1.5 and 2.6 min, respectively. Because the retention time and the absorbance scanning profile of an intermediate product A in the ultraviolet range from 200 to 300 nm were all same to those of standard PA, so the intermediate product A of DMP biodegradation by *C. lunula* was identified as PA.

As DMP reduced, PA was produced correspondingly, with the most rapid increase of PA occurred in day 2 to 3 (Fig. 4a). However the highest level of PA was produced when the initial IC was 11.5 mg l^{-1} .

The accumulation of the intermediate product of PA in the medium caused a sharp decrease in pH between day 2 and 3 (Fig. 4b). Other researchers also reported that PA was an intermediate product in the pathway of phthalate ester biodegradation by numerous microorganisms (Evans, 1997; Wang et al., 1999, 2000) and microalgae (Yan and Liu, 1998; Yan et al., 2002). The product of PA could be further biodegraded by *C. lunula*, which was most obvious when the initial IC was 21.5 mg l^{-1} , where concentration of PA was even lower than those when initial IC was set to 11.5 mg l^{-1} (Fig. 4a).

3.3. Influence of pH on the growth of algae and the biodegradation of DMP

A fast drop in pH occurred between day 2 and 3, which corresponded to the sharp increase of PA and decrease of DMP (Figs. 1b and 4a and b).

Two main contrasting factors were responsible for the change of pH. Firstly the three forms of IC in the medium were CO_2 , HCO_3^- and CO_3^{2-} when NaHCO_3 was added as the carbon source. $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{HCO}_3^-$, and $\text{HCO}_3^- \rightarrow \text{H}^+ + \text{CO}_3^{2-}$. Because CO_2 can mainly be used to support the growth of alga through the photosynthesis and the consumption of CO_2 can cause the decrease of H^+ concentration, which may increase the pH of medium. On the other hand the production of PA from the biodegradation of DMP may release H^+ that may decrease pH of medium. When initial IC was higher, more CO_2 form of IC was used, which could better mitigate the decrease of pH caused by the production of PA. Furthermore when pH was lower than 5, more CO_2 was formed and released into atmosphere from the medium. So IC (NaHCO_3) is a key factor for the degradation of DMP not only because it can enhance the growth as a favorite carbon source, but also because it can maintain a suitable pH for the growth of alga and the biodegradation of DMP.

3.4. The kinetics of the biodegradation

The kinetics of the biodegradation of organic pollutant by microalgae can be described as $-dC/dt = kNr$ (Yan et al., 1995). C is the concentration of organic compound; k is a second-order rate constant; N is the density of algal cells; and r is the growth rate of microalgae. When r is substituted by dN/dt ,

$$C = -0.5kN^2 + C_0 \quad (1)$$

Eq. (1) fitted experimental data well. From the modeling, k were calculated to be 67.0, 7.1, and $3.2 \text{ mg l}^{-1} \text{ N}^{-2}$ when initial IC were 0.5, 11.5, and 21.5 mg l^{-1} , respectively. Although k was apparently declined with the increase of IC, the overall

Fig. 3. HPLC profiles of PA, DMP and an intermediate product A of DMP biodegradation by *C. lunula* at day 6: (a) standard PA of 50 mg l⁻¹ and DMP of 100 mg l⁻¹; (b) at initial IC of 0.5 mg l⁻¹; (c) at initial IC of 11.5 mg l⁻¹ and (d) at initial IC of 21.5 mg l⁻¹.

biodegradation rate of DMP increased because of the increase in the square of microalgal cell density. As IC increased, the alga used more inorganic carbon than organic carbon for growth, making the degradation rate constant for organic carbon declined. At the same time, the increase of IC overwhelmingly increased the growth, making the overall biodegradation rate increased.

4. Conclusions

Biodegradation of DMP by *C. lunula* was enhanced when initial IC increased. PA was produced as an intermediate product during the biodegradation of DMP, which caused a sharp decrease in pH of culture solution and inhibited both the growth of *C. lunula* and the biodegradation of DMP. However the increase of

Fig. 4. Effect of IC on the production of PA during the biodegradation of DMP (a) and the change of pH of culture medium (b).

initial IC triggered a big increase in growth of alga by supplying a favorite carbon source and mitigating the decrease of pH, which might promote the biodegradation of DMP. Because more IC and less organic carbon of DMP were used to support the growth of alga with the increase of IC, the biodegradation rate constant declined, but the overall biodegradation rate of DMP was increased. So IC was playing a very important role in the biodegradation of DMP by *C. lumula*.

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