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Di-*n*-butyl phthalate removal using mixed cultures in batch reactors

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ABSTRACT

Di-*n*-butyl phthalate (DBP), belonging to the family of phthalic acid esters, is usually used as plasticizer. Application of extensive plastic commodities has made DBP the most frequently identified chemical found in the environment. A recent research concern has been raised that DBP may be a mutagen and carcinogen, and also an endocrine-disrupting chemical. Thus, environmentally friendly deposal and treatment of DBP are critically important. In this research, an acclimatized mixed culture was used to explore the treatments of different DBP concentrations in synthetic and actual wastewaters by batch strategy. The mixed culture could utilize DBP for growth when the initial DBP concentration was lower than 1000 mg l^{-1} in both wastewaters. Initial DBP concentrations between 200 and 1000 mg l^{-1} in the synthetic wastewater could be neutralized at 91–99% removal efficiency. The same concentrations in actual wastewaters contained removal efficiencies between 92 and 100%.

1. Introduction

Phthalate esters (PAEs), one derivative of phthalic acid (PA), are aromatic colorless liquids with high-molecular weight, stability, low-volatility and low-solubility in water (Chang et al., 2004; Chao et al., 2006). These compounds are widely used as resin plasticizers in numerous products (Earls et al., 2003), and as additives in paints and inks (Nalli et al., 2006). Owing to their extensive application, these compounds are widely found throughout the environment such as farmland in peri-urban areas (Zeng et al., 2008), the air of urban areas (Teil et al., 2006) and urban soils (Zeng et al., 2009). PAEs are also potential endocrine-disrupting chemicals and can bio-accumulate via the food chain (Jarošová, 2006).

The main PAE in plastics and river sediments is di-*n*-butyl phthalate (DBP) and di-(2-ethylhexyl)phthalate (DEHP) (Yuan et al., 2002). DBP is commonly used as a plasticizer additive in cosmetics and skin care products because of its oily texture which would increase product flexibility (Lovekamp-Swan and Davis, 2003). DBP disrupts the endocrine system and produces marked changes in the growth and development of male reproductive organs (Chen et al., 2011). Basing on the urinary metabolite concentrations and urinary excretion factors, the highest median intake for DBP is estimated at 2.1 μg kg^{-1} per day (Naarala and Korpi, 2009). Due to its threat to human health, DBP has been classified as a level 4 toxic chemical substance in Taiwan. The USA and EU have also prohibited utilizing DBP in toy manufacture. The reason is that these plasticizers are not bound covalently to the resin, which allows them to migrate into the environment (Cartwright et al., 2000). A substantial amount of research (Wang et al., 2000; Chang et al., 2005, 2007; Zhou et al., 2005) has suggested the importance of DBP biodegradation on solid phases, such as river sediments, sludge, and wetlands. However, research contributing to treat wastewaters containing high DBP concentration is rare. To investigate DBP biodegradation in wastewaters, mixed culture originating from activated sludge and farmland soils is acclimated. The DBP degradation performance in artificial and actual wastewaters is studied using a batch bioreactor strategy.

2. Materials and methods

2.1. The mixed culture sources

The mixed microbial cultures were obtained from activated sludge and agricultural soil. The former culture was obtained from an aeration tank at an industrial wastewater treatment plant located in Central Taiwan, and the latter culture was retrieved from rice paddy fields.

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2.2. Mixed culture acclimation

The sludge (20 ml) and the soil (5 g) were both placed into a 500 ml flask containing 300 ml phosphate buffered medium (PBM) (Wang et al., 2009). After adding 200 mg l^{-1} DBP as the sole carbon source, the flask was then incubated on a shaker (180 rpm) at 30 °C for 7 days. After 7 days, an appropriate amount of the mixed culture was transferred into another flask containing fresh PBM with 400 mg l^{-1} DBP for another 7 days incubation. This procedure was repeated, and each time the DBP concentration was increased by about 200 mg l^{-1} until it reached 1000 mg l^{-1} to obtain the enriched mixed culture used in this research.

PBM also refers to the synthetic wastewater containing the following inorganic compounds (in grams per liter): $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.02; K_2HPO_4, 1.5; KH_2PO_4, 1.5; NH_4Cl, 0.32; trace element solution, 10 mL/L. The composition of the trace element solution included (in mg per liter): $FeSO_4 \cdot 7H_2O$, 300; $MgCl_2 \cdot 4H_2O$, 180; $CoCl_2 \cdot 6H_2O$, 106; $Na_2MoO_4 \cdot 2H_2O$, 34; $ZnSO_4 \cdot 7H_2O$, 40. The medium pH was adjusted to 7 with 5 N NaOH.

2.3. Actual wastewater pretreatment

The actual wastewater was obtained from the influent at an industrial wastewater treatment plant in the Central Taiwan. The wastewater with massive suspended solids was first filtered using 80-mesh sieve. The filtered wastewater was then passed through a 0.45 μm Nylon membrane followed with filtering through a 0.10 μm VacuCapTM90 filter (Gelman Laboratory, Ann Arbor, Michigan, USA). After pretreatment, the wastewater was stored at 4 °C until use.

2.4. Batch reactor experiment

DBP biodegradation in the synthetic and actual wastewaters were investigated by batch bioreactor experiments. These experiments were conducted using a series of 50 ml serum bottles. After biomass preincubation and washing, the pellets of the enriched culture were resuspended in the synthetic wastewater or the actual wastewater with cell concentration between 0.08 and 0.1 OD_{600} units. The working volume of each serum bottle was 10 ml. After adding 200–1000 mg l^{-1} DBP as the carbon substrate and sealing with Teflon/silicon stoppers, the serum bottles were shaken at 180 rpm in the dark at 30 °C. The pH, OD_{600}, ammonia and DBP concentration variations were measured periodically.

2.5. Analytical methods

Before DBP analysis, the pH value of wastewaters stored in serum bottles was measured using a pH meter (Mettler Toledo Seven Easy with electrode Mettler Toledo InLab®413, Switzerland). Twenty ml of methanol was then added into the serum bottle. Thirty ml of this mixture was sonicated for 5 min (Bransonic, model 5510R-MT, Branson Ultrasonics Corp., Danbury, CT). After passing through a 0.22 μm pore size PVDF membrane (Millex®-GV), the filtrate was used for DBP concentration and OD_{600} value analysis. DBP was analyzed using high performance liquid chromatography (HPLC) with a UV detector. HPLC was performed with a Waters system equipped with a Agilent eclipse XDB-C18 (3.5 μm × 4.6 mm × 100 mm) column at a flow rate of 1.0 ml/min. The solvent system ratio was methanol: water = 90:10. The UV detector absorbency wavelength was fixed at 234 nm. Recovery percentage for DBP was 95.8–102.4%. The limit of the detection method was 0.96 mg/l. The OD_{600} was measured using a spectrophotometer (Thermo Spectronic Genesys 10 UV, Thermo Fischer Scientific Inc., Waltham, MA, USA). The ammonia concentration was measured using the indophenol blue method (Keeney and Nelson, 1982).

3. Results and discussion

3.1. DBP removal in the synthetic wastewater

Fig. 1 shows the removal of various DBP concentrations using the acclimated mixed culture in the synthetic wastewater. The removal efficiencies for 200 mg l^{-1} DBP were 36.1% and 64.1% in 8 and 16 h, respectively. There was no lag phase during DBP degradation. When the DBP concentration was increased to 400 mg l^{-1}, 41.8% and 79.6% DBP were removed within 8 and 20 h, respectively. Final DBP removal efficiency achieved 91.1%. Within 8 and 20 h, the mixed culture utilized 38.5% and 71.2% of 600 mg l^{-1} DBP, respectively. At the end of the experiment, the residual DBP was 23 mg l^{-1} referring to 96.3% removal efficiency. When the DBP concentrations were 800 and 1000 mg l^{-1}, the removal efficiencies were 99.6% and 96.8%, respectively. Based on the experiment, higher initial DBP concentrations caused more significant biomass growth and a

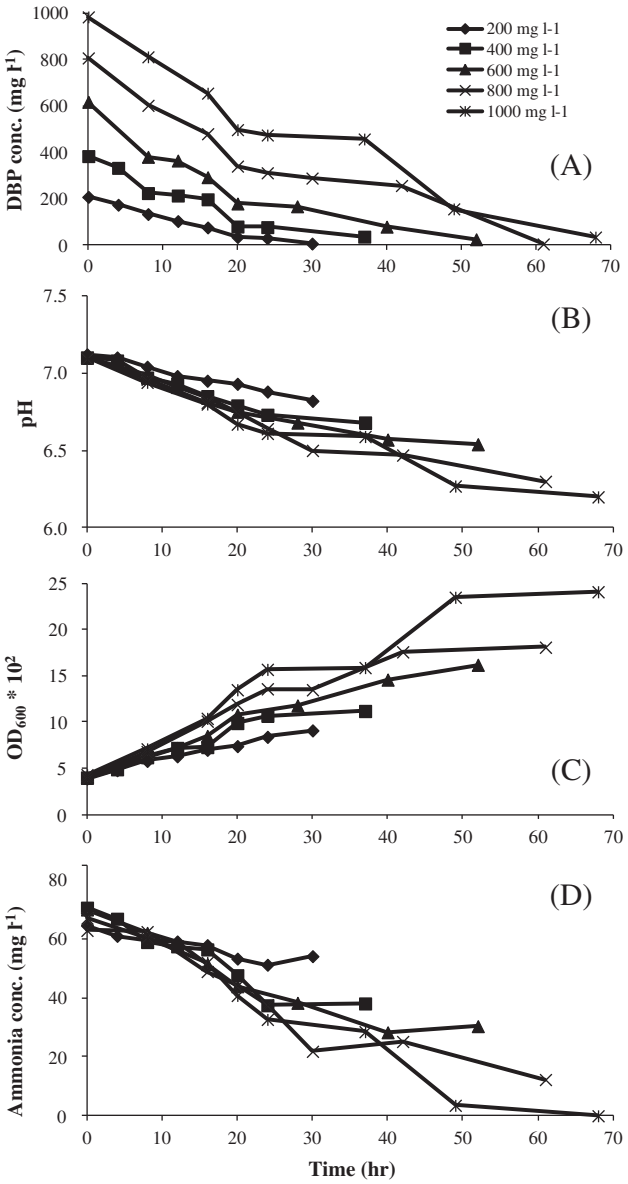

Fig. 1. Time course of the removal of various DBP concentrations by mixed culture in the synthetic wastewater in the batch reactor.