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Di-*n*-butyl phthalate removal by strain *Deinococcus* sp. R5 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 the plasticizer. Extensive and massive plastic commodities use has made DBP the most identified chemical in environment. DBP is also suspected as a mutagen and an endocrine-disrupting chemical. Thus, cautious treatment and deposal of DBP containing wastewater and waste is critically important. In our previous study, potential microbial consortia capable of treating wastewater with 1000 mg l^{-1} DBP were successfully acclimatized. In this research, DBP utilizing strains are isolated from the acclimatized mixed culture. DBP degradation of pure strains is explored in synthetic and actual wastewaters. The results indicated total 9 strains were isolated and 6 strains (T1, T2, T3, R1, R4, and R5) could utilize 500 mg l^{-1} DBP for growth. Among these candidates, strain R5 demonstrated the best DBP removal efficiency, and was identified as *Deinococcus* sp. by 16S rDNA sequence analysis. Strain *Deinococcus* sp. R5 could utilize DBP for growth when the initial DBP concentration was lower than 1000 mg l^{-1} in both wastewaters. DBP removal efficiencies in both wastewaters could achieve 100% within 140 h.

1. Introduction

Phthalate esters (PAEs) are a class of refractory organic compounds widely used as plasticizers in polyvinyl chloride (PVC) plastics (Wang et al., 2000, 2013). These compounds are aromatic colorless liquids with high-molecular weight, stability, low-volatility and low-solubility in water (Chang et al., 2004; Chao et al., 2006; Santino et al., 2013). They are the most common industrial chemicals (Earls et al., 2003) and have become widespread in the environment as they have been found in sediments, natural waters, soils, plants, aquatic organisms and many types of vegetable (Atlas and Giam, 1980; Staple et al., 1997; Teil et al., 2006; Zeng et al., 2008, 2009; Srivastava et al., 2010; Fu and Du, 2011; Kong et al., 2012; Wang et al., 2012; Sun et al., 2013; Yang et al., 2013b). PAEs are also potential endocrine-disrupting chemicals and can bio-accumulate via the food chain (Hens and Caballos, 2003; 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; Srivastava et al., 2010; Yang et al., 2013b). 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 researches (Wang et al., 2000; Chang et al., 2005, 2007; Zhou et al., 2005) have 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. In our previous study, potential microbial consortia capable of treating wastewater with 1000 mg l^{-1} DBP

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Table 1
The physiochemical characteristics of the actual wastewater.

Item	Data
Temperature	22.9 °C
pH	7.21
COD	564 mg l^{-1}
Total suspended solid	118 mg l^{-1}
Conductivity	1429 μs cm^{-1}
Copper	0.175 mg l^{-1}
Zinc	0.319 mg l^{-1}
Nickel	0.849 mg l^{-1}
Total chromium	0.207 mg l^{-1}

were successfully acclimatized (Yang et al., 2013a). In this research, DBP utilizing strains are isolated from the acclimatized mixed culture. DBP degradation of pure strains is explored in synthetic and actual wastewaters.

2. Materials and methods

2.1. Mixed culture sources and acclimation

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.

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 7 days incubation. This procedure was repeated, and each time the DBP concentration increased 200 mg l^{-1} until it reached 1000 mg l^{-1} to obtain the enriched mixed culture.

PBM also refersto 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^{-1}. 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.

2.2. Actual wastewater pretreatment

The actual wastewater was obtained from the influent at the industrial wastewater treatment plant in the Central Taiwan. The physiochemical characteristics of the actual wastewater are listed in Table 1. The industrial wastewater treatment plant received wastewaters from 1018 various scales of factories. Among these factories, there are 16 rubber plants and 88 plastic plants. 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.

2.3. Isolation of pure strains from acclimated mixed strains and the determination of their DBP degradation potential

At stable degradation of DBP, the mixed culture was diluted in the inorganic culture media and a series of the dilution were spread on tryptic soy agar (TSA) and Reasoner 2 agar (R_2A) to obtain single colonies (the plates were incubated at 30 °C for 24 h). The loopfuls of single colony from each plate were streaked onto other fresh TSA or R_2A medium plates to check for purity. After purification process, each strain was checked for its ability to utilize DBP by inoculating them into separate serum bottles containing the inorganic media and 200 or 500 mg l^{-1} DBP. The initial cell concentration was about 0.1 OD_{600} units. After sealing with Teflon/silicon stoppers, the serum bottles were shaken at 180 rpm in the dark at 30 °C. After 7 days, the DBP concentration was analyzed. TSA culture medium components (g l^{-1}) included peptone from casein, 15; peptone from soymeal, 5; NaCl, 5; agar, 15. The R_2A culture medium components (g l^{-1}) are yeast extract, 0.5; protease peptone, 0.5; casamino acids, 0.5; glucose (dextrose), 0.5; starch soluble, 0.5; K_2HPO_4, 0.3; pyruvate sodium, 0.3; $MgSO_4 \cdot 7H_2O$, 0.05.

2.4. Identification of the DBP degrading bacterium

Strain identification was entrusted Mission Biotech Company (Taipei, Taiwan) to carry out.

2.5. Batch reactor experiment

DBP biodegradation in the synthetic and actual wastewaters were investigated by batch bioreactor experiments. The experiments were conducted using a series of 50 ml serum bottles. After biomass of pure strain 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.

2.6. 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

Table 2
Pure strains isolated from DBP acclimated mixed culture and their DBP degrading performance.

Strain number	200 mg l^{-1}	500 mg l^{-1}	Strain number	200 mg l^{-1}	500 mg l^{-1}
	DBP removal efficiency (%)			DBP removal efficiency (%)	
T1	100	80	R1	74	90
T2	86	67	R2	34	–
T3	93	72	R3	9.5	–
T4	40	–	R4	75	87
			R5	98	99