Application of Bioaugmentation Strategy in Biodegradation of Di-n-octyl Phthalate (DOP) in Sequencing Batch Reactor (SBR)

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Abstract. In order to enhance the degradation of Di-n-octyl phthalate (DOP), one bacterial strain was inoculated in SBR. Bioaugmentation by inoculating DOP-degrading bacteria effectively shortened the start-up of SBRs and significantly enhanced DOP degradation in bioreactors (p<0.05). 92.3% DOP removal rate was achieved in consortium bioaugmented SBR, which was double of control SBR. The DOP removal in control reactor was only 37%. The results from this study suggest bioaugmentation is an effective and feasible approach for DOP waste water treatment in practical engineering.

Introduction

Di-n-octyl phthalate (DOP) is one of the most commonly utilized plasticizers and are widely used as building materials, packaging materials, and cosmetics production[1]. DOP is one of most common used PAEs. Three of the phthalic acid esters, namely, di-methyl phthalate (DMP), di-n-butyl phthalate (DBP) and di-n-octyl phthalate (DOP) have been listed as priority pollutants by China National Environmental Monitoring Center and the US Environmental Protection Agency[2]. PAEs have received increasing attention in recent years due to their widespread produce, use and disposal, as a result they are also ubiquitous in environments. Moreover, these compounds are concerning because they have been shown to interfere with the reproductive system of human and animal. Phthalate esters (PAEs) are a prominent group of environmental pollutants and endocrine-disrupting compounds in many environments [3, 4]. In addition, DOP can be taken up by crops and thus enter the food supply chain system, which may harm aquatic organisms and human health[5].

DOP is difficult to be degraded in wastewater treatment plants, because they are designed mainly for removal of nitrogen and phosphorus. Previous studies have revealed that DOP and DBP can be removed by natural processes in natural environments, such as hydrolysis, photo degradation and biodegradation[6, 7]. Due to the low rate of chemical hydrolysis and photolysis of DOP, metabolic breakdown of this widespread pollutant by microorganisms is considered to be the major route[8]. Several PAEs-degrading bacterial strains belonging to the genera Sphingomonas, Pseudomonas, Rhodococcus, Microbacterium and Gordonia have been isolated from different environments, such as active sludge[9, 10].

In the study, after the isolation of DOP-degrading bacteria, the strain was inoculated in SBR system. The biodegradation characteristics and enhanced effects were investigated. The result from this study is expected to provide useful information for DOP bioremediation.

Materials and Methods

Reagents and Chemicals

DOP (99.5% purity) for the experiment was purchased from ChengduKelong Chemical Reagent Co., Ltd. All the chemical reagents were of analytical grade and all solvents (Ethyl acetate and methanol) were of HPLC grade purchased from Tianjingkemiou Reagent Co., Ltd. The MM contained (1L): MgSO₄·7H₂O 0.5 g, K₂HPO₄ 1.70 g, FeSO₄·7H₂O 0.05 g, and NaNO₃ 0.5 g. (NH₄)₂SO₄ 1.0 g, Na₂MoO₄·0.0024 g, CaCl₂·2H₂O 0.04 g, FeCl₃·0.0018 g. The nutrient broth (NB) for bacteria
enrichment consisted of beef extract 3g, peptone 5g, NaCl 5g, pH 7.2. Nutrient agar plates were made using NB supplemented with 2% agar.

**Degradation Experiments of Phenylobacterium ESF-17**

The following environmental factors were assayed to investigate their effects on DOP degradation within 60h of cultivation at a 140 rpm shaking rate. Temperature (10, 15, 20, 25 and 30°C); Initial pH value (4.0, 5.0, 6.0, 7.0, 8.0, 9.0); PAEs (DBP, DOP, DEP, DMP, DEHP and DPP)

**SBR operation**

The bioaugmentation study was carried out in four lab-scale SBR systems. The plexiglass SBRs were built up with 2.2 L working volume. The seeding sludge was collected from a local full-scale municipal wastewater treatment plant with SBR process, and the adding volume of activated sludge was about 0.7 L. One SBR without inoculating DOP-degrading bacteria was as control group, while SBR with single DOP-degrading strain was set as control.

The operating process was as follows: a cycle consisted of 12 h: feeding phase (15 min), aerobic phase (10 h), settling phase (1 h), discharge and idle phase (45 min). The dissolved oxygen (DO) concentrations and pH were controlled at 3.0 mg/L and 7.0, respectively, the operating temperature was maintained at 30±2°C by a water bath instrument.

**Analysis Method**

Concentration of DOP in the supernatant solution was performed using high performance liquid chromatography (HPLC) (Aglient 1200 series). The column temperature was 40°C. The volume of the injected samples was 40μl; Chromatography column was Inertsil ODS-2151-K.6×150 mm.

**Results and Discussion**

**Effects of Temperature on DOP Biodegradation**

The strain was cultivated at condition of 25°C, 500mg/L and pH7, at a 140 rpm shaking rate based on pre-experiment. The effects of temperature on the degradation of DOP in the culture medium were tested after incubation 60 h. The results showed that the optimal temperature for degradation was 25°C (Figure 1). The temperature is consistent with previous reports[11, 12]. The degradation at 25 °C was significantly higher than any other temperatures (P<0.05).

![Figure 1. Effect of temperature on degradation of DOP.](image)

**Effects of Initial pH on DOP Biodegradation**

Figure 2 shows the effects of pH (4.0–9.0) on DOP biodegradation at an initial concentration of 400 mg/L. We observed that the consortium exhibited higher degradation efficiencies and growth rates in alkalinity than in acidity. The highest DOP degradation rate was achieved at pH 8.0 (87.4%). The
reported optimal pH values in degrading of various pollutants by other genus are ranging from 7.0 to 8.0\[13\]. The degradation rate of DOP decreased rapidly when pH decreased 4.0. The results indicate that pH from 7.0 to 9.0 are optimal for DOP degradation and growth.

![Figure 2. Effect of pH and temperature on degradation of DOP.](image)

**Degradation of PAEs by Bacteria**

In order to investigate the degradation ability of the consortium to other commonly used PAEs in environment, the consortium was cultured in MSM supplemented with DBP, DOP, DEP, DMP, DEHP and DPP at 25 °C. Figure 3 presents the degradation rates for six PAEs and the biomass values of the consortium. The lowest biomass along with the lowest degradation rate was observed in DEHP. The consortium could utilize DBP, DOP, DEP, DMP, DEHP and DPP as the sole source of carbon and energy for growth. The degradation rates of DMP, DEP, DBP were higher than that of DOP, DEHP and DPP\[14\]. The result indicated that shorter alkyl chain PAEs were degraded rapidly by consortium, while PAEs with longer alkyl chains were more difficult to be degraded.

![Figure 3. Degradation of other PAEs by isolate.](image)

**Efficiency of Bioaugmentation in SBR**

As shown in Figure 4, the DOP degradation efficiency in bioaugmented reactor is significantly higher than that of control reactor after day 12. At the beginning, the degradation efficiencies between two reactors were not significant. But as the accumulation, the degradation rate in bioaugmented reactor increased quickly. The highest value of 92.3% was achieved, while the degradation rate in control reactor was only 37%. Therefore, bioaugmentation is effective to enhance DOP degradation. Current study mainly focused on PAHs degradation by using bioaugmentation.
technique [15]. The application of bioaugmentation is relatively rare in DOP biodegradation.

![Figure 4. Performance of SBR (bioaugmentation and control).](image)

**Conclusions**

This study investigated the enhanced effect of bioaugmentation in DOP degradation in SBR. The results indicated bioaugmentation could significantly enhance DOP degradation. 92.3% DOP removal rate was achieved. Also, bioaugmentation could effectively short the start-up of reactor. Therefore, bioaugmentation technique has potential to be used in DOP bioremediation in practical engineering.

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