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Biodegradation of naphthalene in the oil refinery wastewater by enriched activated sludge

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ABSTRACT

The main purpose of this paper is to study naphthalene (NAP) biodegradation by acclimated activated sludge, employing the culture-enrichment method in a continuous flow bioreactor of the wastewater treatment process. The effects of various COD loadings and influent flow rates of an artificial wastewater containing 15 mg l^{-1} NAP on the biodegradation rates of the activated sludge will be investigated, in order to determine the biodegradation kinetics and minimum mean cell residence time of the activated sludge. From the experimental results, it was found that the resulting enriched activated sludge follows the growth rate of the Monod type and can biodegrade those COD and NAP loadings in the influents efficiently, and its bio-treatment efficiency on NAPs increases with the decrease of influent flow rate. The sludge volume index (SVI) of the resulting enriched activated sludge meets the design value required by the convectional activated sludge process for the treatment of wastewater.

1. Introduction

Because of their toxicity, mutagenicity and carcinogenicity, polycyclic aromatic hydrocarbons (PAHs) have been listed as priority pollutants by the United States Environment Protection Agency (US EPA) since 1979 (Keith and Telliard, 1979). In Taiwan, PAHs come primarily from large-scale oil refinery plants (Kapley and Purohit, 2009; Sobiecka et al., 2009; Tahhan et al., 2011), and naphthalene (named as NAP below), which is one of the most toxic PAHs, is commonly found in the wastewater that is discharged from local oil refineries (Yuan and Chang, 2007; Chang et al., 2008).

Because of its stable bi-cyclic aromatic structure, NAP is one of the most difficult PAHs for microorganisms to biodegrade (Bauer and Capone, 1988; Juhasz and Naidu, 2000; Farhadian et al., 2008). Microbial biodegradation has been studied extensively over the past two decades as a means of removing PAHs, especially NAP in contaminated oily wastewater (Gomes et al., 2009; Sponza and Gok, 2011). Numerous pure cultures of aerobic microorganisms, such as *Alcaligenes* (Weissenfels et al., 1990), *Pseudomonas* (Ahn et al., 1998), *Bacillus* (Annweiler et al., 2000), *Rhodococcus* (Samanta et al., 2002), *Mycobacterium* (Pagnout et al., 2007), *Sphinogomonas* (Desai et al., 2008) and *Micrococcus* (Jegan et al., 2010) are known to degrade NAP efficiently. Most of these bacterial species were obtained by the culture enrichment method (Haritash and Kaushik, 2009). When the mixture of PAHs was treated by a mixed bacterial culture of *Bacillus* and *Pseudomonas*, Wiesel et al. (1993) found that the degradation rate of the bicyclic compound of naphthalene was always faster than that of the tricyclic or tetracyclic PAHs such as phenanthrene, anthracene and pyrene etc. It was found that NAP degradation by those strains is accomplished through a primary hydroxylation of NAP by the NAP-induced NAP 1,2-dioxygenase. The dioxygenase catalyzed reaction yields 1,2-dihydroxy-naphthalene, which is further reduced to 2-hydroxychromene-2- carboxylate (HCCA) and then to *trans-o*-hydroxy-benzylidenepyruvate (tHBPA) induced by an isomerase enzyme 2-hydroxychromene-2-carboxylate isomerase. A hydratase—aldolase reaction further catalyzes tHBPA to salicylaldehyde by hydration and aldol cleavage (Eaton and Chapman, 1992). However, all of these biodegradation experiments were conducted at the laboratory scale.

For the wastewater treatment process of an oil refinery, the aerobic activated sludge system is preferable because of its cost effectiveness and capacity for high- volume treatment. There are some published studies aimed at examining the degradation of PAHs in activated sludge systems (Moretti and Neufeld, 1989; Cardinal and Stenstrom, 1991; Stringfellow and Cohen, 1999; Garicano et al., 2003; Barret et al., 2010). These researches that have been conducted in this area have sought to accomplish PAH sorption by the sludge biomass in refinery wastewater treatment plants, and to examine the relationship between PAH biosorption

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and biodegradation. The results of these researches indicate that biosorption plays a significant mechanism for PAHs in activated sludge treatment. In order to investigate the biodegradation kinetics of NAP in a real bioreactor, Collina et al. (2005) employed *Pseudomonas putida* M8 strain to degrade NAP in an aerobic slurry bioreactor. In this study, the influence of airflow and agitation rates on volatilization and biodegradation of NAP was investigated. They found that the decrease of NAP followed a pseudo-first-order kinetics, and the time at which the maximum disappearance rate of NAP was observed was not coincident with the time of maximum CO_2 production rate. This time delay was caused by the accumulation of intermediate metabolites before complete mineralization. Under denitrifying condition, Lu et al. (2011) found that though their enriched marine denitrifiers can biodegrade 10 mg l^{-1} NAP (the highest reported NAP concentration that has been biodegraded so far), they will also be inhibited by NAP at 30 mg l^{-1}. All of their experimental works were conducted in the 250 ml batch type serum bottles.

Instead of using a pure culture as the NAP biodegradation strain, the main purpose of the present paper is to study NAP biodegradation in a continuous flow bioreactor by the acclimated activated sludge obtained by the culture enrichment method. The effects of different influent CODs and flow rates of an artificial wastewater containing 15 mg l^{-1} NAP on the biodegradation rates of the activated sludge will be investigated. This investigation will determine the biodegradation kinetics and the minimum mean cell residence time θ_c^M of the sludge.

2. Materials and methods

2.1. Activated sludge

In order to simulate the wastewater treatment process of an oil refinery plant, the present NAP biodegradation study will be conducted in a 3-L CFSTR (constant flow stirred tank reactor). The activated sludge used in the present study was originally obtained from the wastewater treatment plant of Taichung Industrial Park, Taichung, Taiwan. The sludge was maintained in the air aerated part of CFSTR (2 L in volume) accompanied with an Imhoff cone as a clarifier (1 L in volume, Fig. 1). The aeration rate was kept at 3.0 L min^{-1}. The composition of the artificial wastewater, in g l^{-1}, was: Glucose, 5.0; K HPO, 3.46; KH PO, 1.36 and NH Cl, 2.10, which makes the corresponded COD (chemical oxygen demand) value 5300 mg l^{-1} (i.e the mean value determined from three replicates). This medium was added automatically to the tank by a constant flow rate pump (Gilson Co.; Minipuls type) with a hydraulic residence time (HRT) of 14 days for one month. During this period, the pH of the solution was controlled manually at 6.5–7.0 by the addition of 0.01 N NaOH and the temperature was allowed to vary with the room temperature, which was 22–26 °C. Steady-state

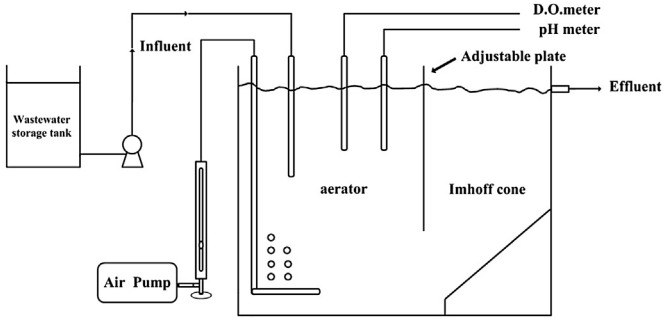

Fig. 1. The schematic diagram of the constant flow stirred tank reactor (CSFTR) adopted in the present paper.

sludge concentrations of mixed-liquor-suspended-solids (MLSS) of 3000 mg l^{-1} and sludge-volume-index (SVI) of 75 were maintained under such conditions (Metcalf and Eddy, 1991); i.e. SVI = % of settable suspended solids × 10000 × $MLSS^{-1}$ (MLSS in mg l^{-1}). The SVI and MLSS of the activated sludge and the COD of the artificial wastewater in CFSTR were all measured by the standard methods of APHA (2012).

2.2. Culture enrichment method

The special nutrient medium adopted in the present study was similar to that described by Edgehill and Finn (1983) for their activated sludge acclimated with pentachlorophenol (PCP) concentration in the range of 40–120 mg l^{-1}. This medium consisted of (in g l^{-1}): glucose, 1.0; K_2HPO_4, 3.46; KH_2PO_4, 1.36; NH_4NO_3, 2.0; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.01; $MnSO_4 \cdot 4H_2O$, 0.003; $FeSO_4 \cdot 7H_2O$, 0.005; Na-EDTA, 0.002. The enrichment procedure was divided into four steps: (1) the feed composition was kept the same as that mentioned above, but HRT was reduced to 12 h and the enrichment period of this step was about 7 days; (2) the glucose concentration was reduced by 25% and a 5 mg l^{-1} concentration of NAP was added in the feed solution; (3) the glucose concentration was reduced to 50% of the original concentration and the NAP concentration was increased to 10 mg l^{-1}; and (4) the glucose concentration was further reduced to 75% of the original value and the NAP concentration was increased to 15 mg l^{-1} (therefore the solution's COD mean value was reduced to 1325 mg l^{-1}). The enrichment period and HRT of the last three steps were kept at 5 days and 10 h, respectively. In all of the above enrichment periods, the values of MLSS, SVI, pH and temperature of the activated sludge solution were adjusted twice a day and all these values were in the allowable ranges of the values mentioned in the previous section. This enriched activated sludge was then employed in the biodegradation experiments under different influent CODs and flow rates of an artificial wastewater containing 15 mg l^{-1} NAP as follows.

2.3. NAP analytical methods

The samples were filtered with filtrate paper (Whatman, GF/C) to remove the activated sludge from the solution, and then shaken with equal volumes of Dichloromethane for 10 min. The organic extract was dried over anhydrous magnesium sulfate, and Dichloromethane was removed with an evaporative concentrator operated at 200 mm Hg and 50 °C. After this procedure, the residual NAP was in a form of solid crystal. These crystal materials were then dissolved completely in 5 ml hexane before analysis by GC (Shimaduz, GC-14A) with an FID detector. A OV-17 capillary column (cross bonded with 50% phenyl polysiloxane phase, 30 m in length and 0.25 mm in I.D.) with nitrogen as the carrier gas was adopted. The oven temperature gradients were: at 5 °C min^{-1} from 50 °C to 250 °C and at 10 °C min^{-1} from 250 °C to 300 °C.

2.4. Biosorption test

The tests of NAP biosorption were done in six 250 ml Erlenmeyer flasks (all were shaken with the frequency of 100 rpm at 25 °C), in which 20 ml of the enriched activated sludge with MLSS = 3000 mg l^{-1} were incubated at pH 7.0. After mixing with 80 mL artificial wastewater of COD = 1325 mg l^{-1} containing 15 mg l^{-1} NAP for 10 min, those NAP concentrations that remained in the wastewater were determined by the above mentioned analytical method. By deducting those residual NAP concentrations from 15 mg l^{-1}, we found that the saturated amount of NAP biosorpted by the present activated sludge is 0.74 × 10^{-3} mg-NAP per mg-MLSS.