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Biodegradation of toluene and dimethyl sulfide in a cocultured biofilter

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Abstract−Biofiltration was performed for 50 days in a perlite-packed biofilter (8 cm I.D.×105 cm height) for the simultaneous removal of toluene and dimethyl sulfide (DMS). Two strains, *Rhodococcus pyridinovorans* PYJ-1 and *Gordonia sihwaniensis* PKL-1, were cocultured in the biofilter. Removal efficiencies of toluene and DMS at an empty bed residence time (EBRT) of 3 min were 80-85% and 40-45%, respectively, for an input concentration of 2.5-3.0 mg/L of toluene and 1.5-2.0 mg/L of DMS. The pH of the perlite column was maintained at 7.0-7.2, and the moisture content varied from 61% at the bottom to 51% at the top. Starting from the same initial cell concentration (2.4×10^6 CFU/g of wet packing) the number of *R. pyridinovorans* PYJ-1 was 2.5 times higher compared with that of *G. sihwaniensis* PKL-1 after 50 days of operation.

Key words: *Rhodococcus pyridinovorans* PYJ-1, *Gordonia sihwaniensis* PKL-1, Biofilter, Toluene, Dimethyl Sulfide

INTRODUCTION

Biofiltration is one of the technologies used for the removal of malodorous and volatile organic compounds. Biofiltration not only requires less capital and operating costs, but is an environmentally benign technology without secondary contamination [Lee et al., 2003; Yudelson and Tinari, 1995]. Biofilters degrade the pollutants by using a consortium of microorganisms. Therefore, we need to apply proper environmental conditions for the growth of microorganisms. Because most of the previous studies utilized a mixture of microorganisms obtained from waste sites, the composition of the microbial mixture was not well investigated [Atoche and Moe, 2004; Yoon and Park, 2000]. Moreover, the removal rate of a target substrate by each strain and the biomass change of each strain during long-term biofilter operation were not well investigated [Yoon et al., 2002].

Many different kinds of microorganisms are present in the microbial consortium from waste sites, so it is practically impossible to simulate the real situation. Recently, we studied a biofilter system which was inoculated with sludge from a wastewater treatment plant [Yoon et al., 2002]. After the biofilter was supplied with gas mixtures of volatile components (BTX, sulfur and chlorinated compounds) for 100 days, more than 80% of the cells in the biofilter column were identified as *Rhodococcus pyridinovorans* PYJ-1 and *Gordonia sihwanienesis* PKL-1 which were utilizing BTX and sulfur compounds, respectively [Jung and Park, 2004; Jung et al., 2004]. This result suggests that an investigation of a model system using these two dominant strains may provide valuable information on the behavior of the actual biofilter system in a much more convenient way. Therefore, we studied a biofilter system inoculated with these two strains to investigate the relationship between the substrate (toluene and DMS) removal rate and the corresponding changes in the microbial population and the composition during 50 days of biofilter operation. Toluene was selected as a representative compound because it is widely used as a solvent and a primary raw material in industries, but biodegradation rate is relatively low. DMS was selected as a typical sulfur compound because it is known to provoke pulmonary and skin disorders, while it is relatively difficult to biodegrade compared to hydrogen sulfide, methanethiol (MT), dimethyl disulfide (DMDS), etc. [Ram et al., 1992].

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MATERIALS AND METHODS

1. Inoculum Preparation

Rhodococcus pyridinovorans PYJ-1 [Jung and Park, 2004] and *Gordonia sihwaniensis* PKL-1 [Jung et al., 2004], which were isolated for degradation of toluene and DMS, were cultivated individually in 250 ml flasks containing 50 ml of the basal mineral salt medium of the following composition (per liter of distilled and deionized water): Na_2HPO_4 4 g, KH_2PO_4 1.5 g, $MgSO_4\cdot7H_2O$ 0.2 g, $CaCl_2$ 0.01 g, $FeNH_4$-citrate 0.005 g. The medium pH was adjusted to 7, and it was autoclaved at 121 °C for 15 min, and stored at 4 °C before use. Reagent grade chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2. Biofilter Setup and Operation

The biofilter consisted of a gas mixture generating system, nutrient supplying system, and a cylindrical three-plate acryl column (I.D. 8.0 cm×105 cm total height) packed with 20 cm of perlite (average diameter: 4 mm) (Won Poong Co., Ltd, Seoul, Korea) in each plate, and a sieve plate (0.1 mm×0.1 mm mesh size) was placed at the bottom of the column (Fig. 1). The temperature of the column was controlled at 32±1 °C by a water jacket. This temperature was optimal in batch experiments for toluene and DMS degradation [Jung and Park, 2004; Jung et al., 2004]. For cell immobilization to the packing material (perlite), cell culture (350 ml each for two strains) at exponential growth phase (OD_{600}=0.8-1.0) was circulated for 3 days from the top of the column to bottom. Mass flow controllers

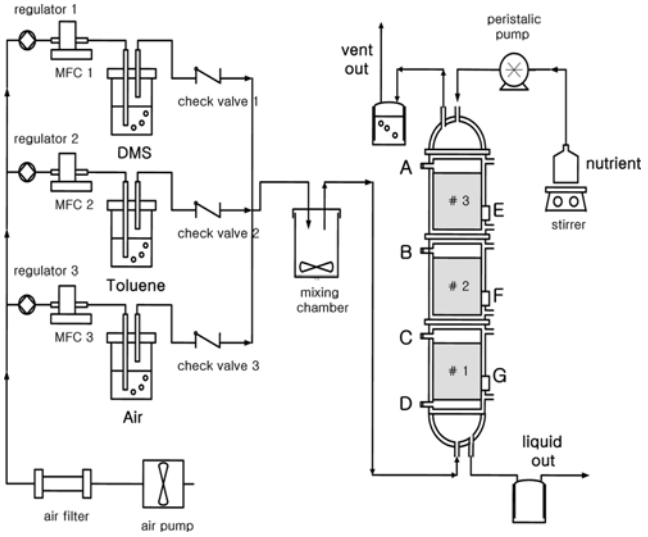

Fig. 1. Schematic diagram of a laboratory-scale biofilter system. MFC, Mass flow controller; A, B, C, D, E, F, G, sampling ports.

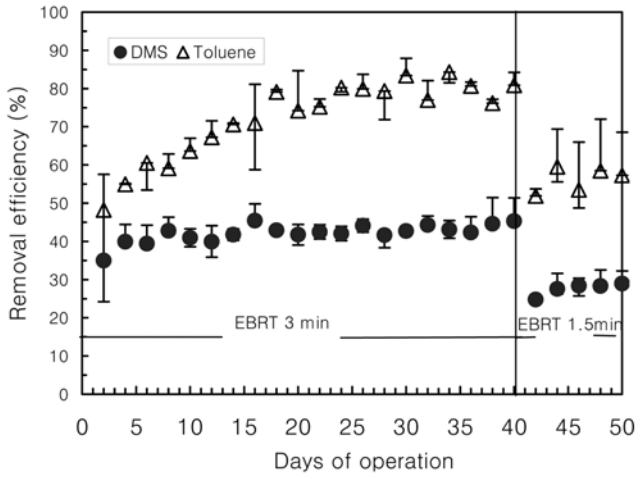

Fig. 2. Toluene and DMS removal efficiencies in the perlite column (EBRT=empty bed residence time).

(FL-280S, Millipore, Billerica, MA, USA) were used to control the concentration and the flow rate of air-toluene-DMS mixture which was filtered through a 0.2 μm pore-sized polycarbonate membrane filter (Midisart2000, Sartorious, Wicklow, Ireland). Air containing 2.5-3.0 mg/L of toluene and 1.5-2.0 mg/L of DMS was supplied to the top of the column at EBRT 3 min from the 3 days of cell immobilization period. Fifty ml of basal mineral salt medium was supplied to the top part of the column once a day. Substrate removal efficiency was calculated as follows.

$$\text{Removal efficiency} = \frac{\text{input concentration} - \text{output concentration}}{\text{input concentration}} \times 100$$

3. Analytical Methods

The toluene and DMS concentrations in the gas sample were analyzed by a gas chromatograph (HP 5890 series II, Hewlett Packard, Palo Alto, CA, USA) equipped with a Flame Ionization Detector (FID) and a 50 m-long Ultra-I capillary column (HP). The oven temperature was maintained at 35 °C for 3 min and raised to 100 °C by 10 °C min^{-1}. The temperatures of the injector and the detector were fixed at 250 and 200 °C, respectively.

Optical density (OD) of the culture was measured at 600 nm by using a spectrophotometer (Spectronic 20D, Milton Roy, Rochester, NY, USA).

Colony forming unit (CFU) in 1 g (moisture weight) of perlite sample from each stage of the column was counted as follows. The sample was vortexed for 3 min in 10 ml of distilled and deionized water, and one hundred microliters of the 10^{-1}-10^{-7} fold-diluted samples was spread on nutrient agar plates (trypton 10 g, yeast extract 5 g, NaCl 10 g, agar 15 g, water 1 L) for 4 days incubation at 32 °C.

RESULTS AND DISCUSSION

1. Removal of Toluene and DMS

A biofilter inoculated with *Rhodococcus pyridinovorans* PYJ-1 and *Gordonia sihwaniensis* PKL-1 was used for the removal of toluene and DMS. Toluene and DMS removal efficiencies in this cocultured biofilter were monitored for 50 days (Fig. 2). Feed concentrations of toluene and DMS were 2.5-3.0 mg/L and 1.5-2.0 mg/L, respectively. EBRT was maintained at 3 min for the initial 40 days, and changed to 1.5 min for the next 10 days. After one to two weeks of transient periods toluene and DMS removal efficiencies were stabilized at 80-85% and 40-45%, respectively. The transient period of this co-culture system was shorter than the biofilter inoculated with activated sludge from wastewater treatment facilities [Yoon and Park, 2000]. Removal efficiency of DMS, a compound difficult to degrade biologically, was lower at input concentrations less than those of toluene. When the gas flow rate was increased (shortening the EBRT from 3 min to 1.5 min), toluene and DMS removal efficiencies decreased to 55% and 30%, respectively.

The moisture content of the packing material is one of the important parameters in biofilter operation because it directly affects the degradation activity of the microorganisms. Moisture contents of the perlite-packed biofilter were 56-61%, 52-58%, and 51-57% in the stages #1, #2, and #3, respectively. These moisture contents were within the appropriate moisture content (45-60%) reported in the literature [Corsi, 1995]. The pH of the biofilter effluent decreased from 7.2 to 7.0 during 50 days of operation. These pH's were within the optimum pH range (6-8) reported in the literature [Gervais et al., 1988]. A negligible pH change during 50 days of operation suggests a good buffering capacity of the perlite.

2. Changes in Substrate Removal Capacity and Microbial Population

The dependence of substrate removal capacity upon the substrate loading is shown in Fig. 3. DMS removal capacity by *G. sihwaniensis* PKL-1 was lower than toluene removal capacity by *R. pyridinovorans* PYJ-1. The maximum removal rates determined by Michaelis-Menten kinetics [Shuler and Kargi, 1992] were 1,137 g toluene/m^3-h, and 288 g DMS/m^3-h, respectively. The profiles of residual substrate concentration within the biofilter bed are shown in Fig. 4. After 20 days of operation substrate removal in the first stage was more than half (54% and 58% for toluene and DMS, respectively) of the total removal in the biofilter. After 40 days, substrate removal

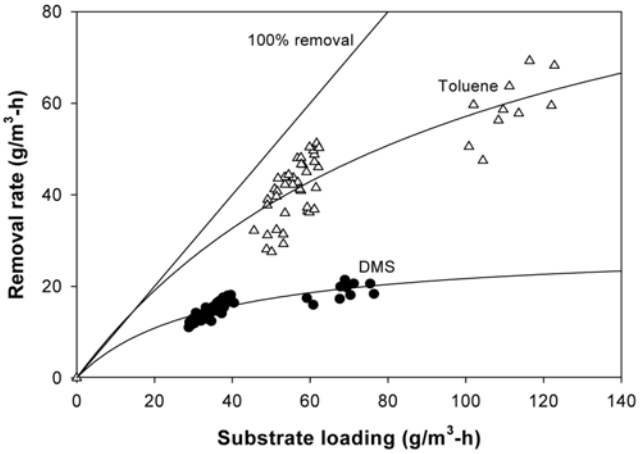

Fig. 3. Effect of substrate loading on the removal rate in the biofilter at 32 °C and EBRT 3.0 min.

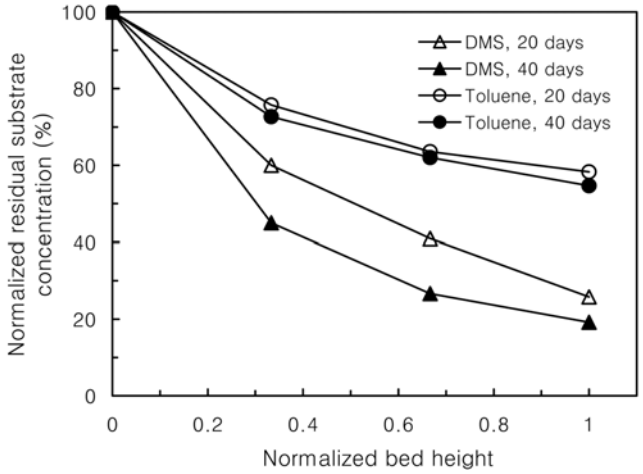

Fig. 4. Normalized residual substrate concentration (%) along the biofilter bed at EBRT 1.5 min.

in the first stage increased to 68% and 60% for toluene and DMS, respectively.

The population of the two strains changed with time (Fig. 5). Initially, the biofilter was loaded with an identical amount (2.4×10^6 CFU/g of wet packing) of *R. pyridinovorans* PYJ-1 and *G. sihwaniensis* PKL-1. During biofilter operation the biomass of *R. pyridinovorans* PYJ-1 increased more rapidly than that of *G. sihwaniensis* PKL-1. After 50 days of operation the biomass of *R. pyridinovorans* PYJ-1 in stage #1 was 2.3×10^7 CFU/g wet packing, which was 2.5 times higher than that of *G. sihwaniensis* PKL-1 in stage #1. Starting from an equal amount of inoculum, the cell mass of *R. pyridinovorans* PYJ-1 was 2.5, 2.6, and 2.9 times larger in stage #1, #2, and #3, respectively, compared with that of *G. sihwaniensis* PKL-1 after 50 days of operation. This difference in the cell mass was apparently responsible for the higher toluene removal efficiency (80-85%) by *R. pyridinovorans* PYJ-1 compared with DMS removal efficiency (40-45%) by *G. sihwaniensis* PKL-1. The larger biomass increase of the two strains in stage #1 compared to other stages was probably due to larger amount of substrate feeding to stage #1. In stages #2 and #3, growth of *G. sihwaniensis* PKL-1 was marginal and the

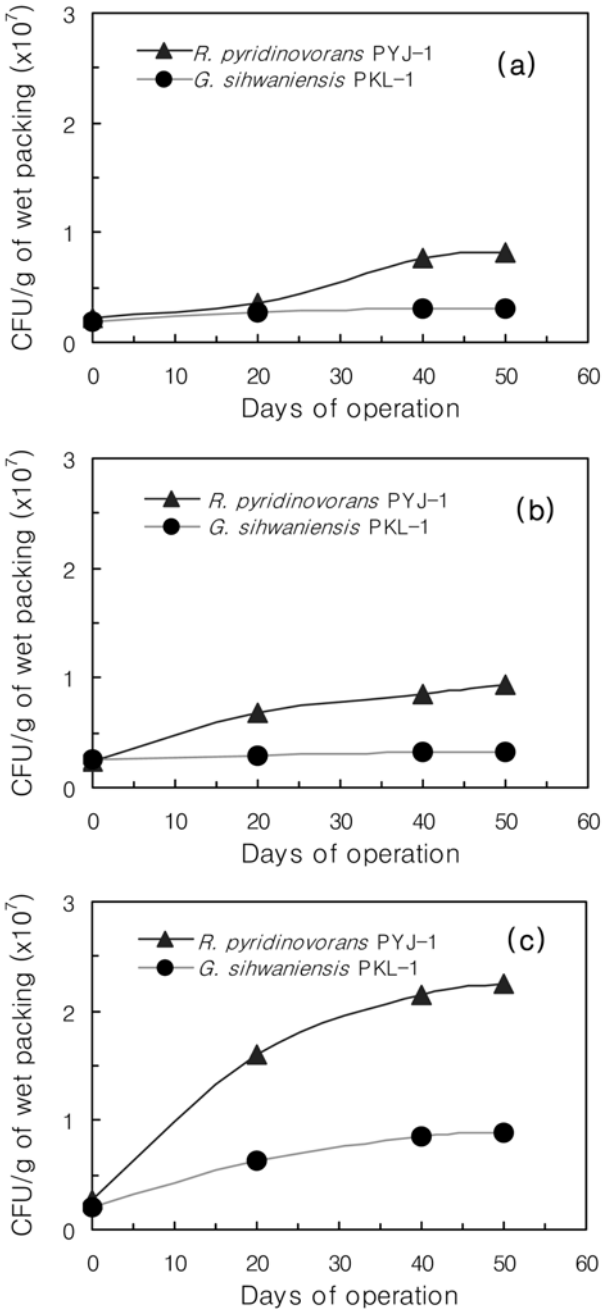

Fig. 5. Changes in microbial population and removal efficiency during 50 days of biofilter operation.
(a) stage #3, (b) stage #2, (c) stage #1

growth of *R. pyridinovorans* PYJ-1 was also not as active as in stage #1. The cell concentrations were 2.2×10^7, 0.8×10^7, and 0.9×10^7 CFU/g wet packing in stages #1, #2, and # 3, respectively. This suggests that stages #2, and # 3 were not well utilized probably because of substrate (toluene and DMS) limitation. Therefore, rates of toluene and DMS removal and their removal efficiencies can be possibly increased if the biofilter is designed to take the feed separately through the feed ports installed in stages #2 and #3 as well as in stage #1. Fig. 5 shows removal efficiencies and the population of the *R. pyridinovorans* PYJ-1 and *G. sihwaniensis* PKL-1 at each stage. Changes of the number of two strains related removal efficiency of toluene

and DMS. Increasing toluene and DMS removal efficiency may be caused by increasing population of the two strains.

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