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Benzene Biodegradation Using the Polyurethane Biofilter Immobilized with *Stenotrophomonas maltophilia* T3-c

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Abstract The benzene removal characteristics of the polyurethane (PU) biofilter immobilized with *S. maltophilia* T3-c, that could efficiently degrade benzene, was investigated. Maximum capacity to eliminate benzene was maintained at 100–110 $g \cdot m^{-3} \cdot h^{-1}$ when space velocity (SV) ranged from 100 to 300 h^{-1}, however, it decreased sharply to 55 $g \cdot m^{-3} \cdot h^{-1}$ as SV increased to 400 h^{-1}. The critical elimination capacities that guaranteed 90% removal of inlet loading of the PU biofilter were determined to be 70, 30, and 15 $g \cdot m^{-3} \cdot h^{-1}$ at SV 100, 200, and 300 h^{-1}, respectively. Based on the result of a kinetic analysis of the PU biofilter, maximum benzene elimination velocity (V_m) was 125 $g \cdot m^{-3}$ of $PU \cdot h^{-1}$ and saturation constant (K_m) was 0.22 $g \cdot m^{-3}$ of benzene (65 $\mu l \cdot l^{-1}$). This study suggests that the biofilter utilizing *S. maltophilia* T3-c and polyurethane is a very promising technology for effectively degrading benzene.

Key words: Benzene, biofilter, polyurethane, *Stenotrophomonas* sp., volatile organic compounds

Volatile organic compounds (VOC) are suspected carcinogens that also have offensive odors [1, 5, 20]. Dispersion of these substances to the ambient air may lead to adverse effects on the environment, and endanger human health and welfare [24, 27, 32]. Considerable quantities are emitted from industrial sources such as printing and coating facilities, foundries, electronics factories, petrochemical refining facilities, metallurgy, laundry and painting manufacturing operations; and environmental foundation facilities such as wastewater treatment plants and night soil treatment plants. They are often found in groundwater due to leaks in underground storage tanks and pipelines, improper waste disposal practices, inadvertent spills, and leaching from landfills. Biofiltration technology has been developed for controlling relatively low concentrations of VOC. Research on biofilters has led to the development of methods to control odors and air pollutants from a variety of industrial and public sources [14, 15, 17, 23, 34, 35, 37].

The selection of proper microorganisms and filter materials in the biofiltration process is a very important factor to obtain high elimination efficiencies [3, 6, 8, 16, 33]. Since microorganisms in biofilters play a vital role in degrading pollutants into water, carbon dioxide, and microbial cells, microbial activity is the main factor for determining the efficiency of the biofilter [8]. Research has recently been conducted on active isolated microorganisms, that are capable of degrading benzene, toluene, ethylbenzene, and xylene (BTEX) to determine what factors are involved in stabilizing and maximizing performance in the initial stage of the biofilter [29, 31]. Microorganisms that degrade BTEX have been identified, and they include *Pseudomonas* spp. [7, 13, 18, 21, 25, 29, 31, 36], *Alcaligens xylosoxidans* [38], *Burkholderia cepacia* [28], *Comamonas testosteroni* [13], *Clavibacter michiganense* [13], *Rhodococcus zopfii* [13], *Mycobacterium vaccae* [12], and thermophilic bacteria *Thermus aquaticus* and *Thermus* sp. [2]. Recently, we isolated a new BTEX-degrading bacterium, *Stenotrophomonas maltophilia* T3-c [19].

In the biofiltration process, the ideal filter materials provide high specific surface areas for the attachment of microorganisms [3, 10]. To obtain and maintain optimal pollutant removal capacity over an extended period, the primary factor is the maintenance of moisture content in the filter materials for sustaining biological activity [3, 10]. High porosity to reduce head loss and to ensure even distribution of incoming gases is also an important property of the material. When loads on the biofilter system are heavy and mineral nutrients are abundant, the biomass may clog the filter materials. Such media clogging can produce large pressure drops and form air channels [10]. Moreover, back pressures on the blower equipment increases,

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resulting in increased electrical demand [10]. Air channeling will limit the amount of contaminant being treated and will negatively affect the performance of a biofilter [10]. As clogging increases, anaerobic zones of activity may develop and odorous products may be generated [10]. Therefore, limiting excessive biomass growth is essential for effectiveness of the biofilter.

In our earlier studies, using a biofilter with porous synthetic materials such as celite stone, lava, activated carbon, and zeolite for the control of BTEX, we demonstrated that decreases in pressure increased clogging and excess biomass during extended periods of operation, which in turn resulted in drastic drops in treatment efficiency (unpublished data). In order to control clogging, backwashing was employed, however, this resulted in air channeling due to an uneven removal of the biofilm (unpublished data). However, backwashing can be effective in shearing off biomass, when porous polyurethane was used as a filter material (unpublished data).

In this study, the degradation of benzene in a polyurethane biofilter inoculated with *S. maltophilia* T3-c was studied. The effects of operational parameters, such as transient increases of the SV, and inlet loading on its performance in the treatment of benzene were examined.

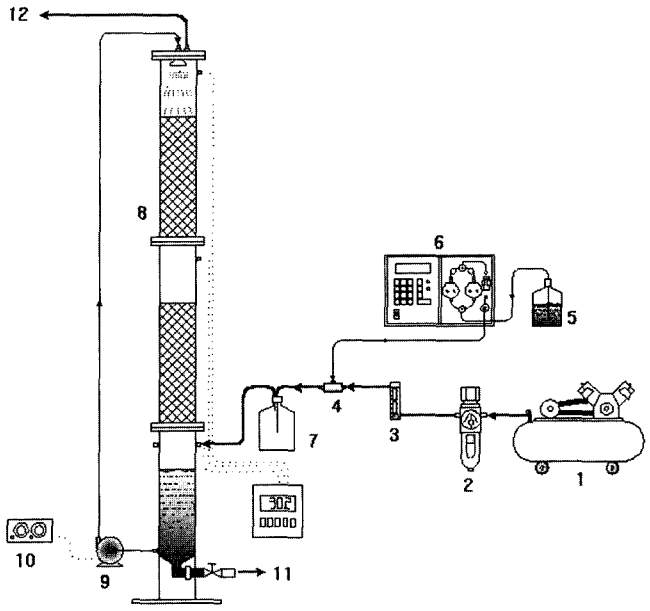

Fig. 1. Schematic diagram of experimental apparatus.
1, air compressor; 2, pressure regulator; 3, flow meter; 4, volatilizing chamber, 5, benzene reservoir bottle, 6, syringe pump; 7, mixing chamber; 8, biofilter; 9, circulation pump; 10, timer; 11, drain; and 12, off-gas vent.

MATERIALS AND METHODS

Bacterium and Medium

S. maltophilia T3-c was used for the biofilter to remove BTEX [19]. This bacterium was cultured in the nutrient medium containing 10 $g \cdot l^{-1}$ of trypton, 5 $g \cdot l^{-1}$ of yeast extract, and 5 $g \cdot l^{-1}$ of NaCl (pH 7.0).

Benzene Degradation of the Polyurethane Biofilter Immobilized with *S. maltophilia* T3-c

The filter material used consisted of a cubic polyurethane foam (Seilsponge, Korea) with a dimension of 1.5×1.5×1.5 cm. Bulk density, water holding capacity, porosity, average pore size, and the surface area of the material were 0.015 $g \cdot cm^{-3}$, 57 g-$H_2O \cdot g^{-1}$, 98.8%, 0.8 mm, and 76.81 $m^2 \cdot g^{-1}$, respectively. The strain T3-c was cultured in 4 l nutrient medium, and harvested by centrifugation at 7,600 ×g for 5 min. The concentrated cell was resuspended in 0.4 l minimal salt medium. The composition of minimal salt medium was as follows ($g \cdot l^{-1}$): KH_2PO_4, 1.5; $Na_2HPO_4 \cdot 12H_2O$, 9; $(NH_4)_2SO_4$, 3; $CaCl_2 \cdot 12H_2O$, 0.01; $MgSO_4$, 0.15. The cell suspension was inoculated in 130 g of the filter material.

Figure 1 shows a schematic diagram of the biofilter used in this study. The biofilter was made of a transparent acrylic resin column with internal side dimensions of 0.12 m and consisted of a drain storage tank, two biofilter-beds, and a liquid distributor. Each biofilter bed was packed with 0.4 m of the filter material that had been immobilized with *S. maltophilia* T3-c. The volume, weight, and density of the packing medium at each biofilter-bed were 4.5 l, 61.5 g, and 13.67 $g \cdot l^{-1}$, respectively. The biofilter was equipped with a nutrient storage tank (0.12 m in diameter × 0.5 m in height) for feeding of the mineral salts. A stainless steel screen (20 × 20 mesh) was placed on the bottom of each the filter bed to support the filter material.

Compressed air flowed through a volatilization chamber before entering the biofilter. The chamber included a stainless-steel rod (0.02 m in diameter and 0.1 m in length), which was connected to a liquid injection system consisting of a peristaltic pump and a liquid benzene storage bottle. Artificial waste gas (benzene vapor) was produced by injecting pure liquid benzene into the volatilization chamber air stream, using a peristaltic pump (0.001–20 $ml \cdot min^{-1}$, M930, Young-Lin Instrument Co., Ltd., Korea). The desired benzene concentration of gas flowing into the biofilter was obtained by adjusting the injection rate of the solution and the flow rate of the air stream.

Benzene removal experiments in respect to the changes of inlet concentration and space velocity (SV) were performed after the biofilter was acclimated by supplying an inlet benzene concentration of 100 $\mu l \cdot l^{-1}$ at SV 100 h^{-1} over two weeks. The biofilter was stabilized within thirty minutes after the changes of inlet concentration and SV (data not shown). Thus, the outlet benzene concentration was measured two hours after the inlet benzene concentrations were changed. When experiments were not performed, the

biofilter was maintained with an SV of 100 h^{-1} and an inlet benzene concentration of 100 $\mu l \cdot l^{-1}$.

Benzene degradation characteristics were evaluated by measuring the inlet and outlet concentrations of benzene after changing the SV and inlet concentration. The concentration of benzene in the inlet gas varied from 60 to 680 $\mu l \cdot l^{-1}$ (0.2–2.2 $g \cdot m^{-3}$), while the SV of the inlet gas in the biofilter varied from 100 to 400 h^{-1}. In order to supply the essential mineral salts to grow the microorganism and avoid drying the filter material, 4-l of a tap water supplemented with the minimal salts was sprayed on the top of the biofilter 5 times per day by a circulating pump. The circulating water in the tank was replaced every two days.

Analytical Methods

Benzene concentrations were measured using a gas chromatograph (HP 5890 series II *plus*, Hewlett Packard Co., U.S.A.), which was equipped with a frame ionization detector and a DB-WAX column (30 m × 0.32 mm × 0.25 μm, J&W Scientific, U.S.A.). The GC oven temperature was programmed to increase from 40 to 150°C at 10 min^{-1}, with a one-min hold at 40°C and a 3-min hold. The carrier gas (He) flow rate was set at 1.5 $ml \cdot min^{-1}$, and the temperature of the injector and the detector was set at 230°C. The detection limit of this procedure was 0.1 $\mu l \cdot l^{-1}$ benzene. Gas phase samples for benzene concentration analysis were taken with gas tight syringes through septa, which was installed in the sampling ports at the gas inlet and outlet from the biofilter.

The cell mass immobilized in the biofilter was measured as follows: The wet weight of the filter materials was measured by subtracting the weight of the dried bottles itself from that of the bottles containing materials sampled from several ports of the biofilter. The dried weight of the filter materials with a cell mass was measured after drying it in the oven at 70°C. The filter materials were then washed with 2 N NaOH, followed by washing with distilled water three or four times in order to completely remove cell mass immobilized in the biofilter. The dried weight of the filter materials without cell mass were then measured after drying the washed materials at 70°C.

Results and Discussion

When 60–680 $\mu l \cdot l^{-1}$ of benzene were supplied at an SV ranging from 100 to 400 h^{-1}, benzene removal characteristics of the PU biofiilter inoculated with *S. maltophilia* T3-c were investigated. Figure 2 shows typical results for benzene degradation by the PU biofilter at SV of 100 h^{-1}. When the inlet concentration of benzene was increased in increments from 60 to 680 $\mu l \cdot l^{-1}$, the outlet concentration of benzene increased as the inlet concentration increased.

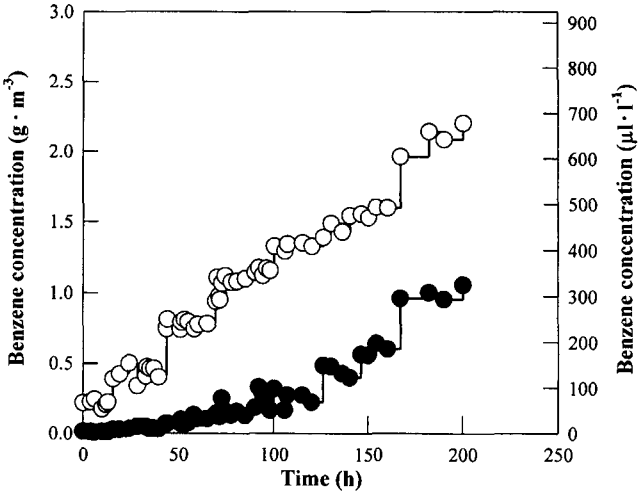

Fig. 2. Typical transient response of the biofilter to step changes in inlet benzene concentration at SV of 100 h^{-1}.
○, inlet concentration; ●, outlet concentration.

Korean clean air regulations stipulate that emission concentrations of benzene within industrial areas must not exceed 50 $\mu l \cdot l^{-1}$. It is anticipated that this limit will be lowered to 20 or even 10 $\mu l \cdot l^{-1}$ in the near future. Figure 3 shows the critical inlet concentrations of benzene necessary to guarantee that the outlet benzene concentrations of the PU biofilter would stay below 50, 20, and 10 $\mu l \cdot l^{-1}$ at various SV. These inlet concentrations are vital in determining a design criteria and an operating condition of the biofilter. Increased SV levels dropped sharply at the point at which inlet benzene concentrations could be maintained under

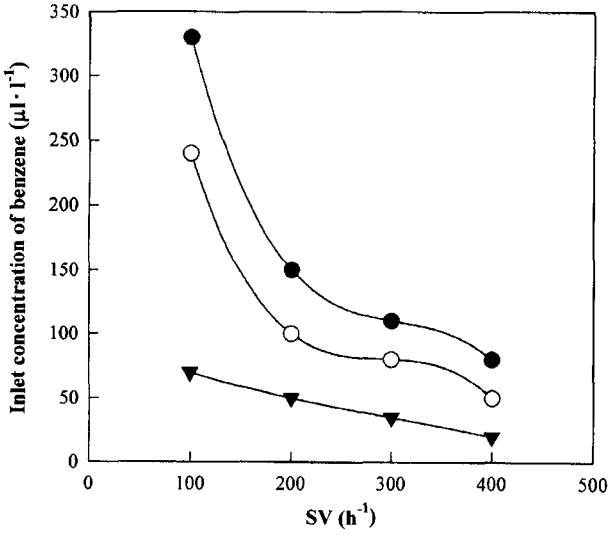

Fig. 3. Relationship of SV to the inlet concentration, which satisfy the outlet concentration required by the regulation of VOC emissions.
Outlet concentration of benzene ($\mu l \cdot l^{-1}$) in the biofilter: ▼, 10; ○, 20; ●, 50.

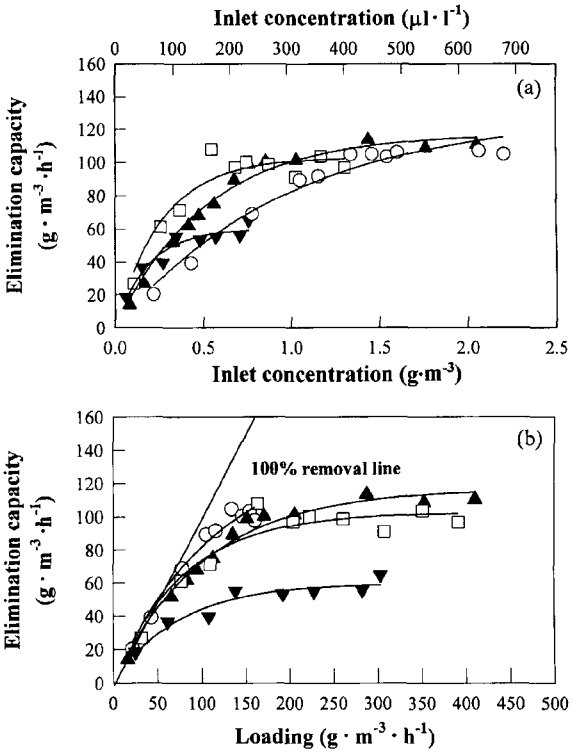

Fig. 4. Relationship between the elimination capacity of inlet benzene concentration (a) and inlet loading (b).
SV (h^{-1}): ○, 100; ▲, 200; □, 300; ▼, 400.

the constant level of outlet concentration. Inlet concentrations which would meet current legal limits (≤50 $\mu l \cdot l^{-1}$) decreased from 330 to 80 $\mu l \cdot l^{-1}$ when the SV was increased from 100 to 400 h^{-1}. Inlet concentrations, which could maintain the outlet concentrations below 20 and 10 $\mu l \cdot l^{-1}$, decreased from 240 to 50 $\mu l \cdot l^{-1}$, and from 70 to 20 $\mu l \cdot l^{-1}$, when the SV was increased from 100 to 400 h^{-1}, respectively. In summary, the retention time of the biofilter for the treatment of benzene containing waste gas should be increased, when legal limits for benzene are lowered.

Figure 4 shows the relationship between the elimination capacity of inlet benzene concentration (Fig. 4a) and inlet loading (Fig. 4b). Elimination capacity increased gradually with increasing inlet benzene concentrations at different SV (Fig. 4a). The kinetics of benzene degradation by *S. maltophilia* T3-c in the biofilter is first-order at low concentrations, zero-order at high concentrations, and fractional-order in the intermediate concentration of benzene in the inlet waste gas. Maximum benzene elimination capacities ranged from 100 to 110 $g \cdot m^{-3} \cdot h^{-1}$ at SVs of 100–300 h^{-1}. However, it decreased to 55 $g \cdot m^{-3} \cdot h^{-1}$, which was half of the former, at an SV of 400 h^{-1}. Inlet benzene concentrations achieving a maximum elimination capacity (Max EC) were 450, 250, 150, and 100 $\mu l \cdot l^{-1}$ at SVs of 100, 200, 300, and 400 h^{-1}, respectively. EC could be maintained after loading reached Max EC at various SVs, regardless of increases in the inlet benzene concentration.

EC increased asymphotically as inlet benzene loading increased [Fig. 4(b)]. When the SV was below 300 h^{-1}, increasing the inlet loading gradually increased EC and reached a maximum of 100–110 $g \cdot m^{-3} \cdot h^{-1}$. Thereafter, at loading more than 130–150 $g \cdot m^{-3} \cdot h^{-1}$, it was maintained regardless of increasing loading. On the other hand, when the SV was 400 h^{-1}, EC deviated from the 100% benzene removal lines even in the low loading. At inlet benzene loading of 100 $g \cdot m^{-3} \cdot h^{-1}$, the EC was only 52 $g \cdot m^{-3} \cdot h^{-1}$. The critical elimination capacities guaranteeing a 90% benzene removal of inlet loading were 70, 30, and 15 $g \cdot m^{-3} \cdot h^{-1}$ when SV were 100, 200, and 300 h^{-1}, respectively. The critical loading here is the load at which the elimination capacity and the loading start to significantly differ.

To determine the maximum removal rate of benzene in the biofilter, a kinetic analysis was employed using the data obtained at SV 100, 200, and 300 h^{-1}. Kinetics of the biodegradation occurring on the biolayer follow the Michaelis-Menten relationship [26]. By assuming the plug flow of gas in the biofilter column and applying the Michaelis-Menten equation, the following equation was obtained, as described previously [4].

$$C_{ln}/R = K_s/V_m + C_{ln}/V_m$$

Where, $R = SV(C_o - C_e)$, the removal rate; $C_{ln} = (C_o - C_e)/\ln(C_o/C_e)$, the log mean concentration; SV, space velocity (h^{-1}); C_o, inlet benzene concentration ($g \cdot m^{-3}$); C_e, outlet benzene concentration ($g \cdot m^{-3}$); V_m, the maximum removal rate ($g \cdot m^{-3} \cdot h^{-1}$); K_s, the saturation constant ($g \cdot m^{-3}$).

Based on the linear relationship between C_{ln}/R and C_{ln} (Fig. 5), maximum removal rate (V_m) and saturation constant (K_s) were assessed from the slope and the intercept, respectively. V_m and K_s for benzene of the biofilter were 125 $g \cdot m^{-3}$ of PU$\cdot h^{-1}$ and 0.22 $g \cdot m^{-3}$ of waste gas (65 $\mu l \cdot l^{-1}$).

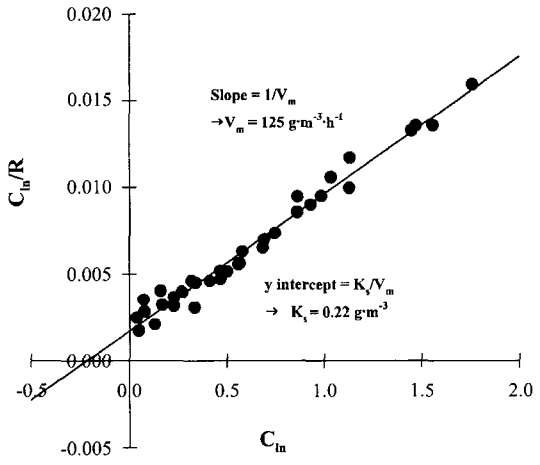

Fig. 5. Kinetic analysis of benzene removal by the biofilter.
The data obtained at SV of 100–300 h^{-1} are used for the analysis.

Table 1. Summary of maximum elimination capacities and critical loading for benzene.

Biofilter medium	EBRT[a] (s)	Critical load ($g \cdot m^{-3} \cdot h^{-1}$)	Maximum EC[b] ($g \cdot m^{-3} \cdot h^{-1}$)	Reference
Compost	-	1	8	9
Compost+GAC[c]	-	-	23	11
Compost+Perlite	-	<5	2~5	30
Compost+Polystyrene beads[d]	240	64	114	22
Polyurethane	9	N.D.	55	This work
	12	15	100	
	18	30	110	
	32	70	105	

[a]Empty bed residence time.
[b]Maximum elimination capacity.
[c]Granular activated carbon.
[d]Trickling biofilter.

The biomass immobilized in the biofilter ranged from 1.5 to 3.0 g-DCW g-PU^{-1} (data not shown): the average biomass immobilized in the biofilter was 2.25 g-DCW·g-PU^{-1} during the operation. Based on the average biomass immobilized in the biofilter, the theoretical value of V_m was 47 μmol·g-$DCW^{-1} \cdot h^{-1}$. Based on the average biomass and Max EC, the specific benzene degradation rate of the biofilter was 38–42 μmol·g-$DCW^{-1} \cdot h^{-1}$ (100–110 $g \cdot m^{-3} \cdot h^{-1}$) at SV of 100–300 h^{-1}, which was nearly the same as the theoretical value calculated using the Michaelis-Menten equation (Fig. 5). On the other hand, it was 20 μmol·g-$DCW^{-1} \cdot h^{-1}$ (55 $g \cdot m^{-3} \cdot h^{-1}$) at SV of 400 h^{-1}, which was half of the theoretical V_m.

From the results of Figs. 4 and 5, it was concluded that the benzene elimination capacity of the biofilter was depent on the inlet benzene loading rather than the inlet flow rate when the SV was below 300 h^{-1}, however, it was affected by the inlet flow rate much more than the inlet loading when the SV was over 300 h^{-1}. Benzene removal rates (max EC of 100–110 $g \cdot m^{-3} \cdot h^{-1}$) of the biofilter is believed to function as the reaction limitation when the SV was below 300 h^{-1}, which provided enough retention time for the microorganism to degrade benzene. However, the mass transfer of benzene from the gas phase to the biofilm immobilized in the carrier functioned as the reaction limitation when the SV was over 400 h^{-1}.

Direct comparison of specific degradation rates of benzene by free and immobilized cells is difficult, because of the difference in operating conditions, including concentration and mass transfer of benzene. Thus, the specific degradation rate of benzene of the immobilized cell of the biofilter could be compared with the maximum specific benzene degradation rates (4.25 μmol·g-$DCW^{-1} \cdot h^{-1}$) of suspended cells of *S. maltophilia* T3-c [19]. The maximum specific benzene elimination capacities of *S. maltophilia* T3-c immobilized in the PU biofilter were 1/100–1/110 of that of the suspended cell, when the SV was below 300 h^{-1}, at which there was no restriction of mass transfer to the biofilm. Possible explanations for this decrease include the following: (1) a biofilm formed in the carrier of the biofilter as a multilayer, which hindered the mass transferring of benzene, and (2) much of the biomass in the biofilter was composed of older or dead cells, even though the total biomass of the biofilter was greater than that of a free cell. On the other hand, free cells were composed entirely of younger cells.

Table 1 shows a comparison of the maximum elimination capacity for benzene under variable operating conditions. The published results about the critical elimination capacity and maximum elimination capacity for benzene of the biofilter are very low: under as much as 5 $g \cdot m^{-3} \cdot h^{-1}$ and under 23 $g \cdot m^{-3} \cdot h^{-1}$, respectively. A possible explanation for this is a diffusion limitation of benzene in the biofilm. The removal performance depends a great deal on the physicochemical properties of the treated pollutants. The pollutant removal performance in biofilters follows the sequence of alcohols> ketones>aromatics>alkanes [9]. For example, the maximum biodegradation rate in biofilters for hydrophilic compounds such as ethyl acetate, methanol, ethanol, and propanol have much higher values of 100–250 $g \cdot m^{-3} \cdot h^{-1}$ (critical load of 30–230 $g \cdot m^{-3} \cdot h^{-1}$). However, maximum elimination capacities of aromatic compounds including BTEX, hydrophobic compounds, and alkanes, such as hexane and isopentane (which have high Henrys law coefficients) are 2–60 $g \cdot m^{-3} \cdot h^{-1}$ and under 10 $g \cdot m^{-3} \cdot h^{-1}$ [9]. The pollutants with high Henry's Law coefficients are difficult to eliminate in a biofilter [9]. The reason is that these pollutants have an unfavorable gas-liquid partition, and the concentration of pollutants in the biofilm is too low to sustain a high biodegradation rate. This will not only affect the biodegradation kinetics, but also the rate of pollutant interphase mass transfer.

The maximum removal capacities (55–110 $g \cdot m^{-3} \cdot h^{-1}$) and critical load (<70 $g \cdot m^{-3} \cdot h^{-1}$) obtained in the present research were significantly larger than the reported values. On the other hand, Lu *et al.* [22] showed that maximum removal capacities of the trickle bed biofilter for benzene was 114 $g \cdot m^{-3} \cdot h^{-1}$, which was similar to values obtained in

this study. However, the retention time of 240s in the trickle bed biofilter reported by Lu *et al.* [22] was 6–20 times longer than the values of 12–36s obtained in this research. Assuming that equal amounts of benzene waste gas was supplied, lower retention times resulted in bigger reactor sizes. From an engineering perspective, the compaction of the reactor is of primary concern. Therefore, considering the investment cost and area, use of a compact biofilter like the one developed and used in this research is preferable.

To conclude, the present study demonstrates that high benzene removal efficiencies can be obtained in a biofilter inoculated with *S. maltophilia* T3-c. Moreover, the results obtained are suitable for the use in developing removal techniques to operate the biofilter using polyurethane filter material for a long period without clogging for the control of benzene in waste-gases.

Acknowledgment

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