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Styrene Degradation in a Polyurethane Biofilter Inoculated with *Pseudomonas* sp. IS-3

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Abstract In a search for bacteria capable of degrading styrene better than previously isolated strains, bacterium IS-3 was isolated from activated sludge and found to be most closely related to *Pseudomonas* sp. Styrene degradation by this strain was tested in liquid cultures and polyurethane-packed biofilters. In liquid cultures, the rate of styrene degradation by this bacterium increased from 24.93 to 76.53 μmol g^{-1} DCW h^{-1} for an initial mass range from 8.7 to 34.8 μmol. The maximum styrene elimination capacity was 580–635 $g/m^3 \cdot h$ at a space velocity (SV) of 50–200/h. The critical elimination capacities guaranteeing 95% removal of the input styrene were determined to be 635, 170, and 38 $g/m^3 \cdot h$, respectively, at SVs of 50, 100, and 200/h. Kinetic analysis revealed that the maximum styrene elimination velocity (V_m) for this biofilter was 1,000 g/m·h, and the saturation constant (K_m) was 454 ppmv. Together, these results suggest that a polyurethane biofilter containing *Pseudomonas* sp. IS-3 could have potential practical applications for the effective removal of styrene gas.

Key words: Styrene, *Pseudomonas* sp., biofilter, polyurethane, elimination capacity, degradation

Styrene, a volatile organic compound (VOC), is an important industrial material involved in the production of plastic, synthetic rubber, synthetic resin, insulation, and other fabricated materials containing molecules such as polystyrene, butadiene-styrene latex, styrene copolymers, and unsaturated polyester resins [14]. Styrene, a colorless liquid/gas that is flammable and foul-smelling, has also been detected in oxy-acetylene flames, cigarette smoke, gasses emitted by brake lining pyrolysis, and stack emissions from waste incineration [9]. Styrene exposure may cause contact-based skin inflammation, irritation of the eyes, nose, and respiratory tract, and may induce a narcotism [8] characterized by headache, fatigue, asthenia, memory defects, dizziness, and decreased reaction velocity and motion capacity. In addition, styrene oxide, a product of styrene metabolism in the body may act as an established mutagen and carcinogen [11]. In 2002, 22,323 tons of styrene was released to the environment, and 538 tons of styrene was reportedly emitted in Korea [15].

Thus, pollution by styrene and other VOCs can be considered a public health risk, and researchers are currently seeking new and improved scrubbing methods. Among a variety of emerging air pollution control technologies [3, 12, 15, 16, 18], biofiltration is an attractive option for the treatment of VOCs, because it is generally cost-effective and does not generate secondary contaminants [6, 17, 29]. Successful biofiltration relies on the elimination capacity of the styrene-degrading organism(s), and the composition of the packing material, which is generally a layer or combination of peat, compost, wood chips, soil, perlite, and/or ceramic. In addition, microbial VOC degradation is influenced by environmental factors such as moisture content, temperature, pH, and accessibility to the target compounds [6, 17]. Thus, a multitude of variables must be taken into account during the generation of an effective biofilter.

Many styrene-degrading bacterial strains have been isolated from contaminated sites and activated sludges; these have included species of *Pseudomonas*, *Rhodococcus*, *Nocardia*, *Xanthobacter*, *Enterobacter*, and *Exophiala* [25]. Individual or mixed cultures of these bacteria have been used for biofiltration-based removal of styrene contamination. To date, the most effective biofiltration rate achieved by a single species-based biofilter was 170 g/m·h with *Pseudomonas* sp. using a peat and ceramic-mixed filter [9], and that by a mixed species was 145 g/m·h using a perlite-packed filter [30].

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Here, we sought to identify a more effective styrene-degrading bacterial strain and use this microorganism to develop an effective styrene biofilter.

MATERIALS AND METHODS

Isolation and Identification of a Styrene-Degrading Bacterium

The activated sludge, obtained from a wastewater treatment plant in Seoul, Korea, was subjected to enrichment culture in minimal salts medium (in g/l: 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) including 10 µl styrene in a serum bottle, and incubated at 30°C with 180 rpm shaking, and then transferred into the same medium three times for further activation. The final culture was spread on LB plates and dominant colonies were selected as major styrene-degrading strains. Among them, a superior strain was isolated through application of the styrene-degrading test as mentioned below in "Measurement of styrene degradation in liquid culture."

The isolated strain (IS-316s) was identified by 16s rDNA analysis. Genomic DNA was extracted with the alkaline lysis method, and the 16s rDNA was PCR amplified with bacterial universal primers 341f (5'-CCT ACG GGA GGC AGC AG-3') and 907r (5'-CCC CGT CAA TTC ATT TGA GTT-3'). The 25 µl PCR reactions contained 1 µl template, 2.5 µl 10×Buffer, 0.5 µg/µl BSA, 200 µM dNTP mixture, 20 pmol of each primer, and 0.5 U/µl Taq polymerase. The reactions were preheated at 95°C for 5 min, followed by 16 cycles of 95°C for 1 min, annealing for 1 min (starting at 63°C and decreasing 1°C every second cycle), and 72°C for 1 min. This was followed by 14 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with an additional 5 min at 72°C as a final extension step. The amplification products were purified using the PCR purification kit (QIAGEN GmbH, Hilden, Germany) and checked for bands of the expected product size (about 550 bp) by agarose gel electrophoresis (100 V, 25 min). The nucleotide sequence was determined by direct sequencing of the PCR product using the T7 primer, and species identification was made by comparison with the GenBank database using the Basic Local Alignment Search Tool (BLAST) [1].

Measurement of Styrene Degradation in Liquid Culture

For analysis of the styrene degradation characteristics of the isolate, the strain was inoculated into 50-ml flasks containing 20 ml minimal salts medium (in g/l: 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) supplemented with 1 g/l of yeast extract and incubated for 2 days at 30°C in a rotary shaker. Cells were then harvested by centrifugation (7,600 ×*g*), washed twice in 40 mM phosphate buffer (Na_2HPO_4-KH_2PO_4, pH 7), and further inoculated into 20 ml mineral salt medium containing styrene in serum bottles with 615 ml headspace volume. The headspaces were held in aerobic conditions, and styrene was introduced for headspace analyses; the initial concentrations of styrene in each headspace were ~200, 400, 600, and 1,000 ppmv after reaching an equilibrium between liquid and gas phase. The bottles were sealed with Teflon-coated mini-nut valves (Supelco, St. Louis, MO, U.S.A.) and incubated at 30°C. At various time points samples of the headspace gas were withdrawn from each bottle using a gas-tight syringe (sample volume: 100 µl) and analyzed for styrene concentration. Headspace sampling continued at frequent intervals until the styrene concentrations in the bottles dropped below the detection limits (0.1 ppmv) of the gas chromatograph. The concentrations of styrene were determined by comparing the standard curve of the chromatogram peak area to those of standardized styrene concentrations.

Biofilter Setup and Operation

The utilized filter material consisted of 1.5 cm^3 cubic polyurethane foam (Seilsponge, Seoul, Korea) with a bulk density, water holding capacity, porosity, average pore size, and material surface area of 0.015 g/cm^3, 57 g-H_2O/g, 98.8%, 0.8 mm, and 76.81/m^2·g, respectively. The IS-3 isolate was cultured in 4 l nutrient medium and harvested by centrifugation at 7,600 ×*g* for 5 min. The concentrated cells were resuspended in 0.4 l minimal salts medium and inoculated onto 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 hexahedron column with internal side dimensions of 0.12 m, and consisted of a drain storage tank, two biofilter

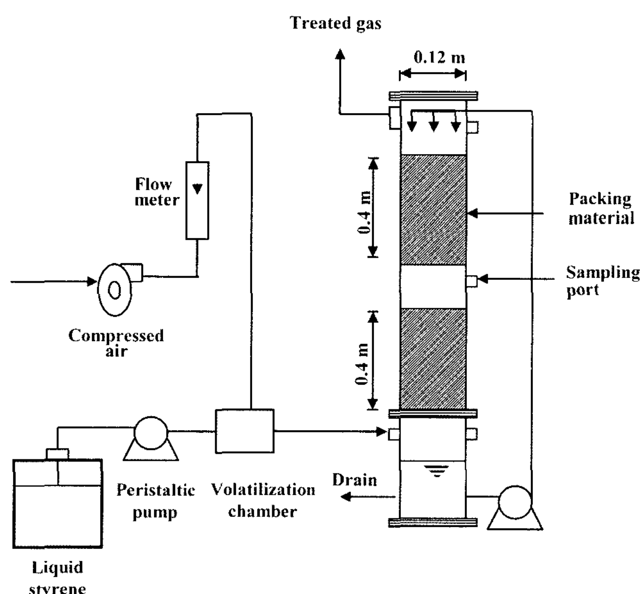

Fig. 1. Schematic diagram of the experimental apparatus.

beds, and a liquid distributor. Each biofilter bed was packed with 0.4 m of isolate-bound filter material. The volume, weight, and density of the packing medium at each biofilter bed were 4.5 l, 61.5 g, and 13.67 g/l, respectively. The biofilter was equipped with a nutrient storage tank (0.12 m in diameter ×0.5 m in height) for feeding of the isolate with minimal salts. A stainless steel screen (20×20 mm mesh) was placed on the bottom of each filter bed to support the filter material.

Compressed air was supplied 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) connected to a liquid injection system consisting of a peristaltic pump and a liquid styrene storage bottle. Artificial waste gas (styrene vapor) was produced by injecting pure liquid styrene into the volatilization chamber air stream with a peristaltic pump (0.001–20 ml/min, M930, Young-Lin Instrument Co., Ltd, Seoul, Korea). The desired concentration of styrene gas flowing into the biofilter was obtained by adjusting the solution injection rate and the air stream flow rate.

For experiments investigating styrene removal by the styrene-degrading bacterium, the biofilter was first acclimated by the application of inlet styrene concentrations from 0 to around 100 ppmv (part per million in volume) at a space velocity (SV) of 50/h (m^3 m^{-3} h^{-1}) for 10 days. The concentration of styrene in the acclimating inlet gas varied from 50 to 3,900 ppmv; the SVs of the inlet gas in the biofilter were set at 50, 100, and 200/h.

The styrene degradation characteristics of the isolated strain (*Pseudomonas* sp. IS-3) were evaluated by measuring the inlet and outlet concentrations of styrene at varying inlet concentrations. To ensure that the microorganisms were supplied with the essential mineral salts and to maintain the moistness of the filter material, 4 l of tap water supplemented with 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.

Analysis of Styrene Concentrations

Styrene concentrations were measured using a gas chromatograph (HP 5890 series II *plus*, Hewlett Packard Co., Palo Alto, CA, U.S.A.) equipped with a frame ionization detector (FID) and a DB-WAX column (30 m×0.32 mm ×0.25 μm, J&W Scientific, U.S.A.). The GC oven temperature was set to 150°C, and the injector and the detector were held at 230°C. The carrier gas (He) flow rate was set at 1.8 mL/min and the split was 100:1, giving a detection limit of 0.1 ppmv styrene. For sampling, gas-tight syringes were inserted through septa in the biofilter gas inlet and outlet sampling ports.

For measurement of the dry cell weight (DCW) immobilized in the biofilter, (1) the wet weight of the filter material was calculated by subtracting the weight of the dry bottle from that of the bottle containing materials sampled from several ports of the biofilter, (2) the microbe-containing filter material was dried in an oven at 70°C and weighed, (3) the filter materials were then washed with 2 N NaOH and rinsed 3–4 times with distilled water for removal of the cell mass immobilized in the biofilter, and (4) the cell-free filter was then dried at 70°C and weighed.

RESULTS AND DISCUSSION

Styrene-Degrading Activity of Strain IS-3

Strain IS-3, the most active strain isolated in this study, was found to be most closely related to *Pseudomonas cf. monteilii* (99% similarity by 16S rDNA sequence comparison).

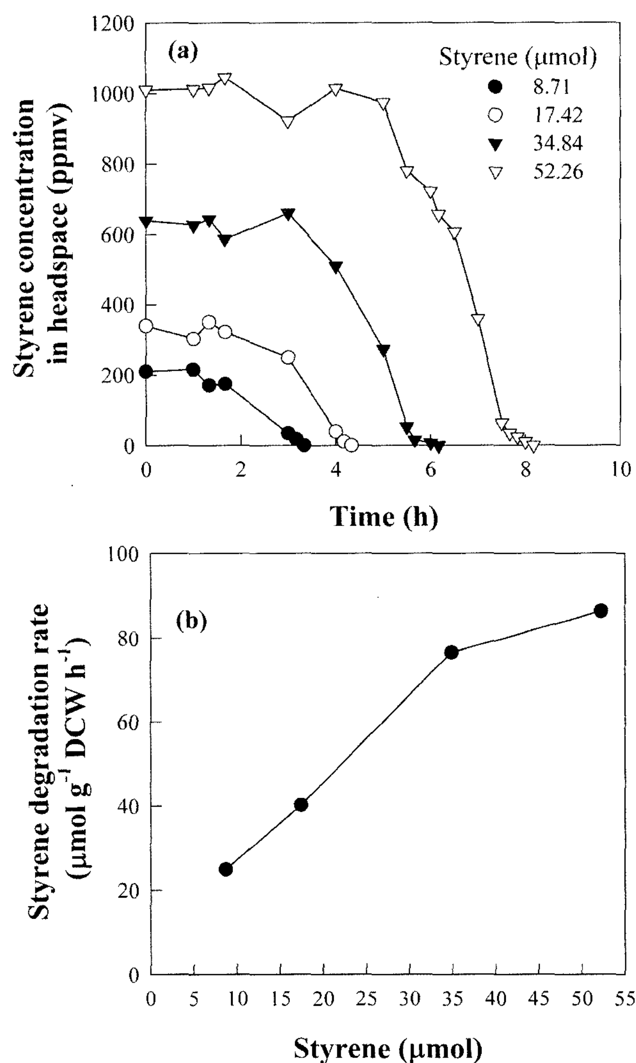

Fig. 2. Styrene degradation by *Pseudomonas* sp. IS-3 in the liquid batch system.
(a) Time profiles of styrene degradation. (b) Styrene degradation rate.

Previous studies have reported styrene degradation by *P. putida* and *P. fluorescens* [18, 19, 25], but this is the first report of styrene degradation by *P. monteilii*. This species is not particularly well-known; it was first identified in 1997 and was reported to be most closely related to *P. putida*, with 54% homology calculated by DNA-DNA hybridization [5]. Once this strain was isolated and identified in our study, we tested its ability to degrade various concentrations of styrene in minimal salt medium. Figure 2, which shows the headspace styrene concentration and styrene degradation rate at each concentration, shows that initial styrene concentrations of 200, 400, 600, and 1,000 ppmv were completely degraded within 3, 4, 6, and 8 h, respectively (Fig. 2a). The ratio of styrene degradation rate to concentration increased sharply from 24.93 to 76.53 μmol g^{-1}DCW h^{-1}, corresponding to initial masses of 8.7 to 34.8 μmol, and the ratio increased more slowly above an initial mass of 34.8 μmol (Fig. 2b). This indicates that approximately 80 μmol g^{-1}DCW h^{-1} or a slightly higher value would be the maximum degradation rate of styrene by IS-3 in the liquid culture.

Styrene Degradation by *Pseudomonas* sp. IS-3 in the Biofilter

The styrene removal efficiencies of the biofilter immobilized with *Pseudomonas* sp. IS-3 were observed at inlet concentrations from around 50 to 4,000 ppmv with an SV of 50/h, from around 50 to 1,500 ppmv with an SV of 100/h, and from around 50 to 900 ppmv with an SV of 200/h (Fig. 3). The removal efficiency remained above 90% up to an inlet concentration of 4,000 ppmv at an SV of 50/h. The removal efficiency decreased gradually to about 80% at 1,500 ppmv with an SV of 100/h and about 65% at 900 ppmv with an SV of 200/h. Thus, this study showed that *Pseudomonas* sp. IS-3 could degrade around 4,000 ppmv styrene; it is possible that the efficiency is even higher, as we did not observe a limiting concentration. Interestingly, in terms of removal efficiency, the space velocity appeared to be more influential than the inlet concentration.

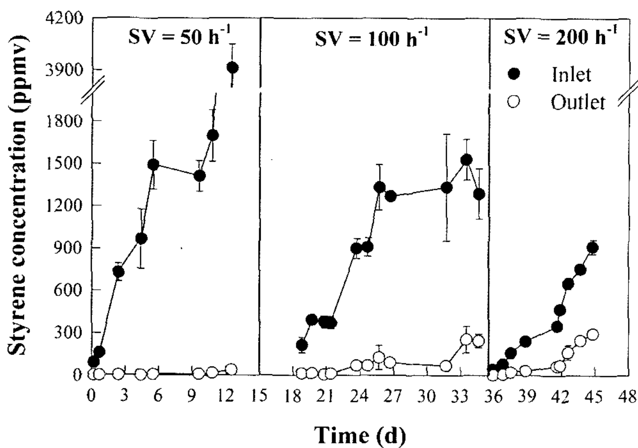

Fig. 3. Time profiles of styrene removal in biofilters inoculated with *Pseudomonas* sp. IS-3.

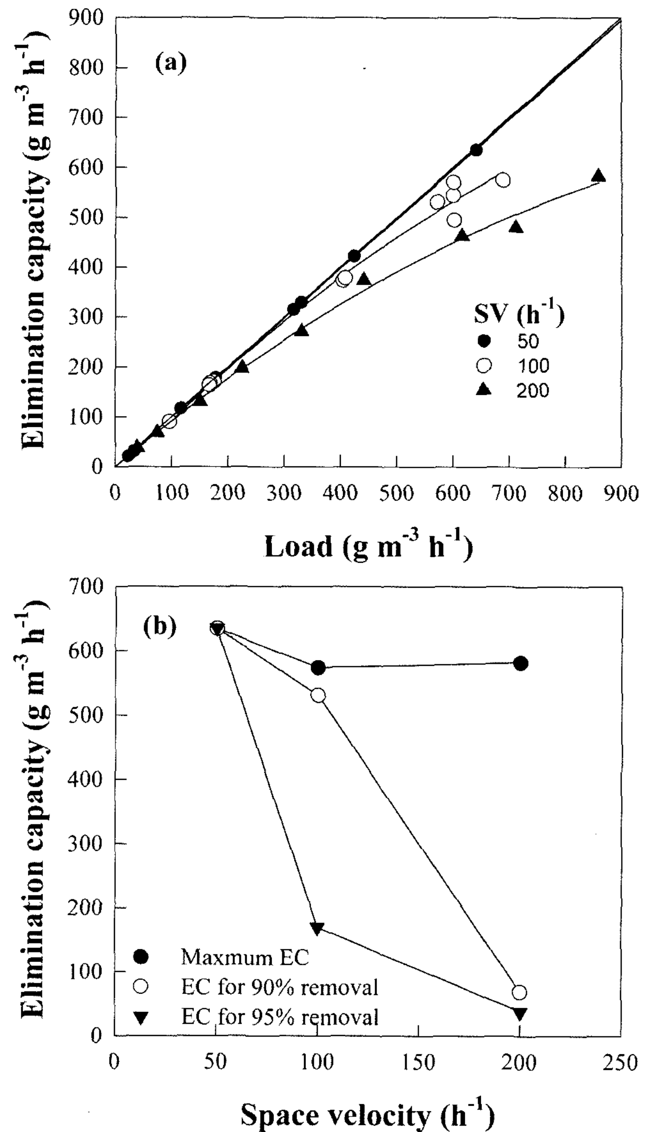

Fig. 4. (a) Relationship between elimination capacity and inlet load, and (b) effect of SV on elimination capacity of styrene by *Pseudomonas* sp. IS-3 in the biofilter system.

Figure 4a shows the styrene gas elimination capacity (removal rate) as a function of influent styrene loading at three different gas flow rates (SV=50, 100, and 200/h). For the SV of 50/h, the elimination capacity increased linearly through the applied range of loading rates, with more than 99% removal efficiency. For SVs of 100 and 200/h, the elimination capacity appeared to be logarithmically related with the input rates; we observed decreasing efficiency with higher loading rates, more significantly at the SV of 200/h than at the SV of 100/h. Thus, it appears that the elimination capacity strongly depends on the inverse of the

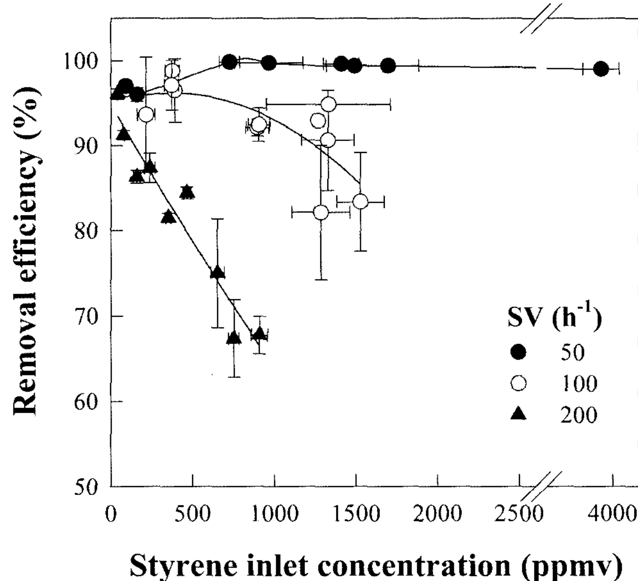

Fig. 5. Effect of styrene inlet concentration on removal efficiency in the biofilter.

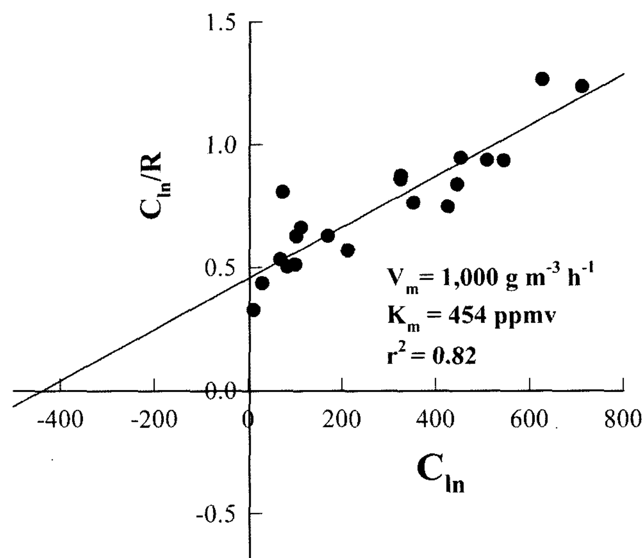

Fig. 6. Kinetic analysis of styrene removal in the biofilter (Note: each axis and the various rates are given in the text).

gas flow rate (Q m^3/h) in this biofilter system. Figure 4b shows the maximum elimination capacity (EC), the EC for 95% removal efficiency, and the EC for 90% removal efficiency with SVs of 50, 100, and 200/h, respectively. The maximum elimination rate of styrene at the SV of 50/h was highest at 635 $g/m^3 \cdot h$. The biofilter maintained the maximum EC at the SV of 50/h, whereas the EC showed a sharp decline in terms of the 95% removal efficiency at the SV of 100/h and the 90% removal efficiency at the SV of 200/h. As shown in Fig. 5, which illustrates the removal efficiencies at three space velocities according to inlet concentration (ppmv), the removal efficiencies at the SV of 50/h stayed consistently high (more than 90%), but those at the other two SVs gradually decreased with increased inlet concentration (more so at the SV of 200/h). Therefore, this biofilter system is capable of 95% styrene removal (maintaining the resulting atmosphere below the 50 ppmv styrene required by the Korean VOC regulations) under an inlet concentration of 4,000 ppmv (or higher) and the SV of 50/h. Meanwhile, the reason that the removal efficiencies at styrene concentrations below 1,000 ppmv at SV 50/h were lower than above 1,000 ppmv might be due to an adjustment period to a new environment of styrene biofilter.

Finally, we applied a kinetic analysis to determine the maximum removal rate of styrene in the biofilter. According to data obtained at SVs 50, 100, and 200/h (Fig. 6), we were able to determine that the biofilm biodegradation kinetics corresponded to the Michaelis-Menten relationship [26]. By assuming the plug flow of gas in the biofilter column and applying the Michaelis-Menten equation, we utilized the following equation (previously reported [2]):

$$\frac{C_{ln}}{R}=\frac{K_m}{V_m}+\frac{C_{ln}}{V_m} \quad (1)$$

where R is the removal rate [$=SV(C_o-C_e)$, where C_o is the inlet styrene concentration (ppmv) and C_e is the outlet styrene concentration (ppmv)], C_{ln} is the log mean concentration [$=(C_o-C_e)/\ln(C_o-C_e)$], V_m is the maximum removal rate ($g/m^3 \cdot h$), and K_m is the saturation constant (ppmv). Based on the linear relationship between C_{ln}/R and C_{ln}, the maximum removal rate (V_m=1,000 $g/m^3 \cdot h$) and saturation constant (K_m=454 ppmv) were obtained from the slope and the intercept as shown in Fig. 6.

Comparison with Other Studies

Comparisons with previously reported styrene elimination capacities revealed that our new biofilter was 4 to 20 times more efficient than previously reported biofilters, including both individual- and mixed-organism strategies (Table 1). Strain IS-3, isolated in this study, appears to have superior styrene-degrading abilities. Mechanistically, *Pseudomonas* sp. represents the most common group of isolates capable of styrene degradation and has been shown to produce styrene monooxygenase, which plays a major role in styrene degradation [25]. Thus, *Pseudomonas* sp. IS-3 may be a styrene monooxygenase producer and/or may produce other enzymes involved in the styrene-degrading pathway. In terms of *Pseudomonas* sp. IS-3 in the biofilter generated in this study, the maximum obtained elimination capacity was about 440% higher than that of the most effective previously reported mixed microbial cultures [30], which yielded an elimination capacity of 145 g/m·h using long-term adapted mixed microbial cultures inoculated on four perlite-packed biofilters. In addition, our biofilter was about

Table 1. Comparison of styrene elimination capacities.

Filter type	Inoculum	Maximum elimination capacity (g/m^3·h)	Ref.
Perlite-packed filter	Enriched fungi culture	70	Cox *et al.*, 1993
Perlite-packed filter	*Exophiala jeanselmei*	91	Cox *et al.*, 1997
Peat filter	Activated sludge	30	Reittu *et al.*, 1997
Pellet filter	Activated consortium	141	Jorio *et al.*, 2000
Coal trickle-bed filter	Activated sludge	70	Lu *et al.*, 2001
Serially aligned, perlite-packed filter	Long-term adapted mixed microbial culture	145	Weigner *et al.*, 2001
Peat-glass bead mixture filter	*Rhodococcus rhodochrous*	63	Zilli *et al.*, 2001
Ceramic filter	*Pseudomonas putida*	90	Okamoto *et al.*, 2003
Peat and ceramic-mixed filter	*Pseudomonas* sp.	170	Jang *et al.*, 2004
Polyurethane filter	*Pseudomonas* sp.	635	This study

370% better than that reported as the most effective biofilter utilizing the sole microbial component (*Pseudomonas* sp.), which was grown on a peat and ceramic-packed filter [13].

In addition to our identification of a superior styrene-degrading bacterium, the utilized filter material (polyurethane) appears to be more effective in styrene degradation than other tested materials. Previously, polyurethane was proved as an excellent filter material for toluene removal; this ability has been associated with its high porosity, suitable pore size, low density, and an ability to absorb water. Thus, our new biofilter combines an effective microbe with an effective filter material to generate an effective styrene removal system that is clearly superior to previously reported biofilters when operated under optimal conditions.

Acknowledgments

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