Korean J. Chem. Eng., **23**(1), 148-152 (2006)

Removal of hydrogen sulfide, benzene and toluene by a fluidized bed bioreactor

Kwang Joong Oh†, Ki Chul Cho, Young Hean Choung, Suk Kyong Park*, Sung Ki Cho* and Donguk Kim*

Department of Environmental Engineering, Pusan National University, Busan 609-735, Korea
*Department of Pharmaceutical Engineering, Inje University, Gimhae, Gyongnam 621-749, Korea
(*Received 11 May 2005 • accepted 5 September 2005*)

Abstract−The dominant off gases from publicly owned treatment works include hydrogen sulfide, benzene, and toluene. In this research, hydrogen sulfide oxidized by *Bacillus cereus*, and benzene with toluene were removed by VOC-degrading microbial consortium. The optimum operating condition of the fluidized bed bioreactor including both microorganisms was 30 °C, pH 6-8, and 150 cm of liquid bed height. The critical loading rate of hydrogen sulfide, benzene and toluene in the bioreactor was about 15 g/m^3 h, 10 g/m^3 h and 12 g/m^3 h, respectively. The fluidized bed bioreactor showed an excellent elimination capacity for 580 hours of continuous operation, and maintained stable removal efficiency at sudden inlet concentration changes.

Key words: Hydrogen Sulfide, Benzene, Toluene, Fluidized Bed Bioreactor, Microbial Consortium

INTRODUCTION

The major odorous compounds of off-gases from publicly owned treatment works (POTWs) include hydrogen sulfide and VOCs such as benzene and toluene [Cox and Deshusses, 2001]. Odorous gases have been conventionally treated by physical and chemical treatments like absorption, adsorption and catalytic oxidation [Cooper and Alley, 1986]. However, these methods have certain problems such as a low removal efficiency, high operating cost, and secondary contamination. Biotreatment of odorous gases depends on effective microorganisms to oxidize off-gases. *Thiobacillus thioparus* [Chung et al., 1996], *Thiobacillus thiooxidans* [Subramaniyan et al., 1998], and *Chlorobium thiosulfatophilum* [Basu et al., 1996] among several microorganisms were used to oxidize hydrogen sulfide. Bacterial strains such as *Rhodococcus rhodochrous* [Deeb and Alvarez-Cohen, 1999], *Ralstonia* sp. [Lee and Lee, 2002], *Pseudomonas* sp. [Oh et al., 1994] or activated sludge [Webster et al., 1996] acclimated in VOCs were used to degrade benzene and toluene. However, cultural conditions of sulfur oxidizer and VOC-degrading microbes were different and separate columns were needed to remove organic and inorganic off gases.

As a bioreactor, the biofilter was widely used to remove several odor gases and showed good removal efficiency [Devinny et al., 1999]. However, it needs maintenance of moisture and pH, and becomes clogged when it operated for long time. Whereas the fluidized bed makes vigorous mixing which increases enhanced gas-biomass contact, and shows excellent removal efficiency in odor treatment [Wright and Raper, 1999; Oh et al., 1998].

In this research, *Bacillus cereus* and VOC degrading microbial consortium were cultured simultaneously in a flask. A single column of a fluidized bed bioreactor including both microbes was used to treat 76-300 μg/L of inlet hydrogen sulfide, 165-700 μg/L of benzene and 190-770 μg/L of toluene at 600 L/h of gas flow rate, and stability of the bioreactor was checked in a continuous operation.

†To whom correspondence should be addressed.
E-mail: kjoh@pusan.ac.kr

EXPERIMENTAL

1. Cells

H_2S was oxidized to SO_4^{2-} by *Bacillus cereus* which was separated from closed coal mines in Hwasoon, Korea. Concentration of sulfate in solution was measured by absorbance at 460 nm after reaction with $BaCl_2 \cdot H_2O$ [Cha et al., 1994]. The aerobic MY microbial consortium used to degrade benzene and toluene was obtained by culturing activated sludge of the Yongho sewage water treatment plant in Busan, Korea at 30 °C, pH 7.0 for 3 months in the following medium [Oh and Kim, 1996] (g/L): 0.5 benzene, 0.5 toluene,

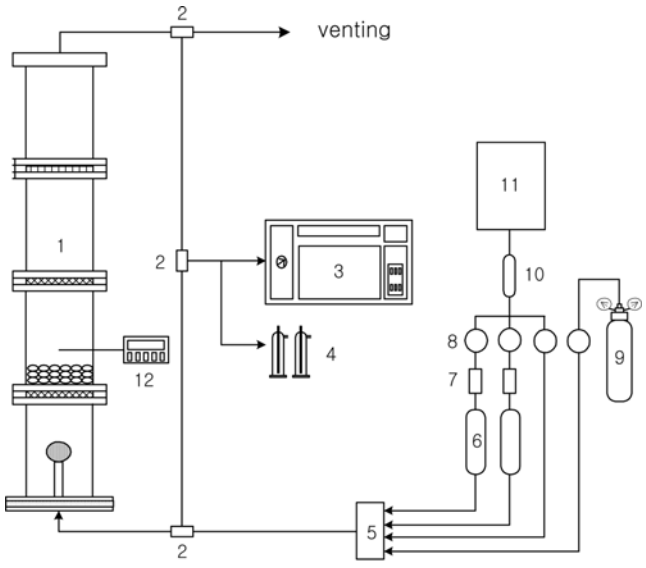

Fig. 1. Schematic diagram of the fluidized bed bioreactor.

1. Fluidized bed bioreactor	7. Check valve
2. Sampling valve	8. Mass flow controller
3. Gas chromatography	9. H_2S gas cylinder
4. Impinger	10. Moisture trap
5. Mixing chamber	11. Air compressor
6. Vaporizer	12. Thermocouple

0.5 $MgSO_4 \cdot 7H_2O$, 0.5 K_2HPO_4, 0.5 $(NH_4)_2SO_4$, 0.01 $CaCl_2$, 0.001 $FeCl_3 \cdot 6H_2O$, 0.001 $MnCl_2$, and 0.0001 $ZnSO_4$. From sequencing of the 16S rDNA (MicroID, Taejon, Korea), the dominant microorganism in the MY microbial consortium was identified as *Rhodococcus rubber*. In a mixed culture of *Bacillus cereus* and MY microbial consortium, the following medium was used (g/L): 0.5 benzene, 0.5 toluene, 8.0 $Na_2S_2O_3$, 0.5 NH_4Cl, 4.0 K_2HPO_4, 4.0 KH_2PO_4, 0.8 $MgSO_4$, 0.5 Na_2EDTA, 0.22 $ZnSO_4$, 0.05 $CaCl_2$, 0.01 $MnCl_2 \cdot 4H_2O$, 0.05 $FeSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$, 0.01 $CuSO_4$, 0.01 $CoCl_2$, 0.5 $(NH_4)_2SO_4$, 0.001 $FeCl_3 \cdot 6H_2O$, and 2.0 yeast extract.

2. Fluidized Bed Bioreactor

The fluidized bed bioreactor used in the experiment is shown in Fig. 1. Hydrogen sulfide was entered from a gas bomb. The benzene and toluene was vaporized in a cylinder-type vaporization equipment where the glass bead was packed to 1/3 of the height to have a uniform distribution of the concentration after liquid benzene and toluene was added. The inlet concentration was adjusted by air flow rate with a Mass flow controller (5850E, Brooks Co., USA) and the gas entered the fluidized bed bioreactor (inner diameter=10 cm, height=210 cm). A gas disperser was installed to reduce the bubble size at the bottom of the bioreactor, and a gas distributor which had 1 mm inside diameter hole was installed over the gas disperser to distribute the inlet gas evenly. The inlet gas fluidized the carriers, and then the bacteria in both carriers and solution oxidized the odor. The gas volumetric flow rate was 600 L/h, whereas the inlet concentration range was 76-300 μg/L for hydrogen sulfide, 165-700 μg/L for benzene, and 190-770 μg/L for toluene. The liquid volume in the column was 11.78 L when the liquid bed height was 150 cm. The minimum fluidization velocity in the bioreactor was 1.0 cm/s. As a cell carrier, 580 g of biosands (Chung woo art system, Korea), which was made of 15% SiO_2 and 85% H_2O, with a diameter of 2.0-3.0 mm, specific surface area of 539 m^2/g, and density of 1,270 kg/m^3 was used in the experiment. Among the various cell carriers, the biosand showed excellent odor removal capacity [Oh et al., 1998]. The concentration of benzene and toluene was measured with gas chromatograph (HP 4890D, Hewlett Packard, USA) by using FID and HP-5MS column. Initial injection temperature was 120 °C, and increased to 150 °C at the rate of 5 °C/min, and the detector temperature was set as 280 °C. As a carrier gas, 1.1 mL/min of N_2 was used. The concentration of hydrogen sulfide was measured by the methylene blue method [Lee, 1995].

In this study, the removal efficiency, inlet loading rate, removal capacity and biodegradation rate were calculated according to the following formulae:

$$\text{Removal efficiency } (\eta) = 100 \cdot (C_{in} - C_{out})/C_{in} \quad (1)$$

$$\text{Inlet loading rate} = C_{in}Q/V \quad (2)$$

$$\text{Removal capacity} = \eta C_{in}Q/(100 \cdot V) \quad (3)$$

$$\text{Biodegradation rate} = (C_0 - C_t)/(t \cdot \text{dry cell weight}) \quad (4)$$

RESULTS AND DISCUSSION

Fig. 2 shows the effect of temperature on the growth of *Bacillus cereus* in a flask culture at pH 7.0, and 30 °C was the optimum growth temperature of the cell. Fig. 3 shows the effect of pH on the growth of *Bacillus cereus* in a flask culture at 30 °C, and pH 7.0 was the optimum pH for the cell growth. *Bacillus cereus* also exhibited V_{max} and K_m at 0.24 g S/L d and 12.09 g/L, respectively.

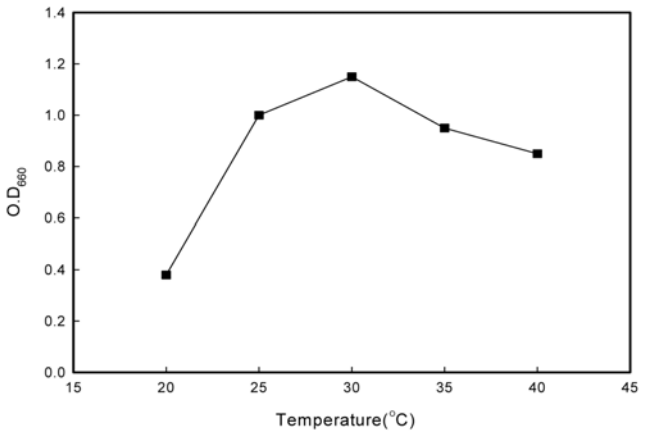

Fig. 2. The effect of temperature on the growth of *Bacillus cereus* in a flask culture at pH 7.0.

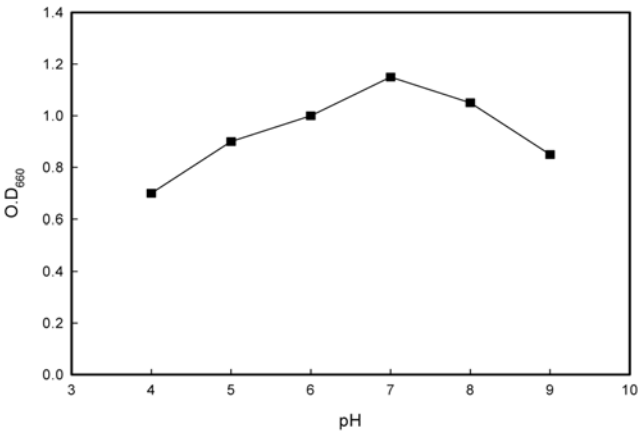

Fig. 3. The effect of pH on the growth of *Bacillus cereus* in a flask culture at 30 °C.

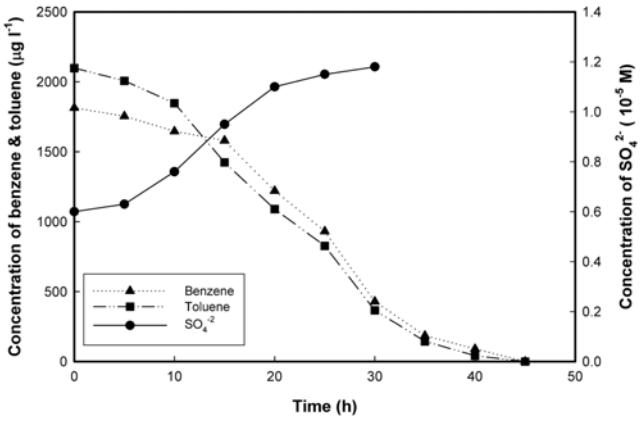

Fig. 4. Simultaneous degradation of thiosulfate, benzene and toluene by VOC-degrading microbial consortium (initial cell concentration=0.25 mg dry cell weight) and *Bacillus cereus* (initial cell concentration=0.22 mg dry cell weight) in a flask culture at 30 °C and pH 7.0.

Fig. 4 shows simultaneous oxidation of thiosulfate, benzene and toluene by VOC degrading-microbial consortium and *Bacillus cereus* in a flask culture at 30 °C and pH 7.0. Oxidation capacity of *Bacillus cereus* was tested by using sodium thiosulfate instead of hydrogen sulfide because the concentration of dissolved hydrogen sulfide in the flask was difficult to measure, and thiosulfate oxidation was the last step to sulfate formation. Thiosulfate was oxidized in 30 h by *Bacillus cereus,* whereas benzene and toluene were degraded completely in 45 h by the MY microbial consortium. The biodegradation rate of hydrogen sulfide, benzene, and toluene was 1.95 mg/L h mg dry cell, 0.86 mg/L h mg dry cell, 0.98 mg/L h mg dry cell, respectively. At a flask culture of *Bacillus cereus* alone in thiosulfate, the complete oxidation took place in 24 h, and the biodegradation rate was 2.21 mg/L h mg dry cell. At a flask culture of microbial consortium alone in benzene and toluene, the same concentration of benzene and toluene was degraded completely in 33 h, and the biodegradation rate of benzene was 1.05 mg/L h mg dry cell and that of toluene was 1.21 mg/L h mg dry cell. Simultaneous culture of VOC degrading-microbial consortium and *Bacillus cereus* oxidized thiosulfate, benzene and toluene completely even though the biodegradation rate of mixed culture was slower than that of separate culture.

Fig. 5 shows the effect of bed temperature on the removal efficiency of hydrogen sulfide, benzene and toluene by a fluidized bed bioreactor. The optimum operating temperature was 30 °C and the removal efficiency of hydrogen sulfide was highest among the inlet gas tested. Fig. 6 shows the effect of solution pH on the removal efficiency, and the bioreactor showed good removal efficiency for all three inlet gases at pH 6-8. The fluidized bed bioreactor showed the maximum removal efficiency when the bioreactor was operated at the optimum cultural condition of cells. Fig. 7 shows the effect of liquid bed height on the removal efficiency of hydrogen sulfide, benzene and toluene. As the liquid bed height increased

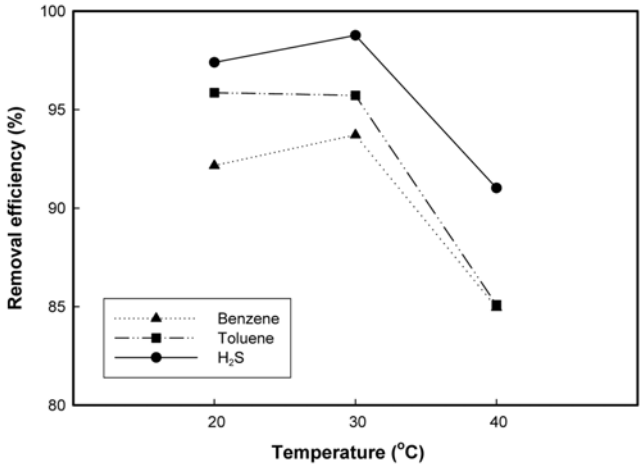

Fig. 5. The effect of bed temperature on the removal efficiency of H_2S, benzene, and toluene by fluidized bed bioreactor at pH 7.0, Q=600 L/h, $C_{in}(H_2S)$=76 μg/L, C_{in}(benzene)=185-200 μg/L, and C_{in}(toluene)=190-220 μg/L.

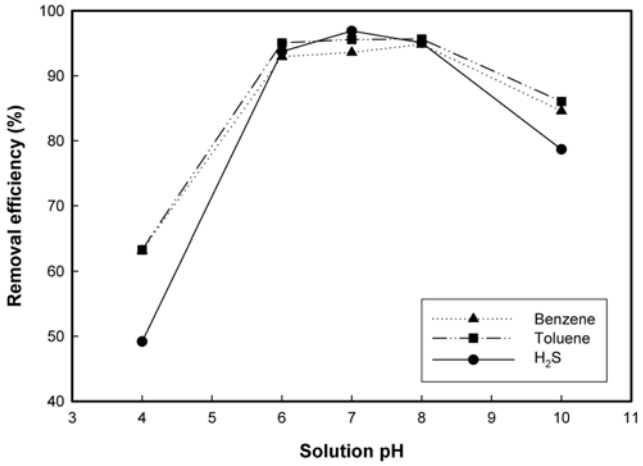

Fig. 6. The effect of solution pH on the removal efficiency of H_2S, benzene, and toluene by fluidized bed bioreactor at 30 °C, Q=600 L/h, $C_{in}(H_2S)$=76 μg/L, C_{in}(benzene)=165-200 μg/L, and C_{in}(toluene)=190-230 μg/L.

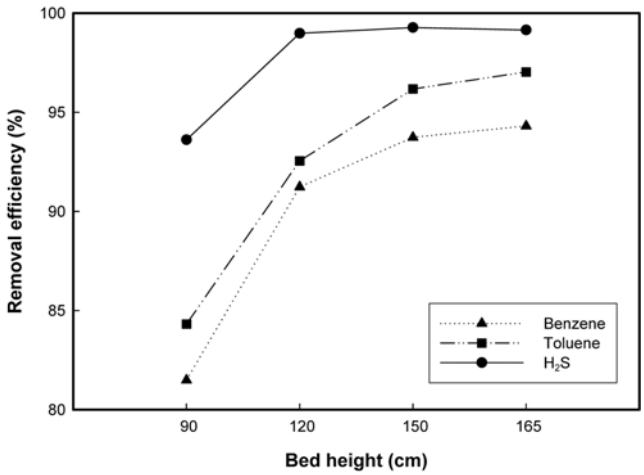

Fig. 7. The effect of liquid bed height on the removal efficiency of H_2S, benzene, and toluene by fluidized bed bioreactor at 30 °C, pH 7.0, Q=600 L/h, $C_{in}(H_2S)$=76 μg/L, C_{in}(benzene)=165-200 μg/L, and C_{in}(toluene)=190-230 μg/L.

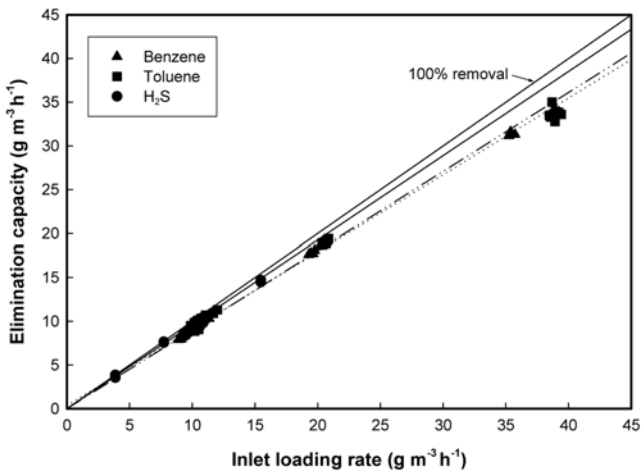

Fig. 8. The elimination capacity of H_2S, benzene and toluene for the inlet loading rate by fluidized bed bioreactor at 30 °C, pH 7.0.

from 90 cm to 165 cm, the removal efficiency was increased significantly due to the increased residence time of gas (42.4 s to 77.7 s) in the solution. However, the increase of the removal efficiency was small between 150 cm and 165 cm of liquid bed height, and the optimum liquid bed height was thought to be 150 cm.

Fig. 8 shows the elimination capacity of hydrogen sulfide, benzene and toluene for inlet loading rate. The diagonal line in the figure means 100% elimination of inlet gas. In this study, the critical loading rate (maximum loading rate which removes 100% of inlet gas) of hydrogen sulfide, benzene and toluene was about 15 g/m^3 h, 10 g/m^3 h and 12 g/m^3 h, respectively. The critical loading rate of the biofilter in selected references was 35-40 g/m^3 h for BTX [Seed and Corsi, 1994] and 100 g/m^3 h for hydrogen sulfide alone [Yang and Allen, 1994]. When a large volume (1 m^3/h) of H_2S and toluene was treated by a biotrickling filter, the removal efficiency of H_2S was about 80% on inlet concentration of 150 ppm H_2S and 1 ppm toluene under neutral pH condition [Cox and Deshusses, 2002]. Most researches of odor treatment by bioreactor have focused on the removal of similar chemicals if not single chemical. When different kinds of chemicals were treated by the bioreactor, the removal efficiency was not satisfactory. In this study, a fluidized bed bioreactor including MY microbial consortium and *Bacillus cereus* in a column showed an excellent removal capacity for combined odor of H_2S, benzene, and toluene.

Fig. 9 shows the inlet and outlet concentrations of hydrogen sulfide, benzene and toluene in the fluidized bed bioreactor for 580 hours of continuous operation. Removal efficiencies of hydrogen sulfide, benzene and toluene were 99%, 93%, and 96% for first two days; however, they were decreased to 92%, 89%, and 93% after 10 days. When solution pH was adjusted and cell medium was added, the removal efficiency of hydrogen sulfide, benzene and toluene returned to the initial level. During the operation, total cell concentration in solution of the bioreactor was maintained at 214-222 mg dry cell/L. At 300 hours of the operation, inlet concentration of benzene was suddenly increased to 700 μg/L and returned to 200 μg/L in 100 hours, while other inlet gases maintained normal condition to see the response of the bioreactor for the environmental change. The outlet concentration of benzene was increased up to 80 μg/L (89% removal efficiency) instantaneously; however, it decreased to normal level when the inlet concentration returned to initial range. The removal efficiency of hydrogen sulfide and toluene was not affected by the fluctuation of inlet benzene. When the concentration of toluene was increased to 770 μg/L after the benzene change, the outlet concentration increased up to 120 μg/L (84% removal efficiency); however, it was returned to normal level when the inlet concentration of toluene came back to the initial condition. When inlet concentration of hydrogen sulfide was increased to 300 μg/L, the outlet concentration suddenly increased to 21 μg/L (93% removal efficiency), and returned to normal concentration like benzene, and toluene after the inlet concentration came back to initial level. From the data, the fluidized bed bioreactor including VOC-degrading microbial consortium and *Bacillus cereus* showed very stable removal capacity for the sudden inlet concentration changes of H_2S, benzene and toluene.

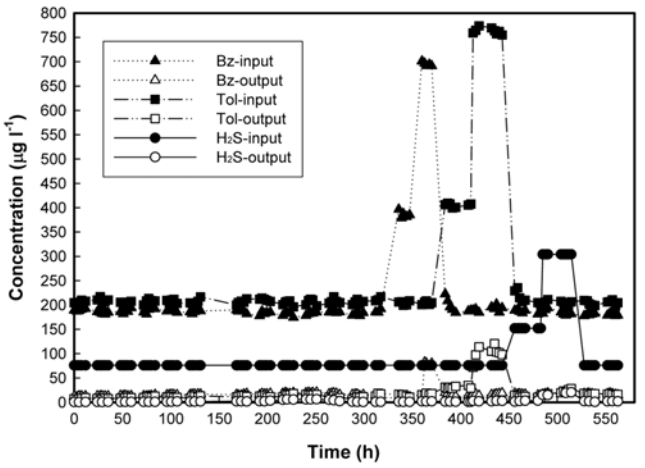

Fig. 9. Inlet and outlet concentration of H_2S, benzene and toluene by fluidized bed bioreactor in continuous operation. Q= 600 L/h, C_{in}(H_2S)=76-300 μg/L, C_{in}(benzene)=165-700 μg/L, C_{in}(toluene)= 190-770 μg/L.

CONCLUSIONS

The simultaneous culture of VOC-degrading microbial consortium and *Bacillus cereus* was successful to degrade hydrogen sulfide, benzene, and toluene completely in 45 hours. The fluidized bed bioreactor including VOC-degrading microbial consortium and *Bacillus cereus* removed 76-300 μg/L of inlet hydrogen sulfide, 165-700 μg/L of benzene, 190-770 μg/L of toluene at 600 L/h of gas flow rate with high efficiency. The critical loading rate of hydrogen sulfide, benzene and toluene in the bioreactor was about 15 g/m^3 h, 10 g/m^3 h and 12 g/m^3 h, respectively. The fluidized bed bioreactor was stable for the sudden inlet concentration changes of H_2S, benzene and toluene in 580 hours of continuous operation.

ACKNOWLEDGMENT

This research was supported by a grant (2001-041-E00388) from Korea Research Foundation.

NOMENCLATURE

C_{in} : inlet concentration [μg/L]
C_{out} : outlet concentration [μg/L]
C_o : cell concentration in flask at t=0
C_t : cell concentration in flask at t=t
K_m : Michaelis constant [g/L]
Q : gas volumetric flow rate [L/h]
t : cell culture time in flask [h]
V : liquid volume of the solution in bioreactor [L]
V_{max} : maximum sulfur oxidation rate [g S/L d]
h : removal efficiency

REFERENCES

Basu, R., Clausen, E. C. and Gaddy, J. L., "Biological conversion of hydrogen sulfide into elemental sulfur," *Environ. Pollution*, **15**, 234 (1996).

Cha, J.-M., Park, Y. and Lee, I. W., "Effect of cultivation condition on growth of the hydrogen sulfide-degrading *Thiobacillus* sp. IW iso-

lated from waste coal mine water," *Korean J. Biotechnol. Bioeng.*, **9**, 287 (1994).

Choi, H.-S. and Myung, S.-W., "Numerical and experimental study on the biofiltration of toluene vapor," *Korean J. Chem. Eng.*, **21**, 680 (2004).

Chung, Y.-C., Huang, C. and Tseng, C.-P., "Kinetics of hydrogen sulfide oxidation by immobilized autotrophic and heterotrophic bacteria in bioreactors," *Biotechnol. Tech.*, **10**, 743 (1996).

Cooper, C. D. and Alley, F. C., *Air pollution control: A design approach*, PWS publishers, Boston (1986).

Cox, H. H. J. and Deshusses, M. A., "Co-treatment of H_2S and toluene in a biotrickling filter," *Chem. Eng. J.*, **87**, 101 (2002).

Deeb, R. A. and Alvarez-Cohen, L., "Temperature effects and substrate interactions during the aerobic biotransformation of BTEX mixture by toluene-enriched consortia and *Rhodococcus rhodochrous*," *Biotechnol. Bioeng.*, **62**, 526 (1999).

Devinny, J. S., Deshusses, M. A. and Webster, T. S., *Biofiltration for air pollution control*, Lewis Publishers, Boca Raton (1999).

Lee, M. H., *Standard testing methods for air pollution*, Shin-Kwang, Seoul (1995).

Lee, S.-K. and Lee, S. B., "Substrate utilization patters during BTEX biodegradation by an o-xylene-degrading bacterium *Ralstonia* sp. PHS1," *J. Microbiol. Biotechnol.*, **12**, 909 (2002).

Lim, K.-H., "The treatment of waste-air containing mixed solvent using a biofilter : 2. Treatment of waste-air containing ethanol and toluene in a biofilter," *Korean J. Chem. Eng.*, **22**, 228 (2005).

Oh, K. H. and Kim, J. C., "Removal of BTX by immobilized microorganisms on granular activated carbon in fixed-film column reactor," *J. Korean Soc. Environ. Eng.*, **18**, 489 (1996).

Oh, K. J., Kim, D. and Lee, I. H., "Development of effective hydrogen sulphide removing equipment using *Thiobacillus* sp. IW," *Environ. Pollution.*, **99**, 87 (1998).

Oh, K.-J., Seo, M.-H., Son, H.-J. and Kim, D., "Removal of hydrogen sulfide in a three phase fluidized bed bioreactor," *Korean J. Chem. Eng.*, **15**, 177 (1998).

Oh, K.-J., Kim, Y.-S., Cho, S.-K. and Kim, D., "Degradation of benzene and toluene by a fluidized bed bioreactor including microbial consortium," *Korean J. Chem. Eng.*, **19**, 1026 (2002).

Oh, Y. S., Shareefdeen, Z., Baltzis, B. C. and Bartha, R., "Interactions between benzene, toluene, and p-xylene (BTX) during their biodegradation," *Biotechnol. Bioeng.*, **44**, 533 (1994).

Park, M.-S., Han, J.-H., Yoo, S.-S., Lee, E.-Y., Lee, S.-G. and Park, S., "Degradation of styrene by a new isolate pseudomonas putida SN1," *Korean J. Chem. Eng.*, **22**, 418 (2005).

Seed, L. P. and Corsi, R. L., *Biofiltration of BTEX contaminated streams: laboratory studies*, Proceedings of 87th Annual Meeting and Exhibition of the Air and Waste Management Association, Pittsburgh, PA (1994).

Subramaniyan, A., Kolhatkar, R., Sublette, K. L. and Beitle, R., "Microbial oxidation of mixtures of methylmercaptan and hydrogen sulfide," *Appl. Biochem. Biotechnol.*, **70**, 995 (1998).

Webster, T. S., Devinny, J. S., Torres, E. M. and Basrai, S. S., "Biofiltration of odors, toxics and volatile organic compounds from publicly owned treatment works," *Environ. Prog.*, **15**, 141 (1996).

Wright, P. C. and Raper, J. A., "Investigation into the viability of a liquid-film three-phase spouted bed biofilter," *J. Chem. Technol. Biotechnol.*, **73**, 281 (1999).

Yamada, H. and Goto, S., "Advantages of counter-current operation for hydrodesulfurization in trickle bed reactors," *Korean J. Chem. Eng.*, **21**, 773 (2004).

Yang, Y. and Allen, E. R., "Biofiltration control of hydrogen sulfide 1: design and operational parameters," *J. Air Waste Manage. Assoc.*, **44**, 863 (1994).