Biodegradation of Hydrogen Sulfide by a Laboratory-Scale Immobilized *Pseudomonas putida* CH11 Biofilter

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A heterotrophic *Pseudomonas putida* CH11 was isolated from livestock farming wastewater and applied for the treatment of H_2S-containing gas. Extensive tests including removal characteristics, metabolic products, and removal efficiencies of H_2S by *P. putida* CH11 were examined in batch and continuous systems. The optimum pH required to remove hydrogen sulfide was found in the range of 6−8. The maximum removal rate and the saturation constant were calculated to be $V_m = 1.36$ g S/day·kg dry bead and $K_s = 45.9$ ppm, respectively. The main metabolic product of H_2S oxidation was determined to be elemental sulfur. When *P. putida* CH11 was immobilized within Ca alginate, the cells exhibited high H_2S removal efficiency, in excess of 96%, at concentrations of hydrogen sulfide from 10 to 150 ppm (flow rates of 36 and 72 L/h). These results suggest that *P. putida* CH11 immobilized within Ca alginate has the potential to be used as a H_2S removal agent.

Introduction

Hydrogen sulfide (H_2S) is a highly odorous, toxic, and corrosive air pollutant. A considerable amount of H_2S is produced by industrial processes such as petrochemical refining, wastewater treatment, food preparation, paper and pulp manufacturing and fuels treatments (Eikum and Storhang, 1986; Yang and Allen, 1994). Because the human threshold exposure limit to H_2S is 10 ppm for 7−8 h periods, excess H_2S must be removed for reasons of health and safety (Buchman and Gibbons, 1974). The physical and chemical processes that have been used to remove H_2S from waste gas and wastewater include activated carbon absorption, ozone oxidation, and incineration (Eby and Wilson, 1969; Barth *et al.*, 1984; Mannebeck, 1986). The major drawbacks of these conventional methods are their relatively high energy requirements, treatment costs, and high disposal costs.

The continuing demand for improved process economy and efficiency has led to investigations into microbiological alternatives to conventional methods (Bohn, 1992). The biofiltration process is most effective when applied to dilute, easily biodegradable waste gases (Leson and Winer, 1991). A number of ways of using biofiltration for H_2S removal by employing some specific packing materials as carriers and breeding appropriate microorganisms to purify waste gases have been studied (Rands *et al.*, 1981; Lee and Shoda, 1989; Leson and Winer, 1991). Biofilters were originally developed by utilizing soils as carriers; however, soils are limited in their effectiveness since they are prone to short-circuiting and clogging (Carlson and Leisner, 1966). Compost is inexpensive and purifies waste gases well, but it suffers from aging effects that create short-circuiting of the biofilter and further decrease the effectiveness of the biofilter (Langenhove *et al.*, 1992). Activated carbons also perform well, but they are too expensive to justify the efficiency difference (Medina *et al.*, 1995). Use of fibrous peat as a packing material has been demonstrated to be preferable to soil, compost, or activated carbon (Leson and Winer, 1991). However, a significant pressure loss occurred when a biofilter packed with microorganism-laden peat was used to treat large quantities of hydrogen sulfide at low concentrations (<20 ppm) (Tanji *et al.*, 1989).

Apart from selecting appropriate packing materials, it is also important to screen an effective species to optimize hydrogen sulfide treatment. Autotrophic and heterotrophic microorganisms have both been used, and there are inherent differences in their nutritional requirements and abilities to catalyze specific reactions. Some chemolithoautotrophic bacteria, such as members of the *Thiobacillus* species, have been seeded into different packing materials and used to metabolize H_2S. The products of H_2S oxidation are dependent on the strain of *Thiobacillus* sp. employed (Sublette and Sylvester, 1987; Cho *et al.*, 1991). Some chemolithoheterotrophic bacteria, such as the *Thiothrix*, *Beggiatoa*, and *Hyphomicrobium* genera, can oxidize hydrogen sulfide into elemental sulfur and store it in their cells. This elemental sulfur will be further oxidized to sulfate (SO_4^{2-}), and the resulting acidity has adverse effects on microbial activity when the concentration of hydrogen sulfide is low (Nelson, 1990). Photoautotrophic bacteria including of *Chlorobium*, *Chromatium*, *Ectothiorhodospira*, and *Rhodobacter* have been used to convert H_2S to elemental sulfur under anaerobic conditions (Kusai and Yamanaka, 1973; Fukumori and Yamanaka, 1979; Then and Truper, 1983). The major disadvantages of the practical use of photoautotrophic bacteria lie in their anaerobic nature and their need for radiant energy. As for the chemoorganoheterotrophic bacteria, *Streptomyces* sp., *Pseudomonas aeruginosa*, *Bacillus brevis*, *Micrococcus* sp., *Xanthomonas* sp., and *Arthrobacter* sp. have also been reported to oxidize H_2S (Wainwright, 1984), but little information, such as desired control mechanisms, proper design, and maintenance of the biofilter, is available. The tall biofilters are generally designed to treat dilute concentrations of H_2S gas even though the problem of pressure loss arises. To eliminate pressure loss and reduce the cost of H_2S treatment, we have developed an innovative biofilter

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S8756-7938(96)00058-6 CCC: $12.00

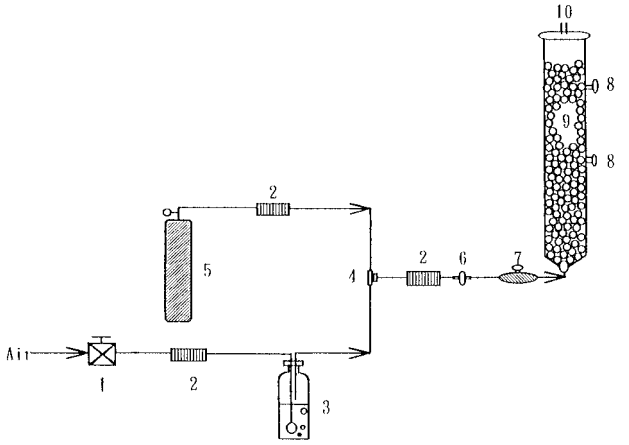

Figure 1. Laboratory-scale experimental biofilter system: (1) air compressor; (2) flow meter; (3) liquid media bottle; (4) three-way valve; (5) H_2S gas cylinder; (6) air filter; (7) inlet chamber; (8) sampling port; (9) glass column; (10) gas outlet.

using Ca alginate to immobilize *Pseudomonas putida* CH11. This biofilter not only reduces the packing height but also enhances the efficiency of H_2S removal, particularly under dilute conditions. A quantitative study of the operation of a heterotropic bacteria biofilter system for H_2S removal was also conducted.

Materials and Methods

Organism and Cultivation. A heterotrophic *Pseudomonas putida* CH11 was isolated from livestock farming wastewater. The bacterium was purified by repeatedly transferring the cells to fresh medium. A plate count broth (Difco) was prepared for cultivation of the isolate, which contained 5 g/L yeast extract, 10 g/L tryptone, and 2 g/L dextrose. In the continuous treatment experiment, a basal medium including 0.2 g/L glucose, 2 g/L KH_2PO_4, 2 g/L K_2HPO_4, 0.4 g/L NH_4Cl, 0.2 g/L $MgCl_2 \cdot 6H_2O$, and 0.01 g/L iron(III) citrate was used. To understand the growth pattern of the isolate, a modified basal medium including 0.4 g/L glucose, 2 g/L KH_2PO_4, 2 g/L K_2HPO_4, 0.4 g/L NH_4Cl, 0.2 g/L $MgCl_2 \cdot 6H_2O$, 0.65 g/L $Na_2S_2O_3$, and 0.01 g/L iron(III) citrate was used. The final pH of the culture was adjusted to 7 by using 2 N NaOH or HCl.

Preparation of Immobilized cells. *P. putida* CH11 grown in 100 mL of plate count broth was harvested by centrifugation (7500*g*, 10 min) and then washed with sterile distilled water three times. The organisms (10^5 cells/mL) were immersed in a sterile 4% Na alginate solution, and then the Na alginate solution containing the cells was dropped into a 4% $CaCl_2$ solution by syringe to immediately form 3 mm diameter immobilized beads. These gel beads were activated by flushing with sterile distilled water for 5 h. The activated beads exhibited excellent mechanical strength in the continuous experiments.

Growth Pattern of *P. putida* CH11. A platinum loop of *P. putida* CH11 from the PCA medium (plate count agar) was inoculated into 100 mL of a modified basal medium in shaken flasks and was incubated at 28 °C by reciprocal shaking (120 strokes/min). Changes in the concentrations of thiosulfate, sulfate, and total organic carbon (TOC), pH, and numbers of cells in the medium were measured periodically.

Apparatus and H_2S Removal Rate for Continuous Operation. A setup of the laboratory-scale experimental biofilter is shown in Figure 1. Glass columns (60 mm ϕ × 25 cm of working height) were packed with cell-laden Ca alginate beads. The packed volume, bead dry weight, and number of cells initially placed in the column were 0.7 L, 0.25 kg, and 10^5 CFU/g dry bead, respectively. The immobilized cells were supplied with the air containing KH_2PO_4/K_2HPO_4 mineral buffer. Relative humidities of between 95% and 100% were routinely and continuously achieved during the operation. In the continuous experiment, H_2S gas at different concentrations was supplied to the column at a flow rate of 36, 72, or 150 L/h. The effect of temperature and flow rate on the removal efficiencies of the biofilter was studied in the ranges of 20−37 °C and 36−150 L/h, respectively. The numbers of microorganisms in the upper and middle layers of the glass column were counted. In addition, the products resulting from the metabolization of H_2S by *P. putida* CH11 were also measured during the continuous experiment.

Kinetic Analysis. The removal rate of H_2S in the immobilized-cell biofilter was carried out using the following equation derived from the Michaelis−Menten equation (Hirai, 1990):

$$1/R = (K_s/V_m)1/C_{ln} + 1/V_m \quad (1)$$

where R (g S/day·kg dry bead) is the removal rate, C_{ln} (ppm) is the logarithmic mean concentration of H_2S at the inlet and outlet of the biofilter, V_m (g S/day·kg dry bead) is the maximum removal rate, and K_s (ppm) is the saturation constant. From the linear equation between $1/C_{ln}$ and $1/R$, V_m and K_s were calculated from the slope and intercept, respectively.

Analytical Methods. Inlet and outlet H_2S gas concentrations in the column were measured continuously by using a Single Point Monitor (MDA Scientific) in the range of 50−1500 ppb or measured periodically by gas detector tubes (GASTEC) in the range of 1−60 ppm. Five grams (wet weight) of cell-laden beads were homogenized in 95 mL of sterilized water, and the pH value of the homogenized solution was measured. Suspended solutions with different diluation ratios were spread on plate count agar plates (PCA). After incubation for 2 days at 28 °C, the number of colonies on the plates was counted. To determine the concentrations of thiosulfate, sulfate, sulfite, sulfide, elemental sulfur, and total organic carbon (TOC), the suspension had previously been filtered through a 0.45 μm membrane filter. Sulfate ion concentration in the filtrate was measured by ion chromatography (Dionex 4500i). Thiosulfate was determined by titration using a standard I_2 solution and a starch indicator (Meites, 1963). Sulfide (S^{2-}) was determined by using an ion-specific electrode. Elemental sulfur was determined by reaction with cyanide to produce thiocyanate, which was quantitated as $Fe(SCN)_6^{3-}$ (Schedel and Truper, 1980). Sulfite was determined by titration using a standard potassium iodide−iodate titrant and a starch indicator (APHA, 1992). TOC was determined by a TOC analyzer (Astro 2001 System 2).

Results and Discussion

Growth Characteristics of the Isolated Microorganism. The growth pattern of *P. putida* CH11 in the modified basal medium is shown in Figure 2. *P. putida* CH11 is a chemoorganoheterotrophic baterium that can oxidize sulfur compounds without obtaining energy from the process (Kuenen *et al.*, 1985). During the initial 5 days of cultivation, both the thiosulfate ($S_2O_3^{2-}$) concentration and the total organic carbon (TOC) concentration decreased, but the cell number and sulfate (SO_4^{2-}) concentration increased (Figure 2). These results indicate the specific uptake rates of thiosulfate and glucose are 3.5×10^{-11} g S/cell·day and 4.6×10^{-11} g C/cell·day,

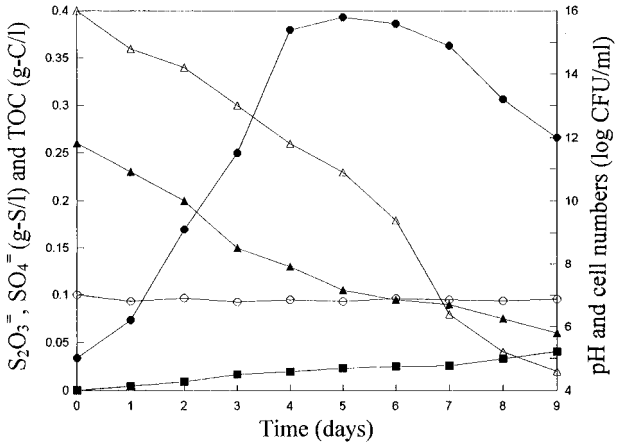

Figure 2. Growth pattern, $S_2O_3^{2-}$, SO_4^{2-}, TOC, pH, and cell number changes in *P. putida* CH11 growth in modified basal medium. Symbols: ▲, $S_2O_3^{2-}$; ■, SO_4^{2-}; △, TOC; ○, pH; ●, cell number.

respectively. Such specific uptake rates for thiosulfate and glucose can be attributed to *Pseudomonas* spp. possessing an extensive biochemical system (Schook and Berk, 1978). The results also indicate that sulfate concentrations increased slowly during the experiment periods, except on the 8th and 9th days. The average conversion rate of thiosulfate during the initial 7 days was 15% (e.g., 0.0255/0.26−0.09), whereas the conversion ratios for the 8th and 9th days were 50%. Because microorganism autolysis can liberate intracellular sulfate (Schook and Berk, 1978), the dramatic increase in sulfate concentration from 15% to 50% may be due to dead *P. putida* CH11, which can be justified by the decay in cell numbers. The 15% conversion ratio implies that *P. putida* CH11 only oxidized a small amount of thiosulfate to sulfate; therefore, a stable and neutral pH value was observed in the growth medium.

The effect of pH on H_2S removal by *P. putida* CH11 is shown in FIgure 3. The H_2S degradation activity at each pH level is expressed relative to the maximum removal rate. *P. putida* CH11 possessed high removal activity (>90%) in the range from pH 6 to 9. The ability of *P. putida* CH11 to remove hydrogen sulfide was less sensitive to pH than *Thiobacillus thioparus* (Cho *et al.*, 1991). From an operational standpoint, the control of pH is a very important parameter in H_2S treatment since the removal efficiency drops to 65% when the culture pH increases to 10. Hence, it is suggested that the optimum pH value for H_2S treatment ranges from 6 to 8.

H_2S Removal Rate in Continuous Operation. Fluctuation in inlet hydrogen sulfide concentration was examined in the range from 10 to 60 ppm at flow rates of 36 and 72 L/h. The H_2S removal efficiency was calculated from the difference in inlet and outlet concentrations. The H_2S removal efficiency in the immobilized *P. putida* CH11 biofilter with or without carbon (glucose) supply at a flow rate of 36 L/h is shown in Figure 4a. The changes in pH and cell number of the biofilter are shown in Figure 4b. The biofilter showed good operational efficiency regardless of whether the flow rate was 36 or 72 L/h (data not shown). At a flow rate of 36 L/h, the average removal efficiency of the biofilter with glucose supply was 97.1% for hydrogen sulfide concentrations ranging from 10 to 60 ppm. When the flow rate was increased to 72 L/h, the average removal efficiency was 95.8%. Such a high removal efficiency lasted for 3 months (data not shown). By contrast, the high H_2S removal efficiency lasted for only 3 days at 36 L/h in the absence of glucose. The removal efficiency of the biofilter

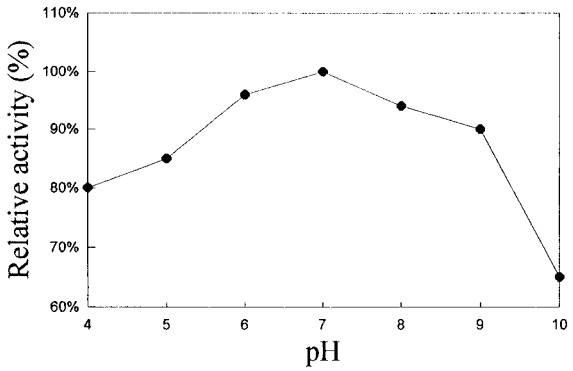

Figure 3. Effect of pH on H_2S degradation by *P. putida* CH11 in basal medium. The ordinate is relative to the maximum degradation rate.

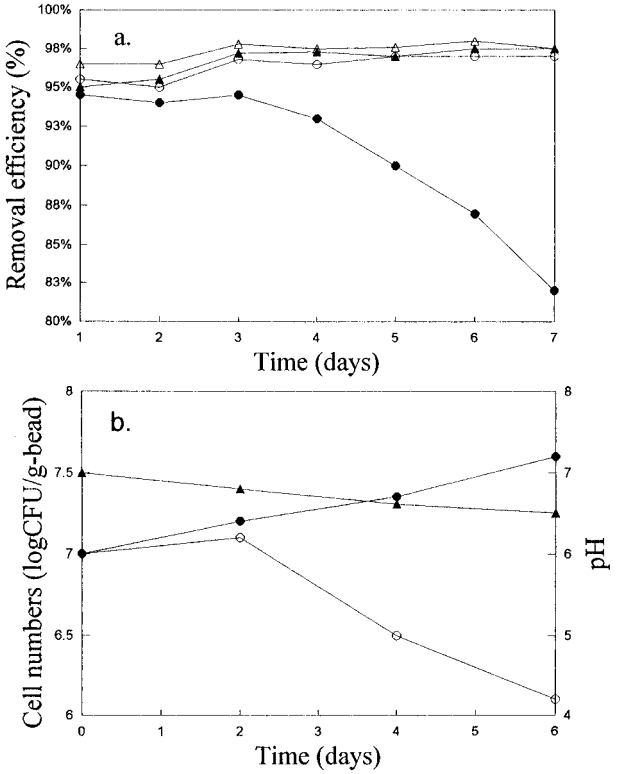

Figure 4. (a) H_2S removal efficiency of the biofilter at a flow rate of 36 L/h for 7 days. Symbols: ▲, 10 ppm; △, 20 ppm, ○, 60 ppm with glucose supply; ●, 60 ppm without glucose supply. (b) Changes in pH and cell number in the middle of the biofilter at 60 ppm of inlet gas. Symbols: ▲, pH; ●, cell number with glucose supply; ○, cell number without glucose supply.

significantly decreased after 3 days of treatment (Figure 4a). These results are due to a decrease in cell number since the paucity of carbon sources limits the growth of *P. putida* CH11 (Figure 4b).

Identification of H_2S Removal Products. To understand the metabolic products of H_2S removal by *P. putida* CH11, the sulfate, sulfide, sulfite, and elemental sulfur concentrations in the upper layer of the biofilter were assessed after 7 days of operation. When the biofilter was operated continually at 60 ppm H_2S at a flow rate of 36 or 72 L/h for 7 days, the biofilter sulfur mass balance was as presented in Table 1. At a flow rate of 36 L/h, the total amount of hydrogen sulfide consumption was 1.905 g S/kg bead, and the total sulfate, sulfide, sulfite, and elemental sulfur accumulations were 0.286, 0.206, 0.152, and 0.977 g S/kg bead, respectively. Therefore, the ratios of hydrogen sulfide conversion to sulfate, sulfide, sulfite, and elemental sulfur were 15%, 12%, 8%,

Table 1. Sulfur Mass Balances in the Biofilter Inoculated with *P. putida* CH11 at 60 ppm H_2S Feed for 7 Days

flow rate (L/h)	H_2S removed (g S/kg bead)	SO_4^{2-} produced (g S/kg bead)	S^{2-} produced (g S/kg bead)	SO_3^{2-} produced (g S/kg bead)	S^0 produced (g S/kg bead)
36	1.905	0.286 (15.0%)[a]	0.206 (10.8%)	0.152 (8.0%)	0.977 (51.3%)
72	3.801	0.543 (14.3%)	0.468 (12.3%)	0.296 (7.8%)	1.859 (48.9%)

[a] Conversion ratio of H_2S removed to varied products.

and 50%, respectively. The identified products accounted for 85% of the total H_2S conversion. The remaining 15% of the conversion product was assumed to be organic sulfur. Note that similar conversion ratios were also found at the high flow rate of 72 L/h. Because the hydrogen sulfide was mainly converted to elemental sulfur, the pH in the biofilter stayed within the neutral range (pH 6.5−7) during the operation period (Figure 4b). The processes of removing hydrogen sulfide developed by Wada *et al.* caused a drop in pH (acidification) that reduced the removal efficiency. Hence, the neutral pH operation condition, while removing H_2S with immobilized *P. putida* CH11 biofilter, appears to be a competitive method.

Shock-Loading Experiments. To examine the adaptability of the biofilter to upset conditions, results obtained by shock loading the H_2S concentration are presented in Figure 5. For the first 7 days, the biofilter was supplied with low concentrations of H_2S (20 ppm), and the removal efficiency stayed at 97.5%. When a shock loading of 200 ppm H_2S on the biofilter was performed for 4 days, a 33% decrease in the removal efficiency was observed during this period. After the 4 day shock-loading operation, the inlet H_2S concentration was adjusted back to 20 ppm for 20 days. The removal efficiency attained an 85% removal efficiency during the first 4 days and came back to 97.5% on the 7th day. The cell numbers in the middle and upper layers on the biofilter as shown in the inset in Figure 5 provide an index of the toxicity of the H_2S concentration to *P. putida* CH11. These cell numbers in the upper layer of the biofilter gradually increased from 10^7 to $10^{8.1}$ CFU/g bead. By contrast, the cell number in the middle layer of the biofilter unexpectedly declined from $10^{7.7}$ CFU/g bead on the 6th day to $10^{7.2}$ CFU/g bead on the 9th day and then gradually increased up to $10^{8.5}$ CFU/g bead after the 9th day. *P. putida* CH11 residing in the middle layer of the biofilter (near the inlet) was more likely to contact high concentrations of H_2S than that residing in the upper layer, which consumed larger quantities of H_2S. Hence, the cells near the inlet are easily damaged by high concentrations of H_2S (200 ppm). Actually, the *P. putida* CH11 used in this experiment was injured when inlet concentrations of H_2S were above 150 ppm, according to the abrupt drop in removal efficiency at this concentration (data not shown). Therefore, the maximum inlet concentration of H_2S needs be controlled below 150 ppm.

Effect of Temperature on H_2S Removal. The effect of temperature on the H_2S removal capacity of the biofilter is shown in Figure 6. Experiments were performed at 20, 25, and 37 °C at a flow rate of 72 L/h. The temperature did not affect the removal capacity of the biofilter when the inlet H_2S concentration ranged from 0 to 60 ppm. Once the inlet concentration increased to 100 ppm, the removal capacity gradually increased with increased temperature. The H_2S removal capacity increased by 20% when the temperature was raised from 20 to 37 °C. The temperature increase did not affect the removal capacity of the biofilter at low inlet concentrations, e.g., 10, 20, and 60 ppm. This phenomenon is due to the performance of the biofilter in the diffusion-limited range (Diks and Ottengraf, 1991). Hence, the low mass

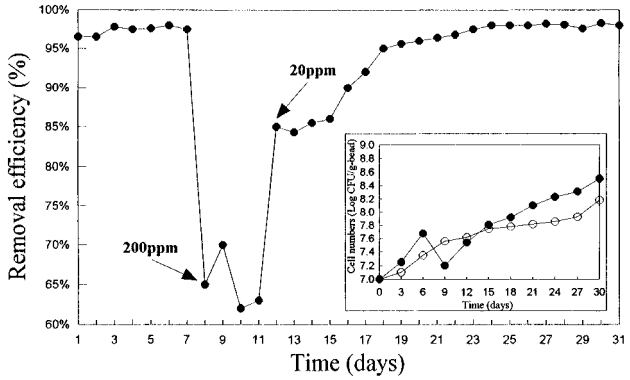

Figure 5. Effect of shock loading on removal efficiency at a flow rate of 36 L/h. Cell numbers in the upper (○) and middle layers (●) of the biofilter are shown in the inset.

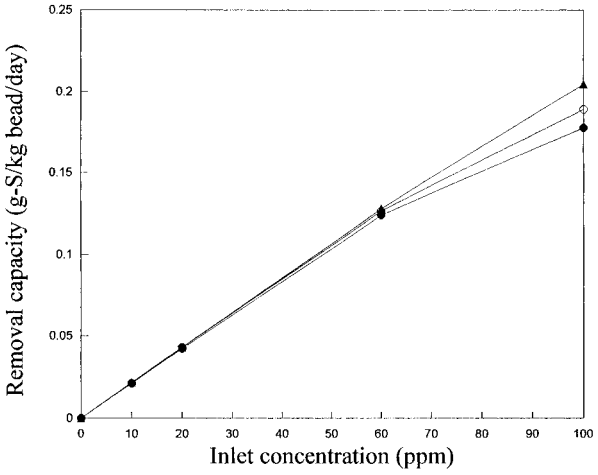

Figure 6. Effect of temperature on H_2S removal capacity. Symbols: ●, 20 °C; ○, 25 °C; ▲, 37 °C.

transfer effect counterbalances the effect of the increased microbial reaction rate.

Effect of Flow Rate on H_2S Removal. H_2S removal capacity as a function of gas flow is shown in Figure 7. The immobilized *P. putida* CH11 biofilter achieved steady state conditions in 7 days. The removal capacity decreased with increases in the hydrogen sulfide flow rate. The removal capacity showed a small difference (5%) at 36 and 72 L/h flow rates. However, a more marked difference (20%) became manifest when the flow rate was raised to 150 L/h. The limitation in H_2S removal was due to mass transfer effects and not to the activity of microbial enzymes at the higher flow rate of 150 L/h (Sublette *et al.*, 1994). Since the gas flow rate strongly influences removal capacity, it should be held below 72 L/h if 90% hydrogen sulfide elimination is required.

Kinetic Analysis. As the preceding studies show, *P. putida* CH11 (immobilized with Ca alginate) has the ability to degrade hydrogen sulfide. To determine the removal rate of hydrogen sulfide in the biofilter, kinetic analysis was performed. The kinetic parameters of the maximum removal rate and the saturation constant for hydrogen sulfide degradation under different conditions were calculated by using the Lineweaver−Burk method (Laidler, 1958; Dawes, 1967) and are shown in Figure 8.

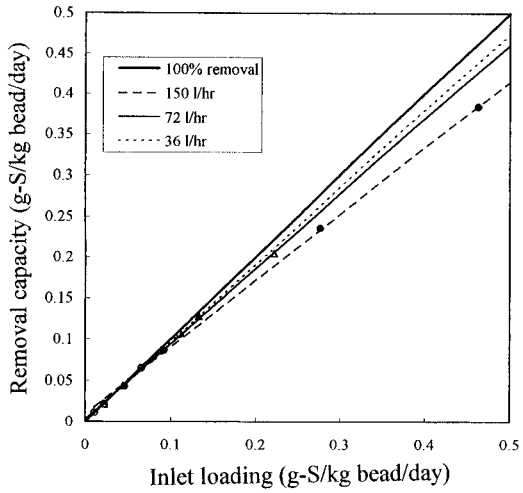

Figure 7. H_2S removal capacity as a function of gas flow rate: line 1, 100% removal curve; line 2, 36 L/h; line 3, 72 L/h; line 4, 150 L/h.

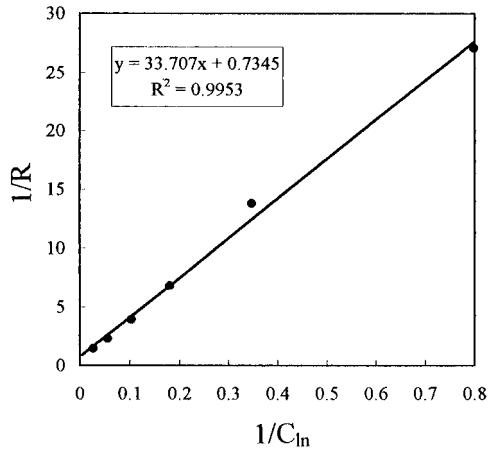

Figure 8. Relationship between H_2S degradation $1/R$ and $1/C_{ln}$ in the biofilter.

The regression equation expressed as $y = 33.707x + 0.7345$. The maximum removal rate and the saturation constant of hydrogen sulfide were calculated to be $V_m = 1.36$ g S/day·kg dry bead and $K_s = 45.9$ ppm respectively, from the slope and intercept of the regression equation. The saturation constant (45.9 ppm) in this study was smaller than the 55 ppm previously reported (Hirai, 1990). If we suppose a physical meaning for K_s analogous to that in enzymatic kinetics, a decrease in K_s suggests an enhancement in biomass affinity for hydrogen sulfide.

Conclusions

The results of this experimental investigation have proved that the immobilized *P. putida* CH11 biofilter has high potency in removing hydrogen sulfide at concentrations as low as 10 ppm or as high as 150 ppm. This biofilter achieved better than 96% removal efficiency at flow rates below 72 L/h. The main product of H_2S oxidation by *P. putida* CH11 was identified as elemental sulfur. Since the neutral element sulfur does not reduce pH and cause acidification of the biofilter, there is a significant advantage in applying this biofilter. The removal capacity of the biofilter was shown to increase with temperature elevation when high inlet concentrations were applied (e.g., 100 ppm). Finally, the low H_2S saturation constant indicates a high biomass affinity for hydrogen sulfide. Therefore, the biofilter packed with immobilized *P. putida* CH11 seems to be an effective method for controlling of H_2S emissions.

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Accepted July 12, 1996.[⊗]

BP960058A

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.