

COMPARISON OF AUTOTROPHIC AND MIXOTROPHIC BIOFILTERS FOR H₂S REMOVAL

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ABSTRACT: We demonstrate that the facultative chemoautotroph *Thiobacillus novellus* CH 3 removes hydrogen sulfide (H₂S) gas in continuous reactors under nutrient-limiting conditions. Extensive tests including removal characteristics, metabolic products, and removal efficiencies of H₂S by *T. novellus* CH 3 were conducted in autotrophic and mixotrophic environments. The optimal pH value and temperature required to remove hydrogen sulfide are found to be pH 7 and 26°C. The biofilter had an H₂S removal efficiency greater than 99.5% under the mixotrophic condition after 10-day operation. The results show that the maximum removal rate and saturation constant were 1.9 g S/d per kg bead and 69.2 ppm, respectively. The main metabolic product of H₂S oxidation was determined to be sulfate, but the conversion ratio was dependent on the growth environment. These results suggest that the mixotrophic potential of *T. novellus* CH 3 biofilter provides a significant advantage in H₂S removal over autotrophic biofilters.

INTRODUCTION

Hydrogen sulfide (H₂S) is a colorless and corrosive air pollutant that is extremely toxic (Roth 1993). It occurs widely in nature and is released by industrial processes, such as petrochemical refining, wastewater treatment, food preparation, paper and pulp manufacturing, and in the treatment of fuels (Eikum and Storhang 1986; Yang and Allen 1994). Excess H₂S must be removed for reasons of health and safety, because it has a great potential to irritate eyes and injure the human central nervous system (Vanhoorne et al. 1995). Conventional treatment of waste gases, wastewater, and ground water containing H₂S use some common technologies. These technologies include activated carbon adsorption, ozone oxidation, incineration, air stripping, and microfiltration (Eby and Wilson 1969; Barth et al. 1984; Mannebeck 1986; Thompson et al. 1995). However, conventional treatment and disposal costs are high and secondary-pollutant issues may arise. The continuing demand for improved process economy and efficiency has led to investigations into microbiological alternatives to conventionally physical/chemical methods (Bohn 1992). Biofilters decontaminate waste gas by passing it through a damp medium that supports a vigorous culture of microorganisms. The biofiltration process can serve as a most effective means when applied to dilute, easily biodegradable waste gases under appropriate sets of conditions (Leson and Winer 1991). Thus, H₂S is an excellent candidate for removal by biofiltration.

A wide range of biofilter bed materials as carriers have been studied (Rands et al. 1981; Lee and Shoda 1989; Leson and Winer 1991). Originally, biofilters are developed with soils as carriers; however, soils are limited in effectiveness because 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 in removing waste gases, but they are too expensive to justify the efficiency difference (Medina et al. 1995). Fibrous peat, used as a packing material, has been shown to perform better than soil, compost, or activated carbon (Leson and Winer 1991); however, a larger space is required when a biofilter packed with microorganism-laden peat is used to treat large quantities of hydrogen sulfide at low concentrations (<20 ppm) (Tanji et al. 1989). Ca-alginate beads have advantages as a biofilter medium in comparison with other commonly used biofilters. Recent work has shown that Ca-alginate beads can have a high microorganism content and prevent microorganism losses (Kokufuta et al. 1982).

The presence of microorganisms is necessary for effective removal of H₂S gas. Although activated sludges (mixed culture) have been used in operating biofilters, acclimation times of at least 1–3 weeks are required (Ottengraf and Van Den Oever 1983). Recently, the use of pure cultures is gaining a lot of attention because it shortens start-up time and enhances removal efficiencies and capacities. Both autotrophic and heterotrophic microorganisms have been employed in pure culture studies, and there are inherent differences in their nutritional requirements and abilities to catalyze specific reactions. Some autotrophic bacteria, such as members of the *Thiobacillus* species have been seeded into bioreactors and used to metabolize H₂S. The products of H₂S oxidation are dependent on the strain of *Thiobacillus* sp. employed (Sublette and Sylvester 1987; Cho et al. 1991a; Chung et al. 1996a). Among heterotrophic bacteria, only *Xanthomonas* sp. and *Pseudomonas putida* have been reported to oxidize H₂S in biofilter systems (Cho et al. 1991b; Chung et al. 1996b). Autotrophic biofilters have shown high affinity for H₂S, but failed to remove reliably low concentrations of H₂S through sustained experiments. However, heterotrophic biofilters have shown opposite tendencies (Huang et al. 1996). Moreover, facultative chemoautotrophs such as *Thiobacillus novellus* possess the unique potential for autotrophic as well as heterotrophic growth. Hence, these bacteria are apparently adaptable to different environments, i.e., autotrophic, heterotrophic, or mixotrophic conditions (Matin 1978). Studies have shown adaptive physiologic/metabolic response of *T. novellus* to mixotrophic environments (Leefeldt and Matin 1980; Perez and Matin 1980), but little engineering information, such as desired control mechanisms, and proper design and maintenance of biofilters for H₂S removal is available.

In this study, we used Ca-alginate to immobilize *T. novellus* CH 3 and studied an H₂S-fed biofilter operated under autotrophic and mixotrophic conditions.

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MATERIALS AND METHODS

Organism Cultivation and Buffer Preparation

The bacteria used in this study were isolated from piggery wastewater. The wastewater was mixed with a pH 7 mineral salts medium containing KH_2PO_4 , 2 g/L; K_2HPO_4 , 2 g/L; NH_4Cl , 0.6 g/L; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.3 g/L; and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 g/L. As the only source of energy, hydrogen sulfide generated by feeding solution of Na_2S and HCl was supplied from 5 to 150 ppm into the Erlenmeyer flasks. Erlenmeyer flasks were closed with rubber stoppers containing inlet and outlet pores and the inlet and outlet gas concentrations were measured regularly. When the outlet gas concentration was nearly zero, the inlet gas concentration was increased to a desired higher level. This process was repeated until a constant level of outlet gas concentration was detectable. At this stage, the process of acclimating microbes was assumed to be completed. One milliliter of bacterial solution was transferred repeatedly to fresh solid medium by spread plate method. The dominant colonies were reserved and further purified until appearance of single colony. The isolated strain was identified as *T. novellus* by the Food Industry Research and Development Institute in Taiwan. During continuous-treatment experiments, the autotrophic inflow medium included KH_2PO_4 , 1.2 g/L; K_2HPO_4 , 1.2 g/L; NH_4Cl , 0.4 g/L; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.2 g/L; and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g/L. The mixotrophic inflow medium was obtained by supplementing the autotrophic inflow medium with 0.2 g glucose. The final pH of the culture was adjusted to 7 using 2 N NaOH or HCl . In batch experiments, for measurement of the pH effect on sulfide removal by *T. novellus* CH 3 under autotrophic and mixotrophic conditions, the *T. novellus* CH 3 were suspended in phosphate buffers in the pH range of 5.5–8.0 at 30°C. The removal rates were measured at sulfide concentrations between 0 and 10 mM. For each pH value, the values of the saturation constant K_s and the maximum removal rate V_m were calculated using Michaelis-Menten equation via the linear regression method.

Preparation of Immobilized Cells

T. novellus CH 3, grown in 100-mL nutrient broth, was harvested by centrifugation ($7,500 \times g$ for 10 min). The organisms were washed three times with sterile distilled water, followed by immersing in a sterile 4% Na-alginate solution (10^5 cells/mL) and then mixing with a 4% CaCl_2 solution. Upon mixing, 3-mm-diameter immobilized beads were formed immediately. These gel beads then were activated by flushing with sterile buffer solution for 5 h. The activated beads exhibited excellent mechanical strength in the continuous experiments.

Apparatus and H_2S Removal for Continuous Operation

A description of the laboratory-scale experimental biofilter was described previously by Chung et al. (1996c). Glass columns (60 mm $\phi \times$ 40 cm of working height) were packed with cell-laden Ca-alginate beads. In each column, 0.5 kg dry beads were packed in a column of 1.44 L. Initial cell numbers in each column were counted to be approximately 10^7 colony-forming units (cfu) per 1 g of dry bead. In the continuous experiment, H_2S gas at different concentrations (10, 20, and 60 ppm) was introduced to the biofilter at a flow rate of 36 or 72 L/h. The H_2S was supplied from a gas cylinder, diluted with compressed air, forced through a filter unit (0.45 μm), and then passed through the humidification bottle into the bottom of biofilter. The effect of temperature on the volumetric oxidation rates and removal efficiencies of the biofilters was studied in the range of 10–30°C controlled by refrigerated

circulator circulating water via heat exchanger and while the flow rates were from 18 to 185 L/h. The products from the metabolization of H_2S by *T. novellus* CH 3 also were analyzed during the continuous experiment.

Kinetic Analysis

The H_2S removal rate in the immobilized-cell biofilter was calculated using the following equation derived from the Michaelis-Menten equation (Hirai et al. 1990):

$$\frac{1}{R} = \frac{K_s}{V_m} \times \frac{1}{C_{in}} + \frac{1}{V_m} \quad (1)$$

where R (g S/d per kg dry bead) = removal rate; C_{in} (ppm) = logarithmic mean concentration of H_2S at the inlet and outlet of the biofilter; V_m (g S/d per kg dry bead) = maximum removal rate; and K_s (ppm) = half-saturation constant. With the use of the linear relationship between $1/C_{in}$ and $1/R$, V_m and K_s were calculated from the slope and intercept.

Criteria for Design of Scale-Up Biofilter

The target concentrations of H_2S at the biofilter outlet were presumed as 0.1 and 1 ppm. The maximum inlet concentrations and critical H_2S loads needed to satisfy this effluent concentration (0.1 or 1 ppm) were obtained at various space velocity (SV) according to the following equation (Tiwari et al. 1992):

$$SV = \frac{\alpha}{(C_0 - C_e)} \times V_m \times \frac{C_{in}}{(K_s + C_{in})} \quad (2)$$

where SV (1/d) = F ($1/S_a L$); F = gas flow rate (m^3/d); S_a = column cross section (m^2); L = packing height (m); C_0 = inlet concentration (ppm); C_e = outlet concentration (ppm); and α = conversion coefficient (kg dry bead/g S). Let C_e be 0.1 or 1 ppm in (2), and the maximum C_0 can be estimated at various SVs. The critical loads (g S/d per kg bead) of the biofilters can be obtained using (3)

$$\text{critical load} = \frac{SV \times C_0}{\alpha} \quad (3)$$

Analytical Methods

Inlet and outlet H_2S gas concentrations in the biofilter were measured continuously using a single point monitor (MDA Scientific) in the range of 50–1,500 ppb or periodically measured by gas detector tubes (GASTEC, Tokyo, Japan) in the range of 1–60 ppm. During continuous experiments, the variation of H_2S concentration at steady state was found to be within $\pm 5\%$. The H_2S outlet concentration was reported as average values from 12 assays. Five grams (wet-weight) of cell-laden beads was dissolved in 95 mL of 0.1 M sodium citrate solution and the sulfur compounds and their amounts in the solution were determined. Sulfate ion concentrations in the solution were measured by ion chromatography (Dionex 4500i). Sulfite was determined by titration using a standard potassium iodide-iodate titrant and a starch indicator [American Public Health Association (APHA) 1992]. Sulfide was determined using an ion-specific electrode (SCHOTT, Germany).

RESULTS AND ANALYSIS

Effect of pH on Sulfide Degradation

At six different pH values ranging from 5.5 to 8, the kinetics of H_2S removal were measured at concentrations between 0 and 10 mM in autotrophic and mixotrophic batch cultures. The pH effects on the sulfide oxidation kinetics of *T. novellus* CH 3 are presented in Table 1. For each pH value, the saturation constant K_s and the maximum removal rate V_m were calculated

TABLE 1. Effect of pH on Saturation Constant and Maximum Removal Rate of *Thiobacillus novellus* CH 3 for Sulfide Degradation in Autotrophic and Mixotrophic Environments at T= 26°C

pH (1)	Autotrophic Environments		Mixotrophic Environments	
	K_s (ppm) (2)	V_m (g S/d per kg bead) (3)	K_s (ppm) (4)	V_m (g S/d per kg bead) (5)
5.5	185.6	0.76	152.3	0.89
6	138.3	1.08	111.3	1.26
6.5	86.8	1.79	77.8	1.74
7	84.3	1.85	74.5	1.81
7.5	89.3	1.73	80.4	1.68
8	139.5	1.13	100.5	1.34

from (1) using linear regression. Trends of pH versus K_s profiles indicate that K_s decreases with the increase of pH. The optimal pH value for sulfide removal was 7 regardless of autotrophic or mixotrophic growth conditions. From an operational standpoint, the control of pH is an important parameter in sulfide treatment, and this study suggests the optimal pH value is approximately 7. The saturation constants under mixotrophic conditions were lower than those under autotrophic conditions. Thus, in mixotrophic environments, *T. novellus* CH 3 exhibited higher enzymatic affinity for sulfide than it did in autotrophic environments. Our results are in agreement with previous studies, which have shown that *T. novellus* had higher cell-growth rates and glucose-transport activity in mixotrophic environments than in autotrophic environments (Matin et al. 1980; Perez and Matin 1982).

Effect of Temperature on H₂S Removal

Fig. 1 shows the H₂S oxidation rate of *T. novellus* CH 3 in autotrophic and mixotrophic environments ranging in temperature from 10 to 30°C. When the biofilters were supplied with 60 ppm H₂S at a flow rate of 36 L/h, the H₂S oxidation rate increased with the temperature, reaching its maximum at approximately 26°C. The effect of temperature on H₂S oxidation rate was almost the same whether *T. novellus* CH 3 was in an autotrophic or mixotrophic environment. In general, the relationship between reaction rate and temperature can be approximated using the Arrhenius equation

$$K_T = K_{20}\theta^{(T-20)} \quad (4)$$

where K_T = reaction rate at $T^\circ\text{C}$ (g S/m³ per day); K_{20} = reaction rate at 20°C (g S/m³ per day); θ = temperature-dependency coefficient; and T = temperature (°C). We obtained θ on

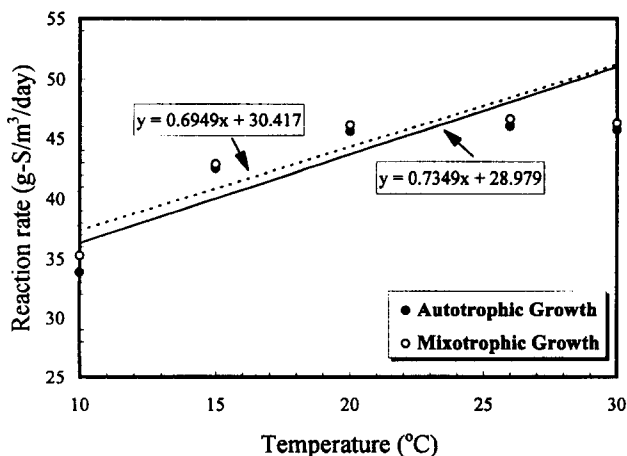


FIG. 1. Effect of Temperature on H₂S Oxidation Rate of *Thiobacillus novellus* CH 3 Biofilters in Autotrophic (Solid Line) and Mixotrophic (Dash Line) Environments

the basis of experimental results using linear regression analysis from Fig. 1 and the values were

$$\theta = 1.023 \quad \text{under autotrophic conditions}$$

$$\theta = 1.025 \quad \text{under mixotrophic conditions}$$

There was no significant difference in θ between the autotrophic and the mixotrophic biofilter at the temperature between 10 and 26°C.

H₂S Removal Rate in Continuous Operation

Fluctuations in inlet H₂S concentrations were examined in the 10–60 ppm range at flow rates of 36 and 72 L/h. The H₂S removal efficiency was calculated from the difference in inlet and outlet concentrations. The H₂S removal efficiencies of the immobilized *T. novellus* CH 3 biofilters in autotrophic and mixotrophic environments at 36 L/h are shown in Figs. 2(a and b), respectively. The variation ranges in the figures were between maximum and minimum removal efficiency for different inlet concentrations. These results demonstrated that the mixotrophic biofilter achieved a steady-state condition (10 days) earlier than did the autotrophic biofilter (13 days). The mixotrophic biofilter showed excellent operational efficiency (>99.5%) regardless of whether the flow rate was 36 or 72 L/h (data not shown). By contrast, a slightly lower H₂S removal efficiency (>98.3%) for the autotrophic biofilter was observed. The higher H₂S removal efficiency achieved by the mixotrophic biofilter appears to be the contribution of enzymatic affinities. The data suggest that the mixotrophic potential of the *T. novellus* CH 3 biofilter could provide significant advantages over the autotrophic biofilter.

Effect of Residence Time on H₂S Removal

The H₂S removal efficiency as a function of residence time at inlet concentration of 60 ppm is shown in Fig. 3. The *T. novellus* CH 3 biofilter achieved a steady-state condition in 72

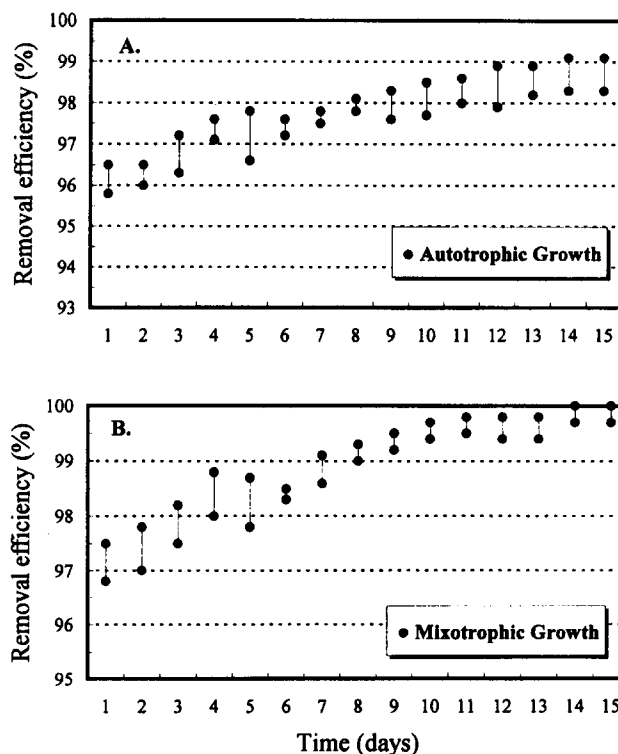


FIG. 2. H₂S Removal Efficiencies by *Thiobacillus novellus* CH 3 Biofilters: (a) in Autotrophic; (b) in Mixotrophic Environments at 36 L/h with Inlet Concentrations of 10–60 ppm at pH 7

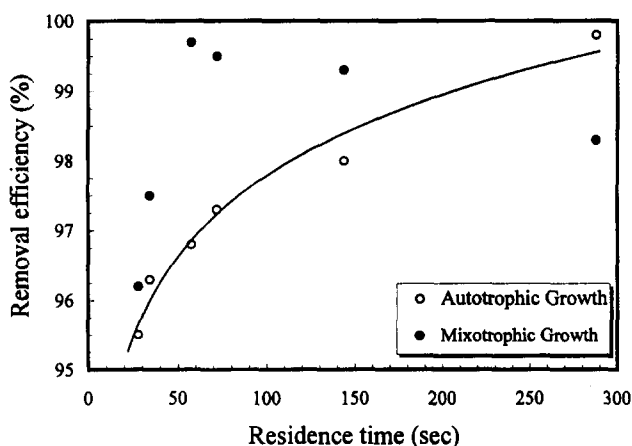
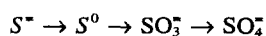


FIG. 3. H₂S Removal Efficiency as Function of Residence Time at Inlet Concentration of 60 ppm

h. The removal efficiency of the autotrophic biofilter decreased significantly with decreases in H₂S residence time. By contrast, the mixotrophic biofilter exhibited a different removal pattern. The removal efficiency increased with H₂S residence time in the range of 28–58 s (equivalent to flow rates from 185 to 90 L/h). When the residence time was longer than 58 s, the removal efficiency decreased with further increases in the H₂S residence time. Under all operating conditions except for residence time of 288 s, the mixotrophic biofilter achieved higher removal efficiencies than the autotrophic biofilter. The cells obtained energy derived from hydrogen sulfide and glucose oxidation in mixotrophic environments. Based on our results and previous studies, it appears that glucose served as an additional carbon source besides CO₂ during mixotrophic growth. Leefeldt and Matin (1980) showed that at long residence times some 30–50% more glucose was used in energy generation by *T. novellus*; hence, less H₂S was utilized by the oxidation to provide energy. By contrast, during short residence times less glucose (approximately 15%) was used in energy generation suggesting that *T. novellus* would oxidize more H₂S to provide energy for survival (e.g., 58 s) (Leefeldt and Matin 1980).

Identification of H₂S Removal Products

To understand H₂S removal by *T. novellus* CH 3 in various physiological situations, the sulfate, sulfite, and sulfide concentrations in the middle layer of the biofilter were monitored after 72 h of operation. Elemental sulfur also was measured but it was always below detection limits (0.1 mg/L). Table 2 shows the mass balance of sulfur in the biofilter, after the biofilter was operated continuously with the inlet of 60 ppm H₂S at residence times of 58 or 288 s for 3 days. When the biofilter was operated in mixotrophic environments, the predominant metabolic product was sulfate, which thus accounted for 97.6% of the total H₂S conversion at a residence time of 58 s. At a residence time of 288 s, sulfate accounted for only 73.5%. When the biofilter was operated under autotrophic environments, the same metabolic products were found; however, H₂S almost was converted to sulfate regardless of the residence time, e.g., 99.3% at 58 s and 98.0% at 288 s. The bioconversion of aerobic sulfide removal pathway by *Thiobacillus sp.* was suggested by Buisman et al. (1990)



It is, therefore, indicated that the nearly complete oxidation of sulfide under autotrophic conditions at all residence times was an artifact of sulfide being the only source of energy. Because the residence time strongly influences H₂S removal efficiency,

TABLE 2. Sulfur Mass Balances in Biofilters in Mixotrophic and Autotrophic Environments at 60 ppm H₂S at pH 7

Residence time (s) (1)	H ₂ S removed (g S/kg bead) (2)	SO ₄ ²⁻ produced (g S/kg bead) (3)	SO ₃ ²⁻ produced (g S/kg bead) (4)	S ⁰ produced (g S/kg bead) (5)
(a) Mixotrophic Biofilter				
58	1.103	0.989 (97.6%)	0.018 (1.8%)	0.004 (0.4%)
288	0.200	0.147 (73.5%)	0.049 (24.5%)	0.005 (2.5%)
(b) Autotrophic Biofilter				
58	0.983	0.976 (99.3%)	0.008 (0.8%)	0.003 (0.3%)
288	0.203	0.199 (98.0%)	0.003 (1.5%)	0.001 (0.5%)

the optimal residence time should be held at either 288 or 72 s, depending on which biofilter condition is present.

Kinetic Analysis

As the foregoing studies show, immobilized *T. novellus* CH 3 possessed an excellent ability to degrade H₂S, especially in mixotrophic environments. Kinetic analysis was performed to determine the enzymatic affinities for H₂S in the experimental biofilters. Results obtained using regression methods are shown in Fig. 4, along with corresponding regression equations. The H₂S half-saturation constants of autotrophic and mixotrophic biofilters were 78.2 and 69.2 ppm, respectively. The maximum removal capacities were 2.0 and 1.9 g S/d per kg bead. Note that the half-saturation constant (69.2 ppm) of the mixotrophic biofilter was smaller than that of the autotrophic biofilter (78.2 ppm). If we infer a physical meaning for *K_s*, analogous to enzymatic kinetics, a decrease in *K_s* suggests an enhancement in biomass affinity for H₂S. Hence, the *T. novellus* CH 3 biofilter operation in the mixotrophic environment enhanced H₂S removal over autotrophic operations. In addition, the enzymatic affinity in the continuous trials (i.e., half-saturation constants are 69.2 and 78.2 ppm, respectively) were higher than those in the batch experiments (i.e., half-saturation constants are 74.5 and 84.3 ppm, respectively), regardless of whether the biofilter was operated under autotrophic or mixotrophic conditions. From a microbial physiological viewpoint, the studies brought into focus differences in the regulation of bacterial enzyme expression in different environments. This observation is in accordance with Leefeldt and Matin (1980) who showed that the enzymes in *T. novellus* may be repressed under nutrient-excess conditions of batch culture and activated under the nutrient-limited conditions of continuous culture.

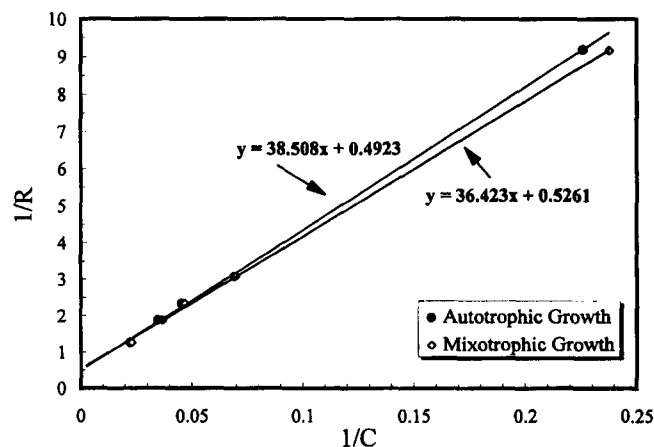


FIG. 4. Relationship between H₂S Degradation 1/R and 1/C in Biofilters

Criteria for Design of Scale-Up Biofilters

Complete H₂S removal can be achieved only at less than critical inlet load. If this critical inlet load is exceeded, H₂S is detected continuously at the outlet of the biofilter. The system load is defined as the amount of inlet gas per unit of time per weight of packing material (g S/d per kg bead). Thus, inlet gas concentrations play an important role in the design of a scale-up biofilter if the weight of the packing material and the gas-flow rate are constant. Finding the maximum inlet concentration and the optimal inlet load therefore becomes important for the operation of a biofilter. The relationship between the maximum inlet concentration and space velocity for H₂S removal is shown in Figs. 5(a and b). The values shown in Fig. 5 also are listed in Table 3. At a space velocity of 64 h⁻¹ (i.e., flow rate, 72 L/h), the mixotrophic biofilter tolerated higher loads than autotrophic biofilter. Although mixotrophic biofilter removed more H₂S than autotrophic biofilter, the former seems to be superior to the later only when a low emission

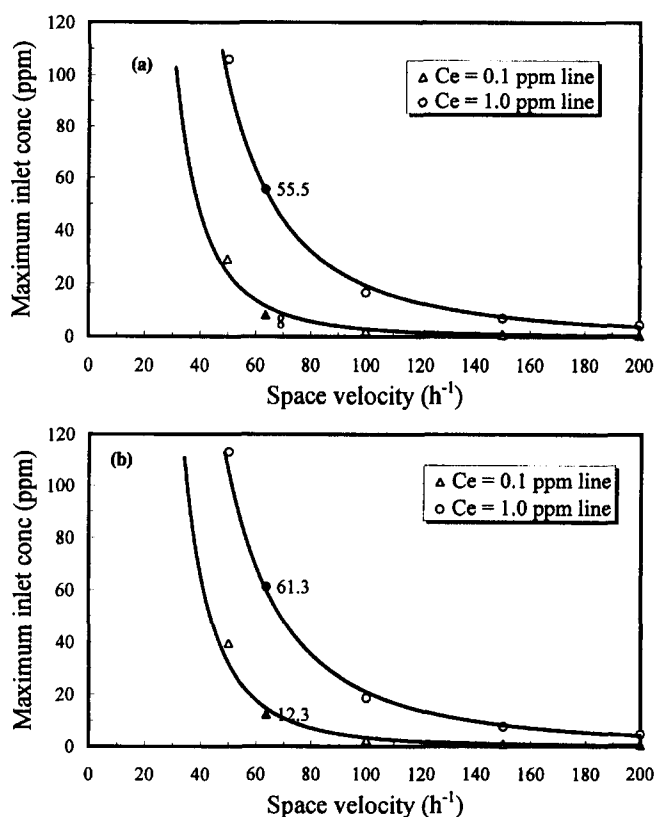


FIG. 5. Relationship between Maximum Inlet Concentration and Space Velocity for H₂S Removal by *Thiobacillus novellus* CH 3 Biofilters: (a) in Autotrophic; (b) in Mixotrophic Environments

TABLE 3. Maximum Inlet Concentrations and Critical Loads in Mixotrophic and Autotrophic Biofilters at Target Emission Concentrations of 0.1 or 1 ppm at 64 h⁻¹

Emission concentration (ppm) (1)	Maximum inlet concentration (ppm) (2)	Critical load (g S/d per kg bead) (3)
(a) Mixotrophic Biofilter		
0.1	12.3	0.070
1	61.3	0.346
(b) Autotrophic Biofilter		
0.1	8.0	0.045
1	55.5	0.313

concentration (<0.1 ppm) was required. Therefore, the mixotrophic biofilter operations were superior to the autotrophic biofilter operations when strict emission concentration constraints were imposed.

CONCLUSIONS

The optimal condition of H₂S removal by *T. novellus* CH 3 biofilter was found at pH 7 and 26°C. In continuous trials, the mixotrophic biofilter achieved steady-state condition in a shorter time and exhibited higher efficiency in removing H₂S than the autotrophic biofilter. The mixotrophic biofilter achieved greater than 99.5% removal efficiency after 10-day operation even at H₂S concentration as low as 10 ppm. The main product of H₂S oxidation by *T. novellus* CH 3 was identified as sulfate. The formation ratio of sulfate to sulfide in the mixotrophic biofilter depended on the residence time of H₂S. Longer residence time benefited the H₂S removal in the autotrophic biofilter, but the optimal residence time was found to be approximately 58 s in the mixotrophic biofilter. The efficiency of sulfide oxidation and the products formed suggest that different physiological regulatory mechanisms affect enzyme synthesis and expression in the mixotrophic biofilter under various H₂S flow rates. When a biofilter concurrently received glucose and H₂S, it exhibited a higher H₂S affinity than a biofilter receiving H₂S only. From a design standpoint, the mixotrophic biofilter possesses a removal capacity similar to that of the autotrophic biofilter when high emission concentration is permitted. However, the mixotrophic biofilter is superior to the autotrophic biofilter, when a stringent emission limit is required.

ACKNOWLEDGMENT

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APPENDIX I. REFERENCES

- American Public Health Association (APHA). (1992). *Standard method: examination of water and wastewater*, 18th Ed., American Public Health Association, New York, N.Y.
- Barth, C. L., Elliott, F. L., and Melvin, S. W. (1984). "Using odour control technology to support animal agriculture." *Trans. ASAE*, 27(8), 859-864.
- Bohn, H. (1992). "Consider biofiltration for decontaminating gases." *Chem. Eng. Progress.*, 88, 35-40.
- Buisman, C. J. N., Geraats, B. G., Ijspeert, P., and Lettinga, G. (1990). "Optimization of sulfur production in a biotechnological sulfide-removing reactor." *Biotechnol. Bioengr.*, 35, 50-56.
- Carlson, D. A., and Leisner, C. P. (1966). "Soil beds for the control of sewage odors." *J. Water Pollution Control Fed.*, 38(11), 829-834.
- Cho, K. S., Hirai, M., and Shoda, M. (1991a). "Degradation characteristics of hydrogen sulfide, methanethiol, dimethyl sulfide and dimethyl disulfide by *Thiobacillus thioarvus* DW44 isolated from peat biofilter." *J. Ferment. Bioengr.*, 71(6), 384-389.
- Cho, K. S., Kuniyoshi, I., Hirai, M., and Shoda, M. (1991b). "A newly isolated heterotrophic bacterium, *Xanthomonas* sp. DY 44, to oxidize hydrogen sulfide to polysulfide." *Biotechnol. Lett.*, 13(12), 923-928.
- Chung, Y. C., Huang, C., and Tseng, C. P. (1996a). "Operation optimization of *Thiobacillus thioarvus* CH11 biofilter for hydrogen sulfide removal." *J. Biotechnol.*, 52, 31-38.
- Chung, Y. C., Huang, C., and Tseng, C. P. (1996b). "Microbial oxidation of hydrogen sulfide with biofilter." *J. Envir. Sci. and Health*, A31(6), 1263-1278.
- Chung, Y. C., Huang, C., and Tseng, C. P. (1996c). "Biodegradation of hydrogen sulfide by a laboratory-scale immobilized *Pseudomonas putida* CH11 biofilter." *Biotechnol. Prog.*, 12, 773-778.
- Eby, H. J., and Wilson, G. B. (1969). "Poultry house dust, odour and their mechanical removal." *Agric. Waste Mgmt. Proc., Cornell Univ. Conf. on Agric. Waste Mgmt.*, Syracuse, N.Y., 303-308.
- EiKum, A. S., and Storhang, R. (1986). "Odour problems related to waste water and sludge treatment." *Odour prevention and control of organic sludge and livestock farming*, V. C. Neilsen, J. H. Voorburg, and P. L.

- Hermite, eds., Elsevier Applied Science Publishers, London, England, 12–18.
- Hirai, M., Ohtake, M., and Shoda, M. (1990). "Removal kinetic of hydrogen sulfide, methanethiol and dimethyl sulfide by peat biofilters." *J. Ferment. Bioengr.*, 70(5), 334–339.
- Huang, C., Chung, Y. C., and Hsu, B. M. (1996). "Hydrogen sulfide removal by immobilized autotrophic and heterotrophic bacterial in the bioreactor." *Biotechnol. Tech.*, 10(8), 595–600.
- Kokufuta, E., Matsumoto, W., and Nakamura, I. (1982). "Immobilization of *Nitrosomonas eruopaea* cells with polyelectrolyte complex." *Biotechnol. Bioengr.*, 24, 1591–1603.
- Langenhove, H. V., Bendinger, B., Oberthur, R., and Schamp, N. (1992). "Organic sulfur compounds: persistent odorants in biological treatment of complex waste gases." *Biotechniques for air pollution abatement and odour control policies*, A. J. Dragt and J. V. Ham, eds., Elsevier Press, Amsterdam, The Netherlands, 177–182.
- Lee, S. K., and Shoda, M. (1989). "Biological deodorization using activated carbon fabric as a carrier of microorganisms." *J. Ferment. Bioengr.* 68(6), 437–442.
- Leefeldt, R. H., and Matin, A. (1980). "Growth and physiology of *Thiobacillus novellus* under nutrient-limited mixed conditions." *J. Bacteriol.*, 142(2), 645–650.
- Leson, G., and Winer, A. M. (1991). "Biofiltration: an innovative air pollution control technology for VOC emission." *J. Air and Waste Mgmt. Assoc.*, 41(8), 1045–1054.
- Mannebeck, H. (1986). "Covering manure storing tanks to control odour." *Odour prevention and control of organic sludge and livestock farming*, V. C. Neilsen, J. H. Voorburg, and P. L. Hermite, eds., Elsevier Applied Science Publishers, London, England, 188–192.
- Matin, A. (1978). "Organic nutrition of chemolithotrophic bacteria." *Annu. Rev. Microbiol.*, 32, 433–468.
- Matin, A., Schleiss, M., and Perez, R. C. (1980). "Regulation of glucose transport and metabolism in *Thiobacillus novellus*." *J. Bacteriol.*, 142(2), 639–644.
- Medina, V. F., Webster, T., and Devinny, J. S. (1995). "Treatment of gasoline residuals by granular activated carbon based biological filtration." *J. Envir. Sci. and Health*, A30(2), 407–422.
- Ottengraf, S. P. P., and Van Den Oever, A. H. C. (1983). "Kinetics of organic compound removal from waste gases with a biological filter." *Biotechnol. Bioengr.*, 25, 3089–3102.
- Perez, R. C., and Matin, A. (1980). "Growth of *Thiobacillus novellus* on mixed substrates (mixotrophic growth)." *J. Bacteriol.*, 142(2), 633–638.
- Perez, R. C., and Matin, A. (1982). "Carbon dioxide assimilation by *Thiobacillus novellus* under nutrient-limited mixotrophic conditions." *J. Bacteriol.*, 150(3), 46–51.
- Rands, M. B., Cooper, D. E., Woo, C. P., Fletcher, G. C., and Rolfe, K. A. (1981). "Compost filters for H_2S removal from anaerobic digestion and rendering exhausts." *J. Water Pollution Control Fed.*, 53(10), 185–189.
- Roth, S. H. (1993). *Hydrogen sulfide. Handbook of hazardous material*. Academic Press, Inc., New York, N.Y.
- Sublette, K. L., and Sylvester, N. D. (1987). "Oxidation of hydrogen sulfide by continuous cultures of *Thiobacillus denitrificans*." *Biotechnol. Bioengr.*, 29, 753–758.
- Tanji, Y., Kanagawa, T., and Mikami, E. (1989). "Removal of dimethyl sulfide, methyl mercaptan, and hydrogen sulfide by immobilized *Thiobacillus thioparus* TK-m." *J. Ferment. Bioengr.*, 67(4), 280–285.
- Thompson, M. A., Kelkar, U. G., and Vickers, J. C. (1995). "The treatment of groundwater containing hydrogen sulfide using microfiltration." *Desalination*, 102(2), 287–291.
- Tiwari, R. S., Cho, K. S., Kirai, M., and Shoda, M. (1992). "Biological deodorization of dimethyl sulfide using different fabrics as the carriers of microorganisms." *Appl. Biochem. Biotech.*, 32, 135–148.
- Vanhoorne, M., Rouck, A., and de Bacquer, D. (1995). "Epidemiological study of eye irritation by hydrogen sulphide and/or carbon disulphide exposure in viscose rayon workers." *Ann. Occup. Hyg.*, 39(3), 307–315.
- Yang, Y., and Allen, E. R. (1994). "Biofiltration control of hydrogen sulfide 1. Design and operational parameters." *J. Air Waste Mgmt. Assoc.*, 44, 863–868.

APPENDIX II. NOTATION

The following symbols are used in this paper:

- C_e = outlet concentration (ppm);
 C_{in} = logarithmic mean concentration of H_2S at inlet and outlet of biofilter (ppm);
 C_o = inlet concentration (ppm);
 F = gas flow rate (m^3/d);
 K_s = half-saturation constant (ppm);
 K_T = reaction rate at $T^\circ C$ ($g S/m^3$ per day);
 K_{20} = reaction rate at $20^\circ C$ ($g S/m^3$ per day);
 L = packing height (m);
 R = removal rate ($g S/d$ per kg dry bead);
 S_a = column cross section (m^2);
 T = temperature ($^\circ C$);
 V_m = maximum removal rate ($g S/d$ per kg dry bead);
 α = conversion coefficient (kg dry bead/ $g S$); and
 θ = temperature-dependency coefficient.