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Removal of Hydrogen Sulfide and Methylmercaptan Using *Thiobacillus* in a Three Phase Fluidized Bed Bioreactor

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Abstract A three phase fluidized bed bioreactor immobilized with *Thiobacillus* sp. IW was tested to remove hydrogen sulfide and methylmercaptan with high loading rate. In a single gas treatment, the bioreactor removed 92–98% of hydrogen sulfide with loading rate of 15–66 g/l/h and removed 87–98% of methylmercaptan with loading rate of 14–60 $gl^{-1}h^{-1}$. In the mixed gas treatment, the removal efficiencies of hydrogen sulfide and methylmercaptan maintained at 89–99% for various inlet loading rates and were not affected by the inlet loading ratio of both gases in low loading rates. When the inlet concentration of methylmercaptan increased 3.8 times and was maintained for 30 h to observe the response of the bioreactor to sudden environmental change, the removal efficiency of methylmercaptan was maintained at an average of 91%.

Key words: Hydrogen sulfide, methylmercaptan, *Thiobacillus*, fluidized bed bioreactor, removal efficiency, inlet loading rate

Odors from petrochemical plants, pulp manufacturing plants, and waste water treatment plants contain many toxic chemicals. Among them, sulfurous compounds including hydrogen sulfide (H_2S) and methylmercaptan (CH_3SH) are the most abundant [2, 22, 25]. Sulfurous gases are conventionally removed by absorption, adsorption, and catalytic oxidation, however, these methods have many problems such as low removal efficiency, high operating costs, and secondary contamination [1, 8]. In recent researches, biological processes have been used to remove sulfur compounds at high removal efficiency without significant secondary contamination. To remove sulfurous odors biologically, sulfur compounds are either oxidized by aerobic bacteria or reduced by anaerobic bacteria [18].

Aerobic microbes oxidize sulfurous compounds to sulfur or sulfate depending on oxygen content and enzymatic activity [12]. Major advantages of the bioreactor using aerobic bacteria are such that the sulfur compounds are oxidized very quickly, the apparatus of the bioreactor is simple, and the operating cost of the bioreactor is low. Aerobic bacteria used in recent researches include *Thiobacillus* [20, 23], *Hyphomicrobium* [7], and *Pseudomonas* [5]. Among these, *Thiobacillus* species are most widely used due to the fact that they oxidize a wide variety of sulfurous compounds and grow rapidly in culture media. Oxidation reactions of sulfur compounds in general are as follows:

$$H_2S+2O_2 \rightarrow SO_4^{2-}+2H^+$$
$$2\,CH_3SH+7O_2 \rightarrow 2\,SO_4^{2-}+2CO_2+2H_2O$$
$$S_2O_3^{2-}+H_2O+2O_2 \rightarrow 2SO_4^{2-}+2H^+$$

SO_4^{2-} generated in the solution of the bioreactor can be precipitated by the addition of $CaCO_3$ to yield $CaSO_4$ in later stages.

Anaerobic microbes convert hydrogen sulfide to sulfur or sulfate depending on the light energy absorbed. The bioreactor using anaerobic microorganisms removes hydrogen sulfide at a very high concentration. However, it has the disadvantage of requiring a strong light source and high concentration of CO_2. Anaerobic microbes commonly used in desulfurization are *Chlorobium* sp. [6, 15].

In aerobic treatments, hydrogen sulfide was extensively tested with various types of bioreactors and the removal efficiencies obtained were 90–99% in general [10]. However, most experiments were carried out in low concentration of hydrogen sulfide and with low gas flow rates. In mixed gas treatments, including several combinations of hydrogen sulfide, methylmercaptan, $(CH_3)_2S$, and $(CH_3)_2S_2$, the removal efficiency was much lower than that in a single gas treatment [4, 9, 24].

The common type of bioreactor used in aerobic treatment is the biofilter which is suitable for lower gas flow rate [17]. The major problem of the biofilter system in treatment of a high concentration of gases is oxygen limitation due to the rapid consumption of oxygen by cells. When the rate of volumetric gas flow entering the bioreactor increases, the

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carrier in the biofilter should be carefully chosen so as to have enough space for gas flow, otherwise the removal efficiency decreases due to short contact time and incomplete mixing [19]. However, in fluidized bed bioreactors, the solution is mixed well with inlet gas and the mass transfer of both sulfurous gas and oxygen to cells increased significantly as entering gas fluidizes the carriers and the bubble size reduces sharply [14]. Therefore, the fluidized bed bioreactor has a great potential to treat a large volume of gases [11].

In this study, the high loading rate of hydrogen sulfide and methylmercaptan was removed in both single and mixed gas treatments using a three phase fluidized bed bioreactor, in which *Thiobacillus* sp. IW is immobilized on biosands. The removal efficiency of hydrogen sulfide and methylmercaptan was measured for the inlet loading rate, and the stability of the bioreactor was tested for when the concentration of inlet methylmercaptan was increased suddenly.

MATERIALS AND METHODS

Microorganisms and Culture Medium

Thiobacillus sp. IW [3], which was isolated from acid drainage water from coal mines (Hwa-Soon, Korea) by Prof. In-Wha Lee of Chosun University, was used. *Thiobacillus* sp. IW showed optimum growth at 30°C and pH 7.0, and was cultured in the following medium (g/l): 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 $CoCl_2$, and 2.0 yeast extract. The basic medium and yeast extract solution were autoclaved separately for 15 min and the pH of the mixture was adjusted to 7.0 with 1 M HCl.

Immobilization of Microorganism

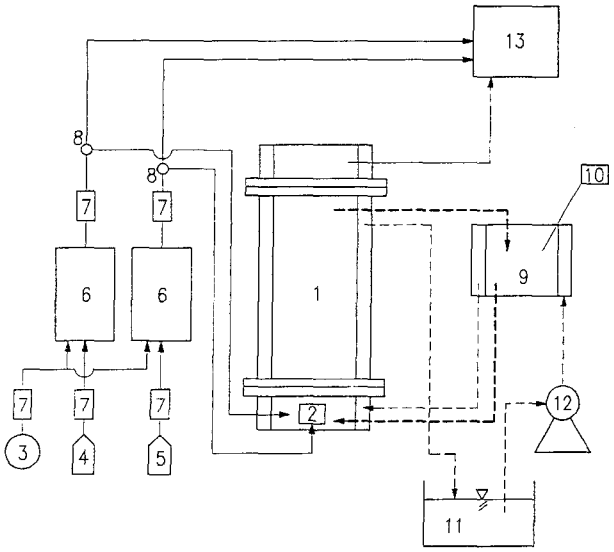
To maintain high efficiency and reduce loss of cells in the bioreactor, the cells were immobilized in biosands which showed the highest removal efficiency in the previous study [16]. The physical properties of the biosand used in this study are shown in Table 1. After *Thiobacillus* sp. IW was cultured for 24 h at 30°C and pH 7.0 in shaking flasks (150 rpm) with biosands, cells and biosands were inserted in the bioreactor. The amount of biosands in the bioreactor was an important factor for the removal efficiency: optimum mass of biosands was L/D=1.0 [21]; 101 g biosands/5 cm diameter. To adjust the cells in the fluidized bed bioreactor, low concentrations of hydrogen sulfide and methylmercaptan were introduced and the bioreactor reached steady state in 7 days of the operation. The steady state cell concentration was approximately 6×10^8 cells/ml and the cells immobilized on biosands before adding to the bioreactor were about 1×10^8 cells/g biosand.

Table 1. Physical properties of the biosand.

Composition	15% SiO_2+85% H_2O
Media size (mm)	2.0-3.0
Density (g/m^3)	1.27
Specific surface area (m^2/g)	539
Total pore area (m^2/g)	589
Pore volume (m^3/g)	740

Three Phase Fluidized Bed Bioreactor

In the present study, the three phase fluidized bed bioreactor, as shown in Fig. 1, was used to remove hydrogen sulfide and methylmercaptan. Hydrogen sulfide from a gas tank was diluted with air in a mixing chamber and entered the fluidized bed bioreactor (inner diameter= 5 cm, height=130 cm) through a 8 mm tube, whereas diluted methylmercaptan entered the bed through the gas sparger (diameter=3.3 cm, length=8 cm) to reduce the bubble size. The inlet gas fluidized carriers and bacteria in both carriers and solution oxidized hydrogen sulfide and methylmercaptan. The outlet gas left through a tube on the top of the bioreactor. To increase the working volume and dissolved oxygen of the solution, an aeration unit (inner diameter= 14 cm, height=15 cm) was installed and the solution in the unit was recirculated into the bioreactor. The total working



1. Fluidized bed bioreactor
2. Gas sparger
3. Air compressor
4. Gas tank (5% H_2S+95% N_2)
5. Gas tank (1% CH_3SH+95% N_2)
6. Gas mixing chamber
7. Flow meter
8. Three way valve
9. Aeration unit
10. Aerator
11. Water bath
12. Pump
13. Gas chromatograph

Fig. 1. Schematic diagram of a three phase fluidized bed bioreactor.

liquid volume including the solution in the bioreactor and aeration unit was 2 l. To maintain a controlled temperature, water at a constant temperature was circulated outside of the bioreactor and aeration unit. The distributor which had 85 holes of 1-mm diameter was installed at the bottom of the column for a uniform gas distribution and prevention of the loss of carriers. The operating condition of the bioreactor was set for the optimum growth condition of the cells.

Analytical Methods

The inlet and outlet concentrations of hydrogen sulfide and methylmercaptan were measured by a gas chromatograph (Donam Instrument, Korea) equipped with pulse discharge detector (Valco Instruments Co., Houston, U.S.A.) and GS-Q column. The oven temperature was increased from 40°C to 110°C in 12 min, and the injector temperature was maintained at 110°C and detector temperature at 160°C. As a carrier gas, 99.999% He was used at a gas flow rate of 8 ml/min. The calibration curve to convert the peak area of GC plot to concentration was obtained for hydrogen sulfide and methylmercaptan using the standard gas (Korea Research Institute of Standards and Science).

To measure the removal efficiency with high loading rate, the range of inlet hydrogen sulfide concentration chosen was 40- 1,100 ppm, the inlet methylmercaptan concentration was 36- 1,000 ppm, and the volumetric gas flow rate selected was 120 l/h. The removal efficiency and inlet loading rate of sulfur compounds were calculated according to the following formulae;

$$\text{Removal efficiency}=(C_{in}-C_{out})/C_{in}\times 100\ [\%]$$

$$\text{Inlet loading rate}=C_{in}Q/V\ [\text{mg l}^{-1}\text{h}^{-1}]$$

To measure the concentration of SO_4^{2-} generated by oxidation of sulfur compounds, 2 ml of 5% $BaCl_2 \cdot 2H_2O$ was mixed with 2 ml of solution and the absorbance of the precipitate was measured at 460 nm [13].

RESULTS AND DISCUSSION

Single Gas Treatment

Figure 2 shows the removal efficiency of hydrogen sulfide for the inlet loading rate in a single gas treatment. The removal efficiency was 97- 98% at an inlet loading rate of 21- 67 mg/l/h and reduced to 92% at higher loading rates. In Fig. 3, the removal efficiency of hydrogen sulfide was measured for 6 days, where the efficiency was at 95- 98% throughout the operation, and the cell concentration in the bioreactor was also maintained at a constant level.

Figure 4 shows the removal efficiency of methylmercaptan in a single gas treatment. When methylmercaptan was added to the bottom of the bioreactor through a tube, the removal efficiency of methylmercaptan was lower than that of hydrogen sulfide at the similar loading rates (Figs. 2 and 4), which had been also observed by other researchers [4]. To increase the removal efficiency of methylmercaptan, the gas sparger to reduce the bubble size was installed at

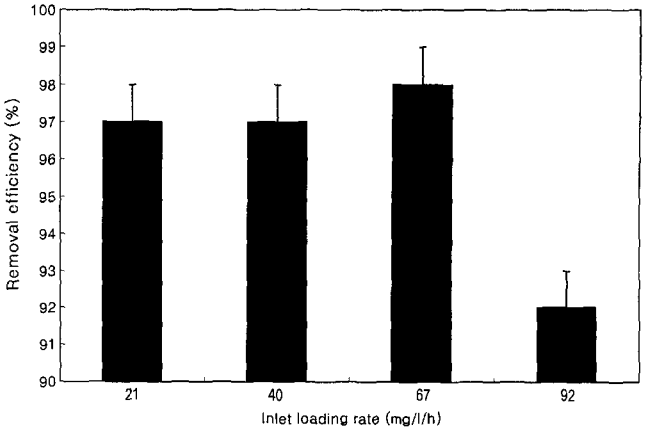


Fig. 2. Removal efficiency of H_2S in a single gas treatment from 10th to 22nd days of the operation.
C_{in}=250- 1,100 ppm, Q=120 l/h.

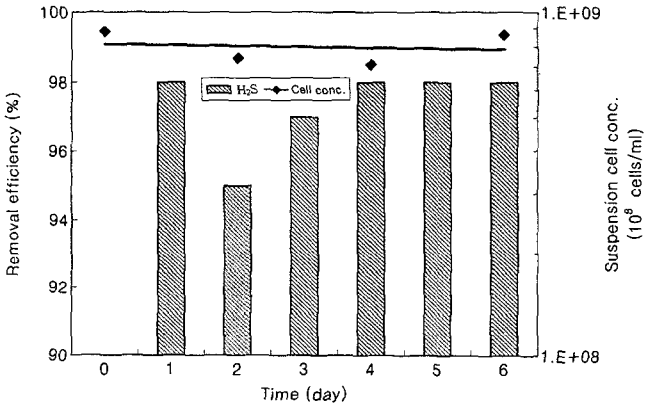


Fig. 3. Removal efficiency of H_2S in a single gas treatment with time course from 25th to 31st days of the operation.
C_{in}=540 ppm, Q=120 l/h.

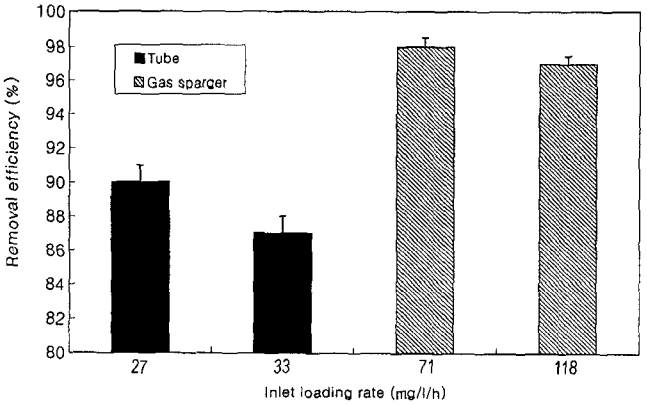


Fig. 4. Removal efficiency of CH_3SH in a single gas treatment from 34th to 47th days of the operation.
C_{in}=220- 1,000 ppm, Q=120 l/h.

the bottom of the bioreactor and the removal efficiency increased significantly. Figure 4 indicates that the bubble size is a very important factor in the removal efficiency of the bioreactor and the fluidized bed bioreactor has an advantage in that aspect.

Mixed Gas Treatment

The removal efficiency of hydrogen sulfide and methylmercaptan in the fluidized bed bioreactor immobilized with *Thiobacillus* sp. IW was compared with the result without the cells in Fig. 5. The removal efficiencies of hydrogen sulfide and methylmercaptan in the bioreactor containing *Thiobacillus* sp. IW were 99 and 98%, respectively, and those without the cells were 28 and 19%, respectively, which was due to the absorption of sulfur compounds into the solution. The difference in the removal efficiencies (71% for hydrogen sulfide, 79% for methylmercaptan) was due to contribution of the cells in the bioreactor. Figure 5 shows that a three phase fluidized bed bioreactor is very effective in removing the mixed hydrogen sulfide and methylmercaptan gases.

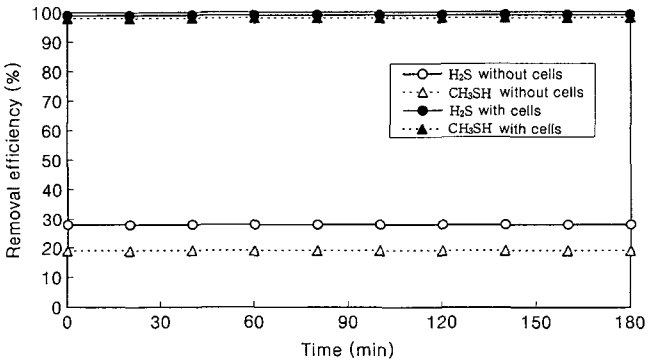


Fig. 5. Removal efficiency of the bioreactor with or without *Thiobacillus* sp. IW.
C_{in} (H_2S)=50 ppm, C_{in} (CH_3SH)=93 ppm, Q=120 l/h.

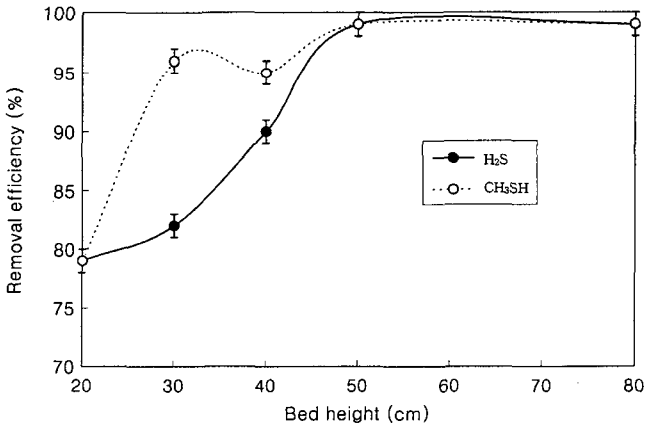


Fig. 6. The effect of bed height on the removal efficiency for mixed gas treatment from 50th to 60th days of the operation.
C_{in} (H_2S)=40-380 ppm, C_{in} (CH_3SH)=83-360 ppm, Q=120 l/h.

Figure 6 shows the effect of liquid bed height on the removal efficiency of the mixed gases. As the liquid bed height increased from 20 cm to 80 cm, the removal efficiencies of hydrogen sulfide and methylmercaptan increased from 79% to 99%. The removal efficiency of methylmercaptan was higher than that of hydrogen sulfide at 20-40 cm depth, which was considered to be due to the fact that the methylmercaptan entered through the gas sparger so that the bubble size of the gas becomes smaller, whereas the hydrogen sulfide entered the bioreactor through a tube so that the bubble size became relatively large. However, at the liquid bed higher than 50 cm, the solution and gas bubbles were mixed well and the residence time of the gas was large enough to achieve 99% of the removal efficiency. In this study, the bed height of the bioreactor was chosen to be 50 cm.

Figure 7 shows the removal efficiencies of hydrogen sulfide and methylmercaptan at various loading ratios of hydrogen sulfide and methylmercaptan. The removal

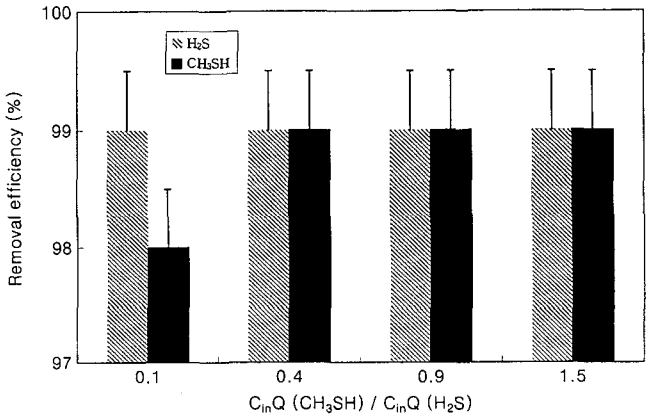


Fig. 7. The effect of gas loading ratio on the removal efficiencies of CH_3SH and H_2S in mixed gas treatment from 65th to 77th days of the operation.
C_{in} (H_2S)=180-380 ppm, C_{in} (CH_3SH)=36-360 ppm, Q=120 l/h.

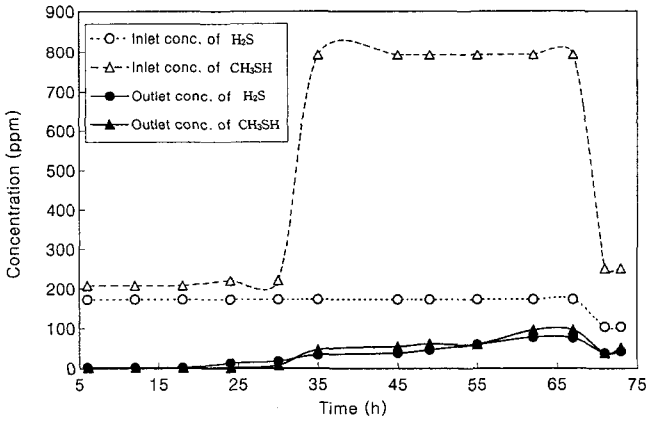


Fig. 8. The response of the bioreactor for concentration change of inlet CH_3SH from 80th to 83rd days of the operation.

efficiencies of both gases were 98-99% regardless of loading ratio in each loading rate below 33 mg/l/h. In higher loading rates, the efficiency decreased to 92-97% for 32-119 mg/l/h of hydrogen sulfide and 89-92% for 100-108 mg/l/h of methylmercaptan.

To examine the response of the bioreactor for sudden loading rate changes, the inlet concentration of methylmercaptan was increased suddenly from 208 ppm to 792 ppm and maintained for 30 h while the inlet concentration of hydrogen sulfide remained unchanged, as shown in Fig. 8 and Table 2. In the lower inlet concentration of 170 ppm (H_2S) and 208 ppm (CH_3SH), the outlet concentration of both hydrogen sulfide and methylmercaptan was negligible up to 22 h (the removal efficiency of both gases was 99%) and increased slightly up to 33 h. When the inlet concentration of methylmercaptan increased to 792 ppm while the inlet concentration of hydrogen sulfide maintained constant level, the outlet concentration of both gases increased steadily; however, the removal efficiency of methylmercaptan remained at an average of 91% for 30 h. Although the inlet concentration of hydrogen sulfide was not changed during this time period, the removal efficiency of hydrogen sulfide was reduced significantly to an average of 68%. Figure 8 indicates that the removal efficiency of hydrogen sulfide was strongly affected by the increase of methylmercaptan concentration. The removal efficiency of total sulfur compounds was 87% in average during the high loading rate period. Although the loading rates of hydrogen sulfide and methylmercaptan were later reduced to near initial conditions, the outlet concentrations of both gases were still high.

During the operation, dissolved oxygen remained at a saturated level due to active fluidization, and the pH was reduced slightly as generated SO_4^{2-} accumulated in the solution of the bioreactor. The cell concentration of the solution in the bioreactor also reduced steadily and its concentration at the end of the operation was about 10% of the initial concentration, which explains the lower removal efficiencies of hydrogen sulfide and methylmercaptan when the inlet concentration of methylmercaptan regained its initial condition. Compared with Fig. 3, which shows the constant cell concentration and the removal efficiency in the single gas treatment, the inlet loading rate of sulfur compounds in a mixed gas treatment was much higher, so that the growing condition of the bacteria becomes deteriorated and the cell concentration reduced steadily during the operation of the bioreactor. When sulfide content in the solution becomes high, it is toxic because it combines with the iron of cytochromes and other essential iron-containing compounds in the cell [18].

Table 2. Time course of cell concentration, SO_4^{2-}, pH, and dissolved oxygen concentration of the solution in the mixed gas treatment.

Time (h)	Suspended cell conc. ($\times 10^8$ cells/ml)	SO_4^{2-} ($\times 10^{-5}$ M)	pH	Dissolved oxygen (mg/l)
0	6.2	-	7.0	8.7
24	1.7	1.0	7.0	10.5
48	-	1.9	6.6	9.0
72	0.66	2.4	6.1	9.1

In conclusion, a three phase fluidized bed bioreactor immobilized with *Thiobacillus* sp. IW could remove a high loading rate of hydrogen sulfide and methylmercaptan efficiently in both single and mixed gas treatments. When the inlet concentration of methylmercaptan increased suddenly in a continuous operation of the bioreactor, the removal efficiency of methylmercaptan was maintained at a high level; however, the removal efficiency of hydrogen sulfide became lower.

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Nomenclature

C_{in}=inlet concentration of sulfurous gas [ppm]
C_{out}=outlet concentration of sulfurous gas [ppm]
D=diameter of the bioreactor [cm]
L=height of packed carriers before fluidization [cm]
Q=volumetric gas flow rate of sulfurous gas [l/h]
V=volume of the solution in both bioreactor and aeration unit [l]

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