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# Hydrogen Sulfide Gas Treatment by a Chemical-Biological Process: Chemical Absorption and Biological Oxidation Steps

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# ABSTRACT

In order to remove high concentrations of hydrogen sulfide ( $H_2S$ ) gas from anaerobic wastewater treatments in livestock farming, a novel process was evaluated for  $H_2S$  gas abatement involving the combination of chemical absorption and biological oxidation processes. In this study, the extensive experiments evaluating the removal efficiency, capacity, and removal characteristics of  $H_2S$  gas by the chemical absorption reactor were conducted in a continuous operation. In addition, the effects of initial Fe<sup>2+</sup> concentrations, pH, and glucose concentrations on Fe<sup>2+</sup> oxidation by *Thiobacillus ferrooxidans* CP9 were also examined. The results showed that the chemical process exhibited high removal efficiencies with  $H_2S$  concentrations up to 300 ppm, and nearly no acclimation time was required. The limitation of mass-transfer was verified as the rate-determining step in the chemical reaction through model validation. The Fe<sup>2+</sup> production rate was clearly affected by the inlet gas concentration as well as flow rate and a prediction equation of ferrous production was established. The optimal

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operating conditions for the biological oxidation process were below pH 2.3 and  $35^{\circ}$ C in which more than 90% Fe<sup>3+</sup> formation ratio was achieved. Interestingly, the optimal glucose concentration in the medium was 0.1%, which favored Fe<sup>2+</sup> oxidation and the growth of *T. ferrooxidans* CP9.

Key Words: Hydrogen sulfide; Ferric sulfate; Thiobacillus ferrooxidans; Glucose.

## **INTRODUCTION**

Wastewater from livestock farming often produces odors and toxic gases that bring many environmental problems. Hydrogen sulfide  $(H_2S)$ , the major component of these gases, is hazardous to human health and can cause extensive corrosive damage to pipelines and equipments when improperly processed.<sup>[1]</sup> In addition, it has great potential to irritate the eyes and injure the developing central nervous systems.<sup>[2]</sup> In comparison with hydrogen sulfide (5-60 ppm) produced from industrial processes such as petroleum refining, food processing, pulp manufacturing, and the treatment of fuels,<sup>[3-7]</sup> concentrations of H<sub>2</sub>S as high as 150-300 ppm have been found in emissions from anaerobic wastewater treatment in livestock farming. The coexistence of corrosive  $H_2S$  gas with methane during the anaerobic emission process has often destroyed the recycling equipment for methane gas. Hence, greenhouse and other toxic gases may be randomly discharged. Thus, control of H<sub>2</sub>S gas emissions is not only essential to mitigate environmental impacts and to protect public health, but it can also increase methane recovery for energy recycling. Because of its toxicity and corrosiveness,  $H_2S$  is listed as a candidate for priority control by the environmental protection agencies in many countries.

Chemical scrubber, physical adsorption, electrochemical treatment, and biofiltration techniques have been used to purify  $H_2S$  from waste gas and wastewater.<sup>[8–10]</sup> Although the chemical and physical treatments have proven effective, high costs and secondary pollution products are a fatal defect.<sup>[8]</sup> Biofiltration is an efficient and inexpensive method of  $H_2S$  removal, but its low oxidation rate in treating high inlet  $H_2S$  concentrations and fixed operating conditions (pH, humidity,  $O_2$  content) result in low flexibility.<sup>[11]</sup> Hence, a highly efficient process of  $H_2S$  abatement from gas streams needs to be developed, which based on combines the best aspects of both chemical and biological processes. This chemical-biological treatment system can be used to purify gases containing  $H_2S$  and prevent corrosive damage to methane gas recycling equipment. One potential advantage of this process is that sulfide converts to elemental sulfur, which can be easily separated from the liquid phase. Moreover, it allows the treatment of anaerobic gases (due to its double-stage design) and high  $H_2S$  concentrations (avoiding direct contact of the gas with the biomass and avoiding the build up of high concentrations of sulfide in solution by rapidly reacting it with iron).

The process for  $H_2S$  gas treatment is based on two steps, the first corresponding to absorption with the chemical solution and the second to biological oxidation with *Thiobacillus* spp. In the first stage, ferric sulfate is used as an oxidizing agent, which rapidly reacts with  $H_2S$  gas and is reduced to ferrous sulfate.

the first stage :  $H_2S + Fe_2(SO_4)_3 \rightarrow S + 2FeSO_4 + H_2SO_4$ 

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In the secondary stage, the ferrous sulfate solution is sent to an aerobic bioreactor, where microorganisms will oxidize the ferrous iron to ferric iron. The ferric sulfate solution is then recycled into the reactor in the first stage to repeat the cycle.

the secondary stage :  $2H_2SO_4 + 4FeSO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$ 

Combining the high reactivity of a chemical process and the regeneration capacity of a biological process creates a closed absorption-oxidation-regeneration system. Indeed, the main characteristics that can be highlighted are low operating costs, simple equipment, no expensive chemical requirements, and no waste production. Finally, the process can operate at ambient pressure and temperature. In this study, the feasibility of the chemical absorption and biological oxidation processes in treating  $H_2S$  are separately examined in detail.

# MATERIALS AND METHODS

# Apparatus and H<sub>2</sub>S Removal with Continuous Operation

The reactor consisted of a glass cylinder with an inner diameter of 6.0 cm, fitted with a thermostatic jacket. It was 60 cm high, including a 40 cm-high packed bed filled with



*Figure 1.* Schematic of the chemical absorption system. (1) reactor; (2) flow meter; (3) three-way valve; (4) regulator; (5) air filter; (6) air compressor; (7)  $H_2S$  gas cylinder.

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small, anti-corrosive, and porous glass cubes of 6 mm size. Ferric sulfate solution of different concentrations (5 or 10 g/l) was added to the glass cylinder, and the initial pH was 1.5. The  $H_2S_{(g)}$  from the gas cylinder was diluted with compressed air and flowed upwards through the bottom of the reactor. A flow meter and valve were used to respectively monitor and control the gas flow through the reactor. A schematic of the experimental setup of the chemical absorption system is shown in Figure 1. The simulated  $H_2S$  concentrations were in the range of 50–200 ppm in the continuous experiment. The flow rates of gases were controlled at 50–200 l/h (with a residence time of 9–36 s), and the operating temperature was maintained at 30°C. In all of these experiments, values of a set of parameters versus time were measured including pH, Fe<sup>2+</sup> concentration, Fe<sup>3+</sup> concentration, total iron concentration (Fe<sub>Tot</sub>), and H<sub>2</sub>S concentration.

# Definition of Removal Efficiency and Capacity and Analysis Method of H<sub>2</sub>S Gas

The removal efficiency of H<sub>2</sub>S was determined according to Eq. 1.

$$\mathbf{R}(\%) = (\mathbf{H}_2 \mathbf{S}_{in} - \mathbf{H}_2 \mathbf{S}_{out}) / \mathbf{H}_2 \mathbf{S}_{in} \times 100\%$$
(1)

where R (%) = removal efficiency;  $H_2S_{in}$  (ppm) = concentration of  $H_2S$  at the inlet and  $H_2S_{out}$  (ppm) = concentration of  $H_2S$  at the outlet. The removal capacity of  $H_2S$  was calculated according to Eq. 2.

$$C(g - S/m^3/h) = Q(l/h) \times C(g - S/l)/V(m^3)$$
 (2)

where C  $(g-S/m^3/h)$  = removal capacity; Q (l/h) = gas flow rate; C (g-S/l) = removed concentration of H<sub>2</sub>S in the reactor and V  $(m^3)$  = volume of ferric sulfate solution.

Inlet  $H_2S$  gas concentrations of the reactor were periodically measured using gas detector tubes (Kitagawa, Japan) and ranged from 12.5–500 ppm. Outlet  $H_2S$  gas concentrations of the reactor were periodically measured by gas detector tubes (Kitagawa, Japan) and ranged from 1–60 ppm or 0.1–4 ppm. Four independent measurements were recorded and later averaged as the final  $H_2S$  concentration.

#### **Model Validation**

The rate-determining step of the chemical absorption process was due to masstransfer limitations. Identification of the absorption regime for  $H_2S$  in the chemical absorption reactor was verified using the following equation presented by Pagella et al.<sup>[12]</sup> and it was generally applied in the diffusional regime:

$$R(\%) = 1 - exp[-k_1 aV/mG] \times 100\%$$
(3)

where R (%) = removal efficiency;  $k_1$  = mass transfer coefficient (m/sec); a = specific interface area (m<sup>2</sup>/m<sup>3</sup>); V (m<sup>3</sup>) = reactor volume; m = partition coefficient and G = gas flow rate (*l*/h). By comparing the theoretical curve and experimental results, the rate-determining step was revealed.

#### **Microorganism and Cultivation**

*Thiobacillus ferrooxidans* CP9 was isolated from acid mine drainage and identified by the procedures of cell lysis, DNA extraction, PCR amplification, cloning and sequencing compared with the EMBL database.<sup>[13]</sup> The similarity degree of the strain is 100% with *Thiobacillus ferrooxidans* by identifying partial 16S rRNA gene. The KBU growth medium developed by Khalid et al. (1993) was used in all the experiments.<sup>[14]</sup> The medium contained ferrous iron (normally 5 g Fe<sup>2+</sup>/l), 0.8 g/l (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.4 g/l KH<sub>2</sub>PO<sub>4</sub> and 0.18 g/l MgSO<sub>4</sub> in water. Different ferrous ion concentrations were used when specified. The final pH of medium was adjusted to 1.5, 2.0, or 2.3 using 1 N H<sub>2</sub>SO<sub>4</sub> solution. The *T. ferrooxidans* CP9 was gram-negative, motile, and shaped like a short-rod with round ends. The colony of *T. ferrooxidans* CP9 was dark-brown and 1–3 mm in size after a seven-day cultivation at 30°C.

# Effect of Fe<sup>2+</sup> and pH on Fe<sup>2+</sup> Oxidation by *T. ferrooxidans* CP9

A platinum loop of *T. ferrooxidans* CP9 was inoculated into 100 ml of a KBU growth medium in shaken flasks and was incubated at 40°C by reciprocal shaking (200 strokes/min). The initial cell numbers were determined as  $9 \times 10^4$  CFU/ml. To examine the effect of substrate concentration on Fe<sup>2+</sup> oxidation by *T. ferrooxidans* CP9, the KBU growth medium was prepared at various ferrous iron concentrations (5, 10 or 20 g Fe<sup>2+</sup>/l). The pH of the medium was adjusted to 1.5, 2.0 or 2.3 to evaluate the effect of pH on Fe<sup>2+</sup> oxidation by *T. ferrooxidans* CP9. Changes in the concentrations of ferric iron, ferrous iron, total iron, pH, and numbers of cells in the medium were measured periodically. The data was obtained from two or more duplicate tests.

# Effect of Carbon Source and Temperature on Fe<sup>2+</sup> Oxidation by *T. ferrooxidans* CP9

*T. ferrooxidans* CP9 was inoculated into 100 ml of KBU growth medium in 250 ml shaken flasks until the initial cell numbers were  $9 \times 10^4$  CFU/ml. To examine the effect of carbon source on Fe<sup>2+</sup> oxidation by *T. ferrooxidans* CP9, the KBU growth medium was prepared at various glucose concentrations (0, 0.1 or 1%). The KBU growth medium was incubated at 30, 35 or 40°C by reciprocal shaking (200 strokes/min). The pH of the medium was maintained at 2.0 to prevent the occurrence of iron compound precipitation and to sustain the activity of *T. ferrooxidans* CP9. The initial ferrous iron concentration in the medium was kept at 10 g Fe<sup>2+</sup>/l. Changes in the concentrations of ferric iron, ferrous iron, and total iron, and in pH and numbers of cells in the medium were monitored periodically. Data were obtained from at least two duplicate tests.

# **Analysis Methods**

Ferrous ion concentration was determined using titration against 0.017 M potassium dichromate in the presence of *N*-phenylanthranilic acid as an indicator.<sup>[15]</sup> Total iron (Fe<sub>Tot</sub>) was measured by atomic absorption (AA). Ferric iron concentration

was estimated by subtracting the ferrous iron concentration from the total iron concentration. The pH value was measured using a pH meter. The cell numbers of *T. ferrooxidans* CP9 were determined by the serial dilution method on solid KBU growth medium.

## **RESULTS AND DISCUSSION**

# Effect of Inlet H<sub>2</sub>S Concentration on H<sub>2</sub>S Removal Efficiency

Various  $H_2S$  concentrations (50, 100, 200 and 300 ppm) were introduced into the chemical absorption reactor to examine the performance of the chemical process at a



*Figure 2.* Kinetics of  $H_2S$  gas removal using a 5 g/l Fe<sup>3+</sup> solution. The inlet  $H_2S$  concentration was 100 ppm (A) or 300 ppm (B) at 100 l/h.

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gas flow rate of 100 *l*/h (with a residence time of 18 s). The kinetic results of continuous oxidation of 5 g/l Fe<sup>3+</sup> solution at 100 ppm or 300 ppm H<sub>2</sub>S are shown in Figure 2. When the inlet H<sub>2</sub>S concentration was maintained at 100 ppm, the removal efficiency was 60% in 30 min and remained at this level until experiment end. Even though the biological oxidation usually has a higher H<sub>2</sub>S removal efficiency than chemical process, it requires long acclimation periods (>7 days) and is restricted to applications with low concentrations of pollutants.<sup>[16,17]</sup> When the inlet concentration was removed. Similarly, the removal efficiency was in the range of 60%–70% when 50 ppm H<sub>2</sub>S was introduced (data not shown). Therefore, the removal efficiency of H<sub>2</sub>S was dependent on the inlet H<sub>2</sub>S concentration to the chemical absorption process. The rate-determining step for the chemical absorption process was determined to the



*Figure 3.* Changes in pH values and iron concentrations in the reactor using a 5 g/l Fe<sup>3+</sup> solution to remove H<sub>2</sub>S gas. The inlet H<sub>2</sub>S concentration was 100 ppm (A) or (B) 300 ppm.

limitation of mass-transfer, not reaction limitations. Hence, reduction in the flow rate of the inlet gas could elevate  $H_2S$  removal efficiency.

## Effect of Inlet H<sub>2</sub>S Concentration on pH and Iron Concentration

Changes in pH and iron concentration of the reactor under various operating conditions are shown in Figure 3. When 100 ppm H<sub>2</sub>S was introduced into the chemical absorption reactor, 10% of Fe<sup>3+</sup> was consumed, which was close to the amount of Fe<sup>2+</sup> production when the total iron concentration remained constant. The results indicated that the chemical absorption process was very stable. No precipitations of iron compounds occurred to interfere with the biological regeneration process.<sup>[1]</sup> In addition, the chemical absorption reactor could operate for 85 h at 100 ppm H<sub>2</sub>S or for 24 h at 300 ppm H<sub>2</sub>S according to the equation of Fe<sup>3+</sup> consumption (y = -0.0538x+4.5941 or y = -0.1942 x+4.7241) if Fe<sup>3+</sup> was not regenerated. Besides, the result of Figure 3 also indicated that the change of pH value in the reactor versus time was insignificant regardless of whether inlet H<sub>2</sub>S concentration changed. (The experimental data of 50 ppm and 200 ppm were not shown.) Theoretically, the pH value in the reactor should gradually decrease according to the reaction equation:  $H_2S+2$  Fe<sup>3+</sup> $\rightarrow$ 2 Fe<sup>2+</sup>+S+2H<sup>+</sup>. However, the system possesses a high pH buffer capacity and can operate steadily for a long time. The relationship between the inlet H<sub>2</sub>S concentration and the Fe<sup>2+</sup> production rate shows a linear behavior and can be expressed by a regression equation (y = 0.0007 x - 0.011). Therefore, this regression equation can be used to evaluate the feasibility of operating system under different H<sub>2</sub>S loads when  $Fe^{2+}$  is oxidized in the biological regeneration reactor.

# Effect of Gas Flow Rate on H<sub>2</sub>S Removal Efficiency

The effect of the gas flow rate on  $H_2S$  removal in the chemical absorption reactor is shown in Figure 4. In this experiment, the gas flow rate was gradually raised from 50 *l*/h to 200 *l*/h. The temperature was maintained at 30°C, and the Fe<sup>3+</sup> concentration was controlled at 10 g/l in the chemical absorption reactor. This data revealed that the  $H_2S$ removal efficiency decreased with an increasing gas flow rate. When the gas flow rate was below 100 l/h (with a residence time of 18 s), the removal efficiency exceeded 85%. When the gas flow rate was adjusted upwards to  $200 \ l/h$ , the removal efficiency still remained 65%. Since the rate-determining step of  $H_2S$  removal is the limitation of mass-transfer, the low gas flow rate favored  $H_2S$  removal. In addition, the  $H_2S$  removal efficiency increased by about 25% when the  $Fe^{3+}$  concentration increased from 5 g/l (Figure 2) to 10 g/l (Figure 4). The variations in pH values are insignificant when the gas flow rates are between 50 and 200 l/h (data not shown). The pH values in the chemical absorption reactor dropped to around 1.0 while the system had continuously operated for 96 h at 200 l/h. The relationship between the gas flow rate and the Fe<sup>2+</sup> production rate at an inlet concentration of 200 ppm indicated that the  $Fe^{2+}$  production rate increased linearly with gas flow rates in the range of from 50 to 200 l/h. A regression equation (y = 0.0015 x - 0.0171) with a high correlation coefficient (0.995) was obtained using regression methods. Apparently, the Fe<sup>2+</sup> production rate was highly affected by the inlet gas concentration and gas flow rate. The H<sub>2</sub>S removal



*Figure 4.* Effect of the gas flow rate on the H<sub>2</sub>S removal efficiency using a  $10 \text{ g/l} \text{ Fe}^{3+}$  solution at 200 ppm H<sub>2</sub>S.



Figure 5.  $H_2S$  removal capacity and removal efficiency as a function of the gas flow rate for different gas flow rates.

capacity and efficiency as a function of gas flow rate are shown in Figure 5. The reverse tendency was observed for  $H_2S$  removal capacity and efficiency. The removal efficiency decreased and the removal capacity increased with increasing gas flow rates. According to the regression equation, if the gas flow rate was reduced to 25 *l*/h, a removal efficiency would be 94.4%. Thus, the regression equation of the removal capacity could be used to design the reactor size when the inlet concentration and gas flow rate were constant.

# **Model Validation**

In this study, we show that the chemical absorption process is limited by masstransfer considerations. In order to identify the absorption regime in the reactor, a model validation was performed. To obtain a  $k_1a$  value, a well-known diagram was used, in which the value for the constant is plotted as a function of the gas flow rate,<sup>[18]</sup> and that value was estimated to be 0.09 s<sup>-1</sup>. The partition coefficient (m) was determined to be about 0.82 in this chemical absorption system. The predicted and actual removal efficiencies are plotted as a function of the gas flow rate and are shown in Figure 6. The results indicated that the model prediction and experimental results had a similar tendency. Therefore, this suggests that the chemical absorption process should operate in a diffusional regime. However, errors of about 1.5% to 2.5% were found between the predictions and actual results. The actual removal efficiencies were slightly lower than the predicted values, due to accumulation of the elemental sulfur



Figure 6. Relationship between the model prediction and experimental data.

(an oxidative product of hydrogen sulfide) interfering with the reaction process. Thus, when the solid-liquid separation is established, the problem should be avoided.

# Effect of Fe<sup>2+</sup> and pH on Fe<sup>2+</sup> Oxidation Rate by *T. ferrooxidans* CP9

Effects of pH and initial Fe<sup>2+</sup> concentration in the range of 5 to 20 g/l on Fe<sup>2+</sup> oxidation by T. ferrooxidans CP9 were examined. Figure 7 shows that the Fe<sup>2+</sup> oxidation rate is affected by initial Fe<sup>2+</sup> concentration and pH. When initial Fe<sup>2+</sup> concentration was controlled at 5 g/l, the Fe<sup>2+</sup> oxidation rates by *T. ferrooxidans* CP9 were similar at pH 1.5 and 2.0, while the highest oxidation rate (0.26 mM/hr) was found at pH 2.3. No significant statistical differences between Fe<sup>2+</sup> oxidation rates were found at different pH values (P>0.05) when initial  $Fe^{2+}$  concentration was elevated to 10 or 20 g/l. Therefore,  $Fe^{2+}$ oxidation rate was affected by initial Fe<sup>2+</sup> concentration, and a high initial Fe<sup>2+</sup> concentration resulted in a fast Fe<sup>2+</sup> oxidation rate. When initial Fe<sup>2+</sup> concentration was 10 or 20 g/l, the average oxidation rate of Fe<sup>2+</sup> reached 0.48 and 1.02 mM/hr, respectively. The regression equation (y = 0.054x - 0.075) and its correlation coefficient (0.991) between initial Fe<sup>2+</sup> concentration and oxidation rate indicated that both were in a good linearity relationship (data not shown). According to Michaelis-Menten theory,<sup>[19]</sup> ferrous iron oxidation by T. ferrooxidans CP9 is presumed to be the first-order kinetic when ferrous iron concentration is below or equal to 20 g/l. Hence, the increasing  $Fe^{2+}$  concentration to achieve the maximum  $Fe^{2+}$  oxidation rate should be available. Besides, the  $Fe^{2+}$  oxidized ratio by T. ferrooxidans CP9 was maintained between  $38\% \sim 40\%$  under the same pH



*Figure 7.* Effect of initial  $Fe^{2+}$  concentration and pH on  $Fe^{2+}$  oxidation rate by *T. ferrooxidans* CP9.

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*Figure 8.* Change in  $Fe^{3+}$  concentration at different pH conditions when initial  $Fe^{2+}$  concentration was controlled at 20 g/l.

condition (data not shown). Thus, the phenomenon of substrate inhibition did not occur. Jones and Kelly (1983) carried out an extensive study of the substrate inhibition of Thiobacillus ferrooxidans grown in a similar medium and found it occurred at Fe<sup>2+</sup> concentrations above 5 g/l. T. ferrooxidans CP9 isolated by us exhibited higher ferrous iron oxidation activity than the Thiobacillus ferrooxidans studied by Jones and Kelly.<sup>[20]</sup> Figure 8 shows the change in Fe<sup>3+</sup> production at different pH conditions with initial Fe<sup>2+</sup> concentration controlled at 20 g/l. When initial Fe<sup>2+</sup> concentration was 5 or 10 g/l, the Fe<sup>3+</sup> production was dependent on pH value (data not shown). If the initial Fe<sup>2+</sup> concentration increased to 20 g/l, the high pH condition would cause less Fe<sup>3+</sup> production (Figure 8). Besides, the amounts of  $Fe^{3+}$  production and  $Fe^{2+}$  oxidation were not equal (data not shown). Therefore, the oxidation of ferrous iron exceeded the formation of ferric iron, and it was not in accord with the mass balance law. Hence, it was presumed the high pH condition (e.g. pH>2.0) would bring about the reaction of chelation and precipitation. In fact, the jarosite (ferric hydroxysulfate) precipitates were observed in the shaken flask. Ferric iron precipitation had a detrimental effect on H<sub>2</sub>S gas removal if the chemical absorption and biological regeneration processes were combined. First, it would diminish the available ferric iron in solution that served as absorbent for  $H_2S$ . Secondly, precipitates created kinetic barriers because of the slow diffusion of reactants and products through the precipitation zone. Hence, we proposed that it was necessary to maintain the pH value in the biological oxidation process below pH 2.3.

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# Effect of Carbon Source on Fe<sup>2+</sup> Oxidation by *T. ferrooxidans* CP9

*Thiobacillus ferrooxidans* has been considered strictly autotrophic.<sup>[21]</sup> Few studies find *Thiobacillus ferrooxidans* capable of heterotrophic growth or that it possesses the ability to oxidize ferrous iron in a heterotrophic condition. In this study, different



*Figure 9.* Effect of glucose concentration on  $Fe^{2+}$  oxidation (A), and  $Fe^{3+}$  production (B) when *T. ferrooxidans* CP9 was cultivated at pH 2.0 and temperature controlled at 35°C.

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Temperature (°C)	Cell number (CFU/ml)		
	Glucose (0 %)	Glucose (0.1 %)	Glucose (1 %)
30	$5.4 \times 10^{7}$	$3.9 \times 10^{8}$	$1.6 \times 10^{7}$
35	$7.2 \times 10^{7}$	$5.1 \times 10^{8}$	$2.1 \times 10^{7}$
40	$3.9 \times 10^{7}$	$2.8 \times 10^8$	$3.5 \times 10^{7}$

*Table 1.* Effect of glucose concentration and temperature on cell number (CFU/ml) of *T. ferrooxidans* CP9 after 144 days operation (initial  $Fe^{2+}$  concentration = 10 g/l, pH 2).

glucose concentrations were employed to evaluate the effect on  $Fe^{2+}$  oxidation by T. ferrooxidans CP9 at different temperatures. The results of Figure 9 showed that changes in the concentration of Fe<sup>2+</sup> oxidation and Fe<sup>3+</sup> production were related to glucose concentrations if T. ferrooxidans CP9 was cultivated at pH 2.0 and temperature was controlled at 35°C. Similar tendency was also observed at 30°C (data not shown). These data suggested that the presence of 0.1% glucose would favor Fe<sup>2+</sup> oxidation and  $Fe^{3+}$  production by T. ferrooxidans CP9, which reached 5.0 g/l at the end. This result was higher than with the similar operation at 40°C (1.3 g/l). In contrast, addition of 1% glucose resulted in inhibition of Fe<sup>2+</sup> oxidation and Fe<sup>3+</sup> production. However, differing results were found when the operating temperature was conducted 40°C (data not shown). Changes in the concentration of  $Fe^{2+}$  oxidation and  $Fe^{3+}$  production were independent on glucose concentrations because high temperature offset the positive effect of glucose on  $Fe^{3+}$  production by *T. ferrooxidans* CP9. Therefore, the average activity of T. ferrooxidans CP9 to oxidize Fe<sup>2+</sup> was highest at 0.1% glucose (0.74 mM/ hr) and lowest at 1% glucose (0.29 mM/hr) when the operating temperature was below 40°C. Table 1 shows the cell numbers of T. ferrooxidans CP9 at different concentrations of glucose and at different temperatures after 144 days of operation. These results clearly indicated optimal glucose concentration (0.1%) simulated the growth of T. ferrooxidans CP9. Thus, T. ferrooxidans CP9 has been shown to utilize not only ferrous iron but also glucose. These findings have only been observed in a few reports. When 0.1% glucose was added into the Fe<sup>2+</sup>-containing medium, bacterial numbers increased about sevenfold.

# Effect of Temperature on Fe<sup>2+</sup> Oxidation Rate by *T. ferrooxidans* CP9

Effects of temperature on the Fe<sup>2+</sup> oxidation rate and Fe<sup>3+</sup> formation ratio at different temperatures were examined. In this study, when the initial Fe<sup>2+</sup> concentration and pH were controlled at 10 g/l and 2, respectively, the optimal operating temperature was 35°C regardless of glucose concentration (Figure 10A). Fe<sup>2+</sup> oxidation rate at 30°C was higher than it was at 40°C. Figure 10B shows the effect of the operating temperature on the Fe<sup>3+</sup> formation ratio. Apparently, this ratio was relatively low (about 40%) at 40°C, and high formation ratios were observed at 30°C (84%), and 35°C (88%); therefore, most of ferrous iron was not successfully transformed to ferric iron. According to the concept of chemical thermodynamic, the high reaction temperature would favor the reaction of chelation, complexation, and



*Figure 10.* Effect of temperature on  $Fe^{2+}$  oxidation rate (A) and  $Fe^{3+}$  formation ratio (B). The initial  $Fe^{2+}$  concentration and pH were controlled at 10 g/l and 2, respectively.

precipitation. The results of various jarosite precipitates have been discovered by Jensen and Webb (1995), and they are observed in the shaken flasks in this experiment.<sup>[22]</sup> Besides, high temperature caused the relatively low cell numbers (Table 1) and decreased the oxidation activity of *T. ferrooxidans* CP9 (Figure 10A).

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Hence, the optimal reaction temperature for *T. ferrooxidans* CP9 to oxidize ferrous iron was 35°C. This result was similar to that reported by Nemati and C. Webb (1996) and Juszczak (1995).<sup>[23,24]</sup>

# CONCLUSION

The results of this study have demonstrated that the chemical absorption and biological oxidation processes have a high potency to apply in removing hydrogen sulfide. This chemical process can achieve a removal efficiency of more than 85% in a very short time (30 min) in comparison with biotreatment systems for which the residence time was longer than 18 sec. The limitation of mass-transfer was verified to be the rate-determining step in the chemical absorption process by model validation. The results of chemical absorption indicated that low gas flow rates and high Fe<sup>3+</sup> concentrations favored H<sub>2</sub>S removal by chemical absorption reactor. The biological oxidation process could achieve up to a 90% Fe<sup>3+</sup> formation ratio under optimal reactive conditions. Fe<sup>2+</sup> oxidation rate was strongly affected by the initial Fe<sup>2+</sup> concentration, glucose concentration, and temperature. High pH growth conditions (e.g. pH > 2.0) would bring about the reactions of chelation and precipitation. The optimal glucose concentration and temperature simulated the activity of Fe<sup>2+</sup> oxidation by *T. ferrooxidans* CP9 and increased the growth of *T. ferrooxidans* CP9.

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#### REFERENCES

- Rai, C.; Taylor, M. Microbial sweetening of sour natural gas using mixed cultures. Environ. Prog. 1996, 15 (1), 6–11.
- Vanhoorne, M.; Rouck, A.; Bacquer, D. Epidemiological study of eye irritation by hydrogen sulphide and/or carbon disulphide exposure in viscose rayon workers. Ann. Occup. Hyg. 1995, 39 (3), 307–315.
- EiKum, A.S.; Storhang, R. Odour problems related to waste water and sludge treatment. In *Odour Prevention and Control of Organic Sludge and Livestock Farming*, 1st Ed.; Applied Science Publishers: London, 1986; 12–18.
- 4. Yang, Y.; Allen, E.R. Biofiltration control of hydrogen sulfide 1. Design and operational parameters. J. Air Waste Manage. Assoc. **1994**, *44* (2), 863–868.
- 5. Langenhove, H.V.; Wuyts, E.; Schamp, N. Elimination of hydrogen sulphide from odorous air by wood bark biofilter. Water Res. **1986**, *20* (7), 1471–1476.
- Chung, Y.C.; Huang, C.; Tseng, C.P. Reduction of H<sub>2</sub>S/NH<sub>3</sub> production from pig feces by controlling environmental conditions. J. Environ. Sci. Health **1996**, *A31* (4), 139–155.
- 7. Cho, K.S.; Hirai, M.; Shoda, M. Enhanced removal efficiency of malodorous gases

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in a pilot-scale peat biofilter inoculated with *Thiobacillus thioparus* DW44. J. Ferment. Bioeng. **1992**, 73 (8), 46–50.

- Barth, C.L.; Elliott, F.L.; Melvin, S.W. Using odour control technology to support animal agriculture. T. ASAE 1984, 27 (3), 859–864.
- Mannebeck, H. Covering manure storing tanks to control odour. In *Odour Prevention and Control of Organic Sludge and Livestock Farming*, 1st Ed.; Applied Science Publishers: London, 1986; 188–192.
- Chang, M.B.; Treng, T.D. Gas-phase removal of H<sub>2</sub>S and NH<sub>3</sub> with dielectric barrier discharges. J. Environ. Eng.-ASCE **1996**, *122* (1), 41–45.
- Bohn, H. Consider biofiltration for decontaminating gases. Chem. Eng. Prog. 1992, 88 (7), 35–40.
- Pagella, C.; Silvestri, P.; De Faveri, D.M. H<sub>2</sub>S gas treatment with *Thiobacillus ferrooxidans*: overall process performance and the chemical step. Trans. Inst. Chem. Eng. **1996**, 74 (5), 123–132.
- 13. Pizarro, J.; Jedlicki, E.; Orellana, O.; Romero, J.; Espejo, R.T. Bacterial populations in samples of bioleached copper ore as revealed by analysis of DNA obtained before and after cultivation. Appl. Environ. Microbiol. **1996**, *62* (4), 1323– 1328.
- Khalid, A.M.; Bhatti, T.M.; Umar, M. An improved solid medium for isolation, enumeration and genetic investigations of autotrophic iron and sulphur-oxidizing bacteria. Appl. Microbiol. Biotechnol. 1993, 39 (2), 259–263.
- Vogel, A.I. Vogel's Textbook of Quantitative Chemical Analysis, 1st Ed.; Longman: London, 1989; 287–310.
- Chung, Y.C.; Huang, C.; Li, C.F. Removal characteristics of H<sub>2</sub>S by *Thiobacillus novellus* CH3 biofilter in autotrophic and mixotrophic environments. J. Environ. Sci. Health **1997**, A32 (6), 1435–1450.
- Chung, Y.C.; Huang, C.; Liu, C.H. Feasibility testing of fluidized-bed bioreactor for remediating waste gas containing H<sub>2</sub>S or NH<sub>3</sub>. J. Environ. Sci. Health 2001, A36 (4), 509–520.
- 18. Perry, R.H. Chemical Engineer's Handbook, 6th Ed.; McGraw-Hill, 1984; 341-350.
- Hirai, M.; Ohtake, M.; Shoda, M. Removal kinetic of hydrogen sulfide, methanethiol and dimethyl sulfide by peat biofilters. J. Ferment. Bioeng. 1990, 70 (2), 334–339.
- Jones, C.A.; Kelly, D.P. Growth of *Thiobacillus ferrooxidans* on ferrous iron in a chemostat culture influence of product and substrate inhibition. J. Chem. Technol. Biotechnol. **1983**, *33* (4), 241–261.
- Colmer, A.R.; Temple, K.L.; Hinkle, M.E. An iron-oxidizing bacterium from the acid mine drainage of some bituminous coal mines. J. Bacteriol. **1950**, *59* (3), 317– 328.
- 22. Jensen, A.B.; Webb, C. Ferrous sulphate oxidation using *Thiobacillus ferrooxidans*: a review. Process Biochem. **1995**, *30* (3), 225–236.
- Nemati, M.; Webb, C. Effect of ferrous iron concentration on the catalytic activity of immobilized cells of *Thiobacillus ferrooxidans*. Appl. Microbiol. Biotechnol. 1996, 46 (3), 250–255.
- 24. Juszczak, A.; Domka, F.; Kozlowski, M.; Wachowska, H. Microbial desulfurization of coal with *Thiobacillus ferrooxidans* bacterium. Fuel **1995**, *74* (5), 725–728.

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