Treatment of High H₂S Concentrations by Chemical Absorption and Biological Oxidation Process

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ABSTRACT
The feasibility of a chemical absorption and a biological oxidation process to remove high H₂S concentrations (500–1500 ppm) was evaluated. The experiment included the effects of gas retention time (GRT) and H₂S concentration on H₂S removal efficiency, Fe³⁺ oxidation rate, and Fe²⁺ production rate in the chemical absorption reactor. The effects of carbon source and liquid flow rate on pH value, Fe²⁺ oxidation rate, Fe³⁺ production rate, total iron concentration, and growth of Thiobacillus ferrooxidans CP9 in the biological oxidation reactor were also examined. The optimal operating conditions for the individual process and design guidelines for the serial processes were established. The results of this study indicated that a long GRT could elevate H₂S removal efficiency under all operating conditions. However, a high H₂S concentration (e.g., 1,500 ppm) resulted in a significant difference. H₂S removal efficiency stayed above 99.5% for the first 10 h of reaction, but it then decreased because of the decreasing ferric iron concentration in the liquid. The presence of 0.1% glucose favored T. ferrooxidans CP9 growth as well as the Fe²⁺ oxidation rate and prevented the occurrence of jarosite precipitates in the biological oxidation process. In addition, the presence of glucose brought about different Fe²⁺ oxidation patterns (linear or curved type) and recovery percentages of total iron (95 and 74%). The results suggest that the liquid flow rate in the biological oxidation reactor was controlled at 3 mL/min, the volume ratio of biological reactor to chemical reactor was 13.5:1 when 150 g-S/m³/h of inlet H₂S loading was introduced to the system.

Key words: hydrogen sulfide; ferric sulfate; Thiobacillus ferrooxidans; biological oxidation

INTRODUCTION
Removal of hydrogen sulfide (H₂S) from the anaerobic wastewater treatment process is necessary for reasons of safety, corrosion-resistance, and health (Roth, 1993). Gas-phase treatment of H₂S has conventionally been accomplished independently using technologies based on physical, chemical, electrochemical, and biological processes, such as activated carbon adsorption, ozone oxidation, dielectric barrier discharges, and biofil-

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HIGH H2S CONCENTRATIONS BY CHEMICAL ABSORPTION

Tritiation (Barth et al., 1984; Cho et al., 1991; Chang and Treng, 1996). Although physical, chemical, and electrochemical methods can achieve satisfactory removal efficiencies, their high operating and disposal costs, as well as secondary pollution issues, are fatal defects (Bohn, 1992). Several biological methods, including biotrickling filters, biofilters, and bioscrubbers for H2S removal from some industrial and agricultural emission sources have been proposed (Chung et al., 1996; Anders and Colin, 1995), but the longer acclimated period (>7 days) and lower oxidative rates of these methods are major disadvantages (Yang and Allen, 1994). Moreover, limited application in aerobic conditions and a relatively low inlet H2S concentration (<150 ppm) further restrict their usage (Bohn, 1992). The waste gases contain high H2S concentrations (500–1,500 ppm) often emitted from various industrial processes or agricultural activities (Yang and Allen, 1994), especially in the process of the anaerobic digestion of organic compounds (Rands et al., 1981). In the mixture gases emitted from anaerobic treatment of organic compounds, the utilization of biogas (e.g., methane) to generate electricity in an internal combustion engine has caused significant corrosion problems because of the presence of a high H2S concentration (Pagella et al., 1996). However, few research reports have presented or focused on the elimination of these concentrations (e.g., 1,500 ppm) (Chung et al., 2003). We believe a combined chemical–biological process for H2S removal would be advantageous in the treatment of high H2S concentrations. The process for H2S gas treatment is divided into two steps: ferric sulfate [Fe2(SO4)3] is used as an oxidizing agent, which can rapidly react with a high concentration of H2S gas under anaerobic conditions and is reduced to ferrous sulfate (FeSO4) in the first stage. In the secondary stage, the ferrous sulfate solution is sent to an aerobic bioreactor, where inoculated Thiobacillus spp. will oxidize the ferrous iron to ferric iron. The ferric iron is then recycled into the reactor in the first stage to repeat the cycle.

Thiobacillus ferrooxidans has been proven to have significant applications in the bioleaching of sulfide minerals, treatment of acid mine drainage, and desulfurization of coal (Jensen and Webb, 1995). Therefore, a similar bioreaction should have beneficial applications in treating hydrogen sulfide-bearing gases by regenerating ferric ion (Fe3+) as in the following equation:

\[ 4 \text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ \rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O} \]

Thiobacillus ferrooxidans is generally regarded as a chemolithotrophic bacterium, with the ability to oxidize ferrous iron (Fe2+) in the acidic solution and couple the energy to support carbon dioxide fixation and growth. This ability is particularly suited for regeneration of Fe3+ ion in the chemical absorption reactor. Recently, Chung et al. (2003) found that Thiobacillus ferrooxidans can oxidize Fe2+ not only in chemolithotrophic conditions, but also in chemoheterotrophic conditions, which application of appropriate concentrations of carbon source will favor Fe2+ oxidation. The other crucial factor affecting Fe2+ oxidation by Thiobacillus ferrooxidans is Fe2+ concentration. High concentrations of ferrous iron (>5 g/L) have shown an inhibitory effect on cell growth and rate of ferrous iron oxidation (Jones and Kelly, 1983). However, we have demonstrated Thiobacillus ferrooxidans CP9 isolated from acid mine drainage can utilize 20 g/L ferrous iron, and no inhibitory effect was observed (Chung et al., 2003). Therefore, it can significantly elevate the Fe2+ oxidation rate or Fe3+ production rate (Chung et al., 2003).

In this study, we further evaluate the efficiency of the separate chemical absorption process in removing high H2S concentrations, and we examine the regenerative capacities of ferric sulfate during the separate biological oxidation process. Effects of gas retention time and H2S concentration on H2S removal efficiency, Fe3+ oxidation rate, and Fe2+ production rate in the chemical absorption reactor were illustrated. Also, effects of liquid flow rate and carbon source on the biological oxidation process to oxidize Fe2+ were evaluated. The design guidelines were established when the chemical and biological processes were in series.

MATERIALS AND METHODS

Micro-organism and medium preparation

Thiobacillus ferrooxidans CP9 was isolated from acid mine drainage and identified by the procedures of cell lysis, DNA extraction, PCR amplification, and cloning and sequencing compared with the EMBL database (Pizarro et al., 1996). The T. ferrooxidans CP9 was Gram-negative, motile, and shaped like a short rod. The optimal pH value, temperature, and concentration of glucose for the growth of T. ferrooxidans CP9 were 2.0, 35°C, and 0.1% (1 g/L), respectively (Chung et al., 2003). To prevent the formation of jarosites, the M16 medium developed by Kim et al. (2002) was used in the bioreactor. The medium contained ferrous iron (normally 5 g Fe2+/L), 3 g/L (NH4)2SO4, 0.1 g/L KCl, and 0.2 g/L MgSO4 in water, and the initial pH was 2.0. To count the cell number of T. ferrooxidans CP9, the KBU medium suggested by

ENVIRON ENG SCI, VOL. 23, NO. 6, 2006
Khalid et al. (1993) was used. The medium contained 5 g Fe$^{2+}$/L, 0.8 g/L (NH$_4$)$_2$SO$_4$, 0.4 g/L KH$_2$PO$_4$, 0.16 g/L MgSO$_4$, and 10 g/L gellan gum in water, and the pH of the medium was adjusted to pH 2.0 by 0.1 N H$_2$SO$_4$.

**Immobilization procedure**

Granular activated carbon (GAC) 4.5-mm in diameter with a surface area of 1,250 m$^2$/g, and obtained from Cherng Tay Corporation Ltd. in Taiwan was used as the packing material for the bioreactor. *T. ferrooxidans* CP9, grown in 1,000 mL M16 medium for 7 days, was harvested by centrifugation (12,000 $\times$ g for 2 min). The precipitates were drawn and mixed with 1,000 ml M16 medium in a 2-liter PVC tank for enriched growth. The pH of GAC (0.6 kg) was first regulated to 2.0 by 1 N H$_2$SO$_4$ and then mixed with the above solution to develop an immobilized cell biofilm. During the cultivation period, the aseptic air was supplied through a 0.22-$\mu$m filter, and the sterile M16 medium was replaced every 5 days until the cell number of *T. ferrooxidans* CP9 was up to $10^7$ CFU/g-dry GAC. After 2 months, the cell-laden GAC in the tank was transferred to the bioreactor.

**Chemical absorption process and apparatus for H$_2$S removal**

A scheme of the separate chemical absorption system is shown in Fig. 1. The glass column (6 cm $\varnothing$ × 60 cm) was packed with 40 cm of anticorrosive glass beads (ID: 6 mm) and filled with 500 mL ferric sulfate solution (15 or 20 g Fe$^{3+}$/L). The initial pH value of the system was regulated at pH 2.0. A perforated sieve plate at the bottom of the column allowed the waste gas containing H$_2$S to flow in. The 10,000 ppm (0.0139 g/L) of H$_2$S gas, supplied from a gas cylinder, was first diluted to different concentrations with compressed air, passed through an air filter (pore size 0.22 $\mu$m, LIDA 3000-06, made in the USA), and then pumped upward through the column at the bottom. In the chemical absorption experiment, the simulated H$_2$S concentrations ranging from 500 to 1,500 ppm (0.000695 to 0.00209 g/L) were introduced to the chemical absorption reactor at various gas retention times (36–60 s) at room temperature to evaluate H$_2$S removal efficiency and the operational characteristics of the system. In all of these experiments, values of a set of parameters versus time were measured including pH, Fe$^{2+}$ concentration, Fe$^{3+}$ concentration, total iron concentration (Tot Fe), and H$_2$S concentration. Data were obtained from at least two duplicate tests.

**Biological process and apparatus for Fe$^{3+}$ regeneration**

The setup of the separate biological oxidation system is shown in Fig. 2. 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 40 cm cell-laden GAC supported by a perforated sieve net 10 cm from the bottom of the column. The M16 medium containing 15 or 20 g Fe$^{2+}$/L stored in the nutrient tank was continuously recirculated by a peristaltic pump at 3–12 mL/min to supply nutrient to the attached cells. If it was required, 0.1% (1 g/L) glucose was also added. The nutrient solution
flowed upward through the reactor in a direction parallel to the aseptic air. The gas flow rate was controlled at 100 L/h, and the reactor temperature was controlled at 35 ± 1°C. At steady state, the liquid volume in the biological regeneration reactor remained about 500 mL. The biological regeneration reactor contained a sampling port in the middle of the column for the analysis of chemicals and micro-organisms. Changes in the concentrations of ferric iron, ferrous iron, pH value, Tot Fe, and the cell numbers of *T. ferrooxidans* CP9 in the GAC beads and in the liquid phase were monitored periodically. Data were obtained from at least two duplicate tests.

**Analytical methods**

Inlet H₂S gas concentrations in the reactor were periodically measured by gas detector tubes (Kitagawa, Japan) in the range of 12.5–500 ppm or 100–2,000 ppm. Outlet concentrations were measured by gas chromatograph equipped (GC) with a flame photometric detector (FPD) and a glass packed column coated with 25% β',β'-oxydipropionitrile at 80°C. The detection limit for H₂S gas was 0.1 ppm. In all continuous experiments, H₂S concentrations were recorded when the variation of H₂S concentrations was within ±5% in 10 min. A total of six data were recorded, and the average was taken to be the H₂S outlet concentration. Ferrous ion concentration was determined using titration against 0.017 M potassium dichromate in the presence of *N*-phenylnthranilic acid as an indicator (Vogel, 1989). Total iron (Tot Fe) concentration was measured by atomic absorption (AA). Ferric iron concentration was estimated by subtracting the ferrous iron from the total iron concentration. The pH value in the reactor was measured using a pH meter. For microbial analyses, 0.5 g of GAC was taken from sampling port of biological oxidation reactor mixed with 5 mL of sterile water. The sample was vortexed for 3 min, and the cell numbers were enumerated on KBU growth medium by traditional plate-counting methods. The inoculated plates grew for 5 days in an incubator at 35 ± 0.5°C. The dark-brown colony in the medium was indicated as *T. ferrooxidans* CP9 (Kai *et al.*, 1990).

**RESULTS AND DISCUSSION**

**Effect of gas retention time on H₂S removal by chemical absorption system**

Longer gas retention times (GRT) are due to the fact that the ferric iron solution in the chemical absorption reaction takes a longer time to react with hydrogen sulfide. Gas retention time plays an important role in H₂S removal (Pagella *et al.*, 1996). The effect of gas retention time on H₂S removal and the change of Fe³⁺ concentration in the separate chemical absorption reaction at different inlet concentrations (500, 1,000, and 1,500 ppm) are shown in Figs. 3, 4, and 5. Figure 3 shows that a retention time of 36 s significantly affected H₂S removal efficiency, but little difference was observed between 45 and 60 s during the whole operating period. If the chemical absorption system must maintain above 95% removal efficiency at 500 ppm of inlet H₂S gas, the system could operate for 45 or 65 h for different GRTs. Figure 3B indicates that the fastest Fe³⁺ consumption rate occurred at a gas retention time of 36 s because the largest amount of H₂S gas was introduced per time unit compared to the GRTs of 45 and 60 s. The effects of GRT 45 and 60 s on Fe³⁺ consumption rate are insignificant as is their effect on H₂S removal efficiency (Fig. 3B). A comparison of Fig. 3A and B demonstrates that when the Fe³⁺ concentration in the chemical absorption reactor was maintained above 6.8 g/L, the H₂S removal efficiency was higher than 95%. This study could be used as a design guideline and an operating principle in removing H₂S at 500 ppm.

When inlet H₂S concentration was raised to 1,000 ppm, similar results were observed (Fig. 4). Once the GRT was reduced to a critical value (e.g., 36 s), its effect would become remarkable. In addition, as the ferric iron is used as an oxidizing agent to react with H₂S gas, the pH of the system gradually decreased according to the equation

\[
2FeSO_4 + H_2S \rightarrow S + 2FeSO_4 + H_2SO_4
\]

However, the change in pH in the chemical absorption reactor was between 1.5 and 2.0 during the whole operating period (data not shown). According to mass balance of iron ion, the recovery ratio for total iron achieved 99.6%, estimated by initial total iron concentrations divided by the sum of soluble Fe²⁺ and Fe³⁺ compounds after reaction. The iron precipitation did not interfere with the reaction’s progress.

When inlet H₂S concentration was further elevated to 1,500 ppm, different patterns of H₂S removal and consumption rates of Fe³⁺ concentration were found at GRTs from 36 to 60 s, as shown in Fig. 5. Although the H₂S concentration was high, a removal greater than 99.5% was achieved in 10 h when GRTs were between 36 and 60 s. Such results are impossible using traditional biological methods (Oh *et al.*, 1998). In the study, a long gas retention time significantly favored H₂S removal. Thus, the highest H₂S removal efficiency was observed at GRT 60 s at the same operation time. This suggested that high H₂S concentrations required long GRTs to produce H₂S oxidation for the chemical absorption process. Hence, the H₂S removal patterns were not similar at Figs. 4 and 5 (GRT 45 and 60 s). Since the change of pH in
the reactor was at 1.5–2.0, connecting the biological oxidation system did not inhibit the growth of *T. ferrooxidans* CP9 (Chung et al., 2003). The change of soluble Fe$^{2+}$/H$_{1001}$ and Fe$^{3+}$/H$_{1001}$ concentrations was also analyzed, and the recovery ratio for initial total iron (19.6 ± 0.3 g/L) achieved 99.2% on average (data not shown). Therefore, although we used Fe$^{3+}$/H$_{1001}$ concentrations higher than those of Pagella et al. (1996) to remove 1,500 ppm H$_2$S, we found none of the iron precipitation or interference with the reaction progress that they did. Besides, as shown in Fig. 5A and B, when the Fe$^{3+}$/H$_{1001}$ concentration in the chemical absorption reactor remained above 9.0 g/L, the H$_2$S removal efficiency reached 95%. This study of the Fe$^{3+}$/H$_{1001}$ depletion rate in the separate chemical stage could be used as a design guideline for removing H$_2$S gas if the Fe$^{3+}$/H$_{1001}$ regeneration rate in the biological oxidation process is known. In other words, the depletion of Fe$^{3+}$/H$_{1001}$ in the chemical stage will be regenerated in the biological stage when two stages are combined into a continuous system. Thus, a successful integrated system combining chemical absorption and biological oxidation stages will be achieved.

**Figure 3.** H$_2$S removal efficiency by chemical absorption reactor (A) and Fe$^{3+}$/H$_{1001}$ concentration in the reactor (B) at different gas retention times. The initial pH and Fe$^{3+}$/H$_{1001}$ concentration were controlled at 2.0 and 14.7 ± 0.3 g/L when 500 ppm H$_2$S was introduced into the reactor.
Criteria for designing a biological oxidation reactor

The chemical absorption process was responsible for removing H$_2$S gas using a ferric iron solution, and the regeneration of ferric iron from ferrous iron was performed by the biological oxidation process. Since the biological reaction was generally slower than the chemical reaction, the required volume of the biological oxidation reactor was dependent on the production rate of ferrous iron by the chemical absorption reactor when other operating factors were constant. The relationships between inlet H$_2$S loading, gas loading, and Fe$^{2+}$ production rate in the chemical absorption process are shown in Fig. 6A and B. The inlet H$_2$S loading was defined below:

\[
\text{Inlet H}_2\text{S loading} = \frac{F \times C}{V}
\]
where $F$ is the gas flow rate (L/h), $C$ is the gas concentration (g-S/L), and $V$ is the volume of the ferric iron solution (m$^3$). The gas loading was defined below:

$$\text{Gas loading} = \frac{F}{A}$$

where $F$ is the gas flow rate (m$^3$/h), and $A$ is the cross-section area of the chemical absorption reactor (m$^2$). The experiments were conducted by introducing different H$_2$S concentrations (500–1,500 ppm) and gas flow rates (30–90 L/h) into the chemical absorption reactor with a constant packing volume. The regression equation ($y=0.0027x$) and its correlation coefficient (0.9844) between inlet H$_2$S loading and Fe$^{2+}$ production rate indicated that both enjoyed a good linear relationship. In addition, gas loading and Fe$^{2+}$ production rate also enjoyed a good relationship. These equations, therefore, can be used as a criterion to design a biological reactor of Fe$^{3+}$ regeneration. For example, the inlet H$_2$S concentration is measured when the gas flow rate and the reaction volume of

Figure 5. H$_2$S removal efficiency by chemical absorption reactor (A) and Fe$^{3+}$ concentration in the reactor (B) at different gas retention times. The initial pH and Fe$^{3+}$ concentration were controlled at 2.0 and 19.6 ± 0.3 g/L when 1,500 ppm H$_2$S was introduced into the reactor.
the ferric iron solution are constant. The Fe$^{2+}$ production rate can be estimated by the regression equation. When the Fe$^{2+}$ oxidation rate by the biological oxidation process is determined, the appropriate volume for the bioreactor can be set up.

Effect of carbon source on Fe$^{2+}$ oxidation and Fe$^{3+}$ production in a biological oxidation system

To ensure the Fe$^{2+}$ oxidation in the reactor was mainly derived from biological rather than chemical oxidation, the biological reactor was operated under aseptic conditions at an air flow rate of 50 L/h for 252 h. The results indicated that the Fe$^{2+}$ concentration (initial 20.0 g/L) decreased with the operating time and leveled off on the 48th h (Fig. 7A). The stable Fe$^{2+}$ concentration (18.3 g/L) was maintained until experiment end. This demonstrated that only 8.5% Fe$^{2+}$ could be chemically converted when 20.0 g/L of Fe$^{2+}$ was used in the biological reactor. This result was similar to the observation of Nemati and Webb (1997). Because *T. ferrooxidans* CP9 exhibited a higher capacity for Fe$^{2+}$ oxidation under heterotrophic than autotrophic conditions in the shake flask (Chung et al., 2003), the phenomenon should also occur in a semicontinuous reactor.

**Figure 6.** Relationship between inlet H$_2$S loading and Fe$^{2+}$ production rate (A) and relationship between gas loading and Fe$^{2+}$ production rate (B).
The results in Fig. 7A show the changes of Fe\(^{2+}\) and Fe\(^{3+}\) concentrations in the biological oxidation reactor with/without glucose addition. When the biological reactor was supplied with 0.1% glucose, the Fe\(^{2+}\) oxidation rate reached 12.8 mM/h (0.72 g-Fe\(^{2+}\)/h/L) and the Ks value was 32 mg/L, estimated by the Monod equation. The oxidation rate of this study is faster than the 7.2, 7.8, 9.6, and 10.8 mM/h obtained by different reactor configurations, support matrixes, and microbial species (Grishin et al., 1988; Garcia et al., 1989; Carranza and Garcia, 1990; Armentia and Webb, 1992). However, when glucose was not added to the biological reactor, Fe\(^{2+}\) oxidation rate fell to 11.3 mM/h (0.63 g-Fe\(^{2+}\)/h/L), and the Ks value was 46 mg/L. A strong apparent decrease of Ks was clearly found when glucose was added to the biological reactor. Although these results were similar with those obtained by shake flask, the values in the semicontinuous reactor were 25-fold higher (Chung et al., 2003). As expected, the 11.3 mM/h Fe\(^{3+}\) production rate was close to the 12.8 mM/h of Fe\(^{2+}\) oxidation rate when the biological oxidation reactor was provided with 0.1% glucose. However, the curve of Fe\(^{3+}\) production first rose and then leveled off when \textit{T. ferrooxidans} CP9 grew in autotrophic conditions. After about a 12-day operating period, Fe\(^{3+}\) production was stagnant. Figure 7B shows changes in total iron concentration (Tot Fe) and pH values in the biological oxidation reactor with/without glucose addition. The pH values in the biological reactor with/without glucose addition ranged
from 1.9 to 2.1 and from 2.1 to 2.4, respectively. A low pH distribution was observed when to the biological reactor was added 0.1% glucose during the whole operating period. This might have been due to the presence of CO₂, which *T. ferrooxidans* CP9 had metabolized from glucose. In fact, a considerable concentration of CO₂ was detected in the outlet once glucose was added, compared to the control, which had no additional glucose (data not shown). Because high pH conditions (e.g., pH > 2.1) have been demonstrated to favor the reactions of Fe³⁺ chelation and precipitation, jarosite precipitates occurred (Jensen and Webb, 1995; Chung et al., 2003). Thus, visible precipitates (ferric hydroxysulfate) were found in the bioreactor to which no glucose was added, and they interfered with Fe²⁺ oxidation by *T. ferrooxidans* CP9. Glucose increased the growth of *T. ferrooxidans* CP9 in a continuous bioreactor as shown in Table 1. This growth was about 16-fold what it would have been without glucose addition, which might be another reason for the high Fe²⁺ oxidation rate by *T. ferrooxidans* CP9 under heterotrophic conditions (see Table 1). This finding has only been observed in few reports (Nemati and Webb, 1996; Chung et al., 2003). If the chemical absorption and biological regeneration processes were connected in series, they would actually have a detrimental effect on H₂S gas removal because of the diminishing of the available ferric iron serving as an absorbent for H₂S and creating kinetic barriers of mass diffusion. Hence, the total iron concentrations gradually decreased, and the Fe³⁺ concentration slowly increased after a 12-day operation when the bioreactor had not been provided with glucose (Fig. 7B). In fact, total iron (Tot Fe) recovery was about a 95 and 74% in the cases with/without glucose, respectively. Therefore, the presence of 0.1% glucose was able to effectively elevate the system performance.

**Effect of liquid flow rate on Fe²⁺ oxidation and cell number of the biological oxidation system**

A long liquid retention time increases the Fe²⁺ oxidation by *T. ferrooxidans* CP9 due to the fact that the microbe in the biological oxidation reaction can take a longer time to make contact with Fe²⁺ solution and nutrient. It may, however, affect the H₂S removal capacity in the chemical absorption reactor when the chemical absorption and biological oxidation processes are connected in series (Teruyuki, 1996; Chung et al., 2003). Hence, liquid flow rate plays a crucial role in H₂S removal. The effect of liquid flow rate on Fe²⁺ oxidation in the biological oxidation reactor provided with 0.1% glucose is shown in Fig. 8A. The results indicated that Fe²⁺ oxidation rate increased with decreasing liquid flow rate. The highest oxidation rate was 0.031 g-Fe²⁺/L/h at 3 mL/min, and the lowest was 0.016 g-Fe²⁺/L/h at 12 mL/min among these liquid flow rates. These results were one order of magnitude higher than in the work of Teruyuki (1996). Although 3.0 to 7.5 g/L of ferrous iron has showed various inhibitory effects on *T. ferrooxidans* growth and the rate of the ferrous iron oxidation by *T. ferrooxidans* (Jones and Kelly, 1983; Juszczak et al., 1995; Gomez et al., 1996; Nemati and Webb, 1997), no inhibitory effect on Fe²⁺ oxidation by *T. ferrooxidans* CP9 in the presence of high Fe²⁺ concentration (19.6 ± 0.3 g/L) was observed in our bioreactor. These results could provide valuable information in designing a combination biological–chemical system. For example, when 150 g-S/m³/h of inlet H₂S loading was introduced to the chemical absorption reactor, the theoretical Fe²⁺ production rate was calculated as 0.405 g-Fe²⁺/L/h according the regression equation in Fig. 6. When the liquid flow rates in the biological oxidation reactor were controlled at 3 or 12 mL/min, a bioreactor with a volume 13- or 25-fold, respectively, that of the chemical reactor was required when the cell numbers of *T. ferrooxidans* CP9 were maintained at 10⁸ CFU/g-dry GAC (see Table 1).

To evaluate the scrubbing effect of the liquid flow rate on the immobilized *T. ferrooxidans* CP9, the cell numbers in the liquid phase were determined. Figure 8B shows the change of cell number in the liquid phase at different liquid flow rates on the 20th day. No significant statistical differences between the cell numbers of *T. ferrooxidans* CP9 and liquid flow rates were found (*p >

**Table 1.** Change in the cell number with/without glucose addition.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>+0% Glucose</th>
<th>+0.1% Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(CFU/g-dry GAC)</td>
<td>(CFU/g-dry GAC)</td>
</tr>
<tr>
<td>0</td>
<td>3.34 × 10⁶ (±2.03 × 10⁶)</td>
<td>2.83 × 10⁷ (±1.91 × 10⁷)</td>
</tr>
<tr>
<td>7</td>
<td>4.69 × 10⁷ (±1.04 × 10⁷)</td>
<td>8.61 × 10⁷ (±2.65 × 10⁷)</td>
</tr>
<tr>
<td>14</td>
<td>5.28 × 10⁷ (±2.41 × 10⁷)</td>
<td>3.06 × 10⁸ (±2.81 × 10⁸)</td>
</tr>
<tr>
<td>21</td>
<td>5.45 × 10⁸ (±2.92 × 10⁸)</td>
<td>8.94 × 10⁸ (±3.41 × 10⁸)</td>
</tr>
</tbody>
</table>

*Mean ± 1 standard deviation (n = 3).*
indicating that a good biofilm existed in the range of 3–12 mL/min.

**CONCLUSIONS**

The results of this study suggest that the chemical absorption process could effectively remove very high H$_2$S concentrations in a short time. This process was capable of 95% removal efficiency when Fe$^{3+}$ concentrations were kept at 6.8 g/L for 500 ppm of inlet H$_2$S concentration, and 9.0 g/L for 1,500 ppm of inlet H$_2$S concentration in the chemical absorption reactor. In addition, the presence of glucose was able to effectively elevate the performance of the biological oxidation process for Fe$^{3+}$ regeneration by increasing the cell numbers of *T. ferrooxidans* CP9 and preventing the occurrence of the jarosite precipitates. Thus, the design guidelines were established for a combination of the chemical and biological processes. Also, the results showed that the system should be very feasible because key factors affecting system performance—including pH, Fe$^{2+}$ production rate, Fe$^{3+}$ oxidation rate, gas retention time, and liquid flow rate—have been fully examined. Also, a high Fe$^{2+}$ tolerant strain, *T. ferrooxidans* CP9, used in the bioreactor should contribute greatly to the success of this work.

**ACKNOWLEDGMENTS**

This work was supported by a grant from the National Science Council.
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