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SHORT COMMUNICATION

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A novel regeneration of iron citrate solution by biooxidation of iron-oxidizing bacteria

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Abstract Liquid phase oxidation process using chelated iron solution is among the most promising techniques for the hydrogen sulfide removal due to its double advantage of waste minimization and resource recovery. Regeneration of chelated iron is a core reaction in this process. Regeneration of chelated iron in acidic solution is very difficult. In this paper, a novel regeneration of iron citrate in acidic solution by biooxidation of iron-oxidizing bacteria was reported firstly. By using such a process, the influence of iron-oxidizing bacteria on the regeneration rate was investigated. The results demonstrated the regeneration rate with the new technology was increased significantly. The process may contribute to the biooxidation of iron-oxidizing bacteria. Application of this novel process increased the regeneration rate under the optimum conditions, suggesting the iron citrate regeneration process may be a feasible and economical method in application.

Keywords Iron citrate · Hydrogen sulfide · Biooxidation · Iron-oxidizing bacteria

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Introduction

Everyday huge amounts of toxic gas pollutants are released to the atmosphere. The dominant among them are volatile organic compounds, carbon and nitrogen oxides, ammonia, methane as well as sulfur compounds. Particularly hazardous for human health and for the natural environment are the inorganic sulfur compounds such as H_2S. They originate from the economic and industrial activity of man, including industrial plants emission, animal farms, septic tanks, waste purification plants as well as from natural sources such as volcano eruptions, thermal springs and bogs. A large amount of effort has been undertaken to remove H_2S from various gaseous streams [6–8]. In recent years, gas desulfurization processes based on iron and chelated iron chemistry has received increasing attention [9, 11]. The iron and chelated iron approach is generally considered as a fast, safe, green and promising technique over other options for the H_2S removal due to its double advantage of waste minimization and resource recovery in a wide range of pH 1–9 [4, 11, 18].

In chelated iron process, iron is presented in chelated iron (FeL, where L is chelating agents such as NTA, EDTA or HEDTA [5, 16]). In operation, the H_2S is firstly contacted with aqueous chelated-Fe(III) solution ([Fe] = 5 mM–0.5 M) [12] and converted into elemental sulfur which is then separated by filtration or sedimentation. The reaction incorporated in the chelated iron process is summarized as follows with (g), (aq) and (s) representing the gaseous, aqueous and solid states, respectively:

$$H_2S_{(g)} + 4H_2O_{(l)} \rightleftharpoons H_2S_{(aq)} \quad (1)$$

$$H_2S_{(aq)} \rightleftharpoons HS^- + H^+ \quad (2)$$

$$2Fe^{3+}L + HS^- \rightarrow 2Fe^{2+}L + H^+ + S \quad (3)$$

Since the active chelated iron ($Fe^{3+}L$) is converted into inactive chelated iron ($Fe^{2+}L$), the latter component has to be regenerated into its ferric form through the direct oxidation of air. A mildly alkaline (pH 7–9) chelated-Fe(III) solution is often used in present operation because chelated-Fe(III) could regenerate better through the direct oxidation of air at this pH.

$$O_{2(g)} + H_2O_{(l)} \rightleftharpoons O_{2(aq)} \quad (4)$$

$$2Fe^{2+}L + 1/2O_{2(g)} + H_2O_{(l)} \rightarrow 2Fe^{3+}L + 2OH^- \quad (5)$$

Thus, the overall process is:

$$H_2S + 1/2O_2 \rightarrow H_2O + S \quad (6)$$

So far, the greatest challenge in the chelated iron process is related to the step of catalyst regeneration [3, 16]. It is the key step to determine the efficiency of whole process because the solubility of oxygen is extremely low in the liquid phase, which results in slower regeneration speed and lower regeneration efficiency. There are various potent works that have been carried out [4, 7, 12]. However, the regeneration process is typically accomplished through the direct oxidation by air in reactor, which has many demerits, such as poor mass transfer efficiency, larger equipment volume, and higher energy consumption, especially in acidic solution. In addition, the chelating agents are easily degraded in mildly alkaline conditions (pH 7–9) because of miscellaneous microbial contamination. Acidic liquid can greatly inhibit microbial contamination so as to maintain the stability of chelated iron [11, 18]. It is critical to carry out researches focused on developing alternate regenerative processes in acidic solution.

Acidithiobacillus ferrooxidans is an obligate chemolithotrophic bacterium, which has the ability to oxidize ferrous iron to ferric iron, a reaction that supplies energy for growth of the iron-oxidizing bacteria [13].

$$4Fe^{2+} + 4H^+ + O_2 \xrightarrow{A.\ ferrooxidans} 4Fe^{3+} + 2H_2O \quad (7)$$

There are many research reports about the oxidation of iron and application [14, 17, 18]. However, there are few in scientific literature about biooxidation regeneration of chelated iron in acidic solution. It is unknown whether stimulation of chelated iron can be achieved using *A. ferrooxidans*. To answer this and to obtain a better insight in the regeneration of chelated iron, a new technology of chelated iron citrate biooxidation by iron-oxidizing bacteria is described. The main object of this research is to develop a novel regeneration process of iron citrate, strengthen regeneration efficiency in acidic solution and expand the application scope of pH. The new regeneration technology will provide theoretical and experimental basis for industrial applications.

Materials and methods

Microorganism and cultivation

Acidithiobacillus ferrooxidans was used in this work. The bacteria were cultured in 9K medium proposed by Silverman and Lundgren [15]: $(NH_4)_2SO_4$, 3 g/L; KCl 0.1 g/L; K_2HPO_4 0.5 g/L; $MgSO_4{\cdot}7H_2O$ 0.5 g/L; $Ca(NO_3)_2{\cdot}4H_2O$ 0.01 g/L and 0.15 mol/L $FeSO_4{\cdot}7H_2O$ (about 44.5 g/L) at an initial pH of 1.7–1.9 in an orbital shaker at 31 °C. The cultures were shaken constantly at 150 r/min on an orbital shaker until they nearly reached their stationary growth phase (2.0×10^8 cells/mL). 2 L cultures were centrifuged at $230\times g$ for 5 min to remove unsolvable precipitation. Then the cells were harvested at $14{,}700\times g$ for 15 min, washed three times with 0.01 N H_2SO_4. Finally, the cells were resuspended in a small volume (20 mL) of 0.01 N H_2SO_4 (1.1×10^{10} cells/mL) and used for this study.

Chemicals

The chemicals used for making nutrient medium for the *A. ferrooxidans* were of laboratory grade, and the chemicals used for analysis were of AR grade. The iron solution medium contains $(NH_4)_2SO_4$, 3 g/L; KCl 0.1 g/L; K_2HPO_4 0.5 g/L; $MgSO_4.7H_2O$ 0.5 g/L; $Ca(NO_3)_2{\cdot}4H_2O$ 0.01 g/L and 0.10 mol/L $FeSO_4{\cdot}7H_2O$ at an initial pH of 1.9 [11, 19]. The sodium salts of citrate were supplied as chelating agents. The molar ratio of chelating agent to Fe(II) was 1:1.

Shake flask experiments

Four different process methods with shake flask experiments were conducted and their oxidation processes were compared. Shake flask experiments were carried out in 250-mL Erlenmeyer flasks. The flasks were filled with 150 mL iron solution medium: iron citrate solution (Group a), iron citrate solution inoculated with *A. ferrooxidans* (Group b, about 1.0×10^8 cells/mL). The cultures were shaken constantly at 150 r/min in an orbital shaker at 31 °C.

Bioreactor and operation

The continuous oxidation of chelated iron by *A. ferrooxidans* was studied in a bioreactor. The main part of the bioreactor consisted of a glass column in Fig. 1 (60×4.25 cm). In the experiments, the chelated iron solution was pumped from the storage tank to the inner of the reactor. The air was dispersed through tiny bubbles via a gas distributor. The airflow rate was 0.1–1 L/min. Both gas and liquid streams contacted counter-currently in the bioreactor, in which the chelated iron solution was regenerated by *A. ferrooxidans*. The temperature was maintained at 31 °C.

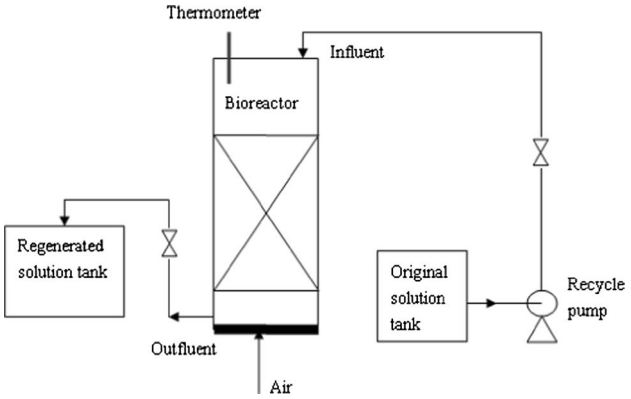

Fig. 1 A schematic of the laboratory-scale reactor for biooxidation of iron citrate. Chelated iron was regenerated by *A. ferrooxidans* in bioreactor

The pH of the reactor influent was 2.0. Prior to entering the reactor, air was led through a humidifier in order to minimize evaporation. The reactor was operated for 15 days. The regeneration rate η is applied to characterize the regeneration efficiency of chelated iron desulfurization solution, which is defined as: $\eta = (C_{in} - C_{out})/C_{in} \times 100\ \%$, in which C_{in} and C_{out} are the Fe^{2+} L concentrations in the inlet and outlet of the electrochemical regeneration device (mol/L), respectively. All the observations presented in this paper in the form of figures are the average value of three sets of experiments. The percentage deviation in the observations with respect to the average values was calculated and indicated using deviation bars across the average values in the figures.

Analytical procedures

The concentrations of Fe(II)L and Fe(III)L were analyzed with potassium dichromate method [20]. Oxidation was monitored by determining Fe^{2+} concentrations using titration against 0.009 N potassium dichromate in the presence of *N*-phenylanthranilic acid as an indicator. The bacterial concentration was determined by direct counting using a cell counter (haemocytometer) of 0.1 mm depth and 1/400 mm^2 area. The pH of solution was measured using PHBJ2260 pH meter.

Results and discussion

Biooxidation of ferrous iron citrate with shake flask experiments

Biooxidation of ferrous iron is a principal characteristic for *A. ferrooxidans*, a mesophile acidophile, can obtain energy for growth and maintenance from oxidation of ferrous iron

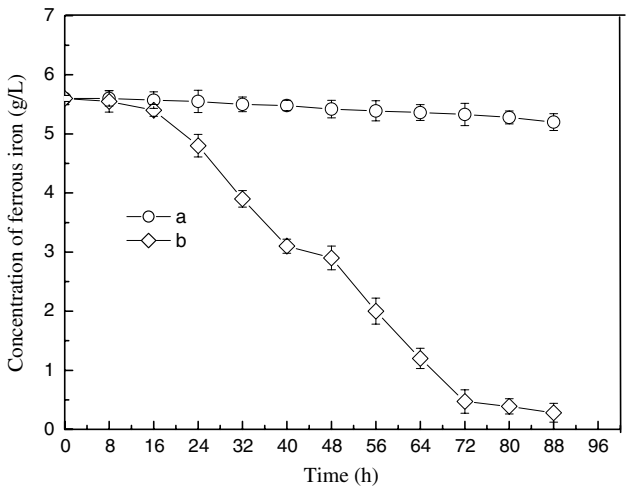

Fig. 2 Bioxidation of ferrous iron citrate with shake flask experiments. Group *a* iron citrate solution was free of iron-oxidizing bacteria, Group *b* iron citrate solution inoculated with *A. ferrooxidans*

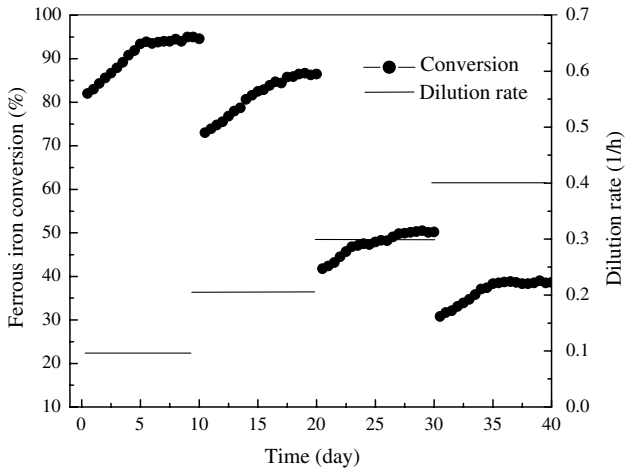

Fig. 3 Long-term performance of reactor. 40-day operation was carried out to investigate the biooxidation efficiency of iron citrate. Following the achievement of 80 % conversion of Fe(II) continuous feeding was initiated at different residence time. Steady-state operation was considered to be established when Fe(II) concentration varied by less than 5 %

to ferric iron at pH 1.5–3.0 with an optimum value about 2.0 [2, 18]. It is very interesting that it has great ability for oxidation of chelated iron citrate. As plotted in Fig. 2, the ferrous iron concentration in medium decreased gradually along the reaction time in Group b. At day 3 Fe(II) concentration in Group b was about 0.47 g/L. About 92 % of the initial ferrous iron citrate was oxidized within 72 h. However, only 5.3 % ferrous iron were oxidized in Group a. The reaction efficiency was very poor. Direct oxidation of ferrous iron citrate by O_2 could occur faintly in acidic solution. This means chelated iron oxidation was affected

greatly by *A. ferrooxidans*. Iron citrate regeneration has a tight relation to biocatalysis of *A. ferrooxidans*. *A. ferrooxidans* could gain energy for the growth from this repeating reaction:

$$4Fe^{2+}L + 4H^{+} + O_2 \xrightarrow{A.ferrooxidans} 4Fe^{3+}L + 2H_2O \quad (8)$$

The experimental results confirmed the validity of the biooxidation method for enhancing regeneration of chelated iron.

Biooxidation of iron citrate in the laboratory-scale reactor

A set of laboratory-scale operations was carried out at different operating parameters to investigate the biooxidation efficiency of iron citrate in reactor (in Fig. 3). Before startup, the reactor was operated in batches. Following the achievement of 80 % conversion of Fe(II), continuous feeding was initiated at a residence time of 10 h. The effluent stream was collected and Fe(II) concentration was measured periodically. In this case, Fe(II) concentration in bioreactor was decreasing continuously. Steady-state operation was considered to be established when Fe(II) concentration varied by less than 5 %. After about 5 days of operation about 93 % conversion of Fe(II) was achieved. After that, the reactor was operated under stable conditions. Our previous study showed that the biooxidation rate was limited by the dilution rate and air flow rate greatly [19]. The influence of dilution rate and air flow rate on chelated iron regeneration behavior was examined in the reactor during the 40-day operation. The pH, temperature and airflow rate were maintained at 1.9, 31 °C, 0.5 L/min. Results indicated that the regeneration efficiency increased with increasing dilution rate in reactor. The time course of the ferrous iron conversion ratio at different dilution rates as shown in Fig. 3. A maximum oxidation rate of 1.02 g L h^{-1} was achieved at dilution rate of 0.2/h or higher. The trend was similar with ferrous iron oxidation rate discussed previously since increasing the dilution rate led to a higher ferrous iron oxidation rate [18]. During the operation, few jarosite crystal deposits could be found in reaction. This is very different from the biooxidation of ferrous iron since iron in 9K medium would facilitate jarosite and Schwertmannite precipitation [10, 19]. The precipitation would have a negative effect on the transport of substrate because of kinetic barriers [1]. The result indicated that chelated iron citrate could decrease jarosite and Schwertmannite precipitation.

The effect of inlet air flow rate on biooxidation regeneration rate at a dilution rate of 0.2/h was also investigated. With increasing inlet air flow rate, the oxidation rate first increases rapidly and then changes slightly. Air flow rate strengthens the micro-mixing efficiency of the air and solution, resulting in excellent mixing and higher mass transfer rate. When inlet air flow rate increases to higher than 0.58 L h^{-1}, the regeneration rate increases slowly. The reason may be that the residence time of air becomes shorter in solution and the gas–liquid mass transfer efficiency decreases with a too large inlet air flow rate. During the long-time operation, few other bacteria were detected in bioreactor except *A. ferrooxidans*, which indicated that miscellaneous microbial contamination in chelated iron solution would decrease greatly in the acidic medium.

Conclusion

The catalyst regeneration is the key step controlling efficiency of chelated iron process. In the present study, a novel bio-regeneration of iron citrate technology was investigated. The oxidation process exhibits better regeneration performance for the iron citrate solution due to the indirect catalyzing of *A. ferrooxidans*. The optimal dilution rate and inlet air flow rate were 0.2/h and 0.58 L h^{-1} in reactor. This process offers an opportunity for high efficiency and continuous regeneration of chelated iron acidic solution. Moreover, miscellaneous microbial contamination on chelated iron solution would decrease greatly. These will potentially have a great market for industrial applications.

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