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Comparison of Fe^{2+} oxidation by *Acidithiobacillus ferrooxidans* in rotating-drum and stirred-tank reactors

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Abstract: Fe^{2+} oxidation by *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) under different solid contents by adding inert Al_2O_3 powder was examined in rotating-drum and stirred-tank reactors. The results show that the bioactivity of *At. ferrooxidans* in the stirred-tank is higher than that in the rotating-drum in the absence of Al_2O_3 powder, but the biooxidation rate of Fe^{2+} decreases markedly from 0.23 g/(L·h) to 0.025 g/(L·h) with increasing the content of Al_2O_3 powder from 0 to 50% (mass fraction) in the stirred-tank probably due to the deactivation of *At. ferrooxidans* resulting from the collision and friction of solid particles. The increase in Al_2O_3 content has a little adverse effect on the bioactivity of *At. ferrooxidans* in the rotating-drum due to different mixing mechanisms of the two reactors. The biooxidation rate of Fe^{2+} in the rotating-drum is higher than that in the stirred-tank at the same content of Al_2O_3 powder, especially at high solid content. The higher bioactivity of *At. ferrooxidans* can be maintained for allowing high solid content in the rotating-drum reactor, but its application potential still needs to be verified further by the sulfide bioleaching for the property differences of Al_2O_3 powder and sulfide minerals.
Key words: Fe^{2+}; *Acidithiobacillus ferrooxidans*; oxidation; bioactivity; solid content; rotating-drum reactor; stirred-tank reactor

1 Introduction

Acidithiobacillus ferrooxidans (*At. ferrooxidans*) is one of the most important bacteria used in the bioleaching of sulfide ores [1,2]. The ability of *At. ferrooxidans* to oxidize ferrous ion and reduce sulfur compounds to ferric ion and sulfate respectively can result in the solubilisation of metals from their sulfides, which is generally referred to as the indirect leaching mechanism [3]. The oxidation rate of Fe^{2+} is an important indicator of the bioactivity of *At. ferrooxidans*. The high bioactivity of *At. ferrooxidans* means fast regeneration of Fe^{3+}, which is favorable to the bioleaching of sulfide minerals.

Some researches related to the oxidation of Fe^{2+} by *At. ferrooxidans* were performed in the bioreactors with different support particles such as polyurethane foam [4], activated carbon [5] and chitosan beads [6]. The Fe^{2+} oxidation was separated from the bioleaching process of sulfide minerals in these investigations. Although Fe^{2+} biooxidation was also extensively studied in stirred-tank reactors [7] and shake flasks [8], no related investigation was reported on rotating-drum reactors, which can be viewed as a potential alternative to stirred-tank reactors.

The mixing and suspension of ore pulp in a rotating-drum is achieved by the direct lifting of baffle plates, whereas the mixing of ore pulp in a stirred-tank is driven by high-speed impellers. HERRERA et al [9] found that the total concentration of iron in the rotating-drum reactor was 2.3 times higher than that in the stirred-tank at high pulp densities in the bioleaching of refractory gold concentrates, but its gas−liquid mass transfer performance was not satisfactory for the lack of an efficient gas sparger [10]. The numerical simulation reported by LIU et al [11] showed that the increase of aeration rate could increase the volumetric averaged gas holdup in a rotary drum. JIN et al [12] improved the

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rotating-drum reactor by fitting a modified gas sparger to enhance its gas−liquid mass transfer performance and evaluated it by examining gas−liquid mass transfer, solid particles distribution and power consumption, but its application potential still needs to be verified further by the bioleaching of sulfide minerals.

In this work, the Fe^{2+} oxidation by *At. ferrooxidans* was investigated at different solid content of inert Al_2O_3 powders in rotating-drum and stirred-tank reactors firstly. The effects of Al_2O_3 content, inoculum concentration and baffle angle on the Fe^{2+} biooxidation were probed in the rotating-drum, and the results were compared with those obtained in the stirred-tank. The changes of pH, redox potential (φ_h) and Fe^{2+} concentration were measured in the biooxidation processes of Fe^{2+} for indexing the bioactivity of *At. ferrooxidans*.

2 Experimental

2.1 Al_2O_3 powder

Al_2O_3 powder, with the fraction of more than 93% particles below 135 μm, was added as inert material to study Fe^{2+} oxidation at different solid contents. The Al_2O_3 powder was soaked in and washed several times with acidified distilled water (pH 1.5), and then dried in an oven at 105 °C before the experiments.

2.2 Microorganism and medium

The bacterial strains of *At. ferrooxidans* used in the experiments were obtained from Institute of Microbiology, Chinese Academy of Sciences. The 9K medium developed by SILVERMAN and LUNDGREN [13] was used for the cell growth with the composition as follows: 3 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4·7H_2O$, 0.5 g/L K_2HPO_4, 0.15 g/L K_2SO_4, and 0.01 g/L $Ca(NO_3)_2$, and the Fe^{2+} concentration was controlled by adding $FeSO_4·7H_2O$ according to the experimental schedule. The initial pH of the medium was adjusted to 2.0 with 6 mol/L H_2SO_4. All the reagents used in the experiments were analytical grade. Before the experiments the bacterial strains of *At. ferrooxidans* were cultured in the 9K medium for 3 months by subculturing repeatedly. The inoculum of *At. ferrooxidans* was prepared in an air-lifting reactor with the working volume of 1.8 L, which was then inoculated after culturing for 22 h, respectively, in the rotating-drum and stirred-tank reactors according to the experimental plan.

2.3 Bioreactor configuration

The rotating-drum bioreactor (13 L in working volume) was described by CONG et al [14] and the drum was made of Plexiglas® with a thickness of 10 mm. A schematic diagram of the reactor and its dimensions are shown in Fig. 1(a). Eight straight baffles were arranged at equal distances around the circumference of the bioreactor. To improve the gas−liquid mass transfer performance of the rotating-drum bioreactor, three single channel microfiltration ceramic membranes with a pore size of about 1.0 μm were used as sparger [15], and their relative positions are shown in Fig. 1(a). The oxidation of Fe^{2+} by *At. ferrooxidans* in the bioreactor was examined to evaluate its application potential for bioleaching.

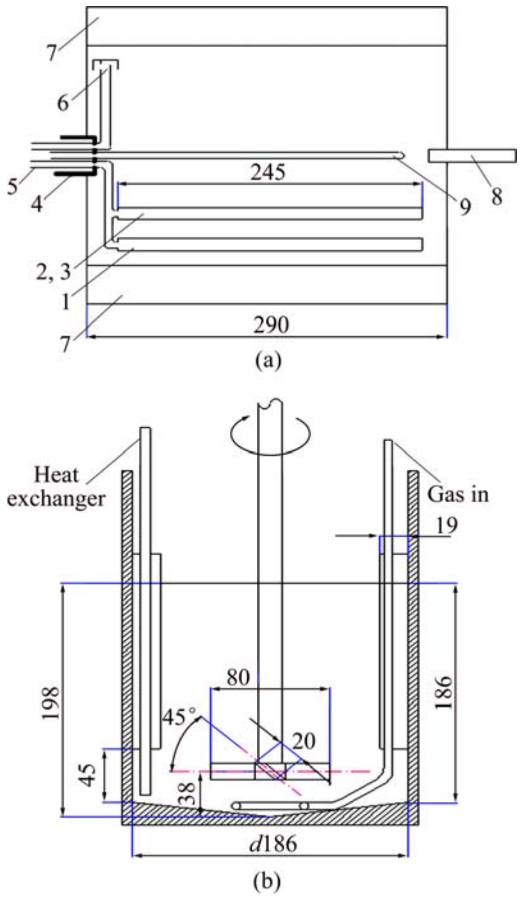

Fig. 1 Schematic diagrams of rotating-drum reactor with fitting gas sparger and stirred-tank reactor (Unit: mm): 1−3—Gas sparger; 4—Stationary shaft; 5—Gas intake; 6—Gas outlet; 7—Baffle; 8—Probe; 9—Heat exchanger

For the sake of comparison, a stirred-tank reactor (4.5 L in working volume), which had 4 baffle plates installed on the inner wall of the stirred-tank (Fig. 1(b)), was also used in the oxidation of Fe^{2+}. A 4-bladed 45° downward pitched blade turbine (impeller diameter of 0.1 m) was installed with off-bottom clearance of 38 mm in order to efficiently suspend the particles. Gas sparging was achieved by using a circular stainless sparger (below turbine) with nine 0.8 mm holes (facing downwards). The temperatures of tap water/slurry in both the rotating-drum and stirred-tank bioreactors were controlled at 35 °C with circulating water from a thermostat during all experiments.

2.4 Experimental procedure

The growth of *At. ferrooxidans* in the rotating-drum and stirred-tank in the absence of Al_2O_3 powder was investigated. The operating conditions in the reactors were pH value of 1.85, inoculum concentration of 10%, temperature at 35 °C and aeration rate of 1 L/(L·min).

Fe^{2+} oxidation by *At. ferrooxidans* was performed at different solid contents by adding different amount of inert Al_2O_3 powder in two reactors. The values of pH, redox potential (φ_h) and Fe^{2+} concentration were monitored during the biooxidation process. The effects of aeration rate and rotational speed of the drum on the distribution of solid particles and gas mass transfer were investigated in the previous work [12]. Unless otherwise specified, the drum rotating speed was 3.33 r/min and the impeller rotating rate in the stirred-tank was 600 r/min with the same aeration rate of 1 L/(L·min) in two reactors. The inoculum concentration was 10% of the total solution volume and the temperature was controlled at about 35 °C.

2.5 Analytical methods

During the oxidation of Fe^{2+} by *At. ferrooxidans*, the values of pH and φ_h were measured by a pH/ORP electrode and the dissolved oxygen (DO) concentration of the systems was monitored by a DO electrode plugged into the reactors. The solution samples were withdrawn at the predetermined intervals. The residual concentration of Fe^{2+} was analyzed by titrating with a standardized $K_2Cr_2O_7$ solution using sodium diphenyl-amine sulphonate as an indicator [16].

3 Results and discussion

3.1 Growth of *At. ferrooxidans*

The growth of *At. ferrooxidans* was compared in the two reactors. The changes in pH, φ_h and Fe^{2+} concentration in the liquid medium in the absence of Al_2O_3 powder are shown in Fig. 2. Compared with the stirred-tank, the rotating-drum has the lower oxidation rate of Fe^{2+} and slower changes of pH and φ_h, which means that the bioactivity of *At. ferrooxidans* is higher in the stirred-tank in the absence of Al_2O_3 powder. The results indicate that the better mixing and oxygen mass transfer can be achieved in the stirred-tank at 600 r/min of the impeller rotational speed, compared with the rotating-drum at 3.33 r/min of the drum rotational speed. It is considered that the fluid shear from rotating impellers even at high speed in the stirred-tank does not affect the bioactivity of *At. ferrooxidans*, which is consistent to the result reported by LIU et al [17].

It is also found from Fig. 2 that the obvious decrease in pH is presented in the latter period of the experiments. The reason for the decrease in pH may be the formation of precipitates such as jarosite or/and iron hydroxide due to the high Fe^{3+} concentration during the later stage of the oxidation process [18]. The related reaction is shown as follows:

$$X^{+}+3Fe^{3+}+2SO_4^{2-}+6H_2O \longrightarrow XFe_3(SO_4)_2(OH)_6+6H^{+} \quad (1)$$

where X=K^+, Na^+, H_3O^+ or NH_4^+. The precipitates can lead to the passivation of the surface of sulfide ores

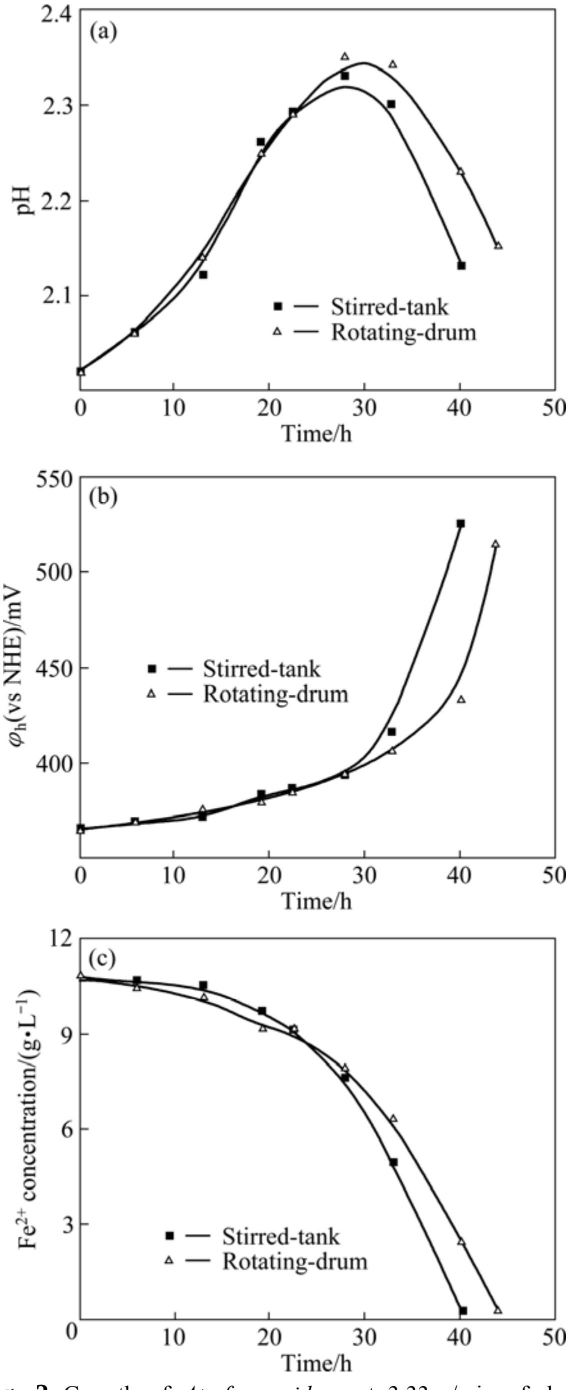

Fig. 2 Growth of *At. ferrooxidans* at 3.33 r/min of drum rotational speed in rotating-drum and at 600 r/min of impeller rotational speed in stirred-tank in absence of Al_2O_3 powder

during the sulfide bioleaching [19]. The decrease in pH value is too little to decrease the oxidation of Fe^{2+} by *At. ferrooxidans* in this work.

3.2 Effect of solid content

The biooxidation of Fe^{2+} by *At. ferrooxidans* at the solid content of 15% and 30% (mass fraction) of Al_2O_3 powder in the two reactors is shown in Fig. 3. The results show that the addition of Al_2O_3 can reduce the apparent bioactivity of *At. ferrooxidans* by prolonging the oxidation period of Fe^{2+} in both the reactors, compared with that in the absence of Al_2O_3 powder. It is found from Fig. 3 that the changes of pH, φ_h and Fe^{2+} concentration in the rotating-drum are larger than those in the stirred-tank, which means that the higher bioactivity of *At. ferrooxidans* can be maintained in the rotating-drum in the presence of Al_2O_3.

However, it is worth noting that the oxidation period of Fe^{2+} at 30% Al_2O_3 content is shorter than that at 15% Al_2O_3 content, and the lag time of Fe^{2+} oxidation at 30% Al_2O_3 is less than that at 0 and 15% Al_2O_3. The possible reasons for the results are that no metal ions are introduced to affect the bioactivity of *At. ferrooxidans* when only adding Al_2O_3 powder, which has less effect on Fe^{2+} oxidation than sulfide minerals. In addition, the state of inoculum can also affect the oxidation of Fe^{2+} by

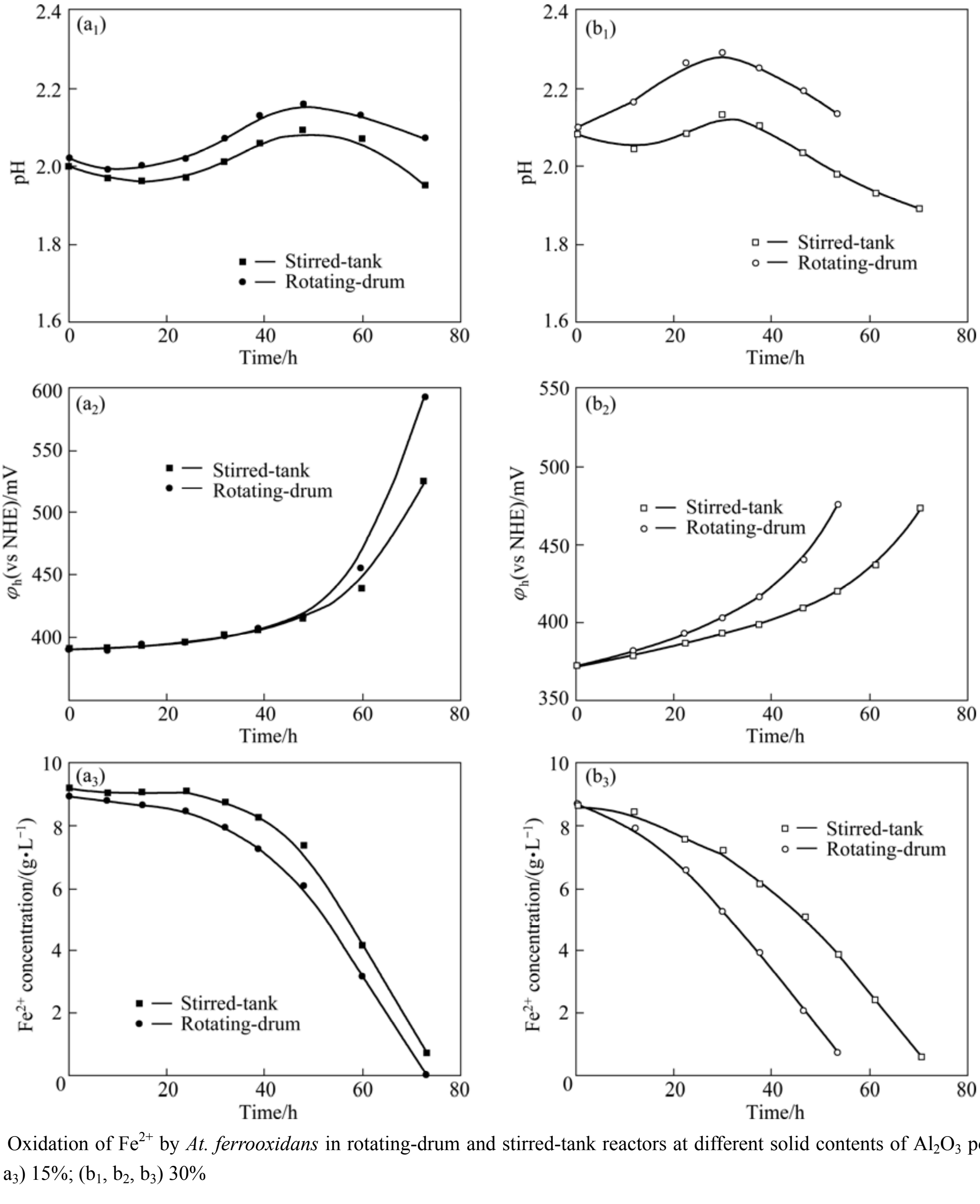

Fig. 3 Oxidation of Fe^{2+} by *At. ferrooxidans* in rotating-drum and stirred-tank reactors at different solid contents of Al_2O_3 powder: (a_1, a_2, a_3) 15%; (b_1, b_2, b_3) 30%

At. ferrooxidans at different solid contents. Compared with the stirred-tank, the rotating-drum gives higher oxidation rate of Fe^{2+} by *At. ferrooxidans* even at higher content of Al_2O_3, which is very favorable to the industrial bioleaching of sulfide minerals where the high solid content is allowed.

3.3 Inoculum concentration

The effects of inoculum concentrations on the biooxidation of Fe^{2+} by *At. ferrooxidans* were examined in two reactors. Under the same content of 30% Al_2O_3, the changes in pH, φ_h and Fe^{2+} concentrations at the inoculum concentrations of 5% and 10% (volume fraction), are shown in Fig. 4. The results indicate that two reactors behave similarly at the same inoculum concentration as pH, φ_h and Fe^{2+} concentration are concerned. Moreover, the lag period for the oxidation of Fe^{2+} increases as the concentration of inoculum decreases from 10% to 5%. The bacterial population available for the oxidation of Fe^{2+} by *At. ferrooxidans* is too low at the inoculum concentration of 5% in the two reactors. It is suitable to increase the inoculum concentration of *At. ferrooxidans* for the Fe^{2+} biooxidation since the advantages of shortened lag phase and increased oxidation rate of Fe^{2+} are favorable to the enhancement of bioleaching of sulfide minerals. The similar results were also reported by WANG et al [20].

3.4 Effect of baffle angle

In order to examine the effect of the inner structure of the rotating-drum on the oxidation of Fe^{2+} by *At. ferrooxidans*, the deflection angle of all baffles was adjusted to about 20° with the same orientation, which leaned backwards to the rotating direction of the drum. The ways to install the baffles with 0º and 20º in the rotating-drum are shown in Fig. 5. The Fe^{2+} oxidation by *At. ferrooxidans* was examined in the rotating-drum after the baffle angle was adjusted to 20° when the Al_2O_3 content increased from 0 to 50%, and the similar experiments were performed in the stirred-tank by adding different amount of Al_2O_3.

The changes of φ_h and Fe^{2+} concentrations under different solid contents of Al_2O_3 during Fe^{2+} biooxidation in the two reactors are shown in Fig. 6. The results from Figs. 6(a_1) and (a_2) indicate that the Fe^{2+} oxidation rate decreases gradually with the increase of solid content of Al_2O_3 and φ_h presents the similar changes in the stirred-tank. However, the changes of Fe^{2+} oxidation and φ_h are not affected markedly by the increase of Al_2O_3 content in the rotating-drum (shown Figs. 6(b_1) and (b_2)). LIU et al [17] confirmed that the main factor to decrease the bioactivity of *At. ferrooxidans* was the collision and friction between solid particles, instead of the fluid shear from the rotating impellers with high speed. It is

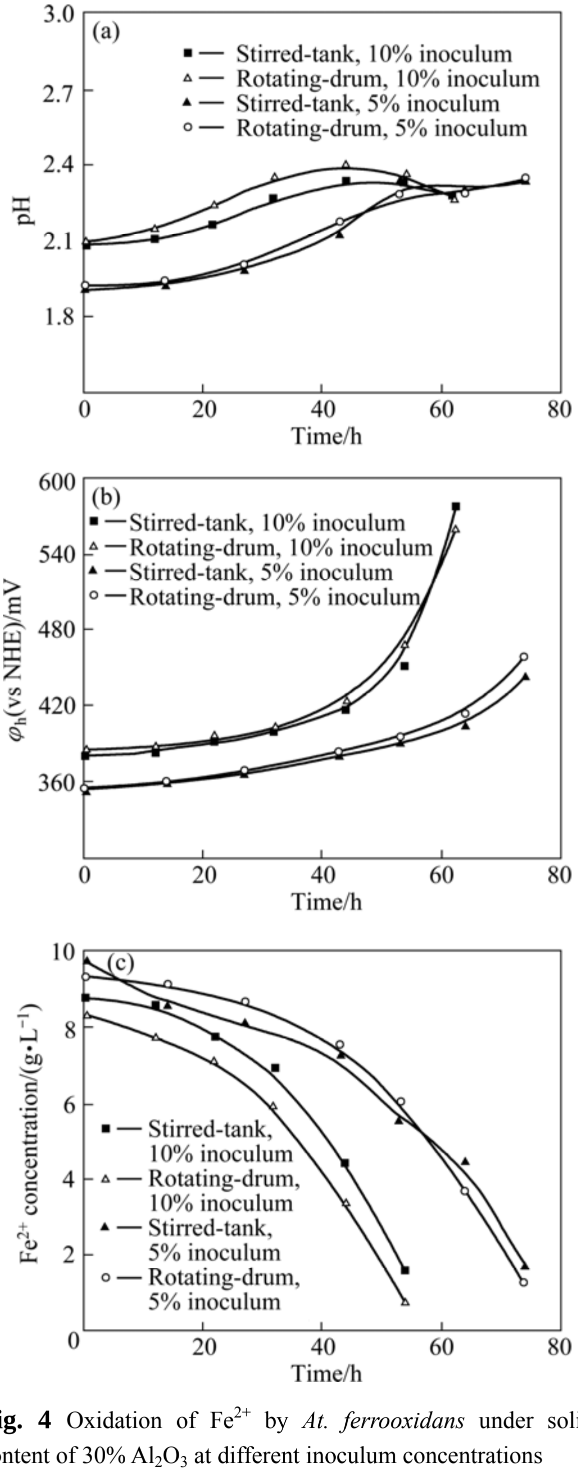

Fig. 4 Oxidation of Fe^{2+} by *At. ferrooxidans* under solid content of 30% Al_2O_3 at different inoculum concentrations

presumed that the collision and friction between solid particles in the stirred-tank increase rapidly with the increase of Al_2O_3 content, which results in the obvious decrease of Fe^{2+} oxidation rate due to the deactivation of *At. ferrooxidans*. The effect of high Al_2O_3 content on the bioactivity of *At. ferrooxidans* in the rotating-drum is lower than that in the stirred-tank due to their different pulp mixing mechanisms, and the rotating-drum reactor is more favorable to the application in sulfide

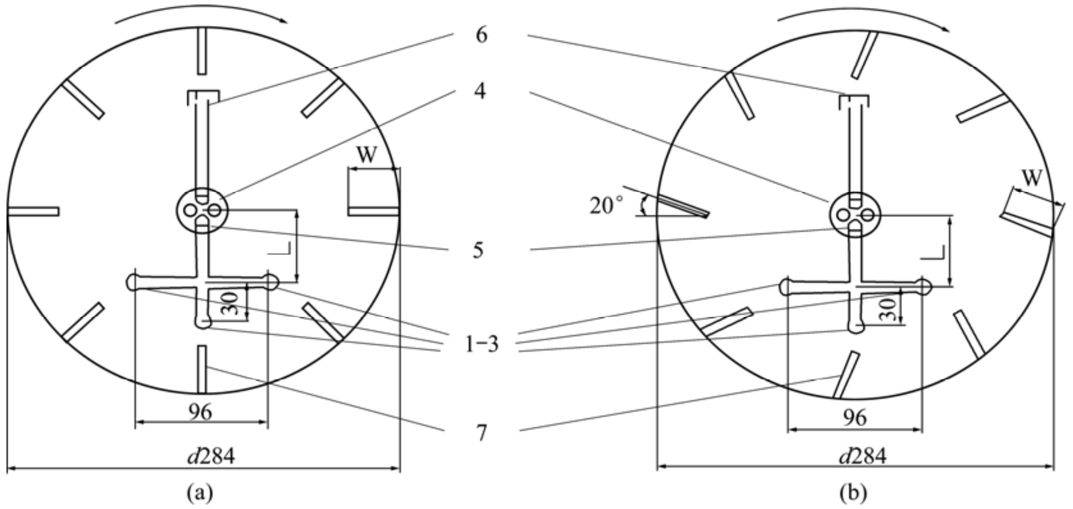

Fig. 5 Cross sections of rotating-drum reactor before (a) and after (b) angle adjustment of baffle plates (Unit: mm): 1−3—Gas sparger; 4—Stationary shaft; 5—Gas intake; 6—Gas outlet; 7—Baffle

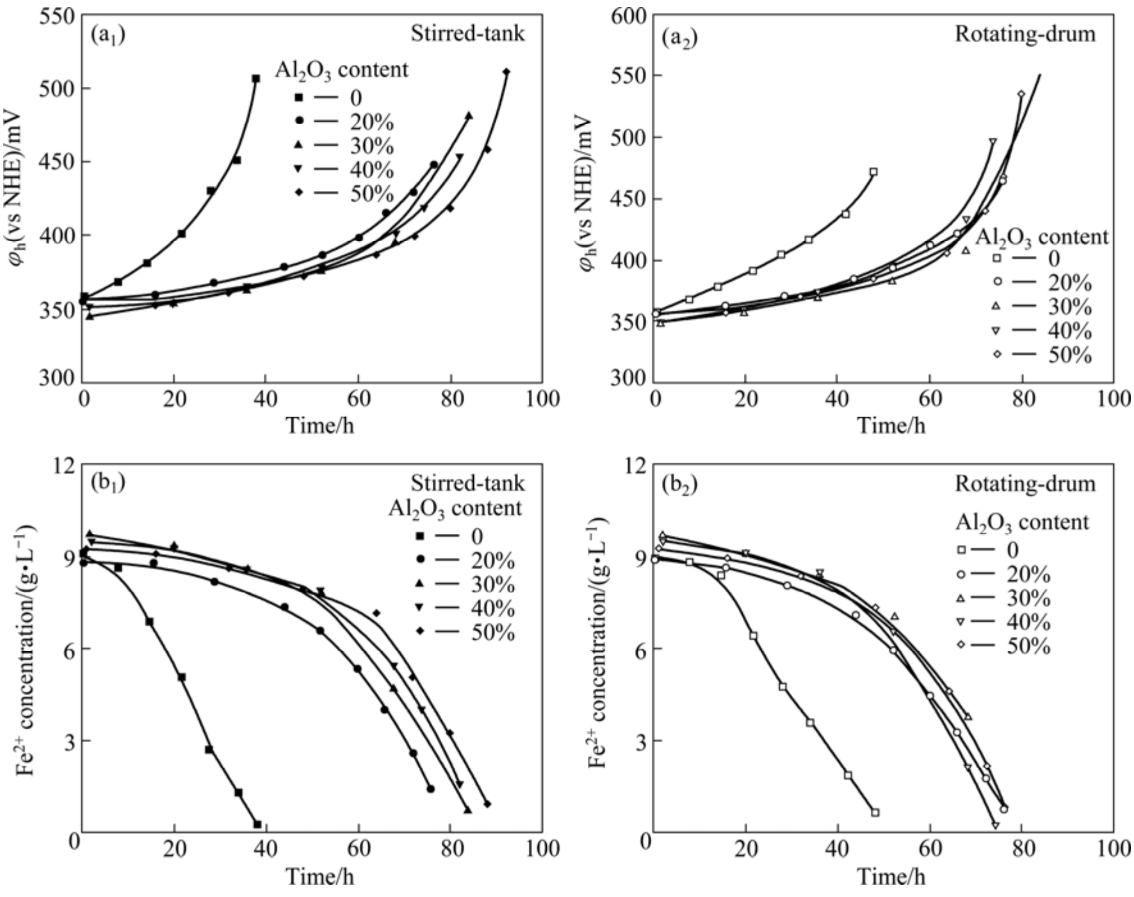

Fig. 6 Changes of φ_h and Fe^{2+} concentrations at different solid contents of Al_2O_3 in rotating-drum and stirred-tank reactors during biooxidation processes of Fe^{2+}

bioleaching for allowing higher solid contents.

In the bacterial oxidation of sulfide minerals, the gas−liquid transfer of O_2 and CO_2 often become the reaction rate determining factor [21]. It is found that the dissolved O_2 level is maintained at 1−3 mg/L at different solid contents, indicating that the dissolved O_2 seems not to be a limiting factor for the oxidation of Fe^{2+} by *At. ferrooxidans* at the aeration rate of 1 L/(L·min) in the two reactors. Although the increase of the solid content can lead to the decrease of dissolved O_2 level in the stirred-tank for deteriorating the slurry mixing and gas-liquid transfer [22], the actual solid content suspended in the medium may be less than the amount of Al_2O_3 added because the particles of Al_2O_3 are not suspended fully in the stirred-tank at 600 r/min of the impeller rotational speed. The dissolved O_2 level in the rotating-drum may be affected slightly by the increase of Al_2O_3 content due to gas pre-dispersion by the modified gas sparger, which is favorable to maintaining the high dissolved O_2 level at high solid content for suppressing the diffusion and coalescence of small bubbles.

The results shown in Fig. 6 also indicate that the oxidation rate of Fe^{2+} by *At. ferrooxidans* decreases with increasing the Al_2O_3 content from 0 to 50%. DEVECI [23,24] showed that the viability of microbes decreased much rapidly when the solid content exceeded 20% in stirred-tank bioreactor. However, the oxidation of Fe^{2+} seems not to be affected markedly as expected when the content of Al_2O_3 powder is higher than 20% in the stirred-tank reactor. The possible reason should be that the impeller speed of 600 r/min (about 2.5 m/s) under aeration might be too low to suspend all the solid particles at high solid content (>30%) in the stirred-tank. Thus, the real solid content of the slurry system is lower than the nominal density calculated by the added amount of Al_2O_3. In contrast, the excellent mixing and gas mass transfer in rotating-drum reactors are easily achieved even at high solid content by optimizing the operating parameters such as the drum rotational speed and aeration rate [12], which do not produce the adverse effect on the bioactivity of microorganisms for different mixing mechanisms of the two reactors.

In order to improve the mixing and gas−liquid mass transfer of the reactor, the deflection angle of all baffles in the rotating-drum was adjusted to about 20º with the same orientation. However, considering the differences of inoculum state and measurement error in batches experiments, it is found that the oxidation of Fe^{2+} by *At. ferrooxidans* is not affected markedly by the angle adjustment of baffle plates in the rotating-drum when comparing the results shown in Figs. 3 and 6. It is presumed that the possible reason is the smaller working volume of the rotating-drum; moreover, the small changes in the actual height of the baffle plates after the angle adjustment of 20º do not markedly affect the performance of mixing and gas−liquid mass transfer in the rotating-drum.

The results in this work show that the oxidation rate of Fe^{2+} by *At. ferrooxidans* in the rotating-drum is higher than that in the stirred-tank, particularly at high solid content. Compared with the stirred-tank, the rotating-drum can provide the excellent mixing and gas mass transfer even at high solid content for their different mixing mechanisms, which means that the higher solid content is allowed in the rotating- drum reactor used in the bioleaching of sulfide minerals for the lower collision and friction of solid particles. However, the effect of scale-up on solids mixing and gas mass transfer efficiency of rotating-drum reactor needs to be further investigated, and the application performance of the reactor should be verified further for the bioleaching of sulfide minerals because of the differences of inert Al_2O_3 powder and sulfide minerals in physicochemical properties.

4 Conclusions

1) A higher bioactivity of *At. ferrooxidans* was observed in the stirred-tank than that in the rotating-drum in the absence of Al_2O_3 powder , but the oxidation rate of Fe^{2+} decreased markedly from 0.23 g/(L·h) to 0.025 g/(L·h) with increasing the content of Al_2O_3 powder from 0 to 50% in the stirred-tank probably due to the deactivation of *At. ferrooxidans* resulting from collision and friction of solid particles.

2) The high content of Al_2O_3 has less effect on the bioactivity of *At. ferrooxidans* in the rotating-drum than that in the stirred-tank due to their different mixing mechanisms. The higher bioactivity of *At. ferrooxidans* can be maintained in the rotating-drum even at the higher Al_2O_3 content, which is favorable to the sulfide bioleaching for allowing the higher solid content.

3) The application performance of rotating-drum reactor for the bioleaching of sulfide minerals still needs further verification because of the property differences of inert Al_2O_3 powder and sulfide minerals.

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转鼓和搅拌槽反应器中氧化亚铁硫杆菌氧化 Fe^{2+} 的比较

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摘 要：研究转鼓和搅拌槽反应器中氧化亚铁硫杆菌在不同 Al_2O_3 粉末含量下对 Fe^{2+} 的氧化。结果表明：未添加 Al_2O_3 粉末时，氧化亚铁硫杆菌在搅拌槽中的生物活性比在转鼓中的生物活性高。当 Al_2O_3 粉末含量从 0 增加到 50% (质量分数)时，Fe^{2+} 的生物氧化速率从 0.23 g/(L·h) 显著降低到 0.025 g/(L·h)，可能是搅拌槽中的固体颗粒碰撞和研磨作用导致氧化亚铁硫杆菌失活。转鼓中 Al_2O_3 的含量增加对氧化亚铁硫杆菌的生物活性仅有较小的负面影响，这是由于两个反应器不同的混合机制所致。在相同的 Al_2O_3 含量下，Fe^{2+} 在转鼓反应器中的生物氧化速率比在搅拌槽中的生物氧化速率更高，尤其在较高的固体含量下，表明转鼓反应器能允许较高的固体含量和维持较高的生物活性。由于 Al_2O_3 粉末与真实硫化矿具有不同的物理化学性质，因此转鼓反应器用于硫化矿生物浸出的可行性还需进一步验证。

关键词：Fe^{2+}；氧化亚铁硫杆菌；氧化；生物活性；固体含量；转鼓反应器；搅拌槽反应器

(Edited by Wei-ping CHEN)