Ferrous Sulphate Oxidation Using *Acidithiobacillus Ferrooxidans* Cells Immobilized in Ceramic Beads

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The immobilization of *Acidithiobacillus ferrooxidans* cells on ceramic beads as carrier is described. The effects of ferrous ion concentration and dilution on the kinetics of ferrous ion oxidation in a packed-bed bioreactor were studied. In a medium containing 13.91 g of ferrous ion per litre, the fastest oxidation rate was 4.21 g L^{-1} at a dilution rate of 0.8 h^{-1}. The corresponding conversion was $X = 70$ %. At ferrous ion mass concentrations greater than $\gamma = 8.34$ g L^{-1} and dilution rates greater than $D = 0.8$ h^{-1}, the rate of ferrous-iron-oxidation reached a plateau or decreased, depending on how much the ferrous ion mass concentration or dilution rate was in excess.

Key words:
Acidithiobacillus ferrooxidans, biooxidation of ferrous ion, ceramic beads, immobilization, packed-bed bioreactor

Introduction

Biooxidation of ferrous ion plays an important role in the mobilization of metals from sulphidic ores. In the process, two mechanisms, indirect and direct mechanisms, are known to be responsible for the biodissolution of sulphide minerals. The direct dissolution of minerals is caused by the attack on sulphide minerals by the enzymatic system of bacteria oxidizing them to sulphates. By the indirect mechanism, the ferric iron produced by microorganisms from oxidation of ferrous iron serves as a leaching agent that reacts chemically with the minerals.[1–4] By the indirect mechanism, the bioleaching process can be divided into two processes: the chemical oxidation of sulphide by ferric iron, and the bacterial regeneration of leach agent. This mechanism suggests that the biological and chemical process can be studied and optimized separately. Biological oxidation of ferrous iron, though very attractive compared with chemical alternatives, because of its lower environmental impact and economic reasons, has not yet been widely used commercially.[5] One of the main reasons is the rate of biological oxidation of ferrous iron. The natural tendency of *Acidithiobacillus ferrooxidans* to grow on surfaces makes it an ideal organism for cell immobilization. Therefore, the immobilization of bacteria is a way to improve the bioleaching rate.[6]

There are many materials that have been used for immobilizing *Acidithiobacillus ferrooxidans* (formally *Thiobacillus ferrooxidans*), which is a widely studied microorganism, using adhesion or entrapment. For example, materials such as iron-exchange resin, activated carbon,[7] glass beads, low grade ore,[8] nicked alloy fibre[9] and siliceous stone particles[10] were used for adhesion of bacteria, whereas materials such as the calcium alginate,[11] agar, κ-carrageean, gerlite[12] and poly(vinyl alcohol) (PVA)[6] were used to entrap the bacterial. Other carriers, such as polyurethene foam,[13] combine the advantages of both adhesion and entrapment. However, none of them is practical on the industrial scale due to the expenditure and low efficiency.

The aim of the work described here was to investigate the continuous oxidation of ferrous iron by bacteria immobilized on a support of ceramic beads in a packed-bed bioreactor. Effects of ferrous iron mass concentration on the process of the oxidation were investigated.

Materials and methods

Microorganism and medium

A strain of *Acidithiobacillus ferrooxidans* from the Beijing Research Institute of Chemical Engineering and Metallurgy was used throughout the study. We used a modification of 9K medium, originally developed by *Silverman* and *Lundgren*.[14]

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Our medium contained 5 g of Fe^{2+} per litre instead of 9 g L^{-1}. The initial pH value of the medium was adjusted to 1.6 using 6 mol L^{-1} H_2SO_4. In order to study the kinetics of biooxidation in the packed-bed bioreactor, different mass concentrations of Fe^{2+} in the form of ferrous sulphate in the range of about $\gamma = 3$–20 g L^{-1} were used.

Ceramic beads

Ceramic beads (particle diameter between d_p = 1.6 and 2.5 mm) were kindly provided by the Beijing Jiarui Environmentel Protection Corporation. The ceramic beads were soaked in an aqueous solution of sulphuric acid having a pH of 2 for 24 h, and then washed until neutral with deionized water.

Packed-bed bioreactor

The bioreactor consisted of a Polymethyl Metacrylat (PMMA) column having a height of h = 36 cm and a diameter of $d = 30$ mm, which was packed with ceramic beads for bacterial support, the PMMA column had an inlet for fresh medium and one for compressed air at the bottom, and one for effluent and exhaust air at the top. The working volume of the bioreactor was $V = 220$ mL and the height of the support bed in the bioreactor was 32 cm, with the operating temperature maintained at $T = 30$ °C using a water jacket. The bioreactor was aerated at $Q = 0.3$ L min^{-1}, and the feed rate of the fresh medium was regulated with a peristaltic pump.

Immobilization procedure

The ceramic beads treated with sulphuric acid were packed into the column. The bacteria were fixed onto the carrier matrix during batch-mode operation of the reactors. The bioreactor was started with a $\varphi = 10$ % inoculation. The number concentration of cell was $C = 1.52 \cdot 10^7$cells mL^{-1}. The culture medium was 9 K medium with $\gamma = 5$ g L^{-1} Fe^{2+} at pH 1.6. The aeration flow rate was set at $Q = 0.3$ L min^{-1}. During the process run in recycling batch mode, the medium in the system was replaced with 500 mL fresh medium when ferrous iron had been oxidized over 95 %. The operation was repeated another five times. Fresh medium was not inoculated except the first time operation. Fig. 1 shows the schematic diagram of the reaction system. During the batch-mode operations, valves 2 and 8 were closed.

Kinetic studies

After the cells were first immobilized and subjected to a recycling batch mode for ferrous-iron oxidation, the bioreactor was then switched to continuous mode and the feeding rate was adjusted by a peristaltic pump. In this time, valve 5 was closed and valves 2, 7, and 8 were opened (in Fig. 1). When the ferrous iron concentration in the effluent stream changed less than 5 %, the bioreactor was assumed to be in steady-state and the rates of ferrous iron oxidation were constant. This process would spend time equal to at least three times the theoretical resident time of the bioreactor. Ferrous and ferric iron concentrations were determined in the process of biooxidation. Experiments were performed with medium containing 3 to 20 g L^{-1} of Fe^{2+}. For each medium, different dilution rates in the range of $D = 0.1$–0.8 h^{-1} were established respectively at each feed rate to estimate the kinetics of ferrous iron oxidation.

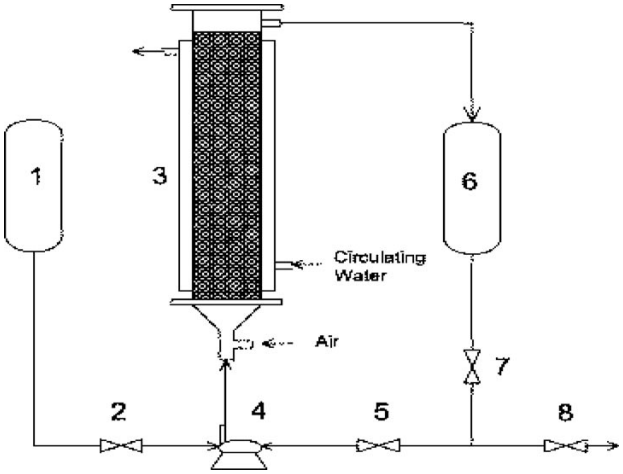


Fig. 1 – *Schematic diagram of the experimental set-up 1. 6. solution reservoir, 2. 5. 7. 8. valves, 3. packed-bed, 4. peristaltic pump*

Analytical methods

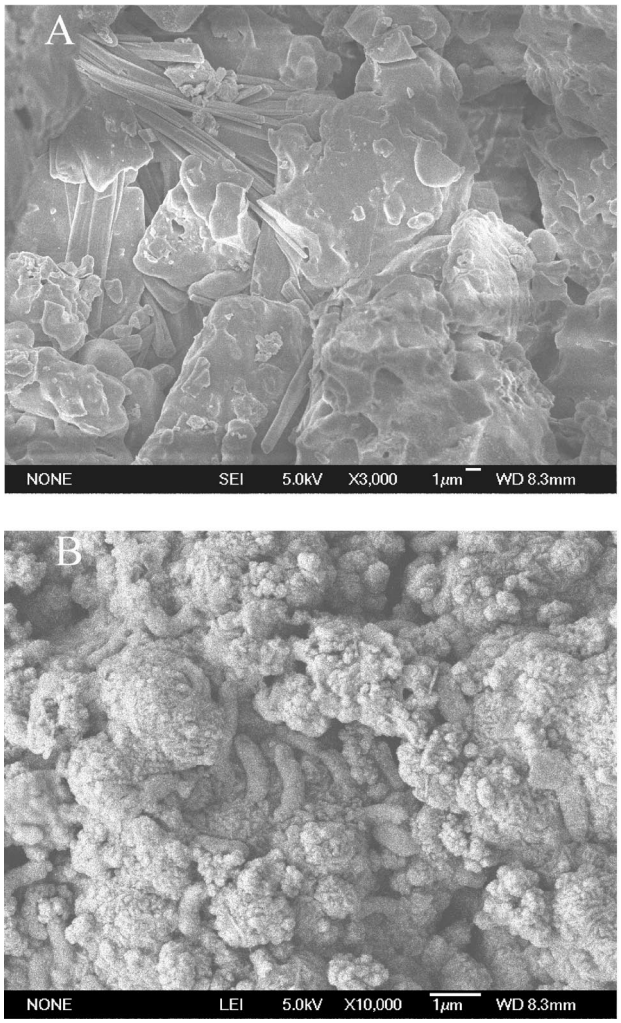
The ferrous iron oxidation rate was monitored by determining ferrous iron concentration at various intervals. The spectrophotometric method of *Herrera* et al.[15] was adopted.

Scanning electron microscopy

Biomass support particles taken from the bioreactor were lightly blotted on quantitative filter paper. The particles were then dried at room temperature (25 °C) for 2 h. The dried samples were coated with gold, and examined using a JEOL JSM-6700F scanning electron microscope.

Results and discussion

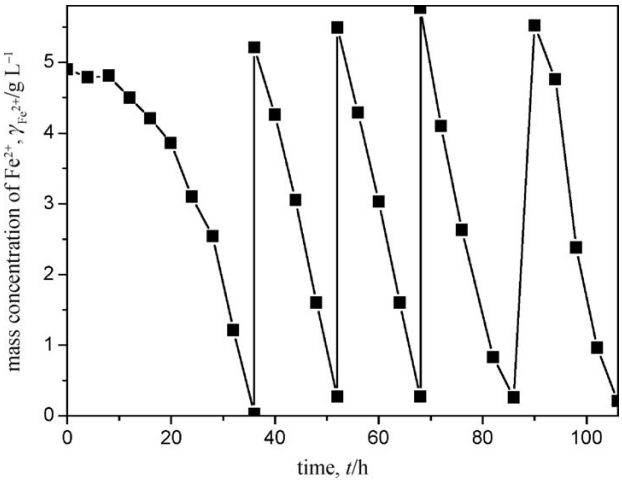
Immobilization of *A. ferrooxidans* on ceramic beads was successfully achieved. Fig. 2 shows that the cells and precipitates accumulated on the sur-



F i g . 2 – *Scanning electron micrographs of biomass support particles removed from the bioreactor. A. fresh support particles, Bar, 1 μm, × 3,000. B. after termination of continuous operation mode, Bar, 1 μm × 10,000.*

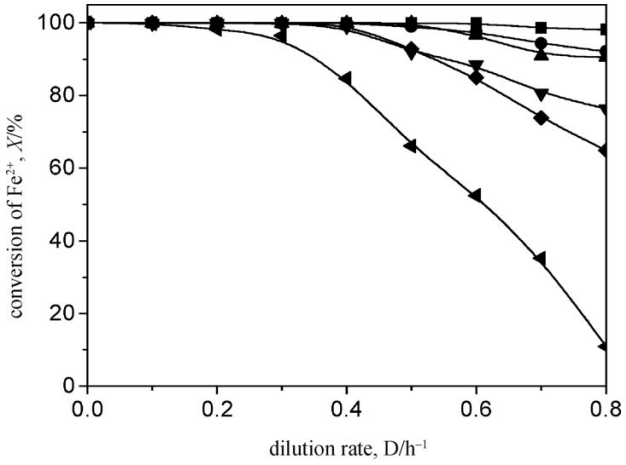
face of the beads. The precipitates promoted the adhesion of *A. ferrooxidans* cell. In fact, the particles were embedded in the aggregate of ferric precipitates and some were even completely covered by these compounds in the column. Some researchers suggested the layer of precipitates could be an effective adsorbent for bacteria.[13] Fig. 3 presents the oxidation of ferrous iron in the column when the medium and cells were in the packed column. In the first recycling batch operation, it took about 36 h to oxidize ~ 95 % of the ferrous iron, whereas in the second and third recycling batch operation it took 20 and 16 h, respectively. In subsequent operations, it took about 16 h for ~ 95 % of the ferrous ion to be oxidized.

It is likely that the *A. ferrooxidans* attached to the ceramic beads because of their natural tendency to adhere to surfaces. The cell adhesion to carrier surface is associated with a microbial aggregate, consisting of microorganisms and extracellular polysaccharide substances, which hold the cells to a surface and each other.[13] First, the bacteria absorb to the surface. Subsequently, the bacteria form bridges of extracellular polymeric substances, binding them to the surface and to each other.[16] Gomez et al. suggested that *A. ferrooxidans* cells combine with the precipitates and adhere to the carrier surface.[9] Although some researchers suggested that the ferric iron hydrates and jarosites played an important role in immobilization of the cell, whether they benefited the activity of the cell should be studied further. The cell combined with or even embedded into the precipitates, but there were still many cells scattered on the surface (in Fig. 3). It is well known that the rough surface was beneficial to cell immobilization. Ceramic beads have a porous surface, large surface area, are inert, as well as low-cost, which is why they represent a suitable support in this type of experiment.

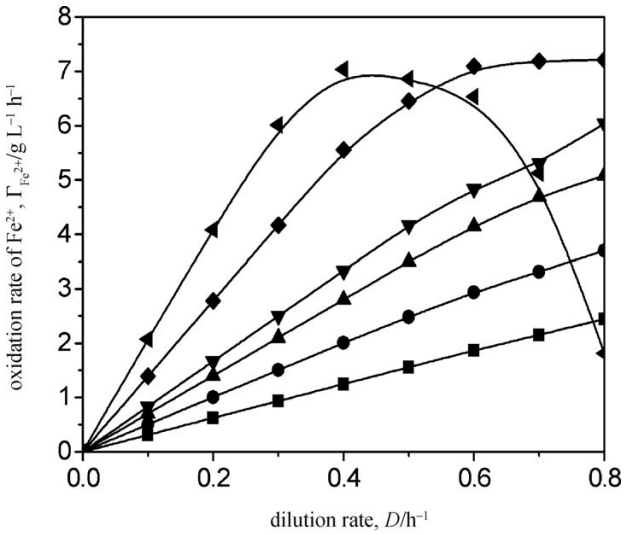


F i g . 3 – *Oxidation of ferrous iron by* A. ferrooxidans *in recycling mode*

After a constant rate of ferrous iron oxidation was achieved in the successive medium-recycling batch runs, the reactor was transferred into continuous operation. The continuous operation was carried out at different dilution rates (D) based on the total volume of the bioreactor. The relationships of conversion (%) ferrous iron oxidized to dilution rate, and the rate of ferrous iron oxidation to dilution rate are shown in Figs. 4 and 5, respectively. Figure 4 shows that increasing dilution rate led to a decrease in conversion (%) ferrous iron oxidized. As can be seen, when the mass concentration of the Fe^{2+} was low, at most up to $\gamma = 7.01$ g L^{-1}, even at a high dilution rate conversion of ferrous iron was achieved. When the mass concentration of ferrous iron in the feed solution was raised to $\gamma = 13.91$ g L^{-1}, the oxidation rate improved up to $\Gamma = 7.21$ g L^{-1} h^{-1},



F i g 4 – *Continuous oxidation of ferrous iron by immobilized A. ferrooxidans with different ferrous iron mass concentrations in the packed-bed bioreactor showing the effect of dilution rate on the conversion of ferrous iron.* ■*: 3.12 g* L^{-1}, ●*: 5.02 g* L^{-1}, ▲*: 7.01 g* L^{-1}, ▼*: 8.34 g* L^{-1}, □*: 13.91 g* L^{-1}, ◀*: 20.78 g* L^{-1}

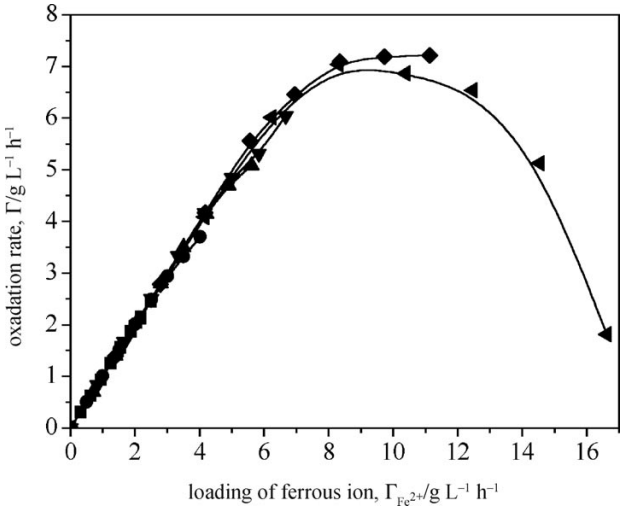


F i g . 5 – *Continuous oxidation of ferrous iron by immobilized A. ferrooxidans with different ferrous iron mass concentrations in the packed-bed bioreactor showing the effect of dilution rate on the oxidation rate of ferrous iron.* ■*: 3.12 g* L^{-1}, ●*: 5.02 g* L^{-1}, ▲*: 7.01 g* L^{-1}, ▼*: 8.34 g* L^{-1}, □*: 13.91 g* L^{-1}, ◀*: 20.78 g* L^{-1}

but the conversion was only around 70 %. Fig. 5 shows that at low dilution rates the oxidation rate increased proportionally with the dilution rate. The highest oxidation rate obtained in this group of experiments was $\Gamma = 7.21$ g L^{-1} h^{-1} at a dilution rate of $D = 0.8$ h^{-1} with a medium containing 13.91 g L^{-1} ferrous iron. When a medium containing $\gamma =$ 8.34 g L^{-1} or less ferrous iron was applied, the oxidation rate was proportional to the dilution rate of the bioreactor. For a medium containing mass concentration of ferrous iron beyond $\gamma = 13.91$ g L^{-1} the ferrous iron oxidation rate reached a plateau and decreased with further increases in the mass concentration of ferrous iron in the feed solution. In addition, when the ferrous iron in the feed solution was $\gamma = 20.78$ g L^{-1}, there was a dramatic decrease in the conversion and the oxidation rate of ferrous iron with increasing the dilution rate.

In our study, the oxidation rate achieved at $\Gamma = 7.21$ g L^{-1} h^{-1} when ferrous iron concentration mass in the feed solution was $\gamma = 13.91$ g L^{-1} and the dilution rate was $D = 0.8$ h^{-1}. Under these conditions, 70 % of ferrous iron in the feed solution was oxidized. This oxidation rate was higher than those reported by others. For instance, with the glass beads and activated carbon as the carrier, the oxidation rate achieved $\Gamma = 0.6$ and 3.1 g L^{-1} h^{-1}.[7,8] Using the ion-exchange resin as carriers, the oxidation rate of 3.3 g L^{-1} h^{-1} was observed,[8] and with Poly (vinyl alcohol) cryogel an oxidation rate of $\Gamma = 3.1$ g L^{-1} h^{-1} was determined.[6] While polyurethene foam BSP covered with activated carbon as carriers, an oxidation rate of $\Gamma = 21.9$ g L^{-1} h^{-1} was achieved, but only 21 % of the ferrous iron was oxidized.[13]

The kinetic results for continuous oxidation of ferrous iron at different mass concentrations of ferrous iron are presented in Fig. 6. As can be seen, when the loading of ferrous iron was less than 1.7 g L^{-1} h^{-1}, increasing the loading of ferrous iron resulted in a linear increase of the oxidation rate. From the data presented in Figs. 5 and 6, one should note that the maximum oxidation rates of ferrous iron were observed at a ferrous iron loading of 8.3 g L^{-1} h^{-1}, corresponding to a dilution rate of 0.4 h^{-1} when the medium contained ferrous iron



F i g . 6 – *Continuous oxidation of ferrous iron by immobilized A. ferrooxidans with different ferrous iron mass concentrations in the packed-bed bioreactor showing the effect of loading rate on the oxidation rate of ferrous iron.* ■*: 3.12 g* L^{-1}, ●*: 5.02 g* L^{-1}, ▲*: 7.01 g* L^{-1}, ▼*: 8.34 g* L^{-1}, □*: 13.91 g* L^{-1}, ◀*: 20.78 g* L^{-1}

13.91 and 20.78 g L^{-1}. This was the critical loading of ferrous iron and further increase beyond this value resulted in the inhibition of activity of *A. ferrooxidans*.

It is well known that the growth rate and the oxidation rate of *A. ferrooxidans* are influenced by the concentration of ferrousiron. *Kelly* and *Jones* reported that the activity of cells was inhibited when the ferrous iron mass concentration was 5.6 g L^{-1} or higher.[17] *Barron* and *Luecking* achieved maximum growth of *A. ferrooxidans* in a medium containing 2–3 g L^{-1} of Fe^{2+} and growth inhibition occurred at higher concentrations.[18] *Nikolov* and *Karamanev* also found the inhibitory effect of ferrous iron when they studied *A. ferrooxidans* oxidation ferrous iron reaction kinetics by re-suspension of the bacteria obtained from a biofilm reactor.[19] Our kinetic results with media containing different concentration of Fe^{2+} showed that the activity of immobilized cells of *A. ferrooxidans* were also affected by the concentration of ferrous iron. The activity of ferrous iron oxidation by immobilized *A. ferrooxidans* was unaffected by the mass concentration of ferrous iron in the range of $\gamma = 0$–8.34 g L^{-1}. However, increasing the mass concentration beyond 8.34 g L^{-1} resulted in a decrease in the oxidation rate.

Conclusion

The kinetic study of this work showed that *A. ferrooxidans* could be immobilized on ceramic beads in a packed-bed reactor, resulting in an improved oxidation rate of ferrous iron by the organism. After the bacteria were immobilized, in continuous operation with a packed-bed bioreactor, a maximum ferrous iron oxidation rate of $\Gamma = 7.21$ g L^{-1} h^{-1} was achieved at a dilution rate of $D = 0.8$ h^{-1} or higher, and 70 % of the ferrous iron was oxidized. The work volume of the bioreactor was $V =$ 220 mL. The reliable performance and the oxidation rate of ferrous iron confirmed the suitability of ceramic beads as carriers for *A. ferrooxidans* immobilization.

ACKNOWLEDGEMENT

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List of symbols

C – number cell concentration, cell mL^{-1}

c – (amount) concentration, mol L^{-1}

D – dilution rate, h^{-1}

d – diameter, mm

d_p – particle diameter, mm

h – height, mm

Q – volume flow rate, L min^{-1}

T – temperature, °C

t – time, h

V – volume

X – conversion, %

Γ – oxidation mass rate, g L^{-1} h^{-1}

γ – mass concentration, g L^{-1}

φ – volume fraction, %

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