

Ferrous ion oxidation by *Thiobacillus ferrooxidans* immobilized on activated carbon

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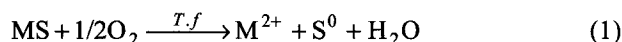
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Abstract: The immobilization of *Thiobacillus ferrooxidans* on the activated carbon particles as support matrix was investigated. Cycling batch operation results in the complete oxidation of ferrous iron in 8 d when the modified 9 K medium is set to flow through the mini-bioreactor at a rate of 0.104 L/h at 25 °C. The oxidation rate of ferrous iron with immobilized *T. ferrooxidans* is 9.38 g/(L·h). The results show that the immobilization of *T. ferrooxidans* on activated carbon can improve the rate of oxidation of ferrous iron. The SEM images show that a build-up of cells of *T. ferrooxidans* and iron precipitates is formed on the surface of activated carbon particles.

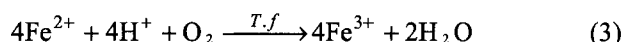
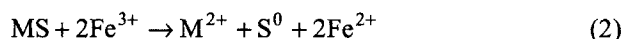
Key words: *Thiobacillus ferrooxidans*; immobilization; activated carbon; biooxidation; precipitate; bioreactor

1 Introduction

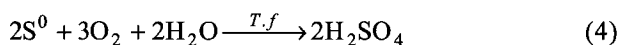
One of the main applications of biotechnology to hydrometallurgy is based on the ability of bacteria such as *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans* to oxidize sulfide minerals to soluble components[1]. Both direct and indirect mechanisms of bacterial leaching have been proposed. In direct mechanism, the bacteria attach on the surface of the mineral, and attack the mineral at the point of attachment by the enzymatic system of bacteria, oxidize them to sulfate. In indirect mechanism, the role of bacteria is to oxidize ferrous ions to ferric ions, and the ferric ions serve as a strong leaching agent of sulfate in the minerals. The direct mechanism of bacterial leaching sulfide mineral can be represented by the following overall reaction:



Whereas the indirect mechanism requires the presence of ferric ions in solution:



In both cases, the bacteria can oxidize elemental sulfur to sulphate acid:



At the iron oxidation step, a high density of cells of bioleaching bacteria is essential for rapid oxidation. For this reason, many studies of *T. ferrooxidans* have been carried out to improve the rate of Fe(II) oxidation.

The immobilization technology of cells and enzymes has rapidly developed and has many applications[2]. The immobilization of cells is characterized by maintaining a stable high density of cells in or on the support matrix. There are many matrices used to immobilize the cells of *T. ferrooxidans* by means of adhesion and entrapment[3,4]. For example, sand[5], ion-exchange resin, activated carbon[6], nickel alloy fibre[7] and low-grade ore[8] have been used as adhesion matrices, and agar, polyurethane foam[9], calcium alginate[10], κ -carrageenan and gerlite have been used as entrapment matrices[8].

Because the immobilization of *T. ferrooxidans* can increase the density of cells and improve the oxidative rate of Fe(II) and sulfide, it has been applied to treat acid mine-drainage, bioleach metal sulfide and remove H₂S from sour gases.

In order to improve the kinetics of bacterial leaching

of sulfide concentrates such as sphalerite and chalcopyrite, the IBES (Indirect Bioleaching with Effects Separation) can be separated into chemical and biological reactions using different reactors so that both stages can be individually optimized[11–13]. In the stage of chemical oxidation of metal sulfide, ferric irons are the lixiviant agent (Eqn.(2)). In the stage of biological reaction, the bacteria oxidize ferrous iron and elemental sulfur (Eqns.(3) and (4)) to ferric iron and sulphuric acid respectively. The regenerated ferric iron is then used in new chemical oxidation.

The aim of this study is to determine the effect of activated carbon used for immobilization of *T. ferrooxidans* on the rapid iron oxidation.

2 Experimental

2.1 Microorganism and culture medium

The bacterial strain used in this study (*T. ferrooxidans*) was obtained from the Dabaoshan Mines of Guangdong, China. *T. f* culture was maintained on modified 9K medium with a reduced iron content: $(\text{NH}_4)_2\text{SO}_4$ 3.0 g/L; K_2HPO_4 0.5 g/L; KCl 0.1 g/L; $\text{Ca}(\text{NO}_3)_2$ 0.01 g/L; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 22.5 g/L. The bacterial cultures were activated and maintained at 30 °C using a bacterial incubator shaker at 200 r/min. Subcultures were carried out every 3 d to keep the bacteria in the logarithmic phase of growth.

2.2 Support material

The granular activated carbon (2–4 mm size fraction) was used as the immobilization matrix. The activated carbon immobilizes *T. ferrooxidans* by adhesion.

2.3 Immobilization procedure

Cells of *T. ferrooxidans* were immobilized on activated carbon according to following procedure: 25 g activated carbon which was washed with H_2SO_4 solution of pH 1.5 was added into a 500 mL flask containing 240 mL 9K medium and 10 mL *T. ferrooxidans* culture in the logarithmic phase of growth. The flask was shaken at 200 r/min on an orbital shaker at 30 °C. When total ferrous iron oxidation conversion was obtained, the flask was drained and the media was replaced with 250 mL fresh 9K medium. Several batches were carried out until the speed of ferrous iron oxidation was stable[14].

2.4 Analytical method

The oxidation of ferrous sulfate by *T. ferrooxidans* was monitored by determining the remainder ferrous iron concentration at various intervals. The method of potassium bichromate titration was used. 2 mL sample was placed in a 50 mL beaker. Then 10 mL of 30%

mixture acid of phosphoric acid and sulfuric acid with ratio of 1:1 was added. This sample was titrated with potassium bichromate until the solution changed in color from achromaticity to purple. A calibration curve of known Fe(II) concentration was used to calculate the iron concentrations. The concentration of Fe(III) was estimated by subtracting the Fe(II) concentration measured regularly at an interval throughout the leaching period from the total iron concentration.

2.5 Mini-reactor experiment

25 g of inoculated activated carbon was packed into a glass column of 5.2 cm in length with a diameter of 4.1 cm. 5 L of 9K medium containing 110 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was set to flow through the column at a rate of 0.104 L/h at 25 °C. The working volume in column was 12.5 mL. The ferrous sulfate oxidation by immobilized *T. ferrooxidans* was monitored by measuring ferrous concentration. The experiment continued until the rate of ferrous oxidation was 100%.

2.6 Preparation of samples for SEM observation

The activated carbon samples were removed from mini-reactor in 1 cm below the top at the termination of experiment. The samples were gently washed with sulfuric acid of pH 2.0 and fixed for 1 h with 2.5% glutaraldehyde dissolved in 0.05 mol/L sulfuric acid. After fixation, the samples were rinsed for three times with sulfuric acid of pH 2.0, followed by dehydrated and air-dried. The samples were then fixed on specimen stubs, gold-coated and then observed with SEM at 30 kV.

3 Results and discussion

3.1 Liquid culture oxidation

In order to study the oxidation of ferrous sulfate by immobilized cells of *T. ferrooxidans* on activated carbon, the liquid culture oxidation was carried out in a 250 mL flask containing 100 mL 9K medium (Fe(II) concentration is 4.5 g/L) with 10%(volume fraction) inoculum. The original density of *T. ferrooxidans* was about 5.0×10^6 cells/mL. The culture was incubated at 30 °C on a rotary shaker at 200 r/min. As shown in Fig.1, it needed 28 h for all ferrous irons to be oxidized, and the density of bacteria reached 5.2×10^7 cells/mL in the logarithmic phase of growth.

When the ferrous iron was completely oxidized, the culture was filtered using Millipore filter of 0.22 μm . The used filter was rinsed with 10 mL uninoculated 9K medium in order to collect the total cells of *T. ferrooxidans*. The number of cells in the logarithmic phase of growth in 100 mL 9K medium was about 5.2×10^9 cells. After this 10 mL inoculum (7.5×10^9 cells) was placed in a 250 mL flask containing 90 mL 9K medium

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