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Immobilization of *Acidithiobacillus ferrooxidans* with complex of PVA and sodium alginate

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Abstract

A new *Acidithiobacillus ferrooxidans* cell immobilization technique utilizing the complex of PVA solution and sodium alginate solution crosslinked by $Ca(NO_3)_2$ as entrapment medium is reported. The mixture of *A. ferrooxidans* suspension and the entrapment complex were extruded into a solution of $Ca(NO_3)_2$ (1–5%) to form beads, then the beads were frozen at −20 °C for 1–2 days and thawed at room temperature. The forming mechanism, characteristic of this immobilized beads and the factors affecting activity of immobilized cells were also discussed. A maximum oxidation rate of 4.6 g Fe^{2+}/(L h) was achieved in batch cultures by these immobilized cells. Precipitation formed during culture process was analyzed. The forming mechanism of this precipitation and how this precipitation affects the whole system were also discussed. In addition, the immobilization technique is operated simply, and the gel beads have high stability even under non-sterile conditions. So its application on an industrial scale would be more practicable.

Keywords: *Acidithiobacillus ferrooxidans*; Poly vinyl alcohol (PVA); Sodium alginate; Precipitation

1. Introduction

Acidithiobacillus ferrooxidans is an obligate chemolithotrophic bacterium, which has the ability to oxidize ferrous ions to ferric ions, a reaction that supplies energy for the growth of *A. ferrooxidans*. So the bacteria is particularly suited for bioleaching of minerals since the bioleaching of sulphide occurs via two steps: the chemical oxidation of the sulphide by Fe^{3+} and the bacterial regeneration of Fe^{3+} as leaching agent [1]. The bioreaction is widely used for the treatment of acid mine drainage [2] and hydrogen sulphide gases [3], however, the slow reaction rate of bioleaching limits its application commercially.

The biological oxidation rate can be achieved with immobilized *A. ferrooxidans*. Therefore, immobilized *A. ferrooxidans* is widely studied in attempts to increase ferrous ion oxidation rate [4].

Although various natural and synthetic polymers have been used, including ion-exchange resin, activated carbon [5], polyurethane foam BSP [6], nicked alloy fiber [7], sand [8], calcium alginate [4], agar, κ-carrageean and gerlite [9], each has its drawbacks. Natural polymers (agar, κ-carrageean, gerlite, calcium alginate) possess poor mechanical strength and durability. Conversely, synthetic polymers have strong mechanical strength and durability but are often toxic to microorganisms. The maximum oxidation rate which has been reported is not more than 3.3 g Fe^{2+}/(L h). The slow kinetics restricts its commercial application greatly.

Recently, a promising type of synthetic polymer, poly vinyl alcohol (PVA), which is cheap and nontoxic to microorganisms, has been used for cell immobilization. A simple and economical technique of cell immobilization with PVA is PVA–boric acid method. But the maximum biological oxidation rate which has been reported for the methods is only

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1.89 g Fe^{2+}/(L h) [10], and two potential problems with this technique, however, are the agglomeration of PVA gel beads and the toxicity of saturated boric acid which have not been completely eliminated until now [11].

In this research, a new *A. ferrooxidans* cell immobilization technique using the complex of PVA solution and sodium alginate solution crosslinked by $Ca(NO_3)_2$ rather than saturated boric acid and $CaCl_2$ solution is described. The combination of sodium alginate and PVA has already been reported in PVA–boric acid method, but usually the concentration of sodium alginate is below 0.4%. Generally, sodium alginate used with such low concentration would just play a role of preventing the agglomeration of PVA gel beads [12], which has nothing to do with the improvement of PVA network structure. In this study, the concentration of sodium alginate in complex was enhanced to 0.9%, and $Ca(NO_3)_2$ was firstly taken as both crosslinking agent and coagulating agent. The technique, which has not been previously reported, can not only avoid the agglomeration of PVA gel beads and the toxicity of saturated boric acid simultaneously but also could efficiently improve and control PVA network structure. This kind of three-dimensional structure would substantially benefit the growth of *A. ferrooxidans*, the transfer of substrate, and greatly promote biological oxidation rate. The main objective of this research was to investigate the feasibility of this method and the biooxidation capacity of Fe^{2+} by immobilized cells, at the same time, to discuss the factors affecting activity of immobilized cells and precipitation behavior during culture process.

2. Materials and methods

2.1. Microorganism and medium

A strain of *A. ferrooxidans* (originally isolated from acid mine drainage by researchers from *College of Life Science, Lanzhou University*) was used throughout the study. The 9K medium with a reduced iron content (10.5 g $Fe2^{+}$/L) was employed. The pH was adjusted to 1.8 with diluted H_2SO_4. The purity of $FeSO_4 \cdot 7H_2O$ was above 99%, whereas all other reagents were of analytical scale.

2.2. Preparation of cell suspension

A. ferrooxidans was grown in 2 L 9K medium and shaken at 150 r/min on an orbital shaker at 31 °C for 1–3 days until the media changed in color from pale green to orange/red. After cultivation, the media was centrifuged at 1500 r/min for 10 min to remove unsolvable substance. Then, the cells were harvested by centrifugation at 12,000 r/min for 15 min. At last the cell pellets were washed for three times with dilute H_2SO_4 (pH 2.0) and the volume of cells' suspension was about 30 mL.

2.3. Immobilization procedure

PVA, 27 g, with 1800 ± 100 degree of polymerization and 2.7 g sodium alginate were diluted with deionized water to 270 mL and heated until dissolved. The solution was cooled down and then mixed with 30 mL of *A. ferrooxidans* suspension. The final mixture contained about 9.0% PVA, 0.9% sodium alginate with 10^9 *A. ferrooxidans* cells per mL. Four different crosslinking methods were used to form four different kinds of PVA beads for comparison in this study. The mixture was extruded as drops (4.0 mm in diameter) into a solution of calcium nitrate (1–5%) and calcium chloride (1–5%), then immersed for 1 h to form PVA–calcium nitrate beads (group C_1) and PVA–calcium chloride beads (group C_2). Then they were frozen at −20 °C for1–2 days. During this period they were thawed at −1 to 3 °C after been frozen for about 12 h. Finally, the beads were thawed at room temperature for further use. PVA–boric acid beads (group C_3) were prepared by dripping the mixture into saturated boric acid solution and immersing for 1 h. Phosphorylated PVA beads (group C_4) were prepared by dripping the mixture into saturated boric acid solution for 20 min and then transferring the beads to a 1.0 M sodium dihydrogenphosphate solution and immersing for 1 h [13]. The beads were washed with deionized water for several times and then stored at 4 °C until further use.

2.4. Relative mechanical strength test

A 4-blade 45 °C turbine and a cylindrical beaker with four baffles were used to determine the relative mechanical strength of the PVA beads [14]. The beaker was 12 cm in diameter, 17 cm in height, and divided into four equal regions by the baffles (1.1 cm wide each). Fifty PVA–calcium nitrate beads (group C_1) or others were added to the beaker and the water level was adjusted with deionized water to 5 cm in height. The agitation speed was determined by a tachometer (LutronDT-2234A) and controlled from 500 r/min to 3000 r/min. The PVA beads were agitated in the beaker for 5 min and the surviving beads were counted. Each run was performed in triplicate.

2.5. Biological activity test

Four different PVA beads weighted 12 g, respectively, were inoculated to four shake flasks of 500 mL, each containing 250 mL 9K culture medium (Fe^{2+} concentration was 10.5 g/L) and were cultured at 31 °C. Three days later, the cultures were inoculated into fresh medium. Several consecutive batches were run on a "draw and fill" basis until the formation of minor amounts of pale yellow precipitation that adsorbed on immobilized beads, which implied that *A. ferrooxidans* has passed the lag phase. Then the PVA beads were transferred into fresh 9K medium and Fe^{2+}concentration was determined at intervals. The beads of each group were well proportioned in size, with a working volume of 20 mL. Fe^{2+} oxidation rate was taken as the symbol of biological activity.