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Bioleaching of copper oxide ore by *Pseudomonas aeruginosa*

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Abstract: Bioleaching is an environmentally friendly method for extraction of metal from ores. In this study, bioleaching of copper oxide ore by *Pseudomonas aeruginosa* was investigated. *Pseudomonas aeruginosa* is a heterotrophic bacterium that can produce various organic acids in an appropriate culture medium, and these acids can operate as leaching agents. The parameters, such as particle size, glucose percentage in the culture medium, bioleaching time, and solid/liquid ratio were optimized. Optimum bioleaching conditions were found as follows: particle size of 150-177 μm, glucose percentage of 6%, bioleaching time of 8 d, and solid/liquid ratio of 1:80. Under these conditions, 53% of copper was extracted.

Keywords: copper ore treatment; copper oxides; bioleaching; organic acids; heterotrophic bacteria; *Pseudomonas aeruginosa*

1. Introduction

Copper extraction by bioleaching has begun since the 1950s, and the first commercial application of this method has been used in Kennecotte Copper [1]. Bioleaching is a good method for copper extraction from low-grade and complex ores [2]. Also, some studies were performed for copper extraction from concentrated ores [3]. The use of bioleaching is growing fast due to its many advantages. Some of these advantages are given as follows: it is an environmentally friendly process and produces less air and water pollution, less energy consumption, low capital investment, and, overall, it is an efficient and economic method in comparison to conventional methods [1-5]. Copper extraction, refractory gold pretreatment, and cobalt extraction are some commercial applications of bioleaching [1,6].

After the exploration of iron and sulfur oxidizing bacteria (like *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*), the application of these bacteria in bioleaching and biooxidation of sulfide ores was investigated, and then, bioleaching was used on a commercial scale. Bioleaching of sulfide copper and other metal ores (especially chalcopyrite and chalcocite) has been studied by many researchers [6]. Watling [5] has reviewed the bioleaching of sulfide minerals with emphasis on copper sulfides.

Bioleaching of oxide ores can be performed by heterotrophic microorganisms. These microorganisms need a source of organic carbon to grow up and in appropriate conditions produce various organic acids. These acids operate as a leaching agent. In published studies, the extraction of metals, such as zinc, nickel, copper, and also radionuclide from contaminated soil, was investigated by fungi like *Penicillium simplicissimum* and *Aspergillus niger* [7-11].

The aim of this study was to investigate the feasibility of copper oxide ore bioleaching by *Pseudomonas aeruginosa* and the optimization of bioleaching conditions. The effects of parameters, such as particle size, glucose percentage in the culture medium, bioleaching time, and solid/liquid ratio in copper extraction, were investigated, and bioleaching conditions were optimized.

2. Materials and methods

2.1. Ore sample

A representative sample of copper oxide ore from Taroom Copper Mine in Zanjan, Iran, was used in these studies. X-ray diffraction (XRD) analysis of the head sample indicated that quartz, oligoclase, chlorite, malachite, laumontite, and illite were the main minerals in the sample. The ore was crushed, ground, and then screened using the ASTM standard sieves series. Chemical analysis of the

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sample was performed by atomic absorption spectroscopy (AAS). The copper content in each particle size fraction is shown in Table 1.

Table 1. Copper content in each particle size fraction

Particle size fraction / μm	Copper content / wt%
−105	3.4
105-150	3.1
150-177	3.2
+177	3.2

2.2. Analysis

The mineralogical composition of the ore sample was obtained using a Philips 1140 X-ray diffractometer (K_α radiation: 0.154 nm, 40 kV, 30 mA). The copper content of the ore sample and working solutions was performed by a Unicam 939 spectrophotometer, and the morphological study of the ore sample and bioleaching residue was carried out by XL30 Philips scanning electron microscopy (SEM).

2.3. Bacterial strain and culture medium composition

A strain of *Pseudomonas aeruginosa* (PTCC 1074) was obtained from the Persian type culture collection. The culture medium consists of the following chemicals: peptone (2% (w/v)), yeast extract (0.2%), glucose, KH_2PO_4 (0.075%), and $MgSO_4{\cdot}7H_2O$ (0.03%) [12]. Peptone and yeast extract were obtained from Liofilchem (Canada), and all other chemicals were purchased from Merck (Germany) and were used without further purification.

2.4. Counting of bacteria

Counting of bacteria during bioleaching was performed by standard plate count (SPC) method. The benefit of this method in comparison to other common methods is that only living bacteria are counted. The number of bacteria under bioleaching studies is shown in Fig. 1.

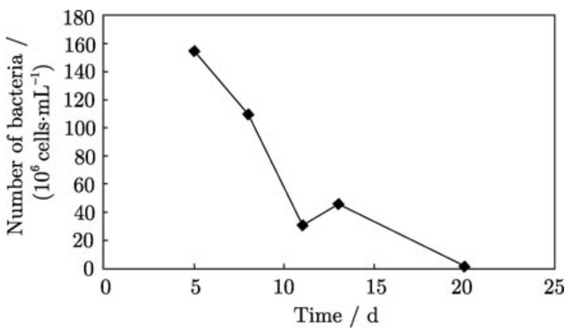

Fig. 1. Bacterial population during bioleaching.

2.5. Bioleaching studies

The culture medium was prepared and autoclaved. About 300×10^6cells/mL bacteria were added to a 500 cm^3 Erlenmeyer flask containing 80 cm^3 medium with 20vol% inoculum volume. This flask was incubated at 37°C and shaken at an 80 r/min shaking rate by a shaker incubator. After one day, the sterilized ground ore was added to the solution, and the flask was incubated. After a specific time, a clear liquid was obtained by filtration and centrifuging of solution (2000 r/min for 20 min). Then, the copper content of the filtrate was analyzed by AAS. The copper extraction percentage (E) was calculated using

$$E = \frac{\text{Copper content in the solution}}{\text{Copper content in the sample}} \times 100\% \quad (1)$$

Each experiment was repeated twice. The obtained results were reproducible within 5%. In the experiments, the role of the parameters, such as particle size, glucose percentage in the culture medium, bioleaching time, and solid/liquid ratio in copper bioleaching by *Pseudomonas aeruginosa*, was investigated, and the optimized conditions for maximizing copper extraction were determined.

3. Results and discussion

3.1. Effect of particle size

The effect of particle size on the copper extraction was studied using four particle size fractions, namely, -105 μm, 105-150 μm, 150-177 μm, and +177 μm. As presented in Fig. 2, the copper extraction was approximately the same in all size fractions. Therefore, 150-177 μm was selected as the optimum particle size fraction.

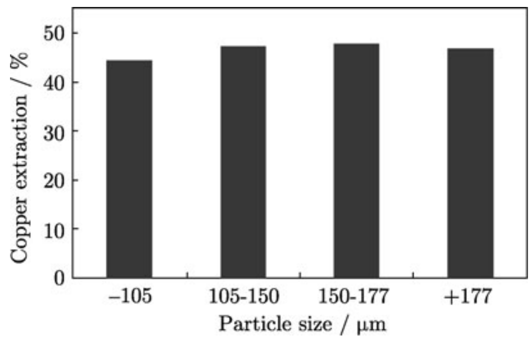

Fig. 2. Effect of particle size on the bioleaching (bioleaching time = 5 d, solid/liquid ratio = 1:80 g/mL, and glucose percentage = 6%).

The pH changes during these experiments are shown in Fig. 3. At first, because of adding bacteria to the solution and without adaptation of bacteria with the medium, the pH value slightly increased. After one day, the pH value decreased to about 4.5 in each particle size fraction. Then, it reached to about 4 after 6 d. According to these results, the pH changes in each particle size fraction were almost the same, and particle size had not significant effect on the pH changes.

3.2. Effect of glucose percentage in culture medium

One of the components of the culture medium was glucose. According to production of organic acids by bacterial metabolism, the amount of produced acids is related to accessible glucose for bacteria [13]. Also, for economic reasons, the minimum percentage of glucose must be selected. Bioleaching experiments were performed by 2%, 4%, 6%, and 8% of glucose in the culture medium. Due to the obtained results, 6% of glucose was selected as the optimum (Fig. 4).

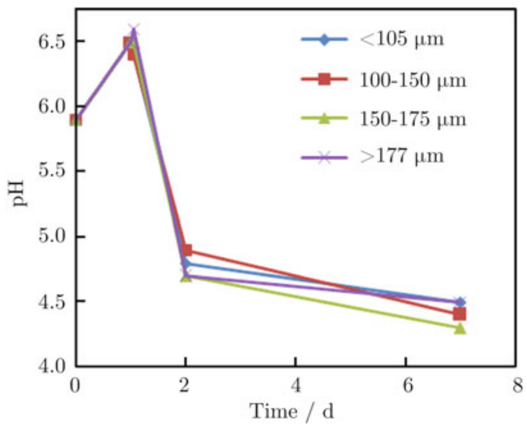

Fig. 3. The pH changes in different particle size fractions.

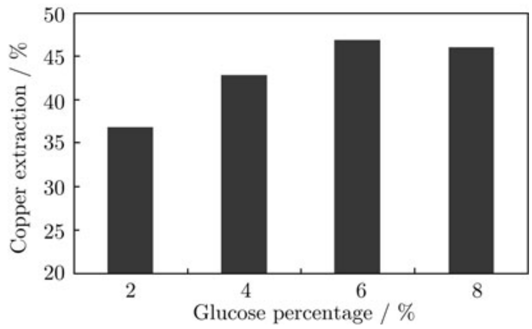

Fig. 4. Effect of glucose percentage in the culture medium (particle size = −105 μm, bioleaching time = 5 d, and solid/liquid ratio = 1:80 g/mL).

As shown in Fig. 5, an increase in glucose percentage from 2% to 8% during 5 d bioleaching causes a significant decrease in pH value. These results demonstrate that production of acids by bacteria is related to available glucose in the culture medium.

3.3. Effect of solid/liquid ratio

The effect of solid/liquid ratio on bioleaching was investigated, and the experimental results are shown in Fig. 6. Generally, the bioleaching rate was increased by decreasing the solid amount. In fact, as 1 g of ore was added to the solution, the maximum copper extraction was obtained. Therefore, the ratio of 1:80 was selected as an optimum ratio of the solid/liquid.

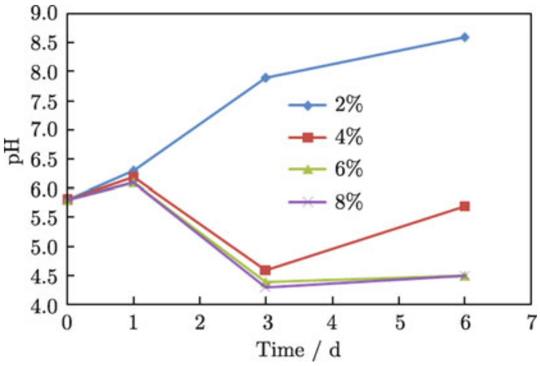

Fig. 5. pH changes at different glucose percentages in the culture medium.

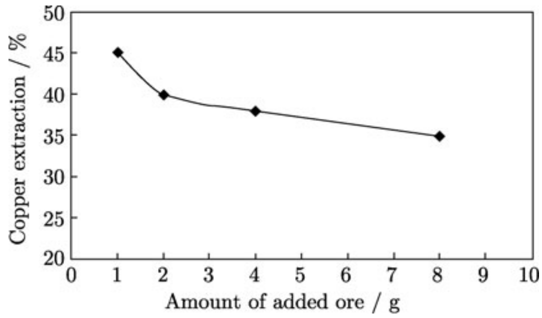

Fig. 6. Effect of solid/liquid ratio on the bioleaching (particle size = −105 μm, glucose percentage = 6%, and bioleaching time = 5 d).

3.4. Effect of bioleaching time

Generally, while bacteria are growing and generating organic acids, the copper extraction increases. After a determined time, the active bacterial population decreases, and hence, the copper extraction decreases. Therefore, the time must be selected that the solution contains an appropriate amount of bacteria. The experiments were performed at a determined time, and then, the copper extraction was calculated. Fig. 7 shows that after 8 d, the copper extraction was decreased. It seems after 8 d, *Pseudomonas aeruginosa* has absorbed copper ions from the solution. Thus, 8 d was the optimum bioleaching time.

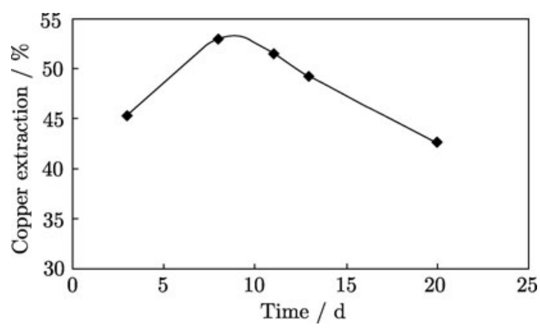

Fig. 7. Effect of bioleaching time on the bioleaching (particle size = −105 μm, solid/liquid ratio = 1:80 g/mL, and glucose percentage = 6%).

3.5. SEM study

As shown in Fig. 8, the surface morphology of the mineral was changed during bioleaching. Some parts of the mineral were dissolved by the reagent produced during bacterial activity, and porosity of the mineral was increased.

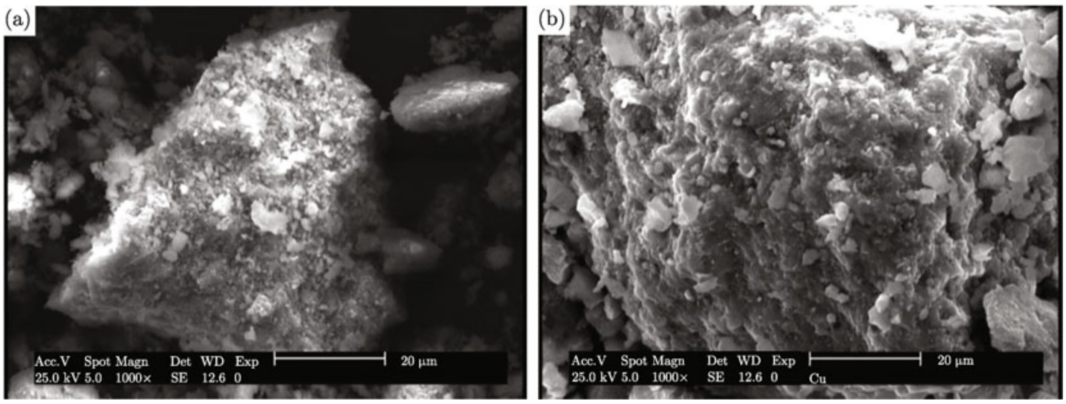

Fig. 8. SEM images of ore sample: (a) before bioleaching and (b) after bioleaching.

4. Conclusions

In the present study, *Pseudomonas aeruginosa* was used for heterotrophic bioleaching of copper oxide ore. According to the experimental results the following conclusions were obtained.

(1) Bioleaching of copper oxide ore by *Pseudomonas aeruginosa* is technically feasible.

(2) *Pseudomonas aeruginosa* is a heterotrophic bacterium, and it produces variant organic acids from glucose-containing medium. These acids serve as leaching agents and dissolve the copper content of the ore.

(3) The optimum bioleaching conditions were the particle size of 150-177 μm, the glucose percentage of 6%, the bioleaching time of 8 d, and the solid/liquid ratio of 1:80.

(4) Under optimum conditions, 53% of copper was extracted.

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