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Research Note

Efficiency of Copper Bioleaching of Two Mesophilic and Thermophilic Bacteria Isolated from Chalcopyrite Concentrate of Kerman-Yazd Regions in Iran

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A mesophilic iron oxidizing bacterium, Acidithiobacillus ferrooxidans, has been isolated (33°C) from a typical chalcopyrite copper concentrate of the Sarcheshmeh Copper Mine in the region of Kerman located in the south of Iran. In addition, a thermophilic iron oxidizing bacterium, Sulfobacillus spp., has been isolated (60°C) from the Kooshk Lead and Zinc Mine near the city of Yazd in the mid-west region of Iran. The variation of pH, ferrous and ferric concentration and cell growth on the time and effects of some factors, such as temperature and initial ferrous concentration, on the bioleaching of chalcopyrite concentrate obtained from Sarcheshmeh Copper Mine, were investigated. Bioleaching experiments were carried out batch wise using 5% (v/v) inoculum and 2% (w/v) of concentrate. The control tests were conducted using the sterilized concentrate and without the inoculum. The results obtained showed the importance of the influence of all the tested variables on the efficiency of the copper bioleaching. The effects of temperature and initial ferrous iron concentration were found to be considerable. Maximum copper recovery was obtained when the thermophilic culture had been used. Copper dissolution reached up to 80% using Sulfobacillus spp., while this value was 72% when Acidithiobacillus ferrooxidans was used at pH = 1.5. The initial Fe^{2+} concentrations for the tested mesophilic and thermophilic strains were 7 and 9 g/L, respectively. The test duration was 30 days.

INTRODUCTION

The supply of high grade ore in the world is becoming more and more scarce, making the processing of more complex ores necessary. Conventional mineral processing of complex sulfide ores, carried out by differential flotation, often produces high-grade concentrates but causes environmental pollution and accompanying penalties. Therefore, much effort has been directed towards developing a hydrometallurgical process suitable for the treatment of ores; most of the proposed methods are complex and expensive [1].

Among the hydrometallurgical processes, biohydrometallurgical techniques seem to be among the best alternatives for treatment of these types of ore. These methods, which have been applied industrially to copper and uranium production, use bioassisted heap, dump and in situ technologies, and have been applied successfully to the extraction of gold from refractory sulfide-bearing ores and concentrates [2-4]. However, for other metal concentrates, this technology remains a promising alternative against conventional pyrometallurgical extraction processes. This is the case for the treatment of chalcopiritic concentrates, which represent a more complicated situation, due to the natural refractivity of chalcopyrite.

Several studies with mesophilic microorganisms, such as Acidithiobacillus ferrooxidans and Leptospirillum ferrooxidans, have shown very slow copper leaching rates [5,6]. However, when thermophilic microor-

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ganisms are used, leaching rates are considerably enhanced, due to high temperatures, higher metal tolerance capacity and the metabolic characteristics of these type of microorganisms [7,8].

Initially, chalcopyrite can be oxidized by dissolved oxygen, according to Equation 1, in an acidic solution. According to Equation 2, the role of the bacteria is to regenerate the oxidant ferric ion in the bulk phase, from the ferrous iron, which results from the chemical oxidation of the metal sulfide in the ore by ferric iron (Equation 3).

$$CuFeS_2 + O_2 + 4H^+ = Cu^{2+} + Fe^{2+} + 2S^0 + 2H_2O, \quad (1)$$

$$4Fe^{2+} + 4H^+ + O_2 = 4Fe^{3+} + 2H_2O, \quad (2)$$

$$CuFeS_2 + 4Fe^{3+} = Cu^{2+} + 5Fe^{2+} + 2S^0. \quad (3)$$

Most of the studies on bacterial dissolution of sulfide have been carried out using mesophilic microorganisms, especially Acidithiobacillus ferrooxidans. The sulfide dissolution by the thermophilic bacteria has been found to be more of a concern, mainly because of the rather rapid oxidation process by these type of bacteria as compared to that of the other studied bacteria [9-11]. In the present study, the ability of the two mesophilic and thermophilic bacteria isolated from the Kerman-Yazd regions of Iran, on the copper recovery of chalcopyrite concentrate, has been investigated.

MATERIAL AND METHODS

Ore Concentrate

A copper sulfide concentrate supplied by the Sarcheshmeh Copper Mine (Kerman, Iran) was used. Chemical analysis of the sample revealed: 24.74% Cu; 26.39% Fe; 0.68% Mo and 35% S. X-ray diffraction analysis of the ore showed chalcopyrite ($CuFeS_2$) as the major component (57.1%) and pyrite (FeS_2) as the minor one (19.3%). Small amounts of chalcocite (Cu_2S), covellite (CuS) and molybdenite (MoS_2) were also shown. Over 90% of the ore had a particle size of less than 45 μm.

Microorganisms and the Cultivation Media

The mesophilic and thermophilic iron oxidizing bacteria used in this work have been isolated from the Sarcheshmeh Copper Mine and Kooshk Lead and Zinc Mine. These strains were identified by the department of microbiology in IROST (Iranian Research Organization of Science and Technology). According to the report of IROST, the strains were identified as Acidithiobacillus ferrooxidans and Sulfobacillus spp., respectively.

Acidithiobacillus ferrooxidans was grown on a medium containing $FeSO_4.7H_2O$: 33.4g/L, $(NH_4)_2SO_4$: 0.4g/L, $MgSO_4.7H_2O$: 0.4 g/L and K_2HPO_4: 0.4g/L. Sulfobacillus spp. was cultured on a medium containing $FeSO_4.7H_2O$: 44.2g, $(NH_4)_2SO_4$:3g, $MgSO_4.7H_2O$: 0.5 g, K_2HPO_4: 0.5g, KCl: 0.1g, $Ca(NO_3)_2$: 0.01g and Yeast Extract: 0.2 g in 1020 ml solution [12]. The cultures of Acidithiobacillus ferrooxidans and Sulfobacillus spp. were incubated in 500 ml Erlenmeyer flasks, each containing 200 ml of the medium and 10% (v/v) inoculum, on a rotary shaker at 180 rpm at constant temperatures of 33°C and 60°C, respectively. The initial pH of the cultures was adjusted to 1.5 using 1N H_2SO_4. The stock and pre-inoculum cultures were maintained in the same medium under similar conditions. The cultures that were used had been subcultured through several transfers in a concentrate medium, in order to adapt the bacteria to the experimental conditions. The stock cultures were subcultured at two week intervals.

Bioleaching Experiments

Shake flask cultures were prepared with chalcopyrite concentrate obtained from the Sarcheshmeh Copper Mine, Kerman, Iran. Prior to use, the chalcopyrite was washed with 2 M hydrochloric acid to remove any surface oxidized deposits, rinsed repeatedly with distilled water and dried at 60°C. 250 mL of medium solution were put into 500 mL Erlenmeyer flasks and 5 g of chalcopyrite concentrate added to each. The pH was adjusted to three different desired starting values, (1.2, 1.5 and 1.8) and the stoppered flasks autoclaved at 120°C for 30 min at 1 atm. These were inoculated (5%, v/v) when cool, with active cultures of each of the test bacteria. Control samples were made by the addition of 5 ml of 0.5% (v/v) formaline in ethanol to the medium. Deionized water was added to the flasks to compensate for evaporation losses. The cultures that were used had been subcultured through several transfers in a chalcopyrite medium, in order to adapt the bacteria to the experimental conditions. All cultures were incubated, shaken (180 rpm) at 33°C for mesophilic bacteria and at 60°C for thermophilic bacteria and samples were taken at regular intervals to determine total soluble copper, iron, ferric iron, pH and cell number.

The effects of pH on chalcopyrite oxidation by two iron-oxidizing acidophiles were compared by adjusting the initial pH of the culture media to 1.2, 1.5 or 1.8. All inoculated cultures and uninoculated controls were set up in duplicate.

For investigation of the effect of Fe^{2+} concentration, some medium, with various amounts of $FeSO_4.7H_2O$, was made. During the experiments, the pH was kept at 1.5 (except one test for determination of optimum pH) by the addition of 1N H_2SO_4 when necessary. Variations of pH on the time, in the presence of bacteria, were investigated.

Analyses

The quantity of free bacteria in the solution was determined by direct counting, using a Thoma chamber of 0.1 mm depth and 0.0025 mm^2 area with an optical microscope ($\times$1000). Copper and total iron concentrations in the solution were measured by an atomic absorption spectrophotometer (German, model AAS 5EA). The ferric ion concentration in the solution was determined by the sulfosalicylic acid spectroscopy method (Varian Techtron UV-VIS spectrophotometer, model 635) [13]. The ferrous ion concentration was ascertained by a volumetric method, by titration with potassium dichromate [14]. The pH of the supernatant at room temperature was also measured with a pH meter (Metrohm, model 691).

Results and Discussion

Figure 1 shows that the pH decreased initially due to the consumption of acid during the protonic attack of the chalcopyrite, according to the following reaction:

$$CuFeS_2 + O_2 + 4H^+ \rightarrow Cu^{2+} + Fe^{2+} + 2S^0 + 2H_2O.$$

Later, acidity increased because of the oxidation of elemental sulfur by sulfur-oxidizing microorganisms.

Figure 2 shows variations of the ferrous and ferric concentrations against the bioleaching time for mesophilic and thermophilic bacteria. Initial ferrous concentration in the medium was 7 and 9 g/L. On the 30th day, these values reached 1.6 and 2.1 g/L, with mesophilic and thermophilic bacteria, respectively. The changing of the values of ferrous and ferric concentrations in solution indicates microbial activity. As can be seen in this figure, the ability of Sulfobacillus spp. in changing ferrous ions to ferric ones is more than the ability of Acidithiobacillus ferrooxidans. Ferrous concentration is decreased, but, ferric concentration increased, with respect to time.

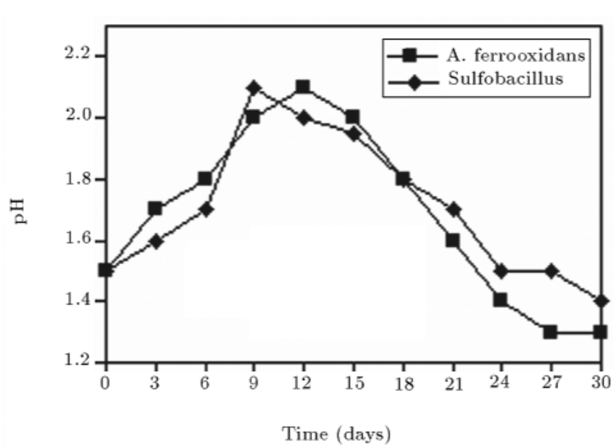

Figure 1. Variation of pH on time: $T = 33^\circ$C and initial Fe^{2+} concentration = 7 g/L for A. ferrooxidans; $T = 60^\circ$C and initial Fe^{2+} concentration = 9 g/L for Sulfobacillus spp.

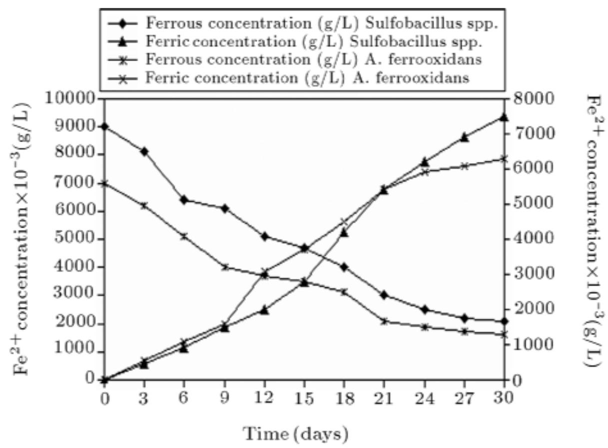

Figure 2. Variation of ferrous and ferric ions concentration on time: $T = 33^\circ$C, pH = 1.5 and initial Fe^{2+} concentration = 7 g/L for A. ferrooxidans; $T = 60^\circ$C, pH = 1.5 and initial Fe^{2+} concentration = 9 g/L for Sulfobacillus spp.

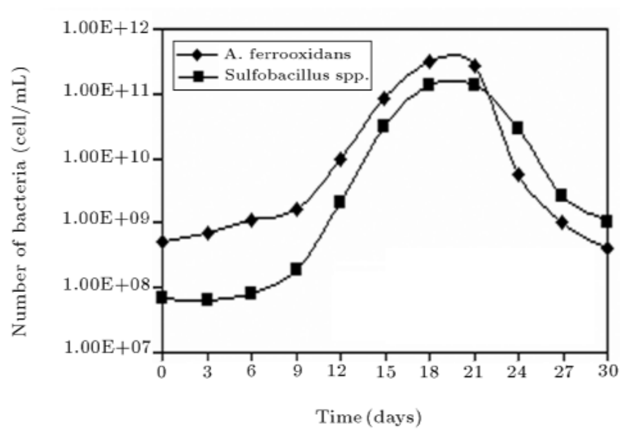

Figure 3. Variation of cell number on time: $T = 33^\circ$C, pH = 1.5 and initial Fe^{2+} concentration =7 g/L for A. ferrooxidans; $T = 60^\circ$C, pH = 1.5 and initial Fe^{2+} concentration =9 g/L for Sulfobacillus spp.

The bacterial growth in shake flasks has been shown in Figure 3. Two stages can be identified from this figure: (a) An increase of the bacteria number detected during the first 15 days for both bacteria (first stage), (b) A death phase, during which the growth of Acidithiobacillus ferrooxidans decreased. The first phase corresponds to the biooxidation of the ferrous initially present in the medium. A growth limitation occurred because the supply of an essential nutrient (ferrous iron) in the medium was decreased.

Figures 4 and 5 indicate that 33°C and 60°C are the best temperatures for the bioleaching of chalcopyrite for Acidithiobacillus ferrooxidans and Sulfobacillus spp., respectively. Most growth of Acidithiobacillus ferrooxidans takes place at 33°C, while the best temperature for growth of Sulfobacillus spp. was obtained as 60°C. As these microorganisms have been isolated at 33°C and 60°C, these temperatures are the optimum for these microorganisms. At other temperatures, for both bacteria, the rate of bioleaching according to these figures, decreased because, at these temperatures, the activity of the bacteria decreased. At higher temperatures than 33°C and 60°C, the solubility of Cu in solution increases, but, the growth of microorganisms will be slowed.

The effect of the initial ferrous iron concentration on the bioleaching kinetics was investigated. Six different media, containing different quantities of Fe^{2+} as ferrous sulfate (0, 2, 4, 5.5, 7 and 9 g/L), were used. According to Figures 6 and 7, under the same conditions for all of the tests, copper extraction reached 10%, 22%, 38%, 49%, 72% and 74% for Acidithiobacillus ferrooxidans and 11%, 26%, 41%, 52%, 71% and 80% for Sulfobacillus spp. after 30 days. In these experiments, the media contained 0, 2, 4, 5.5, 7 and 9 g/L of Fe^{2+} concentration, respectively. These results indicate that an increases in ferrous leads to enhanced activity and bacteria growth and, therefore, higher copper recovery was obtained.

The comparison of the ability of copper recovery between mesophilic and thermophilic bacteria has been shown in Figure 8. As can be observed, the bioleaching ability of Sulfobacillus spp. is more than the bioleaching ability of Acidithiobacillus ferrooxidans. Higher temperature can be one of the reasons for this difference (not shown in figure); because, at higher temperatures, ferrous ions change to ferric ions and, consequently, the extraction of metals will increase. Also, the activity of the microorganisms is an important factor.

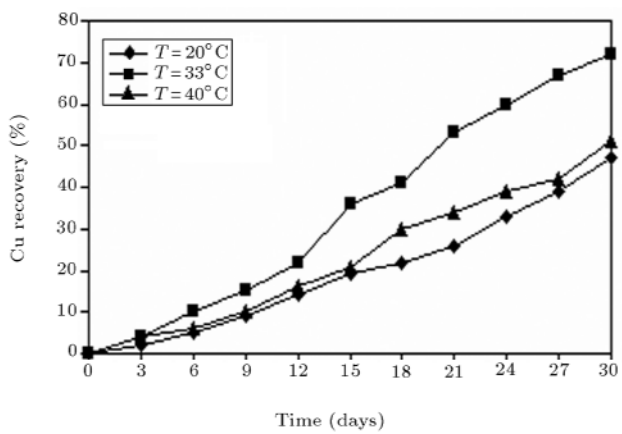

Figure 4. Cu recovery at different temperatures (A. ferrooxidans): pH = 1.5 and initial Fe^{2+} concentration = 7 g/L.

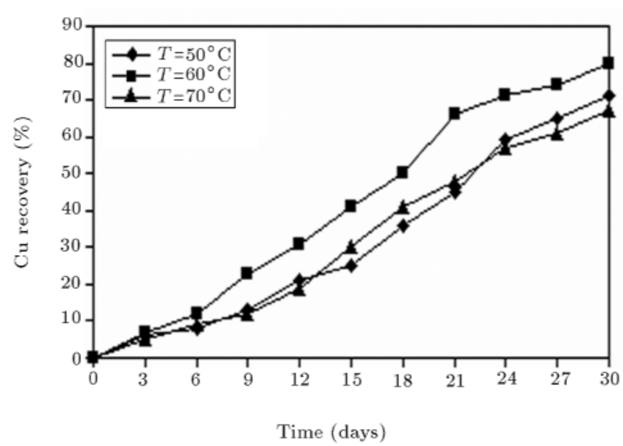

Figure 5. Cu recovery at different temperatures (Sulfobacillus spp.): pH = 1.5 and initial Fe^{2+} concentration = 9 g/L.

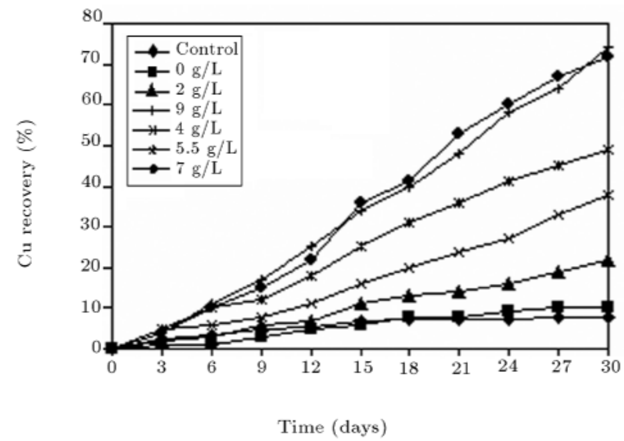

Figure 6. Effect of initial Fe^{2+} concentration on Cu recovery: $T = 33$°C, pH = 1.5 and initial Fe^{2+} concentration = 7 g/L .

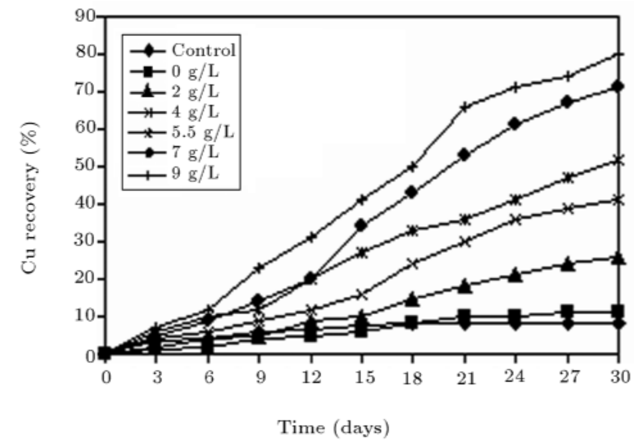

Figure 7. Effect of initial Fe^{2+} concentration on Cu recovery: $T = 60$°C, pH = 1.5 and initial Fe^{2+} concentration = 9 g/L.

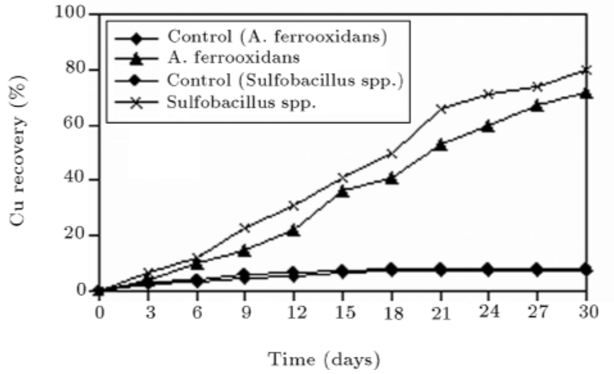

Figure 8. Cu recovery in presence and absence of bacteria (control): $T = 33^\circ$C, pH = 1.5 and initial Fe^{2+} concentration = 7 g/L for A. ferrooxidans; $T = 60^\circ$C, pH = 1.5 and initial Fe^{2+} concentration = 9 g/L for Sulfobacillus spp.

CONCLUSION

The bioleaching ability of the mesophilic and thermophilic bacteria, isolated from the soil of the Sarcheshmeh Copper Mine and from the Kooshk Lead and Zinc Mine in south and mid-west regions of Iran, has been studied in a shake flask. The experimental results obtained showed that temperature and initial ferrous concentration were more effective than other tested factors. The optimum temperature was found to be 33°C and 60°C for mesophilic and thermophilic bacteria, respectively. The best pH was obtained at about 1.5 for both microorganisms, while the optimum level for initial ferrous concentration was obtained at 7 and 9 g/L for mesophile and thermophile strains, respectively. At these conditions, the bacteria has the highest activity and ability for copper recovery from the chalcopyrite concentrate, which were about 72% and 80%, respectively, after 30 days.

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