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Synergistic Copper Extraction Activity of *Acidithiobacillus ferrooxidans* Isolated from Copper Coal Mining Areas

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ABSTRACT

Acidithiobacillus ferrooxidans strains were isolated from mineral wastes in 2 copper coal mining areas in the modified 9K-culture medium containing ferrous ions as the sole source of energy. The isolated bacteria were added to the copper coil mining samples in leaching mini-plants. The total Cu content of samples, which measured by atomic absorption, decreased to 66.7% in sterilized samples and to 20% in non-sterilized samples. The optimum decrease of total Cu content occurred in pH 2. There was a 46.7% increasing in the copper extraction by synergistic activity of natural ferrous oxidizing microorganisms and the isolated bacteria which added to the area of the experiment. The result obtained via comparison of sterilized and non-sterilized samples. The acceleration of copper dissolution showed the advantage of mixed cultures of iron and sulfur oxidizing microorganisms in non-sterilized minerals in low pH values for copper extraction.

Key words: Copper extraction, bioleaching, *Acidithiobacillus ferrooxidans*, synergistic activity, mixed cultures

INTRODUCTION

Nowadays biomining, the use of microorganisms to recover precious and base metals from mineral ores and concentrates has an increasingly important place among the available mining technologies. A large number of the current large-scale bioleaching operations are located in developing countries. Several developing countries have significant mineral reserves and by the simplicity and low capital cost requirement, bioleaching has an especially suitable situation for these countries (Acevedo, 2002). Microbiological aspects should been subjected to far less scrutiny and control, as careful considerations which are made in the design and engineering of biomining operations. In the biomining processes, microbial consortia employed that are dominated by acidophilic, autotrophic iron- or sulfur-oxidizing prokaryotes (Rawlings and Johnson, 2007). Also, heterotrophic eukaryotic microorganisms such as fungi can be used for solubilization of metals from mineral ores by supplementation of organic materials to growth media (Ambreen *et al*., 2002; Hefnawy *et al*., 2002).

Acidithiobacillus ferrooxidans is one of the most studied organisms in microbial leaching. Other iron- or sulfide/sulfur-oxidizing bacteria and some archaea are potential microbial agents for metal leaching at high temperature or low pH environments (Rawlings and Johnson, 2007; Suzuki, 2001).

Different factors including pH, solid particle size and concentration of the solid affect the efficacy of metal extraction by bacteria (Bayat *et al*., 2009). The maximum copper recovery by *Acidithiobacillus ferrooxidans* achieves in mesophilic culture and low pH (Wang *et al*., 2008). In the present study, the copper extraction using *Acidithiobacillus ferrooxidans* isolated from copper coal mining areas of Iran analyzed and the effect of mixed culture of the bacterium with natural iron oxidizing bacteria on copper extraction quality compared to the single culture usage in pilot-scale tanks.

MATERIALS AND METHODS

Microorganisms and growth media: Two isolates of *Acidithiobacillus ferrooxidans* grown on modified 9k medium (M9k) medium (Matsumoto *et al*., 2002). The medium contains (gL^{-1}) $FeSO_4.7H_2O$ 33.3; K_2HPO_4 0.1; $(NH_4)_2SO_4$ 0.4; $MgSO_4.7H_2O$ 0.4. The pH value of the medium adjusted to 2 by H_2SO_4. Inoculated broth cultures were shaken on orbital shaker at 160 min^{-1} at 21°C for 15 days. Ferrous sulfate solutions sterilized by filtration (filters with 0.4 μm in diameters pores) and other solutions sterilized in autoclave. The solid M9k media prepared by adding 2% (w/v) agarose to the broth media.

The bacteria isolated from 2 copper coal mining areas; Mute (area A) and Segzi (area B) copper coal mining areas in Iran. The name and characteristics of the samples are shown in Table 1. The same soils used as samples for bioleaching studies.

Bacterial staining performed after treatment of deposits by diluted hydrochloric acid or Ethylene Diamine Tetra Acetic acid (EDTA).

Total Cu analysis: The total Cu content analyzed by atomic absorption spectroscopy (Varian SpectrAA-300 located in Mesfelezerangi reaserch center, Isfahan, Iran). The standard curves obtained by the analysis of standard solutions of Cu^{2+} in a pH value equal to the value of experimental media in the wavelength of 327.7 nm. The pH adjusted to favorable value by H_2SO_4. Pilot-scale tank bioleaching operation: Acid resistant plastic tanks with pulp at the bottom and the total volume of 2000 mL were designated. The suspension of bacteria grown in M9k medium which contained 10^4 cell per mL as well as 200 mL acidic sterile distilled water (pH=2) were added to the copper coil mining samples in leaching mini-plants daily, after extraction of previous leached liquid from the bottom of the plants. The control medium contained the composition of the inoculated medium except the bacterial solution. The filtrate of the sample and control tanks extracted after 18 days for the sample number 1 and after 50 days for the sample number 2. The total Cu contents of the control and sample filtrates measured by atomic absorption.

Both bacterial isolations and bioleaching studies conduced from August 2007 to December 2009 In Islamic Azad University branch Falavarjan, Falavarjan, Iran.

RESULTS AND DISCUSSION

Bacterial growth: The growth of bacteria on M9K medium broth were detected after 20-30 days by Fe^{3+} deposits formation in the sample media in contrast to control media. The growth in solid M9K media were shown by Fe^{3+} deposits formation along with the inoculation lines.

Stained bacteria: Gram-negative small rods observed among deposits by gram staining after treatment by diluted HCl or EDTA.

Table 1: Characteristics of the samples used for bacterial isolation and the bioleaching studies

Sample	Total Cu^{2+} content (%w/w)
Tailing (number 1)	0.15
Feed (number 2)	2

Table 2: The results obtained in non sterilized samples inoculated with *Acidithiobacillus ferrooxidans*

Sample number	Time (days)	Primary contents of Cu^{2+} (%)	Cu^{2+} contents (%) after treatment
1 (without treatment)	18	0.15	0.15
1 plus bacteria	18	0.15	0.03
2 (ithout treatment)	50	2.0	2.0
2 plus bacteria	50	2.0	0.76

Table 3: The results obtained in sterilized sample number 1 inoculated with *Acidithiobacillus ferrooxidans*

Sample number	Time (days)	Primary contents of Cu^{2+} (%)	Cu^{2+} contents (%) after treatment
1 (without treatment)	18	0.15	0.15
1 plus bacteria	18	0.15	0.1

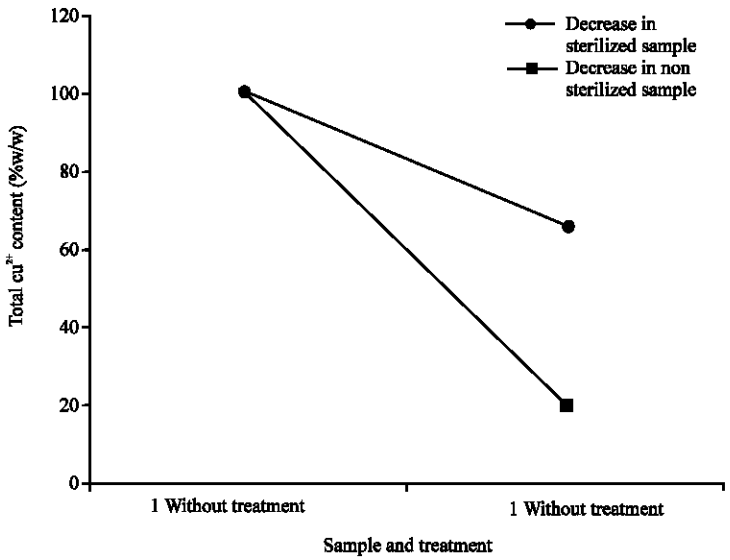

Fig. 1: Comparison of total Cu^{2+} content between sterilized samples and non sterilized samples. In sterilized samples the content falls down to 66.7% and in non sterilized samples to 20% of primarily content

Copper extraction from non-sterilized samples in pilot-scale tanks: The results from samples 1 and 2 are shown in Table 2. The bacteria isolated from Mute mineral ores were added to the samples.

Copper extraction from sterilized samples in pilot-scale tanks: The results from inoculation of the bacteria isolated from the A area mineral ores to the samples number 1, are shown in Table 3.

A comparison of the decrease in total Cu^{2+} amounts in sample number 1 between sterilized and non sterilized conditions is shown in Fig. 1.

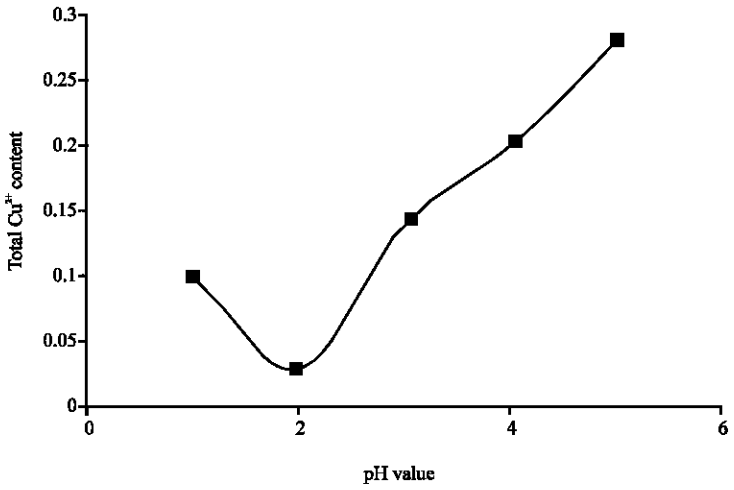

Fig. 2: Decrease in total Cu amount in the range of different pH values. The optimum decrease occurred in pH 2

The effect of pH on total Cu extraction: As shown in Fig. 2, the optimum extraction was in pH 2. The extracted copper resulted in a considerable green color change in M9k medium. *Acidithiobacillus ferrooxidance* is one of the most important bacteria in dissolution of metal sulfides (Suzuki, 2001). Beside industrial bioleaching processes, this bacterium has been used for bioremediation of toxic heavy metals. Takeuchi *et al.* (2003) volatilized mercury from waste water using *Acidithiobacillus ferrooxidance* in low pH.

Most of the studies which carried out in bioleaching at laboratory scales only use one species such as *Acidithiobacillus ferrooxidans*. However, the natural situations such as other microorganisms which are naturally present in the area may involve in the bioleaching process. *Leptosprillum ferrooxidans*, *Acidithiobacillus thiooxidans* and other thermophilic, anaerobic heterotrophic bacteria are the main groups which had synergistic effects in the bioleaching processes (Bayat *et al.*, 2009; Guan-Zhou *et al.*, 2008).

The results of the present study represent increasing values in the copper extraction with the non sterilized mineral soils (Fig. 1) which is because of the activity of the natural microorganisms in the area of the experiment. It is shown that the formation of elemental sulfur on the surface of bioleaching areas can decrease the rate of copper bioleaching (Falco *et al.*, 2003) and the sulfite produced by *Acidithiobacillus ferrooxidans* sulfur oxidizing procedure is a toxic intermediate which inhibits the activity of iron oxidase activity by the bacterium (Sugio *et al.*, 2008). As shown in previous studies in flask scales, other oxidizing bacteria would oxidize the elemental sulfur layers on the surface of extraction residues (Falco *et al.*, 2003; Fowler and Crundwell, 1999).

Qiu *et al.* (2005) used mixed cultures of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus Thiooxidans* for extraction of copper. Copper extraction was 11.25% by mixed cultures, in contrast to 7.75% by pure culture of Acidithiobacillus ferrooxidans after 18 days, in flask scale.

Falco *et al.* (2003) determined the efficacy of covellite bioleaching using pure cultures of *Acidithiobacillus ferrooxidans* or *Acidithiobacillus thiooxidans* and a mixed culture composed of *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, but they showed that copper solubilisation by the *Acidithiobacillus ferrooxidans* culture was nearly the same as that for the

mixed culture (51 versus 55%) after 30 days. In the present study, by the usage of mixed cultures of iron and sulfur oxidizing microorganisms from copper coal mining areas of Iran, in non-sterilized minerals and low pH values we accelerated the copper dissolution in pilot-scale tanks, up to 80%, in contrast to 66.7% using the pure cultures of *Acidithiobacillus ferrooxidans*, which is an elevated extraction value achieved after 18 days.

Yang *et al.* (2009) showed that the quantity of stock culture added and pH are two factors greatly influence bioleaching process. Bayat *et al.* (2009) achieved the maximum extraction of Fe and Zn by *Acidithiobacillus ferrooxidans* at the pH values around 1.3. In the present study, the optimum pH for copper extraction in the presence of natural microorganisms in the non-sterilized samples achieved in pH 2.

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