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Bio-dissolution of copper from Khetri lagoon material by adapted strain of *Acidithiobacillus ferrooxidans*

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Abstract−Bioleaching involves the use of iron and sulfur oxidizing microorganisms to catalyze the dissolution of valuable metals. In this investigation, lagoon material contains 0.39% Cu, in which the major copper bearing mineral is chalcopyrite associated with other minerals present as minor phase. Leaching experiments were carried out using an adapted strain of *Acidithiobacillus ferrooxidans* with various parameters such as presence/absence of iron, pH, pulp density and temperature. Base of the medium was 9 K (without ferrous) Bio-dissolution of copper was found to be maximum, i.e., 80.9% with 9 K^+ (with ferrous) at pH-2.0, 10% pulp-density and 35 °C within an incubation period of 30 days.

Key words: *Acidithiobacillus ferrooxidans*, Khetri Lagoon Material, Bioleaching, Copper

INTRODUCTION

Bioleaching is an emerging technology that is globally applied due to attractive environmental and social benefits. It is widely used for the recovery of copper from sulfide, oxide and carbonate ores with the involvement of microorganisms enhancing the dissolution of metals from minerals [1-3]. This technique is slowly gaining importance in the field of waste treatment [4]. One of the most important copper-bearing sulfidic minerals is chalcopyrite ($CuFeS_2$) [5].

Unlike many other ores, chalcopyrite is known to be particularly recalcitrant to hydrometallurgical processes. On other hand, conventional processes of concentrate produce many problems [6-8] in recovery of metals from wastes and low grade ores. This bio technique is economical and offers reduced environmental pollution. India has recoverable reserves of 53.58 million tones of copper ore capable of yielding 5.29 million tones of copper metal. The sulfide mineral chalcopyrite is the most common copper mineral in the world, comprising the bulk of the known copper reserves: 250 known deposits of copper are present in India: 90% of them are in Singhbhum (Bihar), Malanjkhand (Madhya Pradesh), and Khetri (Rajasthan). There are about 17 working copper mines in India, out of which more than 0.5% copper containing ore is discarded. About 10 million tons of low grade ores are available in India.

Khetri copper complex, Khetri, Rajasthan, processes the crushed copper ore through flotation. Concentrates thus generated are taken to the smelter and tailings are rejected and dumped in tailing ponds. During the process of flotation, plant shutdown occurs for various reasons and the entire material in the flotation cell is rejected. Annually, about 25,000 tons of such material is generated and kept dumped in a lagoon commonly termed as lagoon material. The present investigation is carried out by taking this lagoon material.

Many types of sulfur and iron-oxidizing microorganisms are involved in bioleaching processes [9]. Among them, the most important is *Acidithiobacillus ferrooxidans*, which plays an important role in the bioleaching process. It is a mesophilic acidophile thriving in the temperature range between 25 and 35 °C. It gets energy for growth from the oxidation of either iron or sulfur. The iron must be in the ferrous, or bivalent, form (Fe^{++}), and it is converted by the action of the bacterium into the ferric, or trivalent, form (Fe^{+++}).

Keeping in mind the above aspects, the current studies were performed to test the leach efficiency of *Acidithiobacillus ferrooxidans* in leaching copper from Khetri lagoon material procured from Khetri, Rajasthan, India. Leaching experiments were performed in different shake flasks using an adapted culture of *Acidithiobacillus ferrooxidans* in presence and absence of ferrous sulfate along with variation in their pulp-densities, pH and temperature.

MATERIALS AND METHODS

1. Khetri Lagoon Material (Khetri, Rajasthan, India)

The lagoon material used in this experiment was obtained from Khetri copper complex, Khetri, Rajasthan, India. The samples were dried in air and then passed through a sieve of −200 BSS mesh size. The sieve fraction of the lagoon material obtained was less than 75 micron.

2. Chemical Analysis of Khetri Lagoon Material

The lagoon material was dried and chemical analysis was done after digesting with hydrochloric acid. The result of chemical analysis is presented in Table 1.

3. Microorganism

The microorganism used throughout the present work is a laboratory stock culture of mixed mesophilic acidophilic bacterial consortium, consisting predominately of *Acidithiobacillus ferrooxidans* strains. *A. ferrooxidans* has the ability to achieve optimum growth under strong acid conditions (pH-2.0) by deriving energy for its metabolism from the oxidation of inorganic iron and reduced sulfur [10,11]. The strain was screened for its ability to leach copper at pH of 2.0. Monitoring the iron oxidation rate in 9 K^+ medium accessed the growth of the bacteria.

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Table 1. Chemical analysis of Khetri lagoon material and Malanjkhand low grade ore (values are in percentage)

Name of material	Cu	Ni	Co	Zn	Mn	Pb	Fe^T	Al
Khetri lagoon material	0.395	0.022	0.035	0.305	0.172	0.042	25.13	56.98

4. Media

The *A. ferrooxidans* strain was grown in 9 K^+ medium [12] containing $(NH_4)_2SO_4$-3 g/*l*, $MgSO_4 \cdot 7H_2O$-0.5 g/*l*, KH_2PO_4-0.5 g/*l* & $FeSO_4 \cdot 7H_2O$-44 g/*l*. The pH in the medium was adjusted to 2.0 by adding 1 N sulfuric acid.

5. Adaptation Study

Before the leaching experiments were performed, the laboratory strain was initially activated in 9 K^+ media. For activation the strain was inoculated repeatedly in 9 K^+ media. The activation of microorganism was presumed to be completed when a steady iron oxidation rate was achieved.

The microorganism was initially adapted to copper concentration of 1 g/L. During adaptation study the copper dose was slowly increased. The adaptation was presumed to be completed when the iron oxidation rate was the same in presence and absence of copper in the medium. After activation of microorganism, the same was adapted to copper ore so that during bioleaching the various constituents of the ore would not inhibit the growth of the bacteria.

6. Mineralogy

The lagoon material and low grade copper ore were analyzed by high-resolution synchrotron based X-ray Diffractometer (XRD). The XRD analysis was done in order to determine the major and minor minerals present in the ore material.

7. Shake Flask-leaching Experiments

Leaching experiments were carried out with an adapted strain of *Acidithiobacillus ferrooxidans* in different 250 ml Erlenmeyer flasks at various parameters such as presence/absence of ferrous, pH [1, 1.5, 1.75, 2, 2.25 and 2.50], pulp density [10%, 15%, 20% and 30% (w/v)] and temperature [25 °C, 30 °C, 35 °C, 40 °C, 45 °C, 50 °C]. The flasks were continuously agitated on a rotary shaker (Kuhner Shaker) at 150 rpm for 30 days. The experiments were performed for 30 days to standardize the time period for maximum recovery of copper on a flask stage. Sukla et al. got 30% of copper recovery in 50 days from low grade chalcopyrite of Malanjkhand, India using the same bacterium, *Acidithiobacillus ferrooxidans* [13]. Akcil et al. got 50.3% Cu leaching in 24 days, from chalcopyrite concentrate using *Acidithiobacillus ferrooxidans* [14]. In scale-up processes like column and heap it runs for even more days to achieve maximum recovery [15].

The control set of experiments were carried out with 9 K^+ medium without any inoculum, at 10% pulp density, pH-2, 35 °C and 150 rpm for 30 days. $HgCl_2$ was added to the control set to avoid any contamination. Samples were taken at regular intervals for pH measurement and analysis of copper by AAS (Atomic Absorption Spectrophotometer).

After sampling, loss by evaporation was readjusted by adding freshly prepared medium. After 30 days of incubation the experiments were terminated, as there was no significant recovery of copper.

7-1. Effect of Ferrous

Experiments were carried out to determine the effect of the presence of ferrous sulfate on copper leaching. In one flask the 9 K medium was supplemented with 9 g/*l* of ferrous sulfate and the other flask was devoid of ferrous sulfate. 10ml of adapted inoculum was added and the experiment was conducted at 10% pulp density, pH-2, 35 °C and 150 rpm for 30 days.

7-2. Variation of Pulp Density

The experiments were carried out at different pulp-densities such as 10%, 15%, 20% and 30% (w/v) of the lagoon material. 10 ml of activated inoculum was added and the experiment was conducted for 30 days at pH-2.0, 35 °C and 150 rpm.

7-3. Variation of pH

Acidithiobacillus ferrooxidans is an acidophilic bacterium, so the pH study is very important. Bioleaching experiments were carried out with 9 gram/liter iron at different pH such as 1, 1.5, 1.75, 2.0, 2.25 and 2.5 using 10% (v/v) of the adapted cultures with 105 pulp density Khetri lagoon material at 35 °C and agitated continuously in a rotary shaker at 150 rpm for 30 days.

7-4. Variation of Temperature

In this study, leaching experiments were conducted at different temperatures such as 25 °C, 30 °C, 35 °C, 40 °C and 45 °C using lagoon material at 10% (w/v) pulp density. 10 ml aliquots of late log phase cultures were taken for the experimentation and agitated continuously in a rotary shaker at 150 rpm. The pH of the medium was maintained at 2.0 and incubated for 30 days.

8. Analysis

Chemical analysis and pH of the solution were maintained with 10 N H_2SO_4. Ferrous iron concentration was determined by standard and potassium dichromate solution in an automatic titrator. Copper was analyzed by atomic absorption spectroscopy.

RESULT AND DISCUSSION

1. Adaptation

Before the leaching experiments were performed, the mixed laboratory consortium of autotrophic bacteria was activated by culturing it repeatedly in 9 K media. The activation of microorganism

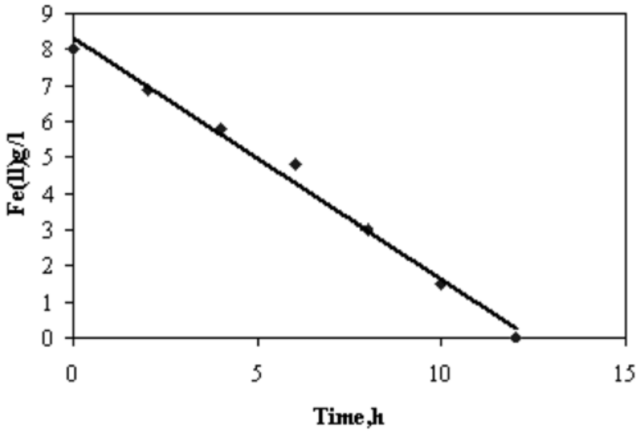

Fig. 1. Determination of iron oxidation rate.

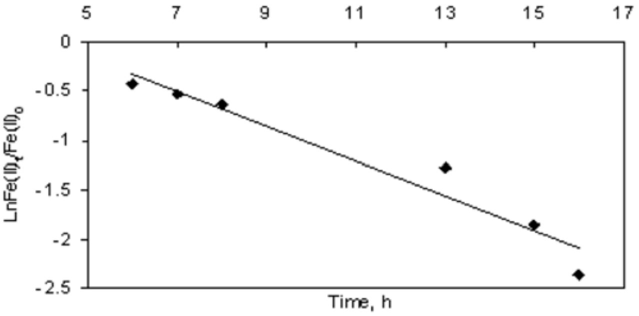

Fig. 2. First order plot.

was presumed to be completed when a steady state of iron oxidation rate was achieved. The result is shown in Fig. 1.

From Fig. 1, it was observed that the bacteria had absolutely no lag phase, indicating the efficiency of the microorganism as well as its adaptability to the growth medium. The iron oxidation rate was 499 mg/L/h. Assuming the iron oxidation to be 1st order, the plot of ln $[Fe(II)_t/Fe(II)_0]$ versus t would give a straight line. The 1st order plot is shown in Fig. 2. The specific reaction rate was calculated to be 0.178 h^{-1}.

1-1. Regeneration Time

Since the rate of bacterial growth is directly proportional to ferrous iron oxidation rate, specific growth rate constant of bacteria, was calculated as the slope of semi logarithmic plots of ferric ion concentration against time for each data. The regeneration time td, was evaluated by using the following relation:

$$t_d=0.693/\mu \tag{1}$$

From Eq. (1) the regeneration time of the activated microorganism was calculated to be 16 hours.

2. Effect of Ferrous

In presence of ferrous the dissolution of copper is higher (80.9%), as shown in Fig. 3. The rate is higher in the above case as leaching occurs by both direct contact of the bacterium and the action of ferric iron. The ferrous supplemented in the medium was converted to ferric due to the action of the bacterium as the bacterium draws energy by oxidizing ferrous. In case of the non-ferrous leaching, the copper extraction percentage was 50.6%.

3. Effect of Pulp-density

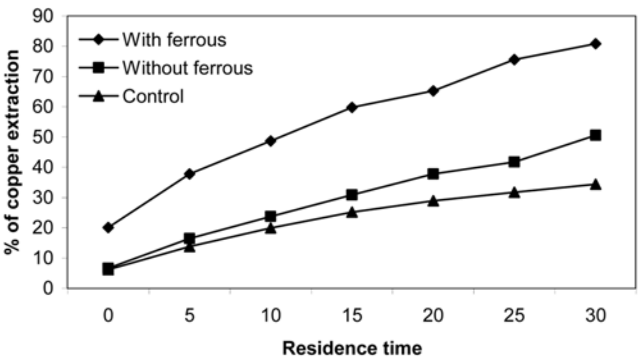

Fig. 3. Effect of presence of iron on extraction of Cu from Khetri lagoon material (Conditions: pH-2.0, temperature, 35 degree centigrade, rotation-150 rpm, residence time-30 days).

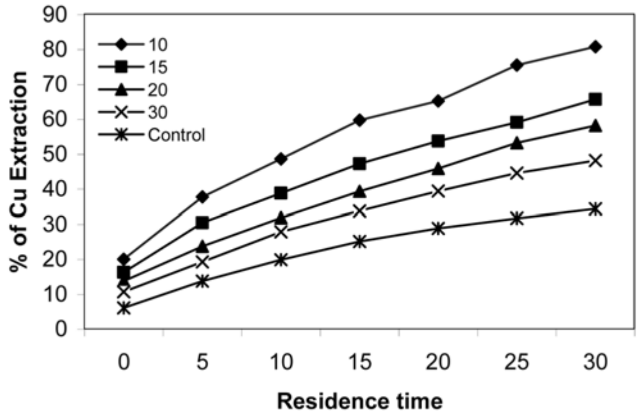

Fig. 4. Effect of Pulp density on extraction of copper from Khetri lagoon material (Conditions: pH-2.0, temperature, 35 degree centigrade, rotation-150 rpm, residence time-30 days).

The bio-dissolution of copper was investigated by varying pulp-densities in the range 10-30% (w/v), pH-2.0 at 35 °C for 30 days. Fig. 4 shows that the bio-recovery of copper was a maximum of 80.9% at 10% pulp-density at 2.0 pH, at 35 °C within 30 days of incubation. The copper extraction at 15%, 20%, and 30% pulp-densities was 65.8%, 58.3% and 48.2%, respectively. In case of control set, maximum 34.4% Cu was recovered in 30 days residence time. From the figure it is clear that the recovery of copper decreased with increase in pulp density. That the Cu leaching rate was very fast initially might be due to dissolution of oxides and secondary sulfides. After the initial faster rate, the leaching rate slowed down. This might be due to the porous nature of raw material in which diffusion is also expected in addition to surface leaching.

4. Effect of pH

From Fig. 5, it can be seen that at pH 2.0 copper recovery was higher, i.e., 80.9% at 10% pulp density. At pH 1, 1.5, 1.75, 2.25 and 2.5-copper recovery by the mesophilic acidophile was 65.4%, 72.9.3%, 73.1%, 70.4% and 50.3.2%, respectively. The bacterium

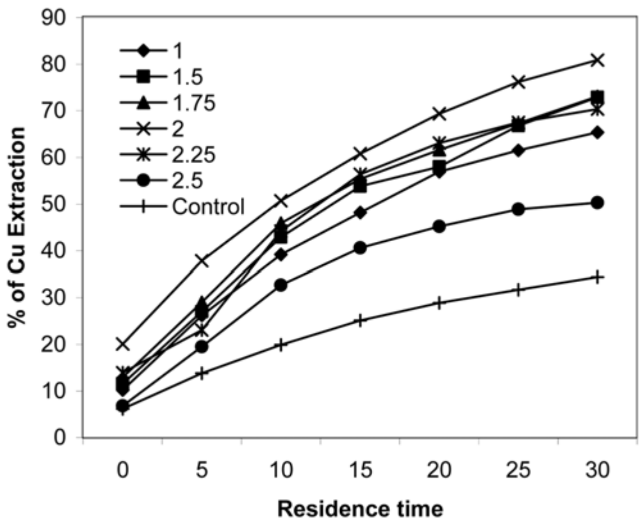

Fig. 5. Effect of pH on extraction of copper from Khetri lagoon material (Conditions: Pulp density-10%, temperature-35 degree centigrade, rotation-150 rpm, residence time-30 days).

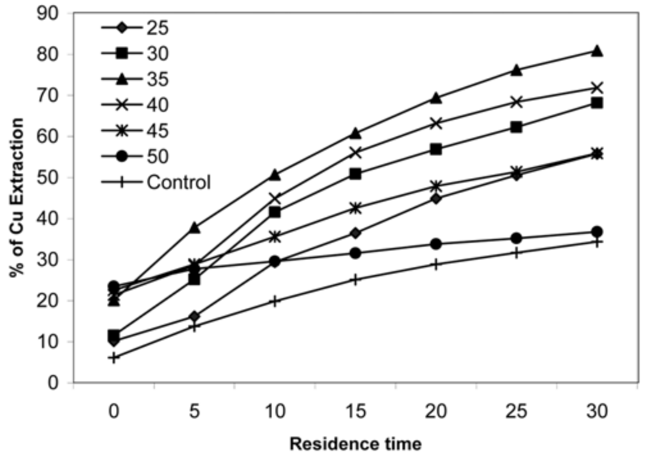

Fig. 6. Effect of temperature on extraction of copper from Khetri lagoon material (Conditions: Pulp density-10%, pH-2, rotation-150 rpm, residence-30 days).

functions best at pH 2.0.

5. Effect of Temperature

It was found that *A. ferrooxidans* culture solubilizes copper actively at temperatures ranging from 30 ºC to 35 or 37 ºC, which is the optimum temperature for the growth of this organism because it is a mesophilic bacterium (operates at room temperature). Copper recovery decreases at very low (around 20 ºC-25 ºC) and very high temperature, i.e., 45-50 ºC. At extreme temperatures the metabolic rate of an organism decreases and it may also happen that at very high temperature the organism does not survive. Copper recovery was a maximum at 35 ºC, i.e., 80.9% and least at 50 ºC (36.8%). At temperatures such as 25 ºC, 30 ºC, 40 ºC, 45 ºC and 50 ºC copper dissolution was 55.6%, 68.2%, 71.9% and 59.4%, respectively, as shown in Fig. 6.

6. Mineralogical Study

The leaching of copper from the Khetri lagoon material is mainly dependent on the type of mineralization. In order to establish the effect of mineralogy, the raw material was examined by optical microscopy and synchrotron X-ray diffraction. The mineralogical studies indicated that Khetri lagoon material contained sulfide minerals like chalcopyrite, hydroxide minerals like goethite, carbonate minerals like siderite, and dolomite and silicate minerals like forsterite, olivine, chamosite, and phologopites. The major copper-bearing minerals phase present in the material was chalcopyrite; whereas the minor copper bearing minerals are goethite, olivine, chamosite, phologopites, siderite, and dolomite etc. Details of the study are given in Fig. 7.

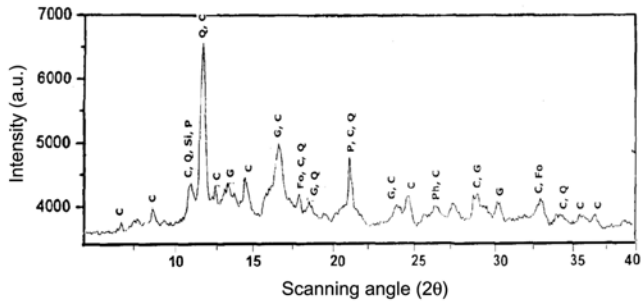

Fig. 7. X-Ray diffraction patterns of Khetri lagoon material.

CONCLUSION

The XRD analysis of raw material of Khetri lagoon revealed that the major copper-bearing minerals phase present in the material was chalcopyrite associated with other minor phase. In this study, the stain *Acidithiobacillus ferrooxidans* was found to be more efficient in solubilizing copper at higher rate in the presence of iron than in the absence of it. The leaching efficiency of copper was found to be maximum of 80.9% with Fe(II) iron and 50.6% of copper leached without Fe(II) iron, respectively. The percentage of leaching is maximum at 10% pulp density, room temperature (35-37 ºC) and pH-2.

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NOMENCLATURE

μ : slope of semi logarithmic plots
t_d : regeneration time

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