



Bioleaching of Copper from Low Grade Ore Bornite Using Halophilic *Thiobacillus Ferroxidans*, N-11

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Abstract

Bioleaching is a process of extracting minerals from ores using microorganisms. The extraction of copper from low grade ores is today's need because of gradual depletion of high grade ore. The conventional methods used for extraction of copper from ore is either Pyrometallurgy or Hydrometallurgy, however both the methods are not free from the environmental pollution problems and economically very expensive, and requires lots of energy. Bioleaching of mineral is the only method considered as most convincing way to solve these problems, requires very less energy and is free from environmental pollution and other problems. By considering this, In the present study Halophilic Thiobacillus ferrooxidans N-11 is explored for bioleaching of copper from low grade ore Bornite. Thiobacillus ferrooxidans N-11 isolated from hyper saline soils of Kolhapur district of Maharashtra, India on 9 K medium. It was identified using Bergey's manual of systematic bacteriology. Bioleaching study was carried out in both shake flask as well as bioreactor. Results showed that in the shake flask Thiobacillus ferrooxidans N-11 tolerates 35 g/L of Bornite when supplemented with 0.5 g/L of Yeast extract. At 120 rpm and 40 °C temperature, about 72% of copper can be extracted after 22 days by shake flask method and 78% can be extracted by bioreactor study in 20 days. Present study indicated the usefulness of Thiobacillus ferrooxidans N-11 in bioleaching of copper from low grade ore Bornite can be used as a potential candidate for bioleaching as a pollution free process.

Key words: Bioleaching, Halophilic, Thiobacillus thiooxidans N-11, Bornite, copper.

Introduction

Bioleaching is a simple and effective process used for metal extraction from low grade ores and mineral concentrates using the chemolithotrophic bacteria. The extraction of copper from low grade ore is to days need because of gradual depletion of high grade ore¹.

The traditional methods used for extraction of copper are either Pyrometallurgy or Hydrometallurgy. However both the methods are not free from environmental problems.

In pyrometallurgical method, the ore is crushed and milled in to a fine pulp and then concentrated by flotation using chemical reagents. The concentrate formed is smelted and electrolytically refined, however refining process creates environmental problems. It releases lots of metal ions in their wastes, it also releases lots of sulphur dioxide during smelting which causes environmental pollution.

In hydrometallurgical method ore concentrate is leached by chemical methods followed by solvent extraction and electro-winning, however this method is not also free from environmental complexity but also from non-competitive economics².

There are many techniques proposed to extract metals but these are not practically suitable, as these requires a very high energy

input as well as most of them creates environmental pollution problem, that also rises the cost of environmental protection throughout the world³.

Biohydrometallurgy or Bioprocessing is a new approach used for extraction of metals this includes bioremediation, biosorption, bioaccumulation and bioleaching,⁴.

Bio processing of mineral is the only method considered as most convincing way to solve these problems. As these processes are easy to operate, requires less energy and they are free from environmental problems and non-competitive economics of conventional methods.

The bacteria most commonly used in bioleaching are of two types. Chemolithotrophic and Heterotrophic. Thiobacillus ferrooxidans is a chemolithotrophic bacterium capable of utilizing ferrous iron as the sole source of energy for its growth⁵. Due to its capacity to oxidize metal sulphides, this bacterium is one of the most important microorganism utilized in industrial operations to recover metals, such as Copper, Uranium and Gold⁶. These organisms causes cytolysis, redoxolysis and acidolysis. Acidolysis is the principle mechanism involved in bioleaching process. In this process organisms produce different acids such as citric, oxalic, and sulphuric acid which helps in metal dissolution process from ores.

By keeping in view this background, in the present study Halophilic *Thiobacillus ferrooxidans* N-11 is explored for bioleaching of copper from low-grade ore Bornite.

Material and Methods

Thiobacillus ferrooxidans N-11 was isolated from hyper saline soil of Kolhapur district of Maharashtra, India on modified 9 K medium as per Silvermann and Lundgren⁷. In brief composition(g/L),Solution-A: (NH₄)₂SO₄(3.0),KCl(0.1),K₂HPO₄ (0.5), MgSO₄.7H₂O(0.5), Ca(NO₃)₂ (10mg/L), 10N H₂SO₄(1ml) Distilled water(700ml).Solution-B Chalcopyrite (40),distilled water (300ml). It was identified by using morphological, cultural, biochemical, methods as per Bergey's manual of systematic bacteriology⁸ and as per MICRO-IS software⁹.

Bornite ore was grinded to -165 / + 300 mesh (58 to 109 u). Initial copper and iron percentage was determined by atomic absorption spectrometry as per APHA¹⁰.

Tolerance: Tolerance of isolate *Thiobacillus ferrooxidans* N-11 to Bornite was determined by inoculating the isolate at concentrations of 10%,20%,30%,40% and 50% and by incubating on rotary shaker incubator at 40⁰C for 48 hours.

Bioleaching procedure: A standard test procedure was followed¹¹. Briefly, 2.0 g of Bornite was added to 50 ml of modified 9 k medium (minus iron) in 250 ml conical flasks. Medium was sterilized at 110⁰C for 10 minutes and was inoculated with 0.1 ml of actively growing culture of *Thiobacillus ferrooxidans* N-11 at initial cell density of 1.0 *10⁷ cells/ ml. Cell density was determined by Petroff-Hauser bacteria counter and as per Nephelometer standards.

Process optimization: Unless otherwise stated the experiments were carried out in 250 ml of flasks with 50 ml of modified 9 K medium. During incubation liquid samples were removed periodically filtered, centrifuged and total Cu⁺⁺, Fe⁺⁺ concentration was determined by Atomic absorption spectrophotometer.

Bioleaching study was carried out in both shake flasks as well as in bioreactor.

Shake flask study: Optimization of temperature: For optimization of temperature inoculated flasks were incubated at

temperatures 20⁰C, 30⁰C, 40⁰C, 50⁰C, 60⁰C. For pH, at pH1.5, 2.5, 3.5, 4.5, 5.5.For Agitation at 40 rpm, 60 rpm, 80 rpm, 100 rpm, 120 rpm, 140 rpm, 160 rpm, 180 rpm, 200 rpm and 220 rpm. For yeast extract with 0.5 g/L,1.0 g/L, 1.5 g/L, 2.0g/L, 2.5 g/ L,3.0g/L, 3.5 g/L, 4.0g/L, 4.5 g/L 5.0g/L, and 5.5 g/L. For optimization of inoculum, culture was added from 1%, 2%, 3%,4% up to 10% v/v with a cell density of 1.0 * 10⁷ cells/ ml. For pulp density flasks with 9 K medium containing Bornite concentration 5%,10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50% was inoculated with *Thiobacillus ferrooxidans* N-11 with a cell density of 1.0 * 10⁷ cells/ ml.

Bioreactor study: For standardization of growth and bioleaching process by *Thiobacillus ferrooxidans* N-11. The parameters which were optimized on shake flask study were determined with fully automatic microprocessor controlled bioreactor model (Biostat B,B Brown international Germany) with 5L capacity.

All parameters viz, Temperature, pH, Agitation, Aeration, were monitored with fully automatic device. Different parameters i.e. Inoculum size,(4% v/v), Temperature (40⁰C), pH (3.5), Agitation (120rpm), Aeration (38%), and Yeast extract (0.5g/L) were kept optimum. During batch run 2 ml quantity of medium was collected after every 24 hours and analysed for growth pattern and concentrations of iron and copper.

Results and Discussion

Table 1 indicates Primary analysis of Bornite. This indicated the 38.2% copper as per table-1.

Table-1
Chemical and mineralogical analysis of Bornite

Elemental/Mineral	Composition %
Cu	38.2
Fe	18.4

Process optimization with respect to shake flask study indicated that the maximum bioleaching observed at Temperature 40⁰C., pH-4.5.,Inoculum size 4% v/v., Agitation 120 rpm., yeast extract 0.5g/L., pulp density of 20 % and time course 22 days. Table-2 and Fig-1 indicates the course of metal extraction during bioleaching process by Shake flask.

Table-2
Chemical and mineralogical analysis of Bornite during bioleaching.(Shake flask study)

Composition Days ↓	1	2	4	6	8	10	12	14	16	18	20	22	24
Element ⇨													
Cu ²⁺⁺	38.2	38.0	36.4	35.1	34.0	32.2	28.6	24.8	18.2	16.4	12.6	11.2	11.1
Fe ²⁺⁺	18.4	18.2	18.0	17.6	17.0	15.5	13.2	11.4	9.2	7.9	6.5	4.3	1.2

Table-3
Chemical and mineralogical analysis of Bornite during bioleaching.(Bioreactor study)

Composition Days↓	Day1	2	4	6	8	10	12	14	16	18	20	22
Element ⇨												
Cu ²⁺⁺	38.2	38.1	37.8	35.9	31.3	27.4	23.2	18.2	14.4	10.3	8.5	8.2
Fe ²⁺⁺	18.4	18.2	18.0	17.1	16.0	14.1	12.3	11.4	9.6	7.2	3.4	1.4

Cu⁺⁺, Fe⁺⁺% Days

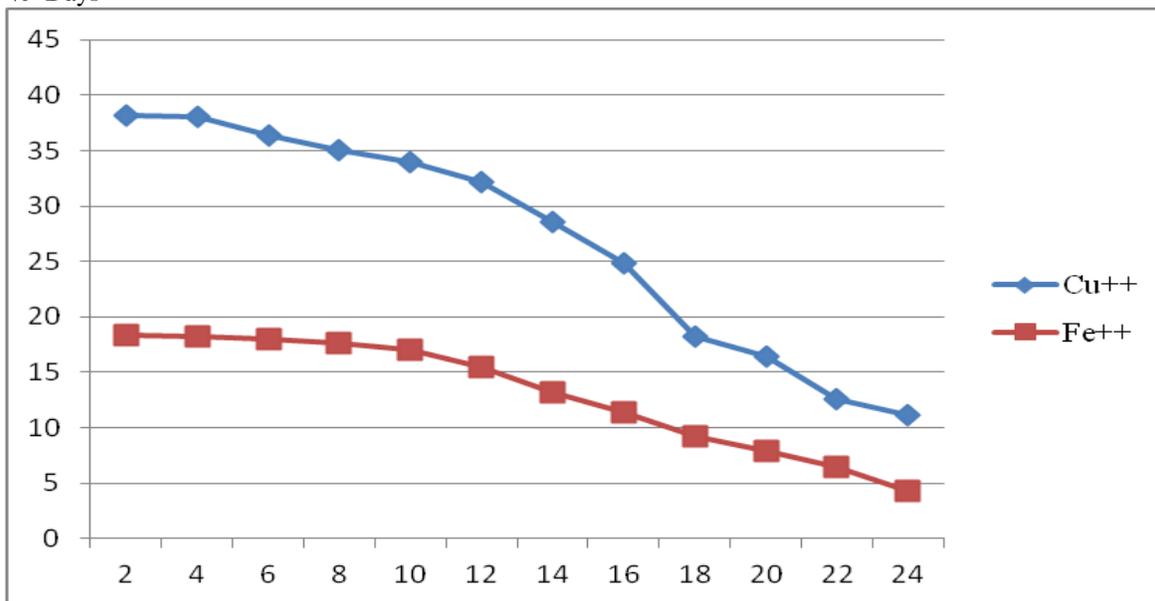


Figure-1
 Course of metal extraction during bioleaching process by Shake flask study

Cu⁺⁺, Fe⁺⁺% Days

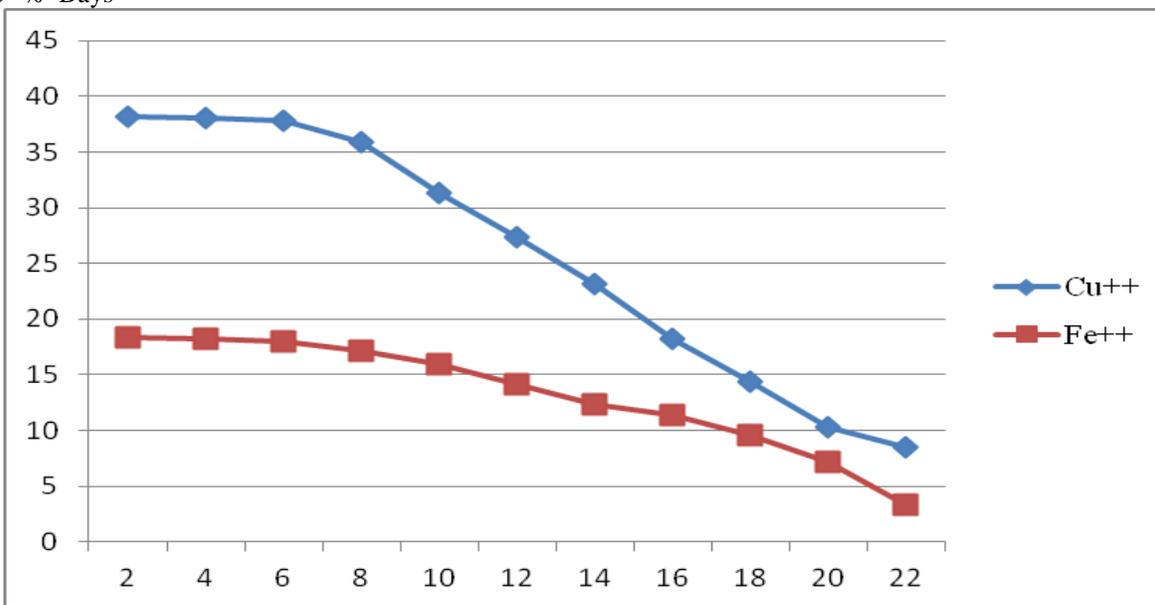


Figure-2
 Course of metal extraction during bioleaching process using Bioreactor

Shake flask study showed that there was an initial lag of 24 hours and a significant Bornite leaching started after 8th days and continued up to 22nd day. The rate then decreased as iron was consumed. After 22nd day a total copper extraction of 72% was achieved by shake flask. table-3 and figure-2 indicates course of bioleaching during bioreactor study. Results indicated that There was a lag of 24 hours as that of shake flask study, the effective leaching started after 6th day of bioleaching and continued up to 20th day of leaching process. The rate then decreased as iron was utilized. Effect of pH was studied by⁷. It was observed that *Thiobacillus ferrooxidans* oxidise iron optimally at pH between 3 to 3.6. My results indicated optimum leaching at pH 3.2 which are similar to that of⁷. Effect of temperature on bioleaching indicated that optimum leaching of sulphide ores by uncharacterised strain at 37^oC¹². My strain gives optimum leaching at 40^oC. Effect of bornite concentration on bioleaching of ore has been studied by several researchers, The rate of metal dissolution decreases with increase in concentration of bornite¹³⁻¹⁴. Effect of inoculum on metal dissolution was studied¹⁵. It was observed that maximum recovery at 3 % of inoculums size. My result indicated maximum copper recovery at 4% inoculums size. It was observed that the optimal pulp density 10% w/v¹³, 5 to 20%¹⁶. There was no significant difference in rate of iron and copper dissolution at 10 to 20%¹⁶. My result indicated the dissolution of copper at 20% w/v pulp density and initial pH of 3.2 at 40 °C with a particle size of 58 to 109 u size.

The percent extraction was found to be about 90% after 12 days¹⁷. My results indicated 72% of copper can be extracted after 22 days and bioreactor study indicated the total extraction of 78% can be achieved in 20 days.

Some researchers isolated bacteria from soil environments and found that the bacteria from soil environments are also equally competent in leaching process^{18, 19}. As my culture is isolated from a saline soil environment, is also have a very good leaching ability. In literature very few reports have been found on bioleaching by halophillic bacteria. Two new species of halotolerant *Thiobacillus* species. *Thiobacillus prosperus* and *Thiobacillus cuprinus* from saline environment²⁰⁻²¹. These organisms are found to be very efficient in bioleaching of copper from Chalcopyrite. Except this information on halophillic organisms no reports have been found on use of *Thiobacillus ferrooxidans*, My report may be the opening of new era for use of Halophillic *Thiobacillus ferrooxidans* N-11 as a potential candidate for bioleaching of copper from a low grade ore Bornite.

Conclusion

Optimum bioleaching process by *Thiobacillus ferrooxidans* N-11 was observed at pH 3.2, Temperature 40^oC, Agitation 120rpm, pulp density 20%, Yeast extract 0.5g/L.

Process may be advantageous over conventional method of copper extraction.

Study opens promising possibilities for optimization of mining process in metallurgy industry.

The isolate *Thiobacillus ferrooxidans* N-11 can also be used in treatment of mineral industrial waste containing high metal concentration, which is difficult to treat by conventional methods.

Present study reports the use of halophillic *Thiobacillus ferrooxidans* N-11 as a bioleaching strain.

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