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Central European Journal of Experimental Biology, 2012, 1 (1):1-6
(http://scholarsresearchlibrary.com/archive.html)

ISSN: 2278–7364

Biomining of copper using Halophilic *Thiobacillus ferroxidans*.N-9

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ABSTRACT

Bio mining is a process of extracting minerals from ores using microorganisms. It is a biochemical process involving interactions between microbes and minerals to recover valuable metals. It is one of the environment friendly process of mining and mineral processing.In the present study 11 different bacterial isolates were isolated from hyper saline soils of Kolhapur district. Isolates were identified using Bergey's manual of systematic bacteriology. All the isolates were investigated for bioleaching of copper using low grade ore Chalcopyrite. Of all isolates, isolate no N-9identified as Thiobacillus ferroxidans.N-9 is found to be most suitable for bioleaching of copper ore in both shake flask as well as bioreactor study. The results showed that in the shake flask the isolate no.N-9 tolerates 40 g/L of Chalcopyrite when supplemented with 0.5 G/L of yeast extract. At 120 rpm and at 40^{o}C temperature,78% of copper can be extracted from 40 g/L of Chalcopyrite after 14 days. Bioreactor study indicated the total extraction of 85% can be achieved in 12 days. Present study indicates the usefulness of isolate N-9 in bioleaching of copper from its low grade ore chalcopyrite.

Key words: *Biomining, Halophilic, Thiobacillus thiooxidans N-9, Chalcopyrite, 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 ore[1].

The conventional 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 hydrometullurgical method ore concentrate is leached by chemical methods followed by solvent extraction and electro-wining, however this method is not also free from environmental complexcity but also from non-competitive economics [2].

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 world [3].

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 active in bioleaching belongs to the genus Thiobacillus, [4,5]. These organisms are chemolithotrophic use iron and reduced sulphur compounds as source of energy [6].

By keeping in view this background, in the present study Halophilic *Thiobacillus ferroxidans* N-9 is explored for bioleaching of copper from low-grade ore chalcopyrite.

MATERIALS AND METHODS

Thiobacillus ferroxidans N-9 Fig-1. was isolated from hyper saline soil of Kolhapur district of Maharashtra, India on modified 9 K medium as per [7]. In brief composition(g/L),Solution-A: $(NH_4)_2SO_4$(3.0),KCl(0.1),K_2HPO_4 (0.5), $MgSO_4.7H_2O$(0.5), Ca(No_3) (10mg/L), 10N H_2SO_4(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 bacteriology by [8] and as per MICRO-IS software[9].

Chalcopyrite ore was grinded to -165 / + 300 mesh (58 to 109 u) Fig-2,3. Initial copper and iron percentage was determined by atomic absorption spectrometry as per [10].

Tolerance: Tolerance of isolate *Thiobacillus ferroxidans* N-9 to chalcopyrite was determined by inoculating the isolate at concentrations of 10%,20%,30%,40% and 50% and by incubating on shaker Fig,4, at 40^0C for 48 hours Fig-5.

Bioleaching procedure:
A standard test procedure was followed[11]. Briefly,2.0 g of chalcopyrite was added to 50 ml of modified 9 k medium (minus iron) in 250 ml conical flasks. Medium was sterilized at 110^0C for 10 minutes and was inoculated with 0.1 ml of actively growing culture of *Thiobacillus ferroxidans* N-9 at initial cell density of 1.0 $*10^7$ cells/ ml. Cell density was determined by Petroff-Hauser bacteria counter and as per Nephlometer 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 20^0C, 30^0C, 40^0C, 50^0C, 60^0C. 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% up to 10% v/v with a cell density of 1.0 * 10^7 cells/ ml. For pulp density flasks with 9 K medium containing chalcopyrite concentration 5%,10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50% was inoculated with *Thiobacillus ferroxidans* N-9 with a cell density of 1.0 * 10^7 cells/ ml.

Bioreactor study: For standardization of growth and bioleaching process by *Thiobacillus ferroxidans* N-9. 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,(5% v/v), Temperature (40^0C), 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

Microorganisms isolated from hypersaline soil were identified as *Arthrobacter* sp,N-1., *Bacillus* sp,N-2.,*Chromobacterium* sp,N-3.,*Planococcus* sp,N-4., *Pseudomonas* sp,N-5., *Micrococcus* sp,N-6., *Peptostreptococcus*,N-7., *Halococcus* sp,N-8., *Thiobacillus ferroxidans* N-9.,*Halococcus* sp,N-10.,*Sulfolobus* sp,N-11.

Primary analysis of chalcopyrite indicated the 32.8% copper as per table-1.

Table-1 Chemical and mineralogical analysis of Chalcopyrite

Elemental/Mineral	Composition %
Cu	32.8
Fe	26.4

Process optimization with respect to shake flask study indicated that the maximum bioleaching observed at Temperature 40^0C., pH-3.5.,Inoculum size 5% v/v., Agitation 120 rpm., yeast extract 0.5g/L., pulp density of 15 % and time course 15 days.

Table-2 and Fig-6 indicates the course of metal extraction during bioleaching process by Shake flask.

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

Composition ⇩ Days ⇨	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Element															
Cu^{2++}	32.8	32.0	30.2	29.4	29.0	28.2	27.4	22.2	18.2	16.3	14.1	12.2	9.1	7.2	7.0
Fe^{2++}	26.4	25.9	25.2	24.6	23.8	22.7	21.2	19.0	17.2	14.3	11.5	9.3	7.4	5.2	3.7

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

Composition ⇩ Days ⇨	Day1	2	3	4	5	6	7	8	9	10	11	12	13
Element													
Cu^{2++}	32.8	31.6	30.2	28.8	27.3	26.4	25.1	20.2	17.4	15.3	11.4	7.1	7.0
Fe^{2++}	26.4	25.2	24.0	23.1	22.0	20.9	19.3	17.4	14.6	10.2	7.1	3.8	3.5

Shake flask study showed that there was an initial lag of 24 hours and a significant chalcopyrite leaching started after 8^{th} days and continued up to 14^{th} day. The rate then decreased as iron was consumed. After 14^{th} day a total copper extraction of 78% was achieved by shake flask.Table-3 and Fig-7 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 7^{th} day of bioleaching and continued up to 12^{th} day of leaching process. The rate then decreased as iron was utilized. Effect of pH was studied by [7] it was observed that *Thiobacillus ferroxidans* oxidise iron optimally at pH between 3 to 3.6. My results indicated optimum leaching at pH 3.5 which are similar to that of [7]. Effect of temperature on bioleaching indicated that optimum leaching of sulphide ores by uncharacterised strain at 37^0 C[12]. My strain gives optimum leaching at 40^oC. Effect of Chalcopyrite concentration on bioleaching of ore has been studied by several researchers, The rate of metal dissolution decreases with increase in concentration of Chalcopyrite [13-15].It was observed that the optimal pulp density 10% w/v[13], 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 15% w/v pulp density and initial pH of 3.5 at 40 ^{o}C with a particle size of 58 to 109 u size.

The percent extraction was found to be about 90% after 12 days[17]. My results indicated 78% of copper can be extracted after 14 days and bioreactor study indicated the total extraction of 85% can be achieved in 12 days.

Some researchers [18,19] isolated bacteria from soil environments and found that the bacteria from soil environments are also equally competent in leaching process. 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 halotolerent *Thiobacillus* species. *Thiobacillus prosperus* and *Thiobacillus cuprinus* from saline environment[20-21]. 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 ferroxidans* ,My report may be the opening of new era for use of Halophilic *Thiobacillus ferroxidans* N-9 as a potential candidate for bioleaching of copper from a low grade ore Chalcopyrite.

Fig: 1 Isolate no. 9, *Thiobacillus ferroxidans*,N-9

Fig:2, Ore Chalcopyrite(Powder form)

Fig:3, Ore Chalcopyrite **Fig:4, Incubator shaker**

Fig:5 Tolerence to Chalcopyrite by *Thiobacillus ferroxidans*,N-9.

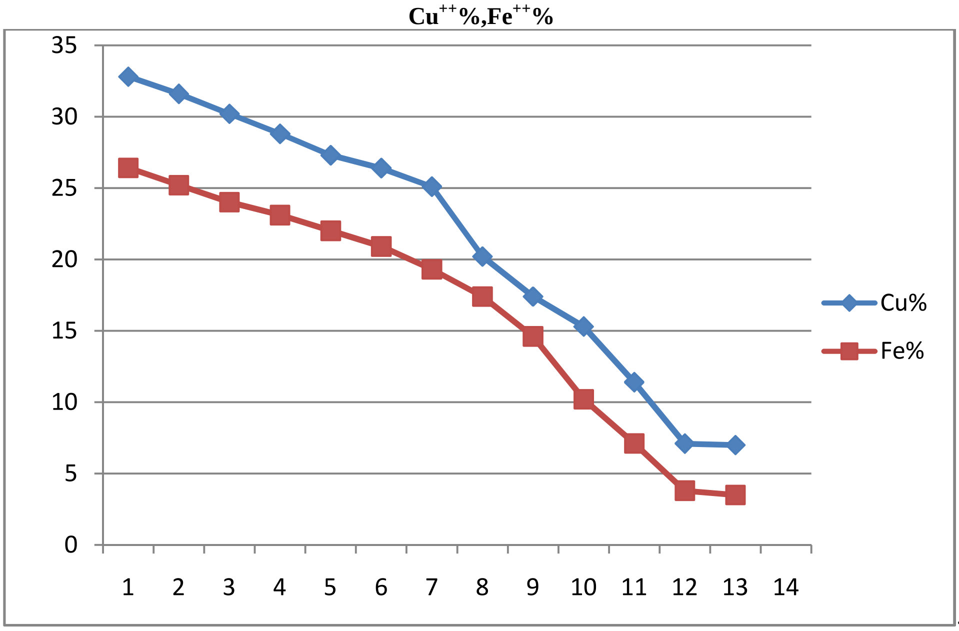

Fig:6 Course of metal extraction during bioleaching process by Shake flask study

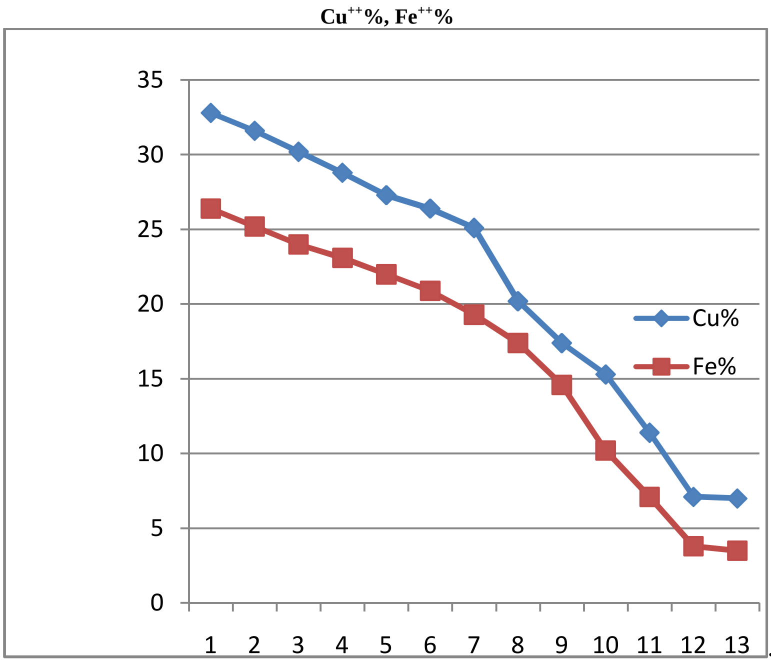

Fig:7 Course of metal extraction during bioleaching process using Bioreactor.

CONCLUSION

Optimum bioleaching process by *Thiobacillus ferroxidans* N-9 was observed at pH 3.5, Temperature 40^{0}C,Agitation 120rpm, pulp density 15%, 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 ferroxidans* N-9 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 ferroxidans* N-9 as a bioleaching strain.

Acknowledgements
I am thankful to Principal ,Rajaram college ,Kolhapur for allowing me to work in laboratories. I am also thankful to Head, Dept. of Geology, Rajaram college for providing me the mineral ore Chalcopyrite for experiments.

REFERENCES

[1] Olson, G.J., Brierly J.A. and Brierly C. L,(**2003**) *Appl. Microbial Biotechnol*. 63 449.
[2] Barrett, J., Hughes M. N., Karavaiko G. I. and Spencer P.A,(**1993**) Metal extraction by bacteria oxidation of minerals, New York. Ellis Horwood.
[3] Watling, H.R, (**2006**) Hydrometallurgy', 84 (1-2) 81.
[4] Vishniac, w., and Santer, M,(**1957**) *Bacteriol. Rev*. 21, 195-213.
[5] Trudinger, P. A.(**1967**) *Rev pure Appl. Chem*. 17, 3-4.
[6] Mossman, D. J., Reimer, T., and Durstling, H,(**1999**) *Can*.26: 131-140.
[7] Silvermann, M.P., and Lundgren, D. G**,(1959**) *Bacteriol*, 77,642.
[8] Williams, S.T., Sharpe, M.E. and Holt, T. J,(**1969**) Bergeys manual of systematic bacteriology', Vol.III, The Williams and Wilkins co. Baltimore.
[9] Portyrata, d. A. and Krichevsky, M.I.(**1992**) *Binary* 4:31-36.
[10] Greenburg, A. E., Clesceri L. S. and Eaton A. D,(**1992**) Standard methods for the examination of water and waste water', 18th ed. Washington: APHA (American Public Health Association).
[11] American society for testing and materials(ASTM)**,(1990**) Test methods for determining the rate of bioleaching of iron from pyrite by *Thiobacillus ferroxidans*, designation E 1357-90, In R. A. Storer (ed.), Annual book of ASTM standards, American society for testing and materials,Philadelphia,Pa.
[12] Ahonen, L., and Tuovinen, O.G,(**1995**) *Hudrometallurgy*, 37, 1-21.
[13] Deng, T.L., Liao, M.X., Wang, M.H., Chen, Y.W., and Belzile, N,(**2000**) *Minerals Engineering*, 13 (14), 1543-1533.
[14] Gomez, C., Blazquez, M.L., and Ballester, A,(**1999**) *Minerals Engineering*,12(1),93-106.
[15] Witne, J. Y. and Phillips**,** C. V,(**2001**) *Minerals Engineering*, 14,25-48.
[16] Ubaldani, S.,Veglio, F., Toro, L., and Abbruzzese, C**,(2000**) *International journal of mineral processing*, 60, 247-262.
[17] Iglesias, N. and Carranza, F**,(1995**) *Minerals engineering*,8(10),1089-1096.
[18] Kanishi, Y., and Sataru, A,(**1992**) *Biotechnol*.12:133-155.
[19] Waksman, S. A. and Joffe, I.S,(**1922**) *J. Bacteriol*.7,239-256.
[20] Huber, H., and Stetter, K.O,(**1990**) *Appl. Environ. Microbiol*. 56, 315-322.
[21] Huber, H. and Stetter, K. O,(**1989**) *Arch. Microbiol*.151, 479-485.