HALOTOLERENT THIOBACILLUS FERROOXIDANS N-13 AS A STRAIN FOR BIOLEACHING OF COPPER FROM LOW GRADE ORE COVELLITE

Dr. NAKADE DHANRAJ. B.
ASSOCIATE PROFESSOR IN MICROBIOLOGY, DEPT. OF MICROBIOLOGY, GOVT. RAJARAM COLLEGE, KOLHAPUR416004 (MAHARASHTRA)

ABSTRACT

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, 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 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.

Environmental pollution and other problems

By considering this, in the present study Halophillic *Thiobacillus ferroxidans* N-13 was explored for bioleaching of copper from low grade ore Covellite. *Thiobacillus ferroxidans* N-13 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 ferroxidans* N-13 tolerates 40 g/L of Covellite when supplemented with 0.5 g/L of Yeast extract. At 200 rpm and 37 °C temperature about 82% of copper can be extracted after 10 days by shake flask method and 88% can be extracted by bioreactor study in 8 days.

Present study indicated the usefulness of *Thiobacillus ferroxidans* N-13 in bioleaching of copper from low grade ore Covellite can be used as a potential candidate for bioleaching as a pollution free process.

Key words: Bioleaching, Covellite, Ores, Saline Soils, Pyrometallurgy.
INTRODUCTION

Bioleaching is a simple and effective process used for metal extraction from low grade ores using the chemolithotrophic bacteria. The extraction of copper from low grade ore is to days need because of gradual depletion of high grade ore Olsen and Brierely,(2003).

There exist a large Stockpiles of low and lean grade ores, it is the need of today to go for low grade ores for extraction of metals. Only the thing is that, the current methods used for extraction of metals from ore are expensive and are not free from problem of environmental pollution.

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 hydrometallurgical method ore concentrate is leached by chemical methods followed by solvent extraction and electro-wining, however this method is not also free from environmental complexity but also from non-competitive economics Barrett et al.(1993).

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 Watling (2006).

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, Vishnia and Santer, (1957), Trudinger (1967). These organisms are chemolithotrophic use iron and reduced sulphur compounds as source of energy Mossman et al. (1999).

By keeping in view this background, in the present study Halophilic Thiobacillus ferroxidans N-13 is explored for bioleaching of copper from low-grade ore covellite.

MATERIAL AND METHODS'

Thiobacillus ferroxidans N-3 Fig-1. was isolated from hyper saline soil of Kolhapur district of Maharashtra, India on modified 9 K medium as per Silvermann and Lundgren (1959). In brief composition(g/L),Solution-A: (NH$_4$)$_2$SO$_4$(3.0),KCl(0.1),K$_2$HPO$_4$ (0.5), MgSO$_4$.7H$_2$O(0.5), Ca(NO$_3$) (10mg/L), 10N H$_2$SO$_4$(1ml) Distilled water(700ml).Solution-B Covellite (40), Elemental iron (2.0) , distilled water (300ml). It was identified by using morphological, cultural, biochemical, methods as per Bergey’s manual of systematic bacteriology by Williams et al.(1989),and as per MICRO-IS software by Portyrata and Krichevosky, ( 1992).

Covellite ore was grinded to -165 / + 300 mesh (58 to 109 µ) Fig-2,3. Initial copper and iron percentage was determined by atomic absorption spectrometry as per Greenberg et al. (1992).

Tolerance

Tolerance of isolate Thiobacillus ferroxidans N-13 to Covellite was determined by inoculating the isolate at concentrations of 10%, 20%, 30%, 40% and 50% and by incubating on shaker Fig,4, at 40°C for 48 hours Fig-5.
Bioleaching procedure
A standard test procedure developed by ASTM, (1990) was followed. Briefly, 2.0 g of Covellite was added to 50 ml of modified 9 k medium (minus iron) in 250 ml conical flasks. Medium was sterilized at 110°C for 10 minutes and was inoculated with 0.1 ml of actively growing culture of *Thiobacillus ferroxidans* N-13 at initial cell density of 1.0 *10^7 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, S 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°C, 30°C, 40°C, 50°C, and 60°C. 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, 2.0 g/L, 2.5 g/L, 3.0 g/L, 3.5 g/L, 4.0 g/L, 4.5 g/L, 5.0 g/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 Covellite concentration 5%, 10%, 15%, 20%, 25%, 30%, 35%, 35%, 40%, 45%, 50% was inoculated with *Thiobacillus ferroxidans* N-13 with a cell density of 1.0 * 10^7 cells/ ml.

Bioreactor study: For standardization of growth and bioleaching process by *Thiobacillus ferroxidans* N-13. 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 (37°C), pH (4.5), Agitation (200rpm), 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 DISCUSSIONS

- 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, *Pseudomonas putida*, N-12., *Thiobacillus ferroxidans* N-13.
Primary analysis of Covellite indicated the 32.8% copper as per table-1.

**Table-1**

<table>
<thead>
<tr>
<th>Elemental/Mineral</th>
<th>Composition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>38.2</td>
</tr>
<tr>
<td>S</td>
<td>18.4</td>
</tr>
</tbody>
</table>

Process optimization with respect to shake flask study indicated that the maximum bioleaching observed at Temperature 37°C, pH-4.5., Inoculum size 4% v/v., Agitation 200 rpm., yeast extract 0.5g/L., pulp density of 40% and time course 10 days.

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

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

<table>
<thead>
<tr>
<th>Composition Days</th>
<th>Day-1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>32.2</td>
<td>28.2</td>
<td>24.4</td>
<td>16.1</td>
<td>11.0</td>
<td>8.2</td>
<td>6.2</td>
</tr>
<tr>
<td>S</td>
<td>13.2</td>
<td>11.4</td>
<td>9.2</td>
<td>7.9</td>
<td>6.5</td>
<td>4.3</td>
<td>1.6</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Composition Days</th>
<th>Day-0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>32.2</td>
<td>27.1</td>
<td>23.8</td>
<td>18.9</td>
<td>10.3</td>
<td>9.4</td>
</tr>
<tr>
<td>S</td>
<td>18.4</td>
<td>12.3</td>
<td>11.4</td>
<td>9.6</td>
<td>7.2</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Shake flask study showed that there was an initial lag of 24 hours and a significant Covellite leaching started after 2nd days and continued up to 10th day. The rate then decreased as sulphide and added iron was consumed. After 10th day a total copper extraction of 82% was achieved by shake flask. Table-3 and Fig-2 indicates course of bioleaching during bioreactor study. Results indicated that there was a lag of 12 hours, the effective leaching started after 24th hour of bioleaching and continued up to 8th day of leaching process. The rate then decreased as sulphide and added iron was utilized. After 8th day a total copper extraction of 88% was achieved by Bioreactor study. Effect of pH was studied by Tuovinen and Kelly (1973), They observed that *Thiobacillus ferroxidans* oxidise sulphide and iron optimally at pH between 2 to 2.5. My results indicated optimum leaching at pH 4.5 which are not similar to that of Tuovinen and Kelly. Effect of temperature on bioleaching was studied by Ahonen and Tuovinen (1991), they observed optimum leaching of sulphide ores by uncharacterised strain at 37°C. My strain also gives optimum leaching at 37°C. 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 metal ore by Deng *et al.* (2000), Gomez *et al.* (1999), Witne and Philips (2001). Deng *et al.* reported the optimal pulp density 10% w/v. Gomez *et al.* reported 5 to 20% , Ubaldani *et al.* (2000) reported that there was no significant difference in rate of iron and copper dissolution at 10 to 20%. My result indicated the dissolution of copper at 40% w/v pulp density and initial pH of 4.5 at 37°C with a particle size of 58 to 109 u size.
Karavaiko et al., (1988) showed that the presence of iron enhances the bioleaching of Covellite, our results also indicated that there was reduction in time of leaching from 18 days to 10 days when medium was supplied with 0.2 % of elemental iron. Iglesias and Carranza (1995) reported that the percent extraction was found to be about 90% after 12 days. My results indicated 82% of copper can be extracted after 10 days and bioreactor study indicated the total extraction of 88% can be achieved in 8 days. My results are somewhat similar to that of Iglesias and Carranza (1995).

Kanishi and Sataru (1992), Waksman and Joffe (1922), 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 halophilic bacteria. Huber and Stetter (1989,1990) isolated two new species of halotolerent *Thiobacillus* species, *Thiobacillus prosperus* and *Thiobacillus cuprinus* from saline environment. These organisms are found to be very efficient in bioleaching of copper from Chalcopyrite. Except this information on halophilic 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-13 as a potential candidate for bioleaching of copper from a low grade ore Covellite.

Cu$^{++}$%, Fe$^{++}$%
CONCLUSIONS

- Optimum bioleaching process by *Thiobacillus ferroxidans* N-13 was observed at pH 4.5, Temperature 37°C, Agitation 200rpm, pulp density 40%, 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-13 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-13 as a bioleaching strain.

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