Bioleaching of low grade granitic chalcopyrite ore by hyperthermophiles: Elucidation of kinetics-mechanism

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Abstract – The current work was off-shoot of an attempt to isolate a thermophilic pyritic sulfur degrading bacteria in selective medium from a coal mine dump. This thermophilic bacteria (archaea) classified as *Sulfolobus* was tested for its feasibility to bioleach copper from a low grade Indian chalcopyrite ore (0.3% copper). With its prevalent ability to preferentially attack pyrite (a major phase in the ore), bioleaching was investigated at various parameters of pH, pulp density (PD), particle size, and temperature. A reflective high 85% copper recovery was obtained using <50 µm particles at 20% (w/w) pulp density, pH 2.0, 75 °C in 30 days. The copper dissolution was facilitated by iron (III) available in the leach liquor because of bacterial oxidation of pyrite present in the ore under acidic conditions. The biogenically generated Fe (III) ions enhanced copper dissolution from the chalcopyrite ore. The bioleaching of copper appeared to follow chemical control kinetic model with the reaction of lixiviant-Fe (III) and acid on the surface of the solid. Phase identification by XRD and SEM study corroborated the above mechanism of copper leaching.

Biohydrometallurgy has been studied extensively since last three decades covering its role for various metals and varied resources [1, 2]. Bioleaching has also faced technical issues in scale-up and commercialization for its slower rate. It was reported that microbes with high tolerance to metal ions promoted leaching efficiency and thus all experiments with pure species compared their efficacy with the ones carried with adapted microbes [3]. Different ores and concentrates have been tested to be amenable to leaching of copper, nickel, zinc, cobalt, uranium by acidophilic microorganisms in single or by mixed cultures of different species of both mesophilic and thermophilic microbes [2]. Temperature and ferric concentration greatly influence the efficiency of the chalcopyrite leaching [5, 6]. In mesophilic conditions, ferric ions are effective for leaching chalcopyrite with bacteria maintaining high ferric to ferrous ratio and a high redox potential [4].

There are two dominant views on the mechanisms involved in bioleaching of copper [7–9]. The first is that the overall leaching process occurs by the microbial oxidation of ferrous to ferric ions followed by the chemical oxidation of the sulfide mineral by the ferric ion. This is known as the indirect mechanism. The second view envisages the microbial catalysis of the overall dissolution of the mineral. It has been proposed that the microorganisms interact with the mineral directly, enhancing the rate of oxidation, over and above that achieved by chemical oxidation. This is known as the direct mechanism. Regardless of the mechanism, it is the enhanced oxidation of the mineral created by the microorganism that creates the acid-solubility required for leaching to take place.

The overall chemical reactions for chalcopyrite are:

\[ 4CuFeS_2 + 11O_2 + 6H_2O \rightarrow 4CuSO_4 + 4Fe(OH)_3 + 4S + 2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4. \]
forms on the mineral surfaces that prevents further leaching [10–13]. This passivating layer could come from one or both of two sources: Fe(OH)₃ tends to form jarosite (KFe₃(SO₄)₂(OH)₆), which coats the unreacted material and forms a passivating layer and/or, the elemental sulfur formed in reaction (1), which also tends to coat the surface. It has been found from fundamental electrochemistry that the newer technology, using thermophilic microorganisms such that the temperature can be raised to 60 °C (140 °F) or higher, destabilizes the passivating layer on chalcopyrite [14–17]. Higher extractions have been achieved [18–20] using extremely thermophilic archaea growing at higher temperatures (60–85 °C). The oxidation reactions involved in the solubilization of mineral sulfides are exothermic; hence the ability of the thermophiles to grow at higher temperatures is advantageous in providing an improved driving force for heat removal. In addition, enhanced oxidation kinetics in terms of rate of reaction and extent of solubilization is a potential advantage of thermophilic bioleaching [21–23]. The extent of leaching of base metals such as copper from refractory sulfidic minerals is greatly increased by the implementation of increased temperature. Thermophiles like Sulfolobus, Acidithiobacillus and Metallosphaerae have been shown to leach chalcopyrite better at 65–75 °C [23,24], in shake flask mode. Application of these thermophiles has been widely reported in metal biodissolution processes, especially in copper bioleaching from chalcocite and covellite [22]. However, a scanty work has been found for low grade chalcopyrite [5,6], which is considered otherwise difficult to be processed by conventional hydrometallurgical route [2].

In this work, an enriched culture of thermophile, Sulfolobus was isolated from the coal mine dumps at 75 °C with the objective of depyratisation of coal. The preferential growth in presence of pyrite made us apply this microbe to a metal rich environment of chalcopyrite (0.3% Cu) from Malanjkhand granitic copper ore and test its feasibility to microbially dissolve copper under various parameters like pH, pulp density, particle size and temperature in presence and absence of bacteria.

1 Materials and methods

1.1 Ore sample

Low tenor granitic hard copper ore (containing 0.3% Cu) was collected in the form of lumps from Malanjkhand copper mine (located in Balaghat, Madhya Pradesh, India). The ore was crushed, ground and passed through a sieve of 50 µm size. The bioleaching experiments were carried out with this material unless stated otherwise. A representative sample was then prepared by coning and quartering for chemical analysis by atomic absorption spectrometer [5]. The phase identification by XRD showed that CuFeS₂ (chalcopyrite), FeS₂ (pyrite) and SiO₂ (quartz) were the major mineral phases and Cu₅FeS₄ (bornite) as a minor mineral phase in the ore. SEM micrograph of the ore is shown in Figure 1, which clearly indicates the presence of Cu, Ni, Co, Fe in predominant alumino-silicate matrix.

1.2 Microorganism

Enriched culture of a thermophilic archaea was isolated from dumps of coal mines, India in medium containing (NH₄)₂SO₄–1.3 g/L, KH₂PO₄: 0.028 g/L; MgSO₄·7H₂O–0.025 g/L; CaCl₂·2H₂O–0.07 g/L; MnCl₂·4H₂O–1.8 mg/L; FeCl₃·6 H₂O–0.02 g/L; Na₂B₄O₇·10H₂O–4.5 mg/L; ZnSO₄·7H₂O–0.22 mg/L; CuCl₂·2H₂O–0.05 mg/L; VOSO₄·2H₂O–0.03 mg/L; yeast extract-1.0 g/L at pH 1.8 and 70 °C. This archaea bacteria was able to oxidize Fe (II) to Fe (III) from its ability to grown proficiently in presence of pyrite. The enriched culture was subsequently adapted thrice on 5% (w/w) chalcopyrite ore (<50 µm) in above medium under conditions of isolation.

1.3 Bioleaching experiments

The leaching experiments were carried out in Erlenmeyer flasks in an incubator shaker. A 200 mL of slurry containing ore (40 g) was inoculated with 10% (v/v) of enriched adapted culture. Conditions such as 70 ± 0.2 °C temperature, pH 1.8, 20% (w/v) pulp density (PD) with shaking at 120 rpm were maintained for leaching in
Fig. 1. SEM of <50 µm fraction of granitic Malanjkhand copper ore with elemental analysis.

High-temperature incubator-shaker (Make-Infors™) unless otherwise stated. A known amount of sample was drawn at 5 days interval for analysis of metals leached out by AAS. All the inoculated sets had their corresponding sterile control sets prepared under the same condition with 0.02 g/L HgCl₂ added as bactericide and ferric ion added to mimic that produced by microbial iron oxidation. Ferrous ion concentration was analysed by titration against N/20 potassium dichromate solution. The pH of the leach solution was maintained daily with 5 M sulfuric acid. Redox potential was measured
against SCE. On completion of the leaching experiment, the slurry was filtered on Whatman Filer Paper (No. 42) and solid residue was dried. The representative sample of the residue was chemically analysed and the phases were identified by XRD and SEM.

2 Results and discussion

2.1 Microbial isolation and culturing

The morphological characteristics of isolate were studied under Motorized material-cum-biological microscope (Make- Leica™). Light and electron microscopy were used in order to investigate the morphological changes of the cells when grown in iron oxidizing medium and also under adaptive environment. The cells were negatively stained and observed with an oil immersion objective of 100/1.6. Bacterial cells growing a oval cells with shining boundaries later grew in 12 days as big haloes of above 10 µm cross-sectional diameter as shown in Figure 2 with a rise in redox potential from 274 mV to 439 mV. Regular sub-culturing in 12 days ensured faster growth kinetics of cells and potency for oxidation of Fe (II) as its concentration decreased from 2.25 g/L to zero in 8 days of incubation. The increase in redox potential may be attributed to the improved oxidation of Fe (II) present in the solution because of bacteria present in it.

2.2 Microbial adaptation

The isolate was grown in presence of 0.5%w/w pyrite in medium with out iron source at similar conditions. The bacteria have a faster growth rate (Fig. 3a) with the artificially supplemented source of pyrite, thus proving its selectivity and initiating our interest to pursue this work. The pyrite grown species were then grown in 5% w/w chalcopyrite ore (<50 µm) in presence of medium at first stage and then without medium in subsequent three stages at pH 1.8–2.0 and 70 °C. There was a visible growth in presence of ore (containing pyrite as a major phase), aiding microbial proliferation (Fig. 3b).

2.3 Optimization of bioleaching parameters

Various parameters like pH, pulp density, particle size and temperature were varied to test their influence on leaching of copper from low grade ore.
2.3.1 Effect of pH

Experiments were conducted by varying pH in the range 1.5–2.5 at 10% (w/w) PD with <50 µm particles at 70 °C as shown in Figure 4. The extraction of copper increases with increase in pH up to 1.8 and descends at 2.0 to 2.5. It was recorded that enriched isolate could leach 78% Cu (Fig. 4a) in 25–30 days of incubation at pH 1.8 (there was hardly any change in recovery after 25 days) with rise in redox potential from 438–653 mV. Whereas, copper bioleaching recorded 58%, 72%, 66% and 60% recoveries at 1.5, 2.0, 2.2 and 2.5 pH respectively. When compared to chemical leaching (Fig. 4b), a maximum recovery of 11.78–16% Cu was observed under same conditions at pH 1.5–1.8. The metal recovery was very low at low pH because of lower oxidation potential of leaching system
and decreased activity of bacteria. Increasing the pH to 2.5 reduced the solubilisation of copper which may be due to the precipitation of high amount of iron (III) as hydronium jarosite [25], which was observed from the XRD phase identification studies. The lower metal bio-recovery (58%) may be further correlated with the lower value of redox potential (509 mV) at 2.5 pH. High metal bio-recovery (78%) at pH 1.8 may be attributed to the enhanced level of oxidation of Cu (II) to soluble form by Fe (III) produced through bio-chemical action on the ore. On the other hand, lower iron oxidation rate in control experiments was responsible for lower metal recovery. The presence of low Fe (II) level and high amount of Fe (III) generated biogenically, yielding high copper recovery suggesting it to follow indirect leaching mechanism.

As the amount of sulphur in the copper ore is 2.83%, auto-generation of acid is expected to be quite significant at pH 1.5–2.0. It was observed that initial acid requirement for leaching was sufficiently high which became significantly low after 5 days of leaching (figure not shown). The sterile control experiment carried out at pH 1.8 consumed 75% more acid than in the bioleaching sets.

2.3.2 Effect of pulp density

Pulp density (PD) for the bioleaching of copper was varied in the range 5–30% (w/w) using ore particles of <50 µm (mixed) size using 10% (v/v) adapted enriched culture, while shaking at 120 rpm at pH 1.8 and 70 °C temperature. Data presented in Figure 5 shows increase in metal dissolution.
Fig. 5. Variation in copper recovery with change in pulp density at pH 1.8 using <50 µm particles at 70 °C (a) Chemical Leaching (CL), (b) Bioleaching (BL).

with time in both chemical and bioleaching experiments. At 5% pulp density, copper extraction of 8% (Fig. 5a) and 52% (Fig. 5b) were obtained in 30 days in chemical and bioleaching respectively. This shows the efficacy of bacteria to work at higher pulp ratio when compared to the results with mesophiles [5, 6]. On further increasing the pulp density to 10% and 20%, the copper bio-recovery was found to increase from 78% to 82% (Fig. 5b) with corresponding chemical leaching (Fig. 5a) of 58% and 68%. The rise in redox potential (Fig. 6) was observed from 443 to 693 mV in bioleaching and 366 to 413 mV in control leaching for 20% PD in 30 days.

At still higher pulp density of 30% (w/v) under the above conditions, the bio-recovery decreased to 74% (Fig. 5b) because of lower ratio of inoculum to substrate which may be attributed to the lack of oxygen availability and increased concentration of metal ions causing toxicity to bacterial growth at higher pulp densities [26]. Copper leaching in sterile set (control) was also lower at higher pulp densities. Thus, in further experiments, 20% pulp density was maintained.

### 2.3.3 Effect of particle size

Studies on effect of particle size (>150 µm, 76–50 µm and <50 µm) as shown in Figure 7 indicates the suitability of fine size fraction (<50 µm) which recorded maximum (82%) copper biodissolution at 20% (w/w) PD, pH 1.8, and temperature 70 °C. It was correlated with the % Cu existing in this fraction which accounted to be 0.32%, whereas it was 0.17% and 0.29% in case of coarser
(<150 μm) and mid-size (76–50 μm) particles respectively. This could be also be attributed to better permeation of the leachant to oxidize the copper sulphide present in the ore and increased surface area. Finer particles were increasingly exposed to lixiviant that dissolved copper from the chalcopyrite phase [27]. The concentration of ferric ions, oxidized by bacterial action on ferrous ions involved in chemically dissolving the metals was much higher in case of leaching of finer size than in case of coarser (<150 μm) and mid-size (76–50 μm) particles. The lower copper recovery of 67% and 70% was recorded for the coarser (<150 μm) and mid-size (76–50 μm) particles (Fig. 7).

Copper bio-recovery may be correlated with the change in concentration of ferrous ions and the redox potential values as plotted in Figure 8. Ferrous ion concentration was high (0.47 g/L) which completely oxidizes to ferric state in 25 days (Fig. 8) for <50 μm size particles whereas different oxidation pattern for Fe (II) was observed for other size fractions. Also, the high value of redox potential (695 mV) was attained for finer particles, which resulted in higher recovery.

2.3.4 Effect of temperature

Rise in reaction temperature (in the range 50–75 °C) led to an increase of copper bioleaching at pH 1.8 and 20% (w/w) PD using 10% (v/v) enriched and adapted culture with <50 μm particles as shown in Figure 9. A maximum metal bio-dissolution of 85% Cu was recorded at 75 °C as compared to other three temperatures i.e. 59% at 50 °C, 63% at 60 °C and 74.2% at 65 °C respectively. The slight rise in recovery of raising the temperature can be attributed to the extremophilic nature and the resistance developed by these species. When compared with the data for chemical leaching sets, a maximum of 38% Cu was recovered at 75 °C, with corresponding recovery of 29% at 50 °C, 31% at 60 °C and 35% at 65 °C respectively in same time period.

During bioleaching at 75 °C, redox potential varied from 452 to 701 mV in 30 days (Fig. 10) inferring to the role of cell interactions with mineral phase and simultaneous rise in redox potential, causing higher metal dissolution. It thus indicates the ability to grown preferentially at high iron concentration in lesser time thereby promoting
the rate of copper bio-dissolution [5, 6]. Chemical leaching was also performed at the optimized temperature to understand the passivation behaviour of chalcopyrite in presence and absence of bacteria, which gave a maximum recovery of 77% (data shown in Fig. 5a). It has been earlier reported that mesophilic bioleaching using adapted enriched culture of *A. ferrooxidans* extracted 75% Cu at 35 °C [2, 5].

### 2.3.5 Kinetics of chalcopyrite bioleaching by *Sulfolobus*

The rate of chalcopyrite bio-dissolution was tested against shrinking core models through diffusion control, chemical control, and mixed control. Kinetic data showed a good fit (Fig. 11) to the diffusion controlled model according to Equation (3)

\[
1 - \frac{2}{3}x - \left(1 - x\right)^{2/3} = k_d t.
\]
The dissolution of copper proceeded by the diffusion of Fe (III) as the lixiviant generated bio-genically, through the porous product layer possibly of the jarosite formed on the ore particles [10–13]. The rate constant values for the diffusion controlled bio-leaching of copper with hyperthermophiles were obtained from Figure 11 as 0.0052, 0.0044, 0.0032, 0.0022, and 0.0018 d⁻¹ at 50 °C (324 K), 60 °C (333 K), 65 °C (338 K), 70 °C (344 K) and 75 °C (348 K) respectively. The plots of rate constant values ($k_d$) with the reciprocal of $r^2$ ($r$ being the size of the particles used) in the temperature range 324–348 K (not given here) which further confirmed that the copper bio-leaching followed the
diffusion controlled model [5]. The activation energy values ($E_a$) were calculated from the Arrhenius plot (Fig. 12) and was found to be 41.2 kJ/mol under the temperature range 324–348 K at 1.8 pH. The kinetics didn’t follow other models and hence not shown.

The XRD data of the leach residue generated in presence of absence of hyperthermophiles vis-à-vis mesophiles was analyzed. At 35 °C, hydronium jarosite [H$_3$OFe$_3$(SO$_4$)$_2$(OH)$_6$] and quartz were present as major phases [28] and chalcopyrite and pyrite as the minor phases. At 75 °C with hyperthermophiles, the presence of pyrite in minor phase couldn’t be accounted due to very low intensity, whereas elemental

Fig. 13. SEM micrographs of leach residue after (a) bioleaching with *Sulfolobus* at 75 °C, (b) bioleaching at 35 °C with *A. ferrooxidans*, (c) chemical leaching with H₂SO₄ at 75 °C.

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Iron was seen. At high temperature without bacteria, along with quartz, pyrite and iron-silicate existed in the major phase.

The mechanism of bio-chemical leaching of copper was further investigated by observing the surface morphology through SEM-EDX (Fig. 13) at various conditions. SEM image of the leach residue at 75 °C with *Sulfolobus* (Fig. 13a) exhibits extensive corrosion and rise in copper dissolution in 30 days with formation of floral patterned structures rich in iron. On the other hand, surface morphology (Fig. 13b) in 30 days leaching at 35 °C with *A. ferrooxidans* doesn’t indicate much corrosion. Extensive formation of hydronium jarosite could be easily
3 Conclusions

- The extremely thermophilic archaea strain isolated from coal mine sample able to degrade pyritic sulfur, was sequentially adapted on chalcopyrite ore of Malanjkhand copper project.
- This enriched and adapted culture was used in the bioleaching experiment at pH 1.8, 20% PD and 75°C temperature in 30 days time with the fine particles of <50 µm size gave 85% copper recovery.
- High metal recovery with the fine size particles (<50 µm) may be attributed by the better permeability of ferric ions biogenically generated by bacterial action on pyrite to dissolve copper. Recovery of copper may be correlated with the values of redox potential acquired during the experiments.
- Under the optimum condition at pH 1.8 and 75°C temperature, rise in redox potential was recorded to be 452–701 mV in 30 days.
- The XRD data of the leach residue generated in presence of absence of hyperthermophiles vis-à-vis mesophiles showed hydronium jarosite [H₃OFe₃(SO₄)₂(OH)₆] and quartz were present as major phases at 35°C and chalcopyrite and pyrite as the minor phases. With hyperthermophiles, along with quartz, pyrite and ferrosilite existed in the major phase. SEM characterization also corroborated the leaching efficiency, and confirmed bioleaching of copper from chalcopyrite ore to follow the indirect mechanism with the involvement of Fe (III) biogenically generated by the bacteria from the ore. These hyperthermophiles can be very useful for exploitation of such reserves from the perspective of the quantity vis-à-vis climate of tropical countries.

References


