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BIOLEACHING OF A LOW GRADE INDIAN CHALCOPYRITE ORE BY MICROBIAL CONSORTIUM

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ABSTRACT

Mesophilic bacteria namely, *Acidithiobacillus ferrooxidans* (*A.ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A.thiooxidans*) were isolated in 9K media from the mine water of Malanjkhhand Copper Project (MCP), India. These strains were used as such (without adaptation) on the ore for the bio-leaching of copper from the low grade chalcopryrite ore (0.27% Cu). With the use of unadapted bacterial consortium in the ratio of 4:1 (*A.ferrooxidans* and *A.thiooxidans*), the maximum copper recovery of 69.4% was obtained in 30 days at pH 1.5, 35°C temperature, 10% (w/v) pulp density with particles of <50 µm size. High copper recovery at pH 1.5 may be correlated with the increase in redox potential from 340-642 mV and increase in bacterial population from 3x10⁷ to 6.07x10⁸ cells/mL in 30 days. This research has shown emphasis on the possibility of achieving high copper extraction in the presence of native strains of *A.ferrooxidans* and *A.thiooxidans*.

INTRODUCTION

Metals are extracted from their ores by different processes depending upon the nature of occurrences, mode of association, mineralogical, physical and chemical characteristics, and grades. World wide reserves of high-grade ores are depleting at an alarming rate due to rapid increase in the demand of metals^[1]. However, there are fairly large stock of lean grade and complex ores and to extract the metals from them using conventional techniques are very expensive because of high energy and capital inputs required. Furthermore, fines generated during mining, milling and other metallurgical operations are to be processed not only to recover the values but also to comply with the stringent environmental issues. In order to recover values from these low-grade ores and fines, an appropriate processing technology is required^[2]. Production of base metals from lean and off-grade ores using bio-hydrometallurgical processes has been widely used all over the world. Apart from recovering copper from chalcopryrite, chalcocite and covellite minerals, bio-hydrometallurgical processes have also been commercially employed for recovering gold from arsenopyrites and uranium from oxidic ores.

It was understood that the Romans made use of bacteria to extract metals from sulfide ores long before the actual importance was understood. Commercial exploiters of copper-bearing ore bodies have almost certainly made use of microorganisms down the centuries, but it was only in the mid nineties that their contribution was recognized. The microbial action on sulfide minerals was explained with the isolation and characterization of the sulfur and iron oxidizing bacterium, *Acidithiobacillus ferrooxidans*^[3]. Commercial application of bacterial leaching

began in the late 1950s at the Kennecott Copper Company's Bingham Canyon Mine^[4]. Since then, most use of these and other microorganisms has expanded worldwide applications particularly in copper dump and heap-leaching applications^[5]. The application of biotechnology to the minerals industry came in light with the commercial success with the mesophilic oxidation of refractory gold ores via the BIOX® process, followed by the recovery of copper from chalcopyrite by thermophiles using the BioCOP™ process^[6-8]. Various researchers have exploited the complex sulfide ore/bulk concentrates using single/mixed culture(s) of mesophilic micro-organisms (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, etc..) in recent past^[9,10]. Implications of acidophilic thermophile-*Acidianus brierleyi*, *Sulfolobus* etc. has off late gained importance for the recovery of copper from chalcopyrite concentrate in various parts of world^[11,12]. In India, attempts were earlier made to treat MCP ores, particularly the overburden material which were essentially a mixed copper oxide-sulphide (chalcopyrite) and lean grade ore^[13-14]. Although, the copper bio-recovery from the mixed ore was observed to be favourable (50-60%) in 60 days on bench scale and 40-50% in column, however the same could not be established in large column and heap leaching experiments conducted at the dump site of MCP. In fact, copper dissolution was found to be 19% on tonnage scale by Agate^[15], 13-15% in 30-40 days by MCP^[16,17]. The bio-leaching of lean grade MCP ore has also been investigated to a limited extent with 75% recovery in 40 days in shake flasks from the quartzitic ore^[18].

In the bio-leaching process, the proper contact of ore with the leach liquor is essential for fluxes of reactants and products, such as bacteria, dissolved gases (O₂ and CO₂), solubilized metals and sulfur species^[19]. In bench scale studies, shake flasks were used for the optimization of bio-leaching parameters^[20]. Ferric iron is an important oxidizing agent in the bacterial leaching of sulfide minerals. Soluble iron species are the main determinants of redox potential, with active iron-oxidizing bacteria, contributing to high Fe³⁺/Fe²⁺ ratio. Precipitation of ferric iron in the leaching system may suppress the metal solubilization by preventing the contact between the leaching agent and the mineral. The bacterial leaching process requires acidic conditions, the acidity often being produced by the oxidation of pyrite and hydrolysis of ferric iron^[21].

Malanjkhand Copper Project (MCP), an open pit mine has around 16.5 million tonnes of ~1% Cu which will not last long. In such a situation, new methods for extracting copper from the low grade ores (2.5 million tonnes) found adjacent to the rich grades requires attention^[22]. As such the bio-leaching of the lean MCP ore requires serious efforts so as to arrive at the conditions suitable for exploitation. In present work, the bioleaching of copper from a low grade granitic chalcopyrite ore of MCP by a mixed culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*.

EXPERIMENTAL PROCEDURE

Copper Ore

Lean grade copper ore (containing 0.27%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 150µm sieve. Representative samples were then prepared by coning and quartering method for each fraction to get chemical analysis by using Atomic Absorption Spectrometer. The chemical analysis of the sieve fractions is given in Table I.

Table I. Chemical analysis of different sieve fractions of copper ore.

Particle size (µm)	Fraction retained, %	Cumulative Fraction retained, %	Composition (%)		
			Cu	Ni	Fe
>150	26.87	26.87	0.17	0.14	4.41
150-75	23.13	50.00	0.27	0.22	4.47
75-50	13.63	63.63	0.29	0.12	3.34
<50	36.37	100.00	0.32	0.12	6.62

The low grade ore is a granitic rock with disseminated sulfides. As regards morphology, sporadic bright patches were seen on the surface of the feldspar matrix of the bulk ore and the preliminary mineralogical study indicated the presence of mineral chalcopyrite in the cracks and fissures in quartz vein. The microscopic study of a typical sample (bakelite mounted and polished section) was identified by Leitz Varian Orthomat Optical Microscope, microphotographs of typical sample of copper ore (Figure 1) showed the distribution of phases.

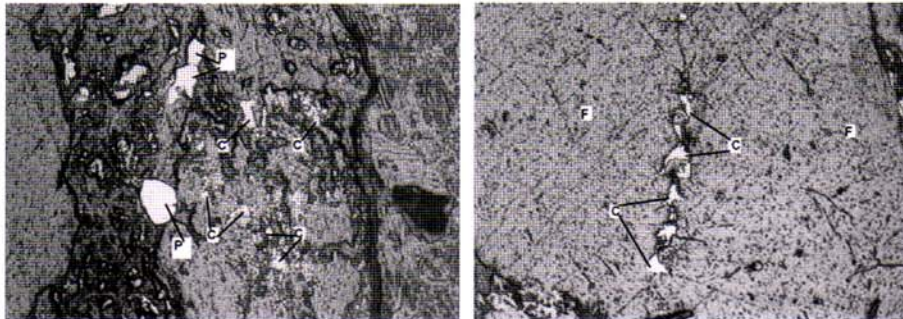


Figure 1. Petrological micrograph of copper ore (100X) [C-Chalcopyrite, P- Pyrite, F-Feldspar]

Chalcopyrite is present in the form of irregular grains in the free form, in veins of quartz which is not continuous. Also, it reveals the presence of pyrite (brighter than chalcopyrite) and chalcopyrite on fractured, filling along fractured zone and within feldspar grains. The bulk ore is slightly pink in appearance; this is due to the high percentage of granite in the ore body. Silica is very high (38%) in the ore. Phase identified by XRD showed major phases as chalcopyrite ($CuFeS_2$), pyrite (FeS_2) and silica (SiO_2) whereas bornite was the minor phase.

Micro-organism and Bio-leaching experiments

The source of bacteria was the mine water, which contained mixed culture of *Acidithiobacillus ferrooxidans* (*A.ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A.thiooxidans*). The bacterium was isolated in 9K and 9K (Silverman and Lundgren) media respectively which provided sufficient nutrients for the growth. *Acidithiobacillus ferrooxidans* (*A.ferrooxidans*) required ferrous sulfate as an energy source for its growth, whereas sulfur was the energy source for *Acidithiobacillus thiooxidans* (*A.thiooxidans*). The oxidation of ferrous sulfate to ferric sulfate by *Acidithiobacillus ferrooxidans* at pH 2.0, 35°C while shaking at 120 rpm was taken as an indication for the growth of *Acidithiobacillus ferrooxidans* (*A.ferrooxidans*). *Acidithiobacillus ferrooxidans* was said to be fully grown, when the ferrous content of the solution became nil. The indication for growth of *Acidithiobacillus thiooxidans* (*A.thiooxidans*) was observed by its cell count and size. The constant readings of redox potential also gave the indication of its growth. The fully grown strain of *Acidithiobacillus ferrooxidans* contained 1.5×10^8 cells/mL and *Acidithiobacillus thiooxidans* contained 3.75×10^8 cells/mL after three times sub-culturing. The enriched culture thus derived from the source mine water were used as such for inoculation in subsequent bioleaching experiments.

Bioleaching experiments were performed in 500 mL Erlenmeyer conical flasks, fitted in an orbital motion incubator shaker. Leaching solutions were inoculated with 10% (v/v) of active and non-adapted *A.ferrooxidans* and *A.thiooxidans* in the ratio 4:1 in all cases, except in sterile/control experimental sets, where mercuric chloride (0.02 g/L) was used as bactericide. General conditions like 35°C temperature, pH 2 and 10% (w/v) pulp density with shaking at 120rpm were used unless otherwise stated. Sterilized distilled water was used for the experiments. The pH of the solution was maintained by using 10N sulfuric acid, and 2N NaOH. Along with the pH adjustment, redox potential (E_h) was measured against SCE and reported as such in the text which was mainly governed by the concentration of ferrous and ferric ions in the solution. During bioleaching experiments, cell counts were also obtained by Petroff Hauser Counter. Ferrous ion was estimated by titrating the sample against potassium dichromate with barium diphenylamine sulfonate as indicator. Samples were mostly taken at the interval of the five days unless stated otherwise to analyze metals and to compute the metal recovery by analyzing the solution by AAS. Solution losses due to titration and cell counting were made up by using distilled water. Upon termination of the leaching experiments, the solid residues were dried and samples were taken for chemical analysis and XRD phase identification. The solid residues were dissolved in HCl-HNO₃ mixture and analyzed by AAS.

RESULTS AND DISCUSSION

For bioleaching of copper ore, a mesophilic consortium of *A.ferrooxidans* and *A.thiooxidans* was inoculated in a ratio of 4:1 with the ore and various process parameters, viz., pH, pulp density, particle size and temperature were optimised. The details are presented in this section.

Effect of pH

Bioleaching of copper was investigated at different pH in the range 1.5-2.5 at a pulp density (PD) of 10% (w/v) and a temperature of 35°C while shaking at 120 rpm. The pH is one of the important factors affecting the bacterial growth as well as its leachability. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* are reported to thrive at an environmental pH in

the range 1.5 to 2.5. The pH in the range 1.5 to 2.5 did not influence the bacterial survival, but at pH less than 1.5 or greater than 2.5, the growth was adversely affected. Data plotted in Figure 2 show that biorecovery of copper was maximum at pH 1.5 for the ore particles of <math><50\mu\text{m}</math> size. Copper recoveries at pH 2 and 2.5 were mainly governed by increase in bacterial oxidation of sulfide mineral. At pH 1.5, the bio-action could be maximum and the same could be ascertained by cell count. The copper recovery of 69.4% was observed at pH 1.5 almost in 30 days, whereas recoveries were 63.8% and 57.6% at pH 2.0 and pH 2.5 respectively. Increased copper recovery at pH 1.5 may be correlated with the increase in redox potential from 340-642 mV, and increase in bacterial population from 3×10^7 to 6.07×10^8 cells/mL. The recoveries of copper at different pH in chemical leaching were 24.1%, 21.9% and 17.5% at pH 1.5, 2.0 and 2.5 respectively (Figure 3). As shown in Figure 4, the redox potential rose from 230 mV and 340 to 642 mV at 1.5 and 2.0pH respectively. However at higher pH of 2.5, the pH value in 30 days reached only the maximum value of 560mV.

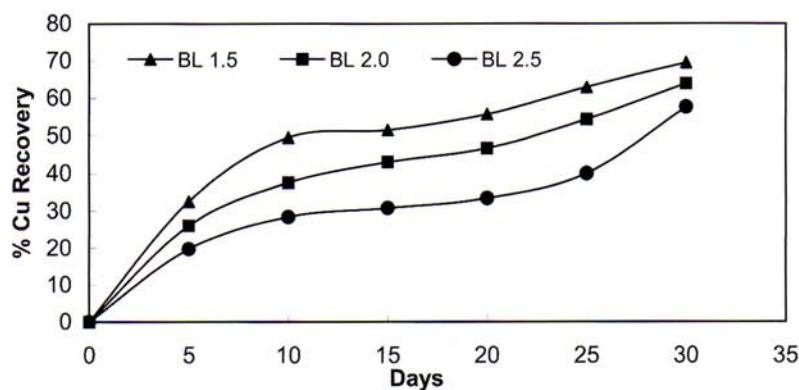


Figure 2. Effect of pH on copper bio-recovery-BL [10% v/v of *A.ferrooxidans*:*A.thiooxidans* in the ratio 4:1, 35°C, 10% (w/v) PD, <math><50\mu\text{m}</math> particles].

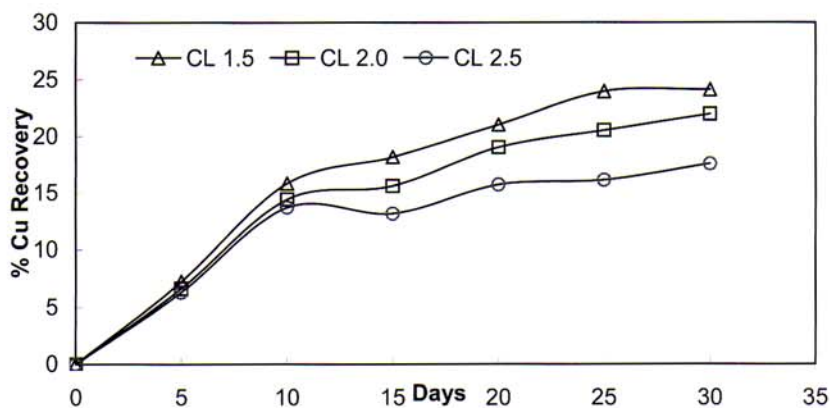


Figure 3. Effect of pH on chemical leaching-CL [35°C, 10% (w/v) PD, <math><50\mu\text{m}</math> particles].

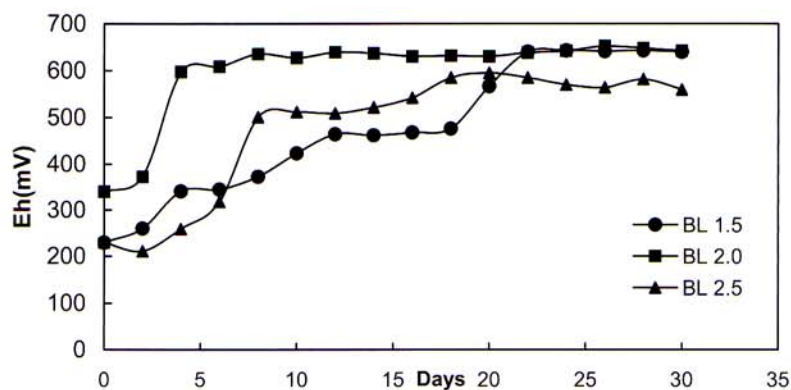


Figure 4. Change in redox potential at varying pH during bioleaching at 10% (w/v) PD.

Effect of Pulp Density (PD)

Biodissolution of copper was investigated at varying pulp densities in the range 5-20%(w/v) at pH 2.0 and 35°C with 150-75 μm size particles using 10% (v/v) isolate of *A.ferrooxidans:A.thiooxidans* as consortium in the ratio 4:1. The plots in Fig.5 and 6 showed clear that biorecovery of copper was relatively more (i.e., 37.6 %) at 5%(w/v) PD in 30 days as compared to 10.6 % metal dissolution in chemical leaching. In general, copper dissolution was not very different at varying pulp densities for 25 days of leaching, although the recoveries were high for low PD in 30 days. This may be attributed to the deficiency of oxygen and CO_2 availability which was essential for *A.ferrooxidans* and *A.thiooxidans* growth and increased concentration of metal ions causing toxicity to bacterial growth at higher pulp densities, on prolonging the leaching.

At 5%(w/v) PD redox potential rose to 589mV for bioleaching in 30 days whereas it was found to be 402mV in chemical leaching at this pulp density. At higher pulp densities viz. 10, 15 and 20% (w/v), there was marginal change in the E_n values from 570-595 in 30 days of bioleaching, with corresponding copper recoveries of 25.9, 18.4 and 19.2%. The high redox potential recorded in 25 days at pH 2 for bioleaching may be attributed to complete oxidation of Fe (II) to Fe (III) whereas; the low redox potential accounted for lesser oxidation of ferrous ions (Fig.7) in chemical leaching and consequently lower metal dissolution in sterile (control) leaching was observed.

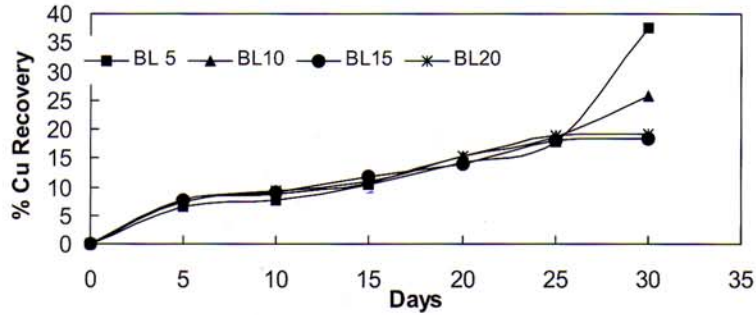


Figure 5. Effect of pulp densities on bio-leaching (BL) of copper ore [10% v/v of *A.ferrooxidans*:*A.thiooxidans* in the ratio 4:1, particle size: 150-75 μ m, 35 $^{\circ}$ C, pH 2.0]

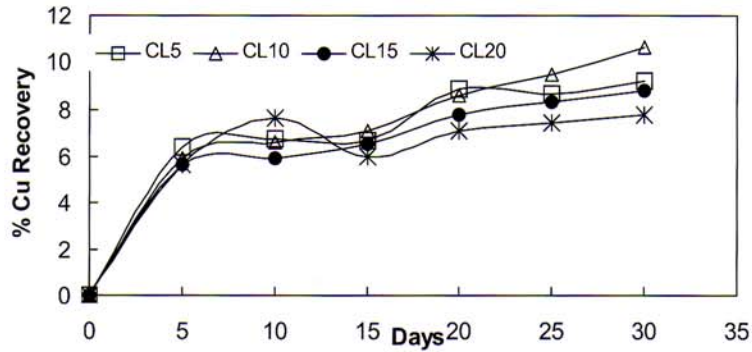


Figure 6. Effect of pulp densities on chemical leaching of copper [particle size: 150-75 μ m, 35 $^{\circ}$ C, pH 2.0]

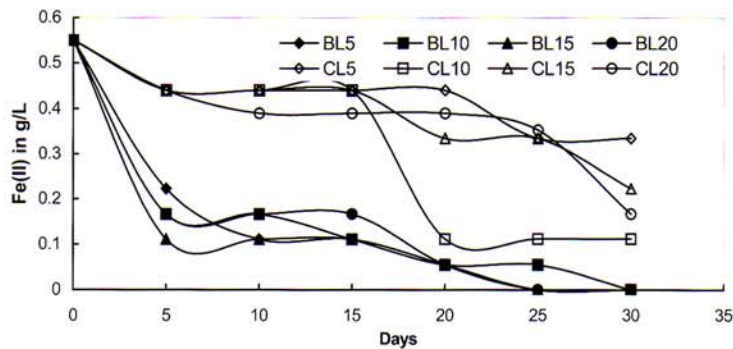


Figure 7. Change in concentration of ferrous ion with time at varying pulp densities [particle size: 150-75 μ m, 35 $^{\circ}$ C, pH 2.0]

Effect of particle size

It may be seen from the results (Figure 8) that the particle size had significant effect on biorecovery of copper when size varied from 150-75 μm to <50 μm resulting in increased recovery of from 37.6% to 63.8% Cu at 2.0 pH in 30 days. This could be mainly due to better permeation of the leachant to oxidize the copper sulfide present in the ore and increased surface area. Finer particles were increasingly exposed to lixiviant that dissolved copper from the chalcopyrite phase. It was also clear that with coarser particles of 150-75 μm size, copper biorecovery was 37.6% with corresponding chemical leaching of 10.6% Cu in 30 days (Fig.9). The redox potential varied in the range 340 to 642 mV in 30 days (Figure10) for the bioleaching with <50 μm particles, whereas E_h of 554mV and 596mV were observed on 30th day in bioleaching experiments with 150-75 μm and 75-50 μm particles respectively. However, in sterile leaching E_h of the solution varied from 312 to 490mV for <50 μm ore particles. High E_h value signified a high metal dissolution for <50 μm ore particles in bioleaching.

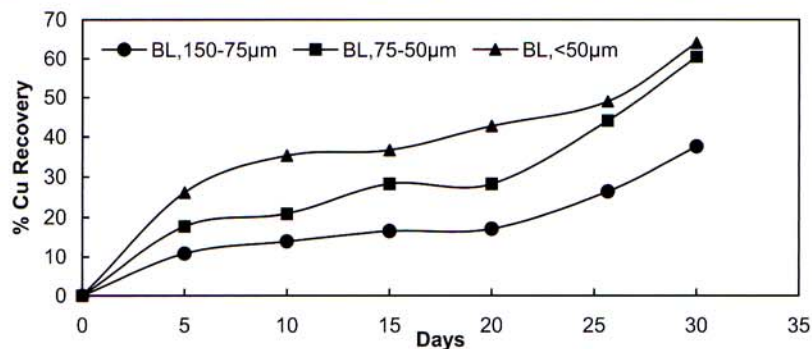


Figure 8. Effect of particle size on bio-leaching of copper [10% v/v *A.ferrooxidans*:*A.thiooxidans* in the ratio 4:1, 10% (w/v) PD, 35°C, pH 2.0, Particle size: 100:150-75 μm , 200:75-50 μm , 300:<50 μm]

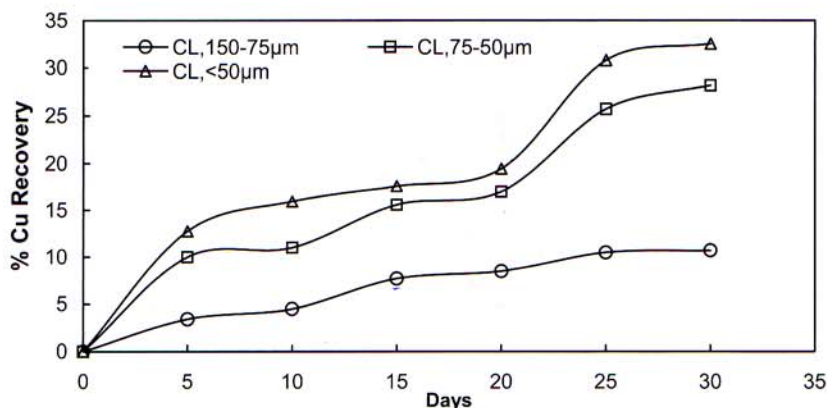


Figure 9. Effect of particle size on chemical leaching of copper [10% (w/v) PD, 35°C, pH 2.0. Particle size- 100:150-75 μm , 200:75-50 μm , 300:<50 μm]

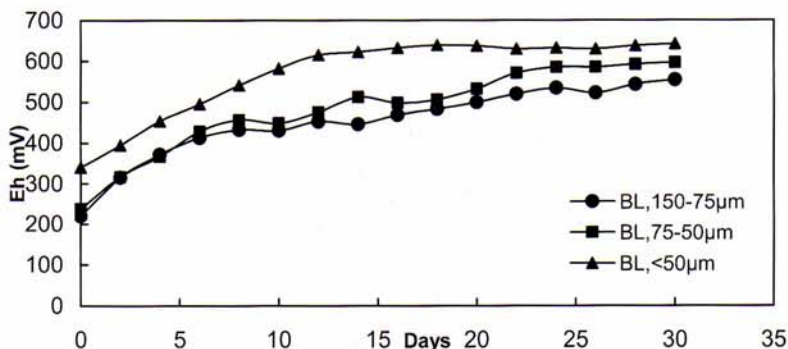


Figure 10. Change in redox potential during bioleaching at varying particle sizes.

Effect of Temperature

As the bacterial strains in the consortium are mesophilic in nature, therefore temperature was varied from 20-35°C in this work. Effect of temperature at 10% (w/v) PD using 10% (v/v) mixed culture of *A.ferrooxidans*:*A.thiooxidans* in the ratio 4:1, at pH 2 with <50µm ore particles was studied. The maximum copper biorecovery was found to be 63.8% at 35°C whereas it was 57.3% at 30°C and 54.1% at 25°C in 30 days as shown in Fig.11. The corresponding chemical leaching was found to be 21.9%, 14.66% and 17.35% (Fig.12). The variation in Fe (II) concentrations and E_h (mV) values in 30 days at 35°C are reported in Fig.13 and Fig.14 respectively. It may be seen that Fe (II) was stable for longer period at lower temperatures viz. 25 & 30°C in bioleaching experiments with low redox potentials of 570 and 635mV respectively. The higher redox potential is associated with faster oxidation of Fe (II) to ferric state resulting in high metal dissolution in bioleaching at 35°C. The cell count (Fig.15) was found higher (4.88×10^8 cells/mL) at 35°C as compared to that of 30°C which may be correlated with high recovery of copper at 35°C.

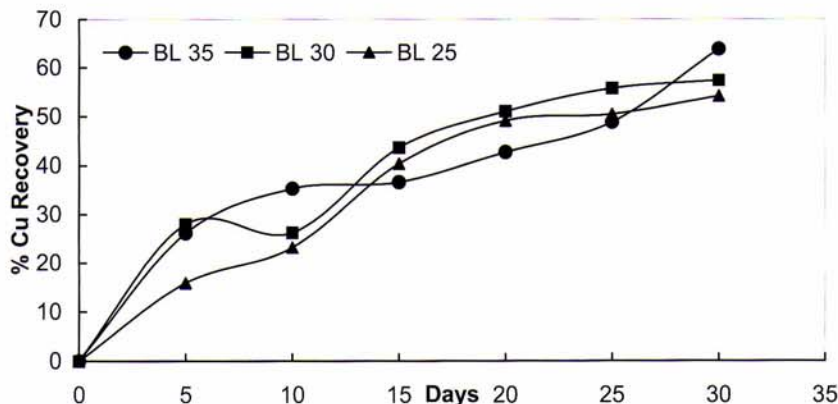


Figure 11. Effect of temperature on bioleaching of copper [10% v/v *A.ferrooxidans*:*A.thiooxidans* in the ratio 4:1, 10% (w/v) PD, pH 2.0, particle size: <50µm].

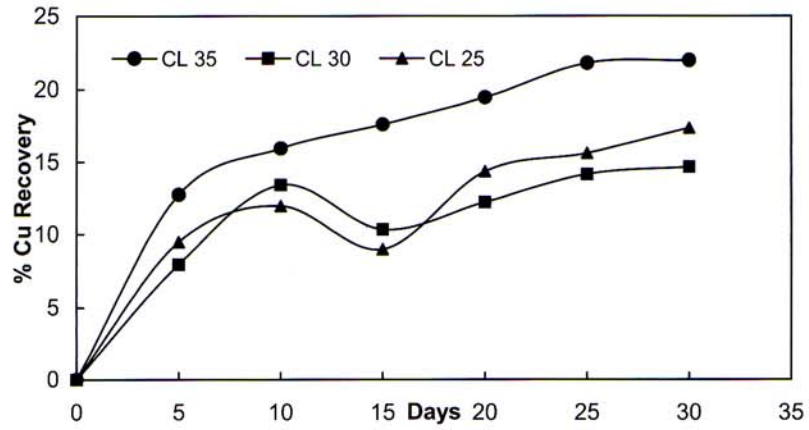


Figure 12. Effect of temperature on chemical leaching of copper [10% (w/v) PD, pH 2.0, particle size: <math><50\mu\text{m}</math>].

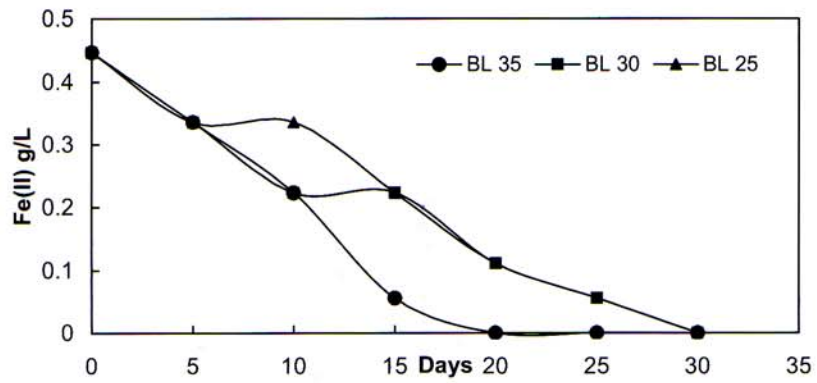


Figure 13. Change in concentration of ferrous ion with temperature for copper bioleaching

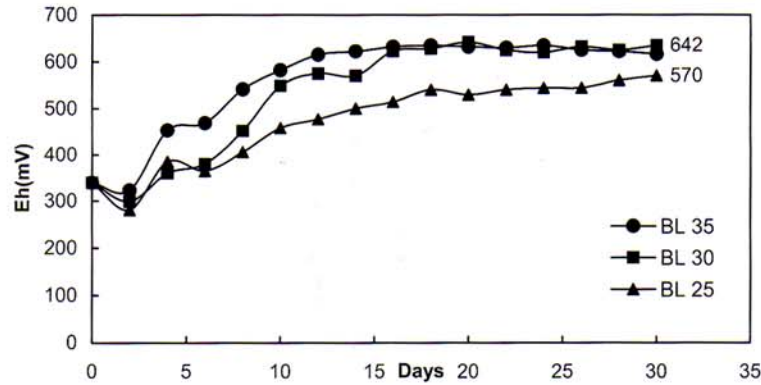


Figure 14. Change in redox potential of the solution during bioleaching of copper at varying temperatures.

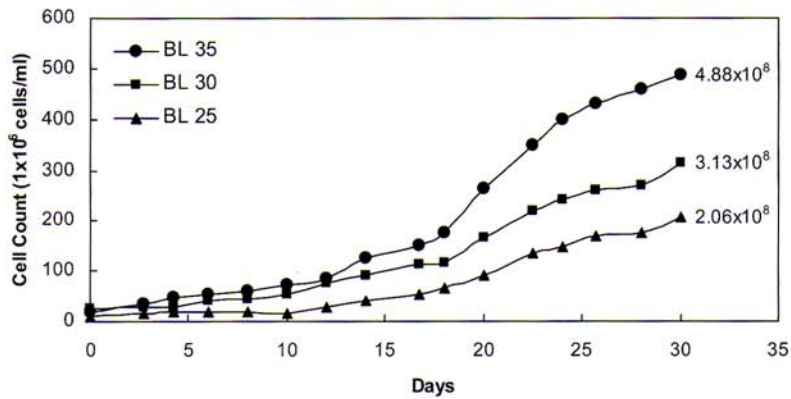


Figure 15. Change in bacterial cell count/mL at various temperatures on <50µm particles.

The bioleaching of copper from chalcopyrite is reported to involve both direct and indirect leaching mechanism. The direct mechanism proceeds through the attachment of *A.ferrooxidans*/*A.thiooxidans* on the mineral surface to oxidize the metal.



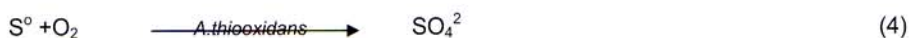
The bio-oxidation of chalcopyrite also involves oxidative ferric reaction, which represents indirect leaching mechanism as:



This apart from dissolution of the metal sulfide produces ferrous iron and elemental sulfur (S^0). It is this ferrous iron and the elemental sulfur that form the substrate for microbial growth according to equations (3) and (4) respectively:



and:



The ferric iron thus formed is hydrolyzed in aqueous solution if pH is higher.



Reaction (2) increases the pH, but the reaction (5) and (6) reduce and stabilizes it. So the extent of ferric iron hydrolysis is dependent on pH.

CONCLUSIONS

Following conclusions may be derived from the present work on the bioleaching of copper ore of MCP:

1. The mesophilic bacteria namely, *Acidithiobacillus ferrooxidans* (*A.ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A.thiooxidans*) were isolated from the mine water in the *Silverman* and *Lundgren* (9K) media. Three times sub-cultured bacteria in the same media are used as such (unadapted) on the ore for bioleaching. Use of enriched microbial culture in the ratio of 4:1 (*Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*) for bioleaching of copper showed an increasing trend with rise in the temperature from 25-35°C and increase in fineness of the ore particle. Increase in pH from 1.5-2.5 and pulp density from 5-20% (w/v) has decreased the copper bioleaching irrespective of the particle size of the ore.

2. Maximum copper recovery of 69.4% is obtained in 30 days with the unadapted/native mixed microbial culture at pH 1.5, 35°C temperature at 10% (w/v) pulp density with the ore particles of < 50 μm size. Increase in copper recovery at low pH (1.5) is associated with increase in redox potential from 340-642 mV in 30 days time and also increase in bacterial population from 3×10^7 to 6.07×10^8 cells/ml. Higher redox potential is indicative of improved oxidation conditions in presence of the microbial strains. In contrast, change in redox potential for control leaching under this condition is found to be low in the range 230-490 mV. The metal recovery is also correlated with the degree of oxidation of Fe (II) with time in presence and in absence of bacteria.

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