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Influence of Ferrous Iron Addition on Silver Catalyzed Bioleaching of Copper from Chalcopyrite using an Isolated Acidithiobacillus Ferrooxidans Strain

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***Abstract*—Shake flask studies on influence of initial ferrous iron on bioleaching of copper from chalcopyrite using novel isolated bacterial strain *Acidithiobacillus ferrooxidans* BMSNITK17 with and without addition of silver catalyst was conducted and reported. Non-catalysed bioleaching of copper yields about 0.9 g/L of with 16 g/L of initial ferrous iron concentration while Silver catalysed bioleaching of copper from chalcopyrite yields about 1.6 g/L of copper with initial ferrous iron concentration of 4 g/L. Variation of pH, ferrous and ferric iron during the study were recorded and discussed. This study confirms the influence of initial ferrous iron concentration on bioleaching of copper.**

***Index Terms*— Bioleaching, Acidithiobacillus ferrooxidans, Chalcopyrite.**

I. Introduction

Hydrometallurgical extraction of valuable minerals from ore by biological method using acidophilic microorganisms relay on its capability and rate of available iron oxidation. Oxidation of iron plays a major role in the dissolution of minerals from ore. Iron in the form of oxidized ferric ions by *Acidithiobacillus ferrooxidans* utilizing ferrous iron present in the environment serves as leaching agent to react with minerals chemically (Karamanev & Nikolov, 1988). Unlike all thiobacillus species, *Acidithiobacillus ferrooxidans* uses ferrous iron as electron donor (Nemati et al., 1998). Exhaust of ferrous iron in the environment directly affects the growth of bacteria as ferrous iron has its role in enhancing oxygen uptake in the metabolic process of organism (Ann P. Wood and Don P. Kelly, 1993).

Potential application of microbes for the recovery of valuable minerals from ore is gaining importance now a day. Copper extraction by bioleaching method is one such process to be focused. More research insight is required on microbial metabolism for effective growth, maintenance and utilization of bioleaching microorganisms in bioreactor. *Acidithiobacillus ferrooxidans* is one of the potential microorganisms used as a tool to recover valuable metals from ore in the bio mining industries. Ferric iron mediated oxidative attack is the key for oxidation process to occur following many enzymatic reactions which is to be studied in depth.

The present investigation focusses on the influence of initial iron concentration in the extraction of copper from

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chalcopyrite using a novel isolated bacterial strain *Acidithiobacillus ferrooxidans* with and without silver catalyst.

II. Literature Review

Chen and team (2022) studied copper leaching from copper sulfide ore using acidithiobacillus bacterial strain and reported maximum leaching of 750 mg/L of copper dissolution claiming the influence of aeration on bioleaching experiments. Being a prime factor in the bacterial metabolism oxygen concentration affects the bioleaching of copper from copper sulfide ore which is demonstrated with kinetic studies in the work (Chen et al., 2022).

Wang and co-workers (2020) studied the synergetic effect of mixed microbial community on bioleaching of copper sulfides claiming 73.7% of copper recovery with *Acidithiobacillus thioxidans* and *Acidithiobacillus ferrooxidans* (Wang et al., 2020).

Yang and team (2019) investigated the influence of silver being the catalyst (0.2% Ag) on bioleaching of copper from chalcopyrite in both bioleaching and chemical leaching claiming the cost effectiveness of bioleaching process with 2.5 g/L copper recovery (Yang et al., 2019).

Rui and team (2020) studied the catalytic effect of silver bearing solid waste on chalcopyrite bioleaching and demonstrated the correlation between temperature and rate of copper extraction claiming the influence of silver on the bioleaching process (Rui et al., 2020).

III. Methodology

A. Sub-culture of Acidithiobacillus ferrooxidans

Acidithiobacillus ferrooxidans (Accession No. MG271840) strain was procured from the Department of Civil Engineering, National Institute of Technology Karnataka, Surathkal (Bhaskar et al., 2019b). Modified 9K media was used for sub culturing. pH of the media was adjusted to 2.5 using 1N H_2SO_4. *Acidithiobacillus ferroxidans* was inoculated to the conical flask containing modified 9K media under sterile condition. Inoculated media was kept in incubator shaker at 30^{o} C and 120 rpm for 14 days. Sub cultured bacteria was stored at 4^{o}C for further use.

B. Preparation of ore sample

Chalcopyrite ore was collected from Ingaldal mining site, Chitradurga, Karnataka. Collected ore was washed neatly, air dried and crushed into powder form using crusher. Fine powder collected were stored for further analysis.

C. Bioleaching experiments

Processed chalcopyrite were added to Modified 9K media at the rate 2 % Pulp density for the bioleaching studies (Wu et al., 2016). pH of the medium was adjusted to 2.5. Initial ferrous iron concentration in the media were maintained at an incremental rate of 2 g/L, 4 g/L, 8 g/L, 16 g/L respectively to which an isolated *Acidithiobacillus ferrooxidans* BMSNITK17 (Accession No. MG271840) inoculum of 1.0×10^{7} cells/ml was added initiating the leaching studies (Bhaskar et al., 2019a). For silver catalysed bioleaching experiments 0.2 g/L of silver chloride was added with initial ferrous iron concentration of 2 g/L, 4 g/L, 8 g/L and 16 g/L respectively. 10 ml samples were drawn at regular intervals for the analysis. All the experiments were conducted in dual with sterile conical flasks on an incubator shaker at 120rpm and 30 ^{o}C.

D. Analytical procedure

Copper content was measured using a spectrophotometer by Bathocuproine method (Pham et al., 2013). Oxidation and reduction potential was measured with a Redox meter (EUTECH make). The pH was monitored by a digital pH meter (HANNA make). Concentration of ferric iron was measured by potassium thiocynate method using UV-Spectrophotometer (Mellon, 1941). Total iron content was measured by 1,10 phenanthroline method (APHA Method 4500-F:, 1992).

IV. Results And Discussions

A. Sub - culture of Acidithiobacillus ferrooxidans

Observed change in color of broth medium from yellow to orange confirms the growth of *Acidithiobacillus ferrooxidans* bacteria with pH drop to 1.9 indicating bacterial iron oxidation. Growth of bacteria strain on solid modified 9k medium is confirmed with the yellow colonies with red rusty iron colored layer (Figure 1). Bacterial strain is positive for both catalase and oxidase tests.

B. Effect of Initial ferrous iron concentration on bioleaching of copper

Initial ferrous iron concentration plays a major role in the metabolic activity of *Acidithiobacillus ferrooxidans* thereby affecting the metal dissolution. Figure 4 presents the dissolution of copper at different initial ferrous iron concentration. Maximum copper dissolution of 0.9 g/L was observed with 16 g/L of ferrous iron concentration without catalyst aid and a dissolution of 1.6 g/L was observed with initial ferrous iron concentration of 4 g/L. Maximum copper dissolution was found within a week of the study.

Figure 2 and Figure 3 presents the variation of pH during the study with and without catalyst aid, in which pH was reduced initially and observed to increase after 6th day of the study. This is in consistent with the copper dissolution observed. Increase in pH after 6th day is attributed to hydrolysis of iron and precipitation of ferric iron in the form of jarosite. After 6th day the rate of copper dissolution remains constant. Nemati and Harrison claims that bacterial growth is inhibited at the pH below 1 and at pH above 4 (HARRISON, 2000). Increase in pH due to hydrolysis may results in bacterial growth inhibition, and precipitated iron as jarosite covers the ore particles denying the bacterial contact to the ore. This may result in the reduced copper dissolution. During the study redox potential observed was in the range of 500 – 600 mV which indicates better bacterial oxidation. High redox potential may be due to high ferric to ferrous ratio resulting from the bacterial reoxidation of ferrous (Bevilaqua et al., 2002). With all the variant of iron, initial ferrous iron is observed to be decreased as the bacteria utilize ferrous iron and converts it into ferric iron gaining the electrons for its metabolism. Figure 2 represents ferrous and ferric iron variation during the study. Iron concentration observed during the study is in consistent with Kelly and Wood. It has been observed the decrease in ferrous iron concentration with parallel increase in ferric iron concentration. Decline in ferrous iron concentration is observed in the first 4 days of the study meanwhile there is an increase in ferric iron concentration. This indicates the bacterial iron oxidation. It is claimed that ferric iron generated during the ferrous oxidation may inhibits the oxidation lowering the substrate saturation co-efficient (Ann P. Wood and Don P. Kelly, 1993).

After 6th day, ferrous iron concentration remains almost constant in the solution indicating the halt in the bacterial activity. Ferric iron concentration in the solution decreases due to ferric precipitation as jarosite. Increase in ferrous iron increases the copper dissolution confirming the dependence of bacterial activity on iron oxidation (Mesa et al., 2002).

Copper dissolution is increased by 0.39 g/L on increase of initial ferrous iron concentration from 2 g/L to 16 g/L confirms the role of iron in copper dissolution by iron oxidizing bacteria. With the ferrous iron concentration of 2 g/L, 4 g/L, and 8 g/L the copper dissolution rate 0.55 g/L, 0.55 g/L and 0.7 g/L respectively. This indicates the lower substrate concentration for bacterial metabolism.

Silver catalyzed copper bioleaching has shown maximum dissolution with ferrous concentration of 4 g/L and the maximum dissolution was observed by 10th day of the study. After 10th day copper dissolution trend remains constant. Observed maximum dissolution at low initial ferrous iron concentration is attributed to increase in formation of ferric iron with increase in initial ferrous concentration which precipitates on ore and prevents further reactions to occur (Muñoz et al., 2007). pH observed during the study rises more than 3 by 8th day marking slow copper dissolution (Munoz). Abdollahi and team claims that minor amount of silver chloride precipitation might be possible with potassium chloride content in 9K medium (Abdollahi et al., 2015). The results obtained in the present study is in consistent with sato and team presenting observed increase in iron concentration with silver being a catalyst after 8th day (Sato et al., 2000).

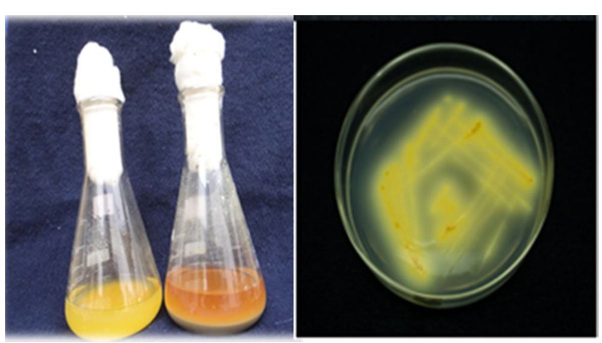

Figure 1: Sub-culturing of Acidithiobacillus ferrooxidans on a) Modified 9k media broth b) Modified 9k solid medium

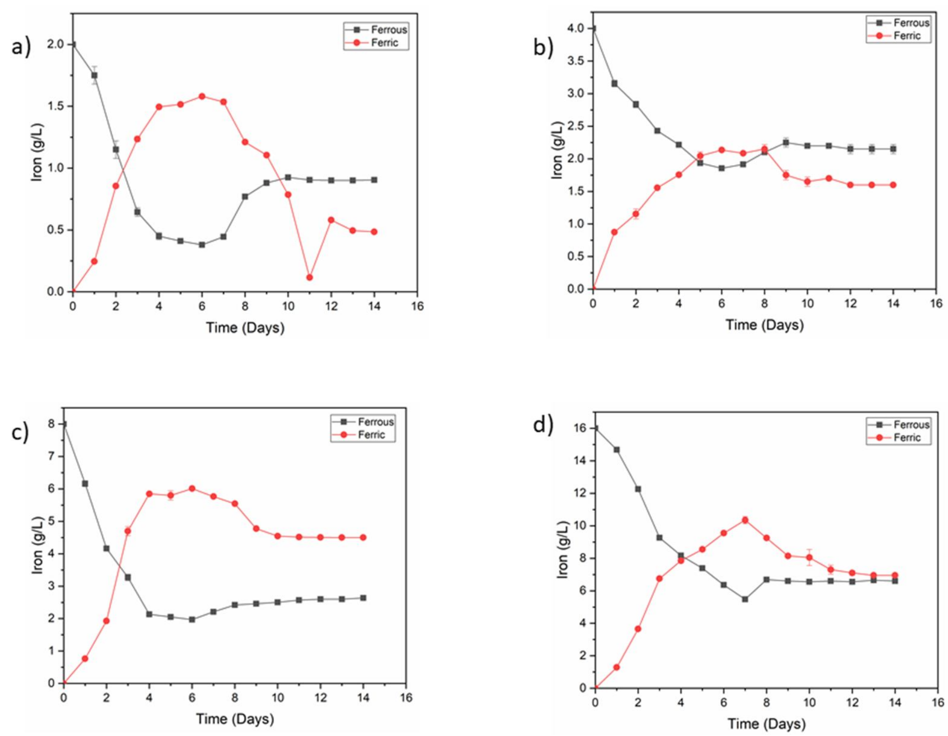

Figure 2: Variation of ferrous and ferric iron during the bioleaching of copper without silver addition at initial iron concentration a) 2 g/L ferrous iron b) 4 g/L ferrous iron c) 8 g/L ferrous iron d) 16 g/L ferrous iron

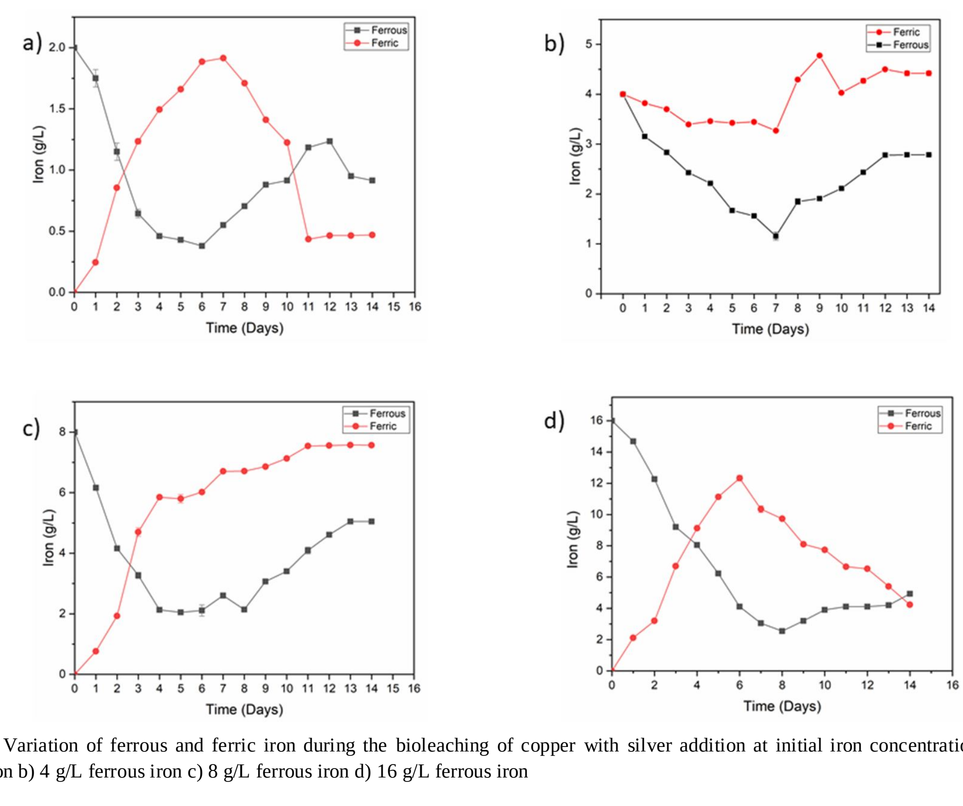

Figure 3: Variation of ferrous and ferric iron during the bioleaching of copper with silver addition at initial iron concentration a) 2 g/L ferrous iron b) 4 g/L ferrous iron c) 8 g/L ferrous iron d) 16 g/L ferrous iron

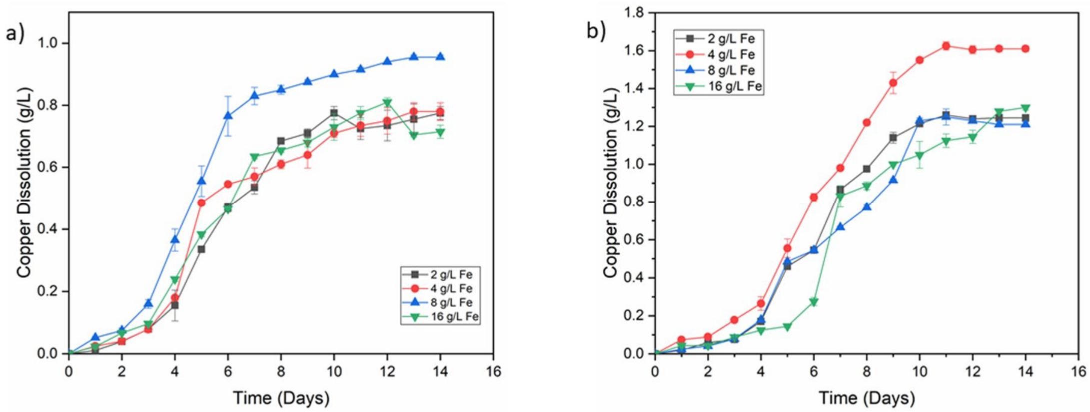

Figure 4: Dissolution of copper at different initial ferrous iron concentration a) Without silver addition b) With silver addition

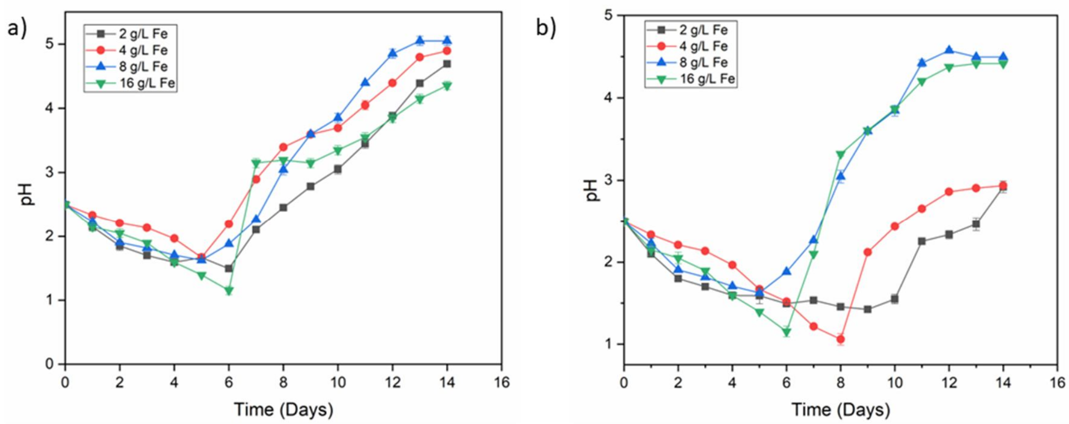

Figure 5: Variation of pH during the bioleaching process a) Without silver addition b) With silver addition

V. CONCLUSIONS

Increase in copper dissolution rate with increase in initial ferrous iron concentration confirms the role of iron in bacterial iron oxidation and copper dissolution. Silver catalysed bioleaching experiments yielded a more copper dissolution than non-catalysed experiments with low initial ferrous iron concentration. Variation of other parameters like pH, oxidation and reduction potential, ferrous and ferric iron concentration indicates their influence on copper dissolution with iron variation. Study confirms the influence of initial ferrous iron concentration on bioleaching of chalcopyrite both with and without silver catalyst.

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