Effect of amino acids on bioleaching of chalcopyrite ore by *Thiobacillus ferrooxidans*

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Accepted 4 November, 2011

Amino acids seem to play a major role during bioleaching of chalcopyrite ore by *Thiobacillus ferrooxidans*. Efficiency of microbial leaching of chalcopyrite by *T. ferrooxidans* was investigated in the presence of L-aspartic acid, L-glutamic acid, L-histidine and L-serine. The bioleaching of copper ion (Cu²⁺) from the low grade ore increased significantly in the presence of L-serine. Although the leaching was increased in the presence of L-aspartic acid, L-glutamic acid and L-histidine during the initial period, it was observed to decrease after a few days. However, in the L-serine supplemented medium, a steady state of leaching was maintained for a reasonable time.

Key words: *Thiobacillus ferrooxidans*, chalcopyrite, amino acid, bioleaching.

INTRODUCTION

Bioleaching is an economical and environment friendly method for the recovery of metals from low grade ores as compared to the conventional metallurgical processes (Rohwerder et al., 2003; Zhou et al., 2009). Increasing demand of industries for metals leads to a quick exhaustion of the best or easiest accessible resources which requires the search for new solutions enabling exploitations of low grade ore. A promising solution seems to be a well recognized process of bioleaching of sulphide ores. Chalcopyrite (CuFeS₂) is the most abundant and commercial interest low grade copper mineral. However, due to slow kinetics of bioleaching of chalcopyrite, the yield of Cu²⁺ is very low (Cordoba et al., 2008). Most work related to bioleaching of chalcopyrite has been done with *Thiobacillus ferrooxidans* bacteria which can oxidize chalcopyrite ore via direct or indirect mechanism (Dopson et al., 2003; Schippers and Sand, 1999; Mukhopadhyay et al., 2008). In spite of optimization of various parameters (Bryner et al., 1954; Devasia et al, 1993; Duncan et al., 1964; Guay et al., 1999; Mier et al., 1995; Third et al., 2002) which influence *T. ferrooxidans* induced Cu²⁺ dissolution from chalcopyrite ore, the efficiency of the process is still very low. The excessively long leach time and usually poor yield, limit the practical application of microbiological leaching of chalcopyrite ore (Cordoba et al., 2008). Previous studies have shown that metal dissolution process from sulphidic ores by *T. ferrooxidans* can be increased in the presence of some amino acids (Groudev and Groudeva, 1993; Groudev et al., 1996; Neunberg and Mandl, 1948; Spasova et al., 2006; Yue hua et al., 2004; He et al., 2009). In this study, we investigated the effect of L-aspartic acid, L-glutamic acid, L-histidine and L-serine during bioleaching of chalcopyrite ore by *T. ferrooxidans*. Attempts have been made to increase the yield of leached copper ion by adding different concentrations of these amino acids.

MATERIALS AND METHODS

Preparation of chalcopyrite sample

Studies were performed on chalcopyrite ore collected from Indian Copper Complex located at Ghatisilla, in Jharkhand state of India. The ore contained Cu (34.6%), Fe (30.51%) and S (34.8%). The mineral was powdered and sieved to below 0.06 mm grain size.

Cultivation of bacteria

*T. ferrooxidans* (strain no.: AIICC 19859) used in this study was
collected from IICB, Kolkata, West Bengal, India. For cultivation of bacteria, 100 ml pre-cultured *T. ferrooxidans* solution was added to 1000 ml 9K medium (Silverman and Lundgren, 1959). The concentration of the final solution was 9.1%. The resulting solution was then kept in a biological oxygen demand (BOD) incubator at 32°C for seven days. The bacterial suspension concentration after seven days was ~$10^5$ cells/ml. This was saved as the inoculum for the leaching experiment throughout this work.

**Bioleaching experiments**

All the experiments were carried out in 250 ml conical flasks containing 2 g dry chalcopyrite powder, 100 ml bacterial inoculum and different concentrations of pure L-amino acids (aspartic acid, glutamic acid, histidine and serine). The initial pH of the medium was adjusted to 2. The flasks were constantly shaken on a rotatory shaker incubator at 32°C and 120 rpm. Throughout the experiment, air was continuously pumped into each of the flasks so that the dissolved oxygen of the medium remained above 6 mg/L. Samples were drawn at regular intervals of two days to estimate the quantity of Cu$^{2+}$ leached from chalcopyrite ore. Copper ion analysis was done in UV-Vis spectrophotometer-2100 (Shimadzu, Japan) at 435 nm (Vogel, 1989).

**RESULTS AND DISCUSSION**

**Effect of L-aspartic acid on bioleaching of chalcopyrite**

The effect of L-aspartic acid on bioleaching of Cu$^{2+}$ from chalcopyrite ore is shown in Figure 1. It can be seen that although L-aspartic acid increased the concentration of

![Figure 1](image-url)
concentration (g/L) of L-glutamic acid

**Effect of L-glutamic acid on bioleaching of chalcopyrite**

Although L-glutamic acid (> 1.0 g/L) increased the concentration of dissolved Cu\(^{2+}\) during initial period, the bioleaching process decreased after a few days. However, the concentration of dissolved Cu\(^{2+}\) in the presence of 0.8 g/L of L-glutamic acid was lower than that of the control solution during the first few days.

**Effect of L-histidine on bioleaching of chalcopyrite**

The effect of L-histidine on bioleaching of Cu\(^{2+}\) from chalcopyrite ore is shown in Figure 2. It can be seen that although L-glutamic acid (> 1.0 g/L) increased the concentration of dissolved Cu\(^{2+}\) during initial period, the bioleaching process decreased after a few days. However, the concentration of dissolved Cu\(^{2+}\) in the presence of 0.8 g/L of L-glutamic acid was lower than that of the control solution during the first few days.
chalcopryite ore is shown in Figure 3. It can be seen that although L-histidine increased the concentration of dissolved Cu\(^{2+}\) during the initial period, the bioleaching process decreased after a few days. Maximum concentration of the dissolved Cu\(^{2+}\) was measured in the presence of 1.6 g/L of L-histidine after eight days.

**Effect of L-serine on bioleaching of chalcopryite**

The effect of L-serine on bioleaching of Cu\(^{2+}\) from chalcopryite ore is shown in Figure 4. It can be seen that, presence of L-serine in the leaching medium significantly accelerated the leaching of Cu\(^{2+}\) from chalcopryite ore by *T. ferrooxidans*. Maximum concentration of Cu\(^{2+}\) was leached from the ore in solutions containing 1.6 g/L of L-serine in only two days. This was two times more than that obtained in the control solution during the same time period. The concentration of dissolved Cu\(^{2+}\) in the presence of more than 2 g/L of L-serine continued to increase with time even after ten days.

Although the bioleaching of Cu\(^{2+}\) from chalcopryite ore increased in the presence of L-aspartic acid, L-glutamic acid and L-histidine during the initial period, it was
observed to decrease after a few days. The decrease may either be due to decrease in bacterial concentration or metabolism rate resulting from the increasing toxicity of metal ions (Natarajan, 1998) in solutions or may be due to formation of copper amino acid salts or complexes (Furia, 1972).

However, L-serine plays a unique role during bioleaching of chalcopyrite ore. Apart from significantly increasing the leaching rate of Cu$^{2+}$ from the ore, it prevents the decrease of Cu$^{2+}$ concentration in leaching.

**Figure 4.** Bioleaching of Cu from chalcopyrite at various concentrations (g/L) of L-serine.
solution with increasing time. So, L-serine can be used as a potential reagent during the bioleaching of Cu²⁺ from chalcopyrite ore by *T. ferrooxidans*. It may be possible that L-serine accelerates the Cu²⁺ leaching process through indirect mechanism (Dopson et al., 2003; Schippers and Sand, 1999) by initially forming stable complexes with the Fe³⁺ (Furia, 1972), obtained in solution by bacterial oxidation of the Fe³⁺ of the chalcopyrite ore and then concentrating the Fe³⁺ on the ore surface.

**Conclusion**

It may be concluded that low concentration of L-serine can act as an effective agent during the bioleaching of Cu²⁺ from chalcopyrite ore by *T. ferrooxidans* solutions. The efficiency of this process may be improved by suitable operational arrangement for continuous removal of the leached Cu²⁺ from the microbial solution, which in turn may help to increase the bacterial metabolic rate by decreasing the Cu²⁺ toxicity.

**ACKNOWLEDGEMENTS**

B. Ghosh gratefully acknowledges M.H.R.D., Government of India and N.I.T., Durgapur for providing the necessary research facilities. B. P. Mukhopadhyay is also thankful to N.I.T. Durgapur for providing the article handling fees.

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