African Journal of Biotechnology Vol. 11(8), pp. 1991-1996, 26 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2162
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

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

B. Ghosh[1, 2], B. P. Mukhopadhyay[1]* and H. R. Bairagya[1]

[1]Department of Chemistry, National Institute of Technology, Durgapur, M.G. Avenue, Durgapur-713209, India.
[2]Department of Chemistry, Dr B.C.Roy Engineering College, Durgapur-713206, India.

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 (Cu^{2+}) 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 ($CuFeS_2$) is the most abundant and commercial interest low grade copper mineral. However, due to slow kinetics of bioleaching of chalcopyrite, the yield of Cu^{2+} 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 Cu^{2+} 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.

*Corresponding author. E-mail: bpmk2@yahoo.com. Tel: +91 0343 2547074. Fax: +91-03432547375/2546753

MATERIALS AND METHODS

Preparation of chalcopyrite sample

Studies were performed on chalcopyrite ore collected from Indian Copper Complex located at Ghatsilla, 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

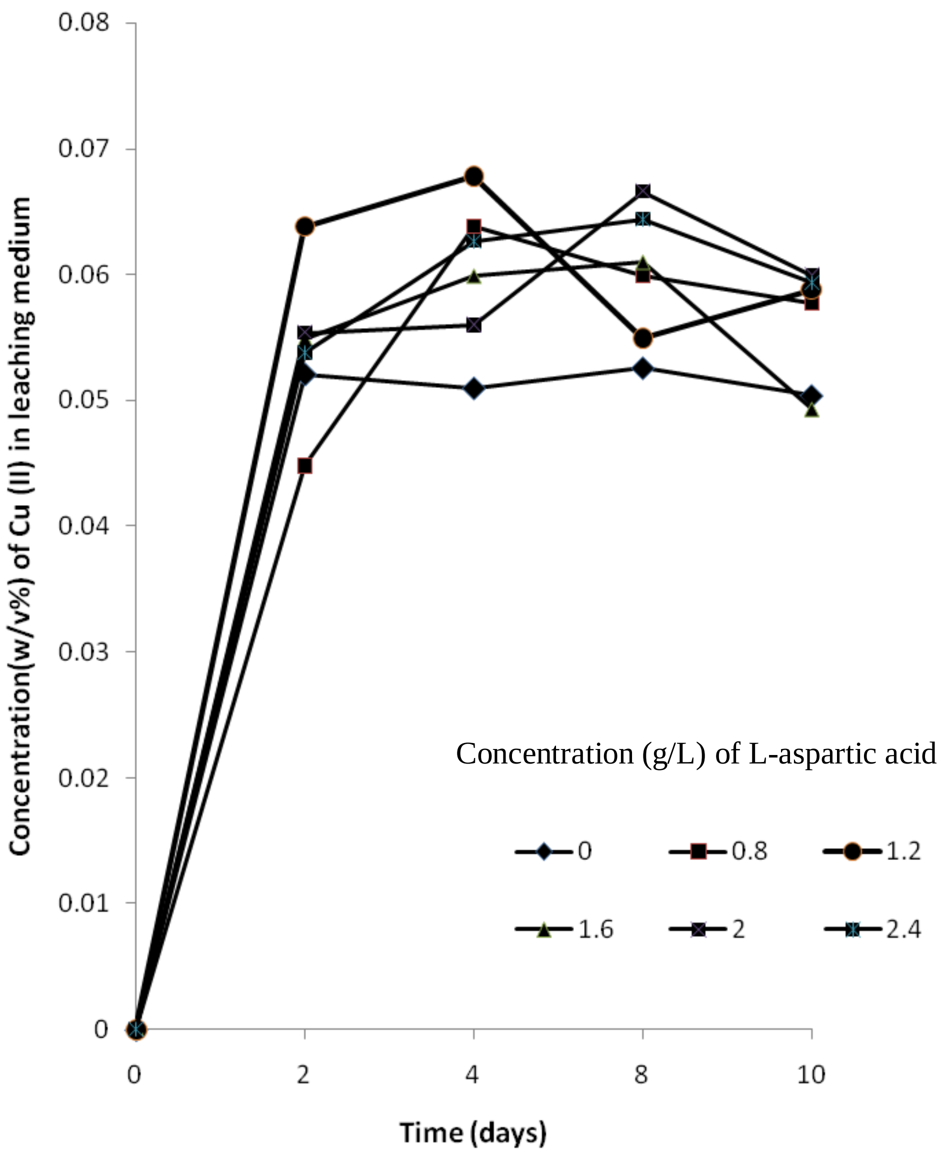

Figure 1. Bioleaching of Cu from chalcopyrite at various concentrations (g/L) of L-aspartic acid.

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

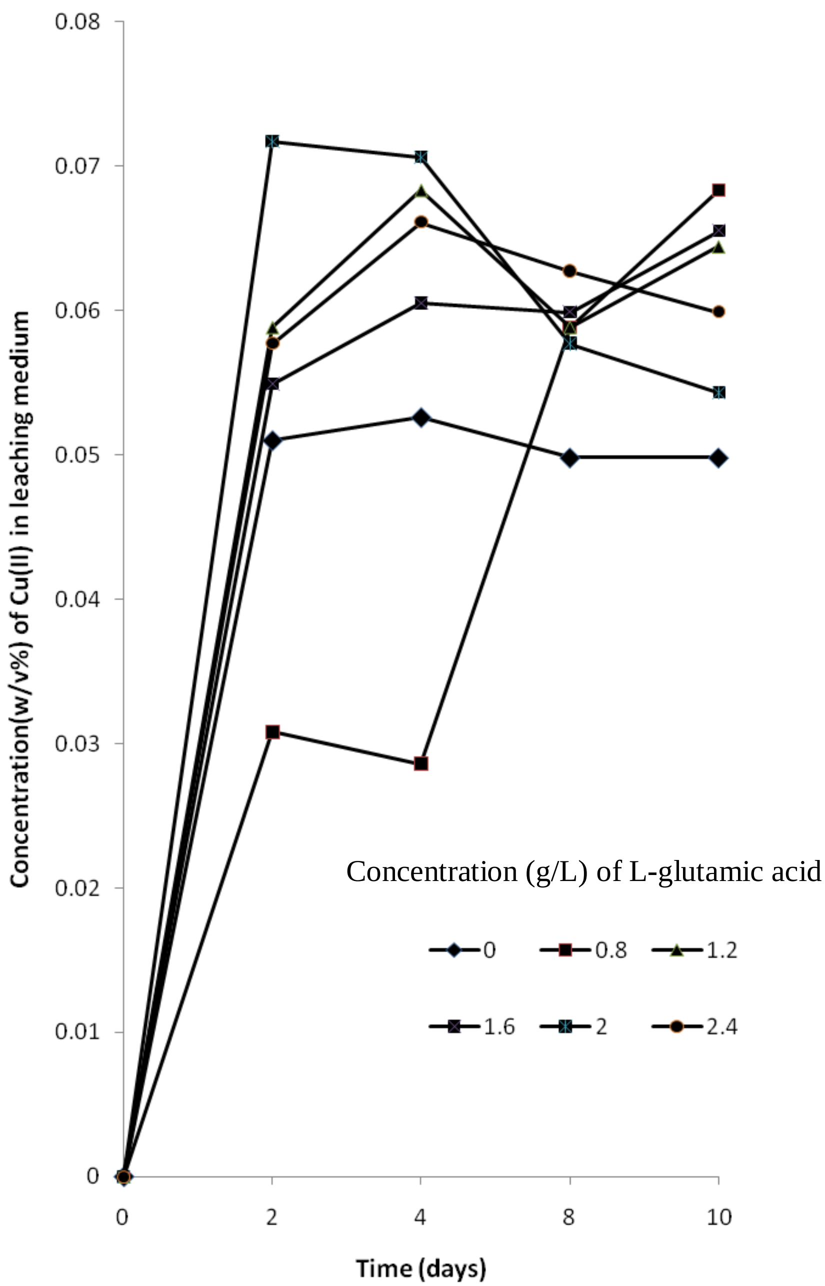

Figure 2. Bioleaching of Cu from chalcopyrite at various concentrations (g/l) of L-glutamic acid.

dissolved Cu^{2+} during initial period, the bioleaching process decreased after a few days. Maximum concentration of the dissolved Cu^{2+} was measured in the presence of 1.2 g/L of L-aspartic acid after four days.

Effect of L- glutamic acid on bioleaching of chalcopyrite

The effect of L-glutamic acid 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.

Effect of L- histidine on bioleaching of chalcopyrite

The effect of L-histidine on bioleaching of Cu^{2+} from

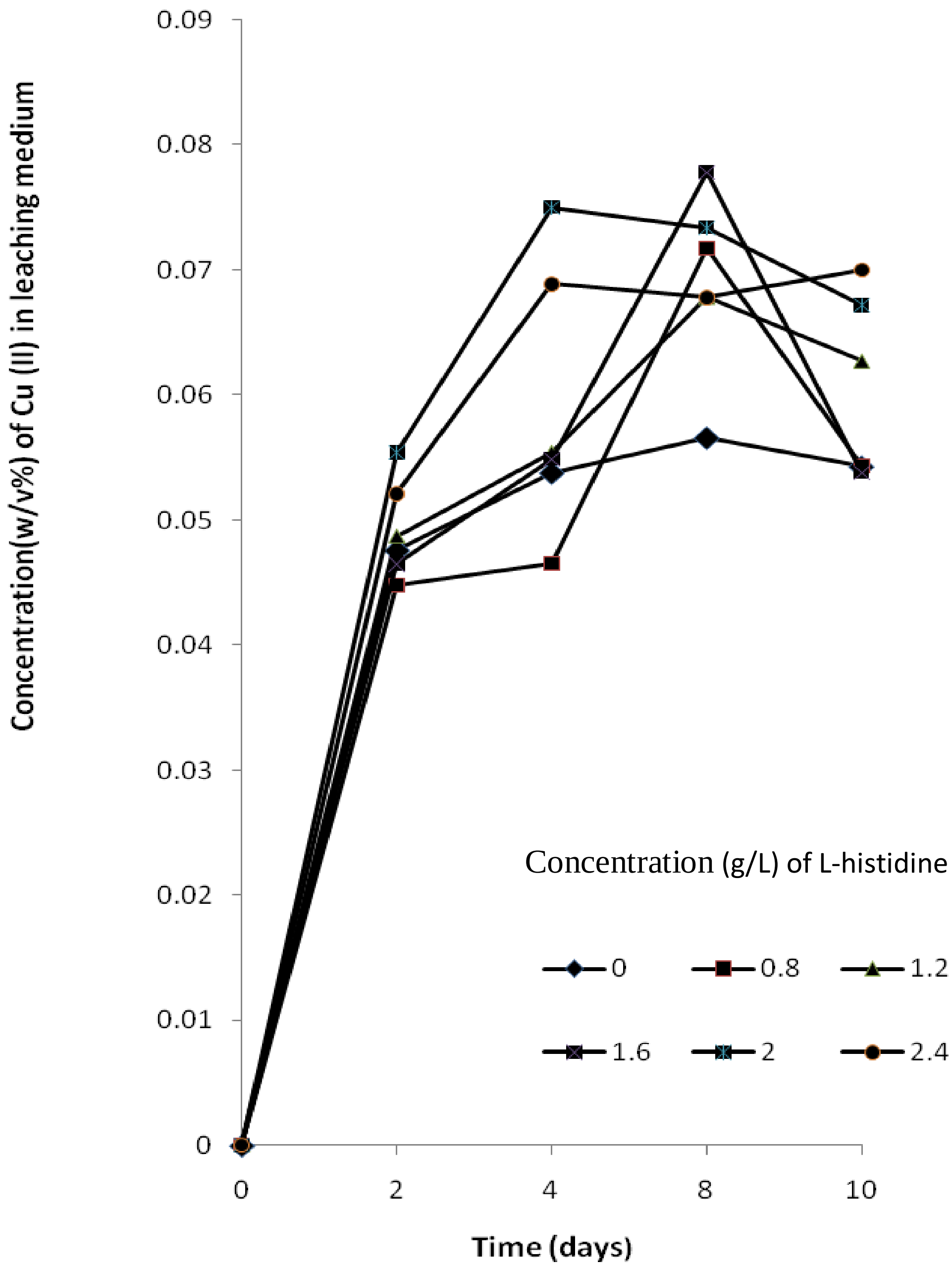

Figure 3. Bioleaching of Cu from chalcopyrite at various concentrations (g/L) of L-histidine.

chalcopyrite 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 chalcopyrite

The effect of L-serine on bioleaching of Cu^{2+} from chalcopyrite 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 chalcopyrite 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 chalcopyrite ore increased in the presence of L-aspartic acid, L-glutamic acid and L-histidine during the initial period, it was

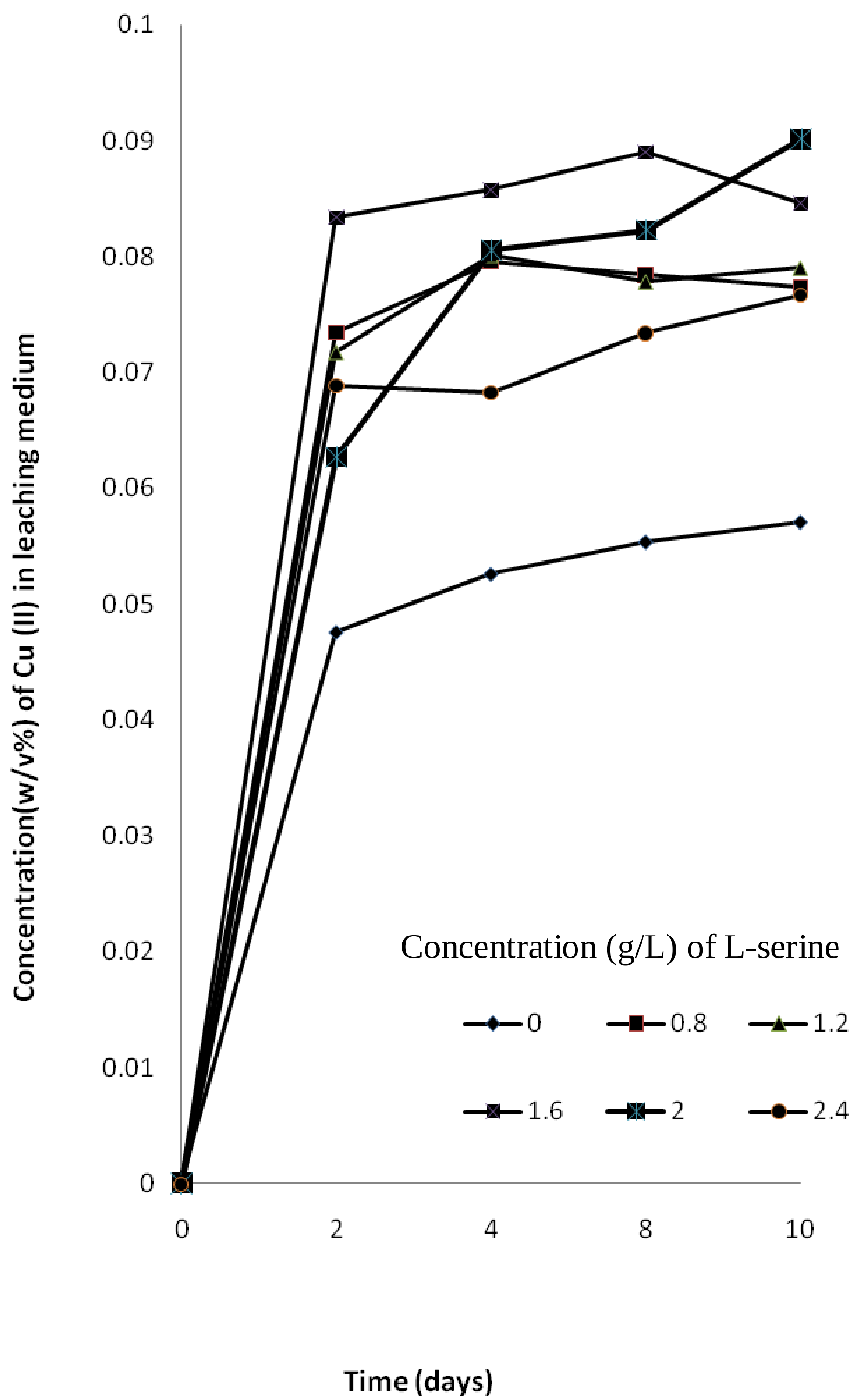

Figure 4. Bioleaching of Cu from chalcopyrite at various concentrations (g/L) of L-serine.

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 solution with increasing time. So, L-serine can be used as a potential reagent during the bioleaching of Cu^{2+} from chalcopyrite ore by *T. ferrooxidans*. It may be possible that L-serine accelerates the Cu^{2+} leaching process through indirect mechanism (Dopson et al., 2003; Schippers and Sand, 1999) by initially forming stable complexes with the Fe^{3+} (Furia, 1972), obtained in solution by bacterial oxidation of the Fe^{2+} of the chalcopyrite ore and then concentrating the Fe^{3+} 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 Cu^{2+} 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 Cu^{2+} from the microbial solution, which in turn may help to increase the bacterial metabolic rate by decreasing the Cu^{2+} 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.

REFERENCES

Bryner LC, Beck JV, Davis DB,Wilson DC (1954). Microorganisms in leaching sulphide minerals. Ind. Eng. Chem. 46: 2587-2592.

Cordoba EM, Munoz JA, Blazquez ML, Gronzalez F, Ballester A (2008). Leaching of chalcopyrite with Fe^{+3} ion (Part IV): The role of redox potential in the presence of mesophillic and thermophillic bacteria. Hydrometallurgy, 93: 106-115.

Devasia P, Natarajan KA, Sathyanarayana DN, Ramananda Rao G (1993). Surface chemistry of *Thiobacillus ferrooxidans* relevant to adhesion on mineral surfaces. Appl. Environ. Microbiol. 59(12): 4051-4055.

Dopson M, Austin CB, Koppineedi PR, Bond PL (2003). Growth in sulphidic mineral environment: Metal resistance mechanism in acidophillic microorganisms. Microbiology, 149(88): 1959-1970.

Duncan DW, Trussell PC, Walden CC (1964). Leaching of chalcopyrite with *Thiobacillus ferrooxidans*: Effects of surfactants & shaking. Appl. Microbiol. 12(2): 122-126.

Furia ET (1972). CRC Handbook of Food Additives (2nd Ed.) CRC Press, USA.

Groudev SN, Groudeva VI (1993). Biohydrometallurgy of gold: present day status and future prospects. Preprints of the XVIIIth International Mineral Processing Congress, Sydney, pp. 1385-1387.

Groudev SN, Spasova II, Ivanov IM (1996). Two-stage microbial leaching of a refractory gold-bearing pyrite ore. Miner Eng. 9: 707-713.

Guay R, Inal OT, Toniazzo V, Mustin C (1999). Chalcopyrite leaching by *Thiobacillus ferrooxidans*: effect of shock activation on chalcopyrite surface characteristics and copper solubilization. International Biohydrometallurgy Symposium IBS-99 'Biohydrometallurgy and the Environment toward the mining of the 21st Century'.

He Z , Gao F, Zhong H, Hua Y (2009). Effects of L-cysteine on Ni-Cu sulfide and marmatite bioleaching by *Acidithiobacillus caldus*. Bioresour. Tech.100: 1383-1387.

Mier JL, Ballester A, Blazquez ML, Gonzalez F, Munoz JA (1995). Influence of metallic ions in the bioleaching of chalcopyrite by *Sulfolobus* BC: experiments using pneumatically stirred columns and massive samples. Miner Eng. 8: 949-965.

Mukhopadhyay BP, Ghosh B, Bairagya HR, Nandi TK, Chakrabarti B, Bera AK (2008). Molecular Modeling of the Ternary Complex of Rusticyanin-Cytochrome c4 -Cytochrome oxidase : An Insight to Possible H- Bond Mediated Recognition & Electron Transfer Reaction in T. ferrooxidans. J. Biomol. Struct. Dyn. 25(5): 543-552.

Natarajan KA (1998). Microbes, Minerals & Environment. Geological Survey India.

Neunberg C, Mandl LAN (1948). Unknown effect of amino acids. Arch. Biochem. 19(2): 149-161.

Rohwerder T, Gehrke T, Kinzler K, Sand W (2003). Bioleaching review (Part A): Progress in bioleaching: Fundamentals and mechanism of bacterial metal sulphide oxidation. Appl. Microbiol. Biotechnol. 63(3): 239-248.

Schippers A, Sand W (1999). Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. Appl. Environ. Microbiol. 65: 319-321.

Silverman MP, Lundgren DG (1959). Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*; an improved medium and a harvesting procedure for securing high cell yields. J Bacteriol.77: 642-647.

Spasova I, Nicolova M, Veglio F, Groudev SN (2006). Leaching of gold from a polymetallic sulphide ore. Annual of the university of Mining and Geology “St. Ivan Rilski”, 49 (I). Geol. Geophys. pp. 213-216.

Third KA, Cord-Ruwisch R, Watling HR (2002). Control of the redox potential by oxygen limitation improves bacterial leaching of chalcopyrite. Biotechnol. Bioeng. 78(4): 433-441.

Vogel I (1989). Vogel’s Textbook of Quantitative Inorganic Analysis. ELBS (Great Britain).

Yue hua HU, Zhr guo HE, Wer xin HU (2004). Effect of two kinds of amino acids on bioleaching of metal sulphides. Trans. Nonferrous Met. Soc. China, 14(4): 794-797.

Zhou HB, Zeng WM, Yang ZF, Xie YJ, Qiu GZ (2009). Bioleaching of chalcopyrite oncentrate by a moderately thermophilic culture in a stirred tank reactor. Bioresour. Technol. 100(2): 515-520.