

Bio-oxidation of a low grade chalcopyrite ore by mixed culture of acidophilic bacteria

A.A. Baba^{1+,*}, E.E. Ezekafor¹, F.A. Adekola¹, R.N. Ahmed³ and S. Panda⁴

¹Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin-240003, Nigeria.

²Hydro & Electrometallurgy Department, Institute of Minerals and Materials Technology, Bhubaneswar 751013, India.

³Department of Microbiology, University of Ilorin, P.M.B. 1515, Ilorin-240003, Nigeria.

⁴Bioresources Engineering Department, Institute of Minerals and Materials Technology, Bhubaneswar 751013, India.

Abstract

A study on the extent of biooxidation of a low grade Chalcopyrite ore using a mixed acidophilic bacterial consortium predominantly of the *Acidithiobacillus ferrooxidans* strain has been carried out. The influence of additive and pH on the bio-oxidation of the ore has been examined. The results of the investigations showed that bio-oxidation was enhanced by the addition of nutrient broth at lower pH. The enhancement in biooxidation of a chalcopyrite ore was 54% and 19% with and without addition of broth in 10 days incubation time, respectively. The study showed that at a lower pH (≤ 4.0), the biooxidation of the ore was higher in presence of broth. Consequently, in presence of nutrient broth, the concentration of Fe and Cu leached were 1200 mg/L and 350 mg/L; and without nutrient broth yielded 580 mg/L and 220 mg/L, respectively within 10 days of incubation and decreases afterwards.

Keywords: Bio-oxidation; chalcopyrite; acidophilic bacteria; nutrient broth.

INTRODUCTION

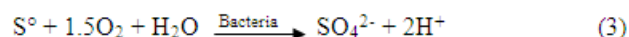
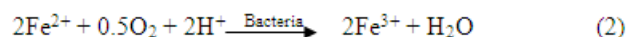
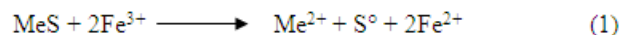
Due to continuing worldwide depletion of metal ore deposits and accumulation of tailings, future sustainable developments require measures to economically use the non-renewable raw materials rich in chalcopyrite ores and to reduce the demand for primary resources. Therefore, new resources for metals must be developed with the aid of novel technologies. In addition, improvement of already existing mining technologies can result in metal recovery from sources that have not been of economical interest [1].

Microorganisms play a predominant role in the solubilization, transport and deposition of metals and minerals in the environment. A better understanding of these processes has allowed furthering characterizing bacterial leaching of metals from ores and innovative microbial based technologies for metals reclamation. Microbial technology presents an economic alternative for today's mining, mineral and waste water treatment industries as high grade mineral resources are depleting, energy costs are increasing and adverse environmental effects are becoming more apparent because of conventional technologies [2]. Thus, the advent of biotechnology in mineral processing has opened up immense possibilities to exploit difficult-to-treat ores. Bioprocessing technologies has; therefore, hold promise as potential substitutes for the conventional technologies in

vogue [3].

The bioleaching of chalcopyrite occurs in the acidic medium that often contains a considerable concentration of Fe (III). It involves electrochemical and chemical reactions of the mineral with the leach solutions and the bacteria [4]. At present, more than 40 bacteria have been discovered [5]. The most often observed to leach chalcopyrite ore are mesophilic, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* [6-9]. Currently, three different mechanisms have been suggested for bacterial oxidation: the direct, indirect and indirect contact mechanisms [8-10].

The direct mechanism assumes that bacteria attach onto the mineral particle surfaces where they achieve mineral dissolution through direct bacterial metabolism. Under the indirect mechanism, mineral dissolution is achieved via oxidative ferric leaching (Equation 1), with bacteria oxidizing ferrous ions to ferric ions, and elemental sulphur to sulphate ions (Equations 2 and 3 respectively).



The third proposed bioleaching mechanism is that of indirect contact in which bacteria attach onto the mineral particle surfaces, where they heighten the ferric ion and acid concentration in the immediate vicinity of the mineral particles (Equations 2 and 3 respectively) [8,10-12]. Thus, it is thought that bacteria attach themselves to mineral particle surfaces by use of extracellular polymeric substances (EPS), and that inside these EPS, higher concentrations of ferric ions accumulate relative to the surrounding media. Hence, it is currently believed that bio-oxidation of

Received: Oct 12, 2011; Revised: Nov 10, 2011; Accepted: Dec 05, 2011.

*Corresponding Author

A.A. Baba
Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin-240003,
Nigeria.

Tel: +23-48035010302.

Email: alafara@unilorin.edu.ng.

chalcopyrite proceeds according to an indirect mechanism [12, 13] and this may be applicable to other sulphide minerals of the form $Me^{2+} S^{2-}$ such as galena [8, 12, 13].

Therefore, the main objective of the present study was to examine the extent of bio-oxidation of chalcopyrite ore by a mixed culture of mesophilic acidophilic bacterial consortium. At present little or no study exist in this area of research, viz-a-viz the ore bacteria leaching from Nigerian origin [14].

MATERIALS AND METHODS

Ore Sample

The sulfidic ore "chalcopyrite" used in this study was collected from Department of Geology and Mineral Sciences, University of Ilorin, Ilorin, Nigeria. The ore is from Kokona origin of Nasarawa State, Nigeria.

Mineralogical studies

The elemental analysis of the ore was carried out by inductively coupled plasma-mass spectrophotometer (ICP-MS). The phase characterization for the mineralogical purity was examined by X-ray diffraction (XRD) and the phase characteristics of the compounds detected was correlated using JCPDS file number for each of the attributed peaks in the X-ray diffractogram. Also, the photomicrograph of the thin section of the ore layer was conducted to support the results of mineralogical purity by XRD and elemental analysis by ICP-MS.

Microorganisms

The microorganisms used in the present study were a mixed culture of mesophilic acidophilic chemolithotrophic bacterial consortium predominantly of the *Acidithiobacillus ferrooxidans* strain. The strain was grown in 9K⁺ medium [15] containing $(NH_4)_2SO_4$: 3 g L⁻¹, KH_2PO_4 : 0.5 g L⁻¹, $MgSO_4 \cdot 7H_2O$: 0.5 g L⁻¹, KCl: 0.1 g L⁻¹, $FeSO_4$: 44.2 g L⁻¹. The solution was prepared in a 1L flask and steam sterilized in an autoclave at 121°C for 15 minutes to get rid of all microorganisms present in the solution [16].

Preparation of Nutrient broth

6.25g of nutrient broth was weighed into 250ml conical flask and dissolved in distilled water. The content was heated for about 10 minutes and was then steam sterilized in an autoclave at 121°C for 15 minutes [16].

Bio-oxidation/Bioleaching Procedure

Two procedures were used for the leaching process. They are (i) nutrient broth aided bioleaching process and (ii) bacterial leaching without broth addition.

Procedure (i): 0.5g of chalcopyrite was weighed and 40ml of distilled water was added to it in a conical flask. 3ml of nutrient broth was supplied to support the life of the organism in leaching medium. To it, 2ml of 1N H_2SO_4 was added (to make the environment acidic since *A. ferrooxidans* lives in acidic environment). 5% inoculums (i.e. 5ml of washed bacteria) were then added. The flask was then placed on 116rpm orbital shaker. This procedure was repeated for 1, 3, 5, 10, 15 and 20 days, respectively.

Procedure (ii): 0.5g of chalcopyrite was weighed into a conical flask. 43ml of distilled water was added followed by addition of 2ml of 1N H_2SO_4 . 5% inoculums (i.e. 5ml of washed bacteria) were added. The flask is then placed on 116rpm orbital shaker. This procedure was repeated for 1, 3, 5, 10, 15 and 20 days, respectively.

At the end of daily investigation, for the two procedures, the solutions was autoclaved, filtered and the residues were air dried and oven dried, then weighed. The pH of the filtrates was also determined using CRISON microPH 2000 pH meter. The atomic absorption spectrophotometric analyses of the filtrates were also determined by PUCK Scientific atomic absorption spectrophotometer, 2010-2011 VGB models.

RESULTS AND DISCUSSION

Results of elemental and phase characterization

Results of elemental analysis by ICP-MS.

The result of the elemental study by ICP-MS for the East of Kokona District of Nasarawa State chalcopyrite ore is presented in Table 1.

Table 1. Result of elemental analysis by ICP-MS.

Element	Cu	Fe	Si	Ti	S	Cd	Pb	W
Composition (%)	7.16	24.87	9.24	3.71	19.74	0.34	2.18	0.0148
Element	Sb	V	Ca	Mg				
Composition (%)	0.1463	0.0024	0.0216	0.00493				

Oxygen (O) and other constituents = 32.57%, obtained by difference.

The result of the ICP-MS analysis from Table 1 indicated the major elements in the ore are Cu, S, Fe, Si, Ti and Pb.

Phase study by XRD

The result of the mineralogical purity of chalcopyrite ore is presented in Table 2.

Table 2. The XRD major diffraction peaks of the Chalcopyrite ore

2θ	d-value	Compounds	JCPDS* File Number	Relative intensity (%)
19.83	1.853	Chalcopyrite (CuFeS ₂)	25-0288	36.4
22.16	1.981	Silica (α-SiO ₂)	11-0695	12.1
23.51	1.741	Iron Sulphide (FeS ₂)	03-0794	7.8
24.23	1.689	Hematite (Fe ₂ O ₃)	13-0534	3.6
26.07	1.581	Cristobalite (SiO ₂)	11-0695	10.1
29.36	1.399	Copper oxide (CuO)	05-0661	8.3
30.87	1.241	Chalcopyrite (CuFeS ₂)	25-0288	11.2
31.43	1.193	Galena (PbS)	18-0378	3.4
38.04	1.089	Cristobalite (SiO ₂)	11-0695A	8.3
40.41	1.011	Ilmenite (FeTiO ₃)	27-1431	5.4
51.57	0.815	Calcium oxide (CaO)	04-0777	6.9

*JCPDS= Joint Committee on Powder Diffraction standards file number.

The results in Table 2 affirmed that the major peaks in the ore include CuFeS₂, α-SiO₂ and FeS₂. Other peaks detected such as

CaO, PbS, FeTiO₃ and Fe₂O₃ were also detected. All these supported the elemental analysis by ICP-MS.

Photo-micrographic examination

The result of the ore thin-layer by photo-micrographic analysis is shown in Fig. 1.

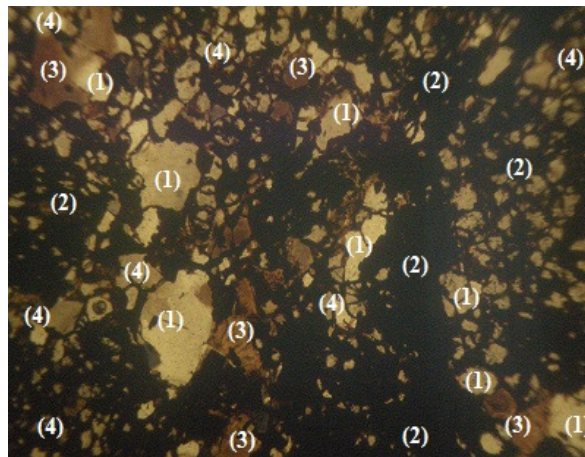


Fig 1. Result of ore thin-layer by photo-micrographic analysis (×40).
 (1) CuFeS₂ (2) FeS₂ (3) α-SiO₂ (4) PbS

From Fig. 1, it is evident that chalcopyrite ore under study comprised majorly of CuFeS_2 portions. Other associated components examined include pyrite (FeS_2), α -quartz ($\alpha\text{-SiO}_2$) and PbS. All these lend the support of both the elemental analysis by ICP-MS and phase characterization by XRD.

Bio-oxidation/Bioleaching studies

Effect of additive

It has been well known that the addition of some ions such as Ag(I) , Sn(II) , Co(II) , Hg(II) and Mn(II) as well as addition of pyrite under certain conditions accelerate copper dissolution from chalcopyrite. However, these additives act as catalyst on the reaction rate of chalcopyrite, which is an alternative method of enhancing or improving the rate of chalcopyrite leaching.

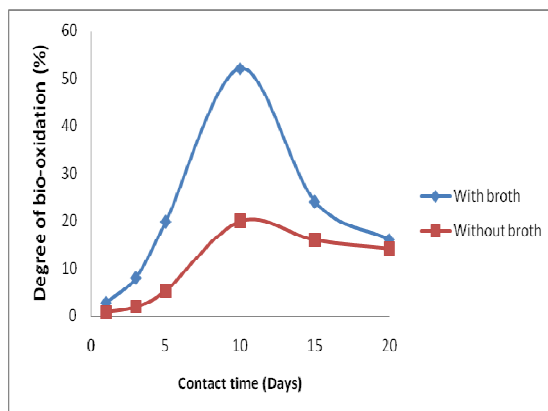


Fig 2. Influence of additive on biooxidation of chalcopyrite.

Fig. 2 shows the degree of biooxidation in presence and absence of broth on chalcopyrite ore. It clearly demonstrates that the bacteria functions well in the presence of broth. The figure showed a maximum of 54% biooxidation in the presence of broth in 10 days time. After that, a drop has been noticed in the degree of biooxidation. This reduction in biooxidation can be due to the fact that oxidizable ferrous ions are no longer present in solution or their concentration has been reduced. In the absence of broth, the degree of biooxidation is lower; that is, a maximum of 19% could be achieved in 10 days and remains constant afterwards.

The indirect mechanism or indirect bacterial contact made by the bacterium *Acidithiobacillus ferrooxidans* oxidizes available ferrous ions into ferric ions. These ferric ions later acts on chalcopyrite as its direct oxidizing agent [17]. Chalcopyrite from previous study dissolves much more readily and with a high yield and extraction rate in the presence of microorganism which is presumably related with changes in electrochemical behaviour of

mineral surface [18].

The rapid change (increase in biooxidation) as shown in Fig. 2, from day 5 to day 10 is due to the rapid increase in the amount of ferrous ion species, which is oxidized into ferric ion by *Acidithiobacillus ferrooxidans* action. This ferric ions acts upon CuFeS_2 to give elemental sulphur with zero oxidation state, oxidized copper from Cu^0 to Cu^{++} and iron to a reduced form of itself.

However, when the quantity of oxidizable Fe^{2+} reduces, a reduction in biooxidation of chalcopyrite is noticed. The leaching process therefore experienced a drop in yield in day 15 to day 20. It shows the effect of depleting ferrous ion in solution.

Effect of pH

The effect of pH on the ore bio-oxidation after leaching is shown in Fig. 3.

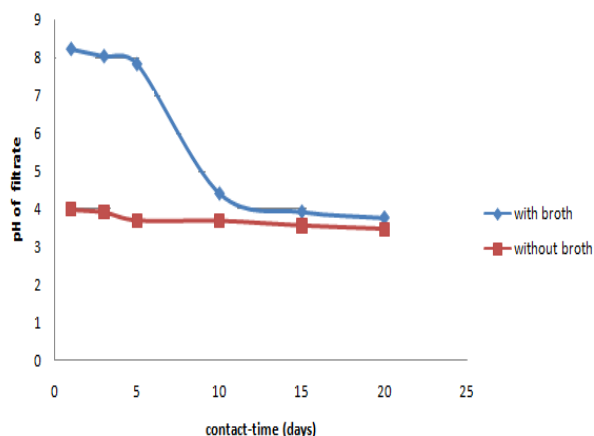


Fig 3. The effect of pH on ore bio-oxidation

From fig. 3, the effect of pH is not so prominent to bio-oxidation without addition of nutrient broth. Throughout the leaching process, the pH value is within the range of 3.98 to 3.46 (without broth). This shows that leaching of chalcopyrite is prominent in acidic medium, and also the use of acidophilic bacteria such as *Acidithiobacillus ferrooxidans* is prominent in acidic medium. Thus, the rate of degree of bio-oxidation increases with low pH. The pH drops from 8.21 to 3.75 as shown in Fig. 3. Thus, leaching is more effective at low pH.

Bioleaching of Cu and Fe in presence of additive

To ascertain the actual quantity of copper and iron (the extent of the metal ions leached) was determined by atomic absorption spectrophotometric analysis. The results of the analysis are shown in Figs. 4 and 5, respectively.

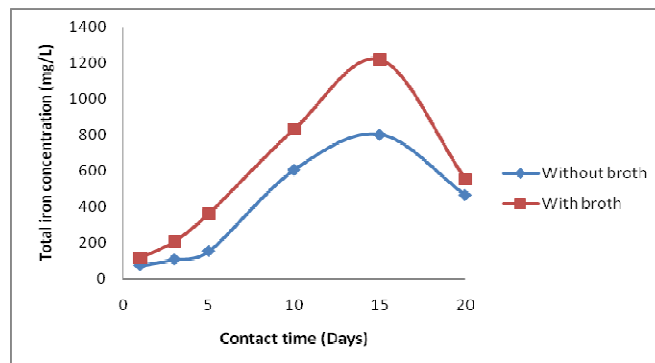


Fig 4. Amount of total iron bio-oxidated into solution over time (*with and without broth*).

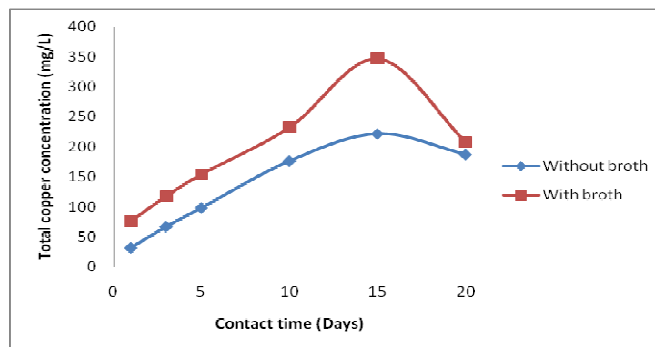


Fig 5. Amount of total copper bio-oxidized over time (*with and without broth*).

Fig 4 shows the yield of biooxidation of iron with and without addition of nutrient broth. In this case, the biooxidation reaction aided by broth addition is more favourable to the production of more iron at a longer or prolonged leaching time [19]. While on the other hand, copper oxidation is feasible in fig. 5 with respect to biooxidation with the addition of nutrient broth. Therefore, figures 4 and 5 clearly indicate that the bioleaching efficiency increased in presence of additive agent.

Conclusions

Experiments on bio-oxidation of chalcopyrite ore have been examined. On the basis of the results obtained in the study, the following conclusions can be drawn:

- I. Bio-oxidation of chalcopyrite ore increases at lower pH (≤ 4.0) in the presence of nutrient broth.
- II. Bioleaching of total copper and iron was enhanced after the addition of nutrient broth.
- III. The concentration of total Fe leached was 1200 mg/L and 580

mg/L and that of Cu were 350 mg/L and 220 mg/L, with and without addition of broth, respectively within 10 days. However, there was a decline in the amount of metal ions (Fe and Cu) afterwards.

ACKNOWLEDGMENTS

A. A. Baba wishes to thank University of Ilorin for permission to honour CSIR-TWAS Fellowship award; Prof. B. K. Mishra, Director, Institute of Minerals and Materials Technology, Bhubaneswar-751013, India for the acceptance as CSIR-TWAS Postdoctoral Fellow and the Management, Academy of Sciences for the Developing World, Trieste, Italy for the Award of 2010 CSIR-TWAS Fellowship for Postdoctoral Research (May, 2011-January, 2012).

REFERENCES

- [1] Ilyas.S., Anwar M. A., Niazi, S.B., Ghauri, M.A., Khalid, A.M. 2007. Microbial leaching of iron from pyrite by moderate bacteria.

- J. Res. (Sci), Pakistan.* 18 (2):159-166.
- [2] Hossain, S.K., Das, M., Ibrahim, S.H., Sheriffa Begum, K.M.M., and Anantharaman, N. 2004. Studies on biohydrometallurgical leaching of iron sulphide using *Thiobacillus ferrooxidans*. *Indian J. Chem. Tech.* 11: 116-120.
- [3] Santhiya, D., Subramanian, S., Natrajan, K.A., Rao, K.H., and Forssberg, K.S.E. 2001, Bio-modulation of galena and sphalerite surfaces using *Thiobacillus thiooxidans*. *Int. J. Miner. Process.* 62: 121-144.
- [4] Walting, H.R. 2006. The bioleaching of sulphide minerals with emphasis on copper sulphide-A review. *Hydrometallurgy.* 84: 81-108.
- [5] Gomez, E., Figueroa, M., Munoz, J., Ballester, A., and Blazquez M.L. 1997. A study of bioleached chalcopyrite surface in the presence of Ag (I) by voltametric methods. *Min. Engr.* 10(1): 111-116.
- [6] Sergio, A., and Jerez, C. A. 2004. Copper ions stimulated polyphosphate degeneration and phosphate efflux in *Acidithiobacillus ferrooxidans*. *Appl. and Envir. Microbiol.* 70 (9): 5177-5178.
- [7] Jiang, L., Zhou, H. Y. and Peng, X. T. 2007. Bio-oxidation of pyrite, chalcopyrite and pyrrhotite by *Acidithiobacillus ferrooxidans*. *Chinese Sci. Bull.* 52 (9): 2702-2714.
- [8] da-Silver, G. 2004. Kinetics and mechanism of the bacterial and ferric sulphate oxidation of galena. *Hydrometallurgy.* 75: 99-110.
- [9] Qui, M., Xiong, S., Zhang, W., and Wang, G. 2005. A comparison of bioleaching of chalcopyrite using pure or a mixed culture. *Min. Engr (Technical note).* 18: 987-990.
- [10] Fowler, T. A., Holmes, P. R., and Crundwell, F. K. 2001. On the kinetics and mechanism of the dissolution of pyrite in the presence of *Thiobacillus ferrooxidans*. *Hydrometallurgy.* 59:257-270.
- [11] Sand, W., Gehrke, T., Jozsa, P. G., and Skippers, A. 2001. (Bio)-chemistry of bacterial leaching-Direct vs. indirect bioleaching. *Hydrometallurgy.* 59: 159-175.
- [12] Boon, M., Snijder., Hansford, G.S. and Heijnen, J.J. 1998. The oxidation kinetics of zinc sulphide with *Thiobacillus ferrooxidans*. *Hydrometallurgy.* 48: 171-186.
- [13] Kringma-Jr, J.G and Silver, M. 1980. Growth of iron-oxidising *Thiobacilli* in the presence of chalcopyrite and galena. *Appl and Envir. Microbiology.* 39 (2): 635-641.
- [14] Baba, A.A., Adekola, F.A., Atata, R.F., Ahmed, R.N., and Panda, S. 2011. Bioleaching of Zn (II) and Pb (II) from Nigerian sphalerite and galena ores by a mixed culture of acidophilic bacteria. *Trans. Nonferrous Metals Society, China.* 21, 2535-2541.
- [15] Silverman, M.P., and Lundgren, D.G. 1959. Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. *Journal of Bacteriology.* 77: 642-647.
- [16] Colmer, A.R. 1962. Relation of the iron oxidizer, *Thiobacillus ferrooxidans*, to thiosulfate. *J. Bacteriol.* 83:761-765.
- [17] Jonylertjunya, W. 2003. Bioleaching of chalcopyrite. *Chemical Engineering.* 37-38.
- [18] Gómez, C., Munoz, J.A., Savillano, M E., Ballester, A., Figueroa, M., Gonzalez, F., and Blázquez, M. L. 1995. Microbial effects on the anodic dissolution of chalcopyrite. *Proceeding of XIX Int. Mineral Processing Congress.* 2: 218-222.
- [19] Schultze, L. E., Sanoval S. P., and Bush, R. P., 1995. Effect of additive on chalcopyrite leaching. *Report of Investigations, RI. 9556 United States Department of the Interior Inc. United State Bureau of Mine.*