ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 5, Issue 10, pp 1378-1382, October 2016

International Journal of Current Advanced Research

ISSN: 2319 - 6475

RESEARCH ARTICLE

BIOLEACHING KINETICS OF CHALCOPYRITE CONCENTRATE USING *LEPTOSPIRILLUM FERRIPHILUM*: EFFECT OF SILVER ION

Venkatesa Prabhu S*., Barathiraja P., Karthik R and Ragul V

Department of Petroleum Engineering, JCT College of Engineering and Technology, Pichanur,
Coimbatore, Tamil Nadu, India

ARTICLE INFO

Article History:

Received 9th July, 2016
Received in revised form 5thAugust, 2016
Accepted 28th September, 2016
Published online 28th October, 2016

Key words:

Chalcopyrite pH; silver ion; copper; iron and *L. ferriphilum*.

ABSTRACT

Bioleaching is a proven eco-friendly biological method to extract metals from their ores. One of the important problems faced in bioleaching is the low leaching rate, which consumes high residence time. In this study, the catalytic effect of silver ion (Ag^+) on bioleaching of copper and iron from chalcopyrite concentrate was investigated using isolated bacteria, Leptospirillum ferriphilum, for enhancing the leaching rate. The bioleaching data were collected for Cu and Fe extraction from chalcopyrite concentrates using different concentrations of silver ion (1−5 mg/L) with the following fixed parameters: initial pH, 1.5; pulp density, 1% (w/v); particle size, 200 µm; and agitation speed, 180 rpm. From the obtained results, it was found that the maximized leaching of Cu (87.73%) and Fe (74.73%) occurred while using 4 mg/L silver ion concentration. The kinetic study on bioleaching data indicated that the rate constants for Cu and Fe leaching were found to be maximum (0.078 and 0.054 d^{-1}, respectively) at Ag^+ concentration 4mg/L. The leaching data were analyzed using shrinking core model for determining the rate-controlling step. It explicated that the diffusion through product layer forms on the mineral surface controls the rate of leaching.

INTRODUCTION

Copper is the second largest metallic chemical element widely used in the world. It is a ductile metal that conducts heat and is resistant to corrosion. This results in the usage of copper as a material of choice in various domestic, industrial, and high-technology applications. The world's production and consumption of copper have increased dramatically in the past 25 years. Copper is obtained from different ore deposits such as chalcopyrite, chalcocite, covellite, bornite, tetrahedrite, cuprite, tennantite, azurite, and malachite. Of these, chalcopyrite is one of the most important sulfide minerals of copper [1,2]. Generally, copper is extracted by pressure hydrometallurgy process; otherwise, it is treated via flotation concentration followed by smelting [3]. This method has several disadvantages such as more chemical usage and high cost, and has also several environmental restrictions. To overcome these problems, the copper industry has moved toward biohydrometallurgical processes. Bioleaching is a technique of biohydrometallurgical processes by which dissolution of minerals due to direct or indirect action of microorganisms takes place [4]. Dissolution of minerals occurs due to the microbial oxidation of insoluble metal sulfides to soluble metal sulfates. This method has many potential advantages over conventional metal extraction techniques because it can be applied to all ore grades and to waste materials produced by conventional ore dressing. In addition, it is an environmentally sound technique as it does not give raise to atmospheric pollution and also requires very low energy.

The microorganisms used in this process are acidophilic bacteria that have the ability to survive at low pH and high metal concentrations, and are capable of iron and/or sulfur oxidation [5]. An iron-oxidizing bacterium (IOB), *Leptospirillum ferriphilum*, is one of the most important bacteria for bioleaching because it can tolerate lower pH, is more extremophile, and can withstand higher cultivation temperature [6]. Thus, it was chosen as a bioleaching microorganism in this study. *L. ferriphilum* obtains energy through oxidation of Fe(II) iron to Fe(III) iron, which acts as oxidizing agent for metal sulfides. The energy transduction is catalyzed by a specific protein called *Iro*protein, which was proposed to be involved in the iron respiratory transport chain [7-10]. The exploitation of chalcopyrite by *L. ferriphilum* for extracting copper in the form of soluble copper sulfate can be described by the following equations.

$$2FeSO_4 + \tfrac{1}{2}O_2 + H_2SO_4 \xrightarrow{L.\ ferriphilum} Fe_2(SO_4)_3 + H_2O \quad \ldots (1)$$

$$CuFeS_2 + 2Fe_2(SO_4)_3 \longrightarrow CuSO_4 + 5FeSO_4 + 2S^0 \quad \ldots (2)$$

$$CuFeS_2 + 4.25O_2 + H^+ \longrightarrow Cu^{2+} + Fe^{3+} + 2SO_4^{2-} + 0.5H_2O \quad \ldots (3)$$

However, a general problem associated with bioleaching is its low leaching rate. It takes approximately 1–2 years to complete the heap or dump leaching operation. This is the major disadvantage for commercialization of bioleaching and hinders its success [11]. Consequently, several engineering efforts should be made on different influencing parameters to improve the leaching rate in bioleaching process.

Though the bacterial growth and bioleaching rate are greatly affected by several parameters such as medium pH, temperature, iron concentration, particle size, agitation speed, and oxidation-reduction potential of medium, the usage of silver ion as catalyst in bioleaching process is attracting significant interest to enhance the leaching rate [12-14]. The mechanism of silver (Ag^+) catalysis in bioleaching was proposed by Miller and Portillo [15]. According to this mechanism, Ag^+ ion rapidly reacts with chalcopyrite to produce ferrous iron, which acts the energy source to IOB (Eq. (4)). Then, the bacterially produced ferric iron reacts with Ag_2S to regenerate ferrous iron {Eq. (5)}. This cyclic process enhances the leaching of Cu during the bioleaching course.

$$CuFeS_2 + 4Ag^+ \longrightarrow Cu^{2+} + Fe^{2+} + 2Ag_2S \quad \ldots (4)$$

$$Ag_2S + 2Fe^{3+} \longrightarrow 2Ag^+ + S^0 + 2Fe^{2+} \quad \ldots (5)$$

In this study, the bioleaching of Cu and Fe from the chalcopyrite concentrates was carried out to investigate the catalytic effect of Ag^+ concentration using the isolate *L. ferriphilum*. In addition, kinetic studies for rate of Cu and Fe leaching based on pseudo-first-order model were carried out. The rate-controlling step was determined using shrinking core model (SCM).

MATERIALS AND METHODS

Chalcopyrite concentrate. The chalcopyrite concentrate was obtained from Rakha copper mines (Singhbum, Jharkhand, India). The concentrates were crushed using laboratory jaw crusher and then classified based on particle size using ASTM sieves. The grounded particles ranging from 100 to 1,200 µm with an average size of 200 µm were used in the bioleaching experiments. Semi-mineralogical studies on mineral by X-ray diffraction analysis revealed that the most important mineral in the concentrate was chalcopyrite ($CuFeS_2$). It was found that the mineral sample was composed of 23.59% chalcopyrite, 23.856% quartz, 10.999% rosickyite, and 41.55% zeolite. The main chemical components of chalcopyrite concentrate are CuO (20.89%), Fe_2O_3 (24.53%) and S (31.02%). The Chemical analysis of chalcopyrite concentrate is shown in Table 1.

Bacterial culture and molecular characterization. The IOB strain, *L. ferriphilum*, used in these experiments was isolated from mine drainage samples of Chitradurga mine sector, Ingaldhal (Karnataka, India). The culture was isolated by multiple-transfer technique using 9K synthetic medium. The medium had the following chemical composition per liter: $(NH_4)_2SO_4$, 3.0 g; KCl, 0.1 g; $MgSO_4.7H_2O$, 0.5 g; K_2HPO_4, 0.5 g; and $Ca(NO_3)_2$, 0.01 g. $FeSO_4.7H_2O$ (44.2 g/L) was used as an energy source in the medium. The initial pH of the media was adjusted to 1.5 using 1 N H_2SO_4. From the developed liquid culture, DNA was extracted in accordance with the manufacturer's instructions on the DNA extraction kit (PCR Diagnostic Kits; Roche, Pleasanton, CA, USA). The PCR amplification was carried out according to the technique described by Ding et al., 2008. From a public database (http://www.ncbi.nlm.nih.gov/), the 16S rRNA sequences of the related reference organisms were downloaded and aligned with the sequence obtained from isolate using software Clustal X, version 1.80. This alignment was used to develop a distance matrix that showed that nucleotide sequences had restriction pattern 99% identical to that of *L. ferriphilum* NR028818. Nucleotide sequences of *L. ferriphilum* isolate were submitted to GenBank (National Center for Biotechnology Information, Bethesda, MD, USA) and accession number KF743135 was obtained.

Experimental procedure. Bioleaching experiments were performed in a 250-mL flask containing 90 mL sterilized ferrous iron-free 9K medium and 10 mL inoculum. The medium was autoclaved at 120 °C for 15 min. The $FeSO_4$ part of the medium was sterilized separately through a 0.2 µm filter and was added as energy source to the leaching medium. Before inoculation, the leaching medium was initialized to pH 1.5 by 1 N H_2SO_4 for active cell growth. To avoid attrition of solids on bacterial cells, 1% (w/v) pulp density of chalcopyrite concentrates was chosen for this study [16]. All the bioleaching experiments were inoculated with actively maintaining culture of *L. ferriphilum* that was constantly renewed. To investigate the effect of Ag^+ on bioleaching, independent experimental runs were conducted with predetermined concentrations of Ag^+. Ag^+ concentrations in the flasks were 1.0, 2.0, 3.0, 4.0, and 5.0 mg/L (added in the form of AgCl). The individual flask was operated at a speed of 180 rpm in an incubator shaker at 313 K. An experiment without inoculum (control) was also maintained at the same experimental conditions with 0.2 g/L $HgCl_2$ as bacterial germicide in the medium (Mehta et al., 1999). Deionized water was added to the flasks to balance evaporation losses to the leaching solution. To ensure the reliability of the process, all the experiments were carried out in triplicates and the mean values of triplicates were considered as results. During the bioleaching process, pH of the leaching medium was assessed every day using a calibrated pH meter (Eutech Instruments, Singapore). At every 2 days interval, 5 mL leaching solution was periodically withdrawn and was centrifuged at 3,000 rpm for 20 min. The supernatant was filtered using Whatman filter paper. It was analyzed for leached Cu and Fe concentration using atomic absorption spectrometer (AA200 model, PerkinElmer, USA) after appropriate dilution whenever necessary. Bioleaching efficiency, denoted by η (%), was calculated using the mathematical expression, $\eta\ \% = [M_{soln} - M_0]/M_T \times 100$, where M_{soln} is the metal concentration in aqueous leach phase at time t during bioleaching, M_0 is the metal concentration at time $t = 0$, and M_T is the total metal concentration available to leach in the chalcopyrite concentrate.

Kinetic approaches on bioleaching studies. The bioleaching process can be considered as pseudo-first-order reaction. General mathematical equation for the rate of pseudo-first-order reaction is as follows [17]:

$$r_A = \frac{dC_A}{dt} = k_A(C_{A,0} - C_{A,t}) \quad \ldots(6)$$

where k_A is the rate constant of bioleaching. Integrating Eq. (6) between the respective limits of time ($t = 0$ d, $C_{A,t} = 0$ and $t = t$d, $C_{A,t} = C_{A,t}$), the resulting mathematical model is given as follows:

$$\ln \frac{C_{A,0}}{C_{A,0} - C_{A,t}} = k_A t \quad \ldots (7)$$

Equation (7) is a simple equation and is widely used to determine the value of k_A. $C_{A,0}$ and $C_{A,t}$ are the total metal concentration in the raw chalcopyrite ore and metal concentration in aqueous phase of leached solution at the particular time *t* during the bioleaching process. Using Eq. (7), a generalized chart ofln($C_{A,0}/[C_{A,0} - C_{A,t}]$)*vs* time on bioleaching data was prepared for predicting the reaction rate constant as the slope of the plot. The identification of rate-controlling step is of immense importance in the kinetic analysis of bioleaching reaction mechanism for further application and modeling of the process [18; 19]. Thus, the determination of rate-controlling step was carried out using SCM. According to SCM, either step, diffusion through the product layer or chemical reaction, may control the overall metal leaching rate [20].The mathematical models of motioned steps are given by Eqs. (8) and (9) [21]:

$$1 + 2(1 - X_A) - 3(1 - X_A)^{2/3} = K_{obs}t \quad \ldots (8)$$

$$1 - (1 - X_A)^{1/3} = k_{obs}t \quad \ldots (9)$$

Where X_A is the fraction of leached metal in the aqueous phase and k_{obs} an observed kinetic constant (time^{-1}) applicable to the respective model. On the basis of the regression analysis of best fit from the charts $1 + 2(1 - X_M) - 3(1 - X_M)^{2/3}$*vs* time and $1 - (1 - X_M)^{1/3}$ *vs* time, the rate-controlling step was identified.

RESULTS AND DISCUSSION

Effect of silver ion on pH during bioleaching. Figure 1 shows the results corresponding to representative curves of pH values during bioleaching at different silver ion concentrations. In the control experiment, there was no significant change in pH. A marginal decrease in pH from 1.5 to 1.39 was observed because of the chemical oxidation of mineral sulfides. In the bioleaching experiments, there was an initial increase in pH from 1.5 to 2.69, 2.75, 2.89, 2.98, and 2.92 in the experiments at 1, 2, 3, 4, and 5 mg/L Ag^+ concentration during the first 6 days as a result of Fe^{2+} oxidation, as stated in Eq. (1), and this was followed by a decrease in pH due to sulfuric acid production, as stated in Eq. (3). The significant reduction (1.45) in the pH value was observed in the experiments at 4 mg/L Ag^+ at the end of 30 days. The final pH values observed at 1, 2, 3, 4, and 5 mg/L Ag^+ were 1.62, 1.58, 1.56, 1.45, and 1.59, respectively. These observations support the results from bioleaching studies by Sand et al., 1995 and Mousavi et al., 2007.

Table 1 − Chemical constituents of chalcopyrite concentrate

Sl. no.	Constituents	Composition (wt.%)
1	Fe_2O_3	24.53
2	CuO	20.89
3	S	31.02
4	MgO	3.03
5	Al_2O_3	0.10
6	K_2O	0.07
7	Na_2O	0.02
8	P_2O_5	0.3
9	TiO_2	0.12
10	SiO_2	13.92
11	MnO	0.40
12	CaO	2.11
13	Loss of ignition	3.49

Effect of Ag^+ on Cu and Fe bioleaching and kinetics. Bioleaching efficiency of Cu and Fe from the chalcopyrite concentrate using *L. ferriphilum* at different Ag^+ concentrations as a function of time is shown in Fig. 2. In the control experiment, 5.73% Cu and 4.73% Fe were solubilized at the end of the 30th day.

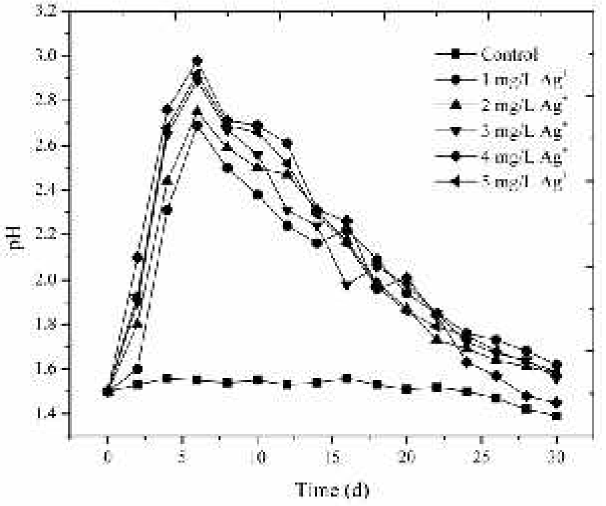

Figure 1 – Effect of silver ion concentration on pH during bioleaching

This was achieved because of solubilization through added sulfuric acid for initializing pH value to 1.5 in the medium. At the end of leaching period, 68.83, 77.24, 80.1, 87.73, and 84.45 % Cu and 58.83, 67.24, 70.91, 74.73, and 72.45 % Fe were leached while using 1, 2, 3, 4, and 5 mg/L Ag^+. It is evident that the leaching of copper increases with the increase in silver ion concentration from 1 to 4 mg/L. Further increase in silver ion concentration did not show higher efficiency of leaching. In contrast, reduced leaching efficiency was observed for the experiment with 5 mg/L Ag^+. It revealed that 4 mg/L is the optimum silver ion concentration for bioleaching of the type of chalcopyrite concentrates used to achieve the maximum leaching efficiency with *L. ferriphilum*. Bioleaching of Cu ranging from 80 to 90% has been reported, depending on the mineral type, experimental conditions, and the presence of microorganisms.

Table 2 − Linear equation of best fit to pseudo-first-order model for Cu and Fe bioleaching and respective regression coefficients

Silver ion (Ag^+) Composition (mg/L)	Cu		Fe	
	Linear equation	R^2	Linear equation	R^2
1	$y = 0.043x$	0.976	$y = 0.033x$	0.944
2	$y = 0.053x$	0.984	$y = 0.040x$	0.977
3	$y = 0.061x$	0.961	$y = 0.046x$	0.965
4	$y = 0.078x$	0.932	$y = 0.054x$	0.868
5	$y = 0.071x$	0.949	$y = 0.051x$	0.907

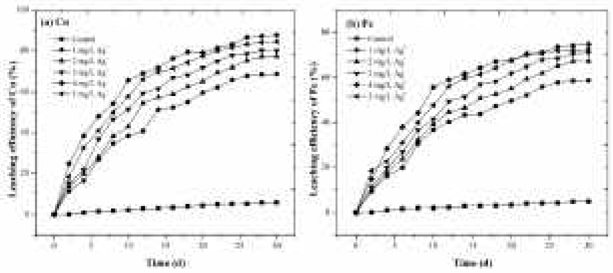

Figure 2 – Effect of silver ion concentration on (a) Cu and (b) Fe leaching

The rate of the reaction is defined as the speed of formation of desired product at the reaction. The rate of Cu and Fe leaching can be described in terms of rate constant. Figure 3 shows a fitting of experimental data to determine the values of rate constant for Cu and Fe leaching at different Ag^+ concentrations.

Table 3 Regression coefficient values of the graphical fit for different controlling step models

Ag^+ Concentration (mg/L)	Ash layer diffusion control model		Chemical reaction control model	
	Cu	Fe	Cu	Fe
1	0.965	0.979	0.949	0.912
2	0.971	0.972	0.953	0.950
3	0.983	0.979	0.907	0.928
4	0.982	0.967	0.825	0.788
5	0.983	0.966	0.872	0.849

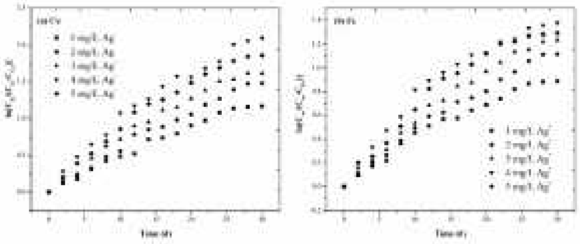

Figure 3 – Fitting of experimental data to pseudo-first order rate equation for leaching rate constant of (a) Cu and (b) Fe

Table 2 shows the equations of linear lines, which are fitted with respect to pseudo-first-order model and corresponding linear regression coefficients for leaching of Cu and Fe. From the table, it is clear that the pseudo-first-order rate kinetic equation is fitted well to the acquired experimental data and that the correlation coefficients of the graphical fitting were also found to be high.

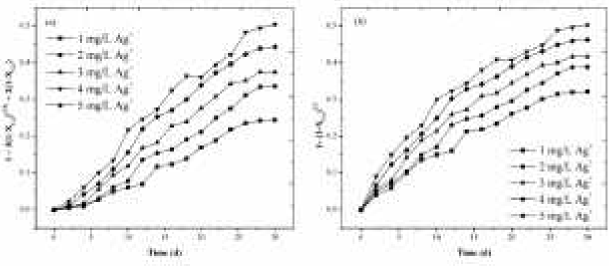

Figure 4 – Fitting of data to (a) ash layer diffusion control and (b) chemical reaction control model for Cu leaching

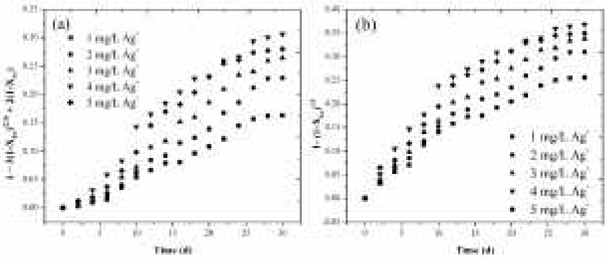

Figure 5 – Fitting of data to (a) ash layer diffusion control and (b) chemical reaction control model for Fe leaching

Because leaching is positively correlated with Ag^+ concentration, the value of rate constant also increases with increasing Ag^+ concentration until 4 mg/L. In the experiments using 1, 2, 3, and 4 mg/L Ag^+, the values of rate constant were found to be 0.043, 0.053, 0.061, and 0.078 d^{-1} and 0.033, 0.040, 0.046, and 0.054 d^{-1} for Cu and Fe bioleaching, respectively. However, the experiment at Ag^+ concentration beyond 4 mg/L (at 5 mg/L) showed a decline in rate constant value to 0.071 and 0.051 d^{-1}. It became evident that while using the optimum Ag^+ concentration of 4 mg/L, the metal leaching was enhanced and the rate of leaching reached to the maximum. The kinetic models of rate-controlling steps, product layer diffusion, and chemical reaction were examined using leaching data to explain the rate-determining mechanism with respect to SCM. The graphical applicability of the SCM is given in Figs. 4 and 5 for Cu and Fe leaching, respectively. Regression coefficient values from the graphical fit of previously mentioned controlling-step models are given in Table 3. From the regression analysis, it is clear that the observed bioleaching data fit better to the diffusion through product layer-controlled SCM.

CONCLUSION

The work was carried out to study the effect of Ag^+ concentration on bioleaching of Cu and Fe from chalcopyrite concentrate using the IOB. The IOB was successfully isolated from Chitradurga mine drainage and subjected to molecular characterization to confirm *L. ferriphilum.*

To study the effect of Ag^+ concentration on bioleaching of chalcopyrite using the isolate, experiments were carried out with different concentrations of Ag^+. The predetermined conditions were the following: initial media pH 1.5, shaking speed 180 r/min, pulp density 1% (w/v), particle size 200 µm, inoculum size 10 mL, and range of Ag^+ concentration in the medium 1−5 mg/L. Experiments were carried out in 250-mL Erlenmeyer flasks with 100 mL working volume. Experimental results showed that 68.83 and 58.83%, respectively, of Cu and Fe were leached with *L. ferriphilum* while using 1 mg/L Ag^+ and that significantly increased up to 87.7 and 74.73%, respectively, while using 4 mg/L Ag^+ at the end of 30 days. The results of kinetic studies indicated that the bioleaching rate increases with an increase in silver ion concentration till 4 mg/L Ag^+. The rate constant values were found to be 0.043, 0.053, 0.061, 0.078, and 0.071 d^{-1} for leaching of Cu, and 0.033, 0.040, 0.046, 0.054, and 0.051 d^{-1} for leaching of Fe at the experiments with Ag^+ concentrations 1, 2, 3, 4, and 5 mg/L. Using SCM, the leaching data were analyzed for determining rate-controlling step. It concluded the rate of metal leaching is controlled by the step of diffusion through the product layer. However, the isolate used was shown to be highly efficient to reach maximum Cu and Fe recoveries from the chalcopyrite mineral.

Acknowledgement

The authors are grateful to the management of JCT College of Engineering and Technology (Coimbatore, Tamil Nadu, India) for providing infrastructure facilities.

References

1. Harahsheh, M.A., Rutten, F., Briggs, D., and Kingman, S. 2006. Preferential oxidation of chalcopyrite surface facets characterized by ToF-SIMS, Applied Surface Science., 252: 7155-65.
2. Seidel, A., Zimmels, Y., and Armon, R. 2001. Mechanism of bioleaching of coal fly ash by Thiobacillus thiooxidants. *Chemical engineering journal*., 83: 123-130.
3. Yohana, R., Antonio, B., Maria, L.B., Felisa, G., and Jesus, A.M. 2003. Study of bacterial attachment during the bioleaching of pyrite, chalcopyrite and Sphalerite. *Geomicrobiology Journal*., 20: 131-141.

4. Rawlings, D.E., David, D., and Du plessis, C. 2003. Biomineralization of metal- containing ores and concentrates. TRENDS in Biotechnology., 21: 38-43.
5. Kalin, P., and Dimitre, K. 2010. Batch Kinetics of ferrous iron oxidation by Leptospirillum ferriphilum at moderate to high total iron concentration, Biochemical Engineering Journal., 50: 54-62.
6. Zeng, J., Geng, M., Liu, Y., Zhao, W., Xia, L., Liu, J., and Qiu, G. 2007. Expression, purification and molecular modelling of the Iro protein from Acidithiobacillus ferrooxidans Fe-1 [J], Protein Expression and Purification, 52:146−152.
7. Romano, P., Blazquez, M.L., Alguacil, F.J., Munoz, J.A., and Ballester, A. 2001. Comparative study on the selective chalcopyrite bioleaching of a molybdenite concentrate with mesophilic and thermophilic bacteria, FEMS Microbiology Letters, 196:71-75.
8. Giaveno, A., Lavalle, L., Chiacchiarini, P., and Donati, E. 2007. Bioleaching of zinc from low-grade complex sulfide ores in an airlift by isolated Leptospirillum ferrooxidans, Hydrometallurgy, 89:117-126.
9. Mousavi, S.M., Jafari, A., Yaghmaei, S., Vossoughi, M., and Roostaazad, R. 2006. Bioleaching of low-grade Sphalerite using a column reactor, Hydrometallurgy, 82:75-82.
10. Chen, S., Qin, W.Q., and Qiu, G. 2008. Effects of Cu^{2+} ions on bioleaching of marmatite Transactions of Nonferrous Metals Society of China, 18:1518-1522.
11. Deveci, H. 2004. Effect of particle size and shape of solids on the viability of acidophilic bacteria during mixing in stirred tank rectors, Hydrometallurgy, 71:385-396.
12. Bhargav, C.P., Devayani, R.T., and Shailesh, R.D. 2012. Development of Leptospirillum ferriphilum dominated consortium for ferric iron regeneration and metal bioleaching under extreme stresses, Bioresource Technology, 118:483-489.
13. Rui-yong, Z., Jin-lan, X., Peng, J., Qian, Z., Cheng-gui, Z., Nie, Z., and Qiu, G. 2010. A new strain Leptospirillum ferriphilum YTW315 for bioleaching of metal sulfides ores, Transactions of. Nonferrous Metals Society of China, 20:135-141.
14. Miller, J.D., and Portillo. 1981. Silver catalysis in ferric sulfate leaching of chalcopyrite, Developments in Mineral Processing, 2:851-901.
15. Aisien, F.A., Ebewele, R.O., and Hymore, F.K.2011. Mathematical model of sorption kinetics of crude by rubber particles from Scrap Tyres, *Leonardo Journal of Sciences,* 18:85-96.
16. Ping, Z., Xin, J.Z., Kun, F.l., Guang, Q., and Ming, Z. 2012. Kinetics of leaching refractory gold ores by ultrasonic- assisted electro- chlorination, *International journal of Minerals, Metallurgy and Materials,* 19:473-80.
17. Naderi, H., Abdollahy, M., Mostoufi, N., Koleini, M.J., Shojaosadati, S.A., and Manafi, Z. 2011. Kinetics of chemical leaching of chalcopyrite from low grade copper ore: behavior of different size fractions, *International journal of Minerals, Metallurgy and Materials*, 18:638-45.
18. Ndukwe, O.C.N., and Uchendu, C.E. 2012. Shrinking core model applied to the bioleaching of iron from silica sand, *International Journal of Academic Research,* 4:183-90.
19. Guohua, G.U., Lijun, S.U., Minglian, C., Xiaojun, S., and Hongbo, Z. 2010. Bio-leaching effects of Leptospirillum ferriphilum on the surface chemical properties of pyrite, Mining Science and Technology, 20:286-291.
20. Seung, J.Y., Ho- Sung, Y., Hee, D.J., Min, J.L., Se-II, L., Seung, T.H., and Hyung, S.P. 2007. Dissolution Kinetics of aluminium can in isopropyl alcohol for aluminium isopropoxide, Chemical Engineering Journal, 133:79-84.
21. Munoz, J.A., Dreisinger, D.B., Cooper, W.C., and Young, S.K. 2007. Silver- catalyzed bioleaching of low grade copper ores. Part II: Stirred tank tests, Hydrometallurgy, 88:19-34.

****