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Bioleaching of Arsenic from Realgar Using Leptosprillum ferriphilum: Effect of Ferrous Iron and Kinetics Aspects

Sundramurthy Venkatesa Prabhu[1*], Bashyam Sasikumar[2]
[1]Department of Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia, venkatchemdata@gmail.com
[2]Faculty of Mechanical and Production Engineering, Arba Minch University, Arba Minch, Ethiopia

ABSTRACT

The eco-friendly technique of metal leaching, called, bioleaching is gaining huge interest in bio-hydrometallurgical process. This method is a promising technology for extraction of several valuable metals. In this investigation, biological extraction of arsenic (As) from realgar was focused. The bacteria, *Leptospirillum ferriphilum* was used as microbial agent. The shaker flask experiments were done at 180 rpm, with 0.3% (w/v) pulp density. The temperature and initial pH were maintained at 313 K and 1.5, respectively. The concentration of Fe(II) was varied from 1−12 g/L. The influence of Fe(II) bioleaching of arsenic was studied. The experiment results explicated that arsenic bioleaching were greatly affected by Fe(II) concentration used in the media. After 30 days, it was observed that 75.42% of arsenic was extracted at maximum when using 6 g/L of Fe(II). Kinetics on the bioleaching data were observed to be the maximum rate constant value (1.4643 d^{-1}) was attained while using the optimum concentration of Fe(II). Shrinking core model (SCM) was used to identify the rate-limiting step. From the study, it was observed that the bioleaching rate of arsenic was controlled by diffusion through ash layer.

Key words: Arsenic, Bacteria, Fe(II), Kinetics, Leaching, *Leptospirillum ferriphilum*, Realgar, Rate controlling step.

1. INTRODUCTION

Arsenic (As) is one of the important minerals that found abundance at the earth's crust. It has been observed in more than two hundred different minerals. Most of the As quantity is exist in the form of sulfide ores such as arsenopyrite (FeAsS), orpiment (As_2S_3), and realgar (As_2S_2) [1]. As can cause poisoning for humans to death if As is swallowed beyond a certain limit since it is readily absorbed. It seriously affects instinctive system, nervous and heart. Exposure of As mainly via the air breathed in can pose to serious threat to induce cancers. However, As can be applied at wide application in various fields. Insects such as fungi and bacteria are eradicated by the As due to its heavy toxicity that led to its potential use as preservatives, in specific, for wood. It is also used for taxonomic sample preservation. As is known to be good n-type dopant in semiconductor devices. Gallium arsenide, the optoelectronic compound of As, is most extensive semiconductor used after doped silicon. As is extracted from the environment by different methods. The extracted As can be subjected in to further appropriate treatment and used for various commercial applications. Realgar is one of the chief sulfide minerals of As that contains major quantity of As. In mordent medical world, realgar is used for the treatment to relapse the chronic myelogenous leukemia and acute promyelocytic leukemia when the dosage of soluble As is in judicious quantity. It is also exploited for curing psoriasis, abdominal pains, and burns [2]. Hence, realgar can be widely used if the concentration of As is reduced to appropriate level [3].

Realgar is the most important source of As. Realgar is commonly subjected into chemical or biological extraction to leach the As. Chemical leaching is expensive and cause environmental pollution. Therefore, biological leaching (bioleaching) is gained huge interest as an alternative method against chemical method [4, 5]. Bioleaching principle is the metal extraction using pure or consortium of sulphur oxidizing and/or iron-oxidizing microbes [6, 7]. Bioleaching can be potential leaching method for As because its cost effectiveness and environmentally-friendly process [8]. In the bioleaching process, Fe(II) is oxidized to Fe(III) by iron-oxidizing bacteria (IOB). The biologically produced ferric iron is acted as a leaching agent of the sulfide minerals to solubilize the target metals. This mechanism is explicated in following equations for the bioleaching of realgar to extract As [9].

$$2Fe^{2+} + 4H^{+} + O_2 \xrightarrow{IOB} 4Fe^{3+} + 2H_2O \quad \ldots. (1)$$

$$As_2S_2 + 2Fe^{3+} \longrightarrow 2As^{3+} + 2\,S^{\circ} + 6Fe^{2+} \quad \ldots (2)$$

Acidithiobacillus and *Leptospirillum* are the extensively employed genus in bioleaching of different metals.

Acidithiobacillus ferrooxidans, *A. thiooxidans*, *A. caldus*, and *Leptospirillum ferrooxidans* are found to be most employed bacteria in bioleaching process. Among them, *Acidithiobacillus ferrooxidans and A. thiooxidans* are observed to be most successful microorganisms for the bioleaching process [10, 11]. *Leptospirillum ferriphilum* is the one of the potential microorganisms under the genus *Leptospirillum.* So far, a few works have been carried out using *Leptospirillum ferriphilum* [12] and researchers showing huge interest on *L. ferriphilum* as it can survive at lower pH and has higher redox potential. The principle objective of this study is to bioleach the As from realgar mineral using *L. ferriphilum*. The impact of energy source (ferrous iron) supplied to the media on the efficiency of As bioleaching was observed. Further, the leaching data were applied to determine the reaction kinetics to measure rate of bioleaching.

2. MATERIALS AND METHODS

2.1 Realgar mineral particles

A sample of realgar mineral was procured from the mine sector of Lingshot province (Zanskar, India). It was powered by lab jaw crusher followed by ball mill. The grounded ore mineral was sieved to isolate the particle size range from 100 to 1,200 µm using standard sieve sets. These selected particles were taken for bioleaching examinations. X-ray diffraction analysis was examined for mineralogical studies to confirm and determine the composition of realgar present in the sample. Quantitative analysis of XRD showed that the mineral sample composed with 12.30% of stishovite, 68.785% of realgar, 2.61% of litharge, 5.78% of tridymite, 3.748% of quartz, and 7.223% of cristobalite.

2.2 Chemical analysis

The realgar mineral sample was under taken to chemical analysis for determining the comprised constituents in the ore. The amount of silica, iron, titanium, and manganese present in the mineral was determined spectrophotometerically using the appropriate reagents, ammonium molybdate, 1,10-phenonthroline, hydrogen peroxide, and periodate, respectively. Amount of sulphur was estimated after precipitation as barium sulphate using standard procedure. Amount of calcium was determined by titration method involving EDTA. Flame photometer was used to analyze the amount of sodium and potassium present in the sample. Amount of As present in the realgar was determined by atomic absorption spectrometer.

2.3 Bacterial strain and media

The bacterial strain, *L. ferriphilum*, was used in this study. The strain was isolated from acid mine province at Copper mines province at Chitradurga, India. The bacteria was developed in 9K medium comprising 0.5 g/L of K_2HPO_4, 0.5 g/L of $MgSO_4.7H_2O$, 3.0 g/L of $(NH_4)_2SO_4$, 0.1 g/L of KCl, and 0.01 g/L of $Ca(NO_3)_2$. 44.2 g/L of $FeSO_4.7H_2O$ was added as the energy source. Bacterial enrichment was carried out in the initial pH value 1.5 at 313 K. Molecular characterization of the strain was found to be 99% identical to *L. ferriphilum.* The Nucleotide sequence of isolated *L. ferriphilum* was deposited in National Centre for Biotechnology Information (NCBI), Maryland, USA. The nucleotide submission was attained the accession number KF743135.

2.4 Bacterial strain and media

To enhance the bioleaching rate, isolated *L. ferriphilum* was adapted to realgar mineral. In order to adapt 15% (volume /volume) of developed culture was sequentially sub cultured in the 9K media with 1% (w/v) of powdered realgar. Suitable conditions such as temperature 313 K, initial pH 1.5, and rotation speed of 200 rpm were maintained for proper growth of the culture. Using H_2SO_4 (5N), the value of initial pH of the media was adjusted. The adapted *L. ferriphilum* was used as inoculum for further bioleaching process. Analytical grade chemicals and purified deionized-water were utilized for the bioleaching examinations.

2.5 Bioleaching experiments

Experiments for As bioleaching were undertaken in 250 mL conical flask. Each flask contained 9K medium (90 mL) and well-developed *L. ferriphilum* cells (10 mL) with initial pH value of 1.5. The bioleaching process was separated into different five parts. The concentration of Fe^{2+} was taken that ranging from 2 -10 g/L in each part. All the conical flasks were agitated at agitation speed 200 rpm with 0.3% (w/v) of realgar particles, and the incubation temperature was maintained at 313 K. In addition, a control study was also carried out under the same conditions without addition of bacteria. $HgCl_2$ (0.2 g/L) was added as bacterial germicide in the control experiment. Owing to compensate the evaporation loss and to maintain the working of 100 mL distilled water was added to required volume. Each experiment was conducted in triplicates for a time period of 30 days. The mean values of the triplicates were calculated and expressed as results.

2.6 Analytical methods

In the bioleaching process, change in media pH media and redox oxygen potential were determined at every day using standard pH meter and platinum electrode with a reference electrode of Ag/AgCl [13], respectively. During the process, 5 mL of the medium sample was collected every 2-day interval and centrifuged at 3500 rpm. From the centrifuged solution, the clear solution was separated by Whatman filter paper. This centrifuged clear solution was subjected to determine the leached-out As. Bio-extracted As concentration of in the aqueous solution was determined using atomic absorption spectrometer (PerkinElmer; AA200 model). The efficiency of arsenic extraction, E_{As} (%), was determined from

the expression $E_{As} = (N_s/N_T) \times 100$ %. Where, N_s is the concentration of As in the aqueous solution at time *t*. N_T refers the total available concentration of As in the realgar.

2.7 Kinetic approaches on realgar bioleaching

The mathematical equation for first-order reaction can be applied for the As bioleaching, the same is given in Eq. (1).

$$r_{As} = \frac{dC_{As}}{dt} = k_{As}(C_{As,0} - C_{As,t}) \quad \text{..... (1)}$$

where k_{As} refers the rate constant for As extraction. While integrating Eq. (1) with the respective limits of time (t = 0 d, $C_{As,t}$ = 0 and t = t d, $C_{As,t}$ = $C_{As,t}$), the outcome relationship between As concentration and time and was obtained [Eq. 2]:

$$\ln\left(\frac{C_{As,0}}{C_{As,0} - C_{As,t}}\right) = k_{As}t \quad \text{---------- (2)}$$

Eq. (2) is therefore applied for determining the value of k_{As}. $C_{As,0}$ and $C_{As,t}$ are the total concentration of As in the original realgar ore and concentration of As in leached clear solution at the time *t*. From the bioleaching data, using Eq. (2), a plot of $\ln(C_{As,0}/(C_{As,0} - C_{As,t}))$ *vs* time was created that provides the k_{As} value as slope. Using the kinetic analysis, the rate controlling step was determined through the shrinking core model (SCM) analysis. In SCM analysis, the expressions for rate control by ash layer diffusion and rate control by chemical reaction were developed and given by Eqs. (3) and (4), respectively:

$$1+2(1 - X_{As}) - 3(1 - X_{As})^{2/3} = K_{ob}t \text{ ---------- (3)}$$

$$1 - (1 - X_{As})^{1/3} = k_{ob}t \text{ ---------- (4)}$$

Where X_{As} refers the fraction of bio extracted As in the aqueous solution. k_{ob} is observed constant (time^{-1}) for the respective model. From the observed plots, $1+2(1–X_{As})–3(1–X_{As})^{2/3}$ *vs* time and $1–(1–X_{As})^{1/3}$ *vs* time, the rate-controlling step was determined based on the regression analysis.

3. RESULTS AND DISCUSSION

3.1 Chemical analysis of realgar

The chemical analysis of realgar showed that raw ore was composed with different constituents as follows (% in w/w): As, 69.2 %; Fe_2O_3, 1.12%; S, 19.1%; MgO, 8.85%; K_2O, 0.10%; Na_2O, 0.15%; TiO_2, 0.11%; SiO_2, 10.35%; and MnO, 0.41%. From the analysis, it has been interpreted that the As consisted at maximum quantity of the mineral.

3.2 pH and redox potential values during bioleaching

Figure 1 shows the value of pH observed in the leachate during biological leaching process. In the control experiment, there was a minimal decrease in pH (1.5-1.46) because the mineral sulphides were chemically oxidized. The pH value increased primarily from 1.51 to 2.52, 2.6, 2.76, 2.4, and 2.1 in the studies in which the ferrous concentration was varied from 2, 4, 6, 8, and 10 g/L, respectively. This pH increases mainly because of the consumption of acid by the realgar. After the second day, the gradual decrement in the medium pH was observed. This decrement in the pH was occurred because of the production of sulphuric acid in the medium by the oxidation of components present in the ore [14]. At the end of the experiment the pH was reduced to 1.52, 1.51, 1.55, 1.49, and 1.48 in the media containing 2, 4, 6, 8, and 10 g/L of Fe(II), respectively.

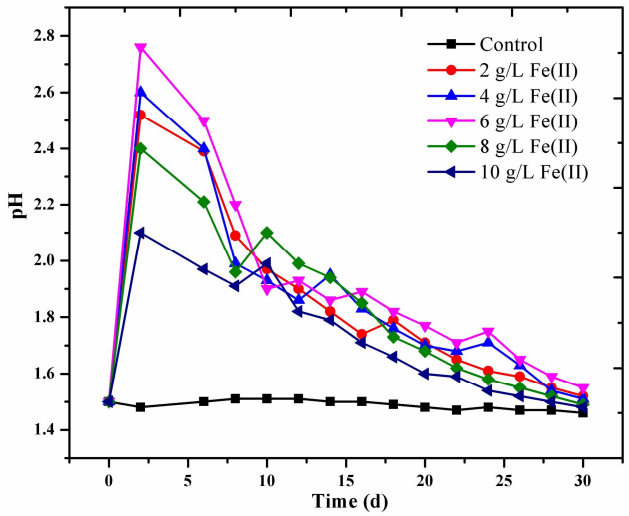

Figure 1: The variation of pH at different concentrations of Fe(II) during bioleaching

Figure 2 shows the observations of redox oxygen potential during the As bioleaching. The increment on redox oxygen potential values from 202mV to 656mV against Ag/AgCl was steady with the Fe(III)/Fe(II) ratio in the bacterial inoculated flasks whereas the ROP value remained constant (200mV) as against Ag/AgCl in the control experiment. The maximum ROP value of 656mV was observed in the flask containing 6 g/L of Fe(II) concentration. This peak for ROP value was found to be high because of the bio-oxidation of Fe(II) to Fe(III) and maintenance of dissolved state of Fe(III) was well supported at 6 g/L of Fe(II) [15].

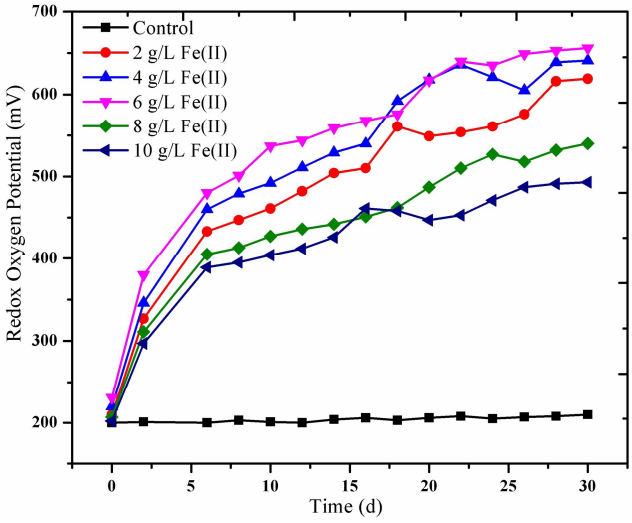

Figure 2: The variation of ROP at different concentrations of Fe(II) during bioleaching

3.3 Effect of ferrous concentration on As bioleaching and kinetics

As bioleaching by *L. ferriphilum* at various ferrous iron concentrations with respect to time is presented in Figure 3. During the experiments, at the control, As was leached about 5.4% after 30 days. However, bio-extraction of As were reached 68.39, 72.22, 75.52, 64.61 and 59.37% in the flasks containing 2, 4, 6, 8 and 10 g/L of Fe(II), respectively. It cleared that the extraction is much associated with concentration of Fe(II) [16]. The experiment results evident that the concentration of 6 g/L for Fe(II) could be an optimal energy source input for the 9K media to the bioleaching process using *L. ferriphilum* to attain the improved efficiency. The speed of bioleaching in term of leaching rate can be explained as rate constant (k_{As}). Figure 4 depicts a fitting of leaching data to observe the rate kinetic constant values for different Fe(II) concentrations [17]. The corresponding values of regression coefficient (R^2) for the data fitting is given in **Table 1**. Since the leaching is positively associated with Fe(II) concentration, the rate constant value also increases with increase in the Fe(II) concentration until 6g/L. In the experiments with 2, 4, 6, 8, and 10 g/L of Fe(II), the values of rate constant were found to be 1.1144, 1.2801, 1.3633 d, 1.0026, 0.9130 d^{-1}, respectively. It is clear that, with the optimum Fe(II) concentration of 6 g/L, As leaching was enhanced and reaches maximum rate. The empirical models of ALDC and CRC were examined to determine the rate-controlling mechanism based on SCM [18]. The graphical fittings of SCM on experimental data was given in Figures 5a and 5b. From the regression values, it clears that the leaching data fit much better to the ash layer diffusion model.

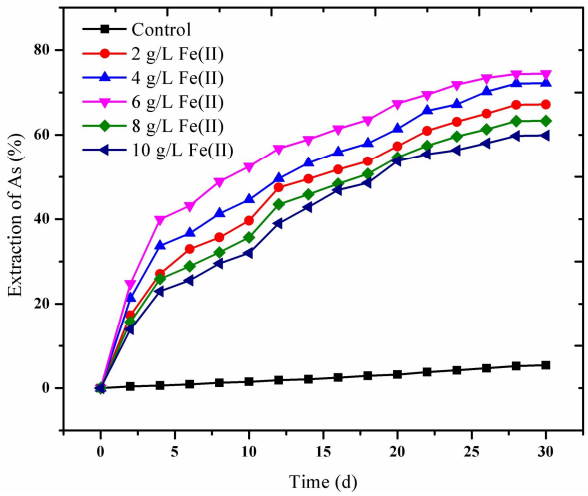

Figure 3: Bioleaching efficiency of As with different concentrations of Fe(II)

Table 1: Regression Coefficient value of SCM Models

Concentration (g/L) of Fe(II)	R^2 value	
	Diffusion by Ash layer Control	Controlled by Chemical Reaction
2	0.9914	0.8627
4	0.9929	0.8359
6	0.983	0.6958
8	0.9878	0.877
10	0.9784	0.8993

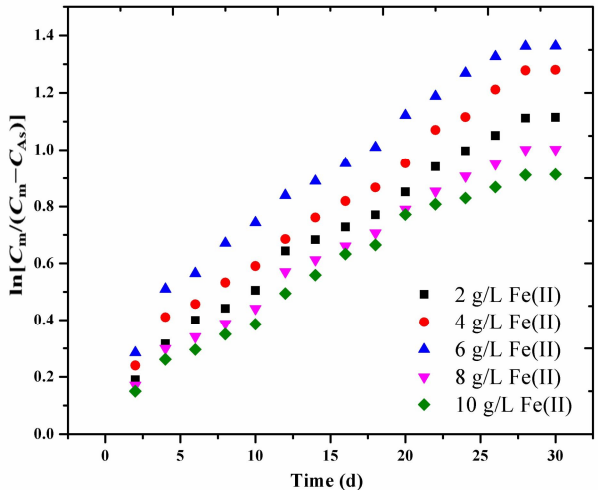

Figure 4: Graphical fitting for value of rate constant at various Fe(II) concentrations.

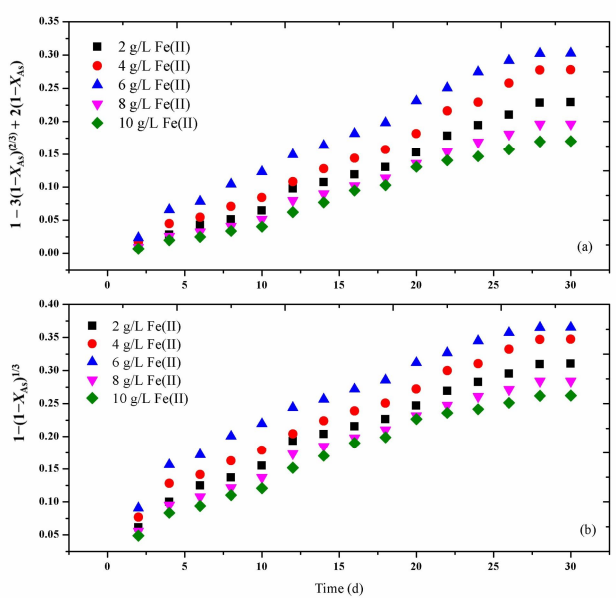

Figure 5: Plot of extraction data to (a) model for ash layer and (b) model for chemical reaction control

3. CONCLUSIONS

A study on the effect of energy source on bioleaching of As from realgar ore using *L. ferriphilum* was carried out. The experimental results showed that the bioleaching process is positively correlated to the concentration of ferrous sulphate used as energy source in the media. Minimum bioleaching efficiency of 67.19% was achieved when the experiment was conducted in the conditions such as initial pH 1.5, agitation speed 200 rpm, temperature 298 K and concentration of Fe(II)

1 g/L. Bioleaching efficiency was observed to be significantly increasing when Fe(II) concentration was increased. Maximum bioleaching efficiency 74.42% was observed when 6 g/L of Fe(II) was used for the experiment at the end of 30 days. The maximum rate constant value was found to be 1.3633 d^{-1} in the flask containing 6 g/L of Fe(II). From the SCM analysis, it was evident that the bioleaching rate is controlled by ash layer diffusion.

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REFERENCES

1. S. Venkatesa Prabhu, R. Baskar, T.A Amare, A.K Gizachew, and G. Amsalu, **Effect of FE(II) Concentration on Bioleaching of Zinc from Sphalerite using Leptospirillum Ferriphilum: Kinetic Aspects**. International Journal of Engineering and Advanced Technology. Vol. 9, no.1, pp.1492 – 1498, October 2019.
2. A. H. Kaksonen, C. Morris, S. Rea, J. Li, K. M. Usher, R. MvDonald, F. Hilario, T. Hosken, M. Jackson, C. du Plessis A, **Biohydrometallurgical iron oxidation and precipitation: PartII-Jarosite precipitates characterisation and acid recovery by conversion to hematite**. Hydrometallurgy, Vol. 147, no.8, pp. 264-272, August 2014.
3. S. Venkatesa Prabhu, R. Baskar, R. Natesan, R. Kavitha, **Bioleaching of Zn from sphalerite using Leptospirillum ferriphilum isolate: effect of temperature and kinetic aspects**. Applied Biological Chemistry, Vol.63, no.44, August 2020.
4. E. Darezereshki, M. Schaffie, M. Lotfalian, S. A. Seiedbaghery, M. Ranjbar, **Use of mesophilic and thermophilic bacteria for the improvement of copper extraction from a low-grade ore**. International Journal of Minerals, Metallurgy and Materials, Vol. 18, no. 2, pp.138-142, April 2011.
5. C. Santhosh, K. H. Kishore, G. P. Lakshmi, G. Kushwanth, P. R. K. Dharma Teja, R. S. E. Ravindran, S. V. Cheerala, M. R. Kumar, **Detection of Heavy Metal Ions using Star-Shaped Microfluidic Channel**. International Journal of Emerging Trends in Engineering Research, Vol. 7, no.12, pp. 768-771, December 2019.
6. S. R. Dave, K. H. Gupta, and D. R. Tipre, **Characterization of arsenic resistant and arsenopyrite oxidizing Acidithiobacillus ferrooxidans from Hutti gold leachate and effluents**. Bioresource Technology, Vol. 99, no. 3, pp.7514-7520, March 2008.
7. L. Falco, C. Pogliani, G. Curutchet, E. Donati, **A comparison of bioleaching of covellite using pure cultures of Acidithiobacillus ferrooxidans or a mixed culture of Leptospirillum ferrooxidans and Acidithiobacillus thiooxidans**. Hydrometallurgy, Vol. 71, no.2, pp. 31-36, October 2003.
8. S. Venkatesa Prabhu, R. Baskar, R. Gurusamy, T. A. Amare, **Bioleaching of Zinc and Iron from Sphalerite Using Leptospirillum Ferriphilum: Kinetic Aspects**. International Journal of Scientific & Technology Research, Vol. 8, pp. 1293- 1300, no. 11, November 2019.
9. A. Giaveno, L. Lavelle, P. Chiacchiarini, E. Donati, **Bioleaching of Zinc from low-grade complex sulphide ores in an airlift by isolated Leptospirillum ferrooxidans**. Hydrometallurgy, Vol. 89, pp. 117-126 March 2007.
10. N. V. S. Pavan Kumar, J. K. R. Sastry, K. S. Rao, **Mining Negative Frequent regular Itemsets from Data Streams.** International Journal of Emerging Trends in Engineering Research, Vol. 7, no.8, pp. 85-98, December 2019.
11. H. Cheng and Y. Hu, **Bioleaching of anilite using pure and mixed culture of Acidithiobacillus ferrooxidans and Acidithiobacillus caldus**. Mineral Engineering, Vol. 20, pp.1187-1190, October 2007.
12. G. H. Jeffery, J. Bassett, J. Mendham, R. C. Denney, **Vogel's textbook of quantitative chemical analysis**, Fifth edition, Longman Group UK Limited, London, pp.845-881, 1989.
13. J. Zhang, X. Zhang, Y. Ni, X. Yang, H. Li, **Bioleaching of Arsenic from medicinal realgar by pure and mixed cultures**. Process Biochemistry, Vol. 42, pp.1265-1271, September 2007.
14. D. P. Lu, Q. Wang, **Current Study of APL treatment in China**. Int J Hematol, Vol. 76, pp.316-325, January 2002.
15. S. M. Mousavi, A. Jafri, S. Yaghmaei, M. Vossoughi, R. Roostaazad, **Bioleaching of low-grade Salpharite using a column reactor**. Hydrometallurgy, Vol. 82, pp.75-82, July 2006.
16. P. Guo, G. Zhang, J. Cao, Y. Li, Z. Fang, C. Yang, **Catalytic effect of Ag^+ and Cu^+ on leaching realgar (As_2S_2)**. Hydrometallurgy, Vol. 106, pp. 99-103. February 2011.
17. P. Zhu, Z. Xin-jiong, L. Kun-fang, Q. Guang-ren, M. Zhou, **Kinetics of leaching refractory gold ores by ultrasonic-assisted electro-chlorination**. International Journal of Minerals, Metallurgy and Materials, Vol. 19, pp.473-481, June 2012.
18. A. Rubio, F.J. Garcia Frutos, **Bioleaching capacity of an extremely thermophilic culture for chalcopyritic materials**. Mineral Engineering, Vol. 15, pp.689-694, September 2002.