



Kinetics of Iron Bioleaching using Isolated *Leptospirillum Ferriphilum*: Effect of Temperature

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Abstract: This study was designed to investigate the effect of temperature on iron bioleaching kinetics using *Leptospirillum ferriphilum*. The bacteria were isolated and subjected to molecular characterization technique for confirming *L. ferriphilum*. Using the isolate, bioleaching data were collected in the temperature range of 298–318 K at an initial pH of 1.5 and 5% pulp density with an average particle size being 300 μm. The results of experiments concluded that leaching efficiency increases with temperature and maximum of 93.85% were observed after 20 days at 313 K. The bioleaching kinetics indicated that the maximum rate (rate constant: 0.1452 d⁻¹) was found in the experiment conducted at the optimum temperature, and the rate-controlling step was “diffusion through ash layer.” The activation energy was calculated to be 37.59 kJ/mol. From the thermodynamic study of the bioleaching system, ΔH° and ΔS° were found to be 0.7399 × 10⁻³ and 28.512 J/mol, respectively.

Keywords: activation energy, bioleaching, *Leptospirillum ferriphilum*, rate kinetics, shrinking core model, thermodynamics.

I. INTRODUCTION

Bacterial leaching (bioleaching) process for extracting metals from their sulfide ores is a promising technology as an alternative to the traditional pyro-metallurgical process because it reduces toxic emissions to the atmosphere, is simple, is applicable to low-grade ores, and is a low cost [1], [2]. In bioleaching, mineral-decomposing microorganisms are used to convert insoluble metal sulfides to soluble metal sulfates that can be readily recovered from the solution [3]–[5]. In the mineral-decomposing process, a Gram-negative consortium is considered as the most important bacteria. The important microorganisms are iron-oxidizing *Leptospirillum ferrooxidans* and *L. ferriphilum*, sulfur-oxidizing *Acidithiobacillus thiooxidans* and *A. caldus*, and iron- and sulfur-oxidizing *A. ferrooxidans* [6].

Revised Manuscript Received on October 30, 2019.

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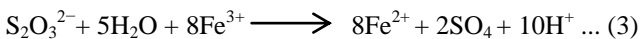
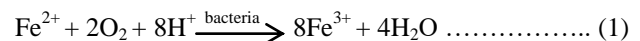
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However, *L. ferriphilum* dominates in bioleaching process because it's potential to meet the requirements for oxidizing iron even at pH below 1, its higher redox potential of the medium, its higher cultivation temperature (moderately thermophiles), and its higher affinity toward sulfide minerals [7].

L. ferriphilum follows an iron-based mechanism, a type of indirect bioleaching. The bioleaching mechanism of pyrite can be explained by stoichiometry Eqs. (1)–(3) [2], [8]. The application of *L. ferriphilum*, which uses Fe²⁺ as its energy source, in the pyrite bioleaching ultimately results in sulfuric acid [Eqs. (2) and (3)]. Sulfuric acid maintains a low pH range favorable to bacterial growth.



One of the important problems of the bioleaching process applied to sulfide ores is the low rate of leaching, which consumes high residence time and hinders success [9]. For this reason, more attention has been paid to enhance the rate of bioleaching. However, temperature considerably affects bioleaching, and many reports have suggested the possibility of using a higher temperature to improve metal leaching rates [10], [11]. Thus, the development of a process with respect to temperature, based on rate kinetics and thermodynamic studies, is important in bioleaching.

The purpose of this study was to investigate the effect of temperature in the conditions favoring to increase the bioleaching rate using *L. ferriphilum*, with respect to iron leaching from pyrite phase of sphalerite ore. The pseudo-first-order kinetic model and shrinking core model (SCM) were applied to assess rate constant and rate-controlling step, respectively. The activation energy and thermodynamic parameters such as a change in Gibbs free energy (ΔG°), change in enthalpy (ΔH°), and change in entropy (ΔS°) were also calculated using Arrhenius equation and Van't-Hoff equation.

II. EXPERIMENTAL DETAILS

A. Mineral concentrate

The mineral concentrate was obtained from the Dariba mine sector in Rajasthan, India. The sample was grounded by ball-mill, and classification of various particle size fractions was carried out using ASTM sieves.



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From the grounded mineral of particles, an average particle size of 300 μm was selected for bioleaching experiments. To identify the semi-mineralogical composition of the raw mineral sample, X-ray diffraction analysis was carried out.

It showed that the mineral composition in the concentrate consisted of 1.80% pyrite (FeS_2), 13.02% sphalerite (ZnS), 0.44% galena (PbS) 82.26% quartz (SiO_2), 0.58% lime (CaO), and 0.94% dolomite [$\text{CaMg}(\text{CO}_3)_2$]. The mineral distribution was determined using microphotographic analysis. The image of the mineral microphotograph is given in Fig. 1, which shows that the pyrite mineral is interlocked with various minerals such as sphalerite and gangue. The chemical analysis of sample showed the following composition (wt%): Fe_2O_3 , 6.12%; ZnO , 40.36%; S , 11.41%; MgO , 8.85%; Al_2O_3 , 6.12%; K_2O , 0.10%; Na_2O , 0.02%; P_2O_5 , 0.24%; TiO_2 , 0.21%; SiO_2 , 16.92%; MnO , 0.41%; CaO , 5.6%; and loss of incineration, 3.67%.

B. Bacterial culture, gene sequencing, and phylogenetic analysis

The iron-oxidizing bacterial culture was isolated from the mine drainage samples collected from Chitradurga pyrite mine province (Ingaldhal, Karnataka, India). The culture was grown by a multiple-transfer technique using DSMZ-leptospirillum medium (Medium 882), which is specific for leptospirillum cultures. The medium had the following chemical composition per liter: 20 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 27 mg KH_2PO_4 , 132 mg $(\text{NH}_4)_2\text{SO}_4$, 147 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 53 mg $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and trace elements of 0.062 mg $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01 mg Na_2MoO_4 , 0.064 mg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.068 mg ZnCl_2 , 0.031 mg H_3BO_3 , and 0.67 mg $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$. The initial pH of the media was adjusted to 1.5 using 1 N H_2SO_4 . The DNA was extracted in accordance with the manufacturer's instructions on the DNA extraction kit (Roche PCR Diagnostic Kits, USA) from the developed liquid culture. The 16S rRNA genes of the isolate were amplified by polymerase chain reaction using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGCTACCTTGTTACGACTT-3'). The PCR amplification was carried out according to the technique described by Moshirabadi *et.al* [12]. From a public database (www.ncbi.nlm.nih.gov/), the 16S rRNA sequences of the related reference organisms were downloaded and aligned with the sequence from isolate using software Clustal X, version 1.80. This alignment was used to develop a distance matrix that showed that nucleotide sequences had a restriction pattern 99% identical to that of *L. ferriphilum* NR028818. Phylogenetic relationships are described in Fig. 2. Nucleotide sequences of *L. ferriphilum* isolate were submitted to GenBank (National Center for Biotechnology Information, Bethesda, MD, USA) and accession number KF743135 was obtained.

C. Inoculum preparation and bioleaching experiments

For improved leaching performance, *L. ferriphilum* isolate was acclimatized with mineral concentrate. For acclimatization, 10% (v/v) culture was sequentially subcultured at every two weeks interval using the medium containing basal salts, as given in DSMZ Medium 882 supplemented with 1% (w/v) mineral concentrates and 7 g/L Fe^{2+} (added as ferrous sulfate). The resulting well-grown acclimatized culture after the fourth subculture was used as inoculum for bioleaching experiments. Bioleaching experiments were carried out in a 250 mL flask containing 90

mL sterilized iron-free medium (Medium 882; DSMZ). Thereafter, 10 mL culture was inoculated and 7 g/L Fe^{2+} ions were added as an energy source. The medium, without FeSO_4 , was autoclaved at 393 K for 15 min. The ferrous part of the medium was separately sterilized through a 0.2 μm filter and was added aseptically to the leaching medium. The bioleaching conditions maintained in the experiments were the following: shaking speed 200 rpm, initial pH 1.5, mineral pulp density 5% (w/v), and temperature range 298–318 K. A controlled experiment without the inoculum was also performed at the same experimental conditions with 0.2 g/L HgCl_2 as bacterial germicide in the medium. To ensure the reliability, the experiments were carried out in triplicates and mean values of triplicates were considered.

D. Analytical methods

During the experiments, pH and redox potential of leaching medium were measured daily using a calibrated pH meter (Eutech Instruments, Singapore) and a platinum electrode against an Ag/AgCl reference electrode, respectively. The level of solubilized iron in the aqueous phase was analyzed using an atomic absorption spectrometer (AA200 model; PerkinElmer) every 2 days. The fresh iron-free nutrient solution of the medium was added to compensate the media loss due to sample collection. Bioleaching of iron was calculated using the following expression:

$$\text{Iron bioleaching (\%)} = \frac{(\text{Solubilized iron at time } t) - (\text{Solubilized iron at time } t = 0)}{\text{Total available iron in raw material}} \times 100$$

E. Kinetic procedure

A general mathematical model for the bioleaching based on pseudo-first-order reaction can be used as follows [13]:

$$r = \frac{dC}{dt} = k(C_0 - C_t) \dots (4)$$

where k is the rate constant of bioleaching, and C_0 and C_t are the iron concentration in the raw concentrate and solubilized iron concentration in the aqueous phase of the medium at time t during bioleaching. Integrating Eq. (4) between the respective limits of time ($t = 0$ d, $C_t = 0$, and $t = t$ d, $C_t = C_t$), the resulting mathematical model is given as follows:

$$\ln\left(\frac{C_0}{C_0 - C_t}\right) = kt \dots (5)$$

Eq. (5) is a linear equation and is therefore extensively used for evaluating the value of k . Using bioleaching data, a plot $\ln(C_0/(C_0 - C_t))$ vs time was prepared for predicting the value of k as the slope of the plot. The activation energy, the minimum amount of energy to bring about the reaction, was determined using Arrhenius equation, which is given as follows [14]:

$$\ln k = \ln A - \frac{E}{R}\left(\frac{1}{T}\right) \dots (6)$$

where E is the activation energy (cal/mol), A is the frequency factor, k is the rate constant of bioleaching, R is the gas constant (J/mol K), and T is the absolute temperature (K). Following Eq. (6), an Arrhenius plot [$\ln k$ vs $(1/T)$] was prepared to determine the activation energy from the slope. The identification of rate-controlling step is of immense importance in the kinetic analysis for designing and understanding of the process [14]. To identify the rate-controlling step, SCM was applied.

According to the SCM, either the step “diffusion through the ash layer” or “chemical reaction” may control the rate of bioleaching [15]. The mathematical models of the earlier mentioned steps are given in Eqs. (7) and (8) [14]:

$$1 + 2(1 - X) - 3(1 - X)^{2/3} = k_0 t \dots (7)$$

$$1 - (1 - X)^{1/3} = k_0 t \dots (8)$$

where X is the fraction of leached iron in the aqueous phase and k_0 is an observed kinetic constant (time^{-1}) applicable to the respective model. Using leaching data, the $1 + 2(1 - X) - 3(1 - X)^{2/3}$ vs time, and $1 - (1 - X)^{1/3}$ vs time graphs were prepared. From these graphs, based on regression analysis of best fit, the rate-controlling step was determined. The ΔG° was determined using the correlation, $\Delta G^\circ = -RT \ln K_c$, where R is the gas constant (8.314 J/mol/K), T the absolute temperature (K), and K_c the equilibrium constant [16]. K_c was calculated as the ratio of leached iron concentration in the aqueous solution to iron concentration in leach residue after attaining the saturation leaching. ΔG° can be correlated with thermodynamic parameters, ΔS° , and ΔH by the Van't-Hoff equation as given below [17].

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \dots (9)$$

Replacing ΔG° by $-RT \ln K_c$ in Eq. 9 and arranging, the following equation can be obtained:

$$\frac{\Delta S}{R} - \frac{\Delta H}{RT} = \ln K_c \dots (10)$$

Using the relationship in Eq.10, ΔH° and ΔS° were determined from the slope and intercept of the linear plot ($\ln K_c$) vs $(1/T)$.

III. RESULT AND DISCUSSION

A. Effect of temperature on pH and redox potential during bioleaching

Fig. 3(a) shows the results corresponding to representative curves of pH values during bioleaching at different temperatures. In the control experiments, without inoculum, a marginal decrease in pH (from 1.51 to 1.41) was observed because of the chemical oxidation of mineral sulfides. During bioleaching, the pH values increased initially from 1.5 to 2.5, 2.6, 2.8, 3.0, and 2.7 in the experiments carried out at 298, 303, 308, 313, and 318 K, respectively, during the first 3 days. This increase in the pH was due to acid consumption by proton attack of sulfide minerals and consumption of H^+ ions during bacterial oxidation of Fe^{2+} ion to Fe^{3+} ion, as mentioned in Eq. 1. A similar increase in the pH during the first few days was observed by Soleimani *et al.*[18]. After the third day, the pH value of the medium began to decrease due to the production of sulfuric acid by oxidation of pyretic phase.

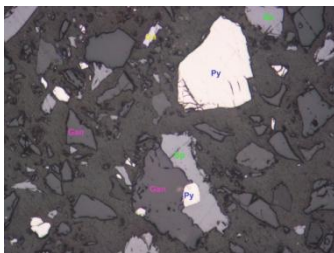


Fig.1. Microphotograph of raw concentrate depicting the mineral distribution
(Py - pyrite, Sp-sphalerite, Gan - gangue)

Though the reduction in the pH value was observed at different temperatures in the experiments, a significant reduction (1.61) was observed at 313 K at the end of 20 days. It was observed that the final pH values at 298, 303, 308, and 318 K were 1.7, 1.64, 1.64, and 1.8, respectively. The redox potential of the leaching medium is one of the key parameters in the bioleaching system because it depends on the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio [19]. It can be correlated with Fe^{3+} concentration as $\text{ORP} = 0.771 + 0.059 \log [\text{Fe}^{3+}/\text{Fe}^{2+}]$. Changes in redox potential (ORP) during bioleaching at experiments with different temperatures are shown in Fig. 3(b). In the inoculated experiments, there is an increase in redox potential from 240 to 591, 252 to 610, 242 to 615, 244 to 642, and 242 to 624 mV at 298, 303, 308, 313, and 318 K, respectively. This indicates the continuous maintenance of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ cycle by *L. ferriphilum* through its unique metabolism. High values of ORP were recorded during 10–20 days’ time due to increased bacterial activity. In the sterile control, the redox potential remained very low and constant at about 255 mV.

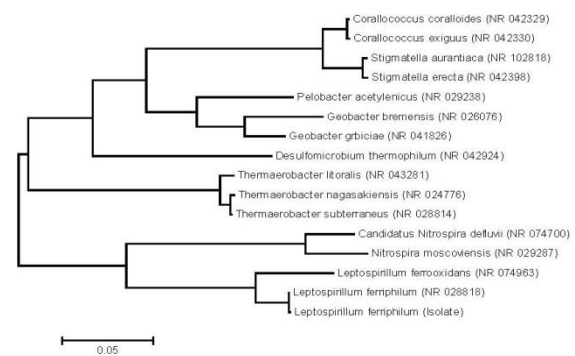


Fig.2. Schematic representation of phylogenetic affiliations of isolate and related reference organisms based on 16S rRNA sequences

B. Effect of temperature on iron bioleaching

Bioleaching efficiency of iron from the pyrite by *L. ferriphilum* at different temperatures as a function of time is shown in Fig. 4. In a control experiment at room temperature (303 K), 9.9% iron bioleaching efficiency was observed at the end of the 20th day. This was accrued by dissolution through added sulfuric acid for initializing the initial pH value to 1.5. Maximum iron leaching efficiencies at 298 K, 303 K, 308 K, 313 K, and 318 K were found to be 71.43%, 80.27%, 90.37%, 93.85%, and 86.31%, respectively. From the experimental runs, it is clear that bioleaching is strongly influenced by temperature. The results showed the increasing trend of bioleaching efficiency with a rise in temperature from 298 to 313 K. In contrast, reduced efficiency of bioleaching occurred with a further increase in temperature to 318 K. It is apparent that 313 K is the optimal temperature for bioleaching system using *L. ferriphilum* to achieve the maximum leaching efficiency. It is well documented that the leaching performance of *L. ferriphilum* is the highest at 313 K [20]. Iron bioleaching ranging from 90% to 100% has been reported elsewhere, depending on the mineral type, experimental conditions, and presence of microorganisms [21].

C. Bioleaching kinetics

The rate of bioleaching can be ascertained in terms of rate constant (k).

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Fig. 5 shows the fitting of experimental data to determine the values of rate constant at different temperatures and the corresponding regression coefficient (R^2). In the experiments at 298 K, 303 K, 308 K, and 313 K, the values of rate constants were found to be 0.0723 d^{-1} , 0.095 d^{-1} , 0.1298 d^{-1} and 0.1452 d^{-1} , respectively. However, the experiment at a temperature beyond 313 K (at 318 K) showed a decline in the rate of constant value to 0.1138 d^{-1} .

While using *L. ferriphilum* at an optimal temperature of 313 K, the leaching efficiency was found to be enhanced and reached to maximum rate due to its sensitiveness with system temperature. From the Arrhenius plot (Fig. 6), the activation energy for bioleaching was calculated to be 37.59 kJ/mol . The kinetic models of “ash layer diffusion control” and “chemical reaction control” were examined to clarify the rate-controlling mechanism of bioleaching with respect to SCM. The plots constructed using “ash layer diffusion control model” and “chemical reaction control model” with bioleaching data are shown in Figs. 7(a) and 7(b). The linear regression analysis for best fit among the plots clearly showed that the observed bioleaching data fit better to the model of “ash layer diffusion control”. During the bio-oxidation of Fe^{2+} , the precipitation of Fe^{3+} iron as jarosite occurred on the mineral surface, which can be seen in the SEM image of leach residue (Fig. 8). This layer, which acts as a diffusion barrier to pyrite oxidation, likely regulates the bioleaching rate. The change in free energy of bioleaching at temperatures 298 K, 303 K, 308 K, 313 K, and 318 K was found to be -2283.90 , -3560.30 J/mol , -5733.17 J/mol , -7091.99 J/mol , and -4965.15 J/mol , respectively. The negative values of ΔG° indicate the spontaneity of the bioleaching process. The values of ΔH° and ΔS° were calculated to be $0.7399 \times 10^{-3} \text{ J/mol}$ and 28.512 J/mol , respectively from the linear plot ($\ln K_c$) vs ($1/T$) (Fig. 9). The positive value of ΔH° suggests the endothermic nature of the bioleaching process. This is supported by the observed increase in the bioleaching efficiency with rising in temperature. The positive value ΔS° indicates an increase in randomness for iron ions at the mineral/solution interface during their leaching.

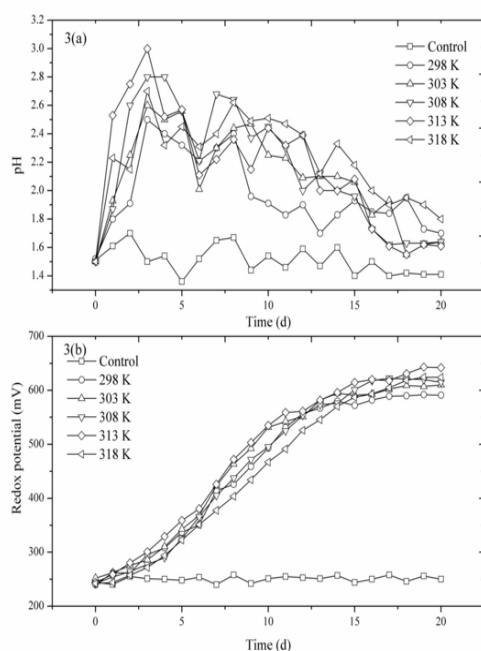


Fig.3. Variation in (3a) pH and (3b) redox potential during bioleaching at different temperatures

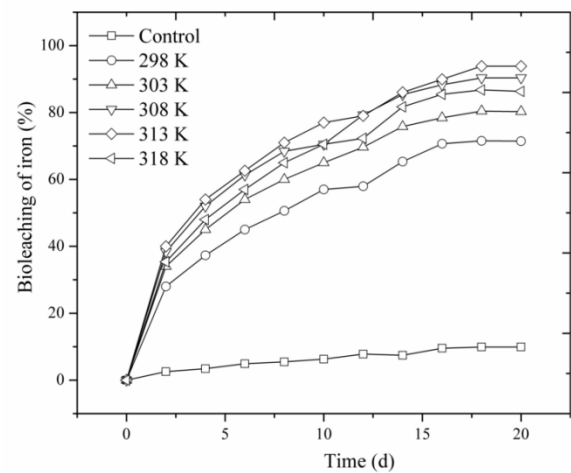


Fig.4. Bioleaching efficiency of iron by *L. ferriphilum* at different temperatures

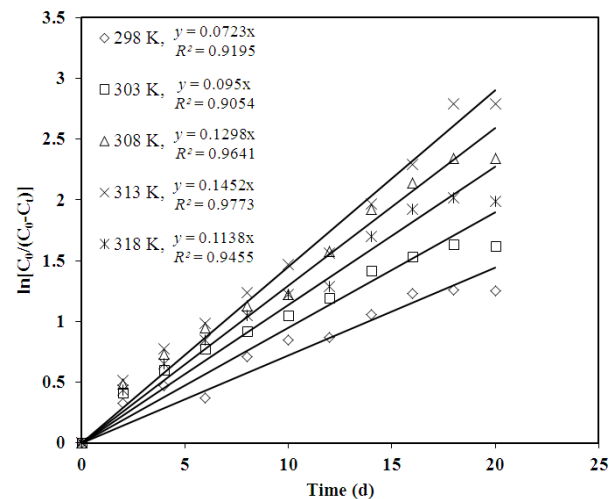


Fig.5. First order rate kinetic plot for iron bioleaching

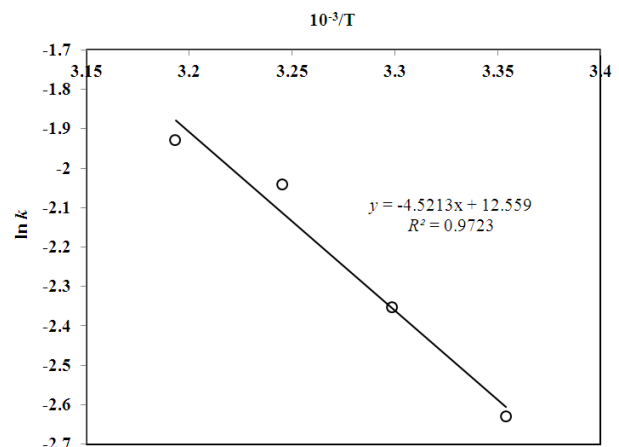


Fig.6. Arrhenius plot for determination of activation energy

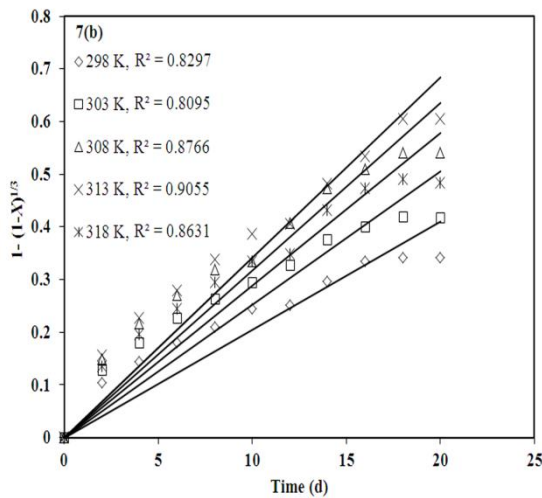
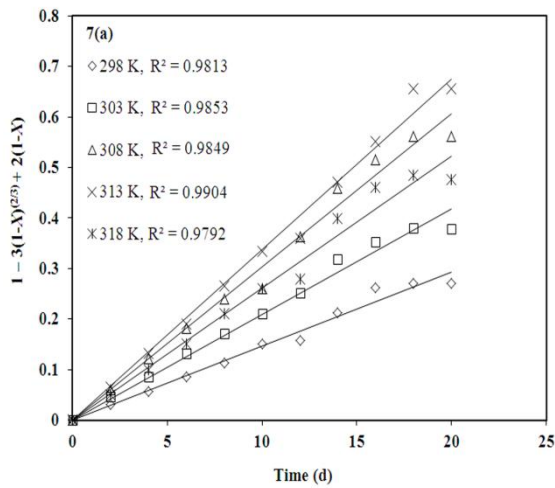


Fig.7. Fitting of iron bioleaching data to (7a) ash layer diffusion control and (7b) chemical reaction control model

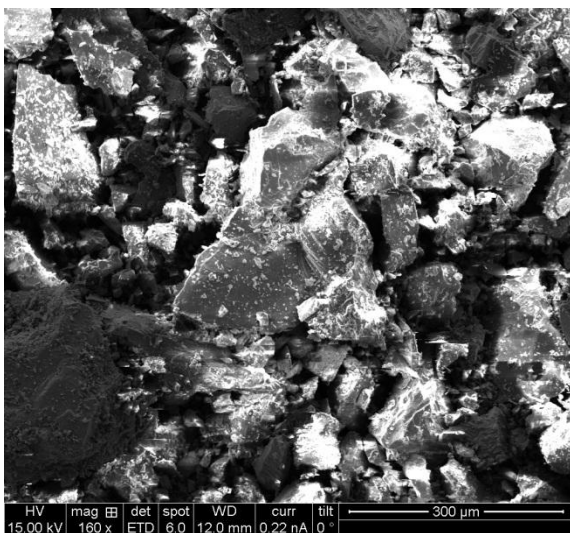


Fig.8. SEM image of leach residue

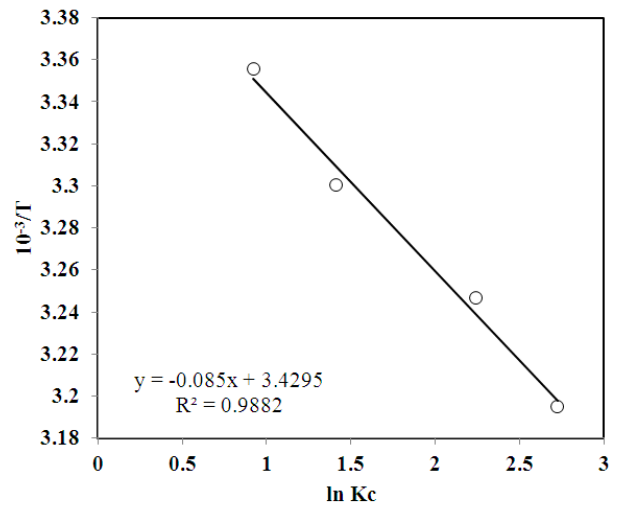


Fig.9. Linear plot based on Van't-Hoff equation

IV. CONCLUSION

The effect of temperature on bioleaching of iron from pyrite using the isolate *L. ferriphilum* was investigated. Using predefined conditions of DSMZ Medium 882, initial media pH 1.5, shaking speed 200 rpm, pulp density 5% at temperature 298 K, bioleaching efficiency of 71.43% iron was achieved with *L. ferriphilum*, which significantly increased up to about 93.85% at 313 K at the end of 20 days. The kinetic studies indicated that the rate of bioleaching increases with an increase in temperature till the optimum temperature of 313 K. The rate constant value was found to be maximum (0.1452 d^{-1}) at the temperature 313 K. It seems that the rate of bioleaching is controlled by the step “ash layer diffusion.” It was found that the activation energy was to be 37.59 kJ/mol. The values of thermodynamic parameters for the bioleaching system, ΔH° , and ΔS° were calculated to be $0.7399 \times 10^{-3} \text{ J/mol}$ and 28.512 J/mol , respectively.

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