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Rate Kinetics on Bioleaching of Realgar Mineral by *Leptospirillum ferriphilum*: Effects of Fe (II) Concentration

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Abstract: The eco-friendly method of metal extraction, bacterial leaching is gaining more important in bio-hydrometallurgical industry. It is a proven method and can be applied for leaching process of several metals. This study was aimed to determine the efficiency of arsenic leaching from realgar mineral using the bacteria, *Leptospirillum ferriphilum.* The experiments were carried out in shaker flask, at fixed parameters 180 rpm, 0.2% pulp density (PD), 313 K at an initial pH of the media 1.5. The energy source, Fe^{2+} concentration was varied from 2−10 g/L. The effect of Fe^{2+} concentration on the kinetics of arsenic extraction was investigated. The experiment results showed that leaching of arsenic was highly influenced by concentration of Fe^{2+} used in the medium. The maximum of 74.42% As was leached after 30 days when using optimum of 6 g/L of Fe^{2+}. Kinetic studies showed that the maximum rate constant value, 1.3633 d^{-1}, was observed when optimum concentration of energy source was used.

Keywords: Arsenic, bioleaching, *Leptospirillum ferriphilum,* realgar, ferrous concentration, rate kinetics.

1. INTRODUCTION

Arsenic (As) is found in the natural environment in some abundance in the Earth's crust and in some quantity in rock, salt, water, and air. It is also present in more than 200 different ores and minerals. Major quantity of As is present in the ores such as realgar (As_2S_2), orpiment (As_2S_3), arsenopyrite (FeAsS) [1]. If a large amount of arsenic is swallowed by humans in a form that is readily absorbed, it can cause rapid poisoning and death. It also affects gut, heart and nervous system. Exposure in the workplace mainly via the air breathed in can cause cancers. However, As has wide application in various fields. The toxicity of As to insect, fungi and bacteria led to its use as wood preservatives. It is also used for taxonomic sample preservation. Arsenic is a common n-type dopant in semiconductor electronic devices. The optoelectronic compound gallium arsenide is most common semiconductor used after doped silicon. Due to these reasons arsenic is extracted from the environment by various methods. The leached arsenic can be further treated and can be used for several commercial applications. Realgar contains major quantity of As and also has many applications. Realgar can be used for the treatment relapsed acute promyelocytic leukemia (APL) and chronic myelogenous leukemia (CML) when the dosage of soluble As is in a precise quantity. It is also used for curing psoriasis, abdominal pains, and burns[2]. Hence, realgar can be widely used therapeutically if As is eliminated or its concentration is reduced in it[3].

Arsenic can be leached from the realgar by chemical and biological method. Chemical method of leaching is expensive and cause environmental pollution. Therefore, bioleaching is used as an alternative method against chemical method[4, 5]. Bioleaching is the process of extraction of metals from ores using iron-oxidizing or sulphur oxidizing bacteria[6, 7]. This method is used for leaching of As because it is an eco-friendly and cost effective method[8]. In bioleaching, ferrous iron is oxidized to ferric iron by iron oxidizing bacteria. This ferric iron is involved in leaching of the minerals. The bioleaching of realgar by iron-oxidizing bacteria can be described by Eq. (1 & 2)[3].

$$2Fe^{2+} + 4H^{+} + O_2 \xrightarrow{L.\ ferriphilum} 4Fe^{3+} + 2H_2O \qquad \ldots (1)$$

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

The bacteria usually employed in bioleaching processes are found to be of genus *Acidithiobacillus* and *Leptospirillum,* such as *Acidithiobacillus ferrooxidans*, *A. thiooxidans*, *A. caldus*, *Leptospirillum ferriphilum,* and *Leptospirillum ferrooxidans.* Among them most widely used microorganism for leaching of arsenic from realgar are *Acidithiobacillus ferrooxidans and A. thiooxidans* [9, 10]. However, only a few works have been carried out using *Leptospirillum ferriphilum*[11]. Recently, researchers showing interest on *L. ferriphilum* as it can survive at lower pH, and has higher redox potential.

Till date, no work has been reported related to bioleaching kinetics of As from realgar using *L. ferriphilum*[3]. The main objective of this work is to leach As biologically from realgar using pure culture of *L. ferriphilum.* The efficiency of arsenic bioleaching is studied by varying the energy source supplied to the media. The leaching efficacy was assessed by measuring parameters such as pH and redox potential.

2. MATERIALS AND METHODS

2.1. Realgar particles: Realgar sample obtained from Lingshot mine (Zanskar, Kashmir, India) was crushed using jaw crusher. The ore was then grounded to size ranging from 100 to 1,200 µm using ball mill and used for bioleaching studies. X-ray Diffraction (XRD) analysis was used for mineralogical studies to confirm the composition of realgar present in the sample. Quantitative

analysis of XRD showed that the ore sample contained 68.785% of realgar, 5.78% of tridymite 2H low, 2.61% of litharge, 3.748% of quartz alpha, 12.30% of stishovite, and 7.223% of cristobalite beta high.

2.2. Chemical analysis: Chemical analysis was carried out to determine the different chemical constituents present in the ore. The amount of silica, iron, and titanium present in the mineral was determined using appropriate reagents, ammonium molybdate, manganese, 1, 10-phenonthroline, hydrogen peroxide, and periodate, respectively by spectrophotometerically[12]. Amount of sulphur was estimated after precipitation as barium sulphate. 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[12]. Amount of arsenic present in the realgar was determined by atomic absorption spectrometer (AA200 model; PerkinElmer) after acid digestion.

2.3. Bacterial strain and media: The *L. ferriphilum* strain was isolated from mine drainage samples of Chitradurga mine province, Ingaldhal (Karnataka, India). The pure microbial population was developed by sub-culturing several times using 9K medium containing $(NH_4)_2SO_4$ (3.0 g/L), KCl (0.1 g/L), $MgSO_4.7H_2O$ (0.5 g/L), K_2HPO_4 (0.5 g/L), and $Ca(NO_3)_2$ (0.01 g/L). $FeSO_4.7H_2O$ (44.2 g/L) was added as the energy source in every sub-culture. Enrichment of samples was done at initial pH 1.5 at 313 K. Molecular characterization of the isolated strain was carried out and it 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 and accession number KF743135 was obtained.

2.4. Inoculum preparation: To enhance the bacterial leaching rate, isolated *L. ferriphilum* was made adapt to realgar mineral. In order to adapt 10% (v/v) of culture was sequentially sub cultured in the 9K media with 1% (w/v) of realgar. Suitable conditions such as temperature 313 K, initial pH 1.5, and rotation speed of 200 rpm were also maintained for proper growth of the culture. The initial pH of the media was adjusted using 5 N H_2SO_4. The culture of adapted *L. ferriphilum* was used as inoculum for bioleaching process. Analytical grade reagents and purified water from Milli-Q system (Millipore) were used for the experiments.

2.5. Leaching experiment: Bioleaching experiments were carried out in 250 mL Erlenmeyer flask, each with 90 mL of the 9K medium and 10 mL of log phase cells of *L. ferriphilum* with initial pH of 1.5. This experiment was divided into five parts. In each part of the experiment, the concentration of Fe^{2+} which is used as energy source in the media is varied ranging from 2 −10 g/L. All the flasks were maintained at the initial pH of 1.5, agitation speed 200 rpm, 0.2% (w/v) of realgar mineral concentration and temperature 313 K. A controlled experiment was carried out under the same conditions but without addition of inoculum and 0.2 g/L $HgCl_2$ was also added as bacterial germicide. Each experiment was conducted in triplicates for a time period of 30 days. The mean value and standard deviation of the triplicates were calculated and expressed as results.

2.6. Analytical techniques: During the bioleaching process, media pH and redox potential were determined at every day using calibrated pH meter (Eutech Instruments, Singapore) and platinum electrode against a reference electrode of Ag/AgCl[13], respectively. For every 2 day interval, 5 mL of the sample from the media were collected and centrifuged at 3000 rpm. The supernatant from centrifuged solution was extracted using Whatman filter paper and preserved at 277 K before determining the leached out arsenic. Concentration of solubilized arsenic in the solution was estimated by atomic absorption spectrometer (AA200 model; PerkinElmer). The bioleaching efficiency of arsenic, E_{As} (%), was calculated using a the mathematical expression $E_{As}\% =$

$(N_{soln}/N_T) \times 100$, where N_{soln} is the As concentration in aqueous phase at time t during bioleaching and N_T the total As concentration in the realgar concentrate.

2.7. Kinetic approaches on realgar bioleaching: The general mathematical model of bioleaching based on first-order reaction can be used as follows[14]

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

Where k_{As} is the rate constant of As bioleaching. Integrating Eq. (1) between the respective limits of time (t = 0 d, $C_{As,t}$ = 0 and t = t d, $C_{As,t}$ = $C_{As,t}$), the resulting equation is given as follows:

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

Eq. (2) is therefore widely used for evaluating the value of k_{As}. $C_{As,0}$ and $C_{As,t}$ are the total arsenic concentration in the raw realgar ore and arsenic concentration in aqueous phase of leachate at the particular time t during the process. Using Eq. (2), a generalized chart of $\ln(C_{As,0}/(C_{As,0} - C_{As,t}))$ vs time on bioleaching data provides the k_{As} value as slope[15].

3. RESULTS AND DISCUSSION

3.1. Chemical analysis of realgar: The results of chemical analysis showed that the composition (wt.%) of raw realgar mineral was as follows : As, 69.2 %; Fe_2O_3, 1.12%; S, 19.1%; MgO, 8.85%; Na_2O, 0.15%; K_2O, 0.10%; TiO_2, 0.11%; SiO_2, 10.35%; and MnO, 0.41%. This analysis interpreted that the As consisted the maximum quantity of the mineral.

3.2 Variation of pH and redox potential: Figure 1 shows the variation of pH in the leachate during bioleaching 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 1.5 to 2.52, 2.6, 2.76, 2.4, and 2.1 in the experiments in which the Fe^{2+} concentration was varied from 2, 4, 6, 8, and 10 g/L, respectively. This increase in the pH is observed due to the consumption of acid by proton attack of realgar. After the second day, gradual decrease in the pH of the medium was observed. This decrease in the pH was due to the production of sulphuric acid in the medium by the oxidation of components present in the ore[16].

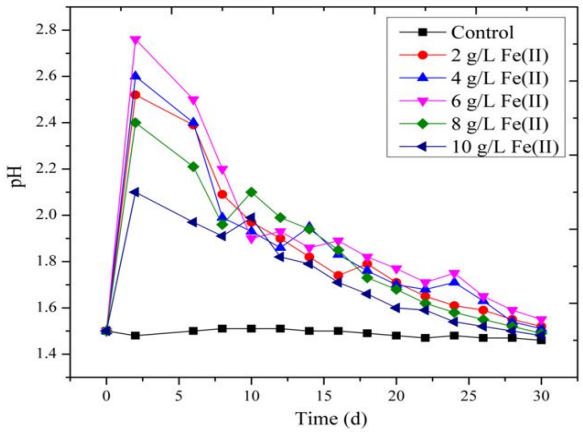

Figure 1: Representation the variation of pH at different concentration of Fe(II) during bioleaching.

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 g/L, 4 g/L, 6 g/L, 8g/L and 10 g/L of Fe^{2+}, respectively.

Figure 2 shows the variation of redox potential during the bioleaching process. The evolution of redox potential values from 202mV to 656mV against Ag/AgCl was steady with the Fe(III)/Fe(II) ratio in solution in the inoculated experiment whereas the oxidation potential remained constant (200mV) as against Ag/AgCl in the sterile control. The maximum redox potential value of 656mV was observed in the flask containing 6 g/L of Fe^{2+}concentration. The redox potential value was found to be high due to the bacterial oxidation of Fe^{2+} to Fe^{3+} and maintenance of dissolved state of Fe^{3+} [5].

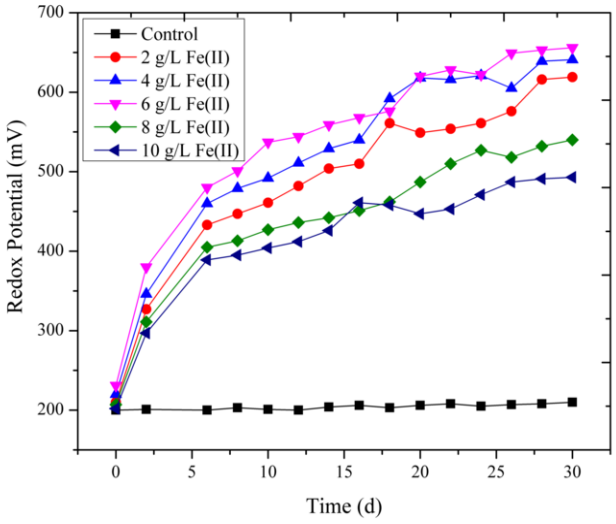

Figure 2: Representation the variation of ORP at different concentration of Fe(II) during bioleaching.

3.3. Effect of energy source on arsenic bioleaching and kinetics: Bioleaching efficiency of arsenic from the realgar ore by *L. ferriphilum* at different energy sources as a function of time is shown in **Figure 3**.

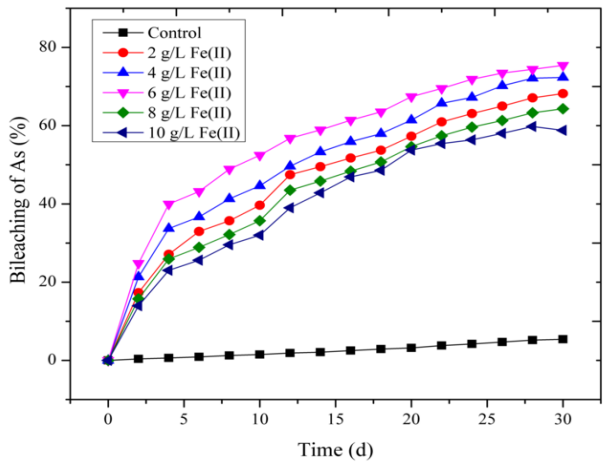

Figure 3: Bioleaching efficiency of arsenic with different concentrations of Fe(II)

After 30 days, the control experiment which was carried out in the absence of inoculum observed to leach 5.4 % of arsenic. Whereas, it was observed that at the end of the experiment, maximum leaching rates of arsenic were found to be 68.19%, 72.32%, 75.42%, 64.32 % and 58.81% in the flasks containing 2, 4, 6, 8 and 10 g/L of Fe^{2+} concentration, respectively. Thus, it illustrates that leaching rate is closely correlated with oxidation of ferrous iron[3]. It is also obvious that 6 g/L of Fe^{2+} is the optimum concentration of energy source that can be supplemented to the 9K media for bioleaching using *L. ferriphilum* for achieving the maximum leaching efficiency. The rate of As bioleaching can be described in terms of rate constant (k_{As}). **Figure 4** shows a fitting of experimental data to determine the values of rate constant at different ferrous concentration.

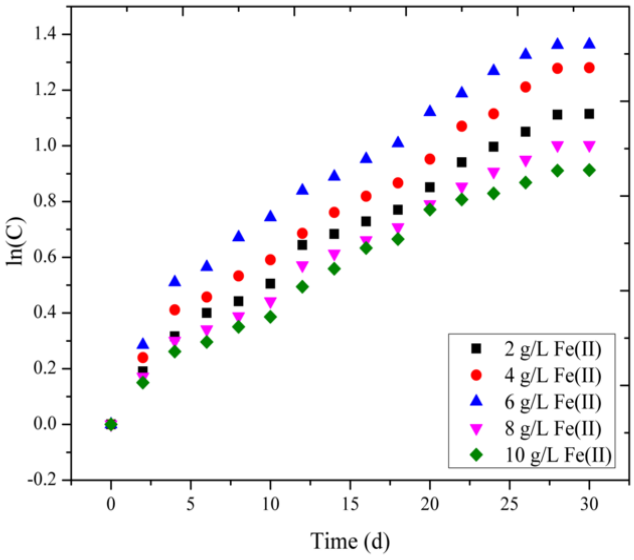

Figure 4: Graphical fitting for value of rate constant at various Fe(II) concentrations.

Since the leaching is positively associated with Fe^{2+} concentration, the rate constant value also increases with increase in the concentration of Fe^{2+} until 6 g/L. In the experiments with 2, 4, 6, 8, and 10 g/L of Fe^{2+}, the values of rate constant were found to be 1.1144, 1.2801, 1.3633, 1.0026, 0.9130 d^{-1}, respectively. It becomes apparent that, while using the optimum Fe^{2+} concentration of 6 g/L, As leaching was enhanced and reaches maximum rate.

4. CONCLUSION

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 68.19% 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^{2+} 1 g/L. Bioleaching efficiency was observed to be significantly increasing when Fe^{2+} concentration was increased. Maximum bioleaching efficiency 75.42% was observed when 6 g/L of Fe^{2+} 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^{2+}.

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