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Effect of FE(II) Concentration on Bioleaching of Zinc from Sphalerite using Leptospirillum Ferriphilum: Kinetic Aspects

Venkatesa Prabhu.S, Baskar.R, Amare T.Adugna, Gizachew Assefa.K ,Amsalu Gosu.A

Abstract: ***The objective of this study was to isolate an acidophilic iron-oxidizing bacterium, Leptospirillum ferriphilum, and explore the impacts of initial Fe(II) concentration on the bioleaching kinetics of zinc retrieval from sphalerite concentrate. L. ferriphilum strain was successfully isolated from Chitradurga mine, Karnataka, India, and molecular techniques for DNA sequencing were applied. The obtained nucleotide sequence was deposited to GenBank and accession number KF743135 was granted. The effect of Fe(II) on the iron-based bioleaching kinetics of zinc leaching using the L. ferriphilum isolate was ascertained under the following experimental conditions: inoculum size, 10% (v/v); bioleaching period, 20 days; system temperature, 301±2 K; initial pH, 3; pulp density 5% (w/v); and Fe(II) concentration in the medium, 1–9 g/L. The results demonstrated that efficiency of bioleaching was highly influenced by concentration of Fe(II) and maximized yield of 87.85% zinc was obtained at 7 g/L. The kinetic study specify that the rate constant estimations of zinc biosolubilization were moderately high at 7 g/L Fe(II), and the kinetic analysis using shrinking core model showed that the leaching rate is constrained by ash layer diffusion step***

Keywords : Bioleaching, L. ferriphilum, Zinc mineral, Rate kinetics, Shirking core model.

I. INTRODUCTION

Zinc is the one among the extensively used metallic chemical elements in the world. After iron, copper, and aluminum, it is the fourth widely used metal. In excess of 11 million tons of zinc is produced annually and its requirement has increased significantly since 2005 worldwide [1]. It is extracted from sphalerite that comprises to a great extent of zinc sulfide in crystalline form. Nowadays, approximately 90% of the world's total zinc is separated from sphalerite through traditional processes such as pressure hydrometallurgy and roast–leach–eletcrowinning [2].

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* Correspondence Author

Venkatea Prabhu. S*, Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia. Email: haiitsvp@gmail.com

Baskar. R, Food Technology, Kongu Engineering College, Erode, India. Email: naturebaskar@gmail.com

Amare T. Adugna, Environmental Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia. Email: amaretiru@gmail.com

Gizachew Assefa. K, Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia. Email: gizenet1@gmail.com

Amsalu Gosu. A, Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia. Email: amsalugosu@gmail.com

Owing to tightening of environmental restrictions, zinc extraction using traditional methods has become highly expensive and difficult. As of late, advancement of biohydrometallurgical procedure for the extraction of zinc from its sulfide minerals has gained interest because these process have several potential advantages (such as less gaseous emission to atmospheric air, no need of roasting and acid plants, simple in operation, applicable to low-grade ores, and cost-effective) over conventional methods. Biohydrometallurgical process is the dissolution of metal species from their sulfide minerals by the catalysis of iron and/or sulfur oxidizing micro-organisms which is commonly known as bioleaching [3]. Sphalerite can be corrupted by acidophilic bioleaching including microorganisms that have the ability to oxidize zinc sulfide to soluble zinc sulfate. In such a way, zinc can be leached into aqueous phase from the insoluble complex minerals [4]. Among the most altogether considered microorganisms, Acidithiobacillus ferrooxidans is important in bioleaching of sulfide mineral [5,6] because it can oxidize both iron and sulfur. However, the members of genera Leptospirillum have been gaining special attention as bioleaching microorganisms because they can tolerate lower pH, have higher redox potential, can withstand higher cultivation temperature, and have higher affinity toward sulfide minerals compared to A. ferrooxidans [7–9]. It is outstandingly known that Leptospirillum ferrooxidans and Leptospirillum ferriphilum are the two most regular species under the genus Leptospirillum; the latter can endure lower pH and is more extremophile [10,11], thus was chosen as the objective bacterium for this study. L. ferriphilum utilize ferrous iron as energy source and leaches zinc from sphalerite through iron-based bioleaching mechanism [12]. Consequently, in this study, special emphasis has been given to explore the impact of initial Fe(II) concentration on the bioleaching of zinc. For this, bioleaching with five modifications of medium containing Fe(II) concentration ranging from 1 to 9 g/L was utilized as feed solution for survey the impact of Fe(II) concentration on bioleaching. This work extends for kinetic study of zinc bioleaching based on the pseudo-first-order kinetic model, and the rate-controlling step was analyzed using SCM (Shrinking Core Model) from the observed data.

II. THEORETICAL

A. Bioleaching Mechanism

Leaching of zinc from sphalerite by *L. ferriphilum* follows iron-based bioleaching mechanism,

a type of indirect mechanism. The leaching mechanism can be explained by the following stoichiometry equations:

$0.5nZnS + Fe^{3+} + H^+ \rightarrow Zn^{2+} + 0.5\ H_2S_n + Fe^{2+}$ (where $n \geq 2$).. (1)

$2Fe^{2+} + 2H^+ + 0.5O_2 \rightarrow 2Fe^{3+} + H_2O$ ……... (2)

$FeS_2 + Fe^{3+} + H_2O \rightarrow Fe^{2+} + S_2O_3^{2-} + 6H^+$……... (3)

$0.5\ H_2S_n + Fe^{3+} \rightarrow Fe^{2+} + H^+ + 0.0625n\ S_8$ ……... (4)

Fe(III) produced via bacterial metabolism can serve as an oxidant for zinc sulfide dissolution (Eq. 1). The resultant Fe(II) from the mineral oxidation is re-oxidized to Fe(III) by *L. ferriphilum* to obtain energy for its growth in the acidic aerobic condition (Eq. 2) [13,14]. The pyritic phase present in the sphalerite can also be oxidized to produce additional Fe(II) and create necessary acidic environment (Eq. 3). During this course, insoluble elemental sulfur (S_8) is liberated and forms a product layer on the surface of the mineral (Eq. 4). In this manner, within the sight of Fe(II) and oxygen, sphalerite can be leached promptly by *L. ferriphilum*.

B. Bioleaching Kinetics

The rate of the reaction is characterized as the speed of formation of desired product at the reaction. General mathematical model applicable for rate of appearance of soluble zinc through bioleaching by assuming the pseudo-first-order kinetics can be used as follows [15]:

$$R_{Zn} = \frac{dC_{Zn}}{dt} = k(C - C_t) \text{……... (5)}$$

where $(C - C_t)$ is the driving force in terms of difference between the maximum available zinc concentration to leach and the zinc concentration existing in the aqueous medium at time t, and k is the kinetic coefficient defined as rate constant. Integrating Eq. (5) between the respective limits ($t = 0$ day, $C_t = 0$ and $t = t$ days, $C_t = C_t$), the resulting mathematical model is given in Eq. (6). It pursues that k value for bioleaching of zinc can be resolved from the slope of the generalized chart $\ln[C/(C - C_t)]$ versus time:

$$\ln\left(\frac{C}{C - C_t}\right) = kt \text{ ……... (6)}$$

To get better understandings of bioleaching mechanism, the kinetics of fluid–particle reaction using SCM was carried out. Based on the SCM, the rate of bioleaching might be constrained by dispersion through product layer formation on the mineral surface, or chemical reaction or diffusion through fluid film between the particle and the medium [16]. These steps can be described using the following linear equations [17]:

$$1 + 2(1 - F) - 3(1 - F)^{2/3} = k_{obs}t \text{ …….(7)}$$

$$1 - (1 - F)^{1/3} = k_{obs}t \text{…….(8)}$$

$$F = k_{obs}t \text{……..… (9)}$$

where F = Fraction of zinc solubilized as a product at aqueous phase. k_{obs} = Observed kinetic constant (time^{-1}). To identify the controlling step, linear regression analysis to the plots $[1 - 3(1 - F)^{2/3} + 2(1 - F)]$ versus time, $[1 - (1 - F)^{1/3}]$ versus time, and $[F]$ versus time was carried out on observed bioleaching data.

III. EXPERIMENTAL

A. Sphalerite Concentrate

The natural mineral, sphalerite, was obtained from the Dariba mines (Rajasthan, India). It was grounded in a laboratory ball-mill, and the classification of different particle size fractions was completed using ASTM sieves. From the grounded concentrate, the particles ranging from 100 to 1200 µm (with an average particle size of 300 µm) were selected for bioleaching experiments. The mineral distribution and quantitative analysis of mineralogical composition were microscopically analyzed and were further confirmed by X-ray diffraction analysis. The total metal constituents, such as zinc, iron, titanium, and aluminum contents, in the concentrates were analyzed after complete acid digestion.

B. Microorganism and Adaptation

Iron-oxidizing bacterial culture was isolated from mine drainage samples of the Chitradurga mine sector in Ingaldhal (Karnataka, India). The isolate was screened from serially diluted mine drainage sample using *Leptospirillum* medium (Medium 882; DSMZ). The medium had the following chemical composition: $FeSO_4.7H_2O$ (20 g/L), $(NH_4)_2SO_4$ (132 mg/L), KH_2PO_4 (27 mg/L), $MgCl_2.6H_2O$ (53 mg/L), and $CaCl_2.2H_2O$ (147 mg/L); and trace elements: $ZnCl_2$ (0.068 mg/L), $MnCl_2.2H_2O$ (0.062 mg/L), $CoCl_2.6H_2O$ (0.064 mg/L), Na_2MoO_4 (0.01 mg/L), H_3BO_3 (0.031 mg/L), and $CuCl_2.2H_2O$ (0.67 mg/L). The underlying initial pH of the media was adjusted to 1.5 using 1 N H_2SO_4. The unadulterated culture was created by subculturing several times on a rotary shaker at 180 rpm at a consistent temperature of 30°C. From the pure culture, cells were gathered by centrifugation at 10,000 rpm for 3 minutes and molecular techniques were applied. The recognition of the isolate was dictated by analyzing 16S rRNA nucleotide sequences. The connection between the isolate and the related species was found by a phylogenetic tree built by software Clustal X. It was determined that the isolate showed a restriction pattern 99% identical to that of the *L. ferriphilum* NR028818. In addition, nucleotide sequences of the isolate were submitted to GenBank (National Center for Biotechnology Information, Maryland, USA) and accession number KF743135 was acquired.

To adjust experimental conditions, *L. ferriphilum* had been subcultured through a few exchanges in the similar media supplemented with sphalerite without ferrous part. For that, 90 mL media supplemented with 0.1% (w/v) sphalerite along with 10% (v/v) inoculum were set up in 250 mL Erlenmeyer flask and incubated at 30°C in a rotary shaker at 180 rpm. In the late logarithmic stage, 10 mL culture medium was transferred to fresh nutrient media containing 0.2% (w/v) sphalerite. In this way, further step-wise adaptation to mineral was made using the fresh media containing mineral concentrations of 0.4, 0.7, and 1.0 (%w/v). It was utilized as inoculum for bioleaching experiments and conserved as stock culture. The stock cultures were subcultured at 2-week interims.

C. Bioleaching Experiments

Bioleaching experiments were completed in 250 mL Erlenmeyer flasks. Each flask comprised 100 mL medium solution [Medium 882; DSMZ without ferrous sulfate and 10% (v/v) inoculum] with 5% (w/v) mineral pulp density. To examine the impact of Fe(II) concentration on the bioleaching, media containing various amounts of Fe(II) concentration were used. The concentrations of Fe(II) (in the form of $FeSO_4.7H_2O$) in the media in each flask were 1, 3, 5, 7, and 9 g/L. The first pH of the leaching medium was changed in accordance with 3.0 using 1 N H_2SO_4 and was shaken with 150 rpm at room temperature (28±2°C). A controlled experiment with 0.2 g/L $HgCl_2$ as bacterial germicide in the medium was also maintained at the same experimental conditions without inoculum. Every one of the analyses were done in triplicates to guarantee the reliability. The mean values for all the triplicates were expressed as results.

D. Analytical Techniques

During the process, pH and oxidation–reduction potential (ORP) of the leaching medium were checked using calibrated pH meter (Eutech Instruments, Singapore) and Pt-Ag/AgCl electrode, respectively, consistently. At every 2-day intervals, 5 mL tests from the flasks were occasionally pulled back and centrifuged at 3000 rpm for 20 minutes. The supernatant was sifted using Whatman filter paper and saved at 4°C to decide the solubilized zinc concentration. The zinc concentration was estimated using atomic absorption spectrometer (AA200 model; PerkinElmer; USA) after appropriate dilution whenever necessary. The fresh iron-free nutrient solution of medium was added to compensate the media loss due to sample collection. Bioleaching efficiency, denoted by $E_{Zn}(\%)$, was calculated using the following mathematical expression

$$E_{Zn}\% = (M_t - M_0) \times 100/M \ldots\ldots (10)$$

where M_t, M_0, and M are the zinc concentration in aqueous phase at time t during bioleaching, the zinc concentration in aqueous phase at zero time, and the total zinc concentration in the sphalerite concentrate, respectively.

IV. RESULTS AND DISCUSSION

A. Mineralogy and Chemical Examination of Zinc Concentrate

Microscopic analysis for mineralogical studies showed that sphalerite and pyrites are the major heavy metal minerals present in the sample with small amounts of galena. During these studies, it was discovered that sphalerite was interlocked with minerals such as quartz, galena, and pyrite. The quantitative analysis of mineralogical composition was completed by X-ray diffraction inspection, which showed that the mineral composition contained 13.02% sphalerite (ZnS), 82.26% quartz (SiO_2), 0.94% dolomite ($CaMg(CO_3)_2$), 1.80% pyrite (FeS_2), 0.58% lime, and 0.44% galena (PbS). The chemical analysis showed the following the composition (wt%) of raw concentrate: ZnO, 40.36%; Fe_2O_3, 6.12%; TiO_2, 0.21%; S, 11.41%; MgO, 8.85%; Al_2O_3, 6.12%; Na_2O, 0.02%; P_2O_5, 0.24%; CaO, 5.6%; K_2O, 0.10%; SiO_2, 16.92%; MnO, 0.41%; and loss of incineration, 3.67%.

B. Effect of Fe(II) on pH and ORP During Bioleaching

The impact of Fe(II) concentration on pH during zinc bioleaching is depicted in Figure 1(a). In the control experiments, no apparent change in pH was observed during the course. Though, a marginal increase in the pH (3.0–3.3) was noticed due to the acid utilization of mineral sulfides. During bioleaching, the pH values increased initially during the first 3 days. This increase was due to proton consumption from the initially added sulfuric acid, as stated by Eq. (1), and acid consumption that also took place with the bacterial oxidation of Fe(II) to Fe(III) ion, thus H^+ concentration decreased initially. Comparable increment in the pH during the initial couple of days was seen by Soleimani *et al.* [18] and Li *et al.* [19]. After the third day, the pH value of the medium started to decrease due to production of sulfuric acid through Fe(III) attack of pyretic phase, as given in Eq. (4). Though reduction in the pH value was observed in experiments with different ferrous concentrations, the highest reduction (2.5) was found in the experiment with 7 g/L Fe(II) at the end of 20 days. At the end of 20 days, pH values were 2.9, 2.7, 2.6, and 2.8 while using initial concentration of 1, 3, 5, and 9 g L^{-1} Fe(II), respectively.

Changes in ORP during bioleaching are presented in Figure 1(b) with respect to time. In control tests, no huge variety in the ORP estimations in the ORP values of the medium was noticed, which indicates the absence of any biological reaction. However, increase in the ORP value from 224 to 320 mV was due to chemical oxidation of ferrous sulfate. Owing to the bacterial catalysis, the oxidation of Fe(II) to Fe(III) results in the release of free electrons, consequently increasing the ORP of the medium during bioleaching. The precipitation of ferric compounds was also found during the hydrolysis of iron. A similar increase in the ORP was observed by Santos *et al.* [20] while treating copper sulfide with *A. ferrooxidans*. The highest ORP value was recorded as 642 mV in the experiment with 7 g/L Fe(II) and the lowest value of 605 mV was found in the experiment with 1 g/L Fe(II). At the end of the 20th day, ORP values at 3, 5, and 9 g/L Fe(II) concentrations were discovered to be 610, 616, and 623 mV, respectively. The trend of change in the ORP values shows that the increase in initial ferrous concentration in the medium is positively correlated to ORP till 7 g/L Fe(II).

C. Effect of Fe(II) on Bioleaching of Zinc

Profiles of zinc bioleaching efficiency from the sphalerite phase by *L. ferriphilum* using different initial Fe(II) concentrations are depicted in Figure 2. Control experiment showed 4.50% bioleaching of zinc. This occurred due to sulfuric acid added for initializing pH value to 3. In the experiments with 1, 3, 5, and 7 g/L Fe(II), the biosolubilization efficiency of zinc was found to be 73.92%, 82.49%, 84.52%, and 87.85%, respectively. With further increase in initial Fe(II) concentration to 9 g/L, the bioleaching efficiency of zinc was found to be reduced to 83.56%. It is apparent that the bioleaching of zinc is positively correlated with increasing initial Fe(II) concentration up to 7 g/L. However, reduced efficiency of metal bioleaching was observed at the flask with 9 g/L Fe(II)

due to the inhibiting impact of initial Fe(II) ions. Thus, it is evident that optimal Fe(II) concentration of 7 g/L is appropriate for iron-based bioleaching of the type of sphalerite used with L. ferriphilum. Approximately 85%–100% of zinc leaching efficiency has been accounted for somewhere else, which relies upon the type of mineral concentrate, microorganism, and conditions present during bioleaching [21-23]. The bioleached residue obtained at the flask using media with 7 g/L Fe(II) was subjected to energy-dispersive X-ray (EDX) analysis to analyze leached out zinc. The spectrum of EDX analysis is given in Figure 3. According to the corresponding EDX analysis, zinc was almost found to be disappeared (0.13 mass percent).

D. Kinetics on Bioleaching

Rate of reaction is estimated in terms of rate constant, which is a numerical measure of the pace of the reaction when all the reactants are brought at the unit concentration. The plotting of experimental bioleaching data to Eq. (6) to determine rate constant for zinc leaching is depicted in Figure 4. Table 1 presents the equations of best-fit linear lines with respect to pseudo first-order model and corresponding linear regression coefficients for zinc bioleaching with different Fe(II) concentrations. It is clear that the pseudo first-order rate kinetic model is well fitted to the experimental data and that the correlation coefficients of the graphical fitting are high (>0.95). From the kinetic study, it was discovered that rate constant esteems of zinc bioleaching at 1, 3, and 5 g/L Fe(II) were 0.070, 0.090, and 0.096 d^{-1}, respectively. The maximum rate constant value of 0.103 d^{-1} was attained using medium containing 7 g/L Fe(II). However, while using Fe(II) concentration beyond 7 g/L, a decline in the rate constant value to 0.096 d^{-1} was observed. It becomes apparent that while using ferrous iron concentration of 7 g/L, the period of lag phase was shortened and bioleaching was increased to the maximum rate

The kinetic models of ash layer diffusion control, chemical reaction control, and liquid film control were tested to explain the rate-controlling mechanism of zinc bioleaching with respect to SCM. The graphical applicability of the SCM on bioleaching data are given in Figure 5(a)-5(c).The linear regression coefficient values from the graphical fit of different controlling step models are given in Table 2. It is obvious that observed bioleaching data remain better to ash layer diffusion-controlled SCM. The diffusion barrier is formation of the elemental sulfur layer (product obtained according to Eq. (4)) on the mineral surface because of the biological reaction, as reported by Deshpande *et al.* [24]. Consequently, diffusion of oxidizing agent, Fe(III), through this layer likely acts as rate-controlling step and regulates oxidation of minerals.

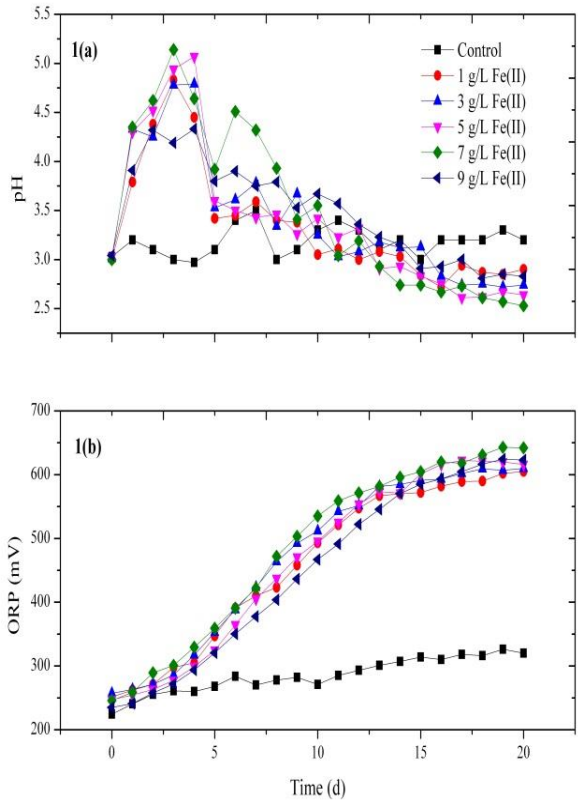

Fig.1. Variation in pH (1a) and redox potential (1b) during bioleaching at different Fe(II) concentrations.

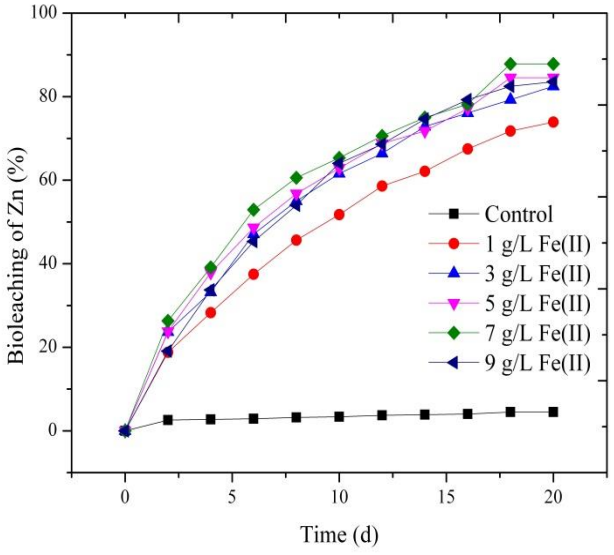

Fig.2. Bioleaching efficiency of zinc by *L. ferriphilum* at different Fe(II) concentrations.

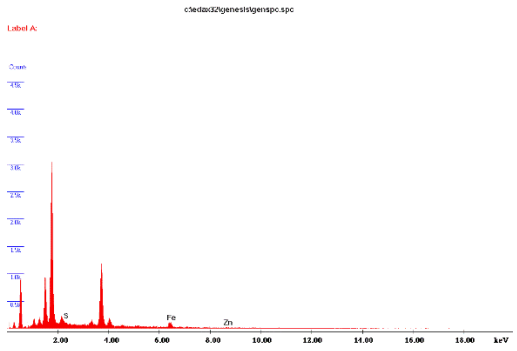

Fig.3. The spectrum of EDX analysis of leach residue obtained from experiment with 7 g/L Fe(II).

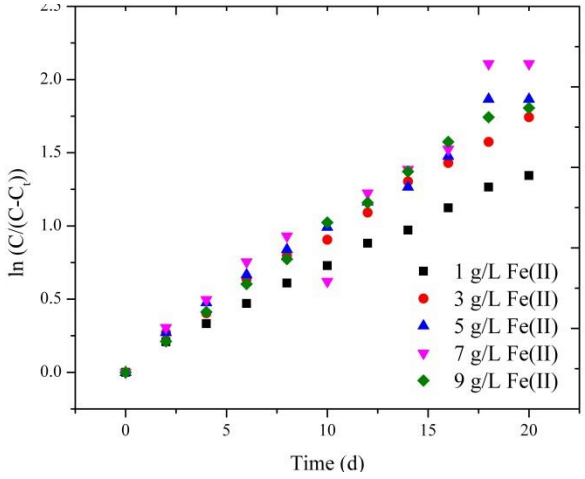

Fig.4. First order rate kinetic plot for zinc bioleaching.

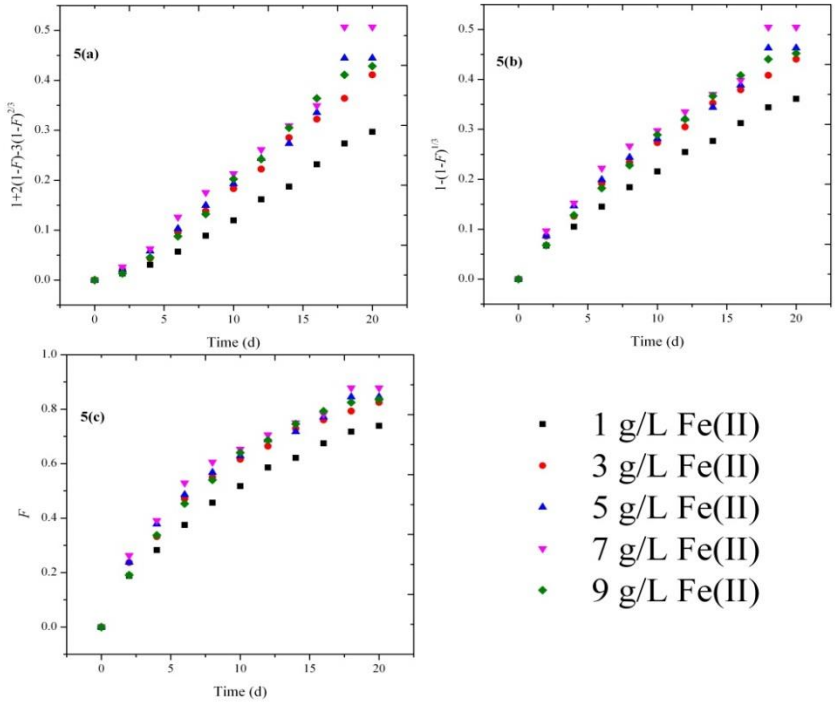

Fig.5. Fitting of zinc bioleaching data to (5a) ash layer diffusion control, (5b) chemical reaction control, and (5c) liquid film control model.

Table I. Linear equation of best fit to pseudo first-order model for zinc bioleaching and respective regression coefficients.

Fe(II) concentration (g/L)	Regression coefficient	
	Linear equation	R^2
1	$y = 0.070x$	0.990
3	$y = 0.090x$	0.989
5	$y = 0.096x$	0.984
7	$y = 0.103x$	0.938
9	$y = 0.096x$	0.994

Table II. Linear regression coefficient values of the graphical fit for different controlling step models.

Fe(II) concentration (g/L)	Ash layer diffusion control model	Chemical reaction control model	Liquid film control model
1	0.968	0.965	0.857
3	0.983	0.949	0.780
5	0.97	0.952	0.767
7	0.961	0.947	0.734
9	0.971	0.968	0.828

V. CONCLUSION

In this study, an iron-oxidizing chemoautotrophic bacterium, L. ferriphilum, was targeted and successfully isolated from the samples collected at acid mine drainage of Chitradurga mine province, India. The genetic analysis was accomplished on enriched pure isolate culture to confirm the isolation of L. ferriphilum, and nucleotide sequence was submitted to NCBI GenBank. The mineral-adapted culture was used to explore the effects of initial Fe(II) concentration on bioleaching of zinc sphalerite. The experiments were completed in shake flasks with media containing five initial concentrations of Fe(II), that is, 1, 3, 5, 7, and 9 g/L. During 20 days of experiment, the results depicted that the zinc bioleaching efficiency has a significant impact on initial Fe(II) concentration used as energy source in the medium. Using predefined conditions of DSMZ Medium 882, initial media pH 3, pulp density 5% (w/v), and room temperature 301±2 K, the highest metal bioleaching yield of 87.85% zinc in the experiment conducted with 7 g/L Fe(II) was obtained. The aftereffects of kinetic study dependent on pseudo first-order model on bioleaching data indicated that the rate constant is extensively influenced by Fe(II). It showed that the highest value of the rate constant was found to be 0.103 d^{-1} with the experiment using 7 g/L Fe(II) among the chosen initial ferrous concentrations. From the SCM analysis, it was affirmed that the rate of zinc bioleaching is controlled by the step of diffusion through ash layer.

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AUTHORS PROFILE

Dr. Venkatesa Prabhu. S received Doctoral Degree in Faculty of Technology in Chemical Engineering specialization from Anna University, Chennai, India and he is having 18 years of Engineering teaching experience, currently teaching Chemical Engineering – Masters Level and PhD of courses at Addis Ababa Science and Technology University, Addis Ababa, Ethiopia. His professional interests focus on Environmental, Biochemical, Food process Engineering, and Enzyme Technology. At present, he is working on the research projects "Process Optimization and Kinetic Studies on Bacterial Leaching of Chromium from Tannery Sludge", and "Investigation of Extraction Techniques for Recovery of Collagen from Tannery Raw Trimming Wastes". He has published 21 reputed research publications. In addition, he serves as referee for the journals for The Journal of Cleaner Production, Fresenius Environmental Bulletin, and Environmental Engineering Research.

Dr. Rajoo Baskar received Doctoral Degree in Faculty of Technology in Chemical Engineering with Biochemical Engineering specialization. He is having 25 years of Engineering teaching experience, currently serving professor & Head, Food Technology – Masters Level and PhD courses at Kongu Engineering, College Erode, India. His professional interests focus on Environmental, Food & Bioprocess Engineering, and Chemical Process Optimization Techniques.

At present, he is supervising four research projects on Food process, and Green Technology. He has published more than 50 reputed research publications.

Dr. Amare T. Adugna working as Assistant professor in the department of Environmental Engineering, college of Biological and Chemical Engineering at Addis Ababa Science and Technology University, and Dean of the college since May 2016. He has obtained PhD from 2iE foundation Ouagadougou, Burkina Faso, West Africa (April 2, 2012 to March 7, 2016) in Science and Technology for Water, Energy and Environment (Specialty: water). His PhD dissertation was on Greywater treatment by vermifiltration for sub-Saharan urban poor. He got His MSc in Environmental Engineering from Addis Ababa University, Addis Ababa, Ethiopia (October, 2006 to November, 2008) and the MSc thesis was on Evaluation of Institutional Biogas Digesters: The Case Study of Kotebe College of Teachers Education. He has been actively participated in teaching, advising (research) and community service. He has been advised more than 15 MSc students and two PhD scholars, and published more than 6 research papers in internationally recognized journals.

Mr. Gizachew Assefa. K received Multiple Master Degrees (MSc in Chemical Engineering from the University of Twente, The Netherlands, MSc in Membrane Materials and Process Engineering, from University of Chemistry and Technology Prague, Czech Republic and MSc in Chemical Engineering specialized in Membrane Engineering from University of Montpellier, France) on July 2015. In addition, he has received MSc degree in Chemical Engineering (Environmental Engineering Stream) in July 2011 from Addis Ababa University. He is having more than 9 years of academic experience. Currently, he is serving teaching and research in Chemical Engineering at Addis Ababa Science and Technology University, Addis Ababa, Ethiopia.

Mr. Amsalu Gosu. A has been graduated in BSc. Degree in Chemical Engineering from Bahir Dar Institute of Technology, Ethiopia in July 2012. He has completed Preparatory Program for Master of Industrial Engineering (in 2014) & MSc Degree (in 2017) in Biochemical Engineering at KU Leuven, Belgium. At present, he is serving as a teaching faculty in Addis Ababa Science and Technology University at Chemical Engineering Department. His research aims at optimization of fermentation and bioprocess technology. Currently, working on the research project entitled “Optimization of Rotenone Extraction from Birbira Plant (Milletia ferruginea) Seed, Alternative Bio-pesticide” funded by Addis Ababa Science and Technology University.

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