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Bioleaching of Zn from sphalerite using *Leptospirillum ferriphilum* isolate: effect of temperature and kinetic aspects

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Abstract

Biological methods for leaching of nonferrous and noble metals from its sulfide ores are widely applied at industrial enterprises of different countries. This process is based on the use of the oxidative activity of acidophilic microorganisms. Since all bio systems are quite sensitive to the temperature, bacterial leaching process also significantly effects. In the present study, the impact of temperature on bacterial leaching of Zn from its sulphide ore, sphalerite, was investigated using ore adapted iron oxidizing bacteria. The bacteria were isolated from mine drainage samples and subjected to gene sequencing. The acquired nucleotide sequence revealed that the isolate was *Leptospirillum ferriphilum*. The nucleotide sequence of *L. ferriphilum* isolate was submitted to National Center for Biotechnology Information (NCBI) and accession number KF743135 was assigned. Using the isolate, the Zn leaching data were collected in the 298–318 K temperature range. The results showed that leaching of Zn increases with temperature until optimum temperature of 313 K and achieves highest leaching efficiency of 96.96% within 20 days. Since bioleaching of minerals have become increasingly applied in different mining industries, there is immense important to analyze mechanistically-based kinetics for the design, optimization, operation, and control of biochemical processes. The kinetic study showed that the rate of Zn leaching was maximized at the optimum temperature. Further, the leaching data were analyzed using shrinking core model which revealed that the rate of leaching was inhibited by diffusion through product layer. Reaction kinetics is also to be contrasted with thermodynamics. Using Arrhenius law of thermodynamics, it was found that activation energy for Zn bioleaching reaction was 39.557 kJ mol^{-1}. Such investigations will be necessitated for designing and implanting the ideal bioleaching system for metal bio-mining industries.

Keywords Activation energy, Bioleaching, Sphalerite, *Leptospirillum ferriphilum*, Rate kinetics shrinking core model, Zn

Introduction

Zn is the fourth most broadly used metal in the world after iron, aluminum, and copper. Inferable from its solid anticorrosive properties and its ability to bond well with other different metals, it has a wide range of uses in applications, such as galvanizing and nonstructural castings, and used as a major component of batteries and alloys. More than 11 million tons of Zn is manufactured annually worldwide, and the demand of Zn has expanded altogether since 2005 [1]. Zn is separated from sphalerite (ZnS), which is one of the most significant Zn-bearing sulfide mineral resources [2]. These days, approximately 90% of the world's all out Zn is produced by customary techniques such as roast–leach–electrowinning and pressure hydrometallurgy from sphalerite [3, 4]. Extracting Zn using traditional methods is difficult and expensive because sphalerite is generally associated

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with other minerals, such as pyrite and galena [5, 6]. Bacterial-assisted leaching (bioleaching) has been proven to be ecofriendly and the most cost-effective option to the traditional procedures without making use of sulfur dioxide that follows as by-product in traditional methods and causes environmental issues [7–9]. Bioleaching can be applied for extracting Zn from sphalerite. Haghshenas et al. [10] have studied experimentation on sphalerite bioleaching by *A. ferrooxidans*. The highest extraction of Zn from sphalerite by bioleaching was obtained at particle size, pulp density and temperature of 38–150 μm, 4% wt/vol and 33 °C, respectively. Ghassa et al. [11] have carried out an investigation on high grade Zn–Pb bearing ore using mixed moderate thermophilic microorganisms. The results showed that the highest zinc recovery, 98.5%, was obtained during 25 days with pulp density 50 (g/L). *A. ferrooxidans*, isolated from native province of lead–zinc tailing, was used to leach Zn from sphalerite by Lei et al. [12]. During 25 days of leaching, they observed 70% of highest leaching at optimized pH value 2. Three different types of zinc sulphides, marmatite, sphalerite and ZnS (synthetically prepared), were studied for comparative bioleaching of Zn using *A. ferrooxidans* and a moderately thermoacidophilic iron-oxidizing bacterium by Shi et al. [13]. They observed that the bioleaching of marmatite concentrate was advantageous over that of the sphalerite and synthetic ZnS which clears that Zn extraction could be influenced by mineral properties. A polymetallic (Cu, Zn, Pb, Fe, Ag, and Au) sulphide concentrate was subjected to bioleaching studies by Vesna et al. [14]. They have used a mixed culture of *A. ferrooxidans*, *A. thiooxidans*, and *Leptospirillum ferrooxidans*. The study showed that Zn had a highest leaching efficiency of 89% which revealed the significant affinity of Zn on biological leaching. A Bioleaching study on lead–zinc tailing dam's sample was carried out by Mehrabani et al. [15] with respect to examine Zn extraction using a mixed culture of mesophile bacteria and mixed culture of moderate thermophile bacteria. They observed that the moderate thermophile bacteria showed significantly higher leaching efficiency than that of the mesophile microbe which indicated the impact of temperature on the Zn bioleaching.

Zinc sulphide ores can be treated by bioleaching through direct or indirect mechanism [16]. In the direct bioleaching mechanism, Zn is extracted by direct oxidation of insoluble zinc sulfide to soluble zinc sulfate, which is catalyzed by sulfur-oxidizing bacteria (SOB) such as *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, as shown in Eq. (1). Indirect bioleaching principally pursues an iron-based mechanism in which ferric ions are produced from bio-oxidation of ferrous ions, which go about as an oxidizing agent to facilitate Zn dissolution from its sulfide mineral [17–19]. Accordingly, bioleaching of sphalerite can be depicted by Eqs. (2) and (3), in which all iron-oxidizing bacteria (IOB), for example, *A. ferrooxidans*, *Acidimicrobium ferrooxidans*, *A. albertensis*, *Alicyclobacillus tolerans*, *Leptospirillum ferrooxidans*, and *Leptospirillum ferriphilum*, are used for cartelization [13]. IOB are capable of re-oxidizing ferrous ions to ferric ions during bioleaching [20, 21]. Regenerated ferric ions can be reused to oxidize sphalerite [22]. Sphalerite usually occurs in association with pyrite (FeS_2). The pyritic phase present with sphalerite can be used as the energy source by IOB, as described in Eq. (4), which enhances Zn leaching and provides a necessary acidic environment for bacterial growth [23].

$$MS + 2O_2 \xrightarrow{SOB} MSO_4 \tag{1}$$

$$2Fe^{2+} + 2H^{+} + 0.5O_2 \xrightarrow{IOB} 2Fe^{3+} + H_2O \tag{2}$$

$$ZnS + 2Fe^{3+} \rightarrow Zn^{2+} + S^{\circ} + 2Fe^{2+} \tag{3}$$

$$FeS_2 + (7/2)O_2 + H_2O \xrightarrow{IOB} Fe^{2+} + 2SO_4^{2-} + 2H^{+} \tag{4}$$

Several studies reported that *A. ferrooxidans* is considered as prime important IOB. Even though it grows well with higher Fe^{2+} concentrations, the members of the genus *Leptospirillum* attain good attention as an alternative to *A. ferrooxidans*. Because *Leptospirillum* members tolerate higher cultivation temperature, lower pH, higher redox potential of the medium, and higher tolerance on of ferric ion concentration compared to *A. ferrooxidans* [24, 25]. These properties make them potential candidates in bio-hydrometallurgical handling of sulfide minerals using IOB. Under genus *Leptospirillum*, two most normal spices correspond to *L. ferriphilum* and *L. ferrooxidans*. Low value of pH can be tolerated by *L. ferriphilum*; they are more extremophile and can withstand higher cultivation temperature [26]. Henceforth, it was picked as the biological agent for this study. Though bioleaching process has been used for several decades, there are several issues still continuing [27]. One of the significant issues of the bioleaching process is still lower rate of leaching, which consumes high residence time compared to the traditional processes and hinders success. In addition, different key factors, such as, types of metal concentrates quantity and nature of metal, nutrients availability during bioleaching, oxygen supply, pH, temperature, solid-ratio, and shear rate during agitation, control microbial growth [28]. Although the bacterial population, leaching effectiveness, and leaching rate appear closely related to the bioleaching conditions based

on the above-mentioned factors, this work is limited to study the effect of temperature on bioleaching of Zn from sphalerite by *L. ferriphilum* isolate. Since bioleaching of minerals have become increasingly applied bio-hydrometallurgical industries, there is immense important to analyze mechanistically-based kinetics for the design, optimization, operation, and control of biochemical processes. There were several laboratory trials of sphalerite bioleaching overseas using moderately thermophilic bacteria, but only a few researches involved the application of *L. ferriphilum* and its bioleaching kinetics [29].

In the present study, the IOB, *L. ferriphilum*, was targeted to isolate and its Zn leaching potential was examined. The emphasis has been given on examining the impact of temperature on bioleaching of Zn from sphalerite using the isolate. Further, the study focused to analyze the rate of Zn leaching with mechanistically-based kinetic, pseudo-first-order model. In addition, the leaching data were analyzed using shrinking core model (SCM) to determine the rate controlling mechanism of the leaching. Also, it was intended to determine the minimum energy required for initiating the Zn bioleaching process. Such a study will be helpful for designing and implanting the ideal Zn bioleaching systems.

Materials and methods

Sphalerite concentrate

Sphalerite concentrate was collected from mines in Dariba, Rajasthan, India. A laboratory jaw crusher was used to crush these samples and a ballmill was used to ground them. ASTM services were used to classify different particles based on size. In bioleaching experiments, the size of normal particle was approximately 300 µm and size of grounded particle was between 100 and 1200 µm. A semi-quantitative investigation for the mineral composition of raw sample was conducted using X-ray diffraction (XRD) strategy. The XRD pattern is shown in Fig. 1. It demonstrates that the concentrate is essentially made up of 82.26% quartz (SiO_2), 0.58% lime, 0.94% dolomite [$CaMg(CO_3)_2$], 13.02% sphalerite (ZnS), 1.80% pyrite (FeS_2), and 0.44% galena (PbS) as major minerals. Chemical analysis of raw ore sample showed the following composition (wt%): ZnO, 40.36%; Fe_2O_3, 6.12%; S, 11.41%; Al_2O_3, 6.12%; MgO, 8.85%; Na_2O, 0.02%; MnO, 0.41%; PbO, 0.10%; P_2O_5, 0.24%; TiO_2, 0.21%; CaO, 5.6%; SiO_2, 16.92%; and 3.67% of loss of incineration.

Microorganism and molecular characterization

From mine drainage samples of Chitradurga, Ingaldhal, a consortium IOB was collected. Samples were enriched at initial pH of 1.5 at 318 K. DSMZ–leptospirillum medium (Medium 882) was used to isolate target bacterium. It is specific to leptospirillum species. The following composition of chemical was present in the medium: $FeSO_4{\cdot}7H_2O$, 20 g L^{-1}; $(NH_4)_2SO_4$, 132 mg L^{-1}; $MgCl_2{\cdot}6H_2O$, 53 mg L^{-1}; KH_2PO_4, 27 mg L^{-1}; $CaCl_2{\cdot}2H_2O$, 147 mg L^{-1}; and trace elements of $MnCl_2{\cdot}2H_2O$, 0.062 mg L^{-1}; $ZnCl_2$, 0.068 mg L^{-1}; $CoCl_2{\cdot}6H_2O$, 0.064 mg L^{-1}; H_3BO_3, 0.031 mg L^{-1}; Na_2MoO_4, 0.01 mg L^{-1}; and $CuCl_2{\cdot}2H_2O$, 0.67 mg L^{-1}. Culture was purified by repetitive subcultures through serial dilutions (from 10^{-1} to 10^{-9}) in test tubes. It was enriched under the following conditions: 250 mL flasks, 100 mL liquid medium with 5% (v/v) inoculum, shaking speed 180 rpm, initial media pH 1.5, and incubation temperature 313 K. After a few subcultures, the culture was viewed as unadulterated when the morphology of the improvement gave off an impression of being homologous under an optical microscope. From this culture media, logarithmic-stage cells were collected by centrifuging at 10,000 rpm, and the DNA was

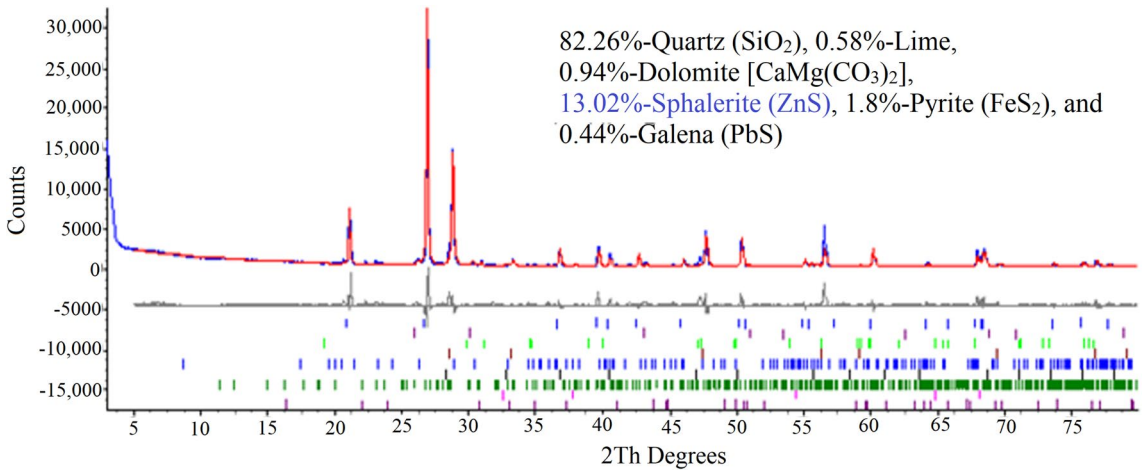

Fig. 1 X-ray diffraction pattern for semi-mineralogical analysis of sphalerite ore

separated using alkaline lysis method. PCR enhancement of 16S rRNA of the disconnected strain was performed in 50 mL culture using primers 27F and 1492R, as indicated by the method reported by Jian-she et al. [30]. PCR results of expected size were extracted from 1.0% agarose gels. The result is shown in Fig. 2. On the basis of homology from 16S rRNA sequence, a phylogenetic tree was used to related species, which was constructed using Clustal X 1.80 software. It revealed 99% of restriction pattern similar to *L. ferriphilum* NR028818. Nucleotide sequence of *L. ferriphilum* isolate was submitted to National Center for Biotechnology Information (NCBI) and accession number KF743135 was obtained for the sequence deposit.

Adaptation to sphalerite concentrate and preparation of working cell culture

In leaching performance, to show signs of improvement, *L. ferriphilum* isolate was made to adjust to sphalerite ore. For that, in a 250-mL Erlenmeyer flask, 90 mL sterilized media (Medium 882, DSMZ) at 1.5 initial pH, which was supplemented by 0.1% (w/v) sphalerite and enhanced by 10% (v/v) inoculum, was set up and hatched at 303 K in a rotary shaker rotating at 180 rpm. In the logarithmic phase (on the seventh day), fresh media containing 0.2% (w/v) sphalerite was used for further growth by adding 10 mL culture medium. Like this, step-wise adapted cultivation was carried out using media that had sphalerite concentrations (w/v) of 0.4, 0.7, and 1.0. For leaching experiments, this adapted culture was used as the working cell culture and it was used as stock culture. At 2-week intervals, subcultures were carried out using the stock culture. All synthetics used in this study were diagnostic evaluation reagents, and filtered water from Milli-Q system (Millipore) was used all through the experiments.

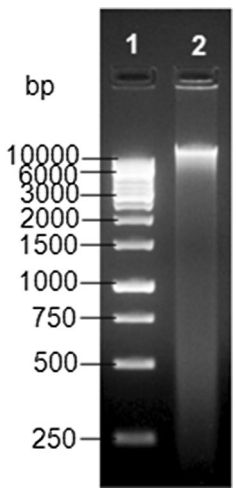

Fig. 2 Agarose gel electrophoresis of 16S-rRNA; Lane: 1-1 Kb DNA ladder; Lane: 2-Genomic DNA (Isolated *L. ferriphilum*)

Experimental procedure

Figure 3 shows the schematic illustration of experimental steps. Bioleaching trials were performed in the 250 mL Erlenmeyer flask with 90 mL sterilized medium and 10 mL log-phase cells of inoculums. The medium consisted of basal salts composition as per DSMZ Medium 882 with 7 g L^{-1} ferrous iron. For 15 min, the medium was autoclaved, without ferrous sulfate at 393 K. Through a 0.2-µm filter, medium's ferrous sulfate portion was cleaned. After being cooled to room temperature, the autoclaved leaching medium was added to ferrous sulfate portion aseptically. In the experimentation, following parameters of bioleaching were maintained: initial media pH value of 1.5, 200 rpm as the speed of shaking, sphalerite ore concentration 5% (w/v), average particle size 300 µm, and a temperature range from 298 to 318 K. Without inoculums, a controlled experiment was also maintained under same condition of experimentation with 0.2 g L^{-1} $HgCl_2$ as medium's bacterial germicide [31]. To guarantee unwavering quality of procedure, all experiments were conducted in triplicates. Mean values of all triplicates were considered as results.

Analytical techniques

During the bioleaching process, pH of the leaching medium was determined once a day by using a calibrated pH meter. At every 2-day interval, 5 mL samples from the leaching solution was collected and centrifuged for 20 min at 3000 rpm. Whatman filter paper was used to purify supernatant from the cells and debris. Further, it was preserved at 277 K until the determination of leached Zn. After appropriate dilution, whenever necessary, concentration of Zn in the leachate was assessed using an atomic absorption spectrometer. Medial loss was remunerated by adding medium's fresh iron-free nutrient solution because of collection of samples. Efficiency of bioleaching of Zn is denoted by η_{zn} (%). Using the mathematical expression $\eta_{zn}(\%) = \left[\frac{M_{soln}-M_0}{M_T}\right] \times 100$, the percent of the leached Zn was computed. M_{soln}, M_0, and M_T are concentrations of solubilized Zn in the leachate at time t (in aqueous phase), concentration of existing Zn at time 0, and total concentration of Zn available to leach in sphalerite, respectively.

Kinetic approaches on bioleaching studies

On the basis of pseudo-first-order reaction, a mathematical model of appearance rate of Zn solubilization is defined as follows [32]:

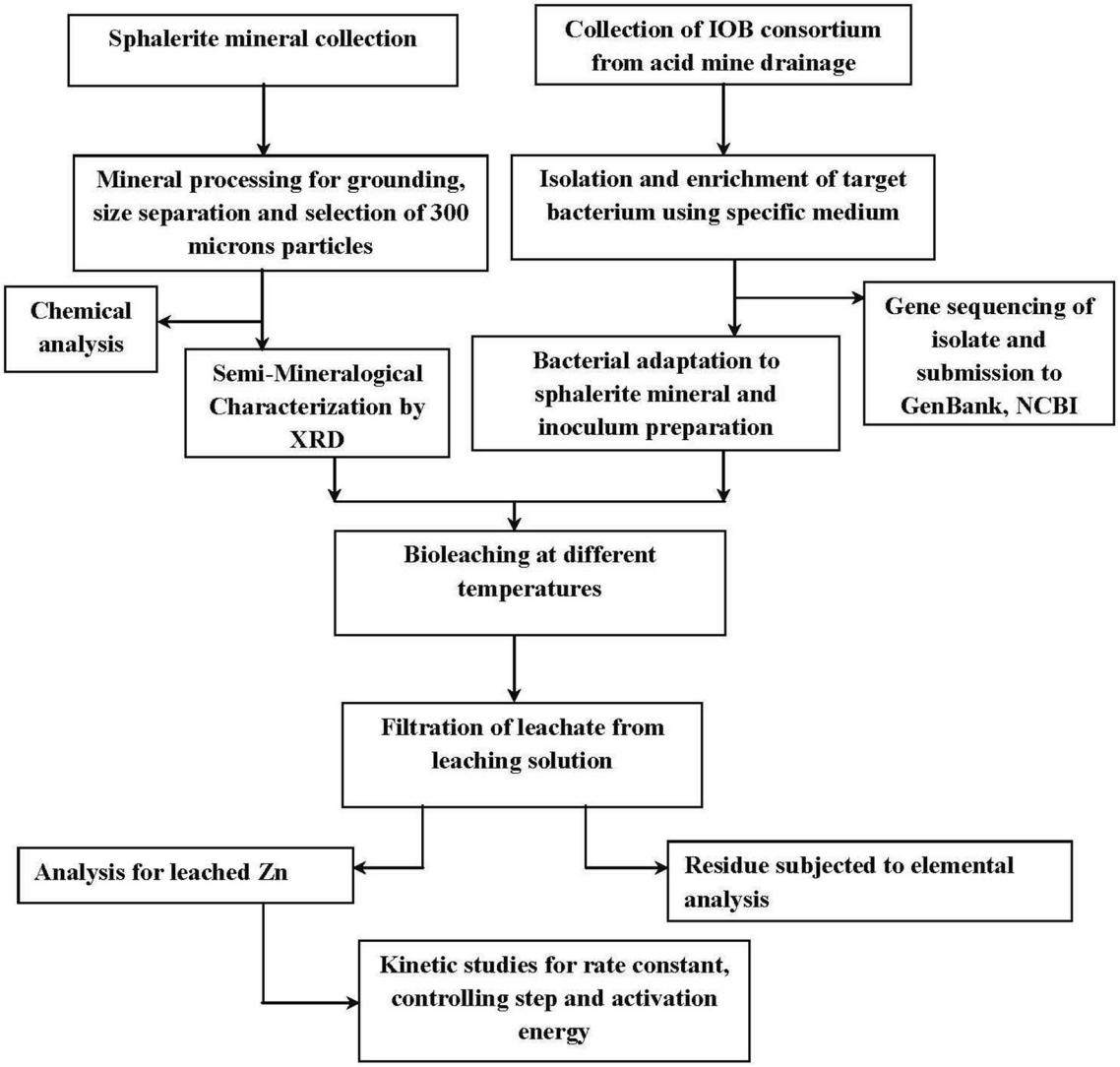

Fig. 3 Schematic representation of bioleaching process

$$r_{\mathrm{zn}} = \frac{\mathrm{d}C_{\mathrm{zn}}}{\mathrm{d}t} = k_{\mathrm{zn}}\left(C_{\mathrm{zn},0} - C_{\mathrm{zn},t}\right) \tag{5}$$

where k_{zn} is the rate constant of Zn leaching. Integrating above equation between time limits $t=0$, $C_{\mathrm{zn,t}}=0$, and $t=t$, $C_{\mathrm{zn},t}=C_{\mathrm{zn},t}$, the resultant mathematical model can be obtained as:

$$\ln\left(\frac{C_{\mathrm{zn},0}}{C_{\mathrm{zn},0} - C_{\mathrm{zn,t}}}\right) = k_{\mathrm{zn}}\mathrm{t} \tag{6}$$

Equation (6) is a linear equation that can be widely used for evaluating the value of k_{zn}. $C_{\mathrm{zn},0}$ and $C_{\mathrm{zn,t}}$ are the total concentrations of Zn in raw sphalerite and concentration of solubilized Zn in aqueous phase at particular time t during the leaching process. Using Eq. (6), a summed up graph of $\ln[C_{\mathrm{zn}},_0/(C_{\mathrm{zn}},_0 - C_{\mathrm{zn}},_t)]$ *vs* time was set up using leaching data for anticipating the reaction rate constant as the incline of the plot. The Arrhenius equation was used to compute minimum amount of energy and activation energy for bringing bioleaching, and it is given in Eq. (7) [33].

$$\ln k_{\mathrm{zn}} = \ln A - \frac{E}{R}\left(\frac{1}{T}\right) \tag{7}$$

where A is the frequency factor, k_{zn} is the rate constant of Zn leaching with respect to temperature, E is the activation energy (J mol^{-1}), R is the empirical gas constant (J mol^{-1} K^{-1}), and absolute temperature is given by T (K). Using Eq. (7), we made an Arrhenius plot of ln k_{zn} *vs* $(1/T)$ to compute activation energy from the acquired slope. While studying bioleaching reaction mechanism, rate-controlling analysis for Zn leaching gains more importance to design and further process applications. Thus, SCM was applied to determine the rate-controlling step for leaching of Zn. As per SCM, Zn leaching rate can be regulated either by diffusion through chemical reaction step or by ash layer step. The developed mathematical models for chemical reaction and ash layer diffusion control step are expressed in Eqs. (8) and (9) [33]:

$$1 - (1 - X_{zn})^{1/3} = k_{obs}t \tag{8}$$

$$1 + 2(1 - X_{zn}) - 3(1 - X_{zn})^{2/3} = k_{obs}t \tag{9}$$

where fraction of leached Zn is represented as X_{zn} and observed kinetic constant ($time^{-1}$) is represented as k_{obs}. To recognize rate-controlling step, charts for $[1+2(1-X_{zn})-3(1-X_{zn})^{2/3}]$ vs time and $[1-(1-X_{zn})^{1/3}]$ vs time were prepared. Rate-controlling step was resolved according to best fit's regression analysis from the above-said charts.

Results and discussion

Effect of temperature on pH and redox potential during bioleaching

Figure 4a shows results that correlate delegate pH value curves with respect to leaching time at various temperatures. Without inoculum, in control experiments, a pH was reduced negligibly (from 1.51 to 1.41) in view of mineral sulfide's chemical oxidation. During bioleaching, H^+ ions are consumed due to proton attack on sphalerite mineral by the available acid in the medium. Further, the bacterial oxidation of ferrous to ferric ions also needs H^+ ions. The same has been explained in Eq. (2). Thus, pH esteems expanded from 1.5 to 3.4 at 298 K, to 3.7 at 303 K, to 3.7 at 308 K, to 3.9 at 313 K, and to 3.4 at 318 K during initial 3 days. In any case, the increase in pH at 313 K showed better bacterial activity with stimulated performance in ferrous oxidation that consumes more H^+ ions compared with other temperatures. Hedrich et al. [34] have observed the same kind of pH increment during initial 2 days. Owing to sulfuric acid production (as presented in Eq. 4), the pH value of the medium began decreasing after the third day. This is due to bacterial oxidation of pyretic phase that is present in the mineral. It showed that the pH values decreased at 298, 303 308, 313, and 318 K to 1.74, 1.62, 1.62, 1.6 and 1.8 K, respectively, toward finish of bioleaching period (20 days). The increment and decrement profiles of pH variation observed in this study support the documentations from Manafi el al. [35], Olivera et al. [36] and Venkatesa Prabhu et al. [33].

Redox potential (E_h) of leaching medium relies on Fe^{3+}/Fe^{2+} ratio and is one among the key factors that decide oxidizing environment in the bioleaching system. It can be correlated with oxidizing agent, Fe^{3+} concentration, as $E_h = 0.771 + 0.059\log (Fe^{3+}/Fe^{2+})$ [37]. Figure 4b shows redox potential variation during bioleaching at various temperatures. From the observed profiles, the E_h value in the non-inoculated medium had maintained at a low level approximately 250 mV. The nil variation in redox potential at control medium cleared that there was no bacterial oxidation of Fe^{2+} to Fe^{3+}. In inoculated investigations, expansion in redox potential from 240 to 591 mV at 298 K, 252 to 610 mV at 303 K, 242 to 615 mV at 308 K, 244 to 642 mV at 313 K, and 242 to 624 mV 318 K were observed. When sphalerite was bioleached by *L. ferriphilum*, from the 3rd day to the 14th day, the E_h values were between 350 and 550 mV which was supported by the observations of Gu et al. [38], and Schippers et al. [16]. During this period, the extraction of Zn increased significantly. This shows, through novel metabolism of *L. ferriphilum*, persistent upkeep of concentration of Fe^{3+}/Fe^{2+}. During the period of 14–20 days, high redox potential was recorded due to enhanced bacterial activity. The leaching was getting lowered when the redox potential stabilized at a high level (above 600 mV).

Many researches have also remarked the essential role of E_h that determines the efficiency of bioleaching. Venkatesa Prabhu et al. [33] have notices the same range of redox potential increments during bioleaching of Zn by *L. ferriphilum*. A high values of E_h at the onset of leaching provokes rapid passivation of Zn. Córdoba et al. [39] reported the effect of E_h on chalcopyrite leaching. They found that the redox potential is one of the crucial factors in the leaching of Cu from chalcopyrite. On other hand, Sandström et al. [40] have observed the bioleaching performance at two different levels of redox potential, 420 mV and 600 mV at 65 °C. They found that bioleaching of chalcopyrite was greatly improved at the lower E_h. High recoveries of Cu were found during chalcopyrite leaching in a small redox potential range of 410–440 mV.

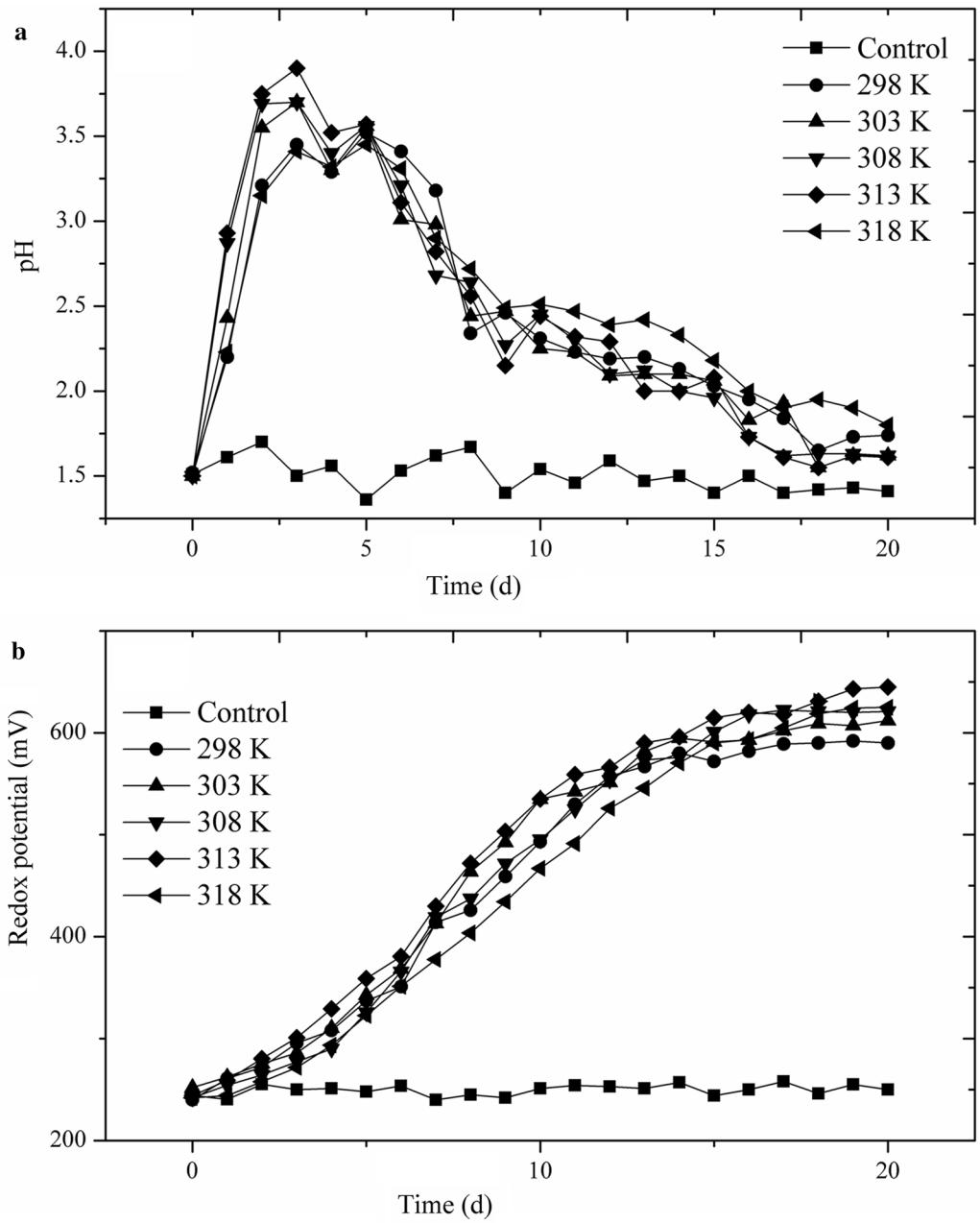

Fig. 4 Variation in **a** pH and **b** redox potential during bioleaching at different temperatures

Effect of temperature on Zn bioleaching

Sphalerite is well known to be recalcitrant to biological leaching, though a number of bio-hydrometallurgical processes for overcoming the issues of passivation have been described in the past 10–15 years [41]. One of the suggested approaches to enhance the rate and efficiency of the bioleaching is optimization of temperature [35]. Figure 5 shows Zn bioleaching proficiency at different temperatures from sphalerite by *L. ferriphilum* as a function of time. At 303 K, in control experiment, 4.92% Zn was leached at the end of the 20th day. This was done by dissolution through sulfuric acid addition to keep up initial value of pH to 1.5 in the medium.

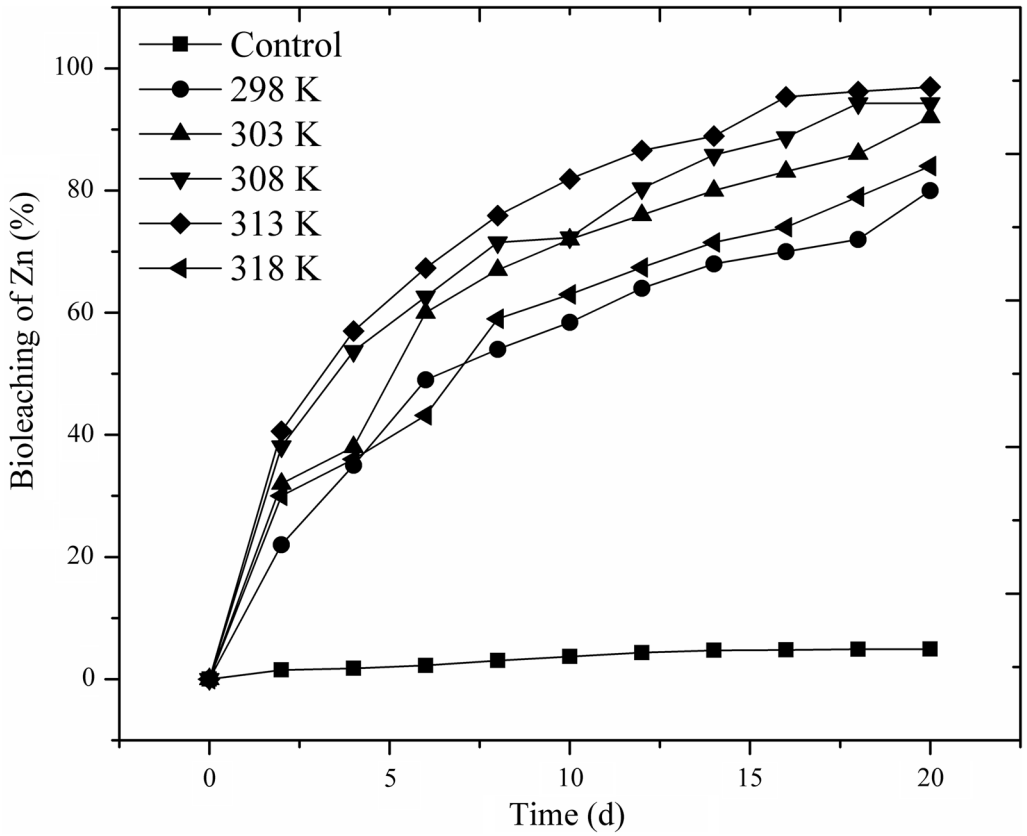

Fig. 5 Bioleaching efficiency of Zn by *L. ferriphilum* at different temperatures

From the experiments, Zn bioleaching efficiencies at 298, 303, 308, 313, and 318 K were observed to be 80.0%, 92.0%, 94.28%, 96.96%, and 84%, respectively. It was observed that temperature affects leaching of Zn firmly. It was also observed that bioleaching trend increases with increase in temperature from 298 to 313 K. Further increment in temperature did not yield any higher proficiency of Zn leaching. Conversely, diminished effectiveness was observed at 318 K. From the bioleaching results, 313 K is perfect temperature while using *L. ferriphilum* to accomplish most leaching proficiency, which supports the reports of the leaching execution of *L. ferriphilum* [38]. Also, the results confirmed the report of Venkatesa prabhu et al. [42] who have documented about better leaching performance of *L. ferriphilum* with respect to temperature. Bioleaching of Zn extending from 90% to 100%, relying on microorganism presence, conditions of experimentation, and type of mineral, has been reported elsewhere. Sajjad et al. [43] have studies the dissolution of Cu and Zn from ore using indigenous IOB consortia supplemented with dried bamboo sawdust. They noticed 90.0% of Zn recovery. Wei et al. [44] have made a comparative bioleaching study using IOB and Sulfur-oxidizing bacteria (SOB) to remove Zn from Pig Manure. But, they found SOB showed good rate of Zn leaching compared to SOB.

The basic substances in the raw mineral sample and leach residue were analyzed to affirm the leached out Zn from the ore using energy-dispersive X-ray spectroscopy (EDX). Spectral patterns of mineral and leach buildup are given in Fig. 6a, b. The results of the EDX analysis demonstrated that O, Si, S, Ca, Fe, and Zn elements were at mass percent 50.53, 38.51, 2.24, 0.68, 0.48, and 7.56, respectively, for raw mineral and 51.83, 29.93, 4.43, 0.88, 4.16, and 0.15, respectively, for leach residue. It showed a huge vanishing of Zn from the ore. Increment in mass the percent at leach residue for S and Fe affirms the arrangement of elemental sulfur and precipitation of jarosite by biological reaction [45, 46].

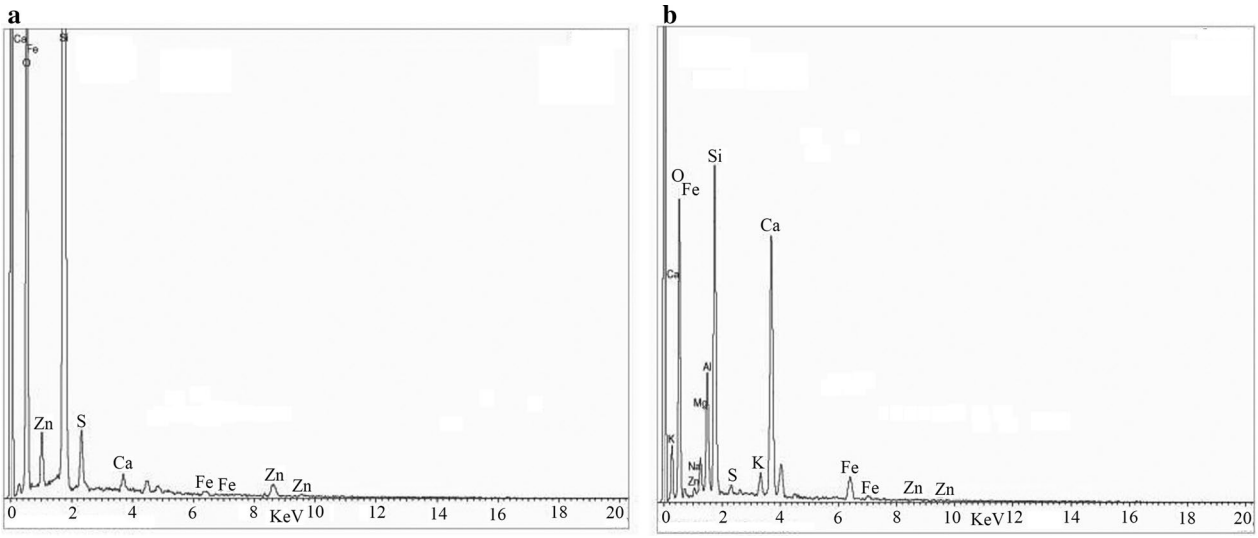

Fig. 6 EDX-spectrum of **a** raw ore and **b** leach residue

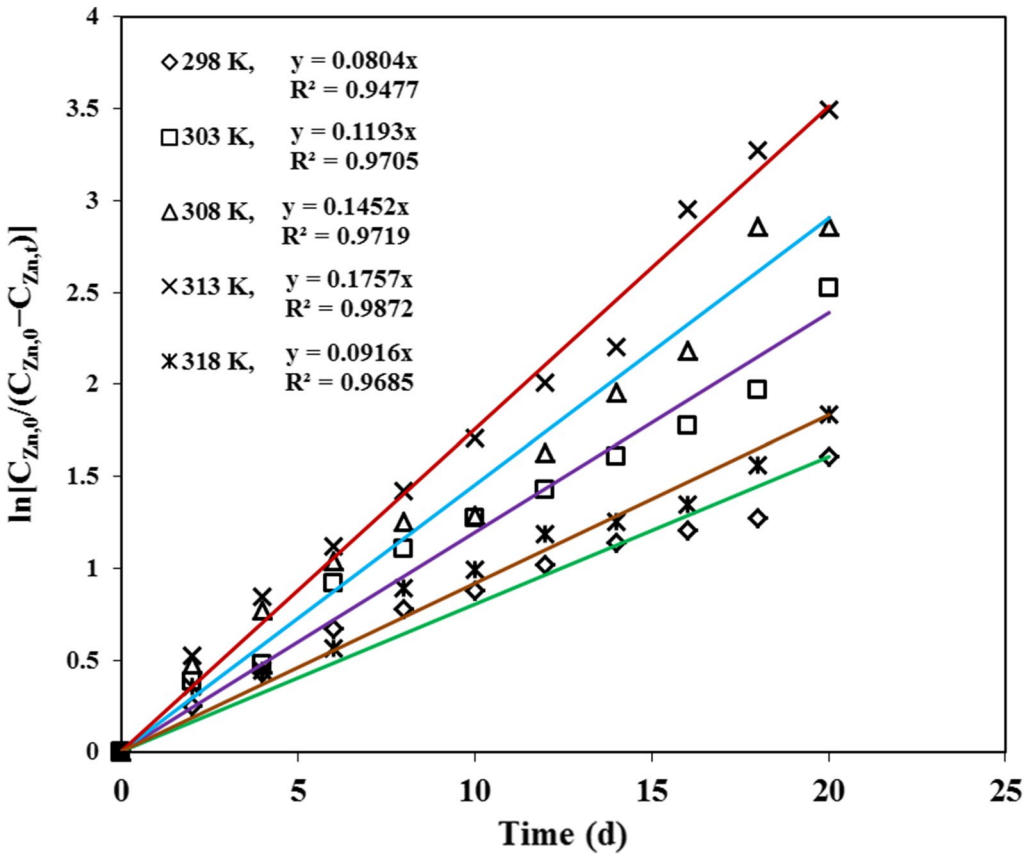

Fig. 7 First order rate kinetic plot for Zn bioleaching

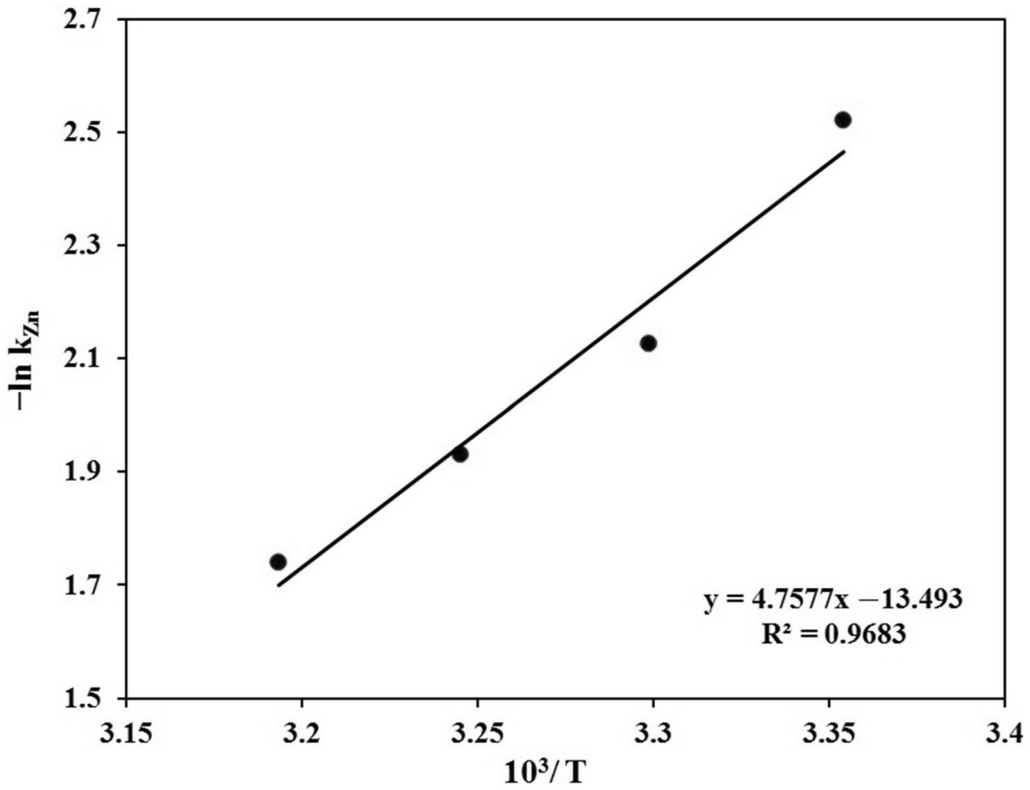

Fig. 8 Arrhenius plot for activation energy determination

Bioleaching kinetics

The Zn leaching rate is described with respect to constant rate (k_{zn}). Figure 7 shows test information's fitting to decide estimations of constant rate at various temperatures as well as relating coefficients of regression (R^2). As leaching decidedly corresponds with temperature, the rate constant value increases with expanding temperature until 313 K. In the experiments, at 298, 303, 308, and 313 K, the values of constant rate were determined to be 0.0804, 0.1193, 0.1452, and 0.1757 day^{-1}, respectively. Decline in rate constant value 0.0916 day^{-1} was observed at temperature 313 K. It is obvious that while keeping up the ideal temperature value of 313 K, leaching is upgraded and reaches the extreme rate. Activation energy for Zn bioleaching was found to be 39.557 kJ mol^{-1}, as shown by the Arrhenius plot (Fig. 8). Mathematical models for chemical reaction control step and ash layer diffusion control were analyzed by substituting leaching data to clarify the rate-controlling step regarding SCM. The visual appropriateness of the chemical reaction control model and ash layer diffusion control model based on bioleaching data are given in Fig. 9a, b. Regression analysis shows that observed bioleaching data can fit better to ash layer diffusion-controlled SCM. Scanning electron microscope (SEM) images of the raw mineral and leach residue are shown in Fig. 10a, b. The SEM image of the leach residue distinctly shows the development of

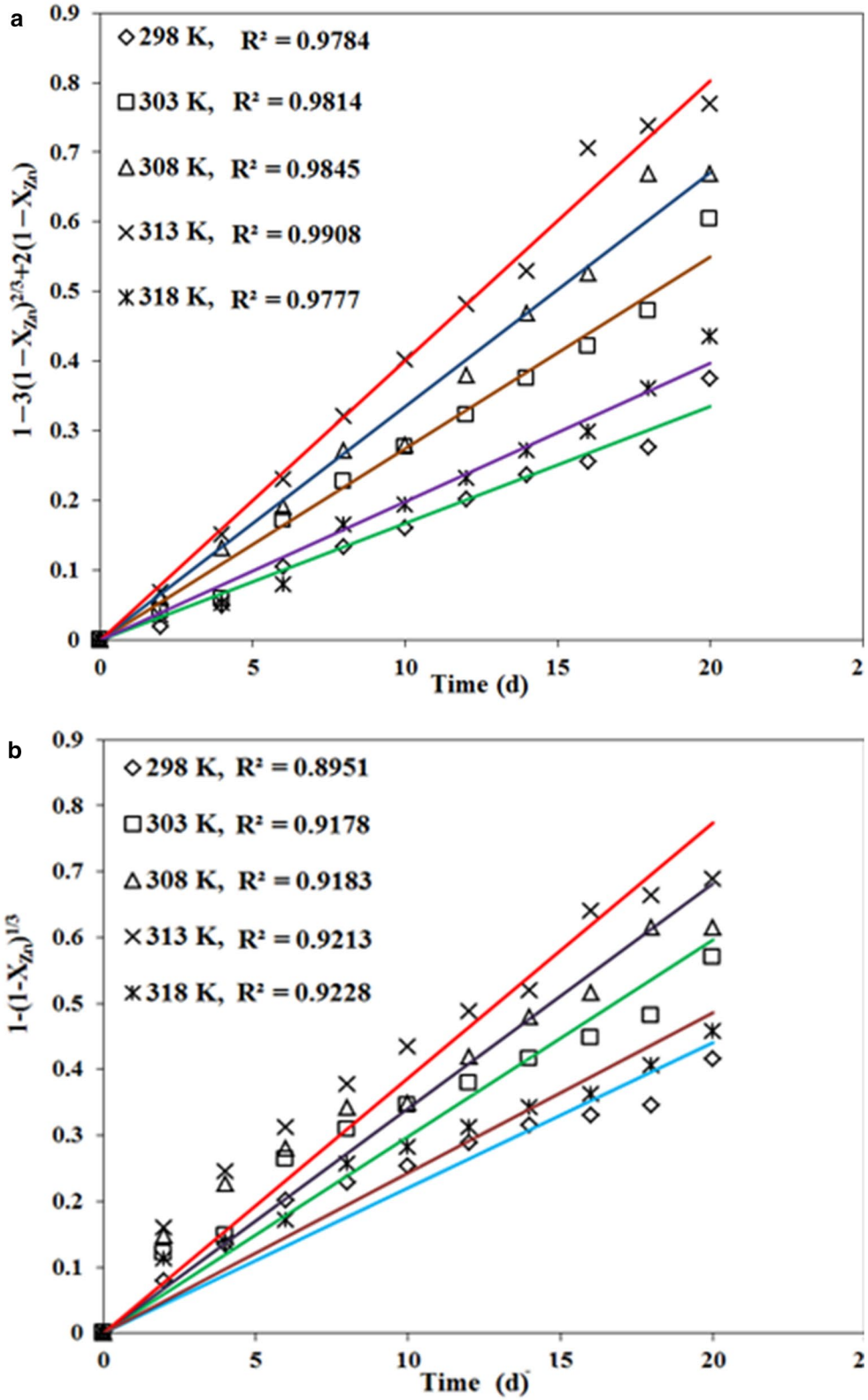

Fig. 9 Fitting of Zn bioleaching data to **a** ash layer diffusion control and **b** chemical reaction control model

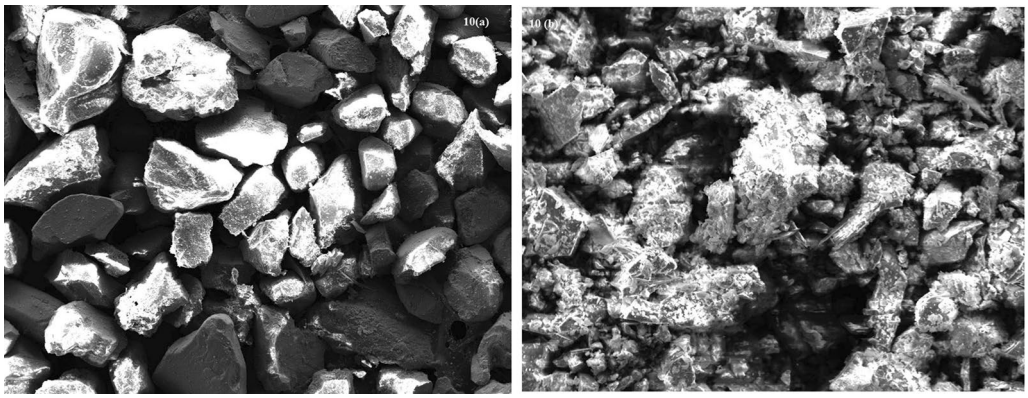

Fig. 10 SEM images of **a** raw ore and **b** leach residue

elemental sulfur layer on the surface of the mineral by the biological reaction (product obtained according to Eq. 3).

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Abbreviations

ASTM	American Society for Testing and Materials
DNA	Deoxyribonucleic acid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EDX	Energy-dispersive X-ray spectroscopy
IOB	Iron-oxidizing bacteria
PCR	Polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SCM	Shrinking core model
SEM	Scanning electron microscope
SOB	Sulfur-oxidizing bacteria
XRD	X-ray diffraction
Zn	Zinc

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Authors' contributions
VPS: microbe isolation, carried out experimental. BR: experimental plan & drafting. NRS: data kinetics & final draft formatting. RK: sphalerite mineral characterization. All authors read and approved the final manuscript.

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Availability of data and materials
The DNA sequences can be found at repositories of Genbank, the link as given below. The experimental data observed have been presented in terms of graphical illustrations https://www.ncbi.nlm.nih.gov/nuccore/KF743135.1.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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