



## Comparison of Copper Dissolution in Chalcopyrite Concentrate Bioleaching with *Acidianus Brierleyi* in Different Initial pH Values

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### Keywords

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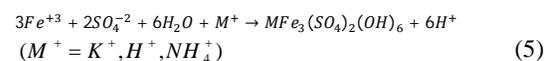
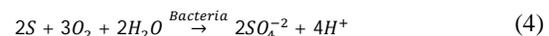
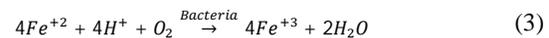
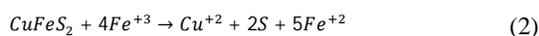
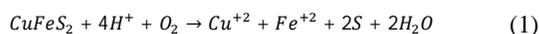
Jarosite precipitation

### Abstract

Although bioleaching of chalcopyrite by thermophilic microorganisms enhances the rate of copper recovery, a high temperature accelerates iron precipitation as jarosite, which can bring many operational problems in the industrial processes. In this research work, the bioleaching of chalcopyrite concentrate by the thermophilic *Acidianus brierleyi* was studied, and the microbial growth, copper dissolution, iron oxidation, and jarosite precipitation were monitored in different initial pH (pH<sub>i</sub>) values. Bacterial growth was greatly affected by pH<sub>i</sub>. While the bacterial growth was delayed for 11 days with a pH<sub>i</sub> value of 0.8, this delay was reduced to nearly one day for a pH<sub>i</sub> value of 1.2. Two stages of copper recovery were observed during all the tests. A high pH<sub>i</sub> value caused a fast bacterial growth in the first stage and severe jarosite precipitation in the later days causing a sharp decline in the bacterial population and copper leaching rate. The copper recoveries after 11 days were 25%, 78%, 84%, 70%, 56%, and 39% for the pH<sub>i</sub> values of 0.8, 1.0, 1.2, 1.3, 1.5, and 1.7, respectively. Sulfur and jarosite were the main residues of the bioleaching tests. It was revealed that the drastic effect of jarosite precipitation on the microbial growth and copper recovery was mainly caused by the ferric iron depletion from solution rather than passivation of the chalcopyrite surface. A slow precipitation of crystalline jarosite did not cause a passive chalcopyrite surface. The mechanisms of chalcopyrite bioleaching were discussed.

### 1. Introduction

Since chalcopyrite leaching is more challenging among other sulfide minerals, bioleaching has drawn much attention among the researchers. Although the initial rate of copper extraction is fast, it diminishes as time passes by, due to the passivation phenomenon. It has been reported that some chemical compounds such as sulfur, polysulfides, and jarosite are mainly responsible for the passivation layer on chalcopyrite [1]. The main reactions in the chalcopyrite bioleaching process have been reported as following [2]:



Although copper recovery is slow (Equation 1), it proceeds more rapidly in the presence of bacterial activity with a direct attack mechanism. Ferric ion is the principal oxidizing agent in the chalcopyrite bioleaching system. Equation 2 demonstrates the so-called indirect leaching of chalcopyrite. Different bacteria used in bioleaching show a diverse capacity of ferrous iron and sulfur oxidation via Equations 3 and 4. Equation 5 shows

how ferric ion can precipitate readily with sulfate and  $M^+$  (where  $M^+$  can be  $K^+$ ,  $H^+$ , or  $NH_4^+$ , all of which may be present in a bioleaching medium) as jarosite [1].

Numerous research works have been performed on the bioleaching of low-grade copper ores containing chalcopyrite and also chalcopyrite concentrate with mesophiles [3, 4] and moderately thermophilic bacteria [5, 6, 3, 7]. Chalcopyrite bioleaching using extremely thermophilic bacteria has been reported to be more capable of achieving a complete recovery of copper from chalcopyrite in fewer days compared with bioleaching with mesophiles [8-10]. Although *At. ferrooxidans* is the most widely studied acidophilic bacteria for the bio-oxidation of refractory gold ores and concentrates, it is less effective in the bioleaching of chalcopyrite. The results of a research on the bioleaching of chalcopyrite using cultures of 11 species of acidophilic Bacteria and Archaea showed that the highest rate of copper solubilization from chalcopyrite was achieved at high temperatures using *Acidianus brierleyi* [1]. *A. brierleyi* has dominated the microbial populations, oxidizing mineral concentrates in pilot-scale bioreactors at Mintek in South Africa [11], and showed the best adaptability and sulfur oxidation ability and pre-dominated in various leaching systems compared with other thermophilic archaea such as *Metallosphaera sedula*, *Acidianus manzaensis*, and *Sulfolobus metallicus* [12].

*Acidianus brierleyi* (DSM 1651) was named for James Brierley, the American bacteriologist who isolated this organism from acidic thermal spring drainage in Wyoming, Yellowstone national park, USA [13]. This Archaeobacteria was first named as *Sulfolobus brierleyi* by Zillig et al. [14] and was later called *Acidianus brierleyi* by Segerer et al. [15] due to the optimum pH range of 1.5-2 for growth. The cell diameter was about 1 to 1.5  $\mu m$ , and the growth was chemolithotrophic through oxidation or reduction of sulfur or by ferrous iron oxidation [15].

Vilcáez et al. [16] have suggested that the use of thermophiles with a higher preference to oxidize elemental sulfur instead of ferrous ion leads to higher copper yields; they reported that additional sulfur in a medium with a high  $Fe^{3+}$  concentration showed the best improvements in the case of bioleaching with *A. brierleyi*. The bioleaching capacity of *A. brierleyi* has been reported to suppress when insufficient initial  $Fe^{3+}$  is provided to trigger the leaching reaction [2]. Konishi et al. [9] have shown that the adsorption of *A. brierleyi* cells to the sulfide surface is attained within the

first 20 min of exposure to the mineral. They also studied the kinetics of *A. brierleyi* growth on the chalcopyrite surface. These authors suggested that the chalcopyrite leaching with *A. brierleyi* was predominantly due to the direct attack by the cells adsorbed on the sulfide surface and that the chemical leaching with ferric iron was insignificant [17]. Liang et al. [18] have studied the bioleaching of chalcopyrite by *A. brierleyi* by an initial pH ( $pH_i$ ) value of 1.5 for all the tests. The tests were performed at a temperature of 68 °C, a rotation speed of 170 r/min, a mineral concentration of 0.2% (w/v), and an initial bacterial concentration of  $1 \times 10^7$  cell/mL. A copper recovery of 60% was obtained after 14 days.

Despite the preference for using thermophiles, with the increase in the temperature, some new difficulties arise. At a higher temperature, the solubility of some compounds decrease and they precipitate more easily at lower pH values resulting in more passivation of chalcopyrite surface and removing the essential nutrients for the bacterial activity of the solution [19]. The most discussed precipitation is related to jarosite (Eq. 5). High temperatures reduce the threshold pH, above which jarosite precipitation takes place [20]. The solution pH has the leading role in this precipitation. It should also be noticed that bacterial activity will be significantly influenced by the solution pH [2].

Extensive attempts have been made for solving chalcopyrite passivation during bioleaching and increasing the rate of copper recovery in bio-heaps and stirred tanks [8]. However, understanding the possible changes that happen in different pH values is essential for effective pH control in a bioleaching solution regardless of the industrial bioleaching methods. Among the operating parameters involved, pH has a vital role in the dissolution and passivation of chalcopyrite in bioleaching for three main reasons. First, the dissolution of chalcopyrite consumes acid. Secondly, the microorganisms are sensitive to the solution pH, which undeniably influences the activity and growth of the microorganisms. Thirdly, pH affects the chemical reactions in the solution, especially the production of jarosite, which is regarded as a critical candidate for chalcopyrite passivation [20].

In this research work, the effects of ( $pH_i$ ) of the solution on the bioleaching of chalcopyrite concentrate by *Acidianus brierleyi* were studied by performing the shake flask tests at six  $pH_i$  values in the range of 0.8-1.7. In the meantime, the bacterial activity, solution pH changes, and mechanisms of chalcopyrite bioleaching were discussed. The main objective of this work was to evaluate the

susceptibility of chalcopyrite concentrate bioleaching by *Acidianus brierleyi* under different solution conditions caused by pH changes. These conditions were characterized by monitoring the parameters involved including copper, total iron, ferric ion, and bacterial concentration along with the pH and ORP of solutions and the solid residue compositions.

No detailed study has considered the effect of pH<sub>i</sub> on the activity of *Acidianus brierleyi*, jarosite precipitation, and the copper dissolution rate. This work will provide some new information for evaluating the behavior of *Acidianus brierleyi* and chalcopyrite bioleaching under different pH<sub>i</sub> values. Also, the rate of jarosite precipitation and its influence on copper leaching was discussed. Such data could be of great importance in a deeper understanding of the bioleaching mechanisms, resulting in more controllable industrial applications such as thermophilic heap bioleaching and bioleaching of concentrates in stirred tanks.

## 2. Materials and Methods

### 2.1. Chalcopyrite concentrate

The copper concentrate was obtained by treatment of the Mazraeh copper ore by magnetic separation followed by flotation tests to remove magnetite, quartz, and pyrite as the main gangue minerals (Figure 1). A dry drum magnetic separator was used to separate the magnetite particles before flotation. The non-magnetic part of the ore was grounded by a laboratory ball mill to liberate the gangue minerals from the chalcopyrite particles. The ground concentrate was sieved to obtain a size fraction of 75 μm (d<sub>80</sub> = 63 μm), and the coarser particles turned back to the mill. Potassium ethyl xanthate (PEX) was used as a chalcopyrite collector in the first flotation stage, while pH was fixed to 11 by lime addition to preventing pyrite flotation. Pyrite removal was of vital significance because it was attended to avoid the possible galvanic interactions in bioleaching tests, which could affect the intrinsic chalcopyrite leaching rate [19, 21]. Therefore, a cleaner flotation stage was performed by adding sodium cyanide as a pyrite depressant.

The interference of the flotation reagents on the bioleaching process has been reported [22]. The presence of unknown oxide minerals in the concentrate can create ambiguity in the interpretation of the results. Therefore, the concentrate was washed consecutively with HNO<sub>3</sub> (1 M), deionized water, and pure acetone to remove the possible fine copper oxide minerals and the flotation reagents. The X-ray powder diffraction

(XRD) analysis indicated that the concentrate mostly included the chalcopyrite particles and a small amount of Quartz (Figure 2). The concentrate copper grade was 32%, analyzed by atomic absorption spectroscopy (AAS).

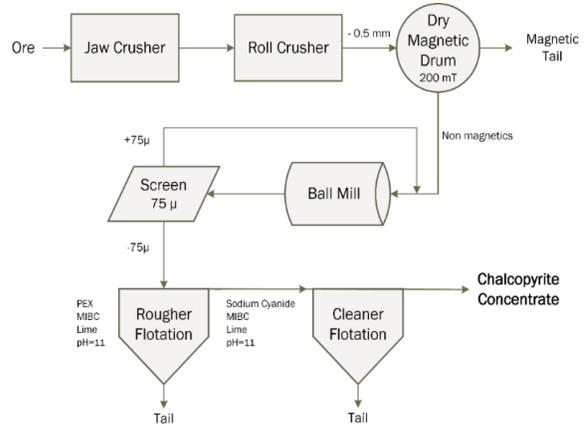


Figure 1. A flowsheet of the laboratory procedure for chalcopyrite concentrate production.

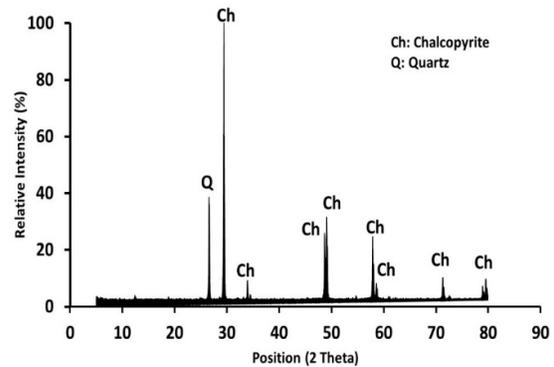


Figure 2. X-ray diffraction pattern of the chalcopyrite concentrate.

### 2.2. Microorganisms and culture medium

The thermophile strain *Acidianus brierleyi* DSM 1651 was obtained from the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The thermophiles were grown aerobically at 60 °C in the appropriate media containing 3.0 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.5 g/L K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 0.5 g/L MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 g/L KCl, 0.01 g/L Ca(NO<sub>3</sub>)<sub>2</sub>, and 0.2 g/L of yeast extract [14]. The original DSM 1651 strain was adapted by the multiple-transfer technique to a medium containing the chalcopyrite concentrate, as the sole energy source.

### 2.3. Apparatus and procedure

All the tests were carried out in 250 mL Erlenmeyer flasks containing 100 mL of the solution (90 mL of the culture medium and 10 mL

of inoculum, 10% (v/v)), and supplemented with 1.0 g of chalcopryrite concentrate (the concentrate/liquid loading ratio was 10 kg/m<sup>3</sup>). The flasks were incubated in a rotary shaker at 120 RPM and a temperature of 60 °C, while the initial cell concentration was around 3×10<sup>7</sup> cells/mL. The water evaporation was compensated with distilled water, and pH<sub>i</sub> was adjusted using a diluted sulfuric acid solution only at the beginning of the experiments, not during the leaching reaction.

#### 2.4. Analytical techniques

In all the leaching tests, the sample solutions were withdrawn for measurement of the copper and cell concentration in the bioleaching solution. The cell concentration was attained by direct counting using a Thoma chamber counter of 0.1 mm depth and 0.0025 mm<sup>2</sup> area under an optical microscope. The fraction of leached chalcopryrite was determined from the copper content in the solution at any time, divided by the original copper content in the concentrate. The copper concentration was obtained using AAS.

The ferric and total iron concentrations in the solution were determined by the 5-sulfosalicylic acid (SSA) spectroscopy method [23]. In this method, the red color formed by the SSA reaction with ferric ion was used to determine the ferric ion concentration. 100 µL of the samples were mixed with 3 mL of the solution of 10% SSA and diluted to 100 mL. The adsorption value of this solution was measured at the 500 nm wavelength using a CesiL 7200 spectrophotometer in order to determine the ferric ion concentration. Then 3 mL of ammonia was added to this solution and the adsorption value of the solution was measured at 425 nm for the total iron concentration analysis.

The solid residues were dried in air and the samples were taken for X-ray diffraction (XRD), scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS), and Fourier transform-infrared (FT-IR) spectroscopy.

### 3. Results and Discussion

#### 3.1. Bacterial growth

Most of the bacteria used in bioleaching preferred the environment pH requirements in acidic solutions. The optimum pH range for the *Acidianus brierleyi* growth has been reported to be 1.5-2 [15]. The shake-flask tests with different pH<sub>i</sub> values (pH<sub>i</sub> = 0.8-1.7) were performed to investigate the bacterial growth and copper recovery. In the first experiments, it was observed that the pH of the bioleaching solution increased as the concentrate leaching consumed acid during the test period.

Therefore, pH<sub>i</sub> of the solutions was chosen to be below 1.5 to make the solution pH values reach an optimum pH range of 1.5-2.0 in the next days.

Figure 3a compares the bacterial concentration changes in leach solutions with different pH<sub>i</sub> values. The overall trend was characterized by a rapid early increase to a peak followed by a drop in the growth rates or nearly constant bacterial population. The test with pH<sub>i</sub> 0.8 showed a slow growth rate and reached a peak within 17 days. According to Figure 4, the trend of pH change in the leaching solution is relatively smooth, reaching pH 1.1 after 20 days. It seems that the highly acidic solution decelerated the bacterial growth, which, however, started after a lag phase of 11 days.

In the test with pH<sub>i</sub> equal to 1.0, pH did not even reach the optimum range lower limit (1.5) during the experiment (Figure 4). Nevertheless, showing a complete growth curve, the bacterial concentration was enhanced compared with the test starting with pH<sub>i</sub> 0.8, and the lag phase decreased to two days. The experiment with pH<sub>i</sub> 1.2 also experienced a typical growth curve even with a shorter lag time. The bacterial growth was rapid in the first four days of the test with pH<sub>i</sub> 1.3 but suddenly stopped and continued stationary. Similarly, for pH<sub>i</sub> values of 1.5 and 1.7, the early fast growth stopped and a sharp decline in the bacterial concentration occurred after the peak.

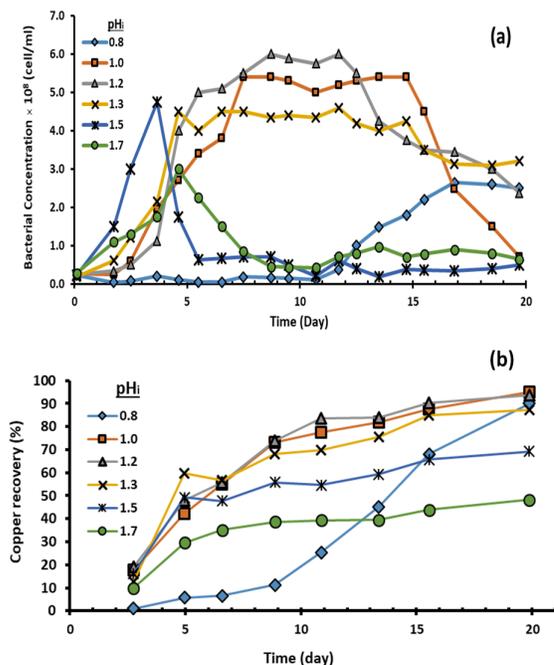


Figure 3. Bacterial concentration (a) and copper recovery (b) in bioleaching tests starting with different pH<sub>i</sub> values.

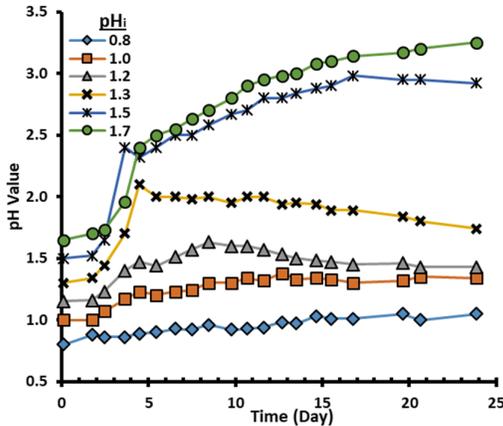


Figure 4. Solution pH changes during bioleaching tests starting with different initial  $pH_i$  values.

### 3.2. Copper extraction

Figure 3b shows the copper recovery during the bioleaching time. Two stages in copper recovery (after the lag phase) appeared in the tests with different  $pH_i$  values, which consisted of an introductory faster copper extraction continued with a slower extraction stage. This data suggests that copper recovery is greatly influenced by  $pH_i$ . Comparison of Figure 3a with Figure 3b shows the compatibility of the copper leaching rate with the bacterial growth rate.

According to Eq. 1, proton consumption occurs when a direct mechanism of chalcopyrite bioleaching proceeds. In the case of indirect bioleaching by ferric ions, pH increase is also a confirmation sign for the bacterial oxidation of ferrous ions (Eq. 3). In the present work, no extra amount of iron was added and the chalcopyrite concentrate was the sole source of ferrous ions. Therefore, it can be concluded that the  $H^+$  consumption rate is proportional to chalcopyrite leaching regardless of the bioleaching mechanism.

### 3.3. Iron oxidation

The chemical ferrous iron oxidation to ferric ion in acidic environments ( $pH \approx 2$ ) is negligible even in the oxygen-saturated solutions [24]. The leaching microorganisms catalyze the ferrous iron oxidation using the enzymes located on their cell wall. This catalytic action is the crucial aspect in the bioleaching process as it contributes to enhancing the rate of electron transfer from the sulfide to the final electron acceptor, oxygen. The leaching microorganisms biologically remove electrons from Fe(II) through a series of electron carriers from the outer membrane to the cytoplasm, where they reduce oxygen to water-consuming protons in the process [25]. The ferric ion and total iron concentrations were monitored during the tests

(Figure 5). The negligible ferric concentration in the first week of bioleaching with a  $pH_i$  of 0.8 shows a poor bio-oxidation of ferrous ions, keeping ORP low and nearly stationary for ten days (Figure 6). It can be assumed that the highly acidic solution prevented bacterial growth (Figure 3) and activity, leading to an inconsiderable copper and iron recovery. On the other hand, a high  $pH_i$  also negatively influences the chalcopyrite bioleaching. In this case, the inadequate ferric ion is not due to weak biological oxidation but it is due to the iron precipitation.

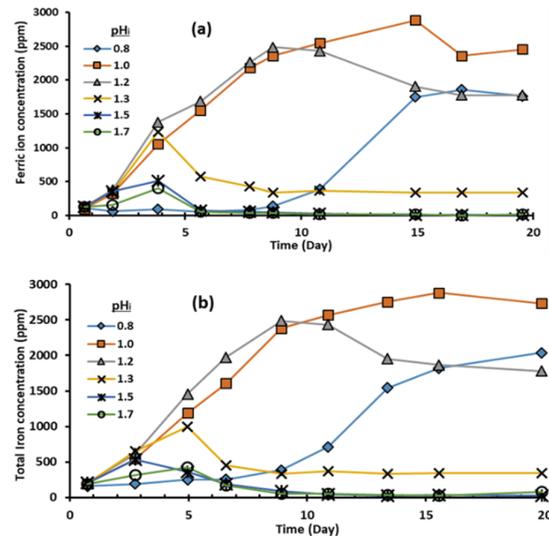


Figure 5. Ferric ion (a) and total iron (b) concentration changes during bioleaching tests starting with different  $pH_i$  values.

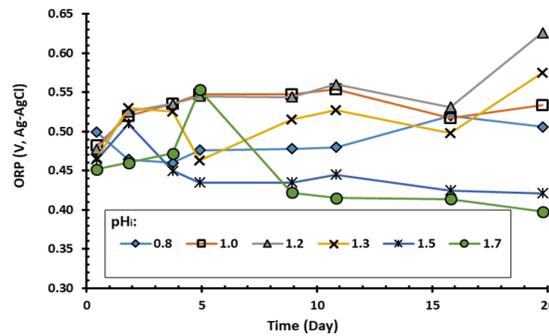


Figure 6. Oxidation-reduction potential changes during bioleaching tests starting with different  $pH_i$  values.

### 3.4. Analysis of residues

According to Table 1, in the tests with  $pH_i$  values of 1.2, 1.3, 1.5, and 1.7, the precipitation of iron occurred. Figure 7 compares the XRD patterns of the residuals remaining from the bioleaching tests with different  $pH_i$  values. The FT-IR and EDS analyses confirmed the presence of potassium

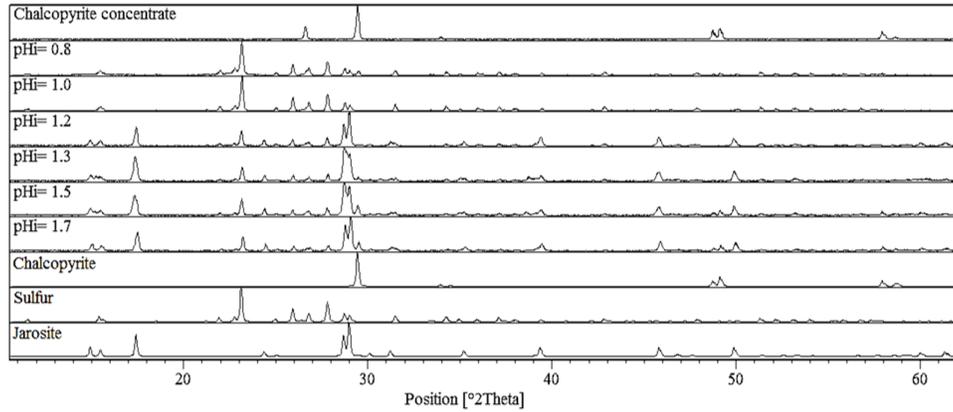
jarosite in the residuals left from the mentioned tests (Figures 8 and 9). Potassium jarosite is a basic ferric sulfate that precipitates according to Eq. 5. Comparing the FT-IR spectra of the residues remaining from the tests with  $pH_i$  1.0 and  $pH_i$  1.5 shows the peaks for specific atomic bonds. The peaks at  $3385\text{ cm}^{-1}$  and  $1005\text{ cm}^{-1}$  stood for OH, 1193, 1085, and 626 for  $\text{SO}_4$ , and 474 and 508 for

$\text{FeO}$ . Therefore, the existence of potassium jarosite in the precipitation was confirmed from this spectrum along with the XRD and EDS results. However, the peak in 1428 probably stands for  $\text{NH}_4^+$  and suggests that a part of jarosite is in the form of ammonium jarosite ( $\text{NH}_4^+$  originated from  $(\text{NH}_4)_2\text{SO}_4$  that was added as the culture medium nutrition) [26].

**Table 1. Weight and composition (XRD) of residuals remaining from bioleaching tests with different  $pH_i$  values.**

$pH_i$	Lag phase (day)	Residual weight (g)	Residual composition <sup>a</sup>
0.8	11	0.34	Ch, S
1.0	2	0.24	S
1.2	1	0.52	S, J
1.3	< 1	0.96	S, J
1.5	< 1	1.12	Ch, S, J
1.7	< 1	1.10	Ch, S, J

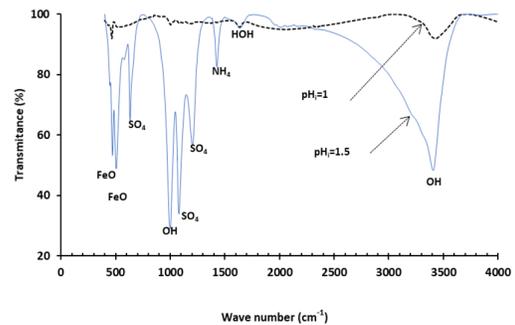
<sup>a</sup>Detected by XRD method: Ch: Chalcopyrite; S: sulfur; J: Jarosite



**Figure 7. XRD patterns of residuals remaining from bioleaching tests with different  $pH_i$  values.**

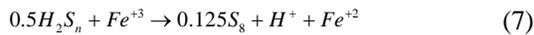
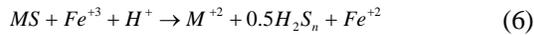
Comparing Figure 4 and Figure 5a, noticeable slight pH falls in the tests at  $pH_i$  1.5 (after three days),  $pH_i$  1.3 (after four days), and  $pH_i$  1.2 (after ten days) were coincident with the ferric precipitation as jarosite. For jarosite formation, two factors must be provided that involve sufficient concentrations of ferric ion and required pH value. The more ferric ion concentration offers the possibility of jarosite precipitation in the lower pH values (Eq. 5). At a low  $pH_i$  value (1.2), the ferric concentration was increased more rapidly than in the experiments with higher  $pH_i$  values (1.5 and 1.7). Therefore, the jarosite precipitation is possible at lower pH values. In these conditions, precipitation occurs gradually and at a slow rate. This continuous jarosite formation generated acid (Eq. 5) and prevented a severe pH rise in the following days. In the tests with higher  $pH_i$  values (1.5 and 1.7), fast and severe jarosite precipitation happened due to the high pH values, and a large portion of the existing ferric ions in solution was

removed. This phenomenon is confirmed by the sudden reduction of bacterial concentration (Figure 3), iron concentration (Figure 5), and ORP (Figure 6). The solution pH increased in the following days due to acid consumption for chalcopyrite dissolution.



**Figure 8. FT-IR spectra of residuals remaining from bioleaching tests with  $pH_i$  values of 1 and 1.5.**

The SEM image of the residual from the bioleaching test at  $\text{pH}_i$  1.5 shows the chalcopyrite structure, depleted of iron and copper, as well as flaky precipitations placed on the remaining surfaces (Figure 9). The EDS analysis of the specific points on the surface of leached chalcopyrite confirmed the presence of sulfur as a single residue. Analysis of the flaky precipitates confirmed the potassium jarosite composition. Although polysulfides ( $\text{XS}_n$ ) or metal deficient sulfides were reported to be among the candidates for chalcopyrite surface passivation [27], in this research work, no sign of any passive layer consisting of these compounds was observed on the surface of the residues at the end of the process. It could be due to the high oxidation potential of the bioleaching solutions (Figure 6), which resulted in complete oxidation of these intermediate phases to metal ions and elemental sulfur. Formation and further oxidation of polysulfide in leaching of sulfide minerals can be presented as the following equations [17]:



The EDS map images confirmed the presence of iron, potassium, sulfur, and a small amount of copper, which is in agreement with the XRD results. The copper map (displaying the remaining chalcopyrite particles) is in exact accompaniment by the sulfur map, which indicates that sulfur is the primary residue from the chalcopyrite particles. The location of iron and potassium dots is almost similar, showing the jarosite precipitates.

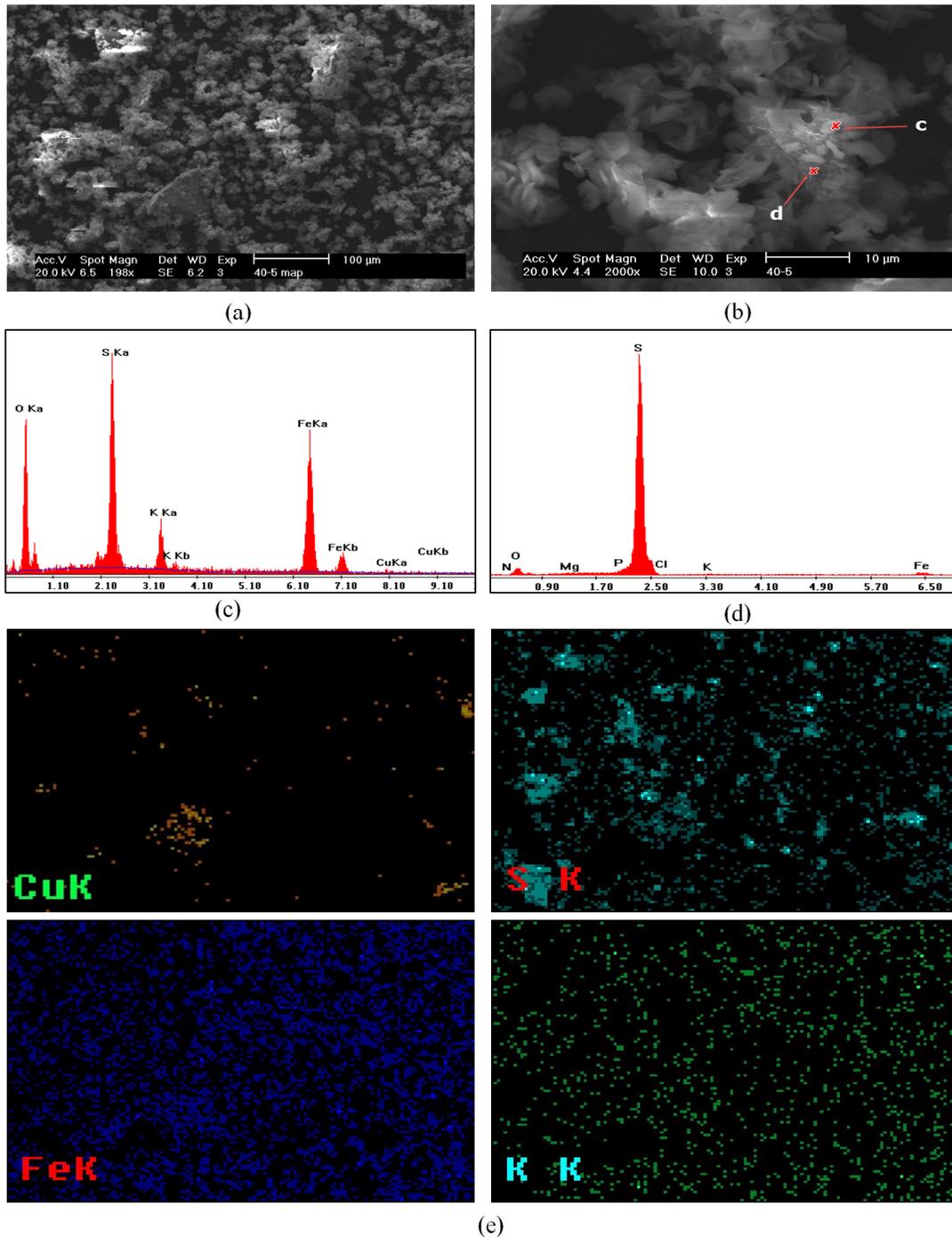
### 3.5. Mechanisms

Vargas et al. [28] have divided the bioleaching process into different parts with unlike control systems. It was stated that at the beginning of bioleaching, with a small population of microorganisms and fresh sulfide mineral, the bioleaching process was only biologically controlled. Improvement of the copper leaching rate could be obtained in this case either through an increase in the bacterial population or by improving their specific activity. New inoculation or more adaptation can be the candidate methods to achieve these objectives, respectively. As the bioleaching advances, the population of bacteria

grows up, and the reactivity of the sulfide mineral decreases. Here, the chemical reactions control the process, either by the kinetics of ferric leaching of the sulfide or by the mass transfer of ferric ions to the sulfide particles.

The results of the tests in the current research work confirmed the two main stages stated by Vargas et al. [28] and revealed the great effect of  $\text{pH}_i$  on both the biological and chemical processes. When a greater  $\text{pH}_i$  value up to 1.5 was applied, the bacterial activity was significantly improved in the first days and thus speeded up the copper extraction in the biologically controlled region (Figure 3). On the other hand, decreasing the ferric ion concentration (according to Figure 5a) caused a hindered copper extraction in the chemical control stage. Using a lesser  $\text{pH}_i$  value, to the contrary, created a lag phase of bacterial growth in the first stage but facilitated the ferric mobility in the second stage. Therefore, there is an opposite effect of  $\text{pH}_i$  in the early days of the process and the later days.

The results obtained showed that although jarosite precipitation happened for all the tests with  $\text{pH}_i$  values of 1.2 to 1.7, its impact on the bioleaching was different. The residual weight became heavier by increasing  $\text{pH}_i$  (Table 1). Severe iron precipitation in the tests with  $\text{pH}_i$  values of 1.5 and 1.7 not only stopped the bacterial growth but also caused a great loss of the active bacterial concentration. Slower iron precipitation in the test with  $\text{pH}_i$  1.3 only prevailed more growth and kept the growth curve stationary. Although jarosite precipitated at  $\text{pH}_i$  1.2, a considerable bacterial concentration and growth with the best copper recovery were gained in this condition. These results proved the possibility of decreasing the iron precipitation negative role by controlling the solution conditions so that precipitation was postponed until after the main copper recovery duration (nearly first week). It is also necessary to keep the precipitation rate as slow as possible. Although jarosite has been reported to be capable of chalcopyrite surface passivation [29], by considering the results of the current research, it may be preferable to use a higher pH by controlled jarosite precipitation. Figure 9 shows the concentrate particle's surface covered with somewhat porous crystals of jarosite. A slower precipitation could also cause a more crystalline jarosite and a lesser passivation.



**Figure 9.** SEM image and EDS analysis of residuals remaining from bioleaching test with  $pH_i$  1.5 (a: SEM image 198X, b: SEM image 2000X, c and d: point EDS analysis, e: elemental map EDS analysis of (a)).

The present results suggest that the negative impact of jarosite precipitation is more about the ferric ion depletion of the solution rather than surface passivation. Comparing the copper extraction, bacterial growth, and ferric ion concentration for the bioleaching tests revealed that when jarosite precipitation occurred intensely (tests with  $pH_i$  values of 1.5 and 1.7), the bacterial population

decreased severely. The reduction in the ferric ion concentration (and consequently, total iron) directly affected both the leaching process and the bacterial activity dramatically.

The ferric/ferrous iron ratio plays a key role in the bioleaching kinetics and influences the rate of both the chemical and biological processes. However, as recognized in Figure 5, in chalcopyrite bioleaching

by *A. brierleyi*, nearly all the iron content of the solution exists in the form of ferric ions due to the high iron oxidation rate by the microorganism. It has been suggested that an increase in the ferric/ferrous iron ratio will increase the rate of chalcopyrite oxidation but will decrease the rate of bacterial oxidation of  $\text{Fe}^{+2}$ , while the opposite effect will occur when the ferric/ferrous iron ratio decreases [28]. When severe jarosite precipitation occurs, ( $\text{pH}_i$  1.5 and 1.7), the ferric content of the solution is removed, leading to a smaller ferric/ferrous ratio. According to the mentioned suggestion, this should alleviate the chemical leaching rate and increase the bacterial oxidation activity. However, as shown in Figure 3, both the copper extraction and bacterial population decreased dramatically. It should be noticed that ferric iron has a vital role in electron transfer in the solution and inside the microorganisms [25], and a minimum amount of ferric ion is required to trigger the energy metabolism. On the other hand, in the case of no additional iron sources (like in the present research work), fewer amounts of ferric ion mean a smaller extent of chemical leaching of chalcopyrite, and consequently, to lessen ferrous iron, as the main energy supply of the microorganisms. In the lack of iron, the bacteria are surprisingly not capable of sulfur oxidation as a substitute of iron, as its energy source, and headed to death, while sulfur grown *A. brierleyi* easily oxidizes sulfur in the absence of iron.

It has been reported that a lower oxidation-reduction potential (400-425 mV) is preferred in chalcopyrite bioleaching with mesophiles and moderate thermophiles [7]. However, in the present work, the best results were obtained in the tests with the  $\text{pH}_i$  values of 1.0 and 1.2, while ORP was in the range of 500-550 mV. These results show that the impact of ORP is primarily dependent on the other parameters such as the  $\text{pH}_i$  value, iron concentration, and the rate and quality of jarosite precipitation.

In the control test with sulfur, as the sole energy source, it was observed that *A. brierleyi* had a great ability in the sulfur oxidation to sulfuric acid, and consequently, decreasing the solution pH, while in the chalcopyrite bioleaching tests, no notable decrease in pH occurred (Figure 4). Therefore, it seems that *A. brierleyi* adapted to the chalcopyrite concentrate prefers to oxidize ferrous ions rather than secondary elemental sulfur that is accessible for the bacteria, confirmed by the SEM and XRD analyses. Ferrous oxidation (Eq. 3) is faster than sulfur oxidation (Eq. 4), and therefore, more protons are consumed by iron oxidation and fewer

are produced by sulfur oxidation, and consequently, the pH rises. These observations are along with the confirmation of the existence of one of the most important members of the sulfur-oxidizing (Sox) enzyme system, SoxB gene, in chalcopyrite bioleaching by *A. brierleyi* [12].

Although the energy yield from oxidizing iron is much lower than that available from sulfur oxidation, ferrous iron is usually used preferentially as an electron donor by acidophiles that can oxidize both iron and sulfur. *At. ferrooxidans* has been reported to prefer the use of ferrous iron instead of reduced sulfur when oxidizing pyrite, in opposition to the thermodynamic sense. The suggested reason is that a reduced sulfur species oxidation happens through a longer electron transport chain and requires to synthesize more enzymes, while energy yields in unit time are similar for both the tetrathionate and ferrous iron oxidation [24]. The extent of microorganisms attached to the mineral surface can be effective, and the sulfur oxidation rates by thermophilic archaea in biofilms have been reported to be completely different from those in suspension [30].

#### 4. Conclusions

In the current research work, we reported the investigation of the effect of initial pH ( $\text{pH}_i$ ) on the chalcopyrite concentrate bioleaching by *Acidianus brierleyi*. The results obtained showed that the bacterial growth was greatly affected by the  $\text{pH}_i$ . At a lower  $\text{pH}_i$ , a greater delay time on the bacterial growth curve was observed (11 days for  $\text{pH}_i = 0.8$ ), and a highly acidic solution decelerated the bacterial growth. As  $\text{pH}_i$  increased, a shorter lag phase appeared but the bacterial activity was disturbed in the later days.

The results obtained suggested a great effect of  $\text{pH}_i$  on both the biological and chemical processes, and consequently, copper recovery. An opposite effect of  $\text{pH}_i$  in the early days of the process and the following days was observed. When a greater  $\text{pH}_i$  up to 1.5 was applied, the bacterial activity was significantly improved in the first days and thus hustled the copper extraction. On the other hand, decreasing the ferric ion concentration caused the hindering of the copper extraction in the second stage. The best copper recovery was achieved with  $\text{pH}_i$  values equal to 1.0 and 1.2.

The thermophilic *Acidianus brierleyi* showed great ability in iron oxidation. The greatest dissolved iron concentration and the smallest residual weight were obtained with  $\text{pH}_i = 1.0$ . Elemental sulfur was the only residue in this test. Iron precipitation was

accelerated in the form of potassium and ammonium jarosite as  $pH_i$  was raised from 1.2 to 1.7. The presence of the flaky jarosite precipitates was confirmed by SEM imaging along with EDS analysis.

The present work proved the possibility of decreasing the jarosite precipitation negative role by controlling the solution conditions so that precipitation happened after the main copper recovery duration. It was also important to keep the precipitation rate as slow as possible. It was discussed that the drastic effect of jarosite precipitation was mainly caused by ferric iron depletion from solution rather than surface passivation.

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## مقایسه انحلال مس در بیولیچینگ کنسانتره کالکوپیریت با استفاده از اسید یانوس بریرلی در مقادیر مختلف pH اولیه

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### چکیده:

اگرچه بیولیچینگ کالکوپیریت با باکتری‌های گرمادوست باعث افزایش سرعت بازیابی مس می‌شود، ولی دمای بالا، نرخ رسوب آهن به صورت جاروسیت را افزایش می‌دهد که می‌تواند باعث بروز مشکلاتی در فرایندهای صنعتی شود. در تحقیق حاضر بیولیچینگ کنسانتره کالکوپیریت با استفاده از باکتری گرمادوست / اسیدیانوس بریرلی انجام شده و مقادیر رشد باکتری‌ها، انحلال مس، اکسیداسیون آهن و رسوب جاروسیت در آزمایش‌هایی با pH اولیه مختلف بررسی شده‌اند. نتایج نشان داد که رشد باکتری‌ها به شدت از مقدار pH اولیه تاثیر می‌پذیرد. در آزمایش با pH اولیه 0.8، رشد باکتری‌ها بعد از یازده روز تاخیر آغاز شد، در حالی که این زمان تاخیر برای pH اولیه 1/2 تقریباً به یک روز کاهش یافت. دو مرحله برای بازیابی مس در همه آزمایش‌ها مشاهده شد. استفاده از pH اولیه بالا باعث رشد سریع باکتری‌ها در مرحله اول و رسوب شدید جاروسیت در روزهای بعدی همراه با کاهش شدید جمعیت باکتری‌ها و نرخ لیچینگ مس شد. بازیابی مس بعد از یازده روز بیولیچینگ با pH های اولیه 0.8، 1.0، 1.2، 1.3، 1.5 و 1.7 به ترتیب برابر 25، 78، 84، 70، 56 و 39 درصد به دست آمد. گوگرد عنصری و جاروسیت مهمترین ترکیبات باقیمانده آزمایش‌ها بودند. مشخص شد که نقش منفی رسوب جاروسیت بر تعداد باکتری‌ها و بازیابی مس، بیشتر به خاطر کاهش شدید یون فریک در محلول اتفاق می‌افتد و رسوب آهسته و بلوری جاروسیت باعث غیرفعال شدن سطح کالکوپیریت نمی‌شود. در ادامه مکانیزم‌های بیولیچینگ کالکوپیریت بحث شدند.

**کلمات کلیدی:** بیولیچینگ، کالکوپیریت، اسیدیانوس بریرلی، pH اولیه، اکسیداسیون آهن، رسوب جاروسیت.