Bioremoval of iron ions from copper raffinate solution using biosynthetic jarosite seed promoted by *Acidithiobacillus ferrooxidans*

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ABSTRACT: The decrease of iron ions concentration from raffinate solution in copper solvent extraction is significant to maintain good current efficiency. In this study, the combined effects of biosynthetic jarosite seed and *Acidithiobacillus ferrooxidans* on iron removal of copper raffinate solution and effect of pH were investigated. Ammonium jarosite seeds biosynthesis was performed efficiently at pH 2 and Fe²⁺ concentration of 50 g·1⁻¹. The percentage of iron removal from raffinate solution at pH of 1.5, 2.5 and 5% seed dosage were 0.81%, 47.38%, and 71.26%, respectively. The iron concentration in raffinate solution was eliminated noticeably up to 71.17% with the incorporation of 10 wt.% jarosite seed and 10% V/V *A. ferrooxidans* together. This was due to the increase in specific solids surface in the solution and magnetic features of *A. ferrooxidans* which promoting the removal of iron. pH 2 and jarosite seed loading of 10% was selected as the optimum conditions for iron removal from the raffinate solution. It was concluded that the recovery of copper from biologically refined raffinate could be increased.

KEYWORDS: *A. ferrooxidans*; Ammonium jarosite; Copper raffinate solution; Iron removal; Magnetosome; Solid surface

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RESUMEN: Biorremoción de iones de hierro de la solución de refinado de cobre utilizando "semilla" de jarosita biosintética promovida por Acidithiobacillus ferrooxidans. La disminución de la concentración de iones de hierro de una solución de refinado procedente de la extracción con solvente de cobre es importante para mantener una buena eficiencia de corriente en el proceso de electrolisis. En este estudio, se investigaron los efectos combinados de la "semilla" de jarosita biosintética y Acidithiobacillus ferrooxidans sobre la eliminación de hierro de la solución de refinado de cobre y el efecto del pH. La biosíntesis de "semillas" de jarosita de amonio se realizó de manera eficiente a pH 2 y una concentración de Fe² + de 50 g·l⁻¹. El porcentaje de eliminación de hierro de la solución de refinado a un pH de 1,5; 2,5 y 5% de dosis de semillas fue de 0,81%, 47,38% y 71,26%, respectivamente. La concentración de hierro en la solución de refinado disminuyó notablemente hasta alcanzar un valor del 71,17% con la incorporación de un 10% en peso de "semilla" de jarosita junto con un 10% V/V de A. ferrooxidans. Esto se debió al aumento en la superficie específica de los sólidos en la solución y a las características magnéticas de A. ferrooxidans que promueven la eliminación de hierro. Se seleccionó un pH 2 y una carga de "semillas" de jarosita del 10% como las condiciones óptimas para la eliminación del hierro de la solución de refinado. Se concluyó que se podría incrementar la recuperación de cobre del refinado biológicamente refinado.

PALABRAS CLAVE: A. ferrooxidans; Eliminación de hierro; Jarosita de amonio; Magnetosoma; Solución de refinado de cobre; Superficie sólida

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1. INTRODUCTION

The impact of iron ions concentration in copper solvent extraction in hydrometallurgy SX-EW processing is well known. Copper raffinate solution with high iron and low copper concentration is an acidic solution that forms in hydrometallurgy solvent extraction. An appropriate concentration of ferric ion is very important in this process (Watling, 2006; Eftekhari et al., 2020). Its high concentration enhances the power of consumption, reduces the efficiency and alters the surface morphology of cathode copper (Gramp et al., 2008; Nurmi et al., 2010). The common techniques for this problem are costly and inefficient (Gan et al., 2017). To set a cost-effective biological method for the elimination of iron was the main purpose of the present study. Therefore, the combined effects of biosynthetic jarosite seed and Acidithiobacillus ferrooxidans (A. ferrooxidans) were studied to control iron in the process.

Jarosite is a crystal structure of alunite mineral subgroup with the general formula [M Fe₃ $(SO_4)_2(OH)_6$], where M= NH₄⁺, H₃O⁺, K⁺, Na⁺, Ag⁺, Hg²⁺ or Pb²⁺ (Hou *et al.*, 2015; Wang *et al.*, 2006; Zhang *et al.*, 2018; Liu and Zhang, 2019; Eftekhari *et al.*, 2020). Biosynthesis of jarosite type compounds is mainly related to bio-oxidation of Fe²⁺ to Fe³⁺ by *A. ferrooxidans.* The rate of biosynthesis depends on concentrations of Fe²⁺, pH value, temperature, dissolved oxygen levels and monovalent or divalent cations (Pogliani and Donati, 2000; Hou *et al.*, 2015; Bao *et al.*, 2018; Eftekhari and Kargar, 2018).

According to studies, jarosite stability is in the form of $K^+ > NH_4^+ > Na^+ > H_3O^+$ (Eftekhari *et al.*, 2020). Therefore, Jarosite can be added to act as a seed crystal to promote the formation of new jarosite on the seed surface (Li *et al.*, 2016; Yang *et al.*, 2020; Eftekhari *et al.*, 2020).

Investigates showed cations like nickel, zinc, copper, cobalt, can be incorporated into jarosite crystals; therefore, some copper ions in solution could precipitate with iron but would be less than co-precipitation that happens with hydroxide precipitation (Dutrizac and Monhemius, 1986; Izadi et al., 2017). This method has some advantages like simplicity, low-cost operation, and protection of equilibrium between sulfuric acid and alkali ions (Gan et al., 2017). Several studies are performed on the removal of different ions in solutions by using chemical precipitation (Pogliani and Donati, 2000; Wang et al., 2006; Gramp et al., 2008; Gaikwad and Gupta, 2008; Nurmi et al., 2009; Albrecht et al., 2011; Lin et al., 2016; Gan et al., 2017; Izadi et al., 2017; Alguacil, 2019) However the lower-cost biological techniques are limited.

This study focused on reducing the concentration of iron from copper raffinate solutions from the Sarcheshmeh copper complex, Iran. The jarosite was synthesized and combined effects of biosynthetic jarosite seed and *A. ferrooxidans* on iron removal were investigated. Also, the effect of pH on iron removal was investigated.

The expected results obtained from this work can improve the efficiency of the de-ironing process in copper bioleaching and reduce manufacturing cost.

2. MATERIALS AND METHODS

2.1. Preparation of A. ferrooxidans

In this study, a pure culture of A. ferrooxidans bacteria was used. The bacteria were separated from acid mine drainage from the Sarcheshmeh copper mine located in the southwest of Kerman, Iran. The bacteria were inoculated into flasks containing 130 ml of 9k medium with the rate 10% v/v. The 9K medium $\begin{array}{l} \text{containing: 3 g}\cdot l^{-1} \ (NH_4)_2 SO_4, \ 0.5 \ g \cdot l^{-1} \ Mg SO_4 \cdot 7H_2O, \\ 0.63 \ g \cdot l^{-1} \ K_2 HPO_4, \ 0.1 \ g \cdot l^{-1} \ KCL, \ 0.014 \ g \cdot l^{-1} \ Ca \ (NO_3)_2 \end{array}$ was used as a basal salt medium. Either 75 g·l⁻¹ of FeSO₄·7H₂O was added into the basal salt medium as bacterial growth energy substrates (Nemati et al., 1998; Nazari et al., 2014). The initial pH of the medium was adjusted to 1.8 by using 1M H₂SO₄. Cultures were run in a thermostatic rotary agitator (130 rpm) at 32 °C and for 7 days. The pH value, oxidation-reduction potential (ORP) and cell density of the microorganism in the solution were monitored in successive intervals, pH and ORP were measured using the pH meter (Mettler Toledo model Mp120) and the Eh meter (WTW model 325, Electrode SenTix ORP 0-100/3 mol·l⁻¹ KCl) respectively. The concentration of cells was determined microscopically using a blood cell counting chambers. A culture of the bacteria contained 1.96×10^8 cell·ml⁻¹, was utilized in the experiments.

2.2. Effects of pH and Fe²⁺ concentration on the synthesis of jarosite seed

Bacteria were inoculated in flasks containing 130 ml 9K medium (10% v/v) and 44.7 g·l⁻¹ FeSO₄·7H₂O was used. The initial pH of each media was adjusted in the range of 1.55, 1.61, 1.67, 1.73, 1.79 and 2 respectively. In order to investigate the effect of Fe²⁺ concentration on the jarosite seeds formation, the initial Fe²⁺ concentration of each media was adjusted in the range of 10 g·l⁻¹, 20 g·l⁻¹, 30 g·l⁻¹, and 50 g·l⁻¹, respectively by FeSO₄·7H₂O. Flasks were incubated at 32 °C on an incubator shaker at 130 rpm. The pH value, concentration of Fe²⁺, total iron and bacterial number of reaction solutions were respectively monitored in successive intervals. The concentrations of Fe²⁺ and Fe³⁺ were determined by titrimetric method with potassium permanganate and atomic absorption spectrometry respectively (AAS), (Varian AA220) (Karamanev *et al.*, 2002). After performing each experiment, the solution was filtrated by 0.45 μ m Whatman filter paper. The solids on the filter paper were washed with dilute sulfuric acid 5%, distilled water, any remaining medium, and dissolved ions and then air-dried naturally (Eftekhari and Kargar, 2018).

The Fe²⁺oxidation efficiency and the amount of Fe³⁺produced were calculated according to equations (1) and (2) (Nurmi *et al.*, 2009):

$$\eta = \left[\rho\left(Fe_{in}^{2+}\right) - \rho\left(Fe_{out}^{2+}\right)\right] / \rho\left(Fe_{in}^{2+}\right) \times 100\%$$
(1)

$$\rho_1 = \rho \left(F e_{in}^{2+} \right) - \rho \left(F e_{out}^{2+} \right) \tag{2}$$

where η is the Fe²⁺ oxidation efficiency; ρ (Fe_{in}²⁺) and ρ (Fe_{out}²⁺) are the initial and final concentrations of Fe²⁺ in the aqueous phase, respectively; ρ_1 is the Fe³⁺ production.

The amount of Fe^{3+} that was precipitated, was calculated according to the following equation:

$$\rho_2 = \rho \left(F e_{tot,in} \right) - \rho \left(F e_{tot,out} \right)$$
(3)

where ρ (Fe_{tot, in}) and ρ (Fe_{tot, out}) are the initial and final concentrations (g·l⁻¹) of total iron in the aqueous phase, respectively; ρ_2 is the Fe³⁺ precipitation.

2.3. Characterization and analytical methods for biosynthetic jarosite

The crystal structure of the jarosite seed was examined by X-ray Powder X-ray diffraction (XRD) (Philips Model -XRG3100-PW 3710). XRD pattern was obtained by scanning speed of 6 deg/ min and scanning angles in 10- 80 deg. The characteristic reflection peaks (d-values) were matched with the peaks in the database of the Joint Committee on Powder Diffraction Standards (Hou et al., 2015). The precipitate was identified by Fourier transform infrared spectroscopy (FTIR)(Nicolet-560), and morphology was observed with a scanning electron microscope (SEM). SEM analysis was performed with a LEO-440i instrument operated at an accelerating voltage of 15 kV. Dried samples were mounted on the Al-stubs with double sided carbon tabs and coated with a slim layer of gold in a Pelco Model 3 Sputter 91000 coater.

2.4. Effects of biosynthetic jarosite seed, *A. ferrooxidans* and, pH on iron removal

The solution was an industrial copper raffinate solution prepared from Sarcheshmeh copper complex raffinate line. The initial pH value of raffinate solution was 1.3. The concentration of Fe and Cu²⁺

present in raffinate was quantified. The influence of pH and jarosite seed on the elimination of iron was performed in 500 ml Erlenmeyer flasks containing 200 ml of the raffinate solution with an initial concentration of 11.1 g·l⁻¹. Jarosite seed was added to the raffinate at various concentrations ranging from 0, 5, 10 and 15 wt.%. The A. ferrooxidans 10 % (V/V) was inoculated in the raffinate solution. The pH was adjusted to 1.5, 2 and 2.5 using $1M H_2SO_4$ and NaOH solutions and the flasks were placed in the rotary incubator shaker (130 rpm, at 32 °C) for 13 days. After performing each experiment, the solution was filtrated with 0.45 µm, Whatman filter paper and analyzed by AAS. The iron removal efficiency was calculated using the following equation (Nur *et al.*, 2015):

Removal efficiency (%) =
$$\frac{C \ 0 - C \ t}{C \ 0} \times 100$$
 (4)

where C_0 and C_t are the initial and final iron concentration in raffinate solution, respectively.

3. RESULTS AND DISCUSSION

3.1. Effect of pH on the synthesis of jarosite seed

A. ferrooxidans in 9k medium with the initial pH of 2 indicated the highest alteration efficiency and significant precipitate (Fig. 1a). The initial pH of 1.61, Fe²⁺ alteration efficiency ascended rapidly. At the pH of 1.61, 1.67 and 2 Fe²⁺ were bio-oxidized completely within 4.5 days. However, Fe²⁺ alteration efficiency of 5 days in other pH values was lower than 90%. Figs. 1(a-b) show the efficiency of Fe²⁺ alteration and a lower scale Fe³⁺ in the first hour. *A. ferrooxidans* metabolized considerable Fe³⁺ after jarosite formation, Fe³⁺ concentration decreased in the medium (Fig. 1b). The amount of jarosite seed precipitation was increased linearly with an increase in pH (Fig. 2).

The inhibition of bio-oxidation activity of A. ferrooxidans at pH value over 2 was related to the formation of a layer of jarosite biofilm on the extracellular polymeric of the organism. Biofilm formation is related to chemical movements of A. ferrooxidans, which is regulated by the quorum sensing (Zhang et al., 2018). Extracellular polymers hinder the diffusion of hydrogen ion. The microorganism extracellular protein may have an important role in oxidation reactions in 9K medium (Yu *et al.*, 2017). The results indicated that the dynamic Fe^{2+} changes and total iron reflects the bio-oxidation capability of the A. ferrooxidans and jarosite seed precipitation. Oxidation of Fe²⁺ to Fe³⁺ shows the activity of A. ferrooxidans for Fe^{2+} as an energy source and synthesis of magnetosomes in cells. The maximum pH was 2 in this range, A. ferrooxidans



FIGURE 1. (a) Variations of iron at different initial pH in 9k medium, Fe^{2+} alteration efficiency and (b) Fe^{3+} concentration.



FIGURE 2. The comparison of jarosite mass at different pH in 9k medium.

formed a greater amount of jarosite and the minimal jarosite precipitate occurred at pH 1.6-1.7. Therefore, appropriate control over the pH value can increase the quantity of jarosite seed formation and would improve the de-ironing process.

3.2. Effect of Fe²⁺ concentration on the synthesis of jarosite seed

In the initial Fe²⁺ concentration of 10 g·1⁻¹, *A. fer*rooxidans demonstrated significant activity and the bacteria population reached 2.5×10^7 cell ml⁻¹ after two days (Fig. 3a). *A. ferrooxidans* disclosed a prolonged lag phase and inhibited growth in the initial Fe²⁺ concentration of 20 g·1⁻¹, 30 g·1⁻¹, and 50 g·1⁻¹ respectively which attributed to the fact that the presence of higher Fe²⁺ concentration in the solution after the bio-oxidation caused faster enhancement of Fe³⁺ precipitation. The lag phase growth of *A. ferrooxidans* could be prolonged by the higher Fe³⁺ concentration (Liu *et al.*, 2009). In 9K medium, jarosite formation began when *A. ferrooxidans* was under their logarithmic phase of growth rate. The amount of jarosite seed and the Fe²⁺ oxidation efficiency was measured (Table 1). Fig. 3b and Table 1 disclosed the relationship between jarosite seed mass and Fe^{2+} concentration. The amount of jarosite seed precipitation was increased linearly with the increase of Fe^{2+} (Fig. 3b).

In some papers, it was mentioned that Fe^{2+} has an important role in the regulation of creating jarosite nuclei and the construction of precipitate (Pogliani and Donati, 2000; Dutrizac and Jambor, 2000; Liu *et al.*, 2009). It can be concluded that the formation of jarosite seed in 9K medium with Fe^{2+} at a concentration of 50 g·l⁻¹ was greater than that in the medium with Fe^{2+} concentration of 10 g·l⁻¹.

3.3. Identification and morphological observation of jarosite seed

The jarosite seed was identified using FTIR technique. The FTIR spectrum of Fe precipitates is shown in Fig. 4, the absorption peak in the region of 2900-3700 cm⁻¹ was ascribed to the O-H stretching; the absorption peak of 1425 cm⁻¹ was attributed to the NH₄⁺ stretching the v4, v3, v2 and v1 vibration of SO₄²⁻ represented in the wave numbers of 628, 1194, 1085, and 1004 cm⁻¹, respectively; the vibration peaks of FeO₆ were observed in the wave numbers of 511 cm⁻¹ and 473 cm⁻¹. The results indicated that the precipitates which bio-mediated during this process are ammonium jarosite with the formula of NH₄Fe₃ (SO₄)₂(OH) ₆.

The process of ammonium jarosite precipitation has three steps: oxidation of Fe^{2+} to Fe^{3+} , the formation of crystal nuclei (embryo) and the growth of crystals of a jarosite group phase (Liu *et al.*, 2009). According to the multi-step of jarosite formation, it is expected that this process is affected by pH, Fe^{2+} concentration and biological factors. The precipitate that is formed in 9K medium usually contains potassium jarosite or a mixture of potassium and ammonium jarosite (Dutrizac and Jambor, 2000).

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FIGURE 3. (a) The growth rate of *A. ferrooxidans* during Fe^{2+} bio-oxidation and (b) Comparison of jarosite mass at different initial Fe^{2+} concentration.

TABLE 1. Jarosite amount and ferrous oxidation rate in different initial Fe²⁺ concentration

Initial Fe ²⁺ , g·l ⁻¹	Fe ²⁺ concentration, g·l ⁻¹	Fe ³⁺ concentration, g·l ⁻¹	Fe ³⁺ /Fe ²⁺	Fe ²⁺ oxidizing efficiency, %	Jarosite amount, g·l ⁻¹
10	0.195	1.805	9.26	90	6.09
20	0.437	3.563	8.16	89.8	10.90
30	1.724	4.276	2.48	71.27	16.65
50	4.386	5.614	1.28	56.14	28.8



FIGURE 4. FTIR spectrum of Fe precipitates mediated with A. ferrooxidans in 9k medium.

9K medium contains a high concentration of NH_4 ions; therefore, the product was mainly ammonium jarosite. So, the quantity of jarosite can be decreased or increased by control of the ammonium ions concentration in the culture medium. Ammonium jarosite had an elemental weight composition of 29.1% Fe, 14.6% NH_4 and 11.2% S.

The crystal structure of precipitate (Fig. 5) was uniformly identified as jarosite seed according to the JCPDS card, given that no considerable difference



FIGURE 5. XRD patterns of Fe precipitates formed in 9k medium.



FIGURE 6. SEM image shows the morphology of jarosite seed produced by A. ferrooxidans in 9k medium.

was observed between the XRD patterns of the precipitate in the standard jarosite pattern and this study. These precipitates were well crystallized according to the sharp peaks and the stable baseline of the XRD spectra.

SEM observation revealed the regular jarosite particles with diameters of $1\mu m$ to $1.5 \mu m$ and smooth surface were dominantly formed in 9k medium (Fig. 6). These results are consistent with XRD findings, confirming that biosynthetic jarosite crystal is uniform with regular and large particles.

The crystals produced by *A. ferrooxidans* (Fig. 6) with a high Fe^{2+} oxidation rate presented as polyhedron-shaped firmly aggregate, and their surfaces were smooth. The higher Fe^{2+} oxidation and Fe^{2+} supply rate in *A. ferrooxidans* inoculated system facilitated polyhedron crystal formation and the increase of particle diameter (Gan *et al.*, 2017). The morphological features of jarosite seeds formed by the biological technique were particularly distinguishable owing to the effect of extracellular substances (Dutrizac and Jambor, 2000). Therefore, the influence of *A. ferrooxidans* and ferric sulfate

pH	Soluble total iron, g·l ⁻¹	Soluble ferrous iron, g·l ⁻¹	Total iron removal, %
1.3*	11.1	6.9	0
1.5	11.01	0.01	0.81
2	5.84	0.02	47.38
2.5	3.19	0.01	71.26

TABLE 2. Effect of the solution pH on iron removal of raffinate solution (5% jarosite seed, 32 °C and 13 days)

* pH of native raffinate



FIGURE 7. The effect of pH on iron removal of raffinate solution using *A. ferrooxidans* and 5% jarosite seed.

concentration has a great effect on the morphologies of ammonium jarosite.

3.4. Effects of pH and *A. ferrooxidans* on iron removal of raffinate solution

According to chemical analysis, the raffinate solution consisted of Cu: $0.26 \text{ g}\cdot\text{l}^{-1}$, Fe: 11.1 g $\cdot\text{l}^{-1}$, Fe²⁺: 6.9 g $\cdot\text{l}^{-1}$ and Fe³⁺: 4.2 g $\cdot\text{l}^{-1}$. The pH control is an important factor for the effective iron removal by the A. *ferrooxidans* bacteria and jarosite seed in raffinate solution. The impact of different pH on iron removal mediated by *A. ferrooxidans* and jarosite seed is shown in Table 2. The percentage of iron removal from raffinate solution at pH of 1.5, 2 and 2.5 was 0.81%, 47.38% and, 71.26%, respectively.

Figure 7 shows the amount of iron removal from raffinate solution at pH 1.3, 1.5, 2, and 2.5 using *A. ferrooxidans* bacteria and 5% jarosite seed percentage. As shown in Fig. 7, The pH of solution noticeably influenced iron removal and 47.38% iron elimination was achieved at pH 2 and 5% seed dosage while no change in iron removal was observed at pH 1.3. The highest amount of iron in the raffinate solution was pH 2.5. At this pH of 7.91 g·l⁻¹ (71.26%) iron was obtained from raffinate.

According to the results with the pH increases, the amount of iron removal from the raffinate solution increases. This is due to the decrease in solubility of ferric iron at high pH values. In this study, optimum pH for iron removal mediated by *A. ferrooxidans* and jarosite seed was pH 2, because of *A. ferrooxidans* had good activity in this pH.

3.5. Effects of jarosite seed and *A. ferrooxidans* on iron removal of raffinate solution

The combined effects of jarosite seed and A. ferrooxidans iron removal is shown in Table 3. The iron concentration $(11.1 \text{ g} \cdot \text{l}^{-1})$ of raffinate solution was decreased biologically in the presence of jarosite seed and A. ferrooxidans. As shown in Table 3, 6.2% of the iron ions were eliminated from solution raffinate in the absence of jarosite seed. Increasing the jarosite seed dosage from 5 to 10 wt.% led to a dramatic increase in iron removal (71.17%) during the period of 13 days. This can be due to the increase in specific solids surface in the solution. As a result, more contact surface is provided for the nucleation and growth of new jarosite crystals. Whereas, no significant rise in iron removal was observed with a further seed addition to 15 wt.%. This may be due to the dissolution of ferrous iron. The highest decrease in iron concentration in the raffinate solution was related to the percentage of seeds of 10%. At this jarosite seeds dosage, 6.55 g·l⁻¹ iron was removed from the raffinate solution.

Figure 8 shows the changes in iron concentration in the raffinate solution over time in two conditions with and without the addition of jarosite seed in the presence of A. ferrooxidans. On the first day, the concentration of iron in the solution increased due to the inoculation of 10% of the A. ferrooxidans bacteria. In the solution containing jarosite seed, after the second day, the concentration of iron in the solution decreased due to jarosite precipitation. This may be due to the bacterium leaving the delay phase after the second day and increasing its activity and production of ferric ions, followed by hydrolysis and initiation of ferric ion precipitation. The minimum concentration of iron remaining was in the solution containing the seed (4.55 g·l⁻¹) on the third day. After the third day, the amount of iron in the solution increased and then was stabilized. This increase in iron concentration in the raffinate solution after day 3 may be due to the re-dissolution of

Jarosite seed loading, Wt.%	Soluble total iron, g·l ⁻¹	Soluble ferrous iron, g·l ⁻¹	Total iron removal, %
0	10.53	0.01	6.26
5	5.84	0.02	47.38
10	4.55	0.04	71.17
15	4.47	0.01	59.72

TABLE 3. Effect of jarosite seed on iron removal of raffinate solution (pH 2, 32 °C and 13 days)



FIGURE 8. Effect of combined effects of 10% jarosite seed and *A. ferrooxidans* on iron removal of raffinate solution.

ferrous ion. In the non-jarosite seed raffinate solution, iron concentration increased on the first day due to bacterial inoculation and then decreased until the fifth day, and after the fifth day, the process was almost constant.

Jarosite precipitation can be used to remove iron and sulfate from extraction solutions during the recovery of Zn^{2+} from ores (Nurmi *et al.*, 2009; Albrecht *et al.*, 2011). The ammonium jarosite and sodium jarosite are commonly used in industry for instance zinc refinery because of their advantages of low cost (Li *et al.*, 2014). For this reason, ammonium jarosite was used in this study.

Iron elimination is very susceptible to process conditions, including temperature, agitate time, filtration rate, oxygen concentration and pH (Gan *et al.*, 2017). The presence of Fe-oxidizing bacteria in iron removal filters is likely to increase the efficiency of iron elimination from acidic water (Florence *et al.*, 2016).

According to the results, magnetic features of *A. ferrooxidans* has a positive impact on improving the rate of precipitation of iron in acidic treatment systems. The magnetosome with diameters between 15 and 70 nm is the origin of the weak magnetotaxis of *A. ferrooxidans*, and could serve as iron storage and detoxifcation compartments (Zhang *et al.*, 2018).

Jarosite seed incorporates iron into its crystal structure and leads to the immobilization of iron. When jarosite concentration is increased to 10 wt%, the incorporation coefficients are remarkably increased (71.17%) in raffinate solution. Adding Jarosite seed acted as a seed crystal to promote the formation of new jarosite in the raffinate solution, thus promoting the removal of iron.

Iron is removed through structural incorporation or substitution. According to the previous literature, metals are immobilized in jarosite by a series of precipitation, co-precipitation and adsorption reactions (Eftekhari *et al.*, 2020)

Izadi *et al.* (2017) investigated the removal of iron by chemical precipitation and reported that the iron removal efficiency was greater with jarosite precipitation (~70%) compared to hydroxide (~20%). Compared to the studies of Izadi *et al.* (2017), this study showed that iron removal by raffinate solution with the combined effects of biosynthetic jarosite seeds and *A. ferrooxidans* is much more efficient (~72%) than the chemical precipitation method.

Florence *et al.* (2016) inspected the iron removal from acid mine drainage using Fe-oxidizing *Ferrovum myxofaciens* and reported that the average iron removal rate is 67%. The results indicated the efficient elimination of iron by the present method. When *A. ferrooxidans* is employed to the Co-precipitation system, it can greatly promote the Fe²⁺ bio-oxidation rate and plays a role as the template of the secondary iron-based mineral, which can increase the metal immobilization rate and efficiency correspondingly.

Based on these findings, we can conclude that the combined effects of biosynthetic jarosite crystals and *A. ferrooxidans*, significantly accelerate the elimination of iron from raffinate solution and the simplicity in operation, low consumption of chemicals and in-situ treatment make the jarosite seed a promising technique.

4. CONCLUSIONS

- The uniform and regular shapes of ammonium jarosite crystals $(NH_4Fe_3 (SO_4)_2(OH)_6)$ were synthesized. Biofilm of *A. ferrooxidans* and ferric sulfate had an effect on the morphologies of jarosite seed. The ideal pH and Fe²⁺ concentration for jarosite seed formation were 2 and 50 g·l⁻¹, respectively.
- Increasing seed dosage to 10% led to approximately 72% iron removal from copper raffinate solution through magnetic features of *A. ferrooxidans*. Increasing the percentage of jarosite seeds in the biological treatment process

increases the rate of removal of iron from the raffinate solution due to the increase in specific solids surface in the solution.

- The pH of solution noticeably influenced iron removal and 47.38% iron elimination was achieved at pH 2 and 5% seed dosage. Consequently, pH 2 and jarosite seed loading of 10% was selected as the optimum conditions for iron removal from the raffinate solution.
- The refining process reduces the amount of ferric iron during direct and indirect leaching and bioleaching. Also, refined raffinate solution is higher loading capacity than other solutions so more copper enters the aqueous phase. As a result, copper recovery increases.

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Conflict of interest

The authors declare that they have no conflict of interest.

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