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Effects of Energy Sources on Rare Earth Elements Bioleaching from Phosphogypsum by *Acidithiobacillus ferrooxidans*

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Highlights

- In acidolysis process, pH is the main factor for improving dissolution.
- The formation of jarosite plays the important role in pH decrease.
- Jarosite also intercepts microbial attachment to the mineral surfaces.
- Elemental sulfur decrease pH to a critical level by forming sulfuric acid.
- Elemental sulfur is more effective energy source.

Abstract1: Effects of different energy sources (ferrous iron, elemental sulfur and their mixture) on the bioleaching efficiency of rare earth elements (REEs) from phosphogypsum (PG) by using Acidithiobacillus ferrooxidans CL (A. ferrooxidans) were investigated in this paper. Potassium and ammonium jarosite was detected in the residues of bioleaching experiments using the ferrous iron and the mixture as the energy source. XRD patterns and SEM images showed that jarosite intercepted the microbial attachment to the mineral surfaces because it was formed as a passivation layer. During the bioleaching process using ferrous iron as the energy source, redoxolysis reaction of PG by ferric iron produced in the oxidation of ferrous iron doesn't perform. In the bioleached residue of using elemental sulfur as the sole energy source, jarosite was not detected and the pH value was 1.25, much lower than that of using the ferrous iron. REEs in PG exist in the form of phosphate and the dissolution of REEs from PG is described as the acidolysis process. The maximum total REEs extraction was found in the test using the elemental sulfur due to the lowest pH value and no formation of jarosite.

Keywords: Bioleaching; Rare earth elements; Energy source; Phosphogypsum; Acidithiobacillus ferrooxidans CL

I. INTRODUCTION

REEs have distinctive physical and chemical properties that enable their use in a broad range of technologies, and recently, many researchers have been interested by extracting REEs from secondary resources such as electronic wastes and industrial wastes. PG, is the main by-product of phosphoric acid production for the fertilizer industry; its REEs content varies between 0.01 and 0.4% (w/w), while the worldwide generation of PG has been estimated to be 200–300 million tons per year [1]. About 100 kt of REEs ends up in PG produced in one year, while current annual world production of REE oxides is about 126 kt [2]. Therefore, it has aroused extensive concern for the recovery of REEs from PG.

Bioleaching technology has the advantages of high metal selectivity and high metal extraction efficiency from low-grade minerals, thereby it is considered to be an appropriate technology for the recovery of REEs from waste materials [3, 4, 5]. *A. ferrooxidans* is one of the most widely used chemoautotrophic bacteria in bioleaching because of its mild growth conditions and oxidizing ability [6]. It is well known that *A. ferrooxidans* can obtain energy for growth through the oxidizing ferrous irons, elemental sulfur or metal sulfide ores, creating metabolic products of ferric iron and sulfuric acid, which can importantly affect the bioleaching process [7].

In a previous study, we recovered some kinds of REEs such as La, Ce, Nd, Y from PG by *A. ferrooxidans* using ferrous sulfate as the energy source [8]. We suggested that during bioleaching process of REEs from PG by *A. ferrooxidans* using ferrous sulfate as the energy source, protons generated by jarosite formation significantly reduced the pH value of the solution, which plays major role to accelerate the dissolution of REEs from PG [8]. However, we didn’t observe the bioleaching performance of REEs from PG by *A. ferrooxidans* in the case of using elemental sulfur as the energy source.

Some studies investigated the effects of using ferrous iron and elemental sulfur as the sole and mixed energy sources on the bioleaching efficiency of valuable metal from minerals. It was found that *A. ferrooxidans* can simultaneously utilize both ferrous iron and elementary sulfur, and iron (II) was more effective than sulfur in the bioleaching of realgar [9]. The mixed energy source of ferrous iron and elemental sulfur could enhance the leaching efficiency of vanadium-bearing shale during the early stages, while elemental sulfur as sole energy source was ultimately more effective [10].

As seen above, the effect of using ferrous iron and elemental sulfur as the sole and mixed energy sources on the bioleaching efficiency of valuable metals are different from the researchers and leached metals, and it would be of significance to study the effects of different energy sources on the bioleaching efficiency of REEs from PG. The purpose of this study is to investigate the performances and mechanisms of REEs bioleaching from PG by *A. ferrooxidans* using ferrous iron, elemental sulfur and their mixture as the energy source. The main objectives of this work are firstly, to explore the best energy source of ensuring the maximum REEs extraction efficiency, and secondly, to investigate the mechanism of bioleaching process in the case of using different energy sources.

II. MATERIALS AND METHOD

A. PG

The PG samples from a waste stack of PG in Jingmen City, Hubei Province, China was ground and sieved to less than 74 μm in particle diameter before use in leach tests. They were digested in a Graphite-digestion device (Hanon, SH230, China) to analyze REEs through a modified method according to the procedure reported previously [11].

Triplicate of 200.0 ± 0.1 mg of PG sample were weighed into Teflon vessels, and 4 mL of HNO_3, 6 mL of HF and 6 mL of $HClO_4$ were added. The sealed vials were heated at 140 °C for 24 h to evaporate to incipient dryness. To remove HF, 1–3 mL of HNO_3 was added and mixed well, then evaporated at 140 °C for 24 h. This process was repeated three times. The residue was transferred quantitatively to a 25 mL volumetric flask, and filtered with 0.22 μm pore size PES filters and used as the sample for the determination of REEs content.

The chemical composition of REEs (La, Ce, Pr, Nd, Sm, Y, Er, Gd, and Dy) in PG were analyzed by inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin Elmer Optima 8300, USA). The mineralogical composition of PG was determined by X-ray diffraction (XRD-7000, Shimadzu).

B. Isolation and growth of microorganism

Autotrophic, acidophilic strain of *A. ferrooxidans* CL was isolated from the acid pit wastewater of Daye copper mine in Hubei Province, China, according to the methods described previously [12, 13].

The 9K basal medium for the culture of *A. ferrooxidans* CL contains of the following ingredients (unit: g/L): $(NH_4)_2SO4$ (3.0), 120 Na_2SO_4 (2.1), K_2HPO_4 (0.5), KCl (0.1), $Ca(NO_3)_2·4H_2O$ (0.01), $MgSO_4·7H_2O$ (0.5). The solution of $FeSO_4·7H_2O$ of 33.3 g/L sterilized with 0.22 μm filter was used as the energy source for the growth of *A. ferrooxidans* CL [14].

The pH of culture medium was adjusted to 2.0 with 1.0 M H_2SO_4. The basal media were autoclaved at 121 °C for 20 min. Erlenmeyer flasks containing 100 mL of sterilized basal medium with 1.0–2.0% (*v*/v) inoculum were incubated in an orbital shaking incubator (bluepard, THZ–98C, China) at 160 rpm in 30 °C. The stable growth stages of *A. ferrooxidans* CL was 60–70 h. At this time, cell density of *A. ferrooxidans* CL is ensured to be in the range of 1.0×10^7–1.1×10^7 cells/mL, which can be used as inoculum for subculture and bioleaching experiments.

C. Design of bioleaching experiments

In order to compare the bioleaching efficiency of REEs by *A. ferrooxidans* CL between different kinds of energy sources, three series of bioleaching processes were performed: bioleaching process using 33.3 g/L of $FeSO_4·7H_2O$ as the sole energy source (test 1); bioleaching process using 10 g/L of elemental sulfur as the sole energy source (test 2); and bioleaching process using the mixture of 33.3 g/L of $FeSO_4·7H_2O$ and 10 g/L of elemental sulfur as the energy source (test 3). The solution of $FeSO_4·7H_2O$ was sterilized with 0.22 μm filter and elemental sulfur was autoclaved at 121 °C for 20 min. Inoculum size of *A. ferrooxidans* CL was set as 1.0% (v/v).

The PG sample was ground and sieved to less than 74 μm in particle diameter, and then sterilized by autoclaving. For each process, 1.0 g of sterilized PG at 1.0% (*w*/*v*) pulp density was added. All bioleaching experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of the relevant culture media in triplicate at 30 °C with a shaking speed at 160 rpm in an orbital shaking incubator over 30 days.

Additionally, control experiment with blank culture media (without the addition of *A. ferrooxidans* CL) was carried out to compare the extraction efficiency of REEs with the bioleaching experiments.

D. Analytical methods

Samples were taken every three days and pH was measured using a pH meter (INESA DDS-307, China). Then, the samples were centrifuged, filtered (0.22 μm) and analyzed by inductively coupled plasma optical emission spectrometer (ICP-OES, Perkin Elmer Optima 8300, USA) for the determination of the leached REEs concentrations and the average values were reported.

Mineralogical compositions of the bioleached residues of test 1, test 2, test 3 were analyzed by X-ray diffraction (XRD-7000, Shimadzu). Microscopic observation of the bioleached residues was conducted by using a scanning electron microscope (SEM, JEOL-IT300, Japan).

III. RESULTS AND DISCUSSION

A. PG characterization

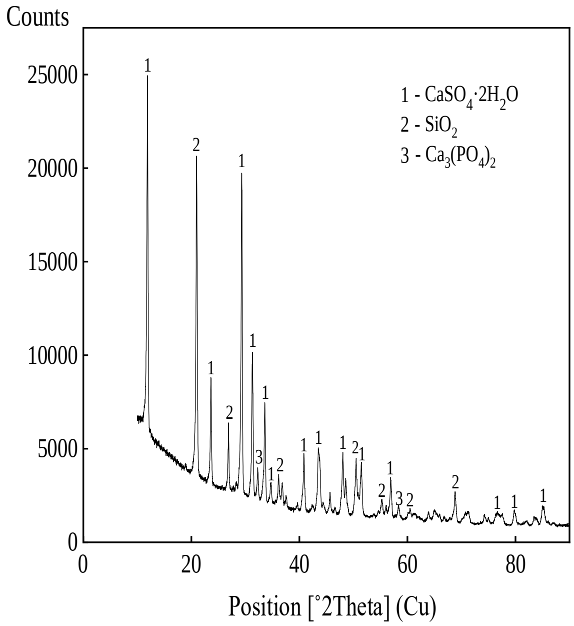

Fig. 1. XRD pattern of PG

The result of XRD analysis of PG used in this work is shown in Fig. 1. The XRD pattern shows that PG mainly consists of gypsum and quartz. The ICP-OES analysis of PG shows that the total content of 9 kinds of REEs in the raw PG was 58.8 mg/kg, including 9.3 mg/kg of La, 13.3 mg/kg of Ce, 15.9 mg/kg of Nd, 13.5 mg/kg of Y, and the other 5 kinds of REEs (Pr, Sm, Er, Gd, and Dy) contents are all less than 3.0 mg/kg. Therefore, in this study, in all of the following leaching experiments, four kinds of REEs (La, Ce, Nd, and Y) which exhibited significant concentrations in the samples were evaluated.

B. Bioleaching Performance of REEs using different Energy Sources

The REEs extraction efficiencies of the control experiment without the addition of *A. ferrooxidans* CL were all less than the results of the corresponding bioleaching experiments. Therefore, this work only discussed on the bioleaching performance of REEs extraction in the biotic experiments.

The effects of energy sources on the bioleaching efficiency of minerals by using *A. ferrooxidans* CL in some publications were different according to the bioleached minerals and elements. For example, the bioleaching performance of arsenic from realgar using ferrous iron as the energy source was more effective than that of using elemental sulfur as the energy source [9], while the bioleaching efficiency of vanadium from vanadium-bearing shale using elemental sulfur as the sole energy source was higher than the ones of the other energy sources [10].

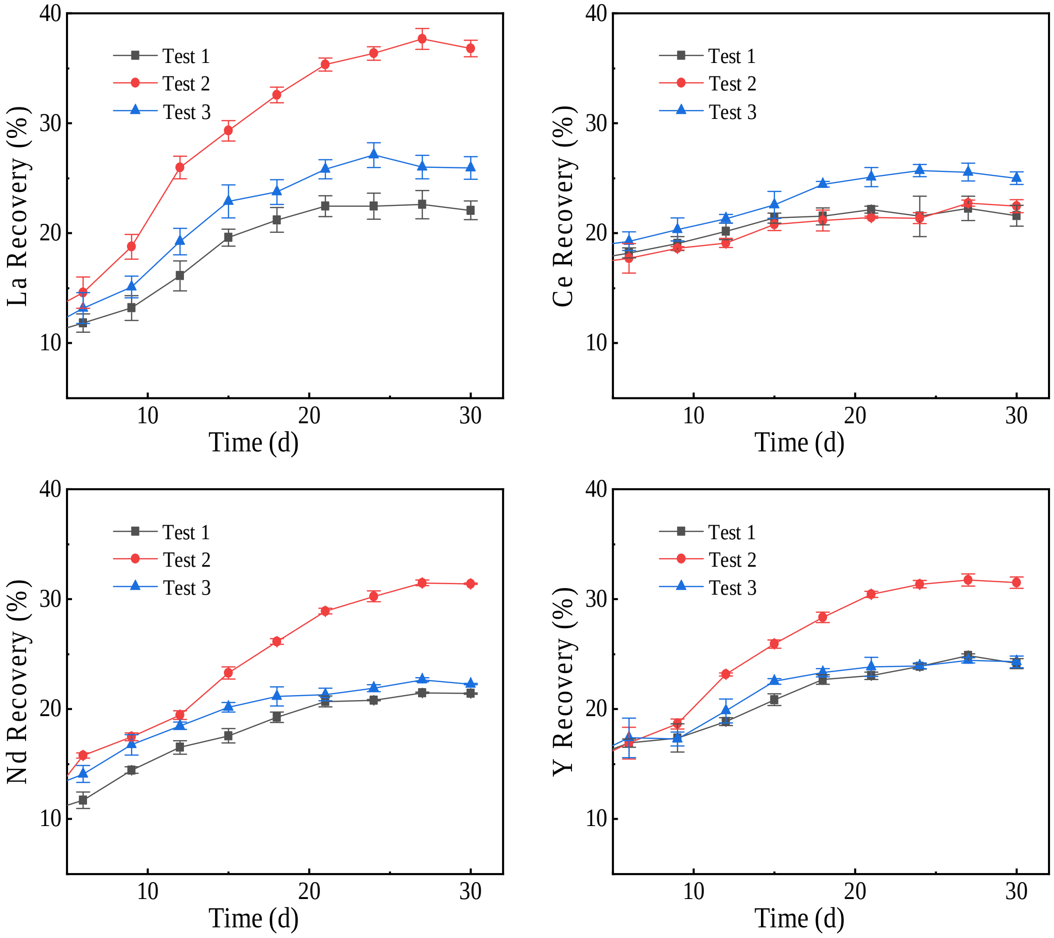

Fig. 2. Changes of REEs extraction under the different energy sources

Fig. 2 shows the REEs extraction results of bioleaching experiments by using different kinds of energy sources. As seen in the Fig. 2, the highest bioleaching efficiencies of three REEs (La, Nd, Y) were found in the test 2 using the elemental sulfur as the sole energy source, which were 36.8%, 31.4%, and 31.5%, respectively. These results were consistent with those of previous report [10]. The maximum leaching efficiency of Ce was obtained in the test 3 used the mixture of ferrous iron and elemental sulfur as the energy source, and this result is the same as the one in the early stage of the vanadium leaching experiment conducted [10]. Bioleaching efficiencies of La and Ce in the test 3 used the mixture energy source were higher than those of test 1 used ferrous iron as the energy source, while the leached efficiencies of Nd and Y of the test 1 and test 3 were similar. That is to say, the lowest extraction efficiency of REEs was found in the test 1 using the ferrous sulfate as the sole energy source, which may be due to its highest pH value of 1.76. As seen above, effects of energy sources on the bioleaching efficiency of REEs from PG are different from those of bioleaching of arsenic from realgar, or vanadium from vanadium-bearing shale. In order to investigate the mechanism of REEs bioleaching process from PG by *A. ferrooxidans* using different energy sources XRD patterns and SEM images of leached residues were analyzed as well as the changes of pH, Fe^{2+} concentration and Fe^{3+} concentration in the following section.

C. REEs Bioleaching Mechanism from PG using Different Energy Sources.

1) XRD analysis of bioleached residues

The mineralogical compositions of the three bioleaching residues were analyzed by XRD and the results are shown in the Fig. 3 (a), (b), and (c).

First of all, $Ca_3(PO_4)_2$ which was detected during the XRD analysis of raw PG was not appeared in the bioleached residues. This means that *A. ferrooxidans*, as the one of the phosphate solubilizing microorganisms, take off the calcium phosphate from PG and dissolved it, which verifies the phosphate solubilizing ability of the *A. ferrooxidans*.

As seen in the Fig. 3 (a) and (c), the potassium and ammonium jarosite ($KFe_3(SO_4)_2(OH)_6$, $NH_4Fe_3(SO_4)_2(OH)_6$) were observed in the leached residues of test 1 and test 3, which used the ferrous sulfate as the energy source. During the metabolism, *A. ferrooxidans* oxidized the ferrous iron into the ferric iron and the culture medium contains the potassium cations and ammonium cations, therefore, formation of jarosite is inevitable during the bioleaching process by *A. ferrooxidans* using ferrous iron as the energy source (Eq. (1) and Eq. (2)) [9].

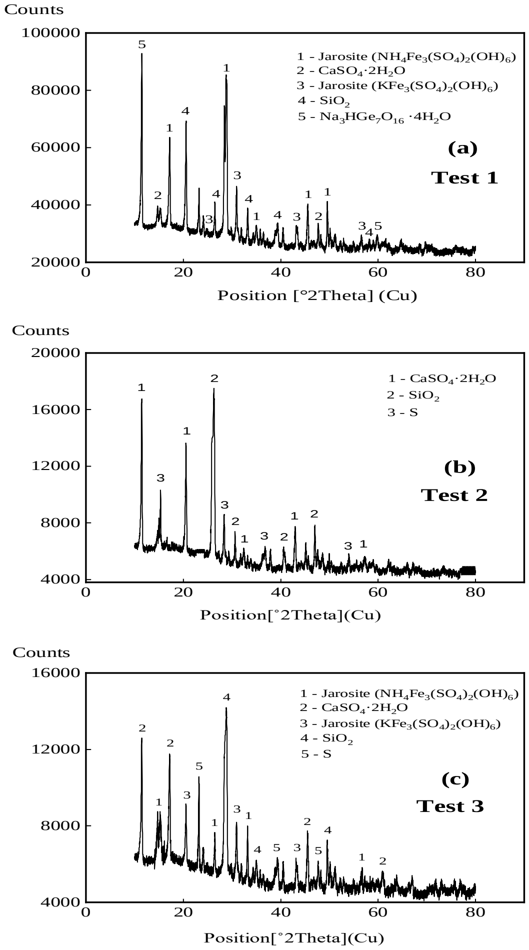

Fig 3. XRD patterns of the residues after bioleaching

$$K^+ + 3Fe^{3+} + 2SO_4^{2-} + 6H_2O = KFe_3(SO_4)_2(OH)_{6(s)} + 6H^+ \qquad (1)$$

$$NH_4^+ + 3Fe^{3+} + 2SO_4^{2-} + 6H_2O = NH_4Fe_3(SO_4)_2(OH)_{6(s)} + 6H^+ \qquad (2)$$

It is the common opinion of previous researchers that jarosite inhibited the microbial attachment to the mineral surfaces and reduced the bioleaching efficiency because it was formed as a passivation layer [10, 15]. Other publication reported that during the formation of jarosite, a lot of protons are produced, which plays a major role to decrease the pH value of the bioleaching process of non-sulfide minerals by using the ferrous iron as the energy source, thus helps the dissolution of REEs from PG [8].

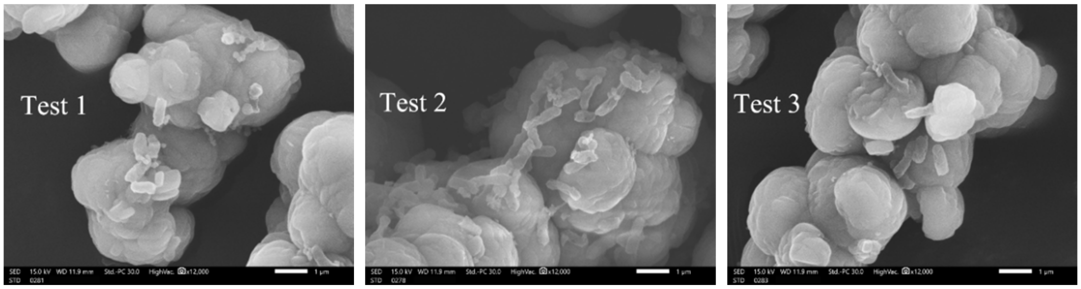

Fig. 4. SEM images of the residues after bioleaching

However, it is clear that under the same conditions of pH value, the existence of jarosite will intercept the attachment between microbes and mineral particles to decrease the bioleaching performance. As seen in the Fig. 3 (b), jarosite was not detected in the bioleached residue of test 2 using the elemental sulfur as the sole energy source, this is because there is neither ferrous iron nor ferric iron in the culture medium of test 2. That is, the higher REEs extraction efficiency of the experiment using elemental sulfur as the sole energy source compared to those of the other tests is referred to the non-formation of jarosite, which led to low leaching efficiency through passivation of metal surface and inhibition of mineral solution.

Additionally, Fig. 4 shows the SEM images of the residues after bioleaching. In the SEM images of three bioleached residues, *A. ferrooxidans* microorganisms which were attached to the mineral can be seen clearly. Microbial cell number attached to the mineral particles in the test 2 was obviously much more than those in test 1 and test 2. During the test 2, jarosite was not formed because elemental sulfur was used as the sole energy source, therefore, these figures can confirm the suggestion of previous literatures that jarosite formation inhibited the microbial attachment to the mineral particles.

2) Changes of Fe2+ and Fe3+ concentrations and pH

The changes of Fe^{2+} and Fe^{3+} concentrations during the bioleaching tests are shown in Fig. 4 (a) and (b). As seen in the figure, variation profiles of Fe^{2+} and Fe^{3+} concentrations in the test 1and test 3, used the ferrous iron and the mixture as the energy source, are similar with each other. It is notable that in the early stage of bioleaching process, the concentration of Fe^{2+} changes greatly. After the first 3 days, the concentration of Fe^{2+} decreased to nearly 0, which can be ascribed to the oxidation of ferrous iron to ferric iron by *A. ferrooxidans*, as shown in Eq (3) [16, 17].

$$4Fe^{2+} + O_2 + 4H^+ = 4Fe^{3+} + 2H_2O \qquad (3)$$

Meanwhile, the concentration of Fe^{3+} rapidly increased in the first three days of experiments and decreased greatly in the next three days, after that it continued to decrease slowly. The increase of Fe^{3+} concentration in the early stage is ascribed to a lot of production of ferric irons by oxidation of ferrous irons as shown in Eq (3), and the decrease in the next stage can be referred to the consumption of ferric irons during the formation of the potassium and ammonium jarosite (Eq (1) and (2)).

Some literatures suggested that the formation of $Fe(OH)_3$ is one of the factors for the decrease of Fe^{3+} concentration [16, 18], but according to our result of XRD analysis for the bioleached residues, $Fe(OH)_3$ was not detected (Fig. 3 (a) and (c)). Other publications insisted that Fe^{3+} with strong oxidizability could chemically oxidize the minerals of zinc, arsenic and vanadium, and at this time, Fe^{3+} is reduced into Fe^{2+}. During this redoxolysis process, the valuable metals could be extracted from minerals. Only metals which exist in the form of sulfide, oxide, or iron compounds in the mineral could be extracted during the redoxolysis process by Fe^{3+}. According to our results, REEs bioleaching efficiencies of the experiments using ferrous iron as energy source are lower than that of other test, and the concentration of Fe^{2+} did not increase after decreased to nearly zero. And according to the analysis of Fe^{3+} concentration change in combination with K^+ and NH_4^+ ions, the decrease of Fe^{3+} concentration is ascribed to the formation of jarosite [8]. Therefore, it can be said that redoxolysis reaction by ferric iron does not perform in the bioleaching process of REEs from PG.

Considering that REEs in PG exist in the form of phosphate, the dissolution of REEs from PG is described by acidolysis process of Eq. (4) [8].

$$(REE)PO_{4(s)} \rightarrow REE^{3+}_{(aq)} + PO_4^{3-} \qquad (4)$$

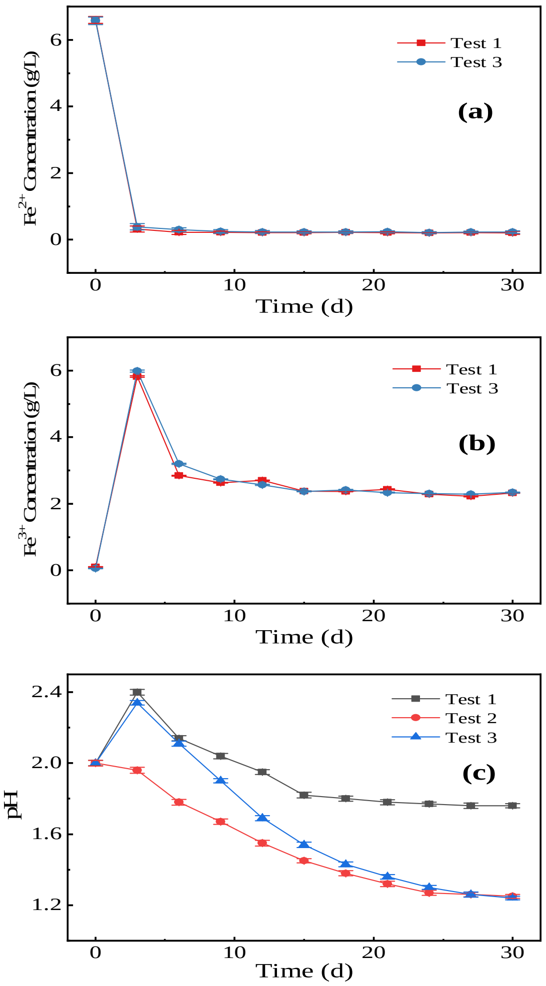

Fig. 5. Changes of (a) Fe^{2+} concentration, (b) Fe^{3+} concentration, and (c) pH

The decisive factors affecting the dissolution of REEs from PG are the pH value of leachate and organic acid secretion during the microbial metabolism [19], and the organic acid is reported to support three times higher phosphate solubilization than that of proton-mediated solubilization [20]. However, *A. ferrooxidans* does not secrete organic acid during its metabolism [8], therefore, the pH value of the leachate is the most important factor that affects the bioleaching efficiency of REEs.

As shown in the Fig 5. (c), in the early stage of test 1, the pH value of solution increased from 2.0 to 2.4, this is because a lot of protons were consumed during the oxidation process of ferrous iron to ferric iron by *A. ferrooxidans* (Eq. (3)). After three days, the pH value of the solution decreased continuously to 1.76, which can be attributed to the production of protons during the formation of jarosite (Eq. (1) and (2)). That is, during the reactions of Eq. (1) and (2), one mole of K^+ cation and NH_4^+ cation produces of six moles of protons respectively, which is the decisive factor of decreasing the pH value of solution in test 1 from 2.4 to 1.76.

The pH value in the test 2 using elemental sulfur as the sole energy source also continued to decrease from 2.0 to 1.25, and this is due to the production of sulfuric acid during the oxidation process of elemental sulfur by *A. ferrooxidans*. This shows that the pH value of bioleaching process by *A. ferrooxidans* using the elemental sulfur as the energy source is much less than that of using ferrous iron as the energy source. The higher REEs extraction efficiency of the test 2 using elemental sulfur as the sole energy source compared with that of the test 1 is referred to the lower pH value of 1.25.

Meanwhile, in the early stage of the test 3 using mixed energy source, the pH value increased from 2.0 to 2.34, this is also because a lot of protons in the solution were used for the oxidation of ferrous iron to ferric iron. After that, the pH value continued to decrease to 1.24. During the bioleaching test 3, both sulfuric acid produced by the oxidation of elemental sulfur and the protons by the jarosite formation affect the decrease of the pH value. However, the pH values of test 3 and test 2 are similar with 1.25 and 1.24, respectively, this means that the quantity of protons produced during the jarosite formation is not enough to decrease the pH value below 1.24.

In conclusion, the extraction efficiency of test 2 using elemental sulfur as the sole energy source is the highest of all, which is referred to its lowest pH value, the decisive factor affecting the REEs bioleaching efficiency from PG, and the non-formation of jarosite which intercepts the microbial attachment to the mineral surface.

IV. CONCLUSION

In this paper, the performance and mechanism of REEs bioleaching from PG by *A. ferrooxidans* using different energy sources such as ferrous iron, elemental sulfur and their mixture. REEs in PG exist in the form of phosphate and the dissolution of REEs from PG can be described by acidolysis process. In the first three days of test, ferrous irons as the energy source were all oxidized to ferric irons and the ferric irons react with the potassium and ammonium cations in the solution to form jarosite. During the formation of jarosite, lots of protons are produced and the pH value decreases, however, jarosite decreases the bioleaching efficiency by forming passivation layer on the surfaces of mineral particles. During the test of using elemental sulfur as the sole energy source, jarosite was not formed and the pH value of leachate decreased to 1.25 by the sulfuric acid produced in microbial metabolism, which promoted the acidolysis of PG to extract the maximum REEs.

Credit authorship contribution statement

Pak Ki Chang: Methodology, Investigation.

Hong Chol: Conceptualization, Writing original draft.

Jon Chol Jin: Data curation

Conflict of Interest:

The authors declare that they have no conflict of interest.

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