FE-SEM Study of Microbially Formed Jarosites by Acidithiobacillus ferrooxidans

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Morphological characterization of jarosite groups formed from Fe(III) biologically oxidized with different numbers of Acidithiobacillus ferrooxidans was conducted using FE-SEM. The higher population of A. ferrooxidans resulted in more distinct jarosite mineral shape, and stronger Raman intensities for potassium jarosite, ammoniojarosite and argentojarosite. The morphology of the jarosites might be dependent on iron-oxidizing activity of A. ferrooxidans.

The technique was applied to identify jarosite compounds formed during microbially mediated dissolution of arsenopyrite by A. ferrooxidans. It is difficult to identify this jarosite compound by X-ray diffraction and Raman spectroscopy because amounts are typically low and the crystallization is poor in minerals formed by microbially mediated oxidation. However, FE-SEM image provided helpful information for identification of jarosite compounds.

The results suggest that morphology would provide useful information for identification and history of jarosite minerals as geochemical samples.

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1. Introduction

Jarosite group minerals (MFe5(SO4)2(OH)6, M: monovalent cation) are well known as secondary minerals often formed in acidic mine environments1,2 and in hydrometallurgical processing containing alkaline cyanidation for gold ore recovery.3 Acidophilic iron-oxidizing bacteria, which is represented by Acidithiobacillus ferrooxidans, accelerate the oxidation rate of Fe(II) by the 6th power of 10 in comparison to abiotic and chemical reaction under acidic conditions.4 Formation of jarosite is favored in mine environments and mine tailings inhabited by iron-oxidizing bacteria. Jarosite can provide information on the geochronology of weathering profiles. Elevated concentrations of potassium (K) in jarosite can be used for age determinations by K-Ar and Ar/Ar methods and isotopic values of sulfur, oxygen, and hydrogen determined. By determining the isotopic characteristics of jarosite, one can use these characteristics to infer water sources, sulfur sources, and paleoclimate conditions during the formation of the mineral. These methods are currently applied to elucidate the formation of barite-fluorite deposits and the weathering of ore deposits.5,6

Recent discovery of jarosite and other iron-bearing sulfate salts in Mars’ Meridiani Planum region by NASA’s Opportunity rover has drawn increased attention to these minerals.7 The identification and history of secondary minerals is often difficult when their quantity is small and is complicated by poor crystallinity. When bioprocesses are involved in formation of the mineral, the problem becomes more significant. Mineral growth history may best be elucidated by using multiple methods of analysis.

We have characterized jarosite group minerals using spectroscopic methods and morphological observation.8–11 In these studies, trace amounts of jarosite compounds can be successfully identified by Raman spectroscopy, based on size of ionic radius involved monovalent cations in jarosite, and poorly crystalline jarosite group can be often detected by Raman. The morphological studies indicated that different jarosite compounds, e.g. NH4, K, Ag-jarosite, have different morphologies, and that morphological character also depends on chemical oxidation rate of Fe(II) ions which are precursor to jarosite compounds.

In the present work, the relationship of cellular population of Acidithiobacillus ferrooxidans to the morphology of microbially formed jarosite was investigated using a field emission type of scanning electron microscopy (FE-SEM). Morphological characterization of microbially-formed jarosite was used to identify trace amounts of secondary jarosite formed during microbial oxidation of arsenopyrite by X-ray diffraction (XRD) and other spectrometric methods.

2. Experimental

2.1 Microorganism

A strain of Acidithiobacillus ferrooxidans (ATCC23270) supplied by American Type Culture Collection was cultivated conventionally at 30 °C in 150 cm3 of 9 K medium ((NH4)2SO4, 3.0 g, KCl 0.1 g, K2HPO4 0.5 g, MgSO4·7H2O 0.5 g, Ca(NO3)2·4H2O 0.01 g, FeSO4·7H2O 44.78 g per one liter, pH 2.0)12 using 500 cm3 Erlenmeyer flasks capped with air-permeable plugs in a rotary shaker at 30 °C. Harvested cells were collected by centrifugation for 20 minutes at 10,000 g and 4 °C, and diluted using distilled and autoclaved water, adjusted to pH 2 with H2SO4. Cell numbers were directly counted by the microscopic observation.

2.2 Microbially mediated synthesis of standard jarosite group minerals by Acidithiobacillus ferrooxidans

Jarosite group minerals were formed through mediated oxidation of Fe(II) by A. ferrooxidans. A 150 cm3 of 160 mmol dm−3 FeSO4·7H2O, adjusted to pH 2.0 using H2SO4 and Li2CO3, was added to a 500 cm3 Erlenmeyer flask with an
air-permeable plug (silico plug). Lithium carbonate (Li₂CO₃) was used because lithium ions are not structurally incorporated in jarosite-type compounds. The medium was inoculated with *A. ferrooxidans* to result in final cell numbers ranging from 10⁶–10⁹ cells cm⁻³. The medium was then cultured at 30°C in a rotary shaker until Fe(II) was completely oxidized. Complete oxidation of Fe(II) was confirmed by spectrometry with 1,10-phenanthroline. Smaller numbers of *A. ferrooxidans* required longer time for the complete oxidation of the Fe(II). A 10⁶ order of magnitude of cells cm⁻³ did not oxidize Fe(II) ions even after 4 weeks, after which the process was abandoned. More than 10⁷ order of magnitude cell numbers oxidized 160 mmol dm⁻³ Fe(II) ions completely within 7 days, although the longer time was required for complete oxidation of Fe(II) with the smaller cell numbers. After confirming complete oxidation of Fe(II), stoichiometric amounts of monovalent cation sources in jarosite compounds, K₂SO₄, (NH₄)₂SO₄, and Ag₂SO₄ were added to the solution ([M⁺] = 53.3 mmol dm⁻³) to lead the following reaction:

\[
M^+ + 3 \text{Fe}^{3+} + 2 \text{HSO}_4^- + 6 \text{H}_2\text{O} = M\text{Fe}_3(\text{SO}_4)_2(\text{OH})_6 + 8 \text{H}^+ 
\]

The solution was aged for 168 hours at 30°C in a rotary shaker since the addition of monovalent cation (M⁺) sources.

### 2.3 Formation of secondary minerals during microbially mediated dissolution of arsenopyrite

Secondary minerals formed during microbially mediated dissolution of arsenopyrite were also prepared to compare with the synthetic jarosite using the characterization technique described. Microbial oxidation of arsenopyrite is expected as follows:

1. Fe₄AsS₄ + 11 O₂ + 6 H₂O = 4 FeSO₄ + 4 H₃AsO₃
2. 4 FeSO₄ + O₂ + 2 H₂SO₄ = 2 Fe₂(PO₄)₃ + 2 H₂O
3. Fe₄AsS₄ + 11 Fe³⁺ + 7 H₂O = 12 Fe²⁺ + H₃AsO₃ + HSO₄⁻ + 10 H⁺

First, arsenopyrite is oxidized by oxygen to release Fe(II) ions in eq. (2). Iron-oxidizing bacteria accelerate the oxidation of Fe(II) ions in eq. (3) and facilitate the formation of Fe(III) ions under acidic conditions, and then lead indirect oxidation of arsenopyrite by Fe(III) in eq. (4) and subsequent jarosite formation involving monovalent cations available in the environment (eq. (1)). It is unlikely that these reactions occur under the condition of high concentrations of arsenopyrite and lead the formation (eq. (1)) of a large amount of jarosite group, because a large amount of the coexisting H₃AsO₃ inhibits the iron-oxidizing activity and growth of *A. ferrooxidans*. It is predicted that a small amount of secondary jarosite-group minerals should be formed. Identification of small quantities is difficult by XRD. Morphology is expected to help identification of such samples.

The arsenopyrite used is from Toroku, Kyushu, Japan. It was ground in a planetary-type ball-mill made of tungsten carbide (Fritsch Japan, P-5). The 38–75 μm diameter fraction was collected by sieving, and measured to have 0.61 m²/g specific surface area determined by the N₂ adsorption three point BET method using a Yuasa Ionics Quantasorb QS-13.

The composition of arsenopyrite was determined to be Fe₄As₅O₁₁·H₂O (1:1:1 molar ratio by X-ray fluorescence spectroscopy (XRF). XRD pattern of arsenopyrite indicates that α-quartz is an impurity in the arsenopyrite. Prior to microbial oxidation, arsenopyrite powder was ultrasonically cleaned to remove the oxidized surface layer once in ethanol, once in 1 mol dm⁻³ HNO₃, three times in H₂O, and once in acetone, and then dried in an aspirator jar to prevent further oxidation.

A 0.1 gram sample of the pretreated arsenopyrite powder was added to a 500 cm³ Erlenmeyer flask containing a 150 cm³ of the basal medium excluding FeSO₄ from the 9 K medium adjusted to pH 2 with H₂SO₄. *Acidithiobacillus ferroxidans* was inoculated with a final concentration of 4.0 × 10⁷ cells·cm⁻³, and then conventionally cultured at 30°C in a rotary shaker for 336 hours.

### 2.4 Characterization of biogenic jarosite compounds

The precipitate was collected by filtration with a 0.2 μm Millipore filter, air-dried at room temperature and kept in a desiccator for XRD, Raman spectroscopy and FE-SEM. The XRD patterns were collected using a JEOL JDX-3500 X-ray diffractometer with a monochromator (Cu Kα, 30 kV, 200 mA; step scanning method; time constant, 0.5 second; angle range, 2.5–65 deg/2θ). Minerals were identified based on the Joint Committee on Powder Diffraction Standards Data (JCPDS).

For biogenic jarosite compounds, crystallite size (L) and lattice strain (ε) were evaluated according to the following equation using XRD data.\(^{(4)}\)

\[
β_{obs} \cos \theta / λ = K / L + 2ε \sin \theta / λ 
\]

in which β_{obs} is the observed full-width at half maximum (FWHM), K a shape factor, equal to 0.89 in the present case, λ a wavelength (λ = 0.15418 nm for CuKα), and θ Bragg’s angle. The β_{obs} values were determined for CuKα₁ by computer fitting using a Cauchy function. By plotting β_{obs} cos θ/λ against sin θ/λ, both ε and L were obtained.

For Raman spectroscopy, excitation was accompanied by light of a single wavelength (514.5 nm) from an Ar ion laser. The incident power was approximately 38 mW at the sample point. The Raman scattered light was detected by a laser Raman spectrometer (JASCO NRS 2000). The samples were diluted to 5 mass% with KBr powder, and 0.30 g of the mixture was compressed to form a disk 10 mm in diameter. The sample stage was rotated during the measurement to avoid heating effects of the laser beam. The laser light was standardized with silicon, using a band maximum at 520 cm⁻¹, polarized parallel to the plane of incidence, and an 80° angle of incidence. The Raman scattered light was collected in the plane of incidence in the direction of normal to the incident laser light. The Raman spectra were obtained from 200 to 1300 cm⁻¹ after three accumulations using 10- and 60-second integration times for the standard jarosite-group compounds and for samples containing arsenopyrite, respectively.

Sample morphologies were observed by FE-SEM (JEOL JSM-6300) at 2–3 kV of accelerated voltage. To avoid differential charging effects, samples examined by FE-SEM were coated by evaporation with platinum.
3. Results and Discussion

3.1 Synthesis of standard jarosite group minerals by mediation of Acidithiobacillus ferrooxidans

XRD patterns for jarosite group microbiologically formed with different cell numbers in the presence K⁺, NH₄⁺, and Ag⁺ ions, showed evidence of potassium jarosite, ammoniojarosite, and argentojarosite, respectively (JCPDS 36-427, 26-1014, 41-1398). Results indicate that peak-width and intensity are independent of cell numbers of A. ferrooxidans.

Based on the results of XRD, lattice strain and crystallite size were calculated as shown in Figs. 1 and 2. Both lattice strain and crystallite size of synthesized jarosite compounds were not sensitive to cell numbers of A. ferrooxidans. These results mean the rate of jarosite formation did not clearly affect its crystallinity.

Raman spectra of biogenic potassium jarosite, ammoniojarosite and argentojarosite with different cell numbers are shown in Figs. 3, 4 and 5, respectively. These spectra are in agreement with the previously reported standards. 10) Eight peaks A-H are observed on each spectrum in Figs. 3, 4 and 5. Peaks A, B, and C are assigned to Fe-O vibrational mode in jarosite compounds. A weak peak D is assigned to γ(C13(O-H)) vibrational mode in jarosite compounds. Strong peaks E and F are assigned to stretching vibration of γ(C23(SO₄²⁻)) and γ(C1(SO₄²⁻)). Weak peaks G and H are assigned to stretching vibration of γ(C3(SO₄²⁻)) in jarosite compounds. A peak F assigned to γ(C23(SO₄²⁻)) was observed at 1006.6 cm⁻¹ for potassium jarosite, at 1006 cm⁻¹ for ammoniojarosite, and at 1011 cm⁻¹ for argentojarosite. Similarly a peak G assigned to γ(C3(SO₄²⁻)) was observed at 1107 cm⁻¹ for potassium jarosite, at 1104 cm⁻¹ for ammoniojarosite, and at 1109 cm⁻¹ for argentojarosite. There are clear peak shifts in the vibration mode of γ(C1(SO₄²⁻)) and γ(C3(SO₄²⁻)) among the different jarosite compounds. Sasaki et al. 10) suggest that these peak shifts are dependent on ionic radius of involved cation, that is, cell parameter of jarosites. The cell parameter c is in order of ammoniojarosite > potassium jarosite > argentojarosite. Chio et al. have reported natrojarosite is also unambiguously identified by micro-Raman. 15) The Raman spectroscopic identification technique was also applied to segnitite (PbFe₃(AsO₄)₂(OH)₆) and alunite (MA₃(SO₄)₂(OH)₆, M: monovalent cation), which are structurally analogical to jarosite mineral group. 16) Figures 3, 4 and 5 also indicate that vibration in the concentration of A. ferrooxidans used in the formation of each biogenic jarosite compound did not cause peak shifts.
The dependence of the Raman peak intensities on cell numbers is shown in Figs. 6, 7 and 8. Data scattering is moderate in Figs. 7 and 8, however, the relationship is mostly positive with more intense Raman peaks observed in biogenic jarosite compounds by larger cell numbers. In the previous report the faster rate of Fe(II) ion oxidation lead the stronger IR intensity of the peaks assigned to the vibration mode of $\nu_3\text{SO}_4^{2-}$ in potassium jarosite, ammoniojarosite and argentoojarosite, when the precursor for jarosite was produced through chemical oxidation of Fe(II) ions by $H_2O_2$ with different oxidation rates. In the present results the difference in the investigated range of microbiological Fe(II) oxidizing rate by $A. ferrooxidans$ is not compared with in case of the chemical oxidation of Fe(II) in the previous work, therefore, the relation would be not clear. In general, intensity of IR and Raman peaks is related to the number of IR- and Raman-actively covalent bond. The results might suggest that the rate of jarosite compound formation affects its molecular structure and fine crystalinity which is not observed by XRD. These results suggest that, Raman spectroscopy might provide information helpful for the identification of jarosite compounds formed by microbial activity of iron-oxidizing bacteria.

Figures 9, 10 and 11 show the FE-SEM images of biogenic potassium jarosite, ammoniojarosite and argentoojarosite with different cell concentrations of $A. ferrooxidans$ used, respectively. Morphologies changed significantly depending on cell concentrations. For potassium jarosite, the morphology is characteristically aggregates of round particles, with more distinct shape and roundness at larger cell concentrations.

Fig. 4  Raman spectra for ammoniojarosite formed by microbial mediation of $A. ferrooxidans$ with different cell numbers. A vertical bar indicates 0.2 cps.

Fig. 5  Raman spectra for argentoojarosite formed by microbial mediation of $A. ferrooxidans$ with different cell numbers. A vertical bar indicates 0.2 cps.

Fig. 6  Relationship between the intensities of Raman peaks for biogenic potassium jarosite and cell numbers of $A. ferrooxidans$ used. Marks of A-H are correspondent to the peaks in Fig. 3.
used (Fig. 9). Ammoniojarosite forms aggregates of cubic crystals, which have more pronounced definition and sharpness at larger cell concentrations (Fig. 10). It is hypothesized that oxidation of aqueous Fe(II) occurred more rapidly in the presence of larger cell concentrations, and that this enhanced the crystallinity of each cube. Biogenic argentojarosite has a rose flower-like morphology (Fig. 11). The figure suggests that crystallinity is more distinct when larger cell concentrations were used, and had more poor definition when cell numbers were smaller. The trend is common among three kinds of biogenic jarosite compounds evaluated here. In light of this finding crystal morphology can provide insight into the identification of jarosites formed under the influence of biogenic activity.

3.2 Identification of secondary minerals during microbially mediated dissolution of arsenopyrite

The dissolution behavior of arsenopyrite has been reported separately. Figure 12 shows an XRD pattern for the arsenopyrite residue after microbially mediated oxidation. Six peaks in the pattern identify arsenopyrite and a single peak identifies quartz which is non-reactive. A sharp peak at $2\theta = 29.4$ is observed and assigned to jarosite compounds, however, difficult to distinguish which kind of jarosite it is. In the range $23–35$ of $2\theta$, a broad peak identifying scorodite ($\text{FeAsO}_4$) is also observed. Scorodite may form according to the following set of reactions in aerobic conditions:

$$\text{H}_3\text{AsO}_3 + 2\text{Fe}^{3+} + \text{H}_2\text{O} = \text{H}_3\text{AsO}_4 + 2\text{Fe}^{2+} + 2 \text{H}^+ \quad (6)$$

$$2\text{H}_3\text{AsO}_3 + \text{O}_2 = 2\text{H}_2\text{AsO}_4 \quad (7)$$

$$\text{H}_3\text{AsO}_4 + \text{Fe}^{3+} = \text{FeAsO}_4 + 3 \text{H}^+ \quad (8)$$

Peaks that may be used to identify jarosite also lie in the range of $2\theta = 23–35$. The broad scorodite peaks may obscure any small jarosite peaks. This is a common difficulty with identification of secondary minerals during sulfide oxidation. Raman spectrum was also analyzed for the sample, however, meaningful peaks could not be identified, probably due to interference by blackish sample of sulfide.

Figure 13 presents an FE-SEM image of the arsenopyrite residue. The image clearly shows round shape that is
characteristic to potassium jarosite. Aggregates of cubic crystal, which is characteristic to ammoniojarosite, were not observed in any field view of the sample. The 9 K medium contains both NH$_4^+$ and K$^+$ ions, therefore, it is possible that ammoniojarosite might be incorporated with the potassium jarosite. However, this image mentions potassium jarosite is preferentially detected unless K$^+$ ion concentration ($4.2 \times 10^{-3}$ mol dm$^{-3}$) is less than one of tenth of NH$_4^+$ concentration ($4.5 \times 10^{-2}$ mol dm$^{-3}$) in 9 K medium. Ivarson et al. have referred potassium jarosite is thermodynamically favored over ammoniojarosite. For that matter, we reported that potassium, because of its ionic radius, is the monovalent cation best suited to stabilize jarosite. The present observation agrees with these earlier findings. It is difficult to interpret from our results the role of varying concentrations of *A. ferrooxidans*. This suggests that morphology could provide an alternative means to identify trace amounts of geochemical samples, and can provide evidence that supports determination of the history of the sample, where conventional methods would require decomposition of samples.
4. Conclusion

Potassium jarosite, ammoniojarosite, and argentjarosite were biogenically synthesized with different populations of *Acidithiobacillus ferrooxidans*. Each jarosite compound was confirmed by XRD to be a single phase. Raman spectroscopy indicated that correlation between activity of *A. ferrooxidans* and Raman intensity was weak but positive. Morphology was remarkably dependent on iron-oxidizing activity of *A. ferrooxidans* in addition of involved cations in jarosite group minerals. Higher *A. ferrooxidans* activity resulted in more sharply defined jarosite minerals.

These results were used to identify jarosite compounds formed during microbially mediated dissolution of arsenopyrite by *A. ferrooxidans*. Jarosite formed by this procedure was difficult to identify using XRD and Raman spectroscopy, due to the trace amounts of jarosite present and the poor crystallinity of these samples. However, FE-SEM imaging clearly showed there is a trend of a positive correlation between the cellular population and morphology, although the relation is not quantitative. These findings suggest that morphology could be means to give helpful information on the history and identification for trace amounts of geochemical samples without decomposition of samples.

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