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RAMAN STUDY OF THE MICROBIALLY MEDIATED DISSOLUTION OF PYRITE BY *Thiobacillus ferrooxidans*

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ABSTRACT

Raman spectroscopy was applied to the microbially mediated dissolution of pyrite by high density (more than 10^9 cells) of *Thiobacillus ferrooxidans*. In the range 600–200 cm^{-1}, peaks corresponding to secondary minerals such as elemental sulfur and jarosite were observed in addition to those of pyrite. Small amounts of elemental sulfur and poorly crystalline jarosite were detected by Raman spectroscopy, but are undetectable by X-ray diffraction and Fourier transform infrared spectroscopy. Ammoniojarosite was predominantly formed in microbially mediated dissolution of pyrite with a high density of cells, whereas jarosite was formed in the sterilized control. This finding is probably ultimately due to the much greater amount of jarosite precursors (Fe^{3+} sulfate complexes) formed by the bacterial oxidation of iron. Initially, and in both cases, the kinetically favored jarosite appeared, but in the presence of bacteria, there was so much precursor present that the available potassium was exhausted. Ammoniojarosite subsequently was formed. In the sterilized control, precursors were less readily formed, and jarosite formed. There was no evidence for direct involvement of bacteria in the formation of jarosite. The changes in mineral compositions are correlated with those in the solutions. Raman spectroscopy was used to follow compositional changes in minerals during the microbially mediated dissolution of pyrite.

Keywords: Raman spectroscopy, *Thiobacillus ferrooxidans*, microbially mediated dissolution, pyrite, elemental sulfur, ammoniojarosite, jarosite.

SOMMAIRE

La spectroscopie de Raman a été appliquée à l'étude de la dissolution de la pyrite en présence d'une forte densité (plus de 10^9 cellules) de *Thiobacillus ferrooxidans*. Sur l'intervalle 600–200 cm^{-1}, des pics correspondant à des minéraux secondaires, tels que soufre élémental et jarosite, sont présents avec ceux attribuables à la pyrite. De faibles concentrations de soufre et de jarosite à cristallinité imparfaite sont repérées par spectroscopie de Raman, mais ne le sont pas par diffraction X ou par spectroscopie dans l'infrarouge avec transformation de Fourier. L'ammoniojarosite est le produit principal de la dissolution de la pyrite en présence d'un grand nombre de cellules, tandis que c'est la jarosite qui apparaît en milieu stérile. Ce contraste est probablement dû à la plus grande proportion de précurseurs de la jarosite (complexes à sulfate de Fe^{3+}), formés suite à l'oxydation bactérienne du fer. Au début, et dans les deux cas, c'est la jarosite, phase favorisée par la cinétique, qui apparaît, mais en présence de bactéries, il y avait une quantité telle de précurseur que le potassium disponible a été complètement épuisé. C'est par conséquent l'ammoniojarosite qui est apparue. Dans le milieu stérile, les précurseurs sont moins aptes à se former, et c'est la jarosite qui est produite. Il n'y a aucune raison de proposer l'implication de bactéries dans la formation de la jarosite. Les changements dans la composition des minéraux montrent une corrélation avec les changements dans la composition des solutions. La spectroscopie de Raman a servi à caractériser ces changements dans les minéraux au cours de la dissolution de la pyrite en présence de bactéries.

(Traduit par la Rédaction)

Mots-clés: spectroscopie de Raman, *Thiobacillus ferrooxidans*, dissolution par intermédiaire de bactéries, pyrite, soufre élémental, ammoniojarosite, jarosite.

INTRODUCTION

The weathering of pyrite is the main culprit in acid mine-drainage. It is well known that iron-oxidizing bacteria, *Thiobacillus ferrooxidans*, play an important role in the formation of acid mine- drainage (Taylor *et al.* 1984). Below pH 2, the oxidation of pyrite, iron and sulfur is expressed by the following equations:

$$FeS_2 + 7/2\ O_2 + H_2O = Fe^{2+} + 2\ SO_4^{2-} + 2\ H^+ \quad \text{(mainly chemical reaction)} \quad (1)$$

$$Fe^{2+} + 1/2\ O_2 + 2\ H^+ = Fe^{3+} + H_2O \quad \text{(bacterial reaction)} \quad (2)$$

$$FeS_2 + 2\ Fe^{3+} = 3\ Fe^{2+} + 2\ S \quad \text{(chemical reaction)} \quad (3)$$

$$FeS_2 + 14\ Fe^{3+} + 8\ H_2O = 15\ Fe^{2+} + 2\ SO_4^{2-} + 16\ H^+ \quad \text{(chemical reaction)} \quad (4)$$

$$2\ S + 3\ O_2 + 2\ H_2O = 4\ H^+ + 2\ SO_4^{2-} \quad \text{(bacterial reaction)} \quad (5).$$

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Reaction (1) proceeds mainly as a chemical reaction, and only to a limited extent as a bacterial reaction (so-called "direct contact mechanism" of *T. ferrooxidans*). The reaction proceeds very slowly, compared with reaction (3) or (4). Under pH 2, oxidation of Fe^{2+} to Fe^{3+} ions by oxygen (reaction 2) is very slow. However, *T. ferrooxidans* can speed up the oxidation of Fe^{2+} ions by five or six orders of magnitude over the rate of the inorganic reaction (Lacey & Lawson 1970), providing a means of rapidly regenerating Fe^{3+} ions in acid solutions. The Fe^{3+} ions released oxidize pyrite and regenerate Fe^{2+} ions *via* reaction (3) or (4) ("indirect contact mechanism" of *T. ferrooxidans*). Singer & Stumm (1970) demonstrated that the rate of oxidation of pyrite by oxygen is too slow to generate acid water in nature, but *T. ferrooxidans* increases the rate of pyrite oxidation through cyclic production of Fe^{3+} ions by the bacteria. Especially in anaerobic environments, the indirect contact mechanism is more important than the direct contact mechanism (Taylor *et al.* 1984).

Previously, using X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR), Konno *et al.*(1991) and Sasaki *et al.* (1993), reported that during microbially mediated dissolution of pyrite with low density (~10^7 cells) of *T. ferrooxidans*, S species on the surface were found to change in a complex manner. Elemental sulfur (S^0) is an intermediate product in the indirect contact mechanism, and jarosite, $KFe_3(SO_4)_2(OH)_6$, is formed as a secondary mineral on pyrite in the following reaction ($M^+ = K^+$):

$$M^+ + 3\,Fe^{3+} + 2\,HSO_4^- + 6\,H_2O = MFe_3(SO_4)_2(OH)_6 + 8\,H^+ \quad \text{(chemical reaction)} \quad (6)$$

in which M^+ is a monovalent cation. However, the jarosite formed was found to be of poor crystallinity, owing to the low density of bacterial cells, and so was not detected by X-ray diffraction (XRD). It is difficult to analyze the spectra derived by XPS for samples containing a high density of cells owing to the differential charging effect (Konno *et al.* 1991). To date, there have been no reports of Raman spectroscopy being applied to study the microbially mediated dissolution of pyrite, though it is considered to be a useful technique for the analysis of samples containing a high density of cells.

In the present work, microbially mediated dissolution of pyrite was carried out using more than 10^9 cells of *T. ferrooxidans*, and the changes in mineral composition were determined by Raman spectroscopy, X-ray diffraction and FTIR, and compared with the changes in redox potential and composition of solutions.

EXPERIMENTAL

Pyrite

Pyrite was supplied from the Yanahara mines (Japan). Its purity is 98.2% and details of the composition have been reported elsewhere (Sasaki *et al.* 1993). The pyrite was ground using a tungsten carbide planetary-type ball mill (Fritsch Co. Ltd., P–5, Germany), and then sieved to obtain the size fraction between 38 and 75 μm. The X-ray powder-diffraction lines for this sample were measured using NaCl as the internal standard, and was found to be identical to that of pyrite. No diffraction lines other than from pyrite were observed with this sample, except for a very weak line due to SiO_2 (d = 0.338 nm), which took no part in dissolution experiments (Sasaki *et al.* 1994).

The pyrite particles were cleaned to remove the oxidized species, as previously reported (Sasaki *et al.* 1995a). The composition of the surface on pyrite after the pretreatment was determined by XPS measurement; it was confirmed that the pretreated surface on pyrite had a stoichiometric composition (Sasaki *et al.* 1995a).

The specific surface-area after the pretreatment was determined to be $0.40 \pm 0.60\ m^2g^{-1}$ by the N_2(g) adsorption BET method on a Quantasorb QS–13 (Yuasa Ionics Co. Ltd.) with a QS–300 cell suitable for low values of the surface area.

Micro-organisms and culture

Iron-oxidizing bacteria, *Thiobacillus ferrooxidans*, were isolated from acid mine-drainage in the Toyoha mines (HUTY8906) (Sasaki *et al.* 1993, 1995b) and cultivated routinely in 150 cm^3 of solution containing the 9K medium (Silverman & Lundgren 1959), adjusted to pH 2 with H_2SO_4, and dispensed into a porous plugged 500-cm^3 Erlenmeyer flask. The 9K medium consists of the following components: 15 mmol dm^{-3} $(NH_4)_2SO_4$, 2.0 mmol dm^{-3} $MgSO_4 \cdot 7H_2O$, 1.3 mmol dm^{-3} KCl, 5.7 mmol dm^{-3} K_2HPO_4, 0.061 mmol dm^{-3} $Ca(NO_3)_2$, and 160 mmol dm^{-3} $FeSO_4 \cdot 7H_2O$ as an energy source. All components of the culture medium were sterilized by autoclaving at 120°C for 20 minutes, and glass assemblies, by heating at 180°C for two hours. All cultures were grown in a rotary shaking culture-apparatus (Takasaki Kagaku Co. Ltd., TB–16) at 30°C. Dissolved oxygen was saturated under conventional conditions in the rotary shaking culture. Iron precipitates such as jarosite, which is formed during the culture of *T. ferrooxidans*, were removed by filtering the cell suspension through a sterile Whatman No. 41 filter paper, and cells were harvested by centrifugation at 18,000 × g for 20 minutes, resuspended in a sterile H_2SO_4 solution (pH 2) and stored in a refrigerator.

Dissolution experiments of pyrite

The 3.75 g of pretreated pyrite was added to a porous plugged 500-cm^3 Erlenmeyer flask containing 150 cm^3 of the 9K medium. Two experiments were carried out: (1) inoculation with 4.20×10^9 cells of *T. ferrooxidans*, and (2) sterilization. Each of the two experiments was carried out four times. Inoculated cells were all active in the logarithmically growing phase, and the number of cells was counted directly by microscopic observation (× 600) using diluted samples where necessary. All these flasks were installed in a rotary shaking culture-apparatus at 30°C

and were incubated for 7 weeks. At intervals, one cm^3 of supernatant fluid was pipetted off and filtrated with a 0.20-μm-pore membrane filter for solution analysis and measurements of pH and redox potential, *E*. The concentrations of Fe, S, and K were measured by inductively coupled plasma – atomic emission spectrometry (ICP–AES: SEIKO Co. Ltd., SPS 1200), and the concentrations of the Fe^{2+} ion were determined by the 1,10-phenanthroline method (Tamura *et al.* 1974). Dilution of the sampled solution was always done with a 0.01 mol dm^{-3} super special grade (SSG) HCl solution. After 1, 3, 5, and 7 weeks, the residues were separated by filtration using a Whatman No. 41 filter paper and vacuum-dried for XRD, FTIR, and Raman spectroscopic analysis.

XRD

The powder X-ray diffraction patterns of samples were collected with a JEOL JDX–3500 diffractometer with a monochromator under the following conditions: Cu*Kα* radiation, 30 kV, 200 mA; step-scanning method; time constant: 0.5 seconds.

FTIR

Infrared spectra were recorded with a FTIR (JASCO VALOR III) using diffuse reflectance infrared Fourier-transform spectroscopy (DRIFTS) with 0.6 (w/w) % of sample precipitate in KBr, under the following conditions: accumulation, 16 times; resolution, 4 cm^{-1}; detector, TGS; range of wavenumbers, 4000–400 cm^{-1}.

Raman spectroscopy

For Raman spectroscopy, excitation was accomplished using 514.5 nm wavelength light from an Ar ion laser. The incident power was about 38 mW at the sample point. The Raman scattered light was detected with a laser Raman spectrometer (JASCO NRS 2000). The pyrite sample was diluted to 5 wt% with KBr powder, and 0.30 g of the mixture was compressed to form a disk 10 mm in diameter. Ammoniojarosite and jarosite also were used as standard samples. They were synthesized by the autoclave method, and the nature of the products was verified by XRD and elemental analysis (Dutrizac & Kaiman 1976). A rotating apparatus was used for the measurement of the disk to avoid heating by the laser beam. The laser light was polarized parallel to the plane of incidence, and the incidence angle was 80°. The Raman scattered light was collected in the plane of incidence in the direction normal to the incident laser light. The Raman spectra were obtained by scanning between 600 and 200 cm^{-1} three times.

Results

The changes with time in the concentrations of dissolved Fe, S, and K species in the presence and absence of 4.20×10^9 cells of *T. ferrooxidans* are shown in Figure 1.

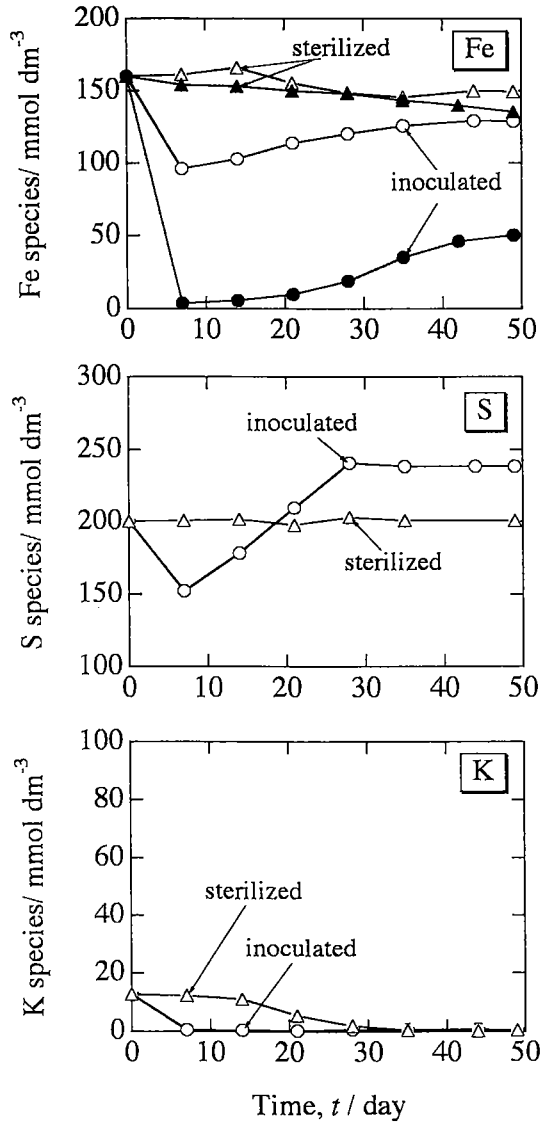

FIG. 1. Time-dependence of dissolved Fe, dissolved S, and dissolved K species in the sterilized experiments and inoculated ones with 4.2×10^9 cells of *Thiobacillus ferrooxidans*. Symbols: ○ and ●: inoculated; △ and ▲: sterilized. In the top figure, open and solid symbols are total Fe and Fe^{2+} ions, respectively.

The data are the average of four experiments. The 9K medium contains 160 mmol dm^{-3} of Fe^{2+} ions, 200 mmol dm^{-3} of S species, and 12.7 mmol dm^{-3} of K^+ ions. In the presence of the micro-organisms, within one week, concentrations of Fe, S, and K species decreased, and rapid oxidation of Fe^{2+} to Fe^{3+} ions was observed. This effect

is due to biological oxidation of Fe^{2+} ions in the presence of *T. ferrooxidans*, and formation of precipitates containing Fe, S, and K species. After one week, concentrations of Fe^{2+} and S species increased, the concentration of Fe^{3+} ions decreased very slowly, and the concentration of dissolved K species remained near zero. This finding indicates that the rate of microbially mediated dissolution of pyrite was faster than that of precipitate formation. In the sterilized control, no changes in the proportion of S species, and only a slow decrease of K and Fe species, were observed.

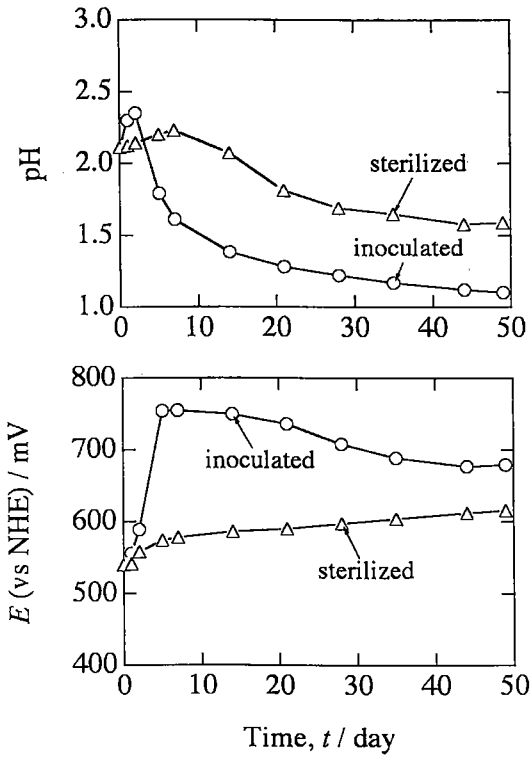

FIG. 2. Changes in pH and E in the inoculated and sterilized experiments.

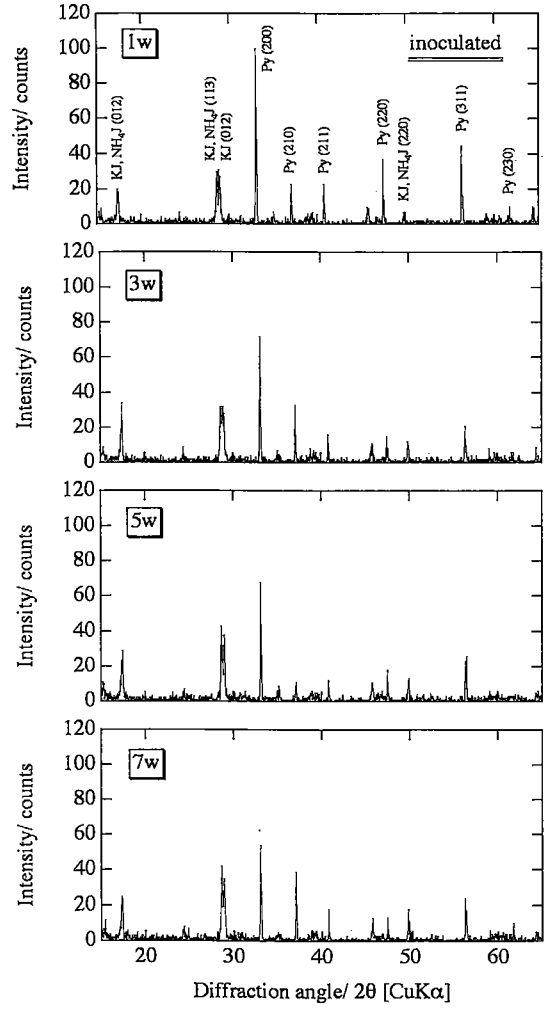

FIG. 3. XRD patterns of pyrite after 1–7 weeks of dissolution in the culture with *Thiobacillus ferrooxidans*. Py: pyrite; KJ: jarosite; NH_4J: ammoniojarosite.

Changes in pH and redox potential E are shown in Figure 2. The data are the average of four experiments. Within the first two days in the inoculated experiments, a rapid increase in pH was observed. After two days, however, pH rapidly decreased to a final value of 1.1. The redox potential E increased rapidly in the first five days, and then decreased slowly with time. With sterile control, changes in pH and E were less pronounced: pH increased slowly in the first week and then decreased after one week, and E increased slowly.

XRD patterns of the samples after 1-, 3-, 5-, and 7-week dissolution in the presence and absence of *T. ferrooxidans* are shown in Figures 3 and 4. These patterns show that in the presence of the micro-organisms, jarosite-type compounds were already formed after one week, and the peak intensities increased with time, whereas the peak intensities of pyrite (JCPDS 6–0710) decreased with time relative to those for jarosite. It is difficult to distinguish ammoniojarosite from jarosite by XRD, since the d-values of the two are very similar (JCPDS 26–1014, 10–443). In the absence of the micro-organisms, intense peaks corresponding to pyrite were observed after one week. They did not decrease significantly with time, and the formation of secondary minerals was not observed (Fig. 4). In all the XRD patterns (Figs. 3, 4), peaks corresponding to elemental sulfur were not detected at all.

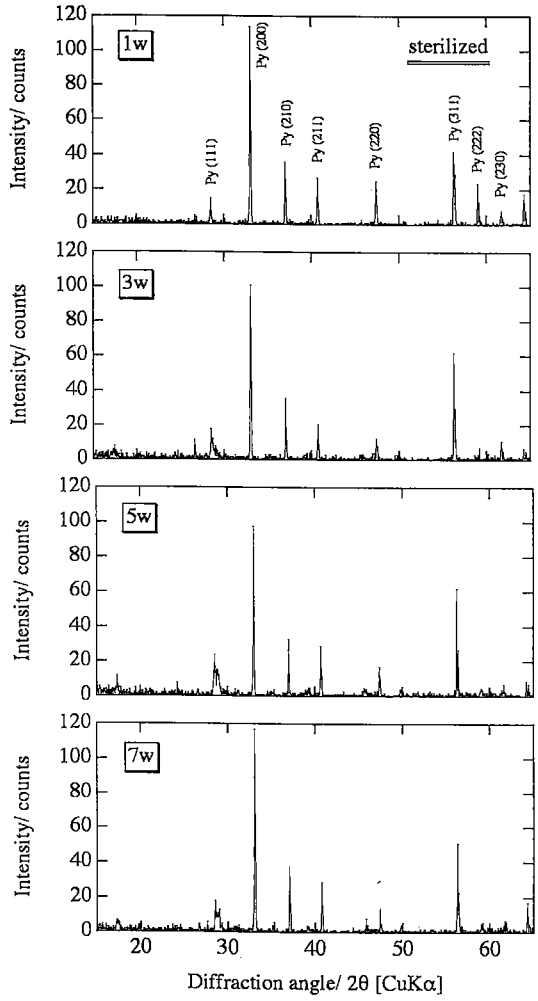

FIG. 4. XRD patterns of pyrite after 1–7 weeks of dissolution in the absence of *Thiobacillus ferrooxidans*. Py: pyrite.

Figure 5 shows FTIR spectra for pyrite after dissolution over the interval 0–7 weeks in the presence of micro-organisms. A spectrum for pure pyrite after pretreatment (0 weeks) has no pronounced peaks between 4000 and 400 cm^{-1}. After one week, the vibration modes of ν_3 (SO_4^{2-}) in jarosite compounds appeared at 1210 and 1080 cm^{-1}, the vibration modes of ν_1 (SO_4^{2-}) in jarosite-type compounds were observed at 1000 cm^{-1}, and the vibration mode of NH_4^+ was also observed at 1422 cm^{-1} (Lazaroff *et al.* 1982), and all of these increased in intensity with time. However, ν_3 (SO_4^{2-}) and ν_1 (SO_4^{2-}) bands in ammoniojarosite are similar to those in jarosite (Lazaroff *et al.* 1982). It is possible that the band at 1422 cm^{-1} due to a N–H vibration mode arises not only from ammoniojarosite, but also from the polyamides derived from the micro-organisms. As for the FTIR spectra, it is difficult to distinguish precisely the different jarosite-type compounds from each other. In the results for the sterilized control (Fig. 6), three peaks were observed, at 1190, 1090, and 1010 cm^{-1} after 3 weeks, and the intensities increased with time, though they were much less than those for the peaks in the interval 1200–1000 cm^{-1} in the inoculated samples, as can be seen from vertical bars in Figures 5 and 6. Peaks at 3400 and ~1630 cm^{-1} in Figure 6 correspond to the deformation mode and the stretching mode of O–H in H_2O, respectively. The peaks around 1422 cm^{-1} were not observed in any spectra in Figure 6.

Figures 7 and 8 show Raman spectra for pyrite after dissolution for 1–7 weeks in the presence and absence of *T. ferrooxidans*, and these can be compared with the standard spectra for pyrite, ammoniojarosite and jarosite shown in Figure 9. The standard spectrum for pure pyrite after pretreatment has two bands at 387 and 353 cm^{-1}, assigned to the vibration mode of Fe–S in pyrite (Fig. 9, top), in good agreement with previous data (Mernagh & Trudu 1993). After one week in the presence of *T. ferrooxidans*, three bands around 430, 305, and 223 cm^{-1} were observed in addition to two bands assigned to pyrite. As can be seen in the standard spectra of ammoniojarosite and jarosite, there are peak shifts around 430, 303, and 224 cm^{-1} with changing composition (marked by asterisks in Fig. 9 bottom). It is clear that the three bands around 427.2, 305.02, and 222.6 cm^{-1} observed for samples after five weeks of dissolution (Fig. 7) must be assigned to Fe–O vibration modes in ammoniojarosite, and not jarosite (Sasaki *et al.*, submitted), though for samples taken in the first three weeks, the three bands observed (Fig. 7) seem to be due to Fe–O vibration modes arising from a mixture of ammoniojarosite and jarosite. The peak intensities of jarosite-type compounds increased with time relative to those of pyrite. After three weeks, one band at 470 cm^{-1} also appeared, and is assigned to elemental sulfur (Mycroft *et al.* 1990). The intensity of the 470 cm^{-1} band increased until five weeks had elapsed, and decreased after five weeks. In the absence of *T. ferrooxidans*, three bands at 433, 302 (broad), and 225.3 cm^{-1} appeared for samples taken after five weeks, in addition to bands from pyrite. No band attributed to elemental sulfur was observed. The three bands correspond predominantly to Fe–O vibration modes in jarosite according to Figure 9 (bottom). The intensities increased relative to those of the pyrite bands, which decreased with time. This finding indicates that small amounts of jarosite were formed preferentially over ammoniojarosite in the sterilized control.

On the basis of peak heights of Raman bands in Figures 7 and 8, the intensities of a band around 430 cm^{-1} (corresponding to ammoniojarosite or jarosite) and a band at 470 cm^{-1} (corresponding to sulfur) relative to a band at 387 cm^{-1} (corresponding to pyrite) were plotted (Fig. 10). In the absence of micro-organisms, relatively small amounts of jarosite-type compounds were formed and increased with time, and elemental sulfur was not

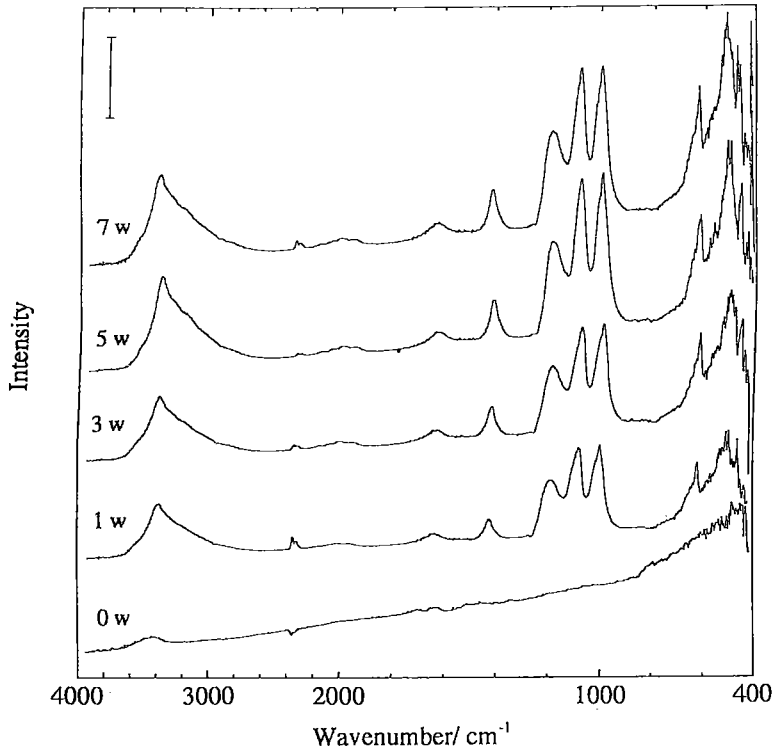

FIG. 5. FTIR spectra of pyrite after 0–7 weeks of dissolution in the culture with *Thiobacillus ferrooxidans*. A vertical bar indicates two Kubelka–Munk units for the samples in interval 1–7 weeks, and one Kubelka–Munk unit for the pure pyrite (0 week).

formed. In the presence of *T. ferrooxidans*, accumulation and consumption of elemental sulfur are clearly observed. Accumulation of jarosite-type compounds was greatly enhanced by the presence of *T. ferrooxidans*.

DISCUSSION

Based on Figures 1, 2, and 10, the process of microbially mediated dissolution of pyrite can be divided to three phases: (1) week 0–1, (2) weeks 1–5, and (3) weeks 5–7.

In the first two days, the micro-organisms oxidized Fe^{2+} to Fe^{3+} ions, with cell growth according to reaction (2), leading to an increase in pH and *E*. As reported previously (Sasaki *et al.* 1993), *E* of the solution in this system is mainly controlled by the redox system $[Fe^{3+}]/[Fe^{2+}]$, and the increase in *E* continued for one week. After two days, the pH decreased, indicating that reaction (4) occurred. The formation of jarosite compounds started [$M^+ = K^+$ and NH_4^+ in reaction (6)], and K^+ ions were completely consumed within one week.

From weeks 1 to 5, the decrease in *E* and pH, and the accumulation of jarosite-type compounds and elemental sulfur were enhanced in the inoculated experiments. This finding indicates that reactions (3), (4), and (6) ($M^+ = NH_4^+$) were enhanced, and that the iron-oxidizing activity of *T. ferrooxidans* declined gradually, since Fe^{2+} ions accumulated with time.

In the last period (weeks 5–7), changes in *E*, pH, and solution compositions became smaller than in the second period, and consumption of elemental sulfur was enhanced in the inoculated experiments. We believe that *T. ferrooxidans* preferred reaction (5) over reaction (2).

Changes in solution composition, pH, and *E* coincided with the process of formation of secondary minerals. The microbially mediated dissolution of pyrite was well explained by the indirect contact mechanism, as shown in reactions (2)–(6), but not by the direct contact mechanism alone. Although the amounts of the secondary minerals formed cannot be estimated exactly from the spectroscopic data, the analysis of the solution gives quantitative information.

The consumption of 12.73 mmol dm^{-3} of K^+ ions in the inoculated samples in the first week (Fig. 1) indicates that 1.91 mmol of jarosite was formed during that time. After five weeks, the three bands in Figure 7 are in good agreement with those corresponding to the vibration modes

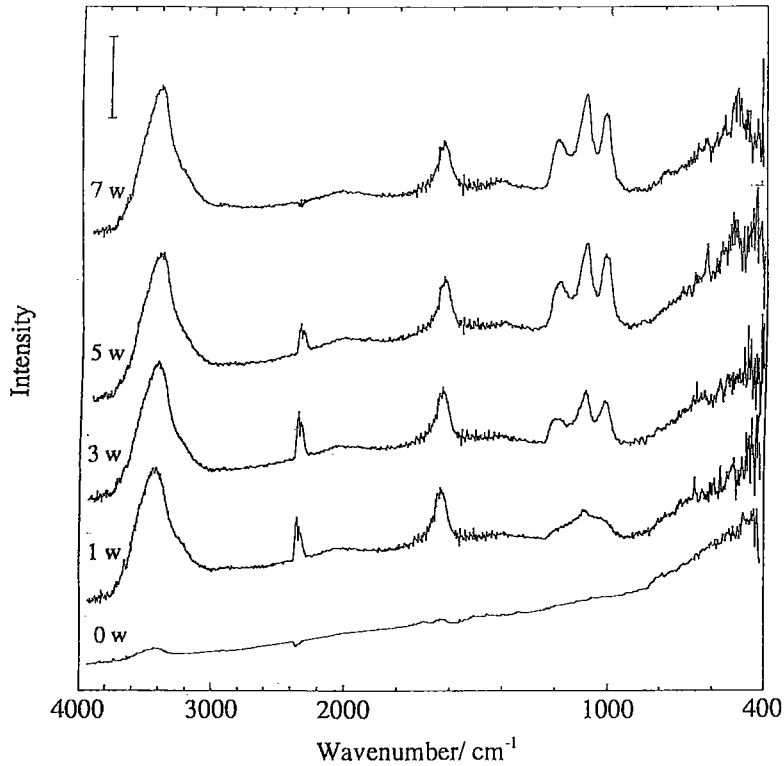

FIG. 6. FTIR spectra of pyrite after 0–7 weeks of dissolution in the absence of *Thiobacillus ferrooxidans*. A vertical bar indicates 0.2 Kubelka–Munk unit for the samples in interval 1–7 weeks, and one Kubelka–Munk unit for the pure pyrite (0 week).

of ν_3 (SO_4^{2-}) in ammoniojarosite (Fig. 9, bottom). It is clear from the composition of the solution and Raman spectra for the inoculated samples (Figs. 1, 3, 5 and 7) that the secondary minerals are predominantly ammoniojarosite, with small amounts of jarosite. The amount of ammoniojarosite formed cannot be estimated, because the concentration of NH_4^+ ions was not determined. However, considering that 160 mmol dm^{-3} Fe^{3+} ions were consumed within one week (Fig. 1), it is reasonable to assume that all of the 30 mmol dm^{-3} of NH_4^+ ions initially present was used to form ammoniojarosite (corresponding to 4.5 mmol of stoichiometric ammoniojarosite). This is not inconsistent with the results of FTIR and XRD (Figs. 3, 5). On the other hand, in the sterilized control, a small amount of jarosite was formed, but not ammoniojarosite. The dominant type of jarosite-group phase in the inoculated system is different from that in the sterilized system.

This phenomenon is not considered a kind of biomineralization. Previously, the author reported that on the basis of X-ray photoelectron spectroscopy (XPS) and X-ray microscopic analysis (XMA), jarosite was formed in microbially mediated dissolution of pyrite with *T. ferrooxidans* (Konno *et al.* 1991, Sasaki *et al.* 1993). The 9K medium contains 30 mmol dm^{-3} of NH_4^+ ions and 12.73 mmol dm^{-3} of K^+ ions as monovalent species. Rates of formation for jarosite-type compounds are related to ionic radii of the monovalent cations, and are reported to be greater for $M^+ = K^+$ than for $M^+ = NH_4^+$ (Ivarson *et al.* 1979). Initial numbers of cells are two orders of magnitude greater in the present work than in previous studies (Sasaki *et al.* 1993). The 9K medium was used in the present work, whereas the basal solution of 9K (excluding 160 mmol dm^{-3} of Fe^{2+} ions from the 9K) was used in the previous work (Sasaki *et al.* 1993). Therefore, precursors (Fe^{3+} sulfate complexes) to the jarosite-group compounds were more easily formed in the present work than in the previous work (Sasaki *et al.* 1993). In the inoculated system, jarosite was considered to have formed more rapidly than in the sterilized system because of the more rapid (bacterially mediated) oxidation of Fe^{2+} ions to suitable precursors of jarosite: initially jarosite was the dominant form, but because of the rapid rate of jarosite formation, all the 12.73 mmol dm^{-3} of K^+ ions in the medium were consumed within one week, and so further precipitation involved mainly the less kinetically favored ammoniojarosite.

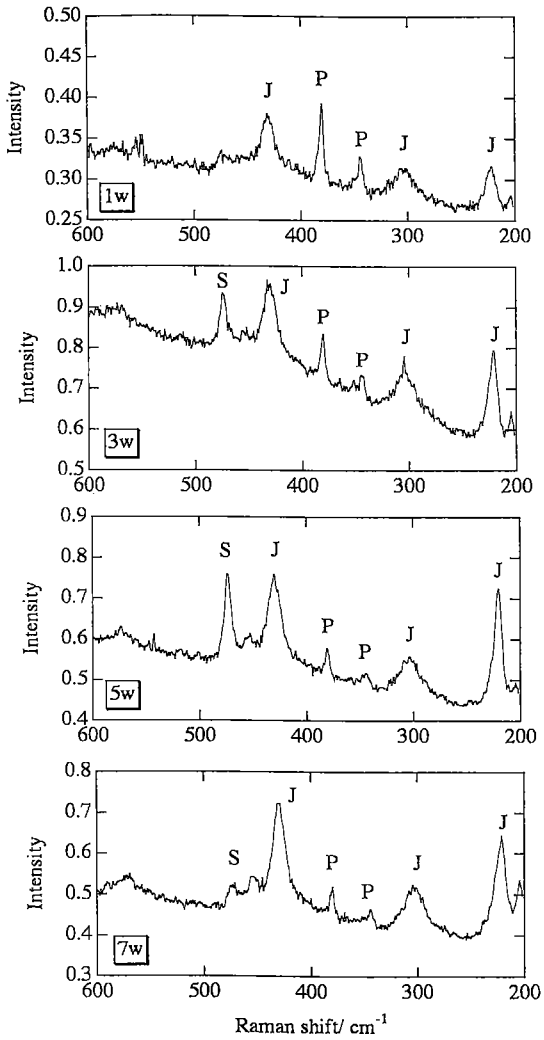

FIG. 7. Raman spectra of pyrite after 1–7 weeks of dissolution in the culture with *Thiobacillus ferrooxidans*. P: pyrite; J: jarosite-group compounds; S: elemental sulfur.

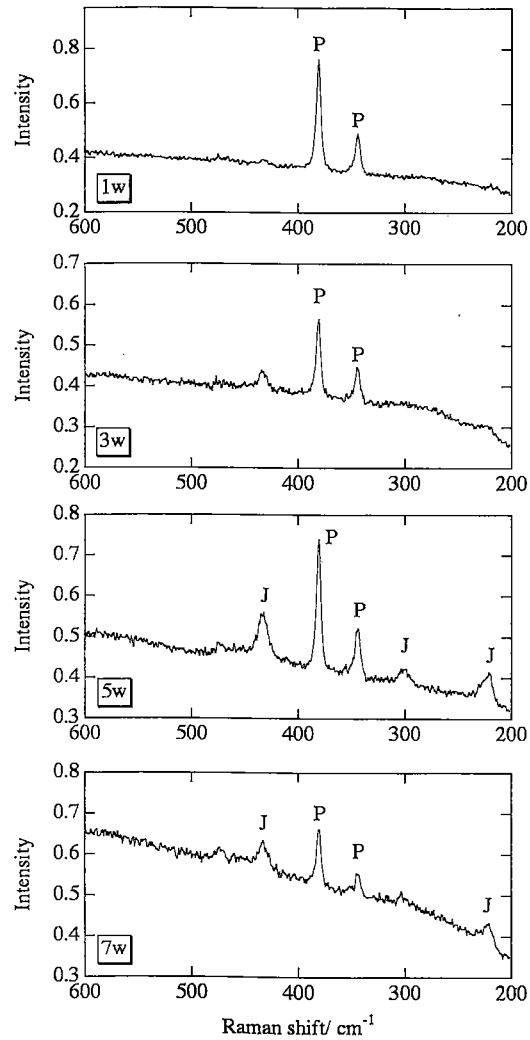

FIG. 8. Raman spectra of pyrite after 1–7 weeks of dissolution in the absence of *Thiobacillus ferrooxidans*. P: pyrite; J: jarosite-group compounds.

Therefore, micro-organisms did not take part directly in the formation of ammoniojarosite and jarosite. Even in the sterilized control, jarosite was formed, though slowly (Fig. 8). Probably the Fe^{3+} ions required for formation of jarosite were formed through the oxidation of Fe^{2+} ions in the 9K medium by air, and jarosite was then chemically formed. Only a small concentration of precursors (Fe^{3+} sulfate complexes) was present, so that the supply of potassium was at all times sufficient for only the kinetically favored jarosite to form. Evidence in support of this conclusion may also be found in another report (Sasaki *et al.* 1995b); the author, using the filtrate obtained after removing bacterial cells from suspensions with a 0.2-μm-membrane filter, has obtained evidence to prove that *T. ferrooxidans* itself makes no direct contribution to the crystallization of argentojarosite [$AgFe_3(SO_4)_2(OH)_6$].

These results suggest that the iron-oxidizing bacteria, *T. ferrooxidans*, are active in acidic mine environments, where different types of jarosite-group phases and elemental sulfur are observed, since the precursors to jarosite (Fe^{3+} sulfate complexes) are formed much more readily bacterially than chemically.

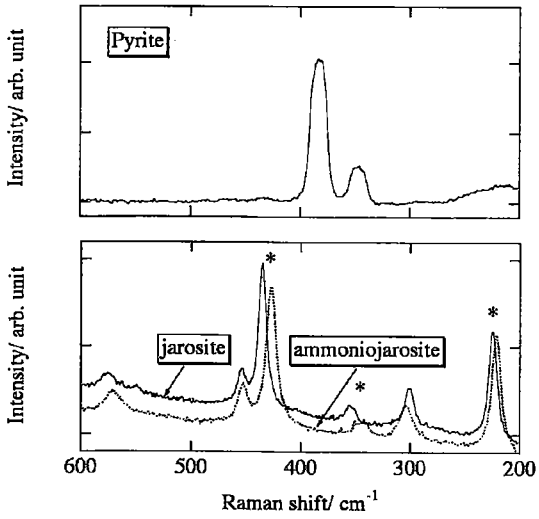

FIG. 9. Raman spectra of pyrite (top), and ammoniojarosite and jarosite (bottom). Marks (*) indicate the peak shift.

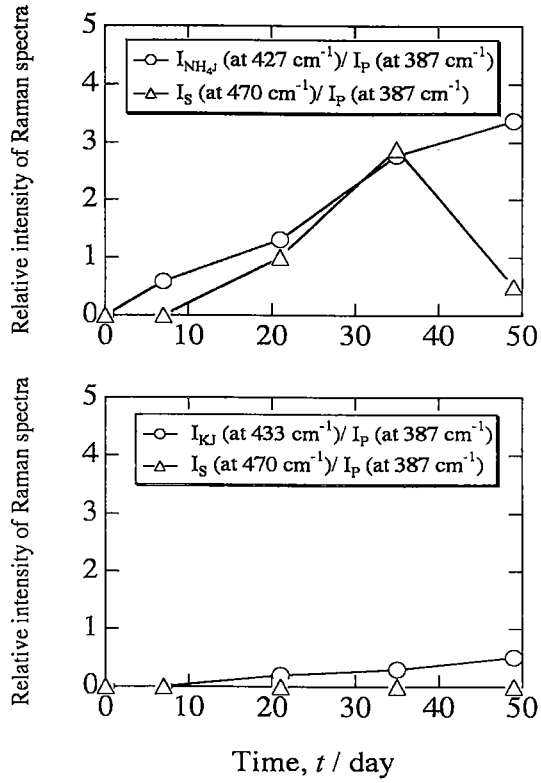

FIG. 10. Relative intensities in Raman spectra: inoculated (top) and sterilized (bottom). I_{NH4J}, I_{KJ}, I_P, and I_S indicate peak intensities of Raman bands at 427 (ammoniojarosite), 433 (jarosite), 387 (pyrite), and 470 (elemental sulfur) cm^{-1}.

CONCLUSIONS

Raman spectroscopy was applied to characterize the microbially mediated dissolution of pyrite with a high density (more than 10^9 cells) of *Thiobacillus ferrooxidans*. This approach provided useful information on the formation of secondary minerals:

(1) In the range between 600 and 200 cm^{-1}, peaks corresponding to the secondary minerals such as elemental sulfur and jarosite-group compounds were observed in addition to those of pyrite. Even small amounts of elemental sulfur and poorly crystalline jarosite, which were undetectable by XRD and FTIR, were detected by Raman spectroscopy.

(2) It is possible to distinguish ammoniojarosite from jarosite based on Raman spectroscopy. Ammoniojarosite was predominantly formed during the microbially mediated dissolution of pyrite with a high density of cells, whereas jarosite was formed in the sterile control and also in the case of low density of cells (~10^7 cells) (Sasaki *et al.* 1993). Initially, the kinetically favored jarosite forms, but if the precursors (Fe^{3+} sulfate complexes) to jarosite-type compounds still remain at significant concentration in the solutions after all the K^+ ions have been consumed to form jarosite, NH_4^+ ions are incorporated into the jarosite compounds, leading to the formation of ammoniojarosite.

(3) The changes in mineral compositions parallel those in the composition of the solutions. Raman spectroscopy was found to be useful in following the subtle changes in composition of minerals and the formation of poorly crystalline minerals during the microbially mediated dissolution of pyrite.

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REFERENCES

DUTRIZAC, J.E. & KAIMAN, S. (1976): Synthesis and properties of jarosite-type compounds. *Can. Mineral.* **14**, 151-158.

IVARSON, K.C., ROSS, G.J. & MILES, N.M. (1979): The microbiological formation of basic ferric sulfates. II.

Crystallization in presence of potassium-, ammonium-, and sodium-salts. *Soil Sci. Soc. Am. J.* **43**, 908-912.

KONNO, H., SASAKI, K., TSUNEKAWA, M., TAKAMORI, T. & FURUICHI, R. (1991): X-ray photoelectron spectroscopic analysis of surface products on pyrite formed by bacterial leaching. *Bunseki Kagaku* **40**, 609-616 (in Japanese, with English abstr.).

LACEY, D.Y. & LAWSON, F. (1970): Kinetics of the liquid-phase oxidation of acid ferrous sulfate by the bacterium *Thiobacillus ferrooxidans*. *Biotech. Bioeng.* **12**, 29-50.

LAZAROFF, N., SIGAL, W. & WASSERMAN, A. (1982): Iron oxidation and precipitation of ferric hydroxysulfates by resting *Thiobacillus ferrooxidans* cells. *Appl. Environ. Microbiol.* 43, 924-938.

MERNAGH, T.P. & TRUDU, A.G. (1993): A laser Raman microprobe study of some geologically important sulphide minerals. *Chem. Geol.* **103**, 113-127.

MYCROFT, J.R., MCINTYRE, N.S., LORIMER, J.W. & HILL, I.R. (1990): Detection of sulfur and polysulphides on electrochemically oxidized pyrite surfaces by X-ray photoelectron spectroscopy and Raman spectroscopy. *J. Electroanal. Chem.* **292**, 139-152.

SASAKI, K., KONNO, H. & INAGAKI, M. (1994): Structural strain in pyrite evaluated by X-ray powder diffraction. *J. Mater. Sci.* **29**, 1666-1669.

_____, TSUNEKAWA, M. & KONNO, H. (1995b): Characterization of argentojarosite formed from biological oxidized Fe^{3+} ions. *Can. Mineral.* **33**, 1311-1319.

_____, _____, _____, HIRAJIMA, T. & TAKAMORI, T. (1993): Leaching behavior and surface characterization of pyrite in bacterial leaching with *Thiobacillus ferrooxidans*. *Shigen-to- Sozai* **109**, 29-35 (in Japanese, with English abstr.).

_____, _____, OHTSUKA, T. & KONNO, H. (1995a): Confirmation of a sulfur-rich layer on pyrite after oxidative dissolution by Fe^{3+} ions around pH 2. *Geochim. Cosmochim. Acta* **59**, 3155- 3158.

SILVERMAN, M.P. & LUNDGREN, D.G. (1959): Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. 1. An improved medium and a harvesting procedure for securing high cell yield. *J. Bacteriol.* **77**, 642-647.

SINGER, P.C. & STUMM, W. (1970): Acid mine drainage: the rate-determining step. *Science* **167**, 1121-1123.

TAMURA, H., GOTO, K., YOTSUYANAGI, T. & NAGAYAMA, M. (1974): Spectrophotometric determination of iron(II) with 1, 10-phenanthroline in the presence of large amounts of iron(III). *Talanta* **21**, 314-318.

TAYLOR, B.E., WHEELER, M.C. & NORDSTROM, D.K. (1984): Stable isotope geochemistry of acid mine drainage: experimental oxidation of pyrite. *Geochim. Cosmochim. Acta* **48**, 2669-2678.

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