Sulfur Chemistry in Bacterial Leaching of Pyrite

AXEL SCHIPPERS, PETER-GEORG JOZSA, AND WOLFGANG SAND*
Universität Hamburg, Institut für Allgemeine Botanik, Abteilung Mikrobiologie, D-22609 Hamburg, Germany
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In the case of pyrite bioleaching by Leptospirillum ferrooxidans, an organism without sulfur-oxidizing capacity, besides the production of tetra- and pentathionate, a considerable accumulation of elemental sulfur occurred. A similar result was obtained for chemical oxidation assays with acidic, sterile iron(III) ion-containing solutions. In the case of Thiobacillus ferrooxidans, only slight amounts of elemental sulfur were detectable because of the organism’s capacity to oxidize sulfur compounds. In the course of oxidative, chemical pyrite degradation under alkaline conditions, the accumulation of tetra- and pentathionate, and thiosulfate occurred. The data indicate that thiosulfate, trithionate, tetrathionate, and disulfane-monosulfonic acid are key intermediate sulfur compounds in oxidative pyrite degradation. A novel (cyclic) leaching mechanism is proposed which basically is indirect.

Bacterial leaching in mine waste containing pyrite leads to the formation of acid mine drainage. Because of its detrimental impact on the environment, pyrite oxidation, which is not yet fully understood, needs to be controlled (6). Bacterial leaching is the oxidation of insoluble metal sulfides by specialized lithotrophic bacteria to sulfuric acid containing dissolved heavy metal ions (5). Two mesophilic bacteria are known to catalyze this process, Thiobacillus ferrooxidans and Leptospirillum ferrooxidans. Since the discovery of bacterial leaching (3), two hypotheses for the dissolution reactions have been discussed: the direct and the indirect attack mechanisms (23).

Doubts concerning the relevance of these mechanisms have arisen because of the results of a study of microbial diversity in uranium mine waste heaps (26), as well as other findings (24). In these heaps, moderately acidophilic thiobacilli like Thiobacillus intermedius or Thiobacillus neapolitanus were detected in higher cell counts than was T. ferrooxidans at sites with mainly neutral pH values. Consequently, the amount of the substrate needed, e.g., elemental sulfur, for the moderately acidophilic thiobacilli could not originate from pyrite oxidation by T. ferrooxidans (24). Other sources of metabolizable substrates were not available. The indirect attack (or leach) mechanism only postulates elemental sulfur and iron(II) ions as intermediates during the oxidation of metal sulfides. Thus, a different pyrite oxidation mechanism had to be taken into account. Goldhaber (10) detected in his chemical studies on pyrite dissolution in the pH range from 6 to 9 the intermediate compounds sulfite, thiosulfate, and tetrathionate. These compounds are known to be suitable substrates for moderately acidophilic thiobacilli. Luther (12), Moses and Herman (14), and Moses et al. (15) proposed an indirect mechanism for the oxidation of pyrite by iron(III) ions. According to this account, thiosulfate and iron(II) ions are formed as the first intermediates. As has been shown, thiosulfate undergoes a series of reactions in acidic solutions starting from disulfane-monosulfonic acid and giving rise to polythionates, elemental sulfur, and sulfite (30, 35).

The aim of the present study was to elucidate the intermediate sulfur compounds occurring in the course of bacterial pyrite oxidation at low pH values. Experiments were run with T. ferrooxidans and L. ferrooxidans and (chemically) with iron(III) ions. The latter were used to consolidate the series of connected reactions into single, comprehensive ones, since the importance of iron(III) ions for the leaching attack is generally accepted.

Some sulfur compounds, like thiosulfate, are unstable in acid solutions, and therefore a sensitive analytical technique is required for their detection. Ionic sulfur compounds (polythionates, sulfite, and sulfite) were detected by ion-pair chromatography (29, 30), and elemental sulfur was detected by reversed-phase chromatography (31). Diode array detection was used to identify compounds by comparison of their UV-absorption spectra with those of standards.

Furthermore, the stability of the respective intermediates in acid solutions was investigated to quantify the contribution of chemical reactions to the mechanism of bacterial leaching.

This paper is based on the results of studies by A. Schippers for a Ph.D. thesis at the Faculty of Biology, University of Hamburg, Hamburg, Germany.

MATERIALS AND METHODS
Organisms and growth conditions. T. ferrooxidans (strain R1) and L. ferrooxidans (ATCC 49879), both originating from Mina Ilba, Baia Mare, Romania (25), were grown in a medium containing iron(II) sulfate (13) at 28°C. Cells were harvested by centrifugation. Cell numbers were determined by counting with a Helber chamber.

Pyrite preparation. The purity of the pyrite used for leaching experiments and for investigations of the chemical stability of polythionates was checked (27). The pyrite was ground, sieved, and washed with boiling 6 N HCl, rinsed twice with deionized water, and rinsed three times with acetone by modifying a procedure described previously (15). Afterwards it was dried at 60°C and sterilized for 24 h at 120°C under nitrogen atmosphere. For all experiments a grain size of 36 to 200 μm was used.

Leaching experiments. For leaching experiments, sterilized 100-ml conical flasks containing 1 g of pyrite and 50 ml of medium without iron(II) sulfate (pH 4.9, sterile filtered) were inoculated with T. ferrooxidans or L. ferrooxidans (10⁶ cells each) according to the procedure described by Mackintosh (13). These bacterial assays as well as sterile control assays containing 10 mM iron(III) sulfate for chemical oxidation of pyrite were shaken (150 rpm) and incubated at 28°C. Experiments were performed in triplicate. Vitality of cells was checked by cell count determination by the most probable number technique with iron(II) sulfate medium. Cross-contamination between morphologically distinct cells of T. ferrooxidans and L. ferrooxidans was checked by microscopy. Aliquots of 1 ml were taken for sampling during shaking of the assay flasks to keep the pyrite particles suspended. The samples were analyzed for ionic sulfur compounds and elemental sulfur by high-pressure liquid chromatography and for iron(II) and iron(III) ions by spectrophotometry (17). Sulfate concentration was determined either indirectly by using atomic absorption spectrophotometry to measure the
bacterium remaining in solution after precipitating sulfate with excess barium chloride (4) or by directly ion chromatography (Dionex, model 2000/SP).

For analysis, the samples were centrifuged in Eppendorf tubes for 10 min (Heraeus, 9,000 rpm). The supernatant was quantitatively decanted, and 250 μl of the supernatant was mixed with 750 μl of M phosphate buffer (pH 7.0) to precipitate iron(II) ions. After removal of the precipitates by centrifugation, the concentrations of thiosulfate and polythionates were measured. For measuring sulfite and sulfate, 100 μl of the supernatant was diluted with 900 μl of a 20 mM phosphate buffer solution instead of phosphate buffer (pH 7.0) to interfere with detection of these compounds. For the determination of iron(II), iron(III), and sulfate ions, the supernatant was also used. Elemental sulfur was extracted three times with ethanol from the pellet of the centrifugation. For this purpose, 1 ml of ethanol was added to the pellet and the mixture was whisked until the pellet was totally suspended. Afterwards, 15 min of ultrasonic treatment ensued; this was followed by centrifugation. The supernatant was quantitatively decanted, and the procedure was repeated two times by addition of fresh ethanol.

The collected supernatants were used for quantification of the sulfur content.

Chemical pyrite oxidation at neutral pH. In order to compare the formation of sulfur compounds caused by pyrite oxidation at low pH values with those at neutral values, 1 g of calcium carbonate as buffering substance was added to assay flasks containing 2.5 g of pyrite in 50 ml of deionized water. The assay flasks were shaken at 150 rpm. Samples were taken at several times as described above.

Ionic sulfur compounds were analyzed without diluting the samples.

Pyrite oxidation by iron(III) in the presence of silver(I) ions. In order to unambiguously detect thiosulfate as the first sulfur compound in the course of pyrite degradation, assays were performed with pyrite and iron(III) ions in the presence of silver(I) ions. Silver(I) ions precipitate thiosulfate, preventing a further oxidation by iron(III) ions. One gram of pyrite was added to a solution containing 100 mM iron(III) nitrate and 500 mM silver nitrate (pH 1.7). Control assays did not contain silver nitrate.

Sulfur compounds were measured after 4 days of incubation (28°C, 150 rpm).

Stability of thiosulfate in bioleaching solutions. The stability of thiosulfate (10 mM) in sterile, acidic iron(III) chloride solutions (20 mM) was investigated. Thiosulfate and iron(III) chloride were separately dissolved. The iron(III) chloride solution was adjusted to pH 1.7. Both solutions were sterile filtered to ensure purely chemical reactions. The reaction was started by mixing the solutions. Samples were taken at different times and diluted 1:4 with a 0.1 M phosphate buffer (pH 7.0) to stop the reaction by precipitating the iron(II) ions with a surplus of phosphate ions. After centrifugation, supernatants were analyzed for the presence of thiosulfate, polythionates, and elemental sulfur. The concentrations of iron(II), iron(III), and sulfate ions were measured at the end of the experiment.

Stability of polythionates in bioleaching solutions. The stability of tri-, tetra-, and pentathionate (10 mM) was investigated in sterile, acidic iron(III) sulfate solutions (20 mM) and in sterile acidic solutions containing 1 g of pyrite. All assays had a pH of 1.9. The assays without pyrite were shaken (150 rpm), and the assays containing pyrite were shaken or vigorously stirred. Samples were taken at several times as described above.

Quantification of sulfur compounds by high-pressure liquid chromatography. The concentrations of inorganic sulfur compounds except sulfate were measured by ion-pair chromatography (30, 31) by using a GPC column (150 by 4.6 mm; Poros columns, Waters) at eluent flow rates of 1 ml/min. The retention times of thiosulfate and polythionates were determined with a water-acetonitrile (75:25 [vol/vol]; Merck) eluent containing 1 mM sodium carbonate and 2 mM tetrabutylammonium dihydrogen phosphate at pH 7.7. Sulfide and sulfate concentrations were determined with a water-acetonitrile (85:15 [vol/vol]; eluent containing 1 mM sodium carbonate and 2 mM tetrabutylammonium hydroxide adjusted to pH 11.0 by the addition of phosphoric acid.

Elemental sulfur (S, homocyes) concentrations were determined by reversed-phase chromatography (31) with a Hypersil-ODS-5 μm column (125 by 4.6 mm) with pure methanol (Merck) as eluent at a flow rate of 1 ml/min.

For analysis of sulfur compounds, a high-pressure liquid chromatography system from Kontron was used. It consisted of a data system 450-MT2/DAD (V, 11.0 V), a gradient former 425, a pump 422, an autosampler 465, and a diode array detector 440. All sulfur compounds were measured by UV detection (sulfite and trithionate at 195 nm, sulfide, thiosulfate, and tetra- and pentathionate at 215 nm, and sulfur at 254 nm). The assignment of the peaks was made by using pure standards. Sulfite, sulfide, elemental sulfur, thiosulfate, and tetrathionate were obtained from Merck. Tri- and pentathionate were gifts of Steudel and coworkers prepared according to Fehér (7).

RESULTS

Biological and chemical pyrite oxidation. In studies on bacterial leaching, pyrite is often used as a model substance for the analysis of reactions, rates, and/or the contribution of various bacteria growing in leach environments (18).

Both organisms, L. ferrooxidans and T. ferrooxidans, solubilized pyrite in equal amounts. The graphs in Fig. 1A and B indicate comparable concentrations of iron(III) ions. Besides elemental sulfur and sulfate, the sulfur compounds tetra- and pentathionate occurred in assays with L. ferrooxidans. This was also noted for the chemical assays with iron(III) ions (Fig. 1C). Sulfide, sulfite, thiosulfate, and tetrathionate were not detected. The formation of sulfur compounds after 7 days is summarized in Table 1. During the course of pyrite oxidation, L. ferrooxidans produced the highest amounts of elemental sulfur, tetrathionate, and pentathionate. The accumulation of these compounds occurred because this bacterium is not able to oxidize sulfur compounds. About 10% of the dissolved (total) sulfur consisted of elemental sulfur, and 0.5% was polythionates. In cultures of T. ferrooxidans, an organism able to metabolize sulfur compounds, polythionates were not detectable at all.

Elemental sulfur occurred in concentrations of about 1% of the total sulfur content.

In the case of chemical pyrite oxidation by iron(III) ions, which is comparable with leaching by L. ferrooxidans (sulfur compounds are not further oxidized), tetrathionate, pentathionate, and elemental sulfur were formed. However, because of the missing biological iron(II) ion oxidation in these chemical assays, iron(II) ions accumulated (Fig. 1C).

In contrast to pyrite oxidation at low pH, thiosulfate, trithionate, and tetrathionate accumulated without the formation of elemental sulfur and pentathionate if pyrite was degraded at a neutral pH of 7.8 to 8.0, as shown in Fig. 2. Tetrathionate was 100 times more abundant than in assays with iron(III) ions at pH 1.9 (Fig. 1C). Sulfide and sulfite were not detectable (as in the assays at low pH) (results not shown).

The first sulfur compound in pyrite degradation. To determine whether elemental sulfur or thiosulfate is the first detectable compound in oxidative pyrite degradation, experiments were carried out with and without the addition of silver(I) ions under acidic conditions with iron(III) ions mimicking bacterial and/or chemical leaching. In the case of thiosulfate being the first detectable compound, a reaction with silver(I) ions would cause the formation of silver thiosulfate, which is an insoluble compound. In acidic solutions, silver thiosulfate is not stable and decomposes to insoluble black silver sulfide (20) which finally can be detected. In the case of sulfur formation, a reaction with silver would not ensue and sulfur would be detectable.

In assays containing silver(I) ions a black precipitate of a silver compound was formed in addition to 1.8 mM sulfate. Elemental sulfur was not detectable. However, in assays without silver(I) ions, 8.6 mM sulfate and 2 mM elemental sulfur occurred. Polythionates could not be measured because of the strong UV adsorption of nitrate ions. To unequivocally show the formation of silver sulfide and to determine the amount, the precipitate was analyzed. The washed precipitate (washed with deionized water) was dissolved in 50 ml of a 20 mM NaOH solution containing 66.4 mmol of sodium cyanide. The analysis by ion chromatography with amperometric detection (Dionex) demonstrated 6.3 mM sulfide. As a consequence, thiosulfate was shown to be the key compound.

Fate of thiosulfate in bioleaching solutions. The chemical fate of thiosulfate was analyzed to study the array of compounds occurring under leaching conditions. In acidic solutions containing iron(III) ions, thiosulfate was rapidly oxidized to tetrathionate, pentathionate, and sulfate, as shown in Fig. 3. The reaction proceeded stoichiometrically. Pentathionate and sulfate were formed in equimolar amounts. Iron(III) ions were reduced to iron(II) species (data not shown).

Stability of polythionates in bioleaching solutions. Since only low concentrations of tetra- and pentathionate were detectable in the experiments at low pH, tests were performed elucidating the chemical fate of polythionates in acidic solu-
tions with or without pyrite. Moses et al. (15) provided evidence for the catalytic role of the pyrite surface in the oxidation of polythionates.

Tetrathionate did not decompose in sterile, acidic, iron(III) ion-containing solutions without pyrite, indicating its chemical stability under these conditions. Only when vigorous stirring was used did a degradation of tetrathionate become detectable, as shown in Fig. 4. About 25% of the tetrathionate was

FIG. 1. Formation of sulfur compounds in the course of pyrite bioleaching by L. ferrooxidans (A), T. ferrooxidans (B), or iron(III) ions (C). Symbols: ×, sulfate; ●, elemental sulfur; ○, tetrathionate; ▲, pentathionate; ◊, iron(II) ions; △, iron(III) ions.
degraded within 19 days in the presence of pyrite to sulfur and sulfate as well as to traces of tri- and pentathionate. Obviously, a close contact between the tetrathionate molecule and the pyrite surface was required for this degradation.

Similar results were obtained concerning the pentathionate degradation. This compound was also stable in sterile, acidic, iron(III) ion-containing solutions without pyrite. Comparable with tetrathionate, more than 50% of the pentathionate was degraded mainly to sulfate, besides elemental sulfur and tetrathionate, within 19 days in the presence of pyrite and when vigorous stirring was used. Pentathionate is less stable in biolaching solutions than teta- or pentathionate. In acidic, iron(III) ion-containing solutions without pyrite, about 90% of the trithionate was degraded to sulfate, tetrathionate, and pentathionate within 19 days. Elemental sulfur did not occur (results not shown), which indicates that trithionate is not a source of sulfur formation.

**DISCUSSION**

In general, the literature about bacterial leaching indicates the occurrence of elemental sulfur in the course of pyrite (16), pyrrhotite (2), arsenopyrite (34), or sphalerite (8) degradation. The indirect leaching mechanism, as cited below, is held to be responsible for the (intermediate) occurrence of elemental sulfur (equations 1, 2, and 3).

\[
\text{FeS}_2 + 2\text{Fe}^{3+} \rightarrow \text{3Fe}^{2+} + 2\text{S}^{0} \quad (1)
\]
\[
3\text{Fe}^{2+} + 0.75\text{O}_2 + 3\text{H}^+ \rightarrow \text{3Fe}^{3+} + 1.5\text{H}_2\text{O} \quad (2)
\]
\[
2\text{S}^{0} + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{SO}_4^{2-} + 4\text{H}^+ \quad (3)
\]

However, these equations cannot explain the formation of tetra- and pentathionate, which has been described before and which was shown to occur in our leaching experiments. Furthermore, polythionates have been detected in the course of pyrite biodegradation of a coal mine spoil (19). Obviously, the classical leaching equations (1 to 3) need to be revised. One possibility for accomplishing this results from the work of Luther (12) and Moses et al. (15). These authors presented evidence for a pyrite oxidation mechanism with thiosulfate as the first sulfur compound intermediate, as shown below (equation 4).

\[
\text{FeS}_2 + 6\text{Fe(H}_2\text{O})_6^{3+} + 3\text{H}_2\text{O} \rightarrow \text{2Fe}^{2+} + \text{S}_2\text{O}_3^{2-} + 6\text{Fe(H}_2\text{O})_6^{2+} + 6\text{H}^+ \quad (4)
\]

The hexaaquairon(III) ion attacks the pyritic sulfide and is reduced to its iron(II) species. Finally, iron(II) ions and thiosulfate are released from pyrite. Thiosulfate is not stable in acidic iron(III) ion-containing solutions. Within seconds a chemical reaction to tetrathionate ensues. An intermediate formation of a colored iron(III)-thiosulfate complex, as described by Williamson and Rimstidt (36), occurs in the course of this oxidation (equation 5).

![Image](http://aem.asm.org/)

**TABLE 1. Formation of sulfur compounds in the case of pyrite biolaching after 7 days**

<table>
<thead>
<tr>
<th>Bioreducing agent</th>
<th>Tetrathionate (mM-S)</th>
<th>Pentathionate (mM-S)</th>
<th>Elemental sulfur (mM)</th>
<th>Sulfate (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. ferrooxidans</em></td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>55</td>
</tr>
<tr>
<td><em>L. ferrooxidans</em></td>
<td>0.12</td>
<td>0.15</td>
<td>6.4</td>
<td>57</td>
</tr>
<tr>
<td>Iron(III) ions</td>
<td>0.04</td>
<td>0.02</td>
<td>0.8</td>
<td>39</td>
</tr>
</tbody>
</table>

* mM-S indicates the concentration of sulfur in these compounds.
Since this reaction proceeds faster than the dissolution of pyrite (equation 4), thiosulfate should be only barely detectable in leaching solutions. Our data are in agreement with this hypothesis. However, Basaran and Tuovinen (1) were able to detect trace amounts of trithionate and thiosulfate in pyrite oxidation experiments with *T. ferrooxidans*. Since thiosulfate is unstable under these conditions, its detection may have been a result of less-specific detection techniques.

In the ensuing reaction, tetrathionate decomposes (in the presence of pyrite) to elemental sulfur, sulfate, and traces of tri- and pentathionate. The occurrence of these compounds is the result of a reaction mechanism involving disulfane-monosulfonic acid (30, 35; for a review, see reference 20). Tetrathionate is hydrolyzed to highly reactive disulfane-monosulfonic acid and sulfate (equation 6).

\[
\text{S}_4\text{O}_6^{2-} + \text{H}_2\text{O} \rightarrow \text{HSSSO}_3^- + \text{SO}_4^{2-} + \text{H}^+ \quad (6)
\]

From this highly reactive compound several reactions may start which finally give rise to the detected compounds elemental
sulfur, thiosulfate, and tri- and pentathionate, as indicated in the following equations (7 to 10).

$$S_3O_3^{2-} + S_2O_3^{2-} + 0.5O_2 + 2H^+ \rightarrow S_3O_6^{2-} + H_2O$$ (7)

$$S_3O_3^{2-} + S_4O_6^{2-} \rightarrow 2S_2O_3^{2-} + S_5O_6^{2-}$$ (8)

$$S_3O_3^{2-} + 1.5O_2 \rightarrow S_3O_6^{2-}$$ (9)

$$4S_3O_3^{2-} \rightarrow S_8 + 4SO_3^{2-}$$ (10)

Thiosulfate, as produced in equation 8, may enter the reaction cycle again. The sulfate in equation 10 is unstable in acidic solutions. It is oxidized in the presence of heavy metal cations to sulfate (as well as traces of sulfur dioxide gas). Possibly *T. ferrooxidans* is participating in this reaction since this bacterium possesses a sulfite oxidase (20, 32, 33). Obviously, all these reactions occur in the direct vicinity of the pyrite surface. The decomposition of polythionates has been discussed as the reaction of an adsorbed molecule on the pyrite lattice (15). Because of the necessity of intimate contact, our experiments required vigorous stirring for the decomposition reaction to take place.

The experimental data concerning the effect of silver(I) ions clearly demonstrate that thiosulfate is the first resulting compound in the case of an acidic iron(III) attack on pyrite. Since silver sulfide instead of elemental sulfur could be detected, evidence for the indirect oxidation mechanism was given. Further evidence for the occurrence of disulfane-monosulfonic acid and the following reactions is given in Fig. 3. In stoichiometric concentrations sulfate is formed together with pentathionate according to the hydrolysis reaction of tetrathionate (equations 6 and 7).

Iron(III) ions are also an effective oxidant of pyrite at neutral pH (14). They are adsorbed to pyrite and accept electrons in the course of pyrite oxidation and reduction to iron(II) ions. The iron(II) ions remain tightly adsorbed onto the pyrite surface and are reoxidized to the iron(III) form by transferring electrons to molecular oxygen. Consequently, thiosulfate formation by iron(III) ion attack on pyrite also takes place at neutral pH. Furthermore, the iron(III) ions also oxidize the thiosulfate being adsorbed to the pyrite surface to tetrathionate (equation 5), which is the primary sulfur compound for disulfane-monosulfonic acid formation (equation 6). Thus, under alkaline or neutral conditions, the same reaction mechanism occurs as under acidic conditions. The formation of the degradation products of disulfane-monosulfonic acid depends on the pH level. Pentathionate and elemental sulfur formation (equations 7, 8, and 10) only occurred at acidic conditions. At neutral pH, high amounts of trithionate (equation 9) and some thiosulfate were detectable.

This series of reactions inherently explains the appearance of all intermediate sulfur compounds in the course of biological and chemical oxidative pyrite dissolution. Summarizing these reactions, a novel oxidative (cyclic) pyrite degradation mechanism is proposed (Fig. 5). The key sulfur compounds are thiosulfate, tetrathionate, disulfane-monosulfonic acid, and tri-thionate. Trithionate is hydrolyzed to thiosulfate and may enter the cycle again. As a consequence, the debate concerning the direct or indirect bacterial leaching mechanism becomes moot. Basically the indirect mechanism is responsible for pyrite dissolution.

A further important fact to be considered is the finding that the leaching bacteria *T. ferrooxidans* and *L. ferrooxidans* carry a considerable load of iron(III) ions complexed in their exopolymer layer (9). These iron(III) ions allow the bacteria to commence the attack on the pyrite surface. For a holistic view, the attachment of leaching bacteria by their exopolymer layer to the substrate surface needs to be taken into account. The exopolymer layer provides a reaction compartment allowing the above-mentioned processes to occur.
The reaction series described supplies the bacteria with iron(II) ions, polythionates, and elemental sulfur. *T. ferrooxidans* is able to metabolize all of these compounds (11, 20). In addition, the existence of sulfur globules (consisting of elemental sulfur and polythionates) in the periplasmic space of *L. ferrooxidans* (in contrast to the data of Sugio et al. [33]). Thus, the functions of these two leaching bacteria are (i) to supply iron(III) ions (complexed in the exopolymer layer) for an attack on the pyrite lattice and (ii) to keep the iron ions in the oxidized iron(III) species. The resulting sulfur compounds differed considerably from those of *T. ferrooxidans* with only iron(II) ion-oxidizing capacity, the concentrations of polythionates were detectable in significant concentrations. A similar result was obtained from the analysis of the chemical assays on pyrite dissolution [by iron(III) ions]. This finding clearly indicates the lack of enzymes for sulfur compound oxidation in *L. ferrooxidans* (in contrast to the data of Sugio et al. [33]). Thus, the functions of these two leaching bacteria are (i) to supply iron(III) ions (complexed in the exopolymer layer) for an attack on the pyrite lattice and (ii) to keep the iron ions in the oxidized iron(III) species. The resulting sulfur compounds will be either further metabolized (in the oxidized iron(III) species. The resulting sulfur compounds differed considerably from those of *T. ferrooxidans* with only iron(II) ion-oxidizing capacity, the concentrations of polythionates were detectable in significant concentrations. A similar result was obtained from the analysis of the chemical assays on pyrite dissolution [by iron(III) ions]. This finding clearly indicates the lack of enzymes for sulfur compound oxidation, like *Thiobacillus thiooxidans* and/or *Thiobacillus acidophilus*.

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