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# Pyrite Oxidation by Thermophilic Archaebacteria

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**Three species of thermophilic archaebacteria of the genera *Sulfolobus* (*Sulfolobus acidocaldarius* and *S. solfataricus*) and *Acidianus* (*Acidianus brierleyi*) were tested for their ability to oxidize pyrite and to grow autotrophically on pyrite, to explore their potential for use in coal desulfurization. Only *A. brierleyi* was able to oxidize and grow autotrophically on pyrite. Jarosite was formed during the pyrite oxidation, resulting in the precipitation of sulfate and iron. The medium composition affected the extent of jarosite formation.**

The use of *Sulfolobus* species in both coal desulfurization (7; C. Chen, Ph.D. thesis, The Ohio State University, Columbus, 1986) and metal ore leaching (10a) has been widely discussed lately. The ability of *Sulfolobus* species to oxidize pyrite, chalcopyrite, and molybdenite is well established (3, 4). The most commonly used microorganism for microbial ore leaching is *Thiobacillus ferrooxidans* (15). However, the thermophilic *Sulfolobus* spp. oxidize pyrite faster than the mesophilic organism *T. ferrooxidans*.

Both *T. ferrooxidans* and *Sulfolobus* spp. have been studied for use in coal desulfurization. *T. ferrooxidans* can remove only pyrite, whereas *Sulfolobus* spp. have been reported to remove organically bound sulfur as well. *Sulfolobus acidocaldarius* has been reported to degrade pure organic sulfur compounds, such as dibenzothiophene (9). The ability to oxidize pyrite, together with the proposed capability to remove organic sulfur, makes *Sulfolobus* spp. very interesting for use in coal desulfurization (9a).

The genera *Sulfolobus* and *Acidianus* belong to the archaebacteria and have been isolated from sulfuric hot springs (14). All species are thermophilic with growth optima around 70°C. They are also acidophilic: *S. acidocaldarius* and *Acidianus brierleyi* have a pH optimum around 2, and *S. solfataricus* has a pH optimum around 4. The bacteria can be cultivated both heterotrophically on yeast extract and autotrophically on sulfur. Some strains are able to gain their energy from the oxidation of ferrous to ferric iron. *A. brierleyi* can grow anaerobically on sulfur, thus producing $H_2S$. *A. brierleyi* also differs from *S. acidocaldarius* and *S. solfataricus* in their DNA base composition. The genus *Acidianus* has recently been approved (13) and includes *A. brierleyi*, formerly called *S. brierleyi*.

The experiments undertaken in this study were done to compare the three different species of archaebacteria, *S. acidocaldarius*, *A. brierleyi*, and *S. solfataricus*, with respect to their ability to oxidize pyrite for potential use in coal desulfurization. Special attention was paid to the problem of jarosite formation.

Pyrite is oxidized by oxygen to ferrous iron and sulfate. The reaction is slow, even at elevated temperatures such as 70°C:

$$FeS_2 + 3.5O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$$

The bacterial pyrite oxidation is considered to be either direct or indirect (7). During direct oxidation, pyrite is solubilized to ferric ion and sulfate:

$$4FeS_2 + 15O_2 + 2H_2O \xrightarrow{\text{Microorganisms}} 2Fe_2(SO_4)_3 + 2H_2SO_4$$

During the indirect process the ferrous ion produced in the pyrite oxidation is oxidized to ferric ion by the microorganisms. Ferric iron is a strong oxidant and readily oxidizes pyrite:

$$FeS_2 + 2Fe^{3+} \rightarrow 3Fe^{2+} + 2S^0$$

Any elemental sulfur formed can be oxidized either by the microorganisms or by $Fe^{3+}$:

$$2S^0 + 3O_2 + 2H_2O \xrightarrow{\text{Microorganisms}} 2H_2SO_4$$

$$S^0 + 6Fe^{3+} + 4H_2O \rightarrow 6Fe^{2+} + SO_4^{2-} + 8H^+$$

The principal difference between the two mechanisms is whether the microorganisms are closely associated with the pyrite surfaces, which is needed for the direct mechanism, or if the microorganisms just provide the chemical reactions with ferric ion. The conversion of $Fe^{2+}$ to $Fe^{3+}$ is slow in the absence of microbial activity and is the rate-limiting step for pyrite oxidation without microorganisms:

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$$

It is possible that both mechanisms take place at the same time. The overall reaction can then be summarized as follows:

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4$$

As can be seen, the production of sulfuric acid lowers the pH in the reaction vessel during pyrite oxidation.

Even at low pH (<2), iron may precipitate as sulfates or hydroxides, which counteracts the solubilization. The amount of reactive $Fe^{3+}$ also decreases. The iron and sulfate may precipitate as hydronium jarosite:

$$3Fe_2(SO_4)_3 + 12H_2O \rightarrow 2HFe_3(SO_4)_2(OH)_6 + 5H_2SO_4$$

If $Na^+$ or $K^+$ is present, sodium or potassium jarosite will be formed prior to hydronium jarosite. Ammonium jarosite can also be formed. The general formula of jarosite can be written as $MFe_3(SO_4)_2(OH)_6$, where M may be $H^+$, $K^+$, $Na^+$ or $NH_4^+$.

## MATERIALS AND METHODS

**Microorganisms.** The microorganisms were purchased from Deutsche Sammlung von Mikroorganismen (DSM), Braunschweig, Federal Republic of Germany. They include *S. acidocaldarius* DSM 639, *A. brierleyi* DSM 1651, and *S.*

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TABLE 1. Growth media (basal salt solution)[a]

| Medium salt | Amt (g/liter) in: | | | | |
|---|---|---|---|---|---|
| | *S. acidocaldarius* medium 88[b] | *S. solfataricus* medium 182[b] | Sulfur-free medium | Leathen medium | *A. brierleyi* medium 150[b] |
| $(NH_4)_2SO_4$ | 1.30 | 2.50 | | 0.05 | 3.00 |
| $KH_2PO_4$ | 0.28 | 3.10 | 3.10 | | |
| $K_2HPO_4 \cdot 3H_2O$ | | | | 0.50 | 0.50 |
| $MgSO_4 \cdot 7H_2O$ | | 0.20 | | 0.50 | 0.50 |
| $CaCl_2 \cdot 2H_2O$ | 0.07 | 0.25 | 0.07 | | |
| $FeCl_3 \cdot 6H_2O$ | 0.02 | | | | |
| $NH_4NO_3$ | | | 1.50 | | |
| $MgCl_2 \cdot 6H_2O$ | | | 0.25 | | |
| KCl | | | | 0.05 | 0.10 |
| $Ca(NO_3)_2$ | | | | 0.01 | 0.01 |

[a] Pyrite or yeast extract was added as the energy source.
[b] Trace element solution contained (in milligrams per liter) the following: $MnCl_2 \cdot 4H_2O$, 1.8; $Na_2B_4O_7 \cdot 10H_2O$, 4.5; $ZnSO_4 \cdot 7H_2O$, 0.22; $CuCl_2 \cdot 2H_2O$, 0.05; $Na_2MoO_4 \cdot 2H_2O$, 0.03; $VOSO_4 \cdot 2H_2O$, 0.03; and $CoSO_4 \cdot 7H_2O$, 0.01. The solution is as recommended in the DSM Catalog. The numbers refer to the catalog numbers of the media.

*solfataricus* DSM 1616. The stock cultures were kept at 70°C in the recommended medium for each microorganism and reinoculated every 3 weeks. The composition of the basal salt medium is given in Table 1, and yeast extract was used as the energy source. The pH was set at 2.0 by adding concentrated hydrochloric acid or concentrated sulfuric acid.

**Chemicals.** The salts were pro-analysis grade and were purchased from E. Merck AG, Darmstadt, Federal Republic of Germany. The yeast extract was obtained from Difco Laboratories, Detroit, Mich., and the bovine serume albumin used as a standard in the protein measurement was obtained from Sigma Chemical Co., St. Louis, Mo. Pyrite with a mass median diameter of 70 μm was obtained from Boliden Kemi AB, Helsingborg, Sweden.

**Experimental procedures.** In the shake flask experiments, 5 ml of cell suspension from the stock culture was added to 100 ml of basal salt medium supplemented with 2% (by weight) pyrite in 500-ml flasks with screw caps or plugs made of aquatic wadding. Yeast extract (1 g/liter), glucose (1 g/liter), ferric iron (0.02 g/liter), or trace element solution (Table 1, footnote) was added to the medium in some experiments. The flasks were incubated at 70°C in a rotary shaker filled with glycerol. The loss of water due to evaporation was adjusted for by adding sterile water after weighing the flasks. Samples were withdrawn every 2 or 3 days. An uninoculated flask was always analyzed as a control. In the experiments with *S. solfataricus* the "sulfur-free" medium was used in addition to medium 182, and for the studies with *A. brierleyi* Leathen medium (2) was used as well as medium 150. The medium compositions are given in Table 1.

The studies of pyrite oxidation by *A. brierleyi* were also carried out in bench scale air lift reactors (volume, 1.7 litres) (Fig. 1). The reactor system consists of four parallel glass reactors with an air supply for aeration and agitation. The air flow was controlled at 1 liter/min by mass flow meters (Brooks Instrument B.V., Veenendal, The Netherlands). Compressed air was used without any oxygen or carbon dioxide enrichment. The temperature was kept at 70°C by a circulating-water bath. Samples were withdrawn from the top of the reactor. Continuous measurements of the pH in the reactors were not possible. The harsh environment of low pH, high temperature, and high concentration of ions

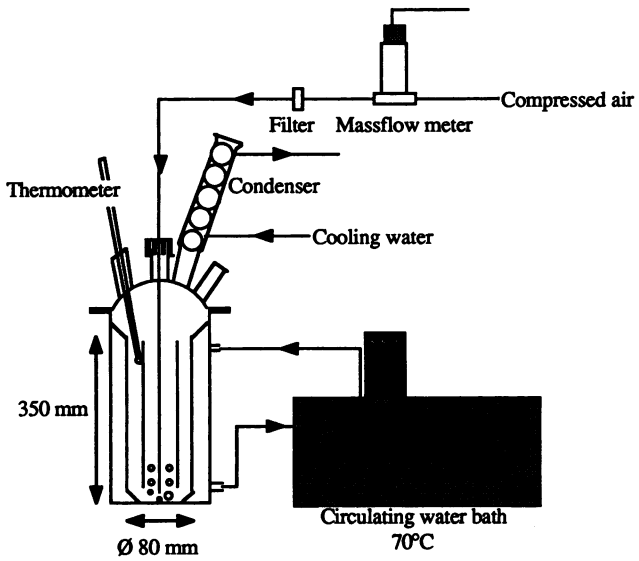


FIG. 1. Schematic picture of the bench scale air lift reactor used in the pyrite oxidation experiments with *A. brierleyi*. The reactor volume was 1.7 litres.

and pyrite particles damaged the glass electrode. The pH adjustment in the reactor experiments (Table 2, runs 2, 3, and 4) had to be done intermittently on samples withdrawn every day by adding the appropriate amount of 2 M NaOH. The reactors were inoculated with 75 ml of cell suspension from the stock culture of *A. brierleyi*. The microorganisms were not adapted to pyrite prior to inoculation in the experimental flasks or in the air lift reactors, except when indicated.

**Analyses.** The reaction liquids were analyzed for pH and sulfate, iron, and protein concentrations after separation of the pyrite particles by filtration. The pH was measured at room temperature with a standard glass electrode. The sulfate concentration was measured turbidimetrically after precipitation with $BaCl_2$ (ASTM D516-68). The iron concentration was determined by atomic absorption spectrometry on a Shimadzu atomic absorption spectrometer. The ferric ion concentration was determined by direct measurement of the $A_{300}$ (1) on a Shimadzu spectrophotometer. The protein concentration in the cell pellet was determined, after cen-

TABLE 2. Pyrite oxidation rates and jarosite formation in air lift reactor experiments with *A. brierleyi*[a]

| Run | Medium | Yeast extract (g/liter) | Pyrite oxidation rate[b] | | Jarosite (g/liter) | Remarks |
|---|---|---|---|---|---|---|
| | | | mg of S/liter per h | mg of Fe/liter per h | | |
| 1 | 150 | 0.5 | 28 | 19 | 5.0 | |
| 2 | 150 | 0.5 | 7 | 0[c] | 21.0 | pH control 2.0 |
| 3 | 150 | 1.0 | 8 | 0[c] | 22.0 | pH control 2.0 |
| 4 | 150 | 0 | 23 | 8 | 21.0 | pH control 1.4 |
| 5 | 150 | 0 | 26 | 17 | 5.3 | |
| 6 | 150 | 0 | 30 | 23 | 5.3 | |
| 7 | 150 | 0 | 24 | 19 | 6.6 | |
| 8 | Leathen | 0 | 23 | 25 | 2.4 | |
| 9 | Leathen | 0 | 24 | 19 | 2.1 | |

[a] Pyrite concentration, 2%. Temperature, 70°C. Aeration, 1 liter/min.
[b] Measured as increase in soluble sulfate and iron concentration.
[c] >98% of the iron was precipitated.

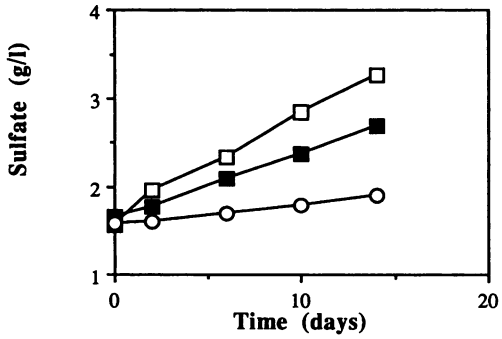


FIG. 2. Shake flask experiments with *S. acidocaldarius*. The pyrite concentration was 2% (wt/vol) in medium 88. Symbols: □, uninoculated control; ■, *S. acidocaldarius*; ○, *S. acidocaldarius* (medium supplemented with 1 g of yeast extract per liter).

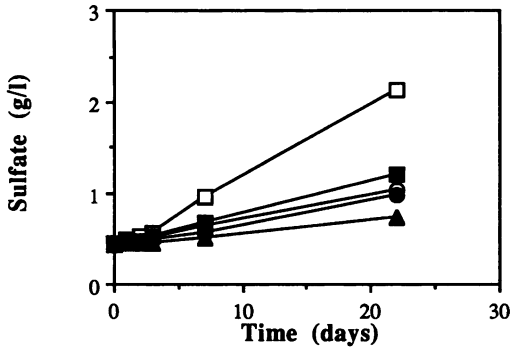


FIG. 3. Shake flask experiments with *S. solfataricus*. The pyrite concentration was 2% (wt/vol) in sulfur-free medium. Symbols: □, uninoculated control; ■, *S. solfataricus* (medium supplemented with 1 g of $FeCl_3$ per liter); ○, *S. solfataricus*; ●, *S. solfataricus* (medium supplemented with 1 g of glucose per liter); ▲, *S. solfataricus* (medium supplemented with 1 g of yeast extract per liter).

trifugation and disintegration of the cells in 0.1 M NaOH at 95°C for 30 min, by the method of Lowry et al. (10). The protein concentration was considered to be a measure of the microbial growth. The microorganisms were not attached to the pyrite surfaces, as could be seen during microscopic studies. The precipitated iron and sulfate were analyzed after dissolution in hydrochloric acid and used as a measure of jarosite formation and determination of the total pyrite oxidation rate. The jarosite was analyzed by X-ray emission (Link System) and X-ray diffraction (X-ray diffractometer no. PW1710; Philips). Chemically synthesized potassium jarosite (6) was analyzed for comparison.

## RESULTS

***S. acidocaldarius* and *S. solfataricus*.** Despite a number of experimental runs, the strains of *S. acidocaldarius* and *S. solfataricus* were not able to oxidize pyrite. In some cases, growth was obtained when the basal salt solution was supplemented with yeast extract, but no increase in pyrite oxidation rate was ever detected compared with the uninoculated control. Reinoculation of the cultures grown in the presence of pyrite and yeast extract in medium without yeast extract resulted in total absence of growth. Hence, there did not seem to be any adaptation of the microorganisms to pyrite. Addition of $Fe^{3+}$, glucose, or trace elements to the basal salt medium with pyrite did not improve the results. In most experimental runs the uninoculated control showed the highest oxidation rate, probably owing to the slightly different medium composition depending on the inoculation. The addition of yeast extract to the medium affects the pyrite oxidation rate in the control; experiments have shown that the pyrite oxidation rate is lowered in the presence of yeast extract. Figures 2 and 3 give examples of the experimental results obtained with *S. acidocaldarius* and *S. solfataricus*, respectively.

***A. brierleyi*.** *A. brierleyi* readily oxidized pyrite without any adaptation problems. Growth was obtained both with and without the addition of yeast extract to the basal salt medium with pyrite. No significant change in growth or pyrite oxidation rate was observed when the yeast extract was added. The results from the experimental runs in the air lift reactors are summarized in Table 2. The pyrite oxidation rates were calculated from the formation of soluble sulfate ions and the liberation of soluble iron. The oxidation rates were defined as the rate of change of the respective species during the exponential growth phase. Figure 4 shows a typical example of an experimental run in terms of sulfate concentration, iron concentration, pH, and protein concentration in the media versus time.

The very first observation is the appearance of a yellow precipitate, which is formed as soon as the pyrite oxidation starts. The results of the X-ray diffraction analysis of the precipitate indicate some kind of jarosite. Jarosite contains sulfate and ferric ion according to the formula $MFe_3(SO_4)_2(OH)_6$. M may be either $H^+$, $K^+$, $Na^+$, or $NH_3^+$ depending on which of these ions are present in the solution. However, sodium jarosite and potassium jarosite are formed much faster than the other types. Chemical analysis of the precipitate gave a ratio of iron to sulfate of approximately 1:0.8. By X-ray emission this ratio was given as approximately 1:1 instead of the theoretical 1:0.67. In chemically synthesized jarosite the ratio of iron to sulfate was 1:1.1. The results of these analyses are summarized in Table 3.

When medium 150 was used, an average of 6 g of jarosite per liter was formed during the oxidation, despite the very low pH. The pH in the beginning was set to 2 and was then lowered to approximately 1 by the formation of sulfuric acid during the oxidation of pyrite. If Leathen medium was used instead of medium 150, the amount of jarosite formed was reduced to 2 g/liter. Leathen medium contains about 1/10 the amount of sulfate and phosphate, 1/20 the amount of ammonium, and 1/5 the amount of potassium as medium 150.

To examine whether the growth of the microorganisms was enhanced when the drop in pH was prevented, we added NaOH to keep the pH at 2 or 1.4 in experimental runs 2, 3, and 4 (Table 2). During these experiments the formation of jarosite increased to 21 g/liter. The iron probably precipitated as $Fe_2(OH)_3$ as well, since the ratio of Fe to S in the precipitate increased with time. During runs 2 and 3, when the pH was kept at 2, the pyrite oxidation rate was lowered.

To study the pyrite oxidation in greater detail, we analyzed the precipitated part of the sulfate and iron after dissolution of the jarosite in 4 M HCl (boiled for 15 min). The apparent oxidation rate was slightly lower than the total oxidation of the pyrite (Table 4). This is due to the formation of the jarosite. When medium 150 was used (run 7), 30% of the iron and 14% of the sulfate precipitated; for Leathen medium these proportions are 18% and 9%, respectively.

Figure 5 shows the correlation between leached sulfate and iron during pyrite oxidation. When analyzing the soluble sulfate and iron concentrations, it appears that more sulfur

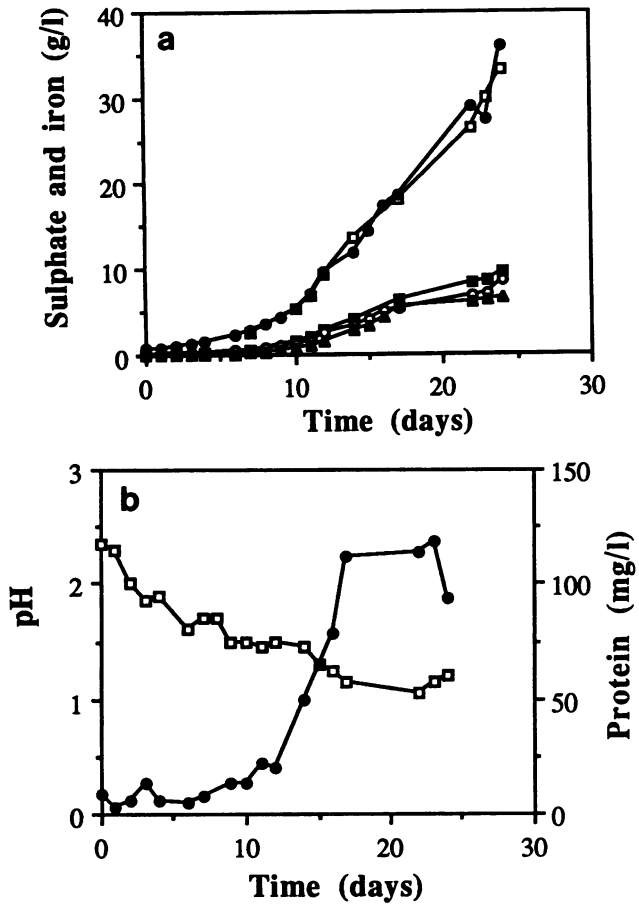


FIG. 4. (a) Pyrite oxidation by *A. brierleyi* in an air lift reactor. Pyrite concentration, 2% (wt/vol) in Leathen medium. The plot shows sulfate and iron concentrations versus time for run 9. Symbols: □, total sulfate produced; ●, soluble sulfate; ■, total iron produced; ○, soluble iron; ▲, soluble ferric iron. (b) Growth and pH change during pyrite oxidation with *A. brierleyi* (run 9). Symbols: □, pH; ●, protein concentration.

than iron is leached from the pyrite. However, the determination of totally leached sulfur and iron reveals that sulfur and iron are leached according to the formula of pyrite ($FeS_2$).

TABLE 3. Composition of the precipitate formed during bacterial oxidation of pyrite

| Expt | Fe:S ratio |
|---|---|
| X-ray emission | |
| Chemically synthesized jarosite | 1:1.10 |
| Pyrite | 1:1.96 |
| Run 1 | 1:0.97 |
| Run 4 | 1:0.97 |
| Chemical analysis | |
| Run 1 | 1:0.73 |
| Run 4 | 1:0.80 |
| Run 5 | 1:0.71 |
| Run 6 | 1:0.69 |
| Run 7 | 1:0.83 |
| Run 9 | 1:1.01 |
| Theoretical value $[MFe_3(SO_4)_2(OH)_6]$ | 1:0.67 |

TABLE 4. Pyrite oxidation rates and jarosite formation during bacterial oxidation of pyrite[a]

| Run | Medium | Pyrite oxidation rate (soluble iron and sulfate) | | Pyrite oxidation rate (total iron and sulfate) | | Jarosite (g/liter) |
|---|---|---|---|---|---|---|
| | | mg of S/liter per h | mg of Fe/liter per h | mg of S/liter per h | mg of Fe/liter per h | |
| 7 | 150 | 24 | 19 | 27 | 24 | 6.6 |
| 9 | Leathen | 24 | 19 | 26 | 22 | 2.1 |

[a] Rates were calculated on the basis of both soluble and total iron and sulfate concentrations in the media (all the pyrite was oxidized).

## DISCUSSION

*A. brierleyi* was the only one of the three species tested in this study that was able to oxidize pyrite. *A. brierleyi* oxidized pyrite without any previous adaptation. The addition of yeast extract was not necessary to obtain good growth and did not improve the oxidation rate. All of the added pyrite was dissolved by the microorganisms during the reaction time except when the pH was maintained at 2 by addition of NaOH. Between 20 and 30% of the sulfate and iron precipitated as jarosite without pH control, and almost all of the iron precipitated as jarosite when pH control was used. The formation of this precipitate counteracted the liberation of soluble sulfate and iron. In addition, the amount of active $Fe^{3+}$ was reduced. By using the low-salt medium (Leathen medium), the amount of jarosite formed decreased without significantly affecting the pyrite oxidation rate. However, the lag phase was somewhat extended. The main difference between medium 150 and Leathen medium is the concentrations of potassium and ammonium. These two can take part in the formation of jarosite, forming potassium jarosite and ammonium jarosite, respectively. The ionic strength and the concentrations of sulfate and iron are just as high in Leathen medium as in medium 150 once the oxidation of pyrite has started.

The decrease in pyrite oxidation rate in the pH-controlled experimental runs was probably due to the precipitation of almost all of the iron formed, so that there was no iron left to take part in the pyrite oxidation. This might imply that the mechanism is indirect rather than direct.

The inability of some *Sulfolobus* strains to oxidize pyrite has been reported elsewhere (11). *S. acidocaldarius* DSM 639 is the type strain of *S. acidocaldarius* and should be the

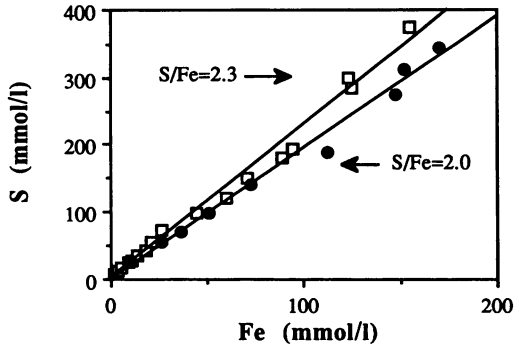


FIG. 5. Correlation between leached sulfur and iron during pyrite oxidation by *A. brierleyi* (run 9). Symbols: □, based on soluble sulfate and iron; ●, based on total sulfate and iron.

same strain as *S. acidocaldarius* 98-3, which has been used in many of the previous studies on coal desulfurization (5). However, the diversity of the *Sulfolobus* genus is great, and the culture may have changed with time, since the microorganisms are stored in yeast extract medium. Most cultures that have been used for pyrite dissolution have been natural isolates rather than the type culture collection strains (9b). The culture used for coal desulfurization had been stored in a coal slurry for several years (Chen, Ph.D. thesis).

**Coal desulfurization by thermophilic archaebacteria.** *A. brierleyi* can be used for the desulfurization of coal, whereas the inability of the tested strains of *S. acidocaldarius* and *S. solfataricus* to oxidize pyrite makes their applicability quite doubtful. *S. acidocaldarius* has been reported to remove pyrite and organically bound sulfur from coal and to degrade pure organic sulfur compounds (8). The strain used in this study was not able to oxidize pyrite. In addition, experiments have shown that this strain of *S. acidocaldarius* is sensitive to compounds leached from coal (12). *A. brierleyi* is able to remove pyrite from coal and to enhance the removal of organically bound sulfur (12b) and does not seem to be sensitive to compounds leached from coal (12).

## ACKNOWLEDGMENTS

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