Growth of Iron-Oxidizing Thiobacilli in the Presence of Chalcopyrite and Galena

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Iron-oxidizing thiobacilli were adapted to grow on a chalcopyrite and a galena ore concentrate. When grown on the chalcopyrite concentrate, the bacteria exhibited a doubling time of 38.4 ± 2.9 h, with a final cellular protein concentration of 185 μg/ml and solubilization of 10.3 g of copper per liter. When grown on the galena ore concentrate, the generation time was 39.6 ± 2.7 h, with a final cellular protein concentration of 120 μg/ml. Galena was converted to lead salts soluble in 1 M ammonium acetate to a concentration of 20.2 g of lead per liter. X-ray diffraction and refractive-image analysis indicated that the smaller-sized particles were favored in this process. Galena was converted to anglesite, and soluble copper was liberated from chalcopyrite with the concurrent formation of jarosite.

The leaching of mineral sulfide ores such as chalcopyrite and galena by iron-oxidizing thiobacilli has been extensively investigated (1, 7, 11, 19) and reviewed (3). Approximately 5% of the total world production and 11.5% of U.S. production of copper (20) are achieved by leaching low-grade copper waste materials. The development of microbiological methods for the reclamation of specific metal ions from low-grade mineral sulfide ores is required as high-grade ore deposits diminish. The bacterial leaching of metals from mineral sulfides is a biochemical oxidation process which can be described by the following equation:

\[ 	ext{MS} + 2\text{O}_2 \rightarrow \text{MOSO}_4 \]

where M is any bivalent metal ion (4, 7). The sulfur moiety of the mineral sulfide is oxidized by the bacteria to sulfate (2, 5).

The action of iron-oxidizing thiobacilli on concentrates of chalcopyrite and galena was examined previously in our laboratories (12, 17). The solid mineral matrix of chalcopyrite is disrupted by bacterial oxidation of the mineral with the formation of soluble copper and iron sulfates:

\[ 2\text{CuFeS}_2 + 8\text{H}_2\text{O} \rightarrow 2\text{CuSO}_4 + \text{Fe}_2\text{(SO}_4\text{)}_3 + 6\text{H}_2\text{O} \] (1)

The ferric sulfate is slowly converted to jarosite, which precipitates:

\[ 3\text{Fe}_2\text{(SO}_4\text{)}_3 + 12\text{H}_2\text{O} \rightarrow 2 \left\{ [\text{Fe}_2\text{(SO}_4\text{)}_3 \cdot 2\text{Fe(OH)}_2] \right\} + 5\text{H}_2\text{SO}_4 \] (2)

Galena can be oxidized directly to lead sulfate, mineralogically known as anglesite:

\[ \text{PbS} + \frac{1}{2}\text{H}_2\text{SO}_4 + \frac{1}{2}\text{O}_2 \rightarrow \text{PbSO}_4 + \text{H}_2\text{O} + \text{S}^0 \] (3)

Both minerals are insoluble in water or weak acid; however, anglesite may be solubilized in alkali or ammonium salt solutions.

This paper deals with the growth of iron-oxidizing thiobacilli in the presence of these minerals. The progressive alterations of these mineral sulfides used by the bacteria as substrates are examined by X-ray diffraction and refractive-image analysis demonstrating physical and chemical changes.

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MATERIALS AND METHODS

Bacteria and growth conditions. Iron-oxidizing thiobacilli originally isolated by Torma (15) from acid mine waters of northern Quebec were adapted to grow on concentrates of chalcopyrite and galena as previously described (12). Two series of 21 250-ml Erlenmeyer flasks, containing 70 ml of modified 9K salts solution of Silverman and Lundgren (13) without ferrous sulfate, 30 ml of distilled water, 6 g of one of the ore concentrates, and an inoculum of 10 ml of an actively growing culture of bacteria adapted to each of the respective ores, were inoculated at 27 ± 1.0°C on a gyratory shaker at 200 cycles/min in a humidity-controlled chamber. Two uninoculated flasks, one containing 10 ml of 1% (wt/vol) thymol in 2% (vol/vol) methanol, served as the controls. The pH of the contents of each growth flask was adjusted to 2.2 daily with concentrated H₂SO₄. Each day, one flask of each series was removed from the shaker. The ore was separated from the supernatant, 10 ml of which was centrifuged at 27,000 × g for the removal of bacterial cells. These were resuspended in modified 9K salts solution, pH 2.2.

Analytical methods. The samples were vigorously
agitated to remove the bacteria from the ores before bacterial cellular protein was determined by the method of Lowry et al. (8) after hydrolysis in 1.0 N NaOH at 100°C for 10 min; bovine serum albumin was used as the standard. The ore concentrate residues were dried in a vacuum oven at 20°C; sizing and mineral composition were determined by image analysis and X-ray diffraction, respectively (10). Soluble copper and lead were determined by atomic absorption spectrophotometry. A portion of the residue from the flasks containing the galena concentrate was treated with 1 M ammonium acetate to solubilize lead sulfate, which was then analyzed by atomic absorption spectrophotometry.

Photographs. Polaroid film was used to record the reflective-image analysis from the Quantimet 720 image analyzer (10).

Chemicals. Bovine serum albumin was obtained from Sigma Chemical Co., St. Louis, Mo. The chalcopyrite and galena concentrates were gifts from A. E. Torma, formerly of the Centre de Recherche Minéraux, Quebec, Quebec, and have previously been described (12).

RESULTS

The increase in cellular protein of a culture of iron-oxidizing thiobacilli in the presence of a chalcopyrite bulk concentrate is illustrated in Fig. 1. The doubling time was calculated to be 38.4 ± 2.9 h. The maximum stationary phase was reached after 200 h, at which time the cellular protein concentration was 185 μg/ml. Within 300 h, 10.3 g of copper per liter was solubilized; as the culture entered the stationary phase, the rate of copper solubilization decreased. Approximately 2 g of copper per liter was solubilized in the control flask without thymol added; this may have been due to the presence of bacteria indigenous to the ore. None was solubilized in the control flask with the bacterial growth inhibitor.

The decrease in the chalcopyrite and the formation of jarosite during growth are shown in Fig. 2. Oxidation of chalcopyrite by the iron-oxidizing thiobacilli released cupric and ferric iron into the solution as their respective sulfates; the former remained dissolved, whereas the latter was subsequently altered to jarosite. Semi-quantitative analysis of the residue indicated that the proportion of chalcopyrite decreased steadily from approximately 80 to 50%; jarosite, which was not detected until the 10th day, increased to comprise 21% of the residue by the 22nd day. The proportions of all other minerals remained relatively constant throughout the experiment, with pyrite assaying at 8 to 15%, sphalerite at 5 to 12%, clay at 4 to 12%, chlorite at 2 to 11%, and quartz at 1 to 10%. The smaller grains of chalcopyrite were affected preferentially, as shown in Fig. 3, which is a plot of the cumulative proportion of this mineral versus the maximum grain size of the original ore and that which had been altered after 15 days of exposure to the bacteria. Photographs of the reflective-image analysis of the original ore and of ore exposed to the bacteria for 10 and 21 days (Fig. 4) clearly illustrates the preferential removal of the smaller particles.

Figure 5 is a growth curve of the iron-oxidizing thiobacillus adapted to and grown on the galena bulk concentrate. A generation time of 39.6 ± 2.7 h was attained. The maximum stationary period was reached after 250 h, at which time the cellular protein concentration was 120 μg/ml. The equivalent of 20.2 g of lead per liter was converted to lead salts soluble in 1 M ammonium acetate over a period of 21 days. No formation of lead salts soluble in 1 M ammonium acetate was observed in the absence of bacteria in either the absence or presence of thymol. Galena, the substrate mineral in the bulk concentrate which was insoluble in ammonium salts, was oxidized to anglesite, which could be dissolved from the residue by 1 M ammonium acetate.

![Fig. 1. Growth of iron-oxidizing thiobacilli in the presence of chalcopyrite. Symbols: (▲) Copper solubilized in the presence of bacteria and (●) bacterial cellular protein; (■) copper solubilized in the absence of bacteria; (▼) copper solubilized in the absence of bacteria and in the presence of thymol.](http://aem.asm.org/)
Semiquantitative analysis (Fig. 6) shows that the proportion of galena in the residue decreased from approximately 50 to 4% during the experiment while anglesite was formed, increasing to approximately 60% in the same period. The proportions of all other minerals remained relatively constant throughout the experiment, with pyrite assaying at 16 to 34%, chalcopyrite at 2 to 6%, sphalerite at 4 to 9%, and clay at 5 to 10%.

Figure 7, which shows the cumulative proportion of the grain sizes of the untreated ore concentrate and that exposed to the culture of iron-oxidizing thiobacilli for 15 days, illustrates that the smaller grains of galena were altered preferentially. Photographs of the reflective-image analysis of the original ore and that exposed to the bacteria for 8 and 21 days show the removal of the smaller grains, with a general decrease in the number of grains of galena and the formation of the lath-shaped particles of anglesite (Fig. 8).

**DISCUSSION**

The alteration of substrate minerals during the growth of iron-oxidizing thiobacilli is clearly shown in this study. Chalcopyrite was oxidized with the solubilization of copper to a concentration of 10.3 g/liter after 15 days and the conversion of the iron in the mineral to jarosite, which comprised 20% of the residue by the end of the experiment. During this period, cellular protein increased to 185 μg/ml, the maximum stationary period being reached after 250 h and the time required for the cellular protein to double being 38.4 ± 2.9 h. Galena was oxidized to anglesite, during which time the cellular protein increased to a concentration of 120 μg/ml, with a doubling time of 39.6 ± 2.7 h, the maximum stationary period being reached after 250 h. In both experiments, the smaller particles were preferentially utilized over the larger.

Margalith et al. (9) suggested that iron-oxidizing thiobacilli might in many instances exist as a heterogeneous culture, and that drastic changes in growth conditions would favor a portion of that population. Thus, under altered environments, quantitative changes in the proportion of the different cell types might occur. This hypothesis is supported by physiological (12) and genetic (6) studies of the iron-oxidizing thiobacilli grown on galena or chalcopyrite. Bacteria grown on a galena concentrate were shown to be unable to consume oxygen in the presence of lead sulfide; chalcopyrite-grown cells were
Fig. 4. Reflective-image analysis photographs of chalcopyrite concentrates during oxidation by iron-oxidizing thiobacilli. The bar represents 25 μm. (A) Control; (B) 10th day; (C) 21st day. 1, Chalcopyrite; 2, pyrite; 3, sphalerite.
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Fig. 5. Growth of iron-oxidizing thiobacilli in the presence of a galena concentrate. Symbols: (△) Lead sulfide converted to lead sulfate in the presence of bacteria and (●) bacterial cellular protein; (■) lead sulfide converted to lead sulfate in the absence of bacteria in the absence or presence of thymol.

unable to metabolize lead sulfide, but showed greater activity toward chalcopyrite and pyrite (12). The molar ratio of the guanosine-plus-cytosine content of the deoxyribonucleic acid of a lead sulfide-grown bacteria was determined to be 54.4%, and that of chalcopyrite-grown cells was shown to be 60.1% (6). These parameters indicate that the two cultures of bacteria used in this study are significantly different from each other.

The mechanism of bacterial action on metal sulfide minerals remains uncertain. Among the parameters of importance are particle size, pulp density, ferric iron concentration, bacterial strain, and the solubility product of the substrate mineral (15, 18). Enough iron is solubilized during the oxidation of chalcopyrite that additional iron is not required. Ferric iron enhances the oxidation of galena, as is demonstrated by the following equation:

\[ \text{PbS} + \text{Fe}_2(\text{SO}_4)_3 \rightarrow \text{PbSO}_4 + 2\text{FeSO}_4 + \text{S} \]  

Both ferrous iron and elemental sulfur can be oxidized by iron-oxidizing bacteria with the pro-

Fig. 6. Alteration of minerals in a bulk galena concentrate in the presence of iron-oxidizing thiobacilli. Symbols: (●) Galena; (△) anglesite.

Fig. 7. Size distribution of galena particles during growth of iron-oxidizing thiobacilli. Symbols: (●) Control; (■) 15th day.
FIG. 8. Reflective-image analysis photographs of galena concentrates during oxidation by iron-oxidizing thiobacilli. The bar represents 25 μm. (A) Control; (B) 8th day; (C) 21st day. 1, Chalcopyrite; 2, galena; 3, sphalerite; 4, pyrite; 5, anglesite.
duction of ferric iron and sulfuric acid (9), which can be used in the conversion of galena to anglesite according to equations 3 and 4. The rate of metal sulfide oxidation by the iron-oxidizing thiobacilli has been shown to be dependent upon the solubility of the mineral (18). Metal sulfides with greater solubilities exhibit faster rates of dissolution in the presence of bacteria. This can be described by the equation:

\[ V = \frac{dM^{2+}}{dt} = AK_{sp} = A(M^{2+})(S^{2-}) \]  

(5)

where \( A \) is a proportionality factor and \( K_{sp} \) is the solubility product constant (18). The solubility product of galena is on the order of 10\(^{-28} \) M, whereas that of chalcopyrite is several orders of magnitude lower. This relationship is not simple and may be modified by factors such as the specific surface area, ferric iron concentration, secondary mineral deposition, and proximity of the bacteria to the minerals.

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LITERATURE CITED