Microbiological Oxidation of Synthetic Chalcocite and Covellite by *Thiobacillus ferrooxidans*

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The microbiological oxidation of synthetic chalcocite and covellite has been investigated using an adapted strain of *Thiobacillus ferrooxidans*. Biodegradation of chalcocite was found to be 90 to 100% and that of covellite 45 to 60%. Optimum conditions for the oxidation of chalcocite were: pH, 1.7 to 2.3; temperature, 35 C; and ferric iron concentration in the range of 0.004 to 0.01 M. For covellite, the optimum conditions were: pH 2.3; temperature, 35 C; and ferric iron concentration in the range of 0.004 to 0.02 M. The energies of activation were determined to be 16.3 kcal (ca. 6.8 x 10^4 J) per mol and 11.7 kcal (ca. 4.8 x 10^4 J) per mol for chalcocite and covellite, respectively.

Since 1947 when Colmer and Hinkle (5) isolated *Thiobacillus ferrooxidans*, many reviews have dealt with the physiology and morphology of this organism, as well as with its involvement in biogemetrallurgical operation (7, 11, 21, 22). Among the sulfide ores that this organism acts upon are pyrite (16), chalcopyrite (2), arsenopyrite (8), zinc sulfide (19, 20), and nickel and cobalt sulfides (A. E. Torma, Canadian patent no. 960, 463, Jan. 7, 1975). In this study the action of two copper sulfides, Cu_2S (chalco-

cite) and CuS (covellite) by an adapted strain of *T. ferrooxidans*, are investigated with the purpose of extending knowledge with a view to future application in copper extraction from ores containing these minerals.

The oxidation mechanism for chalcocite can be expressed by the following equation (9, 13):

\[
\text{Cu}_2\text{S} + \frac{1}{2}\text{O}_2 + \text{H}_2\text{SO}_4 \xrightarrow{\text{Bacteria}} \text{CuS} + \text{CuSO}_4 + \text{H}_2\text{O}
\]

(1)

The CuS, corresponding to the naturally occurring covellite, will be further oxidized (3, 14) by the microorganism according to the following equation:

\[
\text{CuS} + 2\text{O}_2 \xrightarrow{\text{Bacteria}} \text{CuSO}_4
\]

(2)

In studies on the leaching of chalcocite and covellite from natural sources (6), it has been shown that ferric iron is always present and contributes to the solubilization of copper. It was, therefore, of interest to study the effect of ferric iron concentration, as well as the effects of pH and temperature, regarding these substrates.

**MATERIALS AND METHODS**

**Microorganism.** The microorganism *T. ferrooxidans* used in this study was isolated from acid mine water in northern Quebec (18). The organism was adapted to the individual substrates in iron-free 9K nutrient medium described by Silverman and Lundgren (17). At the end of the log phase of bacterial growth, 5 ml of the culture was transferred into a new medium or used as an experimental inoculum.

**Substrates.** Synthetic chalcocite and covellite used in the experiments were procured from Fisher Scientific Co., Fair Lawn, N.J.

**Culture technique.** All experiments were carried out in 250-ml Erlenmeyer flasks containing 5 ml of an 8-day-old, active culture of *T. ferrooxidans*, 70 ml of nutrient medium, and the desired quantities of substrate. The flasks were aerated with air containing 0.2% carbon dioxide (20) and shaken at 250 rpm with a Gyrotory incubator shaker apparatus, model G26, from the New Brunswick Scientific Co., New Brunswick, N.J. Periodically, distilled water was added to compensate for the water loss due to evaporation, and the pH was readjusted with 1 N H_2SO_4 or 1 N NaOH if necessary. The temperature was varied according to the experiment. In the sterile control flasks, 5 ml of a methanol solution containing 2% thymol was added instead of inoculum.

**Analytical methods.** One milliliter of sample was withdrawn from the experimental solutions periodically and analyzed for its metal content by an atomic spectrophotometer, model AA-4, from the Varian Techtron Pty. Ltd., Melbourne, Australia. The sample was replaced with liquid medium containing basal salts only.

Eh measurements were carried out on reaction mixtures with a platinized gold electrode together
with a reference calomel electrode saturated with KCl and a pH meter, model pH-M28, Copenhagen, Denmark, at 21 C.

Mineralogical composition of the residue was determined by X-ray analysis with a Philips diffractometer, model P.W.-1310/61.

Kinetic determinations. The rate of copper solubilization (V) was determined from the linear part of a plot representing the dissolved copper concentration as a function of time. The energies of activation (E_a) and inactivation (E_i) were calculated by the equation:

$$E = \frac{T_1T_2R \ln \left( \frac{V_2}{V_1} \right)}{T_2 - T_1}$$

and the temperature coefficients (Q_10) were calculated from the equation:

$$Q_{10} = \left( \frac{V_2}{V_1} \right)^{\frac{10}{T_2 - T_1}}$$

where $V_1$ and $V_2$ are copper solubilization rates at temperatures $T_1$ and $T_2$, and $R$ is the gas constant.

RESULTS

Effect of pH. The effect of pH on copper solubilization from chalcocite and covellite is shown in Fig. 1. These curves were obtained at 35 C with suspensions containing 1.3% (wt/vol) and 5.3% (wt/vol) solid substrates for chalcocite and covellite, respectively. The optimum pH was found to be from 1.7 to 2.3 for chalcocite and 2.3 for covellite.

Effect of temperature. These experiments were carried out with suspensions containing 1.3% (wt/vol) chalcocite at pH 1.7 and 5.3% (wt/vol) covellite at pH 2.3, respectively. For both substrates the optimum temperature has been found to be 35 C (Fig. 2). $E_a$ and $Q_{10}$, calculated between 25 C and 35 C, were determined to be 16.3 kcal (ca. $6.8 \times 10^4$ J) per mol and 2.4 for chalcocite and 11.7 kcal (ca. $4.8 \times 10^4$ J) per mol and 1.9 for covellite. The $E_i$ values were determined between 40 C and 45 C to be 55.5 kcal (ca. $2.3 \times 10^5$ J) per mol and 61.5 kcal (ca. $2.5 \times 10^5$ J) per mol for chalcocite and covellite, respectively.

Effect of ferric iron. The influence of ferric iron on the rate of copper solubilization has been studied at 35 C and at substrate concentration and pH mentioned in the preceding series of experiments. The optimum concentration of ferric iron was found to be in the range of 0.004 to 0.01 M for chalcocite and 0.004 to 0.02 M for covellite (Fig. 3). At higher concentrations of ferric iron, the rate of copper solubilization de-
creased and jarosite formation was detected.

Effect of substrate concentration. The effect of substrate concentration on the rate of copper solubilization is shown in Fig. 4. These studies were conducted in the presence of chalcocite at pH 1.7, 35 C, 0.004 M Fe³⁺, with substrate concentrations between 0.7 and 3.3% (wt/vol), and in the presence of covellite at pH 2.3, 35 C, 0.004 M Fe³⁺, with substrate concentrations between 0.7 and 12% (wt/vol).

The optimization of substrate concentration on chalcocite was not attempted due to difficulties in maintaining pH. The optimum concentration of covellite was found to be 8% (wt/vol). The highest dissolved copper concentration was 28 g/liter, which was obtained with a suspension containing 12% (wt/vol) covellite.

Eh values increased in the presence of chalcocite from an initial value of 220 to 515 mV and for covellite from 230 to 510 mV. In sterile controls, in the presence of either substrate, the Eh increased from 210 to 330 mV. The Eh is directly related to the Gibbs' free energy change (12) and, thus, to energy available for bacterial action.

**DISCUSSION**

The optimum pH values and temperature derived from this study are in agreement with those obtained in previous investigations for metal sulfide oxidations (4, 9, 14, 19; A. E. Torma, Canadian patent).

The activation energies, 16.3 kcal/mol and 11.7 kcal/mol for chalcocite and covellite, respectively, are in the order of magnitude (12.0 kcal [ca. 5.0 × 10⁴ J] per mol) obtained for zinc sulfide oxidation by the same microorganism (19). The inactivation energies of 55.5 kcal/mol and 61.5 kcal/mol for chalcocite and covellite, respectively, are similar to those obtained for protein denaturation systems (1). The Q₉₀ values of 2.4 and 1.9 for chalcocite and covellite oxidation, respectively, imply that an increase in temperature of 10 C results in an increase of more than double or about double the biodegradation of these substrates.

Only minute concentrations of ferric iron are required to obtain the maximum effect on biodegradation of these substrates. These were found to be in the range of 0.004 to 0.01 M and 0.004 to 0.02 M for the oxidation of chalcocite and covellite, respectively, which is in agreement with previous studies (A. E. Torma, Canadian pat-
ent). Higher concentrations of ferric iron resulted in the deposition of jarosite, due to a partial hydrolysis of ferric sulfate (10), according to the following equation:

$$3 \text{Fe}_2(\text{SO}_4)_3 + 12 \text{H}_2\text{O} \rightleftharpoons 2\text{H}[\text{Fe(SO}_4)_2\text{Fe(OH)}_3] + 5 \text{H}_2\text{SO}_4$$ \hspace{1cm} (5)

This precipitate may cover the solid substrate surface and also result in a decrease in the oxidation of these substrates by the microorganism.

The rate of copper solubilization from covellite shown in Fig. 4 was directly proportional to the substrate concentration until 5.3% (wt/vol); higher substrate concentrations may interfere with the mass transfer of oxygen and carbon dioxide. It is possible that this limitation can be overcome, in part at least, with an increase in aeration and agitation. The rate of oxidation of chalcopyrite was higher than that of covellite.

The change in Eh during substrate oxidation is in agreement with the effect discussed by Moss and Andersen (12).

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LITERATURE CITED