Oxidation of stibnite by *Thiobacillus ferrooxidans*

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Optimum pH, temperature and pulp density for microbiological leaching of museum-grade stibnite mineral has been investigated using a stibnite-adapted strain of *Thiobacillus ferrooxidans*. Optimum conditions were found to be pH 1.75, 35 C and 12 g solid substrate per 100 ml of basal salts medium as the initial dose. The energy of activation was determined to be 16.8 kcal per mole, and the temperature coefficient 2.2. The highest total dissolved-antimony concentration, $[Sb_t] = [Sb^{+3}] + [Sb^{+5}] + [SbO^+] + [SbO_2^+]$, was about 1400 mg/litre, due to relatively low solubility of $(SbO)_2SO_4$ and $(SbO_2)_2SO_4$.

INTRODUCTION

The ability of *Thiobacillus ferrooxidans* to oxidize antimony-bearing complex sulphide minerals (Karavaiko, 1970; Lyalikova et al., 1972), low-grade stibnite (Rossi, 1971) and synthetic antimony sulphides (Silver and Torma, 1974) has been reported. Evidence has been presented that the free energy released by the oxidation of trivalent antimony to higher oxides should be sufficient to support autotrophic growth (Lyalikova, 1971). This finding led to the discovery of a new antimony-oxidizing microorganism *Stibiobacter senarmontii* (Lyalikova, 1974). However, the above references contain limited data on the effects of pH, temperature and substrate concentration, which are important from the point of view of biohydrometallurgical treatment of antimony sulphides.

The stibnite oxidation can be represented by the following equation(Rossi, 1971):

$$Sb_2S_3 + 6\,O_2 \xrightarrow{T.\ ferrooxidans} Sb_2(SO_4)_3$$

The antimony (III) sulphate will be partially hydrolyzed to produce an insoluble antimony (III) oxide sulphate:

$$Sb_2(SO_4)_3 + 2\,H_2O \leftrightarrows (SbO)_2SO_4 + 2\,H_2SO_4$$

MATERIALS AND METHODS

Organisms. Our strain of *T. ferrooxidans* was originally isolated by Torma (1971) from acid mine waters in northern Quebec. It was adapted to a modified nutrient medium of Silverman and Lundgren (1959) in which the stibnite mineral replaced ferrous sulphate as the energy source. For subculturing and for experiments an aliquot of a late-log-phase culture was transferred into a new medium.

Protein determination. Cellular protein was liberated by alkaline digestion (0.1 N NaOH) of the bacteria and estimated by the method of Lowry et al. (1951).

Substrate. The museum-grade stibnite mineral contained (wt/wt) 70.2% antimony, 26.8% sulphur, 0.8% lead and 1.2% iron as the major constituents, and contained the minerals stibnite, pyrite and lead sulphide. This substrate was ball-milled to particles less than 37 microns in diameter.

Manometric technique. The conventional manometric technique (Umbreit, Burris and Stauffer, 1972) was employed using 16-ml Warburg flasks. Each reaction flask contained a total liquid plus solid volume of 2.3 ml: 200 mg of the stibnite mineral and basal-salts medium to a total volume of 2.0 ml, and 0.3 ml of a 10% (wt/vol) bacterial suspension containing 4.7–5.8 mg protein. The center well contained 0.2 ml of 20% KOH. The experiments were carried out at pH 1.8, 35 C and a speed of 130 strokes per min, for 3 h. After 15 min equilibration, the reaction was started by tipping the cell suspension into the main compartment of the flask.

Harvesting. Cells were cultured in Erlenmeyer flasks as indicated under Culture technique. *T. ferrooxidans* cells free of substrate were collected from the decanted leach solutions by centrifugation at 500 rpm (Itzkovitch and Torma, 1976). The supernatant containing the bacteria was carefully removed, then centrifuged at 18000 rpm. The packed cells were resuspended in iron-free nutrient medium (Silverman and Lundgren, 1959) to produce a 10% (wt/vol) suspension and used for experimentation within three days.

Culture technique. All experiments besides those in Warburg vessels were carried out in 250-ml Erlenmeyer flasks on a 250-rpm gyratory shaker. The desired quantity of stibnite mineral and 70 ml iron-free nutrient medium (Silverman and Lundgren, 1959) were placed in the flasks and inoculated with 5 ml of an active and adapted late-log-phase culture of *T. ferrooxidans* (Sakaguchi, Torma and Silver, 1976). The flasks were aerated with air containing

0.2% carbon dioxide (Torma et al., 1972). Periodically, distilled water was added to compensate for evaporation, and the pH was kept at the desired value with 1 N H_2SO_4 or 1 N NaOH. The temperature was varied according to the experiment. In the sterile control flasks 5 ml of a 2% solution of thymol in methanol was added instead of inoculum.

Analytical methods. Periodically a 1-ml sample was analysed for dissolved antimony with an atomic absorption spectrophotometer. The sample was replaced with one ml of liquid medium containing basal salts only.

The mineralogical composition of the leach residue was determined by X-ray diffraction.

Kinetic determination. Extraction curves were drawn representing the dissolved-antimony concentration as a function of time. The rate of antimony solubilization (V) was determined from the initial linear part of each curve, using a linear least-squares fitting method (Draper and Smith, 1968).

The energy of activation (E_a) was calculated from the equation:

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

where R is the gas constant, T_1 and T_2 are absolute temperatures and V_1 and V_2 rates of antimony extraction at T_1 and T_2 respectively.

The temperature coefficient (Q_{10}) was calculated from the equation:

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

RESULTS AND DISCUSSION

Adaptation of T. ferrooxidans to stibnite. Prior to experimentation, the chalcopyrite-grown *T. ferrooxidans* were adapted to the stibnite mineral by successive transfer. For each transfer, early-stationary-phase cells were harvested the protein content of which was determined. In manometric experiments lasting three hours the specific rate of oxygen uptake (μl O_2/h/mg protein) was determined. The data in Table 1 show that after three transfers the adaptation process was already completed. In all further experiments these adapted bacteria were used.

The influence of pH on dissolution of antimony from a high-purity stibnite mineral is shown in Fig. 1. During the lag phase the pH tended to rise and sulphuric acid had to be added to keep the pH constant. However, during logarithmic growth the pH fell and NaOH had to be added. The optimum pH was found to be 1.75.

With sterile controls the yields of dissolved antimony were ca. 5–7 times less than with the corresponding inoculated flasks (Fig. 1).

Table 1. Adaptation of *T. ferrooxidans*, originally grown on chalcopyrite, to stibnite.

Number of transfer	Specific rate of oxygen uptake μl O_2/h/mg protein[1]	
	inoculated	sterile
1	6.05 ± 0.05	2.66 ± 0.03
2	9.17 ± 0.04	2.54 ± 0.04
3	18.63 ± 0.14	2.75 ± 0.06
4	31.02 ± 0.32	2.67 ± 0.05
5	30.97 ± 0.29	2.73 ± 0.04

[1] Each figure is the mean of three individual experiments ± SEM (Standard error of the mean).

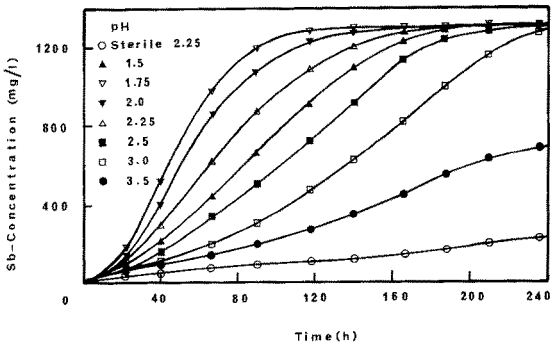

Fig. 1. Effect of pH on antimony dissolution by *T. ferrooxidans* at 35 C, stibnite 6 g per 100 ml medium + inoculum, and 0.2% carbon dioxide in air.

The effect of temperature on antimony dissolution was studied over a temperature range from 20 to 40 C (Fig. 2). The optimum temperature was found to be 35 C. The activation energy (E_a) and the temperature coefficient (Q_{10}), calculated between 25 and 35 C, were 16.8 kcal per mole and 2.2, respectively.

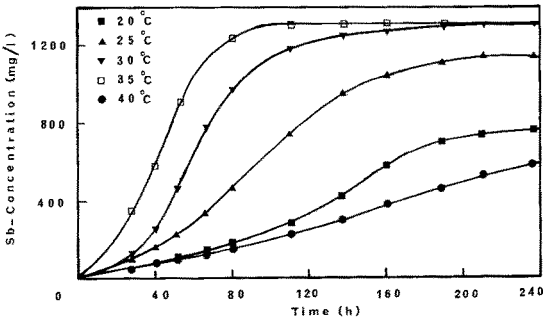

Fig. 2. Effect of temperature on antimony dissolution by *T. ferrooxidans* at pH 1.75, stibnite 6 g per 100 ml medium + inoculum, and 0.2% carbon dioxide in air.

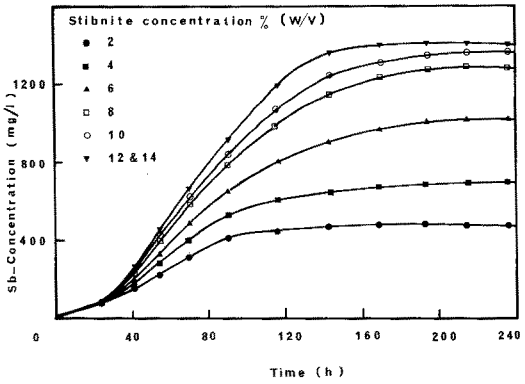

Fig. 3. Effect of amount of stibnite added to 100 ml medium + inoculum on antimony dissolution by *T. ferrooxidans* at pH 1.75, 35 C and 0.2% carbon dioxide in air.

The effect of substrate on the yield of dissolved antimony was studied for initial stibnite concentrations of 2–14 g per 100 ml medium. The optimum value was found to be 12 g per 100 ml (Fig. 3). The highest concentration of dissolved antimony was about 1400 mg/litre, which was obtained with leach solutions containing 12 or 14 g of stibnite per 100 ml medium. The X-ray analysis of the leach residues indicated that it is composed of $(SbO)_2SO_4$, $(SbO_2)_2SO_4$ and unreacted stibnite. Accordingly, the stibnite is oxidized to antimony (III) sulphate, which partially hydrolyzed to antimony (III) oxide sulphate as shown by Rossi (1971). Furthermore, antimony (III) sulphate is partially oxidized to antimony (V) sulphate:

$$Sb_2(SO_4)_3 + O_2 + 2H_2SO_4 \xrightarrow{T.\ ferrooxidans?} Sb_2(SO_4)_5 + 2H_2O$$

which hydrolyzed to insoluble antimony (V) bioxide sulphate:

$$Sb_2(SO_4)_5 + 4H_2O \leftrightarrows (SbO_2)_2\,SO_4 + 4H_2SO_4$$

Further, the X-ray determination revealed that about 5–8% of antimony (III) sulphate is oxidized to antimony (V) sulphate.

Direct involvement of *T. ferrooxidans* in the oxidation of Sb^{+3} to Sb^{+5} could not be established. This phenomenon must be studied separately.

The insoluble antimony oxide sulphates, $(SbO)_2SO_4$ and $(SbO_2)_2\,SO_4$, may precipitate on the surface of the stibnite mineral thereby inhibiting the bacterial action. The conversion of stibnite to antimony sulphates varied between 55 and 64%. However, because of the low solubility of the hydrolysis products, the total dissolved-antimony concentration, which is expressed by the following equation:

$$[Sb_{total}] = [Sb^{+3}] + [Sb^{+5}] + [SbO^+] + [SbO_2^+]$$

has not exceeded the 1400 mg/litre level.

The optimum pH 1.75 found in this study fits well in the range 1.7–2.5 generally obtained in previous investigations for metal sulphide oxidations by *T. ferrooxidans* (Torma, Walden and Branion, 1970; Sakaguchi et al., 1976). The activation energy 16.8 kcal/mole is in good agreement with 16.3 kcal/mole obtained for chalcocite leaching (Sakaguchi et al., 1976). The Q_{10} value of 2.2 implies that an increase in temperature of 10°C results in a more than doubled rate of biooxidation of stibnite.

This study was carried out in the laboratories of the Mineral Research Centre of the Quebec Department of Natural Resources.

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