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INDIRECT BIOLEACHING OF COVELLITE BY *THIOBACILLUS THIOOXIDANS* WITH AN OXIDANT AGENT.

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SUMMARY

High copper extraction by the action of *Thiobacillus thiooxidans* (*T.t.*) on covellite in presence of iron(III) is explained by indirect mechanism, in which *T.t.* only oxidizes the layer of sulphur that covers the sulphide surface allowing sulphide oxidation by iron(III). In cultures on elemental sulphur with iron(III) *T.t.* is not able to use iron(III) as an acceptor of electrons in sulphur oxidation; iron(III) only oxidizes those intermediates which were generated in the aerobic oxidation of sulphur.

INTRODUCTION

T.t. is an important species in the microbial catalysis of sulphide oxidation. Pathways of sulphur oxidation by this bacterium are well studied (Karavaiko *et al.*, 1988), but sulphide oxidation mechanisms remain uncertain.

T.t. is able to oxidize relatively soluble sulphides, such as ZnS, by the direct mechanism. However, insoluble sulphides such as CuS are only lightly attacked. This indicates that *T.t.* only oxidizes sulphide in solution. In contrast *Thiobacillus ferrooxidans*, also important in bioleaching, oxidizes sulphide both in solution and on solid surfaces (Pistorio *et al*., 1994).

Recent work (Donati *et al.*, 1995) has shown catalysis of covellite (CuS) oxidation by *T.t.* when iron(II) is added. This catalysis develops parallel to chemical oxidation of iron(II) (*T.t.* is not able to oxidize iron(II)). This suggests iron(III)-catalysis of the oxidation of very insoluble sulphides by *T.t.*. Moreover, other authors (Karavaiko *et al.*, 1988) have proposed that *T.t.* is able to oxidize sulphur with iron(III) as an

acceptor of electrons. This work attempts to explain and find an eventual link between both mechanisms.

MATERIALS AND METHODS

Substrate. Analytical grade covellite (CuS) of Strem Chemicals was used. For all the tests a 0.1 % pulp density was used (0.1 grams of substrate in 100 ml solution).
Microorganisms. A culture of *Thiobacillus thiooxidans* (Tth04) from Brazil was used. *T.t.* culture was inoculated in the Imai medium (Imai, 1978). When bacteria reached exponential phase of growth medium was filtered (through blue ribbon filter paper) to eliminate sulphur for *T.t.*. Filtrate was directly inoculated at 10 % v/v (bacterial population in the inoculum: $8x10^8$ bact/ml).
Bioleaching Systems. Experiments were carried out in shake flasks at 200 rpm. The temperature was maintained at 28 ^oC. Each flask contained 100 ml of Imai medium with the addition of 6 $g.l^{-1}$ iron(III). The initial pH was 1.8 with no further control. 0.2 g of CuS were added to all systems.
One of these systems additionally contained 10 % p/v powdered elemental sulphur. In sterile systems, an inoculum was replaced by equal volume of sterile medium.
Cultures on elemental sulphur. In these experiments, same conditions as for the bioleaching systems were used. The covellite was replaced by 10 % p/v elemental sulphur.
Analytical procedure. Copper in solution was determined by atomic absorption spectrophotometry (Metrolab 4200 instrument). Iron(II) was determined by titration with 0.01 N $KMnO_4$. Acid production was calculated from the differences in titrations with 0.02 N NaOH.
Bacterial counts were determined using a Nikon microscope with phase contrast attachment and a Petroff-Häuser camera.

RESULTS AND DISCUSSION

a) Covellite bioleaching assays

Figure 1 shows the time profiles of copper(II) and iron(II) concentrations and unattached bacterial population in systems with and without sulphur addition. Copper(II) and iron(II) data were calculated by difference between inoculated and sterile systems. Acid production was 200 mM and 28 mM in the systems with and without sulphur addition, respectively. Iron(II) production was 38 mM in the system with sulphur and 28 mM in the other system.

The high extraction of copper(II) in inoculated systems (65.2 % at 30 days) suggests a catalysis mechanism by *T.t.*. This process should require iron(III) because *T.t.*

is unable to leach CuS in media without iron(III) (Donati *et al.*, 1995).

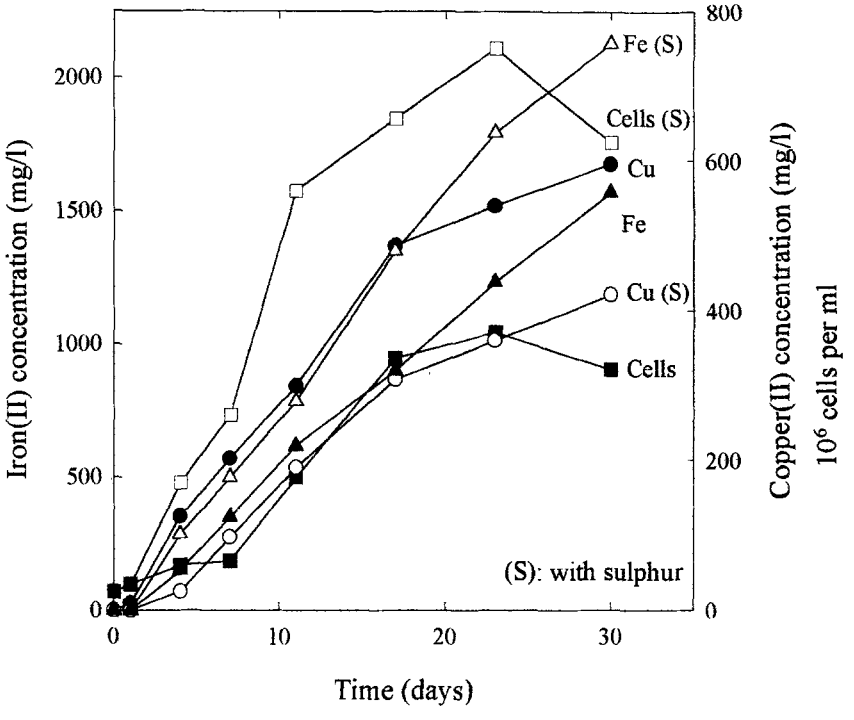

Figure 1. Bioleaching of CuS by *Thiobacillus thiooxidans* in presence or in absence of sulphur

The molar ratio between iron(II) produced and copper(II) released during the first 15 days of experience for the system without sulphur was close to 2 : 1 (Figure 1). This ratio increases after the first 15 days. These observations agree with reaction [1] as the first step of chemical oxidation.

$$2\,Fe^{+3} + CuS \rightarrow 2\,Fe^{+2} + S + Cu^{+2} \qquad [1]$$

followed by a second step of bacteria catalyzed sulphur oxidation:

$$S + O_2 + H_2O \rightarrow SO_3^{2-} + 2\,H^+ \qquad [2]$$

Produced sulphite can be oxidized by O_2 (bacteria catalyzed) or by iron(III) (chemical oxidation or bacteria catalyzed):

$$SO_3^{2-} + \tfrac{1}{2}\,O_2 \rightarrow SO_4^{2-} \qquad [3]$$

$$2\,Fe^{+3} + SO_3^{2-} + H_2O \rightarrow 2\,Fe^{+2} + SO_4^{2-} + 2\,H^+ \qquad [4]$$

Both possibilities agree with the determined 1 : 1 molar ratio between iron(II) and proton (see above). However, according to the behaviour of the iron(II) : copper(II) ratio, iron(III) reduction by sulphite should be more important after the first 15 days of experience.

In conclusion, there is an indirect bacterial action that consists of the removal of sulphur layer on the sulphide surface which allows further chemical oxidation.

Systems with added sulphur showed lower copper(II) extraction. This suggests that the main bacterial fraction was attached to the large additional surface and, as a consequence, the attached fraction to sulphide was smaller. The removal of the sulphur layer on the sulphide should be lower in systems with added sulphur. The proposed mechanism accounts for these observations.

Alternative mechanisms, such as iron(III)-induced direct oxidation of covellite by *T.t.*, were discarded because acid is produced during the process (acid is not produced when sulphide is oxidized to sulphate by oxygen as electron acceptor). Moreover, in complementary experiments with variable iron(III) amounts (data not shown), copper(II) release was also parallel to iron(III) reduction.

b) Sulphur oxidation assays

Figure 2 shows the time profiles of iron(II) and proton concentrations and unattached bacterial population in systems with initial iron(III) and elemental sulfur, along with proton concentrations and unattached bacterial population in a similar system without iron(III). All reported values of iron(II) and proton concentrations were calculated with previous subtraction of the sterile values, assuming equal chemical action in sterile and inoculated systems.

For the sterile systems (data not shown) very low proton yields were determined. Moreover, the iron(II) production was also very low for the sterile system with initial iron(III). Figure 2 shows similar acid yields for inoculated systems with and without iron(III). A lower bacterial growth was observed in the system with initial iron(III) in comparison with that without iron. Additionally, an evident reduction of iron(III) was detected for the former system. These results suggest different mechanisms operating in presence and in absence of iron(III). However, a role of iron(III) as an electron acceptor in the bacterial mechanism was discarded because the experimental ratio between iron(II)

and proton productions was 1.3 while the stoichoimetric ratio for the sulphur oxidation by iron(III) is 0.75.

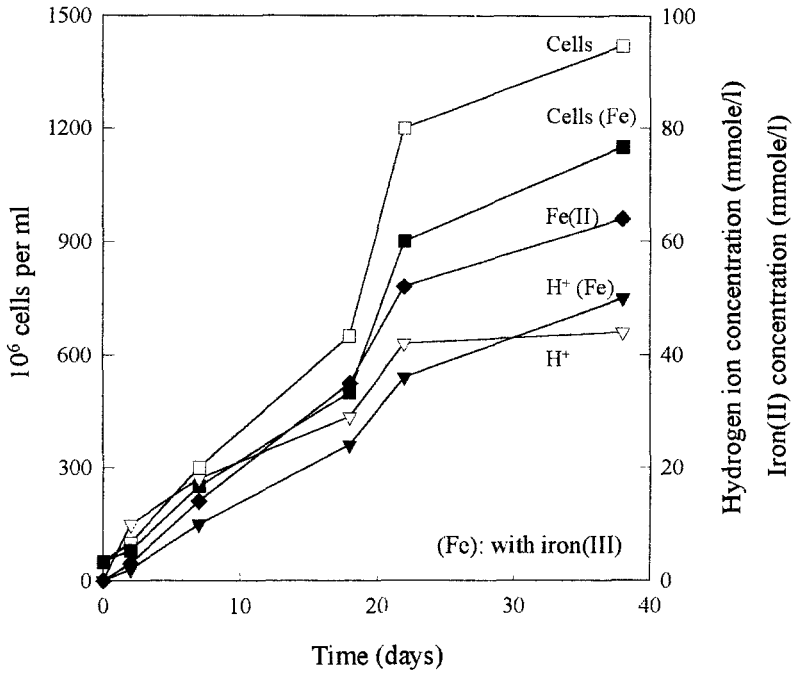

Figure 2. *Thiobacillus thiooxidans* cultures on elemental sulphur in presence or in absence of iron(III)

The first 15 days showed similar bacterial growth in presence and in absence of iron(III), which indicates a similar initial mechanism. Due to the high initial attachment, this mechanism may consist of the sulphur oxidation to sulphite (reaction [2]) or to others intermediates (Shirardi *et al.*, 1993).

After the first 15 days, in the system with iron(III), sulphite in solution may be oxidized by iron(III) (reaction [4]), so the unattached bacterial population has low soluble substrate. On the contrary, in the system without iron(III), sulphite in solution should be oxidized by *T.t.* with oxygen as electron acceptor (reaction [3]) which explains the higher bacterial growth in the system with iron. Consequently, in the iron-free system the bacterial population became higher than in the system with iron(III) after 15 days (Figure 2). Up to 30 days, the rate for acid production in the iron-free system was almost constant although the bacterial growth increased more quickly because the another source of bacterial growth (reaction [3]) does not generate acid.

CONCLUSIONS

T.t. allows the indirect bioleaching of covellite in presence of iron(III). The mechanism consists of an initial chemical attack of iron(III) on the sulphide, producing a layer of elemental sulphur that covers the surface. Then, *T.t.* oxidizes elemental sulphur allowing the further sulphide oxidation by iron(III). *T.t.* does not use iron(III) as an acceptor of electrons in cultures on elemental sulphur with iron. So, the iron(III) reduction observed in these cultures is probably due to the chemical (or bacteria catalyzed) oxidation of intermediates such as sulphite, produced in the bacterial oxidation of sulphur. Anyway, the mechanism of indirect bioleaching of sulphides is very important because it allows higher metal extraction than in a similar chemical system. Moreover, the mechanism of indirect bioleaching by *T.t.* due to its higher acid production in sulphur oxidation, is better than indirect bioleaching by *Thiobacillus ferrooxidans* because the former avoids the precipitation of jarosites.

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