*Proc. Indian natn. Sci. Acad.* **B52** No. 4 pp 519–524 (1986)

# Factors affecting the Growth and Chalcopyrite Oxidation by *Thiobacillus ferrooxidans*

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(Received 1 April 1986; after revision 13 October 1986)

The oxidation rate of a molybdenum contaminated chalcopyrite concentrate by *T. ferrooxidans* depended on the initial acidity as well as the pulp density of the system. There was a lag in chalcopyrite oxidation when ferrous iron grown cells were used. This could be eliminated either by the application of Tween-80 or by the previous adaptation of cells on chalcopyrite. The soluble copper leach curve was synonymous to bacterial growth. Most of the cells remained firmly attached to chalcopyrite particles. In the absence of essential bacterial nutrients the oxidation was negligible. The generation time of the bacterium on chalcopyrite was about 12.5 hr. Existence of an indirect role of cuprous-cupricion system similar to that of ferrous-ferric ion system, which operates in a cyclic manner during chalcopyrite oxidation was revealed.

**Key Words:** *Thiobacillus*, Chalcopyrite, Copper oxidation, Leaching

## Introduction

The involvement of *Thiobacillus ferrooxidans* to solubilise copper from chalcopyrite minerals has been demonstrated and reviewed by many authors (Razzell & Trussell 1963, Bruynesteyn & Duncan 1971, Torma 1977, Lundgren & Silver 1980). The bacterium has the capacity to oxidise the minerals to produce cupric ion and the solvent sulfuric acid, together with regeneration of the oxidising agent ferric ion (Lundgren & Silver 1980). The latter being highly important for leaching operations (Torma 1977, Brierley 1978). Though extensive investigations have been done on the extractions of copper from chalcopyrite minerals by bacterial oxidation, the quantitative data on the kinetics of bacterial oxidation of toxic metal contaminated chalcopyrite mineral is limited (Kingma & Silver 1980).

Since recovery of copper from chalcopyrite has got a great bearing on the industry (Torma 1977), several factors affecting chalcopyrite oxidation by *T. ferrooxidans* were studied.

## Materials and Methods

The chalcopyrite sample (Surda ore, Bihar, India) containing 15% Cu, 20% Fe, 0.002% Mo and 30% S was ground to 63 μm particle size and analytical grade ferrous sulfate (B.D.H., India) were used as substrates for oxidation studies.

The culture of *T. ferrooxidans* (NCIB-8455) obtained from National Chemical Laboratory, Poona, India was maintained on modified 9K medium having a *p*H of 1.6, adjusted with 10(N) $H_2SO_4$ (Mishra et al. 1983). The culture was purified through single colony isolation and was used throughout these studies. When chalocopyrite was used as the energy source instead of ferrous sulfate, the *p*H was adjusted to 2.0. The solid medium was prepared as described earlier (Mishra et al. 1983), and the platings were done after shaking the medium vigorously for 10 minutes to detach cells from chalcopyrite particles.

The cells were grown in liquid medium containing either ferrous sulfate or chalcopyrite depending upon the studies undertaken. The growing cells from the

exponential phase were harvested on Millipore Membrane filter (0.45 μm pore size), washed two to three times with 0.01(N) $H_2SO_4$ and resuspended in the same to contain about $3 \times 10^9$ cells/ml in both the cases. This cell suspension (1.0 ml) was added to 100 ml liquid medium containing requisite amount of chalcopyrite and incubated at 28°C under shaking condition on a gyratory shaker (150 r.p.m.). At regular intervals one millilitre suspension was withdrawn from the growing culture to estimate the water soluble iron and copper by orthophenanthroline (Snell & Snell 1949) and biquinoline (Cheng & Bray 1953) method respectively. Before each estimation, the cells were not separated from the growing suspension, as no marked difference of results was observed. During each phase of estimation copper and iron were determined separately.

## Results

### *Chalcopyrite oxidation by iron grown cells*

*Effect of* pH: The initial *p*H for optimum chalcopyrite oxidation (17 mg Cu per litre per hr) was found to be in the range of 2.0 to 2.5 (figure 1a & b). The acidity increased to a final *p*H value of 1.25 to 1.55 after 280 hr depending on the initial *p*H of the system (figure 1). However, there was an increase in *p*H up to 40 hr followed by a decline. The rate of oxidation was found to increase with increase in *p*H up to 2.0 (69% total copper extraction), after which it slowed down. At the initial *p*H of 1.5 the oxidation was negligible. At *p*H 1.0 no copper release was found to occur. Copper was released from chalcopyrite exponentially after a lag of about 30 hr, and the oxidation rate increased steadily up to about 200 hr. There was no marked change in *p*H value (*p*H 2.0) in the sterile control.

*Effect of pulp density:* The effect of solid to liquid concentration ratio (mass of chalcopyrite × 100/volume of the liquid medium) on chalcopyrite oxidation was studied up to 10% pulp density in the presence of iron grown *T. ferrooxidans*. Above 1% pulp density, there was always a lag period before exponential chalcopyrite oxidation, and the lag increased with increase in the chalcopyrite concentration (figure 2a & b). The average leaching rate increased linearly up to 3% pulp density (20 mg Cu/l/hr) and remained more or less constant below 5%. Above this pulp density, the rate of chalcopyrite oxidation decreased to almost a constant value (12 mg/Cu/l/hr) up to 10%. The optimum pulp density considering both leach rate and percentage of oxidation ranged between 2% to 3%.

*Effect of surfactant:* At 5% chalcopyrite pulp density, the addition of 0.005% Tween-80 reduced the initial lag of oxidation by 70 hr (figure 2a) but the final copper extraction after 180 hr was less than that without the addition of the surfactant (figure 2a).

*Effect of adaptation on chalcopyrite:* Leaching with cells previously adapted on chalcopyrite mineral, showed copper leach curves without any lag (figure 3). This was the only notable difference compared to that of copper leach profile by iron grown cells (figure 2). The rate of leaching was almost similar, but the percentage of oxidation in case of chalcopyrite adapted cells could be achieved earlier i.e. at about 200 hr as against 280 hr with iron grown cells.

*Effect of nutrients on chalcopyrite oxidation:* The absence of nitrogen, phosphate and potassium had a

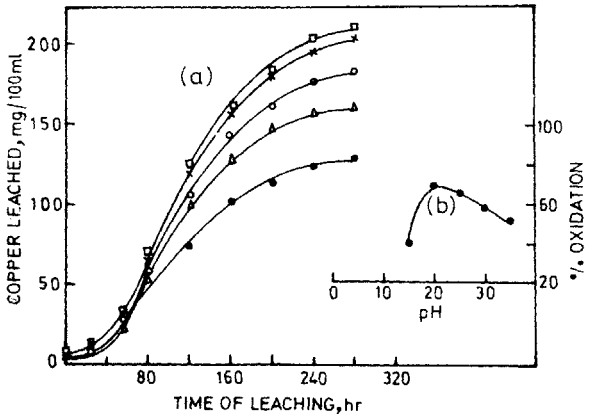


**Figure 1** Effect of initial *p*H on chalcopyrite oxidation by *T. ferrooxidans* at 2% pulp density. (a) Solubilisation of copper at *p*H 3.5(Δ) final *p*H 1.55; 3.0(0) final *p*H 1.50; 2.5(X) final *p*H 1.40; 2.0(□) final *p*H 1.30; 1.5(●) final *p*H 1.25. (b) % oxidation (●) at 280 hr

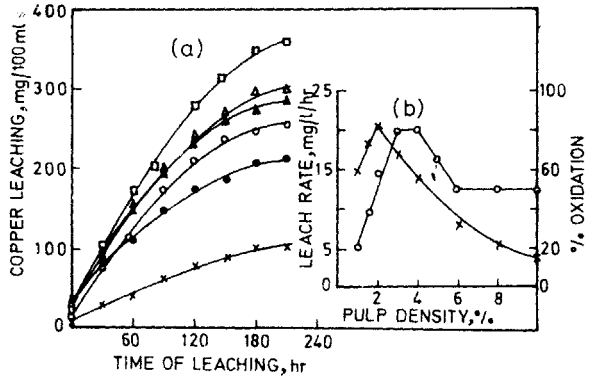


**Figure 2** Effect of pulp density on chalcopyrite-oxidiation by *T. ferrooxidans*. (a) Solubilisation of copper at various pulp densities (%): 1(X); 2(0); 4(□); 5(▲); 6(●); 5 with 0.005% Tween-80 (Δ). (b) copper leach rate (0) and % oxidation (X) at 280 hr

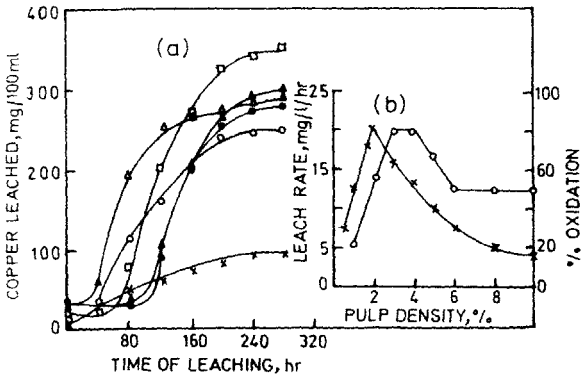


**Figure 3** Effect of pulp density on chalocpyrite-oxidation by *T. ferrooxidans* cells grown on chalcopyrite (a) Solubilisation of copper at various pulp densities (%): 1(X); 1.5(●); 2.0 (0); 4(□) 6(Δ); 8(▲). (b) copper leach rate (0) and % oxidation (X) at 210 hr

drastic effect on chalcopyrite oxidation (table 1). Double the amount of nitrogen source as in complete medium, and absence of magnesium showed nearly similar oxidation rate compared to that of complete medium. One tenth of nitrogen of the complete medium reduced the oxidation by 25%, however, in complete absence of nitrogen source, the oxidation was negligible. Absence of phosphorus the oxidation by 52% and that of potassium by 29% less was encountered as compared to that occurring in complete medium where 68.4% oxidation occurred. Half or one-tenth of phosphorus as present in complete medium does not show such adverse oxidation rate and resulted in nearly 6% to 10% less oxidation than complete medium.

*Growth of* Thiobacillus ferrooxidans *on Chalcopyrite*

The increase in viable cell number during chalcopyrite oxidation is given in figure 4. The rate of cell multiplication and the rate of chalcopyrite oxidation were almost synonymous. The generation time of the bacterium on chalcopyrite was calculated as 12.5 hr (sp. growth rate 0.08/hr) in the range between 0 to 80 hr where growth rate was maximum. After 80 hr the growth slowed down which is also reflected from the copper leach curve. Only 1.1% to 2.0% of the total cells remained as free swimming cells within the supernatant leached solution, and 98% to 99% of the total cells were attached to the chalcopyrite particles.

*Fate of Copper and Iron during Chalcopyrite Oxidation by* T. Ferrooxidans

During chalcopyrite oxidation it was observed that ferric iron ($Fe^{3+}$) always dominated over iron ($Fe^{2+}$) concentration, and the latter never exceeded 8% of the total iron in solution. On the other hand, although the cupric copper ($Cu^{2+}$) always dominated over cuprous copper ($Cu^{+}$) it never allowed the latter to exceed 2.6% of the total soluble copper during active leaching process. Up to about 120 hr the concentrations of cuprous ion and ferrous ion were very low which corresponded to not more than 1.5% of the total soluble copper and 5% of the total soluble iron, respectively. After 120 hr the concentrations of both cuprous and ferrous ion gradually increased. The ratio of $Fe^{3+}$ to $Fe^{2+}$ dropped from 29.0 to 11.2 after 280 hr and, during the same time the ratio of $Cu^{2+}$ to $Cu^{+}$ went down from 75.0 to 38.3. When bacterial activity was terminated at 280 hr by adding 2% (v/v) lysol in the leaching medium, the ferric and cupric ion concentrations dropped sharply. The ratio of $Cu^{2+}$ to

**Table 1** *Effect of mineral nutrients on chalcopyrite oxidation by* T. ferrooxidans *at 2% pulp density*

| Medium | Copper leached (μg/ml) from chalcopyrite (hr.) | | | | | Recovery*** of copper in solution % | Decrease in oxidation over CM (%) |
|---|---|---|---|---|---|---|---|
| | 0** | 40 | 120 | 200 | 280 | | |
| Complete medium* (CM) as reference standard | 60 | 475 | 905 | 1775 | 2125 | 68.44 | — |
| Nitrogen absent | 55 | 59 | 105 | 125 | 125 | 2.31 | 66.13 |
| Phosphorus absent | 56 | 80 | 330 | 475 | 535 | 16.00 | 52.44 |
| Potassium absent | 55 | 75 | 500 | 755 | 1250 | 39.55 | 28.89 |
| Magnesium absent | 58 | 300 | 815 | 1445 | 2000 | 64.44 | 4.00 |
| Nitrogen as 1/10th of CM | 61 | 80 | 525 | 925 | 1392 | 44.00 | 24.44 |
| Nitrogen as double of CM | 60 | 285 | 688 | 1425 | 1977 | 63.55 | 4.89 |
| Phosphorus as 1/10th of CM | 60 | 250 | 635 | 1275 | 1825 | 58.66 | 9.78 |
| Phosphorus as half of CM | 55 | 275 | 675 | 1365 | 1930 | 62.66 | 5.78 |

* CM — denotes modified 9K medium containing (% w/v); N-0.010, Mg-0.004, P-0.008 and K-0.027

** 0 — 0 hr showed this solubility

*** — calculation made after duducting 0 hr values

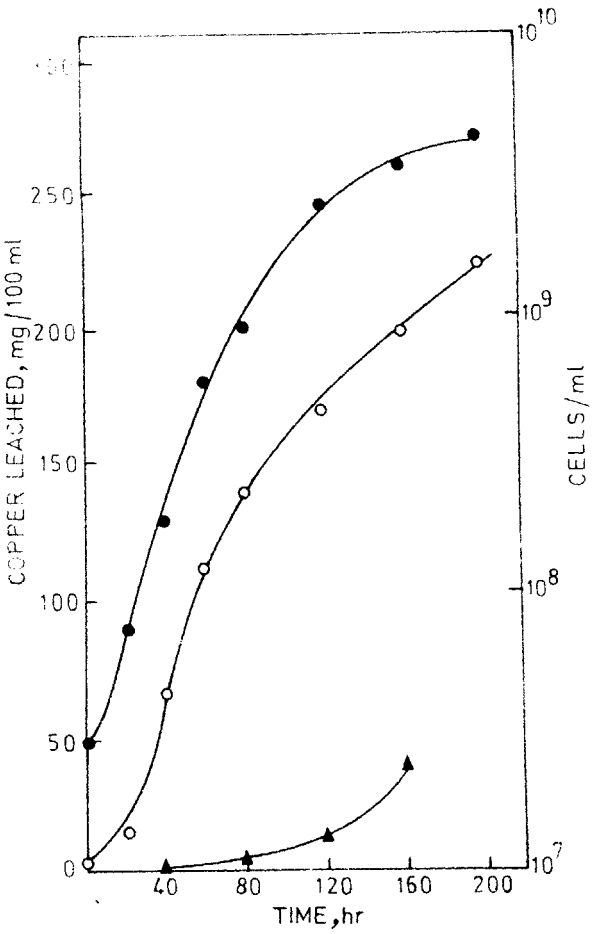


**Figure 4** Solubilisation of copper and increase in cell numbers during growth of *T. ferrooxidans* on chalcopyrite in liquid culture at 2% pulp density and at *p*H 2.0; soluble copeer (0); total viable counts (●); viable count in supernatant (▲)

$Cu^+$ was reduced to 10.2 whereas that of $Fe^{3+}$ to $Fe^{2+}$ dropped to 5.2 (table 2).

## Discussion

*T. ferrooxidans* readily oxidises the reduced iron and copper part of the chalcopyrite. The bacterium has also a greater affinity to oxidise simultaneously the sulfide moiety of this mineral to produce sulfuric acid. The requirement of this acid during oxidation is less than that is produced during the process. As such the chalcopyrite oxidation is accompanied with the reduction of *p*H value. The rate of oxidation was nil below *p*H 1.5. This clearly reflects that the initial acidity below *p*H 1.5 is a rate restricting step in bacterial chalcopyrite oxidation.

The bacterium showed optimum copper release at *p*H 2.0, which is in disagreement with many previous workers (Razzell & Trussell 1963, Bruynesteyn & Duncan 1971, Torma 1977, Kingma & Silver 1980). This might arise due to the variation of the nature of accessory minerals in chalcopyrite under study, as well as the strain used. The initial phase of bacterial chalcopyrite oxidation, is a clear reflection of acid consumption. The increase in *p*H up to 40 hr as observed here was probably due to the consumptions of sulfate ions from the medium.

$$2CuFeS_2 + 8.5O_2 + H_2SO_4 \rightarrow 2CuSO_4 + Fe_2(SO_4)_3 + H_2O$$

It has been suggested earlier (Silverman 1967) that the direct contact between the solid substrates and the cells are required to initiate oxidation. The lag observed here in case of iron grown cells is perhaps due to the requirement of some adaptation period for attachment to chalcopyrite particles. As such chalcopyrite adapted cells showed no lag period for oxidation. Controversial reports have accumulated on the use of surface active agents in microbiological metal extraction process. Duncan et al. (1964) observed that 0.003% Tween-20 was beneficial at 1.5% pulp density of chalcopyrite, although the higher concentration inhibited oxidation. Torma and Gabra (1975) on the other hand contradicted this effect of the surfactants. These authors observed the deleterious effect of surfactant on chalcopyrite oxidation by low oxygen uptake of the cells and reduction of surface tension. We have observed that, the addition of 0.005% Tween-80 at 5% pulp density reduced the lag period of chalcopyrite oxidation by iron grown cells, although the same was inhibitory below this pulp density. Thus surfactant to chalcopyrite ratio is more important than surfactant to medium ratio. The degree of inhibition as observed in our case may be dependent on two factors, viz. concentration of Tween-80 and pulp density of chalcopyrite.

A small fraction of the total cells remained free in the leached solution, which indicates that the cells which were attached to the surfaces of the chalcopyrite minerals are only capable of exponential growth. Due to this reason probably the copper leach curve is synonymous to bacterial growth. The reported data McGoran et al. (1969), Le Roux et al. (1974) for the number of attached cells on solid substrates accounting for more than 90%, is also consistent with our results for chalcopyrite oxidation. The generation time of the bacterium on pyrite as reported earlier is 8 hr (Roy & Mishra 1981), while using molybdenum rich chalcopyrite as the substrate the generation time comes out to about 12.5 hr. The indirect way of cell counting by determining bacterial nitrogen during chalcopyrite oxidation, as reported by McGoran et al. (1969) showed the generation time as 14 hr. Absence of

*Table 2 Fate of copper and iron during chalcopyrite oxidation by* T. ferrooxidans *at 2% pulp density*

| Time (hr) | Copper leached (μg/ml) | | | | | | Iron leached (μg/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | $Cu^{+2}$ | % of the total | $Cu^{+}$ | % of the total | $Cu^{+2}/Cu^{+}$ | Total | $Fe^{+3}$ | %of the total | $Fe^{+2}$ | % of the total | $Fe^{+3}/Fe^{+2}$ |
| 20 | 152 | 150 | 98.68 | 2 | 1.31 | 75.00 | 150 | 145 | 96.66 | 5 | 3.33 | 29.00 |
| 40 | 576 | 568 | 98.61 | 8 | 1.38 | 71.00 | 650 | 625 | 96.15 | 25 | 3.84 | 25.00 |
| 80 | 725 | 715 | 98.62 | 10 | 1.37 | 71.50 | 990 | 950 | 95.95 | 40 | 4.04 | 23.75 |
| 120 | 1015 | 1000 | 98.52 | 15 | 1.47 | 66.66 | 1175 | 1125 | 95.74 | 50 | 4.25 | 23.75 |
| 160 | 1275 | 1255 | 98.43 | 20 | 1.56 | 62.25 | 1885 | 1785 | 94.69 | 100 | 5.30 | 17.85 |
| 200 | 1850 | 1820 | 98.37 | 30 | 1.62 | 60.66 | 2170 | 2015 | 92.64 | 155 | 7.14 | 13.00 |
| 240 | 2085 | 2040 | 97.84 | 45 | 2.15 | 45.33 | 2700 | 2500 | 92.59 | 200 | 7.40 | 12.50 |
| 280 | 2360 | 2300 | 97.45 | 60 | 2.60 | 38.33 | 3050 | 2800 | 91.80 | 250 | 8.19 | 11.20 |
| *280 | 1750 | 1595 | 91.14 | 155 | 8.85 | 10.29 | 2500 | 2100 | 84.00 | 400 | 16.00 | 5.25 |

* Data obtained after addition of lysol 2% (v/v) at 280 hr.

nitrogen, phosphorus and potassium reduced the rate of chalcopyrite oxidation. This finding supports the earlier observations of Torma and Tabi (1973) but not that of Tuovinen et al. (1971), who failed to observe any deleterious effect of iron oxidation in the absence of nitrogen and potassium, while the absence of phosphorus reduced the rate. We have observed that low concentration of phosphate source is essential to attain maximum rate of chalcopyrite oxidation (table 2), although Le Roux et al. (1974) showed reduction in pyrite oxidation with the addition of phosphate. These differences in observations probably arise due to the variations in the sulfide minerals used as stated earlier, and as such identification of the nutrients which are primarily needed for the leaching medium, becomes essential. The reduced rate of chalcopyrite oxidation in the absence of magnesium was not observed which suggests that this requirement is very low and thus could be met through the impurities of chemcials or the carried over materials of the mineral ores. So the supply of identified essential nutrients in the leaching medium should be taken into account which is one of the rate limiting factor of bacterial chalcopyrite oxidation.

The chalcopyrite mineral having copper, iron and sulfide moieties, after oxidation, ends up into ferric ion, cpric ion and sulfate. The former in turn oxidises the sulfide moeity of the mineral to produce elemental sulfur and itself gets reduced to ferrous ion. The bacteria then subsequently oxidise elemental sulfur and ferrous ion. This was true in the present investigation also (table 2). The concentration of ferrous ion increases where growth rate starts to decline i.e., from 120 hr onwards (figure 4). The inhibition of bacterial activity leading to increased ferrous ion concentration from this phase could be due to increased acidity, or by some toxic elements such as molybdenum present in the mineral itself. Although, the leached soluble molybdenum concentration never exceeded 40 μg/ml in any case, the coating of sulfur on sulfide particles of the minerals to restrict bacterial activity is not taken into account as the same has been invalidated earlier (Roy & Mishra 1981). Whatever may be the factor for restricted bacterial activity, interestingly it has been observed that the increase in ferrous ion concentration paralleled with increase in cuprous ion the leached solution (table 3). As iron and copper usually exist in chalcopyrite in both oxidized and reduced valencies (Lundgren & Silver 1980), the possible contribution of these two forms during leaching operation can not be ruled out. Thus apart from redcued iron and sulfur oxidation by *T. ferrooxidans*, an oxidisable metal ($Cu^{+}$) if present in the system might be available to the energy yielding metabolism of this bacterium (Neilson & Beck 1972). The results that are presented here (table 3) also depict such possibility. Hence, in bacterial chacopyrite oxidation the possibility of a similarity to that of ferrous-ferric ion system, and an indirect mechanism involving a cyclic cuprous-cupric ion system, might assist in the release of copper from the mineral. This phenomenon is very important from the industrial point of view, because using *T. ferrooxidans* to any sulfide containing dual metal substrates, the prolonged bacterial activity may presumably occur. As such, the bioleaching operation though has a possibility of being restricted by reaching to a critical concentration of one of the metal ions, still another coexisting substrate may favour the acceleration of further bacterial acitvity.

India possesses a good deal of low grade copper bearing mineral ores. The applicability of such a bioleaching process might prove beneficial in the near future for recovery of both copper and sulfur from these ores.

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