Leaching of chalcopyrite by *Thiobacillus thiooxidans* and oxidized copper ore by *Thiobacillus ferrooxidans* isolated from local environments

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Received 25th February 1988; revised and accepted 31st May 1988

**Introduction**

Metals are released from insoluble minerals directly through oxidative metabolism of microorganisms or solubilized indirectly by chemical oxidants produced as metabolic products of microorganisms. The most important commercial application of microbial mediated metal leaching is extraction of copper containing sulphidic minerals, namely, covellite (CuS), chalcocite (Cu₂S) and chalcopyrite (CuFeS₂). In United States, 18% of total copper produced in 1978 was through bacterial leaching (Wadsworth 1982). Bioleaching of copper in Bulgaria (Groudev et al. 1982) is also practiced with 20 MT (million tonnes) of low grade copper ore being produced from open cut mines.

*Thiobacillus ferrooxidans* and *T. thiooxidans* are the principal microorganisms which play an important role in ore leaching (Ralph 1985; Lundgren *et al.* 1986). *T. ferrooxidans*, having the ability to oxidize sulphur, ferrous iron, cuprous copper, pyrite and iron containing sulphidic ores, has been extensively studied for the bioleaching of copper (Lawrence *et al.* 1984; Agate & Khinvasara 1986). *T. thiooxidans*, having ability to oxidize sulphur and reduced sulphur compounds, generally does not oxidize sulphides. However, it has been used for leaching of both zinc sulphide ores and copper concentrates (Khalid & Ralph 1977; Kelly *et al.* 1979; Groudev 1983; Agate & Khinvasara 1986). Leaching by *T. thiooxidans* and *T. ferrooxidans* of different zinc sulphide ores and carbonate rich German copper shale has been compared (Khalid & Ralph 1977; Groudev 1983; Bosecker *et al.* 1978). As a result of *T. thiooxidans* producing sulphuric acid, Cu, Cr, Zn and V from non-sulphide industrial waste products can be extracted (Bosecker 1983).

Although biohydrometallurgical processes for the extraction of copper are being...
used in many parts of the world, such processes have not been used in Pakistan. Feasibility studies were carried out for leaching of two different copper ores namely chalcopyrite (CuFeS₂) and oxidized copper ore with previously isolated strains of *T. thiooxidans* NB 101 and *T. ferrooxidans* NB 102, respectively. Metal sulphide (such as chalcopyrite) on oxidation in acidic solution results in the production of passive film of sulphur:

\[ \text{MS} + \text{H}_2\text{SO}_4 + 0.5 \text{O}_2 \rightarrow \text{MSO}_4 + \text{H}_2\text{O} + \text{S}^\circ. \]

For this purpose the sulphur oxidizer, *T. thiooxidans* NB 101 was presently studied for removal of this film and generation of acid for further solubilization. On the other hand, oxidized copper ore (devoid of sulphur but with 0.3% Fe) was studied with *T. ferrooxidans* NB 102. In addition, the effect of supplementation of sulphur and chalcopyrite (as energy sources for the bacteria) to this ore on copper release was also studied.

**Materials and methods**

*Ore Samples.* Two different samples of copper ore, namely chalcopyrite from Saindak area of Baluchistan and Oxidized Copper Ore (OCO) from Durash area of Chitral, North Western Frontier Province of Pakistan, were supplied by Prof. Dr. A. Rauf of Gomal University, D.I. Khan. For leaching studies, these samples were ground to pass through a 200 mesh. The oxidized copper ore was amended with chalcopyrite ore and supplemented with sulphur as additional source of energy for bacteria. Various ratios of oxidized copper ore, chalcopyrite ore and sulphur used in these tests are shown in Table 1.

*Bacterial Inoculum.* *T. thiooxidans* NB 101 and *T. ferrooxidans* NB 102 were isolated by enrichment from sewage waters (Khalid & Malik, 1987). These cultures were maintained on the 9K medium of Silverman and Lundgren (1959). In case of *T. thiooxidans*, sulphur 1% w/v (9KS) was supplied instead of ferrous iron as for *T. ferrooxidans* (9KFe).

*Leaching Procedures.* The experiments were carried out in 250 ml Erlenmeyer flasks containing 78 ml of iron-free 9K medium of pH 2.5 and 4 g of finely ground substrate. After autoclaving, 2 ml of inoculum (*T. thiooxidans* for chalcopyrite and *T. ferrooxidans* for OCO) was added. In the control flasks 2 ml sterilized water was added instead of inoculum. The flasks were incubated at 28 ± 2°C on rotary shaker at 100 rev/min.

*Analytical Procedures.* Chalcopyrite ore as well as oxidized copper ore (OCO) were digested in conc. HCl/conc. HNO₃ (1:1, v/v), diluted with deionized water and analysed for Cu and Fe by Varian AA 1475 series atomic absorption spectrophotometer. Samples were taken from leach flasks (control and inoculated) under aseptic conditions and centrifuged at 5000 × g to separate suspended particles. The total volume in the flasks was kept constant by adding sterile distilled water before each sampling. The aqueous layer thus obtained was diluted with deionized water and analysed for soluble copper contents by atomic absorption spectrometry. The pH of the leach solution was also determined.