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# Influence of Nutrients on the Biological Oxidative Activities of a Locally Isolated *Thiobacillus ferrooxidan*

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The strain of *Thiobacillus ferrooxidan* was isolated from the Sorange coal mine water in ferrous sulphate medium. The effects of inorganic nitrogen ($NH_4$+, $NO_3$-) and phosphate on the biological oxidation of copper sulphide ore were examined. It is observed that the iron was solubilized first from $FeS_2$ or $Fe_{1-x}$ S to as $Fe^{++}$ and then oxidized to $Fe^{+++}$. The dissolution of iron $Fe^{++}$ was a chemical reaction whereas the conversion of $Fe^{++}$ to $Fe^{+++}$ was bacteria mediated. The addition and increase in the concentration of phosphate delayed and reduced the conversion of $Fe^{++}$ to $Fe^{+++}$. Ammonium was found to be the preferred source of nitrogen for the isolated bacterium. The nitrogen as $NH_4^+$ increased the $Fe^{++}$ oxidation whereas $NO_3^-$ had negative effect. The solubilization of copper was directly related to the $Fe^{++}$ iron oxidation. Maximum dissolved copper concentration were observed in 6mM ammonium supplemented medium and minimum in 6mM nitrate.

***Key words:*** *Thiobacillus ferrooxidan*, Ammonium nitrate, Ammonium phosphate, Oxidation

## Introduction

Most of the bioleaching processes involve the mesophilic chemoautotrophic, lithotrophic bacteria namely *Thiobacillus ferrooxidan*, *Thiobacillus thiooxidan*, *Leptospirillum ferrooxidan* etc, for the solubilization of copper (Rahman and Gul 1999), gold (Chapman *et al* 1993), zinc (Kraft and Hallberg 1993), uranium (Munoz *et al* 1993) and manganese (Toro *et al* 1993). These bacteria require only inorganic compounds with ferrous iron and sulphur compounds (Rossi 1990, Hallbeck and Pederson 1991). The oxidation of ferrous iron provides energy for growth with $O_2$ as terminal electron acceptor (Stevens *et al* 1986). The requirements of major and micro nutrients and trace metals are fulfilled from the mineral constituents of the ores during bioleaching. Mostly the ores lack nitrogen compounds which are requi-red for most of the crucial metabolic activities. Among the nitrogen sources, ammonium is the preferred source of nitrogen for chemoautotrophic, acidophilic bacteria. The ability of these bacteria to assimilate and fix nitrate nitrogen is variable (Stevens *et al* 1986; Stevens and Tuovinen 1986). Hence, the supplementation of nitrogen sources to the leach solutions may enhance the bioleaching activity in the leaching systems. In the present studies, the effect of ammonium and nitrate as nitrogen source and phosphate as phosphorus source on the oxidation of $Fe^{++}$iron and solubilization of copper from Saindak ore was examined.

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## Materials and Methods

The Saindak ore contains chalcopyrite (CuFeS), copper oxide (CuO), pyrite ($Fe_2S$) and silica ($SiO_2$). The ore used in these studies contained 0.5% copper including 0.038% copper as copper oxide. The ore was ground to-200# particle size fraction.

A culture of *Thiobacillus ferrooxidan* was isolated from Sorange coal mine water of 9K medium. The culture was maintained in mineral salt solution (g $l^{-1}$), ammonium sulphate 3.0, potassium chloride 0.1, dipotassium hydrogen phosphate 0.5, magnesium sulphate 0.5, calcium nitrate 0.01, supplemented with 10% w/v Saindak ore. The pH of the medium was adjusted at 2.0 with 10N $H_2SO_4$.

The inoculum was prepared by growing the culture in mineral salt medium supplemented with 10% w/v -200# size Saindak ore for 15 days at 35±1°C. The culture was harvested by centrifugation and re-suspended in 0.005 M $H_2SO_4$. The density of the inoculum was ~ $5X10^5$ bacteria per ml. 10% v/v inoculum was used throughout the study. The control flask (sterilized by autoclaving) received 10% v/v 0.005 M $H_2SO_4$

The basal salt medium used for the investigation of the effects of nitrogen and phosphorus was 0.5 g $l^{-1}$ magne-sium sulphate, pH 2.0 supplemented with-200# size Saindak ore 10% w/v.

The effects of nitrogen (ammonium and nitrate) and phosphorus were investigated by adding 0, 3, 6 mM ammonium nitrogen as $(NH_4)_2SO_4$, 0, 3, 6 mM nitrate nitrogen as $NaNO_3$ and 0, 2,4 mM phosphate phosphorus as $KH_2PO_4.3H_2O$ in the basal medium. All the experiments were carried out at 35±1°C.

The conentration of total iron and $Fe^{++}$ iron in the leach solution was determined by O-phenanthroline method (Herra *et al* 1989 Muir and Andersen 1977).

The concentration of $Fe^{+++}$ iron in the solution was derived from calculations. $Fe_{total} - Fe^{++} = Fe^{+++}$

The copper in the leached solution was determined by AAS 2380, Perkin Elmer, USA.

## Results and Discussion

The results of the control medium (without nitrogen and phosphorus) after 6 hours (Fig 1) show that the ore contains iron in other froms such as pyrrhotite ($Fe_{1-x}S$) with pyrite ($FeS_2$), the major part of which gives iron in ferrous form in the solution. This ferrous iron primarily provides the iron nutrient source for the growth of *Thiobacillus ferrooxidan*. Initially upto 4 days of incubation the bacteria established itself in the medium and then started the function of oxidation. However, very little oxidation of iron was observed at the 2nd and 4th day. The dissolution of copper also started with the oxidation of $Fe^{++}$ iron.

The iron and copper dissolution depends upon the nutrient present in the medium (Niemela *et al* 1994). The dissolution of iron was comparable in all the experiments upto 4 days but the oxidation of iron was greatly affected by the nutrients present

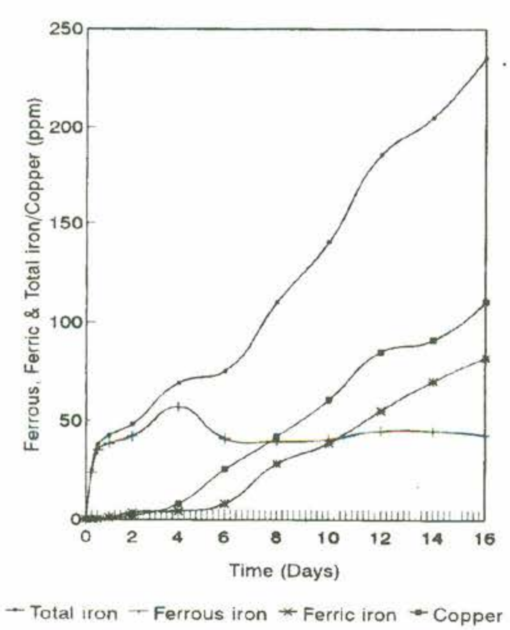


**Fig. 1** Dissolution of Saindak ore in control medium (magnesium sulphate with Saindak ore)

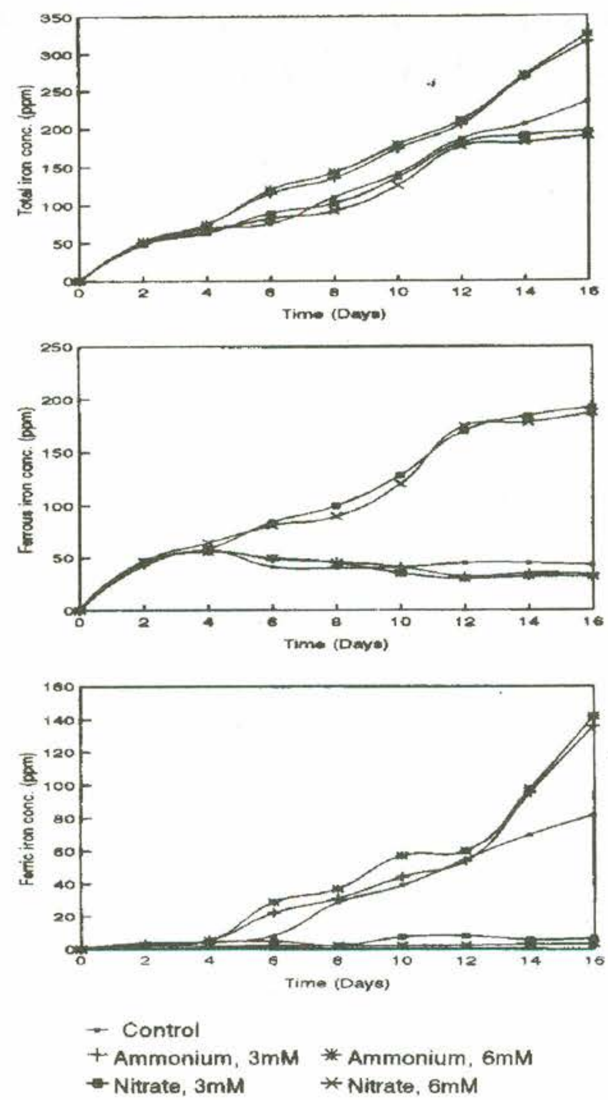


**Fig. 2** Changes in Total, Ferrous and Ferric iron during leaching of Saindak ore in presence of phosphorus.

in the medium (Fig 2). In the presence of $NO_3^-$ as nitrogen source, the oxidation of $Fe^{++}$ iron was very slow and increase in the concentration further reduced the rate of oxidation. Ammonium as nitrogen source enhanced the rate of oxidation of $Fe^{++}$ to $Fe^{+++}$ and the increase in the concentration of ammonium nitrogen further increased the oxidation of $Fe^{++}$. The oxidation of $Fe^{++}$ was higher in the medium not supplemented with any external source of nitrogen than those amended by either 3 and 6 mM nitrate nitrogen. From the Fig 2 it is also clear that the isolated bacterium preferred ammonium nitrogen source for fulfilling its nitrogen requirement. The bacterium had very little activity towards the fixation of nitrate nitrogen. It is also evident (Fig 2) that in the presence of ammonium as nitrogen source, the total iron concentration also increased in the medium. This increase in total iron concentration might be

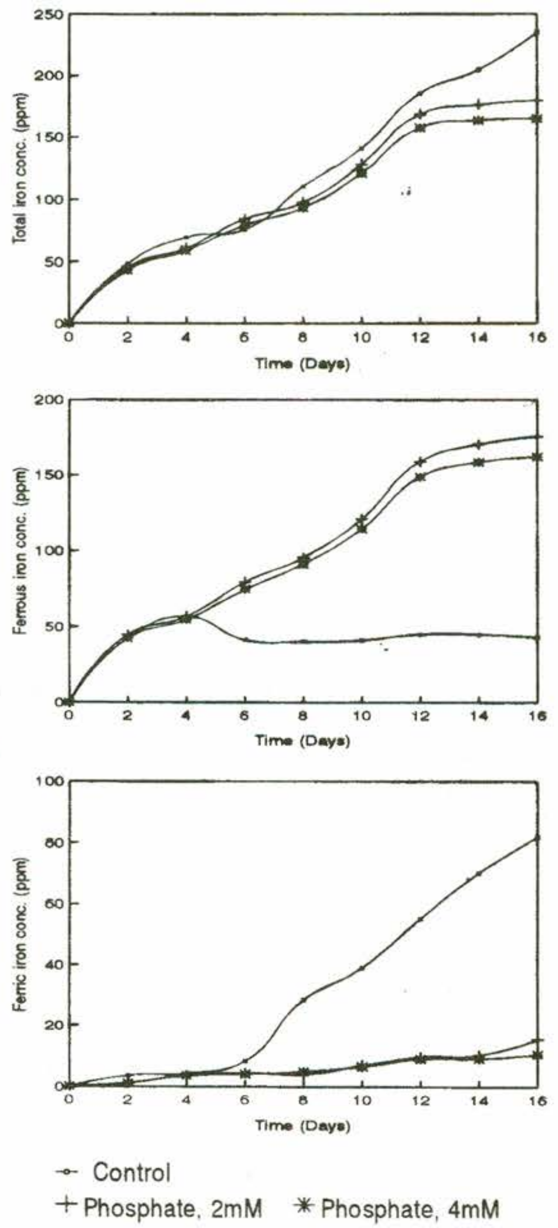


**Fig. 3** Changes in Total, Ferrous and Ferric iron during leaching of Saindak ore in presence of phosphate phosphorus.

due to the enhancement in the metabolic activity of the bacterium and production of sulphuric acid. The slow rate of oxidation could be due to the toxicity of nitrate for bacterium *Thiobacillus*. According to Alexander *et al* (1987) and Ingledew (1990), *Thiobacillus ferrooxidan* and other chemoautotrophic acidophilic bacteria are sensitive to nitrate due to the non specifice permeability of their membranes and intercellular accumulation of nitrates, driven by transmembrane potential. Ingledew (1990) also reported that the nitrate inhibition is associated with the acidification of cytoplasm due to the change in transmembrane potential which is balanced by proton entry.

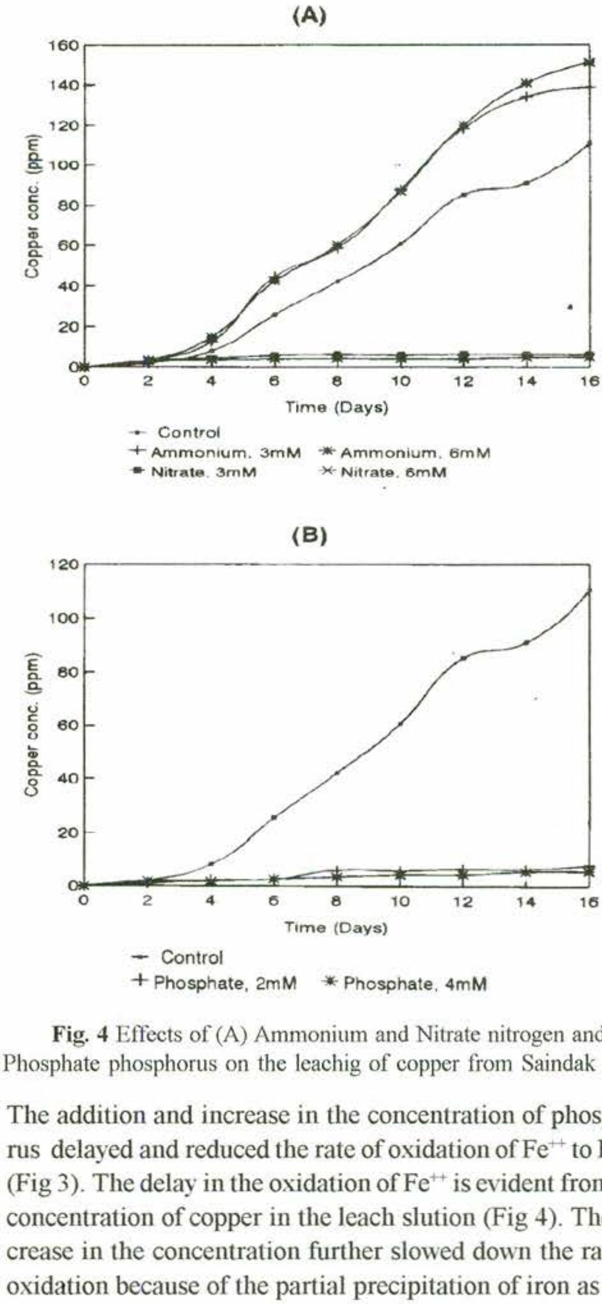


**Fig. 4** Effects of (A) Ammonium and Nitrate nitrogen and (B) Phosphate phosphorus on the leachig of copper from Saindak ore.

The addition and increase in the concentration of phosphorus delayed and reduced the rate of oxidation of $Fe^{++}$ to $Fe^{+++}$ (Fig 3). The delay in the oxidation of $Fe^{++}$ is evident from the concentration of copper in the leach slution (Fig 4). The increase in the concentration further slowed down the rate of oxidation because of the partial precipitation of iron as iron phosphate. This precipitation might not only withdraw iron but also other essential compunds from the medium and thus suppress the bacterial activity and also chemical leaching. Duncan *et al* (1967) reported that at least 16 ppm phosphate is required for chalcopyrite leaching with *Thiobacillus ferrooxidan*. The findings of Silverman and Lundgren (1959) confirmed our results that phosphorus inhibits the oxidation when the phosphate concentration was increased from nil to 14 ppm. The inhibitory effects of phosphate on the pyrite oxidation were also reported by Napier *et al* (1968).

From the comparison of data presented in Figs 2,3 and 4 it is clear that the oxidation of iron by bacteria depends upon the nutrients present in the medium whereas the solubilization of copper from the ore relies upon the oxidized iron ($Fe^{+++}$). The leached copper concentrations were maximum in the medium supplemented with 6mM ammonium and was minimum with 6mM nitrate. The medium supplemented with phosphorus was little better for copper solubilization than nitrate nitrogen (Fig 4). According to Kelly and Tuovinen (1988) and Tuovinen *et al* (1991), the bioleaching processes of metal recovery especially from sulphide ores basically involve the oxidation of ferrous iron and inorganic sulphur compounds as the electron donors. Such processes are utilized for the commercial bioleaching of copper from the ores (Rossi 1990).

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