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Pyrite oxidation by using *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* in pure and mixed cultures

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Abstract

Pyrite oxidation reaction was carried out using acidophilic microorganisms, Thiobacillus ferrooxidans and Thiobacillus thiooxidans in pure and mixed cultures of these bacteria. Bioleaching experiments in this study were carried out at 30°C and different parameters such as pH, Fe (II) and Fe (III) concentration were studied. The results showed that mixed cultures of T. ferrooxidans and T. thiooxidans enhanced the dissolution of Fe from pyrite, whereas T. thiooxidans alone did not oxidize pyrite. Amount of sulphuric acid produced in mixed cultures of bacteria was higher than pure cultures of each bacterium. Enhancement of Fe (III) ions was also observed with mixed cultures of T. thiooxidans and T. ferrooxidans, while this did not occur in pure cultures of T. ferrooxidans. The presence of iron (III) showed a negative effect on the bacterial iron oxidation rate. In the case of pure cultures of T. ferrooxidans, pyrite was nearly completely oxidized to sulphate because of the capacity of this culture to oxidize both iron (II) ions and sulphuric compounds. Ferric ions competitively inhibited ferrous ion oxidation by the bacteria. Also it was observed that volatilization of Fe (II) from pyrite is highly pH-dependent.

Key words*:* Pyrite oxidation, *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*

1. Introduction

The volatilization of metals due to the action of microbes and the subsequent recovery of the metals from solution has deep historical roots that have been extensively reviewed (Olson et al., 2003, Rawlings, 2002). Similarly, an indication of the number and sizes of the operations that employ microbes for the recovery of mainly copper

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(Fernando, 2002), gold (Petre, 2001), cobalt (Hugues et al., 1997) and uranium (Mouns et al., 1995) has also been evaluated. In general, bioleaching is a process described as being the dissolution of metals from their mineral source by certain naturally occurring microorganisms or the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered through it (Olson et al., 2003, Chen, and Lin, 2001). Usually, bioleaching is referring to the conversion of solid metal values into their water soluble forms using microorganisms. New resources for metals must be developed with the aid of novel technologies. In addition, improvement of already existing mining techniques can result in metal recovery from sources that have not been of economical interest until today. Metal-winning processes based on the activity of microorganisms offer a possibility to obtain metals from mineral resources not accessible by conventional mining (Bosecker, 1997). In general, the types of microorganisms found in bioleaching and biodesulphurization processes, are the iron- and sulfur-oxidizing *Acidithiobacillus ferrooxidans* (previously *Thiobacillus ferrooxidans*), the sulfur-oxidizing *Acidithiobacillus thiooxidans* (previously *Thiobacillus thiooxidans*) and *Acidithiobacillus caldus* (previously *Thiobacillus Caldus*), and the iron-oxidizing leptospirilli, *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum* (Coram, and Rawlings, 2002, Foucher et al., 2003, Goebel, and Stackebrandt, 1994, Hallberg, and Lindström, 1994, Vásquez, and Espejo, 1997). In Iran bioleaching experiments carried out by using *T. ferrooxidans*, *T. thiooxidans* and *Leptospirillum ferrooxidans* in copper extraction (Ziloue et al., 2003) and biodesulphurization has not been evaluated. It also evaluates the desulphurization by chemical methods (Ehsani, and Eghbali, 2007). In view of the fact that abundances low grade sulphuric mine in Iran and not economically extraction by chemical methods, bioleaching processes is the most important technique for element extraction from low grade sulphuric ores and decreasing of air pollutant due of chemical methods. Thus in this paper we evaluated the role of *T. ferrooxidans* and *T. thiooxidans* on pyrite biooxidation in pure and mixed cultures of them.

2. Materials and methods

Pyrite was obtained from Ghanat Marvan Mine Kerman, Iran. The mineral composition of ore by XRD method consisted of: 7.46% pyrite, 1.82% sphalerite, 0.1% chalcosite, 0.07% chalcopyrite and 0.02% covelite. According to elemental analysis by XRF method, the ore contained 0.25% Cu and 4.38% Fe. The ore was finely ground to particles of lower than 75 mm in size for shake flask studies.

Microorganisms: a pure culture of *T. ferrooxidans* and *T. thiooxidans* were obtained of Sarcheshmeh copper mine. *T. ferrooxidans* was grown in 9K medium (Silverman, and Lundgren, 1959) and *T. thiooxidans* was grown in 317 medium (Chen, and Lin, 2001), was composed of (in g/l) $(NH_4)_2SO_4$ 0.3, K_2HPO_4 3.5, $MgSO_4.7H_2O$ 0.5, $CaCl_2$ 0.25 and tyndallized sulphuric powder 5.0. Mixed culture of *T. ferrooxidans* and *T. thiooxidans* carried out in 317 medium.

All bioleaching experiments were carried out in 250 ml flasks containing 100 ml specific medium and 5g ore. In all experiments where inoculation was required, a 5% (v/v) inoculum's of an active culture was used and flasks were incubated at 30°C on rotary shaker at 180 rpm. Control samples had no bacterial treatment. Deionized water was added daily to compensate evaporation and during cultivation, the pH was always kept below 2.5.

Ferrous iron content in solutions was measured by atomic absorption (Varian 220 AA). Ferric ion concentration determined with potassium thiocyanate spectrophotometrically method (Oser, 1965). pH of media and leachate solutions was measured by pH–meter (Metrohm 691) and number of bacteria, determined by MPN method (Oblinger, 1975) and produced sulphuric acid content was calculated by titration with a 0.01 N NaOH solution at the initial and final stages of each experiment. All of the data were analyzed statically using Microsoft Excel 2003 for calculating mean and standard error.

3. Results

The results of pyrite bioleaching experiments showed in figures 2, 3 and 4. The results showed that mixed cultures of *T. ferrooxidans* and *T. thiooxidans* enhanced the dissolution of Fe^{2+} from pyrite (Figure 3), whereas *T. thiooxidans* alone did not oxidize pyrite (Figure 4). These figures shown that *T. ferrooxidans* is more than efficient *T. thiooxidans* in pyrite oxidation (Figure 2) and it can be released high content of fe^{2+} from pyrite and *T. thiooxidans* cannot release Fe^{2+} from pyrite. Amount of Fe^{2+} in mixed cultures of bacteria is more than pure culture of *T. thiooxidans* and less than *T. ferrooxidans.* It is clearly that in mixed cultures the presence of *T. ferrooxidans* causes pyrite oxidation. *T. thiooxidans* occupies the active surface of ores thus it decreases the active surface that occupy by *T. ferrooxidans*. Thus efficiency of mixed cultures is less than pure cultures of *T. ferrooxidans*. Content of sulphuric acid produced in mixed cultures of bacteria was higher than pure cultures of each bacterium (Table 1).

Table 1: Sulphuric acid produced in pure and mixed cultures of bacteria (mmol)

T. ferrooxidans + *T. thiooxidans*	0.92
T. thiooxidans	0.36
T.ferrooxidans	0.19

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 4Fe^{3+} + 8SO_4^{2-} + 4H^+$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

Production of sulphuric acid carried out by both bacteria, thus amount of this acid is higher than in pure cultures of each bacterium. *T. ferrooxidans* oxide the pyrite to ferrous ion and elementary sulphure and *T. thiooxidans* able to oxide the elementary sulphure produced by *T. ferrooxidans*. Mixed cultures of bacteria and *T. ferrooxidans* oxide ferrous ion to the ferric ion (Figure 1). Thus ferric precipitation on the surface of pyrite can decrease oxidation of pyrite and the amount of ferrous ion decreases. Also thin layers of sulphure decrease the active surface of pyrite oxidation. Production of sulphuric acid causes decreasing pH and activity of bacteria on lower pH decreasing (Figure 5). It was observed that volatilization of Fe (II) from pyrite is highly pH-dependent.

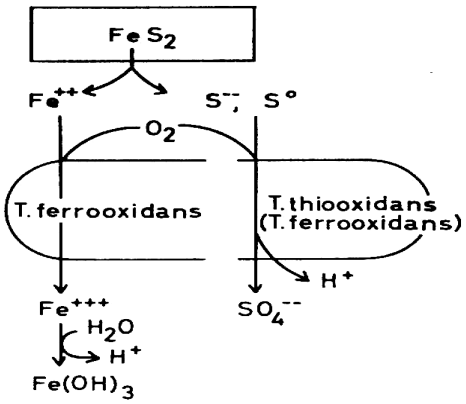

Figure 1: Schematic diagram of activity of *T.ferrooxidans* and *T.thiooxidans*

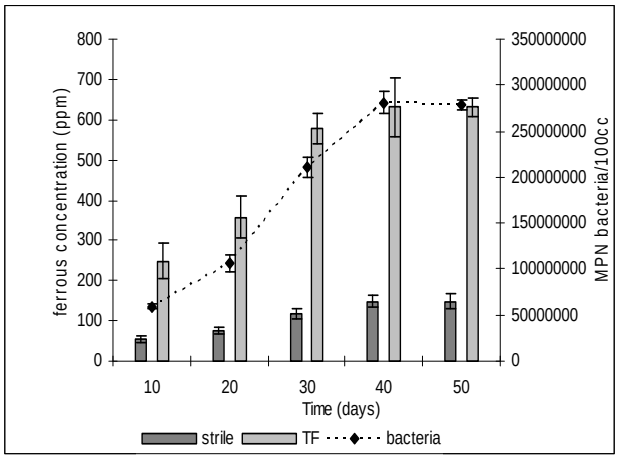

Fig. 2. Ferrous ion concentration and number bacteria in pure culture of *T.ferrooxidans*.

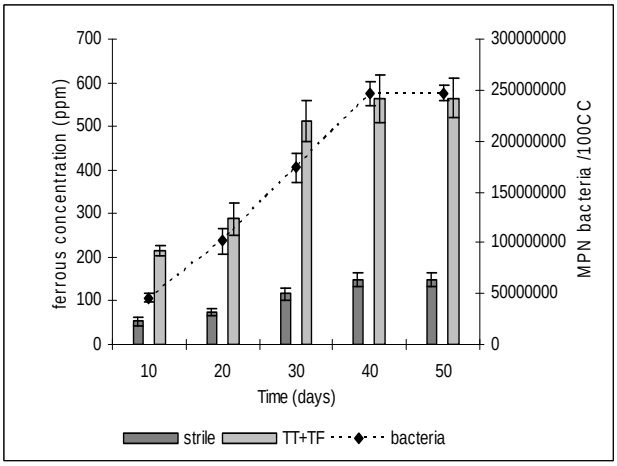

Fig. 3: Ferrous ion concentration and number of bacteria in mixed cultures of *T. ferrooxidans* and *T. thiooxidans*.

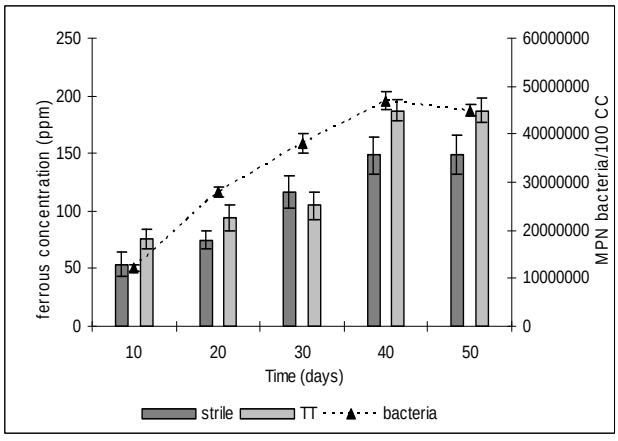

Fig. 4. Ferrous ion concentration and number of bacteria in pure culture of *T. thiooxidans.*

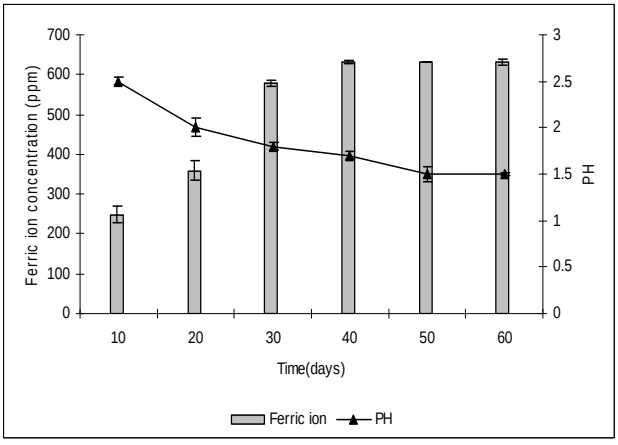

Fig. 5. pH changes and ferric ion concentration in pure culture of *T. ferrooxidans*

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 4Fe^{3+} + 8SO_4^{2-} + 4H^+$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

$$2Fe^{2+} + O_2 + 4H^+ \rightarrow 2Fe^{3+} + 2H_2O$$

$$Fe^{3+} + H_2O \rightarrow FeOH^{2+} + H^+$$

$$Fe^{3+} + 2H_2O \rightarrow Fe(OH)_2 + 2H^+$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+$$

Enhancement of Fe (III) ions content were also observed with mixed cultures of *T. thiooxidans* and *T. ferrooxidans*, while this did not occur in pure cultures of *T. ferrooxidans*. The presence of iron (III) showed a negative effect on the bacterial iron oxidation rate. In the case of pure cultures of *T. ferrooxidans*, pyrite was oxidized to sulphate because of the capacity of this culture to oxidize both iron (II) ions and sulphure compounds. Ferric ions competitively inhibited ferrous ion oxidation by the bacteria (Figure 6).

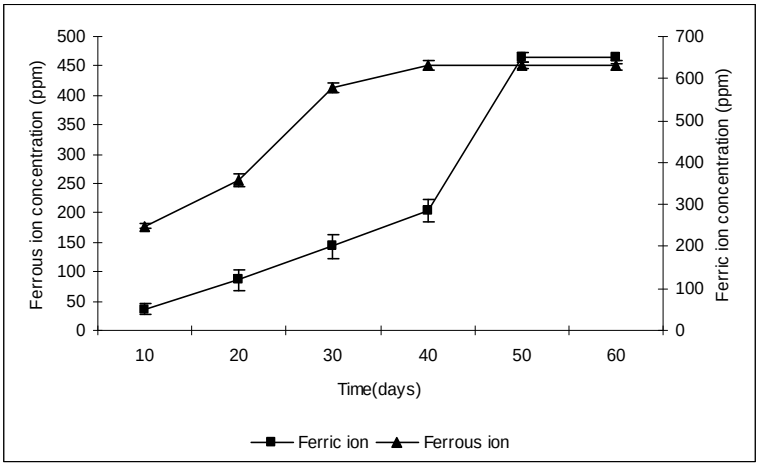

Fig. 6. Ferric and ferrous ion concentration in pure culture of *T. ferrooxidans*

4. Discussion

In spite of the large variety of potential organisms that can be used, the microbes that play the most important roles tend to have certain properties in common The results showed that *T. ferrooxidans* is the most important microorganism for pyrite oxidizing. This organism belongs to the group of chemolithotrophic bacteria. The organism is rod-shaped, non-spore forming, gram-negative, motile, and single-pole flagellated. Carbon dioxide and ammonium is used as carbon and nitrogen sources (Johnson, 1998, Rawlings, 2005). Although *T. ferrooxidans* has been characterized as being a strictly aerobic organism, it can also grow on elemental sulphure or metal sulfides under anoxic conditions

using ferric iron as electron acceptor (Pronk et al., 1992, Ohmura et al. 2002). This bacterium has been more extensively studied than any other biomining organism and was also the first to have its genome sequenced (Barreto et al., 2003). This organism obtains their energy by the oxidation of either iron or reduced inorganic sulfur compounds and volatilization of metals from minerals or their concentrates is believed to be largely a chemical process that is due to the action of ferric iron and protons depending on the mineral being treated. Although some microorganisms are capable of using both energy sources, a combination of iron-oxidizing and sulfur-oxidizing microbes such as *T. thiooxidans* often works best in sulphuric acid production. The production of sulfuric acid in solution means that the organisms are acid tolerant.

Sulphure and iron oxidizing bacteria oxidize the sulphuric ores with two types of mechanisms which are involved in the microbial mobilization of metals has been proposed (Tributsch, 2001, Schippers, and Sand, 1999). Microorganisms can oxidize metal sulphides by direct mechanism obtaining electrons directly from the reduced minerals. Cells have to be attached to the mineral surface and a close contact is needed. The adsorption of cells to suspended mineral particles takes place within minutes or hours. The following equations describe direct mechanism for the oxidation of pyrite.

Direct:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4$$

The oxidation of reduced metals through indirect mechanism is mediated by ferric iron (Fe^{3+}) originating from the microbial oxidation of ferrous iron (Fe^{2+}) compounds present in the minerals. Ferric iron is an oxidizing agent and can oxidize metal sulphides and is (chemically) reduced to ferrous iron which, in turn, can be microbial oxidized again. In this case, iron has a role as electron carrier. It was proposed that no direct physical contact is needed for the oxidation of iron. The following equations describe indirect mechanism for the oxidation of pyrite.

Indirect:

$$4FeSO_4 + O_2 + 2H_2SO_4 \xrightarrow{T.ferrooxidans} 2Fe_2(SO_4)_3 + 2H_2O$$

$$FeS_2 + Fe_2(SO_4)_3 \xrightarrow{chemicaloxidation} 3FeSO_4 + 2S$$

$$2S + 3O_2 + H_2O \xrightarrow{T.thiooxidans} 2H_2SO_4$$

However, the model of direct and indirect metal leaching is still under discussion. Recently, this model has been revised and replaced by another one which is not dependent on the differentiation between direct and indirect leaching mechanisms (Rawlings, 2005). Cells have to be attached to the minerals and in physical contact with the surface, cells form and excrete exopolymers, these exopolymeric cell envelopes contain ferric iron compounds which are complexed to glucuronic acid residues. These are part of the primary attack mechanism, thiosulfate is formed as intermediate during the oxidation of sulfur compounds, sulfur or polythionate granules are formed in the periplasmatic space or in the cell envelope. Thiosulfate and traces of sulphite have been found as intermediates during the oxidation of sulfur. Sulfur granules have been identified as energy reserves in the exopolymeric capsule *T. thiooxidans* chemical

oxidation *T. ferrooxidans*, *L. ferrooxidans thiobacilli* around cells of *T. ferrooxidans* during growth on synthetic pyrite films.

The following equations summarize the oxidation mechanisms thiosulfate mechanism (Figure 7). (Found for FeS_2, MoS_2, and WS_2):

$$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+$$

Polysulfide mechanism (Figure 8) (found for PbS, $CuFeS_2$, ZnS, MnS_2, As_2S_3, and As_3S_4):

$$2MS + 2Fe^{3+} + 2H^+ \rightarrow 2M^{2+} + H_2S_n + 2Fe^{2+}$$

$$H_2S_n + 2Fe^{3+} \rightarrow 0.25S_8 + 2Fe^{2+} + 2H^+$$

$$0.25S_8 + 3O_2 + 2H_2O \rightarrow 2SO_4^{2-} + 4H^+$$

In the case of the polysulfide mechanism, volatilization of the acid-soluble metal sulfide is through a combined attack by ferric iron and protons, with elemental sulfur as the main intermediate. This elemental sulfur is relatively stable but may be oxidized to sulfate by sulfur-oxidizing microbes such as *Acidithiobacillus thiooxidans* or *Acidithiobacillus caldus*. The ferrous iron produced may be reoxidized to ferric iron by iron-oxidizing microorganisms such as *T. ferrooxidans* or bacteria of the genera *Leptospirillum* or *Sulfobacillus*. The role of the microorganisms in the volatilization of metal sulfides is, therefore, to provide sulfuric acid for a proton attack and to keep the iron in the oxidized ferric state for an oxidative attack on the mineral. In thiosulfate mechanism electrons are transferred from the membrane-located cytochrome c 2 (Yarzábal et al., 2002) to rusticyanin and then along one of two paths. The downhill path is via cytochrome c4 (Cyt1) to cytochrome aa3 (Appia-Ayme et al., 1999) or the uphill, reverse electron transport path via cytochrome c4 (CytA1) to a bc1 I complex and a NADH-Q oxidoreductase (Elbehti et al., 2000). *T. ferrooxidans* has up to twelve cytochrome c (Yarzábal et al., 2002) and a variety of cytochrome oxidases some of which appear to play different roles depending on whether iron or sulphure is being oxidized (Brassuer et al., 2004). The NADH is responsible for mercury reduction using a MerA mercuric reductase and the cytochrome aa3 is required to reduce mercury via the unique iron dependent mechanism discovered in *T. ferrooxidans* (Sugio et al., 2003) (Figure 7).

In polysulfide mechanism Thiol groups of outer membrane proteins are believed to transport the sulfur to the periplasm where it is oxidized by a periplasmic sulfur dioxygenase (SDO) to sulfite and a sulfite acceptor oxidoreductase (SOR) to sulfate (Rohwerder et al., 2003). Although other cytochrome oxidases are present, a *ba*3 cytochrome oxidase and a *bc*1 II complex together with a *bd*-type ubiquinol oxidase are believed to play the major roles during sulfur oxidation (Brassuer et al., 2004, Wakai et al., 2004) (Figure 8).

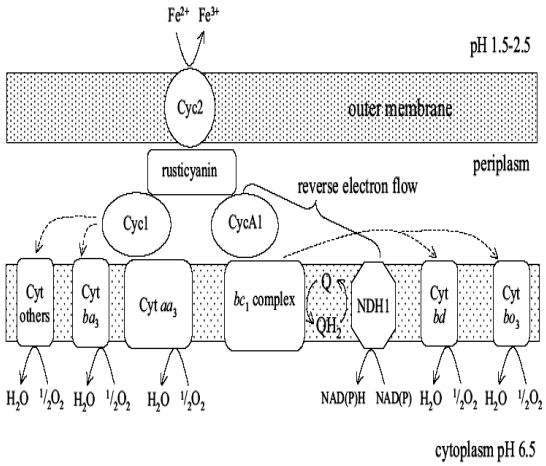

Fig. 7. Model of the iron electron transport pathway of *T. ferrooxidans* (Pronk, et al, 1992; Sugio, et al, 2003; Wakai, et al, 2004)

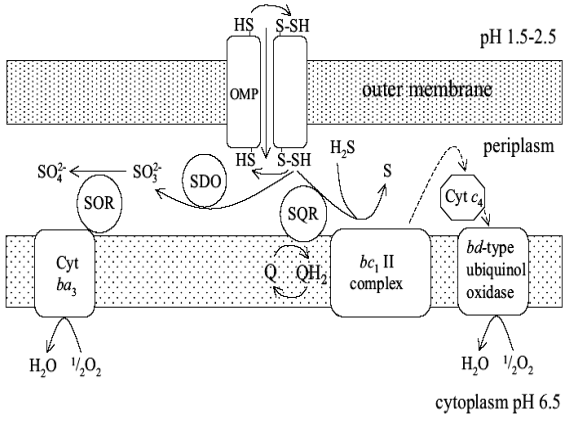

Fig.8. Model of sulphure oxidation electron transport pathway of *T. Ferrooxidans* (Pronk, et al, 1992; Sugio, et al, 2003; Wakai, et al, 2004)

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