Bioleaching of copper from low-grade ore using isolated bacteria and defined mixed cultures

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Abstract

Pure mesophilic bioleaching bacteria were isolated to compare their potential for oxidizing ferrous and sulfur in synthetic media and copper extraction from low grade ore with mixed bacterial community. A total of 160 samples were collected from various sites of different mines. Enrichment and isolation of ferrous-/sulfur- oxidizing bacteria were done in specific media. A total of 68 isolates were screened, 63 of which oxidized Fe^{2+}; the rest oxidized sulfur at different rates. Three important types of bacteria were identified to be Thiobacillus ferrooxidans, Thiobacillus thiooxidans and Leptospirillum ferrooxidans on the basis of morphological and physiological tests. The oxidation characteristics of both sulfur and ferrous were studied in pure isolates and mixed cultures. Oxidation behavior for all pure Fe^{2+}-oxidizing isolates was properly modeled with Monod equation and a specific Fe^{2+}-oxidation rate (μm) of 0.076 to 0.737 / h was reached. The rate of sulfur oxidation for pure sulfur-oxidizing isolates was 9 mg of sulfur / l/ h. Results of ferrous- or sulfur- oxidation for mixed cultures were in agrement with their bacterial community and pure isolates. The role of bacteria in releasing of copper was evaluated for pure isolates and mixed cultures. The results obtained showed that the simultaneous use of three types of isolates leads to more copper release and lower acid consumption compared to other communities. The positive effect of the initial concentration of Fe^{2+} showed that major portion of copper extraction is via indirect reactions.

Keywords: *Bioleaching; Isolation; Thiobacillus spp.; Leptospirillum spp.; Mixed cultures*

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INTRODUCTION

Bioleaching is a dissolution process -catalyzed by suitable microbial strains- of values from rocks or concentrates. It is a simple and effective technology for metal extraction from low-grade ores and mineral concentrates. Metal recovery from sulfidic minerals is based on the activity of chemolithotrophic bacteria, which convert insoluble metal sulfides into metal sulfates (Boon, 1996).

Recently, bioleaching has been widely used in industrial scale because of its economical and environmental advantages. Biooxidation is widely used in the bioleaching of copper from low-grade ores in addition to its application in pretreatment of refractory gold ores and concentrates. Bioleaching of cobalt, nickel and zinc from sulfide ores have been of considerable practical interests (Barrett *et al*., 1993; Mazuelos *et al*., 1999; Bosecker, 1997; Torma, 1977).

Various acidophilic iron- and/or sulfur- oxidizing bacteria interfered in oxidation of mineral sulfides during bioleaching processes. The most extensively studied bacteria are *Thiobacillus ferrooxidans, Thiobacillus thiooxidans, Leptospirillum ferrooxidans, Acidianus brierleyi* and *Sulfolobus acidocaldarius* (Bosecker, 1997; Rowlings, 1997; Sand *et al*., 1992; Battaglia *et al*., 1998). These microorganisms play an important ecological role in nature. They tend to adapt to the local ores in which they are found and may be better suited for more efficient extraction from that specific ore (Shahverdi *et al*., 1997; Asmah *et al*., 1999).

Several big dumps of low-grade copper ore exist

around Sarcheshmeh and Darezar mines and conventional copper extraction methods for this low grade ore are not economically feasible. In order to improve the copper recovery from this type of mineral, the adopted local microorganisms have been applied in copper extraction. In this study, attempts have been made for isolation and evaluation of three major mesophilic mineral oxidizing bacteria to estimate their growth and biooxidation kinetics and the effect of bacterial community on copper bioleaching.

MATERIALS AND METHODS

Isolation and purification: A total of 160 samples including acid mine drainage, pregnant liquor solution and mineral ore surfaces were collected from various sites in Sarcheshmeh and Darezar mining area, Kerman, Iran. Enrichment of 1gr of each sample was done, using series of subculturing under aerobic conditions at 30°C in 100 ml 9K medium for 2 months (Espejo and Ruiz., 1987; Lizama and Suzuki., 1988). We used 9K and 2:2 solid media for single colony isolation and morphological studies (Peng *et al*., 1994). Because of inhibitory effects of agar as an organic compound on growth of bioleaching bacteria, we modified these media using 4.5 and 4 g/l agar-agar ultrapure for 2:2 and 9K solid media, respectively. Sulfur oxidizing bacteria were isolated from enriched samples in Starkey's medium supplemented with 1% (w/v) sulfur powder (Lizama and Suzuki 1988; Konishi *et al*., 1995). Purification of cultures was finalized after various transfers of the isolates on 9K and 2:2 fresh media. Selected isolates were subjected to light and scanning microscopy for morphological characterization.

Preparation of mixed cultures: In order to evaluate the behavior of mixed culture of bacterial species on bioleaching reactions, three different mixed cultures namely; A, B, C were prepared. Culture A consisted of all enriched cultures in ferrous or sulfur oxidizing media. Culture B included 16 purified ferrous oxidizing isolates. Culture C was a mix of three species, *Thiobacillus ferrooxidans, Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans* in proportion of 2:1:1.

Ore: The typical low-grade copper ore was obtained from Sarcheshmeh mine, Kerman, Iran. The mineral composition of ore consisted of: 7.46% pyrite, 1.82% sphalerite, 0.1% chalcosite, 0.07% chalcopyrite and 0.02% covelite. According to elemental analysis, the ore contained 0.25% Cu and 4.38% Fe. The ore was finely ground to particles of < 75 mm in size for shake flask studies.

Ferrous iron and sulfur oxidation: Ferrous iron oxidation experiments were carried out in 250-ml flasks containing 100 ml 9K medium and 5 to 10 ml of the bacterial suspension as inoculum. Flasks were incubated at 30°C on a rotary shaker (NewBrunswick scientific, USA) at 180 rpm. Bacterial ferrous iron oxidation rate was determined calculating the amount of Fe^{2+} remaining in the solution (Mazuelos *et al*., 1999; Wiertz 1993). Sulfur oxidation experiments were initiated by inoculating a 5 ml of an active culture of bacteria to Starkey's medium containing 1% (w/v) sulfur powder at an initial pH of 4 (1N H_2SO_4 was used for pH adjustment). Flasks were incubated at 30°C (no shaking) and sulfate concentration was analyzed during the experiments.

Shake-flask leaching experiments: All experiments were carried out in 250 ml flasks containing 100 ml 9K or 0.9K medium and 5 gr ore. In all experiments where inoculation was required, a 5% (v/v) inoculum of an active culture was used and flasks were incubated at 30°C on rotary shaker (180 rpm). Control samples were made by the addition of 5 ml of 0.5% (v/v) formaline in ethanol. Deionized water was added daily to compensate evaporation and during cultivation the pH was always kept below 2.

Analytical procedures: Samples were periodically withdrawn from flasks for chemical analysis. Copper and total iron contents were measured using atomic absorption spectroscopy (Philips, UK) technique. Ferrous iron was analyzed at 509 nm using visible spectroscopy; o-phenantroline was used as the complexing agent (Wiertz, 1993; Underwood and Day., 1991). Sulfate concentration was indirectly determined by atomic absorption spectroscopy analysis of Ba after precipitation of $BaSO_4$ (Wiertz, 1993; Ahonen and Tuovinen., 1995). In order to determine the activity of bacteria in each flask, *Eh* of the suspensions was measured using a Pt-indicator and a standard calomel reference electrode (Metrohm, Switzerland). Bacterial concentration (free in solution) was determined using a Thoma counting-cell under the optical microscope

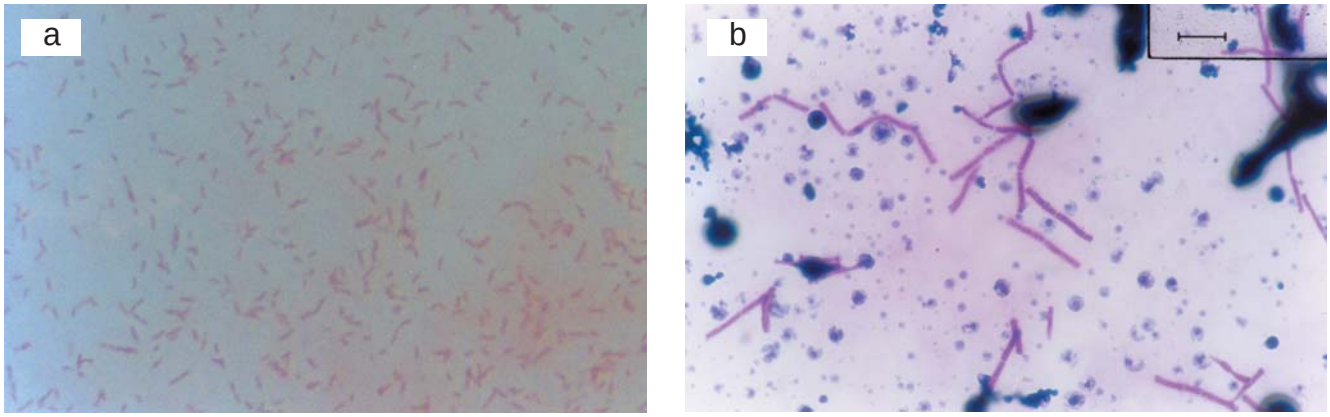

Figure 1. Light microscopic of iron-oxidizing isolates at × 1000 magnification. a) *Thiobacillus ferrooxidans*, b) streamer-like structure.

with a magnification power of × 400 (Batteglia *et al*., 1998).

RESULTS

Isolation of iron and sulfur oxidizing bacteria: In this study, 61 different ferrous-oxidizing bacteria were isolated from the collected samples of Sarcheshmeh and Darezar mines, Iran. Depending on colony appearance, they were classified into 7 different types. The most frequently observed colonies were semi-spheroidal and smooth-surfaced, with a white or yellow band outside and around centre, and a margin with many short projections. All isolates were Gram negative, rod-shaped, very small (1-2 µm in length), seen in singles or pairs (Fig. 1-a); except one type that had entwined filaments forming a streamer like structure (Fig. 1-b). All these isolates were subjected to ferrous oxidation test.

In order to isolate sulfur oxidizing bacteria 15 selected samples (from 160 collected samples) were enriched in Starkey's medium and single colonies isolated on modified 2:2 solid media. Thus, 5 sulfur-oxidizing bacteria were isolated. Their colony appearances were creamy white, entire and smooth. They were Gram-negative, and appear as very small (1-2 µm length), single and rod (Fig. 2).

Ferrous iron and sulfur oxidation: Ferrous oxidation was studied for all bacteria isolates. Results of Fe^{2+} oxidation by 5 purified isolates are shown in figure 3. Isolates of R14-1, F14-1 and F15-2 showed the strongest ability to oxidize ferrous ion, while the other

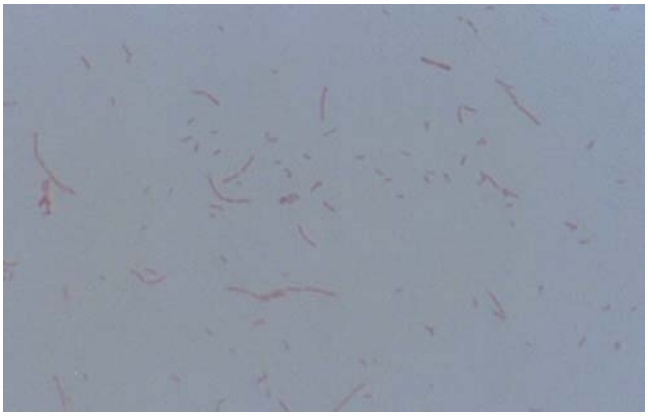

Figure 2. Light micrograph of sulfur-oxidizing isolates (*Thiobacillus thiooxidans*) at × 1000 magnification

species, A1-1 and F1-1 (with streamer-like structures) did not turn out to have such an effect. All small rod-shaped isolates showed similar patterns on the oxidation of Fe^{2+}, being different in rates and lag periods (Fig. 3). As shown in the figure 3, the Fe^{2+} oxidation ability started after about 20 hours and all of Fe^{2+} in solution was oxidized within the next 18 to 32 hours. In contrast streamer-like isolates were unable to oxidize Fe^{2+} for such experiment.

Kinetic parameters for Fe^{2+}-oxidation in purified isolates were obtained using Monod equation. Monod constants, K_s and µm were calculated with non-linear fitting using Statisca software and the results are presented in table 1. Specific oxidation rate varied between 0.076 to 0.737 / h for pure isolates. The ability to oxidize Fe^{2+} in the three mixed cultures was also evaluated. Figure 4 shows the variation in ferrous concentration based on the population of free bacteria in the solution.

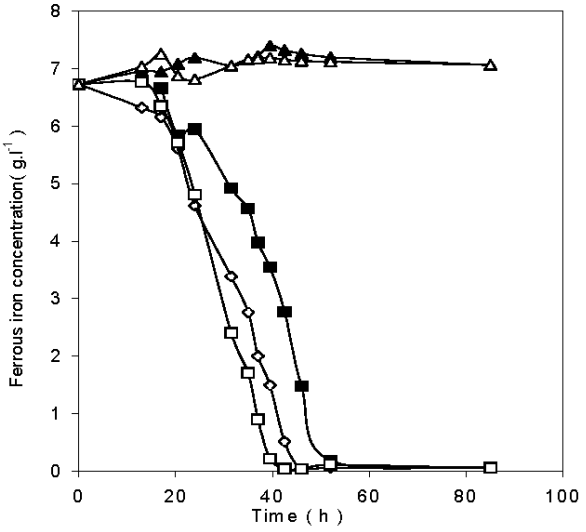

Figure 3. Ferrous oxidation by purified isolates (▲: F1-1, △: A1-1, □: R14-1, ■: F14-1, ◇: F15-2 .

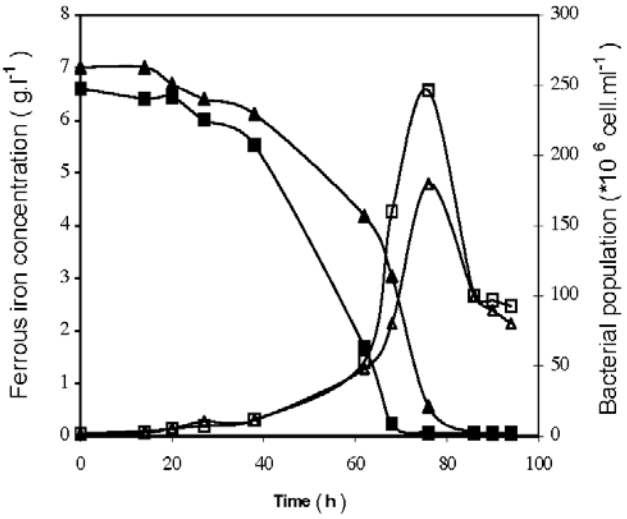

Figure 4. Growth of mixed cultures concomitant with ferrous oxidation in 9K medium (Ferrous concentration: ▲: Culture A, ■: Culture B ; Biomass concentration: Δ: Culture A , □: Culture B).

Table 1: Monod constants of Fe^{2+} oxidation for purified isolates and mixed cultures.

Culture		K_s (mM)	μ_m (hr^{-1})
	R14-1	27.1	0.076
	F14-1	45.2	0.103
	F15-2	255.3	0.737
	P3-1	85.9	0.206
	F12-1	75.7	0.152
	R1-1	26.4	0.112
	Cul.A	103.5	0.127
Mixed cultures	Cul.B	148.6	0.192
	Cul.C	198.5	0.229

The oxidation ability of elemental sulfur can prevent the formation of sulfur layer on the surface of ore and thus, provide favorite conditions for bioleaching of sulfidic ores. The results of sulfur oxidation for pure isolates are shown in figure 5. Figure 6 illustrates the same experiment for mixed cultures. The sulfur oxidation rates for Culture A and Culture C were 13 and 8.3 mg of sulfur / l/ hr, respectively.

Bioleaching of copper ore: Three pure isolates namely, *T. thiooxidans*, *T. ferrooxidans* and *Leptospirillum* were compared with a natural sample (non-inoculated). Then the required conditions such as medium, aeration, temperature, etc. for growth and activity of indigenous bacteria in ore were met.

The influence of initial ferrous iron on leaching of copper was evaluated in 9K and 0.9K media in the presence of different mixed cultures. Initial concentration of ferrous iron was 6.73 and 0.673 g/l for 9K and 0.9K media, respectively. As shown in figures 7 and 8, all mixed cultures in 9K medium extracted relatively more copper as compared to that in 0.9K medium and in individual media. On the other hand, Culture C extracted the most copper compared to other mixed cultures. After 14 days of incubation, Culture C extracted 96 and 91% of copper in ore with 9K and 0.9 K medium, respectively.

The *Eh* of solutions was also determined in these studies. *Eh* of leaching solutions is an important factor affecting bioleaching because of the semi-conductivity properties of minerals. According to the obtained results, the effects of *Eh* on different copper minerals need to be investigated.

DISCUSSION

Isolation of sulfur and iron oxidizing bacteria: Vibrio-shaped bacteria appeared only during continuous bioleaching of pyrite (Battaglia *et al.*, 1998). Based on this experience, two isolates of *Leptospirillum*-like bacteria were enriched from effluent solution of column leaching experiments. Their morphological and biochemical characteristics were found to be resembled to those of the genus *Leptospirillum* (Harrison and Norris, 1985).

In order to evaluate physiological and biochemical characteristics of sulfur-oxidizing isolates, the sulfur and ferrous oxidizing abilities were investigated.

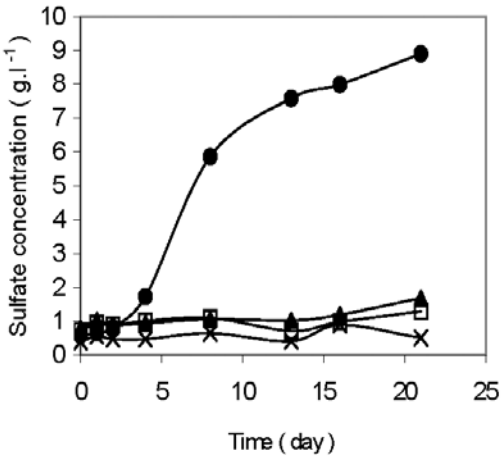

Figure 5. Elemental sulfur oxidation by purified isolates. (□: *T. ferrooxidans*, ●: *T. thiooxidans,* ▲: *L. ferrooxidans,* ×:Control).

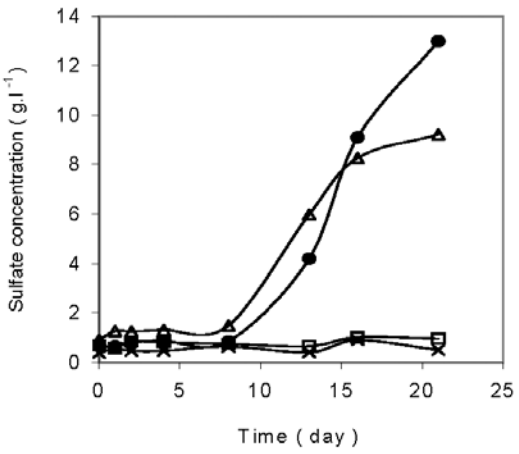

Figure 6. Elemental sulfur oxidation by mixed cultures. (●: Culture A , □: Culture B, Δ: Culture C, ×: Control).

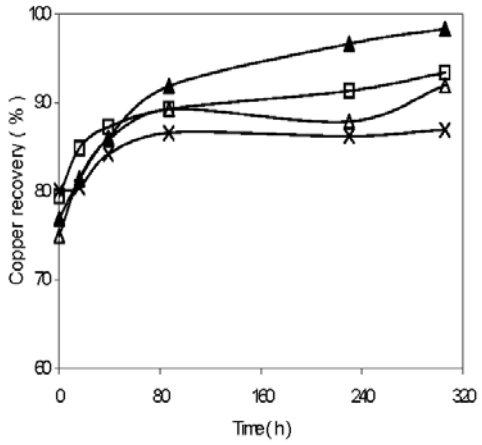

Figure 7. Bioleaching of copper from sulfide ore by mixed cultures in 9K media (Δ: Culture A , □: Culture B, ▲: Culture C, ×: Control).

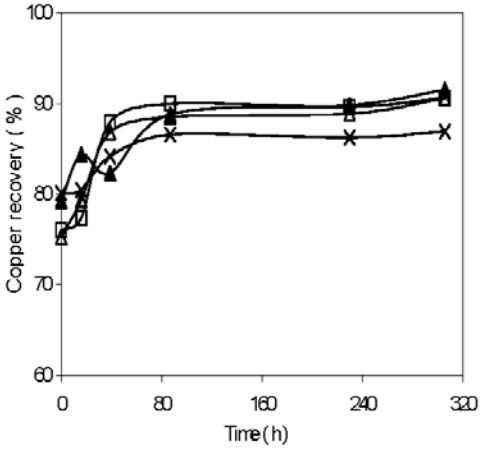

Figure 8. Bioleaching of copper from sulfide ore by mixed cultures in 0.9K media (Δ : Culture A , □: Culture B , ▲: Culture C , ×: Control).

Sulfur-oxidizing bacteria did not have any ferrous oxidation property in 9K media even after 10 days. However, *Thiobacillus ferrooxidans* and *Leptospirillum* isolates could oxidize all of initial ferrous within 3-7 days. The last two isolates had a very low sulfur oxidizing activity in Starkey's medium. Under the same conditions, sulfur-oxidizing bacteria had a very high activity.

Ferrous and sulfur oxidation: Mixed cultures, Culture A, Culture B and Culture C were observed to have lag periods of about 35, 35 and 28 h respectively. The corresponding times for complete oxidation of Fe^{2+} were found to be 85, 70 and 60 hours, respectively. A strong relation exists between the results and bacterial population changes in mixed cultures. The results obtained can be explained by types of the bacterial community formed in mixed cultures. Culture A practically, consisted of all sulfur and Fe^{2+}-oxidizing bacteria (small-rod shape, streamer-like, vibroid-shaped) found during the enrichment step. In Culture B only 16 small-rod Fe^{2+}-oxidizing isolates were noted, having different oxidation abilities. Culture C consisted of a defined mixture of *T. thiooxidans, T. ferrooxidans* and *L. ferrooxidans*. It should be noted that the oxidation ability of *T. ferrooxidans* presented in Culture C was the highest determined. Kinetic parameters for Fe^{2+} oxidation in mixed cultures are shown in Table 1. the longer lag phase and lower oxidation ability of Culture A can be explained by the presence of streamer-like and sulfur oxidizing bacteria, which not only decreased the population of Fe^{2+}-oxidizing bacteria, but also had inhibitory effects on their activity.

The rate of sulfur oxidation was calculated from the

linear part of the oxidation curve. Sulfur oxidizing bacteria exhibited high sulfur oxidation activities (9 mg of sulfur / l / h). The considerable decrease of the pH in the media, from the initial value of 4 to a final pH of 0.5-0.7, is also indicative of the high sulfur oxidizing ability of these bacteria. The increase of sulfate concentration for *Thiobacillus ferrooxidans* and *Leptospirillum* isolates were minimal (less than 300 mg of sulfate/l of solution after 21 days). The results obtained from sulfur oxidation in mixed cultures resembled those expected theoretically, indicating the oxidizing ability of pure isolates as well as their bacterial community.

Bioleaching of copper ore: Releasing of copper from sulfidic copper differs in rates and total extractions for each community. Pure *T. thiooxidans* had no effect on leaching of copper; while pure *T. ferrooxidans* had more effect on leaching of copper than *T. thiooxidans. L. ferrooxidans* stood second to the former. In this experiment, the natural sample had the most extraction of copper compared to pure isolates. Pure *T. thiooxidans* oxidizes elemental sulfur to sulfuric acid only; thus enhancing copper extraction through acid leaching. This will be possible in the presence of an oxidant or biooxidant that elemental sulfur will be produced from indirect mechanism of bioleaching. Pure *T. ferrooxidans* and *L. ferrooxidans* with their Fe^{2+}-oxidizing ability can extract copper via indirect and direct mechanisms. The high concentration of ferrous iron in this ore explains the reason for the indirect mechanism to be the dominant mechanism in copper extraction using these isolates.

According to results in figures 7 and 8, approximately 70 to 80 percent of copper was released in the first day, since the copper in the form of oxide and free were the dominant portion of copper in ore. These results show that the presence of ferric ion at the beginning of the experiment enhances copper extraction mostly via indirect mechanism for this mineralogy. Also, simultaneously the presence of three isolates (*Thiobacillus ferrooxidans*, *Leptospirillum* and *Thiobacillus thiooxidans)* in Culture C resulted in more copper extraction compared to other mixed cultures.

CONCLUSION

In this study, three mesophilic indigenous bacteria were isolated from Sarcheshmeh and Darezar mines of Iran. *T. thiooxidans* oxidizes elemental sulfur with rates as high as 9 mg of sulfur /l/h while *T. ferrooxidans* and *L. ferrooxidans* had no effect on elemental sulfur. Ferrous oxidation kinetic of *T. ferrooxidans* was evaluated using Monod equation. *T. thiooxidans* had no effect on the leaching of copper sulfidic ore by itself, but having it in a mixed with *T. ferrooxidans* and *L. ferrooxidans* lead to more copper release and lower acid consumption compared to pure isolates. It is therefore possible to increase the copper extraction using this mineralogy, strengthening the indirect mechanism by initial introduction of ferric iron in the solution and *Eh* control of the bioleaching solution.

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