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Bioleaching of Cobalt from Cobaltiferous Ore by a Mixed Culture of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*

S. Borhany[1], N. Chaibakhsh[1] and F. Vahabzadeh*

A cobaltiferous ore was subjected to bioleaching by mixed bacterial cultures. Mixed cultures made up of pure *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* strains of the DSM culture collection were tested batchwise for their ability to oxidize the ore sample. These samples were obtained from the Eghlid mine in Iran. The main elemental analysis of the ore, according to the results obtained from the XRF test, were: 33.30% Fe, 0.76% Co, 4.14% S, 0.65% As and 9% Ca. The ore contained about 1% pyrite. For the biological adaptation of *T. ferrooxidans* to the ore sample, various levels of ferrous sulfate were added to the bacterial growth medium (9K medium) and among the different concentrations of $FeSO_4$, the level of 0.4% was selected. From among the three levels for the ore samples, ore at the level of 3% was chosen. Combinations of the two above-mentioned bacterial strains were set at five different levels. Results obtained, based on the amount of Fe(III) formed and on the level of recovered cobalt, show that leaching for a bacterial combination at the ratio of 2/3:1/3 was the most efficient. Moreover, results obtained from the mixed culture of *T. ferrooxidans* and *T. thiooxidans* at the above-mentioned level, show the possibility of an occurrance of smooth biological adjustment for the culture, as compared with that of the monoculture of *T. ferrooxidans*. The amount of formed iron(III) for the mixed culture was about 560.41 ppm by the second day of the incubation while, at the same time, the ability of the *T. ferrooxidans* to oxidize iron(II) was lower, compared to that of the mixed culture. The amount of iron(III) for the monoculture system was about 1/5 of that of the mixed culture. The trend of Fe(III) formation was in good correlation with the amount of cobalt recovered. As cobalt is disseminated in the pyrite matrix, the dissolution rate of cobalt and iron are, therefore, similar and cobalt concentration in aqueous leaching is proportional to iron concentration. The ability of the mixed culture to release cobalt from its main matrix could be graded as being good: recovery of Co for the mixed culture was 97% as compared to 67% recovery, due to the action of the monoculture in the bioleaching experiment.

INTRODUCTION

Cobalt metal shows high strength under extreme temperature and exhibits good conductivity, which is considered an essential characteristic property for many high technological devices in today's industries [1].

At the present time, using biotechnology in extracting valuable metals from different types of ore has been considered as an innovative approach in the mining industry [2]. As an example, one may say that the bacterial refractory of sulfidic gold ore (less than 80% recovery using a conventional cyanidation process) has been used as a pretreatment step for extracting precious gold [3]. In fact, precious metals are frequently locked and finely disseminated in iron sulfide minerals (principally pyrite and/or arsenopyrite) [4].

A few bacterial species are capable of oxidizing sulfide minerals to liberate the costly metal. The major types of bacteria in this regard are: *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, which are obligate chemoautolithotrophs and are active in the acid leaching of pyrites [5,6].

The mutual effect of mixed *Thiobacilli* and *Leptospirilli* populations on cobaltiferous pyrite bioleach-

1. *Department of Chemical Engineering, Amir Kabir University of Technology, Tehran, I.R. Iran.*
*. *Corresponding Author, Department of Chemical Engineering, Amir Kabir University of Technology, Tehran, I.R. Iran.*

ing has been studied and the results obtained showed that bioleaching with *T. ferrooxidans* was only improved by adding *T. thiooxidans* when the initial level of *T. thiooxidans* was greater than the initial level of *T. ferrooxidans* [7]. Several other studies regarding cobalt extraction using *T. ferrooxidans* have been reported in [8-10].

The purpose of this research is to study the performance of the mixed culture(s) of *T. ferrooxidans* and *T. thiooxidans* on the extraction of cobalt from cobaltiferous ore, which was obtained from a local mine, in the batch system.

MATERIAL AND METHODS

Microorganisms

Pure bacterial strains of *T. ferooxidans* 583 and *T. thiooxidans* 2705 were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM). The initial inoculum concentration was set at about 10^7 cells/ml. The medium for the growth of these strains was prepared according to the directions given by the microbial supplier [11] and the temperature of the incubation of the microbes was set at 25°C. In order to have some kind of biological adaptation to the ore sample, *T. ferrooxidans* and *T. thiooxidans* inocula were cultivated in successive basal media, in which ferrous ions had been replaced in a decreasing trend by the ore sample. The final concentration of ferrous iron was set at the level of 0.08% (or 0.4% $FeSO_4.7H_2O$).

Ore

The cobaltiferous ore used in the present study was obtained from a local mine in Eghlid, Iran. According to the results obtained from the XRF analysis, the main components of the ore sample were: 46.8% Fe_2O_3, 15.2% SiO_2, 12.6% CaO, 1.06% Co_3O_4 and 0.86% As_2O_3. Moveover, the main elemental analysis of the ore was: 33.3% (Fe), 0.76% (Co), 4.14% (S) and 0.65% (As) (see the appendix). The ore was ground to particles of less than 150 mesh [12].

Leaching Solution

The solution medium used for the leaching experiments contained 100 mg NH_4Cl, 500 mg KH_2PO_4, 150 mg $CaCl_2.2H_2O$, 500 mg $MgSO_4.7H_2O$, 400 mg $(NH_4)_2SO_4$ and 4 g $FeSO_4.7H_2O$ per 1 liter of distilled water. Before starting the bioleaching experiments, the pH of the solution was adjusted to 1.8 with H_2SO_4 (0.1N). This was partly done to prevent the formation of jarosite during the experiments, since, in an acid environment, ferric iron remains in solution and does not precipitate out [13]. When necessary, appropriate pH adjustment was made during bioleaching using H_2SO_4, 0.1N solution.

Leaching Experiments

Stationary leaching experiments were carried out in a 250 ml Erlenmeyer flask containing 100 ml of leaching medium, plus 3 g cobaltiferous ore. The leaching medium was sterilized in an autoclave at 110°C for 10 minutes and the ore samples added to the medium. Then, the flasks were incubated with an appropriate volume of solutions of *T. ferrooxidans* and *T. thiooxidans*, which were prepared in such a way that the cell concentration for each of these two bacterial cultures met the specifications given in Table 1. The flasks were then incubated in a stationary mode at 30°C.

Analytical Methods

Ferric iron was determined, according to the spectrophotometric method, using ortho-phenantroline [14]. Cobalt was determined by the Nitroso-R salt photometric method [14]. Each set of the bioleaching test was performed in duplicate and the data reported here are the average results taken from each set of the two treatments. The calculated standard deviation stands within the acceptable range (± 0.3).

Table 1. The ratios and cell numbers of the two bacterial species which were used as the initial levels of the inoculum in the leaching experiments.

Bacterial Combination (*T. ferrooxidans*: *T. thiooxidans*) as the Ratio in the Inoculum	Number of Bacterial Cells (*T. ferrooxidans* + *T. thiooxidans*) per ml
(tr[a].1), 1:1	$1.65 \times 10^6 + 1.65 \times 10^6$
(tr.2), 1/2:1	$0.82 \times 10^6 + 1.65 \times 10^6$
(tr.3), 1:1/2	$1.65 \times 10^6 + 0.82 \times 10^6$
(tr.4), 2/3:1/3	$1.10 \times 10^6 + 0.55 \times 10^6$
(tr.5), 1/3:2/3	$0.55 \times 10^6 + 1.10 \times 10^6$

a : treatment

RESULTS AND DISCUSSION

In order to use an ore adapted microbial strain, at first, the experiment with cobaltiferous ore was conducted at 2% ore content. The culture medium was the same as the 9K medium [15] and varied levels of iron (II) were tested. On the basis of observed microbial growth, the level of 0.4% ferrous sulfate ($FeSO_4.7H_2O$) was chosen to be added to the culture medium [12,16]. The ore which was used in the present study contained less pyrite than necessary for carrying out efficient cobalt leaching in a specified short incubation time. Therefore, in the present study, it was possible to make sure that a sufficient ferric iron content could be present in the leaching solution by the addition of ferrous sulfate to the 9K medium [17]. As also reported elsewhere, the optimum leaching of uranium occurred when iron was added to the medium at a level corresponding to 10% of the ore on a molar basis [17]. In the present study, results obtained from the addition of 0.8% ferrous sulfate showed that Fe(III) concentration increased 0.64 and 1.9 times by day 16 for 3% and 5% ore content, respectively (Table 2). Although relatively good results were obtained through the addition of 0.8% ferrous sulfate to the leaching solution, the level of 0.4% ferrous sulfate addition was selected. Therefore, for proper microbial adaptation, 0.4% ferrous sulfate was used (see Table 2). In order to select a proper level of ore content for the bioleaching experiments and, at the same time, considering the limitations that usually exist for a bioleaching test in the shake flask [18], three levels of usable ore content were evaluated using *T. ferrooxidans*. It should be mentioned that *T. ferrooxidans*, which firstly adapted to 2% ore content, was then used to conduct the bioleaching experiments at levels of 3% and 5% ore content. By increasing the concentration of the ore sample from 2% to 3%, the level of bioleaching (amount of Fe(III) formed) was increased (Figure 1). When the sample ore was used at a level of 5%, the variations in pH of the bioleaching solution was not as significant as that of the pH of the solution containing ore at the level of 3% (see Figure 1). Moreover, it has been shown that bacterial population at low levels are unable to oxidize the amount of ferrous iron generated (i.e. solublized) at a high ore concentration, therefore, the rate of mineral oxidation decreases [19]. Also, it has been mentioned in the literature that in order to process ores with a low content of sulfide minerals or ores which are high in acid consuming materials such as calcium carbonate, one may expect to use a heap bioleaching technique [20]. This method provides improved air and fluid (such as acid) flow within the heap and, therefore, in this way, variations in high pH can be controlled. In the present experiment the appropriate pH adjustment during bioleaching was achieved by using 0.1N H_2SO_4 solution. Results obtained from the elemental analysis show that in the present bioleaching experiment the ore contains less than 1% pyrite and has 9% calcium, which probably exists in the form of calcium carbonate.

Table 2. Leaching by *T. frrooxidans*.

Pulp Density (%)	Iron (III)(ppm) FeSO4 Added (%)	
	0.4	0.8
3	807	1325
5	246	713

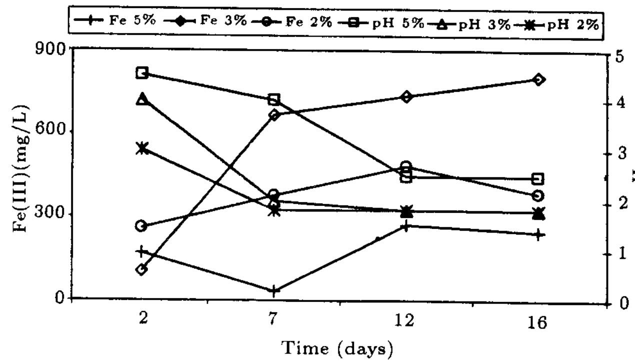

Figure 1. Iron oxidation of the cobaltiferous ore by *T. ferrooxidans* at different pulp densities; also, changes in the pH of the leaching solution.

On the basis of the mechanism of oxidation of ore containing pyrite, the action of a particular strain of bacterial species could be improved rather significantly by using mixed bacterial cultures [7]. Ores have several important physical and chemical characteristics in such a way that each of the chemical constituents of the particular ore can be used by the individual strain of the bacteria. Application of mixed bacterial culture(s) in the bioleaching of valuable metal from an ore containing pyrite has been evaluated as being good [21] (also see Figure 2). In the present study, several ratios of microorganism have been used as the inoculum for the mixed culture (see Table 1). In fact, some preliminary tests were conducted in order to

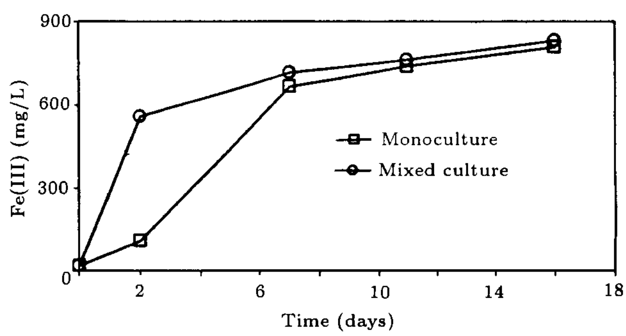

Figure 2. Comparison of two leaching experiments: Monoculare test (*T. ferrooxidans*) and the mixed culture test (*T. ferrooxidans* and *T. thiooxidans*, ratio, 2/3:1/3).

determine the ratio of mixed bacterial cultures, which was finally used for the bioleaching of the ore.

Trends of the time course of Fe(III) formation during bioleaching of the ore sample by the mixed cultures is shown in Figure 3. The efficiency of using mixed bacterial inocula largely depends on the concept that the different bacterial species have different affinities for the substrate in charge as the source of energy for their growth. The suggested mechanism for the microbial oxidation of sulfidic pyrite includes four main steps, in which *T. ferrooxidans* has the ability to solublize the iron(II) fraction of the ore and the subsequent oxidation of iron, as well as sulfur content, would yield $Fe_2(SO_4)_3$ and H_2SO_4. *Thiobacillus thiooxidans* cannot oxidize the iron fraction and is expected to attack the sulfide portion of the ores without any preference for the particular metal involved [16]. In the present study a bacterial monoculture, in the form of *T. ferrooxidans*, showed a proper and typical trend for oxidation of ferrous iron as compared with that of the other inocula (see Figures 2 and 3). By considering the action of bacterial cells from day 16 up to the end of the bioleaching period, the rate of ferric iron formation for treatments 1, 2, 3, 4 and 5 are as follows (mg/L/day): 4.9, -4.3, 9.2, 37.9, 21.1, respectively. At the same time, the percent of cobalt recovery due to the action of bacterial cultures, was calculated and the results are as follows (%): 36.97, 24.58, 45.71, 96.77, 42.45, respectively. The rate of ferric iron formation for treatment 4 is the highest among the rates for all five treatments. For example, the rate for treatment 4 is almost eight times higher than that for treatment 1. As mentioned before, cobalt is mainly disseminated in the fraction of pyrite in the ore sample. The dissolution rate of cobalt and iron are similar and the concentration of cobalt stays proportional to the iron concentration [8]. Therefore, the amount of leachable cobalt, which is determined for the ore sample, corresponds to the rate of Fe(III) formation (Figure 4). One may say that the highest level of ferric iron formation (1364 ppm) has been coupled with the highest percent of leachable

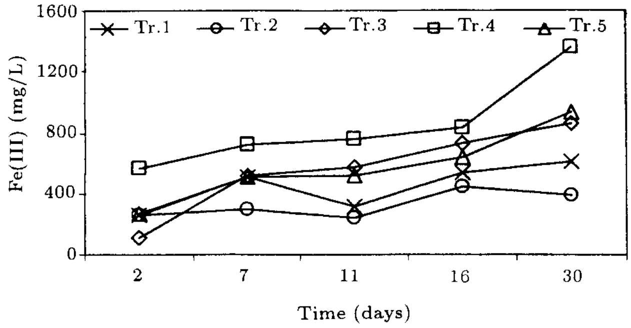

Figure 3. Time course of the leaching by the mixed culture of *T. ferrooxidans* and *T. thiooxidans* at different ratios.

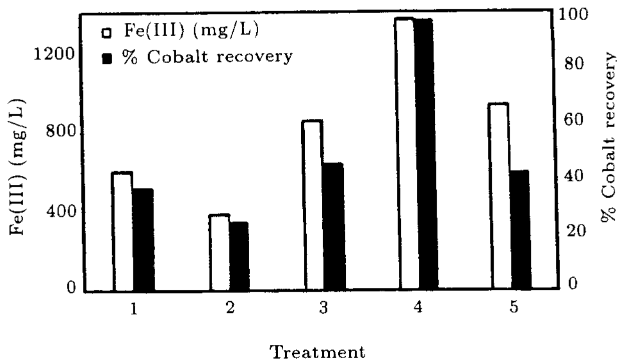

Figure 4. Percent of cobalt recovery along with the amount of ferric iron formation for five different treatments.

cobalt (97%) (see Figure 4). It seems that the initial number of bacterial cells in the inoculum has some important effect on the rate of Fe(III) formation, as well as on the level of cobalt extracted biologically. When a specific cell population in the aqueous leaching solution is reached, that concentration then acts as a limiting factor on the rate of microbial growth and may stabilize the system in such a manner that the oxidation of substrate becomes more favorable [5]. When the number of cells of *T. ferrooxidans* is half that of *T. thiooxidans* (treatment, 2, Table 1), the lowest rate of Fe(III) formation is obtained (see Figure 3). At the same time, the amount of cobalt extracted by using this mixed culture was also the lowest (Figure 4). By changing the ratio of mixed culture to the level of 1:1, little improvement could be observed i.e. 57% increase in Fe(III) formation corresponding to 50% raise in the amount of cobalt leached (see Figure 4). A low level of *T. freeoxidans* cells in the mixed culture, along with a high level of cells for *T. thiooxidans*, somehow reduces the performance ability of *T. ferrooxidans* to oxidize Fe(II), while the presence of higher levels of *T. thiooxidans*, along with a low level of sulfur content in the ore sample, decreases the tendency of bacterial cells to H_2SO_4 formation. Therefore, the pH of the solution did not decrease as much as expected. In the present study, during the first seven days of incubation, the pH of the leaching solutions in all five treatments was changed from the initial value of pH 1.8 to pH 4 by day 2-3. Therefore, it became necessary to add acid (0.1N H_2SO_4) to the leaching solution to make a proper pH adjustment for the bioleaching (pH 1.5-2.5). With the changes in the bacterial cell numbers (an increasing amount of cells for *T. ferrooxidans* and decreasing cell numbers for *T. thiooxidans*) the trend of ferric iron formation was improved and this improvement is mainly observed at the ratio of 2/3:1/3 (see Figure 3, tr. 4). The difference(s) between the leaching behavior among those using only the monoculture of *T. ferrooxidans* and those ore samples leached with the mixed culture of *T. ferrooxidans* and *T. thiooxidans* at the ratio of 2/3:1/3, could be related to a smooth biological adjustment for the mixed culture, as compared with that of the monoculture of *T. ferrooxidans* (see Figure 2). The lag phase is shortened, as compared with that of the typical growth curve for the bacterium. The growth curve for *T. ferrooxidans*, in the form of biomass formation as the function of time, has been shown to be in good correlation with ferric iron concentration during the time of the leaching experiment [15].

The amount of Fe(III) formation by day 2 for *T. ferrooxidans* and the mixed culture of *T. ferrooxidans* and *T. thiooxidans* at the ratio of 2/3:1/3, was 108 and 560 mg/L, respectively. Continuing the incubation of the cultures shows that by day seven, the amount of Fe(III) formation for the monoculture and mixed cultures was 669 and 720 mg/L, respectively (see Figure 2). At the same time, the amount of leachable cobalt for the monoculture and, also, for the mixed culture (bacterial culture was set at the level described above) in this experiment, was found to be 67% and 97%, respectively. The smooth changes in the trend of ferric iron during the first seven days of the bioleaching experiment could be due to the occurrence of proper pH adjustment for the mixed bacterial culture (see Figure 2). Based on the possible existence of correlation between cobalt recovery and Fe(III) formation, this type of biological adjustment and adaptation may lead to a better performance for the bacterial culture and the result would be a higher level of cobalt recovery.

CONCLUSIONS

Results obtained from the bioleaching experiment, which was conducted on a cobaltiferous ore, showed that 97% of cobalt can be extracted from the ore sample by using the mixed cultures of *T. ferrooxidans* and *T. thiooxidans* at the ratio of 2/3:1/3. Because of the low sulfur content of the ore, the bioleaching was only improved by adding *T. thiooxidans*, when the initial concentration of *T. thiooxidans* was lower than the initial concentration of *T. ferrooxidans*. This low level of *T. thiooxidans* causes a proper biological adjustment, as compared with that of the monoculture of *T. ferrooxidans*. As a result of this adjustment, the amount of cobalt recovery is in good correlation with the amount of Fe(III) formation, with an increasing trend for both.

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