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Facilitate of Gold Extracting From Mouteh Refractory Gold Ore Using Indigenouse Bacteria

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ABSTRACT

The term biomining have been coined to refer to the use of microorganisms in mining processes as in the biooxidation of refractory gold minerals. The biooxidation of refractory gold ores presents similar characteristics when compared with roasting and pressure oxidation. Almost without exception, microbial extraction procedures are more environmentally friendly. The isolated bacteria in this study, were included a variety of oxidizing acidophilic autotrophic iron and sulfur oxidizing that named F.O.C.B and C.L.L.B. Biological oxidation with shaking flask method were done in the presence of 1 gr of the ore milled of Mouteh with a particle diameter of 150 microns (100 mesh) in 9K medium without iron , at 30°C and shaking speed 180 rpm, during the 7 days, during this period ferrous ions assessment were performed by colorimetric method with orthophenantrolin. The results showed that F.O.C.B. bacteria reduced the amount of ferrous ion from 0.63 to 0.015 gr/L and C.L.L.B. bacteria from 0.64 to 0.04 gr/L. Also mineral pyrite was removed after 7 days. This study aimed to Optimization of gold extracting from sulfide ore Mouteh using indigenous bacteria.

Keyword: Bioleaching; Isolation; Mouteh; Refractory Gold; Chemolithotrop; Ferrous ion.

1. INTRODUCTION

The term biomining have been coined to refer to the use of microorganisms in mining processes. On the other hand, biooxidation implies the bacterial oxidation of reduced sulfur species accompanying the metals. For many years bioleaching was thought as a technology for the recovery of metals from low-grade ores, flotation tailings or waste material [1, 2]. Today bioleaching is being applied as the main process in large scale operations in copper mining and as an important pretreatment stage in the processing of refractory gold ores [2]. The main advantages of biooxidation of refractory gold ores

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as compared with pyrometallurgy lie in its relative simplicity, low capital costs, low energy input, and in its friendliness towards the environment [3, 4].

The primary biomining organisms have several physiological features in common. hemolithoautotrophs are major organisms in biomining process that are able to use ferrous iron or reduced inorganic sulfur sources (or both) as electron donors [2]. These organisms are acidophilic and most will grow within the pH range 1.5-2.0. This extreme acidophily applies even to those biomining organisms that can oxidize only iron [4, 5].

Chemolithoautotrophic mesophilic bacteria of genera acidithiobacillus and leptosprillum are the most commonly found leaching organisms. Acidithiobacillus is the gram-negative rod shape bacteria with length 1-3 and Width 0.5. Leptosprillum is gram-negative aerobic and spiral shape bacteria that obtained energy requirements from the oxidation of ferrous ions [4, 5]. This study aimed to optimization of gold extracting from sulfide ore Mouteh using native bacteria.

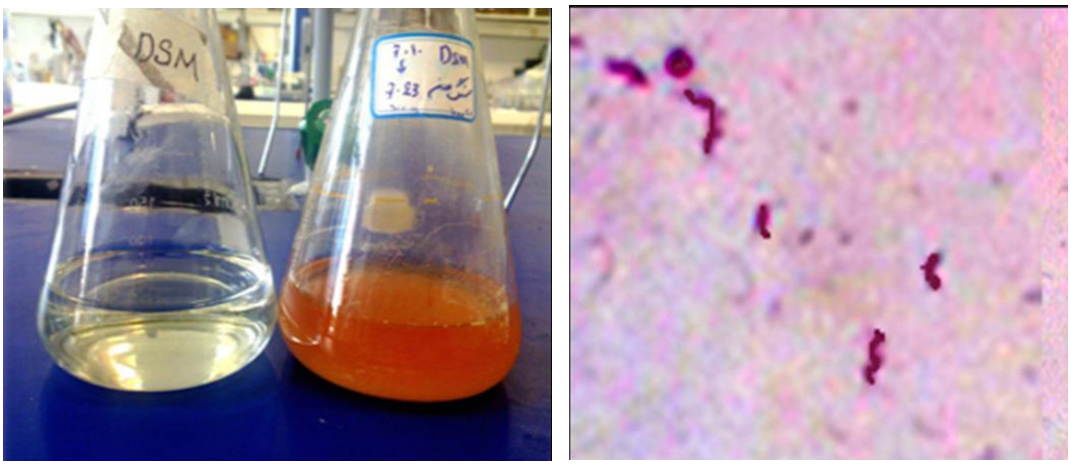
2. MATERIALS AND METHODS

The Chemolithoautotrophic mesophilic iron oxidizing bacteria used in this study have been isolated from the chahkhatoon and senjedeh mines located in mouteh gold Mines complex, Isfahan, Iran. Total of 10 samples collected from Chahkhatoon and Senjedeh minerals and dumps in mouteh gold mine. 10 gr of each sample inoculated in in 250 mL Erlenmeyer flasks containing 90 mL 9K medium (3.0 g/L $(NH_4)_2SO_4$, 0.1 g/L K_2HPO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.1 g/L KCl, and 0.013 g/L $Ca(NO_3)_2.4H_2O$, 44.2 gr $FeSO_4.7H_2O$, 1 mL H_2SO_4 10 N, 1 L D.W.) and DSMZ882 medium (132 mgr $(NH_4)_2SO_4$, 53 mgr $MgCl_2.6H_2O$, 27 mgr KH_2PO_4, 147 mgr $CaCl_2.2H_2O$, 20 gr $FeSO_4.7H_2O$, 50 mL H_2SO_4 (0.25 N), 950 mL D.W). The pH value was adjusted with sulfuric acid to 2 before the inoculation was processed [2, 4].

The presence of iron-oxidizing bacteria in liquid iron medium (9K and DSMZ882) was indicated by the formation of ferric iron and the medium becoming brick red in color. Ferrous iron was analyzed at 509 nm using visible spectroscopy. 1, 10 Orthophenanthroline was used as the complexing agent. For enrichment and refreshing, 10 mL of brick red color flasks was inoculate in 90 mL of 9k fresh media [2, 7 and 8]. We used 9K agar (4 g/L agar-agar ultrapure) and 2:2 solid media (4.5 g/L agar-agar ultrapure) for single colony isolation and morphological studies [6]. For enrichment of pure cultures, single colony of iron-oxidizing bacteria, were picked from the plates by using a sterile inoculating loop and inoculated into 25 mL sterilized vials containing 10 mL liquid iron medium, pH 2.0 and was vortexed to spread the colony. All the cultures were incubated at 30°C until the color of the medium changed to brick red indicating ferrous iron (Fe^{2+}) oxidation by iron-oxidizing bacteria. Such ordinary purification procedures were repeated several times, finally pure cultures were obtained. Selected isolates were subjected to light and scanning microscopy for morphological characterization [7]. Finally leaching experiments were performed in 250 mL agitation flasks for 7 days, in which the initial 1% pulp concentration of 150 μ ore particle size and bacterial inoculation was 10% V/V. Control samples were made by the addition of 10 mL of inactive bacteria. All experiments were done and carried out in rotatory shaker at 180 rpm, 30°C for 7 days. During the leaching, Redox potential and pH were measured daily [6, 8]. Bacterial ferrous iron oxidation rate was determined calculating the amount of Fe^{2+} remaining in the solution by spectrophotometer using 1, 10 orthophenanthroline ferrous complex as an indicator. Sulfate concentration was indirectly determined by atomic absorption spectroscopy analysis of Ba after precipitation of $BaSO_4$ [6, 8]. The chemical composition and particle size distribution of ore was determined prior and after of bioleaching experiments (Table 1).

3. RESULTS

After 3-5 days of incubation in 9K and DSMZ882



(a) (b)

Figure1: *a) DSMZ 882 medium right before and left after bacterial growth C.L.L.B. b) Microscopic images of bacteria C.L.L.B.*

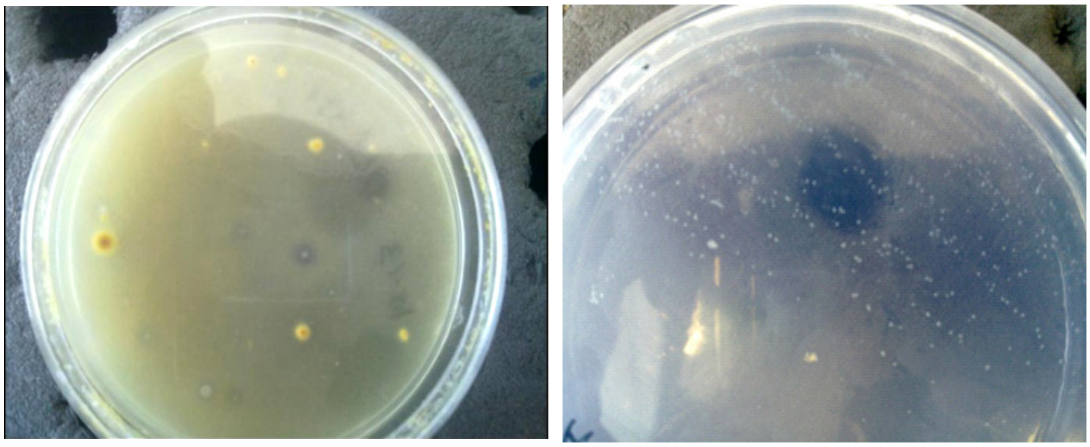


Figure 2: *a) The iron-oxidizing bacteria colonies on 9K agar medium. b) The iron-oxidizing bacteria colonies on 2:2 agar medium.*

Table 1: *Composition of Mouteh pyritic ore concentrate.*

% SiO_2	% Al_2O_3	% FeS_2	% Na_2O	% K_2O	Gold
13.98	2.99	78	0.99	0.27	ppm

media at 30°C and 150 rpm under shaking condition, samples of Chahkhatoon spring became reddish-brown due to bacterial oxidation of Fe^{2+} to Fe^{3+}. After the gram staining different biochemical activities were analyzed. The compound microscopic observations of isolated strains of bacteria revealed that these strains were Gram-negative, motile, very small (1-2 μm in length), rod shape and

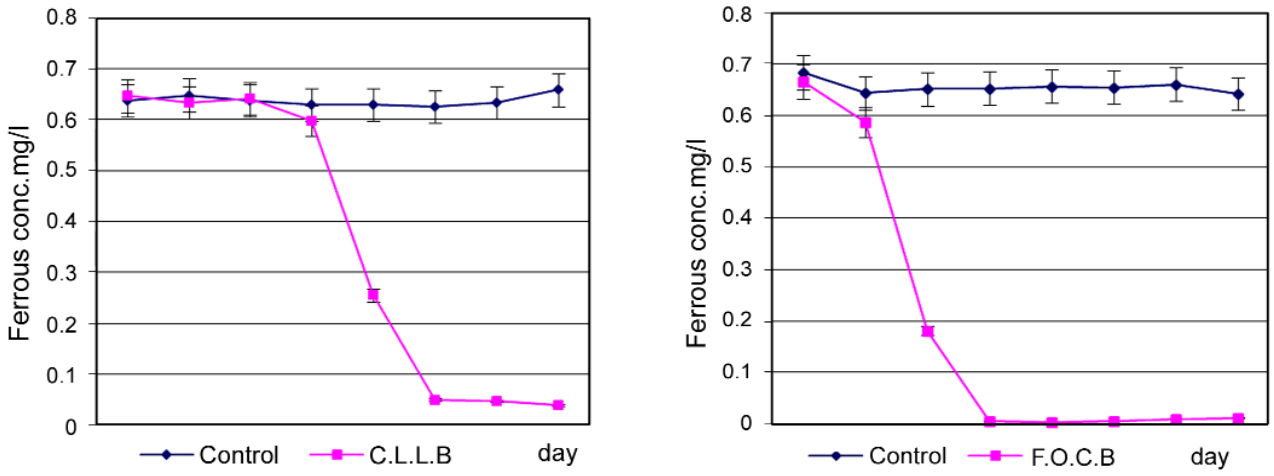


Chart 1: *Right: Mouteh gold sulfide mineral ferrous ion concentration changes in 9k medium iron lacking with F.O.C.B bacteria within 7 days compared with control. Left: Mouteh gold sulfide mineral ferrous ion concentration changes in DSMZ 882 medium iron lacking with C.L.L.B bacteria within 7 days compared with control.*

spiral shape bacteria, singles or pairs bacteria. The most frequently observed colonies in 9K agar medium were semi-spheroidal and smooth-surfaced, with a white or yellow band outside and around centre, and a margin with many short projections. Ferrous oxidation was studied for all bacteria isolates. These bacteria oxidized Fe^{2+} to Fe^{3+} and reduced sulfur compounds produced sulfuric acid which followed a drop in initial pH-value of the medium. Two strains showed the strongest ability to oxidize ferrous ion. Depending on colony appearance, they were classified into 2 different types. These strains were rod-shaped and a spiral shape bacteria was named F.O.C.B. (Ferrous Oxidizing Chakhatoon Bacteria) and C.L.L.B. (Chahkhatoon Leptospirillum like Bacteria) respectively. These bacteria did not grow in culture TSI and NA media. Growth was inhibited at neutral and alkaline pH. Based on morphological and biochemical characteristics of one isolate of Leptospirillum-like bacteria (C.L.L.B.) were found to be resembled to the genus Leptospirillum (Figure 1). Based on morphological and biochemical characteristics of other isolate were found to be resembled to those of the genus species Acidithobacillus ferooxidans.

Oxidation of Ferrous Iron (Fe^{2+}) by F.O.C.B. and C.L.L.B. was conducted in shake flasks containing iron liquid medium (9K Fe^{2+}) containing pH-value of 1.8. It was observed that ferrous iron (Fe^{2+}) was completely oxidized to ferric iron (Fe^{3+}) by the isolated strain during 3-5 days of incubation time at 30°C and 150 rpm. In chemical control flasks, only a negligible amount of ferrous iron was oxidized due to air-oxidation under the same experimental condition. As shown in the chart 1, F.O.C.B. reduced ferrous ions from 0.64 to 0.004 mg/L, but in bioleaching by C.L.L.B. these changes was from 0.63 to 0.015 mg/L. this results, indicates high biooxidation potential of both types of bacteria.

XRD analysis of the after leaching processes for both types of bacteria showed pyrite remove from ore (Figure 3).

4. DISCUSSION

Gold is usually obtained from ores by solubilization with a cyanide solution and recovery of the metal from the solution. In ores known as refractory, small particles of gold covered by insoluble sulfides. The main mineral composition of this ore was pyrite and arsenopyrite, therefore, removal of

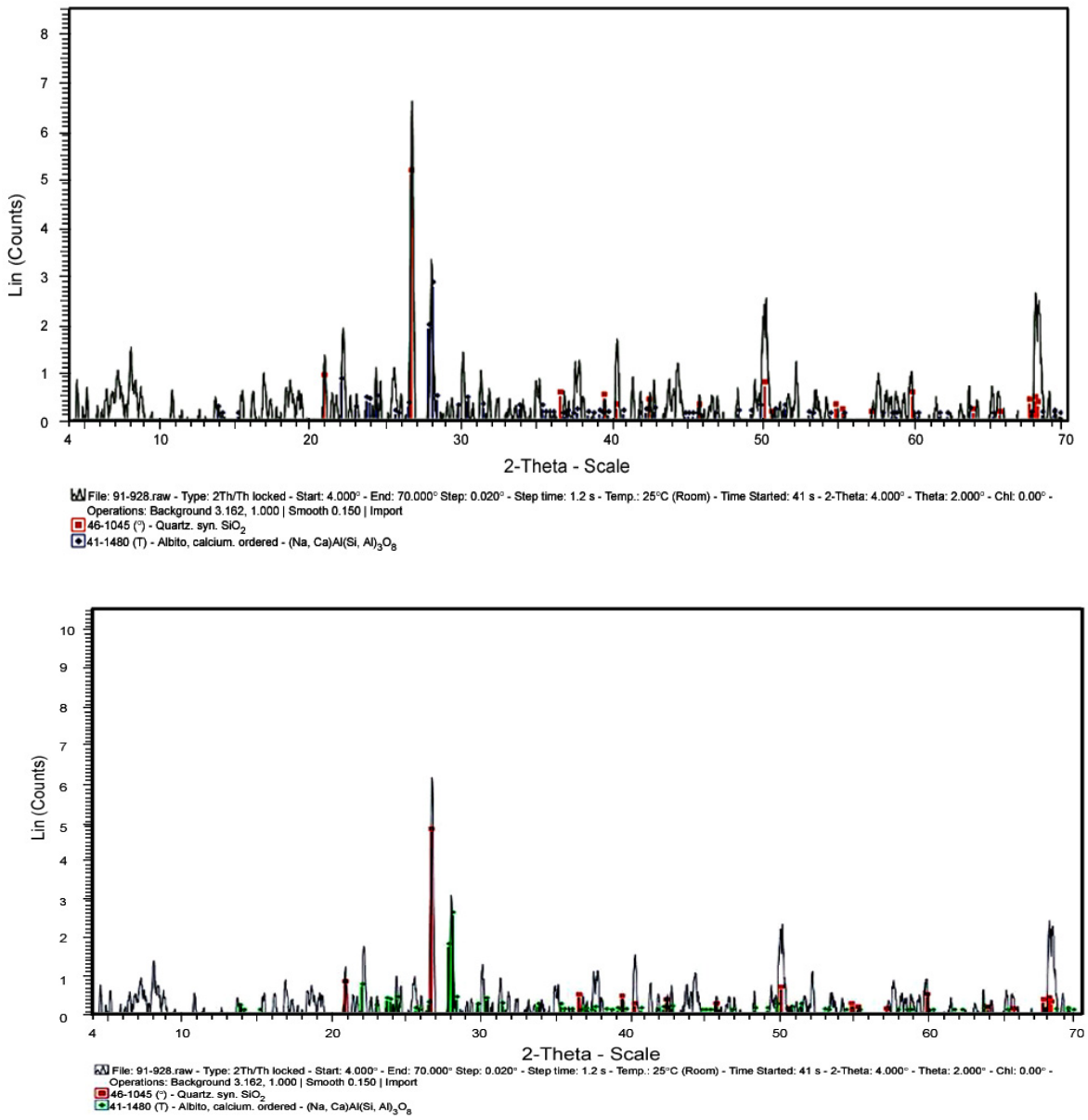


Figure 2: *XRD analysis of gold sulfide ore of Mouteh, a before and b after 7 day's biooxidation by F.O.C.B bacteria. Blue peaks in figure a, indicate the presence of pyrite that in the figure b, have been removed from ore.*

these minerals does it feasible for extracting using cyanide. Several alternative technologies are available, such as pressure oxidation, chemical oxidation, roasting and biooxidation, the latter currently being the alternative of choice. In the biooxidation process, bacteria partially oxidize the sulfide coating the gold microparticles. Micro-organisms belonging to the Thiobacillus and Leptospirillum genera are commonly used, although an increasing interest exists in thermophilic archeons. Gold recovery from refractory minerals can increase from 15-30% to 85-95% after biooxidation. Currently studies are being carried on for the development of processes for the

bioleaching of gold concentrates [6]. In this study, for the first time, the Mouteh gold mine indigenous bacteria were used for bioleaching of Mouteh sulfidic gold ore, whereas in the previous studies (Shahverdi et al. 1378 and 1379 and Meybodi. 1378), were used from thermophilic adapted bacteria isolated from hot springs [8, 10 and 11]. Results indicate high biooxidation potential of indigenous bacteria. 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, Therefore In bioleaching process using indigenous bacteria adaptation Stage has been removed and will spend less cost and time [6].

Chemolithoautotroph bacteria are very sensitive to organic matter including the small quantities of sugar present as impurities in polysaccharide based gelling agents such as agar or agarose. Attempts to use highly purified agars have not been very successful, probably because some of the sugar molecules in the gelling agent are released owing to acid-hydrolysis at low pH, and the released sugars inhibit cell growth, a number of alternative gelling agents have met with partial success, but most of these are difficult to work. 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 [6].

In order to evaluate physiological and biochemical characteristics of sulfur oxidizing isolates, the sulfur and ferrous oxidizing abilities were investigated F.O.C.B and C.L.L.B isolates could oxidize all of initial ferrous within 3-7 days. Based on this experience, one isolates of Leptospirillum-like bacteria (C.L.L.B) were isolated from Chahkhatoon mine in this study. Their morphological and biochemical characteristics were found to be resembled to those of the genus Leptospirillum. Sand (1992) and Rolling (1999) Studies indicate that Leptospirillum-like bacteria are less sensitive to the inhibitory effect of ferric ion and the inhibitory concentration of this ion is more than ten times higher than amount that for Acidithiobacillus ferrooxidans like bacteria. Also the activity of these bacteria increases in mixed cultures compared with single culture [12, 13]. Pachvlvska (2003) results determined, although Acidithiobacillus ferrooxidans can be in relatively high ferric to ferrous iron in comparison with Leptosprillum ferrooxidans has higher growth, but when ferric iron concentration is high, Leptosprillum ferrooxidans will win the competition [9].

The result of this study showed that division time of C.L.L.B. bacteria is longer than F.O.C.B. and is longer time to reach the logarithmic phase. On the other hand, this bacterium tolerance of power in high levels of ferrous ions is greater in comparison with F.O.C.B. bacteria. As result in long-term processes simultaneous use of these bacteria will give better result. The results was equalled with study Sand and Pachvlvska and Rolling [9, 12 and 13].

5. CONCLUSIONS

XRF analysis of mouteh gold ore shows that high value of iron (34.668%) and sulfur (13.686%), created good conditions for the growth of iron and sulfur oxidizing bacteria and it could be one of the causes of high biooxidation potential of both types of bacteria [8].

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