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Optimization of parameters affecting recovery of copper from Sarcheshmeh low-grade sulfide ore using Bioleaching

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Abstract

In this work, the parameters affecting the recovery of copper from the low-grade sulfide minerals of Sarcheshmeh Copper Mine were studied. A low-grade sulfide ore was used with a copper grade of 0.25%, which was about 28% of the mineral oxide, and the sulfide minerals made up the rest. Much more sulfide minerals were found to be pyrite and most of the gangue minerals were quartz, anorthite, biotite, and muscovite. In order to investigate, simultaneously, the solids (10 to 20%) and acidity (1.5 to 2.5) and shaking (110 to 150 rpm), the separation of bacteria from Sarcheshmeh Copper Mine was carried out. After adjustment of the sample, bio-leaching tests were performed in accordance with the pattern defined by the software DX7 in shaking flasks, and the Cu recovery was modeled and optimized using the response surface methodology. The influential parameters were comprehensively studied. The central composite design methodology was used as the design matrix to predict the optimal level of these parameters. Then the model equation was optimized. The results obtained showed that increasing solids (from 10 to 20%) was bad for bacteria. The highest copper recovery was equivalent to 69.91%, obtained after 21 days at 35 degrees using the Acidi Thiobacillus Ferrooxidans bacteria and a K9 medium with a pulp density of 10% and pH 1.5.

Keywords: *Optimization, Copper, Bioleaching, Bacteria, Acidi Thiobacillus Ferrooxidans*.

1. Introduction

World reserves of high-grade ores are declining due to the increasing consumption of raw materials. One of the problems with recovery from low-grade ores by conventional techniques is that it is expensive due to the high energy consumption and the need for high capital costs. Another problem is the high cost of environmental pollution. Due to the reduction in the high-grade mineral reserves, energy efficiency, and environmental standards, application of new methods and changing this industry is essential. Biotechnology is the most promising solution to this problem. Microorganisms gain access to some of the goals of the industry, health, and environmental uses. Recent advances in biotechnology have led to this technique being used as a powerful tool in mineral processing and hydrometallurgy and solving environmental problems [1]. Biomining is the generic term used to describe technologies that utilize biological systems to facilitate the extraction and recovery of metals from ores and waste materials [2-4].

Bioleaching has been developed into a frontier technology for extraction of copper from minerals in the 21st century [5]. Mainly in bioleaching processes, mesophilic bacteria (Acidithiobacillus ferrooxidans, Acidithiobacillus-thiooxidans, and Leptospirillum ferrooxidans) are used in the temperature range of 20-45 °C. The bacteria convert ferrous ions to ferric ions on the surface of the minerals, and oxidation reactions are caused. In fact, the minerals are oxidized by ferric ions through chemical reactions [6].

This process due to many advantages, can be used as an ideal technology in developing countries.The advantages and limitations of using bioleaching technology are shown in Table 1.
Copper, as a precious and strategic metal, has been used by humans from the earliest times. Currently, 25% of the world copper is produced using hydrometallurgy, and 75% of it is produced by pyrometallurgy. However, due to the increasing global demand for copper and reduced supplies of high-grade mines, it is predicted that in the coming years this ratio is reversed [8]. Thus in order to make optimal use of mineral resources, studying the parameters involved in the extraction of copper from low-grade sulfide ores by bioleaching is essential.
The physical, chemical, and microbial factors involved in a leaching environment, thecharacteristics of ore for leaching, and the type of leaching process are the most important factors affecting the microbial oxidation of metals. Some of the influential factors are temperature, pH value, pulp density, bacteria, population and distribution of microorganisms, type of ore, ore chemical composition, particle size, porosity, leaching method, and stirring rate [9, 10].
In this work, the bioleaching process for the extraction of copper from low-grade sulfide ores was evaluated in a sample taken from Sarcheshmeh Copper Mine. The aim of this work was to evaluate the parameters affecting the recovery of copper from low-grade sulfide ores such as temperature, pH, and bacterial population during bioleaching. The copper content of the sample was 0.25%, and about 65% of the copper minerals was sulfide minerals. Gangue minerals in the sample were mostly of silica type. Shaking flask experiments were carried out to achieve the desired goal.

Table 1. Advantages and limitations of bioleaching [7].

Advantages	Limitations
1. Suitable for low grade resources	1. A slow process
2. The possibility of selective leaching	2. Slow ROI (the rate of return on investment)
3. Flexible	3. Low levels of expert's familiarity with this technology
4. Oxide, a mineral with relatively high efficiency	4. Problems due to production of sulfuric acid during some processes
5. Relatively low capital and operating costs	5. Lack of confidence in the industry to bacterial processes
6. Low maintenance cost	6. There are vague hints in connection with this process
7. No need for complex equipment	
8. Perform under ambient pressure and temperature	
9. The possibility of doing it *in situ*	
10. Appropriate for the environment	
11. Simple controls	
12. No need for expertise control	

2. History of bacterial technology in mining industries

Since the beginning of this century, bioleaching has found a special place in the extraction of metals. The use of microorganisms in the dissolution of metal sulfides can be traced back to ancient times. Of course, until 1947, when the first type of mining waste was separated and mesophilic bacteria were identified, this role was still unknown. The Rio Tinto copper mine in Spain was the first mine that used microorganisms for metal extraction in industrial scale [11]. Table 2 lists the names of the mines that produce copper by the bioleaching technique [12].
Bacterial oxidation of sulfide ores for copper recovery has been carried out in Spain, Sweden, Germany, China, and other countries for centuries without knowing its cause. More than fifty years ago, Beek and Bryner succeeded in separating Acidithiobacillus Ferrooxidans and Acidithiobacillus Thiooxidans and determined their role in leaching copper sulfide [13].
Sulfide ore bioleaching happens in an acidic environment that often contains a significant concentration of iron(III) ions. Secondary copper sulfides (chalcocite, covellite) are solved easily in the presence of oxidizing agents such as ferric iron and in an acidic environment, while the primary copper sulfide minerals (chalcopyrite, bornite, enargite) have a hard time dissolve even under these conditions [8].
During 1947-1951, Colmer, Hinkle, and Temple isolated the Thiobacillus bacteria from acidic water out of mines and later was named as Acidi Tiobacillus Ferrooxidans [9]. In fact, the discovery of Acidi Thiobacillus Ferrooxidans started a new era in understanding the phenomena related to the formation and destruction of minerals. Since 1954, after the discovery of microorganisms and their role in the oxidation and

dissolution of the metals widely used in the bioleaching of mineral processing and extraction of metals from low-grade stockpiles, concentrates and scrap industries were also used. The practical application of this science started in 1957, and it is now used in many countries such as America, Chile, Canada, and Australia [14].

Numerous research works and studies have been carried out on bioleaching sulfide minerals in several sectors including chemical principles of bioleaching, mineralogy, microbiology, mechanism of sulfide mineral bioleaching; the heap leaching and reservoir engineering aspects studied are summarized in Tables 3 and 4.

Table 2. Names of world's major mines producing copper through mass bioleaching [12].

Heap bioleaching of copper ores (historical and current)			
Region/mine	**Operation reserves (t)**	**Ore processed (t/day)**	**Cu production (t/year)**
Lo Aguirre, Chile 1980-1996	Heap bioleach 12×10^6 at 1.5% Cu	Oxides/chalcocite 16×10^3	14-15$\times10^3$
Cerro Colorado, Chile 1993-	Heap bioleach 80×10^6 at 1.4% Cu	Chalcocite, covellite 15×10^3	100×10^3
Ivan Zar, Chile 1994-	Heap bioleach 5×10^6 at 2.5% Cu	Oxides/sulfides 1.5×10^3	12×10^3
Quebrada Blanca, Chile 1994-	Heap/dump bioleach 85×10^6 at 1.4% Cu 45×10^6 at 0.5% Cu	Chalcocite 17.3×10^3	75×10^3
Punta del Cobre, Chile 1994-	Heap (bio)leach 10×10^6 at 1.7% Cu	Oxides/sulfides	7-8$\times10^3$
Andacolle, Chile 1996-	Heap/dump bioleach 32×10^6 at 0.58% Cu	Chalcocite 15×10^3	21×10^3
Dos amigos, Chile 1996-	Heap bioleach 2.5%	Chalcocite 3×10^3	-
Zaldivar, Chile 1998-	Heap/dump bioleach 120×10^6 at 1.4% Cu 115×10^6 at 0.4% Cu	Chalcocite 20×10^3	150×10^3
Lomas Bayas, Chile 1998-	Heap/dump 41×10^6 at 0.4% Cu	Oxides/sulfides 36×10^3	60×10^3
Cerro Verde, Peru 1977-	Heap bioleach - at 0.7% Cu	Oxides/sulfides 32×10^3	54.2×10^3
Escondida, Chile	Heap bioleach 1.5×10^9 at 0.3-0.7%	Oxides, sulfides	200×10^3
Lince ll, Chile 1991-	Heap leach 1.8% Cu	Oxides, sulfides	27×10^3
Toquepala, Peru	Heap leach	Oxides, sulfides	40×10^3
Morenci, Arizona 2001-	Mine for leach 3450×10^6 0.28% Cu	Chalcocite, pyrite 75×10^3	380×10^3
Equatorial Tonopah, Nevada 2000-2001	Heap bioleach 0.31% Cu	25×10^3	25×10^3
Gunpowder Mammoth Mine, Australia 1991-	In situ (bio)leach 12×10^6 at ~ 1.8% Cu	Chalcocite and bomite -	33×10^3
Girilambone, Australia 1993-2003	Heap bioleach - at 2.4% Cu	Chalcocite/chalcopyrite 2×10^3	14×10^3
Nifly Copper, Australia 1998-	Heap bioleach - at 1.2%	Oxides/chalcocite 5×10^3	16×10^3
Whim Creek and Mons Cupri, Australia 2006-	Heap bioleach 900×10^3 at 1.1% Cu 6×10^6 at 0.8% Cu	Oxides/sulfides	17×10^3
Mt Leyshon, Australia 1992-1997	Heap bioleach - 0.15%	Chalcocite 1.3×10^3	750
S & K Copper, Monywa, Myanmar 1991-	Heap bioleach 126×10^6 at 0.5% Cu	Chalcocite 18×10^3	40×10^3
PHoenix deposit, Cyprus 1996-	Heap (bio)leach 9.1×10^6 at 0.78% Cu 5.9×10^6 at 0.31% Cu	Oxides/sulfide-	8×10^3
Jinehuan Copper, China 2006-	240×10^6 at 0.63% Cu	Chalcocite, covellite, enargite	10×10^3

Table 3. Some previous studies of bioleaching technology, chemical principles, and bioleaching mineralogy.

Researcher(s)	Year	Topics	Report
Anjum et al.	2012	Bioleaching Technology	Bioleaching process has been impressively developed during the recent decades [15-17].
Pradhan et al.	2013		
Qin et al.	2013		
Harisson et al.	1966	The use of bioleaching in mining industries	They declared the role of iron oxidizing (Acidi Thiobacillus Ferrooxidans) in leaching uranium [12].
Acevedo, 2002 & Badouie, 2004	1950 to 1980		Bioleaching was raised as a suitable technology for recovering copper and other metals from low-grade ores. The most important copper mines were Rio Tinto in Spain with a production of 8000 tons per year and Kanani in Mexico producing 9000 tons per year [12,18].
	1966 to 1980		In Lo Aguirre Mine (Chile) approximately 16,000 tons of ore per day was mined using the bioleaching method, and in the mid-80s, the first factory that worked only with this method began in Minera Pudahuel Chile [12,18].
	Decade 1990		Since the majority of bioleaching factories were established in this decade, it is considered the industrial decade of microbial processes [12,18].
Lazaro and Nicol	2003	Chemical principles (bioleaching process chemistry)	They evaluated the oxidation-reduction potential of chalcopyrite during the bioleaching process and found that the oxidation reaction of sulfide minerals are chalcopyrite and convert ferric ions to elemental sulfur, is sensitive to the redox potential, so that the potential lower dissolution rate increases [19].
Klauber	2003		They recognized four species containing sulfur on the surface of leached chalcopyrite during bioleaching using X-ray photoelectron spectroscopy [20-22].
Klauber et al.	2001		
Parker et al.	2004		
Kinnunen et al.	2003		They suggested that leaching at pH = 1 prevented the formation of jarosite deposits or delayed their formation [23].
Tshilombo et al.	2002		Lack of inaction during bioleaching chalcopyrite can be compensated under controlled thermal (45-65 °C) and electrochemical conditions (V SHE 65/0-45/0). The authors noted that the passive layers composed at 25° prevented the revival of ferric ions on the surface of chalcopyrite polarized strongly [24].
Nazari et al.	2014		They examined variable parameters such as temperature and concentration of iron in the growth medium, and pH for jarosite formation in the presence of bacteria Acidithiobacillus ferrooxidans [25].
Whittington et al.	2003	Bioleaching mineralogy	The use of quantitative mineralogical analysis of feed and residual leaching process is valuable to understand its chemistry during high-pressure acid leaching of nickel laterite, and is corrected as part of a systematic study of leaching to describe the copper sulfide concentrates [19, 26-27].
Gottlieb et al.	2000		Combining the results of automated SEM technique studies with quantitative analysis of X-ray diffraction data, SEM-microprobe data, and elemental analysis of ores and residues can provide new insights into leach chemistry and reaction mechanisms for difficult to process ores [20].

Table 4. Some past research works on microbiology bioleaching.

Researcher (s)	Year	Topics	Report
Mackintosh	1976	Thiobacillus Ferrooxidans	Acidi Thiobacillus Ferrooxidans bacteria capable of fixing nitrogen are available. In the absence of sources of nitrogen, they are also able to supply the energy needs from the atmosphere [12].
Estivenson	1986		
Gostawson & Bruck	1976		Thiobacillus bacteria are capable of oxidation of sulfur in an anaerobic environment and in the presence of ferric ions instead of oxygen as a final electron receiver [12].
Li et al.	2013		Common microbes extracted from acidic mine sites consist of Acidithiobacillus [28, 29].
Rodriguez et al.	2003		
Gonzalez- Toril et al.	2003	Microbiology	Use of new molecular microbiological methods of enumeration and identification of organisms, possible to follow changes in microbial consortia as a function of time or location, and it was introduced as a valuable tool to describe or understand the biodiversity and bacterial leaching processes [30,31].
Okibe et al.	2003		
Williams et al.	1999		They reported that the possibility of utilizing specific mineral bacteria was raised [32].
Atkinson et al.	2000		They evaluated a number of hydrothermal sites and studied microbiologically [33-35].
Burton and Norris	2000		
Plumb et al.	2002		
Readett et al.	2003		Microbial growth can be promoted at heap operations by the addition of nutrients to leach solutions and by creating conditions that result in increased iron concentrations [36].
Robertson et al.	2002		Microbiological studies carried out on a number of mines [37-39].
Keeling et al.	2004		
Demergasso et al	2005		
Dopson et al.	2004		They carried out microbiological studies on acid mine drainage [40].
Hallberg and Johnson	2013		A group of acidophiles, possibly overlooked because they grow in amid pH range (pH 3–6) in an acidmine drainage system, are of particular interest because of their role in promoting the oxidation and precipitation of iron [41].
Das et al.	1997	Microbiology (Acclimatisation to the high total dissolved solids)	Increasing bacterial tolerance to high concentrations of heavy metals is a topic that has been noted [42].
Dopson et al.	2003		The mechanisms of metal resistance in acidophilic microorganisms have been reviewed [43].
Shiers et al.	2005		They used a quantitative batch culture method to investigate adaptation or habituation of a mixed culture of acidophiles to growth media containing increased concentrations of sodium sulfate or sodium chloride. Their results indicated relatively rapid adaptation to sodium sulfate at levels in excess of those normally found in process water. However, concentrations of only 7 g/L sodium chloride inhibited cell replication by more than 50%, and no significant culture adaptation occurred during prolonged exposure [44].
Franzmann et al.	2005		They were concerned with quantifying bacterial tolerance to particular leaching environments and/or with adapting bacteria to have changed conditions [45].

3. Experimental

3.1. Sample preparation

The samples were taken from low-grade sulfide ores in Sarcheshmeh Copper Mine. They were divided into four parts for several times using the cone method. Finally, using a jaw crusher, they were crushed. Then using a rifle (channel divider), the reagent and uniform samples were prepared and used for testing. The representative sample was isolated from the samples by planetary ball mill powder to a size of fewer than 180 microns (80 mesh). In order to determine the size distribution of the powder particles by the ball mill, the dimensional analysis was carried out using laboratory screens (standard ASTM-E11-01). Based upon the gradation curves, d_{80} of grinding sample after 15 minutes was obtained about 83 microns (Figure 1).

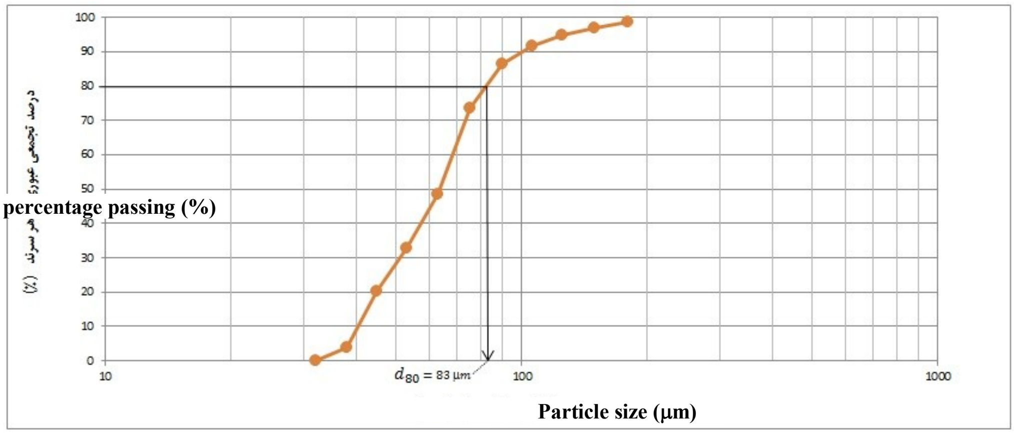

Figure 1. Sample grading chart.

The actual specific gravity of the sample was measured using a 100-mL pycnometer and calculated according to the following equation:

$$\rho = \frac{M_2 - M_1}{(M_2 - M_1) - (M_4 - M_3)} = \frac{32.5460 - 29.4220}{(32.5460 - 29.4220) - (137.9380 - 135.9630)} = 2.72 gr/cm^3 \quad (1)$$

Where M_1 is the weight of the empty pycnometer, M_2 is the weight of pycnometer and dry sample, M_3 is the weight of pycnometer and water, and M_4 is the weight of pycnometer and water and solid sample. Thus the specific gravity of the sample was obtained to be 2.27 g/cm^3.

Predicting the behavior of an ore during the bioleaching process requires a knowledge of the composition of both the quantitative and qualitative. For this reason, the chemical and mineralogical samples analyzed were cognitive. The chemical analysis was performed by atomic absorption spectrometry, and the elemental and oxide analysis was done using X-ray fluorescence. The structural analysis and mineralogical studies were performed using X-ray diffraction, and the study of minerals was done by polished sections using the optical microscopy (Tables 5 to 7). All experiments were performed in the laboratories of the Faculty of Mining, Petroleum, and Geophysics in Shahrood University of Technology.

The XRF analysis results are presented in Table 7, and the XRD results (mineralogical study) were provided in Table 8, which includes the approximate percentage of metallic minerals and each phase formed.

As it can be seen, the sample contained a mixture of minerals, oxide, and sulfide, and the pyrite in the sample was approximately 6%, which plays an important role in the process. The results of microscopic sections are shown in Figure 2. In order to study the mineralogy of the samples more accurately, they were prepared in several sections. According to the results obtained from the cross-sectional study, the participants of this research work included pyrite (FeS_2), sanidine (with the chemical formula $K[AlSi_3O_8]$), biotite (mica group of silicate minerals whose chemical formula is $K(Mg, Fe^{2+})_3(AlSi_3O_{10})(F, OH)_2$), plagioclase, hornblende, calcite, chalcopyrite, chalcocite, magnetite, and quartz as the silica gangue.

Table 5. Results of chemical analysis.

Element	Cu	Fe	S	CuO
Grade (%)	0.25	4.24	2.36	0.07

Table 6. Results of XRF (compounds in sample).

Oxide	SiO_2	Al_2O_3	MgO	Na_2O	MnO	TiO_2	CaO	K_2O	SO_3	P2O5	MoO_3	Fe_2O_3	CuO	L.O.I
Grade (%)	58.24	15.79	1.85	1.64	0.07	0.58	1.93	1.44	5.12	0.22	0.26	7.23	0.35	4.87

Table 7. Mineralogical specimens and a variety of minerals present in sample (XRD test results).

Minerals	Chalcocite	Covellite	Chalcopyrite	Pyrite	Limonite	Sphalerite	Hematite	Magnetite
Chemical formula	Cu_2S	CuS	$CuFeS_2$	FeS_2	FeOOH	ZnS	Fe_2O_3	Fe_3O_4
Wt%	0.083	0.052	0.141	6.198	1.719	0.035	0.087	0.258

Table 8. Acidophile bacteria used in this study.

Organism	Reported growth substrates	Characteristics
Acidithiobacillus ferrooxidans	S oxidation, sulfides	pH range ~ 2-4
Acidithiobacillus thiooxidans	(Af, Fe(**II**) oxidation; Fe(**III**) reduction as a facultative anaerobe)	

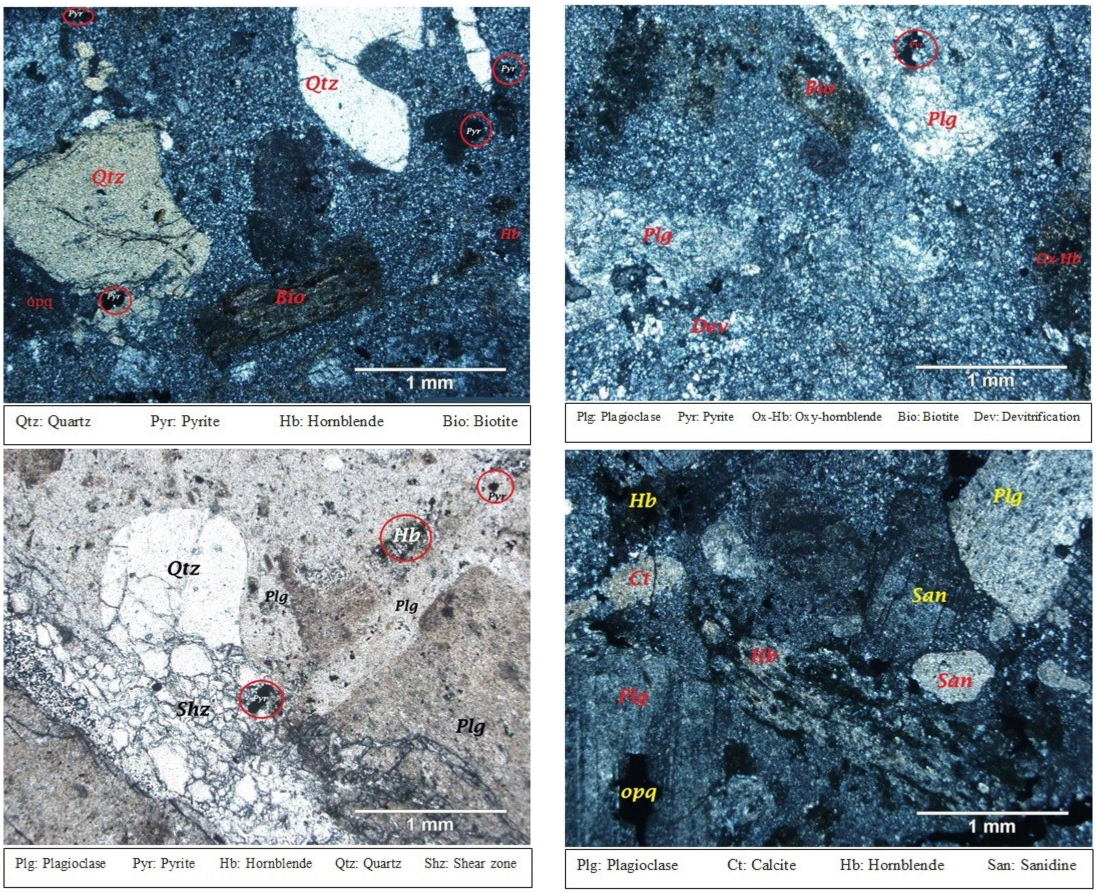

Figure 2. Image of various sections prepared from samples.

3.2. Cultivation of bacteria

The bacteria used for the bioleaching tests were mesophilic (Acidi Thiobacillus Ferrooxidanse and Acidi Thiobacillus Thiooxidanse), which were prepared from the microbial bank of Sarcheshmeh Copper Mine. These bacteria were isolated from acidic mine drainage, and after compatible with low-grade soil, they were kept in the microbial bank of the complex.

To do a primary culture of these bacteria, we used two types of the medium as Norris, and K9, based on the information obtained from the biomining book[1]. The composition and amount of salt in each one of these mediums are shown in Table 9.

The primary cultivation of bacteria was done in 250-mL Erlenmeyer flasks with volume ratio 90% medium and 10% bacterial inoculation in the shaker incubator at 35 °C with a rotation speed of

[1]- Biomining:Theory, Microbes and Industrial Processes, Douglas E. Rawlings (Ed.), Springer, 1997.

130 rpm. The acidity was measured daily and, if necessary, adjusted by sulfuric acid.
Change of the color of the medium to a milky one was a sign of the growth and activity of bacteria. However, for a greater certainty, in order to count the bacteria indirectly, an spectrophotometer (model UV-2100 Manufacturing Co. Unico of America) was used.
Due to the density of the pulp (solid percent) which was one of the factors examined in bioleaching experiments, the bacteria should be used with three pulp densities of 10, 15 and 20% were compliant. Past research works have shown that a sudden increase in the pulp density causes to kill the bacteria, thus increasing the solids was gradual. Bacteria were consistent in the medium initially at a pulp density of 2%. Then this amount rose gradually to 5, 7, 10, 12, 15, 17, and 20%. For bacteria compatibility and to perform bioleaching testing, the culture medium K9 and Norris were used. A comparison between the two medium, K9 and Norris show that the K9 compounds are more than Norris, and so in terms of nutrients they are better than Norris. Also in the primary bacterial cultivation, the efficiency of this medium was better than Norris, so in this study, K9 was chosen as the suitable medium.
Adaptation of these bacteria in different pulp densities was with different combinations of the medium (K9) and bacterial inoculation. This difference was due to the intolerance and resistance of some bacteria against the increased solid in ambient. In compatibility culture, not added any iron sulfate and sulfur to bacteria feed iron and elemental sulfur requirements by itself.

Table 9. Salts of medium used in experiments.

The Salts (gr/L)		$Ca(NO_3)_2.H_2O$	KCl	K_2HPO_4	$MgSO_4.7H_2O$	$(NH_4)_2SO_4$
medium	K9	0.014	0.1	0.63	0.5	3
	Norris	-	-	0.4	0.5	0.4

3.3. Design of experiments

The experimental design methods are commonly used to control the effects of the parameters involved and the process modeling. Its usage decreases the number of experiments, time, and material resources. Furthermore, the analysis performed on the results is easily realized and the experimental errors are minimized. Thus after sample preparation and an initial study, a series of tests were designed by Software Design Expert (Version: 7.0.0-Serial Number: STUDENT) and modeled using the response surface methodology (RSM). The effects of pH, pulp density, and speed mixer incubator on the response (Cu recovery) were modeled. For each one of these factors, two levels are shown in Table 10. The central composite design (CCD) methodology was used as the design matrix to predict the optimal level of these parameters by determining the relationship between the response and the three process parameters. Then the model equation was optimized by maximizing the total Cu recovery within the studied experimental range.

Table 10. Factors and intended levels in bioleaching tests in shaking flasks.

Parameter	Level 1	Level 2
Acidity (PH)	1.5	2.5
Pulp density (solids)	10	15
Stirrer speed incubator	120	140

3.4. Shaking flask tests

In all experiments, using Thiobacillus Ferrooxidans bacterial inoculation, 10% (by volume) was consistent with the time of each test sample taken within 20 days. The tests were performed in 500-mL Erlenmeyer flasks with a final volume of 200 mL of the K9 culture medium in a shaking incubator (model SKIR-601 Company Finetech, Korea) with a temperature of 35 °C at the atmospheric pressure.
The pH values of the flasks were measured daily. In the bioleaching processes, the pH value decreased gradually due to the bacterial activities and production of sulfuric acid by the bacteria. In Figures 3 to 7, charts related to pH changes are shown separately for different pH values. As it can be seen, the pH values during the process on end days were without any change, which can be explained as being due to slower reactions. Changes in pH = 1 was very low because the bacterium used did not grow and died.

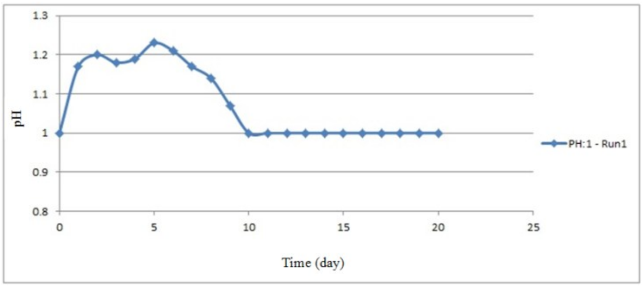

Figure 3. pH shift over time in initial pH = 1.

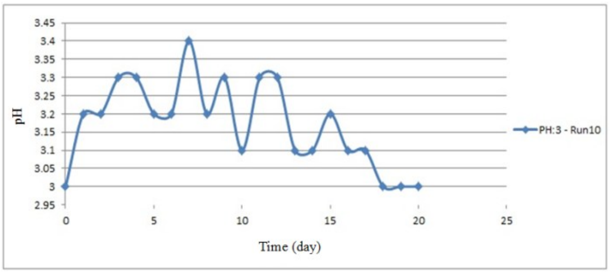

Figure 4. pH shift over time in initial pH = 3.

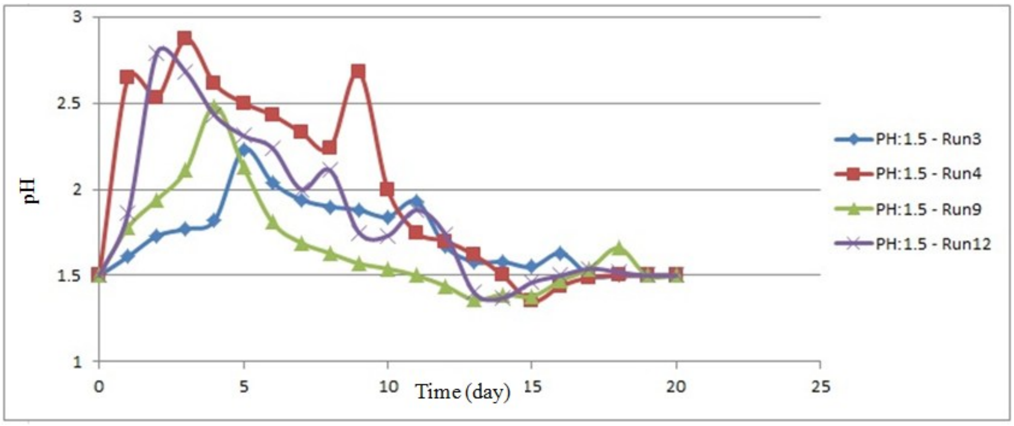

Figure 5. pH shift over time in initial pH = 1.5.

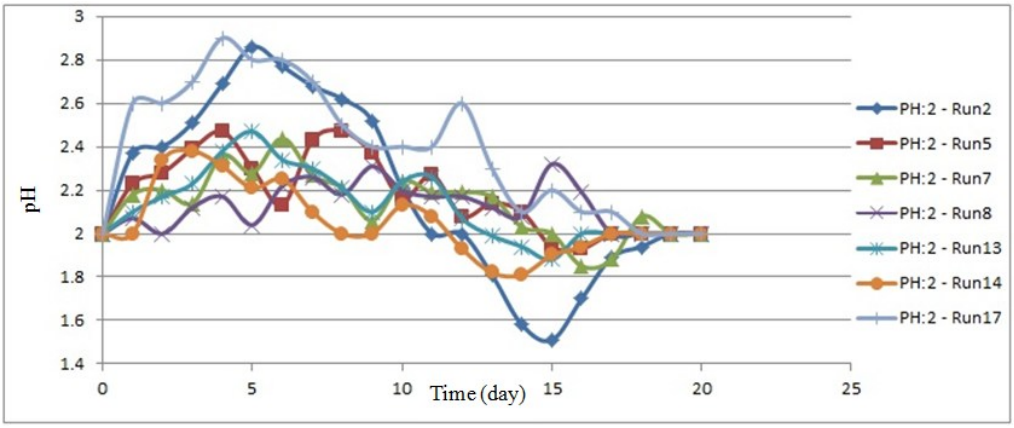

Figure 6. pH shift over time in initial pH = 2.

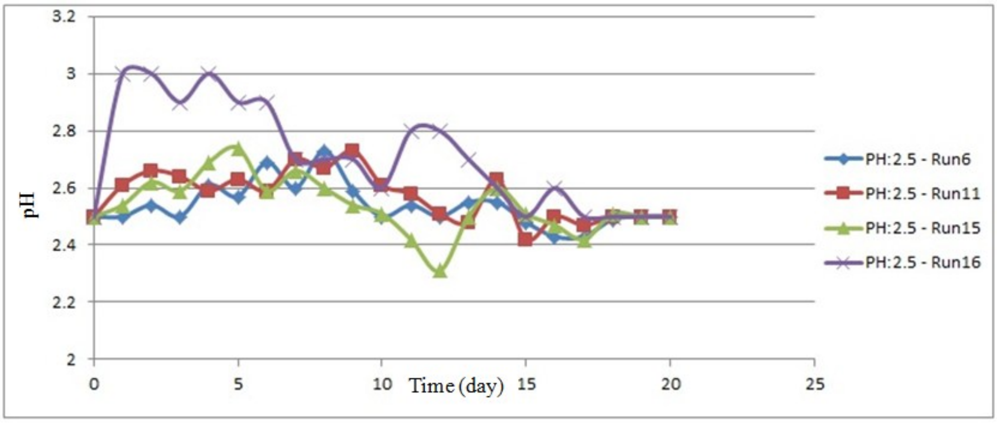

Figure 7. pH shift over time in initial pH = 2.5.

The parameters studied were acidity (pH), pulp density (solids), and stirrer speed incubator. The final copper recovery was the dependent parameter response in the experiments. After 20 days, the liquid samples were collected at Falcon tubes using the centrifuges (Hettich, model EBA 270, made in Germany) with a 4000 rpm spin and the separated solid after drying, prepared for chemical analysis tests using atomic absorption (model: Solarr S Series, Thermal element Co., England), and the recovery results were analyzed using the experimental software.

4. Results and discussion

The response data was analyzed using the experimental software, and the effects of the parameters involved were calculated. The statistical parameters such as F-Value and lack-of-fit were used. The statistical analysis results for the response are summarized in Table 11.

According to the ANOVA table, the Model F-value of 37.40 implies that the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. The values of "Prob > F" less than 0.0500 indicate that the model terms are significant. Also in this case, A, B, C, and AB are significant model terms. The "Lack-of-Fit F-value" of 2.38 implies that the Lack-of-Fit is not significant relative to the pure error. There is a 33.23% chance that a "Lack-of-Fit F-value" this large could occur due to noise. A non-significant lack-of-fit is good. The "Pred R-Squared" of 0.8422 is in reasonable agreement with the "Adj R-Squared" of 0.9010. "Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. A ratio of 18.364 indicates an adequate signal. This model can be used to navigate the design space.

Thus according to the above table, the suggested model based coded values is the following equation:

$$Cu \text{ Recovery} = 58.35 - 5.81A - 6.19B + 2.19C - 2.63AB \quad (2)$$

As it can be seen, the fitted model is significant statistically at the 95% confidence level (impact of the error is less than 0.05) and possibility of effects of the error (0.0001) in the model is less than 0.05.

In Figure 8, the graph of normal distribution of residuals is shown, and in Figure 9, correlation between the predicted values and actual values that obtained from the experiments to validate the model is shown. As it can be seen, the residuals of recovery are along the line, and there are no significant deviations from the normal line. Thus the selected model predicts the copper recovery very well within the various factor levels. A comparison between the results of the tests and the results obtained from the defined model shows that copper recovery is consistent with the model, which increases the confidence of the fitted model.

In order to verify the authenticity of the fitted model to predict the response test, it is necessary to check the error analysis. The results of error analysis are shown in Figs. 10 and 11. As it can be seen in Figure 10, residuals versus predicted values of response do not follow a specific pattern. In the case of residues randomly distributed, shows the accuracy of the model to predict the response. Also in Figure 11, existence of a pattern of residual value versus sequence of testing is investigated. According to the chart, we observe that the amount of residues is scattered randomly, which means that the residuals are independent from the testing sequence, and this is desirable.

In Figure 12, the graph of recovery versus pH variations is plotted. It can be observed that the recovery is greater for lower pH values. More acidity in the environment causes greater dissolution of the ore. In Figure 13, the graph of recovery versus solids is plotted. It is observed that the recovery is greater for a lower pulp density (lower solid percent).

In Figure 14, the chart of recovery versus stirrer speed is plotted. It can be seen that the recovery is higher for higher stirrer speeds. In Figure 15, interaction of pH and solid percent to the recovery of copper in bio-leaching experiments is shown.

According to the results obtained from the model and also the variance analysis, optimization was performed using the software DX7. The optimum levels for the parameters are shown in Table 12.

In order to validate the proposed model, from the proposals with the predicted points by the experimental software to numerical optimization parameters, the first experiment was selected as the best plan and was performed with three repetitions. The average recovery of copper from three experiments was 70.2766.

Table 11. Analysis of variance (ANOVA) of experimental design.

Response	1	CU Recovery				
ANOVA for Response Surface Reduced 2FI Model						
Analysis of variance table [Partial sum of squares-Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	1284.81	4	321.20	37.40	< 0.0001	significant
A-PH	540.56	1	540.56	62.94	< 0.0001	
B-Solid Percent	612.56	1	612.56	71.32	< 0.0001	
C-Speed	76.56	1	76.56	8.91	0.0114	
AB	55.13	1	55.13	6.42	0.0263	
Residual	103.07	12	8.59			
Lack of Fit	95.07	10	9.51	2.38	0.3323	not significant
Pure Error	8.00	2	4.00			
Cor Total	1387.88	16				
Std. Dev.	2.93			R-Squared	0.9257	
Mean	58.35			Adj R-Squared	0.9010	
C.V. %	5.02			Pred R-Squared	0.8422	
PRESS	219.00			Adeq Precision	18.364	

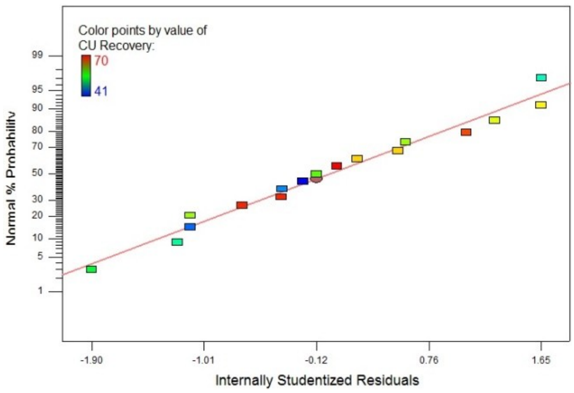

Figure 8. Graph of normal distribution of residuals.

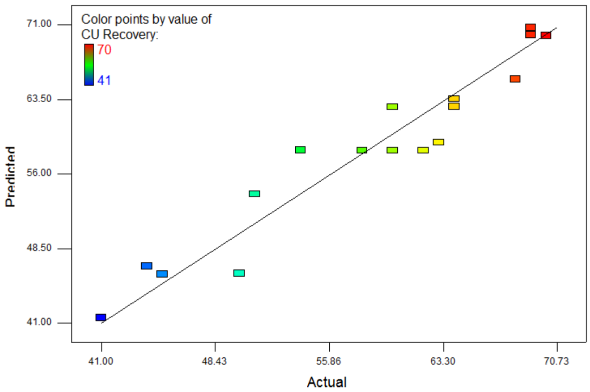

Figure 9. Predicted values to actual value.

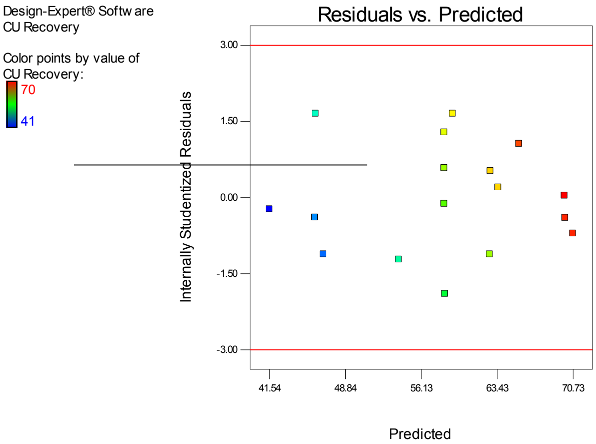

Figure 10. Residual variations *vs.* predicted values of recovery.

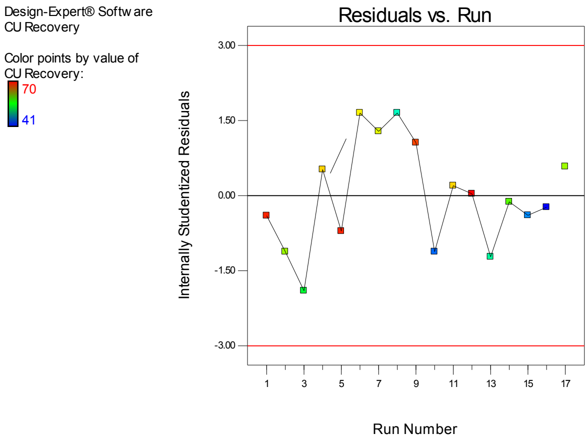

Figure 11. Residual variations *vs.* sequence of tests.

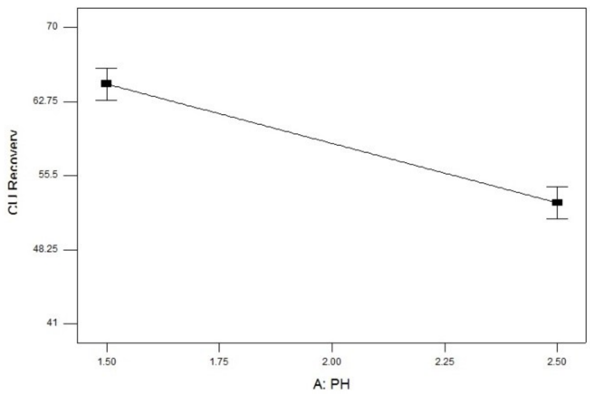

Figure 12. Chart of recovery for various pH values.

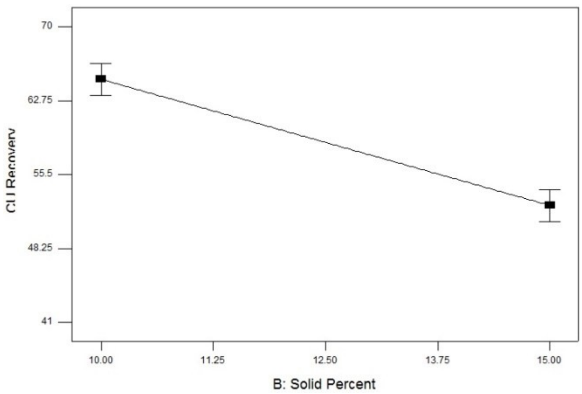

Figure 13. Chart of recovery for various solids.

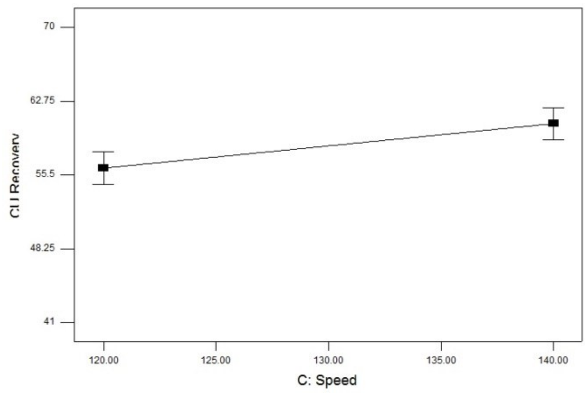

Figure 14. Cu recovery for various speeds of shaking.

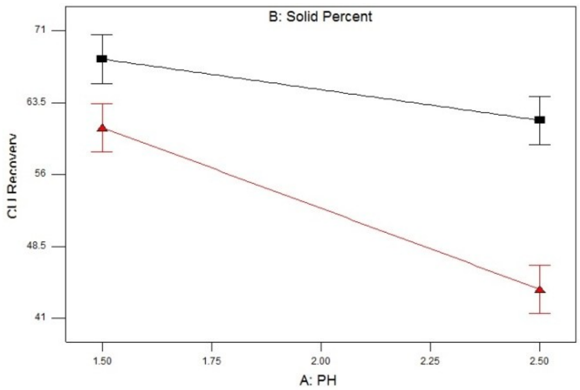

Figure 15. Interaction of pH and solid percent.

Table 12. Optimal levels for parameters.

Constraints						
Name	**Goal**	**Lower Limit**	**Upper Limit**	**Lower Weight**	**Upper Weight**	**Importance**
pH	Is in range	1.5	2.5	1	1	3
Solid Percent	Is in range	10	15	1	1	3
Speed	Is in range	120	140	1	1	3
Cu Recovery	maximize	41	70	1	1	3
Solutions						
Number	**pH**	**Solid Percent**	**Speed**	**Cu Recovery**	**Desirability**	
1	1.50	10.00	140.00	69.9151	0.997	Selected
2	1.50	10.00	140.00	69.8852	0.996	
3	1.50	10.03	140.00	69.8669	0.995	
4	1.51	10.00	140.00	69.849	0.995	
5	1.50	10.01	139.74	69.8488	0.995	
6	1.50	10.10	140.00	69.7686	0.992	
7	1.53	10.00	140.00	69.752	0.991	
8	1.50	10.13	140.00	69.7276	0.991	
9	1.52	10.06	140.00	69.7239	0.990	
10	1.50	10.17	140.00	69.6698	0.989	
10 Solutions found						

5. Conclusions

The results of copper analysis using atomic absorption were performed using the experimental software DX7 and ANOVA with significant differences 95% and in risk of 5%, and in some cases, the risk was 1%. The initial pH value of the medium decreased with time, which was due to the bacterial activity and production of sulfuric acid by them. It was observed that the pH values at the end of the trial period had no change that can be explained due to the slow reactions but at a very low pH value (pH 1), the studied bacteria did

not grow and were destroyed. The recovery results were analyzed by the DX7 software. The parameters pH, solid percent, stirrer speed, and interaction of pH and solids were identified as the factors affecting the process.
To choose the best model, variance analysis of data was performed using the DX7 software. The fitted model was statistically significant at the 95% confidence level, and the possible influence of error in the model was less than 0.05. The adequate precision of the proposed model was 18.364, being highly desirable.
The results of final copper recovery from bioleaching tests, conducted on low-grade sulfide ore samples of Sarcheshmeh Copper Mine by acidi Thiobacillus Ferrooxidanse bacteria in shaking flasks after 21 days in the K9 culture medium at a temperature of 35 °C showed that the maximum copper recovery was 69.91%, corresponding to 10% solids and a stirring speed of 140 rpm with pH 1.5. In order to validate the proposed model, from the proposals with the predicted points by software to numerical optimization parameters, the first experiment was selected as the best plan and with three repetitions performed. The copper recovery results for these three repeats proved the validity of the proposed model.

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بهینه‌سازی پارامترهای مؤثر بر بازیابی مس از کانی‌های سولفیدی کم‌عیار سرچشمه به‌روش بیولیچینگ

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چکیده:

در این پژوهش پارامترهای مؤثر بر استحصال مس از کانی‌های سولفیدی کم‌عیار مس سرچشمه‌ی کرمان مورد بررسی قرار گرفت. کانسنگ سولفیدی کم‌عیار به کار رفته، دارای عیار مس برابر ۰/۲۵ درصد است که حدود ۲۸ درصد آن را کانی‌های اکسیدی و بقیه را کانی‌های سولفیدی تشکیل می‌دهند. بخش عمده‌ی کانی سولفیدی شامل پیریت و کالکوسیت است و کانی‌های باطله بیشتر کوارتز، آنورتیت، بیوتیت و مسکویت است. به منظور بررسی هم زمان درصد جامد (۱۰ تا ۲۰ درصد) و اسیدیته‌ی محیط (۱/۵ تا ۲/۵) به همراه دور همزن (۱۱۰ تا ۱۵۰ دور در دقیقه)، ابتدا جدایش باکتری از معدن مس سرچشمه انجام شد، سپس بعد از سازگاری با نمونه، آزمایش‌های بیولیچینگ طبق الگوی تعریف شده توسط نرم‌افزار DX7، در ظروف لرزان انجام شدند و با استفاده از روش پاسخ سطح، بازیابی مس مدل‌سازی و بهینه‌سازی شد و پارامترهای مؤثر، مورد مطالعه مقایسه‌ای قرار گرفتند. به منظور پیش‌بینی سطوح بهینه این پارامترها، از روش طراحی نقاط مرکزی استفاده شد و در نهایت معادله مدل بهینه ارائه شد. نتایج آزمایش‌ها نشان می‌دهند که افزایش درصد جامد (از ۱۰ به ۲۰ درصد) برای باکتری نامناسب است. بالاترین بازیابی مس پس از ۲۱ روز در دمای ۳۵ درجه و با استفاده از باکتری اسیدی تیوباسیلوس فرو اکسیدانس و محیط کشت K9، در محیطی با دانسته‌ی پالپ ۱۰ درصد و اسیدیته ۱/۵ برابر ۶۹/۹۱٪ به دست آمد.

کلمات کلیدی: بهینه‌سازی، مس، بیولیچینگ، باکتری، اسیدی تیوباسیلوس فرو اکسیدانس.