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The Effect of Different Additives and Medium on the Bioleaching of Molybdenite for Cu and Mo Extraction Using Mix Mesophilic Microorganism

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Abstract

Bioleaching processes for extraction of Cu and Mo from molybdenite cons. are more environmentally friendly and consume less energy than conventional technologies, yet less economically efficient. One necessary step towards arriving at a cost-effective bioleaching process is using appropriate methodology to optimize pertinent factors in such processes. To this end, the present study employed Response Surface Methodology to optimize important factors in a molybdenite bioleaching process by mix mesophilic microorganism using shake flasks. The effect of change in the levels of molybdenite concentration, pyrite and silver ion concentration as additives - in the range 3-9%, 1-5%, and 0-1.2gr/l, respectively - on the rate of Cu and Mo bioleaching was studied using a Central Composite Design. The results showed a statistically significant effect of silver ion and molybdenite concentration, and to a lesser pyrite concentration, on the rate of bioleaching of Cu and Mo. Further, different mediums and additives were evaluated for copper and molybdenum extraction from molybdenite concentrate in bioleaching process. Small amounts of silver (100mgr/l $AgSO_4$) dramatically accelerated the copper dissolution process. Addition of FeS_2 and sulfur with ferrous sulfate accelerated the acidification and raised the oxidation-reduction potential of solution (medium) with an inoculation of 15% (v/v) of active and adapted indigenous mesophilic bacteria, thus resulting in an overall increase in Mo dissolution efficiency.

Keywords: additive, biohydrometallurgy, Cu and Mo dissolution, mixed mesophilic bacteria, modeling and optimization, molybdenite concentrate.

1. Introduction

Identification of the correct optimum values of the effective parameters in biotechnological processes is a prerequisite for their successful commercial exploitation. This is particularly the case when these processes have to compete with alternative chemically or physically based processes [1]. An example of such a process is the bioleaching of sulfide minerals such as chalcopyrite and molybdenite. Alternative hydrometallurgical or pyrometallurgical technologies for extracting Cu from copper sulfide sources are currently available and operating at commercial scale. However, processes based on these technologies are energy-intensive and also cause environmental pollution [2,3]. Bioleaching, on the other hand, is a less energy-intensive and an environmentally friendly process [2-4]; however, it will only be commercially applied for extracting Cu from chalcopyrite or Mo from molybdenite, provided that the process also proves to be economically efficient. Physicochemical parameters identified in previous research as significantly influencing the rate of Cu and Mo dissolution from molybdenite are pH, temperature and initial ferrous iron concentration, ORP, type of microorganism, solid percent, etc. The proper choice of the value of these parameters is necessary if an economic molybdenite bioleaching process is to be developed. The wide range of values used or reported as optimum for the above-mentioned physicochemical parameters can partly be explained by different

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strains of microorganism and, to a lesser extent, by the type of sulfide source employed in these studies. However, such a wide range of values cannot be fully explained by these factors and the optimum level of these parameters might also be influenced by the level of the other important parameters in the molybdenite bioleaching process. In statistical parlance, there might be an interaction between effective parameters in such a process. A survey of previous literature on molybdenite bioleaching provides no clues as to whether such interaction between the important process parameters in a chalcopyrite and molybdenite bioleaching process exists. This is because previous studies have adopted one-factor-at-a-time methodology to optimize the above-mentioned parameters [1, 5-17].

This methodology is very inefficient and provides no information about interactions between parameters in a process. The only methodology capable of providing an answer to this question is factorial design of experiments (DOE), which – through the use of techniques such as Response Surface Methodology (RSM) – is able to simultaneously consider several factors at different levels, and give a suitable model for the relationship between the various factors and the response [18]. However, RSM has been applied in only a few cases to a bioleaching process [19-21]. There are full as well as fractional factorial DOEs; the former gives the most complete information regarding interaction between parameters but the number of experiments becomes excessive when the number of factors or their levels become relatively large. Additionally, higher order interactions are usually statistically insignificant and, consequently, the corresponding information is not very useful [18]. Fractional factorial designs (FFD), such as central composite design (CCD) or Box–Behnken, can give information regarding parameter interactions with the use of less experimentation; however, reliable information about first order interactions can only be obtained from the results of DOEs which are not highly fractionated [18, 19, 22].

The aim of the present work was to identify and quantify the interaction between important physicochemical parameters i.e. solid percent, and additive concentration (silver ion, pyrite, sulfur and ferrous sulfate)) in a molybdenite bioleaching process by using appropriate methodology, namely RSM. Shake flask cultivation was employed as it is the method commonly used for preliminary optimization of parameters in a bioleaching process [20, 21, 23]. The CCFCD (central composite face centered design) was chosen as the design matrix since it allows reliable identification of interaction between factors and provides a model which can be used to predict optimum level of these parameters [18]. We further evaluated the dissolution of copper and molybdenum during bioleaching of molybdenite concentrate in different conditions using a native mixed culture of mesophilic microorganisms.

2. Materials, Methods and Design Procedure

2.1. Response surface methodology (RSM)

Response surface methodology is a collection of statistical and mathematical methods that are useful for modeling and analyzing engineering process. In this technique, the main objective is to optimize the response surface that is influenced by various process parameters. RSM also quantifies the relationship between the controllable input parameters and the obtained response surfaces. The design procedure of RSM is as follows [6, 24-29]:

1. Designing a series of experiments for adequate and reliable measurement of the response of interest;
2. Developing a mathematical model of the second-order response surface with the best fittings;
3. Finding the optimal set of experimental parameters that produces maximum or minimum value of response;
4. Representing the direct and interactive effects of process parameters through 2D and 3D plots.

If all variables are assumed to be measurable, the response surface can be expressed as follows [6, 29-31]:

$$y = f(x_1, x_2, x_3, ..., x_k), \qquad (1)$$

where y is the answer of the system and xi are the variables of action called factors. The objective is to optimize the response variable (y). It is assumed that the variables are independent and continuous and controllable by experiments with negligible errors. It is required to find a suitable approximation for the true functional relationship between independent variables and the response surface. Usually a second-order model is utilized in RSM as follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \varepsilon, \qquad (2)$$

where x_1, x_2, ... , x_k are the input factors that influence the response y. β0, βii (i=1, 2, ... , k), βij (i=1, 2, ... , k; j=1, 2, ... , k) are unknown parameters, and e is a random error. The b coefficients, which should be determined in the second-order model, are obtained by the least square method. In general, Equation 2can be written in matrix form as Equation 3 [6, 30-32]:

$$Y = bX + \varepsilon, \qquad (3)$$

where Y is defined to be a matrix of measured values, X is a matrix of independent variables. b and ε consist of coefficients and errors, respectively. The solution to Equation (3) can be obtained by the matrix approach [6, 30-32]:

$$b = (X^{'}X)^{-1}X^{'}Y, \qquad (4)$$

where X' is the transpose of the matrix X and $(X'X)^{-1}$ is the inverse of the matrix X'X.

The experimental design techniques commonly used for process analysis and modeling are the full factorial, partial factorial and central composite rotatable designs. A full factorial design requires at least three levels per variable to estimate the coefficients of the quadratic terms in the response model. A partial factorial design requires fewer experiments than the full factorial design does. However, the former is particularly useful if certain variables are already known to show no interaction [33, 34].

An effective alternative to factorial design is central composite face centered design (CCFCD), originally developed by Box and Wilson and improved by Box and Hunter [35, 36]. The CCFCD gives almost as much information as a three-level factorial, requires many fewer tests than the full factorial design. It has also been shown to be sufficient to describe the majority of steady-state process responses [33, 37, 38]. Therefore, in this study, it was decided to use CCFCD to design the experiments. The tests required for CCFCD included the standard 2^k factorial with its origin at the center, 2k points fixed axially at a distance, say β, from the center to generate the quadratic terms, and replicate tests at the center; where k is the number of variables. The axial points were chosen such that they allow rotatability [36], ensuring that the variance of the model prediction is constant at all points equidistant from the design center (in central composite rotatable design). Replicates of the test at the center are very important as they provide an independent estimate of the experimental error. Once the desired ranges of values of the variables were defined, they were coded to lie at ±1 for the factorial points, 0 for the center points and ±β for the axial points. As shown in Table 1, the codes were calculated as functions of the range of interest of each factor [31]. In this method, ±β is equal to ±1.

Table 1. Relationship between coded and actual values of a variable

Code	Actual value of ariable
-β	x_{min}
-1	$[(x_{max} + x_{min})/2] - [(x_{max}-x_{min})/2\alpha]$
0	$(x_{max} + x_{min})/2$
+1	$[(x_{max} + x_{min})/2] + [(x_{max}-x_{min})/2\alpha]$
+β	x_{max}

x_{max} and x_{min} = maximum and minimum values of x, respectively; $\alpha = 2^{k/4}$; k= number of variables.

2.2. Concentrate preparation and characterization studies

A representative sample of molybdenite concentrate from the Sarcheshmeh Copper Complex of Iran with a size distribution of 90 wt. % less than 38 μm was used for bioleaching tests. The d_{80} of the sample achieved 33 microns using cyclosizer equipment. Separation of molybdenite from copper bearing minerals was carried out by using a rougher and sequential seven flotation cells as cleaner in four series bank with capacity of approximately 20 tons per day (final concentrate). The chemical analyses of the 5th, 6th and final concentrate are given in Table 2. As shown in the table, there is 0.98% Cu in the 5th cleaner sample which shows highest grade of the copper content in three cleaners. Therefore, the 5th cleaner sample was used in the bioleaching experiments.

Table 2. Chemical analyses of the molybdenite concentrate in three cleaner stages

Component	5th Cleaner	6th Cleaner	Final Cons.
Cu	0.98	0.91	0.44
Fe	1.56	1.43	1.17
Mo	53.84	54	54.88
Total	56.38	56.34	56.49

Semi quantitative X-ray diffraction (SQXRD) technique was used to define the main and the trace minerals in the sample. X-ray powder diffraction patterns were obtained using a Siemens D-500 diffractiometer with Ni-filtered Cu–Ka radiation, and a goniometer speed of 1° 2ɵ/min. The diffraction profiles with a 0.01 precision of d-spacing measurements were conducted from 5° to 70° (2ɵ). The results of the mineralogical studies revealed that molybdenite is the major phrase of the Mo bearing mineral. Also, mineralogical study of the sample showed molybdenite (MoS_2) as the main mineral phase and chalcopyrite ($CuFeS_2$) and pyrite (FeS_2) as the minor which are presented in Figure 1. As it can be observed, chalcopyrite and pyrite can be distinguished in the molybdenite cons. as unwanted minerals. These minerals are liberated from the molybdenite in this size fraction but in some cases, cu bearing minerals and also pyrite are interlocked with molybdenite. Optical microscopy provided information on color, morphology and crystallinity. The preparation procedure is as follows. Small amounts of the sample were dispersed on a glass slide and were then mounted in embedding resin (Entellan (Merck) or Canada balsam) and overlain with a cover slip. This technique was routinely used both in the field and in the laboratory.

For better understanding of the presence of major and minor minerals in the molybdenite concentrate, the SEM–EDS analysis was carried out. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analyses (EDAX) were conducted on the polished and grain samples. The polished sections and grain samples were gold coated to obtain electron conductive surfaces. The examination of the internal structure of the samples, the distribution of mineral phases, and the choice of areas for EDAX-analyses were carried out in a scanning electron microscope using the back scattered electrons (BSE) technique. The results also confirmed the mineralogical observation.

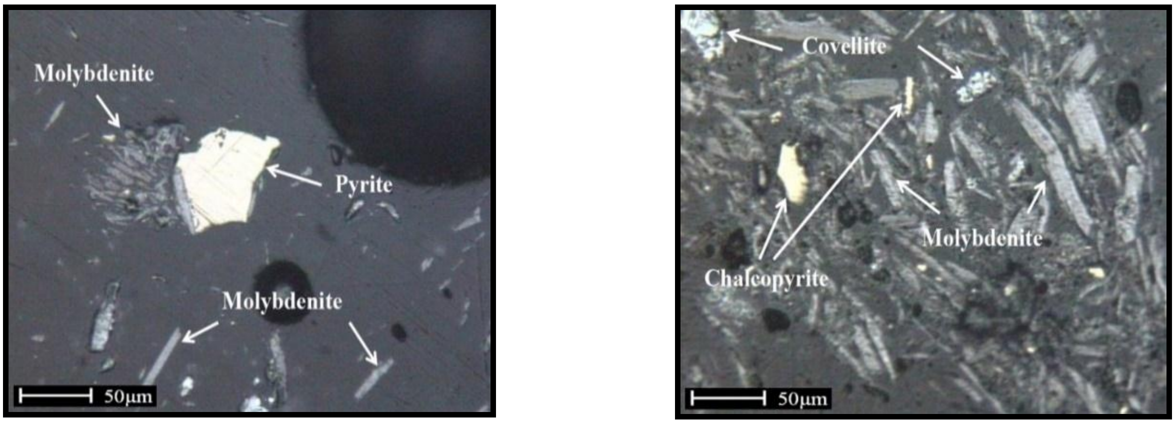

Figure 1a. Mineralogical observations from Molybdenite concentrate with chalcopyrite and pyrite minerals

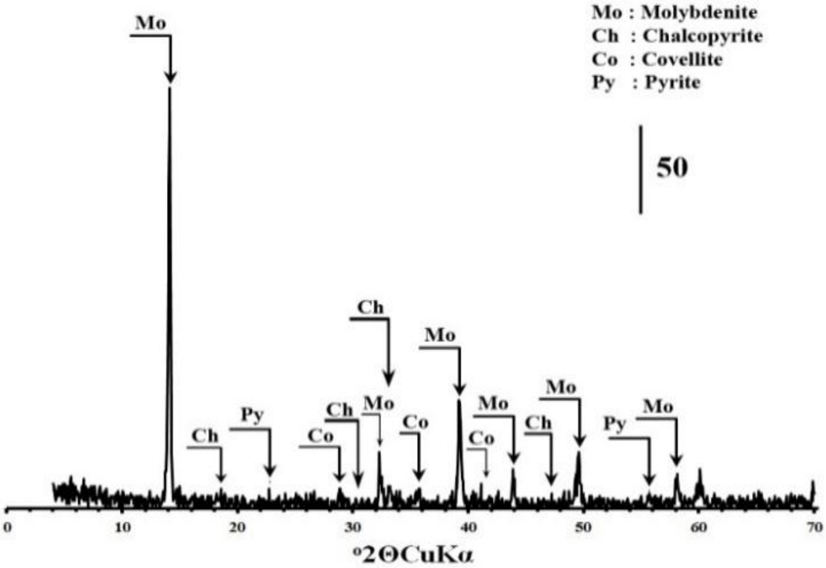

Figure 1b. X-ray diffractogram of molybdenite concentrate with chalcopyrite, covellite and pyrite minerals as impurities.

2.3. Culture media and microorganisms

Local mix strains of mesophilic microorganisms were enriched from mine drainage of the Sarcheshmeh mine and used throughout the present study (Figure 2). To establish resistance of microorganisms to molybdenum, the microorganisms were grown on molybdenite concentrate in 9K medium containing (per liter of distilled water) 3.0g $(NH4)_2SO_4$, 0.5g $MgSO_4{\bullet}7H_2O$, 0.1g KCl, 0.5g K_2HPO_4, 0.01g $Ca(NO_3)_2$, and 44.22g $FeSO_4{\bullet}7H_2O$; the pH was adjusted to 1.8 with H_2SO_4 [39]. The study was performed with mix mesophilic cultures, which were maintained in the microbiology laboratory of the Sarcheshmeh mine. The mesophile culture contains *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* [40, 41]. The cultures were sub-cultured through several transfers in the concentrate medium in order to adapt the microorganisms to the experimental conditions. The growth experiments showed that the isolated indigenous sulfur-iron oxidizing bacterium could grow in a wide pH range of 1.5-5.0 and had a strong ability to oxidize Fe^{2+} or reduced sulfur compounds. The experiments also showed strong adaptation ability to grow in the high amount of molybdenite concentration. In order to reproduce the data, the same bacterial culture was used as inoculum in the study of each variable.

2.4. Adaptation process

The mixed culture was used in adaptation experiments in which the bacteria gradually acclimatized themselves to different molybdenite concentrations as sole energy source. The isolates were adapted and grown with gradually increased concentrations of molybdenite up to 10% (w/v) in 500 mL Erlenmeyer flasks containing 200 mL of nutrients and bacteria over three months. Mix cultures of mesophilic microorganisms were incubated at 32°C in a temperature-controlled orbital shaker (Innova 4200 model, New Brunswick Scientific Company, USA) at 150 rpm. The pH was adjusted to 1.8 by adding pure sulfuric acid and, consequently, bacterial growth was monitored by measuring the oxidation–reduction potential (ORP) using Eh meter (WTW model 323) and pH using a pH meter (Metrohm model 827). Cell counts were determined using a Neubauer chamber counter with a depth of 0.02 mm and area of 1/400 mm^2 under a light microscope. The adapted bacteria sub-cultured in 9K medium were used in the subsequent experiments. (Count, Eh and pH curve). Furthermore, adaptation tests were carried out with 20% of inoculation, $Fe^{2+}/Fe^{3+}=10$ (Ferro: 10 gr, Ferric: 1gr), 2 gr of sulfur and molybdenite concentrate by 1, 3, 5 and 10 gr in 200 mL of the solution. The original counts of the mesophile were 4×10^8. The Eh and pH of mix mesophilic bacteria in the adaptation stages are given in Figure 3. In addition, the counts of mix culture mesophilic bacteria after every adaptation stages were 3.2×10^8, 2.2×10^8, 1.8×10^8 and 1.4×10^8, respectively. It is obvious that by increasing solid concentration, the adaptation rate and also count of bacteria decreases.

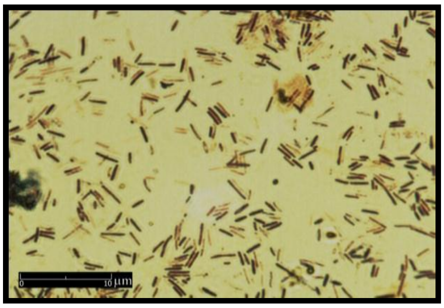

Figure 2. Mixed cultures of mesophilic bacteria, the scale bar is 10µm

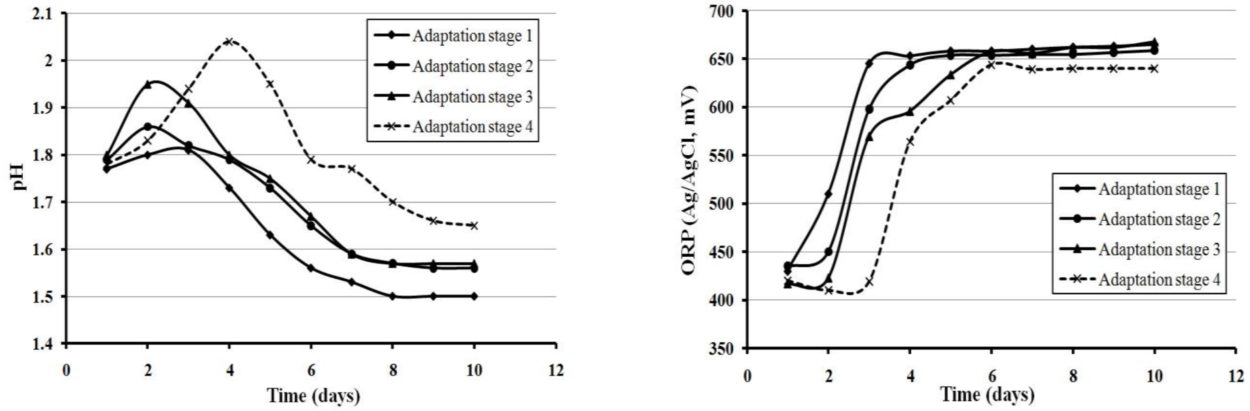

Figure 3. The changes of Eh and pH in adaptation stages for mesophilic bacteria

2.5. Bioleaching tests and sampling procedure

Bioleaching experiments were carried out in 250 mL Erlenmeyer flasks incubated at a constant temperature of 32°C in an orbital shaker agitating at 150 rpm. Each flask contained 85mL of 9Kmedium, different amounts of molybdenite concentrate (3-9% pulp density, unless otherwise stated) and the corresponding additives (pyrite and silver ion) according to each case. After 1 day of chemical conditioning, the pH of the leach suspensions was adjusted to specific values (1.6 or 1.8) with 6M H2SO4, and 15 mL of an active microbial culture, previously adapted to the conditions used, was added. Abiotic control tests received 15mL of 2g/L thymol in methanol (or formaldehyde) instead of the inoculum. The redox potential was measured using a Pt combination redox electrode with an Ag/AgCl reference electrode. The pH was monitored using a gel-filled combination pH probe with an Ag/AgCl reference electrode. Water lost by evaporation was compensated by adding distilled water. Periodically, pH and the redox potential were measured and 5mL of sample was removed from the leach solutions and analyzed for copper, molybdenum and iron by ICP method. The sample removed was replaced with 9K nutrient medium. Bacterial population was determined weekly using a counting chamber and an optical microscope. At the end of the experiments, the solids were filtered and a chemical analysis of the residues was accomplished to complete the mass balance for the calculation of final copper, molybdenum and iron extractions. The oxidation of Fe^{2+} to Fe^{3+} was considered as an indication of its growth. Growth was also monitored through cell count which was determined in a Neubauer counter using Zeiss Biological Microscope. In the bioleaching studies, samples were taken over the 5-day reaction period at pre-determined intervals and analyzed for Cu, Mo and Fe by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). To calculate the fraction of Cu, Mo and Fe leached, the following equation was used [42]:

$$X_i = \frac{(V_0 - \sum_{i=1}^{i-1} v_i)C_i + \sum_{i=1}^{i-1} v_i C_i}{M(\frac{C_M}{100})} \qquad (5)$$

where X_i is the Cu, Mo or Fe extraction corresponding to sample i, V_0 the initial volume of the leaching solution in the reactor (ml), vi the volume of sample i withdrawn from the reactor (ml), C_i the Cu, Mo or Fe concentration in sample i (mg/l), M the initial mass of the molybdenite concentrate in grams added into the reactor, and C_M the Cu, Mo or Fe percentage in the molybdenite concentrate.

Three effective parameters, including molybdenite percent, pyrite percent and silver ion concentration (grl^{-1}), were selected to be applied in designing experiments according to the preliminary experiments. The CCFCD was employed to design the experiments. The range of values for each variable is given in Table 3. The conditions of bioleaching experiments (coded and actual values of levels) and their results are also shown in Table 4. In these experiments, eight tests as full factorial by three factors in high and low levels and six tests in axial points were implemented. Validation of models was finally investigated by performing two replicated tests.

Table 3. Independent variables and their levels for central composite design (CCFCD)

Coded variable level			*Symbol*	*Variable*
low	*center*	*high*		
-1	*0*	*+1*		
3	6	9	A	**Molybdenite percent (%)**
1	3	5	B	**Pyrite percent (%)**
0	0.6	1.2	C	**Silver ion concentration (grl^{-1})**

Table 4. Coded and actual levels of variables with experimental results

Run no.	*Coded level of variables*			*Actual level of variables*			*Observed recovery*	
	A	*B*	*C*	*A (%)*	*B (%)*	*C (grl^{-1})*	*Cu (%)*	*Mo (%)*
1	-1	-1	-1	3	1	0	39.62	3.44
2	+1	-1	-1	9	1	0	42.91	2.06
3	-1	+1	-1	3	5	0	39.62	3.7
4	+1	+1	-1	9	5	0	38.15	1.68
5	-1	-1	+1	3	1	1.2	47.11	0.26
6	+1	-1	+1	9	1	1.2	88.49	0.26
7	-1	+1	+1	3	5	1.2	47.11	0.26
8	+1	+1	+1	9	5	1.2	91.43	0.19
9	-1	0	0	3	3	0.6	94.73	0.39
10	+1	0	0	9	3	0.6	86.45	0.18
11	0	-1	0	6	1	0.6	91.58	0.27
12	0	+1	0	6	5	0.6	90.9	0.2
13	0	0	-1	6	3	0	38.52	1.71
14	0	0	+1	6	3	1.2	42.6	0.27
15	0	0	0	6	3	0.6	84.77	0.33
16	0	0	0	6	3	0.6	88.18	0.37
17	0	0	0	6	3	0.6	89.41	0.13
18	0	0	0	6	3	0.6	87.55	0.35
19	0	0	0	6	3	0.6	89.75	0.27
20	0	0	0	6	3	0.6	86.49	0.12

The changes in pH, ORP, Total Fe concentration and cell counts over time are shown in Figure 4. As it can be seen in Figure 4a, the ore material shows a little acid demand for the first two days before the reaction became acid producing. The bioleaching tests lasted 30 days and pH of the solution was decreased to around pH=1.2 and pH=0.9 in 16 days with and without silver ion, respectively, and remained without changes thereafter. The variation of solution potential in absence and presence of silver ion are presented in Figure 4b. Addition of silver ion to the solution causes the solution potential to decrease, even less than 400 mV. The silver ion presence has two different effects in the bioleaching solution. In this situation, electron transition is done easily and also bacterial population decreases because of the toxicity effect of the silver ion on the bacterial activity. In addition, when the pyrite is added to the solution, ORP of the solution increases even more than 700 mV in 8 days. In all tests, Eh of the solutions was changed within 8 days and remained constant until the end of the bioleaching process. The total iron concentration and microorganism population were remained approximately constant, when silver nitrate, in different values, was added to bioleaching solution (Figure 4c, d). In contrast, the total iron concentration and microorganism population were drastically increased, when pure pyrite, as alternative energy, and iron source were added, especially in low amount of solid percent including molybdenite and pyrite minerals. Furthermore, acidity and ORP of the sterile test solution were approximately 1.8 and 320 (mV.) up to the end of the experiment.

3. Result and discussion

According to the design matrix, 20 tests were conducted in order of the sequence defined by DX7 software. Two responses, i.e., Cu and Mo recoveries, were evaluated. The results are shown in Table 4. The design matrix of the variables in actual units is given in Table 5 along with the predicted and experimental values of responses (Cu, and Mo recoveries). It should be noted that the predicted values of responses were obtained from model equations using the software.

3.1. Model construction for copper recovery

The experimental results in Table 5 were fitted to a model equation by applying multiple regression analysis for copper recovery (using the abovementioned software). The model equation representing the copper recovery (Y1) was expressed as functions of molybdenite

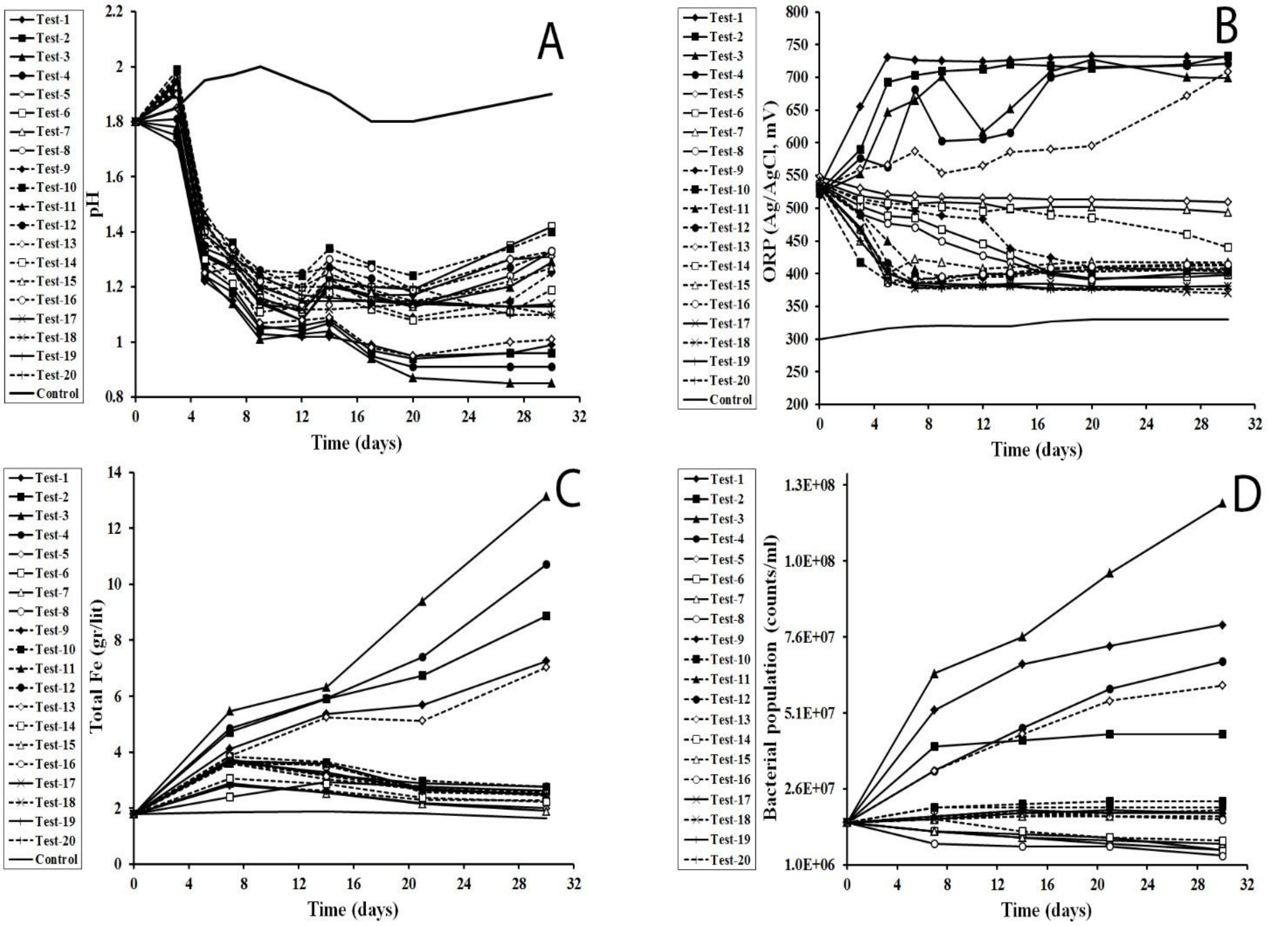

Figure 4. The changes of pH, Eh, Total Fe and cell counts in the mesophilic bioleaching tests

Table 5. Experimental (observed) and predicted values for Cu, and Mo recoveries

Run no.	*Variables*			*Cu recovery (%)*		*Mo recovery (%)*	
	A (%)	*B (%)*	*C (grl^{-1})*	*Observed*	*Predicted*	*Observed*	*Predicted*
1	3	1	0	39.62	40.21	3.44	3.49
2	9	1	0	42.91	41.57	2.06	1.96
3	3	5	0	39.62	38.24	3.7	3.60
4	9	5	0	38.15	38.69	1.68	1.72
5	3	1	1.2	47.11	45.77	0.26	0.16
6	9	1	1.2	88.49	89.08	0.26	0.31
7	3	5	1.2	47.11	47.65	0.26	0.3
8	9	5	1.2	91.43	90.05	0.19	0.09
9	3	3	0.6	94.73	96.32	0.39	0.5
10	9	3	0.6	86.45	88.04	0.18	0.29
11	6	1	0.6	91.58	93.08	0.27	0.37
12	6	5	0.6	90.9	92.58	0.2	0.32
13	6	3	0	38.52	40.11	1.71	1.82
14	6	3	1.2	42.6	44.19	0.27	0.38
15	6	3	0.6	84.77	86.63	0.33	0.19
16	6	3	0.6	88.18	86.63	0.37	0.19
17	6	3	0.6	89.41	86.63	0.13	0.19
18	6	3	0.6	87.55	86.63	0.35	0.19
19	6	3	0.6	89.75	86.63	0.27	0.19
20	6	3	0.6	86.49	86.63	0.12	0.19

concentration (A), pyrite percent (B), and silver ion concentration (C) for coded units as given below:

$$Y_1 = 44.19 + 6.35A + 0.71B - 173.83C - 0.23AB + 20.97AC + 1.93BC + 17.74A^2 + 6.20B^2 - 177.91C^2 + 24.38A^2C + 15.08AB^2 \quad (6)$$

The statistical significance of Equation (6) was checked by F-test. The analysis of variance (ANOVA) for response surface model is shown in Table 6. The p value for this model is less than 0.0001, which is given in Table 6. This indicates that the model is considered to be highly significant statistically.The predicted values of copper recovery obtained from Equation (6) are given in Table 5. The coefficient of multiple determinations, R^2, was found to be 0.9956, which means that the model could explain about 99.56% of the total variations in the system. The high value of R^2 indicates that the equation is capable of representing the system under the given experimental domain. This fact was also confirmed from the plots of the predicted versus observed values for copper recovery in Figure 5.

Table 6. Analysis of variance (ANOVA) of the response surface model to predict copper recovery

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value, Prob > F*	
Model	10815.49	1	983.23	163.52	< 0.0001	Significant
A-Mo solid%	64.41	1	64.41	10.71	0.0113	
B-Pyrite %	2.26	1	2.26	0.38	0.5572	
C-Ag(NO$_3$) grl^{-1}	3864.99	1	3864.99	642.79	< 0.0001	
AB	0.41	1	0.41	0.069	0.7996	
AC	879.48	1	879.48	146.27	< 0.0001	
BC	7.41	1	7.41	1.23	0.2992	
A^2	318.41	1	318.41	52.95	< 0.0001	
B^2	105.79	1	105.79	17.59	0.003	
C^2	5440.24	1	5440.24	904.77	< 0.0001	
A^{2}C	237.75	1	237.75	39.54	0.0002	
AB^2	363.85	1	363.85	60.51	< 0.0001	
Residual	48.1	8	6.01			
Lack of Fit	30.67	3	10.22	2.93	0.1384	Not Significant
Pure Error	17.43	5	3.49			
Cor Total	10863.6	1				

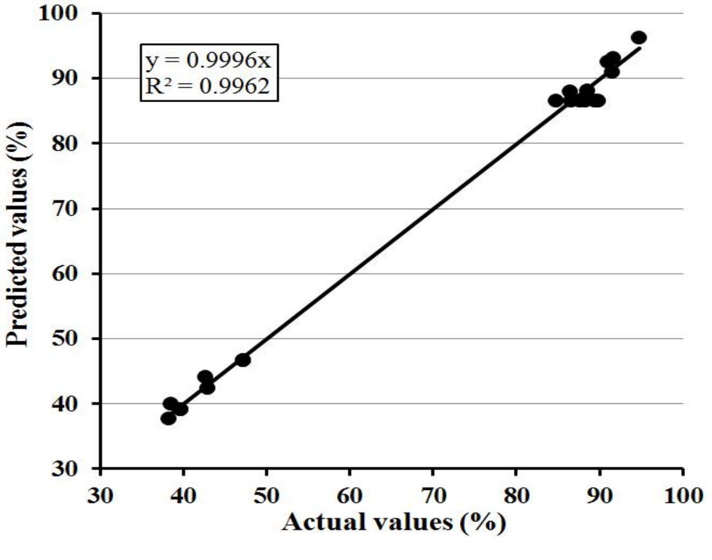

Figure 5. Plot of predicted versus observed values for copper recovery.

3.2. Model construction for molybdenum recovery

The model equation representing the molybdenum recovery (Y2) was expressed as functions of molybdenite concentration (A), pyrite percent (B), and silver ion concentration (C) for coded units as given below:

$$Y_1 = 0.38 + 0.31A - 0.02B + 2.19C \\ - 0.089AB + 0.83AC + 0.012BC - 0.32A^2 \\ + 0.15B^2 + 3.63C^2 - 1.04A^2C - 0.33AB^2 \tag{7}$$

The statistical significance of Equation (7) and ANOVA for response surface model are provided in Table 7. The predicted values of molybdenum recovery obtained using Equation (7) was given in Table 5. The coefficient of multiple determinations, R^2, was found to be 0.9907, which means that the model could explain 99.07% of the total variations in the system. The high value of R^2 indicates that the equation is capable of representing the system under the given experimental domain. This fact was also confirmed by the plots of the predicted versus observed values for molybdenum recovery in Figure 6.

Table 7. Analysis of variance (ANOVA) of the response surface model to predict molybdenum recovery

Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value, Prob > F*	
Model	22.87	1 1	2.08	79.50	< 0.0001	Significant
A-Mo solid%	0.16	1	0.16	5.93	0.0409	
B-Pyrite %	1.734E-003	1	1.734E-003	0.066	0.8033	
C-Ag(NO_3) grl^{-1}	0.61	1	0.61	23.51	0.0013	
AB	0.063	1	0.063	2.41	0.1592	
AC	1.39	1	1.39	53.00	< 0.0001	
BC	3.125E-004	1	3.125E-004	0.012	0.9157	
A^2	0.10	1	0.10	3.85	0.0853	
B^2	0.065	1	0.065	2.47	0.1549	
C^2	2.27	1	2.27	86.72	< 0.0001	
A^2C	0.43	1	0.43	16.46	0.0036	
AB^2	0.17	1	0.17	6.61	0.0331	
Residual	0.21	8	0.026			
Lack of Fit	0.15	3	0.049	3.99	0.0853	Not Significant
Pure Error	0.062	5	0.012			
Cor Total	23.08	1				

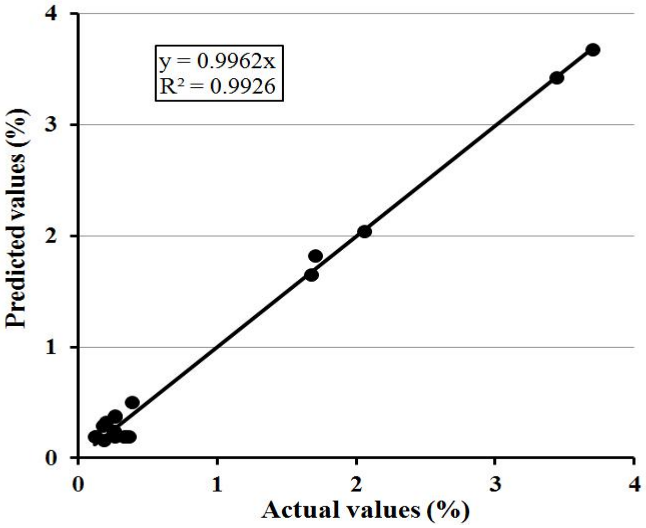

Figure 6. Plot of predicted versus observed values for molybdenum recovery

Effects of variables on the bioleaching of molybdenite concentrate for copper and molybdenum extraction were also investigated. The influence of molybdenite concentration (%), pyrite percent (%), and silver ion concentration (grl^{-1}) on increase and/or decrease of the copper and molybdenum dissolution were further evaluated. The results showed that the amount of silver ion concentration have the most marked effect on the copper dissolution. Indeed, enhancement of silver ion dosage in solution till specific value increased the copper extraction; after that it had negative effect on the bioleaching process because of inhibitory effect of silver ion on the microorganism metabolism. Also, the effect of other parameters on the copper dissolution was less pronounced than that of silver ion concentration. These results are illustrated in detail in Figures 7-10.

The relationship between variables and their influence on the response, the copper and molybdenum extraction, are shown in Figures 7-10. Figure 7 shows the influence of molybdenite concentration (%) and silver ion concentration (grl-1) on the copper dissolution. The figure indicates that the enhancement of silver ion concentration as silver nitrate till 0.1 gr per 100cc of solution increases the copper dissolution; after that it has a negative effect on the bioleaching process and, consequently, decreases the copper extraction. Also, the results showed that variation of molybdenite concentration in this rage has a small effect on the copper extraction efficiency. Figure 8 shows that the presence of pyrite in different values does not have significant influence on the copper dissolution.

Further, Figure 9 presents meaningful influence of molybdenite concentration (%), and silver ion concentration (grl^{-1}) on the molybdenum dissolution. It is obvious that by decreasing molybdenite percentage and silver ion concentration in solution, the molybdenum extraction increases. These graphs confirm that more concentration of molybdenum and silver ions in solution cause detrimental effects on the microorganism metabolism and bioleaching activity for molybdenite dissolution. In others words, Figure 10 indicates that the presence of pyrite as external additive for source of iron and sulfurhas approximately invariable influence on the bio-leaching of molybdenite.

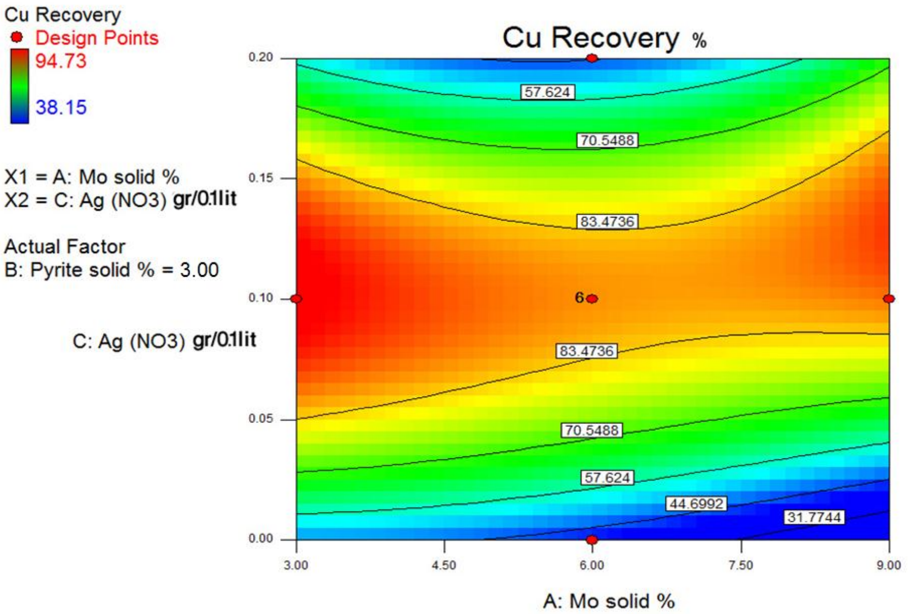

Figure 7. Influence of molybdenite concentration (%), and silver ion concentration (grl^{-1}) on the copper dissolution.

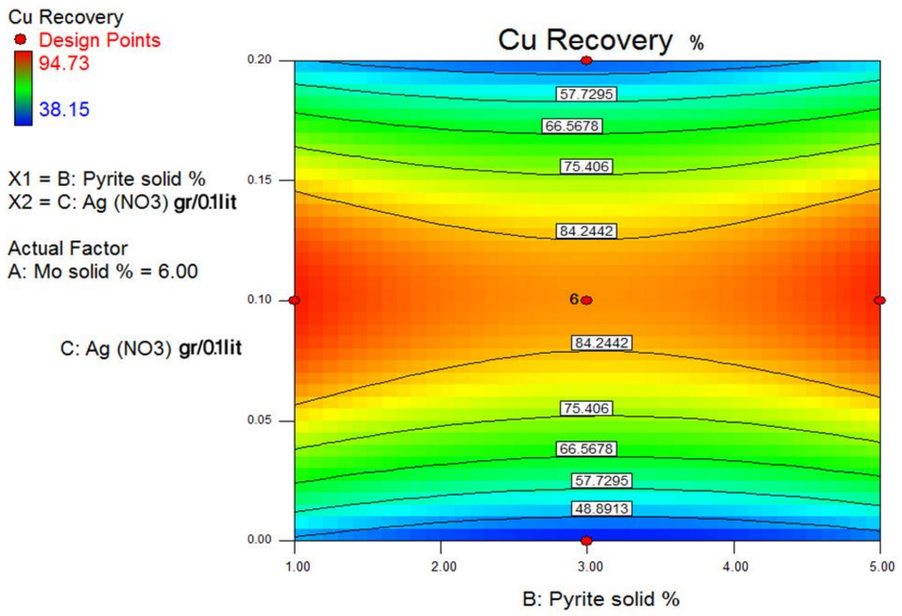

Figure 8. Influence of pyrite percent (%), and silver ion concentration (grl^{-1}) on the copper dissolution.

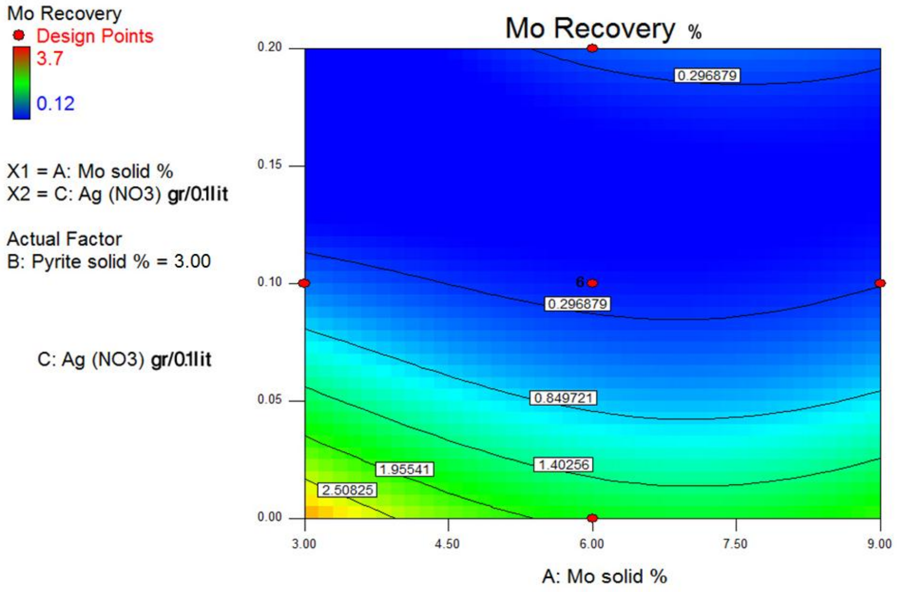

Figure 9. Influence of molybdenite concentration (%), and silver ion concentration (grl^{-1}) on the molybdenum dissolution.

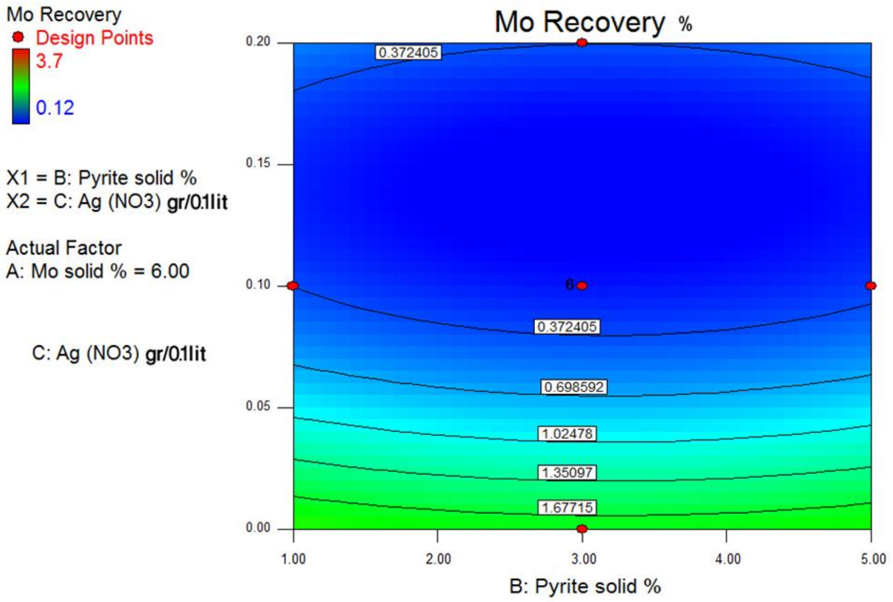

Figure 10. Influence of pyrite percent (%), and silver ion concentration (grl^{-1}) on the molybdenum dissolution.

3.3. Influence of different mediums and additives for Cu & Mo extraction

Different mediums and additives were evaluated for copper and molybdenum extraction from molybdenite concentrate in the bioleaching process. A total of 13 tests were carried out. Table 8 presents these tests and their corresponding conditions. All mesophilic bioleaching tests were performed in 32°C and 150 rpm; pH, inoculation and solid present were set as 1.6, 15% and 3 percent, respectively. First count of bacteria in ml. of solution before inoculation (After adaptation stages) was 0.8*10^8. Different culture mediums were examined with 1gr of sulfur and 5 gr of $FeSO_4$, $7H_2O$ (ferrous iron) as constant amount of additives (Test no.1 to Test no.8), while medium in test no.9 to test no.13 were identical. In test no.9, bioleaching test was carried out with 9k as culture medium and without any additive as external energy sources. 1gr of pure pyrite mineral was added to test no.10 as alternative iron and sulfur source. Sterile test (No.11) was performed with 20cc/lit of formaldehyde as bacterial inhibitor. Amount of 0.5 gr sulfur and 2.5 gr of ferrous iron were added to bioleaching test no.12 as external additives (half amount compare to test no.1). In test no.13, just 100 mg/lit of $AgSO_4$ was added as the sole additive. Various types of mediums and additives are given in Table 8 as Factor-1 and Factor-2, respectively.

Mineral salts media formulations used in this study are given in Table 9. The formulations were pooled and modified from various literature sources, including 9K from Silverman and Lundgren (1959) and others [43-46]. Descriptions of additional media formulations can be found in some sources [47]. Also, the changes in pH, ORP, bacterial population and total iron concentration over time in all tests are presented in Figure 11.

Table 8. Condition of tests including medium and additive

Test	*Factor-1*	*Factor-2 (per 0.1lit)*
Test-1	9k	1gr S+5gr Fe
Test-2	D1	1gr S+5gr Fe
Test-3	D2	1gr S+5gr Fe
Test-4	(A) HP	1gr S+5gr Fe
Test-5	(B) Norris	1gr S+5gr Fe
Test-6	(C) Leathen	1gr S+5gr Fe
Test-7	0.9k	1gr S+5gr Fe
Test-8	M	1gr S+5gr Fe
Test-9	9k	ND
Test-10	9k	1gr Pyrite
Test-11	9k	Formaldehyde
Test-12	9k	0.5gr S+2.5gr Fe
Test-13	9k	100mgr/l $AgSO_4$

Table 9. Nutrient media and their composition

Mineral salts	Concentration (g/l)							
	9K	0.9K	A(HP)	B(Norris)	C(Leathen)	D1	D2	M *
$(NH_4)_2SO_4$	3.0	0.3	0.4	0.2	0.02	0.06	0.01	3.0
$MgSO4{\cdot}7H_2O$	0.5	0.05	0.4	0.2	0.02	0.06	0.01	--
K_2HPO_4	0.5	0.05	0.1	0.2	0.02	0.02	0.01	--
KCl	0.1	0.01	--	--	0.02	0.02	0.01	0.1
$Ca(NO_3)_2{\cdot}H_2O$	0.01	0.001	--	--	--	--	--	--
Conc. H_3PO_4	--	--	--	--	--	--	--	0.5

* This medium was used in the bioleaching process for the first time and inexpensive compare to other medium in the industrial scale.

Figure 11 (A-D) shows the changes in pH, ORP, bacterial population and total iron concentration in bioleaching tests versus time with different kind of culture mediums and additives. The pH was increased in the first five days, and then decreased in the following 25 days; the final pH was stabilized after 17th day of bioleaching at various values depending of tests condition. In all tests with sulfur, ferrous sulfate or pyrite as external additives, the pH trend were decreased drastically even to 1.1 as acidity value (Figure 11-A).

In the tests runs into which 15% of inoculum and 10 gl^{-1} FeS_2 or sulfur and ferrous sulfate were added, a faster decrease in pH was observed and the final pH (1-1.2) was much lower than that of control run (with Formaldehyde) due to the microbial oxidation activity (Equations 8and 9) followed by precipitation of ferric iron in the form of jarosite (Equation 10) [48, 49]. In addition, sulfuric acid could be produced during the oxidation process (Equation 8) using mix culture of mesophilic microorganism [50].

$$2FeS_2 + 2H_2O + 7O_2 \xrightarrow{A.\,ferrooxidans} 2FeSO_4 + 2H_2SO_4 \qquad (8)$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \xrightarrow{A.\,ferrooxidans} 2Fe_2(SO_4)_3 + 2H_2O \qquad (9)$$

$$3Fe^{3+} + X^{+} + 2HSO_4^{-} + 6H_2O \rightarrow XFe_3(SO_4)_2(OH)_6 + 8H^{+} \qquad (10)$$

Where X represents monovalent metallic ion.

Inoculation of indigenous iron-oxidizing bacteria and addition of FeS_2 or ferrous sulfate as alternative iron sources resulted in an overall increase in solution ORP (Figure 11-B); however, the ORP in all the treatment groups with ferrous sulfate increased almost at the same rate and in fact became identical (750 mV) after 7 days of bioleaching, while the ORP of the solution with pyrite was increased gradually and reached to 700mV after 18 days. In contrast, the ORP of the control run was maintained at 450mV during the same bioleaching period. These results demonstrate that inoculation of indigenous iron and sulfur-oxidizing bacteria could accelerate the acidification by oxidizing the reduced sulfur compound to sulfuric acid (Equation 8) and raise the ORP by oxidizing ferrous iron to ferric ion (Equation 9).

In all bioleaching tests to which FeS_2 or ferrous sulfate and sulfur were added as iron and sulfur sources, the bacterial counts was increased. In some cases, the counts reached 0.9*10^8 counts/ml (Tests no.2 and 3). In tests no.9 and 13, the bacterial population could not rise dominantly and remained constant at (0.8-1.6*10^7 counts/ml) because of no energy sources in test no.9 and presence of silver ion in test no.13. In test no.11, enough amount of formaldehyde as bacterial inhibitor was added which in the bacterial counts reached minimum level (0.4*10^6 counts/ml). The increasing or decreasing trends of the microorganism population in these bioleaching tests are demonstrated in Figure 11-C.

The total iron concentration in the bioleaching process for all tests are illustrated in Figure 11-D.

As it can be seen, high amount of iron consisting ferrous and ferric irons were observed in solution, when ferrous sulfate was added as energy source. Also, the total iron concentration in solution was gradually decreased due to jarosite formation whose presence was confirmed using Semi quantitative X-ray diffraction (SQXRD) technique and Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analyses (EDAX). Furthermore, in the test with pyrite as iron source, the total iron concentration was gradually increased because of pyrite bio-oxidation.

The results of molybdenite bioleaching for copper and molybdenum dissolution in the various conditions (Different mediums and additives) are shown in Figure 12.

As shown in Figure 12, the amount of copper extracted from the molybdenite concentrate was considerably improved by the addition of silver ions. Copper, at 77.04%, 87.25%, 92.86% and 96.94%, was dissolved from the concentrate after 11, 18, 25 and 30 days of bioleaching test for 100mgr/l of $AgSO_4$, respectively, compared with only a 15% recovery in the absence of silver or 45% for other additives after even 30 days. The copper recovery in the presence of silver ion was at least twice as much as that in the absence of silver. The catalytic effect of silver ion in the leaching of chalcopyrite by ferric sulfate or with bacteria has been reported [51-54]. Miller and Portillo (1981) explained the catalytic mechanism of silver ion as follow; Ag_2S crystallites precipitate on the surface of chalcopyrite (Equation 11).

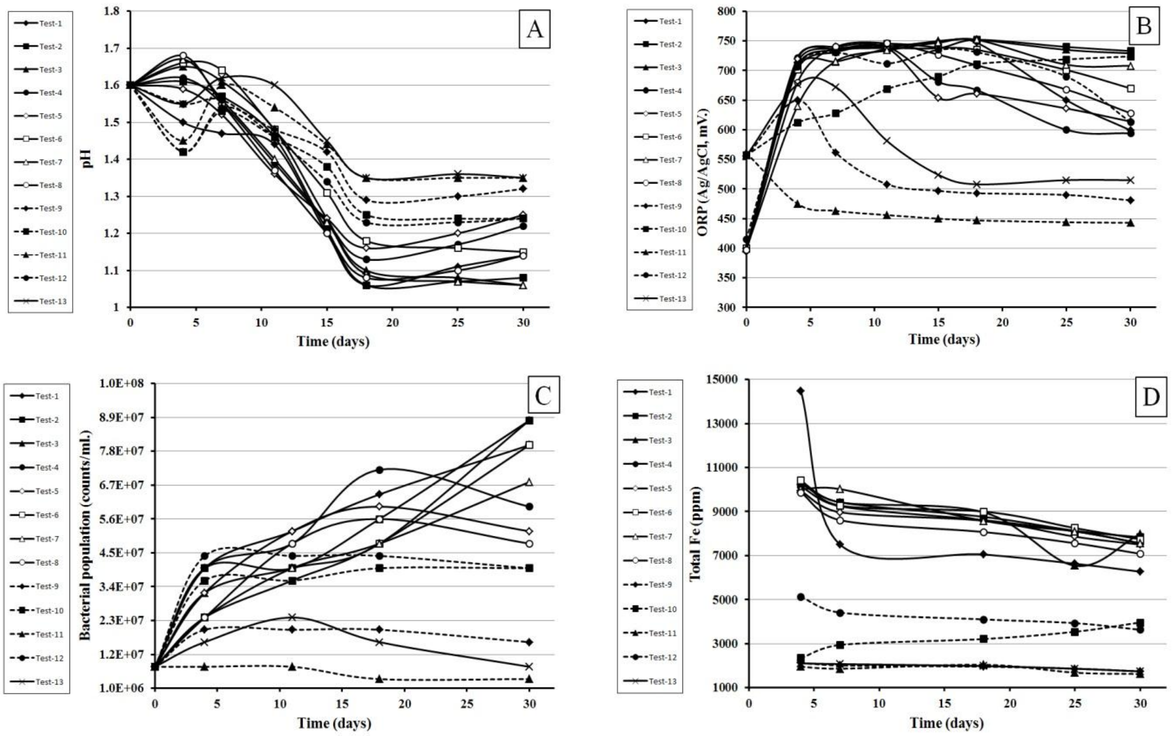

Figure 11. The changes of pH, Eh, cell counts and Total Fe in the mesophilic bioleaching tests

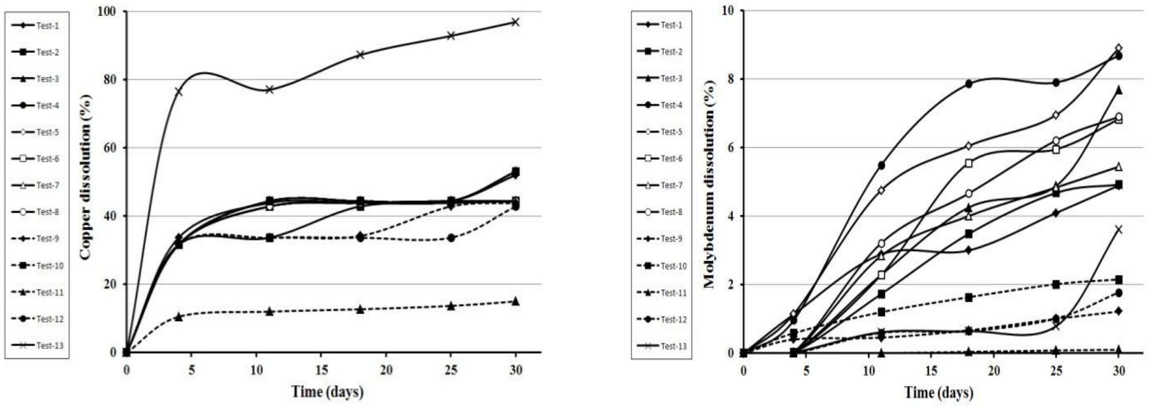

Figure 12. The Cu and Mo extraction in mesophilic bioleaching test

$$4Ag^{+} + CuFeS_2 \rightarrow 2Ag_2S + Cu^{2+} + Fe^{2+} \quad (11)$$

$$Ag_2S + 2Fe^{3+} \rightarrow 2Ag^{+} + 2Fe^{2+} + S^{o} \quad (12)$$

Ferric iron oxidizes Ag_2S to Ag^+ and S^o (Eq. (12)). The silver ion thus released will again react with chalcopyrite. As the elemental sulfur thus produced is less tenacious and porous one, which does not prevent electron transport processes on mineral surface, the dissolution of copper continues. In the silver-catalyzed bioleaching of chalcopyrite, the role of *Thiobacillus ferrooxidans* is to maintain a favorable ratio of Fe^{3+}/Fe^{2+} by oxidizing ferrous iron, and contribute to the ferric-iron dependent oxidation of silver sulfide [52]. The solubility of silver chloride in the water is very low, and its solubility product of silver chloride is 1.78×10^{-10} [55]. If the medium contains chloride ion, for example 9K medium contains 1.32×10^{-3} M chloride ion, silver ion reacts with chloride ion to precipitate as silver chloride [39]. In the presence of 100 mg/l chloride ion in the medium, the addition of 50 mg/l silver ion enhanced the copper dissolution [56]. Silver chloride could act as a catalyst for the bioleaching of chalcopyrite [57]. In all bioleaching tests with silver nitrate or sulfate as additives, when silver bearing components added to 9k culture medium, AgCl formed and precipitated (confirmed with XRD method). The dissolution rate of molybdenite is very slow compare to the dissolution of chalcopyrite in the bioleaching process. As expected, maximum amount of molybdenum extracted from concentrate was 8.9%, compared with the 97% of copper extraction in the mix mesophilic bioleaching process. The maximum Mo dissolution was reported with HP (A) and Norris (B) as culture mediums and 1gr sulfur with 5gr $FeSO_4$, $7H_2O$ as external energy sources.

Furthermore, Cu and Mo extraction in test with medium M were 44.38% and 6.9%, respectively, which can be used instead of other expensive culture mediums, particularly in industrial application.

4. Conclusion

In this study, the central composite face centered design as one of method of the response surface methodology was used for modeling and optimizing the bio-leaching process for molybdenite concentrate which was prepared from Sarcheshmeh copper complex, Iran. The chemical analyses of the concentrate showed that there is 0.98% of Cu in the sample. Mineralogical study of the sample showed molybdenite (MoS_2) as the main mineral phase and chalcopyrite ($CuFeS_2$) and pyrite (FeS_2) as the minor impurities. Of many factors affecting the bio-leaching process, three main parameters i.e. molybdenite concentration (%), pyrite percent (%), and silver ion concentration (grl^{-1}) were considered in a central composite response surface design. The copper and molybdenum dissolution from molybdenite concentrate using bioleaching method were successfully modeled with high correlation coefficient and the influence of molybdenite concentration (%), pyrite percent (%), and silver ion concentration (grl^{-1}) on the increase and/or decrease of the copper and molybdenum dissolution extensively evaluated. The results showed that the amount of silver ion concentration have the most marked effect on the copper dissolution. Indeed, enhancement of silver ion dosage in solution till specific value increased the copper extraction; after that it had negative effect on the bioleaching process because of inhibitory effect of silver ion on the microorganism metabolism. Also, the effect of other parameters on the copper dissolution was less pronounced than that of silver ion concentration. It indicates that the enhancement of silver ion concentration as silver nitrate till 0.1 gr per 100cc of solution increased the copper dissolution and reached to the maximum elimination.

The results showed that variation of molybdenite and pyrite concentration in this rage has a little effect on the copper extraction efficiency. There is meaningful influence of molybdenite concentration (%), and silver ion concentration (grl^{-1}) on the molybdenum dissolution. It is obvious that by decreasing molybdenite percentage and silver ion concentration in solution, the molybdenum extraction increases. It was also confirmed that higher concentration of molybdenum and silver ions in solution causes detrimental effects on the microorganism metabolism and consequences in bioleaching activity for molybdenite dissolution. Also, the presence of pyrite as external additive for source of iron and sulfur has a small influence on the bio-leaching of molybdenite. The dissolution rate of molybdenite is very slow, compared with that of chalcopyrite in the bioleaching process. Expectedly, maximum amount of molybdenum extracted from concentrate was 8.9%, compared with the 97% of copper extraction in the mix mesophilic bioleaching process. The maximum Mo dissolution was reported with HP (A) and Norris (B) as culture mediums and 1gr sulfur with 5gr $FeSO_4$, $7H_2O$ as external energy sources.

Small amounts of silver (100mgr/l $AgSO_4$) dramatically accelerated the copper dissolution process. Addition of FeS_2 and also sulfur with ferrous sulfate accelerated the acidification and raised the oxidation-reduction potential of solution (medium) with an inoculation of 15% (v/v) of active and adapted indigenous mesophilic bacteria, thus resulting in an overall increase in Mo dissolution efficiency. Applying other sources of energy (sulfur, ferrous sulfate or pyrite) was necessary for the bioleaching of molybdenite concentrate with mix mesophilic microorganism. Ferric hydroxy sulphates such as jarosite were formed, when ferrous sulfate and pyrite were added as external additives. The SEM images and XRD result from the remaining particles in the bioleaching tests confirmed the presence of the jarosite which were formed on the molybdenite surface. X-ray Mapping showed that most part of particles is covered with S and Fe bearing components. The XRD result from the remaining particles in the bioleaching tests showed that bioleaching approach can remove most part of the copper bearing minerals and pyrite (were not detected in XRD graph). Bioleaching treatments proved to be a technique to purify molybdenite flotation concentrates by selectively dissolving chalcopyrite.

Acknowledgments

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