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Empirical model for bio-extraction of copper from low grade ore using response surface methodology

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Abstract: The copper extraction in shaking bioreactors was modeled and optimized using response surface methodology (RSM). Influential parameters in the mesophilic bioleaching process of a low-grade copper ore including pH value, pulp density, and initial concentration of ferrous ions were comprehensively studied. The effect of leaching time on the response (copper extraction) at the 1st, 4th, 9th, 14th and 22nd days of treatment was modeled and examined. The central composite design methodology (CCD) was used as the design matrix to predict the optimal level of these parameters. Then, the model equation at the 22nd day was optimized using the quadratic programming (QP) to maximize the total copper extraction within the studied experimental range. Under the optimal condition (initial pH value of 2.0, pulp density of 1.59%, and initial concentration of ferrous ions of 0 g/L), the total copper extraction predicted by the model is 85.98% which is significantly close to that obtained from the experiment (84.57%). The results show that RSM could be useful to predict the maximum copper extraction from a low-grade ore and investigate the effects of variables on the final response. Besides, a couple of statistically significant interactions are derived between pH value and pulp density as well as pH value and initial ferrous ion concentration which are precisely interpreted. However, there is no statistically significant interaction between the initial ferrous ion concentration and the pulp density. Additionally, the response at optimal levels of pH value and pulp density is found to be independent on the level of initial ferrous concentration.

Key words: modeling; optimization; bacterial leaching; response surface methodology; copper extraction; copper ore

1 Introduction

Bioleaching process has been impressively developed during the recent decades [1−8]. Recently, the bioleaching of primary copper sulfide minerals and potential application of this process have been studied in Iran's copper industry as well as the application of mesophilic and thermophilic bacteria for increasing the extraction of copper [9−11]. Sarcheshmeh Copper Complex is one of the largest copper mines in the world, located in SE of Iran. About 20 million tons copper ore per year, containing 0.8% Cu, are mined and processed. Simultaneously, about 1 million tons of low-grade copper ore, a mixture of sulfide and oxide with grade of 0.3% Cu, which is not a suitable feed for pyrometallurgical process, should be treated using hydrometallurgical technique [2,3]. Thus, finding a new environmental friendly processing technology for the retained low grade copper ore is a major task [12]. So, alternative hydrometallurgical and pyrometallurgical technologies are currently available for copper extraction from the sulfide and oxide low-grade copper ore which operate at the commercial scales. Nonetheless, the pyrometallurgical processes consume a large quantity of energy and also cause environmental problems [12−15]. On the other hand, bacterial leaching consumes less energy and is an environmental friendly process [1−3,13−16]. Considerable physicochemical parameters, which have influence on the rate of the copper bio-extraction, have been identified such as pH value, temperature, ratio of ferric to ferrous ions, pulp density, fraction of inoculation, and initial ferrous iron concentration [2,3,10−12].

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In statistical parlance, there could be an interaction between effective parameters in such a process. Examining the previous literatures on sulfide and oxide low-grade ores bioleaching provides no clues that whether such interaction between the important parameters exists or not. This is because in the previous studies, one- factor-at-a-time methodology has been used to optimize the physicochemical parameters [2,3,17−19]. This methodology is inefficient besides gives absolutely no information about the interactions between the parameters in a process. The only methodology which is able to provide an answer to this question is factorial design of experiments (DOE), which, through the use of statistical techniques such as response surface methodology (RSM), is capable of simultaneously considering several factors at different levels, and giving an appropriate model to describe the relationship between the various factors and the response [20]. RSM is a collection of statistical and mathematical methods for modeling and analyzing the engineering problems. In this technique, the principle goal is to optimize the response surface influenced by diverse process parameters. In addition, RSM determines the relationship between the controllable input parameters and the obtained response surfaces [21]. The design procedure for RSM is as follows [22]:

1) Designing a series of experiments for sufficient and reliable measurement of the desirable response;

2) Developing a mathematical model with a second order response surface and a maximum fitting;

3) Finding the most desirable set of experimental parameters which produce a maximum or minimum value of response;

4) Expressing the direct and interactive effects of the process parameters using two- and three-dimensional plots.

RSM has previously been applied in only a few cases of a bioleaching processes [23−27]. Besides, reliable information about first order interactions can only be obtained from the results of DOE. However, higher order interactions between the parameters are usually statistically insignificant and therefore the information about them is not quite useful [20]. Generally, changing one parameter and keeping other ones at a constant level has a huge disadvantage as it does not include the interaction effects among the variables and as a result, it does not show the complete effects of different parameters on the response [28,29]. To overcome this problem, modeling and optimizing studies are performed by using RSM. Besides, theories and essential aspects of RSM have been well explained in Refs. [20,30] associated with this subject. To determine several parameters and their interaction, RSM is often used because it decreases the number of experimental trials. As a result, it takes less effort and time than other applications. It is remarkable that, in recent years, RSM has been applied for modeling and optimizing the several mineral processing researches [26,27,31−35].

Literature review shows that despite there are many researches related to experimental works on bacterial leaching, few researches have also been conducted on the DOEs, and the bacterial extraction of copper from the oxide sulfide ore with an emphasis on the leaching period by using RSM and central composite design methodology (CCD) approaches have not been totally investigated. The aim of the present work is to identify and quantify the important physicochemical parameters, i.e., pH value, pulp density and initial ferrous ions concentration, as well as interactions between them in a sulfide and oxide low-grade copper ore bioleaching process by mixed mesophilic microorganisms, using appropriate methodology such as RSM. This study involved modeling and optimizing the process parameters affecting the total copper extraction as a response. In addition, CCD was used as the design matrix to identify the polynomial model for interpreting the interaction between the influential factors. After modeling and optimizing the parameters, the effect of leaching time on the variables (pH value, pulp density and initial concentration of ferrous ions) as well as on the response (copper extraction) at the 1st, 4th, 9th, 14th and 22nd days was comprehensively examined. Furthermore, the effect of changing variables on the response at the 1st, 4th, 9th, 14th and 22nd days of treatment was also investigated. The trend of copper extraction during the treatment time intervals was investigated.

2 Experimental

2.1 Low-grade copper ore

About 3500 kg low-grade copper ore was obtained from the Sarcheshmeh copper mine with 0.31% Cu content. The particle size distributions of the original ore and the ground sample [36] with three stages crushers and mill were obtained with d_{80} of ~30000 and ~85 μm. In addition, the grade of copper and iron with their distributions in different particle sizes of the original sample were achieved. The results of particle size and grade distributions are shown in Figs. 1(a) and (b), respectively. The representative sample including different size fractions were pulverized for the chemical and mineralogical assays. The chemical composition of this sample includes 0.31% Cu, 6.10% Fe and 1.72% S. The X-ray diffraction (XRD) analysis of the ore shows the presence of quartz and silicates as the main mineralogical components and chalcopyrite as the minor phase. The X-ray fluorescence (XRF) and mineralogical analysis of the representative sample are presented in Tables 1 and 2.

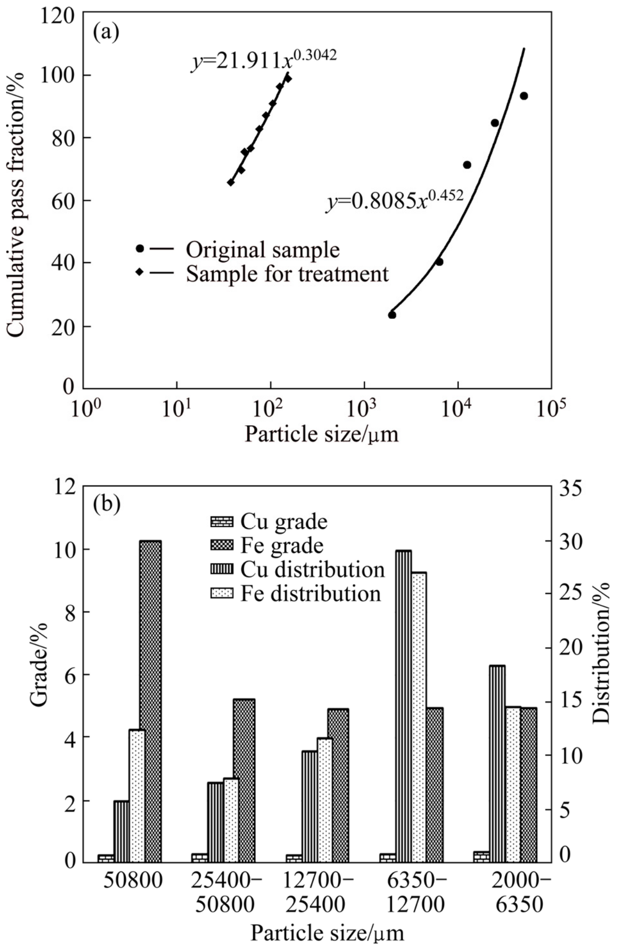

Fig. 1 Particle size distributions of original ore and sample used in treatment (a), and grade of copper and iron with their distribution in different particle sizes of original sample (b)

Table 1 XRF analysis of representative sample (mass fraction, %)

Cu	CuO	Fe	SiO_2	Mo	Al_2O_3
0.32	0.14	8.22	51.36	0.004	15.21
K_2O	TiO_2	MgO	MnO	P_2O_5	CaO
2.66	0.72	2.70	Trace	0.42	0.81

Table 2 Mineralogical analysis of representative sample (mass fraction, %)

FeS_2	MoS_2	FeOOH	Fe_2O_3
12.9	0.01	0.15	0.1

2.2 Microorganisms

A mixed culture of mesophilic bacteria including *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirilum ferrooxidans* was used after an isolation process [37]. Both iron- and sulfur-oxidizing microorganisms in mixed culture were used. These microorganisms were cultivated in 9K medium containing ferrous sulfate and sulfur powder [38]. Consequently, the effects of both iron- and sulfur-oxidizing microorganisms on the leaching kinetics and copper extraction were investigated. Besides, the bacterial cultures were adapted to the elemental sulfur. The cultures were incubated at 32 °C in a temperature-controlled orbital shaker (Innova 4200 model, New Brunswick scientific company, USA) at 150 r/min and the medium acidity was measured by pH meter (model MP120, Mettler Toledo Company, Switzerland). In addition, the bacterial growth was monitored by measuring the oxidation reduction potential (ORP) using a pH/Eh meter (826pH mobile model, Metrohm Company, Switzerland). This was used to observe the oxidation ability of microorganism to oxidize ferrous ion and sulfur.

2.3 Design of experiments by RSM and CCD

The experimental design methods are commonly used for process modeling and incorporate the full factorial, partial factorial and central composite designs. At least, three levels per variable are required at a full factorial design to estimate the coefficients of the quadratic terms in the response model. Therefore, concerning the three independent process variables, a number of experiments as well as replications should be conducted [39]. If all variables are supposed to be measurable, the response surface can be expressed as follows:

$$y=f(x_1,\ x_2,\ x_3,\ \cdots,\ x_k) \tag{1}$$

where y is the response of the system, and x_i denotes the variables of action called factors. The objective is to optimize the response variable (y). It is supposed that the independent variables are continuous and controllable by the experiments with insignificant errors. Also, finding a proper approximation for the true functional relationship between the independent variables and the response surface is necessary [22]. It is obvious that a partial factorial design requires fewer experiments than the full factorial. However, the partial factorial design cannot reveal all interactions between the parameters [32,40]. An efficient alternative to the factorial design is CCD, originally developed by BOX and WILSON [39] and improved by BOX and HUNTER [41]. CCD gives nearly as much information as a three-level factorial and needs quite fewer tests than the full factorial design. Also, it has been shown to be adequate to describe the majority of steady state process responses [42]. The number of tests required for CCD contains the standard 2^k (k is the number of variables) factors with its origin at the center, $2k$ points fixed axially at the distance, for example β

($\beta=2^{k/4}$), from the center to generate the quadratic terms, and replicate the tests at the center. At these tests, k is the number of variables. The axial points are chosen so that they make rotation possible [41], which guarantees that the variance of the model prediction is constant at all points equally distant from the design center. The replicates of test at the center are of much importance as they provide an independent estimation of the experimental error. For three variables, six tests at the center are suggested. Consequently, the total number of tests necessary for the three independent variables is $y=2^3+2\times3+6=20$ [32,41]. After defining the desired ranges of values of the variables, they are coded to lie at ±1 for the factorial points, 0 for the center points and $\pm\beta$ for the axial points. The codes are calculated as functions of the desirable range of each factor, as shown in Table 3 [43].

Table 3 Relationship between coded and actual values of variables

Code	Actual level of variable
$-\beta$	x_{min}
−1	$[(x_{max}+x_{min})/2]-[(x_{max}-x_{min})/2\alpha]$
0	$[(x_{max}+x_{min})/2]$
+1	$[(x_{max}+x_{min})/2]+[(x_{max}-x_{min})/2\alpha]$
$+\beta$	x_{max}

x_{max} and x_{min} are the maximum and minimum values of x, respectively.

When the response data are obtained from the test work, a regression analysis is performed to determine the coefficients of the response model (b_1, b_2, $\cdots$, b_n), their standard errors and their significance. In addition to constant (b_0) and error (ε) terms, the response model incorporates [32]:

1) Linear terms in each variable ($x_1, x_2, \cdots, x_n$);

2) Squared terms in each variable ($x_1^2, x_2^2, \cdots, x_n^2$);

3) First-order interaction terms for each paired combination ($x_1x_2, x_1x_3, \cdots, x_{n-i}x_n$).

Therefore, for the three variables under consideration, the response model is

$$y=(b_0+\varepsilon)+\sum_{i=1}^{3}b_ix_i+\sum_{i=1}^{3}b_{ii}x_i^2+\sum_{i=1}^{3}\sum_{j=i+1}^{3}b_{ij}x_ix_j \tag{2}$$

The coefficients b, which should be determined in the second-order model, are obtained by the least squares method. In general, Eq. (2) can be written in a matrix form:

$$\boldsymbol{Y}=\boldsymbol{bX}+\varepsilon \tag{3}$$

where $\boldsymbol{Y}$ is the matrix of measured values and $\boldsymbol{X}$ represents the matrix of independent variables. The matrices $\boldsymbol{b}$ and ε consist of coefficients and errors, respectively. The solution of Eq. (3) can be obtained by a matrix approach [21,22].

$$\boldsymbol{b}=(\boldsymbol{X'X})^{-1}\boldsymbol{X'Y} \tag{4}$$

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

The coefficients, namely the main effect (b_i) and two-factor interactions (b_{ij}), can be estimated from the experimental results by computer simulation programming applying a least squares method using Design Expert 7 Trial (State Ease, Inc., Minneapolis, MN, USA). To determine the significance of each term in equation as well as to estimate the best fitness the polynomial equation for the response was validated by the analysis of variance (ANOVA) [20,44].

2.4 Experimental design

CCD was chosen to design a series of experiments in order to provide data to determine the relationship between the response (i.e., total copper extraction) and the three process parameters, with the initial pH value of 0.96−2.64, the pulp density of 1.59%−18.41% (ratio of mass to volume), and the initial concentration of ferrous ions of 0−6.27 g/L.

Using the relationships in Table 3, the actual values are given in Table 4. Also, the actual levels of the variables for each 20 experiments are given in Table 5.

Table 4 Actual levels of independent variables used in RSM design.

Independent variable	Symbol	Actual level				
		$-\beta$	−1	0	+1	$+\beta$
Initial pH value	A	0.96	1.30	1.80	2.30	2.64
Pulp density/%	B	1.59	5	10	15	18.41
Initial concentration of ferrous ions/(g·L^{-1})	C	0	1.27	3.14	5.00	6.27

2.5 Shake flask tests, sampling, measurements and analysis

The whole experiments (runs) were carried out in 500 mL Erlenmeyer flasks containing 200 mL solution. The experiments were conducted in a rotary shaker at 140 r/min under different conditions. A mixture of sterilized medium with an appropriate amount of sterilized energy source, a predetermined amount of bacterial inoculum (20 mL) and an adequate quantity of low-grade ore, was added to the leaching vessel and maintained in the rotary shaker incubator. In all tests, the volume reached 200 mL. The pH value of the media was monitored regularly. During the experiments, distilled water was added periodically to the flasks to compensate for evaporation loss. Afterwards, the pH value of the solution was adjusted back to the initial value with 1 mol/L sulfuric acid. The redox potential [45] was recorded at day intervals, and 1 mL sample was removed from the liquid using a pipette to obtain the kinetics

Table 5 Coded and actual levels of independent variables

Run	Coded level of variable			Actual level of variable		
	A	*B*	*C*	Initial pH value	Pulp density/%	Initial concentration of Fe^{2+}/($g \cdot L^{-1}$)
1	0	1.68	0	1.80	18.41	3.14
2	−1.00	−1.00	1.00	1.30	5.00	5.00
3	−1.68	0	0	0.96	10.00	3.14
4	1.68	0	0	2.64	10.00	3.14
5	−1.00	−1.00	−1.00	1.30	5.00	1.27
6	0	0	0	1.80	10.00	3.14
7	0	0	0	1.80	10.00	3.14
8	0	−1.68	0	1.80	1.59	3.14
9	0	0	0	1.80	10.00	3.14
10	−1.00	1.00	−1.00	1.30	15.00	1.27
11	0	0	0	1.80	10.00	3.14
12	0	0	0	1.80	10.00	3.14
13	1.00	−1.00	1.00	2.30	5.00	5.00
14	1.00	1.00	1.00	2.30	15.00	5.00
15	0	0	−1.68	1.80	10.00	0
16	1.00	1.00	−1.00	2.30	15.00	1.27
17	0	0	0	1.80	10.00	3.14
18	1.00	−1.00	−1.00	2.30	5.00	1.27
19	0	0	1.68	1.80	10.00	6.27
20	−1.00	1.00	1.00	1.30	15.00	5.00

information about metals dissolved. The metal concentrations, Cu and total Fe, in the samples were measured, using atomic absorption spectroscopy (AAS). To keep the volume of the culture constant, an equal volume of 9K medium was added after taking each sample. The redox potential was measured using a Pt-combination redox electrode with an Ag/AgCl reference electrode. Then, the pH value was monitored using a gel-filled combination pH probe with an Ag/AgCl reference electrode. The initial bacterial concentration in all the experiments was approximately 10^8 cell/mL. The bacterial population was determined using a counting chamber and an optical microscope (Carl Zeiss model, Axioskop 40, USA). At the end of experiments, the solid was filtered and a chemical analysis of the residues was accomplished to complete the mass balance for the calculation of final copper and iron extractions.

2.6 Effect of leaching time on dependent and independent parameters

The parameter time is one of the important and impressive factors in most natural phenomena. The time of reactions at the bacterial leaching processes is an effective parameter influencing the copper extraction. Considering the time factor and analyzing the process response towards time represent different effects of the independent parameters on the response. The effect of leaching time on the dependent parameter (copper extraction) and independent parameters (pH value, pulp density and initial ferrous concentration) were investigated at the 1st, 4th, 9th, 14th and 22nd days. According to the preliminary tests and based on some experiments conducted by other researches on this ore, the change of copper extraction in the bioleaching treatment after the 22nd days was leveled off, so this time was selected as the final process time [10,46]. It should be noted that the predetermined time for the analysis was selected as 1st, 4th, 9th, 14th and 22nd days, because this ore consists of oxide, primary and secondary sulfide copper minerals with different dissolution rates. So, the effect of cessation time of leaching process was examined at the 1st, 4th, 9th, 14th and 22nd days on the 20 mentioned tests in Table 5. Thus, the purpose of this study was to analyze the independent parameter changes on the response of the bioleaching treatment. To examine the effect of time on the copper extraction, there are two approaches:

1) Considering the time factor as x_4, which turns the run number from 20 to 30, and independently plotting the graph change of copper extraction versus time for the tests.

2) Substituting the results of all runs at RSM and analyzing them after elapsing of a given time.

It is remarkable that the second approach was used to show the changes of independent parameters and their effects on the type of response.

3 Results and discussion

3.1 Statistical analysis

The response data were analyzed using the design experimental software, and the effects for all the model terms were calculated. The statistical parameters such as F-values, lack of fit, and R^2-values were used, and consequently, a convenient quadratic model was accordingly selected. The model terms in Eq. (2) were calculated after the elimination of some insignificant variables and their interactions, which have the lowest F-value. The ANOVA results for the response at the 1st, 4th, 9th, 14th and 22nd days are summarized in Table 6. The model accuracy was checked via lack-of-fit F-test. Lack of fit compares the residual error with the pure error. Lack of fit is not desirable, so a small F value and probability values greater than 0.1 are desired. If a model shows lack of fit, it cannot be used to predict the response [47]. As indicated in Table 6, at the 22nd day, the lack-of-fit of the model (0.633) has a probability above 0.1, and the model is highly significant with very low probability values (<0.0001). Besides, the model presents a very high determination coefficient (0.948). The R^2 value provides a measure of how much variability in the observed response values can be explained by the experimental variables and their interactions. When the R^2 value is closer to 1, the model predicts better response [48]. A very low value of coefficient of variation (1.48) indicates a good precision and reliability of the experiments [49]. Adequate precision (23.598) measures the signal to noise ratio, and a ratio greater than 4 is generally desirable [50]. In addition, the predicted sum of square (PRESS) is a measure of how a particular model has fitted each point in the design [51]. Furthermore, Table 6 presents amounts of p-value for each variable in the polynomial model at the 1st, 4th, 9th, 14th and 22nd days of treatment. The values of "Prob. >F" less than 0.05 indicate that the model terms are significant while the values greater than 0.1 indicate that the model terms are insignificant.

Table 6 ANOVA results for response model at 1st, 4th, 9th, 14th and 22nd days with amounts of p-value for each variable in polynomial model

Source	1st day	4th day	9th day	14th day	22nd day
Model	0.0002	0.1291	0.8246	<0.0001	<0.0001
A	<0.0001	−	−	0.0007	<0.0001
B	0.0505	−	−	0.0003	<0.0001
C	−	−	−	−	0.016
A^2	−	−	−	0.0052	<0.0001
B^2	−	−	−	−	−
C^2	−	−	−	−	−
AB	−	−	−	0.0097	0.003
AC	0.0363	−	−	−	0.029
BC	−	−	−	−	−
R^2	0.6932	0.3608	0.1314	0.7991	0.948
R^2 adjusted	0.6356	0.1904	−0.1788	0.7455	0.924
R^2 predicted	0.4470	−0.1002	−0.4133	0.6268	0.883
Prob. >F	0.0002	0.1291	0.8246	<0.0001	<0.0001
Lack of fit (LOF)	0.1012	0.4650	0.9850	0.2316	0.633
PRESS	72.68	279.71	339.84	149.80	38.620
Adequate precision	12.396	3.654	2.250	12.979	23.598
Coefficient of variation	3.43	6.26	6.49	3.08	1.48

3.2 Significant factor

The observed and predicted values of total copper extraction at the 1st, 4th, 9th, 14th and 22nd days are summarized in Table 7. Considering the effects of the main factors and also the interactions between two-factor, Eq. (2) is summarized as follows:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \tag{5}$$

From the experimental design in Table 5, experimental results in Table 7 and Eq. (5), the second-order response function representing total copper extraction (Y) can be expressed as a function of the three coded process parameters, i.e., initial pH value (A), pulp density (B) and initial concentration of ferrous ions (C). The relationship between the response (total copper extraction) and process parameters at the 22nd day was obtained using coded variables, as presented in Eq. (6):

$$Y_{\text{22nd day}} = 79.10 - 2.79A - 3.08B - 0.86C - 1.46AB - 1.00AC - 1.70A^2 \tag{6}$$

The negative signs in variables of the predicted model indicate that these factors must be kept in low levels in order to maximize the extraction of copper by bioleaching process. Moreover, the models of the bioleaching process for the time of 1st, 4th, 9th, 14th and 22nd days were obtained and their initial formulas are as follows:

$$Y_{\text{1st day}} = 46.25 - 2.21A - 0.91B + 0.50C - 0.87AB - 1.28AC - 0.61BC \tag{7}$$

$$Y_{\text{4th day}} = 52.61 - 1.29A - 0.78B + 0.05C - 0.46AB + 1.72AC - 1.05BC \tag{8}$$

Table 7 Observed and predicted values of total Cu extraction at 1st, 4th, 9th, 14th and 22nd days (%)

Run	1st day		4th day		9th day		14th day		22nd day	
	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	45.31	44.72	49.55	50.84	59.18	60.77	67.81	71.65	75.72	73.92
2	50.39	50.65	51.57	53.00	56.61	57.49	74.43	77.82	81.60	81.95
3	47.96	49.96	56.75	56.26	57.23	58.57	77.11	75.51	79.48	78.98
4	44.11	42.53	51.39	51.92	54.64	57.12	66.22	66.51	69.51	69.58
5	48.35	48.08	59.80	56.44	62.10	59.43	77.78	77.82	82.11	81.67
6	46.35	46.25	50.87	50.84	51.28	60.02	78.30	76.60	80.94	79.10
7	46.81	46.25	49.77	50.84	57.57	60.02	79.31	76.60	79.43	79.10
8	48.12	47.77	48.20	50.84	58.70	59.27	81.77	81.56	84.57	84.27
9	44.21	46.25	54.45	50.84	63.24	60.02	79.52	76.60	79.93	79.10
10	47.42	46.27	54.82	56.44	62.38	61.88	75.22	76.78	76.95	78.42
11	45.94	46.25	48.01	50.84	55.53	60.02	78.21	76.60	78.33	79.10
12	46.93	46.25	56.15	50.84	62.26	60.02	74.67	76.60	77.43	79.10
13	45.63	43.66	58.53	53.86	58.88	58.19	75.65	77.33	78.22	77.27
14	38.75	41.85	47.50	53.86	57.50	57.52	65.14	66.58	67.68	68.21
15	44.70	46.25	52.14	54.91	59.12	61.65	77.82	76.60	80.26	80.54
16	44.30	44.41	53.08	50.42	63.08	59.45	69.59	66.58	71.76	71.93
17	45.71	46.25	49.69	50.84	66.39	60.02	76.94	76.60	78.90	79.10
18	44.75	46.23	50.37	50.42	62.17	60.12	77.17	77.33	80.62	80.99
19	48.23	46.25	57.64	54.91	59.98	58.40	74.09	76.60	76.23	77.65
20	50.97	48.83	51.90	53.00	62.09	59.94	78.32	76.78	79.00	78.71

$$Y_{9\text{th day}} = 59.50 - 0.43A + 0.45B - 0.97C - 0.78AB - 0.39AC + 0.36BC \tag{9}$$

$$Y_{14\text{th day}} = 77.82 - 2.67A - 2.95B - 0.91C - 2.43AB - 0.71AC + 0.44BC - 2.13A^2 - 1.02B^2 - 0.61C^2 \tag{10}$$

$$Y_{22\text{nd day}} = 79.17 - 2.79A - 3.08B - 0.86C - 1.46AB - 1.00AC + 0.11BC - 1.70A^2 + 0.29B^2 - 0.39C^2 \tag{11}$$

After sensitivity analysis and significance of independent parameters over the model, significance and insignificance of each parameter were determined and the constants of Eq. (5) were then obtained. With substituting these constants in Eq. (5) and considering R^2, the model describing copper extraction as a function of time was obtained at the 1st, 4th, 9th, 14th and 22nd days. Figure 2 is the internally studentized residual plots for Y (total copper extraction) at the 22nd day in the model (Eq. (6)). It shows that the distribution of the internally studentized residuals for the response approximately follows the fitted normal distribution and the internally studentized residuals of the response randomly scatters in the internally studentized residual plot. The 3D response surface plots in Figs. 3(a)–(d) which are the simulations from Eq. (6) at the 22nd day describe the effect of the process variables on the total copper extraction. The explanations for these trends are not given here as this is not the main focus of the study. Referring to Table 6, it can be concluded that at the 22nd day, all the three main variables are a clearly good significance while the interaction between the pulp density and the initial concentration of ferrous ions (BC) is insignificant. To improve the model adequacy, the interaction between the pulp density and the initial concentration of ferrous ions (BC), B^2 and C^2 are removed. Table 7 indicates that at the 22nd day, the highest total copper extraction is 84.57% which was obtained in Run 8, with the initial pH value, pulp density and initial ferrous ions concentration of 1.80, 1.59% and 3.14 g/L, respectively. In addition, the lowest total copper extraction is 67.68% which was obtained in Run 14, with the initial pH value, pulp density and initial ferrous ions concentration of 2.30, 15.00%, and 5.00 g/L, respectively.

The three-dimensional and contour plots in Fig. 3 show the effects of initial pH value and pulp density (Figs. 3(a) and (c)) as well as the initial concentration of ferrous ions and initial pH value on the total copper extraction (Figs. 3(b) and (d)) at the 22nd day, while the initial concentration of ferrous ions and pulp density are fixed at their central values, i.e., 3.14 g/L and 10.00%, respectively. In Figs. 3(a) and (c), the total copper

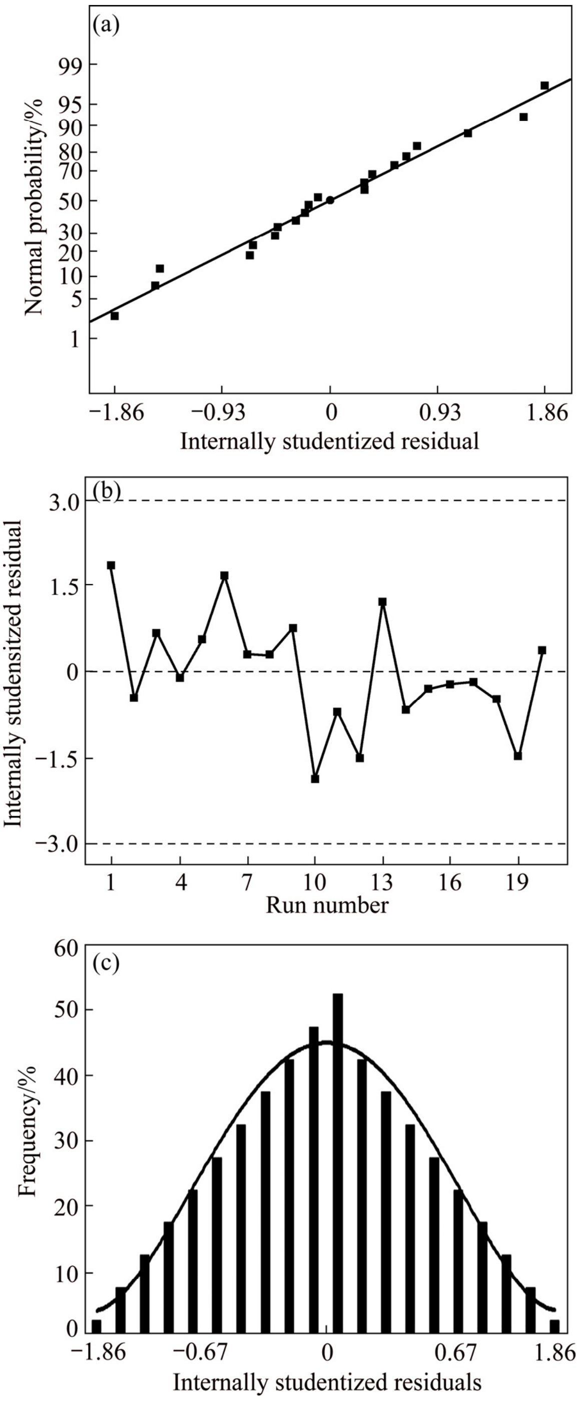

Fig. 2 Internally studentized residual plots for Y (response) at 22nd day in model: (a) Normal plot of residual; (b) Residual vs run number; (c) Histogram of residual

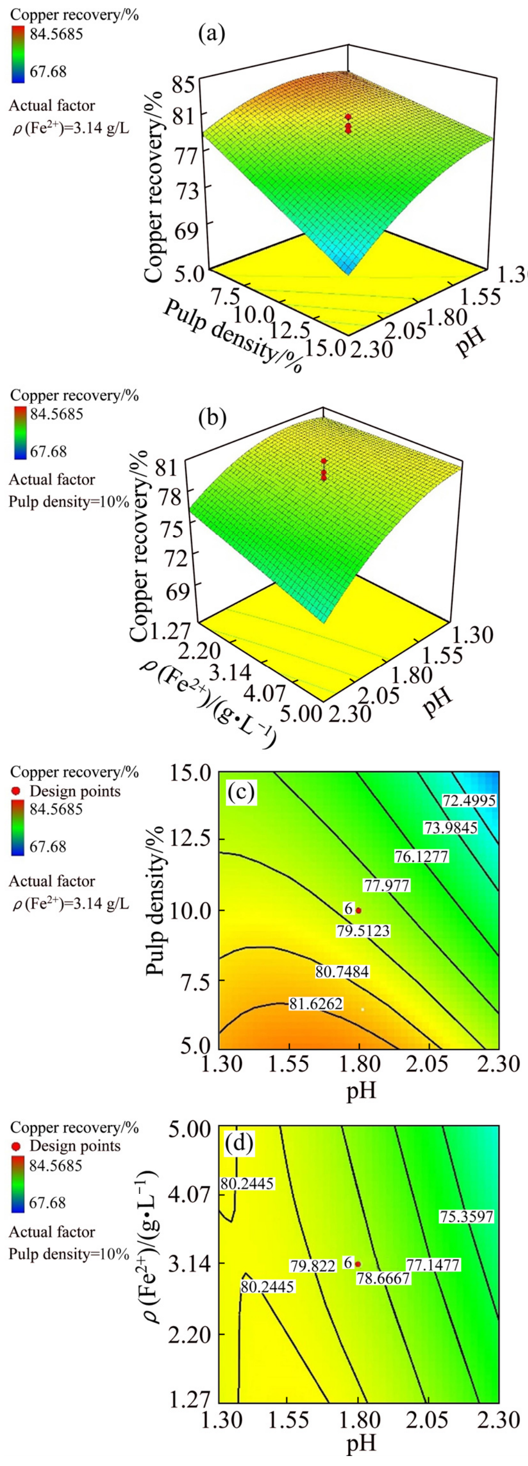

Fig. 3 Response surface plots of effect of initial pH value and pulp density (a) and initial pH value and initial concentration of ferrous ions (b), contour plots of interactions between initial pH value and pulp density (c) and initial pH value and initial concentration of ferrous ions (d)

extraction has a maximum value at the pH value of 1.60. After this point, at a constant value of pulp density, any decrease of pH value decreases the total copper extraction. Moreover, the results indicate that the total copper extraction increases up to 80.46% with decreasing the pH value, but after that, the total copper extraction decreases. Also, considering the initial pH value, an increase of pulp density and initial concentration of ferrous ions causes the total copper extraction to decrease. The three parameters could have an important effect on the total copper extraction. As shown in Table 7, the fractions of copper extraction during the bioleaching experiments are greatly affected by these three parameters.

3.3 Influence of parameters on total copper extraction

The results of shake flasks tests support the idea that commercial bio-hydrometallurgical processes employing mesophilic bacteria for the treatment of the low grade copper ore can be useful. The effect of leaching time on the dependent parameter (copper extraction) and independent parameters (pH value, pulp density, and initial ferrous concentration) at the 1st, 4th, 9th, 14th and 22nd days is shown in Figs. 4−6. It is clear that the leaching time affects the copper extraction kinetics and changes the leaching rate.

The procedure of changes in pH value over the obtained results at the 1st, 4th, 9th, 14th and 22nd days on the copper extraction is presented in Table 8. As illustrated in Fig. 4 and Table 8, with elapsing the leaching time at the first day, the pH value has a negative effect in the form of linear trend with a sharp slope. In fact, an increase of pH value from 1.3 to 2.3 decreases the amount of copper extraction below 4.42%. At the 4th day, the pH value has a negative effect in the form of linear trend with a low inclination, not statistically significant, and in fact with increasing the pH value from

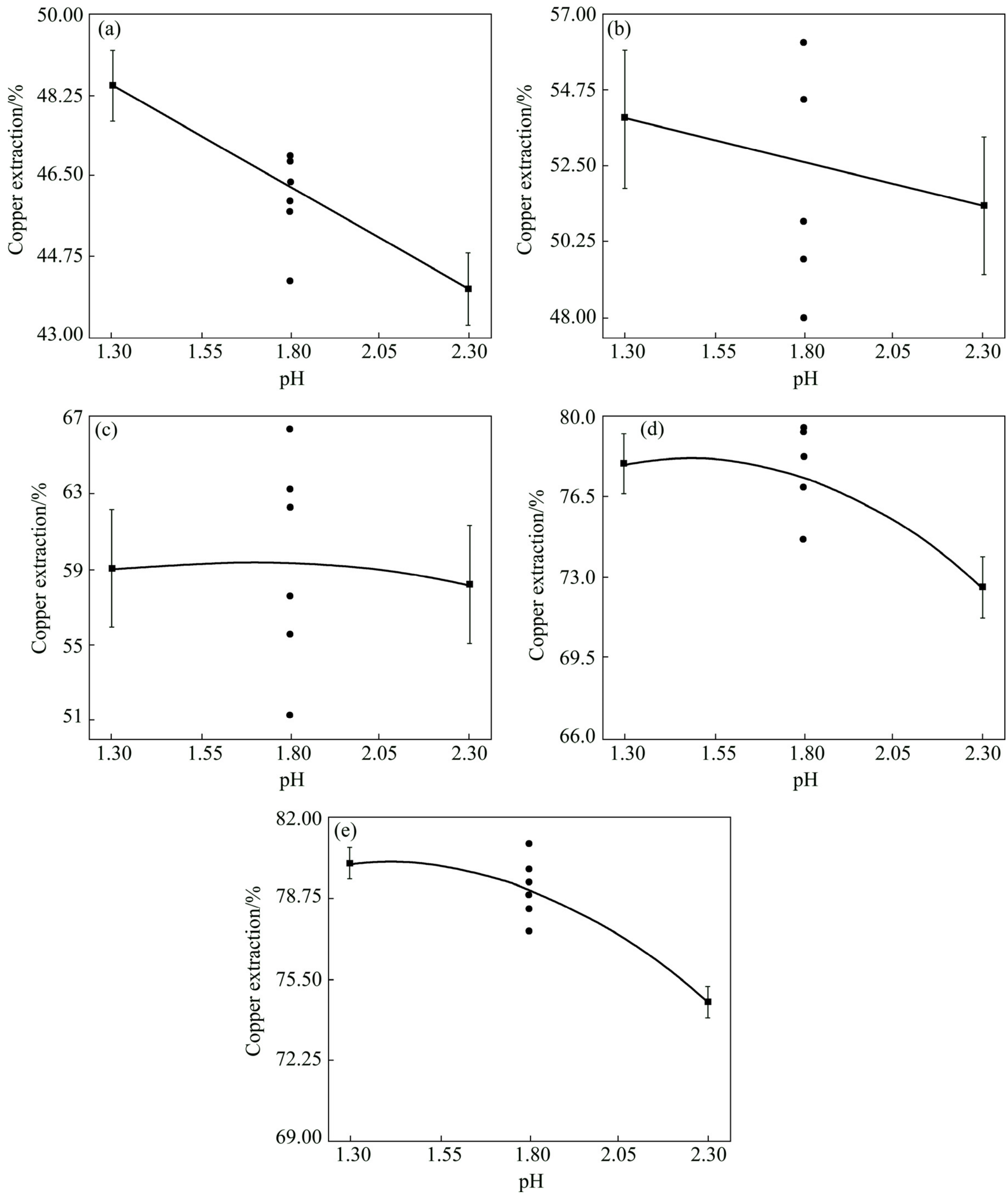

Fig. 4 Copper extraction vs pH value at 1st day (a), 4th day (b), 9th day (c), 14th day (d), and 22nd day (e)

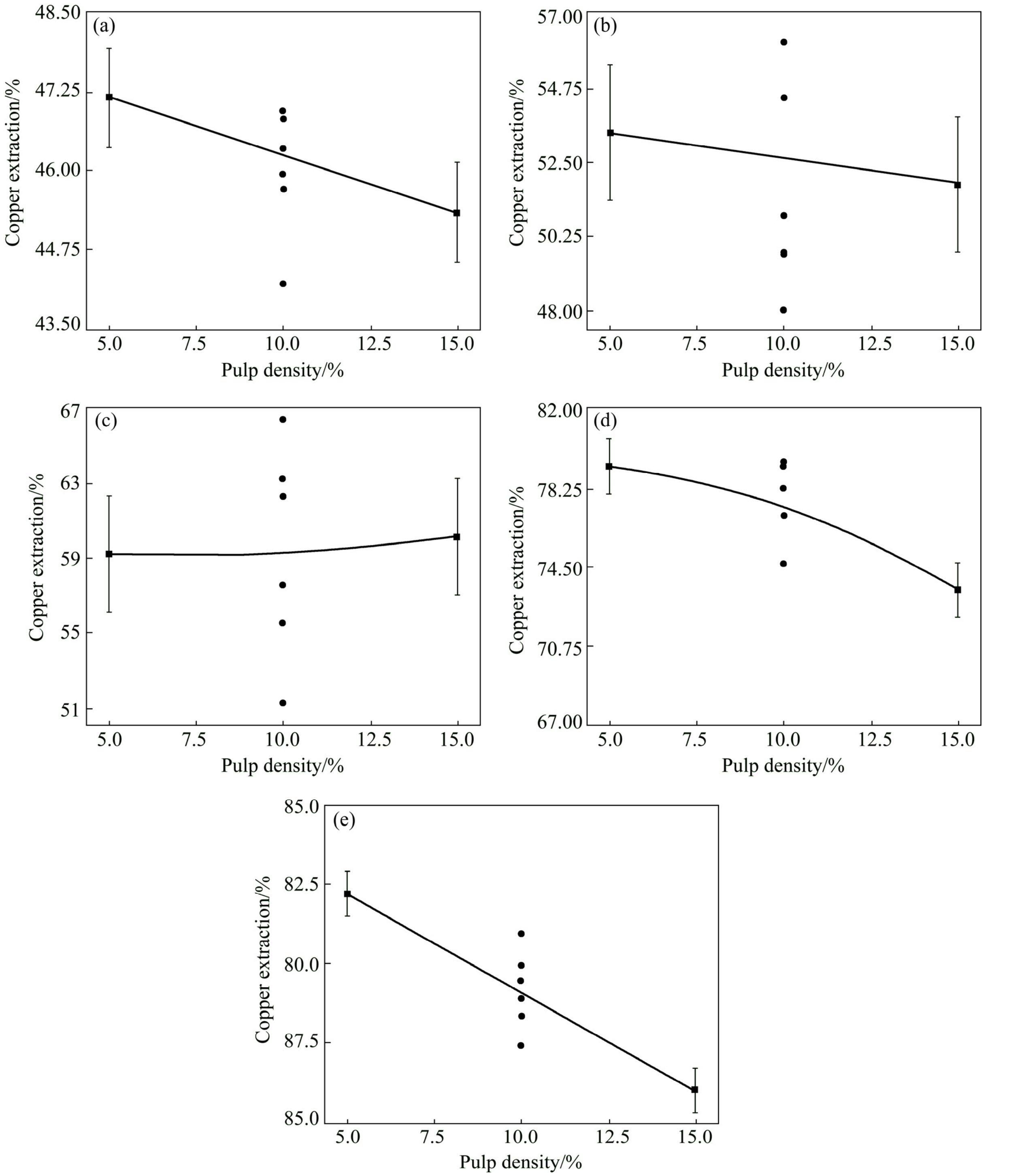

Fig. 5 Copper extraction vs pulp density at 1st day (a), 4th day (b), 9th day (c), 14th day (d), and 22nd day (e)

Table 8 Copper extraction difference during time intervals against variables (%)

Variable	1st day			4th day			9th day			14th day			22nd day		
	E_{Max}	E_{Min}	E_{diff}	E_{Max}	E_{Min}	E_{diff}	E_{Max}	E_{Min}	E_{diff}	E_{Max}	E_{Min}	E_{diff}	E_{Max}	E_{Min}	E_{diff}
pH	44.04	48.46	−4.42	51.32	53.90	−2.58	59.05	58.18	0.87	72.59	77.93	−5.34	74.60	80.19	−5.59
Pulp density	45.34	47.16	−1.82	49.92	51.49	−1.57	60.12	59.23	0.89	73.41	79.30	−5.89	76.02	82.17	−6.15
$\rho(Fe^{2+})/(g\cdot L^{-1})$	46.75	45.74	1.01	52.53	52.66	−0.13	58.92	60.86	−1.94	76.40	78.23	−1.83	78.24	79.95	−1.71

E_{Max}, E_{Min} and E_{diff} are the maximum, minimum and differences of copper extraction, respectively.

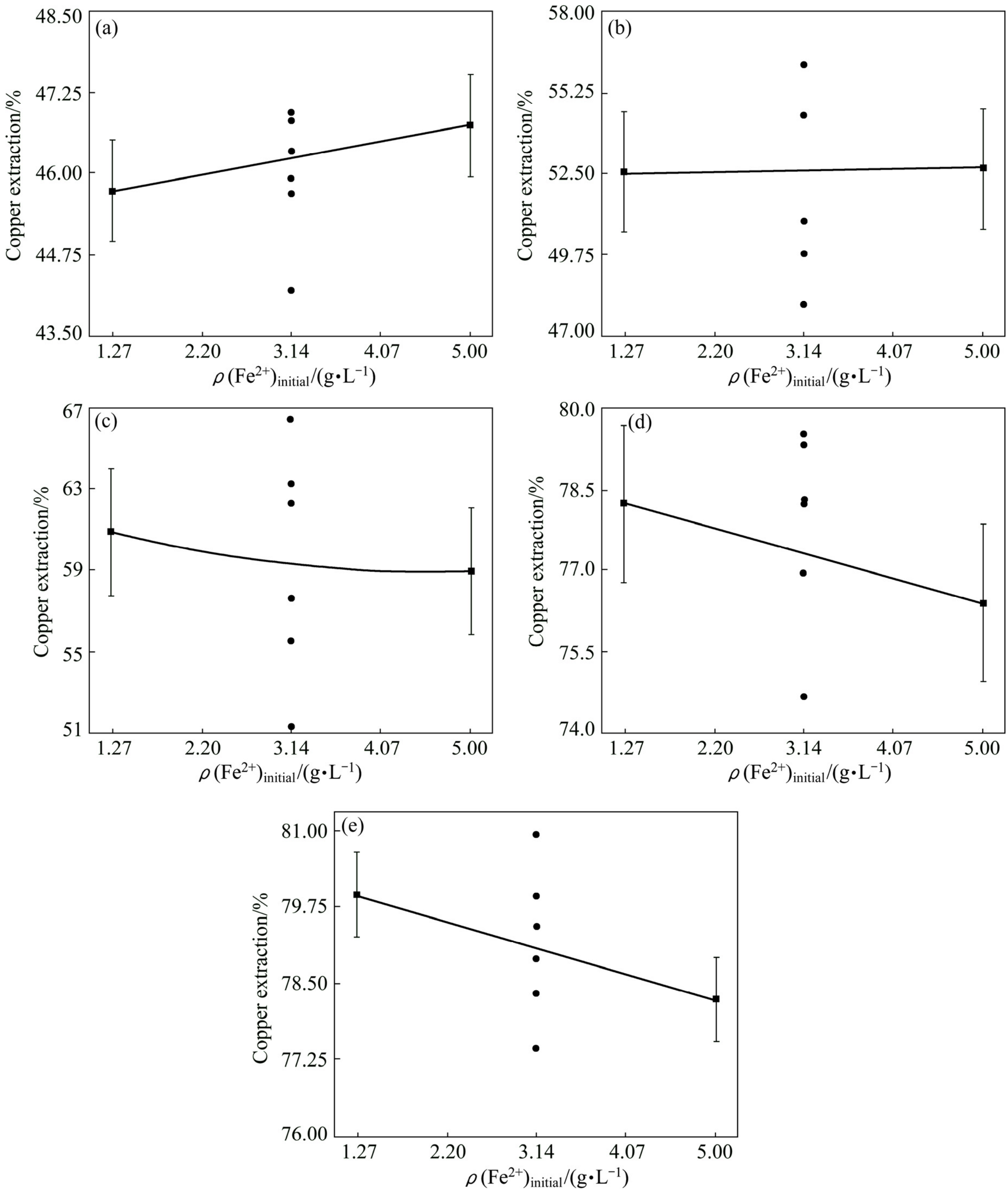

Fig. 6 Copper extraction vs initial concentration of ferrous ions at 1st day (a), 4th day (b), 9th day (c), 4th day (d), and 22nd day (e)

1.3 to 2.3, the amount of copper extraction decreases by about 2.58%. At the 9th day, the effect of pH value changes from linear first order to nonlinear, not statistically significant, and in fact, with increasing the pH value from 1.3 to 2.3, the amount of copper extraction increases by about 0.87%. Finally, at the 14th and 22nd days, the pH value has nonlinearly changed, and in fact, with increasing the pH value from 1.3 to 2.3, the amount of copper extraction decreases by about 5.34% and 5.59%, respectively. The results demonstrate that an increase of pH value decreases the total copper extraction. The acid production by bacteria proves the nonlinear nature of pH value in a bacterial leaching process. The acidity of growth medium significantly affects the growth and activity of acidophilic microorganisms.

As illustrated in Fig. 5 and Table 8, at the 1st day of treatment, the pulp density has a negative effect on the form of linear trend with a slow inclination. In fact, with increasing the pulp density from 5% to 15%, the amount of copper extraction decreases by about 1.82%. At the 4th day, the pulp density has a negative effect on the form of linear trend, not statistically significant and in fact, with increasing the pulp density from 5% to 15%, the amount of copper extraction decreases by about 1.57%. At the 9th day, the effect of pulp density changes from linear first order to nonlinear, not statistically significant and in fact, with increasing the pulp density from 5% to 15%, the amount of copper extraction increases by about 0.89%. At the 14th day, the effect of pulp density has nonlinearly changed, and in fact, with increasing the pulp density from 5% to 15%, the amount of copper extraction decreases by about 5.89%. Finally, at the 22nd day, the pulp density has linearly changed and indeed, with increasing the pulp density from 5% to 15%, the copper extraction decreases by about 6.15% with a sharp inclination. The effect of pulp density on the total copper extraction at the 22nd day is shown in Fig. 5(e).

With the same approaches as pH value and pulp density, the procedure of changes in the initial concentration of ferrous ions over the obtained results at the 1st, 4th, 9th, 14th and 22nd days on the copper extraction was investigated. As illustrated in Fig. 6, at the 1st day of treatment, the initial concentration of ferrous ions has a positive effect in the form of linear trend. In fact, by increasing the initial concentration of ferrous ions from 1.27 to 5.0 g/L, the amount of copper extraction increases by 1.01%. At the 4th day, the initial concentration of ferrous ions shows no any considerable effect. In fact, by increasing the initial concentration of ferrous ions from 1.27 to 5.0 g/L, the amount of copper extraction decreases by about 0.13%. At the 9th day, the effect of initial concentration of ferrous ions changes in the form of nonlinear, not statistically significant and in fact, by increasing the initial concentration of ferrous ions from 1.27 to 5.00 g/L, the amount of copper extraction decreases by about 1.94%. Finally, at the 14th and 22nd days, the initial concentration of ferrous ions has linearly changed, in fact, by increasing the initial concentration of ferrous ions from 1.27 to 5.00 g/L, the amount of copper extraction decreases by about 1.83% and 1.71%, respectively.

3.3.1 Effect of initial pH value

The pH value affects the bioleaching process in several ways. An appropriate pH value enhances the desired reactions. Also, keeping the pH value in an appropriate amount is important for those microorganisms which are sensitive to this parameter. The effect of pH value change on the leaching efficiency in the initial pH value range of 0.96−2.64 were studied (Fig. 7). It can be seen that the pH value in all

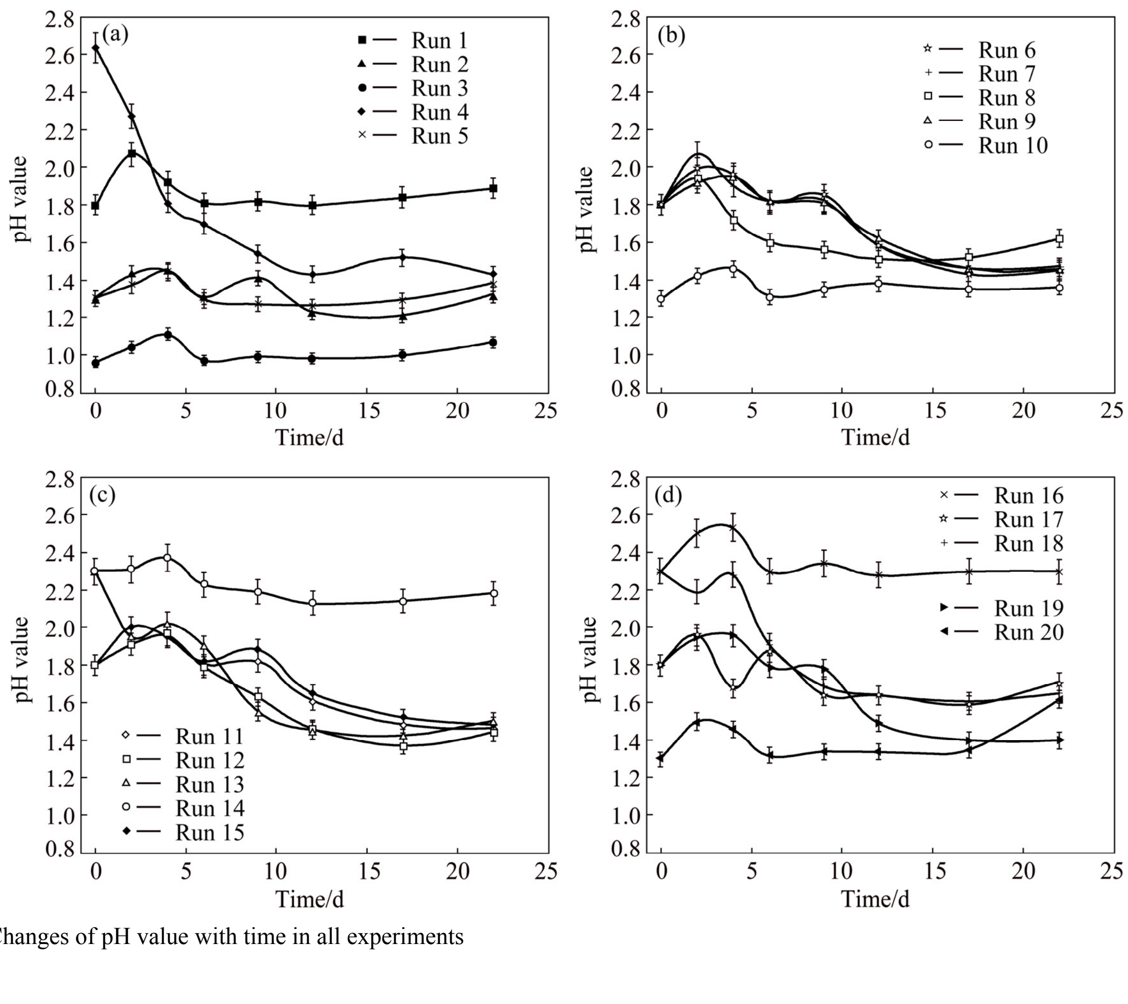

Fig. 7 Changes of pH value with time in all experiments

levels of the experiments increases for a few days and then decreases after a while. This pH value trend is attributed to two main reasons.

1) The increase of pH value is a reflection of the consumption of acid according to Eq. (12), and then, with the production of acid through the microbial oxidation of elemental sulfur, according to Eq. (13), the pH value decreases again.

2) The oxidation of ferrous ions to ferric ions consumes acid and the pH value increases. Furthermore, the pH value is buffered by iron hydrolysis resulting in the formation of jarosite (Eq. (14)) [52].

$$2FeSO_4+H_2SO_4+(1/2)O_2\xrightarrow{\text{Bacteria}}Fe_2(SO_4)_3+H_2O \tag{12}$$

$$S^0+H_2O+(3/2(O_2\xrightarrow{\text{Bacteria}}H_2SO_4 \tag{13}$$

$$X^{+}+3Fe^{3+}+2SO_4^{2-}+6H_2O= XFe(SO_4)_2(OH)_6+6H^{+} \tag{14}$$

where X^+ can be $(NH_4)^+$, K^+, Na^+ and Ag^+.

One may interpret that at the beginning of the experiments, the amount of acid required for the leaching solution was available due to the existence of initial acidity in each experiment. Moreover, due to the presence of oxide minerals in the ore, the copper extraction is caused with the pH value decreasing in the tests. As time progresses, the amount of solution acidity decreases and consequently the pH value has no significant effect on the copper extraction. On the other hand, bacteria reaches the appropriate growth and count. In addition, the procedure of H^+ production by bacterial activity is gradually accelerated and the solution acidity provided by the bacterial activity is enough to maintain the copper extraction. The total copper extraction at the 22nd day is presented in Table 7 and the effect of initial pH value on the total copper extraction at this day is shown in Fig. 4(e). Figure 4(e) shows that higher copper extraction is obtained at lower pH values. According to this results, the maximum amount of copper extraction is obtained at pH value of 1.3. On the other hand, the total copper extraction increases with decreasing the pH value from 2.3 to 1.3, which is probably due to the higher iron oxidation and bacterial activity at lower pH values [53].

The iron oxidation rate decreases with an increase of initial pH value. This results in a lower ferric concentration in the leaching medium. As a result, the total copper extraction decreases as the ferric concentration in the leaching medium decreases at higher pH value. This phenomenon is probably due to the iron precipitation process. The iron precipitation rate increases as the pH value increases [54]. The faster decrease of pH value under higher initial pH values can be attributed to the higher iron precipitation as jarosite and H^+ production. To confirm the iron precipitation reaction, the leaching residues were analyzed by XRD. The XRD data prove the presence of ammonium jarosite $(NH_4)Fe_3(SO_4)_2(OH)_6$ in the leaching residue (data not given). The ammonium ions presented in 9K medium may facilitate the formation of ammonium jarosite. The jarosite is found to be impervious, forming a thin layer on the solid particles [55]. This can be reported as a bioleaching process, which occurs via either intra-particle diffusion or diffusion through a product layer formed during the course of dissolution process. In addition, the decrease of leaching rates at pH values higher than 2.5 may occur due to the inhibition of bacterial activity since bacteria have a higher affinity to be absorbed on jarosite at higher pH values [56]. The growth of bacteria at pH value of 0.96 is inhibited, while at pH value of 2.64, leaching is more inhibited, probably by some jarosite formation or the formation of layers of elemental sulfur on the mineral surface. Otherwise, Run 3 shows that the resistance of bacteria at lower pH values less than 1 which the mesophilic microorganisms do not lose their activity and consequently, results in a considerable copper extraction. The copper dissolution rate is remarkably affected at pH value of 2.64, with little influence in the pH value range of 1.3−2.3.

3.3.2 Effect of pulp density

The bioleaching experiments in shake flasks were carried out using various pulp densities. The range of pulp density was from 1.59% to 18.41%. It is obvious that the pulp density has a significant effect on the leaching efficiency. The results confirm that the ability of the mesophilic bacteria to oxidize low grade copper ore is dependent on the pulp density. With the same approach as pH value, the procedure of change in pulp density over the obtained results at the 1st, 4th, 9th, 14th and 22nd days on the copper extraction is presented in Table 8. As illustrated in Fig. 5, at lower pulp densities, the leaching rates and total copper extraction are considerable, but with an increase of pulp density, the total copper extraction decreases. Hence, it is possible to obtain a higher level of copper extraction at pulp densities lower than 5%. A couple of reasons for the inefficient bioleaching at high pulp densities can be explained as follows.

1) The higher mechanical stress on the microorganisms that may be caused at higher ore concentrations [57,58].

2) The rate of oxygen supply from the gas phase is exceeded by the microbial demand, as is pointed out by the fact that the rates of leaching at 15% and 18.41% of pulp densities are approximately the same [57−60]. It is believed that high pulp density results in the decrease of copper extraction because of the limitation of air distribution and oxygen mass transfer in the process.

The change of redox potential (ORP) with time is shown in Fig. 8. As well illustrated, the redox potential is also considerably dependent on the pulp density. At lower pulp densities (≤5%), most of the copper is extracted, but at higher pulp densities (≥15%), only about 74% of the copper (the average of total copper extraction ranges from 67.68% to 76.95%, at −1 level in Tables 5 and 7) is extracted even after 22 days of treatment. The rate of leaching is constantly affected by the metal ions toxicity and ferric ion concentration which is gradually built up in the leaching system. The metal toxicity affects both the rate and degree of iron oxidation by bacteria at high pulp density [61]. At pulp densities of 1.59% and 5%, the redox potentials increase continuously from 396 to final values of 716 and 721 mV, respectively. At a pulp density of 10%, during the active phase of the bioleaching process, the redox potential increases continuously from 398 to 655 mV, but with slower rates than those at pulp densities of 1.59% and 5%. Applying pulp densities of 15% and 18.41% shows a relatively constant redox potential between 395 and 426 mV. As shown in Fig. 8, the redox potential at 1.59%, 5%, and 10% pulp densities increases to 716, 721 and 655 mV during only 4, 9 and 11 days, respectively, whereas the redox potential values are still less than 430 mV at 15% and 18.41% pulp densities even after 22 days of incubation. The results obtained at a pulp density of 1.59% show higher bio-extraction of copper compared with the tests that were carried out in the range from 5% to 18.41%. The shake flask tests confirm that more than 84% of Cu extraction can be obtained from low grade copper ore at a pulp density of 1.59% in a short period of time. The results of bioleaching tests at pulp densities varying from 1.59% to 18.41% show that the maximum copper extraction is obtained at the 22nd days (84.57%) using mesophilic bacteria when the pulp density is 1.59%.

3.3.3 Effect of initial concentration of ferrous ions

The oxidation of ferrous ion is one of the energy sources for the growth and metabolism of the evaluated bacteria in the bioleaching systems. Bacterial growth is directly related to the bioleaching process. The variation in ferrous ion concentration in the leaching medium is also important to predict the leaching kinetics. The bacteria use ferrous ion as the nutrient and convert it to ferric ion as a metabolite. As an oxidant, the ferric ion reacts with sulfide matrix and thereafter, it is reduced to ferrous ion. The oxidation−reduction cycle is continued in the entire leaching process. To evaluate the effect of Fe concentration on the leaching kinetics, the initial ferrous ion concentration was varied from 0 to 6.27 g/L.

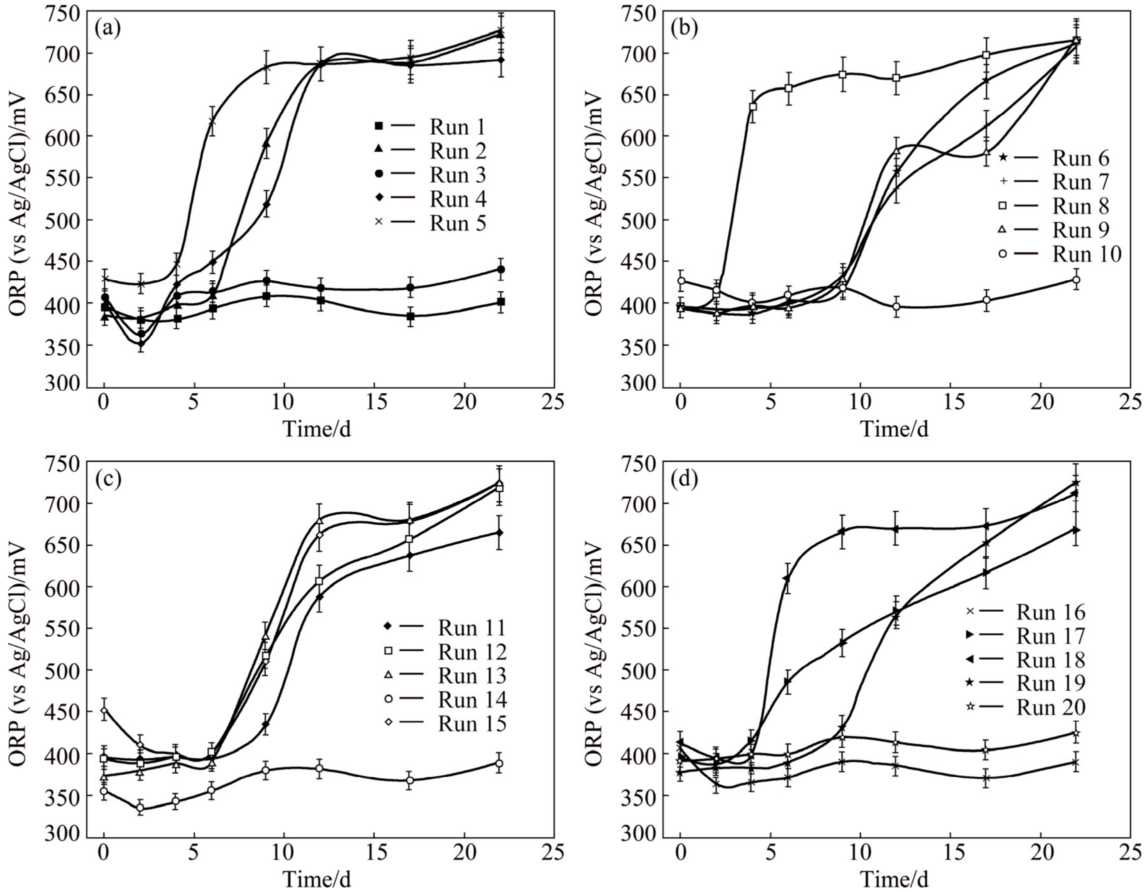

Fig. 8 Changes of redox potential with time in all experiments

According to the results, the total copper extraction decreases as the initial ferrous concentration increases. The results of these experiments explain that at the 1st day, some of this ion is converted into ferric ion due to the abundance of free ferrous ions in the solution and according to Le Chatelier's principle. The ferric ion attacks the ore structure as an oxidant agent and causes copper extraction (Fig. 6(a)). However, as time progresses and pyritic ore structure breaks, the amount of ferrous ion in the environment highly increases. In addition, by an increase of bacterial population, the amount of ferric ion increases in the environment as well. Moreover, the total iron in the solution increases, which results in iron precipitation reaction. The copper extraction eventually decreases (Figs. 6(b)−(e)). The effect of initial concentration of ferrous ions on the total copper extraction at the 22nd day is shown in Fig. 6(e). As illustrated, higher extraction rates are obtained at low initial concentrations of ferrous ions, i.e., 1.27 g/L. Also, high ferrous ion concentrations may negatively affect the bacterial activity. It is observed that the leaching rate and total copper extraction decrease with an increase of initial ferrous concentration. The negative effect of increasing the initial ferrous concentration may be due to the enhanced iron precipitation rate. The precipitated iron is observed to be ammonium jarosite ($(NH_4)Fe_3(SO_4)_2(OH)_6$) which forms an impervious product layer on the reactant [62]. Ammonium jarosite impedes the leaching process which involves either the diffusion inside the reactive matrix or through product layer formed during the course of dissolution process. In addition, it is suggested that jarosite controls the solubility of ferric ion as the main oxidant in the bioleaching process [63,64]. Therefore, the rate of metal dissolution decreases with the increase of jarosite at higher ferrous ion concentrations. Besides, the pH value of the leaching media decreases as the concentration of ferrous ion increases due to the production of large amounts of acid during the leaching process. The amount of acid can only be compensated by the hydrolysis of ferric ion produced during the bacterial iron oxidation reaction [56].

3.4 Optimization of parameters

The levels of three influential factors were optimized to achieve the maximum copper extraction after 22 days of mixed mesophilic bioleaching tests on the sulfide and oxide low-grade ore, using the proposed second order polynomial model (Eq. (6)). One of the main goals of this study is to find optimal values of parameters to maximize the total copper extraction derived from the mathematical model equation. The quadratic model equation was optimized using quadratic programming (QP) to maximize the total copper extraction within the studied experimental range. This model predicts that the maximum copper extraction is 85.98% after 22 days of bacterial leaching under the pH value of 2.0, pulp density of 1.59%, and with the absence of ferrous ions at the initial stage. Whereas the maximum copper extraction is 85.17% in the experiment with the similar condition (0.81% upgrading in the total copper extraction). Since the predicted response by the model is appropriate, this confirms the suitability of the regression model for the predictive purposes. The second order polynomial equation indicates that the three factors do not have the same effect on the response.

4 Conclusions

1) The pulp density and pH value are the major experimental parameters affecting the copper extraction after 22 days of incubation process.

2) After 22 days of treatment, the level of pH value during the process is found to depend on both levels of pulp density and initial concentration of ferrous ions.

3) There is no statistically significant interaction between the initial ferrous ion concentration in the bacterial leaching media and the pulp density at the 22nd day.

4) Decreasing the pulp density in the optimal level, 1.59%, has the effect of shifting the optimal level of pH value to higher values. Furthermore, this also diminishes the importance of pH value control during the process.

5) Taking the advantage of quadratic programming, the initial pH value of 2.0 and the pulp density of 1.59% with the absence of ferrous ions at the initial stage are determined as the preferable levels of the parameters to achieve the maximum copper extraction of 85.98% which is 84.57% in the tests.

6) After modeling and optimizing the process parameters, the effect of leaching time on the independent parameters as well as on the copper extraction was examined.

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从低品位铜矿石中生物浸出铜的基于响应面方法的经验模型

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摘　要：使用响应面方法对在摇动的生物反应器里浸出铜进行模拟和优化。采用中温细菌生物浸出低品位铜矿石，研究浸矿过程参数的影响，包括 pH 值、矿浆浓度、亚铁离子起始浓度。检测了浸出 1、4、9、14 和 24 天后的铜浸出率，对浸出时间对浸出率的影响进行建模。采用中心组合设计法(CCD)建立模型，以预测最佳参数值。采用二次方程建模对实验范围内浸出 22 天的参数进行优化，以达到最大铜浸出率。在最佳条件下(初始 pH 值 2.0，矿浆浓度 1.59%，亚铁离子浓度为 0)模型预测的铜浸出率为 85.98%，这非常接近实验结果(84.57%)。对 pH 值和矿浆浓度之间以及 pH 值和亚铁离子浓度之间的相互作用的影响进行研究。结果显示，亚铁离子起始浓度和矿浆浓度之间没有明显的相互作用，而且发现在最佳 pH 值和矿浆浓度下，铜的浸出率与亚铁离子起始浓度值没有关系。

关键词：建模；优化；细菌浸出；响应面方法；铜浸出；铜矿

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