JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2013. Vol. **7**(2), p. 871-879

Optimization of Bioleaching Conditions of a Low Grade Complex Nickel-copper Concentrate using Response Surface Methodology

Rong Cheng*, Rongbo Shu, Xiangwen Liao, Houming Liu and Changliang Wang

Institute of Multipurpose Utilization of Mineral Resources, Chinese Academy of Geological Sciences, Chengdu - 610 041, China.

(Received: 09 March 2013; accepted: 19 April 2013)

The increased demand has generated renewed interest in lower grade or complex ores. In this study, a low grade complex nickel-copper concentrate was subjected to bioleaching. To develop the process technology, response surface methodology (RSM) coupled with center composite design (CCD) was applied to optimize the bioleaching conditions of the concentrate. Based on the models, pulp density was considered as the most significant component since its contribution reached 55.78% for Ni extraction and 15.26% for Cu extraction. The terms of pulp density and pH in the model indicated unfavorable effects on the Ni extraction, which defied the experimental phenomenon of Cu extraction. On the other hand, the interaction effect of pulp density and pH was favorable on the Ni recovery, whereas Cu extraction would be least influenced by the effect of interaction. The optimal conditions for Ni extraction and Cu extraction were quite different.

Key words: Bioleaching, Nickel-copper concentrate, Center composite design, Chalcopyrite, Pentlandite.

The demand for nickel is growing worldwide, driven by the world stainless steel market which consumes 67% of primary nickel, due to increased demand from China[1]. The increased demand has generated renewed interest in exploration to discover new resources and the development of new processes suited to lower grade or complex ores. Bioleaching of sulfide minerals is a mature technology with many industrial applications. Recently, Cameron *et al.*,[2] investigated the technical feasibility of using elevated-pH bioleaching on a Canadian low-grade ultramafic nickel sulfide ore and found that nickel extraction from pentlandite was relatively insensitive to acidity at low pH but sensitive at high pH. Likewise, Zhen *et al.*,[3] studied the bioleaching of a Chinese low grade nickel-bearing sulfide ore using mixed mesophiles and found that the tolerance of the mixed bacteria to magnesium in solution could be improved markedly from 10 g/L to 25 g/L Mg. On the other hand, Santos *et al.*,[4] studied on the bioleaching of a low grade complex nickel-iron concentrate containing pentlandite together with significant levels of pyrrhotite and minor amounts of chalcopyrite. The external addition of Fe (II) showed no effect on the extraction of nickel, emphasizing the importance of pyrrhotite dissolution in the first step of bioleaching. Yang *et al.*,[5] investigated the bioleaching behavior of the metals (Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{3+} and Mg^{2+}) of a Chinese low grade nickel-copper-cobalt sulfide ore and the co-precipitation of copper and nickel ions with the jarosite formation was also studied. Other recent investigations of bioleaching of a low-grade copper-nickel sulfide ore from Radio Hill focused on the impact of

* To whom all correspondence should be addressed.
Tel.: +86 28 8559 2350; Fax: +86 28 8559 4582;
E-mail: chengrong008899@aliyun.com

aeration and pH on copper and nickel extraction during column and heap leaching[1, 6, 7].

In the present, a low grade complex nickel-copper concentrate was subjected to bioleaching. To develop the process technology, single-factor experiment was applied to optimize the bioleaching conditions of the concentrate. Unfortunately, the traditonal method is time-consuming and has a poor performance for optimizing a large number of parameters. Besides, it might lead to erroneous conclusions, since interactions between factors are missed[8]. Response surface methodology (RSM) coupled with center composite design (CCD), is a comprehensive and multi-functional tool for designing experiments, building models, and analyzing the effects of multiple factors and their interactions[9,10]. RSM is the most popular technique used to find the optimal conditions by using quadratic polynomial model and is applied as a consequence of a screening or diagnostic experiment[11,12]. The CCD requires much fewer tests than the full factorial and has been shown to be sufficient to describe the majority of steadystate responses[13]. However, very few works have focused on using CCD in bioleaching technology. The interactions between pH value, pulp density and inoculum size still need more investigation to understand environmental effects.

To simplify the calculations and for uniform comparison, the bioleaching process of the low grade complex nickel-copper concentrate was investigated for nickel ions extraction and copper ions extraction via CCD. During the process, three main factors (pH value, pulp density and inoculum size) were systematically researched and optimized for high extraction. The experimental results were analyzed statistically by the analysis of regression models, model components, 3D response surfaces and optimized studies.

MATERIALS AND METHODS

Concentrate samples and characterization

The low grade complex nickel-copper concentrate obtained from Danba County, Sichuan Province, China, was used in the test. The chemical analysis of the concentrate is shown in Table 1. Using XRD, the major phases in the concentrate were found to be calcopyrite, pentlandite and pyrrhotite, together with lesser amounts violarite, covellite, and calcocite *et al*. The main minerals, calcopyrite, pentlandite and pyrrhotite were not completely liberated exhibiting very fine and intimately associated grains. The concentrate samples used for flask leaching were screened and further ground to -0.074 mm (≥ 90%).

Table 1. Chemical composition of the concentrate sample

Ni (%)	Cu (%)	Fe (%)	S (%)	Mg (%)	SiO_2 (%)
5.3	2.21	35.52	16.18	3.71	6.55

Microorganism and medium

A mixed moderate thermophiles was obtained from a mine in Sichuan province and previous biooxidation studies have shown that the bacteria are capable to oxidize ferrous iron and sulfur (Data not shown). The microorganisms were cultured in 250 mL shake flasks using an orbital incubator with a stirring speed of 200 revolutions per minutes (rpm) at a constant temperature (48°C). A sterilized basal salts (9K) medium, composed of 3.0 g/L $(NH_4)_2SO_4$, 0.1 g/L KCl, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g/L K_2HPO_4 and 0.01 g/L $Ca(NO_3)_2$, was used for bacteria growth, with ferrous ions (44.7 g/L $FeSO_4{\cdot}7H_2O$) as the energy source. Giaveno and Donati[14] found that the mixed culture was more efficient under all conditions tested. So, prior the bioleaching experiment, the bacteria were sub-cultured into iron-free 9K medium supplemented with the concentrate (-0.074 mm ≥ 90%) as the energy source. The resulting mixed culture was used as inoculums for the shake flasks experiments.

Bioleaching experiments

An appropriate amount of concentrate powder (-0.074 mm ≥ 90%) was initially added to basal salts medium without ferrous ions and the flasks were placed in an orbital shaker at 200 rpm and 48°C. The pH value of acid leaching in the first 3~5 days was controlled at pH 0.8~1.0 with concentrated sulfuric acid since the ore contained high levels of acidconsuming minerals, then an amount of inoculums with the cell density of about 10^8 cell/mL was added into the conical flask for bioleaching. The pH value of the mixtures, pulp density and inoculum size were controlled according to the experimental design (Table 2). Water lost by evaporation was supplemented periodically by adding sterile water until the mass of the flask equaled its initial mass. Samples (3 mL) were removed for analysis and the sample volume was replaced with an equal volume of fresh 9K media.

Table 2. Coded levels for independent factors in the experimental design

Factors	Symbol	Coded levels				
		-1.68	-1	0	1	1.68
pH value, /	X_1	1.0	1.2	1.5	1.8	2.0
pulp density, % (w/v)	X_2	1.59	5	10	15	18.41
inoculum size, % (v/v)	X_3	6.59	10	15	20	23.41

Analytical techniques

Aqueous metal concentrations (Ni, Cu and total iron) were determined using ICP-AES (a Perkin-Elmer Optima 5300V device). Ore samples were digested in aqua regia (HNO_3: HCl=1:3) analysis by ICP-AES. The pH (Mettler Toledo SG8) was controlled and the redox potential (Mettler Toledo SG2 LE501) recorded.

Experimental design and evaluation

Based on the results obtained by the classical approach, the factors were selected, and the ranges of these variables were decided (Date not shown), while the optimum growth temperature of the bacteria was at 48°C. Further, RSM coupled with CCD was employed to optimize the bioleaching process and investigate the relative and interactive effects of pH value, pulp density and inoculum size on the Ni extraction and Cu extraction. In brief, CCD was performed with three chosen independent variables at five levels, as shown in Table 2. According to statistical theory, three-factor, five-level CCD requires 20 sets of experiments, which include eight factorial points (cubic points), six axial points (star points), and six replicates at the center point.

Design Expert software (trail Version 8.0.5.0, Stat-Ease Inc., Minneapolis, MN, USA) was used to design the experiments and analyze the data. The obtained experimental data were fitted to the following quadratic polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} x_i \beta_{ij} x_j + \varepsilon \quad ...(1)$$

where Y is the response variable; i and j are the index numbers for the patterns; β_0 is the constant coefficient; β_i, β_{ii} and β_{ij} are the coefficients for the linear, quadratic and interaction effects, respectively; x_i and x_j are the coded variables; and ε is the error.

In Eq. (1), the coded variables were obtained according to the equation below:

$$x_i = (X_i - X_0) / \Delta X_i \quad ...(2)$$

where x_i is the coded value of the independent variable, X_i is the actual value of the independent variable, X_0 is the actual value of the independent variable at the center point, and ΔX_i is the step change value.

RESULTS AND DISCUSSION

Regression model and statistical analysis

The multiple regression analysis has been employed to study the relationships between the three effect factors and the responses (Table 3). Twenty sets of runs were randomly carried out to minimize the effects of the uncontrolled factors.

After regression analysis of the experimental data using Design Expert software, the following second-order polynomial equation in the coded form was obtained, which could give the predicted value of Ni extraction and Cu extraction:

$$P_1 = 0.98 + 0.036x_1 - 0.070x_2 + 0.011x_3 + 0.032x_1x_2 - 9.748\times10^{-3}x_1x_3 + 0.017x_2x_3 - 0.026x_1^2 - 0.031x_2^2 + 1.701\times10^{-3}x_3^2 \quad ...(3)$$

$$P_2 = 0.52 + 0.044x_1 + 0.066x_2 - 2.876\times10^{-3}x_3 + 0.067x_1x_2 + 0.01x_1x_3 - 0.039x_2x_3 + 0.07x_1^2 + 0.13x_2^2 + 3.581\times10^{-3}x_3^2 \quad ...(4)$$

where P_1 and P_2 are the responses denoted as the predicted Ni extraction and Cu extraction.

Table 3. Experimental results of the central composite design

Run	Factors			Response values (Ni extraction)		Response values (Cu extraction)	
	X_1 (coded)	X_2 (coded)	X_3 (coded)	Observed	Predicted	Observed	Predicted
1	1.5 (0)	10 (0)	15 (0)	0.951	0.980	0.507	0.520
2	1.8 (1)	5 (-1)	10 (-1)	1.000	1.014	0.489	0.588
3	1.5 (0)	10 (0)	15 (0)	0.975	0.980	0.505	0.520
4	1.8 (1)	15 (1)	20 (1)	0.919	0.941	0.908	0.869
5	1.8 (1)	15 (1)	10 (-1)	0.892	0.904	0.935	0.932
6	1.0 (-1.68)	10 (0)	15 (0)	0.849	0.846	0.670	0.644
7	1.5 (0)	10 (0)	15 (0)	1.000	0.980	0.502	0.520
8	1.8 (1)	5 (-1)	20 (1)	1.000	0.983	0.552	0.681
9	1.5 (0)	18.41 (1.68)	15 (0)	0.806	0.775	0.868	0.999
10	1.5 (0)	10 (0)	6.59 (-1.68)	0.991	0.966	0.579	0.535
11	1.2 (-1)	5 (-1)	10 (-1)	1.000	0.987	0.534	0.654
12	1.5 (0)	10 (0)	15 (0)	0.975	0.980	0.493	0.520
13	1.2 (-1)	5 (-1)	20 (1)	1.000	0.994	0.624	0.707
14	2.0 (1.68)	10 (0)	15 (0)	0.987	0.967	0.846	0.792
15	1.5 (0)	1.59 (-1.68)	15 (0)	1.000	1.010	0.968	0.777
16	1.5 (0)	10 (0)	15 (0)	0.988	0.980	0.520	0.520
17	1.2 (-1)	15 (1)	10 (-1)	0.726	0.749	0.781	0.730
18	1.5 (0)	10 (0)	15 (0)	1.000	0.980	0.548	0.520
19	1.5 (0)	10 (0)	23.41 (1.68)	1.000	1.003	0.561	0.525
20	1.2 (-1)	15 (1)	20 (1)	0.831	0.824	0.646	0.627

And x_1, x_2 and x_3 are the coded terms for the three independent variables denoted as pH value, pulp density and inoculum size, respectively. It could be obtained that the predicted Ni extraction and Cu extraction from different batches by analyzing Eqs. (3) and (4). Moreover, from Table 3, a wide variation ranging from a minimum of 0.749 to a maximum of 1.014 occurred for the predicted Ni extraction. And a wide variation ranging from a minimum of 0.520 to a maximum of 0.999 occurred for the predicted Cu extraction. From these results, it was demonstrated that Ni extraction and Cu extraction was strongly dependent on the choice of the variables in the study.

The residual plots were examined for the model adequacy for each metal extraction values. In Fig. 1 the normal % probability and studentized residual plot is shown for two metal extraction percentages. All of the normal probability plots show how well the model satisfies the assumptions of the ANOVA where the studentized residuals measure the number of standard deviations separating the actual and predicted values[15]. On the other hand, Eqs. (3) and (4) may be used to visualize the effects of experimental factors on metal extractions by using the actual and predicted metal extraction percent graphs.

Effects of model components and their interaction on metal extractions

The sectoral diagram (Fig. 2) showed the percentage contributions for the first-order, quadratic and interaction terms according to the sum of squares (Date not shown). For Ni extraction (Fig. 2a), the first-order terms exhibited the highest percentage of total contribution of 71.45%, and followed by the quadratic terms of total contribution of 19.38%. The rests of the contributions from the interaction terms were less than 10% and recognized as insignificant components for predicting Ni extraction. To summarize, the first-order independent variables had more direct effects on the regression model than the others. For Cu extraction (Fig. 2b), the quadratic terms exhibited the highest percentage of total contribution of 47.08%, and followed by the interaction terms of total contribution of 26.82% and the first-order terms of total contribution of 26.10%.

Table 4. Different conditions found by the models and verification of the models

Run	pH value	pulp density	inoculum size	Predicted		Observed	
	/	% (w/v)	% (v/v)	Ni	Cu	Ni	Cu
1	1.5	10	15	0.980	0.520	0.992	0.514
2	1.8	15	20	0.941	0.869	0.921	0.924
3	1.8	15	10	0.904	0.932	0.896	0.962
4	1.0	10	15	0.846	0.644	0.874	0.674
5	2.0	10	15	0.967	0.792	0.925	0.815

The three terms were more than 10%, respectively, and recognized as significant components for predicting Cu extraction. However, the quadratic independent variables had more direct effects on the regression model than the others.

Fig. 3 showed standardized effects of the individual variables and their interactions on the dependent variable (metal extraction) using a Pareto chart. The bars in the Pareto chart represent the absolute values of estimated effects on the response. Furthermore, the vertical line of the chart is equal to the 95% confidence level. If the bar crosses the vertical line, the corresponding term is regarded as a statistically significant factor. Among the studied terms (Fig. 3a), the bar for x_1x_3, x_2x_3, x_3 and x_3x_3 in the chart did not cross the vertical line, indicating Ni extraction would be least influenced by the interaction between x_1 and x_3 and the interaction between x_2 and x_3. And the effect of x_3 was also regarded as a secondary factor. Meanwhile, it was revealed that the interaction between x_1 and x_2 was significant. In other words, pH and pulp density had different effects on Ni extraction. On the other side, the negative terms in the model (x_2, x_2x_2 and x_1x_1) indicated unfavorable or antagonistic effects on the Ni extraction, whereas the positive terms (x_1 and x_1x_2) showed favorable or synergistic effects on the Ni extraction. As seen in Fig. 3b, the effects of x_2, x_2x_2 and x_1x_1 were regarded as the positive and significant factors, which defied the effects on Ni extraction. It demonstrated that the optimal condition for bioleaching Ni was an adverse condition for bioleaching Cu. And the maximum Ni extraction and Cu extraction would not obtain at one same leaching condition. Moreover, the bar for x_1x_3, x_2x_3, and x_1x_2 in the chart did not cross the vertical line, indicating Cu extraction would be least influenced by the effect of interaction, which was also confirmed by the sectoral diagram (Fig. 2b).

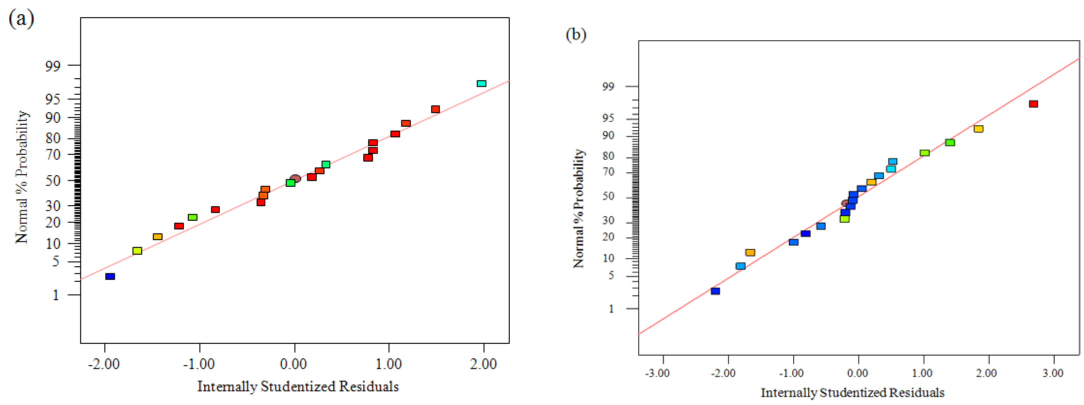

Fig. 1. The studentized residuals and normal % probability plot for (a) nickel and (b) copper extraction

Three-dimensional response surfaces for metal extractions

Three-dimension (3D) response surfaces

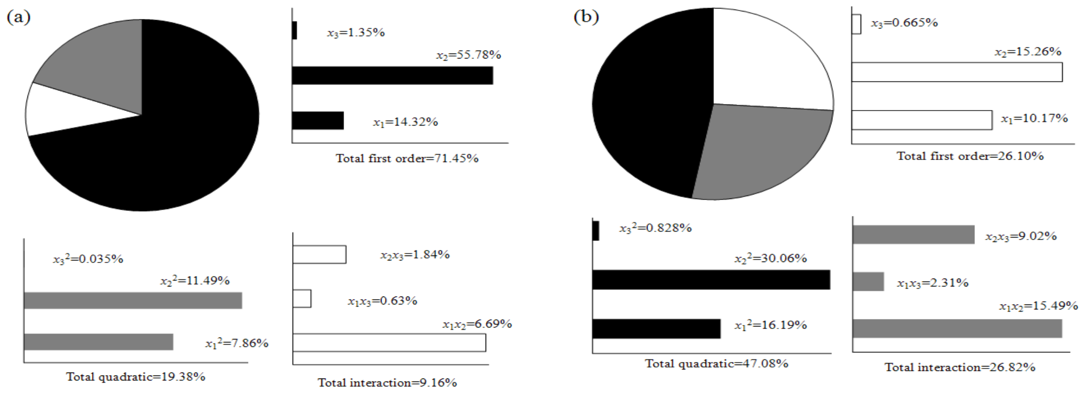

Fig. 2. Percentage contributions of model components from the regression model: (a) Ni; (b) Cu

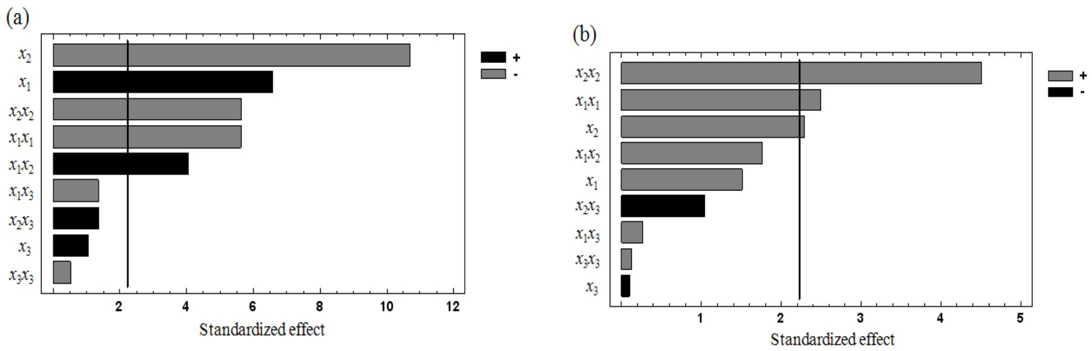

Fig. 3. Pareto chart of standardized effects for the model: (a) Ni; (b) Cu. The vertical line indicates the 95% confidence level. Factors with statistically significant are those crossed the line

were performed to elucidate the individual and mutual effects of the experimental variables on the response (Figs. 4 and 5).

It is clean that the leached Ni is markedly higher in lower pulp density (Fig. 4a). In the shake flask leaching, the movements and mechanical actions of ore particles on bacteria may disturb their growth, and in a higher pulp density these more effective actions would decrease the extracted yields in bioleaching. On the other hand, some produced metal ions would cause damage to cells at certain concentrations[16]. From Fig. 4b and c, the morphology indicated inoculum size acted as an insignificant factor for Ni extraction, which was also confirmed by the sectoral diagram (Fig. 2a) and the Pareto chart (Fig. 3a). During leaching the pH of the pulp was controlled by adding sulfuric acid solution regularly. It seems that a lower pH may hinder the growth of bacteria in the one side, and it is favorable to prevent ferric ions in leachates from precipitation and then to the bioleaching. As seen in Fig. 4a and b, nickel extraction showed limited dependency on pH in the range of pH 1.0-2.0, which was similar to previous reports. Several studies on the (bio)leaching of nickel from pentlandite and pyrrhotite has been shown to be less dependent on pH[17-19].

Fig. 5a shows the proportional increase of Cu extraction with increase in pulp density and pH value. It was hypothesized that high pulp density resulted in high concentration of total iron (Date not shown) was responsible for the phenomenon. Under such conditions, iron-oxidizing bacteria present would be unlikely to assist significantly in the dissolution of chalcopyrite. In the presence of enough ferric and ferrous ions, chalcopyrite is reduced to cupric ions according to

$$CuFeS_2+3Cu^{2+}+3Fe^{2+}=2Cu_2S+4Fe^{3+} \quad ...(5)$$

$$2Cu_2S+8Fe^{3+}=4Cu^{2+}+2S+8Fe^{2+} \quad ...(6)$$

As the reaction goes on, sulfur layer passivation occurring would prevent the leaching of chalcopyrite. The bacterial flora in the bioleaching system of chalcopyrite consisted mainly of sulfur-oxidizing organisms at relatively high pH value. Although the experimental phenomenon was opposite to general reports that low-pH conditions are considered to be favorable for the dissolution of chalcopyrite[20], Riekkola-Vanhanen *et al.*,[18] observed copper extraction from chalcopyrite to be faster at high pH value. From Fig. 5b and c, the morphology indicated inoculum

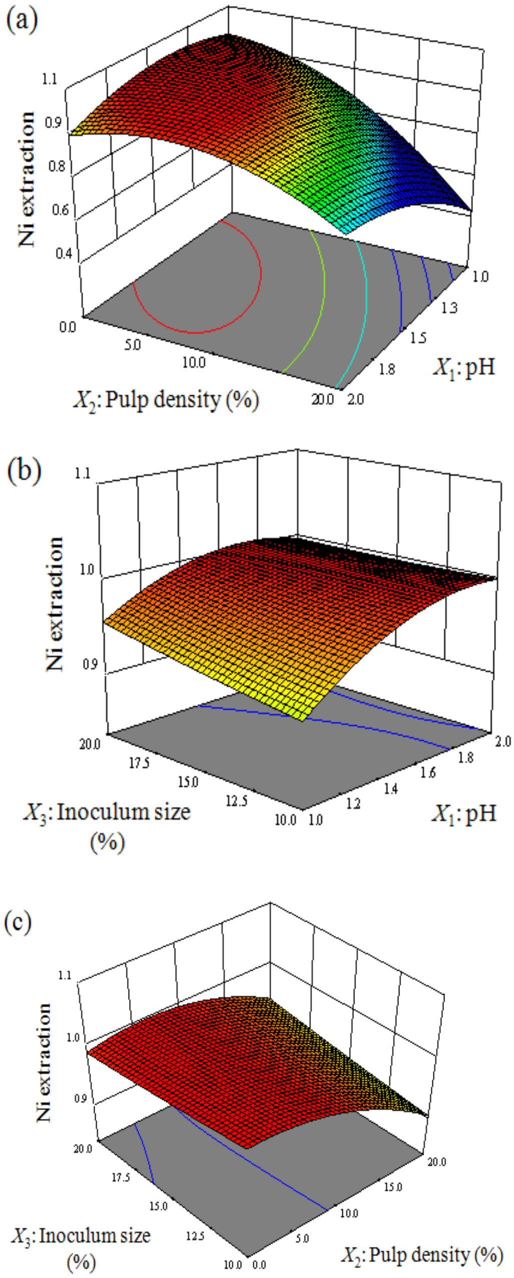

Fig. 4. Ni extraction in 3D response surfaces:
(a) effects of pH value and pulp density,
(b) effects of pH value and inoculum size,
(c) effects of pulp density and inoculum size

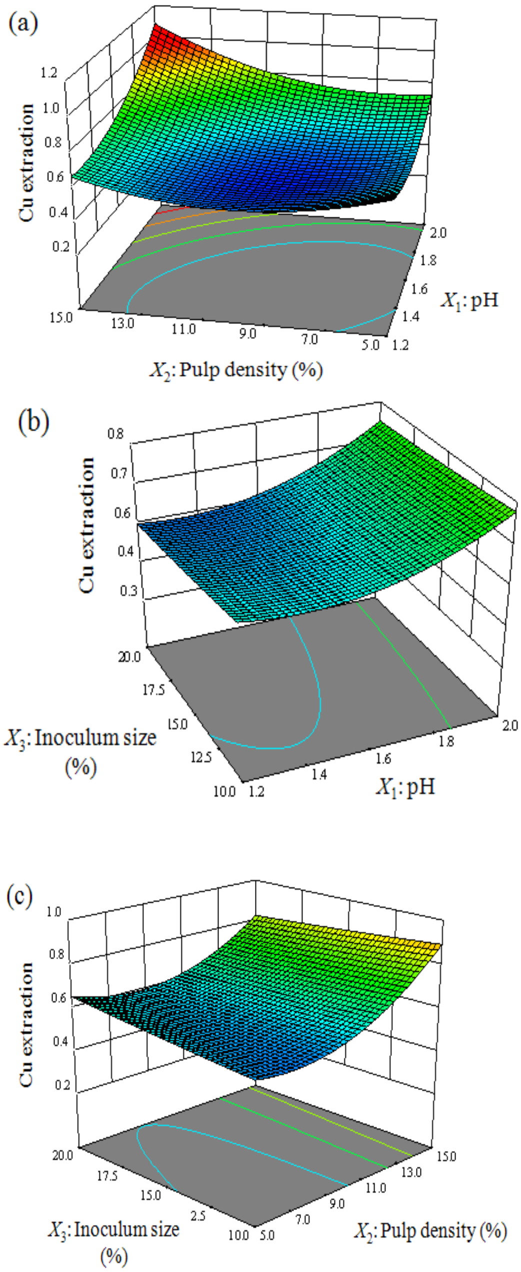

Fig. 5. Cu extraction in 3D response surfaces:
(a) effects of pH value and pulp density,
(b) effects of pH value and inoculum size,
(c) effects of pulp density and inoculum size

size acted as a secondary factor for Cu extraction, which was consistent with the conclusions of the sectoral diagram (Fig. 2b) and the Pareto chart (Fig. 3b).

The results obtained in this study demonstrated that the best conditions for Ni extraction and Cu extraction were quite different. However, it is infeasible to realize the synchronous control of two different conditions for bioleaching a complex nickel-copper ore within a single batch. Therefore, using some active methods, such as catalysis of Ag^+, to promote chalcopyrite oxidation under optimal condition of Ni recovery is advised. And relevant research will be discussed in the next report.

Optimized studies for maximizing metal extractions

One of the main objectives was to optimize the bioleaching process to maximize the metal extraction of the low grade complex nickel-copper concentrate. Accordingly, three variables (pH value, pulp density and inoculum size) have been studied through the regression model obtained by RSM. To confirm the adequacy and the validity of the optimization procedure, five sets of validation experiments were performed under different conditions from the model. It was found that the experimental values matched well with their predictive counterparts (Table 4). It was revealed that the statistical method of RSM was successfully applied to optimize the process parameters and study the effects of the test variables on the metal extraction.

CONCLUSION

A relationship between metal extraction and bioleaching process was investigated using response surface methodology (RSM). Based on the models, pulp density was considered as the most significant component since its contribution reached 55.78% for Ni extraction and 15.26% for Cu extraction.

The negative terms in the model (pulp density and pH) indicated unfavorable or antagonistic effects on the Ni extraction, which was opposite to the experimental phenomenon of Cu extraction. On the other hand, the interaction effect of pulp density and pH was favorable on the Ni recovery, whereas Cu extraction would be least influenced by the effect of interaction.

Though high concentration of total iron and sulfur-oxidizing organisms would promote chalcopyrite oxidation, the optimal conditions for Ni extraction and Cu extraction were quite different. So, using some active methods to promote chalcopyrite oxidation under optimal condition of Ni recovery is advised.

ACKNOWLEDGEMENTS

The authors would like to thank the financial support from the China Geological Survey (grant no. D1106).

REFERENCES

1. Watling, H.R. The bioleaching of nickel-copper sulfides. *Hydrometallurgy*, 2008; **91**: 70-88.
2. Cameron, R.A., Lastra, R., Mortazavi, S., Bedard, P.L., Morin, L., Gould, W.D., Kennedy, K.J. Bioleaching of a low-grade ultramafic nickel sulphide ore in stirred-tank reactors at elevated pH. *Hydrometallurgy*, 2009; **97**: 213-20.
3. Zhen, S.J., Yan, Z.Q., Zhang, Y.S., Wang, J., Campbell, M., Qin, W.Q. Column bioleaching of a low grade nickel-bearing sulfide ore containing high magnesium as olivine, chlorite and antigorite. *Hydrometallurgy*, 2009; **96**: 337-41.
4. Santos, L.R.G., Barbosa, A.F., Souza, A.D., Leão, V.A. Bioleaching of a complex nickel-iron concentrate by mesophile bacteria. *Miner. Eng.*, 2006; **19**: 1251-8.
5. Yang, C., Qin, W., Lai, S., Wang, J., Zhang, Y., Jiao, F., Ren, L., Zhuang, T., Chang, Z. Bioleaching of a low grade nickel-copper-cobalt sulfide ore. *Hydrometallurgy*, 2011; **106**: 32-7.
6. Maley, M., van Bronswijk, W., Watling, H.R. Leaching of a low-grade, copper-nickel sulfide ore 2. Impact of aeration and pH on Cu recovery during abiotic leaching. *Hydrometallurgy*, 2009; **98**: 66-72.
7. Maley, M., van Bronswijk, W., Watling, H.R. Leaching of a low-grade, copper-nickel sulfide ore. 3. Interactions of Cu with selected sulfide minerals. *Hydrometallurgy*, 2009; **98**: 73-80.
8. Abdel-Fattah, Y.R., Saeed, H.M., Gohar, Y.M., El-Baz, M.A. Improved production of *Pseudomonas aeruginosa* uricase by optimization of process parameters through statistical experimental designs. *Process Biochem.*, 2005; **40**: 1707-14.
9. Chen, J., Huang, P.T., Zhang, K.Y., Ding, F.R.

Isolation of biosurfactant producers, optimization and properties of biosurfactant produced by *Acinetobacter* sp. from petroleum-contaminated soil. *J. Appl. Microbiol.*, 2012; **112**: 660-71.

10. Meng, H., Hu, X., Neville, A. A systematic erosion-corrosion study of two stainless steels in marine conditions via experimental design. *Wear*, 2007; **263**: 355-62.
11. Coman, G., Bahrim, G. Optimization of xylanase production by *Streptomyces* sp. P12-137 using response surface methodology and central composite design. *Ann. Microbiol.*, 2011; **61**: 773-9.
12. Song, D., Chen, Y., Zhu, M., Gu, Q. Optimization of bacteriocin production by a new *Lactobacillus* Strain, ZJ317, using response surface methodology. *J. Pure Appl. Microbiol.*, 2012; **6**: 1507-15.
13. Obeng, D.P., Morrel, S., Napier-Munn, T.J. Application of central composite design to modeling the effect of some operating variables on the performance of the three product cyclone. *Int. J. Miner. Process.*, 2005; **76**: 181-92.
14. Giaveno, A., Donati, E. Bioleaching of heazlewoodite by *Thiobacillus* spp. *Process Biochem.*, 2001; **36**: 955-62.
15. Körbahtý, B.K., Rauf, M.A. Determination of optimum operating conditions of carmine decoloration by UV/H_2O_2 using response surface methodology. *J. Hazard. Mater.*, 2009; **161**: 281-6.
16. Watling, H.R., Elliot, A.D., Maley, M., van Bronswijk, W., Hunter, C. Leaching of a low-grade, copper-nickel sulfide ore. 1. Key parameters impacting on Cu recovery during column bioleaching. *Hydrometallurgy*, 2009; **97**: 204-12.
17. Cameron, R.A. Bioleaching of low-grade nickel sulphide ore at elevated pH. PhD Thesis, 2011; University of Ottawa.
18. Riekkola-Vanhanen, M., Sivela, C., Viguera, F., Tuovinen, O.H.: Effect of pH on the biologicalleaching of a black schist ore containing multiple sulfide minerals. In: Biohydrometallurgy: Fundamentals, Technology and Sustainable Development, Part A (Ciminelli VST, Garcia O, ed). Amsterdam: Elsevier, 2001; pp 167-74.
19. Ahonen, L., Tuovinen, O.H. Bacterial leaching of complex sulfide ore samples in bench-scale column reactors. *Hydrometallurgy*, 1995; **37**: 1-21.
20. Cameron, R.A., Lastra, R., Gould, W.D., Mortazavi, S., Thibault, Y. Bioleaching of six nickel sulphide ores with differing mineralogies in stirred-tank reactors at 30°C. *Miner. Eng.*, 2011; **97**: 213-20.