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Full Length Research Paper

Bioleaching of metal ions from low grade sulphide ore: Process optimization by using orthogonal experimental array design

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The present work was aimed at studying the bioleachability of metal ions from low grade sulphide ore containing high amount of carbonaceous materials by selected moderately thermophilic strain of acidophilic chemolothotrophic bacteria, *Sulfobacilllus thermosulfidooxidans*. The bioleaching process was optimized by constructing L_{25} Taguchi orthogonal experimental array design and optimization of variable proportions of process parameters. Five factors were investigated and twenty five batch bioleaching tests were run under lower, medium and higher levels of these factors. The parameters considered for shake flask leaching experiments were initial pH (1.8, 2, 2.5, 3, 3.5), particle size, (50, 100, 120, 200, 270 µm), pulp density (1, 5, 10, 15, 25%), temperature (40, 45, 47, 52, 57°C) and agitation (100, 120, 180, 220, 280 rpm). Statistical analysis (ANOVA) was also employed to determine significant relationship between experimental conditions and yield levels. The experimental results for selective leaching showed that under engineered leaching conditions; pH 1.8, particle size 120 µm, pulp density 10%, temperature 47°C and agitation 180 rpm, the percent bioleachabilities of metals were Zn 72%, Co 68%, Cu 78%, Ni 81% and Fe 70% with an inoculum size of 1.0 ×10^7 /mL.

Key words: Mineral economics, process optimization, bioleaching, sulphide ores, bacteria.

INTRODUCTION

Various regional geological surveys conducted in the recent past have confirmed the occurrence of ores of copper, gold, silver, platinum, chromium, iron, lead and zinc in various regions of China. Most of these minerals occur as low grade-ores and hence, are not fit for extraction of metal values by conventional metallurgical techniques.

Metal-winning processes based on the activity of microorganisms, offer a possibility to obtain metal ions from mineral resources not accessible by conventional techniques. Microbes such as bacteria and fungi convert metal compounds into their water-soluble forms and are biocatalysts of this process called microbial leaching or bioleaching (Olson et al., 2003; Ilyas et al., 2008). For many years, *Acidithiobacillus ferrooxidans* were considered to be the most important microorganisms in the bioleaching of metal ions from ores (Bosecker e, 1997; Kelly and Wood, 2000).

The ability of *A. ferrooxidans*, *Leptospirillm ferrooxidans* and other microorganisms to solubilize metal sulfides in their habitats has been successfully applied in bioleaching of metal ions from ores. However, the relatively slow process kinetics is the major bottleneck in the widespread use of these microorganisms at the commercial level. In this regard, the use of moderately thermophilic bacteria with their ability to operate at temperatures of 45 - 55°C has great potential for improving the kinetics of metal extraction from sulphide minerals.

A. ferrooxidans is a mesophilic chemolithotrophic prokaryote that obtains its energy from the oxidation of ferrous iron, elemental sulfur, or partially oxidized sulfur

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compounds (Hiroyoshi et al., 1999; Rawlings, 2002). Only a few biochemical investigations of the oxidative dissimilatory metabolism of sulfur compounds have been reported for *A. ferrooxidans* (Acosta et al., 2005). In contrast, *Surfobacillus thermosulfidooxidans* are moderately thermophilic bacteria having a highly versatile metabolism and may grow as autotroph, heterotroph, mixotroph, or chemolithotroph (Torma and Banhegyi., 1984; Toni and Johnson., 1998). The rates of bioleaching of metal ions from ores by moderate thermophiles have been demonstrated to be higher than mesophiles and in another case even higher than extreme thermophiles (Das et al., 1999; Deveci et al., 2004).

But the effectiveness of bioleaching is also highly dependent on the physical, chemical and biological factors in the system. The maximum yield of metal leaching may be achieved when these parameters are considered and optimized collectively (Bosecker, 1997).

Taguchi method has been shown to be an effective means for the improvement of the productivity in research and development. It has found much application in a wide range of industrial fields because of its universal applicability to all fields, particularly where co-optimization is considered a necessary parameter (Çopur et al., 2003). The Taguchi experimental design was therefore, used to determine the optimum leaching conditions for maximizing the percent metal ions dissolution. Accordingly, the effect of experimental parameters such as pH, particle size, pulp density, temperature and agitation, were investigated using an L_{25} (5^5) orthogonal array.

The main objective of the present study was to optimize the bioleaching process by constructing orthogonal array design to evaluate the efficiency of moderately thermophilic bacteria *S. thermosulfidooxidans* for solubilization of metal ions from low grade ore and to apply metal adapted cells in achieving enhanced metal bioleaching rates from the above mentioned ore.

MATERIALS AND METHODS

Collection of ore

The samples of ore were obtained from Daye copper ore deposits, Hubei, China and large pieces were crushed in a jaw-crusher separately. Then, all pieces were ground to relatively smaller particles using disc-grinding machine. The final grinding of the ores was carried out using ring grinder (FRITSCH Pulverisette, Germany). In order to separate the particles according to their sizes, ASTM sieves were used.

Analysis of ore samples

Finally, powdered sample (1.0 g) of ore was refluxed with 100 mL of aqua regia in round bottom flask for one hour. The solution was allowed to cool at room temperature and was filtered through Whatman No.42 filter paper. The concentration of metal ions was determined by atomic absorption spectrophotometer (VarianAA-400) and the percentage of these metal ions in the ore sample was calculated.

Microorganism and culture conditions

An acidophilic chemolothotrophic bacterial culture of *S. thermosulfidooxidans* strain RDB was collected from Reko Diq copper ore deposits, Pakistan (GQ228448). Iron-tryptone soya broth (FeTSB) medium, developed by Johnson et al. (1987) was used to obtain the growth of *S. thermosulfidooxidans*. The FeTSB medium composed of (g/L): $MgSO_4$. $7H_2O$, 0.50; $(NH_4)_2SO_4$, 0.15; KCl, 0.05; KH_2PO_4, 0.05; $Ca(NO_3)_2$, 0.01 and TSB, 0.25. The solution pH was adjusted to 1.8 using sulfuric acid and autoclaved at 121°C and 15 psi for 15 min. Filtered sterilized ferrous sulfate solution was added to the solution to a final concentration of 50 mM before inoculation. After obtaining rich growth, the cell mass of cultures was harvested by centrifugation at 10,000 rpm for 20 min. The cell pellet was washed twice with autoclaved distilled water having pH adjusted at 1.8 with 2.0 M sulfuric acid and finally it was suspended in sterilized distilled water and preserved at 4°C for inoculation in further experiments. The above mentioned liquid medium was supplemented with 0.5% (w/v) agarose to prepare solid media. The flasks were inoculated with 1 mL of inoculum containing 1×10^7 cells/mL and incubated at 47°C in a rotary shaker at 180 rpm. While in the logarithmic phase of growth, 1 mL of culture from each flask was transferred to fresh medium containing 20 mM of each metal ion. In this way, further step-wise transfers were made to the media containing next higher metal concentrations, that is, 30, 40, 50, 100, and 200 mM. Finally, the cells were harvested by centrifugation and inoculum was prepared as described earlier in this section.

Orthogonal experimental array

An orthogonal experimental array design was constructed for this study that consisted of L_{25} (5^5) orthogonal array (Dehghan et al., 2009). The degree of influence of individual parameters and the best co-optimization combination of these parameters for bioleaching of metal ions from sulphide ore with moderately thermophilic bacterial culture was observed.

Experimental parameters and their levels were determined in the light of preliminary tests and inoculum size was kept at 1.0×10^7/mL. In the orthogonal array method, selection of factors was based on previous knowledge of bioleaching. Based on these experimental conditions reported by other researchers for the bioleaching of low grade sulfide ores, preliminary tests were performed and significant factors that can affect the metal ions dissolution were identified as pulp density, initial pH, particle size, temperature and agitation. The most significant factors were chosen for optimization, using a five-level orthogonal array with L_{25} (5^5) matrix, which denotes five parameters each with five levels, since it is the most suitable for the conditions being investigated and each experiment was repeated twice under the same conditions to monitor the effects of noise sources of the laboratory medium in the bioleaching process. The exact levels were selected using the levels obtained from previous results. In the proposed method, possible interactions between variables were not in the matrix, and the focus was placed on the main effects of the five most important factors. Low, medium and high levels of the factors are given in Table 1. The order of experiments was obtained by inserting parameters into columns of orthogonal arrays, L_{25} (5^5), chosen as the experimental plan given in Table 2, but the order of experiments was made random in order to avoid noise sources which could take place during an experiment and affect results in a negative way. Table 2 is an L_{25} orthogonal array, a table of integers whose column elements represent the low, medium and high levels of the column factors. Each row of orthogonal array represents a run, which is a specific set of factor levels to be tested (Safarzadeh et al., 2008).

Bacterial leaching studies

Experiments on bioleaching of metal ions from ore were conducted in

Table 1. Quantitative value of coded parameter levels.

Coded factors	Parameters	Levels				
		1	**2**	3	4	5
A	Temperature-A (℃)	40	45	47	52	57
B	Initial pH-B	1.8	2.0	2.5	3.0	3.5
C	Particle size-C(µm)	50	100	120	200	270
D	Agitation-D(rpm)	100	120	180	220	280
E	Pulp density-E(%)	1	5	10	15	25

Table 2. L_{25} (5^5) experimental work plan.

Experiment No.	Parameters and their level				
	A	B	C	D	E
1	1	1	1	1	1
2	1	2	2	2	2
3	1	3	3	3	3
4	1	4	4	4	4
5	1	5	5	5	5
6	2	1	2	3	4
7	2	2	3	4	5
8	2	3	4	5	1
9	2	4	5	1	2
10	2	5	1	2	3
11	3	1	3	5	2
12	3	2	4	1	3
13	3	3	5	2	4
14	3	4	1	3	5
15	3	5	2	4	1
16	4	1	4	2	5
17	4	2	5	3	1
18	4	3	1	4	2
19	4	4	2	5	3
20	4	5	3	1	4
21	5	1	5	4	3
22	5	2	1	5	4
23	5	3	2	1	5
24	5	4	3	2	1
25	5	5	4	3	2

250 mL Erlenmeyer flasks contained 100 mL Fe-TSB medium in each flask at different levels of various factors (temperature, pH, pulp density, particle size and agitation). After sterilization by autoclaving at 121℃ and 15 psi pressure for 15 min, twenty five batch bioleaching tests were run with different combinations of factors and levels for a period of 30 days.

All flasks were weighed before sampling and any decrease in volume due to evaporation during incubation was compensated by adding corresponding volume of sterilized distilled water. During the course of the leaching experiments, 3.0 mL of sample was taken from each flask periodically and its pH, ferrous, ferric, total iron concentration and redox potential was noted. The sample was then filtered through Whatman No. 1 filter paper to remove solid particles and centrifuged at 10,000 rpm for 10 min to remove bacterial cells and supernatant was analyzed for nickel, copper, cobalt, zinc and iron concentration.

Analytical methodology

Free bacteria in solution were counted by direct counting, using a counting chamber with an optical microscope (×1000). Soluble metal ions in the leached solutions (Ni, Co, Cu, Zn and Fe) were measured using an atomic absorption spectrophotometer (Varian AA-400). The solid residues were air dried and samples were taken for chemical analysis and X-ray diffraction (XRD). The ferric and total iron concentration in the solution was determined by spectrophotometry using the 5-sulfosalicylic acid and 1-10 orthoph-

Table 3. Chemical analysis of ore for metal content.

Metals	Metal ions conc. % (w/w)
Fe	7.8 ± 0.04
Cu	8.0 ± 0.04
Ni	1.2 ± 0.06
Co	0.3 ± 0.03
Zn	0.8 ± 0.04

enanthroline methods. The ferrous iron concentration was determined by a volumetric method by titration with potassium dichromate. The pH of the bioleaching medium was monitored at room temperature with a pH meter calibrated with a low pH buffer. The redox potential (Eh) of the leaching solution was measured with a Pt electrode in reference to a saturated Ag/AgCl electrode.

RESULTS AND DISCUSSION

Chemical and mineralogical analysis of ore

Chemical analysis of ore used in these studies was carried out to determine the concentration of various metal ions present. The major metal ions in the ore were found to be Zn, Ni, Co, Cu and Fe as shown in Table 3.The mineralogical analysis of ore sample showed that, main sulphide minerals were chalcopyrite (12%), violarite (1.8), pyrite (5.5%) and sphalerite (1.4%).The main gangue minerals were magnesite (20%), siderite (12%) and dolomite (5%) with lesser amount of sphaerocobaltite (0.3%).

Bioleaching studies

Preliminary leaching experiments were conducted at pH 2.5, temperature 45°C, particle size 50 -150 µm and agitation of 180 rpm. After adjustment of initial acid demand, the experimental flasks were inoculated with 1.0 mL inoculum (1×10^7 cells/mL) of *S. thermosulfidooxidans* aseptically. The percent metal ions recovery was very low (data not shown) so, an orthogonal experimental array was constructed for optimization of bioleaching process and optimization of parameters prior to bioleaching while size of inoculum was kept at 1×10^7 cells/mL on the basis of some previous research (Ilyas et al., 2007). Corresponding leaching efficiencies with two replications obtained under the candidate conditions are displayed in Table 4.

The collected data were then analyzed by Origin pro 7.5 software to evaluate the effect of each parameter on the optimization criteria. Maximum amount of metal ions dissolution was defined as optimization criterion. In the view of above, mean response calculation was performed to achieve the defined aim. For better realizing of the experimental conditions related to each response, the corresponding conditions were brought in each row of that Table. Taguchi recommends analyzing the mean response for each run and uses graphs of the marginal means of each factor, as shown in Figures 1a, b, c, d, and e, but these graphs are only used to make the trend of each factor more understandable and it is incorrect to use these graphs to predict other values which have not experimented. The usual approach is to examine the graphs and select the maximum value. In Figure 1a, the effects of controllable factors on mean responses for metals of sulphide ore are displayed.

Bioleaching process, which causes acidification and solubilization of heavy metal ions, is mainly governed by pH of the leaching medium. The temporal change of pH depends on the buffering capacity of the leaching medium. The optimum pH for bioleaching with moderately thermophilic culture of *S. thermosulfidooxidans* was found to be 1.8 as shown in Figure 1b. At pH values "between" 2.0 - 3.5 the decline of percent recovery could be due to the formation of hydronium jarosite on concentrate particles. The jarosite is responsible for creation of diffusion barrier of reactants and products, which leads to deceleration of kinetics of the ferrous and sulfur oxidation reactions. Low leachability obtained at higher pH are due to cessation of bacterial growth and sulfur oxidation reactions because of the presence of high concentration of protons as well as heavy metal ions (Chen and Lin, 2001).

Since, particle size influences the surface area, it is an important factor in determining the kinetics of the leaching reactions (Ahonen and Tuovinen, 1995). Increased surface area of the particle helps in increasing the specific contact area between bacteria, liquid phase and substrate. According to Figure 1b, particle size of 120 µm was found to be optimum. However; a further decrease in particle size adversely affected the activity of the cells. This could be due to the presence of very fine particles, which apparently damaged the structure of cells, thereby resulting in their inability to oxidize the ore particles.

Pulp density is one of the physical parameter that strongly influences the metal ions dissolution. The major practical limitations that are seen in industrial operation are high pulp density that causes reagent starvation and insufficient stirring which results in liquid and mass transfer resistance. The low metal ions solubilization at smaller pulp densities are attributed to low accessibility of the substrate. Hence, it is essential to standardize optimum pulp density in order to gain maximum leachability. Optimum pulp density was found to be 10% as shown in Figure 1c.

The optimum temperature was found to be 47°C as shown in Figure 1d. Like all biochemical and chemical processes, the rates of leaching reactions are also temperature dependent. Hence, it is considered as an important environmental parameter that influences bacterial activity in bioleaching operations (Ahonen and Tuovinen., 1990). Bacterial cultures have an optimum temperature for growth above which the oxidation reaction becomes inhibited or stop.

Agitation is a major factor in bioleaching processes. Optimum velocity of agitation is required to attain proper

Table 4. Percentage (%) bioleaching efficiency of metals and experimental factors.

Runs	Experimental factors					Bioleaching efficiency (%)				
	A	B	C	D	E	Copper	Zinc	Nickel	Iron	Cobalt
1	1.8	50	1	40	100	40	38	45	35	27
2	1.8	100	5	45	120	50	43	49	40	36
3	1.8	120	10	47	180	79	72	79	70	68
4	1.8	200	15	52	220	20	18	23	16	14
5	1.8	270	25	57	280	10	8.1	14	7.2	5.3
6	2.0	50	1	40	100	35	30	40	27	23
7	2.0	100	5	45	120	35	31	39	27	23
8	2.0	120	10	47	180	30	28	33	25	21
9	2.0	200	15	52	220	40	38	45	35	29
10	2.0	270	25	57	280	50	48	55	40	35
11	2.5	50	1	40	100	20	16	24	12	10
12	2.5	100	5	45	120	70	67	74	63	58
13	2.5	120	10	47	180	40	35	44	31	27
14	2.5	200	15	52	220	40	35	43	31	27
15	2.5	270	25	57	280	15	12	17	11	8.7
16	3.0	50	1	40	100	15	12.3	16.8	11	8.2
17	3.0	100	5	45	120	12	10	15	8.5	6.6
18	3.0	120	10	47	180	17	14.2	20	10	12.5
19	3.0	200	15	52	220	10	8.3	12	5.8	4.4
20	3.0	270	25	57	280	30	27	32	25	23
21	3.5	50	1	40	100	8.2	5.5	10.3	4.3	4.0
22	3.5	100	5	45	120	10	8.4	12	5.5	4.2
23	3.5	120	10	47	180	15	13	17	5.2	4.0
24	3.5	200	15	52	220	15	13	17	5.0	4.0
25	3.5	70	25	57	280	10	8.5	12	5.7	4.0

Bioleaching efficiency (%) of all metals ions is mean response of two replications with an error of ± 0.5.

aeration, homogeneous solid suspension, pH and temperature uniformity, heat and mass transfer (nutrients, oxygen, and carbon dioxide). High agitation velocity reduced the dissolution of metal ions due to excessive mixing that led to high turbulence of ore particles with the cells, which adversely affected the bacterial growth and ultimately resulted in decreased levels of leachabilities (Witne and Philips, 2001; Shi and Fang., 2005).

At low rpm, oxygen and mass transfer limitations led to decreased dissolution of metal ions. Hence, an agitation of 180 rpm for a pulp density of 10% was considered optimum as in Figure 1e. So the optimum conditions for enhanced metal ions dissolution were found to be pH 1.8, particle size 120 µm, pulp density10%, temperature 47℃ and agitation speed 180 rpm.

Statistical analysis of variance (ANOVA) was performed to see whether the process parameters are statistically significant. The F-value for each parameter, that was a ratio of the squared deviations to the mean of the squared error, indicates which parameter has a significant effect on the leaching efficiency. According to these results, initial pH, temperature and agitation speed have significant effect on mean response for metal ions leaching. The F-value for these factors is greater than the extracted F-value from the table at 95.0% confidence interval. This means that, the variance of all these factors is significant compared with the variance of error and all of them have meaningful effect on the responses for metal ions. Summary of statistical results in the form of *P* values are given in Table 5. Finally, using these findings and modeling significant effects by the Taguchi method, results for all combination of levels were predicted. These predictions were confirmed by some experiments and then bioleaching was carried out under optimized conditions.

In the present study, the initial pH of the bioleaching medium was adjusted at 1.8 and pH profile was studied after every 24 h and rise in pH was compensated by adding 2.0 M sulfuric acid. At the fourth day when no increase in pH was observed, inoculation was then carried out. After inoculation, the pH remained stable for three days and can be attributed to delayed phase of bacterial growth and then started decreasing due to the hydrolysis of the metal ions that were released in the medium due to microbial action. No further significant change in the pH was observed after the 23rd day, while in case of uninoculated control, the rise in pH was 1.8 - 3.0 as shown in Figure 2.

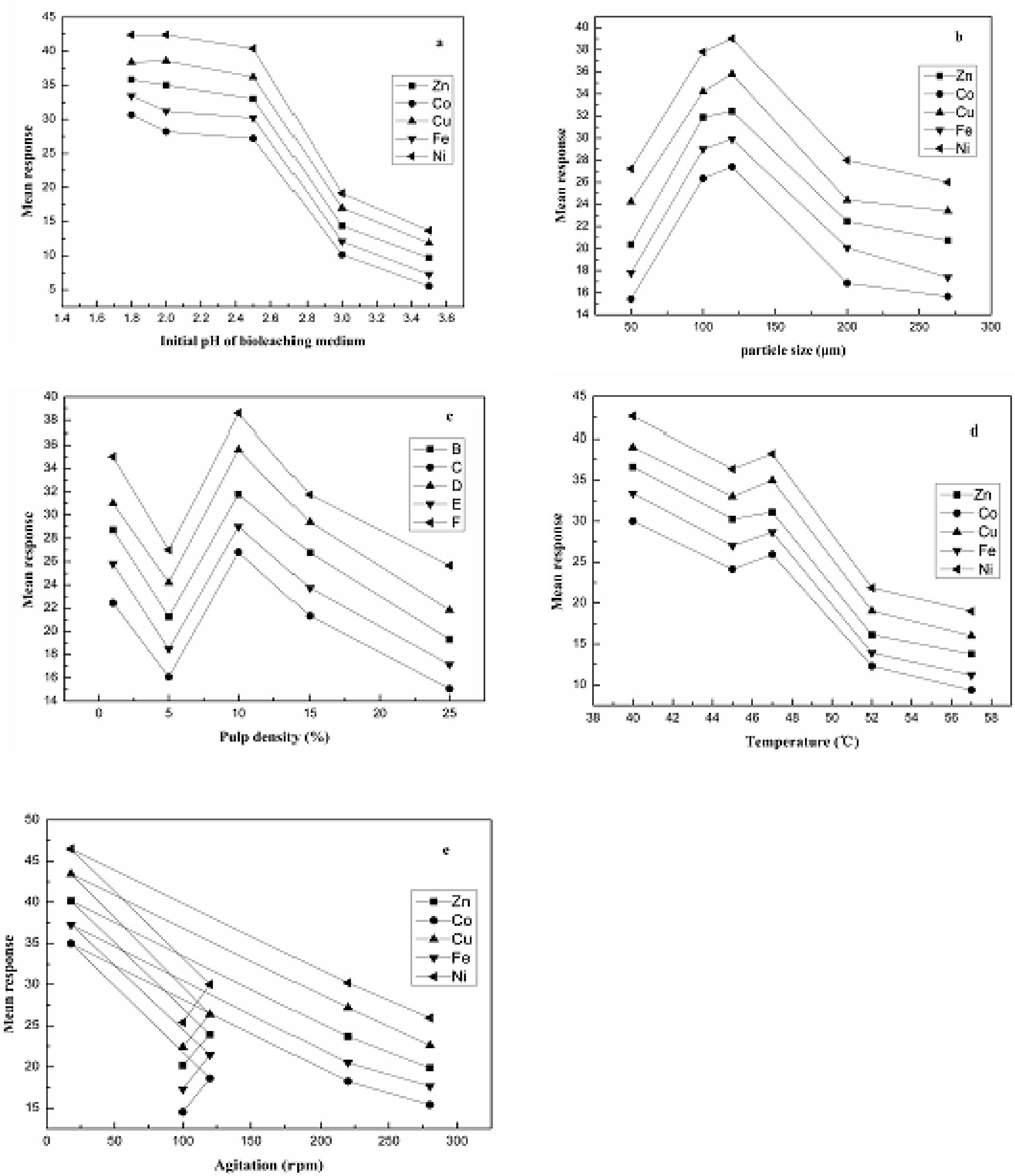

Fig.1.Effects of controllable factors on mean responses for percent metal ions dissolution

Table 5. Summery of statistical results.

P-value	Zn	Co	Cu	Fe	Ni
A	0.003	0.009	0.009	0.005	0.003
B	0.045	0.048	0.050	0.041	0.045
C	0.036	0.030	0.038	0.032	0.037
D	0.001	0.002	0.002	0.002	0.002
E	0.003	0.003	0.003	0.003	0.003

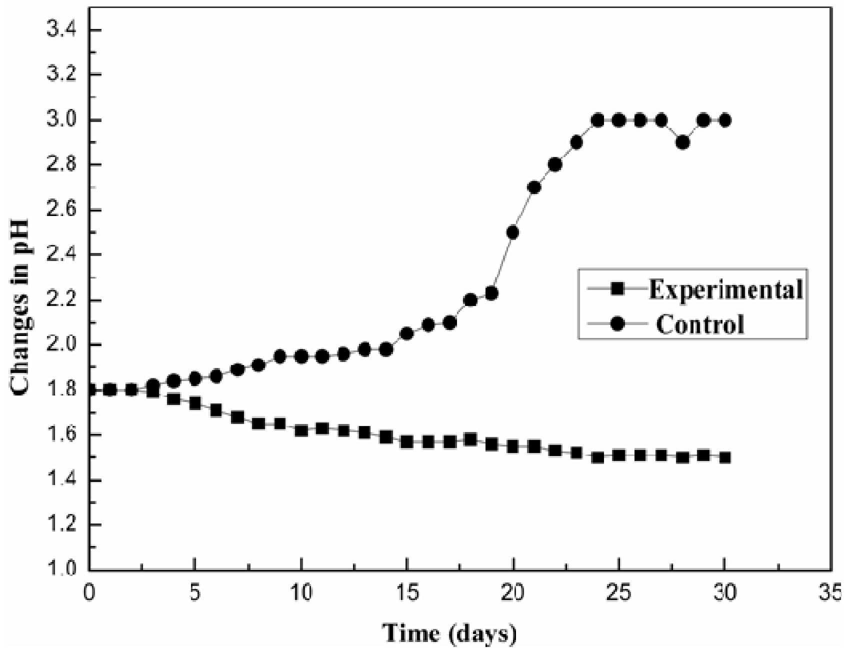

Figure 2. Changes in pH during bioleaching studies. All values are means of two replications with an error of ± 0.03.

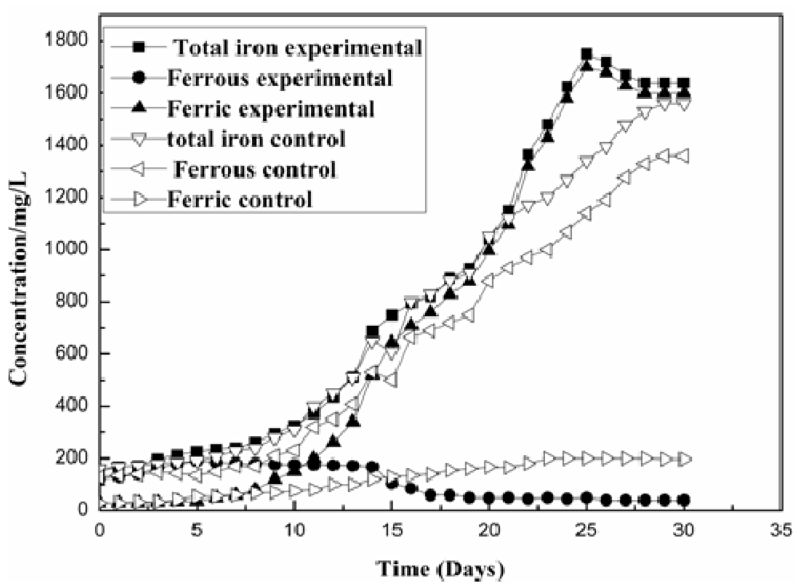

Figure 3. Changes in total iron, ferrous and ferric concentrations during bioleaching studies. All values are means of two replications with an error of ± 0.05.

Iron species present in the flasks were monitored and results are presented in Figure 3. It can be seen from the figures that total iron and ferrous concentration at the start increase in a quite linear pattern but ferric concentration increased very slowly during the first six days and redox potential of effluents also increased very slowly as shown in Figure 4, coinciding with low growth of bacterial cultures and domination of acid leaching stage.

However, from day 7- day 15, the ferrous ions concentration started to decreased while ferric ions concentration began to increase linearly and from day 16 - day 25, concentration of ferric ions increased up to 1720 mg/L and high value of redox potential (592 mV) was observed. This trend was attributed to good bacterial activity and efficient bioleaching stage instead of acid leaching. However, during the last days, concentration of ferric ions and value

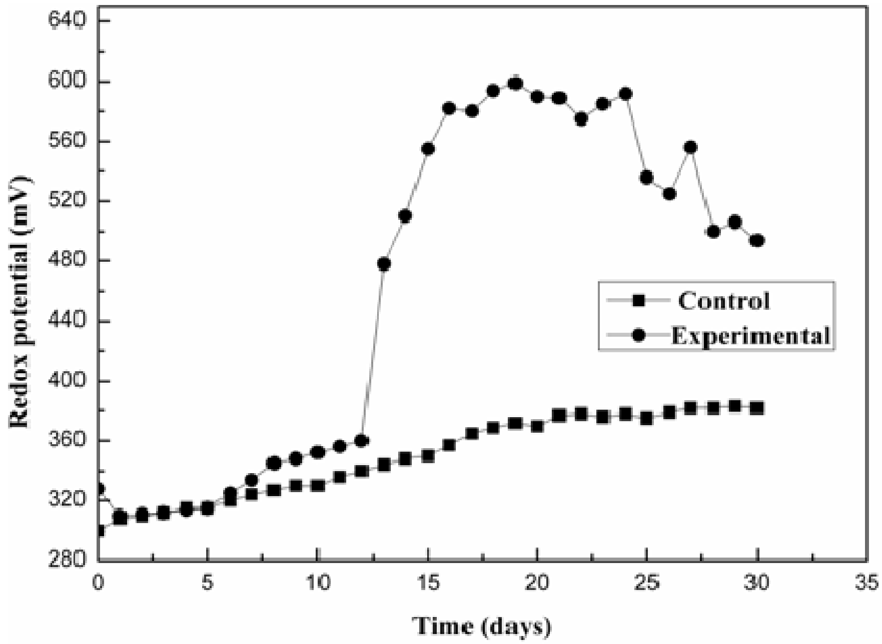

Figure 4. Changes in redox potential during bioleaching studies. All values are means of two replications with an error of ± 0.03.

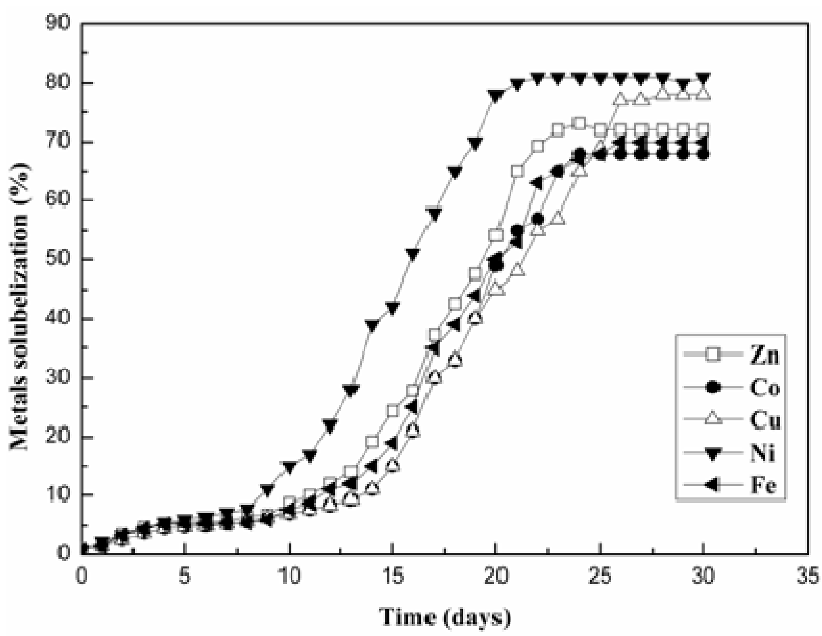

Figure 5. Percent metal ions solubilization under optimized conditions of bioleaching. All values are means of two replications with an error of ± 0.04.

of redox potential decreased, indicative of regressive bioleaching and deceleration phase of bacteria. During this whole process, about 72% of Zn, 68% (Co), 78% (Cu), 81% (Ni) and 70% (Fe) metals leached out as can be seen from Figure 5.

Then, the bioleaching was carried out with metal ions adapted moderately to thermophilic culture of *S. thermosulfidooxidans* under already optimized conditions. Further enhancement in percent metal ions dissolution was observed (Zn 76%, Co 72%, Cu 84%, Ni 86% and Fe 75%) as shown in Figure 6. The percent leachability of metal ions in control flasks were very low (Zn 5%, Co 8%, Cu 8%,

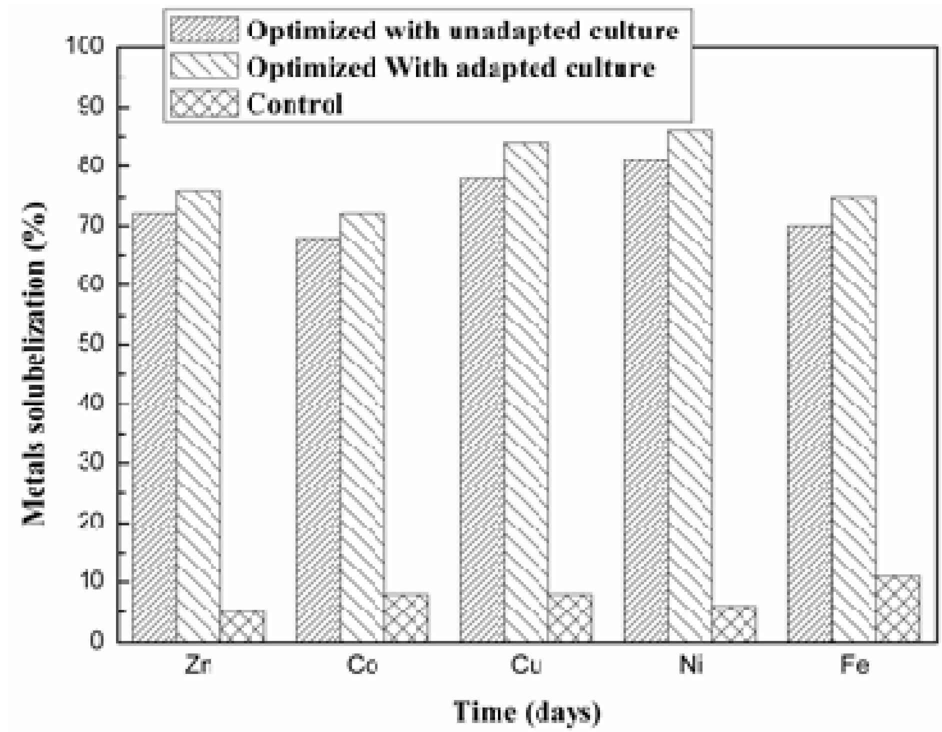

Figure 6. Enhancement in metal ions solubilization by using adapted bacterial culture with optimized process conditions. All values are means of two replications with an error of ± 0.04.

Ni 6% and Fe 11%).

Conclusions

Orthogonal array design was constructed for the bioleaching of metal ions from low grade sulfide ore. Optimization of process parameters was carried out by choosing L_{25} orthogonal array matrix and the effect of proposed parameters (initial pH, particle size, pulp density, temperature and agitation) on the responses for metals (copper cobalt ,nickel, zinc and iron) percent dissolution was evaluated. The optimization criterion was defined as maximum metals percent dissolution. So, pH 1.8, particle size 120 µm, pulp density 10%, temperature 47°C and agitation 180 rpm were recommended for optimal selective bioleaching conditions. Based on the achieved optimum condition, Zn 72%, Co 68%, Cu 78%, Ni 81% and Fe 70% were leached out with moderately thermophilic culture of *S. thermosulfidooxidans*. The most effective parameter for maximum dissolution of metals was found to be initial pH of bioleaching medium, temperature and agitation, respectively. Further conditions like adaptation of bacterial cultures to high concentration of metals also enhance the bioleaching efficiency of bacteria. Percent dissolution of metals was increased; Zn 76%, Co 72%, Cu 84%, Ni 86% and Fe 75%, respectively. So, overall optimization of bioleaching process will contribute to implement the process on industrial scale with maximum percent yield of metals from low grade ores and low capital costs.

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