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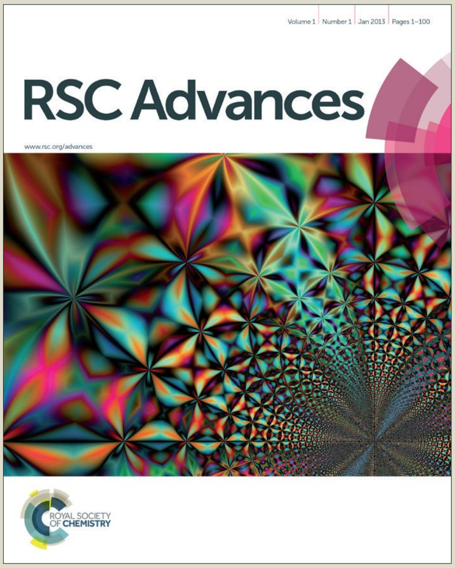

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Bioleaching of fuel-oil ash using *Acidithiobacillus thiooxidans* in shake flasks and a slurry bubble column bioreactor

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Abstract

This work investigated a bioleaching process to remove V, Ni and Cu from fuel-oil ash (FOA) using *Acidithiobacillus thiooxidans*. Adaptation of the bacterium to tolerate up to 4% (w/v) of the FOA particles suspended in the culture medium was carried out. The response surface methodology (RSM) based on a statistical analysis was used to optimize initial sulfur concentration, initial pH and ash concentration in shake flask cultures first. The maximum V recovery (96.4%), Ni recovery (100%) and Cu recovery (99.2%) at the ash concentration of 1%, initial sulfur of 9 g/L and initial pH of 1 were achieved. Risk assessments of FOA before and after bioleaching using the toxicity characteristic leaching procedure (TCLP), synthetic precipitation leaching procedure (SPLP) and total threshold limit concentration (TTLC) were evaluated. The results indicated that the bioleaching process detoxified FOA and the bioleached solid residue was well within the regulatory limits for disposal. In order to scale up the bioleaching process, some experiments were also performed in a bubble column bioreactor. The changes in growth characteristics in the bioleaching process were also studied. Maximum recoveries for V, Ni and Cu were 77.6%, 89.7% and 65.7%, respectively at an ash concentration of 1% after eight days of incubation. These recovery rates were lower than those for shake flasks,

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but they suggested that this process could be operated in a bioreactor after optimizing the bioreactor operating conditions.

Keywords: Bioleaching; Fuel-oil ash; *Acidithiobacillus thiooxidans*; Risk assessments; Bioreactor

1. Introduction

Industrial furnaces produce large amounts of ash residues that have to be disposed in landfills. These residues usually contain large amounts of metals that may be extracted economically for useful applications.[1,2] Fuel-oil furnaces produce ashes with a concentration of 2 –10% vanadium (V) and varying amounts of nickel (Ni) and copper (Cu).[3,4] V is a valuable metal used for energy storage in lithium batteries, in titanium and iron alloys, or as V_2O_5 (a catalyst) for the production of sulfuric acid, just to name a few applications. The global V consumption is about 88000 million tone/year and its current market price is about US$20 per kg.[5]

There are different ways for the extraction of metals such as hydrometallurgy and pyrolysis. However, these methods are not economical and they cause environmental problems. Biological methods for the extraction of metals known as bioleaching have various advantages, including simpler, cheaper, more environmental friendly and more flexible to use it for more source materials than traditional methods.[6] Bioleaching has been applied to remove heavy metals from different wastes such as spent catalysts[7], electronic wastes[8] and municipal waste incineration fly ashes[9] with high efficiencies. *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* are the most common mesophilic species that have been used in bioleaching processes.[6] The mechanism for the bioleaching of metal with different valence is according to following reaction[10]:

$$M_2O_x\,(s) + xSO_4^{2-}\,(aq) + \;2xH^+(aq)\;\rightarrow\;M_2(SO_4)_x\,(aq)\;+\;xH_2O\,(aq) \qquad (1)$$

in which x is the valence of the metal M (V, Ni and Cu in this work) in the metal oxide (M_2O_x) and $M_2(SO_4)_x$ is the soluble form of the bioleached metal. To replenish sulfuric acid, *A. thiooxidans* can be used to convert elemental sulfur that can come from an inexpensive unrefined waste,[11]

$$S^0(s) + 1.5O_2(aq) + H_2O(aq) \xrightarrow{A.\ thiooxidans} SO_4^{2-}(aq) + \;2H^+(aq) \qquad (2)$$

Several parameters affect bioleaching such as inoculum size, reaction time, pH, initial substrate concentration and pulp density.[12-16] Some parameter values can lead to growth inhibition in the bioleaching process, which decreases the recovery of metals.[17,18] Thus, parameters optimization in this process can be desired.

The response surface methodology (RSM) is an efficient strategy based on the statistical methods in which the optimal conditions, role of each component and interactions between the parameters of the process are determined. Other advantages include savings in experimental time, number of tests required and manpower.[19–21]

Laboratory-scale studies of bioleaching of solid wastes are often performed in shake flasks. Subsequent bioreactor studies are desired for scale-up.[22, 23] In an industrial mineral ore treatment process, bioleaching is usually used in large mechanically agitated bioreactors. However these reactors are limited to solid loadings of 20% and it consumes a large amount of energy to agitate the suspension of ground particles. Furthermore, the high shear and turbulence generated by agitation can damage the microorganims.[24,25] Some researchers have suggested using bubble columns as an alternative[23-25] because they are energy efficient and hydro-dynamically gentle toward microbes with good heat transfer and mass transfer characteristics.[23]

There are some reports about metals extraction form FOA using different mineral acids as chemical leaching agents in the literature,[26-29] but this study is the first attempt for the bioleaching of metals from FOA using *At. thiooxidans*. Experiments in this study were done in shake flasks and in a bubble column bioreactor despite often studies in this field were done only in the shake flasks. Doing experiment in the bubble column bioreactor have many advantages such as low shear condition, simple design, low energy requirement and great mass and heat transfer.[30] Therefore the aim of this study was to evaluate the effects of controllable factors such as initial sulfur concentration, initial solution pH and initial ash concentration in the bioleaching process and to identify their interactions using RSM. The feasibility of a bubble column bioreactor application in the bioleaching of V, Ni and Cu from FOA using adapted *At. thiooxidans* was also investigated. Furthermore, toxicities of the ash before and after bioleaching were assessed.

2. Materials and methods

2.1. Materials

2.1. 1. FOA sample

The FOA sample was provided from the Shahid Salimi power plant, Mazandran, Iran. It was ground and passed through No. 200 sieve to yield particle sizes less than 75 μm.

2.1.2. Microorganism, culture medium and shake flask culture

At. thiooxidans (PTCC 1717) was provided by the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The culture medium composition (in g/L) was 2 $(NH_4)_2SO_4$, 0.25 $K_2HPO_4.3H_2O$, 0.25 $MgSO_4.7H_2O$, 0.1 KCl, and 10 S° in distilled water. The autotrophic bacterium utilized CO_2 in the air supply as the carbon source. The inoculum percentage was 10% (v/v) with bacterial population around 10^7 cells/mL in the inoculum. 50 mL culture medium was added to each 250 mL Erlenmeyer flask and its pH was adjusted to 2 using a concentrated H_2SO_4 solution. The culture medium did not need sterilization due to this low pH that favored the growth of the acidophilic *At. thiooxidans*. The flasks were shaken at 160 rpm in an orbital shaker incubator (WIS-20R, WiseCube, South Korea) at the optimum growth temperature of 32 °C.[31]

2.1.3. Analytical methods

The culture medium pH and Eh (Redox potential) in flasks containing the leaching solution (i.e., the inoculated culture medium) during incubation were monitored using a portable pH meter (Model 713, Metrohm, Switzerland) and an Eh meter (Model 713, Metrohm, Switzerland). The cell numbers in the liquid phase was counted using a hemocytometer under a phase-contrast microscope at 40X (Zeiss Standard 25, Germany). X-ray fluorescence (XRF) was used to analyze the chemical composition in the FOA sample, which yielded (g/kg ash): V 49.0, Cu 1.4, Ni 15.6, Fe 370.8, Cr 8.3 and Zn 2.0.

At the end of the tests, the leaching solution was filtered through Whatman No. 42 filter paper (pore size 2.5 μm) to separate the solids from the liquid. The analysis of metals in the bioleaching solutions was carried out using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Vista-pro, Australia).

The sulfur oxidation rate is a key index used to determine the growth of the microorganism in the bioleaching process.[32] The amount of sulfate in the medium was measured using the turbidimetry method according to the procedure in standard methods.[33] The percentage of sulfur oxidation during bioleaching was calculated using Eq. (3),

$$\text{Sulfure oxidation (\%)} = \frac{\text{Final Sulfate (mg/L)} - \text{Initial Sulfate (mg/L)}}{\text{Stoichiometric sulfate concentration (mg/L)}} \times 100 \qquad (3)$$

in which the stoichiometric sulfate concentration is 3000 mg/L when 1 g/L of sulfur is completely oxidized.

2.2. Experimental methods

2.2.1. Cell adaptation

The FOA sample contained metals that were cytotoxic to the bacterium. To determine the maximum tolerance of the bacterium, the FOA concentration was increased stepwise in a series of sub-cultures. The inoculum percentage was 10% (v/v) and distilled water was added to the flask to compensate for evaporation during the aerobic incubation. The adaptation of the cells began at 0.1 g and continued until 4 g of FOA in 100 mL of growth medium in shake flask cultures. At concentrations of FOA greater than 4%, the cell concentrations decreased dramatically, indicating that the highest tolerated concentration was 4%. Once there was no improvement in bacterial growth, adaptation was stopped.

2.2.2. Experimental design

The response surface methodology (RSM) is a collection of mathematical and statistical methods applied to develop, improve and optimize the experimental processes.[34] In order to investigate the effects of initial pH, initial sulfur and ash concentration on process efficiency and also to obtain optimal conditions; RSM was combined with the central composite design (CCD). CCD was used to calculate the total number of trials as $2k + n_a + n_0$, where k is the number of factors studied in the experiment, n_a the number of axial points and n_0 the number of center points.[35] Each factor was varied at five different levels based on RSM as shown in Table 1. The experimental design was performed using Design Expert 7.1.4 (Stat-Ease, Minneapolis, MN, USA) software in the statistical analysis of data and in the development of the quadratic empirical model:

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i<1}^{3} \sum_{j=1}^{3} \beta i_j X_i X_j + \varepsilon \qquad (4)$$

where y is the expected value of the response variable, β_0 , β_{ii} and β_{ij} the model parameters, X_i and X_j the coded factors evaluated. In this study, y represents the metal extraction percentage in the different empirical models.

Analysis of variance (ANOVA) was used to evaluate the statistical significance of the models and the interaction between the process variables and the responses for polynomial models with a 95% confidence level. Finally, the optimal values of the critical parameters were obtained by analyzing surface plots and by searching a module in a dedicated RSM program.[35]

2.2. Ash toxicity assessments

Toxicity assessments were carried out for the untreated and bioleached FOA samples. Different extractants were used as specified by the assessment methods to obtain leachate solutions from the ash samples. The conditions for chemical leaching are summarized in Table 2. TCLP was conducted to simulate materials in a landfill for a number of years with the assumption of acidic pH found in most landfills. It was used to determine the mobility of both organic and inorganic compounds present in the liquid, solid and multiphase wastes from the leachate that materials would produce. TCLP tests were done based on USEPA Method 1311.[36] SPLP and TTLC were calculated according to USEPA Method 1312[37] and USEPA Method 3050B[38]. All tests were done in shake flasks at 30 rpm. Prior to the analysis by ICP-OES, samples were filtered through 0.45 μm filters. Results were compared with their respective threshold limits.

2.3. Bubble column bioreactor study

In order to evaluate the feasibility of the process for scale-up, tests were performed in a 2.4 L bubble column bioreactor made of glass. Fig. 1 shows the schematic of the bioreactor. The column's internal diameter was 7.5 cm and height was 55 cm. It was fitted with a water jacket to maintain the culture medium temperature at 32 °C. Water was heated in a bath and circulated through the jacket. Air was sparged from the bottom of the bioreactor using a compressor, which produced a recirculation flow pattern in the culture medium. The aeration rate was adjusted to 1 vvm using a rotameter. The initial pH of culture medium in the bioreactor adjusted to 2 by adding a concentrated H_2SO_4 solution. The FOA sample and the inoculum (10% of the culture medium volume) were added to the medium at the same time. The sulfur concentration, cell count, Eh and pH were monitored during incubation.

3. Results and discussion

3.1. Cell adaptation

Adaptation started at 0.5 g of the FOA in 100 mL of fresh growth medium. In order to maintain good bacterial activity and a large cell concentration in the culture medium, the highest initial sulfur concentration (10 g/L) was used. After seven days, the cell count reached 10^9 cells/mL compared with the initial cell count of 10^6 cells/mL. This cell concentration did not change and thus it was concluded that the microorganism could tolerate 0.5% FOA.

Adaptation continued by adding adapted cells from the previous round as inoculum at 10% (v/v) to a new flask containing fresh culture medium and 1 g of FOA. At the beginning, the cell number dropped and then started to increase. After 10 days the cell count reached 10^7–10^8 cells/mL. After successive sub-culturing like this, eventually it was found that an ash concentration greater than 4% decreased the cell concentration to below 10^6 cells/mL after one month of incubation. Thus 4% was considered the highest tolerable concentration for the bacterium to FOA. The cell adapted in the 4% ash was selected as working cell culture for the remaining experiments using various ash concentrations in the range of 0.5% to 4%.

3.1.1. pH variation

The pH variation of the culture medium in shake flasks vs. ash concentration during the bioleaching process is shown in Fig. 2(a). Results indicate that the pH decreased from the initial pH of 2 to below 1. This was because of the bioconversion of S^0 for the production of H_2SO_4 as the bioleaching agent. Fig. 2(a) also shows that pH on day 9th was lower when the ash concentration was lower. This was because the lower consumption of H_2SO_4 for bioleaching led to acid accumulation. With a much higher ash concentration, viscosity of the solution increased considerably and it resulted in the decrease of O_2 transfer from the gaseous phase to the aqueous phase.

3.1.2. Sulfate production variation

Fig. 2(b) shows the variation of the sulfate content in the flasks. As can be seen in the figure, bioleaching of FOA using sulfur as an energy source led to an increase in sulfate concentration in the medium. The highest sulfate concentration in the medium was observed for the initial ash concentration of 0.5% and the sulfate concentration increased from the initial 1.80 g/L to 12.26 g/L at the end of the day 9th bioleaching process. The amount of sulfur oxidation percentage with different ash loadings for the bioleaching process is presented in Table 3. The amount of oxidation sulfur percentage decreased when the initial ash concentration in the medium was increased. This was due to decrease sulfur to ash ratio and thus less conversion of sulfur that is a growth inhibitor in the system.[39] Rising sulfur to ash ratio, increased the availability of bacteria to adsorption on the sulfur surface by means of van der Waals forces, and allowed for increased subsequent oxidation.[6]

3.2. Statistical analysis

CCD was used to fit the model and the experimental conditions, and the results are shown in Table 1. A total of 20 runs in shake flasks were required for this procedure. For each run, the recovery of V, Ni and Cu was recorded. The data was statistically analyzed using ANOVA and the result calculated for each model is being presented in Table 4. Results showed that the calculated p-values of the metal recovery model are less than 0.05, indicating that models were statistically significant at a 95% confidence level.

The coefficient of variation (CV), also known as relative standard deviation (RSD), is a standardized measure of dispersion of a probability distribution or frequency distribution. It is defined as the ratio of the standard deviation to the mean, and it measures the reproducibility of the model. A model can be considered reasonably reproducible if CV is less than 10%. Signal-to-noise ratio (S/N) which indicates an adequate level of precision to navigate through the design space is considered desirable if it is greater than four.[2,33] The results in Table 4 showed that the precision for each model was greater than four.

3.3. Data fitting of the models

The empirical relationship between metal recovery and the three test factors in coded terms obtained by applying multiple regression analysis on the experimental data are shown below:

$$V\ recovery\ (\%) = 97.40 + 0.79A + 4.23B - 4.03C - 1.12AB - 3.49AC + 2.53BC + 3.76A^2 - 4.01B^2 - 0.84C^2 \quad (5)$$

$$Ni\ recovery\ (\%) = 96.95 - 4.76A + 5.37B - 1.18C + 4.61AB + 4.86AC - 2.33BC - 0.48C^2 \quad (6)$$

$$Cu\ recovery\ (\%) = 97.35 - 2.41A + 2.50B - 3.49C + 3.48AB + 3.92AC - 1.02BC \quad (7)$$

where A, B and C denote initial pH, initial sulfur (g/L) and ash concentration percentage, respectively.

It should be noted that polynomial models are reasonable approximations of the true functional relationship over relatively small regions of the entire space of independent variables. According to Table 5, the relatively high R^2 and R^2_{adj} values indicate a satisfactory correlation between the experimental data and the predicted data, thus confirming the robustness of the model.

3.4. Contour plots for metal recovery data

3.4.1. V recovery

Fig. 3(a and b) shows the two dimensional response plots of the relationship between various parameters for V recovery. The interaction effect of the initial sulfur and initial pH at a constant ash concentration of 1.6% is presented in Fig. 3(a). In this figure, decreasing pH to about 1.5 while increasing sulfur content resulted in increased bacterial growth, which, in turn, resulted in increased H_2SO_4 production that served as the oxidant agent in the bioleaching process.[16] Fig. 3(a) shows that increasing initial sulfur and decreasing initial pH both increased V recovery. The maximum V recovery (99%) was observed at initial pH of 1.5 and initial sulfur content of approximately 7 g/L. Fig. 3(b) shows the relationship between ash concentration and initial sulfur for V recovery at constant initial pH of 1.7. According to Fig. 3(b), V recovery increased when initial sulfur was decreased from 8 to 2 g/L and ash concentration was decreased from 1.6 to 0.5%. A maximum V recovery about 99% was observed at the initial sulfur concentration about 7 g/L and ash concentration of 0.5%.

3.4.2. Ni recovery

Fig. 4(a) shows the interaction between initial sulfur and ash concentration. The maximum Ni recovery was observed at ash concentration of 1% and initial sulfur of 8 g/L at initial pH of 2.2. The relationship between initial pH and ash concentration was shown in Fig. 4(b). Results indicated that lower initial pH and ash concentration increased Ni recovery. A maximum recovery for Ni (near 100%) was observed at ash concentration of 1% and initial pH of 1.5 and initial sulfur of 4 g/L.

3.4.3. Cu recovery

Fig. 5 shows the effects of different parameters on Cu recovery. According to results from the interaction between initial sulfur and ash concentration (Fig. 5a) and interaction between initial sulfur and initial pH (Fig. 5b), it is evident that increasing initial sulfur, decreasing initial pH and decreasing the ash concentration had a positive effect on the Cu recovery. This outcome was also consistent with Eq. (7). At a low ash concentration (i.e., low viscosity), oxygen and carbon dioxide were more effectively dissolved and transferred to bacteria, resulting in increased bacterial growth and, therefore, improved metal recovery. In Fig. 5(b), a maximum recovery of Cu greater than 99% was observed with initial pH of 1.5, initial sulfur of 5.5 g/L and ash concentration of 1%.

3.5. Process optimization and validation

It should be noted that the goal of optimization is to find a good set of conditions to receive a maximum or minimum level of response. The desirability function approach is one of the most widely used methods in industry for the optimization of multiple response processes. RSM seeks to find the best local maximum by starting from several points in the design space. In this study, the goal was the simultaneous maximization of V, Ni, and Cu recoveries. The optimum conditions obtained from the models were initial pH of 1, initial sulfur of 9 g/L and ash concentration of 1 g/L. To test the suitability of the optimized model conditions, an experiment was performed with these optimum values. The metal recovery values were found to be 96.4% for V, 100% for Ni and 99.2% for Cu, which fell within the predicted ranges of 91-100% for V, 98-100% for Ni, and 95-100% for Cu, respectively using the 95% confidence interval. Table 6 compared results obtained in this work at optimal condition with those of reported in the literature. There is an enhancement for the vanadium and nickel recovery from FOA using *At. thiooxidans* at proposed optimized condition obtained in this study in comparison with those reported in previous studies.

3.6. Toxicity assessment tests

In this study, TCLP, SPLP and TTLC test results of FOA before and after bioleaching are listed in Table 5. The metal concentrations in the ash sample before bioleaching were much higher than the regulatory limits set by USEPA. Using bioleaching with the optimum operating conditions, V, Ni and Cu concentrations were reduced well below the regulatory limits. Thus, the bioleaching process detoxified the FOA, making it safe for disposal.

3.5. Bioreactor results

In order to study scale-up feasibility, some experiments were performed in a 2.4 L bubble column bioreactor. Fig. 6 (a-c) shows the results of the bioreactor study with initial sulfur of 9 g/L and initial pH of 1. This figure shows that in general metal recoveries increased over time for ash concentrations ranged from 1% to 4%, and a higher ash loading decreased metal recoveries. The maximum recoveries for V, Ni and Cu were 77.6%, 89.7% and 65.7%, respectively with ash concentration of 1% at the end of 8 days of incubation. These recovery rates were lower than those for shake flasks, but they suggested that this bioleaching process could be operated in a bioreactor after optimizing the bioreactor operating conditions.

The changes in pH, amount of sulfate produced and bacterial cell count during the 8-day bioleaching process with an ash concentration of 1% are shown in Fig. 7. After one day, the pH peaked at 1.7. It began to decrease afterwards and reached 1.1 at the end of the process. The planktonic increased during the bioleaching process, which was accompanied by increased total sulfate concentration in the medium, which reflected the sulfate production.

4. Conclusion

This work examined the bioleaching of V, Ni and Cu from FOA using *At. thiooxidans* in shake flasks and in a bubble column. Adaptation of this bacterium to FOA was carried out. The optimum conditions for metal recoveries were initial pH of 1, initial sulfur of 9 g/L and ash concentration of 1%. The maximum recoveries were 96.4% for V, 100% for Ni and 99.2% for Cu, respectively for shake flask tests. The toxicity test results proved that the bioleached FOA were safe for discharge. The reactor results suggested that the process can be scaled up, but to short the process time and reach higher metals concentration the bioreactor operating conditions should be optimized before scaled up.

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Tables and figures captions

Table 1. Experimental plan based on CCD and the results of metals recoveries.

Table 2. Toxicity assessment test procedure.

Table 3. Experimental sulfate production and sulfur oxidation percentage.

Table 4. ANOVA for response surface models applied.

Table 5. Toxicity assessment tests of before and after bioleaching of FOA.

Table 6. Comparison of the obtained results for V and Ni recovery in this work with those reported in previous studies.

Fig. 1. Schematic of bubble column bioreactor used for the bioleaching study: (A) aeration pump; (B) sparger; (C) water bath and (D) jacket.

Fig. 2. Variation of different parameters in the medium with the different ash concentration during time (a) the pH variation (b) sulfate production variation.

Fig. 3. Contour plots of the interactive effects for V recovery (a) for initial pH and initial sulfur at the constant ash concentration 1.6% (b) for initial sulfur and ash concentration at the constant initial pH 1.7

Fig. 4. Contour plots of the interactive effects for Ni recovery (a) for initial sulfur and ash concentration at the constant initial pH 2.2 (b) for initial pH and ash concentration at the constant sulfur 4 g/L.

Fig. 5. Contour plots of the interactive effects for Cu recovery (a) for initial sulfur and ash concentration at the constant initial pH 2.2 (b) for initial pH and initial sulfur at the ash concentration 1%.

Fig. 6. Metals recovery in different ash concentration versus time under optimal condition (a) V recovery (b) Ni recovery (c) Cu recovery.

Fig. 7. Growth characteristics of *At. thiooxidans* cells versus time under optimal conditions.

Table 1

	Factors			Responses		
Run	Initial pH	Initial Sulfur conc. (g/L)	Ash conc. % (w/v)	V recovery (%)	Ni recovery (%)	Cu recovery (%)
	Actual value (Coded value)					
1	1.8 (0)	5.5 (0)	2.1 (0)	85.1	88.4	85.2
2	2.2 (+1)	2.8 (-1)	1.0 (-1)	94.1	83.0	93.0
3	1.0 (-1.68)	5.5 (0)	2.1 (0)	100	90.9	88.8
4	1.3 (-1)	8.2 (+1)	3.2 (+1)	100	60.03	62.0
5	1.8 (0)	5.5 (0)	4.0 (+1.68)	57.9	80.9	73.0
6	1.3 (-1)	2.8 (-1)	1.0 (-1)	100	100	100
7	1.8 (0)	1.0 (-1.68)	2.1 (0)	54.0	89.2	87.7
8	2.2 (+1)	8.2 (+1)	3.2 (+1)	62.0	100	100
9	2.2 (+1)	8.2 (+1)	1.0 (-1)	100	100	100
10	1.3 (-1)	2.8 (-1)	3.2 (+1)	69.6	79.9	77.0
11	1.8 (0)	5.5 (0)	2.1 (0)	86.1	95.3	93.6
12	1.8 (0)	5.5 (0)	0.2 (-1.68)	100	100	100
13	1.8 (0)	5.5 (0)	2.1 (0)	83.0	91.5	89.6
14	1.3 (-1)	8.2 (+1)	1.0 (-1)	100	100	100
15	1.8 (0)	10.0 (+1.68)	2.1 (0)	95.7	99.5	93.9
16	1.8 (0)	5.5 (0)	2.1 (0)	83.9	89.4	90.0
17	2.2 (+1)	2.8 (-1)	3.2 (+1)	46.4	100	94.1
18	1.8 (0)	5.5 (0)	2.1 (0)	95.2	96.0	92.0
19	2.5 (+1.68)	5.5 (0)	2.1 (0)	93.7	100	99.8
20	1.8 (0)	5.5 (0)	2.1 (0)	89.0	95.0	91.0

Table 2

Test	**TCLP**	**SPLP**	**TTLC**
Extractant	$C_2H_4O_2.H_2O$	H_2SO_4, HNO_3, H_2O	HNO_3, H_2O_2, HCl, H_2O
Leaching solution volume (mL)	2000	2000	100
Ash sample (g)	100	100	1
Leaching time (h)	18	18	48

Table 3

Initial ash concentration %(w/v)	Overall sulfate production (g/L)	Sulfur oxidation (%)
0. 5	12.26	87.2
1	6.13	43.6
2	4.90	34.9
3	4.46	31.8
4	3.30	23.5

Table 4

Response	**Model**	**p-value**	**C.V. (%)**	**Adeq. Precision**	**R^2**	**Adjusted R^2**
V recovery	Full quadratic	0.0008	7.92	12.79	0.91	0.83
Ni recovery	Reduced quadratic	<0.0001	3.95	16.98	0.91	0.86
Cu recovery	2FI	< 0.0001	3.74	17.43	0.91	0.86

Table 5

Metal	TCLP (mg/L)		TCLP Limit (mg/L)	SPLP (mg/l)		SPLP Limit (mg/L)	TTLC (g/kg)		TTLC limit (g/kg)
	Before	After		Before	After		Before	After	
V	210	17	24	2690	45	n.s.	215	1.8	2.4
Ni	458	4	20	515	9.2	n.s.	40	0.52	2
Cu	37	0.3	20	53	3.13	n.s.	4.3	0.044	2.5

n.s.: not stated

Table 6

Solid waste	**Leaching agent**	**Recovery (%)**	**Ref.**
Oil fired fly ash	HCl (Chemical)	58% V 86% Ni	29
Oil-fired fly ash	H_2SO_4 Chemical)	65% V 60% Ni	40
Heavy oil fly ash	H_2SO_4 (Chemical)	68% V	28
Spent catalyst	Acid produced by *At. thiooxidans*	32% V 88% Ni	41
Ore residue	Organic acid produced by *A. niger*	44% V	42
Converter slag	Biosurfactant produced by *P. putida*	90% V	43
Fuel-oil ash	Acid produced by *At. thiooxidans*	96% V 100% Ni	This study

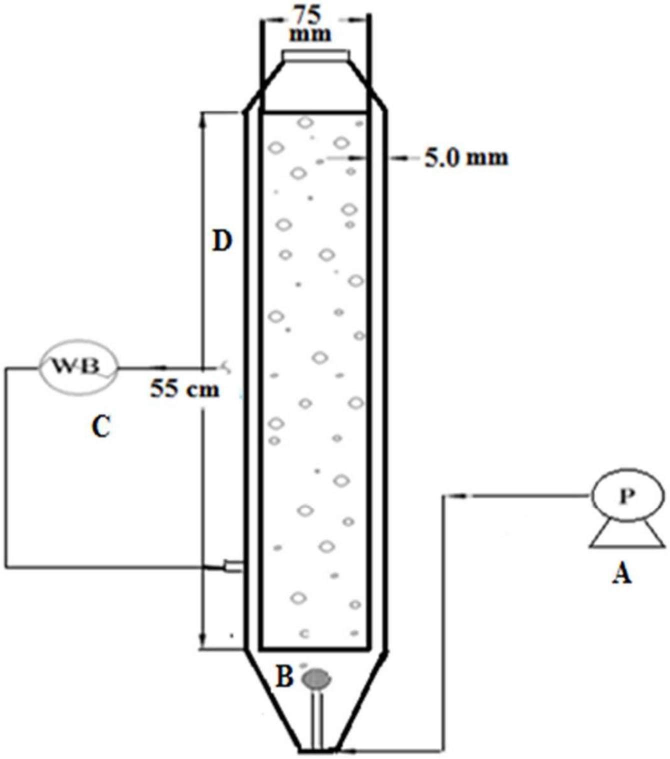
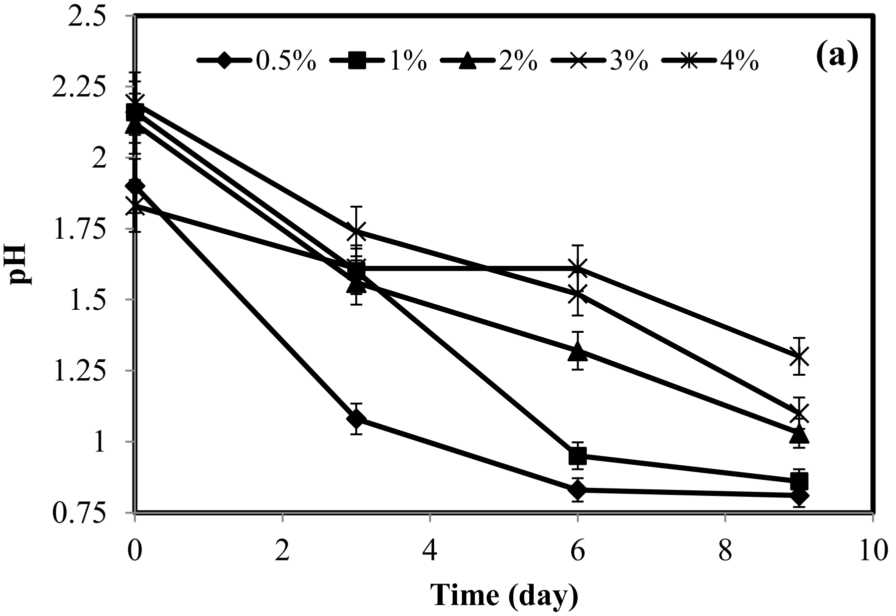


Fig. 1


2.5
2.25
2
1.75
1.5
1.25
1
0.75
pH
0
2
4
6
8
10
Time (day)
0.5%
1%
2%
3%
4%
(a)

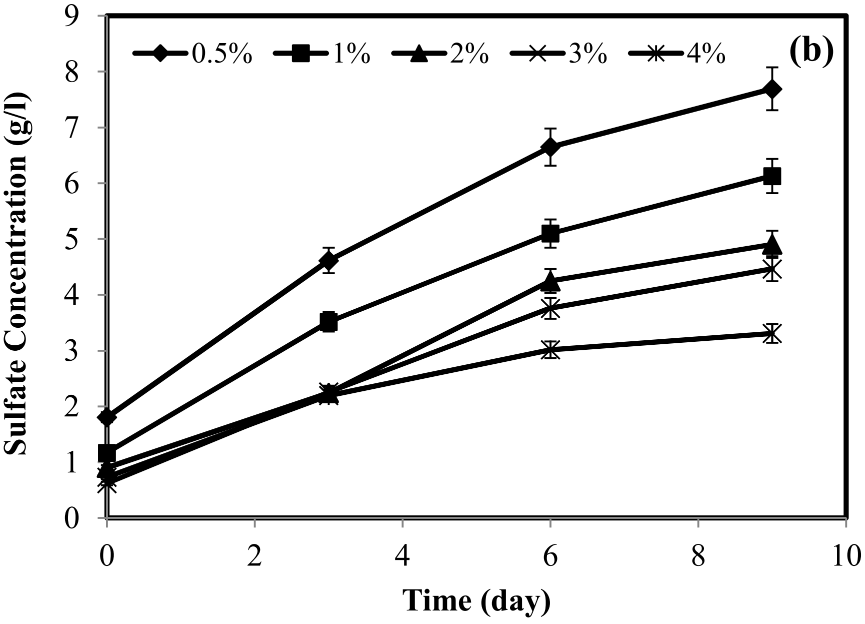

9
8
7
6
5
4
3
2
1
0
Sulfate Concentration (g/l)
0
2
4
6
8
10
Time (day)
0.5%
1%
2%
3%
4%
(b)

Fig. 2

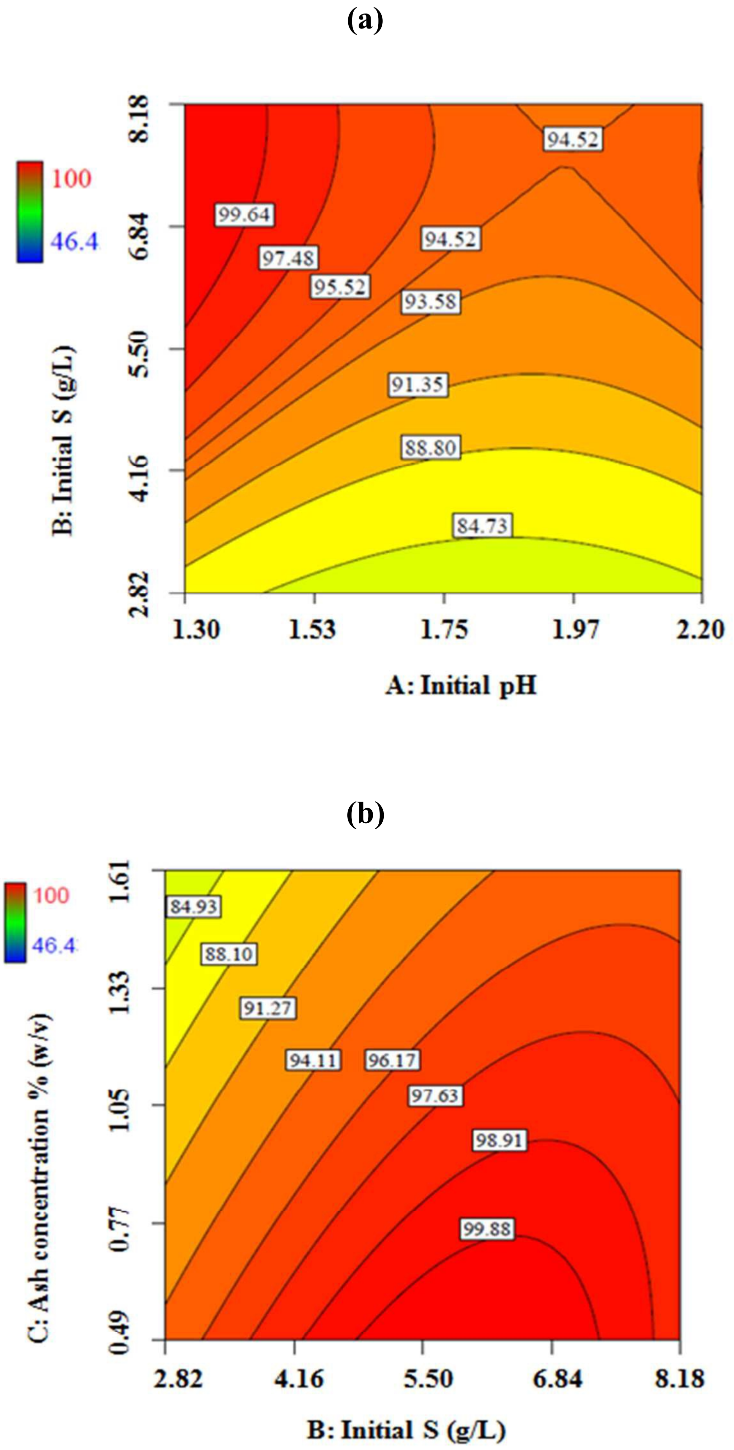

(a)
100
46.4
8.18
6.84
5.50
4.16
2.82
B: Initial S (g/L)
94.52
99.64
94.52
97.48
95.52
93.58
91.35
88.80
84.73
1.30
1.53
1.75
1.97
2.20
A: Initial pH
(b)
100
46.4
1.61
1.33
1.05
0.77
0.49
C: Ash concentration % (w/v)
84.93
88.10
91.27
94.11
96.17
97.63
98.91
99.88
2.82
4.16
5.50
6.84
8.18
B: Initial S (g/L)

Fig. 3

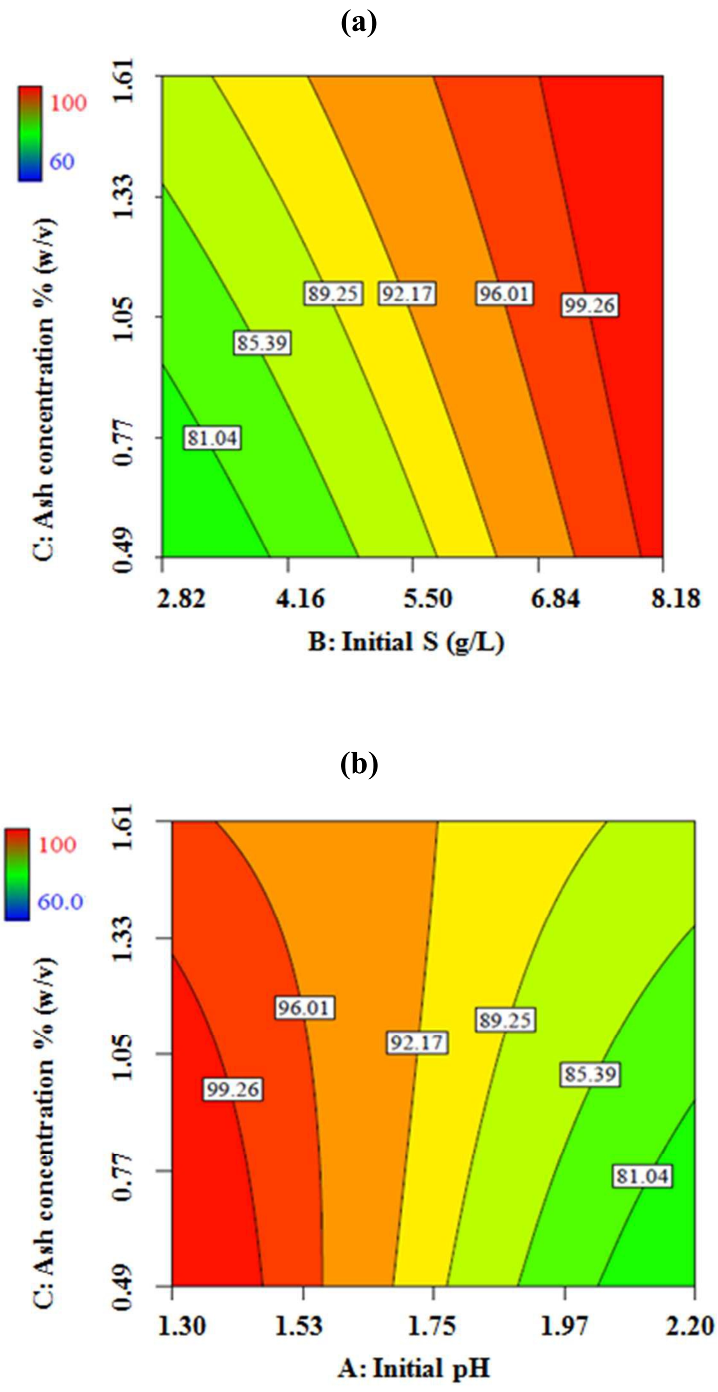

(a)
100
60
C: Ash concentration % (w/v)
1.61
1.33
1.05
0.77
0.49
89.25
92.17
96.01
99.26
85.39
81.04
2.82
4.16
5.50
6.84
8.18
B: Initial S (g/L)
(b)
100
60.0
C: Ash concentration % (w/v)
1.61
1.33
1.05
0.77
0.49
96.01
92.17
89.25
99.26
85.39
81.04
1.30
1.53
1.75
1.97
2.20
A: Initial pH

Fig. 4

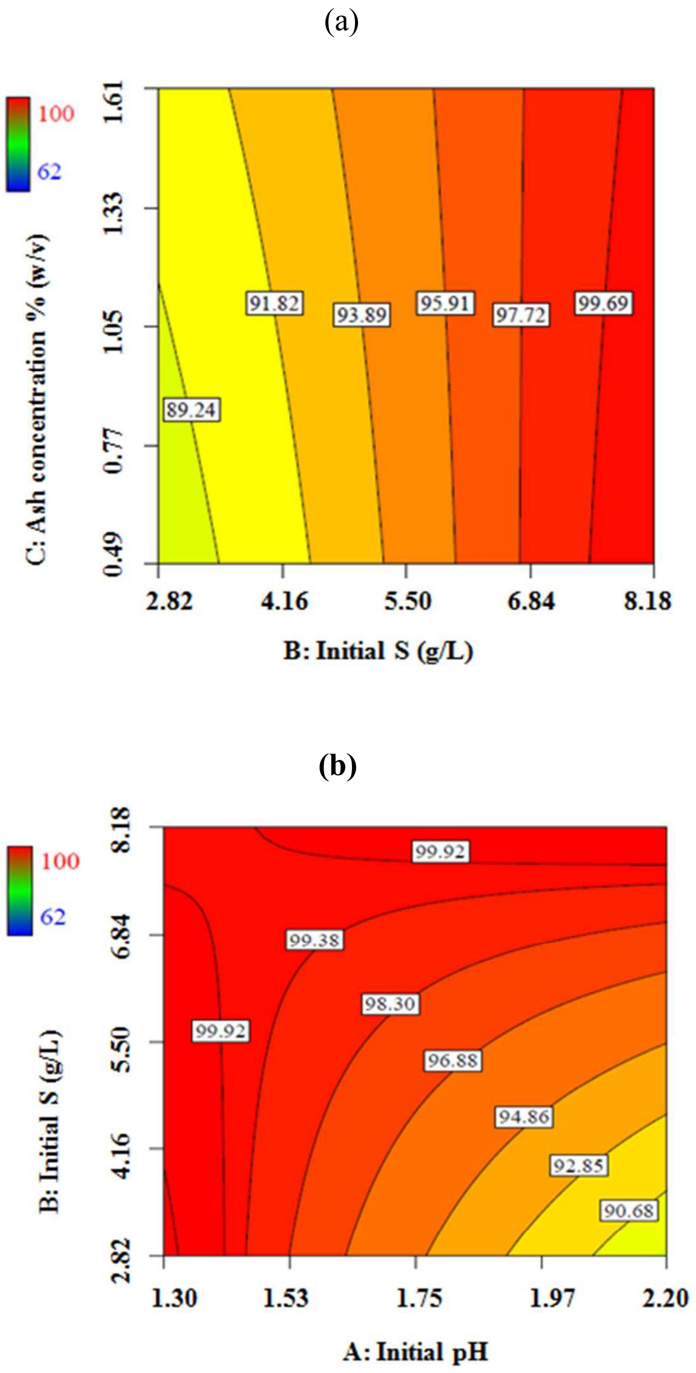

(a)
100
62
C: Ash concentration % (w/v)
1.61
1.33
1.05
0.77
0.49
91.82
93.89
95.91
97.72
99.69
89.24
2.82
4.16
5.50
6.84
8.18
B: Initial S (g/L)
(b)
100
62
B: Initial S (g/L)
8.18
6.84
5.50
4.16
2.82
99.92
99.38
98.30
99.92
96.88
94.86
92.85
90.68
1.30
1.53
1.75
1.97
2.20
A: Initial pH

Fig. 5.

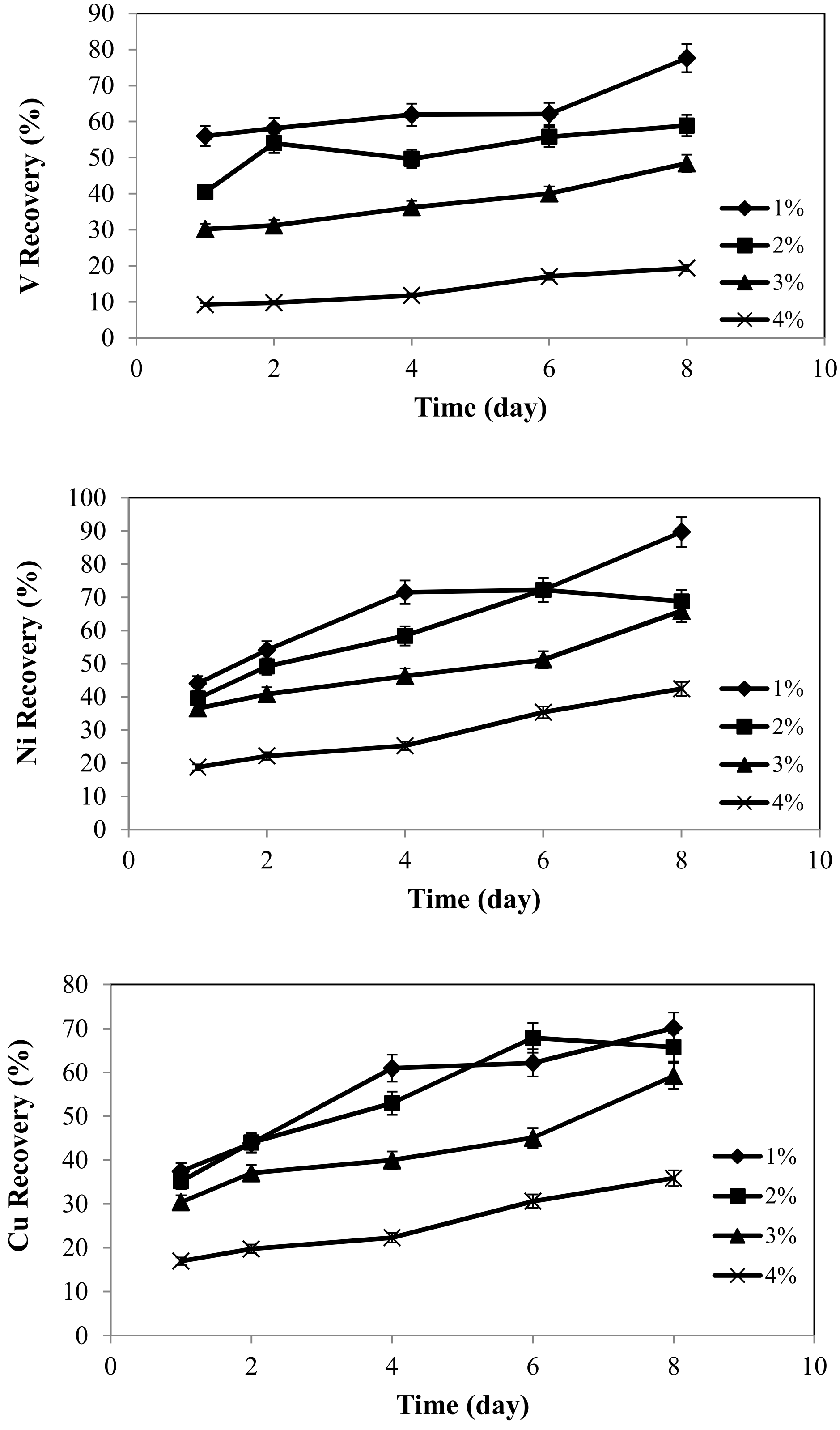
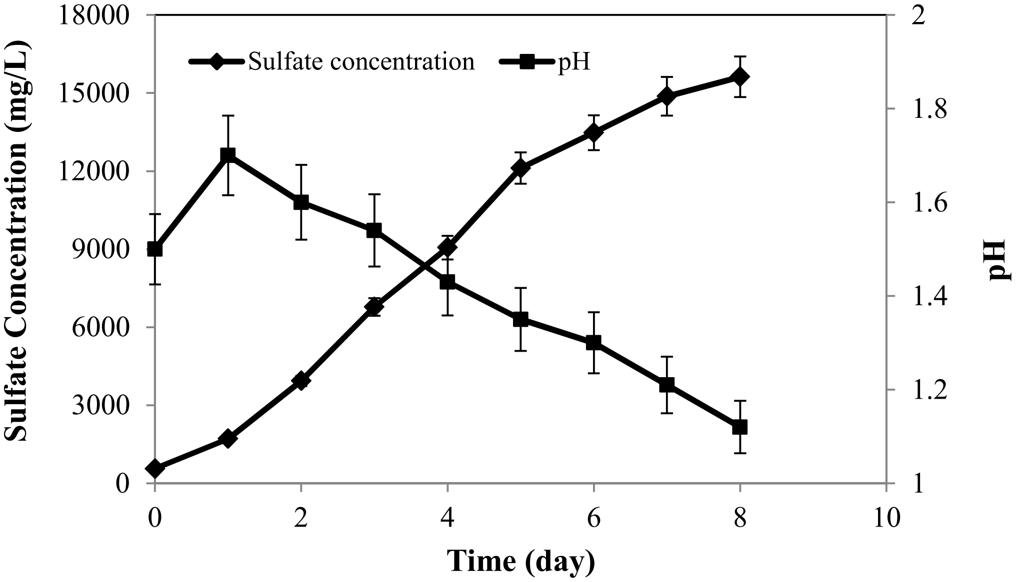

V Recovery (%)
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
Time (day)
1%
2%
3%
4%
Ni Recovery (%)
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
Time (day)
1%
2%
3%
4%
Cu Recovery (%)
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
Time (day)
1%
2%
3%
4%

Fig. 6.

(a)


18000
15000
12000
9000
6000
3000
0
Sulfate Concentration (mg/L)
Sulfate concentration
pH
2
1.8
1.6
1.4
1.2
1
pH
0
2
4
6
8
10
Time (day)

(b)

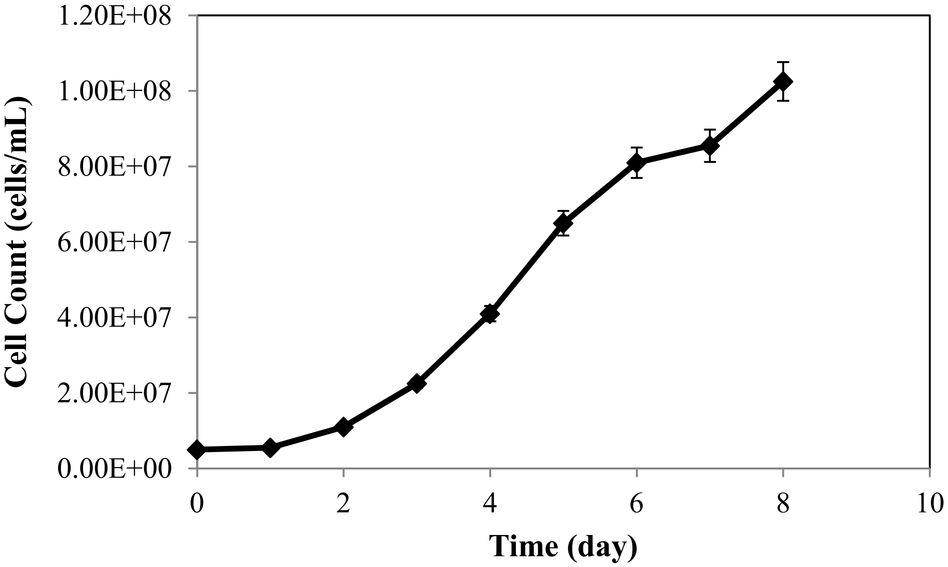
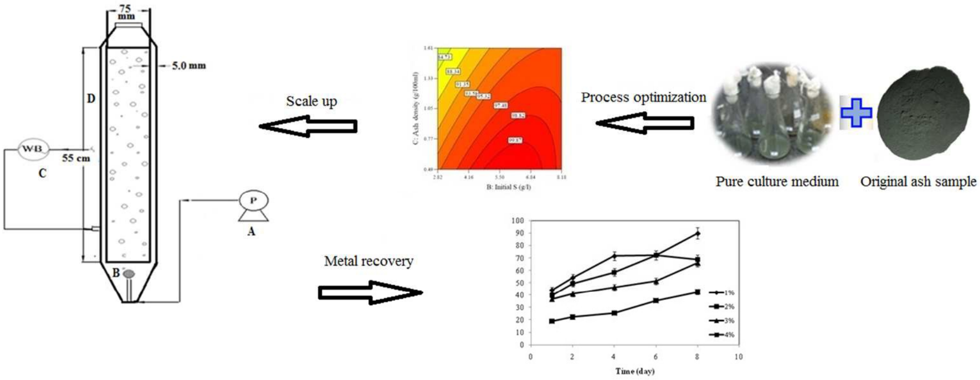

1.20E+08
1.00E+08
8.00E+07
6.00E+07
4.00E+07
2.00E+07
0.00E+00
Cell Count (cells/mL)
0
2
4
6
8
10
Time (day)

Fig. 7



330x131mm (96 x 96 DPI)