An Optimized Integrated Process for the Bioleaching of a Spent Refinery Processing Catalysts

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ABSTARCT: Bioleaching of spent refinery processing catalysts using adapted indigenous strains of *Acidithiobacillus ferrooxidans* to recover Ni and Mo, and the separation of Ni from bioleaching liquor using iron magnetic nanoparticles were optimized in this study. Bioleaching optimization was performed using central composite design (CCD) method to optimize temperature (32 to 38ºC), initial pH (1.5 to 2), particle size (-30 to -190µm sieve fraction) and pulp density (0.1 to 0.5%). The maximum values for Mo and Ni extraction were determined to be 62.72 and 92.34%, respectively, at 34 ℃, -90.38µm sieve fraction, 0.22 % pulp density and initial pH of 1.8 under controlled pH condition. The results of bioleaching study suggest the shaking bioreactor as a suitable tool to optimize pulp density at low values for scale up purposes. The optimum pH value for Ni adsorption from synthetically made Ni solutions was found to be 8. The Ni adsorption increased with increasing temperature and the mass of the adsorbent. The percentage of Ni adsorption from a bioleaching liquor contained 30 ppm Ni and 5g/l magnetic nanoparticles was determined to be 97% after 1 hour at 34ºC and pH 8. An integrated process for bioleaching of Ni and Mo from spent catalysts and adsorption of Ni using magnetic nanoparticles has been proposed in this study.

Key words: Spent Catalysts, Bioleaching, *Acidithiobacillus ferrooxidans*, Central Composite Design

INTRODUCTION

Metal contaminants available in soils, groundwater, sediments, surface water (Furimsky, 1996; Manju *et al.,* 2014; Gonzalez-Corrochano *et al.,* 2013; Nasrabadi *et al.,* 2010; Serbaji *et al.,* 2012; Nwabanne and Igbokwe, 2012) indoor and outdoor air (Amouei Torkmahalleh *et al.,* 2012a, 2012b, 2012c, 2013) have become a serious environmental concern. Spent catalysts are currently one of the environmental issues that are classified as hazards by the U.S. Environmental Protection Agency (USEPA) because of the presence of toxic metals such as Ni, V, Mo, Co, and Al (Marafi and Stanislaus, 2008). The high cost and the negative environmental impact of conventional methods for the extraction of metals from waste spent catalysts necessitate investigating on the alternative methods such as bioleaching

technology (Aung and Ting, 2005). The advantages of bioleaching include reduction of energy, low operating cost and less environmental impact. *A. ferrooxidans* is one of the most common bacteria for metal dissolution which belongs to the genus Bacillus (Aung and Ting, 2005). Few studies investigated the ability of the pure culture of *A. ferrooxidans* in bioleaching of spent catalysts (Mafi Gholami *et al.,* 2011; Bredberg *et al.,* 2004; Pradhan *et al.,* 2009; Pradhan *et al.,* 2010; Pradhan *et al.,* 2013). Among these studies, limited data are available for the optimized values of the bioleaching parameters such as pH, temperature, pulp density and particle size (Mafi Gholami *et al.,* 2011; Pradhan *et al.,* 2009; Pradhan *et al.,* 2010). Different optimum conditions were reported for the bioleaching of spent catalysts using pure

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culture of *A. ferrooxidans* that could be due to the differences in the characteristics of the spent catalysts and the strain of the bacteria. For instance, Mafi Gholami et al. (2011) reported the optimum temperature and pH for the bioleaching of a spent catalyst to be 40°C and 1.5, while Pradhan et al. (2010) determined the optimum temperature and pH to be 35°C and 2.5, respectively.

Typically, bioleaching factors (parameters) such as temperature and pH are optimized properly in shaking bioreactors (shake flask cultivation) which is cheaper and less time consuming compared to the stirred bioreactors (Haghshenas *et al.,* 2012; Chauhan *et al.,* 2013). The idea of using shaking bioreactors is to find the optimum values, and apply them for the larger scales. Pulp density is suggested to be optimized in stirred bioreactors which provide much better gas transfer rates, and make it possible to employ higher pulp density compared to the shaking bioreactors (Haghshenas *et al.,* 2012). However, pulp density can still be studied in shaking bioreactors to understand its possible interactions with other bioleaching parameters. This type of information is valuable to design of large scale bioleaching processes. So far, two methodologies were used for the optimization of the spent catalysts bioleaching. The first one is one-factor-at- a time (Pradhan *et al.,* 2009; Pradhan *et al.,* 2010) which is inefficient in providing some information regarding the interaction between the process parameters. Alternatively, modeling and optimization of process parameters can be conducted statistically using design of experiments through response surface methodologies (RSM) which is suitable for understanding the interactions between parameters. Design of experiments methodology is able to simultaneously consider several factors at different levels, and suggest a suitable model to correlate the factors and the response (Montgomery, 2006). Simate et al. (2009) using central composite rotatable design method showed that pH, particle size, pulp density and substrate type were statistically significant operating parameters in the extraction of nickel from nickel laterites, while bacterial inoculum size was not. Among previous research on the bioleaching of spent catalysts, so far, Mafi Gholami et al. (2012) and Amiri et al. (2012) employed central composite design (CCD) approach to optimize the extraction of heavy metals from spent catalysts using *Aspergillus niger.* Also, Amiri et al. (2011) used CCD method to optimize the extraction of heavy metals from spent catalysts using *Penicillium simplicissimum.* A survey of the literature shows that no study was performed to optimize the bioleaching of spent catalysts using pure culture of *A. ferrooxidans* through design of experiments

methodology. The bioleaching of spent catalysts results in the dissolution of toxic elements in the bioleaching liquors. Therefore, it is imperative to design metal separation units to remove the toxic ions from the bioleaching effluent streams. Removal of heavy metals from aqueous solutions was investigated using sulfur reducing bacteria (SRB) and fungi (Sayer *et al.,* 1999; Kolmert and Johnson, 2001; Lioyd and Lovley, 2011; Bosio *et al.,* 2008). The main idea in those studies was to precipitate heavy metals using microbial generated sulfides, phosphates or oxalates. These bioprecipitation processes have some practical limitations such as high operational cost, toxicity of heavy metals for SRB and clogging of SRB biofilm by precipitated heavy metals (Bosio *et al.,* 2008). Thus far, the only study that investigated the separation of the toxic metals in the bioleaching liquors of spent catalysts was performed by Bosio et al. (2008). In their study, the extraction of nickel from bioleaching liquors was examined by precipitating nickel with sulfide that was generated by *Desulfovibrio* cells. The highest precipitation efficiency of nickel at room temperature and under optimum conditions was found to be 79%. Separation of toxic metals from bioleaching liquors of spent catalysts using different methods is absolutely open for investigation.

The first objective of this investigation was to maximize Mo and Ni extraction using an appropriate method, namely CCD method, from Iranian FCC spent catalysts using adapted indigenous strain of *A. ferrooxidans.* In this study, temperature, initial pH and size fraction were studied to determine their optimum values that are useful for process scale up. Pulp density was studied to understand its effect on metal extraction, and to find out its possible interaction with other aforementioned parameters. Also, this study investigated the adsorption of Ni from synthetic Ni solutions and bioleaching liquors using iron magnetic nanoparticles. The impact of pH and temperature on Ni adsorption was studied, and a new integrated process involving bioleaching and adsorption units was proposed.

MATERIALS & METHODS

The indigenous iron oxidizing bacteria, *A. ferrooxidans* was provided by Sarcheshmeh copper mine (Kerman, Iran), and cultured in a 9K medium (Silverman and Lundgren, 1959) containing 3g/L $(NH_4)_2$ SO_4, 0.1g/L KCl, 0.5g/L K_2HPO_4, 0.5g/L $MgSO_4.7H_2O$, 0.01g/L $Ca(NO_3)_2$ and 44.8g/L $FeSO_4.7H_2O$. The medium, minus $FeSO_4$, was autoclaved at 120°C for 20 minutes. The $FeSO_4$ medium was separately sterilized through a 0.2 µm filter, and was added aseptically to the iron free medium. The bacterial growth was monitored by cell

counting using a nevox microscope on the neuber lam. The serial subculturing adaptation method (Haghshenas *et al.,* 2009a) was employed in this investigation to adapt *A. ferrooxidans* cells to 5% pulp density. Initial growth was observed at 0.1% pulp density. Then, the grown cells suspended in the bioleaching solutions were subcultured in the new bioleaching media with a higher level of pulp density (in steps of 0.1 % (w/v)). When adaptation was established, subculturing to the higher level of the pulp density was performed until the adapted cells to 5% pulp density were achieved (Noori Felegari et al. 2012). The adaptation experiments were conducted in 9 K media supplemented with the appropriate amount of catalysts with 10mL inoculums size, in a rotary shaker at 180 rpm and 32°C. The adaptation process lasted for 5 months. The adapted *A. ferrooxidans* cells to 0.5% spent catalysts were used for the bioleaching experiments.The spent refinery catalysts were provided by Tehran Oil Refinery Company, Iran. The elemental analysis of the spent catalysts was performed by an inductively coupled plasma optical emission spectrometer (ICP-OES). The following mass fraction values were obtained: Ni: 3.1%, Mo: 7.1%, S: 1.23 %, Co: 0.006% and V < 0.002%. The catalysts were sieved to obtain the proper size fractions for the optimization experiments (30μm to 190μm). The catalysts were pretreated in a hot air oven at 90°C for 2 hours. Previous studies employed different pretreatment procedures to remove hydrocarbons and organic materials attached to catalysts particles (Beolchini *et al.,* 2010, Bosio *et al.,* 2008) which could inhibit Fe/S oxidizing bacteria metabolism (Beolchini *et al.,* 2010). However, the bacteria used in the current study were adapted to the spent catalysts which were pretreated (at 90°C for 2 hours) prior to the adaptation experiments as described above. The adapted bacteria were also utilized successfully in the bioleaching experiments conducted in our recent study (Noori Felegri *et al.,* 2012) indicating that the bacterial cells are able to grow in the presence of the organic compounds adsorbed to the spent catalysts, after adaptation. The elimination of the pretreatment procedure could be cost effective for the industrial scale bioleaching. All experiments were carried out in 250 mL Erlenmeyer flasks containing 90 mL of bioleaching solution. In the bioleaching experiments, 9 K media were supplemented with the appropriate amount of spent catalysts to give the desired pulp density. All of the bioleaching optimization experiments in this study were conducted with the 10mL inoculum size and at 180 rpm. Temperature, initial pH, size of the catalysts and pulp density were optimized in the bioleaching experiments for the range of 32 to 38°C, 1.5 to 2, -30 to -190μm sieve fraction and 0.1 to 0.5%, respectively. Previous studies demonstrated that

as sphalerite pulp density increases, the amount of the attached cells increases (Haghshenas *et al.,* 2009a; Cerruti *et al.,* 1998). Therefore, in this study, to exclude this effect a low level of pulp density values (0.1% to 0.5%) was chosen. Similarly, Mafi Gholami et al. (2011) studied the effect of pulp density on the bioleaching of spent catalysts using pure culture of *A. ferrooxidans* at such low level of pulp density (0.15% to 0.35%). Our preliminary studies showed that bacterial population (approximately 4.3×10^7 cells/mL) and Ni extraction (over 90%) were significant after 7 days of bioleaching. Therefore, for the rest of our study, samples were taken from the bioleaching liquors after 7 days that might also be reasonable for industrial application, where 7 days residence time might be reached with recirculation of flows (Beolchini *et al.,* 2010).

During the bioleaching experiments, make-up distilled water was added periodically to the flasks to compensate for the evaporation loss, and after that pH of the solutions was adjusted back to its initial value (1.6 to1.7) with 0.1 to 0.2 mL of concentrated sulfuric acid solutions. Sterile control (9K media without the microorganisms) was also run to determine the contributions from the chemical leaching of nickel and molybdenum. Analytical Determinations: A pH meter (Metller Toldo Company, Germany) with glass membrane electrode was used to measure pH. The extraction of Ni and Mo was defined as the ratio of the mass of the metals determined in the bioleaching liquor to the mass of the metals in the non-bioleached spent catalysts. Alternatively, to check metal concentration obtained using the aforementioned definition, the extraction of the metals was defined as the ratio of the difference in mass of the metals in the non-bioleached spent catalyst to the mass of the metals in the catalysts residue divided by the mass of the metals in the non-bioleached spent catalysts. No significant differences in the metal extraction values calculated using the two definitions were observed. To determine the mass of the Ni or Mo in the catalysts residue, at the end of each bioleaching experiment, the bioleaching liquors were filtered using Whatman 41 filter papers to collect the spent catalysts residue. Then, the filter papers were kept at 40°C in an oven for 24 hours to dry the residue. The spent catalysts were digested using concentrated hydrochloric acid and nitric acid, in a ratio of 3:1. After cooling, the digestate was filtered through Whatman41 filter paper and made up to 100 mL using 50% HCl to perform the metal analysis (Beolchini *et al.,* 2010). The images of the non-bioleached and bioleached spent catalysts were taken using scanning electron microscope (SEM)-energy dispersive X-ray analysis. The most popular response surface method (RSM) is CCD. Central composite is designed to estimate the

coefficients of second-order polynomials called "quadratic". Using RSM, the optimum leaching condition having satisfactory extraction performance can be achieved with minimum number of experiments. This ability eliminates the need for experimentally studying all possible combinations of the process variables. To determine a critical point (maximum, minimum, or saddle), it is necessary for the polynomial function to contain quadratic terms according to the following equation:

$$(1)$$

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_{ii} x_i \sum_{\langle i \langle j}^{k} \beta_{ii} x_i^2 + \sum_{1 \langle i \langle j}^{k} \beta_{ij} x_i x_j + \varepsilon$$

where $k, \beta_0, \beta_i, x_i, \beta_{ii}, \beta_{ij}$, and å represent the number of variables, a constant term, coefficients of the linear parameters, variables, coefficients of the quadratic parameters, coefficients of the interaction parameters and a residual associated to the experiments, respectively (Bezera *et al.,* 2008; Mehrabani *et al.,* 2010). The parameters and their levels that were studied by CCD method are shown in Table 1.

All bioleaching experiments were repeated three times. To produce magnetic nanoparticles (Tombacz, 2007), 10.81g $FeCl_3.6H_2O$ and 3.97g $FeCl_2.4H_2O$ were separately dissolved in 40 mL hydrochloric acid solutions (2M). An ammonium solution (0.7M, 500ml) was deoxygenated using an argon flow for 30 minutes. Then, the iron chloride solutions were added to the ammonium solution while stirring (200 rpm). Upon mixing, the color of the solution turns to black indicating the formation of the iron magnetic nanoparticles. The precipitates were rinsed with water, and added to 500 mL of deionized water.BET and Transmission Electron Microscopy (TEM) analyses were performed to understand the shape and size of the magnetic nanoparticles. The prepared magnetic nanoparticles were utilized to examine the Ni adsorption from pure Ni solutions. Pure Ni solutions were made synthetically using $NiSO_4.6H_2O$ salt with the concentration of 4.0 ppm Ni, and 50 mL of such solutions were used in the adsorption experiments. Sodium sulfate salt was used as a background electrolyte. The ionic strength of the nickel solutions was adjusted by sodium sulfide.

Percentage of Ni adsorption is defined by equation 1.

% Ni adsorption =((initial concentration of Ni- final concentration of Ni) in the adsorption solution ✕100)/ (initial concentration of Ni in the adsorption solution)

Equation 1
    Adsorption experiments were performed in a stirred tank (adsorption tank) at 250rpm equipped with pH and temperature controllers. To perform the optimization experiments the adsorption tank was set at the desired pH and temperature. Then the synthesized Ni solution was added to the tank following with addition of the magnetic nanoparticles such that the concentration of the nanoparticles was adjusted at 5g/L.

    Duplicate adsorption experiments were conducted in the present study. Variability is defined as "absolute (value of sample 1 – value of sample 2) / average (value of sample1 and value of sample 2)".

RESULTS & DISCUSSION
    Figs 1a and 1b show the SEM images of the spent catalysts before bioleaching and after bioleaching, respectively, using *A. ferrooxidans* that was adapted to 0.5% spent catalysts in our recent study (Noori Felegari *et al.,* 2012). Non-bioleached (fresh) catalysts have smooth surfaces with variations in the particle size, while the bioleached catalysts show small broken particles with the consistent particle size that could be the effect of the bioleaching. Similar morphology was observed by Mafi Gholami et al. (2011) for the non-bioleached and bioleached spent catalysts using *A. ferrooxidans*. Sixty bioleaching experiments were designed using CCD method. Table 2 shows the experimental conditions and their responses. The

Table 1. The levels of variables for the CCD study

Name	- alpha	-1 Level	Center	+1 Level	+ alpha
Initial pH	1.44	1.65	1.80	1.95	2.15
T (^oC)	29.3	32.0	34.0	36.0	38.7
Size (µm)	16.3	70.0	110.0	150.0	203.6
Pulp density (%)	0.065	0.2	0.3	0.4	0.53
E (pH)		-1(controlled pH)		+1 (uncontrolled pH)	
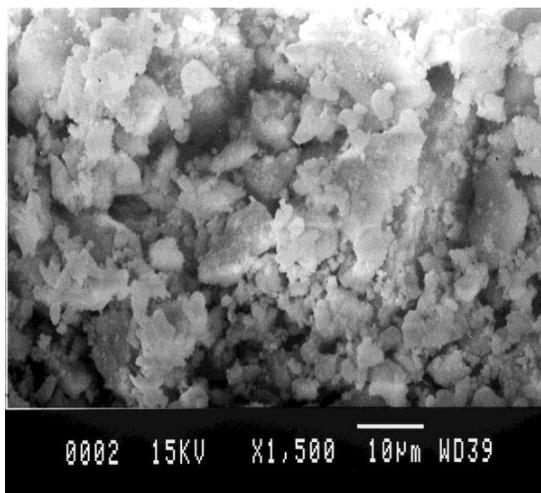
Fig. 1. (a) SEM images of non-bioleachedspent catalysts
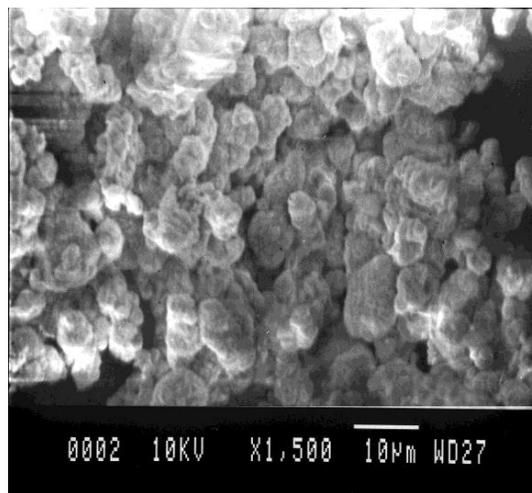
Fig. 1. (b)Bioleached spent catalystsin this study

quadratic models for the prediction of the response variables (Ni and Mo extraction) are given by equations 2 and 3.

$$\text{Ni Extraction} = +89.73 - 1.56{\times}A - 1.47{\times}B - 1.06{\times}C - 6.32{\times}D - 2.53{\times}E - 1.64{\times}B^2 - 1.96{\times}D^2 \qquad (2)$$

$$\text{Mo Extraction} = +60.78 - 7.30{\times}D - 2.23{\times}E + 2.66{\times}A{\times}B - 1.47{\times}A^2 - 1.32{\times}B^2 - 2.97{\times}D^2 \qquad (3)$$

In equations 2 and 3, "A" represents the initial pH, "B" is the temperature, "C" is the particle size, "D" represents the pulp density, and "E" refers to the condition under which the pH of the bioleaching liquors is either controlled or uncontrolled. "E" with value of -1, represents the condition under which pH is kept constant as its initial value over the bioleaching period (controlled pH), and "E" with value of +1, corresponds to the condition under which pH varies during the bioleaching (uncontrolled). For example, in Table 2, at raw 4, initial pH is 1.7 and pH is controlled referring to the condition that initial pH was 1.7, and it was kept 1.7 by adding sulfuric acid during the bioleaching. On the other hand, at raw 5, the initial pH is 1.7 and pH is uncontrolled corresponding to the condition that initial pH was 1.7, but it changed during the bioleaching. Equations 2 and 3 reveal that temperature, pulp density, particle size and initial pH are the important factors to recover Ni and Mo. No interaction between the parameters was observed for Ni and Mo extraction, in particular with respect to pulp density, except a positive interaction between initial pH and temperature for Mo extraction indicated by equation 3. This interaction means that the optimum level of temperature for maximizing Mo extraction in the bioleaching of the spent catalysts depends on the level of the initial pH and vice versa. Mafi Gholami et

al. (2012) used CCD method for the bioleaching of spent catalysts using *Aspergillus niger*. They reported a positive linear interaction between pH and temperature for Mo extraction, and no interaction between pulp density with other process parameters for Ni and Mo extraction. Negative quadratic and linear effects by pulp density were observed for Mo and Ni extraction (equations 2 and 3). The decrease in the extraction values of Mo and Ni with increasing pulp density could be due to several possible reasons. First reason could be the increase in the concentrations of dissolved toxic metals with increasing pulp density that negatively affects the microbial activity (Bayraktar, 2005, Aung and Ting, 2005). Second possible reason would be the increase in the level of the damage to bacterial cells at higher pulp density values as a result of the attrition of the bacterial membrane (Xia et al. 2008). However, the first and second reasons are not applicable for the current study since *A. ferrooxidans* employed in this investigation was already adapted to 0.5% pulp density. It is expected that under the studied pulp density range, the adapted *A. ferrooxidans* cells tolerate metal toxicity and shear stress provided by the catalysts particles (Noori Felegari *et al.,* 2012). The most possible reason for this observation could be the insufficient oxygen and carbon dioxide transfer for the growth of *A. ferrooxidans* with the increasing pulp density (Noori Felegari *et al.,* 2012; Mousavi *et al.,* 2007; Gomez *et al.,* 1999; Pradhan *et al.,* 2009; Haghshenas *et al.,* 2009b).

Equations 2 and 3 indicate the importance of the controlling pH in the bioleaching of spent catalysts using *A. ferrooxidans*. As shown by equations 2 and 3, the controlled pH bioleaching (E= -1) showed higher Mo and Ni extraction values compared to the

Table 2. Central Composite Design (CCD)

Standard Runs	Actual levels of variables					Responses	Recovery %Mo	Recovery %Ni
	Initial pH	T(⁰C)	Particle Size	Catalyst Concentration (g/l)	pH		%Mo	%Ni
1	1.8	34	34	110	0.3	Controlled	94.7	63.2
2	1.8	34	34	110	0.3	Controlled	95.2	63.7
3	1.7	32	32	150	0.4	Controlled	78.2	43.4
4	1.7	36	36	150	0.4	Controlled	76.3	42.7
5	1.7	32	32	70	0.4	Uncontrolled	81.3	45.7
6	2	36	36	70	0.4	Uncontrolled	73.5	41.8
7	1.8	34	34	110	0.3	Controlled	94.9	63.2
8	1.7	36	36	70	0.2	Uncontrolled	92.3	63.8
9	2	36	36	150	0.4	Uncontrolled	69.8	37.2
10	1.8	34	34	190	0.3	Uncontrolled	90.7	56.1
11	1.8	34	34	190	0.3	Controlled	92.33	61.2
12	1.7	32	32	150	0.2	Controlled	95.3	66.1
13	1.8	34	34	110	0.1	Controlled	95.4	63.9
14	1.8	34	34	110	0.3	Uncontrolled	85.1	57.2
15	1.7	32	32	150	0.2	Uncontrolled	91.2	59.8
16	1.7	32	32	70	0.2	Controlled	95.7	67.2
17	1.8	34	34	110	0.3	Controlled	94.6	63.2
18	1.7	36	36	150	0.2	Uncontrolled	89.3	57.3
19	1.8	34	34	110	0.3	Uncontrolled	84.3	56.8
20	1.7	32	32	70	0.2	Uncontrolled	93.1	64.9
21	1.7	36	36	70	0.2	Controlled	93.4	64.9
22	2	32	32	70	0.2	Uncontrolled	91.2	58.1
23	2	32	32	70	0.2	Controlled	93.1	59.8
24	2	32	32	70	0.4	Uncontrolled	75.4	42.1
25	1.8	34	34	110	0.3	Uncontrolled	84.7	57.1
26	2	36	36	70	0.4	Controlled	78.5	57.3
27	1.8	34	34	110	0.5	Controlled	76.3	42.1
28	1.8	34	34	110	0.3	Uncontrolled	84.6	57.3

Table 2. Central Composite Design (CCD)

29	2	32	150	0.2	Controlled	93.1	64.1
30	1.7	32	70	0.4	Controlled	83.2	46.4
31	2	32	150	0.4	Uncontrolled	72.1	38.7
32	1.5	34	110	0.3	Controlled	94.7	62.1
33	1.8	34	110	0.3	Uncontrolled	84.9	57.4
34	1.7	36	70	0.4	Controlled	81.3	41.7
35	2	36	70	0.2	Controlled	89.9	56.7
36	1.8	34	110	0.3	Controlled	94.6	63.4
37	1.8	34	30	0.3	Uncontrolled	92.4	62.3
38	2	32	150	0.4	Controlled	76.1	40.7
39	1.8	34	110	0.1	Uncontrolled	96.3	64.4
40	2	36	70	0.2	Uncontrolled	86.3	54.1
41	1.7	36	150	0.2	Controlled	92.4	62.1
42	1.8	38	110	0.3	Uncontrolled	81.2	51.3
43	1.7	36	150	0.4	Uncontrolled	71.2	38.3
44	1.8	34	30	0.3	Controlled	96.2	64.3
45	1.8	30	110	0.3	Uncontrolled	87.3	61.3
46	2.1	34	110	0.3	Controlled	93.1	60.7
47	1.7	36	70	0.4	Uncontrolled	76.3	38.1
48	2.1	34	110	0.3	Uncontrolled	85.2	54.1
49	2	36	150	0.2	Controlled	91.8	63.5
50	1.7	32	150	0.4	Uncontrolled	89.2	58.1
51	1.8	34	110	0.5	Uncontrolled	71.2	39.8
52	2	32	70	0.4	Controlled	79.3	39.9
53	1.8	34	110	0.3	Controlled	94.7	63.4
54	1.8	34	110	0.3	Uncontrolled	84.5	63.4
55	1.5	34	110	0.3	Uncontrolled	85.3	57.3
56	2	36	150	0.2	Uncontrolled	86.7	61.3
57	1.8	30	110	0.3	Controlled	92.4	65.1
58	2	36	150	0.4	Controlled	74.7	56.1
59	2	32	150	0.2	Uncontrolled	76.5	42.3
60	1.8	38	110	0.3	Controlled	83.4	58.9

uncontrolled pH bioleaching (E= +1). This observation shows the importance of controlling pH during bioleaching of spent catalysts in bioreactor cultivations. This conclusion is well supported by the fact that no interaction between pulp density and initial pH was observed in this study. Negative quadratic effect and linear effects by temperature was observed for Ni extraction as well as a negative quadratic effect by temperature for Mo extraction. Equation 2 shows that particle size had a negative linear impact on the Ni extraction. As the size of the catalyst particles decreases, their surface area increases, and, thus, the mass transfer rates of Ni from catalyst particles to the bioleaching lixivia increase.

Table 3 presents the results of the analysis of variance (ANOVA) for the developed models. The results indicate that the two fitted models are significant in 95% confidence level (p-value < 0.05). The low values of P determined for the regression as well as the fact that the lack of fit of the model was not significant (Table 3) revealed that the quadratic equation is capable of representing the system under the given experimental domain. As indicated in Table 3, the F-values of 8.96 and 7.55 for the two models, imply that the models are significant. In this case A, B, D, E, B^2, D^2 and D, E, AB, A^2, D^2 are the dominant terms. The "Lack of Fit, F-value" of 330.72 and 10.90 imply that the lack of fits are substantial. There is only 0.01% chance that the large "Lack of Fit, F-value" could occur because of the noise. The objective of the response surface optimization is to find a desirable location in the design space. This could be a maximum, minimum, or an area where the response is stable over a range of the factors. In this research, a simultaneous optimization technique was used (by DX7 software) for optimization of multiple responses (Aslan and Cebeci, 2007; Mehrabani *et al.,* 2010). Table 4 shows the maximum extraction values for Mo and Ni under the optimum conditions. The maximum values for both Mo and Ni extraction were predicted to be 67.4 and 96.95%, respectively, at approximately 34 ℃, -90.38μm sieve fraction (particle size), 0.22 % pulp density and initial pH of 1.8 (case 1). The decrease in the extraction of Mo and Ni at the higher temperature than 34℃ occurs as a result of the bacterial inactivity. The reduction in the extraction of Mo and Ni at pH values beyond 1.8 could be due to the formation of jarosite (Nemati *et al.,* 1998). Case 2 represents the condition under which Ni extraction was maximized separate from Mo, and case 3 shows the condition under which Mo extraction was maximized separate from Ni. The one-factor-at- a-time method that was implemented in our recent study to experimentally optimize the bioleaching parameters (Noori Felegari *et al.,* 2012) may not be able to find the optimum conditions if interactions between parameters exist. Whereas, CCD model used for our current study is able to find the interactions between parameters, and, consequently, find the global optimum. Figs 2a and 2b illustrate the effects of pulp density and initial pH on Ni extraction, and the impact of pulp density and temperature on Mo extraction, respectively. Fig. 2a shows that for the entire range of initial pH, Ni extraction decreases as pulp density increases. Fig. 2b indicates that at all pulp density values, the maximum Mo extraction is achieved at almost 34℃.

To validate the predicted optimum conditions with the experimental data, the final step of the experiments was performed at 34℃, -90 μm sieve fraction, 0.22% pulp density and initial pH of 1.8 and under the controlled pH condition. The results showed that 62.72 and 92.34% of Mo and Ni were recovered after 7 days that are in a good agreement with the predicted values (Table 4). Hence, our presented model is successful in predicting the responses. The maximum Mo and Ni extraction values at 34 ℃, -90 μm sieve fraction, 0.22% pulp density and initial pH of 1.8 and under the controlled pH condition by chemical leaching of the spent catalysts (control experiments) in the current study were found to be 25 and 75%, respectively, after 7 days. The optimum pH and temperature determined in the current study are close to the optimum pH and temperature for the growth of *A. ferrooxidans* (Mousavi *et al.,* 2006). This similarity in the optimum values suggests that the bioleaching process of the spent catalysts is more driven by the bacterial leaching mechanism (Fe^{+3} attack) compared to the chemical leaching mechanism (proton attack). The identification of interaction between pH and temperature for Mo extraction from spent catalysts in the current study strongly recommends future studies to implement design of experiments for optimization of the parameters of spent catalysts bioleaching for maximizing Mo extraction. On the other hand, since no interactions between the bioleaching parameters were found for Ni extraction, one-factor-at-a-time methodology can be used for bioleaching optimization to maximize Ni extraction. No interactions between pulp density and other parameters were observed in the current study implying that shaking bioreactors can be used to optimize the key parameters for bioleaching of spent refinery catalysts using *A. ferrooxidans*. Increasing the pulp density in a bioleaching process potentially decreases the gas transfer rates which affect bacterial activity and consequently the metal extraction (Haghshenas *et al.,* 2012). The pulp density employed in this study is much lower than its values for the industrial bioreactors. However, it is still useful to

Table 3. The results of the analysis of variance (ANOVA)

Source	Ni					Mo				
	Sum of squares	df	Mean squares	F Value	P-Value Prob>F	Sum of squares	df	Mean squares	F Value	P-Value Prob>F
Model	2986.29	20	149.31	8.96	<0.0001	3970.11	19	208.95	7.55	<0.0001
A-initial pH	117.5	1	117.5	7.05	0.0114	65.1	1	65.1	2.35	0.1330
B-T	103.55	1	103.55	6.22	0.017	22.55	1	22.55	0.81	0.3722
C-Size	54.4	1	54.4	3.27	0.0785	17.89	1	17.89	0.65	0.4263
D-pulp density	1916.48	1	1916.48	115.05	<0.0001	2559.38	1	2559.38	92.45	<0.0001
E-pH control	383.55	1	383.55	23.02	<0.0001	297.04	1	297.04	10.73	0.0022
AB	-	-	-	-		226.31	1	226.31	8.17	0.0067
A2	-	-	-	-		118.76	1	118.76	4.29	0.0448
B2	147.38	1	147.38	0.015	0.005	95.78	1	95.78	3.46	0.0702
D2	210.27	1	210.27	12.62	0.001	484.33	1	484.33	17.49	0.0002
Lack of fit	648.98	29	22.38	330.7	<0.0001	1074.5	30	35.82	10.90	0.0002

Table 4. Optimum process conditions and their results

Case	Response	Target	Initial(pH)	T(°C)	Size (µm)	Pulp density (%)	E (pH)	%Ni	%Mo
1	Ni Mo	Maximize Maximize	1.8	33.83	90.38	0.22	-1	96.95	67.4
2	Ni Mo	Maximize None	1.8	34.89	78.64	0.20	-1	96.81	-
3	Ni Mo	None Maximize	1.8	33.50	113.08	0.21	-1	-	67.27
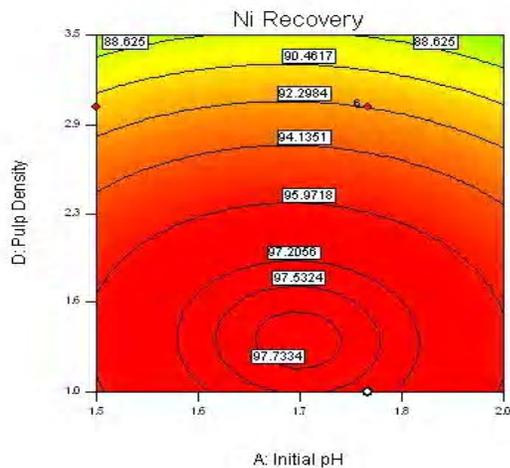
Fig. 2. (a) Effect of temperature and pulp densityon Ni recoveryat-110 µm sievefractionand pH 1.8
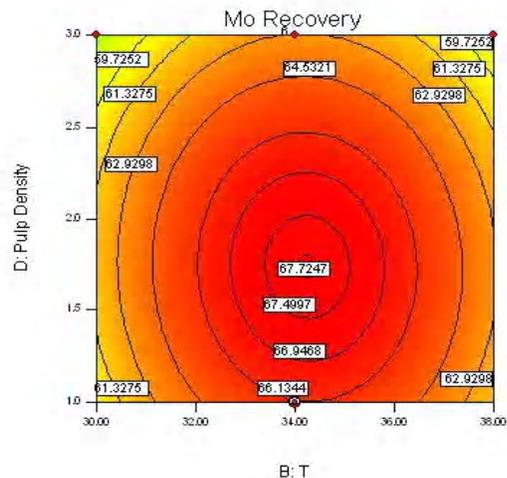
Fig. 2. (b) Effect of temperature and pulp densityon, MO recoveryat-110 µm sievefraction and pH 1.8

interpret the pulp density results obtained in a shaking bioreactor even if it has interactions with other process parameters. The reason behind this fact is that high pulp density in the shaking bioreactors simulates the low gas transfer rates condition for bacterial growth in the stirred bioreactors and large scale bioleaching, and low pulp density simulates the high gas transfer rates condition (Haghshenas *et al.,* 2012). The 0.1 to 0.5% pulp density utilized in this study simulates the high and sufficient gas transfer rates condition during the bioleaching of spent catalysts in industrial bioreactors. The presented optimum conditions and the obtained maximum Mo and Ni extraction values in the current study, are very close to the corresponding values observed experimentally in our recent study (Noori Felegari, et. al. 2012) through one-factor-at-a-time methodology implying that the impact of the interaction between studied parameters are not strong as shown by equations 2 and 3.

The surface area, mean pore diameter and the total pore volume of the nanoparticles were determined to be 44.36 m^2/g, 1.99 nm and 0.071 cm^3/g, respectively. The TEM image of the synthesized iron magnetic nanoparticles shows that the particles had spherical shape with the consistent size distribution (Fig. 3). The size of the nanoparticles was determined to be between 10 to 40 nm with the mode diameter of approximately 25 nm. Fig. 4 indicates the effect of pH on Ni adsorption from the synthesized Ni solution after 1 hour, at 25°C, 250 rpm and in the presence of 5g/L adsorbent. Fig. 4 reveals that the extent of Ni adsorption increases with increasing pH showing maximum Ni adsorption at pH 8. At pH 8, in average 97% of the Ni was separated from the synthesized Ni solution indicating the key effect of pH in this process. At high pH values, the hydroxyl groups available at the surface of the nanoparticles become negatively charged, and, hence, the extent of the cation adsorption such as Ni increases. Oliviera, (2007) reported that Ni precipitates as $Ni(OH)_2$ at pH values greater than 8 implying that no Ni precipitation occurred in this study up to pH 8. Fig. 5 presents the influence of temperature on Ni adsorption from the synthesized Ni solution after 1 hour, at pH 8, 250 rpm and in the presence of 2g/L adsorbent. Fig. 5 shows that temperature has positive effect on the adsorption of Ni. As temperature increased the Ni adsorption increased such that at 70°C, in average 88% of Ni was separated from the solution. Fig. 4 reveals that at 25°C and pH 8, 97% of Ni in the solution was separated, while Fig. 5 shows that at the same temperature and pH, approximately 72% of Ni was adsorbed. This discrepancy in Ni adsorption was due to the higher amount of adsorbent utilized for the pH study compared to the temperature study implying the positive influence of the adsorbent concentration on

Ni adsorption. Experiments were conducted to examine the separation of Ni from a real bioleaching liquor sample prepared from our bioleaching experiments in the presence and absence of magnetic nanoparticles. Before performing the adsorption study using the synthesized nanoparticles, the biomass and spent catalysts residue in the bioleaching liquor were removed by filtration. The bioleaching liquor contained 30 ppm Ni and 5g/l of the nanoparticles was added to the liquor. The average percentage of Ni adsorption was found to be 98% at 34°C and pH 8. Ni adsorption without addition of nanoparticles at pH 8 resulted in no changes in the Ni concentration after 1 hour compared to its initial concentration (data not shown). This observation ensures us that the reduction in Ni concentration during our Ni adsorption experiments is due to the adsorption by magnetic nanoparticles and not precipitations of Ni ions. The influence of the impeller speed was studied on the Ni adsorption, and the results showed no significant impact (data not shown). Moreover, adsorption of Mo was found to be inefficient using magnetic nanoparticles (data not shown) due to the paramagnetic characteristics of molybdenum.Bosio et al. (2008) proposed a two-step process to separate Ni from the bioleaching liquors of spent catalysts. They employed Desulfovibrio sp. cultures to produce sulfides at the first step, and through the second step, NiS was precipitated. They reported the highest percentage of Ni adsorption to be 79% at room temperature and under optimum condition. The Ni adsorption considerably decreased to 29% when the initial Ni concentration was almost doubled suggesting that one of the limitations of this method would be lower efficiency at higher initial Ni concentrations. In addition, the possibility of sulfide volatilisation through production of H_2S can be added to the limitations of the process proposed by Bosio et al. (2008). The adsorption of Ni using magnetic nanoparticles presented in the current study resulted in the removal of 97% of Ni at room temperature and pH 8. In addition, increasing the initial Ni concentration from 4 to 30 ppm, showed no effect on the adsorption of Ni such that at both cases, 97 to 98% of Ni was adsorbed at pH 8.

To make an environmentally clean solution, the magnetic nanoparticles and the adsorbed Ni should be removed from the adsorption solutions. To accomplish this goal, the solution was passed continuously through a glassy tube (magnetic tube) surrounded by a magnetic field. The magnetic nanoparticles were attached to the tube's wall, and completely separated from the solution. To recover nanoparticles and separate them from Ni, they were suspended in a solution at pH 2. Fig. 6 shows the schematic diagram of the proposed integrated process

Fig.3. TheTEM imagetaken from iron magnetic nanoparticles synthesized in this study
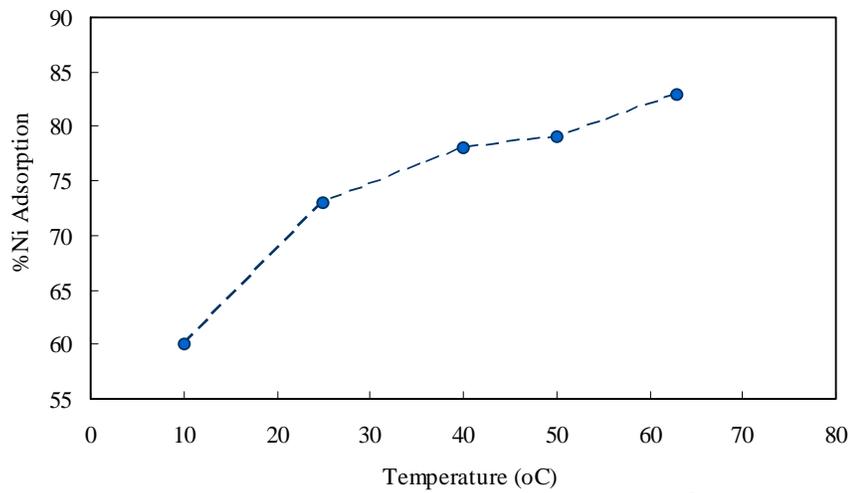
Fig. 4. Effect of pH onNi adsorptionusingironmagneticnanoparticles (5g/L)at 25°C and 250 rpm (after 1 hour), variability<5%
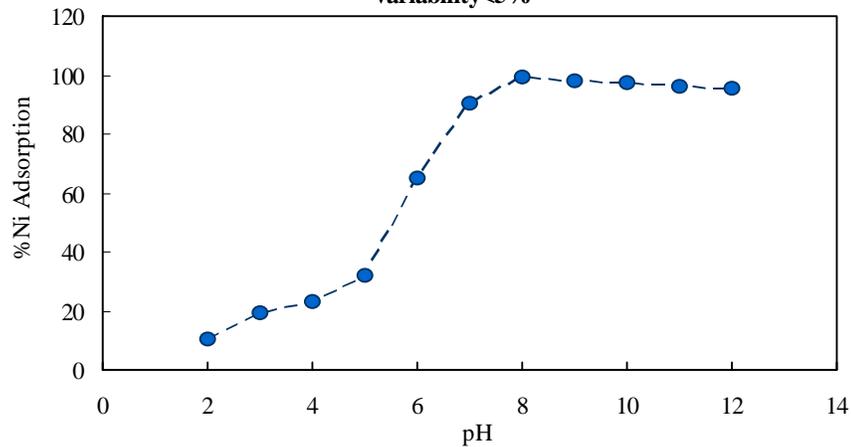
Fig. 5. Effect of temperatureon Niadsorption byiron magneticnanoparticles(5g/L) at 25°C and 250 rpm (after1 hour), variability<7%
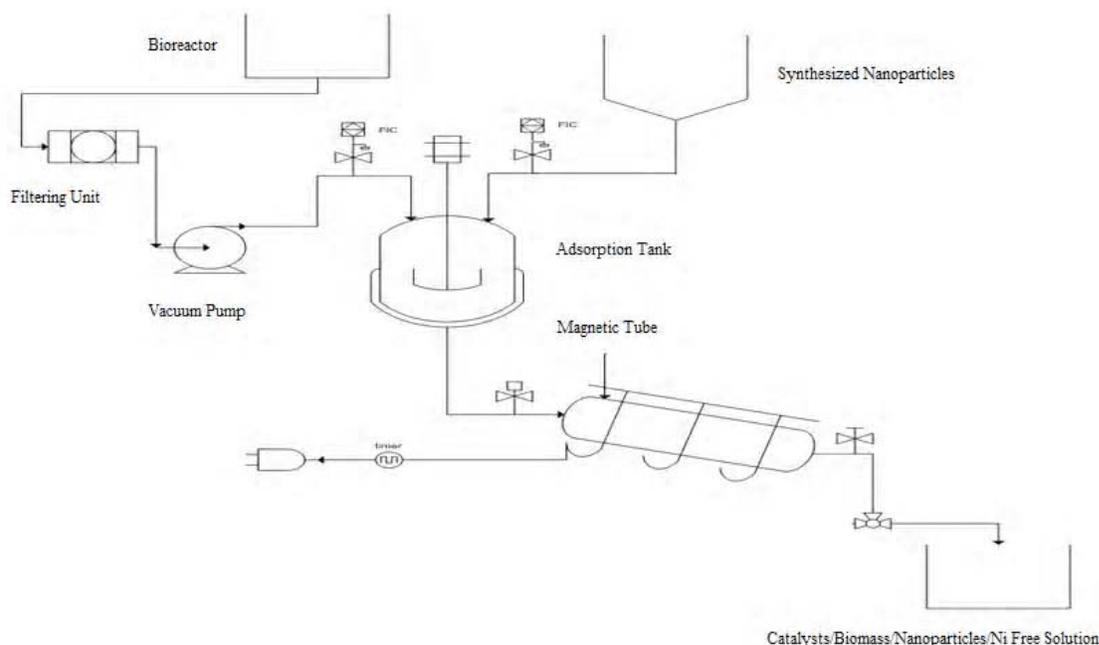
Fig. 6. Theschematicdiagram of the proposedintegrated process in this study

in this study. The bioleaching liquor passes through a filtering unit by a vacuum pump to remove spent catalysts and biomass. Then, the catalysts/biomass free solution is introduced to the adsorption tank. Simultaneously, the synthesized nanoparticles are entered to the tank to perform the adsorption process at the controlled pH and temperature. Afterward, the adsorption solution passes through a magnetic tube to remove nanoparticles and adsorbed Ni from the adsorption solution. The solution is entered a filtering unit to remove any metal precipitates that might be formed in the presence of ferrous ions at the pH of the adsorption tank (Bartzas *et al.,* 2006). At the end, a catalysts/biomass/nanoparticles/Ni free solution is collected in a storage tank. The proposed integrated process in this study needs further investigations for the process design, optimization and scale up purposes. For instance, a proper separation unit such as filtration or centrifuging for biomass and spent catalysts removal should be selected and optimized. In particular, adsorption of multiple metals and their chemistry at different pH values should be studied. It is also interesting to add activated carbon to the adsorption tank to adsorb Mo (Beolchini *et al.,* 2010). A feasibility study is required to economically evaluate the proposed process in the current study under optimum condition, and compare with other proposed processes (Chauhan *et al.,* 2013; Haghshenas *et al.,* 2012; Mafi Gholami *et al.,* 2012; Montgomery, 2006; Simate *et al.,* 2009).

CONCLUSION

An integrated processes involving bioleaching of Ni and Mo from refinery spent catalysts using pure culture of *A. ferrooxidans*, and subsequent separation of Ni from the bioleaching liquor using iron magnetic nanoparticles was examined and optimized in this study. The present study provides new information for toxic metal removal after bioleaching of spent catalysts. The current study presented quadratic models for the extraction of Ni and No from spent refinery catalysts that were successful in predicting the responses. This study observed no interactions between pulp density and other bioleaching parameters indicating the suitability of shaking bioreactors to optimize pulp density at low values. High percentages of Ni adsorption (over 97%) observed in the present investigation using magnetic nanoparticles indicating that this technique could be an efficient alternative for the bioprecipitation methods examined in the previous studies.

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