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BIOLEACHING OF TRACE METALS FROM COAL ASH USING MIXED CULTURE OF *ACIDITHIOBACILLUS ALBERTENSIS* AND *ACIDITHIOBACILLUS THIOOXIDANS*

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Abstract

The bioleaching of zinc, manganese, chromium, iron and copper from coal ash (fly and bottom) from coal ash pond using mixed culture of *Acidithiobacillus albertensis* and *Acidithiobacillus thiooxidans* was studied. Using the Box-Behnken experimental design, there are three variable parameters used namely: initial bacterial inoculum, % pulp density and weight of sulfur added. After fifteen days of bioleaching, the maximum percentage metals leached were 56.69% Zn, 70.68% Mn, 79.86% Cr, 70.74% Fe and 69.32% Cu at different variable parameters. Also, the solution has an initial pH value of 2 was decrease from a range of 1.39 to 0.61 due to the oxidation of sulfur via microbial activity. Based on the statistical analysis, the experimental data fit the quadratic model for all metal leached. Moreover, using numerical optimization the significant parameters are pulp density and initial mass of sulfur. Using the optimal parameters, the maximum percent metal leached were 56.38% Zn, 70.88% Mn, 85.01% Cr, 74.44% Fe and 74.46% Cu.

Keywords: Bioleaching, *Acidithiobacillus albertensis*, *Acidithiobacillus thiooxidans*, Coal ash, Trace metals.

1. Introduction

Coal ash is generated by the combustion of coal for energy production by power plants. Coal ash produces fly ash and bottom ash upon combustion and may contain significant amount of trace metals. In the global perspective, coal-based power plant produces approximately 750 million tons of coal ash per year and only 50% of coal ashes are being utilized and recycled [1, 2]. However, coal ash can be used as aggregate for construction materials, blended cement, mining application, road construction fills and embankments in roads [3]. On the other hand, most of the coal ash still remains in lagoon or ash ponds for disposal.

Due to the increasing amount of coal ash being underutilized and its substantial trace metals amount, it can be used as a secondary resource for metal extraction for further industrial application. In the recent years, new and innovative green technology is emerging called bioleaching or biological leaching [4]. Bioleaching is an eco-friendly process of using microorganisms (bacteria or fungi) to extract soluble and extractable elements (metals) via the production of acids (inorganic and organic) from secondary sources or materials that can be possibly recovered and used [5, 6].

This research used two different types of sulfur-oxidizing bacteria namely: *Acidithiobacillus albertensis* and *Acidithiobacillus thiooxidans*. The optimal growth condition of *A. albertensis* is at 25-30°C at pH of 3.5-4.0 while *A. thiooxidans* is at 28-30°C at pH 2.0-3.0. These microorganisms are chemolithoautotrophs. Also, mixed culture is used because *A. albertensis* has the capability to grow at higher pH in contrast with *A. thiooxidans* [7]. Moreover, these two microorganisms uses inorganic source for growth and metabolism such as sulfur and carbon dioxide. The metal solubilization is done by the in-situ production of inorganic acid which is sulfuric acid [8]. There are many studies that were conducted using *A. thiooxidans* in bioleaching of different secondary materials in pure and mixed culture with *A. ferrooxidans*, however for *A. albertensis* there is only one conducted by Xia et al. [9].

The main focus of this study is to determine the percent metal leached from coal ash collected in an ash pond using a mixed culture of *A. albertensis* and *A. thiooxidans*. The metals under investigations are iron, copper and zinc which are base metals and manganese and chromium which are strategic metals. Moreover, Box-Behnken design of experiment was used to determine the optimum condition for the extraction of metals and its interaction with variable parameters. The variable parameters are amount of initial inoculum, pulp density and amount of sulfur against the response factor which is the percent metal leached.

2. Materials and Methods

2.1. Microorganism

The microorganisms used in this study were *A. albertensis* (ATCC 35403) and *A. thiooxidans* (ATCC 19377). These microorganisms were provided by Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures in Germany.

2.2. Coal ash from coal ash pond

The coal ash (mixture of fly and bottom) was provided by a private coal fired power plant in the Philippines that uses imported and local coals as a raw material on a Circulating Fluidized Bed (CFB) Boiler. The coal ash was collected in the company's coal ash pond. The as-received coal ash was oven dried at 105°C for 24 hours. The dried sample was sieved using a particle size passing through #200 mesh or 0.075 microns. The particle size was constant through the experiment. Moreover, the ash is alkali in nature with pH of 10-11.5.

2.3. Growth of the microorganism

A. albertensis and *A. thiooxidans* were cultured in a media consisting of macronutrients and mineral salts. The modified culture media contains the following ingredients: 0.2 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.25 g $CaCl_2$, 3 g KH_2PO_4 and 10 g sulfur dissolved in 1 liter of deionized water [10] and autoclaved at 121°C for 15 minutes. The inoculum was incubated in a rotary shaker (IKA KS 4000i control) at 160 rpm and 30°C. The bacteria were incubated for 10 days prior to the bioleaching experiment. Using Perkin Elmer Lambda 10 UV/Vis Spectrometer, the microbial density has an absorbance of 0.233 per ml of inoculum.

2.4. Bioleaching experiment

The bioleaching experiments were performed in a 250 ml Erlenmeyer flask with constant parameters of 100 ml medium at 160 rpm and 30°C in an orbital shaker. The variable parameters are initial inoculum (1 ml, 3 ml and 5 ml), pulp density (1, 3 and 5 grams of ash per 100 ml medium) and weight of sulfur added (1, 2 and 3 grams per 100 ml medium). Using Design Expert Software, Tables 1 and 2 show the Box-Behnken experimental design for the three variable parameters and their coded values. The response factor is the percent metal leaching for zinc, manganese, chromium, iron and copper.

Table 1. Box-Behnken Design of Experiment.

Run	A	B	C
1	0	0	0
2	1	0	-1
3	-1	0	-1
4	0	0	0
5	-1	-1	0
6	-1	1	0
7	0	-1	1
8	0	0	0
9	1	-1	0
10	0	0	0
11	1	0	1
12	-1	0	1
13	1	1	0
14	0	-1	-1
15	0	1	-1
16	0	0	0
17	0	1	1

Table 2. Coded values at different parameters.

Variable	-1	0	1
A: Initial inoculum (ml)	1	3	5
B: Pulp Density (%w/v)	1	3	5
C: Weight of Sulfur (g)	1	2	3

The medium with the ash (at different pulp density) were autoclaved at 121°C for 15 minutes and cooled. Upon the addition of ash and medium, the pH of the solution is 6.5-8. Then pH of the solution was adjusted to 2 using 1M H_2SO_4 to favor the growth of the bacteria. Then, the appropriate amount of sulfur (different weight) was added in the flask. Finally, the initial inoculum (different volume) was added into the solution. Leached solution was filtered using a 0.45 micrometer cellulous acetate filter. Few drops of concentrated nitric acid were used to acidify the solution and stored at 4°C prior to metal analysis using ICP-OES. Bioleaching experiments were done for fifteen days and in duplicate runs.

2.5. Analytical methods

X-ray diffraction (XRD) analysis was performed on a Bruker D8 Advance diffractometer equipped with energy dispersion Sol-X detector with copper radiation (CuKα, $\lambda = 0.15406$ nm). The acquisition was recorded between 2° and 80°, with a 0.02° scan step and 1 s step time. Samples were previously dried at 25°C and crushed prior to XRD analysis.

Moreover, the metals were analyzed using Perkin Elmer Optima 8300 Induced Couple Plasma – Optical Emission Spectrometer (ICP-OES). Using different wavelengths of 324 nm for Cu, 238 nm for Fe, 213 nm for Zn, 257 nm for Mn and 267 nm for Cr to measure the concentration of metals in the solution. Prior to analysis, few drops of concentrated nitric acid were added. The concentrations of metals were reported as mg/kg.

3. Results and Discussion

3.1. Mineralogical data – XRD

Figure 1 shows the XRD analysis of the coal ash sample. The minerals identified in the coal ash samples are quartz-SiO_2, melilite, anhydrite, tricalcium aluminate, lime-CaO, periclase, mullite and rutile. The major elements are calcium, aluminum, iron and silicon. On the other hand, the trace elements are chromium, manganese, zinc, copper and titanium. These minerals were also identified by several other authors Demir et al. [11]; Skordas et al. [12]; Ahmaruzzaman [13]; Hareeparsad et al. [14]; Akar et al. [15]; Neupane and Donahoe [16].

The total metal content in the ash are 0.96 mg/kg Zn, 4.13 mg/kg Mn, 0.81 mg/kg Cr, 269.5 mg/kg Fe and 2.22 mg/kg Cu.

3.2. pH profile

Figure 2 shows the pH profile for the 17 experimental runs. From the initial pH value of 2, the pH values of all the samples were decreased from a range of 0.61 to 1.39. The decrease of pH value is an indirect indication of the growth of the bacteria and its production of sulfuric acid via the oxidation of sulfur. These results coincide with the studies conducted by Ishigaki et al. [17], Moura et al. [18] and Pradhan et al. [19].

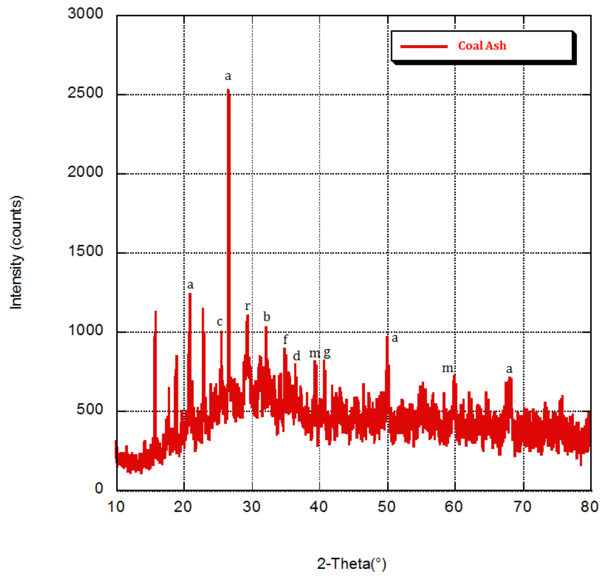

Fig. 1. XRD analysis for coal ash (a: Quartz-SiO_2, b: Melilite, c: Anhydrite, d: Tricalcium aluminate, f: Lime-CaO, g: Periclase, m: Mullite, r: Rutile).

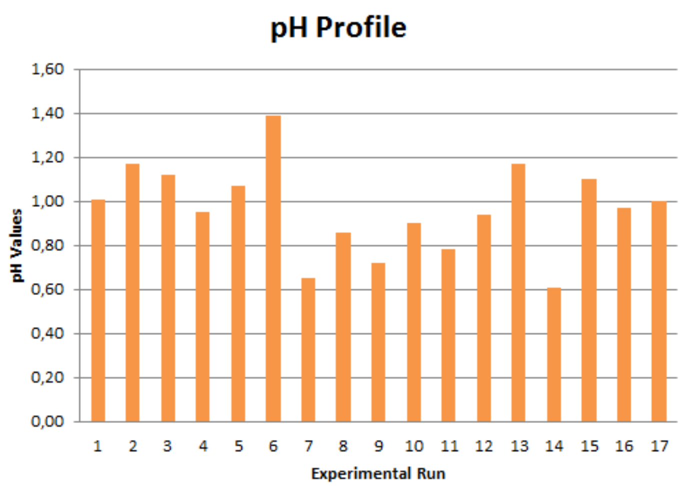

Fig. 2. pH values versus the experimental runs.

3.3. Box-Behnken design: bioleaching experiment

An optimization design was conducted using a Box-Behnken Design which is one of response surface methodology. The Box-Behnken design is suitable for exploring quadratic response surfaces and generating second degree polynomial model or equation, thus obtaining optimum parameters using small experimental runs [20, 21]. Table 3 shows the response factor (% metal leached) for zinc, manganese, chromium, iron and copper in each trial.

After the bioleaching experiments, the highest percent metal leached were as follows: 59.69% zinc, 70.68% manganese, 79.86% chromium, 70.74% iron and 69.32% copper. The highest leaching for zinc and copper occurred at 5 ml initial inoculum, 5% pulp density and 2 g sulfur. For chromium and iron, it occurred at 3 ml initial inoculum, 5% pulp density and 3 g sulfur. And for manganese it occurred at 3 ml initial inoculum, 5% pulp density and 1 g sulfur.

In previous studies, the rate of metal leaching or solubilization decreases as the solid concentration or pulp density increases. This is because to the high pH conditions thus, having high buffering capacity of the material. Also, inhibition of bacterial activity is due to the amount of toxic metal present in the material [22-26]. However, this problem has been addressed by adjusting the initial pH value of the solution to favor the microbial growth. Thus, the highest leaching of metal occurred at 5% pulp density as seen in Table 3.

Table 3. Coded values for Box-Behnken design.

Run	A	B	C	% Zn	% Mn	% Cr	% Fe	% Cu
1	0	0	0	33.30	49.24	56.36	50.17	42.16
2	1	0	-1	34.90	48.97	55.56	43.45	39.72
3	-1	0	-1	37.61	51.36	58.71	49.93	48.63
4	0	0	0	36.09	50.57	58.61	53.52	44.60
5	-1	-1	0	10.90	18.65	22.73	19.21	16.41
6	-1	1	0	53.94	68.87	71.81	50.99	56.38
7	0	-1	1	11.38	20.71	30.14	30.52	16.85
8	0	0	0	36.19	49.35	58.29	52.45	47.58
9	1	-1	0	12.05	20.93	30.20	28.00	18.43
10	0	0	0	38.58	54.44	63.25	56.13	47.43
11	1	0	1	36.62	66.81	41.92	57.40	44.00
12	-1	0	1	48.54	55.72	69.32	35.05	51.74
13	1	1	0	**56.69**	69.47	77.90	65.36	**69.32**
14	0	-1	-1	13.46	24.48	36.19	36.18	18.62
15	0	1	-1	56.44	**70.68**	79.07	65.13	67.24
16	0	0	0	37.41	51.76	60.78	54.01	49.83
17	0	1	1	50.71	68.91	**79.86**	**70.74**	59.15

Using Design Expert 6.0.8, the statistical results of the ANOVA for the response surface for quadratic models of zinc, manganese, chromium, iron and copper are presented in Table 4.

Table 4. Statistical results of the ANOVA.

Statistical results	Zn	Mn	Cr	Fe	Cu
Model F-value	100.71	146.68	65.94	18.23	14.15
Model prob > F	<0.0001	<0.0001	<0.0001	0.0005	0.001
Lack of fit F-value	1.11	0.52	1.39	7.37	7.38
Lack of fit prob > F	0.4442	0.6933	0.3677	0.0417	0.0416
R squared	0.9923	0.9947	0.9883	0.9591	0.9479
Adj R squared	0.9825	0.9879	0.9734	0.9065	0.8809
Adequate Precision	28.941	34.894	26.604	15.539	12.266

According to the statistical data presented in Table 5 for quadratic models, the Model F-values 18.23, 100.71, 146.68, 65.94 and 14.15 implied that the models were significant and there was 0.01% for zinc, manganese and chromium, 0.05% for iron, and 0.10% for copper chance that a 'Model F-value' could occur due to noise. The prob>F values for the models which were <0.0001 indicated that the models were statistically significant with a confidence interval of 99.99% for zinc manganese and chromium. On the other hand, 99.95% and 99.90% confidence interval were obtained for iron and copper, respectively. The 'lack of fit' tests compare the residual error to pure error from the replicated experimental points in the design. The p-values greater than 0.05 mean that the model lack of fit is insignificant.

Based on Table 4, the values R^2 are evaluated as 0.9923, 0.9947, 0.9883, 0.9591 and 0.9475 while the adjusted R^2 values are 0.9825, 0.9879, 0.9734, 0.9065 and 0.8809 for the models for zinc, manganese, chromium, iron and copper, respectively. The R^2 and adjusted R^2 values in this study ensured a satisfactory adjustment of the quadratic model to the experimental data. Moreover, adequate precision is the measurement of signal to noise ratio and values greater than 4 is desirable [27].

3.4. Percent metal leached model

Using Design Expert 6.0.8 software, the following quadratic equation for percent zinc, manganese, chromium, iron and copper leached were obtained (Eqs. (1-5)):

$$\%\ \text{Zn leached} = -0.44 - 1.29A + 15.71B + 0.015C + 0.016A^2 - 0.75B^2 - 0.33C^2 + 0.10AB + 0.54BC - 0.46AC \quad (1)$$

$$\%\ \text{Mn leached} = 7.43 + 0.96A + 19.88B - 5.47C - 0.32A^2 - 1.33B^2 + 0.45C^2 - 0.11AB + 0.75AC + 0.25BC \quad (2)$$

$$\%\ \text{Cr leached} = 25.20 + 2.48A + 19.15B - 18.32C - 0.74A^2 - 1.46B^2 + 2.70C^2 - 0.086\,AB + 1.78AC + 0.86BC \quad (3)$$

$$\%\ \text{Fe leached} = 25.52 + 6.97A + 13.86B - 20.32C - 1.58A^2 - 1.51B^2 + 3.44C^2 + 0.35AB + 1.39AC + 1.41BC \quad (4)$$

$$\%\ \text{Cu leached} = 14.08 + 1.95A + 14.60B - 11.89C - 1.04A^2 - 1.02B^2 + 1.64C^2 + 0.68AB + 1.37AC + 0.81BC \quad (5)$$

The significance of the each coefficient was determined by the p-values through the statistical analysis. The smaller the p-value shows a higher significance of the coefficient. For the percent zinc and manganese leached, the significant coefficients are B (% pulp density) and B^2. On the other hand, for percent chromium leached, the significant coefficients are B, C (amount of sulfur), B^2 and interaction of AC (initial inoculum x amount of sulfur). Also, for percent iron leached, the significant coefficients are B, A^2 (initial inoculum^2) and B^2. However, for percent copper leached, there are no significant factors among the parameters. The coefficients with p-values less than 0.05 are considered significant.

3.5. Optimization of parameters

According to the numerical optimization of Design Expert 6.0.8, the process optimum conditions are shown in Table 5.

For all metals, the common optimum pulp density is 5%. Higher amount of ash in the solution can be attributed to a possible increase in metal extraction. Also, increasing the amount of sulfur in the solution would increase the metal extraction due to the production of sulfuric acid via the oxidation of sulfur done by the microorganism. However, for the initial inoculum it varied from 1.88 to 4.97 ml. Based on the statistical analysis, initial inoculum (A) is not a significant factor in the bioleaching experiment. Using the numerical optimization, the maximum percent metal leached are as follows: 56.88% Zn, 70.88% Mn, 85.01% Cr, 74.44% Fe and 74.46% Cu after fifteen days of bioleaching.

Table 5. Optimum process condition for different parameters.

	Initial Inoculum (ml)	% Pulp Density	Weight Sulfur (g)	% Metal leached
Zinc	2.20	5	1	56.88
Manganese	1.88	5	1	70.88
Chromium	4.97	5	3	85.01
Iron	4.08	5	3	74.44
Copper	4.56	5	3	74.46

Based on the study of Ishigaki et al. [17], bioleaching of municipal solid waste incineration (MSWI) fly ash using mixed culture of sulfur-oxidizing and iron-oxidizing bacteria exhibit a metal extraction of 67% Cu, 78% Zn and 100% Cr and Cd. Aslo, Siedel et al. [26] investigated on the mechanism of bioleaching of coal fly ash using *Thiobacillus thiooxidans*. Results show the maximum leachability of aluminum and iron were approximately 100% and 80% respective. Moreover, Krebs et al. [25] studied bioleaching of MWSI fly ash using pure and mixed culture of *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*. The data show extraction of over 80% Cd, Cu and Zn, 60% Al and 30% Fe and Ni. Based on the previous studies, it is comparable with the numerical optimization.

4. Conclusion

Based on the bioleaching experiment using mixed culture of *A. albertensis* and *A. thiooxidans*, the percent metal leached are 56.69%, 70.68%, 79.86%, 70.74% and 69.32% for zinc, manganese, chromium, iron and copper respectively. Also, form the initial pH of 2, the pH value of the solution decreases from a range of 1.39 to 0.6. Based on statistical analysis, the experimental data fit the quadratic model based on Box-Behnken design of experiment. Moreover, the optimum parameters for bioleaching are 5% pulp density and 3 grams sulfur. Also, initial inoculum is not a significant factor based on statistical analysis. At optimum parameters, the metal leached are 56.88% Zn, 70.88% Mn, 85.01% Cr, 74.44% Fe and 74.46% Cu.

For future works, the researchers will focus on bioleaching of pure culture of *A. albertensis*. Furthermore, the effects of higher pulp density and weight of sulfur in the bioleaching of coal ash. And, bioleaching kinetics at optimum conditions will be investigated.

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