

**BIOLEACHING FOR THE RECOVERY OF METALS
PEMISAHAN UNSUR LOGAM DENGAN BIOLEACHING****Rita Susilawati**

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ABSTRACT

A simple laboratory experiment has been conducted in order to demonstrate the ability of bacteria to leach the metal elements from insoluble ores or solid substrates. The experiment was conducted in a leaching column that was set up using 5 cc syringe that contained 1 cc of glass wool and 3.5cc of mine tailings. The basal salt was used as an inoculation media while mine tailing was used as a leaching subject and a source of bacterial leaching. Bacterial leaching was isolated using the most probable number (MPN) technique. Overall, the results of the experiment showed the capability of bioleaching process to recover metals from the mine tailings.

Keywords: bacteria, bioleaching, metals

SARI

Kemampuan bakteri untuk memisahkan unsur-unsur logam (bioleaching) dari tailing diperlihatkan melalui eksperimen sederhana yang dilakukan di laboratorium. Eksperimen dilakukan dengan menggunakan kolom yang dibuat dari syringe berukuran 5cc yang telah diisi oleh 1cc benang kaca dan 3.5cc tailing. Tailing digunakan sebagai objek pemisahan dan juga sumber dari bakteri peluruh logam, sementara garam basal digunakan sebagai media untuk merangsang pertumbuhan bakteri tersebut. Isolasi bakteri pemisah logam yang terkandung dalam tailing dilakukan dengan menggunakan teknik MPN (Most Probable Number). Secara umum, eksperimen yang dilakukan berhasil menunjukkan kemampuan bakteri dalam memisahkan logam-logam yang terkandung dalam tailing.

Kata kunci: bakteri, bioleaching, logam

INTRODUCTION

In general, bioleaching is a process described as being the use of microorganisms to transform elements (metal) from insoluble ores or solid substrates so that the elements can be extracted from a material when water is filtered through it. In this process, the mobilization of metal ion occurred either directly by the metabolism of leaching bacteria or indirectly by ferric iron (Fe^{3+}) that is regenerated from ferrous iron (Fe^{2+}) by bacterial oxidation (Escobar *et al.*, 2010). Bioleaching is mainly applied in the recovery of low grade sulfide ores and concentrates, that cannot be processed economically by conventional methods (Suzuki, 2001). Bioleaching is also used as a natural bioremediation control to heavy metal pollution in sulfuric mine waste (Southam & Beveridge, 1992). A variety of acidophilic and chemoautolithotrophic bacteria such as *Thiobacillus thiooxidans* (sulfur) and *Thiobacillus ferrooxidans* (both iron and sulfur) as well as *Leptospirillum ferrooxidans* facilitate metal solubilization from solid wastes or other solids (Escobar *et al.*, 2010 ; Suzuki, 2001 ; Mielke *et al.*, 2003). The bacteria can catalyze the oxidation of elemental sulfur or reduced sulfur compounds to sulfuric acid and obtain energy from the oxidation of elemental sulfur or reduced sulfur compound, and cause bioacidification and

solubilization of heavy metals (Southam & Beveridge, 1992). Examples of metals extracted by bioleaching are Copper from Chalcopyrite, Zinc from Sphalerite, Uranium from Uraninite and Gold from gold bearing Arsenopyrite.

Using simple laboratory experiment, the paper aims to demonstrate the capability of bioleaching process to recover metal ions from mine tailings. The paper also discusses several key factors that may need to be considered for optimizing the recovery of metals through bioleaching.

METHODS

Material

In this experiment, mine tailings was used as a leaching subject and a source of bacterial leaching. Mine tailings sample was provided by Geomicrobiology Lab at the School of Earth Sciences, University of Queensland, Australia and no information provided about the source location of the tailing. The basal salt was used as an inoculation media, which containing (per L of deionized water): 0.4 gr $(\text{NH}_4)_2\text{SO}_4$, 0.1 gr K_2HPO_4 , 0.4 gr $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.33 gr $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 18 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, pH 2.3. Bioleaching experiment was carried out in a simple leaching column that was set up using 5 cc syringes. The syringe contained 1 cc of glass wool and 3.5 cc of tailings (Figure 1).



Figure 1. Leaching column for bioleaching experiment (left) and tubes prepared for enumeration of iron oxidizing bacteria (right)

Enumeration of Bacteria using Most Probable Number (MPN) Method

In the laboratory, bacterial leaching which are the dominant iron and sulfur oxidizing bacterium from the tailings were isolated using the MPN technique (Cochran, 1950). One gram of mine tailings subjected to tenfold serial dilution (10^{-1} to 10^{-5}) and diluted into 5 steriles 16x100 mm test tubes (with push caps) containing 9 mL of basal salt media. Inoculation was done with transferring 0.5 mL of each fold dilution into 50 sterile 13x100 mm test tubes (with push caps), containing 4.5 mL of basal media. The tubes are then divided into 2 groups. The first 25 tubes prepared for enumeration of iron oxidizing bacteria (e.g: *Acidithiobacillus ferrooxidans*) where additional 0.5 mL sterile $FeSO_4 \cdot 7H_2O$ (33.3 gr/100mL, pH 2.3) was added into each tubes as a source of energy. The second group also consist of 25 tubes and prepared for enumeration of sulfur oxidizing bacteria (e.g: *Acidithiobacillus thiooxidans*) where a thin film of S^0 was added into each tubes after the bacteria have been inoculated. All culture tubes were then incubated for 2 weeks under stationary conditions at room temperature. At the end of inoculation, tubes were scored for positive growth and iron and sulfur oxidizing bacteria population density was calculated.

Bioleaching Experiment

Table 1. Result of bacterial enumeration using MPN method

	Dilution fold				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Iron oxidizing bacteria	0	0	0	0	0
Sulfur oxidizing bacteria	4	3	3	4	3
MPN of sulfur oxidizing bacteria	$0.45 \times 10^2 \times 2 = 90$ MPN/gr of tailings				

Bioleaching Experiment

In this experiment, the function of the bacteria is to create a chemical environment suitable for metal extraction. In order to figure out the influence of bacteria on leaching chemical

Four mL of basal salts (pH = 2.3) was added to the bioleaching column (Figure 1) at the start of the experiment (T=0) while 2 mL was added in each subsequent week to promote the growth of iron and sulfur oxidizing bacteria. The column was incubated for 4 weeks in room temperature. The leachate was collected each week for inductively coupled plasma atomic emission spectroscopy (ICP AES) elemental analysis. The pH of pool leachate was also recorded each week.

Carbonate Analysis

The amount of carbonates in the tailings was determined using a simple gravimetric procedure as described by Bauer *et al.* (1972). The method estimates soil capacity to neutralize acid, or to produce CO_2 on treatment with acid (HCl), and then calculate the weight of carbonate present on the assumption that it is all present as $CaCO_3$ (Calcite).

RESULTS

Bacterial Enumeration

Positive growth was only observed in sulfur oxidizing bacterial tubes (Table 1), while all iron oxidizing bacterial tubes reveal negative results. Using MPN method, it is calculated that the number of sulfur oxidizing bacteria present in the tailings was 90 MPN/gr of tailings.

environment, the pH of the leachates was analyzed regularly as well as the metal ion leached each week (Table 2). The amount of Iron and Sulfur in the media added to the column (C2 and C3) was also calculated (Table 3), which are 0.9 ppm and 0 ppm for sulfur and iron respectively.

Tabel 2. ICP EMS results of leachate solution

Date collected	Time (days)	Composition of the leachate from columns with 3.45gr tailings (ppm)										
		pH	As	Cu	Ni	Pb	Zn	Ca	Fe	Na	S	Si
Aug. 28, 2013	0.00	7.15	0.12	0.28	0.50	0.63	127.16	421.71	0.00	3476.07	5445.37	2.61
Sept. 4, 2013	7.00	6.67	0.10	0.14	0.34	0.17	83.11	439.37	0.02	1117.57	3144.35	5.18
Sept. 11, 2013	14.00	5.52	0.09	0.14	0.32	0.25	59.63	469.84	0.00	162.09	1300.58	3.55
Sept. 18, 2013	21.00	6.02	0.07	0.08	0.28	0.03	50.64	511.52	0.00	70.10	943.19	3.41

Table 3. Iron and sulfur in media

Element	Media	%wt element	C1(ppm)	C2(ppm)	C3(ppm)
Fe	FeSO ₄ .7H ₂ O (18 mg/L)	6.50	1.17	0.00	0.00
S	MgSO ₄ .7H ₂ O (400 mg/L)	13.01	52.04	0.21	0.10
S	FeSO ₄ .7H ₂ O (18 mg/L)	11.53	2.08	0.01	0.00
S	(NH ₄) ₂ SO ₄ (400 mg/L)	24.27	97.07	0.39	0.19
Total S			151.18	0.60	0.30

C1= concentration in 1 L media; C2=concentration in 4 mL media added to the column;
C3= concentration in 2 mL of media added to the column

Whereas the basal salt media has acidic pH, the leachate at time 0 shows neutral pH. Overall, there is a tendency that the pH of the leachate decreased, from 7.15 at time 0 to 6.02 at day 21 (Figure 2). During the course of 21 days, 26.71% of Zinc leached from the column while negligible

amount of Arsenic, Copper and Lead recovered from the column (Table 4). Except for Calcium, our data shows the decreasing trend in the amount of elements leached during this experiment (Figure 3).

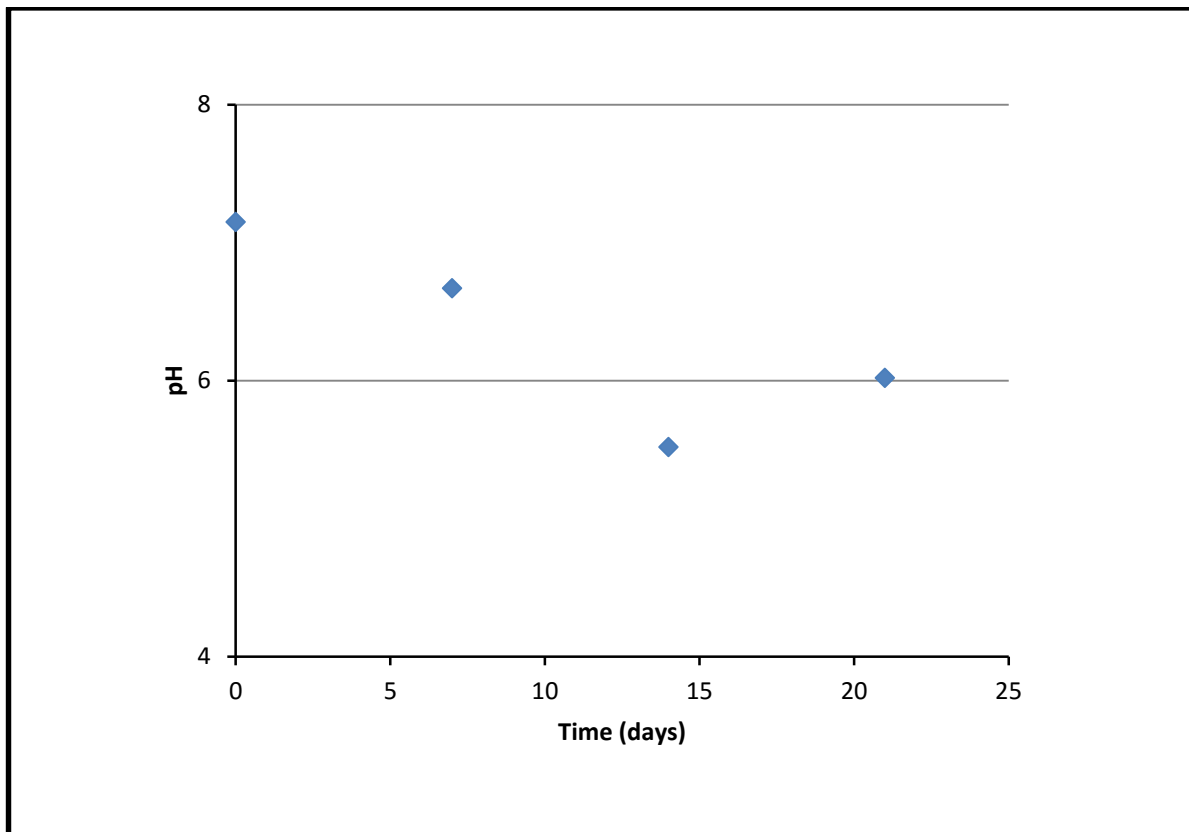


Figure 2. pH of the leachate monitored during the experiment

Tabel 4. Element leached from 3.45 gr tailings

	As	Cu	Pb	Zn
Original concentration of element in 3.45 gr tailings (ppm)	1100	4000	9000	1200
Time (days)	Element leached %			
	As	Cu	Pb	Zn
0	0.01	0.01	0.01	10.60
7	0.01	0.00	0.00	6.93
14	0.01	0.00	0.00	4.97
21	0.01	0.00	0.00	4.22
cumulative	0.03	0.02	0.01	26.71

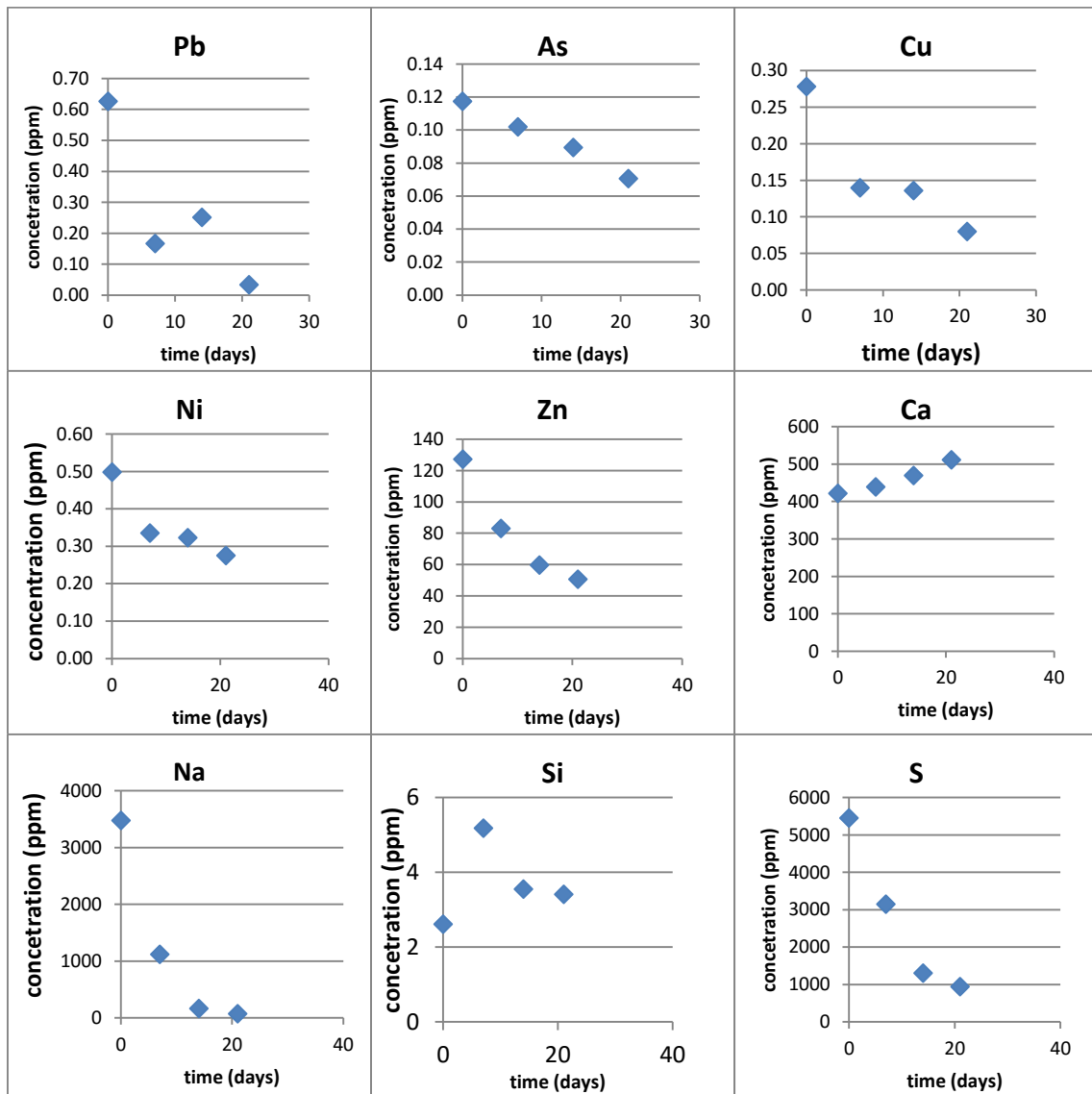


Figure 3. Concentration of elements released from the leachate solution monitored every week

Carbonate Experiment

The amount of carbonate present in the tailings (with the assumption all carbonate present in the form of CaCO₃), calculated following the formula (Bauer, 1972):

Weight of CO₂ = W₂ - W₃, Percent of CaCO₃ equivalent = (W₂- W₃) x 227.4/(W₂-W₁)

where :

- W₁ = Weight of HCl, beaker and tray
- W₂ = Weight of HCl, beaker, tray and original tailings
- W₃ = Weight of HCl, beaker, tray and tailings after being reacted with HCl for 90 minutes

Based on the above formula, the percentage of carbonate present in the tailings is 31.88 (Table 5).

Tabel 5. Carbonate analysis

Original tailings (mg)	1045.43
W1 (mg)	28896.94
W2 (mg)	29942.37
W3 (mg)	29795.80
%wt Carbonate	31.88

Minerals in the tailings

In this experiment, no XRD analysis conducted to identify mineral present in the tailings. However, the ICP EMS data analysis reveals the presence of some element that may associated with several Sulphides, Oxides, Silicates and Carbonate minerals as listed in Table 6.

Table 6. Possible mineral present in the tailings

Sulphides	Oxides	Silicates	Carbonate, Sulphates and Salts
Pyrite FeS ₂	Hematite Fe ₂ O	Quartz SiO ₂	Calcite CaCO ₃
Pyrrhotite FeS	Limonite FeO.OH.nH ₂ O		Dolomite CaMg(CO ₃) ₂
Chalcopyrite CuFeS ₂			Cerussite PbCO ₃
Arsenopyrite FeAsS			Halite NaCl
Sphalerite (Zn,Fe)S			Gypsum CaSO ₄ . 2H ₂ O
Galena PbS			

DISCUSSION

In this experiment, bacterial enumeration using MPN methods reveals very low concentration of iron oxidizing bacteria. The outcome with all negative tubes in each dilution of iron oxidizing bacteria tubes suggests bacterial count less than 0.18 MPN/gr of tailings. Low concentration of bacteria, thought to be related to sampling issue. Bacterial mostly attached to its substrate (tailings). Solid particle of the tailings that sank to the bottom of the tubes is more likely has high concentration of bacteria. Inoculum mostly sampled from the middle of the tubes; as such the bacterial concentration in 0.5 mL inoculum taken from those part possible are very low.

In general, the result of this experiment which is shown by the concentration of the element found in the leachate during the course of 21 days of experiment, as well as the changes of the pH of the leachate over time, suggest that biogenic process occurred in this column and that chemical reactions is unlikely working alone in this system. The process is explained below.

When tailings sample containing sulfide mineral and its indigenous iron and sulfur oxidizing bacteria is contacted with basal medium pH 2.3, two possible bioleaching

mechanisms can be occurred, which are direct and indirect mechanisms (Sand *et al.*, 2001 ; Suzuki, 2001). In direct mechanism, microorganism will directly oxidize and solubilize metals (reaction 1). In this process, the sulfur is biologically oxidized to sulfate without any detectable intermediate occurring (reaction 2). In contrast, in indirect mechanism, sulfide mineral is oxidized by ferric iron which is regenerated from ferrous iron. Ferrous iron can be generated from pyrite oxidation (reaction 5) and can be re oxidized to ferric iron by iron-oxidizing microorganisms (reaction 4). In this mechanism, microorganism has a role simply in regeneration of ferric iron from ferrous iron. Ferric iron can also be hydrolyzed (reaction 6) and can chemically react with pyrite to generate ferrous iron and elemental sulfur (reaction 7). Under acidic conditions the oxidations of sulfide minerals by iron oxidizing bacteria can be 10⁶ times faster than the abiotic rate (Escobar *et al.*, 2010).

- a) $MS + H_2SO_4 + 1/2O_2 \rightarrow M^{2+} + SO_4 + S^0 + H_2O$ M=divalent metal
- b) $S^0 + 3 \frac{1}{2} O_2 (ac) + H_2O \rightarrow SO_4^{2-} + 2H^+$
- c) $MS + 2Fe^{3+} \rightarrow M^{2+} + 2Fe^{2+} + S^0$
- d) $2Fe^{2+} + 1/2O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$

- e) $2 \text{FeS}_2 + 3 \text{O}_2 (\text{ac}) + 2\text{H}_2\text{O} \rightarrow 2\text{FeSO}_4 + 2 \text{H}_2\text{S}$ chemical reaction
- f) $\text{Fe}^{+3} + 3 \text{H}_2\text{O} \rightarrow \text{Fe} (\text{OH})_3 (\text{s}) + 3\text{H}^+$ chemical reaction
- g) $\text{FeS}_2 (\text{s}) + \text{Fe}_2 (\text{SO}_4)_3 \rightarrow 3\text{Fe}^{2+} + 3\text{SO}_4 + 2\text{S}^0$ chemical reaction

The pH of the column decrease from time 0 to day 14, however on day 21 the pH is slightly increase. Overall, during the experiment the pH of the column stand in the range of 5 to 7. The decreasing pH indicates that acid was generated in the column. The oxidations of ferrous iron to ferric iron increase the ferric iron concentration. As ferric iron accumulates, hydrolyses occurred and acid produced which further lower the pH of the column environment (reaction 6). Acid can also be produced from sulfur oxidation (reaction 2). The increase of the pH at day 21 is likely caused by accumulation of ferrous iron, which consumes a lot of acid to produce ferrous iron (reaction 4). The occurrence of carbonate mineral in the tailings, which is quite significant (31.88%), is also thought to act as a buffer which may prevent further acidic condition of the column. Carbonate minerals may form protective layer on mineral surface that can decrease the metal dissolution process (Deveci *et al.*, 2003). Study has noticed that the occurrence of carbonate minerals such as Calcite (CaCO_3), Dolomite [$\text{CaMg}(\text{CO}_3)_2$], Ankerite [$\text{Ca}(\text{Fe},\text{Mg})(\text{CO}_3)_2$] and Siderite (FeCO_3) in sulfide bearing mineral deposit can help to prevent the formation of low pH drainage from the waste contaminant area (Al *et al.*, 2000).

In this experiment, almost no Iron was found in the leachate, suggesting that Iron was heavily used in the oxidation process of other mineral. As significant amount of Zinc found in the leachate, it is quite likely that Iron was used for the oxidation of Sphalerite. Furthermore, among other element, Sulfur concentration in the leachate is the greatest, even after Sulfur from the media has been taken into account (Table 3), suggesting that a lot of Sulfur produced during the biosolubilization of metal sulfide (reaction

3). On the other hand, the increasing Ca concentration in the leachate solution each week was thought to be related to the pH of the leachate. The decreasing of pH was likely increase the solubility of Ca. Study on Ca and Mg concentrations in the leachate from permanent meadow soils in Western Lithuania also showed the same phenomena where Ca solubilization increased with decreasing pH (Butkute R, 2006).

Negligible amount of Lead, Arsenic and Copper leached from the column (Table 2), suggest that the low concentration of Iron and Sulfur oxidizing bacterial in the column are too low to drive the bioleaching process of all base metal in the tailings. Bacterial leaching is very important to accelerate the metal leaching. For instance, the Copper solubilization has increase significantly when more stock culture added to the bioleaching system, suggesting that the large amount of bacteria can accelerate the Copper leaching (Yang *et al.*, 2009). It should be noted that the ferrous ion can also be oxidized to ferric ion only by oxygen from air, but this process was very slow (Yang *et al.*, 2009). The poor solubilization of Lead is common, as under bioleaching condition, Lead forms Anglesite (PbSO_4), a poorly soluble complex with sulfate which tend to accumulates in residual solid (Viera *et al.*, 2007).

The pH of the column is also thought not favorable for optimal growth of microbial leaching which then slow the bioleaching process. The optimum pH for the growth of acidophilic bacteria involved in bioleaching ranges from 1.5 to 3.0. High proton concentration is needed to drive certain chemical reactions during bioleaching which can only be provided if the pH maintained to be acidic. Additionally, high precipitation of Jarosite may also occur in pH over 2.0. Jarosite has been known can influence the mechanisms of Copper extraction from the ores during bioleaching process (Gentina and Acevedo, 2003). Acid neutralization by carbonate minerals may also cause a reduction in the concentration of dissolved metals (Al *et*

al., 2000). In neutral pH, metals partition from the aqueous phase to mineral surfaces mostly occurred due to precipitation, co-precipitation and adsorption processes (Al *et al.*, 2000). As the surfaces of carbonate minerals are highly reactive, the concentration of dissolved metals in the system will be influenced by co-precipitation and adsorption reactions at carbonate mineral surfaces (Al *et al.*, 2000).

In this experiment, considerably higher concentration of Zinc is also observed in the leachate. Approximately 27 % of Zinc leached during 21 days of experiment. Within this condition, all Zinc in 3.45 gr tailings can be expected to solubilize in approximately 79 days. The higher solubilization rate of Zinc compare to other elements is thought to be related to sulfide mineral electrode potential. Sulfide minerals vary in susceptibility to bio oxidation (Olson *et al.*, 2003). Dissolution of metal sulfides depends on their electrochemical potential. The mineral with the lowest potential tends to oxidize first (Olson *et al.*, 2003). Study noticed that sulfides mineral like Sphalerite (ZnS) have low electrode potentials which make this mineral readily bio oxidized (Olson *et al.*, 2003). However, it is observed that the concentrations of Zinc leached from the column decrease over time. Study noticed that when zinc sulfide is oxidized by ferric ions, a product layer of elemental sulfur is formed on the surface of the mineral and the diffusion of ferrous ions across this sulfur layer becomes the rate-limiting step (Viera *et al.*, 2007). In the absent of sulfur oxidizing bacteria, the passivation of the Sphalerite surface by a layer of elemental sulfur may slow the leaching process, however in the presence of sulfur oxidizing bacteria this condition may remain occurred if the rate of elemental sulfur oxidation is inadequate (Viera *et al.*, 2007). The decreasing concentration of sulfur leached overtime in this experiment, occurred as some sulfur possibly layered the surface of mineral as explained above. To increase the recovery of Zinc during bioleaching of sulfides ores, Saririchi *et al.* (2012) has suggested to do the continuous

inoculation of the ores with the solution of *A. ferrooxidans* type which should be maintained until the bioleaching process become self-sustaining.

Finally, in order to achieve optimum performance of the bioleaching column in this experiment, several factors should be taken into account. These factors are temperature, acidity, oxidizing conditions, availability of nutrients, oxygen and carbon dioxide as well as surface area and presence of toxic ions (Deveci *et al.*, 2003).

Bioleaching processes are temperature and pH dependent, with optimum metal dissolution occurring in a particular range where the bacterial strain is most active. The acidity of the bioleaching environment is possibly controlled by the oxidation of iron, sulfur and metal sulfides as well as dissolution of carbonate minerals (Kumar & Nagendran, 2007 ; Suzuki, 2001). The optimum growth of the bacteria can be maintained with provision of nutrient salts. Metal dissolution will also depend to the quantity of nutrients and the availability of substrate (Olson *et al.*, 2003 ; Saririchi *et al.*, 2012 ; Viera *et al.*, 2007 ; Yang *et al.*, 2009).

Most bacterial leaching are autotrophic aerobes. The activity of these bacteria depends largely on the availability of oxygen and CO₂ which are important to complete the cycle of they respiration. The oxygen levels below 1-2 mg/L can negatively affect the oxidizing activity of bacteria (Deveci *et al.*, 2003). The surface area (particle size) may improve the bioleaching rate while increasing concentrations of ions such as Cl⁻ may also negatively affect the oxidative activity of bacteria (Viera *et al.*, 2007). High concentration of Ag, anions of Te, As and Se (50-100mg/L) has been proven to have inhibitory effect on the iron oxidizing activity of the bacteria (Deveci *et al.*, 2003). Moreover, the salinity of process water may also suppress the bioleaching activity of mesophilic bacteria (Deveci *et al.*, 2003). In addition, mineral species as well as ore composition and structure also

affect the rate and extension of the metal extraction by bioleaching from reduced sulfur compounds. For instance, the secondary copper minerals are easier to bioleach than the primary ones (Gentina and Acevedo, 2003).

CONCLUSIONS

This experiment has demonstrated the capability of bioleaching process for recovery of metals from the tailings. While the amount of Lead, Arsenic and Copper leached from the column are negligible, significant amount of Zinc leached from the column. Low concentration of iron and sulfur oxidation bacteria in the inoculum thought to be the major factor that suppresses the bioleaching activity in this experiment. Adding more microbial leaching or nutrient to the column, may increase the performance of the bioleaching column. It is also important to maintain the pH to be in the range of 1.5 to 2. Other than nutrient and pH, temperature, oxidizing conditions, oxygen and carbon dioxide, surface area as well as the presence of toxic ions are several key factors that may need to be considered for optimizing the recovery of metals through bioleaching process.

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