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Use of LixKit® to Improve the Bioleaching of a Polymetallic Concentrate in Stirred Tank Bioreactors

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ABSTRACT

El Toqui mine is located 1,350 kilometers from Santiago, in Alto Mañihuales community, belonging to the municipality of Coyhaique, in the XI Aysén Region. This region has a well-known history of polymetallic mineralization. Currently, El Toqui mine produces a polymetallic sulfide concentrate containing copper, zinc, cobalt, iron and arsenic. The present study was conducted to establish the possible application of bioleaching to recover valuable metals from this polymetallic sulfide concentrate. Shake flask culture experiments were carried out to adapt a mixed culture of the moderately thermophilic microorganisms (*Sulfobacillus acidophilus* and *Sulfobacillus thermosulfidooxidans*) to this concentrate (stirring rate of 100 rpm and the temperature of 45 °C, with the addition of yeast extract). The initial bioleaching experiments were performed in small stirred tank bioreactors (7 liters) at 45°C and at a pulp density of 10% and 20% (wt/vol) during 60 days. For continuous improvement of operating parameters during the bioleaching tests, the LixKit® method was used to determine the metabolic activity of microorganisms. The extraction of copper, zinc, iron and arsenic shows an increasing trend during the first 30 days of bioleaching. Instead, at the end of 60 days of bioleaching, only copper and arsenic showed an increase in their level of extraction. Greater recovery to iron, cobalt and zinc and lower copper recovery was achieved. The metabolic activity of the microbial consortium reaches its peak at approximately 25 days of bioleaching, becoming progressively decay until the end of the test. Using these results, the leaching process was successfully scaled up to 200 L bioreactor with a pulp density of 10% and 20%. This shows that the LixKit® test enables a rapid monitoring of the metabolic activity of the microbial population, a continuous improvement of the operational parameters and an easy scale-up of the process.

Keywords: Bioleaching; El Toqui mine; copper; zinc; cobalt; Sulfobacillus; LixKit®; stirred tank bioreactors.

INTRODUCTION

Presently, there is great interest in developing methods of extracting metals more efficient, since the discovery of new deposits of high grade or low grade reservoirs are too few to match the expected growing demand (Watling, 2014). With the decreasing high grade ore reserves and increased concern regarding the effect of mining on the environment, biomining technology, is now being developed as a main process in the mining industry to meet the demand (Kundu & Kumar, 2014).
Biomining has been studied for over 50 years and is an established technology for metal recovery. Conventional biomining is usually performed in heaps or dumps and is most commonly used for the recovery of copper or uranium from low-grade ores. It is applied in large-scale heap bioleaching operations located in Chile, Peru and Australia. Another way to perform biomining is the continuous stirred tank reactor technology (CSTR) (Acevedo, 2000; Lindström at al., 2003; Astudillo & Acevedo, 2008; Kundu & Kumar, 2014). It is a commercial reality for the treatment of refractory gold ore concentrates (Arrascue & van Niekerk, 2006). Currently, there are different industrial plants in South Africa, Brazil, Australia, Ghana and Peru using this technology. There is little information in the literature about the industrial application of CSTR for metal recovery from concentrates. One example of this is the use stirred tank reactor to base metals is the bioleaching of a cobalt-rich pyrite concentrate (Morin & d'Hugues 2007).
El Toqui mine is located 1,350 kilometers from Santiago, in Alto Mañihuales community, belonging to the municipality of Coyhaique, in the XI Aysén Region, Chile. This region has a well-known history of polymetallic mineralization. Currently, El Toqui mine produces a polymetallic sulfide concentrate containing copper, zinc, cobalt, iron and arsenic. The present study was conducted to establish the possible application of bioleaching in stirred tank bioreactors to recover valuable metals from this polymetallic sulfide concentrate. To improve the operating parameters during the bioleaching tests, a real-time method for detecting active microorganisms was used (Cotoras & Viedma, 2011 and 2013).

METHODOLOGY

Microorganism and culture medium

Sulfobacillus acidophilus and *Sulfobacillus thermosulfidooxidans* strain kindly supplied by Professor Blanca Escobar from the Universidad de Chile, Santiago, was used. The microorganism was cultured in modified Kelly medium (Norris, 1989, Olson y Harvey, 2011) with concentrate at pulp densities of 8% to 20% w/v.

Flotation concentrate

The flotation polymetallic concentrate was supplied by Minera El Toqui, Nyrstar, Alto Mañihuales, XI Aysén Region. Chile. Chemical analysis of the sample revealed: 10.18% Zn; 0.53 %Pb; 24.84% Fe; 1.37% Cu; 11.43% As; 1.19% Co; 0.30% Bi; 119.7 Au g/t and 80 Ag g/t. Microscopic analysis of the polymetallic concentrate showed following major components (wt.%): pyrite (FeS_2) 24.27%, sphalerite (ZnS) 20.86%, arsenopyrite (FeAsS), 19.57%, chalcopyrite ($CuFeS_2$) 11.56%, galena (PbS) 1.16% and pyrrhotite ($Fe_{1-x}S$) 0.74%. Over 80% of the concentrate had a particle size of less than 70 μm.

Culture adaptation

Experiments were performed in 100 mL Erlenmeyer flasks in modified Kelly medium with El Toqui flotation concentrate at 8% of pulp density. Flasks were inoculated with 10% of active culture. Initial pH was adjusted to 1.8 with sulfuric acid. Cultures were run in a thermostatic rotary agitator (LabTech, Korea) at 45 °C and 100 rpm.

Laboratory-scale 7 L stirred tank bioreactor

Batch experiments with 10% and 20% solids were carried out in a 7 L laboratory-scale polypropylene stirred tank bioreactor thermostated at 45°C. pH was maintained under 1.8 by addition of H_2SO_4 (20% v/v) when necessary. Air (0.35 – 0.50 vvm) was injected beneath a turbine (rotation speed from 100 to 290 rpm) at the bottom of the bioreactor.

Pilot-scale 200 L stirred tank bioreactor

Batch experiments with 10% and 20% solids were carried out in a 200 L fiberglass stirred tank bioreactor, with polypropylene inner cover, thermostated at 45°C. This bioreactor was three baffles to improve mass transfer. pH was maintained under 1.8 by addition of H_2SO_4 (50% v/v) when necessary. Air (0.125 - 0.150 vvm) was injected beneath a marine propeller (rotation speed at 1300 rpm) at the bottom of the bioreactor. A sequential series of four batches was performed using a semicontinuous culture method. 180 L of the bioreactor content were withdrawn and replaced with fresh medium and concentrate. In this way it was possible to have a microbial culture actively growing.

Bioluminescence-based bacterial counting (LixKit®)

The quantification of active bioleaching microorganisms was performed using the LixKit® (Biohidrica, Chile) according to the manufacturer's instructions (Viedma, 2010). A volume of 10 mL of the microorganism culture to be assayed was passed through a filter holder with a 0.22 μm membrane using a syringe. The second stage of this assay consists in the removal of the agents that are inhibitory for the bioluminescence reaction. It was carried out by sequentially washing of the previously concentrated acidophilic microorganisms. In order to do that, 20 mL of solution 1 were passed through the filter and after that the filtrate was discarded. Then the membrane was rinsed with 20 mL of solution 2 and the filtrate was discarded again. Finally, using moderate pressure over a syringe plunger air is allowed to pass through the membrane to remove the remaining solution). A swab was wetted by immersion in solution 2, and was rubbed against the surface of the membrane, keeping a constant pressure. The third stage of the assay corresponds to the extraction of intracellular adenosine-triphosphate (ATP) from the acidophilic microorganisms and the performance of the bioluminescence reaction. Finally, emitted light was immediately measured in a luminometer (Kikkoman Lumitester PD-20, Japan). The values obtained are expressed in relative light units (RLU) (Cotoras & Viedma, 2011 and 2013).

Analytical methods

Soluble concentration of copper, cobalt, zinc, iron and arsenic was measured using an inductively coupled plasma spectrophotometer. Eh and pH were measured with a Cole-Parmer potentiometer.

Chemicals

All chemicals were of analytical grade and were purchased from commercial sources.

RESULTS AND DISCUSSION

Adaptacion of the cultures in shake flask

Shake flask culture experiments were carried out to adapt these moderately thermophilic microorganisms (*Sulfobacillus acidophilus* and *Sulfobacillus thermosulfidooxidans*) to the concentrate. Three successive cultures were performed in in modified Kelly medium with El Toqui flotation concentrate. For both moderate thermophilic bacterial strains presented active microbial growth on this concentrate and a significant change in the concentrate aspect from black color to a reddish color after approximately 6 days of culture were observed. A mixed culture of the strains *Sulfobacillus acidophilus* and *Sulfobacillus thermosulfidooxidans*, adapted after three subcultures in concentrate, was used as inoculum for the bioleaching experiments in a 7.0 liters stirred tank reactor.

Bioleaching of the polymetallic concentrate in a 7.0 liters stirred tank bioreactor

The initial bioleaching experiments were performed in small stirred tank bioreactors (7 liters) at 45°C and at a pulp density of 10% and 20% (wt/vol) during 60 days. For continuous improvement of operating parameters during the bioleaching tests, the LixKit® method was used to determine the metabolic activity of microorganisms. In practice, this means that the operating parameters were adjusted in real time, depending on the observed change in microbial activity determined by this assay. Figure 1 shows the copper, cobalt, zinc, iron and arsenic release from the polymetallic concentrate by moderate thermophilic bacteria strains in a 7.0 liters stirred tank bioreactor at 45°C. Arrows indicate the times at which an increase in the stirring speed in the bioreactor was made. The intensification in the agitation was performed in order to maintain a high microbial activity, according to measurements made with the LixKit® test. Overall, it appears that the extraction of all metals is produced, although they are clearly different in its trends and recovery levels obtained after a month bioleaching (approximately between 13 and 59%). In the case of cobalt, it is observed that the concentration stabilizes after day 15, whereas the other metals maintain their upward trend during this experiment time. Instead, at the end of two months bioleaching, only copper and arsenic have an increased level of extraction.

Figure 2 shows the measurement of the metabolic activity of the microorganisms in the bioreactor. This result shows a significant initial increase in microbial activity, measured as ATP, during the development of this bioleaching experiment. The metabolic activity of the microbial consortium reaches its peak at approximately 25 days of bioleaching, becoming progressively decay until the end of the test.

Figure 3 shows the pH and Eh measurements in the bioreactor. It was necessary to adjust the pH value by adding sulfuric acid during the first 7 days and then maintained in the desired range. The redox potential, Eh, was increasing during the test, which shows an oxidative activity in the bioreactor.

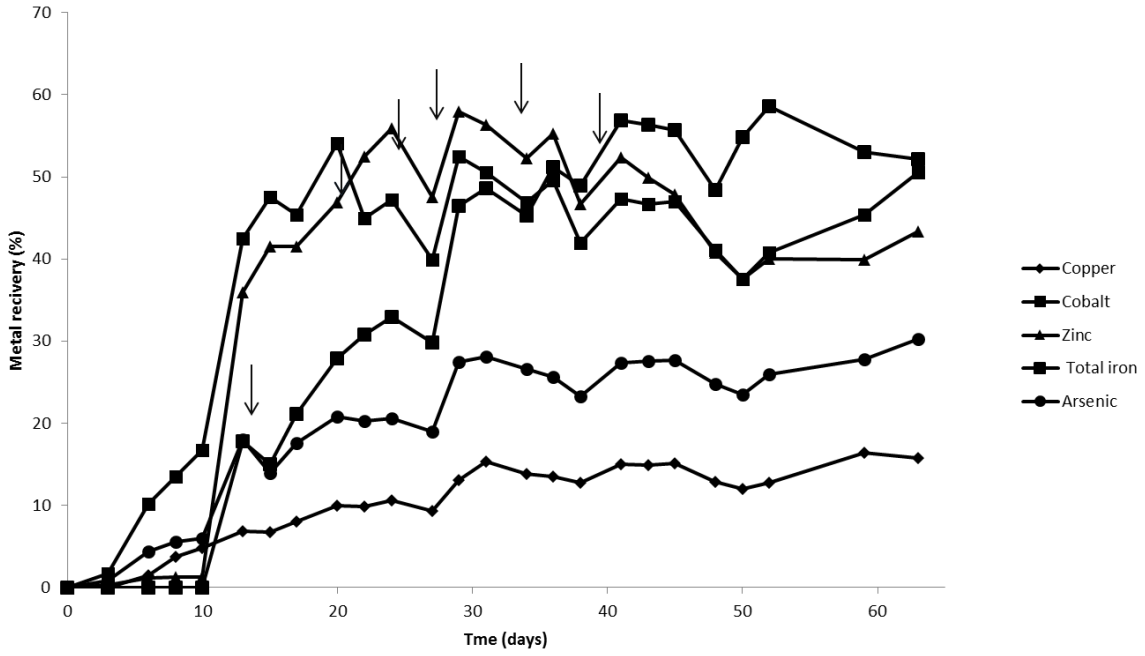

Figure 1 Copper, cobalt, zinc, iron and arsenic release from the polymetallic concentrate by moderate thermophilic bacteria strains in a 7.0 liters stirred tank bioreactor at 45°C. Arrows indicate the times when an increase in the stirring speed in the bioreactor was made (100 rpm Initial arrows 140, 210, 250, 270 and 290 rpm, respectively)

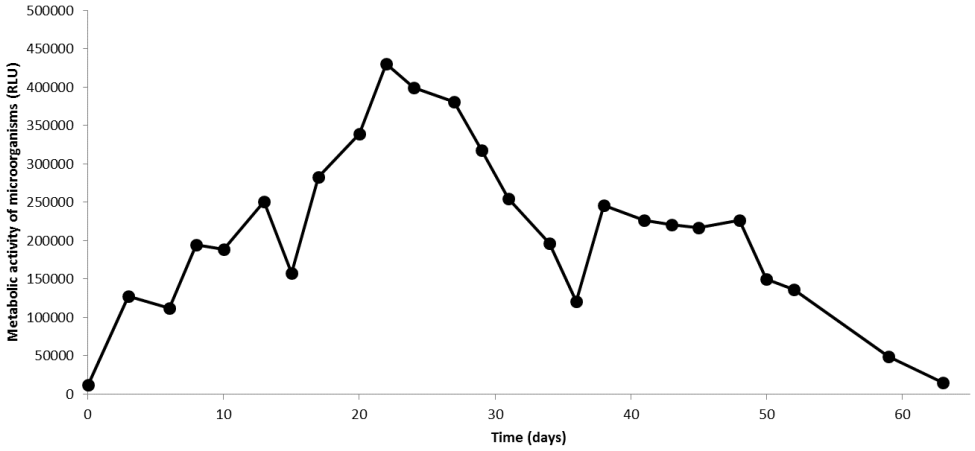

Figure 2 Microbial metabolic activity during bioleaching concentrated in stirred tank bioreactor of 7.0 liters at 45°C.

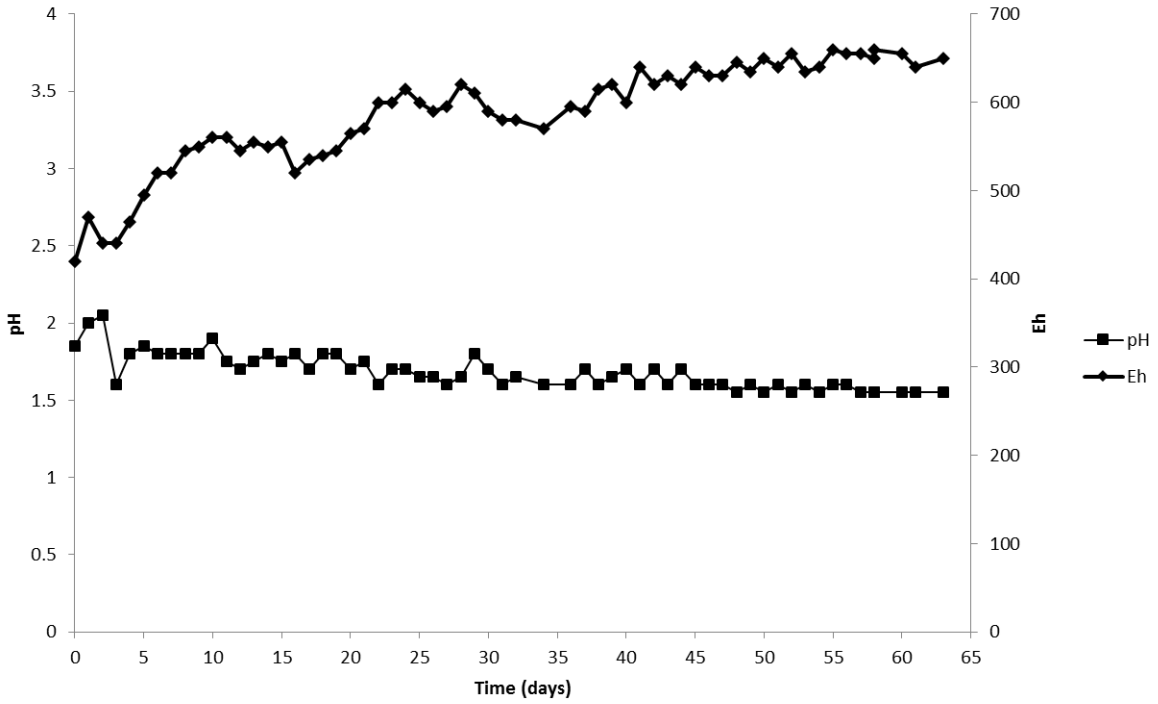

Figure 3 pH and Eh value during bioleaching concentrated in stirred tank bioreactor of 7.0 liters at 45°C.

In summary, an effective bioleaching of zinc and cobalt is achieved in only 23 days of operation. Furthermore, an experiment using a pulp density of 20% showed that the concentrations of copper, cobalt, zinc, iron and arsenic in the solution were clearly superior to those achieved using 10% pulp density (data not shown). These results were important because they allowed the use a shorter operation time and a higher pulp density in the following bioleaching experiments in 200 L stirred tank bioreactor.

Bioleaching of the polymetallic concentrate in a 200 liters stirred tank bioreactor

Using these results, the bioleaching process was successfully scaled up to 200 L stirred tank bioreactor with a pulp density of 10% and 20% (Table 1). Similarly as in bioleaching experiment in the 7.0-liter bioreactor, in all these bioleaching batches the microbial activity was determined using the LixKit® method to optimize operational parameters in real time. In the first batch, using 10% pulp density and 21 days of operation time, it was possible to extract copper, cobalt, zinc, iron and arsenic. The obtained concentrations were similar to those achieved in the experiment in the 7.0 liter bioreactor with 10% pulp density levels and 21 days. The second batch was made with 20% pulp density for 23 days. In Table 1 it can be seen that the concentrations reached are greater than those achieved with 10% pulp density. In the case of copper, cobalt and zinc, the concentrations almost doubled. The next two batches (3 and 4) were performed with 20% pulp density for only 14 days. In these experiments similar or even higher concentrations than in the batch 2 were obtained. This

result could be caused by high bacterial growth achieved by a semicontinuous culture system. More studies are needed to optimize the process of bioleaching of this concentrate.

Table 1 Copper, cobalt, zinc, iron and arsenic release from the polymetallic concentrate by moderate thermophilic bacteria strains in a 200 liters stirred tank bioreactor at 45°C.

	Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
Batch duration (days)	21	23	14	14
Pulp density (%)	10	20	20	20
Copper (g/L)	0.192	0.412	0.445	0.393
Cobalt (g/L)	0.591	1.171	2.749	2.575
Zinc (g/L)	5.791	11.020	16.326	16.871
Total iron (g/L)	6.925	8.705	8.871	12.027
Arsenic (g/L)	2.552	2.967	2.642	5.063

CONCLUSIONS

- Bioleaching of copper, zinc, cobalt, iron and arsenic from a polymetallic sulfide concentrate from the mine Toqui in a small stirred tank bioreactor (7 liters) at 45 °C and at a pulp density of 10% was achieved.
- Using these results, the bioleaching process was successfully scaled up to 200 L bioreactor with a pulp density of 10% and 20%.
- The LixKit® test was a useful tool for rapid monitoring of the metabolic activity of the microbial population, continuous improvement of the operational parameters and easy scale-up of the process.

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NOMENCLATURE

RLU relative light units
rpm revolutions per minute
vvm volume per volume per minute

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