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Variation in microbial community from predominantly mesophilic to thermotolerant and moderately thermophilic species in an industrial copper heap bioleaching operation

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ABSTRACT

A comprehensive monitoring program has been performed at an industrial bioleaching heap at the Escondida mine in Chile since 2006, in order to study possible changes to the indigenous microbial population. In the initial stage of the bioleaching heap operation, the microbial population was dominated by mesophilic microorganisms. When the height of the heap increased, the microbial population changed from predominantly mesophilic to thermotolerant and moderate thermophilic microorganisms. The results of molecular analyses indicated that under these conditions *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans* are the most abundant microorganisms. Those results obtained show that the highest ferrous iron oxidation activity at the third lift occurs at the same operational temperature window as that of the thermotolerant and moderate thermophiles. An increase in the expression of genes (including *rfa*F1) associated with cell envelope biogenesis was indicative of the active growth of *L. ferriphilum*. We conclude that the increasing heap height and the consequent increase in temperature within the heap triggered a change in the microbial community from mesophilic to moderate thermophilic microorganisms. Further, this shift had a relevant impact on the metallurgical performance of the industrial bioheap process at Escondida mine.

1. Introduction

Heat generated by exothermic chemical reactions within a heap is transported via convection in gaseous and liquid phases and via conduction through the heap. The accumulation of heat in the heap is determined by the balance of the leaching rate, the water transport rate and the gas transport rate (du Plessis et al., 2007; Leahy et al., 2005; Petersen and Dixon, 2007). Hence, the temperature inside the bed increases with depth. Then a shorter heap has effectively less volume per unit cross sectional area, within which heat is generated, meaning that heat is removed more easily without raising temperatures as high as those found in a taller heap (Moreno et al., 1999; Petersen and Dixon, 2007). Several studies have been carried out with regards to heat balances around dump and copper sulfide heap leaching operations (Brierley and Briggs, 2002; de Klerk Batty and Norton, 2005; Dixon, 2000; Leahy et al., 2007; Marsden et al., 1995; Petersen and Dixon, 2002a). Heat transport is especially important in copper sulfide heap leaching, in which one or more highly exothermic sulfide oxidation reactions take place. Primarily, those bacteria which catalyze the oxidation reactions are sensitive to temperature. Secondly, many oxidation reactions, that are central to copper recovery from secondary ores, are strongly temperature-dependent. Commercial heap leaching operations are mainly used to leach low grade ores, resulting in low mineral concentration per unit volume heaps and hence low heat generation rates (Petersen and Dixon, 2002b). Moreover, some of these are Run of Mine (ROM) processes in which a heterogeneous distribution of solution and air and the occlusion of minerals in larger particles may further decrease the heat generation (Petersen and Dixon, 2002b).

Although different monitoring devices and techniques have been developed for measuring multiple process variables including heat at various depths and locations in the heap (Dew et al., 2011; Gericke et al., 2011; Petersen and Dann, 2010; Petersen et al., 2011; van Buuren, 2010), most of them are not operationally practical due to the technical and economic barriers. The physico-chemical characteristics

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of the leach liquor including the dynamic of the microbial community could be considered as an indirect and suitable indicator of heat inside the heap.

Molecular methods has been successfully used in an industrial setting to monitor the microbial community composition (Demergasso et al., 2005, 2010; Galleguillos et al., 2008; Remonsellez et al., 2009). However, metabolic changes in the microbial population during the leaching cycle have recently been studied (Demergasso et al., 2010; Galleguillos et al., 2008; Rawlings and Johnson, 2007; Remonsellez et al., 2009). In recent years, the advent of new sequencing and molecular quantification technologies has enabled rapid genome sequencing and more accurate determination of gene expression in single microbial strains (Leimena et al., 2012) or communities (Goltsman et al., 2009; Leimena et al., 2013), contributing new insights into the role, adaptation and responses of microbial populations to different environmental conditions (Bertin et al., 2011; Hazen et al., 2013). These methods enable a quantitative tool for detecting the response of microbial communities to environmental factors (Hazen, 2013) and operational changes. The quantitative determination of gene expression through reverse transcription followed by real time PCR analyses (RT-qPCR) allows an accurate determination of the expression of target genes to infer what metabolic functions are triggered by organisms under certain conditions or stimuli. The most often used protocols to analyze those data obtained by RT-qPCR are the ΔΔ Ct and Pfaffl (Galleguillos et al., 2013; Livak and Schmittgen, 2001; Pfaffl, 2001).

Cell division is achieved by the macromolecular machinery called the divisome (Takada et al., 2014). The divisome has been studied mainly in *Escherichia coli* and to a lesser extent in *Bacillus subtilis* (Errington et al., 2003). As two new cell poles need to be formed at the end of the cell division process, all the cell envelope layers (including the membrane, the wall and the outer membrane) must be remodeled during this process (Takada et al., 2014). Therefore, those genes related to cell envelope biogenesis and cell division would be suitables marker for microbial growth inside the bioleaching system.

Heap bioleaching at the Escondida mine (base dimensions, 2000 m wide by 5000 m long) is divided into 40 leaching strips (125 × 18 × 2000 m). A schematic drawing of the heap with three lifts and eight strips is shown in Fig. 1 with each strip generating its own pregnant leaching solution (PLS). However, since there are no inter-lift liners between the strips, contamination from the adjacent strips is expected, particularly after the second lift was built. PLS samples are always collected at the bottom of the heap (first lift); therefore when the heap is operating with more than one lift, the irrigation solution passes through the lifts until reaching the bottom of the heap. Forced aeration is supplied through blowers located at the base of the heap. Each leaching strip has its own individual irrigation and forced aeration system (Soto et al., 2013).

The microbial succession of heap bioleaching at the Escondida mine has been studied previously in the first and second lifts of low grade copper sulfide ore (Demergasso et al., 2010; Remonsellez et al., 2009; Galleguillos et al., 2008; Soto et al., 2013). The second and the third lifts of the heap were set up during 2008 and 2010, respectively.

It has also been shown that bioleaching reactions are carried out mainly by prokaryotes attached to the sulfide mineral within the heap in order to provide Fe^{3+} ions and protons (Schippers, 2007). Further, as previously mentioned, the use of PLS as an indicator of the microbial communities within an industrial bioheap was largely argued before (Remonsellez et al., 2009). However, tracking the dynamics of the microbial population in the bioleaching system using PLS requires a collection of adequate database to visualize meaningful trends.

This paper summarizes the results of a six-year monitoring program (2006–2012) which was conducted at the Escondida sulfide heap bioleaching plant. Its aim was to assess the variation in the microbial community from predominantly mesophilic to thermotolerant and moderate thermophilic, using complementary technical approaches (both culture dependent and independent) as potential operational indicators of heap temperature, in response to changes in the heap height and consequently PLS temperature. The effect of ore mineralogy on PLS temperature, microbial community and copper recovery in the second lift of the heap has been reported elsewhere (Soto et al., 2013).

2. Materials and methods

2.1. Heap bioleaching and solution sampling

The Escondida mine is located 170 km south-east of Antofagasta, Chile. The industrial heap bioleaching was built with ROM ore characterized as low-grade sulfide minerals, with more detailed information being available in Soto et al. (2013). All samples were taken from the PLS collector installed in each strip (Fig. 1).

2.2. Physico-chemical monitoring of PLS samples

Physico-chemical parameters including pH, PLS temperature (°C) and Eh (mV) were measured daily. Redox potentials were measured using an Orion 3 STAR (Thermo Scientific) voltmeter with an Ag/AgCl reference electrode and then adjusted against a standard hydrogen electrode. The concentrations of Cu^{2+} (g/L) and total Fe (g/L) was assessed

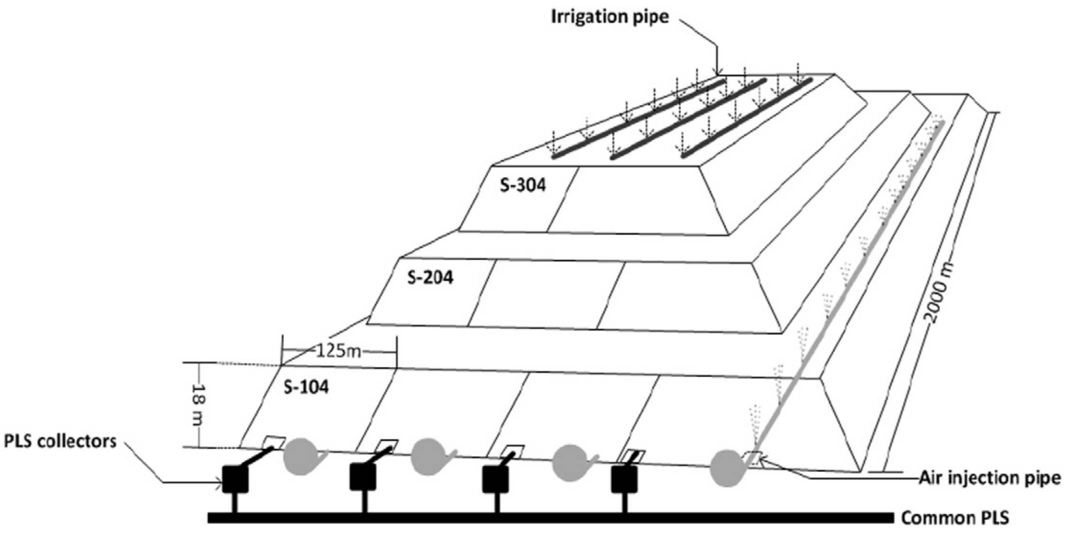

Fig. 1. Schematic view of heap bioleaching at the Escondida mine.