Abstract

The aim of this study was to assess biotic and abiotic conditions for a better understanding of the biological and chemical reactions in the bioleaching processes. For this, bioleaching experiments were carried out with Zn-Pb-Ag mine tailings using two column reactors, each operating under abiotic and biotic conditions, respectively. *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Acidithiobacillus thiooxidans* were used to inoculate the biotic column. Both columns were equipped with moisture and oxygen sensors and build on top of two scales. A peristaltic pump ensured a constant medium inflow rate of 2.3 L/m²s. Oxidation-reduction potential, pH, oxygen, metals, moisture, sulfate and isotopes measurements were used analyze the composition of the leached material and to differentiate leaching from chemical and biological reactions. The pH behaviour was similar in both columns with an increase from 4.2 to 5.4 after 18 days of experiment, which may be due to the presence of carbonate shales and gangue mineral. The ORP decreased similarly in both columns, but slightly faster in the abiotic experiment. Oxygen concentration at the top of the columns was lower in the biotic column, denoting bacterial activity consuming oxygen. Sulfur and oxygen isotopic composition of formed sulfate might give relevant information about the processes involved on the sulfide mineral.

Keywords: bioleaching, sphalerite, tailings, column reactor, sulfide mineral

Introduction

The increasingly decrease in the grade of the ore deposits has turned exploitation of minerals into a big challenge for the mining industries. This decrease has been generating bigger amounts of mining waste material (Falagán et al. 2006). Tailings are finely ground rock particles with a big reactive potential, which may contain metals in relatively high concentrations (Falagán et al. 2006). The high amount of these metals contained in the tailings has attracted a lot of attention of scientific and industrial areas on finding a profitable and environmental friendly technology of exploiting these lost resources. Since physical and chemical processes have shown many drawbacks like intensive energy consumption and secondary pollution (Ye et al. 2016), bioleaching have been gaining a lot of strength in processing sulfide minerals contained in the tailings. Bioleaching is a process in which acidophilic and chemolithotrophic microorganisms convert insoluble solids to soluble and extractable forms by oxidizing the reduced elemental sulfur and/or oxidizing ferrous iron as energy source (Heydarian et al. 2017). This technology can be utilized for a broad variety of different types of sulfide minerals, including: pyrite (FeS₂), chalcopyrite (CuFeS₂), arsenopyrite (FeAsS), sphalerite (ZnS), pentlandite ((FeNi)₉S₈) and pyrrhotite (FeS) (Mousavi et al. 2006).

From the bioleaching methodologies, heap bioleaching is widely applied due to its operational and economic advantages (Yin et al. 2018). Heap bioleaching consist of dumping low grade or mine tailings in a drained area, which is irrigated by a water-based inoculum of oxidizing microorganisms. While significant attention has been given to the geotechnical and hydraulic aspects of heap bioleaching, the optimal microbial conditions in the process is poor understood. In laboratory scale, heap bioleaching can be
simulated by irrigated column reactors, once it is capable of reproducing one of the possible paths of a liquid percolating through a mass of material by gravity (Mousavi et al. 2006). This simulation enables a specific experimentation of different physicochemical and biological parameters and leaching conditions (Johnson, 2008; Manafi et al. 2013; Wang et al. 2014). Among the most important parameters and conditions cited are: pH, temperature, particle size, initial ferrous and ferric iron concentration (Pina et al. 2004; Haghshenas et al. 2011), dissolved oxygen, oxidation and reduction potential (ORP), bacterial strain and cell concentration (Hu et al. 2016). All these parameters and conditions are fundamental for a better comprehension of the biological activity and occurring mechanism in the reactor.

In order to achieve success and reduce the costs of heap bioleaching, it is crucial to improve our knowledge of the biological reactions existing in the process (Gericke 2012). Previous studies have proposed direct and indirect mechanisms to explain the solubilisation of sulphide minerals by oxidising microorganisms. Direct mechanism describes the attachment of the microorganism and the oxidation of the mineral surface through enzymatic reactions. The indirect mechanism is summarized by the constant regeneration of the leaching agent through the bacterial enhanced oxidation of ferrous ions to ferric ions, whether by thiosulphate or polysulphide depending on the composition of the leached mineral (Ghassa et al. 2014). On the other hand, recent studies defend that the degradation of the sulphide mineral only happens because of the presence of the ferric ions oxidized by the bacteria in the glycocalyx or extracellular polymeric substance (EPS) created on the mineral surface by the attached bacteria (Gericke 2011; Ye et al. 2016). Regardless the mechanism, it is believe that the use of bacteria can considerably accelerate the dissolution rates of mineral sulphides (Pisapia et al. 2007 and Bruynesteyn and Hackl1982), but it is not clear at which extend. So a better understanding of the bio-oxidation mechanism in order to define more profitable heap leaching conditions is needed.

While Scanning Electron Microscopy (SEM) images of bacterial attachment to metal particles already proved that both mechanisms could happen at the same time in the same reactor it is still hard to compare the efficiency of each mechanism (Johnson 2010). Pisapia et al. (2007) studied the isotopic fractionations between sulfates and pyrite to constrain the oxidation pathways occurring during acid mine drainage formation. They found that at least two-pyrite bio-oxidation pathways occurred, which depend on the period occurring the oxidation process. This technique can help to explain the chemical and mineralogical nature and the oxygen isotopic composition of compounds formed on the mineral surface in bio-oxidation and compare it to the abiotic oxidation process. The information acquired with this comparison can be utilized in bigger scale heap bioleaching projects.

Therefore, the aim of this study is to assess biotic and abiotic conditions for a better understanding of the biological and chemical reactions in the bioleaching processes in fine-grained Zn-Pb-Ag tailing material.

Methods

Samples of tailings from Century Mine in north-west Queensland were used in this study, one of the largest Australia’s open cut zinc mine before its closure in August 2015. The sedimentary exhalative Zn-Pb-Ag deposit possessed average grades of approximately 10.2% Zn, 1.5% Pb and 36 g/t Ag. Mineralization comprises fine-grained sphalerite with minor galena and pyrite (Broadbent, Myers and Wright 1998). Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) showed abundant fine-grained and sub-μm-scale material, with silica and quartz phases, pyrite, jarosite, and sphalerite (Kerr 2017). Particle size distribution test was realized using ASTM sieves and hydrometer test resulting on a distribution of 7% clay, 76% silt and 17% sand in the tailing material. In virtue of the high amount of fine particles present in the material, a mixture of 40% tailings to 60% sterile sand on weight percentage was established to avoid permeability problems. Both materials were sterilized using a
The pH of 4.9 of the sand tailing material mixture was defined using a ratio 1:2.5 to deionized water with a Sper Scientific benchtop pH meter. The bulk density of the mixed material was calculated and defined as 1.72 g/cm³.

Iron-oxidising bacteria (Acidithiobacillus ferrooxidans and Leptospirillum ferrooxidans) and sulphur-oxidising bacteria (Acidithiobacillus thiooxidans) were mixed and used in the bioleaching experiments at concentrations of 4x10⁷ cells/ml counted with a phase contrast microscope (Olympus EC-40) and a Hauser Scientific™ counting chamber. Bacterial growth was realized in 300ml Erlenmeyer flasks containing 100ml solution using a 9K modified medium containing: 20g FeSO₄·7H₂O, 0.1 g KCl, 0.5 g K₂HPO₄, 0.5 g MgSO₄·7H₂O, 2.42 g (NH₄)Cl and 3.22g NaSO₄ in 1 L of distilled water. The pH of the culture medium was adjusted to pH 2.0 with 1 M H₂SO₄, filtered (0.45 µm) and mixed with the bacteria on a 10 ml bacteria to 90 ml medium ratio. The bacteria were incubated in a 125 rpm shaker in a heating room at 30°C.

The experiments were carried out in two column reactors fabricated from 5mm thick acrylic cylinder glued on an acrylic plate and build on top of two Ohaus Navigator XL 20000 scales. The height of both columns is 50cm and the internal diameter 15cm. Special attention was given at the packing of the column in order to avoid preferential flow and clogging of the column. Therefore, the columns were dry packed carefully on 0.5cm layers and compacted and scarified after each layer to help integration of the fine particles. At the bottom of the columns, a sand layer of 4cm was packed with use of a geotextile between the two different soil materials to avoid the escape of the fine particles from the columns and also for filtering the collected leachate.

Before packing the columns, a set of six oxygen sensors from Presense GmbH and two EC-5 moisture sensors from ECHO equipped each of the two columns. Of the six spot oxygen sensors in each column, three were the SP-PS-3 for a measurement range of 0-100% oxygen in dissolved or gaseous

![Figure 1 Detailed scheme of the experiment.](image)
phase and three SP-PSt-6 for a low range of 0-5% oxygen content. The six optical sensor spots were attached to the inner wall of the cylinder and are being measured twice a week in a contactless and non-invasive way by the use of an optic fibre connected to a Fibox 4 oxygen meter. The two EC-5 moisture sensors were installed in each column using cable glands responsible for avoiding the leakage of the columns. All four sensors installed in both columns were connected to a HOBO datalogger and configured with a measurement interval of 10 minutes. The drawing below (Figure 1) helps to understand the set-up of the experiment.

The bioactive column was inoculated pouring 2.1 L of the modified 9K medium with the different strains of bacteria aiming to achieve the desired bacterial concentration. The control column was filled with 2.1 L of the modified medium and sodium azide (0.5g/L) to eliminate any remaining endemic species present in the tailing material. This bactericide was used in the control column every two weeks to ensure that no microorganism starts growing up again in the abiotic column. The columns were left closed for 36 hours inoculating. After this period they were leached and the leachate was recirculated to guarantee the attachment of the bacteria to the tailings.

After the inoculation, both columns were fed with an irrigation medium composed of 0.111 g NH4Cl and 0.176 g K2HPO4 per liter with the use of a peristaltic pump (Watson Marlow™ 323) at a flow rate of 2.3 L/m²s. This predominantly saturated condition had to be established in order to guarantee enough head pressure to maintain a constant outflow rate. Considering the inflow rate, the bulk density, the volume and the porosity of the tailing mixture material, a retention time of 60 hours was calculated. The solution was dripped on the top of a 5 cm sand layer packed on the top, which guaranteed a homogeneous distribution of the liquid through the tailing material. Two times a week samples have been collected from the leachate bucket of the two columns for the analysis of the liquid. The sampling interval was defined based on the retention time.

Ph and ORP (oxidation and reduction potential) is being measured using a Sper Scientific Benchtop pH/mV meter IC860031. Sulfate is going to be measured using a Thermo Fisher Dionex ICS-1100 Ion Chromatograph (IC). Metals are going to be measured with an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) Perkin Elmer Optima 7300DV after filtering and digesting the samples with nitric acid. SEM is going to be used to generate images in order to check the attachment of the bacteria to the mineral surface and to the metals precipitated. Samples were processed at the Centre for Microscopy and Microanalysis at the University of Queensland on a Hitachi TM3030 SEM. Isotope analysis of sulfur and oxygen isotope ratios of the leached sulfate are going to be determined by continuous flow isotope ratio mass spectrometry using an elemental analyser ($\delta^{34}$S) or a Thermo-Finnigan TC/EA ($\delta^{18}$O) coupled to a gas source mass spectrometer.

**Results and discussion**

Despite the low pH of 2.0 of the irrigation medium, the pH of the leachate increased similarly in both columns (Figure 2a). In column 1, the pH varied from 4.2 to 5.4, while in column 2 the variation during the 5 weeks was from 4.4 to 5.8. This increase may be due to the presence of carbonate shales and the gangue mineral siderite contained in the mine deposit, which has the capacity of buffering the acidity of the leachate (Broadbent, Myers and Wright 1998). The lower values of the pH in the bioactive column might be a result of the acidity produced by the microorganisms.

The ORP also decreased similarly in both columns, but slightly lower in the abiotic experiment (Figure 2b). After day 18, both pH and ORP displayed steady state conditions in both columns. The slight increase in ORP in the biotic column might be an indication of biological activity (Olson et al. 2003).
Figures 3 and 4 showed the data acquired by the 3 normal and 3 low range oxygen sensors in column 1 and 2. Both figures present a sharp decrease after the first day, which can be explained by the saturation of the tailings by the medium, pushing the trapped oxygen out of the columns. The only difference in oxygen concentration between the columns was observed at the top of the column in the abiotic experiment (Figure 4a), where a higher concentration might explain the lack of bacterial activity that can consume oxygen. The consumption of the oxygen by the aerobic microorganisms inoculated in the biotic column is key for the oxidation of ferrous iron into ferric iron and for the production of acids (Baba et al. 2011).

Comparable to the oxygen sensors, the moisture sensors have been delivering stable measurements with values between the range of 0.18 to 0.19 for the bottom sensors and 0.16 to 0.18 for the upper sensor showing that...
the moisture conditions of both columns are equal and maintained constant with the in- and outflow rate (data not shown). Further analysis will support the results presented and will help us to clearly observe the differences between bioleaching under biotic and abiotic conditions. These analyses include metals content, and isotope analysis. This analysis will also help to determine the relevance of the biological activity and better comprehend the bio-oxidation process at the mineral surface.

**Conclusions**

This study maintains a clear distinction between the biotic and the abiotic column aiming to understand the biological and chemical reactions existing in both columns and consequently, estimating the influence of using microorganisms through the metal leaching discrepancy between both columns. The outcome of the present study will provide clear evidence on the effect of bacterial activity in the dissolution of mineral sulfide contained in Zn-Pb-Ag tailing material in lab-scale simulations of heap leaching.

**References**


