Hydrometallurgy xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Hydrometallurgy

journal homepage: www.elsevier.com/locate/hydromet

Effect of high sulfate concentrations on chalcopyrite bioleaching and molecular characterisation of the bioleaching microbial community

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 30 June 2016
Accepted 13 July 2016
Available online xxxx

Keywords:
Bioleaching
Chalcopyrite
Salt tolerance
Sulfate

ABSTRACT

The efficiency of chalcopyrite bioleaching in the presence of a high sulfate background was evaluated using acidophilic microorganisms that were previously enriched from naturally salty and acidic environments and subsequently adapted to increasing sulfate concentrations. Bioleaching with mesophilic, moderately thermophilic and thermophilic microbial consortia was tested with 100, 40 and 80 g L^{-1} sulfate, as determined by the maximum tolerable sulfate concentration observed for the adapted enrichment cultures. Copper extraction was greatest at 45 and 60 °C (22% and 48%, respectively) and lowest at 30 °C (16%). Quantitative X-ray diffraction (QXRD) of the concentrate and bioleach residues revealed complete disappearance of pyrrhotite and a significant reduction of pyrite at all temperatures. Significant chalcopyrite leaching occurred at 45 and 60 °C; however, no chalcopyrite was leached at 30 °C. Bioleaching did not plateau for any of the cultures after 31 days, and it is possible that higher leaching yields could have been achieved with prolonged and optimised leaching. The extraction of DNA from the cultures was hindered most likely due to the presence of high sulfate and magnesium concentrations, in combination with metals and acidity. Preliminary results indicated that *Acidithiobacillus caldus* and *Leptospirillum ferriphilum* were detected in the 30 °C culture, but this could not be reproduced regardless of the method chosen for sample preparation or DNA extraction. No DNA was successfully extracted from the 45 or 60 °C cultures. Further method development for sample preparation and DNA extraction from chalcopyrite bioleaching cultures with high salt concentrations, metals and other potential inhibitors is still required.

1. Introduction

As the world faces a decline in easily accessible and economically viable mineral resources, a shift towards targeting lower grade resources and resource recovery from secondary sources such as electronic scrap has commenced. In 2009, Mudd estimated that high grade and quality ores would only last for the next 20 to 40 years (Mudd, 2009). Lower grade resources with increasing impurity concentrations, in combination with the scarcity of, competition for and restrictions placed on the use of potable water for mineral processing, has resulted in increased processing costs and decreased efficiency and yield of production.

In an effort to improve yields from lower grade mineral resources, the industry is investigating alternative mineral processing techniques to cope with increased impurity concentrations in the process streams. Chloride and sulfate anions can be introduced via the use of saline/brackish process waters (i.e. seawater, saline groundwater, recycled process waters), or from impurities contained within lower grade minerals that dissolve and are subsequently recycled back into the process (Gahan et al., 2010). The ability to deal with increasing contaminants in mineral processing without compromising the leaching efficiency and yield, and quality of value recovered by leaching, have been the focus of recent research studies.

Bioleaching has previously been employed to recover value from a number of low grade sulfide and oxide ores, which would otherwise be considered uneconomic to process via traditional hydrometallurgical methods (Johnson, 2013). However, despite the advantages associated with bioleaching of low grade ores such as chalcopyrite (Pradhan et al., 2008; Rawlings and Johnson, 2007), the efficiency of bioleaching is dramatically reduced in moderately brackish/saline water (Watling et al., 2010; Deveci et al., 2008; Shiers et al., 2005; Suzuki et al., 1999). Accumulation of saline anions such as chloride and sulfate, can acidify microbial cells responsible for bioleaching, resulting in loss of activity and ultimately, cell death (Zammit et al., 2012; Gahan et al., 2010; Simmons and Norris, 2002; Alexander et al., 1987).

Previously, research has indicated that as little as 1.5 g L^{-1} chloride can inhibit microbial activity within a bioleaching heap (du Plessis et al., 2007); however, bioleaching microorganisms can withstand greater chloride concentrations in laboratory pure cultures (Gahan et al., 2010; Nicolle et al., 2009; Davis-Belmar et al., 2008; du Plessis et al., 2007; Shiers et al., 2005; Kamimura et al., 2005; Blight and Ralph,

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http://dx.doi.org/10.1016/j.hydromet.2016.07.006
0304-386X/

2004; Kamimura et al., 2003; Huber and Stetter, 1989). In contrast to chloride, acidophilic bioleaching microorganisms have been shown to tolerate significantly higher concentrations of sulfate ranging from 100 to 136.5 g L^{-1} (Rea et al., 2015; du Plessis et al., 2007; Shiers et al., 2005; Blight and Ralph, 2004). The tolerance to sulfate is largely dependent on temperature and the growth substrate, with mesophilic bioleaching microorganisms tolerating higher concentrations than thermophiles, and with sulfur oxidisers able to tolerate much higher concentrations than iron oxidising acidophiles (Rea et al., 2015). In a heap and stirred tank process environment, sulfate concentrations can exceed 130 and 145 g L^{-1}, respectively (Watling et al., 2010). Therefore, it is likely that bioleaching efficiency would be compromised in the presence of such high sulfate concentrations.

Most microorganisms typically adopt mechanisms to cope with increasing salt concentrations. Some are able to produce compatible solutes that help to prevent desiccation and lysis of the cell (Dartnell, 2011; Santos and da Costa, 2002). These mechanisms have previously been identified in common bioleaching microorganisms such as *Acidithiobacillus ferrooxidans*, *At. thiooxidans* and *At. caldus* (Guo et al., 2013; Norris et al., 2010; Csonka, 1989; Kieft and Spence, 1988). Studies looking at the adaptation of non-salt tolerant microbial strains to increasing salt concentrations have also been undertaken and suggest that prolonged adaptation may improve tolerance to extreme growth conditions (Rea et al., 2015; Zammit et al., 2012).

Previously, Rea et al. (2015) bio-prospected naturally occurring salt tolerant microorganisms from groundwater, surface water, sediments and soils from the Western Australian Wheatbelt for potential application in bioleaching processes where fresh water is scarce. The Wheatbelt area is highly saline and acidic due to clearing of native vegetation, disturbance of pyritic soil profiles, rising of the acidic groundwater table, salinisation of sediments, and mobilisation of heavy metals (Degens et al., 2012; Hatton et al., 2003; Wood and Kelly, 1991). Enriched acidophilic iron and sulfur oxidising microorganisms were adapted to increasing sulfate concentrations, and the study identified some iron and sulfur oxidising cultures with higher sulfate tolerance than has been previously reported (Rea et al., 2015).

The current study aimed to determine the impact of sulfate concentrations on the efficiency of chalcopyrite bioleaching at mesophilic (30 °C), moderately thermophilic (45 °C) and thermophilic (60 °C) temperatures with cultures that were enriched from saline and acidic samples or sourced from culture collections, and subsequently adapted to increasing sulfate concentrations. The efficiency of chalcopyrite bioleaching in elevated sulfate concentrations at 30, 45 and 60 °C were compared to that of previous studies undertaken at similar temperatures with non-sulfate backgrounds. The molecular diversity of the sulfate tolerant bioleaching cultures was also investigated, and the impact of high sulfate backgrounds on the consistency and quality of DNA extractions were thoroughly discussed.

Ultimately, the adaptation and identification of salt tolerant acidophilic bioleaching cultures has the potential to reduce the requirement for fresh, potable water, whilst also improving the bioleaching efficiency of low grade minerals with anionic impurities.

2. Materials and methods

2.1. Culturing and adaptation

Previously, environmental cultures were enriched from samples obtained from the South-West of Western Australia (WA), in an area called the Wheatbelt (Rea et al., 2015). In addition to enriched environmental cultures, pure cultures were revived from the CSIRO Biotechnology culture collection and obtained from other international culture collections for adaptation and bioleaching studies (Rea et al., 2015). The successive adaptation of these iron- and sulfur-oxidising enrichment and culture collection cultures to increasing increments of sulfate in the form of $MgSO_4 \cdot 7H_2O$ was previously described (Rea et al., 2015).

Mixed inocula were prepared for mesophilic, moderately thermophilic and thermophilic bioleaching cultures. Briefly, equal volumes of each adaptation culture grown at the corresponding temperature (30, 45 or 60 °C) were added to a 250 mL flask and mixed. A 10% (v/v) aliquot of mixed inoculum was added to each bioleaching culture flask, which contained basal medium (g L^{-1}: KH_2PO_4, 0.25; $(NH_4)_2SO_4$, 1.5; $MgSO_4 \cdot 7H_2O$, *x*; pH 1.8). The final sulfate concentration (as denoted by *x*) for each of the bioleaching cultures were dictated by the maximum tolerable sulfate concentration observed for the individual iron and sulfur oxidising cultures (Rea et al., 2015; Table 1). Cultures were grown on 3% (w/v) chalcopyrite concentrate (Mount Isa Mines, QLD, Australia). The cultures were also supplemented with a small amount (0.1 g L^{-1}) of yeast extract as previously described by Rea et al. (2015) in order to support the growth of potential heterotrophic bioleaching microorganisms in addition to the chemolithoautotrophic microorganisms that may be present in these microbial cultures.

Bioleaching cultures were incubated at 30, 45 or 60 °C for three successive subcultures (1 week of growth) prior to commencement of bioleaching tests in order to adapt the sulfate tolerant microorganisms to the presence of the chalcopyrite ore as a growth substrate instead of soluble ferrous and elemental sulfur.

Chalcopyrite concentrate used for culturing and bioleaching tests was obtained from Mount Isa Mines (MIM), washed in 100% ethanol and then rinsed three times in deionised water to remove organic contaminants that may have remained in the concentrate following the flotation process (Rea et al., 2015). The washed concentrate was recovered by filtration (Whatman paper No. 4), dried at 50 °C and then sterilised by tyndallisation (1 h at 100 °C for 3 successive days).

2.2. Bioleaching tests

After three successive subcultures, bioleaching cultures grown at 30, 45 and 60 °C were scaled up to 1 L. Aliquots of each scaled up culture were sonicated for 5 min in 1 min cycles to detach cells from mineral particles. The ore was then separated from solution by low speed centrifugation (1600 × *g*, 1 min) and cells were harvested from supernatant at 21,800 × *g* for 30 min. Cells were resuspended in 50 mL of basal medium with either 100, 40 or 80 g L^{-1} of sulfate, dependent on the temperature of growth (Table 1).

Bioleaching tests were undertaken with basal medium (pH 1.8) containing various concentrations of sulfate and 3% (w/v) chalcopyrite concentrate. The tests were also supplemented with 0.1 g L^{-1} yeast extract to mimic the growth conditions of the adapted cultures (Rea et al., 2015). The 30, 45 and 60 °C bioleaching tests were inoculated with 10% v/v inoculum. The minimum density of cells added to each bioleaching test was 5×10^8 cells mL^{-1}. The bioleaching tests were undertaken in duplicate at 30, 45 and 60 °C with shaking at 150 rpm. Over a period of 31 days, samples were collected at days 0, 3, 7, 10, 14, 19, 26 and 31 for chemical analyses. Abiotic controls were also run concurrently at each temperature.

Total cells were enumerated in triplicate under phase contrast using a Thoma ruled haemocytometer (100× objective). Solution pH and redox potential were measured immediately after sampling using a

Table 1
Sulfate concentrations used for bioleaching experiments as determined by the maximum tolerable sulfate concentration observed for the individual iron and sulfur oxidising cultures (Rea et al., 2015).

Concentration (g L^{-1})	Mesophilic (30 °C)		Moderately thermophilic (45 °C)		Thermophilic (60 °C)	
	Fe	S	Fe	S	Fe	S
$MgSO_4 \cdot 7H_2O$	225	350	100	225	175	225
Mg^{2+}	22.20	35	9.87	22.20	17.27	22.20
SO_4^{2-}	87.68	136.7	38.97	87.68	68.20	87.68
SO_4^{2-} for bioleach tests	100		40		80	

Please cite this article as: Boxall, N.J., et al., Effect of high sulfate concentrations on chalcopyrite bioleaching and molecular characterisation of the bioleaching microbial comm..., Hydrometallurgy (2016), http://dx.doi.org/10.1016/j.hydromet.2016.07.006