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Overcoming the bacteriostatic effects of heavy metals on *Acidithiobacillus thiooxidans* for direct bioleaching of saprolitic Ni laterite ores

Hee-Chan Jang, Marjorie Valix *

School of Chemical and Biomolecular Engineering, University of Sydney, NSW 2006, Australia

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ABSTRACT

In this study, the adaptation of *Acidithiobacillus thiooxidans* to heavy metals leached from saprolitic Ni laterite ores from Riverina, Australia, was performed by gradual acclimatisation. The microorganism was cultivated in heavy metals (Ni, Co, Fe, Mg, Cr and Mn) with total concentrations of 2400 to 24,000 ppm equivalent to total dissolution of 1 to 10% (w/v) pulp densities of the saprolitic Ni laterite ore. Adaptation evolution mapped from its tolerance index was found to be dependent on metal concentration, acid generation, and period of adaptation. Bio-stimulation of cell growth and acid production was promoted by heavy metal stress on the bacteria. Pre-established heavy metal tolerance of the bacterial strain improved the leaching rate in its early phase; 20% and 7% increase in Ni and Co metal recoveries were observed in using adapted bacteria. However heavy metal tolerance was also achieved by the bacterium during the leaching process, albeit delayed by a lag phase. These results confirm the robust nature and suitability of *A. thiooxidans* in direct bioleaching of Ni ores.

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1. Introduction

Nickel (Ni) is a valuable metal mined from two main ore types: sulfides and laterites (oxide-type ores). In 2015, despite an oversupply of Ni and its weak price, mining industries around the world continue to bring on new Ni mining and processing projects in anticipation of a turnaround in the global economy (Geological Survey, 2016). In addition, the decline in discovery of new sulfide deposits has motivated the exploration and exploitation of low-grade Ni laterite ores. Australia, in particular, is one of the world major producers of Ni with an abundant source of Ni laterites (Dalvi et al., 2004; Mudd, 2010). In the past few years, the use of Ni laterite minerals have dominated the Ni production in Australia (Mudd, 2010). However, its low-grade and mineralogical complexity require intensive hydrometallurgical or pyrometallurgical processing such as high pressure acid leaching (HPAL) and reduction roasting based on the Caron process (Mudd, 2009; Oxley and Barcza, 2013). The long-term demand of Ni continue to push the interest for a more sustainable, cost-effective and eco-friendly alternatives to these conventional physiochemical extraction processes (Mishra et al., 2005).

Biomining is considered a promising technology in the extraction of metallic values from Ni laterite ores in particular when grades are sub-economic for the conventional technologies (Johnson, 2008; Simate et al., 2010). Laterite oxides have been shown to be amenable to the actions of heterotrophic fungi and to acidophilic *Acidithiobacilli* spp. The fungi generates organic acids and the acidophilic bacteria generates sulfuric acids, both which facilitate metal leaching (Gadd, 2001; Tzeferis, 1994; Valix et al., 2001b). However, the direct "in-situ" bioleaching environment amidst the abiotic stresses has been the major challenge for a successful application of this technology (Simate et al., 2010). The remarkable adaptability of chemoautotrophic microorganisms has the potential to contribute to enhanced biomining efficiencies of low-grade Ni laterites. In particular, *Acidithiobacillus thiooxidans* is an acidophilic mesophile that is tolerant to extreme acidic environments (at a pH of 1 or below). Its metabolite, mainly sulfuric acid, can play an important role in dissolution of host minerals and also maintaining pH levels below the secondary reactions that hinders metal extraction from lateritic ores (Saidan et al., 2012). Management of microbiology within the leaching process continues to be a key area of research in this field (Johnson, 2008). Achieving stable populations of microbes with the required functionality is crucial for efficient bioprocessing. This, however, is challenged by the many possible inhibitory factors, foremost is the bacteriostatic and in some cases biocidal effects of leached heavy metals (Watling, 2008). Microorganisms have a biphasic response to heavy metals (Bae and Chen, 2004). At low levels, heavy metals stimulate the growth of organisms. However at higher concentrations, heavy metals can inhibit the growth and metabolism of microorganism. The effect of these toxins can either be considered: i) bactericidal where the effect is lethal and organisms are unable to grow even when toxins are removed; or ii) bacteriostatic where organism growth is inhibited, but is reversible (Randall et al., 2013; Seil and Webster, 2012). In this study because organisms are able to acclimatise to heavy metals the toxin effects was considered bacteriostatic. The bacteriostatic effect of

* Corresponding author.
E-mail addresses: hee-chan.jang@sydney.edu.au (H.-C. Jang), marjorie.valix@sydney.edu.au (M. Valix).

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Table 1
Elemental composition of Ni saprolitic ores.

Metal components	wt%
Mg	17.09
Fe	5.81
Ni	0.92
Mn	0.18
Co	0.056
Cu	0.02

heavy metals such as Ni^{2+} and Cu^{2+} involve binding of these metal ions to the cell surface and thus, disrupting the enzyme catalysed sulfur oxidation and reproduction (Hu et al., 1996; Maeda et al., 1996). This study examined and compared the evolution of heavy metal adaptation of *A. thiooxidans* in two environments: sequential acclimatisation to predetermined heavy metal concentration steps and in the bioleaching of saprolitic Ni laterite ores. This was performed to establish the potential merit of pre-established adaptation of the microorganism to heavy metals on its growth, acid production and its ability to dissolve the metallic fractions of Ni oxide ores.

2. Material and methods

2.1. Ore preparation and characterisation

The saprolitic Ni laterite ore was obtained from Riverina, Australia, and prepared in-house. The raw ores were milled using the TEMA mill (T 100 mill ball) and screened between 63 and 180 μm. The sieved ores were then sampled using coning and quartering method for mineralogical and elemental compositional analysis and the remainder was used for the leaching studies.

Mineralogical analysis of the saprolitic ore was conducted using a Siemens D5000 X-ray diffractometer (XRD). The study revealed the presence of serpentine $(Mg,Fe,Ni)_3Si_2O_5(OH)_4$, goethite (Fe,Ni)O(OH), hematite Fe_2O_3, and quartz SiO_2. No separate nickel bearing mineral phase was found in the saprolitic minerals.

Elemental analysis of the saprolitic ore (Table 1) was performed using PANalytical PW2400 Sequential wavelength-dispersive X-ray fluorescence (WD-XRF) Spectrometer with rhodium end-window tube. The sample was prepared in 40 mm glass bead at 1050 °C for 15 min (Norrish and Hutton, 1969). The SuperQ software was used with PANalytical WROXI (wide-ranging-oxides) calibration to determine the elemental concentrations (in weight percent). These data were then used to calculate the equivalent elemental compositions that would be present in the given pulp densities (Kelloway et al., 2014). For example the metal composition in pulp density of 1.0%, the mass of the ore is 1.0 g in 100 mL of leaching solution. The metal composition present in 1.0 g of ore was calculated using the XRF analysis of the ore in mg and divided by 0.1 L to give metal concentrations in terms of ppm.

Table 2
Elemental concentration (ppm) of various pulp densities of saprolitic ores.

Pulp density (%, w/v)	1	2	5	10
Metal components	Equivalent heavy metal concentration (ppm)			
Mg	1709	3418	8545	17,090
Fe	581	1162	2905	5810
Ni	92	184	460	920
Mn	18	36	90	180
Co	5.6	11.2	28	56
Cu	2	4	10	20
Total	2407.6	4815.2	12,038	24,076

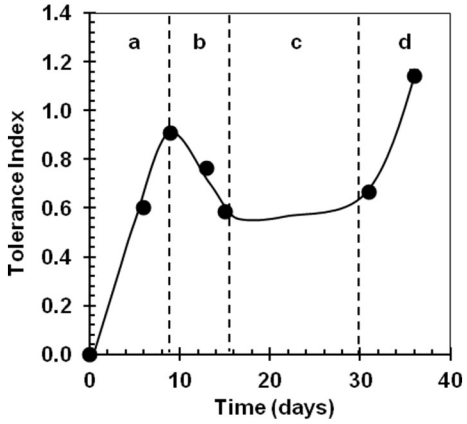

Fig. 1. The growth phases of *A. thiooxidans* in the presence of 1% heavy metals relative to control grown in absence of heavy metals. Legend: a - rapid growth; b - retarded growth; c - similar growth; d - enhanced.

2.2. Heavy metal adaptation

The *A. thiooxidans* (ATCC 8085) used in this study was grown aerobically in shake-flask using a modified ATCC Medium 125, consisting of 1 L distilled water, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.25 g/L $CaCl_2$, 0.2 g/L $(NH_4)_2SO_4$, 3.0 g/L KH_2PO_4, and 5.0 mg/L $FeSO_4$, with 25 g/L S^0 as the energy source. The pH of the medium was then adjusted to 1.5 with 10 M H_2SO_4. The salt solution was filter-sterilised using 0.45 μm membrane-filtration, while S^0 was sterilised separately by tyndallisation. The growth medium was inoculated with 1×10^8 cell/mL of bacterial strains. The inoculated culture was then incubated in a shaker at 30 °C, 150 rpm for 35 days. The bacterial strain was adapted by subculturing 1% (v/v) of 100 mL fermentation broth and gradually exposing them to growth medium containing higher heavy metal (HM) concentrations from 2400 to 24,000 ppm (see Table 2) for 140 days (with 35 days for each HM concentration). The bacterial growth population was monitored by cell counts using Nebauer Counting Chamber and its acid production by pH measurement using Dual Channel pH-ORP-Temperature Sensor. The adaptive evolution of bacterium was mapped by measuring the growth tolerance index (TI) with time (Eq. (1)) (Valix and Loon, 2003). The TI method of measuring the bacteriostatic effect was used as it is able to measure tolerance development of a microorganism over an extended period of time through sequential exposure to the toxins unlike minimum inhibitory concentration (MIC) approach,

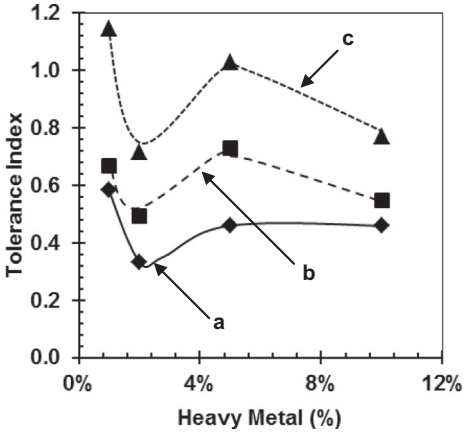

Fig. 2. Development of relative heavy metal tolerance of *A. thiooxidans* with growth phase: (♦) a - retarded growth (Day 13); (■) b - similar growth (Day 15); (▲) c - enhanced (Day 35).

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