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Effect of saline stress on fungi metabolism and biological leaching of weathered saprolite ores

V. Thangavelu, J. Tang, D. Ryan, M. Valix *

Department of Chemical Engineering, The University of Sydney, NSW 2006, Australia

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Abstract

Biological leaching of nickel laterite ores is based on the use of heterotrophic microorganisms and their metabolic products to dissolve nickel and cobalt from oxide minerals. High salinity of water supplies and soils in the vicinity of nickel laterite ore bodies can be a major challenge in the application of bio-leaching process in situ. Salt stress can imbalance the osmotic potential in fungi cells generating a water deficit and the influx of sodium may lead to metabolic toxicity. The purpose of this study is to examine salt tolerance development of *Aspergillus foetidus* using gradual acclimatization technique to salt concentrations up to 2% and to assess the use halotolerant microorganisms in leaching weathered saprolite ores under saline conditions. It has been observed that salinity stress affects the growth but not the energy metabolism of the organism. Kinetic of metal leaching, nature of secondary reactions and metal dissolutions were also influenced by salt stress.

Keywords: Oxide ores; Bio-leaching

1. Introduction

Nickel laterite ores remain important to the future supply of Ni and Co as they contain the bulk of known nickel and cobalt reserves (80% and 90–95% respectively) (Canterford, 1971; Valix et al., 2001a,c). Basic research that will deliver a step change in technology is required for these deposits to be worked economically. Current commercial extractions of Ni and Co from nickel laterite ores are energy intensive and operational costs are high. Despite these difficulties, processing of laterite deposits continues to grow strongly. There is a need for viable processes that can address the economic and environmental restrictions currently associated with the processing of nickel laterite.

Microbial leaching of oxide ores has the potential to offer a much needed step change in the technology for processing laterite ores. Biological leaching of low-grade nickel laterite is based on a non-traditional leaching of oxide minerals using heterotrophic microorganisms. The organisms solubilise metals by excreting organic acids; these acids then form complexes with heavy metals. The use of fungi has dominated most studies; these fungi have been found to solubilise metals more efficiently than bacterial microorganisms (Bosecker, 1985; Castro et al., 2000). *Aspergillus* and *Penicillium* species are considered the most efficient in acid production and are thus used in most bio-leaching tests (Crueger, 1990). In this study, *Aspergillus foetidus* was used in bio-leaching tests. The use of microorganisms and their metabolic products has a number of advantages: it has lower energy requirements than conventional hydrometallurgical and smelting process; it is more flexible and selective; and it resolves environmental concerns about the toxicity of chemicals and their containment during the metal extraction processes (Lundgren et al., 1986).

Fungi are heterotrophic eukaryotes which exhibit resilience in diverse and extreme environments. Environmental

* Corresponding author. Tel.: +61 2 9351 4995; fax: +61 2 9351 2854.
E-mail addresses: jtang@chem.eng.usyd.edu.au (J. Tang), mvalix@chem.eng.usyd.edu.au (M. Valix).

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stress, such as those which the organism will experience in in situ bio-leaching operations, however will change the structure or metabolism of the organism in order to protect itself. Exposure of the fungi cells, for example, to saline stress implies both exposure to specific cation toxicity (Na^+) and osmotic stress. Such ions are toxic to cells due to their ability to inhibit specific metabolic pathways (Posas et al., 2000). It is therefore necessary to establish these effects and in particular their effects on the bio-leaching of nickel laterite ores, the application of interest in this study. The presence of high salinity in most underground water sources close to laterite ore bodies, in particular, Western Australia is of concern. In this study, abiotic factor arising from the presence of high salinity was of interest (Laing et al., 1988). To circumvent the effect of salinity stress to *A. foetidus*, the organism was adapted to tolerate the presence of salt by gradual acclimatization which has previously been proven to successfully develop fungi organism with tolerance to certain heavy metals (Valix et al., 2001a). The metabolism and the bio-leaching properties of the adapted organism were established in this study.

2. Experimental

2.1. Ore material

The laterite ore used in this study is weathered saprolite obtained from New Caledonia. This saprolite is distinguished by its rich magnesium silicate content. The sample was prepared by milling to produce a mean particle size of 53 μm. The chemical composition of the ore is reported in Table 1.

2.2. Microorganism

The microorganism used in this study is *A. foetidus* (FRR5041). This microorganism was obtained from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Food Science & Technology Culture Collection, Australia.

2.3. Adapting fungi to saline stress

2.3.1. Media preparation

In this study, Czapek yeast extract agar (CYEA) was used as the growth medium for the *A. foetidus* and is represented in Table 2.

Salt (NaCl) was added to the CYEA growth media prior to sterilization of the solution in an Oualtex autoclave at 121 °C for 20 min. The sterilized medium was removed from the autoclave at about 60 °C then poured into petri dishes in a GELAIRE 20BHEN2006D laminar flow hood which was previously sterilized by ultraviolet (UV) light. The medium was then inoculated with the fungi strain using a sterilized loop.

Table 2
Czapek yeast extract agar (CYEA)

Component	g/0.25 l
Distilled water	0.25
K_2HPO_4	0.25
$NaNO_3$	0.75
$MgSO_4 \cdot 7H_2O$	0.125
KCl	0.125
$FeSO_4 \cdot 7H_2O$	0.0025
Sucrose	7.5
Yeast extract	1.25
Agar	3.75
Trace elements (ml)	0.25

Trace elements consist of (w/v) 1% $ZnSO_4 \cdot 7H_2O$ and 0.5% $CuSO_4 \cdot 5H_2O$.

2.3.2. Salinity tolerance adaptation

Strain adaptation was conducted through a series of repeated sub-culturing of the fungi to different salt concentrations. Acclimatization was conducted in petri dishes, 8 cm in diameter, containing the growth medium with salt concentration from 0 or the control to 2000 ppm. The plates were inoculated by placing a 4 mm circular paper previously dipped into the growing fungi strain. The inoculated plates were incubated at 30 °C for 9 to 18 days. The growth was monitored by measuring the spread of the culture from the point of inoculation or centre of the colony to the end of the longest hyphae. The tolerance index, an indication the organism response to salt stress was calculated from the growth of strain exposed to the salt divided by the growth in the control plate. The growth of the fungi strain was mapped according to procedures outlined by Valix et al. (2001a) (see Fig. 1). Fungi subjected to abiotic factors responded with a growth pattern characterised by five stages: (a) lag phases which occur at the beginning of the inoculation, where very little growth occurs, (b) rapid growth rate but absolute growth is often suppressed relative to the control, (c) decline in growth rate, (d) leveling of the tolerance index indicative that the rate of growth of fungi with metals and control are similar and finally (e) second growth phase were the absolute growth rate often exceeds the control (tolerance index greater than 1). Strains demonstrating growth at phase "e" were sampled and further exposed to a higher salt concentration.

2.4. Bio-acid production

To establish the metabolism of the halotolerant *A. foetidus* organism, the strain was used in bio-acid production

Table 1
Chemical analysis of weathered saprolite ore

Sample	Ni (wt.%)	Co (wt.%)	Fe (wt.%)	Mn (wt.%)	Mg (wt.%)	SiO_2 (wt.%)
Weathered saprolite	2.25	0.035	8.1	0.13	15.8	34.9