Integration of Scientific and Industrial Knowledge on Biohydrometallurgy

Edited by
Nicolas Guilliani, Cecilia Demergasso, Raquel Quatrini,
Francisco Remonsellez, Carol Davis-Belmar,
Gloria Levican, Pilar Parada,
Carlos Barahona and Rebekah Zale

TRANS TECH PUBLICATIONS
Effects of individual use, mixed culture and sulfur addition on the effectiveness of nickel laterite ore bioleaching with Penicillium verruculosum and Galactomyces geotrichum (Conference Paper)

Mubarak, M.Z.a, Astuti, W.b, Chaeun, S.K.c

a Department of Metallurgical Engineering, Institut Teknologi Bandung, Jl. Ganesha 10, Bandung 40132, West Java, Indonesia
b Mineral Processing Division of Indonesian Institute of Sciences (LPI), Jl. Ir. Soemarmo Km. 16, Tanjung Bintang, Lampung Selatan, Lampung, Indonesia
c Laboratory of Bioenvironment, Mining and Environmental Bioengineering, School of Life Sciences and Technology, Institut Teknologi Bandung, Jl. Ganesha 10, Bandung 40132, West Java, Indonesia

Abstract

In the present study, the influences of individual use of Penicillium verruculosum and Galactomyces geotrichum on the nickel extraction from Indonesian laterite ore are compared with that of the fungi mixed culture. The effect of sulfur addition in the mixed culture of Penicillium verruculosum and Galactomyces geotrichum on the sulfate ion formation was also investigated. Shake flask bioleaching assays have been performed in a medium growth of Indonesian food production waste over the course of 28 days. It was observed that the mixed culture of Penicillium verruculosum and Galactomyces geotrichum had a higher effect in nickel extraction compared with the use of individual fungi for both seppellite ore. For seppellite ore, the fungi species of Galactomyces geotrichum demonstrated a greater nickel extraction than Penicillium verruculosum. For limonite ore, the extraction of nickel by Penicillium verruculosum was slightly more effective than the mixed culture and Galactomyces geotrichum. The addition of elemental sulfur in the mixed culture was found to generate sulfate anion. The concentration of sulfate increased with time from 1.6 g/l to 8.3 g/l within 28-days of incubation. © (2013) Trans Tech Publications, Switzerland.

Author keywords

Extraction; Fungal bioleaching; Laterite ore; Nickel; Sulfur

Indexed keywords

Elemental sulfur; Food production; Galactomyces geotrichum; Laterite ores; Nickel extraction; Nickel laterite; Penicillium verruculosum; Sulfate anions

Engineering controlled terms: Bioleaching; Fungi; Nickel; Ore treatment; Ores; Rocks; Soils; Sulfur

Engineering main heading: Extraction

References (5)

Jain, N., Sharma, D.K.

1 Biohydrometallurgy for nonsulfidic minerals - A review


doi: 10.1080/01490450490275371

View et Publisher

http://www.scopus.com/record/display.uri?eid=2-s2.0-84886777984&origin=resultslist&sort=plf&src=sst1+astu&st2=wid1nl&nl=1&rl=20&nl=1&count=f...
Mubarak, M.Z., Astuti, W., Chaerun, S.K.
Changsha, China

Mubarak, M.Z., Astuti, W., Chaerun, S.K.
Bodrum-Turkey

Tang, J., Velix, M.
Article 5

Department of Metallurgical Engineering, Institut Teknologi Bandung, Jl. Ganesha 10, Bandung 40132, West Java, Indonesia
© Copyright 2013 Elsevier B.V., All rights reserved.
Integration of Scientific and Industrial Knowledge on Biohydrometallurgy

Volume 825
doi: 10.4028/www.scientific.net/AMR.825

Info
Authors: Nicolas Guillani, Cecilia
Editors: Demergasso, Raquel Quatrini, Francisco Remonsellez, Carol Davis-Belmar, Gloria Levican, Pilar Parada, Carlos Barahona and Rebekah Zale
Category: Selected, peer reviewed papers from the 20th International Biohydrometallurgy Symposium (IBS2013), October 8-11, 2013, Antofagasta, Chile
Pages: 600
## Description


The main focus of this collection of peer-reviewed articles is biohydrometallurgy. This is the field of microbial ecology which is the key to answering central questions concerning not only the diversity and behavior of micro-organisms in commercial operations, but also possible applications in biohydrometallurgy of extremophiles coming from very different environments.

The 134 papers are grouped as follows:

- Chapter 1: Microbial Ecology, Geomicrobiology and Bioprospecting in Natural and Mining Environments;
- Chapter 2: Omics, Molecular Genetics and Biochemistry of Microorganisms in Mining Processes;
- Chapter 3: Industrial Biohydrometallurgy: Studies, Practices and Operation;
- Chapter 4: Biohydrometallurgy as a Remediation Strategy.

**Review from Ringgold Inc., ProtoView:** About 180 selected and peer-reviewed papers cover microbial ecology, geomicrobiology, and bioprospecting in natural and mining environments; -omics, molecular genetics, and the biochemistry of microorganism in mining processes; studies, practices, and operations in industrial biohydrometallurgy; and biohydrometallurgy as a remediation strategy. Among specific topics are microbial ecology in extreme acidic pit lakes of the Aberian Pyrite Belt in southwestern Spain, galactose as an inducer of the production of extracellular polymeric substances by *Acidithiobacillus ferrooxidans*, evidence for widespread dissimilatory hydrogen metabolism among acidophilic bacteria, immobilizing new isolated iron oxidizing bacteria on natural carriers, and the influence of pyrite on the galvanic assisted leaching of chalcocite concentrates.

**Buy this volume**
Useful Links

Subscriptions (/ACCOUNT/SUBSCRIPTIONS)
FOR EDITORS (/INFO)
LOG IN (/ACCOUNT/LOGON?
RETURNURL=%2FAMR.825%2FBBOOK)
MY CART (/PAYMENT/CART)
SEARCH (/PAPER/SEARCH)
CONTACT US (/HOME/CONTACTS)
TERMS AND CONDITIONS (/HOME/TERMSANDCONDITIONS)
POLICY AND ETHICS (/HOME/POLICYANDETHICS)
CONFERENCE ETHICS AND QUALITY CONTROL (/HOME/CONFERENCEETHICSANDQUALITYCONTROL)
DISTRIBUTORS (/DISTRIBUTOR)

Recent Themes

- Engineering and Technology on Non-Ferrous Metals (/KEM.682)
- Wear and Contact Mechanics II (/KEM.681)
- Properties and Testing Techniques of Inorganic Materials (/KEM.680)
Effects of Individual Use, Mixed Culture and Sulfur Addition on the Effectiveness of Nickel Laterite Ore Bioleaching with *Penicillium verruculosum* and *Galactomyces geotrichum*

M.Z. Mubarok\(^1\)^, W. Astuti\(^2\) and S.K. Chaerun\(^3\),\(^c\)

\(^1\)Department of Metallurgical Engineering, Institut Teknologi Bandung, Jl. Ganesha 10, Bandung 40132, West Java, Indonesia

\(^2\)Mineral Processing Division of Indonesian Institute of Sciences (LIPI), Jl. Ir. Sutami Km. 15, Tanjung Bintang, Lampung Selatan, Lampung, Indonesia

\(^3\)Laboratory of Biogeosciences, Mining and Environmental Bioengineering, School of Life Sciences and Technology, Institut Teknologi Bandung, Jl. Ganesha 10, Bandung 40132, West Java, Indonesia

\(^a\)email:zaki@mining.itb.ac.id, \(^b\)email:widi004@lipi.go.id, \(^c\)email:skchaerun@gmail.com

**Keywords:** Fungal bioleaching, nickel, laterite ore, sulfur, extraction

**Abstract.** In the present study, the influences of individual use of *Penicillium verruculosum* and *Galactomyces geotrichum* on the nickel extraction from Indonesian laterite ore are compared with that of the fungus mixed culture. The effect of sulfur addition in the mixed culture of *Penicillium verruculosum* and *Galactomyces geotrichum* on the sulfate ion formation was also investigated. Shake flask bioleaching assays have been performed in a medium growth of Indonesian food production waste over the course of 28 days. It was observed that the mixed culture of *Penicillium verruculosum* and *Galactomyces geotrichum* had a higher effect in nickel extraction compared with the use of individual fungi for both saprolite ore. For saprolite ore, the fungi species of *Galactomyces geotrichum* demonstrated a greater nickel extraction than *Penicillium verruculosum*. For limonite ore, the extraction of nickel by *Penicillium verruculosum* was slightly more effective than the mixed culture and *Galactomyces geotrichum*. The addition of elemental sulfur in the mixed culture was found to generate sulfate anion. The concentration of sulfate increased with time from 1.6 g/l to 8.3 g/l within 28-days of incubation.

**Introduction**

Bioleaching has been regarded as one of alternative methods for extraction of nickel and cobalt from laterite ore. Since the laterite ore contains no sulfur needed for the energy source of chemolithotrophic microorganism, the bioleaching of nickel laterite ore usually utilizes heterotrophic microorganisms either bacteria or fungi which requires organic carbon as the source of energy for their life[1]. For bacteria, the genus of Thiobacillus and Pseudomonas have been reported to perform effectively for bioleaching of oxide minerals, while fungi species that have been widely used are from the genus of *Penicillium* and *Aspergillus*[1,2]. Principally, metals dissolution takes place through the reaction of the host minerals with organic acids generated by the microorganism activities. Several major types of organic acids secreted by the fungus metabolisms are citric, oxalic, lactic and gluconic acids. Citric acid was the most dominant organic acid generated by fungal consortia isolated from a mine area in South-East Sulawesi Province of Indonesia during 28-day of shake flask bioleaching assays of nickel laterite ore with a media of local food and sugar processing wastes (i.e. tofu, tempeh and molasses)[3]. Series of agitation leaching tests of saprolite and limonite ore samples by using several commercial organic acids also exhibited the highest nickel extraction by citric acid in comparison to oxalic, lactic and acetic acids [4].

The effectiveness of metals extraction during fungal bioleaching are dependent on the types of fungi used, culture media for the grow of the fungi, physico-chemical parameters of the leaching system (temperature, pH of the leachate, oxygen supply) as well as the mineralogical composition,
particle size distribution of the ore and slurry density[1]. Tang and Valix demonstrated that secondary reaction and adsorption of dissolved nickel onto gangue minerals, acid activity, pulp density and the capability of the acids to dehydroxylate the main gangue phase are decisive factors which determine nickel recovery by fungi metabolic acids [5]. In the present work, the effectiveness of individual and mixed cultur of *Penicillium verruculosum dan Galactomyces geotrichum* in bioleaching of Indonesian nickel laterite ore is discussed. The possibility of sulfate generation by the additions of sulfur elemental into the mixed culture of the fungus is also elucidated.

**Materials and Method**

The ore samples used in the investigation are limonite and low grade saprolite ore (LGSO) types received from a mine area in Pomaelas, South-East Sulawesi Province of Indonesia. After sampling, analysis of particle size distribution, drying and surface moisture determination, chemical composition of the ore sample was measured by X-Ray Fluorescence (XRF) Analysis. In the present study, the ore sample used had a particle size distribution of -100+200 mesh. Chemical composition of the ore sample is presented in Table 1. Predominant minerals in each ore type were clarified by X-Ray Diffraction (XRD) Analysis. The XRD analysis results reveal that the most predominant mineral in limonite ore sample was goethite (FeOOH) and quatz, while chrysotile and serpentine (3MgO.2SiO₂.2H₂O) were identified as the most predominant minerals in saprolite ore.

**Table 1. Chemical composition of the ore sample used in the investigation**

<table>
<thead>
<tr>
<th>Component</th>
<th>Limonite Wt%</th>
<th>LGSO Wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiO</td>
<td>1.65</td>
<td>1.76</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>40.9</td>
<td>8.18</td>
</tr>
<tr>
<td>CoO</td>
<td>0.20</td>
<td>0.035</td>
</tr>
<tr>
<td>Cr₂O₇</td>
<td>2.03</td>
<td>0.39</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>8.06</td>
<td>1.39</td>
</tr>
<tr>
<td>MgO</td>
<td>6.02</td>
<td>32.23</td>
</tr>
<tr>
<td>SiO₂</td>
<td>25.69</td>
<td>41.14</td>
</tr>
<tr>
<td>MnO</td>
<td>1.00</td>
<td>0.14</td>
</tr>
<tr>
<td>CaO</td>
<td>0.39</td>
<td>0.05</td>
</tr>
<tr>
<td>Na₂O</td>
<td>0.17</td>
<td>-</td>
</tr>
<tr>
<td>K₂O</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>LOI</td>
<td>13.56</td>
<td>14.63</td>
</tr>
</tbody>
</table>

The fungus consortia of *Penicillium verruculosum* and *Galactomyces geotrichum* were isolated from a nickel mining area in South Sulawesi Province of Indonesia. The fungus isolation was performed by serial dilution agar plating method in Fe-agar medium for three days. Biomolecular identification of the fungus isolate was conducted by amplification method in the area of Internal Transcribed Spacer (ITS) from ribosom DNA (rDNA) using Polymerase Chain Reaction (PCR) for primary couple of ITS 5 and ITS 4. The fungus isolate growth was conducted in 150 mL of Potato Dextrose Broth (PDB) which was rotated in a roating shaker for three days at room temperature. Domestic food and sugar production wastes (i.e. tofu, tempeh and molasses) were utilized as a media for 28-day shake flask bioleaching assays of the ore samples.

Series of the shake flask direct bioleaching assays were carried out by using 250 ml conical flasks in a rotary shaker at room temperature for 28 days. The effects of individual use and a mixed isolate of *Penicillium verruculosum* and *Galactomyces geotrichum* on the nickel extraction level was evaluated. The effect of sulfur elemental addition on the sulfuric acid generation was evaluated by adding 5% (w/w) elemental sulfur in the mixed isolate. The shake flask bioleaching assays were conducted by using ore particle size distribution of -100+200 mesh, 5% slurry density and shaking speed of 180 rpm. The leachates were periodically withdrawn from the flasks for pH measurement and dissolved metal concentrations analysis with Atomic Absorption Spectrophotometer (AAS). High-performance liquid chromatography (HPLC) analysis was performed to characterize the type of organic acids generated by the microorganisms, while sulfate ions concentration generated by sulfur oxidation within 28 day of the leaching was determined by titration method.
Result and Discussion

Based on the results of ITS 4F and ITS 5R biomolecular identification, it was found that the isolated fungi were *Penicillium verruculosum* dan *Galactomyces geotrichum*. The fungi consortia can be cultured in either chemical medium (i.e. Fe broth) as well as in the mixed of tofu and tempeh processing wastes and molasses. The investigation results show that the use of the mixed culture of *Penicillium verruculosum* and *Galactomyces geotrichum* perform sinergistic and higher effect in nickel extraction compared with the use of individual fungi for saprolite as illustrated in Fig. 1.a., while for limonite ore the extraction of nickel by *Penicillium verruculosum* was slightly more effective than the mixed culture and *Galactomyces geotrichum* (Fig. 1.b). *Galactomyces geotrichum* is a newly species being used for nickel bioleaching from laterite ore. Until recently, the bioleaching of nickel laterite ore mostly uses the fungi from the genus of *Aspergillus* and *Penicillium*.

![Figure 1. Profiles of Ni extraction a function of time using individual and mixed culture of *P. verruculosum* and *G. geotrichum* (a) saprolite ore and (b) limonite ore](image)

It was found that the mixed culture of *Penicillium verruculosum* and *Galactomyces geotrichum* promotes the oxidation of sulfur elemental to sulfate ions and resulted in a significant increase of sulfate ion concentration until approximately 8000 ppm within 28-day of incubation. Profiles of sulfate concentration and solution pH during 28-day of shake flask assays of saprolite ore are presented in Fig. 2.a. The increase of sulfate concentration still tends to occur by the extension of leaching duration beyond 28 days. In contrast with the investigations without the addition of sulfur, the solution pH at the presence of sulfur tends to decrease by leaching duration and slightly lower than the initial pH of the leachate after 28 days (Fig.2.a). However, the higher acidity by sulfuric acid generation did not result in the increase of nickel extraction in comparison with the condition without sulfur addition due to ore passivation by un-oxidized sulfur. The XRD spectra of the leaching residue from the 28-day shake flask assays of saprolite ore indicated the presence of residual elemental sulfur on the surface of mineral (Fig.2.b). It can be easily understood that an optimum sulfur dosage must be provided in order to give sufficient sulfuric acid generation with minimum risk of mineral passivation due to residual sulfur blocking effect on the surface of the ore.
Figure 2. (a) Profiles of sulfate concentration and solution pH during 28-day shake flask assays of saprolite ore (b) XRD spectra of the initial saprolite ore and the leaching residue in the presence of sulfur elemental.

Conclusion

The growth of *Galactomyces geotrichum* and *Penicillium verruculosum* can be conducted in a media of the mixed of tempeh and tofu processing wastes and molasses. Under specified condition, a single use of *Galactomyces geotrichum* in the shake flask bioleaching assays give a better nickel extraction than *Penicillium verruculosum* for saprolite ore and slightly lower nickel extraction for limonite. The use of mixed culture of *Penicillium verruculosum* and *Galactomyces geotrichum* has significantly higher effect in nickel extraction compared with the utilization of individual fungi for saprolite ore. In addition to the organic acids, the fungi consortia demonstrates the ability of promoting elemental sulfur oxidation to generate sulfuric acid and maintaining the leach acidinity. *Galactomyces geotrichum* is a newly fungus species being used for nickel bioleaching from laterite ore. The investigation results give notable insight for further using of *Galactomyces geotrichum* and its mixed culture with *Penicillium verruculosum* for nickel laterite ore bioleaching.

References


