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Effects of Individual Use, Mixed Culture and Sulfur Addition on the Effectiveness of Nickel Laterite Ore Bioelaching with *Penicillium verruculosum* and *Galactomyces geotrichum*

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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 fungis 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 chemolitotropic microorganism, the bioleaching of nickel laterite ore usually utilizes heterotropic microorganisms either bacteria or fungis 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 fungis metabolisms are citric, oxalic, lactic and glukonic acids. Citric acid was the most dominant organic acid generated by fungal concortia 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 fungis used, culture media for the grow of the fungis, 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 fungis 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 Pomalaa, 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 Fluoroscence (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 ($3MgO.2SiO_2.2H_2O$) were identified as the most predominant minerals in saprolite ore.

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

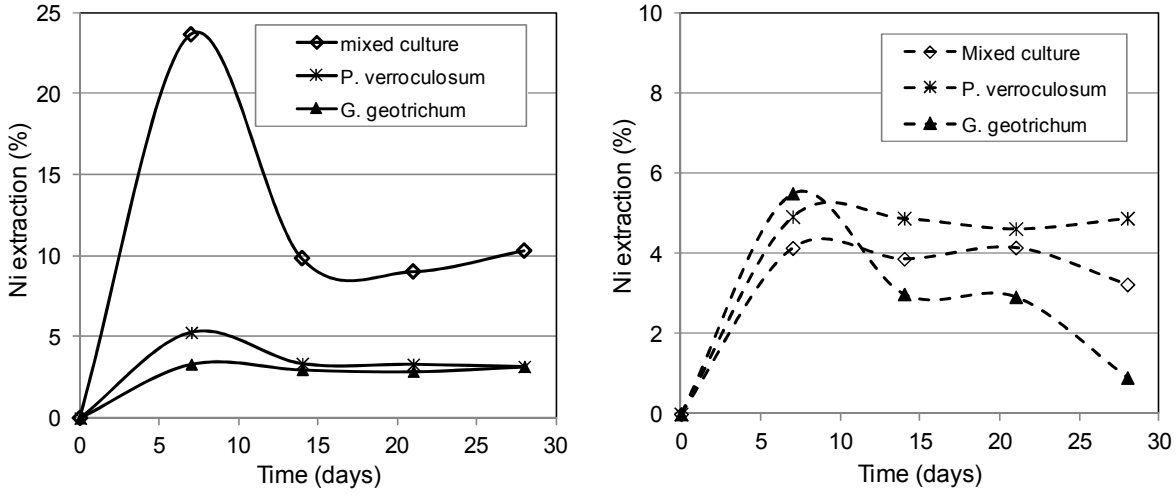
Component	Limonite	LGSO
	Wt%	Wt%
NiO	1.65	1.76
Fe_2O_3	40.9	8.18
Co_2O_3	0.20	0.035
Cr_2O_3	2.03	0.39
Al_2O_3	8.06	1.39
MgO	6.02	32.23
SiO_2	25.69	41.14
MnO	1.00	0.14
CaO	0.39	0.05
Na_2O	0.17	-
K_2O	0.03	0.01
LOI	13.56	14.63

The fungis concortia of *Penicillium verruculosum* and *Galactomyces geotrichum* were isolated from a nickel mining area in South Sulawesi Province of Indonesia. The fungis isolation was performed by serial dilution agar plating method in Fe-agar medium for three days. Biomolecular identification of the fungis 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 fungis 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

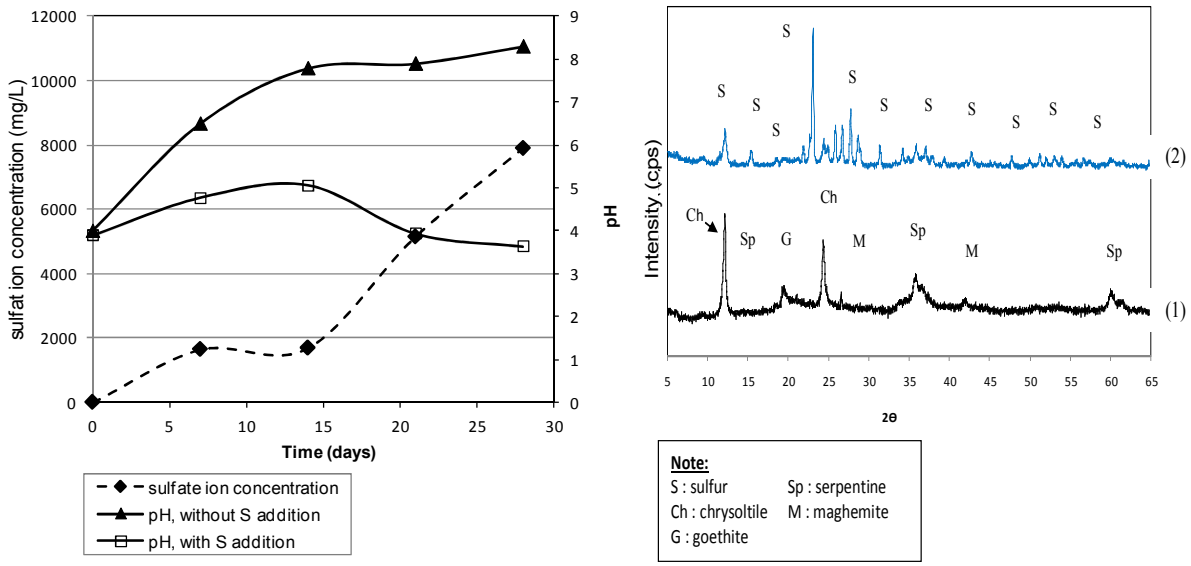
Based on the results of ITS 4F and ITS 5R biomolecular identification, it was found that the isolated fungis were *Penicillium verruculosum* dan *Galactomyces geotrichum*. The fungis concortia can be cultured in either chemical medium (i.e. Fe broth) as well as in the mixed of tofu and tempeh processsing 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 fungis from the genus of *Aspergillus* and *Penicillium*.



(a) (b)

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

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 ini 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.



(a) (b)

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 fungsi concortia demonstrates the ability of promoting elemental sulfur oxidation to generate sulfuric acid and maintaining the leachate acidity. 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.

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