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Short Communication

Bioleaching of Primary Nickel Ore Using *Acidithiobacillus ferrooxidans* LR Cells Immobilized in Glass Beads

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Abstract: Sulphide minerals are one of the most important sources of value metals. For several years, a large number of hydrometallurgical and biotechnological processes have been developed to leach low-grade sulphide ores and the conditions are well established. However, the management of microorganisms in the bioleaching process is not easy to handle. In this paper, the use of immobilized cells of *Acidithiobacillus ferrooxidans* LR in glass beads in bioleaching of primary nickel ore was evaluated. The column experiments inoculated with immobilized cells of *A. ferrooxidans* LR showed the same efficiency than the conventional method using free cells and is promising for application on a larger scale as it ensuring integrity and activity of biomining microorganisms and reduce process costs.

Keywords: Acidithiobacillus ferrooxidans LR; cell immobilization; primary nickel ore; bioleaching

1. INTRODUCTION

Nickel usually occurs in nature as sulphide or oxide forms and its concentrates commonly contain different proportions of iron sulphides (pyrite and pyrrhotite) and nickel (pentlandite). The nickel extraction from low-grade ores have been conducted in biohydrometallurgical process employing chemolithoautotrophic microorganisms which are capable of promoting redox reactions and solubilise the metal of interest [1, 2]. The proposed reactions for pentlandite microbial dissolution are as follows (Equations 1 to 4):

$$4(NiFe)_9S_8 + 93O_2 + 58H_2SO_4 \rightarrow 36NiSO_4 + 18Fe_2(SO_4)_3 + 58H_2O \quad (1)$$

$$(NiFe)_9S_8 + 18Fe_2(SO_4)_3 \rightarrow 9NiSO_4 + 45FeSO_4 + 8S^0 \quad (2)$$

$$2FeSO_4 + 1/2O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O \quad (3)$$

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \quad (4)$$

The acidophilic bacterium *Acidithiobacillus ferrooxidans* has a great importance on bioleaching of metal sulfide ores essentially due to its iron(II) oxidizing capacity. Adhered cells on low-grade ore are capable of regenerate iron(III), which are responsible by metal sulfides dissolution [3, 4].

Bioleaching process usually occurs in the presence of native microbial activity and also requires an addition of a consortium of mesophilic and/or thermophilic microorganisms that act simultaneously in iron and sulfur oxidation and solubilising precious and base metals [5]. A leaching solution containing specific biomining bacteria is conventionally produced in bioreactors and its inoculation on ore heaps and dumps are done by irrigation [6].

An alternative to reduce volume and costs of transport of leaching solutions and to improve a uniform inoculation of the ore could be the immobilization of biomining microorganism cells [7]. Microbial cells immobilization has also the advantage

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of allows more cells per volume of inoculum and can reduces damages against environmental toxic conditions [8]. Therefore, the objective of this present study was immobilize *A. ferrooxidans* cells in glass beads and evaluate its utilization as innoculum in mesophilic bioleaching of primary nickel ore experiments.

2. MATERIAL AND METHODS

Ore samples

A primary nickel ore, kindly provided by Mineração Serra da Fortaleza (Grupo Votorantim, Brazil), was studied. Subsamples of the bulk ore for column tests were crushed with a jaw crusher at particle size of 2 mm (9 mesh). Size fractions between 0.10 and 0.14 mm (150-100 mesh) were used in Erlenmeyer flasks experiments.

Microorganisms and culture conditions

Three microbial cultures were used in the tests. *Acidithiobacillus ferrooxidans* strain LR, *Lepstospirillum ferrooxidans* strain ATCC53992 and *Acidithiobacillus thiooxidans* strain FG-01 were originally isolated from uranium mine effluents in Brazil [9]. Ferrous sulphate mineral salts medium (TK medium) comprising 0.5 g L^{-1} $(NH_4)_2SO_4$; 0.5 g L^{-1} K_2HPO_4; 0.5 g L-1 $MgSO_4.7H_2O$; 1.5 g L^{-1} $FeSO_4.7H_2O$ [10] at pH 1.8 was used to maintain *A. ferrooxidans* LR and *L. ferrooxidans* ATCC53992 and to grow cells for bioleaching experiments. For *A. thiooxidans* FG-01, the same medium as used replacing ferrous sulphate by elemental sulphur (10 g L^{-1}) at initial pH 2.8. A consortium of these three bacteria was used in bioleaching experiments in columns.

Immobilization of A. ferrooxidans LR on glass beads

Glass beads (particle size between 2 and 4 mm) were used as supports for *A. ferrooxidans* LR immobilisation. Before use, glass beads were pre-treated with concentrated HF and washed until neutral. The cell immobilization were carried out in 500 ml Erlenmeyer flasks containing 200 ml of TK medium with glass beads (10% , w v^{-1}) at initial pH 1.8 and with an initial bacterial population of 1.0 x 10^7 cells per ml. Erlenmeyer flasks were incubated at 150 rpm and 30 ±2 ºC. When iron(II) was exhausted, the medium was replaced for a fresh solution four times consecutively.

Bioleaching experiments on Erlenmeyer flasks

The leaching experiments were carried out in 500 ml Erlenmeyer flasks containing 200 ml of diluted Modified Kelly Medium (MKM) comprising 0.08 g L^{-1} $(NH_4)_2SO_4$; 0.08 g L^{-1} $MgSO_4.7H_2O$; 0.008 g L-1 K_2HPO_4 [11] at pH 1.8. The flasks were sterilized by autoclaving (20 min, 121 ºC) and inoculated with *A. ferrooxidans* LR (10%, v/v) after addition of primary nickel ore (10%, w v^{-1}). Erlenmeyer flasks were incubated at 150 rpm and 30 ±2 ºC during 30 days. Samples (20 ml) were periodically withdrawn for measurements of pH and redox potential (an $Ag^o/AgCl$ reference) and for analysis of dissolved Ni and Fe (by atomic absorption spectrometry).

Bioleaching experiments in columns

The 120.0 g primary nickel ore was placed in glass columns (5.0 x 20.0 cm tall). A shallow layer of quartz (5.0 g) was placed above and below the ore to support and to disperse the leach solution and aeration air. Columns containing immobilized cells were disposed as illustrated in Figure 1.

The columns consisted of *Column A:* not inoculated ore (abiotic control); *Column B:* ore inoculated with *A. ferrooxidans* LR immobilized cells on glass beads and *Column C:* ore inoculated with 1.2 x 10^8 consortium cells per gram, consisted of *L. ferrooxidans* and *A. thiooxidans* in the presence of *A. ferrooxidans* LR immobilized cells on glass beads; *Column D:* ore inoculated with 1.2 x 10^8 consortium cells per gram, consisted of *A. ferrooxidans* LR, *L. ferrooxidans* and *A. thiooxidans*.

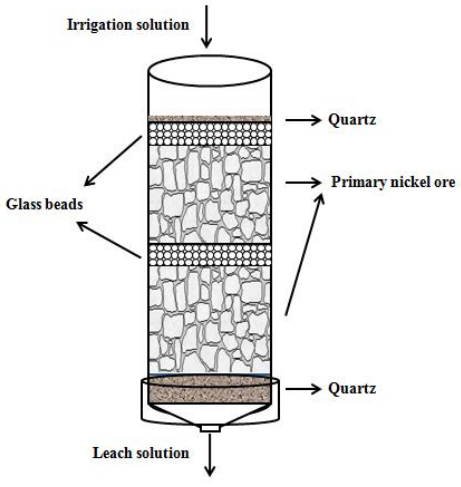

Figure 1. Experimental device to bioleaching columns containing immobilized cells (columns B and C).

Irrigation solution, comprising 500 ml of diluted MKM (5x), was applied to the top of the ore at a rate of 6.0 ml min^{-1} and was recycled in all experimental columns. Leach solution was maintained at pH 1.8-2.0 with 5M H_2SO_4 addition. Bioleaching experiments were carried at room temperature (25 ±2 ºC). The pH and redox potential of the leach reservoir were measured 4 to 5 times weekly and solution samples (20 ml) were removed for analysis of dissolved Ni (by atomic absorption spectrometry).

3. RESULTS AND DISCUSSION

Primary nickel ore bioleaching in Erlenmeyer flasks

The chemical composition (w w^{-1}) of primary nickel ore sample indicated 0.29% Ni, 11.9% Fe and 0,002%Co. Scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDS) analyses of the concentrate revealed pentlandite [$(NiFe)_9S_8$] as the main sulfide phase. The bioleaching curve of primary nickel ore in agitated submerged cultures in the presence of *A. ferrooxidans* LR is shown in Figure 2. In only 16 d, the nickel extraction was 55.1% (33.5 mg Ni^{2+}) while not inoculated flasks presented 21.3% (12.3 mg Ni^{2+}) of metal solubilisation, which represent the effect of leach solution and the possible action of microorganisms inherent ore [12]. These indicated that *A. ferrooxidans* LR inoculation increased the nickel extraction in 2.6-fold besides decreased the time expended to be initiated the metal solubilization. Watling [1] described a nickel extraction corresponding to 40% in 20 d, using different strain of *A. ferrooxidans*. In this case, extraction yields only enhanced through the nickel sulphide inoculation with thermopile species as *Sulfolobus metallicus*

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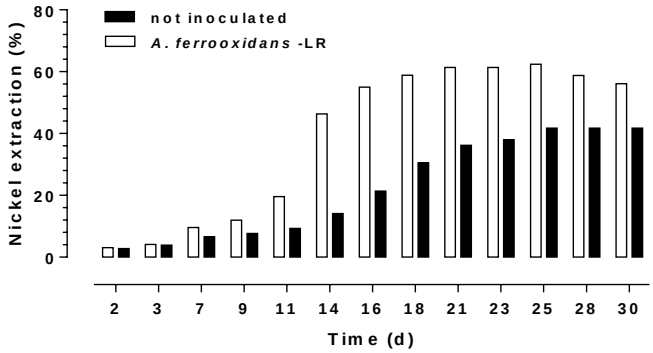

Figure 2. Nickel extraction of submerged bioleaching experiments in the presence of *A. ferrooxidans* LR cells.

A lower increment in metal extraction after 25 d, 56% (32.6 mg Ni^{2+}) to inoculated ore against 41.7% (24.2 mg Ni^{2+}) to the control experiments, can be explained by the capacity of *A. ferrooxidans* strains in only partially oxidize the pentlandite and also by the unadaptation of bacterial cells to higher concentrations of nickel [1,13].

This fact was also demonstrated to a nickel-adapted *A. ferrooxidans* strain, which recovered 43% of nickel compared with 27% in the abiotic control in only 6 d in the presence of commercial reagent nickel sulphide. In this experiment, nickel concentration in solution was only 1.4 mM, similar to the results obtained in the present paper (1.9 mM Ni). However, around 50% of the bacteria attached to the mineral surface could be exposed to higher localised metal concentrations [14]. The metal solubilization gradually increases during bioleaching course, and nickel–tolerance is essential to the bacterial nickel dissolution [1].

Primary nickel ore bioleaching in columns using immobilized cells

The dispersed (agitated) and heap solid (stationary) are the two fundamental systems that have been used for bioleaching processes. Submerged bioleaching experiments are necessary to the evaluation of effectiveness of the process using the chosen strain. The stationary bioleaching in columns constitutes a fixed bed reactor where the leaching solution containing bioleaching-bacteria cells circulates through the ore bed in higher particles sizes [1,4]. This kind of experiment is essential to the development of industrial technologies to nickel heap bioleaching to moderate capital investment with low

operating costs and simple operating procedures [15].

In heap bioleaching process, an important parameter is the effective external area of the solid particles wetted by the leaching solution and colonized by bacteria cells. The use of immobilized particles is important to reduce volume of leaching solutions and to improve a uniform inoculation, but the bacteria cells must detach and colonize the heap [7].

In immobilization process, *A. ferrooxidans* LR had available ferrous sulfate from TK medium [10] as energy source for growth from the oxidation of iron(II). Usually, these iron-oxidizing bacteria adhere to inert support by biomass immobilization associate to biofilm formation. XRD analysis had suggested that a large amount of porous jarosite [$KFe_3(SO_4)_2(OH)_6$] and/or ammonium jarosite [$NH_4Fe_3(SO_4)_2(OH)_6$] can precipitates in the biofilm and contributes to cell adsorption [16].

Surface modification of glass beads are also necessary to create imperfections on smooth surface that could favours bacterial adhesion and biofilm colonization [17]. Figure 3 shows the photos of glass beads supports before and after fluoridric acid treatment and after bacterial colonization and jarosite precipitation

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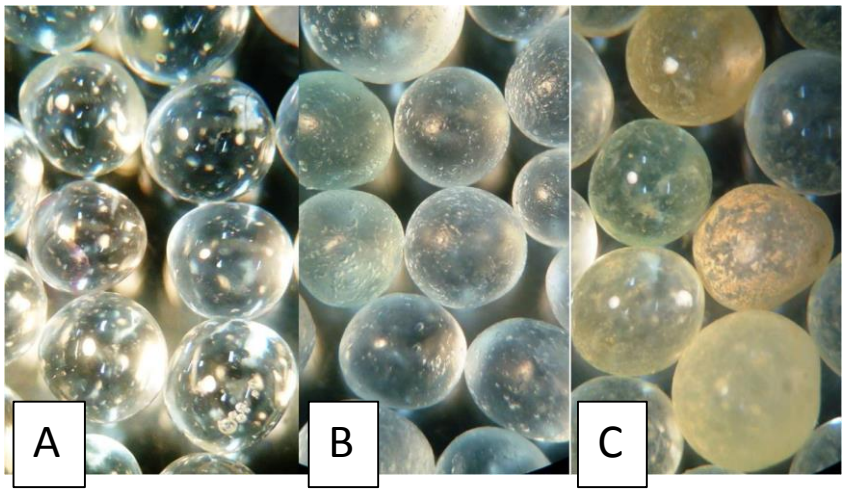

Figure 3. Photos of glass beads (80x) before (A) and after HF treatment (B). After *A. ferrooxidans* LR cells immobilization (C) is also possible observe jarosite precipitation.

The bioleaching curves of primary nickel ore extraction conducted in columns are shown in Figure 4. The nickel extraction in abiotic control (column A) is derisive, only 3.5%, in comparison to another columns, showing that the presence of a consortium of biomining bacteria is important to increase the yield extraction (4.5-fold in the present experiment) in this conditions.

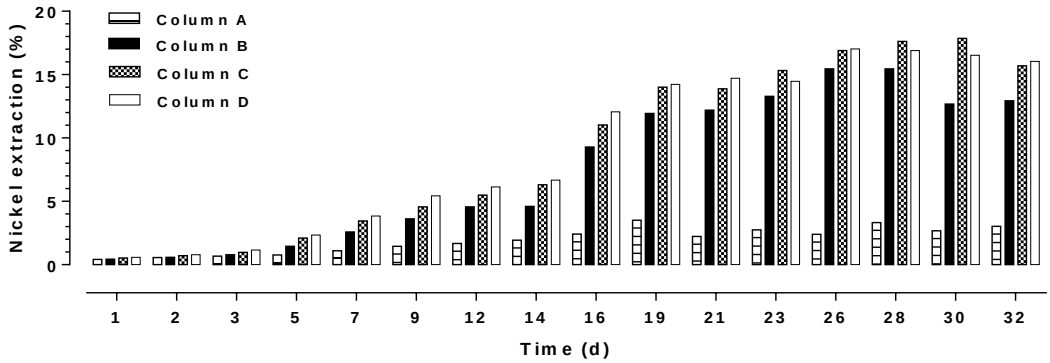

Figure 4. Nickel extraction of column bioleaching. *Column A:* not inoculated ore; *Column B:* not inoculated ore + *A. ferrooxidans* LR immobilized cells on glass beads; *Column C:* inoculated ore + *A. ferrooxidans* LR immobilized cells on glass beads; *Column D:* inoculated ore with a mixed consortium.

After 28 d, the nickel extraction was around 17% (~60 mg Ni^{2+}) in both columns (C and D) inoculated by a mixed consortium of bacteria, almost the same in the column B, which was inoculated with *A. ferrooxidans* LR immobilized cells on glass beads (15.5%, 53.8 mg Ni^{2+}). In this case, the use of a consortium of *L. ferrooxidans* and *A. thiooxidans* in the presence of immobilized *A. ferrooxidans* LR cells presented the same efficiency as the use of *A. ferrooxidans* LR strain alone as immobilized catalyst.

In the column experiments, the lowest percentage of nickel extraction in comparison to Erlenmeyer flasks (56%, 32.6 mg Ni^{2+}) is expected, since higher particle size, the lack of homogenization carried out by stirring, uncontrolled temperature, e.g. can negatively influence the metal solubilization in bioleaching process [1,18]. Nickel recoveries up to 90% have been described to pilot-scale column, using black schist ore as nickel sulphide source, in about 300 days of leaching tests [19].

Use of *A. ferrooxidans* LR immobilized cells on glass beads as inoculum had satisfactory yields of nickel extraction, as well as represented an economy of 5.7% of sulphuric acid required to the metal solubilization. Whereas in the experiments C and D the consumption of H_2SO_4 was 143.8 kg/t ore, in the column B this requirement was 135.6 kg/t ore. The lower acid consumption is also imprescindible to minimize the leach process costs as well the environmental impacts [15].

In conclusion, the immobilization of biomining microorganisms represents an alternative and sustainable inoculation method for bioleaching processes since enhances the inoculation efficiency, minimize acid consumption, and also enhances the tolerance to nickel and other heavy metals in solution. Further studies are necessary in order to develop new systems of chemolithoautotrophic microorganism immobilization to generate higher metal extraction yields and more comprehension of this bioprocess.

4. ACKNOWLEDMENTS

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