Indirect bioleaching of Co and Ni from iron rich laterite ore, using metabolic carboxylic acids generated by P. putida, P. korensis, P. bilaji and A. niger

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A R T I C L E   I N F O

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A B S T R A C T

Indirect bioleaching of Co and Ni from iron rich laterite ore was studied, using spent media produced by four fungal and bacterial species including Pseudomonas putida, Pseudomonas korensis, Aspergillus niger and Penicillium bilaji. HPLC analysis showed that the main carboxylic acids in spent media were gluconic, citric and oxalic acids. Gluconic acid (with 10.8-14.4 g/l) was the predominant metabolite in spent media of Aspergillus niger and Pseudomonas species, whereas citric acid (with 6.2 g/l) was the main metabolite for Penicillium bilaji. Bioleaching kinetics was assessed at 45, 75 and 90 °C. Results indicated that the highest recovery rate of Ni and Co obtained from spent medium of Pseudomonas putida were 90.6 and 71.98%, achieved at 90 °C after 3 h. The corresponding activation energy for Ni and Co solubilization using spent medium of Pseudomonas putida were 41.82 and 43.91 kJ/mol, indicating that solubilization rate of Ni and Co from iron rich laterite ore is governed by a chemically controlled reaction mechanism.

1. Introduction

Complex mineralogy and limited application of existing technologies have caused difficulties in processing of nickel-bearing laterites. Extraction of cobalt and nickel from laterites in commercial scale is energy-consuming and associated with high operating costs (Valix et al., 2009; Behera et al., 2011; Behera et al., 2012). Therefore, economic and environmentally friendly replacement technologies need to be developed (Valix et al., 2009). In general, the most important reactions that occur for nickel ions in laterite dissolution are proton absorption, reduction and complexation/chelation. In proton absorption, protons produced by organic acids have a significant effect on dissolution of the mineral, the reduction reaction accelerates release of nickel, and the organic acid complexing with metal ions in the solution decreases activity of the metal, therefore the apparent solubility of the mineral accordingly increases (Simate et al., 2010).

A number of advantages have been mentioned for biological leaching of low grade ores over the traditional techniques of leaching including its relative simplicity, lower operational costs and energy consumption, as well as its environmentally friendly process (Li et al., 2010; Simate et al., 2010; Li et al., 2014; Ahmadi et al., 2015; Mahmoud et al., 2017). Bioleaching is the utilization of microorganisms and their metabolic products to dissolve metals from low-grade reserves (Sahu et al., 2011). The application of microorganisms depends on their capability to produce hydroxy-carboxylic acids (gluconic, citric, pyruvic, tartaric and lactic acids) and other metabolites that are produced within the cultivation medium which depends on microorganism resistance to heavy metals (Simate et al., 2010; Behera et al., 2011). However, until now, bioleaching of non-sulfuric minerals has not been developed on a commercial scale (Gadd, 2010; Urik et al., 2015; Jafari et al., 2018).

In recent years, extraction of metals from laterite ores has been studied using organic acid metabolites generated by microorganisms (Li et al., 2010; Behera et al., 2011). Although metal leaching using organic acids is considered as an effective method for selective extraction of specific metals from laterite ores, problems such as the long time required for leaching and low metal yield should be resolved prior to industrialization. The most effective organic acid for the Ni extraction from laterite ores of serpentinite type is citric acid however, because of the low reactivity of citric acid with goethite, this acid is not efficient to dissolve nickel from laterite ore of the limonite type. However, this method is not suitable for nickel extraction since nickel is surrounded by goethite in limonite laterite ore (Li et al., 2010). Autotrophic and heterotrophic microorganisms have the ability to dissolve nickel from laterite ore (Sahu et al., 2011). It seems that chemoorganotrophic
bioleaching of oxide ores have a high ability for processing of low-grade laterite ore and therefore their bioleaching process needs to be optimized (Chaerun et al., 2017). The use of heterotrophic microorganisms to leach non-sulfide minerals is very common (Sahu et al., 2011; Mubarok et al., 2013). Among heterotrophic bacteria, *Pseudomonas* species are effective in leaching the non-sulfide minerals. Since, non-sulfide ores have no energy sources for the use of microorganisms, when a carbon source exists to provide microorganism energy and growth, these ores can be dissolved through heterotrophic bacteria and fungi. Organisms use the carbon source and produce organic acids and compounds with at least two hydrophilic reactive groups (for instance, phenol derivatives) in the cultivation medium, called metabolic products. Secondary metabolites generated by heterotrophic organisms that use organic carbon to generate energy, react with mineral surfaces. In addition to formation of several organic acids such as citric acid, acetic acid, α-ketogluaric acid and oxalic acid, these metabolites also have proteins, amino acids and exopolysaccharides, which can dissolve metals through different mechanisms (Sahu et al., 2011). Organic acids play an important role in the overall process of dissolution because they provide both the protons and anions to form metal complexes (Behera et al., 2011; Sahu et al., 2011). Laterite oxides can react with heterotrophic fungi and *Acidithiobacillus* acidophilic species. Acidophilic bacteria produce sulfuric acid and fungi produce organic acids, which both assist dissolution of metals (Jang and Valix, 2017). Fungal metabolism converts sucrose or other carbohydrates into diverse products including organic acids, which leads to decreasing the pH. Accumulation of organic acids by the microorganisms decreases the pH (Behera et al., 2011). In many investigations, two species of *Aspergillus* and *Penicillium* fungi have been used for bioleaching of laterites (Boecker, 1986; Boecker, 1989; Franz et al., 1991; Boecker, 1997; Coto et al., 2001; Valix et al., 2001a; Valix et al., 2001b; Valix et al., 2001c; Coto et al., 2003; Coto et al., 2005; Behera et al., 2012). These two species are the most effective organisms for dissolution of laterites (Mubarok et al., 2013; Valix et al., 2001a). *Aspergillus foetidus* and *Aspergillus niger* are species of *Aspergillus* which are commonly used for extraction of nickel and cobalt from laterite ore (Mubarok et al., 2013). The dissolution behavior and kinetics of nickel-bearing laterite are influenced by different parameters such as pulp density, temperature, particle size, and acidity of the leaching solution (Petrus et al., 2018). Available studies in the literature related to the laterite bioleaching are summarized in Table 1.

Based on the results of previous investigations, the most efficient leaching agent was citric acid, while oxalic acid had the least efficacy on leaching of nickel laterites. Parameters affecting the bioleaching process include ultrasonic waves, salinity of the culture medium and type of the growth medium of the microorganism, pulp density, pH, particle size, species type, temperature, solid percentage, etc. Optimization of these parameters can greatly increase the dissolution rate and the nickel and cobalt recoveries in nickel bearing laterites (Sukla and Panchanadikar, 1993; Tzeferis, 1994; Swamy et al., 1995; Le et al., 2006; Thangavelu et al., 2006; Mubarok et al., 2013; Pawlowska and Sadowski, 2017; Petrus et al., 2018).

Nickel is considered as an important and strategic metal however, despite its frequent applications, very few investigations have focused on its extraction process. Here, the available research have been carried out in the field of nickel ore leaching, while bioleaching of these ores is less well-studied.

In this study, for the first time, the biological dissolution mechanism of cobalt and nickel elements from iron-rich laterite ore was investigated using spent medium produced by four species include *Pseudomonas koreensis*, *Pseudomonas putida*, *Aspergillus niger* and *Penicillium bilaiji*. The organic acids produced by the microorganisms were in contact with the laterite sample previously heated at 500 °C for 2 h. Leaching experiments were performed at different temperatures, and the data was used to determine the activation energy for the nickel and cobalt dissolutions. Finally, the kinetics of the biological dissolution process (type of kinetic model and activation energy) was assessed. Importantly, nickel bioleaching for the laterite sample studied in this research has not been investigated. This laterite sample has low magnesium and high Fe₂O₃. Consequently, due to the change in mineralogical composition, the bioleaching process would be different. Bioleaching is considered an environmentally friendly method (Mahmoud et al., 2017). Therefore, results of this study can help to achieve a more environmentally friendly process for the nickel and cobalt extractions, and increase nickel grade to provide stainless steel and metal alloys benefiting from the high resistance and fracture toughness of nickel especially at high temperatures.

2. Material and methods

2.1. Sample and characterisation studies

A representative sample of laterite from Kanshargh mine (located east of Sarbisheh in southern Khorasan province, Iran, with a proved reserve of 3,700,000 tons) was prepared. The laterite sample was rich in nickel and cobalt with high iron content. Elemental analysis showed that the average nickel, cobalt and iron contents in this sample were 1.74%, 0.14% and 40.83%, respectively. Results of the particle size analyzes based on wet method (using Particle Size Analyzer, Micro Tec Plus) indicated a fine-grained laterite sample with particle size ranging from 0.1 to 100 μm. The d₅₀, d₈₀ and d₉₀ of the sample were 2.5, 8.6, and 25.2 μm, respectively. The chemical composition of the laterite sample analyzed by XRF (MAGIX-PRO) is presented in Table 2. The laterite sample had high iron content (61.4%) with 3% of NiO, 0.2% of Co₂O₄, 9.2% silicon dioxide and 5% aluminium oxide. Results of XRD analysis (MDP 3000, Counting time: 0.5 s, Step size: 0.02, Anode: Cu, Voltage: 40 Kv, Flow: 30 mA, and 2θ: 4–90°) for this sample revealed that goethite (FeOOH), calcite (CaCO₃), hematite (Fe₂O₃), and quartz (SiO₂) are the dominant crystalline phases (Fig. 1), and ore is nickel ferrous laterite of goethite type.

Thermogravimetric Analysis (TGA, TG 209F3 NETZSCH) and Differential Thermal Analysis (DTA) were used to assess thermal behavior of the laterite sample in the air atmosphere (results are presented in Fig. 2). Heating of the laterite sample resulted in conversion of goethite as the main phase of mineralogy to hematite through reaction (1) (Teir et al., 2007):

\[
2\text{FeOOH} \rightarrow \alpha\text{Fe₂O₃} \tag{1}
\]

As shown in Fig. 2, the TG/DTA curve represents two main endothermic peaks at 300 °C and 700 °C related to structural changes. The first major peak at about 300 °C is related to dihydroxylation of goethite to hematite (Maccarthy et al., 2016). The endothermic peak at around 700 °C is associated with elimination of the hydroxyl group and partial degradation of silicates such as lizardite (Maccarthy et al., 2016). The sample was heated at 200, 350, 500 and 650 °C for 2 h in a furnace (Nabertherm®), after which leaching experiments were performed. Here, at each pre-calcination temperature, the prepared samples with 20% solid content were leached using 3.5 M sulfuric acid at 60 °C for 2 h (the stirring speed of 400 rpm). Nickel and cobalt recoveries at different temperatures were determined with and without calcination prior to leaching. Ultimately, as shown in Table 3, the highest nickel and cobalt recoveries as well as the lowest iron dissolution rate obtained for the sample calcined for 2 h at 500 °C prior to leaching. The first endothermic peak occurred at around 300 °C, which shows conversion of the goethite to hematite, which is easier to dissolve. This can explain why Ni and Co dissolutions were higher at 300 °C compared with 200 °C (Table 3). At the calcination temperature of 650 °C, the nickel and cobalt recoveries were lower than those of 500 °C where the second endothermic peak of TG/DTA graph recorded. Based on the TG/DTA graph, the optimal calcination temperature prior to leaching should lie between 300 and 650 °C, while data presented in Table 3 shows 500 °C as the optimal temperature. In addition, results of BET
analysis showed that the specific surface area, total volume and average diameter of the pores before the calcination of the laterite sample were 40.72 m$^2$/g, 0.073 cm$^3$/g and 7.17 nm, which were altered to 183.84 m$^2$/g, 0.25 cm$^3$/g and 5.40 nm after calcination at the optimal temperature (500 °C). In other words, after calcination, the peaks associated with Ni and Co were shifted to lower angles, and microstructural changes occur due to transformation of the laterite sample. BET analysis (TriStar II Plus Micromeritics) is based on measurement of the amount of nitrogen gas absorbed and desorbed by the material. The surface area increased by 4.5 times, the total volume of pores increased by 3.4 times (due to the exhaust gases), and the average diameter of the pores decreased by 1.3 times (caused by shrinkage at high temperatures). BET analysis (TriStar II Plus Micromeritics) is based on measurement of the amount of nitrogen gas absorbed and desorbed by the surface of the material at a constant liquid nitrogen temperature (77 K). Nickel, cobalt and iron grades at a calcination temperature of 500 °C prior to leaching under the above conditions were 2.3, 0.17 and 32.66% respectively, which were considered as feed grades in all the leaching tests.

Biswas et al. showed that after calcination, the ore grains agglomerate, and microstructural changes occur due to transformation of goethite to hematite. FTIR spectrum of calcined ore leach solution showed that the peaks associated with Ni–O and Co–O were shifted relative to those of the raw ore (Biswas et al., 2013a). Substantial increase in porosity facilitates better recovery of Ni and Co due to the higher surface area and total volume of pores.

**Table 2**

Chemical composition of the laterite sample analyzed by XRF (wt%).

<table>
<thead>
<tr>
<th>Formula</th>
<th>SiO$_2$</th>
<th>Al$_2$O$_3$</th>
<th>Fe$_2$O$_3$</th>
<th>CaO</th>
<th>MgO</th>
<th>SO$_3$</th>
<th>MnO</th>
<th>Cr$_2$O$_3$</th>
<th>NiO</th>
<th>Co$_3$O$_4$</th>
<th>L.O.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td>9.2</td>
<td>5.0</td>
<td>61.4</td>
<td>4.0</td>
<td>0.4</td>
<td>0.4</td>
<td>0.7</td>
<td>0.9</td>
<td>3.0</td>
<td>0.2</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Fig. 1. Results of XRD analysis of the laterite sample.
increased surface area for exposure to the leaching solution (Biswas et al., 2013b).

2.2. Apparatus and test procedure

Indirect bioleaching experiments at atmospheric pressure were performed in 1-l glass reactor (No. 1, Fig. 3). Silicon oil bath that was electrically heated (No. 2), Heidolph mechanical stirrer (Model HPS-55, Germany) (No. 3), Magnetic stirrer (Multi stirrer DM-8 Scinics, Japan) (No. 4), Pyrex glass door which was removed at the time of feed entry (No. 5), thermometer (No. 6), periodic sampling from input (No. 7), reflux condenser to prevent solution evaporation at high temperatures (No. 8), and the thermostat (No. 9) were different components of this reactor.

Using atomic absorption spectrometer (Varian SpectrAA20), the solution was analyzed at the end of the reaction time to determine concentration of the nickel and cobalt. The experiments were carried out in duplicates, some were randomly repeated for the third time, and finally the obtained results were averaged.

Indirect bioleaching experiments were performed using organic acids produced by fungal and bacterial species. To generate the metabolite, 10 ml of inoculated microorganism was added to 250 ml of liquid cultivation media (in a 1-l erlenmeyer). A pH meter (METTLER TOLEDO, MP120 Basic Portable pH / mV / °C Meter) was utilized to measure and adjust the pH of the media using sulfuric acid. The fungal species were Aspergillus niger and Penicillum bilaji while bacterial species were Pseudomonas putida and Pseudomonas koreensis delivered by the biological research department of the Water and Soil Institute in Karaj, Iran. The PDB cultivation medium was then applied to cultivate the fungus and the modified SP medium for bacterial culture (Table S1 in the appendix). It should be mentioned that for preparation of both

Table 3
Dissolutions of Nickel, Cobalt and Iron at different calcination temperatures prior to leaching (S/L = 0.2, concentration of sulfuric acid = 3.5 M, stirring speed = 400 rpm, leaching temperature = 60 °C, leaching time = 2 h).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ni recovery (%)</th>
<th>Co recovery (%)</th>
<th>Fe dissolution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.8</td>
<td>24.8</td>
<td>23.4</td>
</tr>
<tr>
<td>200</td>
<td>11.6</td>
<td>28.3</td>
<td>31.6</td>
</tr>
<tr>
<td>350</td>
<td>31.9</td>
<td>33.6</td>
<td>49.7</td>
</tr>
<tr>
<td>500</td>
<td>39.3</td>
<td>34.7</td>
<td>44.2</td>
</tr>
<tr>
<td>650</td>
<td>28.2</td>
<td>30.1</td>
<td>33.9</td>
</tr>
</tbody>
</table>

Fig. 2. Result of TG/DTA analysis of the laterite sample.

Fig. 3. Schematic design and real picture of the reactor used for indirect bioleaching tests.
media, glucose was used as the carbon source. Since production of organic acids and reduction of the medium pH were our research goal, calcium phosphate (Ca₃(PO₄)₂) was not added to the culture medium. The autoclave was used to sterilize the culture media and culture dishes of microorganisms. Species were cultured under a laminar hood in two types of solid and liquid mediums. After cultivation, separation of fungi and bacteria from their metabolites was performed using a centrifuge (Heraeus Labofuge 400 R) for 20 min at 4000 rpm, and their supernatants used for HPLC analysis. The pure gluconic, citric, lactic, and oxalic acids prepared by Merck Co were applied as HPLC standards. The HPLC analysis with a Sykam HPLC device equipped with an Adept 4100CE high pressure pump and diode array detection was performed to determine the type and concentration of citric, gluconic, oxalic and lactic acids generated by microorganisms in the culture media. Isolation of acids was achieved using an ODS C18 column (250 mm × 4.6 mm, 5 μm particle size) with a mixture of acetonitrile (95: 5 v/v) and acidic water (pH adjusted using sulfuric acid on 2%) at 210 nm for 20 min with a flow rate of 0.8 ml/min. The dynamic range was between 5 mg/l and 1000 mg/l with a relative standard deviation of less than 0.73% (n = 4). Peaks of oxalic acid and gluconic acid were almost overlapping and separation was performed using the HPLC instrument algorithm. Lactic acid was not one of the identified acids in the produced metabolite by the studied microorganisms in the spent media. The produced metabolites were used for indirect bioleaching tests. First, 150 ml of the produced solution by fungi or bacteria was fed into the reactor and then 10 g of laterite was added to the reactor vessel after adjusting the agitation speed and reaching to the desired temperature. The pH of all the bioleaching solutions with sulfuric acid was set to 0.5 at the beginning of the experiment. A brief summary of the experiment parameters are listed in Table 4.

Table 4
Parameters of the indirect bioleaching experiments.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Solids content (g)</th>
<th>pH</th>
<th>Agitation speed (rpm)</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td>10</td>
<td>0.5</td>
<td>390</td>
<td>45, 75, 90</td>
<td>15, 35, 70, 120, 180</td>
</tr>
<tr>
<td>P. bilaji</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. putida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. koreensis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5
Concentration and type of the organic acids produced by microorganisms.

<table>
<thead>
<tr>
<th>Specie type</th>
<th>Type of produced acids</th>
<th>Acid concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td>Citric acid</td>
<td>9.96</td>
</tr>
<tr>
<td></td>
<td>Gluconic acid</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>Oxalic acid</td>
<td></td>
</tr>
<tr>
<td>P. putida</td>
<td>Citric acid</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>Gluconic acid</td>
<td>12.94</td>
</tr>
<tr>
<td></td>
<td>Oxalic acid</td>
<td>1.05</td>
</tr>
<tr>
<td>P. koreensis</td>
<td>Gluconic acid</td>
<td>10.8</td>
</tr>
<tr>
<td></td>
<td>Oxalic acid</td>
<td>0.07</td>
</tr>
<tr>
<td>P. bilaji</td>
<td>Citric acid</td>
<td>6.23</td>
</tr>
<tr>
<td></td>
<td>Gluconic acid</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td>Oxalic acid</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Figure 6: Recovery of Ni and Co with time for different bacterial species and temperatures.

- **A**: P.putida, 45°C, 75°C, 90°C, Leaching by H2SO4, 90°C
- **B**: P.koreensis, 45°C, 75°C, 90°C
- **C**: P.bilgai, 45°C, 75°C
- **D**: A.niger, 45°C, 75°C

(caption on next page)
Fig. 5. Nickel (left side) and cobalt (right side) bioleaching of laterite by A and E) *Pseudomonas putida* bacteria; B and F) *Pseudomonas koreensis* bacteria; C and G) *Penicillium bilaji* fungi; and D and H) *Aspergillus niger* fungi (pH = 0.5, agitation speed = 390 rpm, solid percentage = 6.67% w/v).

Fig. 6. Iron dissolution rate by metabolites of *Pseudomonas putida, Pseudomonas koreensis, Penicillium bilaji* and *Aspergillus niger* (pH = 0.5, agitation speed = 390 rpm, solid percentage = 6.67% w/v).

Table 6
Equations and mechanisms of dissolutions of minerals (from MacCarthy et al., 2014).

<table>
<thead>
<tr>
<th>Eq. no.</th>
<th>Model</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$k_t = 1 - (1 - X)^{1/2}$</td>
<td>Chemical reaction control</td>
</tr>
<tr>
<td>2</td>
<td>$k_t = 1 - (1 - X)^{7/2}$</td>
<td>Mixed control model by shrinking core model (diffusion control; chemical reaction control)</td>
</tr>
<tr>
<td>3</td>
<td>$k_t = \left[1 - (1 - X)^{3/2}\right]^{2/3}$</td>
<td>Diffusion through product layer</td>
</tr>
<tr>
<td>4</td>
<td>$k_t = -\ln(1 - X)$</td>
<td>Mixed control model (surface reaction control; and diffusion through sulfur layer)</td>
</tr>
<tr>
<td>5</td>
<td>$k_t = 1 - \frac{2X}{3} - (1 - X)^{7/2}$</td>
<td>Diffusion control</td>
</tr>
<tr>
<td>6</td>
<td>$k_t = \frac{1}{2}\ln(1 - X) + \left[(1 - X)^{3/2} - 1\right]$</td>
<td>Interfacial transfer and diffusion across the product layer</td>
</tr>
<tr>
<td>7</td>
<td>$k_t = 1 - 3(1 - X)^{7/2} + 2(1 - X)$</td>
<td>Diffusion of hydrogen ions through a product layer by shrinking core model</td>
</tr>
<tr>
<td>8</td>
<td>$k_t = 1 - (1 - 0.45X)^{1/4}$</td>
<td>Surface chemical reaction by shrinking core model</td>
</tr>
</tbody>
</table>
2.3. Kinetics tests

Prior to the kinetics tests, some preliminary experiments were performed to determine optimal parameters of laterite chemical leaching using organic acids (these experiments are not included in this report). Based on these experiments, the pH of pulp at the beginning of all experiments and the agitation speed were set to 0.5 and 390 rpm, respectively. It should be noted that although the optimum solid-liquid

Fig. 7. Kinetics modeling of nickel dissolution from the laterite sample at 45 °C, 75 °C and 90 °C by A and E) *Pseudomonas putida*; B and F) *Pseudomonas koreensis*; C and G) *Penicillium bilaji*; D and H) *Aspergillus niger*. Left side: chemical control model; Right side: diffusion control model.
ratio was 0.1 in chemical leaching, a lower solid content was applied due to the lower concentrations of the produced organic acids in the bioleaching tests. Therefore, 10 g of laterite (which was calcined prior to leaching at 500 °C) was added to 150 ml of metabolites (6.67% w/v). Leaching experiments with spent media produced by two species of bacteria (*Pseudomonas putida* and *Pseudomonas koreensis*) and two species of fungi (*Aspergillus niger* and *Penicillium bilaji*) were performed at different temperatures including 45, 75 and 90 °C. Sampling of leaching solution was performed at predetermined time points of 15, 35, 70, 120 and 180 min of test. All chemicals used in the experiments were pure and de-ionized water was used to prepare all the solutions. To perform kinetics studies, 5 ml samples were taken at different time points for elemental analysis and the solution loss was compensated using metabolite produced by the fungus or bacteria. A control test was carried out.

Fig. 8. Kinetics modeling of cobalt dissolution from the laterite sample at 45 °C, 75 °C and 90 °C by A and E) *Pseudomonas putida*; B and F) *Pseudomonas koreensis*; C and G) *Penicillium bilaji*; D and H) *Aspergillus niger*. Left side: chemical control model; Right side: diffusion control model.
at optimal temperature using sulfuric acid only to evaluate the effect of sulfuric acid on the nickel and cobalt recoveries. In the control test, the same amount of sulfuric acid was used to adjust the pH of the metabolite solution.

3. Results and discussion

3.1. Indirect bioleaching using spent media

Variation of the pH for each medium as a function of the cultivation time is presented in Fig. S1. The pH of the PDB medium with sulfuric acid on the nickel and cobalt recoveries. In the control test, the same amount of sulfuric acid was used to adjust the pH of the metabolic solution. Several unknown peaks were appeared in the HPLC chromatograms presented in Fig. 4.

Results of the bioleaching experiments of laterite sample at different temperatures for the dissolutions of nickel and cobalt are shown in Fig. 5. To assess the influence of sulfuric acid on the cobalt and nickel recoveries, a control test was conducted with sulfuric acid only, at 90 °C. In this experiment, the same amount of sulfuric acid (max. 1.8 ml) was used to adjust the pH of the metabolic solution. The results indicated that metabolites of all four species were able to properly dissolve nickel and cobalt from the laterite ore. The highest amount of nickel and cobalt recoveries were obtained with Aspergillus niger, Pseudomonas putida, Pseudomonas koreensis, and Pseudomonas putida bacteria. Similarly, the lowest recovery of nickel was found with fungi and the lowest recovery of cobalt with Aspergillus niger fungi. Iron dissolution rates for four species at 45 °C, 75 °C and 90 °C are presented in Fig. 6, indicating increase of dissolution rate of iron as a result of temperature increase. As shown in Fig. 6, dissolution of iron by the metabolites of these species at 45 °C, 75 °C and 90 °C is presented in Fig. 6, indicating increase of dissolution rate of iron as a result of temperature increase. As shown in Fig. 6, dissolution of iron by the metabolites of these species at 45 °C, 75 °C and 90 °C of nickel and 31.08% of cobalt. As shown in Fig. 5, dissolutions of iron by the metabolites of these species at 45 °C, 75 °C and 90 °C of nickel and 31.08% of cobalt. As shown in Fig. 5, dissolutions of iron by the metabolites of these species at 45 °C, 75 °C and 90 °C of nickel and 31.08% of cobalt. As shown in Fig. 5, dissolutions of iron by the metabolites of these species at 45 °C, 75 °C and 90 °C of nickel and 31.08% of cobalt. As shown in Fig. 5, dissolutions of iron by the metabolites of these species at 45 °C, 75 °C and 90 °C of nickel and 31.08% of cobalt.
nickel and cobalt were not effective with sulfuric acid only (without the usage of metabolite carboxylic acids) at 75 °C and 90 °C.

Results presented in Table 5 indicate that A. niger fungus produces more citric acid than P. bilaji fungus however, nickel and cobalt recoveries were higher in P. bilaji. Therefore, citric acid was not the most efficient leaching agent for cobalt and nickel dissolutions from laterite ore. For the four studied species, it seems that the amount of gluconic acid produced by microorganisms plays an important role in leaching of iron-rich laterites. Moreover, the higher amount of oxalic acid produced by microorganisms generally resulted in greater recoveries of nickel and cobalt. However, the observed peaks of gluconic acid have overlap with those of oxalic acid and therefore, analysis results may be uncertain. It was found that the larger amounts of gluconic acid produced by the studied microorganisms result in higher Ni and Co recoveries, while the higher amounts of citric acid produced by the studied microorganisms lead to lower recoveries rates. Table 5 also shows that the citric acid content in Aspergillus niger is higher compared with the other three studied species. Metabolic carboxylic acids seem to act differently from organic carboxylic acids that may be explained by the role of unknown peaks in HPLC chromatograms and their effects on increasing the nickel and cobalt recoveries. The detail mechanism of heterotrophic bioleaching is still under discussion (Behera et al., 2011, 2012). Therefore, further investigations are needed to characterize the reaction mechanism during the process by identifying and quantifying various types of organic acids produced (Behera et al., 2011).

To prepare the microorganism cultivation media, molasses could be used as a substitute for glucose to reduce the production costs of biogenic organic acids (Kirimura et al., 2011; Astuti, 2015).

3.2. Kinetics studies

Leaching of minerals can be described using various kinetic models. One of the main developed models for expression of dissolution process in non-catalytic liquid-solid environments is the shrinking core model (Garabaghi et al., 2009; Tang et al., 2010). The choice of kinetics model is based on the assumption that the particle size does not change with calcining and leaching operations (Lima et al., 2014). Table 6 presents a set of dissolution mechanisms with their equations, where x is the fraction reacted, k is the kinetic constant of the reaction, and t is the reaction time (Ghassa et al., 2017).

To describe the kinetics mechanism governing the indirect microbial dissolution of the laterite sample, shrinking core models (equations presented in Table 6) were fitted to the laboratory data from indirect microbial leaching, and the best fit on the data with the highest correlation coefficient was determined as the dissolution mechanism. The shrinking core model considers that the particle maintains its volume while its non-reactive core continuously shrinks with the reaction time and leads to the formation of a porous layer (MacCarthy et al., 2014). During the indirect bioleaching tests, the initial particle size is constant. In heterogeneous liquid-solid reactions, soluble reactants penetrate through common surfaces and/or within the solid porous layer, and then chemical reactions occur. The reaction rate is controlled by the reactant penetration through a soluble boundary layer, either by diffusion from a layer of solid product or by the rate of chemical reaction at the core surface of non-reactive particles (Levenspiel, 1972; Maccarthy et al., 2016). The slowest stage determines the rate of the leaching reaction (Maccarthy et al., 2016). Among different models presented in Table 6, the two models of diffusion control and chemical control (presented with the Eqs. (2) and (3)) had the best fit for all the four studied species on the data obtained for nickel and cobalt dissolutions.

\[ k_1 = 1 - \frac{2}{3}X - (1 - X)^{\frac{2}{3}} \]  

(2)

\[ k_1 = 1 - (1 - X)^{\frac{2}{3}} \]  

(3)

Application of the kinetics model of the diffusion control reaction and surface chemical control reaction at different temperatures for nickel and cobalt extractions using the four microorganisms are demonstrated in Figs. 7 and 8. As shown in Figs. 7 and 8, two models of chemical control and diffusion control are well fitted on the data of nickel and cobalt dissolutions. The first part of the graphs in Figs. 7 and 8 has the largest slope (the highest constant of the reaction rate). These slopes (constant values of the reaction rate) are used to determine the activation energy.

The constant of the reaction rate and the correlation coefficients of nickel and cobalt dissolutions at various temperatures are given in Tables 7 and 8. Regarding data presented in Figs. 7 and 8, and correlation coefficients listed in Tables 7 and 8, both chemical control and diffusion control equations fit well on the data of Ni and Co dissolutions. The obtained results indicate that the constant of the reaction rate increases with temperature rise. Thus, it can be concluded that the process is highly temperature-dependent, and is therefore under chemical control (Tang et al., 2010).

The Arrhenius relationship was used to determine activation energy values (E_a). Using the Arrhenius equation \[ k = A \exp \left( - \frac{E_a}{RT} \right) \], and plotting ln k versus 1/T provides a line that its gradient equals E_a/R. In this equation, k is rate constant, E_a is activation energy (kJ/mol), R is ideal gas constant (8.314 J/mol. °K), T is absolute temperature (K), and A is exponential function coefficient. The plot was designed for two control models, and activation energy values were accordingly calculated. Plots used to calculate the activation energy of nickel and cobalt dissolutions using metabolites produced by the four studied microorganisms are presented in Figs. 9 and 10.

Figs. 9 and 10 indicate that the plots for nickel and cobalt in chemical control have large correlation coefficients ranging from R^2 = 0.916 to R^2 = 0.9984 for nickel, and from R^2 = 0.9491 to R^2 = 0.9995 for cobalt. Using the Arrhenius equation, activation energy values for the chemical control model were between E_a = 37.33 kJ/mol and E_a = 41.82 kJ/mol for nickel, and between E_a = 37.83 kJ/mol and E_a = 43.91 kJ/mol for cobalt. In addition, regarding data presented in Figs. 9 and 10, activation energy values for the diffusion control equation were between E_b = 72.91 kJ/mol and E_a = 77.82 kJ/mol for nickel, and between E_b = 68.09 kJ/mol and E_a = 86.65 kJ/mol for cobalt.

Generally, for diffusion control, the activation energy was less than 20 kJ/mol and for chemical control greater than 40 kJ/mol (Habashi, 1999; Uçar, 2009). Since the equation of chemical control was well fitted to the laterite dissolution data at different temperatures and the activation energy is within the range of chemical control reaction, chemical control is more descriptive than the diffusion control for dissolution rate of the studied laterite sample.

3.3. SEM/EDS analysis

Surface analysis was conducted to investigate on the change of the surface of laterite particles during indirect microbial leaching. Results of the SEM (SEM, FEI QUANTA 450) micrograph together with the results of elemental EMS (BRUKER XFLASH 6/10) of Si, Ni, Co, Fe, Al and O for the bioleaching feed are reported in Fig. 11A. The corresponding data for the leached solid product under optimum conditions by Pseudomonas putida and Pseudomonas koreensis bacteria, Aspergillus niger as well as by Penicillium bilaji fungus are shown in Fig. 11B-11E. As the SEM results show, most particles were round and spherical. Under
Fig. 10. Graph presenting $-\ln k$ against $1000/T$ to calculate the activation energy of cobalt dissolution in indirect bioleaching treatment; Right side: chemical control model. Left side: diffusion control model.
the optimal conditions, the particle surface for leaching feed and leached solid product were not significantly different. The table of elemental EDS for Si, Co, Ni, Fe, Al and O reported in Fig. 11 reveals that the amounts of Ni and Co in the remaining solid after the indirect bioleaching process were declined. However, the amount of Si before and after leaching treatment remained almost unchanged. These findings indicate that leaching using metabolites of microorganisms was able to solve most of the Ni and Co elements without solving the Si and with no gel production.

4. Conclusion

For the first time, this research investigated on the indirect bioleaching of nickel and cobalt from iron-rich laterite ore type, using four different species of fungus and bacteria. Results of the HPLC analysis confirmed presence of citric acid (1.3–10.0 g/l), gluconic acid (4.5–14.4 g/l) and oxalic acid (0.07–1.05 g/l) in most of the samples, while none of the species produced lactic acid. Although, the concentration of the organic acids produced by microorganisms was very low, they had the ability to properly extract nickel and cobalt from the laterite ore and bring them into the soluble phase. The highest recoveries of nickel and cobalt at 90 °C (90.6% and 71.98%) were achieved by Pseudomonas putida. Regarding the activation energy, the chemical control equation had more descriptive capability to explain experimental data obtained from nickel and cobalt through indirect bioleaching. Some fatty acids with low molecular weight (C1-C5), although with low concentrations, are most likely to be present in the metabolites produced by microorganisms that increase nickel and cobalt extractions. Further research is needed to clarify possible contribution of side products of metabolites on bioleaching dissolutions of nickel and cobalt.

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Author contributions

Hadi Abdollahi conceived and designed the experiments; Marzieh Hosseini Nasab performed the experiments; Marzieh Hosseini Nasab, Hadi Abdollahi, Mohammad Noaparast and Mohammad Ali Amoozegar analyzed the data; Marzieh Hosseini Nasab and Hadi Abdollahi wrote the paper. Mohammad Noaparast is responsible for ensuring that the descriptions are accurate and agreed by all authors.

Declaration of Competing Interest

The authors claim that they have no conflict of interest.
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