Direct and Indirect Bio-Leaching of Co and Ni from an Iron-Rich Laterite Ore using *Delftia Acidovorans* and *Acidithiobacillus Ferrooxidans*

Marzieh Hosseini Nasab[1*], Mohammad Noaparast[2] and Hadi Abdollahi[2]

1. Mining Department, University of Sistan and Baluchestan, Zahedan, Iran,
2. School of Mining Engineering, College of Engineering, University of Tehran, Tehran, Iran

Abstract

In this research work, the bio-leaching of Co and Ni from an iron-rich laterite ore is assessed using the acidophilic heterotrophic (Delftia acidovorans) and autotrophic (Acidithiobacillus ferrooxidans) bacteria. The metabolic products of the acidophilic bacteria play an important role in bio-leaching. The results obtained from the indirect bio-leaching indicate the highest nickel recoveries of up to 83.65% and 80.18%, respectively, by the supernatants of Acidithiobacillus ferrooxidans and Delftia acidovorans, both measured at 90 °C for 3 h with a stirring speed of 370 rpm and S/L of 0.1, while the corresponding cobalt recovery rates have reached 86.93% and 83.94%, respectively. The iron dissolution rates in these conditions for the two studied bacteria are 64.34% and 54.41%, respectively. The nickel and cobalt extractions by the indirect bio-leaching of Delftia acidovorans are, respectively, 29.84% and 23.75% higher than those for the direct bio-leaching, performed at 30 °C and 150 rpm of an incubator shaker for 30 days. For the indirect bio-leaching, the chemical control has a larger influence on the dissolution rate of the iron-rich laterite compared to the diffusion control. The activation energies of nickel and cobalt in the chemical control model are 40.07 and 39.08 kJ/mol, respectively.

1. Introduction

In the nature, nickel can be found in both the sulfide and oxide/laterite forms [1]. The demand for nickel is increasing worldwide [2]. About 85% of the world's total nickel reserves has been found in the laterite ores, which highlights the importance of this ore as nickel deposits for the future [1, 3]. Nickel and cobalt are incorporated into the network structure of precipitated-hydrated iron oxides through either absorption or substitution with iron [4]. In order to extract the nickel of goethite or other host minerals, the strong bond between oxygen and ferric iron should be broken [1, 5, and 6]. Regarding the disadvantages of the traditional methods used for extraction of nickel and cobalt from laterite ores (e.g. pyrohydrometallurgy and hydrometallurgy), bio-leaching has recently gained more attention in mineral processing. This is mainly due to its eco-friendly, energy efficient, and economic process. Importantly, bio-leaching can

also be used for extraction of metals from low-grade ores [1, 6].

The use of bacteria has been shown to facilitate the reductive dissolution of ferric iron-bearing minerals in laterite ores, and thereby, releases the metal content (including nickel) along with the ferric iron-bearing minerals into the solution. The previous studies have assessed the effects of several parameters including the pH, sulfur content, substrate type, cell nature of the bacteria, and temperature on the nickel dissolution process [7, 8]. In particular, the rate of metal dissolution has been found to be influenced by the rate of acid production as well as the physical contact between the metal and the bacterium [9]. In an acidic medium (e.g. by acidophilic microorganisms), a low pH accelerates the dissolution of nickel [10-12]. In addition, the organic acids generated by the acidophile cells can help feeding the

✉ *Corresponding author: (M. Hosseini Nasab).*

microorganism by producing carbon dioxide [10]. In addition, the metabolic products of acidophiles (mainly sulfuric acid) have been proved to play an important role in bio-leaching [9]. The produced sulfuric acid keeps the pH at an optimum level, and thereby, improves the leaching of nickel laterites [6]. Amongst the commonly used bacteria in bio-leaching, *Acidithiobacillus ferrooxidans* (*At.ferrooxidans*), as an acidophile bacterium has been considered as the only pattern microorganism for investigation of the biochemical cycle and electron transport in the iron oxidation phenomena [13-15]. This bacterium uses several sources of energy including molecular hydrogen, H_2S, S^0, as well as Fe^{2+} and inorganic sulfur compounds in order to oxidize iron and sulfur [13, 16, and 17]. Importantly, oxidation of sulfur by *At.ferrooxidans* produces sulfuric acid, which accelerates the dissolution of metals [14]. Moreover, reduction of the soluble ferric iron by the bacterium accelerates the dissolution rate of the minerals [5, 12, and 18]. The reductive dissolution of some of ferric iron-containing minerals (including goethite) using heterotrophic acidophilic bacteria has been previously reported [19-23]. However, for the first time, Hallberg *et al*. (2011) have shown that *At.ferrooxidans* can facilitate the reductive dissolution of ferric iron-containing minerals [12]. In a later study, Johnson (2012) has reported that in anaerobic bioreactors, *At.ferrooxidans* could enhance the reductive dissolution of asbolane (an oxyhydroxide mineral in the laterite containing manganese and cobalt) [4].

Nickel ions strongly inhibit sulfur dioxygenase, which could otherwise accelerate the oxidation of elemental sulfur to sulfite. They also prevent sulfite oxidase, and therefore, limit the oxidation of sulfite to sulfate. Nickel surrounds the plasma membrane, and therefore, blocks the entry of both sulfite and sulfate, which consequently prevents the cell growth [24]. Although nickel inhibits the bacterial physiological functions, some bacteria can be readily adapted to high concentrations of nickel [2]. It is known that in the bio-leaching process, the mesophiles are more tolerant to nickel than thermophiles and moderate thermophiles [2]. Simate and Ndlovu (2008) have shown that bacteria from the *Acidithiobacillus* genus can be used to efficiently extract nickel from the minerals [6]. Moreover, *At.ferrooxidans* has been proved to be more tolerant to nickel than *Acidithiobacillus thiooxidans* [2]. It has also been reported that between 30 $^\circ$C and 37 $^\circ$C the cultivation media containing *At.ferrooxidans* can more effectively dissolve nickel than the media containing the

Aspergillus species [1]. Interestingly, they showed that adding a ferrous iron supplement could considerably enhance the leaching process by *At.ferrooxidans* in the cultivation media. Another important factor that can affect the bio-leaching process is the concentration of the metal in the medium. Natarajan and Iwasaki (1983) gradually increased the Ni^{2+} concentration from 5 g/L to 50 g/L, and could successfully adapt *At.ferrooxidans* to higher metal concentrations in the media containing ferrous ions [25]. Johnson *et al*. (2000) have shown that most heterotrophic acidophiles have the ability to reduce the ferric irons [26]. Leaching of metals using the heterotrophic microorganisms can generally produce the metabolites containing organic acids and amino acids [27]. The leaching recovery, while using the heterotrophic microorganisms, mainly depends on the organic metabolites that can decrease the pH of the cultivation medium [1]. Amongst different heterotrophic acidophiles, the *Delftia* genus has a particular importance in biotechnology [28, 29]. It belongs to the β-proteobacteria group that is metabolically diverse and can be isolated from various nutrition sites including soil, seawater, activated sludge, and mineral sites [30]. *Delftia acidovorans* shows phenotypic similarities with the *Pseudomonadaceae* family [31]. It is a nickel-tolerant species [32], which is also able to withstand high concentrations of chromium and lead [29]. Importantly, this bacterium is able to oxidize elemental sulfur and thiosulfate to sulfate [33]. In very few studies including Garcia-Moyano *et al*. (2015) and Heinzel *et al*. (2009), the *Delftia acidovorans* strain has been found while investigating the microbial population of AMD (Acid Mine Drainage), and has been identified using the gene libraries prepared from the 16S rRNA gene [34, 35]. However, the application of *Delftia acidovorans* in mineral processing is very new. Das *et al*. (2015) have used the secondary metabolic products of *Delftia acidovorans* (delftibactin) in order to synthesize the gold nanoparticles from electronic waste [36]. The *Delftia acidovorans* AR has also been used for the bio-treatment of metal-containing wastewater in fixed bed bioreactors, showing promising capabilities for removing the Cd(II), Zn(II), and Cr(VI) ions. Moreover, *Delftia acidovorans* is capable of destroying complex organic materials including 2-(4-sulfophenyl) butyrate (SPB) [37]. However, the application of chemolithotrophic bacteria to recover nickel from the laterite ores has been less well-investigated. This is probably due to the lack of sulfide content in the ore in order to

produce the required amount of sulfuric acid [6, 18]. In addition, to date, *Delftia acidovorans* or its metabolic products have not been used to leach nickel or cobalt, and therefore, the kinetics of these processes are unknown.

In the previous investigations, the researchers have used different boi-leaching procedures and a varity of different bacteria in order to extract nickel and cobalt from the laterite ores. Ghosh *et al*. have reported 53% and 65% Ni recovery from double-step and indirect bio-leaching of the chromite overburden using *Aspergillus humicola* SKP102 [38]. Marrero and co-workers found the reduction of iron, nickel, cobalt, and manganese from laterite overburden more efficient when *At.thiooxidans* was used in an aerobic process compared to the anaerobic reduction using *At.ferrooxidans* [39]. Some studies have also used the two species of *Aspergillus* and *Penicillium* fungi for bio-leaching of laterites [40-42]. Moreover, the mineral type in the ore has been found to be a major determinant of the adsorption capacity, and the pH control during bio-leaching is critical for achieving an optimized leaching process [43].

Hosseini Nasab *et al*. have performed a series of design of experiments and kinetic studies in order to assess the dissolution of nickel and cobalt from the iron-rich laterite when using different organic acids or sulfuric acid. These investigations have revealed an optimal condition when using 5 M, 0.1, 370 rpm, 90 °C, and 2 h, respectively, for the concentration of sulfuric acid, solid to liquid ratio, stirring speed, temperature, and process time. This resulted in the highest recovery rates of nickel and cobalt (95% and 86%). The design of experiments identified the optimal concentrations of the acid compound (1 part of gluconic acid, 2 parts of lactic acid, and 3 parts of citric acid) to be 3.18 M, with S/L = 0.1, pH = 0.5, and stirring speed = 386 rpm. Moreover, the kinetic studies have suggested a 120-minutes process time at 75 °C as the conditions resulting in the highest nickel and cobalt recovery rates (25.5% and 37.6%) [44, 45]. Hosseini Nasab *et al*. have also investigated the kinetics of the double-step bio-leaching of Ni and Co from the iron-rich laterite when using the supernatant metabolite products of *Salinivibrio kushneri* as a halophilic bacterium. They reported that the nickel and cobalt recovery rates could reach 58.40% and 60.6%, respectively, after 3 h bio-leaching at 90 °C [46].

In this work, we aimed to assess the effect of adding Fe^{2+} and Fe^{3+} ions on the dissolution of the iron-rich laterite samples using sulfuric acid as the solubilizing agent. In addition, the influence of

using the two new strains of *At.ferrooxidans* and *Delftia acidovorans* on the biological dissolution of the iron-rich laterite was investigated. For the first time, the performance of *Delftia acidovorans*, as a heterophropic acidophilic bacterium, for the dissolution of nickel and cobalt from an iron-rich laterite sample was compared against *At.ferrooxidans* as one of the most common acidophilic autotrophic bacteria. A comparison was made during the direct and indirect (spent medium) bio-leaching based on the recovery rates of nickel, cobalt, and iron. For the indirect bioleaching, the kinetics of the process at different times and temperatures was studies in order to determine the optimum parameters. Moreover, the activation energy and type of the controller model for each bacteria during the indirect bio-leaching were determined.

2. Materials and Methods
2.1. Sample and characterization studies

The laterite sample used for this research work was obtained from the Kanshargh mine (located in the east of Sarbisheh, South Khorasan Province, Iran, with an estimated reserve of 3,700,000 tons). The average grades of nickel, cobalt, and iron in the sample were 1.74%, 0.14%, and 40.83%, respectively. This sample is known to be rich in nickel and cobalt with a high iron content. The results obtained from the particle size analysis (Micro Tec Plus Analyzer Particle Size) showed that the laterite sample used in this work was finer than 38 microns. The pre-calcined sample was used as the feed in all the leaching experiments for two main reasons. It is known that pre-calcination converts goethite (FeOOH) to hematite (Fe_2O_3). It also develops nickel in the fine pores or cracks, which can be more easily dissolved by the microorganisms [1]. Before pre-calcination, d_{25}, d_{50}, and d_{80} of the sample were 2.5 μm, 8.6 μm, and 25.2 μm, which were changed to 5.6 μm, 15.1 μm, and 30.1 μm after calcination. The elemental analysis of the laterite sample after pre-calcination in the furnace at 500 °C for 2 h showed that the average grades of nickel, cobalt, and iron in this sample were 2.3%, 0.17%, and 32.66%, respectively. The XRD analysis (MPD 3000) indicated that goethite, calcite, hematite, and quartz were the most important crystalline phases. The results of XRF analysis (MAGIX-PRO) showed a high iron content (61.4% of Fe_2O_3) together with 3% NiO, 0.2% Co_3O_4, 9.2% SiO_2, and 5% Al_2O_3 in the sample.

2.2. Bacteria and cultivation

The *Delftia acidovorans* and *At.ferrooxidans* bacteria were extracted from the acid drainage (bottom of the tailing dump) of the Sungun copper mine located in the east Azerbaijan Province, Iran. The microbial population of the acid drainage was low due to the high toxicity of the environment. A sample was taken using 50 mL of region water in 50 mL sterile falcon, which was then centrifuged in $4000 \times g$ for 20 min. For the initial cultivation, 5 mL of the falcon bottom liquid was inoculated to 100 mL of an acidic culture medium. Doublly distilled water was used in order to prepare all the culture media, while sulfuric acid was used for the pH adjustment. The compounds and sterilization

conditions of each culture medium as well as the reason for choosing them are described in Table 1. The flasks containing the inoculated culture media were shaken at 150 rpm in the refrigerated incubator shaker for 3–5 weeks, while the temperature was set at the optimal value (Table 1). After the growth was confirmed, lams were prepared in order to perform the gram staining. To do this, 5 mL of the growth medium was centrifuged at 11500 rpm for 20 min in order to produce the required biomass. The lam was prepared from the settlements of the falcon. Then the gram staining was done based on the Hucker method [47] using $\times 100$ optical magnification of the microscope.

Table 1. Culture media used for *At.ferrooxidans* and *Delftia acidovorans*.

Culture media	Components of culture medium (g/L)	Heating conditions ($^\circ$C)	Sterilization conditions	Microorganism
9K	$(NH_4)_2SO_4$: 3 $MgSO_4.7H_2O$: 0.5 KCl: 0.1 K_2HPO_4: 0.5 $Ca(NO_3)_2$: 0.01 $FeSO_4.7H_2O$: 44.4 (50)	30-34	20 psi-15 min (Iron sterilization using 0.22 μm filter)	*At. ferrooxidans*
9K+Glucose	Glucose: 10 $(NH_4)_2SO_4$: 3 $FeSO_4.7H_2O$: 0.01 $MgSO_4.7H_2O$: 0.5 KCl: 0.1 KH_2PO_4: 0.5 $Ca(NO_3)_2$: 0.01	27-30	20 psi-15 min (Iron sterilization using 0.22 μm filter)	*Delftia acidovorans*

In order to preapare 1 L of the 9K+Glucose medium, two samples (each of 50 mL) were used to dissolve the glucose and the ferrous iron sulfate. These samples were filtered using a 0.22-μm filter, and added to the solution made from all the components of the culture media (that were autoclaved). Since the optimal pH levels for the *At.ferrooxidans* and *Delftia acidovorans* growth in the 9K and 9K+Glucose mediums were 1.8-2 and 3.5, respectively [34, 48], the pH of the culture medium was adjusted to the proper level using 5 mol/L of sulfuric acid. When using *At.ferrooxidans* for the laterite bio-leaching, the energy source (sulfur, also ferrous iron oxide in the form of iron sulfate) was provided.

In the presence of the reducing agents (for example Fe^{2+}), the electron transport at the surface of the oxidized material produces an oxidized Fe^{2+} species that can be more easily attacked by the acid [49]. The nickel-bearing laterite ores do not contain sulfur, and therefore, it should be added to the chemolithotrophs for leaching the laterite ores [6, 50]. A culture medium with 1% sulfur and 5%

ferrous iron sulfate, as the energy source for *At.ferrooxidans* [1], was prepared at the initial pH of 1.8. All the direct leaching experiments as well as the preparation of the supernatant were performed in 250 mL flasks under sterile conditions. 10 mL of the incubated liqued containing the bacteria grown in the culture media (with around 1×10^9 cells/mL) was used as an inoculum liquid in order to prepare 100 mL of the final volume. To do this, 10 mL of the no-filtered suspension (containing the bacteria) as well as 90 mL of the culture medium of each bacterium were added to each flask. The flasks were then placed in an incubator shaker (150 rpm and 30 $^\circ$C) for 3-5 weeks for the medium containing *At.ferrooxidans*, and about 2 weeks for the medium containing *Delftia acidovorans*. The pH, ORP, and bacterial counts in the flasks were measured on a daily basis. The pH and ORP were measured using a portable Eh/pH-meter (METTLER TOLEDO, MP120 Basic Portable pH/mV/$^\circ$C Meter). After reaching the pH of 1-1.2, the flasks were placed in the refrigerator for use as the inoculum liquid for the

direct experiments, whereas the filtrates of the solutions were used as the supernatant in the indirect experiments. It should be mentioned that both bacteria could tolerate 10 g of laterite in 100 mL of the solution, and thus there was no need to adapt the bacterium in the laterite sample (containing nickel and cobalt).

2.3. Methodology of bio-leaching experiments

The control tests of the direct bio-leaching were performed for both bacteria. 10 g of the autoclaved laterite sample was added to 100 mL of the bacterial culture medium with bactericide. Here, $HgCl_2$ was used as bactericide in the control tests of the laterite sample and the 9K culture medium. For the tests where the laterite sample and the 9K+Glucose culture medium were used, the eukaryotic antibiotic served as the bactericide. The pH was initially adjusted at 1.8 using 5 mol/L of sulfuric acid. Throughout the whole direct experiments (for 30 days), no further adjustment of the pH was performed. Sampling for the control tests was identical to that of the main direct experiments, which were performed on the 5th, 10th, 15th, 20th, and 30th days of the tests. Then the samples were analyzed by atomic absorption spectrometry (Varian Spectr AA.20) in order to determine the nickel, cobalt, and iron contents. To determine the Fe^{3+} content, the amount of Fe^{2+} in the sample was subtracted from the total iron detected by atomic absorption analysis. The concentration of Fe^{2+} was determined using potassium dichromate and following the titration technique. The volumes extracted for sampling and titration of the tests were compensated using the same volume of the culture medium. The pH, ORP, and bacterial counts were performed regularly over 30 days of treatment. Every day, the decrease in the solution volume due to the measurements and evaporation was compensated by distilled water. The required acid to recover the initial pH of the sample (1.8) was determined and considered as the acid was consumed by the laterite.

The turbidity and discoloration of the inoculated liquid media were assessed within 3-5 weeks (or longer, if necessary). The reddish-brown color as well as the formation of iron species in the medium containing high concentrations of iron(III) sulfate were used as the indications for oxidation of iron(II) ions to iron(III) ions, and growth of the iron-oxidizing microorganisms. In the medium containing *Delftia acidovorans*, the turbidity observation was used to determine the optimum growth. Meanwhile, during the bacterial growth, the pH and ORP as well as the bacterial counts were measured by biological microscopy (Axiostar Plus model) every other days. When the bacterial count reached 5×10^8 cells/mL, the bacteria were isolated by centrifugation (Heraeus model) at $4000\times g$ for 20 min, and the supernatant products were extracted and used for the indirect (spent medium) bio-leaching experiments. The direct and indirect parallel experiments were repeated for three times, and the results obtained were averaged. At the end of the leaching experiments, the flask contents were filtered, and the biomass together with the ore residue were washed with 2% sulfuric acid. The samples obtained during the experiments were properly diluted in order to determine the percentage of the leached nickel and cobalt as well as the dissolution rate of iron by the studied bacteria using an atomic absorption spectrometer. The surface analyses were performed in order to evaluate the changes in the laterite surface during the bacterial leaching. The SEM analysis (FEI QUANTA 450) and EDX elemental mapping (BRUKER XFLASH 6/10) for Si, Ni, Co, Fe, Al, and O were performed on the feed sample as well as on the leached residue obtained under the optimum conditions.

The indirect leaching experiments were carried out in a 1-L glass reactor at the atmospheric pressure (No. 1 in Figure 1). It was floated in an electrically heated silicone oil bath (No. 2 in Figure 1). The reactor was equipped with a mechanical stirrer (Heidolph, HPS-55, Germany) that had a digital control unit and a Teflon impeller (No. 3 in Figure 1). A magnetic stirrer (Multi-stirrer DM-8 Scinics, Japan) was used as the base of the device (No. 4 in Figure 1). The temperature was measured continously by a thermometer (No. 6 in Figure 1). Using a thermostat (No. 9 in Figure 1), the reactor temperature was controlled within a ±0.1 °C accuracy. A reflux condenser was also installed in order to prevent the evaporation of the solution, especially at high temperatures (No. 8 in Figure 1). The periodic sampling was performed from the access point 7 depicted in Figure 1. In order to place the laterite sample into the reactor, the Pyrex glass lid was removed, and the feed was added into the solution by a glass funnel (No. 5 in Figure 1).
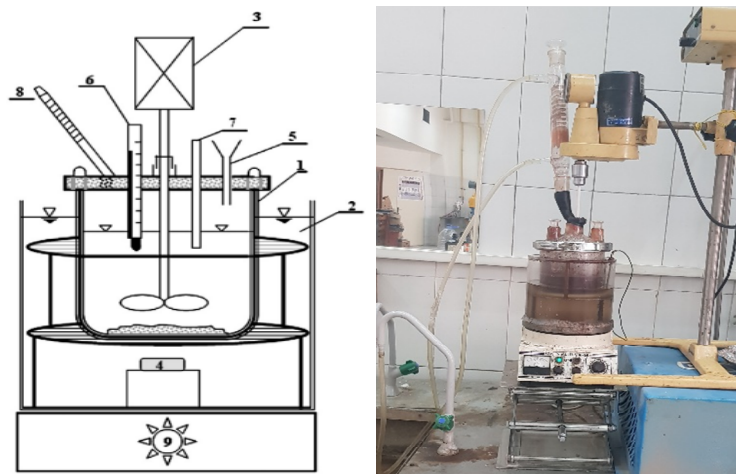
Figure 1. A schematic layout and a real photo of the reactor used for the indirect bio-leaching experiments.

2.4. Kinetic mechanism of dissolution process for indirect bio-leaching experiments

Leaching of the minerals can be represented using different models. The shrinking core model is one of the main models developed in order to describe the kinetics of the dissolution process in a non-catalytic solid-liquid environment [51, 52]. Table 2 lists different reaction rate constants that can be used to describe different mechanisms of dissolution for the minerals using the shrinking core model [53].

Table 2. Reaction rate constant for different mechanisms of mineral dissolution [53]. X is the reacted fraction, *k* is the kinetic constant of the reaction, and t is the reaction time.

Eq. No.	Model	Mechanism
1	$K_t = 1 - (1-X)^{\frac{1}{3}}$	Chemical reaction control
2	$K_t = 1 - (1-X)^{\frac{2}{3}}$	Mixed control model by shrinking core model (diffusion control; chemical reaction control)
3	$K_t = [1 - (1-X)^{\frac{1}{3}}]^2$	Diffusion through product layer
4	$K_t = -\ln(1-X)$	Mixed control model (surface reaction control; diffusion through sulfur layer)
5	$K_t = 1 - \frac{2}{3}X - (1-X)^{\frac{2}{3}}$	Diffusion control
6	$K_t = \frac{1}{3}\ln(1-X) + [(1-X)^{\frac{-1}{3}} - 1]$	Interfacial transfer and diffusion across the product layer
7	$K_t = 1 - 3(1-X)^{\frac{2}{3}} + 2(1-X)$	Diffusion of hydrogen ions through a product layer by the shrinking core model
8	$K_t = 1 - (1 - 0.45X)^{\frac{1}{3}}$	Surface chemical reaction by the shrinking core model

In order to determine the kinetic mechanism governing the microbial dissolution of the laterite sample, the shrinkage model (equations in Table 2) was fitted to the experimental data obtained from the microbial leaching. Here, the model resulted in the best fit (with the highest correlation coefficient) was selected in order to determine the dissolution mechanism. The shrinking core model assumes that the solid particles retain their initial volume, while their inactive cores continuously shrink with the reaction time and form a porous layer [54]. The bio-leaching process requires four consecutive steps: (1) diffusion of the attacking species from the solution into the reactant; (2) diffusion of the reactants into the solid matrix; (3) chemical reaction; (4) transformation of the resultant species to the solution [35, 55, 56]. The slowest step will determine the rate of the leaching reaction [56]. The severe shacks during the process prevent the first and last steps to considerably impact the reaction rate [7]. Therefore, in this work, the diffusion control (Eq. 5 in Table 2) and the chemical reaction control (Equation 1 in Table 2) mechanisms were investigated in order to describe the reaction using the shrinkage core model.

3. Results and discussion

3.1. Molecular identification and phylogenetic analysis of bacterial strain

The complementary identifications of the *Delftia acidovorans* and *At.ferrooxidans* strains were performed by amplification and sequencing of the 16S rRNA gene. For this purpose, a bacterial clone was first isolated from the acid drainage, and the required biomass was prepared. After extracting the genomic DNA of the strain, gene amplification of the small ribosomal subunit was performed using the 16S rRNA primers in a polymerase chain reaction. The genomic DNA extraction and 16S rRNA gene amplification were confirmed using the agarose gel electrophoresis (EV243 model). The final product of the polymerase chain reaction (PCR) was sequenced, and the results obtained were used for the phylogenetic analysis [46].

In order to assess the quality of the extracted genomic material, 5 µL of the PCR product of the extracted DNA was loaded on 0.8% agarose gel using the 27F and 1492R primers. A single band at around 1200 bp was selected as the appropriate band, and was then sequenced. This stage was repeated for three different clones of the gene (numbers 1, 2, and 3 in Figure 2).

The results of the *Delftia acidovorans* strain were identical to those for *Delftia acidovorans* 2167^T (JOUB01000005). However, the complementary identification of the *At.ferrooxidans* strain showed 99.33% similarity to *At.ferrooxidans* $ATCC23270^T$ (CP001219). Both *Delftia acidovorans* and *At.ferrooxidans* were gram-negative bacteria (see the microscopic image in Figure 3).
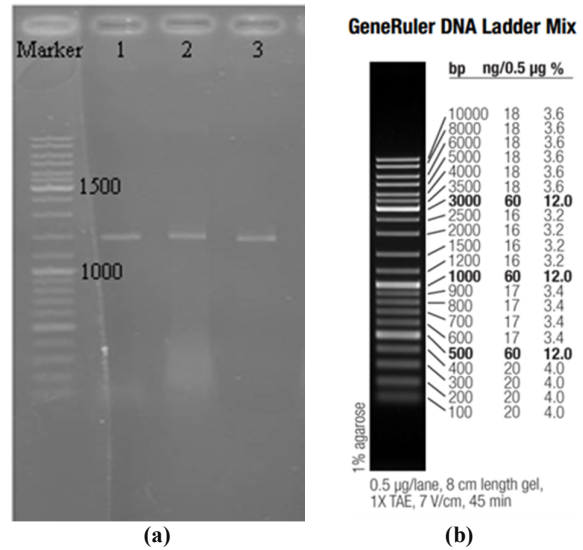
Figure 2. An exemplary gel electrophoresis of 16S rRNA of the extracted genomic bands and the corresponding reference gel electrophoresis for the polymerase chain reaction products. A) DNA ladder; B) gel electrophoresis of 16S rRNA.
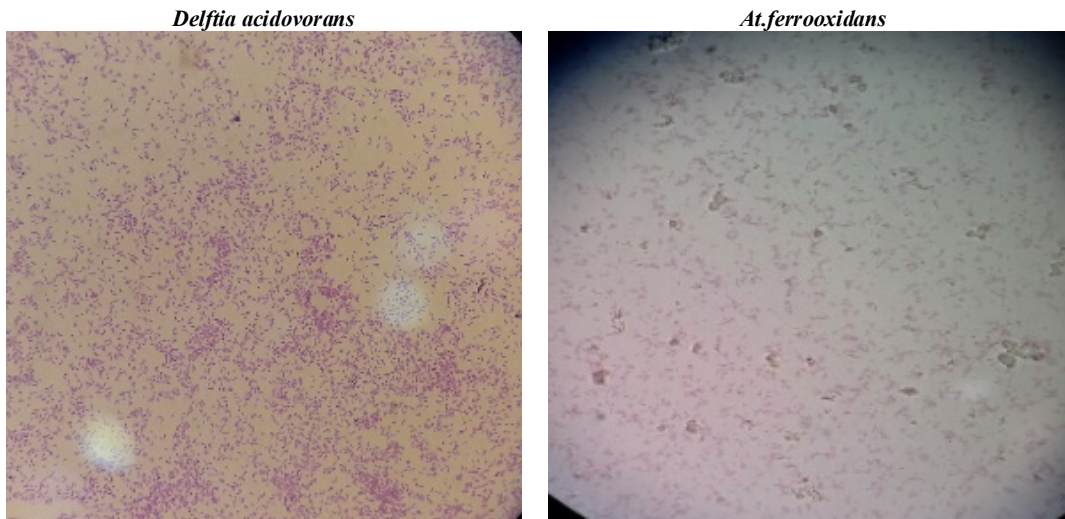
Figure 3. Microscopic images of *Delftia acidovorans* and *At.ferrooxidans* (*1000x magnification*).

3.2. Leaching of sample by sulfuric acid in presence of Fe^{2+} and Fe^{3+} additives

In order to investigate the role of the ferric and ferrous iron on the dissolution of the studied laterite sample, a number of experiments were performed at 30 °C, S/L = 0.1, stirring speed = 370 rpm, and 5 mol/L of sulfuric acid. Ferrous iron ($FeSO_4.7H_2O$) and ferric iron ($Fe_2(SO_4)_3$ and $FeCl_3$) with 0, 25, and 50 g/L were used as the additives.

As shown in Table 3, adding 50 g/L of Fe^{+2} to 5 mol/L of sulfuric acid at 30 °C, S/L = 0.1, and stirring speed = 370 rpm to 5 mol/L sulfuric acid

resulted in 18.32% and 19.57% increase in the nickel and cobalt recoveries and 62.7% increase in the iron dissolution rate. This can be explained by the fact that the Fe^{2+} ion is a reducing agent, and therefore, its higher concentration in the medium decreases the oxidation/reduction potential, thus shifting the condition towards reduction [13, 16, and 17]. This enhances the dissolution of nickel and cobalt since laterite is an oxidized ore and requires a reductive medium to be dissolved. Moreover, hematite and iron oxide minerals can be more easily degraded in a reductive medium since the oxygen of these minerals can be more readily

separated. The reductive medium can also help the nickel and cobalt presented in laterite to be more easily dissolved in the presence of acid [4, 39, and 26]. Here, the Fe^{3+} ions produced by the *At.ferrooxidans* bacteria can infiltrate into the laterite and reduce it to Fe^{2+} [4]. This reduction produces acid, which can help the dissolution of cobalt and nickel [14]. The results presented in Table 3 support the mechanisms explained above, and thus endorse using the acidophilic *At.ferrooxidans* bacteria in the biological dissolution of nickel and cobalt from the iron-rich laterite.

Table 3. Leaching of the laterite sample by 5 M sulfuric acid with different additives (30 °C, S/L = 0.1, stirring speed = 370 rpm).

Additive (g/L)	Ni recovery (%)	Co recovery (%)	Fe dissolution (%)
0	16.81	19.58	9.42
25 Fe^{+2} ($FeSO_4.7H_2O$)	30	37.96	48.37
50 Fe^{+2} ($FeSO_4.7H_2O$)	35.13	39.15	72.12
25 Fe^{+3} ($Fe_2(SO_4)_3$)	14.83	18.59	24.17
50 Fe^{+3} ($Fe_2(SO_4)_3$)	14.41	20.04	39.57
50 Fe^{+3} ($FeCl_3$)	17.48	21.49	49.10

3.3. Direct bio-leaching of sample by two studied bacteria

The direct bio-leaching experiments were performed in the incubator shaker at 150 rpm and 30 °C for 30 days. For both bacteria, the control tests (2.3) were also carried out in order to provide

a basis for comparison of the main test results. The results of dissolution of Ni, Co, and Fe for both the main and control tests are presented in Figure 4. It is clear that for both bacteria, the amounts of dissolved nickel and cobalt in the main experiments are three times greater than those in the control tests.
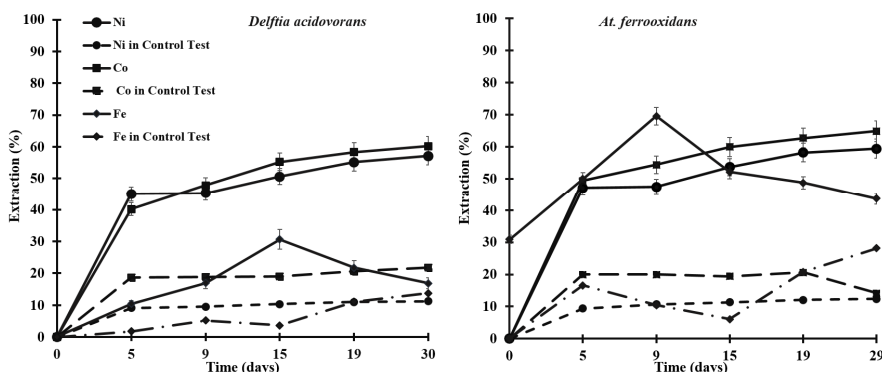
Figure 4. Nickel and cobalt recovery and iron dissolution versus time for both studied bacteria during direct bio-leaching.

The highest recovery rates of nickel and cobalt by *Delftia acidovorans* at the end of the experiments were 57.09% and 60.19%, respectively, while the iron dissolution rate at this stage was 16.84%. However, the highest dissolution of iron (30.62%) was observed after 15 days. When using *At.ferrooxidans,* the terminal recovery rates for nickle and cobalt were 59.41%

and 64.85%, respectively, with the iron dissolution of 43.60%. Here, the highest dissolution rate of iron (69.55%) was observed after only 9 days. These results obtained indicate very similar nickel and cobalt recovery rates for both species; however, the iron dissolution was much lower when using *Delftia acidovorans* compared with *At.ferrooxidans.* This shows the superiority of

Delftia acidovorans over *At.ferrooxidans* for the dissolution of iron-rich laterite containing nickel and cobalt. A possible reason for the low iron dissolution when using *Delftia acidovorans* may originate from the lower amount of iron used in order to preparare the culture medium for this bacterium.

The concentration of Fe^{3+} can be determined by comparing the total amount of Fe obtained from the atomic absorption analysis *vs.* the amount of Fe^{2+} obtained from the titration technique (Figure 5).

Compared with the control tests of *At.ferrooxidans,* the amount of released ferrous iron was much higher in the main experiments, which was caused by the acid produced by the bacteria. For the main experiments, the higher Fe^{2+} level at the first sampling day (day 5) when using *At.ferrooxidans* can be explained by the use of 50 g/L of hydrated ferrous sulfate in order to prepare the culture medium. However, over time, Fe^{2+} was gradually converted to Fe^{3+}, as shown for both bacteria. Conversion of Fe^{2+} to Fe^{3+} enhances the production of biological sulfuric acid, which concequently decreases the pH. For *Delftia acidovorans*, the concentration of the ferrous iron decreases between the days 5 and 9, possibly due to iron absorption in the ore. For the two bacteria, the concentration of Fe^{3+} was gradually increased by time until 15 days of bio-leaching for *Delftia acidovorans* and 9 days of bio-leaching for *At.ferrooxidans*, and after that, the iron deposition resulted in a decline in Fe^{3+}. Generally, the ORP and pH followed the opposite trends, indicating a decrease in pH due to an increased ORP. Here, for both bacteria, the oxidation of Fe^{2+} to Fe^{3+} due to the bacterial activity increases the ORP; however, compared with *Delftia acidovorans,* *At.ferrooxidans* was more successful in pH reduction. This is mainly due to using 50 g/L of hydrous ferrous sulfate in the culture medium of *At.ferrooxidans,* which facilitates the reduction reaction producing biological sulfuric acid.

The bacterial population decreased during the first period of the experiments (Figure 5), which may be due to the bacterial uptake of the laterite surface. However, the number of bacteria generally increased until the end of 30 days, which was accompanied by a pH decrease. Nickel is mainly bounded with goethite [57]. The bacteria reduce the ferric iron content of the goethite minerals in the laterite ore, and consequently, destruct the goethite and release the nickel [12]. Due to the higher bacterial activity at lower pH values, the acid concentration (H^+ ions) is also expected to be high (pH = $-\log_{10} [H^+]$). The H^+ ions cause the nickel dissolution (Equation (1)):

$$FeO(OH) + 3H^+ \rightarrow Fe^{3+} + 2H_2O \qquad (1)$$
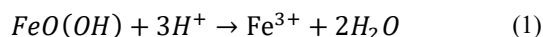
Since both the studied bacteria were acidophilic, any changes in the pH (or H^+) can affect the bacterial growth, and thereby, the leaching efficiency, and thus assessment of the pH in this research work was crucial. It should be mentioned that laterite is an alkaline ore, and this is why the pH of the pulp increased immediately after adding the laterite sample; however, it decreases again after adaptation of the bacterium (Figure 5).

The acid consumption as well as the pH changes over time are presented in Figure 6.

The laterite sample had a higher acid absorption at the beginning; however, after 2 weeks, it did not consume any acid. Simate and Ndlovu (2008) have found that regardless of the amount of the bacterial inoculation, the rate of acid production through sulfur oxidation is higher than the rate of acid consumption by the bacteria [6]. Here, the extreme rate of acid consumption at the beginning of the test can be due to the alkaline pH of the laterite and the presence of the acid-consuming minerals. Acid consumption in the reductive dissolution (Equation 2, 1.67 mol per one mole of goethite) was significantly lower than the non-reductive acid dissolution (Equation 1, 3 mol per one mole of goethite) [5, 18].

$$6\alpha - FeO.OH + S^0 + 10H^+ \rightarrow 6Fe^{2+} + 8H_2O + SO_4^{2-} \qquad (2)$$
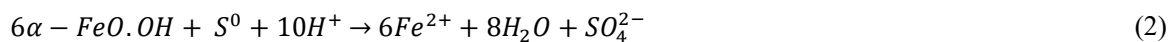
The release of the ferric iron from the ferric-bearing oxides in an acidic solution using acidolysis (Equation 1) is a very slow reaction. It has been shown that the release of ferric iron (by the chelating agents or by the reduction of ferric iron to ferrous iron), and thus the imbalance between ferric iron in the solution and the solid phase can accelerate the release of ferric iron [18].

The accompanied nickel and cobalt can be dissolved by H^+ (Equations (3) and (4)) and remain in the aqueous phase in the form of a sulfate complex.
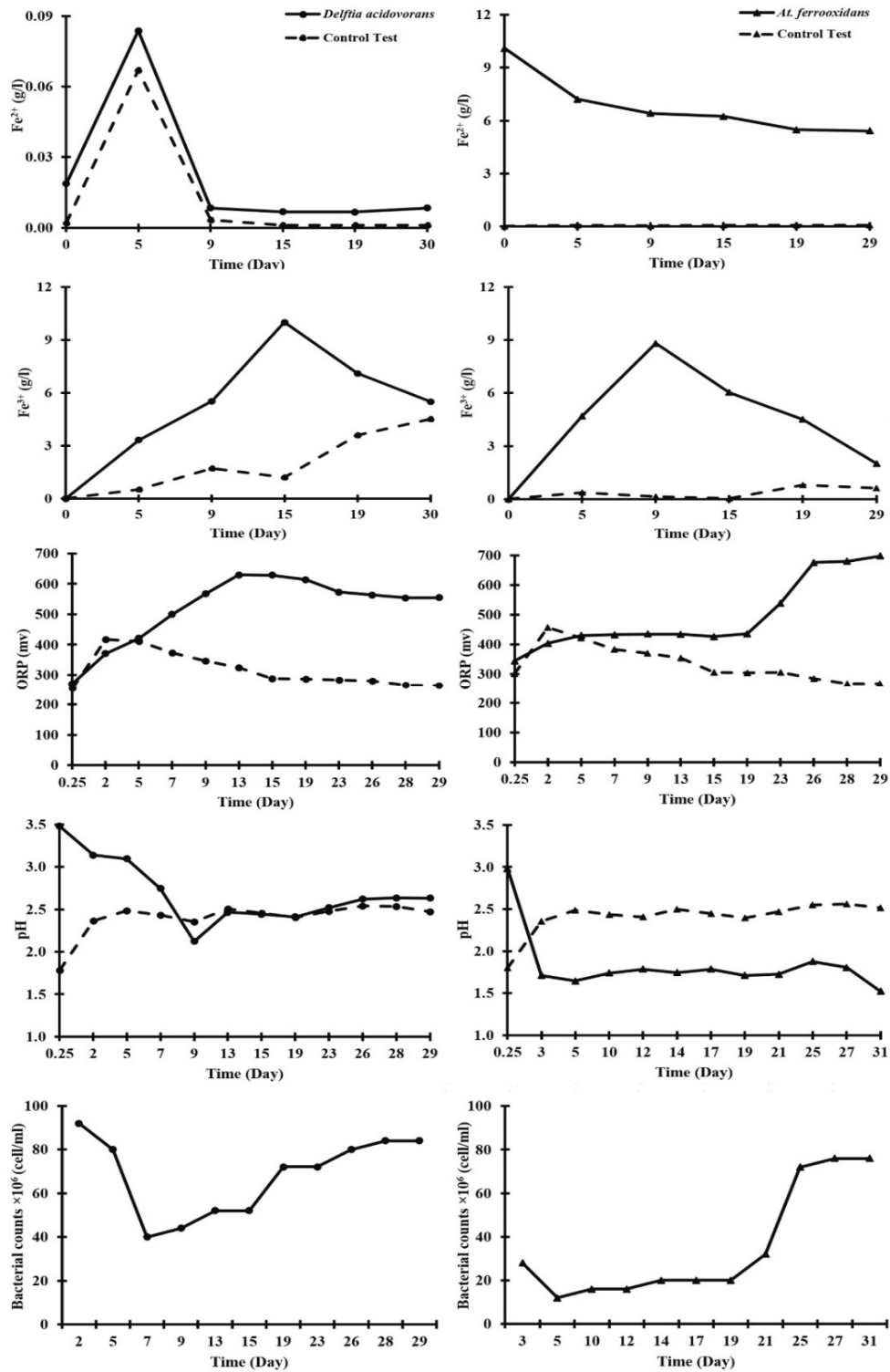
$$NiO(OH)_s + 3H^+ \rightarrow Ni^{2+} + 2H_2O \qquad (3)$$

$$CoO(OH)_s + 3H^+ \rightarrow Co^{2+} + 2H_2O \qquad (4)$$
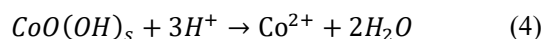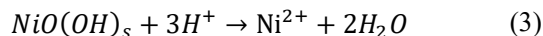
Figure 5. Growth curves of the two studied bacteria versus time in direct bio-leaching (at 30 °C and 150 rpm).

3.4. Indirect bio-leaching of sample by two studied bacteria

The results of the indirect bio-leaching tests showed that both species were well able to extract cobalt and nickel from the laterite structure (Figure 7). The highest nickel recovery by the *At.ferrooxidans* and *Delftia acidovorans* supernatants were 83.65% and 80.18%, respectively, while the highest cobalt recoveriy rates were 86.93% and 83.94%, all observed at 90 °C. The corresponding iron dissolution rates were

64.34% and 54.41%, respectively. At this temperature, the nickel and cobalt recoveries reached a plateau after 2 h. Therefore, for the studied laterite sample, 2 h and 90 ℃ were considered as the optimal time and temperature for the nickel and cobalt dissolution in the indirect bio-leaching.
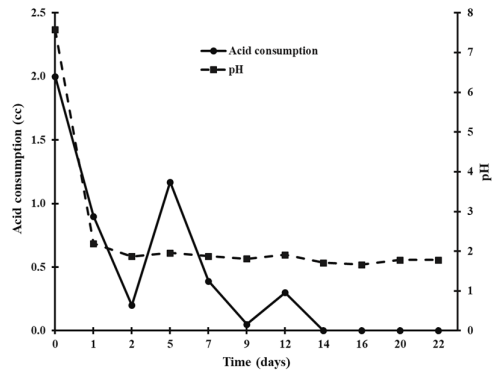

Figure 6. Acid consumption and pH changes throughout the process for the laterite sample.
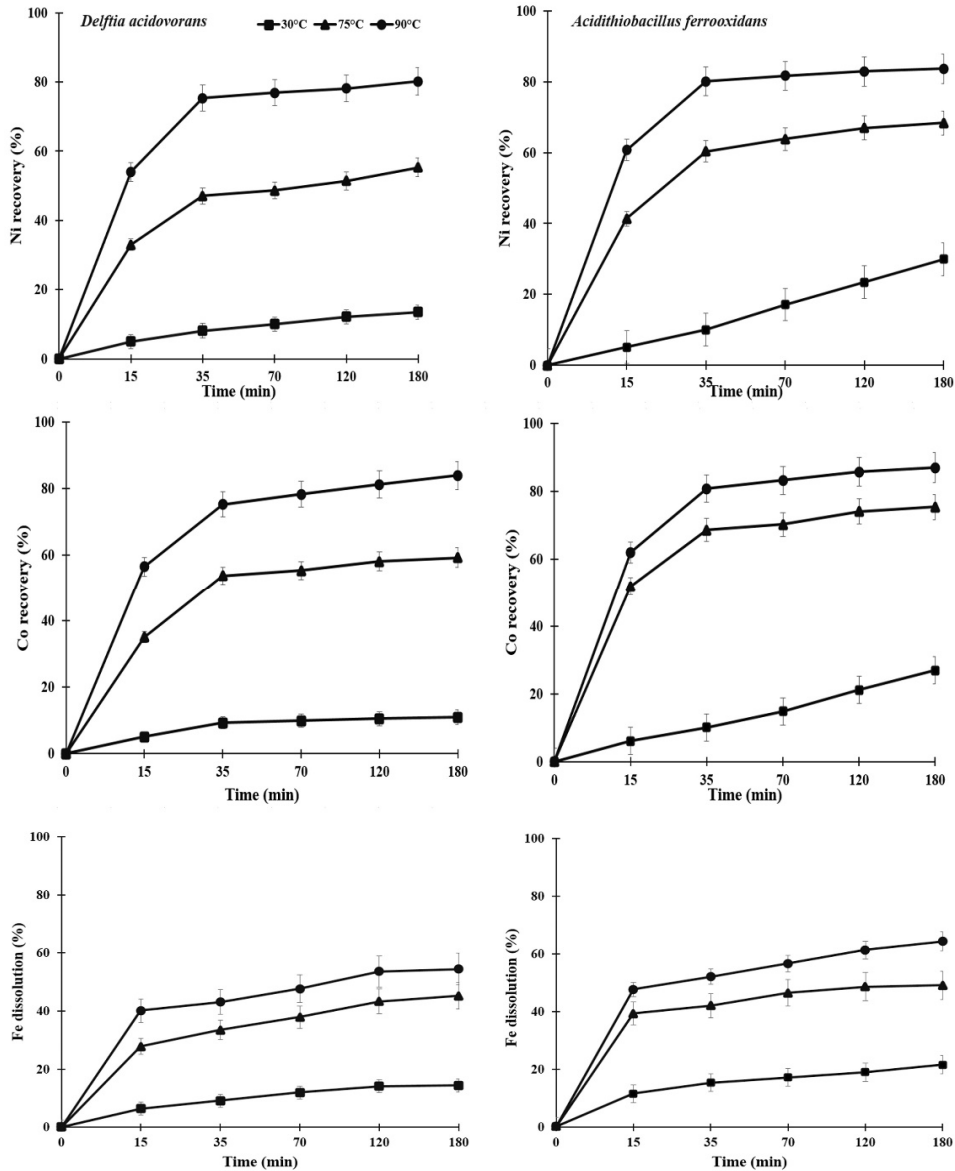

Figure 7. Nickel and cobalt bio-leaching from the laterite sample by *Delftia acidovorans* (left diagrams) and *At.ferrooxidans* (right diagrams) metabolites (stirring speed = 370 rpm, solid percentage = 10%, w/v).

The results obtained showed that for both *At.ferrooxidans* and *Delftia acidovorans,* the indirect bio-leaching at high temperatures and short time periods can more efficiently dissolve nickel and cobalt from the studied laterite sample compared to the direct bio-leaching at low temperatures for a long time. In the spent medium bio-leaching by the *Delftia acidovorans* supernatant, the nickel and cobalt extractions at 90 °C for 2 h were 29.84% and 23.75% higher than those for the direct bio-leaching at 30 °C and for 30 days. It should be mentioned that due to the use of high temperatures in the indirect bio-leaching, the iron dissolution rate is 23.79% more than the direct bio-leaching, which is a disadvantage for the indirect bio-leaching technique. However, for the industrial applications, the indirect bio-leaching method can be used at higher temperatures in order to accelerate the dissolution of metals, while the disolved iron can be removed using the well-known techniques [58-60].

3.5. Kinetic studies of indirect bio-leaching

From the models presented in Table 2, the diffusion and chemical control models (Equations 5 and 6) had the most appropriate fits with the highest correlation coefficients for the nickel and cobalt dissolution data obtained for the two studied bacteria (Figure 8).

$$k_t = 1 - \frac{2}{3}X - (1 - X)^{\frac{2}{3}} \qquad (5)$$

$$k_t = 1 - (1 - X)^{\frac{1}{3}} \qquad (6)$$

The first part of the diagrams in Figure 8 with the highest slope (reaction rate constant) can be used in order to determine the activation energy. The reaction rate constants as well as the correlation coefficients of the kinetic models used to describe nickel and cobalt dissolution by *At.ferrooxidans* and *Delftia acidovorans* at different temperatures are listed in Table 4.

Based on the data presented in Figure 8 and Table 4, both the diffusion and chemical control equations fit well to the experimental data. The reaction rate constants increase with an increase in temperature (Table 4), which indicates a highly temperature-dependent and thus a chemically controlled process [52].

The Arrhenius equation ($k = A \exp(-E_a/RT)$) was used in order to calculate the activation energy (E_a) for the process, where k is the rate constant, R is the ideal gas constant (8.314 J/mol.K), T is the absolute temperature (K), E_a is the activation energy (kJ/mol), and A is the coefficient of the exponential function. In order to calculate the activation energy, $-\ln k$ was plotted against 1/T to obtain a line with the slope of E_a/R. This was performed for the two control models, and thereby, the corresponding activation energies were calculated (Figure 9).

The chemical control models for the nickel and cobalt dissolution using *Delftia acidovorans* had acceptable correlation coefficients (Figure 9, $R^2 = 0.999$ for nickel and $R^2 = 0.9997$ for cobalt). For the chemical control model, the activation energy values for nickel and cobalt were $E_a = 40.07$ kJ/mol and $E_a = 39.08$ kJ/mol, while for the difusion control model, the corresponding values were $E_a = 75.82$ kJ/mol and $E_a = 70.67$ kJ/mol. Similarly, when using the *At.ferrooxidans* supernatant, in a chemical control model, R^2 for nickel and cobalt were 0.999 and 0.9867, respectively, while the corresponding activation energies were $E_a = 39.66$ kJ/mol and $E_a = 40.66$ kJ/mol.

Table 4. Reaction rate constants and correlation coefficients of the kinetic models used to describe the nickel and cobalt dissolution by *Delftia acidovorans* and *At.ferrooxidans* at different temperatures.

			1st period: Chemical		2nd period: Chemical		1st period: Diffusion		2nd period: Diffusion	
		Temperature (°C)	R^2	K	R^2	K	R^2	K	R^2	K
Delftia acidovorans	Ni	30	0.9460	0.0008	0.9656	0.0001	0.9950	0.00002	0.9836	0.00001
		75	0.9168	0.0059	0.9919	0.0003	0.9993	0.0009	0.9877	0.0001
		90	0.9441	0.0114	0.9920	0.0003	0.9999	0.0030	0.9922	0.0001
	Co	30	0.9794	0.0009	0.9540	0.00004	0.9708	0.00003	0.9630	0.000003
		75	0.9558	0.0069	0.9585	0.0002	0.9952	0.0012	0.9611	0.00008
		90	0.9184	0.0115	0.9892	0.0006	0.9966	0.0030	0.9910	0.0003
At.ferrooxidans	Ni	30	0.9926	0.0010	0.9939	0.0005	0.9490	0.00003	0.9981	0.00007
		75	0.9425	0.0081	0.9271	0.0004	0.9995	0.0017	0.9335	0.0002
		90	0.9033	0.0130	0.9354	0.0002	0.9890	0.0037	0.9366	0.0001
	Co	30	0.9551	0.0010	0.9963	0.0004	0.9910	0.00003	0.9951	0.00006
		75	0.9006	0.0100	0.9535	0.0004	0.9920	0.0024	0.9551	0.0002
		90	0.9174	0.0130	0.9561	0.0005	0.9947	0.0037	0.9572	0.0002
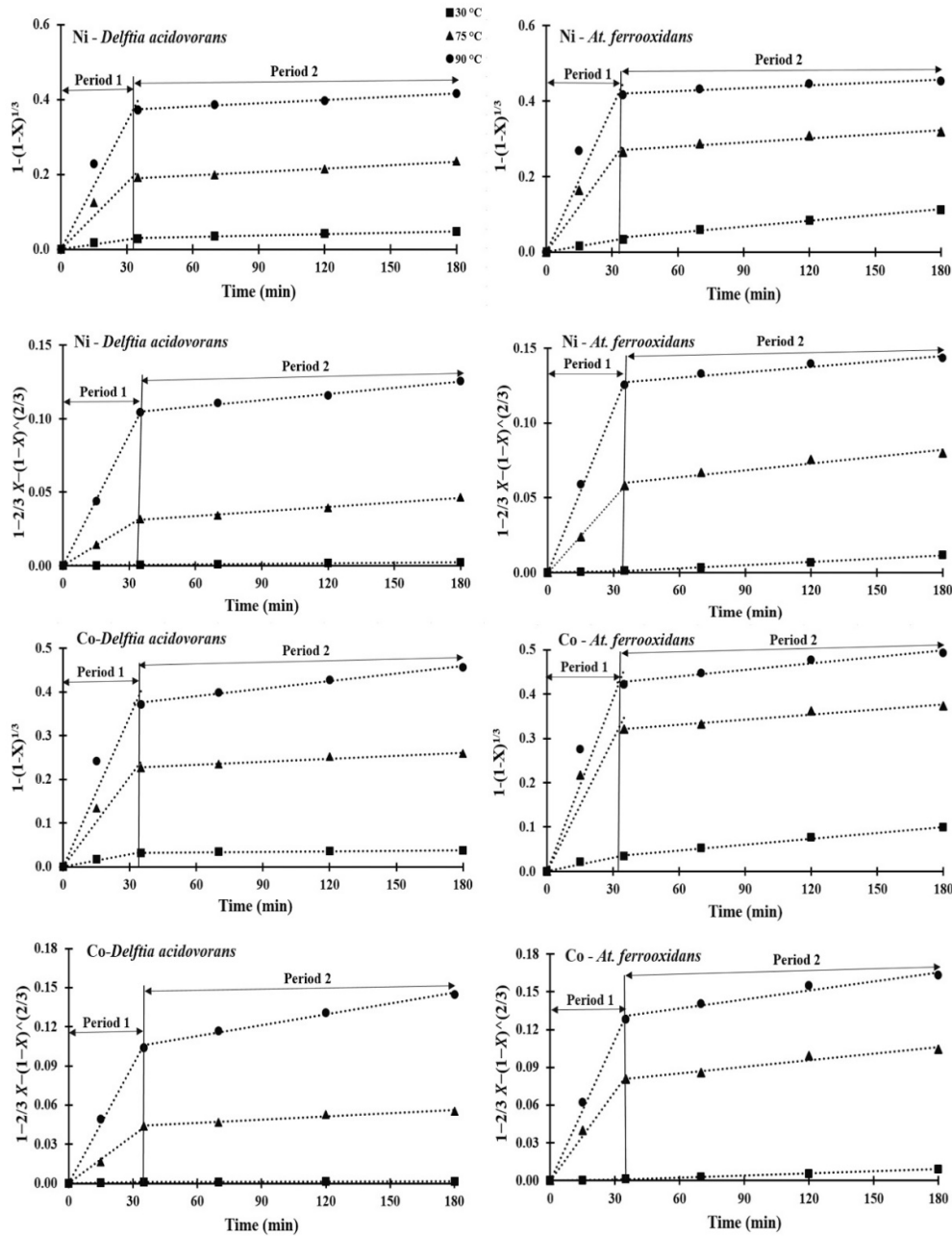
Figure 8. Kinetic modeling of the nickel and cobalt dissolution from the laterite sample by the two studied bacteria at different temperatures.

The activation energy for the diffusion control models is generally lower than 20 kJ/mol, whereas for the chemical control models, it is higher than 40 kJ/mol [61, 62]. In this work, both the chemical and diffusion control equations fitted well to the laterite dissolution data obtained at different temperatures. However, the activation energy is well within the range of a chemically controled reaction, and therefore, it can be concluded that the chemical control model is more descriptive for the studied bio-leaching process than the diffusion control. This can be justified by the relatively low rate of acidolysis, which can be caused by the structural changes of laterite and deposition of metals [7].
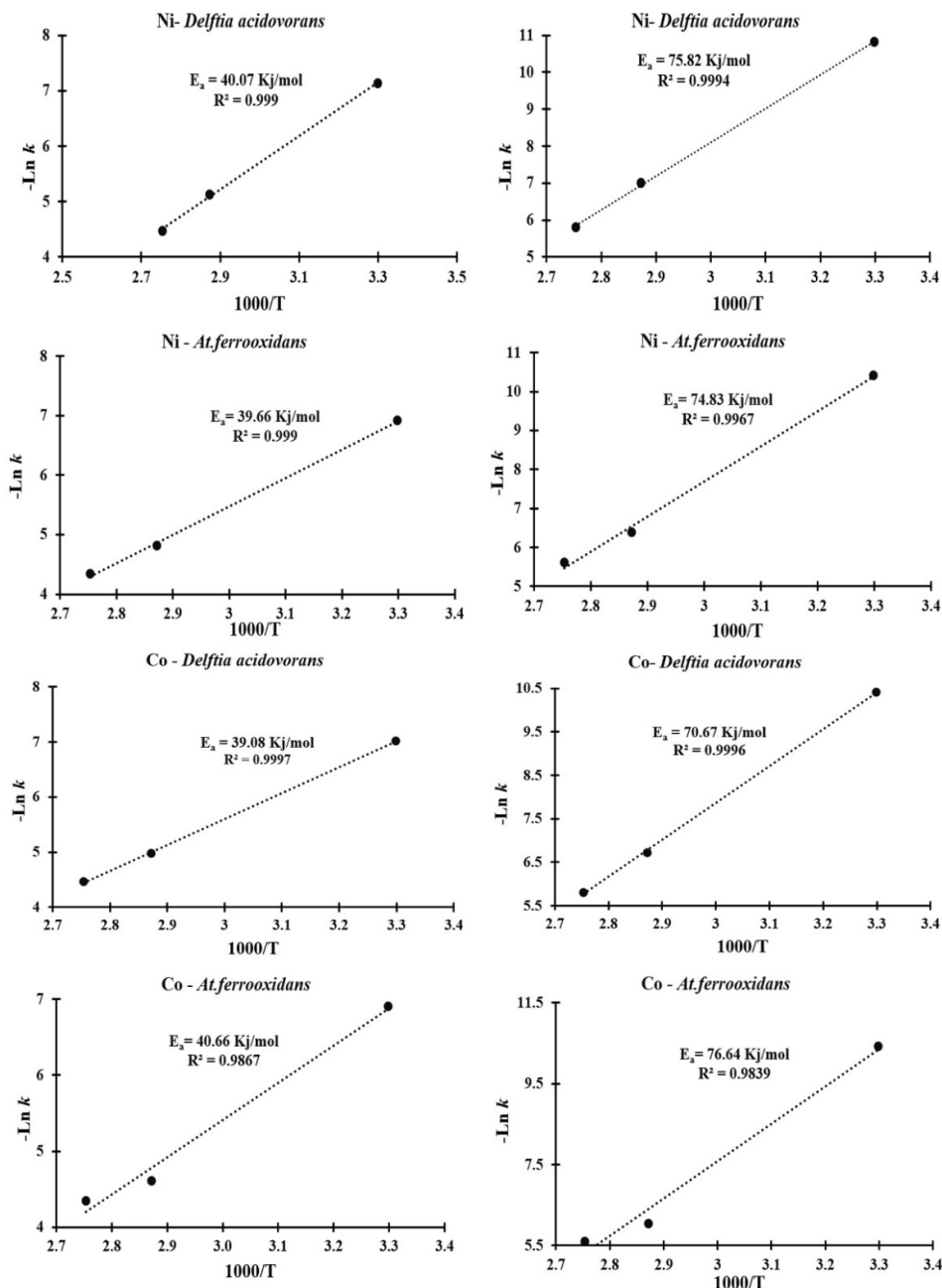
Figure 9. –ln *k* plotted against 1000/T to calculate the activation energy of the cobalt and nickel dissolution for both studied microorganisms; left: chemical control, and right: diffusion control models.

3.6. SEM/EDX analysis

The results obtained for the SEM and EDX elemental mapping of Ni, Si, Co, Al, Fe, and O for the feed sample as well as for the solid product leached under the optimum conditions are reported in Figure 10. As it could be seen, in the SEM graphs, most of the particles were flat; however, some round particles were also observed. The surface of the particles in the feed was not very different from that of the solid product leached under optimum conditions; however, there were small differences in their brightness.

The results obtained from the EDX elemental mapping of Si, Ni, Co, Fe, Al, and O in Figure 10 show a drastic decline in the Ni and Co contents of the residual solids after bio-leaching. However, the Si content did not change considerably after bio-leaching, which indicated that bio-leaching with sulfuric acid was able to dissolve most of the Co and Ni without dissolving Si and any gel production.
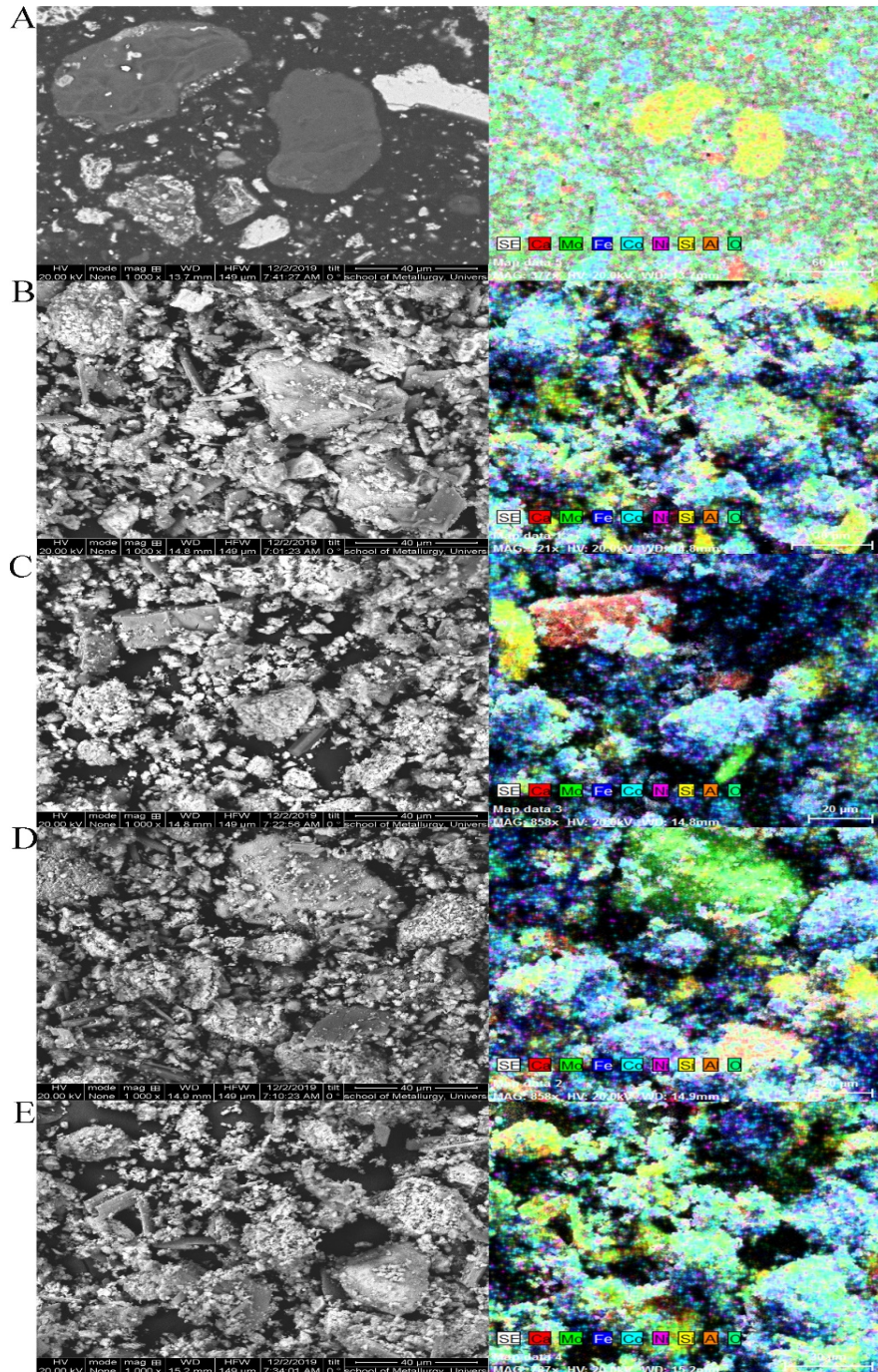
Figure 10. Left: SEM analysis, right: EDX mapping. A): feed sample calcined at 500 °C; Direct and indirect bioleaching, respectively, under optimal conditions by B and C): *Delftia acidovorans*; D and E): *At.ferrooxidans*.

4. Conclusions

The results presented in this work indicated that the laterite dissolution under an acidic condition could effectively dissolve nickel and cobalt. In particular, adding hydrous ferrous sulfate to sulfuric acid could increase the nickel and cobalt recovery rates in the bio-leaching of iron-rich laterite using *At.ferrooxidans* and *Delftia acidovorans*. More importantly, however, this work showed that using *Delftia acidovorans* and its metabolites could be used as better alternatives to *At.ferrooxidans* due to their lower iron solubility, while the nickel and cobalt recovery remained identical. The maximum nickel recoveries obtained were 83.65% and 80.18% by the supernatants of

Acidithiobacillus ferrooxidans and *Delftia acidovorans*, respectively, while the corresponding cobalt recovery rates reached 86.93% and 83.94% at 90 °C for 3 h with stirring speed = 370 rpm and S/L = 0.1. The iron dissolution rates in these conditions for the two studied bacteria were 64.34% and 54.41%, respectively. The highest recovery rates of nickel and cobalt by *Delftia acidovorans* at the end of the direct bio-leaching experiments (at 30 °C and 150 rpm of the incubator shaker for 30 days) were 57.09% and 60.19%, respectively. When using the *At.ferrooxidans* supernatants, the terminal recovery rates for nickle and cobalt were 59.41% and 64.85%, respectively. For the indirect bio-leaching, the chemical control was shown to have a larger influence on the dissolution rate of the iron-rich laterite compared to the diffusion control. The activation energies of nickel and cobalt in the chemical control model were 40.07 kJ/mol and 39.08 kJ/mol, respectively. In general, the economic process and the higher metal recovery rate when using the acidophilic bacteria compared to the use of other bacteria types highlights the advantages of using the acidophilic bacteria in the nickel and cobalt dissolution from the laterite in the future applications.

References

[1]. Mohapatra, S., Bohidar, S., Pradhan, N., Kar, R.N., and Sukla, L.B. (2007). Microbial extraction of nickel from Sukinda chromite overburden by *Acidithiobacillus ferrooxidans* and *Aspergillus* strains. Hydrometallurgy, 85: 1–8.

[2]. Watling, H.R. (2008). The bioleaching of nickel-copper sulfides. Hydrometallurgy, 91: 70–88.

[3]. Ilyas, S., Ranjan Srivastava, R., Kim, H., Ilyas, N., and Sattar, R. (2020). Extraction of nickel and cobalt from a laterite ore using the carbothermic reduction roasting-ammoniacal leaching process. Separation and Purification Technology, 232: 115971.

[4]. Johnson, D.B. (2012). Reductive dissolution of minerals and selective recovery of metals using acidophilic iron- and sulfate-reducing acidophiles. Hydrometallurgy, 127-128: 172–177.

[5]. du Plessis, C.A., Slabbert, W., Hallberg, K.B., and Johnson, D.B. (2011). Ferredox: A biohydrometallurgical processing concept for limonitic nickel laterites. Hydrometallurgy, 109: 221–229.

[6]. Simate, G.S. and Ndlovu, S. (2008). Bacterial leaching of nickel laterites using chemolithotrophic microorganisms: Identifying influential factors using statistical design of experiments. International Journal of Mineral Processing, 88: 31–36.

[7]. Rastegar, S.O., Mousavi, S.M., and Shojaosadati, S.A. (2014). Cr and Ni recovery during bioleaching of dewatered metal-plating sludge using *Acidithiobacillus ferrooxidans*. Bioresource Technology, 167: 61–68.

[8]. Johnson, D.B. and Falagan, C. (2016). *Acidithiobacillus ferriphilus* sp. nov., a facultatively anaerobic iron- and sulfur-metabolizing extreme acidophile. International Journal of Systematic and Evolutionary Microbiology, 66 (1): 206–211.

[9]. Chang, J.H., Hocheng, H., Chang, H.Y., and Shih, A. (2008). Metal removal rate of *Thiobacillus thiooxidans* without pre-secreted metabolite. Journal of Materials Processing Technology, 201: 560–564.

[10]. Nordstrom, D.K., Alpers, C.N., Ptacek, C.J., and Blowes, D.W. (2000). Negative pH and extremely acidic mine waters from Iron Mountain, California. Environmental Science & Technology, 34 (2): 254–258.

[11]. Johnson, D.B. and Hallberg, K.B. (2003). The microbiology of acidic mine waters. Research in Microbiology, 154 (7): 466–473.

[12]. Hallberg, K.B., Grail, B.M., du Plessis, C.A., and Johnson, D.B. (2011). Reductive dissolution of ferric iron minerals: A new approach for bio-processing nickel laterites. Minerals Engineering, 24: 620–624.

[13]. Golyshina, O.V., Pivovarova, T.A., Karavaiko, G.I., Kondrateva, T.F., and Moore, E.R. (2000). *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the Archaea. International Journal of Systematic and Evolutionary Microbiology, 50 (3): 997–1006.

[14]. Wu, A., Zhang, Y., Zheng, C., Dai, Y., Liu, Y., Zeng, J., Gu, G., and Liu, J. (2008). Purification and enzymatic characteristics of cysteine desulfurase, IscS, in *Acidithiobacillus ferrooxidans* ATCC 23270. Transactions of Non-ferrous Metals Society of China, 18: 1450-1457.

[15]. Simate, G.S., Ndlovu, S., and Gericke, M. (2009). Bacterial leaching of nickel laterites using chemolithotrophic microorganisms: process optimization using response surface methodology and central composite rotatable design. Hydrometallurgy, 98: 241–246.

[16]. Gray, N.F. (1996). A substrate classification index for the visual assessment of the impact of acid mine drainage in lotic systems. Water Research, 30 (6): 1551–1554.

[17]. Williams, K.P. and Kelly, D.P. (2013). Proposal for a new class within the phylum Proteobacteria, *Acidithiobacillia* classis nov., with the type order Acidithiobacillales, and emended description of the class Gammaproteobacteria. International Journal of Systematic and Evolutionary Microbiology, 63 (8): 2901–2906.

[18]. Marrero, J., Coto, O., Goldmann, S., Graupner, T., and Schippers, A. (2015). Recovery of Nickel and Cobalt from Laterite Tailings by Reductive Dissolution under Aerobic Conditions using *Acidithiobacillus* Species. Environmental Science & Technology, 49: 6674−6682.

[19]. Sahu, S., Kavuri, N., and Kundu, M. (2011). Dissolution kinetics of nickel laterite ore using different secondary metabolic acids. Brazilian Journal of Chemical Engineering, 28 (2): 251–258.

[20]. Behera, S.K., Panda, S.K., Pradhan, N., Sukla, L.B., and Mishra, B.K. (2012). Extraction of nickel by microbial reduction of lateritic chromite overburden of Sukinda, India. Bioresource Technology, 125: 17–22.

[21]. Mubarok, M.Z., Kusuma, H., Minwal, W.P., and Chaerun, S.K. (2013). Effects of several parameters on nickel extraction from laterite ore by direct bioelaching using *Aspergillus niger* and acid rock drainage from coal mine as an organic substrate. Advanced Materials Research, 825: 356–359.

[22]. Jang, H.C. and Valix, M. (2017). Overcoming the bacteriostatic effects of heavy metals on *acidithiobacillus thiooxidans* for direct bioleaching of saprolitic Ni laterite ores. Hydrometallurgy, 168: 21–25.

[23]. Hosseini Nasab, M., Noaparast, M., Abdollahi, H., and Amoozegar, M.A. (2020). Indirect bioleaching of Co and Ni from iron-rich laterite ore using metabolic carboxylic acids generated by *P. putida*, *P. koreensis*, *P. bilaji*, and *A. niger*. Hydrometallurgy, 193: 105309.

[24]. Nogami, Y., Maeda, T., Negishi, A., and Sugio, T. (1997). Inhibition of Sulfur Oxidizing Activity by Nickel Ion in *Thiobacillus thiooxidans* NB1–3 Isolated from the Corroded Concrete. Bioscience, Biotechnology, and Biochemistry, 61 (8): 1373-1375.

[25]. Natarajan, K.A. and Twasaki, T. (1983). Role of galvanic interactions in the bioleaching of Duluth gabbro copper-nickel sulphides. Separation Science Technology, 18: 1095.

[26]. Toni, D.B.J. and Bridge, A.M. (2000). Reductive Dissolution of Ferric Iron Minerals by *Acidiphilium* SJH. Geomicrobiology Journal, 17 (3): 193–206.

[27]. Coto, O., Galizia, F., Hernandez, I., Marrero, J., and Donati, E. (2008). Cobalt and nickel recoveries from laterite tailings by organic and inorganic bio-acids.

Hydrometallurgy, 94: 18–22.

[28]. Morel, M.A., Iriarte, A., Jara, E., Musto, H., and Sowinski, S.C. (2016). Revealing the biotechnological potential of *Delftia* sp. JD2 by a genomic approach. AIMS Bioengineering, 3 (2): 156–175.

[29]. Ubalde, M.C., Brana, V., Sueiro, F., Morel, M.A., Martinez-Rosales, C., Marquez, C., and Castro-Sowinski, S. (2012). The versatility of *Delftia* sp. isolates as tools for bio-remediation and bio-fertilization technologies. Current Microbiology, 64: 597–603.

[30]. Brana, V., Cagide, C., and More, M.A. (2016). The sustainable use of *Delftia* in agriculture, bioremediation, and bioproducts synthesis. In: Castro- Sowinski, Susana, editor. Microbial models: from environmental to industrial sustainability, Springer, 227–247.

[31]. Barrionuevo, M. and Vullo, D.L. (2012). Bacterial swimming, swarming, and chemotactic response to heavy metal presence: which could be the influence on wastewater bio-treatment efficiency? World Journal of Microbiology & Biotechnology, 28: 2813–2825.

[32]. Zou, G., Papirio, S., van Hullebusch, E.D., and Puhakka, J.A. (2015). Fluidized-bed denitrification of mining water tolerates high nickel concentrations. Bioresource Technology, 179: 284–290.

[33]. Robertson, L.A. and Kuenen, J.G. (1999). The colorless sulfur bacteria, the prokaryotes: an evolving electronic resource for the microbiological community. 3rd Ed. NY: Springer-Verlag.

[34]. Garcia-Moyano, A., Austnes, A., Lanzen, A., Gonzalez-Toril, E., Aguilera, A., and Ovreas, L. (2015). Novel and Unexpected Microbial Diversity in Acid Mine Drainage in Svalbard (78° N), Revealed by Culture-Independent Approaches. Microorganisms, 3 (4): 667–694.

[35]. Heinzel, E., Hedrich, S., Janneck, E., Glombitza, F., Seifert, J., and Schlomann, M. (2009). Bacterial Diversity in a Mine Water Treatment Plant [J]. Applied and Environmental Microbiology, 75 (3): 858–861.

[36]. Das, S., Dong, B., and Ting, Y.P. (2015). Gold Bio-dissolution from Electronic Scrap and Biomineralization of Bacterial Gold Nanoparticles. Advanced Materials Research 1130, 668-672.

[37] Schulz, S., Dong, W., Groth, U. and Cook, A.M. (2000). Enantiomeric degradation of 2-(4-sulfophenyl) butyrate via 4-sulfocatechol in *Delftia acidovorans* SPB1. Applied and Environmental Microbiology, 66: 1905–1910.

[38]. Ghosh, S. and Paul, A.K. (2016). Bio-leaching of nickel by *Aspergillus humicola* SKP102 isolated from Indian lateritic overburden. Journal of Sustainable Mining, 15 (3): 108-114.

[39]. Marrero, J., Coto, O., and Schippers, A. (2017). Anaerobic and aerobic reductive dissolutions of iron-

rich nickel laterite overburden by *Acidithiobacillus*. Hydrometallurgy, 168: 49-55.

[40]. Alibhai, K., Dudeney, A.W.L., Leak, D.J., Agatzini, S. and Tzeferis, P. (1993). Bio-leaching and bio-precipitation of nickel and iron from laterites. FEMS microbiology reviews, 11 (1-3): 87-95.

[41]. Valix, M., Tang, J.Y., and Cheung, W.H. (2001). The effects of mineralogy on the biological leaching of nickel laterite ores. Minerals Engineering, 14 (12): 1629–1635.

[42]. Yang, Y., Ferrier, J., Csetenyi, L., and Gadd, G.M. (2019). Direct and indirect bioleaching of cobalt from low-grade laterite and pyritic ores by *Aspergillus niger*. Geomicrobiology Journal, 36 (10): 940-949.

[43]. Valix, M., Usai, F., and Malik, R. (2001). The electro-sorption properties of nickel on laterite gangue leached with an organic chelating acid. Minerals Engineering, 14 (2): 205-215.

[44]. Hosseini Nasab, M., Noaparast, M., and Abdollahi, H. (2020). Dissolution optimization and kinetics of nickel and cobalt from iron-rich laterite ore using sulfuric acid at atmospheric pressure. International Journal of Chemical Kinetics 52, 283–298.

[45]. Hosseini Nasab, M., Noaparast, M., and Abdollahi, H. (2020). Dissolution of nickel and cobalt from iron-rich laterite ores using different organic acids. Journal of Mining and Environment, 11 (3): 779-797.

[46]. Hosseini Nasab, M., Noaparast, M., Abdollahi, H., and Amoozegar, M. A. (2020). Kinetics of two-step bioleaching of Ni and Co from iron-rich laterite using supernatant metabolites produced by *Salinivibrio kushneri* as a halophilic bacterium. Hydrometallurgy, 195: 105387.

[47]. Murray, R.G.E. and Stackebrandt, E. (1995). Taxonomic Note: Implementation of the Provisional Status Candidatus for Incompletely Described Procaryotes. International journal of systematic bacteriology, 45 (1): 186–187.

[48]. Ciftci, H. and Atik, S. (2017). Microbial leaching of metals from a lateritic nickel ore by pure and mixed cultures of mesophilic acidophiles. Metallurgical Research & Technology, 114: 508.

[49]. McDonald, R.G. and Whittington, B.I. (2008). Atmospheric acid leaching of nickel laterites review Part I. Sulfuric acid technologies. Hydrometallurgy, 91: 35–55.

[50]. Simate, G.S., Ndlovu, S., and Walubita, L.F. (2010). The fungal and chemolithotrophic leaching of

nickel laterites-Challenges and opportunities. Hydrometallurgy, 103: 150–157.

[51]. Gharabaghi, M., Noaparast, M., and Irannajad, M. (2009). Selective leaching kinetics of low-grade calcareous phosphate ore in acetic acid. Hydrometallurgy, 95 (3): 341-345.

[52]. Tang, A., Su, L., Li, C., and Wei, W. (2010). Effect of mechanical activation on acid-leaching of kaolin residue. Applied Clay Science, 48 (3): 296-299.

[53]. Ghassa, S., Noaparast, M., Shafaei, S.Z., Abdollahi, H., Gharabaghi, M., and Borumand, Z. (2017). A study on the zinc sulfide dissolution kinetics with biological and chemical ferric reagents. Hydrometallurgy, 171: 362-373.

[54]. Onal, M.A.R. and Topkaya, Y.A. (2014). Pressure acid leaching of Caldag lateritic nickel ore: an alternative to heap leaching. Hydrometallurgy, 1 (42): 98-107.

[55]. Levenspiel, O. (1972). Chemical engineering reaction. Wiley-Eastern Limited, New York.

[56]. MacCarthy, J., Nosrati, A., Skinner, W., and Addai-Mensah, J. (2016). Atmospheric acid leaching mechanisms and kinetics and rheological studies of a low-grade saprolitic nickel laterite ore. Hydrometallurgy, 160: 26-37.

[57]. Mohapatra, P., Bhoja, S.K., Kumar, C.R., and Jena, B. (2016). Lateritic nickel mineralization and its extraction techniques-a review. Dr. SK Sarangi, 17 (1): 37–43.

[58]. Changa, Y., Zhaoa, K., and Pesicb, B. (2016). Selective leaching of nickel from pre-reduced limonitic laterite under moderate HPAL conditions-Part I: Dissolution. Journal of Mining and Metallurgy Section B Metallurgy B 52 (2), 127–134.

[59] Nayanthika, I.V.K., Jayawardana, D.T., and Gunathileka, B.M. (2018). Sorption of Fe ions in aqueous media by laterite clay: A study of pH dependency. Journal of Geological Society of Sri Lanka 19, 35-46.

[60]. Wang, K., Li, J., McDonald, R.G., and Browner, R.E. (2018). Iron, aluminium, and chromium co-removal from atmospheric nickel laterite leach solutions. Minerals Engineering 116, 35–45.

[61]. Habashi, F. (1999). Kinetics of metallurgical processes. Metallurgie Extractive Quebec.

[62]. Ucar, G. (2009). Kinetics of sphalerite dissolution by sodium chlorate in hydrochloric acid. Hydrometallurgy. 95 (1): 39-43.

بیولیچینگ مستقیم و غیرمستقیم کبالت و نیکل از سنگ معدن لاتریتی غنی از آهن با استفاده از *Delftia acidovorans* و *Acidithiobacillus ferrooxidans*

مرضیه حسینی نسب[1]*، محمد نوع پرست[2] و هادی عبداللهی[2]

۱- بخش مهندسی معدن، دانشگاه سیستان و بلوچستان، زاهدان، ایران

۲- دانشکده مهندسی معدن، دانشگاه تهران، تهران، ایران

چکیده:

در این تحقیق، بیولیچینگ کبالت و نیکل از سنگ معدن لاتریتی پرآهن با استفاده از دو باکتری اسیدوفیل هتروتروف (*Delftia acidovorans*) و اتوتروف (*Acidithiobacillus ferrooxidans*) مورد برر سی قرار گرفت. محصولات متابولیکی باکتری‌های ا سیدوفیل، نقش مهمی در بیولیچینگ ایفا می‌کنند. نتایج حاصل از بیولیچینگ غیرمستقیم در C° ۹۰ به مدت ۳ ساعت با دور همزنی ۳۷۰ دور در دقیقه و S/L برابر با ۰/۱، بالاترین بازیابی‌های نیکل با استفاده از سوپرناتانت *Acidithiobacillus ferrooxidans* و *Delftia acidovorans* را بترتیب ۸۳/۶۵ و ۸۰/۱۸ درصد نشان داد، در حالیکه بازیابی‌های کبالت بترتیب به ۸۶/۹۳ و ۸۳/۹۴ درصد رسید. نرخ‌های انحلال آهن در این شرایط برای دو باکتری مورد مطالعه، بترتیب ۶۴/۳۴ و ۵۴/۴۱ درصد بودند. انحلال نیکل و کبالت با استفاده از روش بیولیچینگ غیرمستقیم برای باکتری *Delftia acidovorans* بترتیب ۲۹/۸۴ و ۲۳/۷۵ درصد بیشتر از روش بیولیچینگ مستقیم با استفاده از این باکتری بود که در C° ۳۰ به مدت ۳۰ روز در انکوباتور شیکر با ۱۵۰ دور در دقیقه انجام شد. برای بیولیچینگ غیرمستقیم، کنترل شیمیایی در مقایسه با کنترل نفوذی، تاثیر بیشتری بر نرخ انحلال لاتریت پرآهن داشت. انرژی‌های فعالسازی نیکل و کبالت در مدل کنترل شیمیایی به ترتیب kJ/mol ۴۰/۰۷ و ۳۹/۰۸ بودند.

کلمات کلیدی: لاتریت، باکتری اسیدوفیل، سوپرناتانت، سینتیک، کنترل شیمیایی.