



Bioleaching of Lateritic Nickel Ores

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Abstract

Despite nickel-bearing sulfide deposits having a large share of the world's nickel extraction, lateritic ore deposits contain more than 70% of the world's nickel reserves. Considering the limitations of producing nickel from sulfide reserves, the use of oxide reserves (laterites) for the production of nickel will be of great importance in the future. In this chapter, the applications of nickel and cobalt in various industries were described. Nickel and cobalt are mainly used in alloys of other metals. In addition, the most effective methods for extracting nickel and cobalt from lateritic nickel ores were examined. Due to the need for high energy, pyrometallurgical methods, as well as acid leaching, which uses a high amount of acid, are rarely used today. Therefore, the bacterial and fungal leaching methods (bioleaching), which is another hydrometallurgical process, and their mechanisms were explained. Bioleaching is a new prospective method for extracting valuable elements from hard-to-treat ores. The benefits of bioleaching low-grade ores are numerous in comparison to traditional methods due to their simplicity, using unskilled labor, low capital and operating costs, low energy consumption, and also the lowest negative environmental effects. In this processing operation, metals are dissolved from low-grade deposits by using microorganisms and their metabolic products. In addition, the final concentrations of iron in PLS can be decreased by biological methods. The most effective factors in the bioleaching process such as pH, size of sample particles, type of microorganism species, type of

substrate, amount of inoculation, type of produced metabolic acid, the pulp solid to liquid ratio, salinity, temperature, and leaching time were explained. Heterotrophic bacteria such as *Aspergillus*, *Penicillium*, *Pseudomonas*, and *Delftia* were also successful at dissolving laterites, in addition to autotrophic bacteria such as *At.ferrooxidans* and *At.thiooxidans*. The presence of O₂ is considered a key factor in increasing the bio-reduction dissolution of nickel and cobalt of iron-containing minerals. In addition, high temperature, low density, and pH gained a higher dissolution rate of nickel and cobalt. The main mechanisms for autotrophic acidophilic (iron-oxidizing) and iron-reducing (dissimilatory iron-reducing bacteria) were acidolysis and redoxolysis. In general, biological dissolution and chemical control, respectively, had a greater effect compared with chemical dissolution and diffusion control on the dissolution rate of nickel and cobalt from the laterites. It was found that optimizing factors that affect the bioleaching of nickel and cobalt from nickel-containing laterites greatly increased the dissolution rate, recovered nickel and cobalt, and reduced iron dissolution.

Keywords

Laterite · Nickel · Cobalt · Bioleaching

1 Application of Nickel in Various Industries and Characteristics of This Element

Nickel is a strategic metal with a wide range of industrial and metallurgical uses (Sadat et al., 2016). The use of nickel has now spread to a wide range of sectors, including steel alloying elements, space applications, and rechargeable batteries (Buyukakinci, 2008). Nickel is highly corrosion-resistant in contact with air, seawater, and organic and

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non-oxidizing acids; on the other hand, it is being eroded by solutions of aqueous ammonia. Nickel dissolves in dilute mineral acids; in contrast, it is insoluble in pure nitric acid. Among ionic states -1 , 0 , $+2$, $+3$, and $+4$, the ionic number $+2$ is the most important form of nickel ion. In aqueous solutions, this metal is stable in the $+2$ state (Buyukakinci, 2008). Nickel is most commonly used in alloys with other metals. This metal increases the strength, stiffness, and corrosion resistance of alloy metals. The other important application of nickel alloy is making stainless steel which uses more than 60% of the world's nickel to make it stronger and more resistant to corrosion. Nickel alloy production has the second rank in the world for using nickel. Because of their great thermal resistance and fracture toughness, nickel alloys have a wide variety of uses (Buyukakinci, 2008).

2 Types of Nickel Ores

In iron meteorites, nickel is typically found in the form of kamacite and taenite alloys. Nickel ores are divided into two categories (Sadat et al., 2016):

- (1) Pentlandite $[(\text{Ni}, \text{Fe})_9\text{S}_8]$ is the major nickel mineral associated with this type of ore, which is linked to mafic and ultramafic rocks (magmatic sulfide deposits).
- (2) Near-surface laterite deposits formed on olivine-rich host rocks after extensive weathering.

To date, most of the nickel ores are being extracted from the sulfide type minerals and sulfide deposits have been the main source of nickel for a long time as a result of the ease of nickel processing due to its sulfide-independent mineral (Dalvi et al., 2004; Sahu et al., 2011). Despite the limitations in nickel extraction from these sources, oxide ores (laterites) are becoming more and more prevalent (Dalvi et al., 2004; Pawlowska & Sadowski, 2017; Sahu et al., 2011; Valix et al., 2009). Over 70% of nickel reserves around the world come from laterite deposits (Dalvi et al., 2004; Pawlowska & Sadowski, 2017; Petrus et al., 2018; Sahu et al., 2011), recent advancements have weakened the supremacy of sulfide ores and allowed laterites to be used in new studies (Krstev et al., 2012). As a result, laterites will play a larger role in nickel and cobalt production in the future, accounting for 80% of nickel reserves and 90–95% of cobalt reserves (Valix et al., 2009). It's worth noting that laterite deposits account for just 40% of global nickel output at the moment (Petrus et al., 2018). The great majority of cobalt sources are found in laterite ores. A variety of catalytic processes, renewable batteries, super alloys, and chemical processes have put cobalt in high demand globally. Hence, laterite ore extraction is becoming more essential (Li et al., 2010). Pure nickel is not found in nature. Among the

nickel ores, only pentlandite (sulfide $[(\text{Ni}, \text{Fe})_9\text{S}_8]$, garnierite (hydrated silicate $[(\text{Ni}, \text{Mg})_6\text{Si}_4\text{O}_{10}(\text{OH})_{10}]$), and nickel limonite oxides ($[(\text{Fe}, \text{Ni})\text{O}(\text{OH})]$) have economic importance (Buyukakinci, 2008; Kim et al., 2010).

3 Methods of Processing Nickel from Laterites

Nickel is extracted from laterite ores by pyrometallurgical and hydrometallurgical methods. Figure 1 depicts flowsheets for pyrometallurgical and hydrometallurgical nickel extraction from laterites.

In the following, each of the laterites processing methods will be briefly explained.

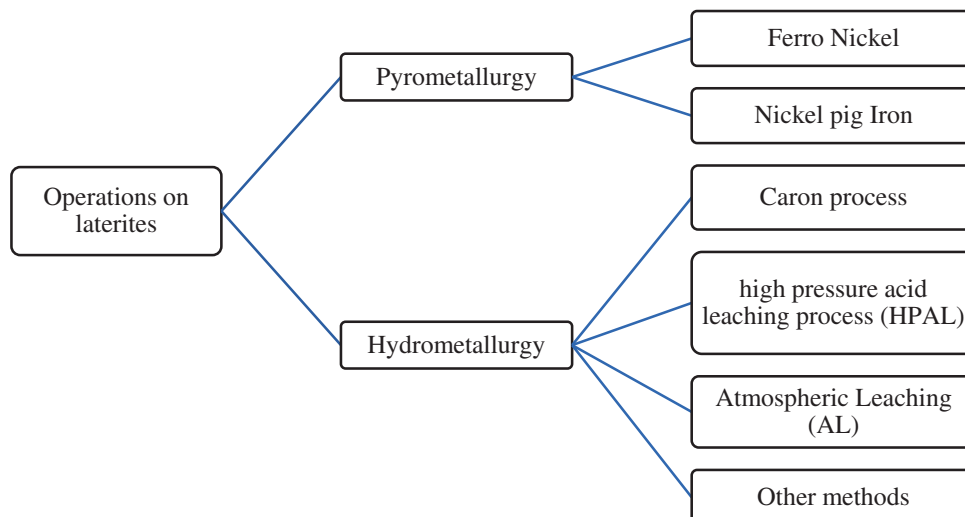
3.1 Pyrometallurgical Operations of Laterites

The main stages of pyrometallurgical processes consist of drying, calcination, reduction, and electric melting furnaces (Buyukakinci, 2008). This method is more suitable for saprolite (garnierite) deposits which contain lower amounts of iron and cobalt compared to limonite deposits. The Ni/Co ratio of feed of the melting section is usually around 40 (Fatahi et al., 2014). The minimum acceptable nickel content in this method with a cheap energy source is 1.7% and otherwise is 2.1% (Dalvi et al., 2004). Laterite ores have high humidity (more than 45%) in addition to the water in the form of hydroxides. Drying operations are carried out to lower the ore's moisture content to reduce problems in the following processes. During the melting stage, nickel is selectively reduced and high-grade metal is produced (Buyukakinci, 2008). Because of their high iron content, limonite ores are not suited for this procedure. Furthermore, laterites have a high humidity level that requires significant amounts of heat (around 800 to 900 °C) to dry. For this reason, the production costs will be increased per ton of nickel (Buyukakinci, 2008). As a result, hydrometallurgical processes are better for the treatment of high-grade iron limonite laterites, however, pyrometallurgical methods are better for magnesium-rich saprolite laterites processing (Kursunoglu & Kaya, 2016).

3.2 Hydrometallurgical Operations of Laterites

The hydrometallurgy method or the combination of hydrometallurgical and pyrometallurgical operations of the laterites depends on the chemical homogeneity and mineralogical distribution of the laterites. In laterites, nickel can be found in goethite, serpentine, smectite, manganese oxides

Fig. 1 Operations on laterites (Buyukakinci, 2008)



as well as cobalt (Buyukakinci, 2008). Hydrometallurgical processes are more commonly used for limonite or garnierite laterite ore. Although the nickel of saprolite laterites (highly weathered) is often richer than limonite ores, besides, their high magnesium content leads to high acid consumption (Dalvi et al., 2004).

Various hydrometallurgical methods have been studied on laterites. At the commercial level, only two methods are used to extract industrially nickel and cobalt which are the Caron process and the acid leaching process that uses sulfuric acid (Buyukakinci, 2008).

The Caron process includes reduction with high temperature and then ammonia leaching with ammonium carbonate (Buyukakinci, 2008). The acceptable range of nickel grade in these ores in the Caron method is 1–1.7% (Buyukakinci, 2008). The first step in the manufacturing process is drying the ore to reduce its moisture content. Then, the ore is reduced in the calcination plant using airless petroleum fuel at a temperature of about 700 °C. In the end, the extraction method with an organic solvent has been used to separate nickel and cobalt in an ionic solution. A concentrated ammonia carbonate solution is then used to wash the nickel from the solvent. The problem with the Caron process is its high-energy consumption which economically increases the cost of production. Therefore, the use of the Caron process has been limited due to the higher costs per ton of nickel production compared to the pressure acid leaching (Buyukakinci, 2008). Figure 2 shows the complete steps of the Caron process.

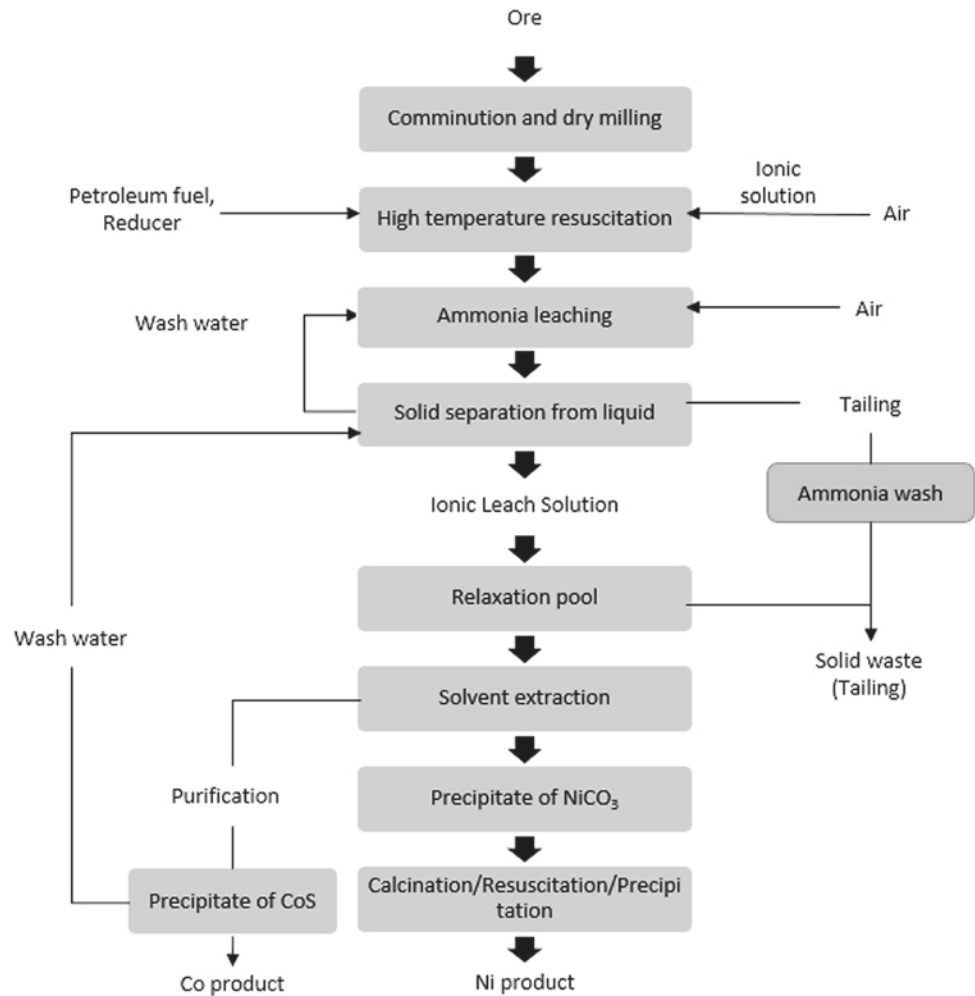
Pressure acid leaching has been applied to the Caron process to reduce high-energy consuming phases in direct ore leaching. Direct leaching eliminates the need for drying and reduction steps, which then leads to energy savings (Buyukakinci, 2008). The acceptable nickel content in this method is 1–5% (Dalvi et al., 2004). Also, the crushed

ore is dissolved directly with sulfuric acid at high pressure and temperature, which leads to the dissolution of goethite. Then, the iron precipitates rapidly in the form of hematite. This precipitation reduces the ratio of iron to nickel in the solution. It has also been reported that when the acidity at the reaction temperature is less than 0.1 mol/L, nickel and magnesium can precipitate as a mixture of sulfated salts. High magnesium oxide increases acid consumption and consequently increases the costs of the operation process (Buyukakinci, 2008). One of the ways to extract nickel metal from this method is electrolytic extraction. However, the presence of by-products such as copper, cobalt, and zinc in nickel solutions from pressure acid leaching proves the importance of this method (Moskalyk & Alfantazi, 2002).

The cost of titanium autoclaves used in the procedure, as well as the corrosion of high-pressure valves, are the main disadvantages of pressure acid leaching (Buyukakinci, 2008). The high amount of required sulfuric acid, the difficulty of maintaining the permeability of the substrate under acidic leaching conditions, and the need for the proper time and water management are other disadvantages of this method (Kursunoglu & Kaya, 2016). Pressure acid leaching is currently the most acceptable operation for producing nickel from low-grade laterites in the absence of rival methods (Buyukakinci, 2008).

Laterite acid leaching at atmospheric pressure has been proposed as a substitute for pressure acid leaching. It is used for relatively low-grade nickel (less than 1.5% nickel), limonite, and saprolite leaching due to a low investment cost. In addition, the recovery of nickel and cobalt in this method is higher compared to heap leaching (Buyukakinci, 2008; Kursunoglu & Kaya, 2016). This process is performed at temperatures below 100 °C for a maximum of 12 h (Kursunoglu & Kaya, 2016). Direct leaching of laterites with organic and inorganic acids using agitation and

Fig. 2 Complete flowsheet of the Caron process (Nicol & Zainol, 2003)



heap leaching are two of the proposed leaching methods (Buyukakinci, 2008). Leaching using different acids has been studied; nevertheless, for reasons especially economic, sulfuric acid is preferred. Nickel extraction from lateritic ores is influenced by the type of nickel-bearing minerals, leaching temperature, and sulfuric acid concentration. Studies on the leaching kinetics of laterites have shown that magnesium dissolved rapidly during leaching (Beukes et al., 2000). The magnesium in serpentine is the largest consumer of acid in the leaching process of serpentine-containing laterites (Whittington et al., 2003a). Therefore, nickel in limonite or saprolite compounds is easily soluble in sulfuric acid (Buyukakinci & Topkaya, 2009). However, the nickel in nontronite laterites dissolves incompletely. In these laterites, some nickel is extracted without destroying the structure of this type of laterites which is either in the form of nickel ion exchange from nontronite or due to the presence of nickel-rich minerals that are rapidly soluble in nontronite ores (Whittington et al., 2003b). The dissolution of nickel linked with iron has been described as a time-dependent process that frequently necessitates the

total dissolution of iron (Buyukakinci & Topkaya, 2009). It is noteworthy that the solubility of lizardite, magnetite, hematite, and goethite in sulfuric acid, which are from iron minerals, decreases, respectively. Above all, the solubility of magnetite and hematite are almost equal (Liu et al., 2009). According to research, preheating laterites increases porosity and surface area, making it more appropriate for leaching, in addition to modifying the mineralogical composition. When raw ore and roasted ores are leached at different temperatures, the results reveal that increasing the roasting temperature to 300 °C gives optimal nickel recovery, whereas more heating is detrimental for nickel recovery (Li et al., 2009). Laterites leaching with sulfuric acid consume large amounts of acid. However, researches have shown that it is better to reduce the amount of calcite using a strong magnetic field despite the significant amount of calcite mineral in laterite ores which of course depends on the size of the feed particles (Agatzini-Leonardou et al., 2004). In addition, the presence of calcite harms the permeability of the ore in heap leaching due to gypsum formation (Agatzini-Leonardou & Zafiratos, 2004).

Heap leaching uses different methods such as diluting sulfuric acid solution at a limited temperature, extraction of nickel and cobalt from the leaching solution with solvent extraction and electrolysis, and purification of the leaching solution by chemical precipitation at atmospheric pressure (Buyukakinci, 2008). In HPAL and heap leaching, the final product is NiSO_4 , which is used primarily for the production of batteries. Iron, a critical component that influences acid consumption, makes selective metal recovery from leaching solutions difficult. Due to the high concentration of residual acid, substantial volumes of neutralizing agents such as limestone are required during the iron removal from the atmospheric leaching process. In addition, the high initial Fe/Ni ratio in the leaching solution can cause severe loss of nickel and cobalt during iron removal wherefore increasing the cost of the product per ton of nickel production (Buyukakinci & Topkaya, 2009). The Vieille Montagne (V.M) process has been proposed for iron precipitation in the form of goethite from an aqueous solution of laterites ore leaching using air as the oxidizing agent. Therefore, this method does not require the reduction of ferric iron ions at the start, and the iron concentration in the final solution might be less than 1 g/L. The oxidation of ferric ions and the loss of nickel in solid waste are both influenced by the pH value. The oxidation rate of ferric ions increases with increasing the pH, in contrast, leads to the loss of more nickel in the solid waste. At pH between 2.5 and 3, the oxidation rate of ferric ion is about 53.8 mg/min. L, and nickel loss is 4.1%. Therefore, some nickel co-precipitates with iron in the precipitation process. The precipitated nickel cannot be recovered by leaching using weak acid (Chang et al., 2010).

In agitation leaching, first, the ore is crushed then milled, and at the end dissolved in a leaching tank. The temperature can be regulated by heating the tank, which will cause the metal to dissolve faster in the acidic solution, speeding up the process. Then, the ionized solution is collected to recover the metal (Buyukakinci, 2008). The results of the studies showed that the leaching time extension of the saprolite laterites causes the loss of nickel in the solid residues of waste, on the contrary, these laterites can be dissolved at low temperatures with a longer time and more free acid (Luo et al., 2009). In addition, the influence of sulfuric acid concentration on nickel dissolution rate has been studied. The amount of nickel dissolved increases by 60% with rising sulfuric acid concentration at 95 °C; The highest nickel dissolution (99.2%) was achieved at 95 °C in 120 min of leaching with a solution of 60% sulfuric acid concentration (Girgin et al., 2011). Another way to improve the atmospheric leaching of laterites using sulfuric acid is to blow SO_2 gas as a reduction agent. When Cu (II) is used as a catalyst, the rate of leaching increases as well. The particle size does not affect the leaching process

in this case. However, nickel recovery increases from 74 to 90% which leads to increases of iron recovery from 47 to 73% in this case an optimal mode must be selected (Das & Lange, 2011). Another method includes three main steps of sulfating-roasting-leaching. With this method, 75% to 78% of nickel can be extracted and only 5% to 10% of iron is dissolved in the solution (Li et al., 2010).

Metal-rich leaching solution selectively precipitated to recover the metal using various methods. These methods include hydroxide precipitation, sulfide precipitation, or a combination of both methods (Buyukakinci & Topkaya, 2009). For example, it has been suggested that calcium hydroxide $[\text{Ca}(\text{OH})_2]$ can be used to remove magnesium from the leached sulfate solution, which precipitates magnesium in the form of hydroxide $[\text{Mg}(\text{OH})_2]$, and calcium added in the form of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). This method can remove rapidly 90 to 99% of magnesium from the solution. Temperature and precipitation amount are two of the most critical elements in this process (Karidakis et al., 2005). Under certain conditions, nickel metal can also precipitate into one of the oxides, hydroxide, and even pure metal forms. According to Fig. 3, nickel precipitates at pH between 6 and 12, and the Eh value determines the chemical composition of the precipitate (Beukes et al., 2000).

Atmospheric leaching versus pressure leaching has been challenged for several years and has a long list of strengths and weaknesses summarized in Table 1.

Among the three well-known hydrometallurgical processes for recovering nickel from lateritic ores with the least amount of energy expenditure, agitation leaching at atmospheric pressure is the least energy-intensive, followed by heap leaching, ferronickel, and high-pressure acid leaching, based on the available evidence and hypotheses. Furthermore, the Caron process consumes double as much

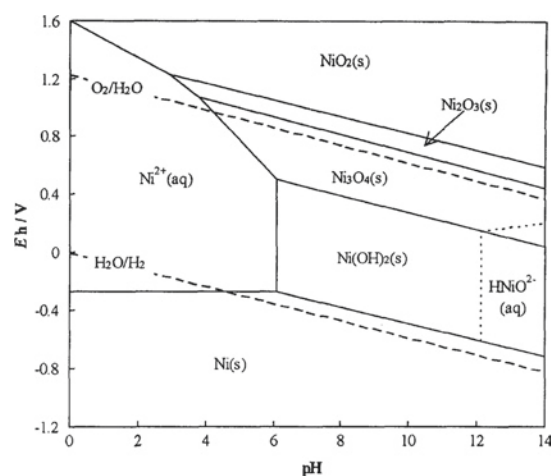


Fig. 3 Eh-pH diagram for Ni-H₂O at 25 °C temperature and 1 atm pressure (Beukes et al., 2000)

Table 1 Compared to pressure acidic leaching, the flaws and strengths of atmospheric leaching (tank and heap leaching) (McDonald & Whittington, 2008a)

Strengths	Weaknesses
Common, cheap, and ordinary technical equipment	Consume more acid to achieve acceptable nickel recovery
Lower maintenance costs	Consume more lime
Faster development	Not applicable for all types of stones
Ease of starting and stopping	Lack of economic justification for the present time
Less specialized worker for construction, operation, and maintenance	Slower extraction kinetics and longer retention time
Lower energy consumption	Produce a more contaminated solution with a lower Ni/Fe ratio
Easier process control	Extraction of less nickel and cobalt
Ease of water recycling in the process	The presence of jarosite in the tailings is a potential environmental problem
Applicable for low grades	
Suitable for remote and undeveloped places	
Fewer problems related to precipitation and corrosion	
Economical due to the availability of cheap sulfuric acid	
Use seawater to provide sodium to remove iron	

energy as other approaches (Kursunoglu & Kaya, 2016; Norgate & Jahanshahi, 2011).

The extraction of nickel-containing laterites by using organic acids is still in its early stages. The extraction of clay minerals is greatly improved by the use of citric and oxalic acids (Agatzini-Leonardou et al., 2004). The highest nickel recovery rate and magnesium selectivity are achieved by citric acid. Using the chelation mechanism, this acid generates a soluble ligand complex (Petrus et al., 2018). In addition, studies have shown that tank leaching is probably more effective than heap leaching. However, more research is still needed to improve metal recovery (Agatzini-Leonardou et al., 2004). In the alkaline leaching method to remove silica, silicon is first dissolved using concentrated sodium hydroxide. Then, magnesium is removed from the solid residue of the previous step using carbonation as magnesium carbonate. Finally, nickel is obtained as an oxide using ammonium carbonate (McDonald & Whittington, 2008b). Figure 4 shows a complete flowsheet of this process. This method has been used in the laboratory for a relatively high-grade silicate laterite sample. Table 2 shows the grades at different stages of this process.

Another proposed method for processing nickel-bearing laterites as shown in Fig. 5 includes milling, calcination, and magnetic separator. The magnetic property of laterite may change with calcination because the Curie temperature of magnetite is about 575 °C (Kim et al., 2010). Generally, increases in the temperature to 600 °C for limonite provide optimal recovery, while reduction of weathered saprolite at 800 °C is desirable. Forsterite is formed when weathered saprolite is reduced following calcination at temperatures above 700 °C. The formed phases at 800 °C in laterite minerals do not return to their original state upon cooling (Valix & Cheung, 2002).

There has been a development of a nickel extraction method for low-grade nickel-containing laterites with a grade of less than 2%. In this method, an electrochemical operation is used. In an electrolytic cell, nickel is electrochemically leached and then separated into two halves by a PVC separator. The cell current density varies in the range of 3.2–32 mA/cm². In the presence of SO₂ gas, nickel dissolves in iron-containing laterites through electrolytic reduction. In electrolysis, the reductive dissolution of iron is suspected to be a contributing factor to nickel extraction from laterites by electrochemical leaching. The experimental procedure is shown in Fig. 6 (Lee et al., 2005).

This method, unlike the hydrometallurgical method, is applicable at limited temperatures and a low cost. Also, it can be used for any type of magnesium-bearing or iron-bearing laterite. Nickel recovery in this method is between 50 and 75% and depends on the type of nickel and the amount of iron. The used electrodes and the cell design have a significant impact on extraction efficiency and energy consumption. On the other side, in this method, it is necessary to minimize the pollution caused by the release of SO₂ gas into the environment during electrolysis and exposure to dissolution wastes caused by nickel extraction (Lee et al., 2005).

Metal demand has increased as a result of industrialization and population development, while high-grade (sulfide) ores have declined, however, there are reserves of low-grade and low-profit for extraction in large volumes. It is very expensive to recover metals from low-grade reserves using conventional methods due to high-energy consumption and a high cost of capital. The high level of pollution caused by low-grade reserves is another major drawback of these reserves. Biotechnology is a more promising solution to these problems than pyrometallurgy or hydrometallurgy. In biotechnology, chemical reactions take place under gentle conditions and usually without the addition of toxic chemicals. Bioleaching techniques produce aqueous solutions, which are easier to process and regulate than gaseous wastes (Krstev et al., 2012).

Fig. 4 Leaching flowsheet of a laterite sample using sodium hydroxide (McDonald & Whittington, 2008b)

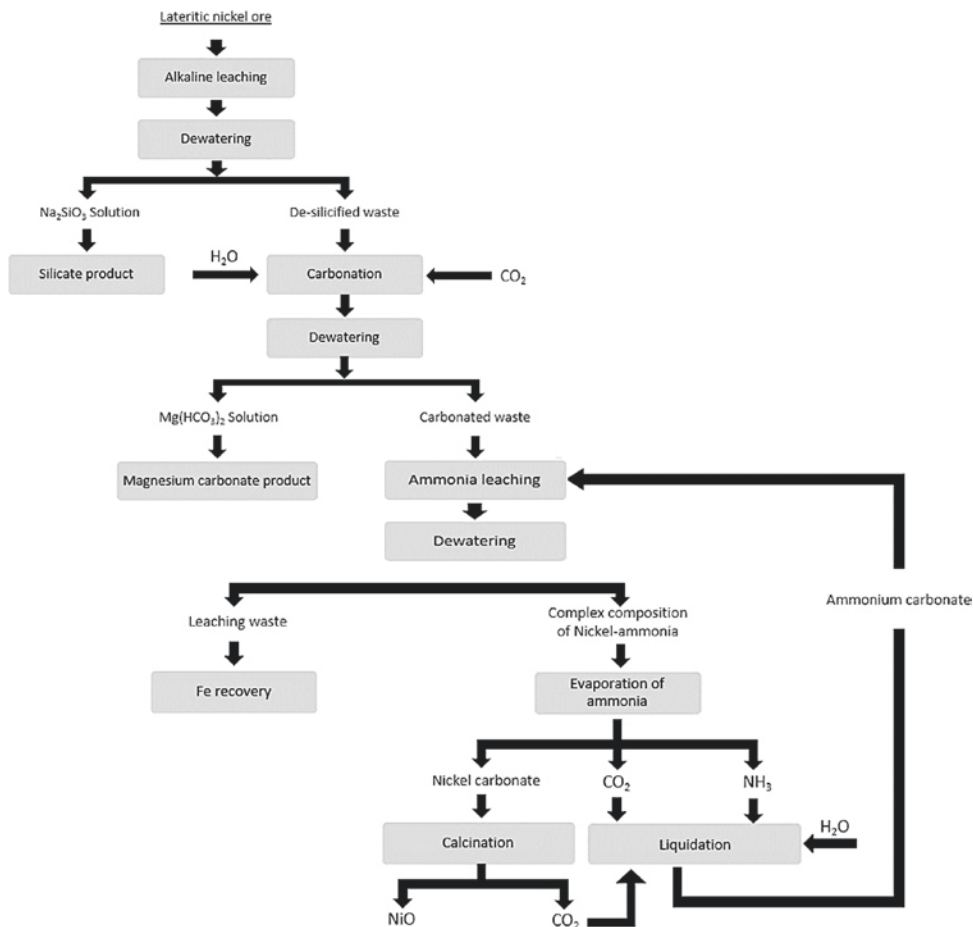


Table 2 Grade in different stages of leaching of laterite samples using sodium hydroxide (McDonald & Whittington, 2008b)

Sample	Grade (%)				
	MgO	Fe ₂ O ₃	SiO ₂	NiO	Cr ₂ O ₃
Feed	27.2	15.8	49.3	0.98	0.63
De-silicified waste	39.78	23.87	6.51	1.41	0.28
Carbonated waste	8.22	51.11	12.52	2.96	0.57
Residual waste	12.46	59.72	13.05	0.23	0.86

4 The Mechanism for Bacterial and Fungal Leaching (Bioleaching) of Laterites

Biological leaching has emerged as a promising new approach for recovering important elements from difficult-to-process ores in recent years which is the process of dissolving metals from low-grade deposits using microorganisms and their metabolic products (Li et al., 2010; Sahu et al., 2011). Microorganism-based processes make it feasible to recover metals from mineral deposits that are now inaccessible by conventional mining methods. During

leaching processes, bacteria and fungi convert metal compounds into water-soluble forms by using biocatalysis (Krstev et al., 2012). At low metal concentrations, bacterial leaching is possible and takes little energy. Even when the extraction recovery is above 85–90%, this procedure is environmentally friendly (Krstev et al., 2012).

There are two mechanisms by which bacteria promote metal solubilization: the contact (direct) mechanism, which involves oxidation by microorganisms attaching to the metal substrate's surface and the non-contact (indirect) mechanism, which involves mineral oxidation by the oxidizing agent, which is usually ferric ion Fe (III) produced by bacteria in the solution (Borja et al., 2016; Santos et al., 2006). In the first mechanism, the bacteria adhere to the mineral surface, then produces compounds that break sulfur-metal and sulfur-sulfur bonds (Santos et al., 2006). Cysteine plays an important role in this mechanism, especially in the bio-oxidation of pyrite (Borja et al., 2016; Santos et al., 2006). On the other hand, bacteria oxidize Fe (II) to Fe (III) and consume electrons in the second operation. Fe(III) is a strong oxidant that can oxidize most sulfide minerals (Santos et al., 2006).

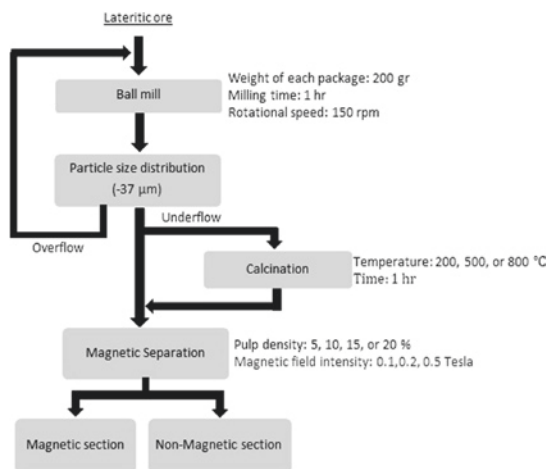


Fig. 5 Proposed flowsheet for nickel-bearing laterite processing (Kim et al., 2010)

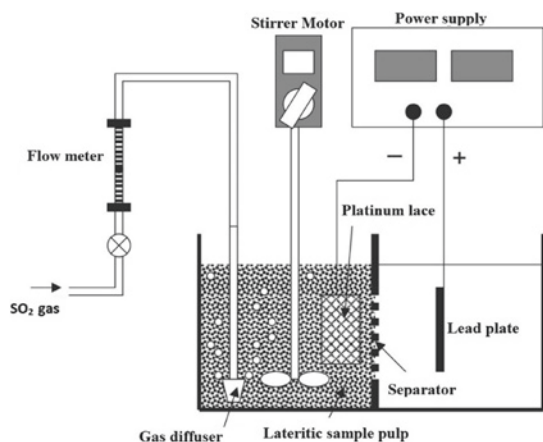
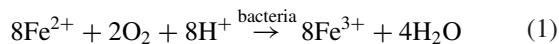


Fig. 6 Schematic of laboratory equipment for the extraction of nickel from nickel-containing laterites (Lee et al., 2005)

Biological oxidation of ferrous iron to ferric iron is shown in reaction (1): (Borja et al., 2016).

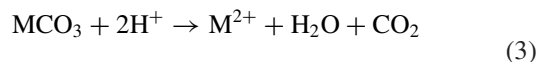
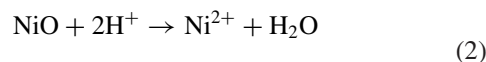


Fungal species, unlike chemolithotrophic species, require organic additions as a source of energy (Borja et al., 2016). Fungal leaching of metals is generally an indirect process. This process results in the production of organic acids, amino acids, and other metabolites (Simate et al., 2010). The following bioleaching processes are used by fungi to dissolve metals: (1) acidolysis (acid–base reaction), (2) redoxolysis (reduction processes), (3) chelation/complex formation, and (4) bioaccumulation (Dusengemungu et al., 2021; Simate et al., 2010). The first three steps are triggered by fungi's metabolites, whereas the fourth occurs when fungi take metal ions from the solution and disrupt the equilibrium between solids and dissolved metal, resulting in the metal's ongoing dissolution (Simate et al., 2010). The

controlling process in fungal metal leaching is acidolysis. Metal detachment and metabolite formation are supported by the quick protonation of oxygen atoms covering the metal's complex surface and their interaction with water; protons stimulate solubilization processes without neutralizing them (Borja et al., 2016).

Therefore, the main processes that occur for nickel ions during the dissolution of laterite are listed below:

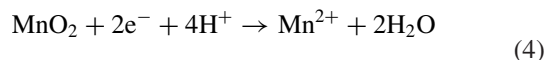
(A) Acidolysis (Proton attack) (Simate et al., 2010)



That M can be Fe, Mg, Al, Mn, Ca, etc.

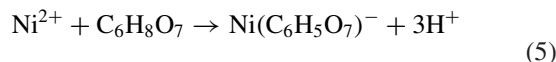
In Eq. (2), organic acids produce protons that ease the mineral's dissolution. In Eq. (3), the acid reacts to any carbonate in the ore, which increases the release of nickel. Particularly, this reaction necessitates the generation of an excessive amount of acid.

(B) Reduction (Simate et al., 2010)



The reduction of soluble manganese in Eq. (4) can lead to an equilibrium between Mn^{4+} in the solid phase and Mn^{2+} in the soluble phase, which somewhat accelerates the dissolution of the mineral and also the release of nickel.

(C) Complexation/chelation (Simate et al., 2010)



Equation (5) shows how metal ions are inhibited by organic acids increases the mineral's apparent solubility while the metal's activity is diminished (Simate et al., 2010).

The ability of microorganisms to create hydroxyl-carboxylic acids (citric, lactic, gluconic, pyruvic, and tartaric acids) and other metabolites eliminated from the culture media, as well as their resistance to heavy metals, determines their usage) (Simate et al., 2010). For example, citric acid is made by fermenting sucrose with the *Aspergillus niger* fungus, which generates a soluble ligand complex via the chelation mechanism. Metabolic citric acid is a type of citric acid produced by *Aspergillus niger's* excretory activity (MCA) (Petrus et al., 2018). The brucite layer of clay ores contains nickel from silicate ores, which forms weak bonds and dissolves quickly. Goethite and hematite have different adsorption capacities for nickel, based on surface

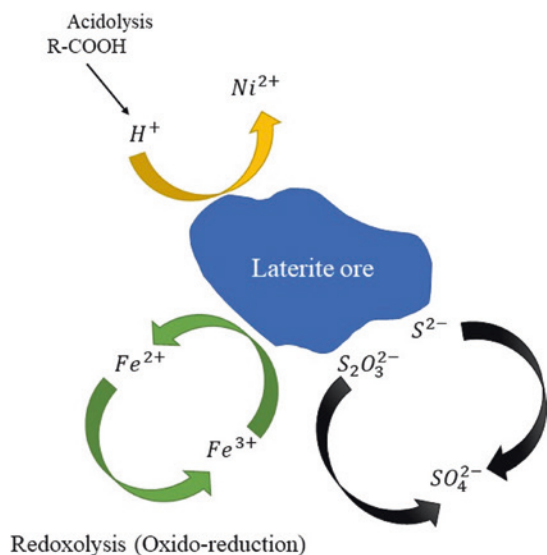


Fig. 7 Main mechanisms for bioleaching of laterites

area; for example, the lower the surface area, the greater the adsorption capacity) (Simate et al., 2010).

Figure 7 depicts the mechanisms of bacterial and fungal leaching. The mechanism for autotrophic acidophilic (iron-oxidizing) and iron-reducing (dissimilatory iron-reducing bacteria) bacterial leaching has been found.

4.1 Laterite's Bioleaching

The processes of nickel-based laterites have been impeded by the complicated mineralogy and limited applicability of existing technologies, hence, making nickel and cobalt extraction from commercial-grade laterites energy-intensive and costly. Therefore, alternative technologies that are technical, economic, and environmentally friendly are needed (Valix et al., 2009).

Metals have been recovered from laterite ores in recent years using organic acid metabolites generated by microorganisms. Nevertheless, several technical challenges must be overcome before industrialization, including a long leaching duration and low metal recovery. The organic acid is a good choice for extracting specific metals from laterite ores due to its high selectivity. Citric acid is the most effective organic acid for extracting nickel from serpentine-type laterite ores. On the contrary, it cannot dissolve nickel from limonite-type laterite ores because of its low reactivity with goethite where nickel is embedded in a goethite structure ($\text{FeO}(\text{OH})$) (Li et al., 2010). Nickel-bearing laterite is not able to participate in the bacterial oxidation of primary chemolithotrophs because it does not contain ferrous iron and a significant amount of reduced sulfur (Krstev et al.,

2012). Autotrophic and heterotrophic microorganisms can extract nickel from ore (Sahu et al., 2011). Industrial-scale techniques for the heterotrophic leaching of oxides, carbonates, and silicates lacked progress early on and are still awaiting development. Since low-grade nickel ores processing by conventional methods requires a lot of energy and high operation costs, for the recovery of nickel from laterite ores, the most environmentally friendly and economical technologies were used such as chemoorganotrophic bioleaching which is required to develop for use in industrial applications. More research is needed to improve the procedures for processing low-grade lateritic ores, as it appears that chemoorganotrophic bioleaching of oxide ores has a lot of potential (Chaerun et al., 2017).

The use of heterotrophic bacteria in the leaching of non-sulfide ores has recently attracted a lot of attention (Mubarok et al., 2013; Sahu et al., 2011). There has been evidence of heterotrophic bacteria leaching non-sulfide minerals successfully by *Pseudomonas* subtypes. Because non-sulfide ores lack an energy source for microorganisms when there is a carbon supply for energy and growth, heterotrophic bacteria and fungus can dissolve them. The metabolic products produced by organisms can contain organic acids and compounds that are formed from at least two hydrophilic reactions. Mineral surfaces are affected by secondary metabolites, which are made by organotrophic organisms that make energy from organic material. These metabolites generate exopolysaccharides, amino acids, and proteins, as well as many organic acids such as acetic acid, citric acid, oxalic acid, and ketoglutaric acid, which can be used to dissolve metals through various methods. The protons and anions of metal complexes present in organic acids make them useful for leaching. (Sahu et al., 2011).

Heterotrophic fungi and *Acidithiobacilli* species can react with lateritic oxides. The sulfuric acid made by acidophilic bacteria dissolves metals more readily than the organic acids made by fungi. Particularly, the environment in the in-situ and direct bioleaching method has a very important role in the success of this technology. In particular, *Acidithiobacillus thiooxidans* is a mesophilic acidophil that can tolerate severely acidic environments ($\text{pH}=1$ or less). Its metabolite, predominantly sulfuric acid, can help in the dissolving of host minerals as well as pH regulation, which can help prevent metal extraction from lateritic ores) (Jang & Valix, 2017). The most effective organisms for dissolving laterites are *Aspergillus* and *Penicillium* species (Tang & Valix, 2004, 2006). Nickel and cobalt can be extracted from laterite ores using the species *Aspergillus foetidus* and *Aspergillus niger* exclusively from *Aspergillus* species (Mubarok et al., 2013).

5 Importance of Bioleaching Method Among Other Methods of Processing Nickel from Laterites

When compared to conventional processing technologies (high temperature/pressure), the biological processing method for extracting base metals from oxidized ores such as limonite offers significant advantages (in terms of environmental and carbon effects) (Smith et al., 2017). Table 3 summarizes the benefits and drawbacks of several nickel recovery techniques.

A variety of uses are available in the mineral industry for biological leaching of low-grade ores because of its relative simplicity, low operational costs, low investment costs, low input energy requirements, and absence of harmful environmental implications (Ahmadi et al., 2015; Li et al., 2014; Mohapatra et al., 2007; Simate & Ndlovu, 2008; Simate et al., 2010).

5.1 The Merits and Demerits of Bacterial and Fungal Applications

The microbes used in biomining have been designed to work under extreme environments, such as low solution pH, high temperature, and high heavy metal concentrations. Bioleaching, as an ecologically friendly method, will play a key role in minerals extraction more sustainable in the near future by selectively targeting exposed mineral grains (Borja et al., 2016).

Filamentous fungi are utilized in bioleaching because of their capacity to release organic acids that cooperate in the solubilization of metal ions from the solution phase while tolerating high concentrations of metal ions. The value of these organic acids is that they keep the pH low, which is necessary for a better bioleaching process, and they offer needed protons. In the fight to improve low-grade mineral bioleaching, fungi's great adaptability and tolerance to

Table 3 Advantages and disadvantages of various processes for nickel recovery (Simate et al., 2010)

	Advantages	Disadvantages
Bioleaching	<ul style="list-style-type: none"> • Low-temperature process • Done at atmospheric pressure • Low need for process control • Can be used for laterite ores with low-grade nickel • Operation ability at a low cost • Requires low investment costs 	<ul style="list-style-type: none"> • Need for a long time • It has only been successful on a semi-industrial scale
High-pressure acid leaching	<ul style="list-style-type: none"> • Proven technology • Short Residence Time Distribution (RTD) 	<ul style="list-style-type: none"> • Along with environmental concerns • Expensive administration • High-temperature process • Run at high pressures • Need more process control • Need for an oxygen plant to provide the needed oxygen • Only effective for certain types of laterite ores with certain grades (often limonite) • Need for high investment costs
Caron process	<ul style="list-style-type: none"> • Proven technology 	<ul style="list-style-type: none"> • Along with environmental concerns • Expensive administration • High-temperature process • Only effective for certain types of laterite ores with certain grades (limonite or a mixture of limonite and saprolite) • Requires a variety of reactants • Need for high investment costs
Melting	<ul style="list-style-type: none"> • Proven technology 	<ul style="list-style-type: none"> • Along with environmental concerns • Expensive administration • High-temperature process • Effective only for certain types of laterite ores with certain grades (often saprolite) • Need for high investment costs

environmental pressures such as metal contamination have been recognized as a benefit (Dusengemungu et al., 2021).

Long extraction times, the further process for produced by-products, and metal toxicity to biomining microorganisms are the key obstacles that bioleaching of mineral ores and concentrates encounters. New strategies should focus on generating new catalysts that can increase microbe-mineral interactions while also speeding up the kinetics. In laterite bioleaching, the catalyst should perform two key functions: (1) activation of the mineral surface for faster interactions with microorganisms; and (2) continual provision of nutrients or electrons to the bacteria. Those functions might help to speed up the kinetics (Borja et al., 2016).

Technological advancements should be made on both the process and equipment development fronts. However, further testing is required to make biohydrometallurgy suitable for mineral extraction.

6 Research Background (Leaching and Bioleaching) of Laterite Samples

Nickel bioleaching is a complicated process that is influenced by some factors including temperature, pH, bacterial type, sulfur content, and cell concentration (Chang et al., 2008). Many researchers studied the bioleaching of nickel-bearing laterite ores by changing these parameters and reached an acceptable recovery for a nickel. Some of the more new studies are presented in the following:

Gadd (2001) stated that organic acids generated by fungi have a reciprocal influence on the supply of hydrogen ions for the acidolysis of minerals and the creation of metal complexes, due to their chelating capacity (Gadd, 2001). Valix et al. (2001a) investigated the leaching of a variety of minerals, including saprolite, weathered saprolite, limonite, and nontronite. Their study showed that *Aspergillus* and *Penicillium* species were the most effective organisms and chemical dissolution was shown to be less successful than biological dissolution which increases the dissolution rate of nickel from saprolite or silica-rich phases. The accompaniment of nickel with goethite prevents the effective nickel recovery of limonite. On the other hand, cobalt dissolves easily from the limonite. Cobalt recovery is low from saprolite due to metal adsorption on the gangue rather than a lack of mineral solubility. The effective and selective dissolving of particular species of cobalt and nickel (not observed in the chemical dissolution process) leads to the conclusion that biological activity impacts the dissolution process itself in addition to bio-acid generation (Valix et al., 2001a). A study by Tang and Valix (2004) investigated nickel and cobalt solubility in limonite and weathered saprolites. The results showed that nickel and cobalt dissolution is influenced by the host minerals' structure and the ability

of the acid to dehydroxylate these gangue minerals. The effect of electrosorption is minimized by adjusting the pulp pH below the pH_{IEP} (zero charge point) of the gangue in the ore, which can improve nickel recovery by up to 200%. This phenomenon has the least effect on the dissolution of cobalt. pH_{IEP} is 6.6 for limonite and 3.8 for weathered saprolite. Dissolution efficiency depends on the concentration of hydronium (H_3O^+) rather than the acid's type. Hydronium concentration is determined by the strength of the acids or how readily they dissolve in solution. Citric, malic, and lactic acids are listed in order of acid resistance. Weathered saprolites have the lowest solid to liquid ratio, which is linked to a larger amount of acid-neutralizing magnesium minerals. In weathered saprolites, the higher ability of nickel to be dissolved compared to cobalt is because in weathered saprolites, nickel is more reactive with the serpentine phase and cobalt is partly associated with goethite. Similarly, cobalt dissolves more readily in limonite than nickel because cobalt is connected with serpentine minerals in limonite and nickel with goethite (Tang & Valix, 2004). Swamy et al. (2005) showed that the bioleaching processes of lateritic nickel ores can be improved by ultrasound due to the increased growth of *Aspergillus niger* with ultrasound. Ultrasound speeds up the transfer of oxygen and nutrients to the cell (Swamy et al., 2005). Tang and Valix (2006) used fungal metabolic acids to dissolve low-grade limonite and nontronite ores. Cobalt had a maximum recovery of 90%, while nickel had a maximum recovery of 33% (Tang & Valix, 2006). Le et al. (2006) extract nickel and cobalt in New Caledonian weathered saprolite ores containing goethite, hematite, titaniferous pyrite, and nickel-ferrous pyrite, using *Aspergillus foetidus*. The extraction of nickel and cobalt was 28% and 31%, respectively (Le et al., 2006). Thangavelu et al. (2006) improved Le et al. (2006) research by adding NaCl to the bioleaching process while using the same fungus, resulting in a 45% improvement in nickel extraction (Thangavelu et al., 2006). Researchers Doshi and Mishra (2007) investigated nickel extraction from laterite ores containing nickel. Nickel was coupled with goethite in the laterite nickel ore, while cobalt was first found in the manganese mineral phase. The ore was soft and agglomerated with very fine porous particles with a very high surface area. Goethite, hydrated iron oxide ($\alpha\text{-FeOOH}$), and dissolved residue including some jarosite were found in the analyzed laterite ore. The results of this study showed that nickel extraction decreases with increasing pulp density. With a pulp density of 2%, the maximum nickel extraction was achieved in 15 days and the percentage of nickel extracted was influenced by bacterial activity (Doshi & Mishra, 2007).

To dissolve nickel-bearing laterites, Simate and Ndlovu (2007) used a culture media containing a combination of chemolithotrophic bacteria (*Acidithiobacillus ferrooxidans*,

Acidithiobacillus caldus, and *Leptospirillum ferrooxidans*). Nickel was recovered from low-grade chromite ores using a culture media of a combination of acidophiles which *At. ferrooxidans* was predominating. It was found that a higher concentration of ferrous ions in the culture media resulted in a higher nickel recovery. Adaptation can improve an organism's structure to tolerate heavy metals. Heavy metal tolerance is highest in *Penicillium funiculosum* and *Aspergillus foetidus*. Generally, nickel and cobalt are not tolerated well by *penicillium simplicissimum*. Using the statistical design method of experiments, the effective factors in the bacterial dissolution of nickel-bearing laterites were examined in this work. Results showed that the amount of inoculation was not statistically significant, while the other factors were significant. A weak interaction between variables was observed in the study area. Findings show that recovery is highest at low pH, low pulp density, and big particles. Furthermore, nickel recovery was influenced by particles smaller than 38 microns and the sulfur substrate outperformed the pyrite substrate (Simate & Ndlovu, 2007). Mohapatra et al., (2009a) also examined the effect of laterite ore in various forms such as raw ore, calcined ore, and calcined ore and then soaked in dilute sulfuric acid, before bacterial dissolution. The results of this study showed that the highest percentage of nickel extraction with calcined ore at 600 °C for 31 days was obtained at a pH of 1.5, which was able to extract 77.23% of nickel. The minimum nickel extraction was in the raw ore state which extracted only 9.47% of nickel (Mohapatra et al., 2009a). Simate et al. (2009a) studied the efficacy of using elemental sulfur or pyrite in the bacterial breakdown of nickel-bearing laterites. Hybrid culture media (cultured in standard nutrient 9 K medium) including *At. ferrooxidans*, *A. caldus*, and *Leptospirillum ferrooxidans* were studied. The results revealed that in the presence of bacteria, the dissolution rate of nickel-bearing laterite was high at low pH and high ORP. In the presence of pyrite substrate, nickel recovery was marginally higher than in the presence of sulfur substrate. Consequently, bacterial influence on the dissolution of minerals that cannot participate in the bacterial oxidation process of primary chemolithotrophs appears to be determined by the interaction between the bacteria and the added substrate. In other words, in the early stages of leaching, bio-oxidation of the substrate is considered as a potential factor (Simate et al., 2009a). Nickel was extracted from the laterite ores of the Sukinda mines in Orissa in India with biological dissolution using *Aspergillus niger* by Mohapatra et al. (2009a). To match the experimental model reduction of the second-order system, the experiments were carried out using a central composite design method. The model is significant at a level of less than 0.01%, according to the F-value (for example 99.99% confidence level). Based on a

pulp density of 8.75%, a sucrose content of 10.04 g/L, and a 33.8 °C temperature for 37.5 days, the maximum extraction predicted for nickel was 31.34% (Mohapatra et al., 2009b). Büyükakinci and Topkaya (2009) examined the leaching of lateritic nickel ores under atmospheric pressure using agitation leaching. It turned out that particle size did not affect agitation leaching at atmospheric pressure. Finally, nickel recovery was 93.1% and cobalt recovery was 75% (Büyükakinci & Topkaya, 2009). Valix et al. (2009) report that the high salinity of water and soil near laterite ores caused the use of fungi in in-situ bioleaching. To address this issue, *Aspergillus foetidus* adjusts to saline environments over time to make itself more resistant to salinity. In this study, the effect of this adaptation on fungal growth, metabolism, and leaching behavior of weathered saprolite ores with an average size of 53 microns was studied. This saprolite was rich in magnesium silicate. To increase *Aspergillus foetidus*' salinity tolerance, its gradual adaptation to salinity can be exploited. Thus, compared to conditions of 0 ppm salt, salinity slowed fungal metabolism, reduced bio-acid production, and reduced sucrose to bio-acid conversion efficiency. Nickel and cobalt had less metal dissolution in the first leaching stage and some metals (Co and Fe) experienced an adsorption delay (5–8 days), but high salinity (500 ppm NaCl) more effectively overcomes secondary reactions and metal loss, resulting in higher metal recovery. High salinity also stabilizes the selectivity of nickel over cobalt and iron during a long time. High salt concentrations appear to have two impacts in general. Where salinity is likely to be effective, precise salinity control requires optimization of metal recovery using bioleaching process (Valix et al., 2009). Simate et al. (2010) discussed new advances in fungi (chemoorganotrophic) and chemolithotrophic microorganisms. This study found that non-sulfide ores could be reacted with heterotrophic bacteria and fungi. The research on fungal leaching for nickel-bearing laterites is summarized in Table 4 (Simate et al., 2010). The most effective dissolving agent is citric acid, while oxalic acid has the least effect. One explanation for this rating may be the precipitation of nickel oxalate by oxalic acid, which has extremely low solubility (Simate et al., 2010).

Sahu et al. (2011) studied the dissolution kinetics of nickel-bearing laterite ores in a batch bioreactor using aqueous acid solutions containing three metabolic acids: citric acid, oxalic acid, and acetic acid. In nickel-bearing laterite ores, mineralogical studies have revealed that there is no discrete mineral phase from nickel. Iron is found in goethite, which is the host rock or principal phase in raw laterite ore which contains the majority of nickel. The laterite ore also contained hematite, nickel ferrite (NiFe_2O_4), garnierite, and quartz. Ashes-layer-diffusion control is a type of

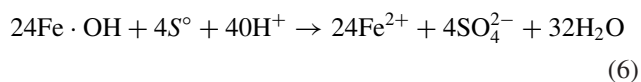
Table 4 A research summary on fungal leaching of nickel-bearing laterites (Simate et al., 2010)

Ore/minerals	Type of fungus or species	Types of acids	Ni recovery (wt. percentage)	References
Calamine, garnierite	<i>Aspergillus niger</i>	Citric, oxalic	Up to 78	Castro et al. (2000)
Saprolite, limonite, nontronite	<i>Aspergillus, Penicillium</i>	Not specified	Up to 36	Valix et al. (2001)
Saprolite, weathered saprolite, limonite, nontronite	<i>Aspergillus, Penicillium</i>	Not specified	Up to 35	Valix et al. (2001b)
Weathered saprolite	<i>Aspergillus foetidus</i>	Not specified	28	Le et al. (2006)
Chromite slag	<i>Aspergillus</i>	Citric, oxalic, gluconic	Up to 34	Mohapatra et al. (2007)

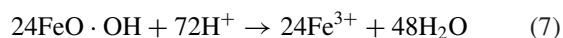
heterogeneous reaction that includes the reaction of laterite ore particles (which are present in the goethite core) with metabolic acids such as citric acid, oxalic acid, and acetic acid. The shrinking core model was found to be compatible with laboratory evidence. Through the ash layer of the ore spherical particle, citric acid, oxalic acid, and acetic acid showed different diffusion coefficients. Effective diffusion coefficients of acids, besides activation energy profiles, can be useful for the development of basic prototypes to simulate, control, and increase the scale of biological leaching processes for nickel ores (Sahu et al., 2011). Krstev et al. (2012) reviewed heat-resistant ores or low-grade nickel oxides (laterites) in Macedonia by magnetic separation–flotation method, and production and melting of ferronickel. At the same time, this study pays attention to the possibility of dissolution by chemolithotrophic microorganisms (bioleaching). The results showed that the average grade obtained from the separation–flotation process in the form of nickel concentrate was 80–90% nickel and the recovery was 80–85%. The average recovery for nickel leaching was 87–90% while 93–95% for its bioleaching (Krstev et al., 2012). *Aspergillus niger*, along with acid mine drainage from a coal mine, was used as organic substrate (for carbon and as a source of fungi nutrients) by Mubarok et al. (2013) for direct bioleaching of nickel from Indonesian Pomalaa laterite ore. Goethite and quartz were the main minerals of limonite ores. The type of organic acids generated by the microbes was determined using high-performance liquid chromatography (HPLC). The optimal amount of acid mine drainage should be determined to increase nickel extraction and reduce the simultaneous dissolution of iron and magnesium. Saprolite ore dissolves better with fungal metabolite acids than limonite, according to the findings. The distribution of coarse particle size (+177 –250 microns) in both saprolite and limonite ores resulted in superior nickel extraction than the finer particle size of these ores. In the finer particle size distribution, the reduction in nickel extraction may be due to the adsorption of dissolved nickel ions into the gangue minerals and at the same time the higher dissolution of the metal impurities. The findings revealed that *Aspergillus niger* can grow on an acid mine

drainage organic substrate derived from a coal mining location. The addition of elemental sulfur did not affect the nickel extraction rate (Mubarok et al., 2013).

Economical metals are also present in oxidized ores (such as nickel in laterite). On the other hand, because these ores cannot have oxidative dissolution, the ferric iron minerals present in these ores can be dissolved by reducing the iron and releasing the metals. Johnson et al. (2013) used the acidophilic bacterium *At. ferrooxidans* capacity to be permanently anaerobic which oxidation of sulfur is linked to ferric iron reduction in the goethite of nickel limonite ores. When anaerobic culture media were placed in bioreactors temperature and pH were adjusted, *At. ferrooxidans* accelerated the reductive dissolution of goethite and thus increase the dissolution rate of nickel in the ore. The quantity of ferrous iron in the solution was strongly correlated with the amount of nickel in the solution; on the other hand, goethite was found to be the source of the majority of the nickel. Furthermore, the proportion of sulfuric acid needed to keep the pH of the leach solution at 1.8 and the amount of dissolved iron (1.75:1) was close to the theoretical value (1.7:1) in Eq. (6), indicating that the predominant form of dissolved sulfate at pH 1.8 is HSO_4^- instead of SO_4^{2-} (Johnson et al., 2013).



In contrast, when iron dissolution proceeds entirely through acidic dissolution (according to Eq. 7), the ratio of proton consumption to the amount of dissolved iron is 3:1.



In addition to the dissolution of iron and nickel, the concentration of dissolved cobalt and manganese also increases during the bioleaching of the laterite ore. Bio-processing of lateritic ores under anaerobic conditions contained higher amounts of dissolved manganese, nickel, iron, and cobalt within 20 days compared to aerobic conditions. Anaerobic conditions accelerate the reduction of ferric iron to ferrous iron by using elemental sulfur as a source of electrons provided by the *At.ferrooxidans* bacterium (Johnson et al.,

2013). Fatahi et al. (2014) examined the extraction of nickel from laterites using agitation leaching at atmospheric pressure. In this study, sulfuric acid and nitric acid were used for dissolution. As a result, 83% of recovery was achieved in the optimal conditions (Fatahi et al., 2014). MacCarthy et al. (2014) performed acid leaching at atmospheric pressure using 98% sulfuric acid on a laterite sample. The results showed that all pulps have non-Newtonian behavior and the maximum recovery was 87.78% for goethite-siliceous nickel-bearing laterites and 55.42% for goethite nickel-bearing laterites (MacCarthy et al., 2014). Önal and Topkaya (2014) replaced the pressure acid leaching with heap leaching by studying a sample of Çaldağ laterite ores. Variable parameters included acid to ore ratio, temperature, and time. Nickel had a maximum recovery of 94.1%, and cobalt had a maximum recovery of 94%. It was also found that nickel extraction is temperature sensitive and cobalt extraction is acid sensitive (Onal & Topkaya, 2014).

Microbial processing of lateritic reserves still needs to be improved so that this processing method can be applied on an industrial scale. Microorganisms play an essential role in the biological processing of minerals because they accelerated the extraction of metals from minerals. Therefore, the discovery of physiological and biomolecular mechanisms involved in this interaction of microbes and minerals is inevitable. Advances in nickel extraction from laterites were discussed by Behera and Mulaba-Bafubandi (2015). Application of heterotrophic microbes (fungi of the *Aspergillus* and *Penicillium* genus and bacteria of the *Bacillus* and *Pseudomonas* species), chemolithotrophic microorganisms (mainly *Acidithiobacillus*, *Leptospirillum*, and *Sulphobacillus*), and iron-degrading and reducing bacteria (mainly from *Shewanella*, *Geobacter*, and *Geovibrio* species) was examined in this study. The use of chemolithotrophic microorganisms for the processing of oxidized minerals (laterites) using conventional bio-oxidation leaching is not used because oxidized minerals do not have the nutritional support (reduced sulfur compounds or divalent iron compounds) for such microbes. However, the microbial reduction process, which uses DIRB and chemolithotrophic bacteria, provides a clearer path to the development of a technical process that extracts nickel from laterites in an economically viable manner (Behera & Mulaba-Bafubandi, 2015). Based on the results of Astuti et al.'s (2016) studies, it was determined that citric acid is more efficient than other organic and inorganic acids for saprolite leaching in Indonesia. They compared the efficiency of citric acid with that of other acids (lactic acid, oxalic acid, sulfuric acid, hydrochloric acid, nitric acid, as well as the combination of citric acid and sulfuric acid). Citric acid, as well as sulfuric acid, separately provided a recovery of 72%, while nitric acid and hydrochloric acid with a

ratio of 1 + 1 provided a recovery of 43–45%. Furthermore, recovery with a 0.75 + 0.25 ratio using citric acid and sulfuric acid yielded 80% in one sample and 100% in the other. Sulfuric acid was also discovered to be superior to citric acid for leaching laterites containing goethite. Nickel was recovered from goethite as effectively as serpentine with inorganic acids such as sulfuric acid, nitric acid, and hydrochloric acid. Although citric acid is a weak acid, it is an excellent metal chelator and binder through the mechanism of acidolysis and complexolysis. With the combination of citric acid and sulfuric acid, nickel recovery and selectivity are both high, and acid consumption is lowered, making this system environmentally friendly (Astuti et al., 2016). The selective dissolution of nickel from limonite laterites was studied by Chang et al. (2016) under moderate pressure conditions. It was found that hematite precipitation lowers the iron concentration to less than 1 g/L when nickel is recovered at 95.2% under optimal conditions (Chang et al., 2016). Kursunoglu and Kaya (2016) studied leaching at atmospheric pressure on a sample of Caldag laterite ore. The nickel and cobalt recoveries were respectively 91.9% and 93.5%, while the iron recovery was 80.5%. The results showed that crushing finer than 0.150 mm did not affect nickel recovery, while the highest cobalt extraction was obtained with the smallest particle sizes. The nickel, asbolane, goethite/hematite, and clay phases were detected using XRD and SEM-EDX. Furthermore, the nickel extraction kinetics revealed a 51.6 kJ/mol activation energy. Thermal pretreatment, high mineral acid concentrations, and high-temperature leaching are methods used to recover significant quantities of nickel and cobalt (Kursunoglu & Kaya, 2016). Chaerun et al. (2016) evaluated nickel recovery from limonite and saprolite ores using bioleaching with agitation tank reactors with native bacteria and a combination of iron-oxidizing bacteria including *Alicyclobacillus ferrooxydans*, *Comamonas testosterone*, and *Pantoea septic* species, which was isolated from Indonesian ore. With a 10% pulp density, coarse limonite, and weathered saprolite ore were tested for their pulp density. The bioleaching process was done by measuring the content of iron, nickel, and the pH of the leaching solution. In addition, the FDA hydrolytic activity was used to calculate the overall bacterial enzyme activity. FDA hydrolytic activity was 17.2 µg fluorescein/mL for limonite and 16.9 µg fluorescein/mL for saprolite. At pH = 2.5–3.5, the amount of nickel and iron dissolved for limonite ore (30% Ni, 5.6% Fe) was higher than their amount in biocontrol (1% Ni, 0.1% Fe) but in the saprolite ore, a species of hybrid bacterium, was only able to bioleach 2.5% of nickel and 6% of iron (similar to limonite). In biological control, the pH of the environment remained relatively constant (about 6) (Chaerun et al., 2016).

Most researchers used the terms single-stage bioleaching (fungi and ores enter media simultaneously), two-stage bioleaching (fungus was added first, then ore after metabolite production began), and spent medium bioleaching to describe bioleaching with fungi (biometabolites were produced by fungi after a determinate time). The three different methods of fungi bioleaching have been described by Chaerun et al. (2017) as direct bioleaching (one-stage bioleaching), semi-direct bioleaching (two-stage bioleaching), and indirect bioleaching (spent medium) (Chaerun et al., 2017).

Fungal metabolic organic acids were used to indirectly bioleaching low-grade nickel-bearing laterite ores with *Aspergillus niger* due to the following reasons:

- (1) Chemoorganotrophic microorganisms are better suited for the treatment of ores that are not sterile and cannot be sanitized, due to their ability to flourish in less selective media and higher tolerance than acidophilic chemolithotrophs.
- (2) It seems that the growth of chemoorganotrophic microorganisms on the ore causes the degradation of produced acidulantes/ligands.
- (3) Organic acids, unlike inorganic acids such as sulfuric acid, are less corrosive.
- (4) Nickel is extracted from laterite ores with a low nickel content using *Aspergillus niger* which is caused by the adsorption of dissolved nickel ions into the hyphae of the fungus (Chaerun et al., 2017).

In this study, the parameters include sources of organic carbon (molasses and starch) as substrate, sources of nitrogen and phosphorus ($(\text{NH}_4)_2\text{SO}_4$, KH_2PO_4), micronutrients (MgSO_4 , MnSO_4), and aeration which improve the bio-production of fungal metabolic organic acids, were optimized. Chaerun et al. (2017) also used limonite and saprolite from an Indonesian mine to measure the types of organic acids produced and their effects on nickel leaching processes, as the types of organic acids produced by fungi differ with the sources of metabolized carbon (Chaerun et al., 2017). *Aspergillus niger* produces fungal metabolic organic acids which were used in the experiment to demonstrate greater selectiveness of nickel bioleaching compared to iron (S_{Fe}) and magnesium (S_{Mg}) at different temperatures (25, 65, and 95 °C). Hence, leaching with organic acid created by bio-production was strongly selective for magnesium and relatively non-selective for iron. *Aspergillus niger* produced the highest amount of organic acid with starch as a carbon source when it was incubated with KH_2PO_4 , $(\text{NH}_4)_2\text{SO}_4$, and MgSO_4 under aeration conditions, which was then used for indirect bioleaching. At a pH of 1.4, starch produced more organic acids than molasses after 16 days, although the rate of synthesis was slow so it will need further studies for industrial uses. Saprolite and limonite ores both

dissolved nickel at a maximum efficiency of 88.9% and 84.9%, respectively (Chaerun et al., 2017).

Jang and Valix (2017) studied the gradual adaptation of *A. thiooxidans* to dissolve heavy metals from Riverina-related nickel-bearing saprolitic laterites in Australia. For heavy metal adaptation trials, the media was sterilized. The adaptation, drawn in terms of the adaptation index, depended on the metal concentration, acid production, and adaptation period. Simulation of cell growth and acid production proceeded using the effect of heavy metals on bacteria. The use of adapted bacteria increased nickel recovery by 20% and cobalt recovery by 7%. The results obtained in this study confirmed the strong structure and high stability of *A. thiooxidans* in the direct bioleaching of nickel-bearing ores. Acid production improves metal stability in solution and lowers metal adherence to the cell wall, as well as its toxic effects. Furthermore, as compared to unadapted bacteria, adapted *A. thiooxidans* demonstrated up to a tenfold improvement in cobalt leaching selectivity (Jang & Valix, 2017).

Several studies have discussed leaching processes using various organic acids, but only a few types of research have been done on the effect of surface area and particle size distribution on the leaching process. Pawlowska and Sadowski (2017) study focused on how microorganisms produced citric acid and added citric acid impact the specific surface area and particle size distribution of minerals chemically. The effect of chemical citric acid and filtered citric acid on nickel extraction following cultivation of *Aspergillus niger* was also compared in this article. Besides, laterite ore was acquired from the Szklary deposit in Poland. The microbes were cultivated for four days before being autoclaved and filtered to eliminate the dead biomass. It was observed that higher nickel recovery is the main factor of higher particle surface area. The highest recovery in nickel extraction was obtained for the 1 mol/dm³ (67%) concentration of citric acid. After leaching, the specific surface area was 159 m²/g. After leaching with citric acid, a particle size distribution study revealed an increase in average particle size, owing to a drop in the percentage of the smallest grains. Polish laterites also disintegrated according to the shrinking core-shrinking particle model (Pawlowska & Sadowski, 2017).

Marrero et al. (2017) demonstrated anaerobic reduction dissolution of iron, nickel, cobalt, and manganese from laterites in laboratory bioreactors using *At. ferrooxidans* and *A. Thiooxidans*. The chemical mobility of ferric ions in inoculated anaerobic bioreactors occurred at pH=0.8. After 7 days, the pH needed to be increased from 0.8 to 1.8 to increase the dissolution rate with a biological decrease in ferric ions. Aerobic reactors by maintaining the pH at 0.8 (inoculated or not inoculated) resulted in more release of ferric ions in laterites than anaerobic reactors. The process of aerobic reduction dissolution was much more efficient

than the anaerobic reduction solution for extracting total iron and ferrous iron, as well as for dissolving manganese, cobalt, and nickel. The presence of O_2 is considered a key factor in increasing the bio-reduction dissolution of iron-containing minerals (Marrero et al., 2017). Rasti and Rajabzadeh (2017) studied the geochemical and mineralogical characteristics of nickel-containing laterites of serpentine origin, the application of the lateralization process, and the classification of nickel-containing laterites related to the Fars region in Iran (Rasti & Rajabzadeh, 2017). For low-grade nickel ores in the Sarbisheh region, Javanshir et al. (2018) used atmospheric pressure leaching. The ore has a high content of iron and low content of nickel, with quartz, goethite, and dolomite being the major crystalline phases; the nickel-ferrous laterite ore was of the goethite type. The results of their study showed that goethite and hematite were the main minerals of the sample and nickel are scattered throughout the ore area. Magnetic separation was used to pre-concentrate the ore in this investigation, although it was unsuccessful. Hematite (the main mineralization phase) was formed when the laterite ore was heated, which was easier to dissolve, so calcination was an effective step to increase nickel extraction. TG/DTA analysis showed two endothermic peaks, dihydroxylation of goethite to hematite occurred at 280 °C and XRD analysis before and after calcination confirmed this conversion. Leaching with sulfuric acid obtained higher nickel values than leaching with hydrochloric acid (Javanshir et al., 2018). Li et al. (2018) examined the amount of nickel and cobalt extraction from limonitic laterites by changing the type and concentration of acid as well as the calcination temperature. Phosphoric acid and sulfuric acid were the selected acids for this selective leaching. The maximum recovery was 98.7% for nickel and 89.8% for cobalt, and 98.3% of the iron was converted to insoluble iron. After calcination, recovery was 95.9% for nickel and 91.4% for cobalt. In addition, 92.4% of iron was converted to insoluble iron. This study determined that the formation of iron phosphate with phosphoric acid was more effective than that using sulfuric acid and hydrochloric acid (Li et al., 2018). Petrus et al. (2018) used limonite-type laterite nickel ores from the Pomalaa region of southern Sulawesi, Indonesia. *Aspergillus niger* under optimal conditions was used to produce metabolic citric acid as the dissolving agent. The results showed that the maximum metal recovery increases with decreasing pulp density. The pulp density (solid to liquid ratio) of 5% obtained maximum recovery; the recovery rate at this pulp density was 1.63% for Ni, 0.47% for Al, 0.23% for Fe, and 1.09% for Mg. These recovery values were lower than those obtained from synthetic citric acid, which dissolved about 90% of nickel. This necessitates a change in the dissolution mechanism involving metabolic citric acid. This modification is done by adding H_2O_2 to decrease metals such as Fe and Mn, or

by fine-tuning the metabolic citric acid synthesis pathway to produce higher acid concentrations. The shrinking core model of leaching is illustrated by the effect of particle size on leaching process. Three steps affect the reaction rate:

- (1) Diffusion through a coating on the particle's surface,
- (2) Diffusion through the particle's ash layer,
- (3) Surface reaction on the particle's unreacted section.

The rate of the leaching reaction is determined by the slowest stage. In this research, the diffusion-controlled reaction rate in the leaching process. The maximum metal recovery occurred with a particle size of 125–149 μ and the recovery rate was 1.37% for Ni, 0.72% for Al, 0.1% for Fe, and 0.62% for Mg. Due to the broader radius of the ash layer generated for larger particles, which prevents the reactant from adsorbing to the reaction region and the product from desorbing to the liquid section, the recovery rate was lower in particle sizes larger than 200 microns than in 125–149 microns. The reaction rate increases with smaller particle sizes, which means a bigger surface area, but as the reaction rate increases and complex metal molecules form on the reaction area's surface, a space barrier is generated for the result to be desorbed (Petrus et al., 2018).

Giese et al. (2019) conducted bioleaching experiments using the heterotrophic species *Bacillus subtilis* on a Brazilian sample of nickel-containing laterite ores. The ore mainly contained lizardite (32.6% MgO) and 1% NiO (0.85% Ni). The results of SEM analysis showed that the primary nickel-bearing mineral was goethite. Discontinuous bioleaching experiments showed that 8.1% Ni (0.7 mg nickel per gram of ore) was dissolved using *Bacillus subtilis* within 7 days. In this study, diluted laterite with water was heated using a microwave as a pretreatment method. The biological extraction of nickel was raised from 8 to 26% using this pretreatment approach (2.3 mg nickel per gram of ore) (Giese et al., 2019). Yang et al. (2019) investigated the performance of direct or indirect bioleaching of cobalt and nickel from laterite and pyrite ores using *Aspergillus niger*. Imported laterite ores came from Kazakhstan, while pyrite ores came from Finland. Average cobalt recoveries from laterites and pyrite ores using direct bioleaching were obtained $65.9 \pm 1.8\%$ and $4.9 \pm 2.7\%$, respectively, and average nickel recoveries were obtained $30.9 \pm 0.6\%$ and $10.9 \pm 6.2\%$, respectively. Recovery of cobalt from indirect laterite bioleaching was significantly lower in the absence of fungal biomass compared to cobalt recovery in direct bioleaching. The most important bioleaching phase in laterites was cobalt-containing goethite, according to the X-ray powder diffraction (XRPD) study of mineral phases before and after bioleaching. Direct and indirect bioleaching yielded no significant changes in cobalt-containing synthesized goethite. As a result, Yang

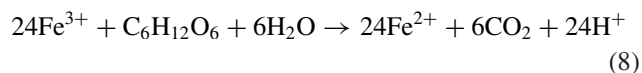
et al. proposed that bioleaching of laterites occurs in two stages:

- (1) Cobalt-containing goethite was exposed to direct fungi-mineral interaction.
- (2) Organic acids and metabolites produced by *Aspergillus niger* were used to dissolve cobalt-containing goethite.

The metal recovery process also revealed an inconsistent pattern of Co and Fe bioleaching from laterites (Yang et al., 2019).

Mulroy (2019) studied the geochemistry and mineralogical phases of nickel and cobalt oxides containing Mn (III/IV) and the microbiological interactions with these phases. The ore samples were inoculated with glucose to stimulate native microbial assemblies, while the asbolane mineral was inoculated with a pure culture medium of *Geobacter sulfurreducens* and acetate. Synchrotron-based XAS was used to determine the chemical properties of Co, Ni, and Mn crystals in both natural and synthetic samples before and after microbiological activity. The results showed that in fresh ore, Ni (II) compared to Co (III) is more diverse. Ni (II) is either incorporated in aluminum-bearing parts of lithiophorite or adsorbed on the edges of manganese oxide. The extent to which this occurs indicates oxidation/reduction conditions and microbial effects on the various laterite horizons. The microbial consortium reduces Mn (III / IV) and Co (III) to divalent forms. *G. sulfurreducens* has been shown to effectively degrade synthetic asbolane and release Co and Ni in solution. Then, the precipitation of Mn, Co, and Ni as carbonate phases represents a way to form products that are often used as intermediates in the various industrial process (Mulroy, 2019). Stankovic et al. (2020) reviewed the most important hydrometallurgical processes for the extraction of nickel from laterites. The results of this study evaluated high-pressure acid leaching (HPAL), Caron process, heap leaching (HL), atmospheric leaching (AL), direct nickel, Ferredox reductive bioleaching, and hydrochloric acid leaching (Neomet process). Using the direct nickel process (DNP), nickel ores are leached with nitric acid at 110 °C under atmospheric pressure in agitation tanks. The Direct Nickel Group of Australia, which processes Indonesian laterite ores, created the process. The duration of leaching is 2–4 h and the recovery of nickel and cobalt is more than 95%. DNP is a complicated technique that requires multiple pH adjustments throughout the purification stage. As a result of hydrolysis, which takes place at a pH of 2 to 3, ferrous iron is first removed. As a by-product, the hematite cake that collects on the filter is sold. To raise the pH and precipitate aluminum hydroxide, magnesia pulp (MgO) is used to treat the iron-free solution. To create products containing aluminum, aluminum hydroxide is filtered. The residual solution, which contains a small

amount of aluminum, is prepared for the mixture hydroxide precipitation (MHP). In a two-stage circuit, the magnesia pulp is used to increase the pH once more, and the hydroxide mixture containing the majority of the nickel and cobalt recovered from the feed is precipitated. Eventually, nitrogen oxide gases are recovered during a series of adsorption steps to form nitric acid, which is returned to the leaching cycle (Stankovic et al., 2020). The Canadian Neomet company invented chloride leaching and the Neomet process. In tanks at atmospheric pressure and temperatures of 100–110 °C, hydrochloric acid is used to leach nickel and cobalt from limonite and saprolite ores. In this method, the recovery of nickel and cobalt is high (more than 90%). The removal of nickel from laterites is enabled by the biological reduction of oxidized iron and manganese minerals. Some anaerobic microorganisms include heterotrophic and autotrophic acidophilic bacteria. In hypoxic environments, these bacteria can use Fe³⁺ as an electron acceptor instead of oxygen in a respiratory chain that converts ferric iron (Fe³⁺) to ferrous iron (Fe²⁺), as shown in Eq. (8) (Stankovic et al., 2020).



The reductive bioleaching process takes place at 30 °C and in a mildly acidic environment (pH=1.7–2). As a result, when compared to conventional hydrometallurgical processes for extracting nickel and cobalt from laterites (excluding heap leaching), this process uses less energy and requires less expensive equipment, hence the Ferredox process's capital and operational costs may be lower. The consumption of sulfur and sulfuric acid has an impact on the economics of this process. Laterites are the part of oxidized ores so, unlike sulfide ores, they do not tend to catalyze the oxidative dissolution of ferric iron (Hallberg et al., 2011; Johnson, 2012). A strong bond between oxygen and ferric iron must be broken for the nickel in goethite or other host nickel minerals to be soluble. Several hydrometallurgical and pyrometallurgical methods have been used to dissolve the oxygen-iron bond (Mohapatra et al., 2007; Plessis et al., 2011; Simate & Ndlovu, 2008), but these methods require high energy or high costs of reactant and high capital costs for equipment. Hydrometallurgical processing methods result in the simultaneous dissolution of gangue minerals, raising the process complexity and increasing the cost of recovering precious metals and removing unwanted elements. A low-cost method for breaking the oxygen-iron bond in goethite can significantly reduce the economic costs of processing limonite containing nickel and cobalt. Oxygen-iron bond breakage is performed by the Ferredox process (Plessis et al., 2011). The Ferredox process with the reductive dissolution of goethite (the most important host mineral for nickel-bearing limonite) using elemental sulfur

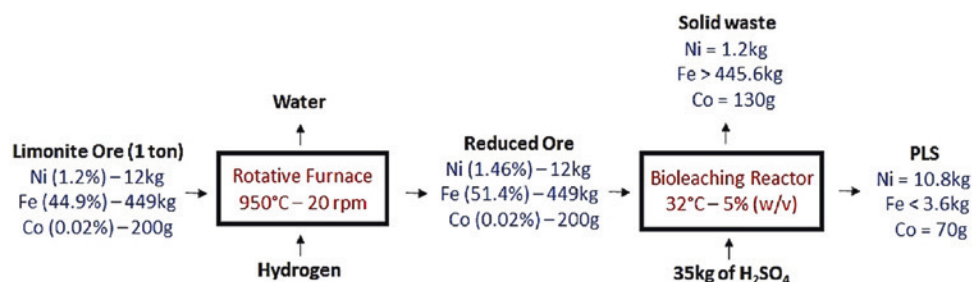
was identified as a reducing agent in environmental conditions (Johnson, 2012; Plessis et al., 2011). Reductive dissolution of nickel-bearing limonite led to the fundamental development of biomining. In short, this method, in contrast to the traditional method (oxidative dissolution, the use of ferric iron produced by bacteria), was used for the biological processing of metal ores (Johnson, 2012). The process, which was performed using pyrometallurgical methods at 800 °C, is performed at a temperature of 30 °C, which indicates the potential of microorganisms. It has been shown that indirect reductive dissolution of minerals, including asbolane and goethite, can be applied more broadly to the processing of oxidized ores (Johnson, 2012).

As pointed out before, the only commercial hydrometallurgical processes for the extraction of nickel and cobalt from laterites are HPAL and heap leaching. Hydrometallurgical plants have become more efficient with the production of cobalt, which is lost in pyrometallurgical processes. HPAL provides excellent recovery rates of nickel and cobalt and is quick to leach, however, it is complex and requires a large investment. HPAL is more expensive than pyrometallurgical activities in some instances, and it requires a higher operating cost, while heap leaching is more simple, with significantly lower operating costs and investment costs than HPAL and pyrometallurgy, however, the leaching rate is very slow. The possibility of obtaining cobalt and other valuable elements from laterite reserves makes hydrometallurgical processing a more appealing process, in contrast, pyrometallurgical processing extract 80% of nickel from laterite reserves (Stankovic et al., 2020). Santos et al. (2020) studied the biological leaching of three limonite-type laterite ores from Greek mines under reductive conditions using a consortium of acidophilic bacteria in stirred tank bioreactors at pH=1.5 and 35 °C (using elemental sulfur as an electron source). *At. ferrooxidans*^T, *At. ferrooxidans*, species of *CF3*, *At. ferriphilus*^T, *At. ferridurans*^T, and *Sulfobacillus (Sb.) thermosulfidooxidans*^T are among the acidophilic bacteria used in this work. In this study, the target metal, which is cobalt, was successfully bioleached (40–50% within 30 days). Some other metals, such as iron, were recovered between 2 and 48%. In most cases, the concentration of dissolved cobalt was highly correlated with the concentration of dissolved manganese.

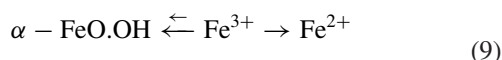
This was under the finding of manganese (IV) minerals as a major source of cobalt. Extensive mineralogical analysis for these three limonite samples before and after biological processing revealed significant changes between these ores and showed that mineralogical and elemental changes could significantly affect the adaptability of the ore with reductive bioleaching (Santos et al., 2020). Esther et al. (2020) used Dissimilatory Iron Reducing Bacteria (DIRB) to remove iron impurities from various ores in bioremediation to eliminate the toxicity of heavy metals and toxic pollutants. This study aimed to investigate the effect of a consortium of anaerobic bacteria that decompose and reduce iron (III) on different phases of iron in lateritic nickel ores. Such changes are useful for better recovery of metals such as Ni and Co using bioleaching or acid leaching in later stages. 70% of nickel and 81% of cobalt are recovered using DIRB-treated acidic leaching of ore, while more than 54% of nickel and 57% of cobalt are recovered using acid leaching of untreated chromite overburden (COB). Therefore, COB pretreatment using DIRB can improve nickel recovery (Esther et al., 2020).

Two of the most important challenges in implementing the acid leaching process at atmospheric pressure to extract nickel from laterites are the high acid consumption and the high final concentrations of iron in PLS. In a study conducted by de Alvarenga Oliveira et al. (2021) using pyrometallurgical and biohydrometallurgical operations, a new process for the extraction of nickel from laterites was proposed. In the laboratory, nickel limonite ores are reduced with hydrogen gas in a rotary furnace at 900 °C until all goethite is converted into metallic iron then reduced sample bioleached with 5% solid by mesophilic microbes cultured on Fe²⁺ (*At. ferrooxidans*) at 32 °C and pH<3. According to experiments conducted using 35 kg of H₂SO₄ per ton of reduced ore, researchers discovered that Fe, Ni, and Co will leach as Eh production from bacteria increases. Nickel dissolved at a rate of 92%, whereas cobalt at 35%. During these laboratory conditions, the concentration of iron in the aqueous phase produced was less than 5 mg/L due to the precipitation of Fe³⁺ as jarosite. A lower concentration of total iron in the aqueous phase of the leach was also observed (less than 200 mg/L), which was much lower than HPAL's reported values (Alvarenga Oliveira et al., 2021) (Fig. 8).

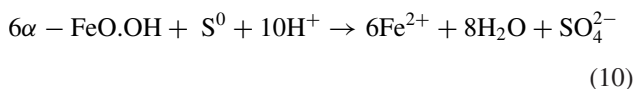
Fig. 8 Flowsheet and mass balance for the proposed procedure in nickel extraction (per ton of limonite ore) (Alvarenga Oliveira et al., 2021)



Acidophiles are a group of extremophiles whose optimum growth pH is 3 and below 3 (Gomes & Steiner, 2004; Rampelotto, 2013; Rothschild & Mancinelli, 2001). Organic acids inside acidophilic cells as respiratory chain separators are harmful, but the cell cleverly combines protons with organic acids inside the cell and converts organic acid to carbon dioxide (Nordstrom et al., 2000). Low pH strengthens and intensifies the population of acidophilic prokaryotes as well as increases the solubility of metals (Johnson & Hallberg, 2003; Nordstrom et al., 2000). Iron-reducing acidophiles use ferric iron as an electron acceptor (rather than oxygen) and need an electron donor (sulfur, hydrogen, or an organic molecule, depending on the species type) to reduce ferric iron (Hallberg et al., 2011; Johnson, 2012; Marrero et al., 2015). Due to the bacteria's ability to decrease the amount of soluble ferric iron, a balance between ferric iron in the mineral phase and ferric iron in the soluble phase is created, thus the mineral dissolution rate accelerated according to Eq. (9) (Hallberg et al., 2011):



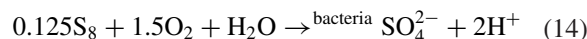
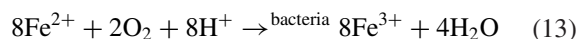
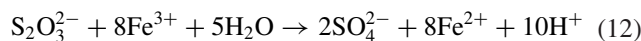
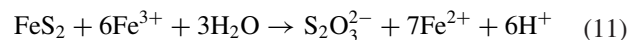
The reduction of goethite with sulfuric acid produced an alkaline (proton-consuming) reaction, as shown by Eq. (10) (Hallberg et al., 2011; Plessis et al., 2011):



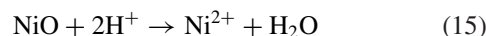
For the reduction of ferric iron minerals in laterite ores, bacteria can release metals (particularly nickel) and ferric iron minerals into the solution. By doing the process in an acidic environment (for example, acidophilic microorganisms), it is possible to keep the pH of the solution low and thus the extracted nickel remains in the solution and its recovery is facilitated (Hallberg et al., 2011; Johnson, 2012).

The physical contact between bacteria and metals produced by their extracellular metabolites is the basis for metal dissolution by chemolithotrophic bacteria such as *At. Ferrooxidans* (Chang et al., 2008). In bioleaching, metabolites (mostly sulfuric acid) that suggest exponential growth play a crucial role. The pace of bioleaching is limited by acid generation. Metals dissolve when they come into physical contact with bacteria and when they produce acid, so acid production and bacteria can influence metal dissolution (Chang et al., 2008). Gram-negative chemolithotrophic, mesophilic, and voluntary anaerobic bacteria *At. ferrooxidans* have been the subject of extensive genetic, genomic, and phylogenetic studies and therefore have been the only model microorganisms to study the biochemical cycle and electron transfer during iron oxidation (Golyshina et al., 2000; Wu et al., 2008). This bacterium which oxidizes iron and sulfur (Hallberg et al., 2011; Mohapatra et al., 2007), uses energy sources that include Fe

(II), H₂S, S⁰, reduced inorganic sulfur compounds, and molecular hydrogen. Oxidation of sulfur produces sulfuric acid, which gives interesting properties of *At. ferrooxidans*. These bacteria grow under the temperature conditions of mesophiles (Wu et al., 2008). The use of chemolithotrophs in bioleaching, which uses sulfur and sulfide minerals such as pyrite as energy sources, eventually produces sulfuric acid during the oxidation process [Eqs. (11)–(14)] (Simate & Ndlovu, 2008):



Nickel-bearing laterite ores do not contain sulfur, so sulfur needs to be added to chemolithotrophs in the leaching of laterite ores (Simate & Ndlovu, 2008; Simate et al., 2010). For the growth of bacteria and to be able to effectively leach nickel-bearing laterites, the produced sulfuric acid maintains the desired pH level (Simate & Ndlovu, 2008). Biogenic sulfuric acid can be produced using chemolithotrophs such as *At. ferrooxidans* (Simate et al., 2010). The property of hydrogen ions to displace metal cations from the ore matrix is the reason that metal dissolution is enhanced (Eq. 15) (Simate & Ndlovu, 2008).



The use of chemolithotrophic bacteria in nickel recovery from laterite ores has been relatively unknown until recently, owing to the ore's lack of sulfide concentration, which is required to make sulfuric acid (Simate & Ndlovu, 2008). There is uncertainty over whether ferric iron reduction is an indirect reaction from inorganic reduced sulfur compounds formed during sulfur oxidation or a direct reaction from sulfur oxidation enzymes (Marrero et al., 2015).

Simate et al. (2009) expressed that the use of a culture media with a combination of chemolithotrophs could improve the dissolution of nickel from nickel-containing laterites. This is due to the competition of using oxygen in iron-oxidizing bacteria which can lead to the reductive dissolution of ferric iron in nickel laterites and destabilize these ions (Simate et al., 2009b). As well as inhibiting sulfur dioxygenase, nickel ions make the conversion of elemental sulfur to sulfite faster, as well as making the conversion of sulfite to sulfate easier. Nickel surrounds the plasma membrane and prevents the entry of both enzymes, in other words, it prevents cell growth. While nickel interferes with the physiological functions of bacteria such as sulfur and iron oxidation, some bacteria can quickly adapt to high

nickel concentrations (Watling, 2008). Simate and Ndlovu (2008) expressed that bacteria of the *caldu*s genus extract nickel more efficiently than fungi by producing sulfuric acid through oxidizing elemental sulfur (Simate & Ndlovu, 2008). It has been shown that mesophiles can tolerate nickel in bioleaching environments better than thermophiles and moderate thermophiles. *At. ferrooxidans* are more tolerant of nickel than *A. thiooxidans* (Watling, 2008). Mohapatra et al. (2007) expressed in their study that the culture media contained *At. ferrooxidans* dissolve nickel more efficiently at temperatures of 30–37 °C than *Aspergillus* species and are unable to dissolve nickel at higher temperatures such as 45 °C. Optimal bacterial growth temperature of *At. ferrooxidans* is 30 °C. The results of their research showed that increases in ferrous-iron supplementation increase the leaching efficiency of *At. ferrooxidans*. Also, after 28 days of leaching, the maximum dissolution of nickel at 2% pulp density was equal to 40% and at 10% pulp density was equal to 24% (Mohapatra et al., 2007).

Although bacteria may not respond well to abrupt changes in metal ion concentrations, they can adapt to gradually increasing concentrations over time to increase their tolerance to metals. Natarajan and Iwasaki (1983) examined the growth of *At. ferrooxidans* in ferrous iron medium as nickel concentration increased gradually from 5 to 50 g/L of Ni²⁺ (Watling, 2008). Iron soluble in leaching leachate is often present in the form of iron, which makes the oxidation of bacterial ferrous ions a highly efficient process in bioleaching reactors. There is concern that the high concentration of iron in bioreactor leachates could affect the bio-oxidation of ferrous ions in acidophiles. Kawabe et al. (2003), Ojumu et al. (2006), and Molchanov et al. (2007) showed interest to study the effect of ferric ions on the oxidation of ferrous ions using *At. ferrooxidans* (Watling, 2008).

Acidiphilium was the first acidophilic heterotroph to be found to accelerate ferric iron reduction differently, and *Acidiphilium* SJH subsequently was found to be a strong reducing agent of iron and was able to use amorphous and crystalline ferric iron minerals as well as soluble ferric iron as an electron acceptor. *Acidiphilium* SJH:

- (1) The ability to sustain extremely low pH levels is essential (a pH of at least 1.8 is necessary since the ferric iron reduction is an acid-producing reaction).
- (2) Able to use a wide range of soluble electron sources with low molecular weight.
- (3) Capable of reducing ferric iron in the presence of oxygen, removing the need for bioreactors to operate in an anaerobic environment.
- (4) The inability of this species (as opposed to *At. ferrooxidans*) to oxidize ferrous iron, thus avoiding the fundamental problem of creating a cycle for iron (Johnson, 2012).

Coto et al. (2008) studied the recovery of nickel and cobalt from laterite wastes using organic and inorganic bio-acids. The results of their study showed that the recovery of metals in the two-stage experiments using biological sulfuric acid (79% of nickel and 55% of cobalt) was higher than that of biological citric acid, but the amount of recovery obtained in the two-stage experiments were lower than one-stage experiments after 15 days (100% of nickel and 80% of cobalt) (Coto et al., 2008). Hallberg et al. (2011) state that nickel and cobalt can be recovered from limonite ores by reducing goethite with *At. Ferrooxidans* (Hallberg et al., 2011; Plessis et al., 2011). After 14 days, 70% of the nickel had dissolved from the ore. In this study, initially, the pH of the culture media was raised to 1.8, and culture was performed at 30 °C with aeration for better growth of bacteria on sulfur. After the cultivation of the bacteria and reaching the number of bacteria to more than 5×10^8 cells per mL, the leaching process was performed under anaerobic conditions (Hallberg et al., 2011). Cabrera et al. (2005) used *At. ferrooxidans* to investigate the oxidation kinetics of ferrous ions to build a model that included the effect of metal ions (Watling, 2008). Although Pronk et al. (1991) were the first to reveal that *At. ferrooxidans* could reduce soluble ferric iron, it wasn't obvious until Hallberg et al. (2011) that this bacterium could also facilitate the reductive dissolution of ferric iron-containing rocks (Johnson, 2012). According to Johnson's (2012) findings, *At. ferrooxidans* facilitate the reductive dissolution of asbolane ((Ni,Co)_xMn(O,OH)₄·nH₂O) in anaerobic bioreactors by producing ferrous iron. According to the study, the sulfuric acid utilized to maintain the appropriate acidity of the leach solution contains more than 90% of the sulfur used in the reductive solution for the processing of nickel-bearing laterites. The remaining used sulfur (10%) is as an electron donor to reduce ferric iron (Johnson, 2012). Simate and Ndlovu (2008) showed that using sulfur compared to pyrite as a substrate leads to higher nickel recovery due to higher acid production. It was also discovered that independent of the number of bacteria inoculated, the rate of acid production by bacteria via sulfur oxidation is larger than the rate of acid consumption (Simate & Ndlovu, 2008). *Desulfovibrios* are among the identified heterotrophs in the SRB (Kelly & Wood, 2000). SRBs are involved in the reduction of sulfates to hydrogen sulfide, which then binds the hydrogen sulfide produced to the metals to form an unstable solution precipitate. Then the metals in a stable state are removed from the solution (Luptakova & Kusnierova, 2005). A study by Johnson and McGinness (1991) indicated that many heterotrophic acidophiles are capable of reducing ferric iron (Toni & Bridge, 2000). Metal leaching by heterotrophic microorganisms is typically a side effect of the microbes' production of organic acids, amino acids, and other metabolites (Coto et al., 2008). Heterotrophic bacteria' leaching

efficiency is determined by the formation of organic metabolites, which are expelled in the culture medium and cause a pH drop (Mohapatra et al., 2007). In the presence of reducing agents (e.g., Fe^{2+}), electron transfer at the surface of the oxidized substance forms highly reactive oxidized forms of Fe (II) (McDonald & Whittington, 2008a). The *Delftia* genus is of particular importance in biotechnology (Morel et al., 2016; Ubalde et al., 2012). One of the nickel tolerant species is this one (Zou et al., 2015). The phenotypic similarities between *Delftia acidovorans* and the *Pseudomonadaceae* family are shown (Barrionuevo & Vullo, 2012).

Newsome et al. (2020) noted that so far, the knowledge of how the biogeochemical cycle affects the behavior of cobalt has not been able to develop new technologies for cobalt recovery from ores. Metal reduction is stimulated by the addition of inexpensive organic substrates with simple access (acetate or glucose). At the end of the experiment, the amount of cobalt that could be easily recovered (soluble or extracted with acetic acid) increased from less than 1% to more than 64%, as well as manganese, with only a small fraction of iron which is transferred to a phase that is easily recoverable. The addition of organic substrates encouraged the growth of native prokaryotes, which are virtually exclusively associated with recognized manganese (IV) / iron (III) reductants, especially Clostridiales, according to microbial community sequencing. It was also discovered that *Penicillium* fungi can produce useful organic acids for the leaching of nickel and cobalt from laterites. The results demonstrated that the biogeochemical cycle of manganese using microorganisms is likely to affect the environmental behavior of cobalt in laterites. Interestingly, four laterites from different continents (Acoje, Alda, Piau, and Shevchenko) showed comparable behavior. A novel biological processing technique involves treating the laterites with an organic substrate to reduce the metal, then rinsing with acetic vinegar to remove it. Organic substrates are not only eco-friendly and may be generated from waste carbon substrates, but they also precipitate a little quantity of iron oxide, resulting in less waste production (Newsome et al., 2020). Hosseini Nasab et al. (2021) researched the Sarbisheh (Iran) laterite sample. The supernatant of two fungi, *Aspergillus niger* and *Penicillium bilaji*, and two bacteria, *Pseudomonas putida*, and *Pseudomonas korensis*, were used to explore indirect bioleaching of nickel and cobalt. The most prominent carboxylic acids in the supernatant of these bacteria and fungi were gluconic acid, citric acid, and oxalic acid, according to HPLC analyses. Gluconic acid (10.8–14.4 g/l) was the most important metabolic acid in *Aspergillus niger* supernatant and *Pseudomonas* species, while citric acid (6.2 g/l) was the most important metabolic acid in *Penicillium bilaji*. The maximum nickel and cobalt recovery from *Pseudomonas*

putida supernatant was 90.6% and 71.98%, respectively, after 3 h at 90 °C. The resulting activation energies also indicated a greater effect of chemical control compared to diffusion control on the dissolution rate of nickel and cobalt from the studied laterite sample (HosseiniNasab et al., 2020a). In another study, the two-stage bioleaching kinetics of nickel and cobalt from iron-rich laterite ores were investigated using the metabolic products of the supernatant *Salinivibrio kushneri* as a halophilic bacterium. In this study, recovery of nickel and cobalt was reported 58.4% and 60.6% after 3 h of bioleaching at 90 °C, respectively (HosseiniNasab et al., 2020b). In another study on the same sample, Hosseini Nasab et al. (2021) studied the bioleaching of nickel and cobalt from an iron-rich lateritic sample using the *Delftia acidovorans* (heterotroph) and *At. ferrooxidans* (autotrophs) acidophilic bacteria. The findings revealed that acidophilus bacteria metabolic products play a significant role in the bioleaching of this sample. Highest nickel recovery using *Delftia acidovorans* and *At. ferrooxidans* supernatants were 80.18% and 83.65%, respectively, and cobalt was 83.94% and 86.93%, respectively. The dissolution rate of iron in these conditions was 54.41% and 64.34% for the two studied bacteria, respectively. The extraction of nickel and cobalt in the indirect bioleaching method using *Delftia acidovorans* supernatant was 29.84% and 23.75% higher than the direct bioleaching method (Hosseini Nasab et al., 2021).

Major investigations relating to the nickel laterite leaching by microorganisms are summarized in Table 5.

Figure 9 summarizes the bioleaching process of the laterites as schematically by iron-oxidizing and iron-reducing bacteria, acidophilic bacteria, and fungi.

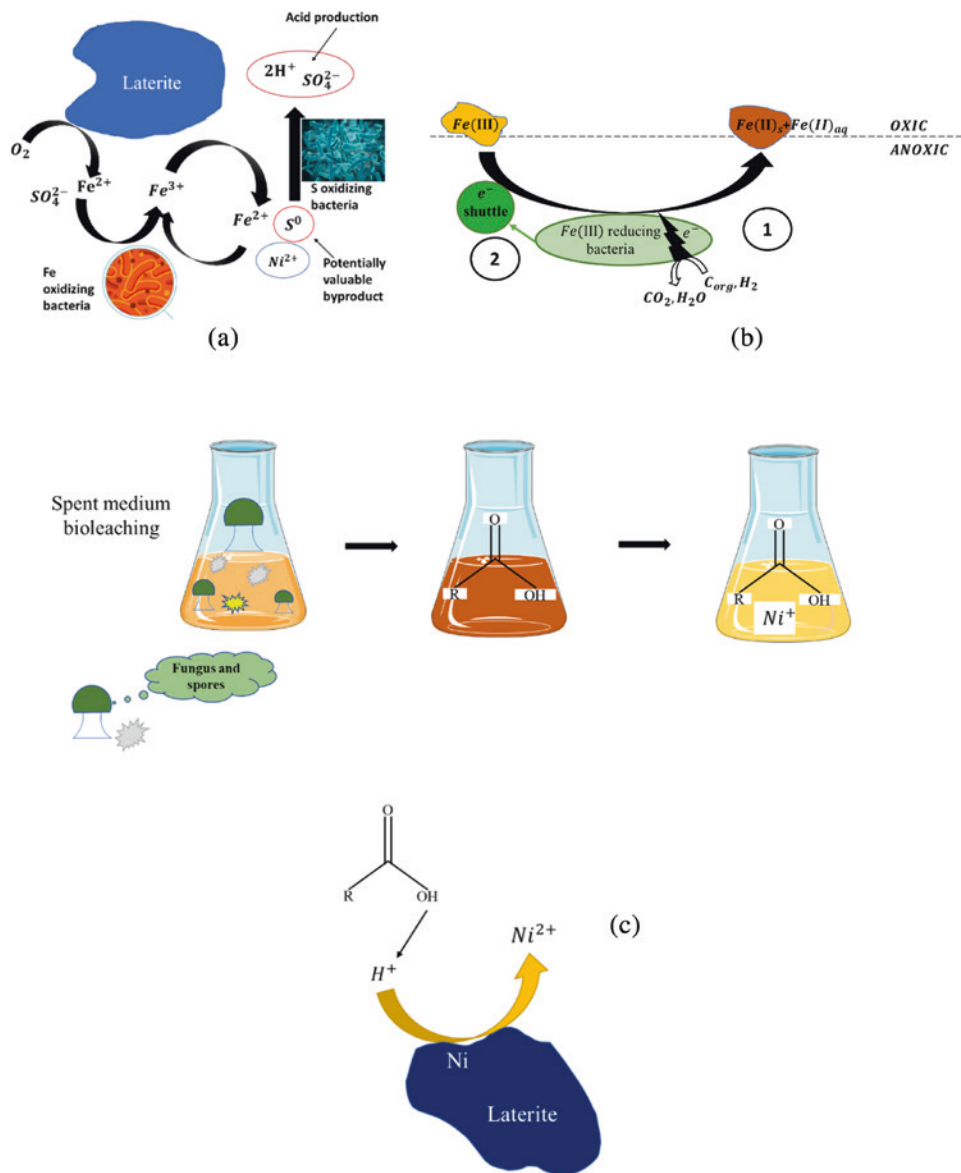
7 Conclusions

Bioleaching is mostly used in the rehabilitation of the environment and the recovery of valuable metals. Researchers are interested in metal recovery from mineral ores, metal concentrates, and mine tailings. According to the research on the biological dissolution (bacterial and fungal leaching) of laterites, it can be concluded that the most parameters affecting the bioleaching process of nickel and cobalt from the laterites include the effect of ultrasonic waves, the salinity of the culture media, and growth medium, density, pH, particle size, and species type of microorganism. The optimization of these parameters will substantially aid in increasing nickel and cobalt dissolving rates and recovery from nickel-bearing laterites. Among the studied parameters, the change of species type has attracted more attention compared to other parameters. Heterotrophic fungi and bacteria can react with laterite. New studies have focused on indirect bioleaching by metabolites produced

Table 5 Summary of results of previous studies on bioleaching of lateritic ore

Studied subject	Result	References
Fungal bioleaching	Chemical dissolution was shown to be less successful than biological dissolution	Valix et al. (2001)
Fungi metabolic acids leach low-grade nickel ores	Cobalt is more soluble in limonite than nickel	Tang and Valix (2004)
Chemolithotrophic bacteria are used in bioleaching (<i>Acidithiobacillus ferrooxidans</i>)	Nickel extraction is reduced by increasing pulp density	Doshi and Mishra (2007)
Statistical design of experiments for bacterial leaching	The maximum recovery was found when the pH and the pulp density were low and the particles were large	Simate and Ndlovu (2007)
Nickel and cobalt biological leaching	The highest Ni and Co leaching efficiencies were achieved using a consortium primarily composed of <i>At. ferrooxidans</i>	Mohapatra et al. (2009b)
Bioleaching with halotolerant <i>Aspergillus foetidus</i>	The presence of salt slows down microbial metabolism, produces less bio-acid, and reduces the conversion of sucrose to bio-acid	Valix et al. (2009)
A new technique for bio-processing nickel laterites	Bioleaching of Ni laterites by <i>At. ferrooxidans</i> . Leaching was carried out under anaerobic circumstances. Over 70% of the nickel in the ore had been dissolved	Hallberg et al. (2011)
Bioleaching with <i>Aspergillus niger</i> filtrated culture	An oxalic acid concentration of 87% was found in the filtrate of a 21-day culture at a pulp density of 2% at 80 °C, while 67% of Ni and 65% of Co were recovered from roasted ore samples	Biswas et al. (2013a)
<i>Aspergillus niger</i> was used to leach chromite-bearing laterite overburden	Bioleaching recovered 65% Ni and 59% Co from the roasted ore, while oxalic acid brought back 26.87% Ni and 31.3% Co	Biswas et al. (2013b)
Biological leaching by mixotrophic bacteria	Nickel and iron were present in greater quantities in limonite ore (30% Ni and 5.6% Fe) than the biocontrol (1% Ni and 0.1%)	Chaerun et al. (2016)
Direct bioleaching uses the fungi's metabolic organic acids generated by <i>Aspergillus niger</i>	The rate of synthesis was slower for starch at pH = 1.4 than molasses after 16 days, despite producing more organic acids	Chaerun et al. (2017)
To overcome the bacteriostatic effects of heavy metals through direct bioleaching	The use of modified bacteria resulted in a 20% increase in nickel recovery and a 7% increase in cobalt recovery (<i>Acidithiobacillus thiooxidans</i>)	Jang and Valix (2017)
A lateritic ore is bioleached by mesophilic acidophile cultures and mixed cultures	A mixed culture of mesophilic acidophiles recovered 97% of Ni and 95% of Co	Ciftci and Atik (2017)
Chemical and bioleaching effects on laterite surface area	The maximum Ni recovery of 67% was achieved by adjusting citric acid content, surface area, and particle size	Pawłowska and Sadowski (2017)
Laterite is chemically and biologically leached by <i>Aspergillus niger</i>	For bio-catalytic processes containing 25% sucrose and 1% solids, nickel, cobalt, and iron recovered at 95, 3%, 74.3%, and 50%, respectively	Ciftci et al. (2018)
Redox cycling of manganese and cobalt in laterites	A new biological processing technique suggests treating laterites with an organic substrate to reduce the metal, followed by rinsing them with vinegar to remove cobalt	Newsome et al. (2020)
Using the metabolic carboxylic acid produced by <i>Aspergillus niger</i> , <i>Penicillium bilaia</i> , <i>Pseudomonas putida</i> , and <i>Pseudomonas koreensis</i> , this study examined the indirect bioleaching of Co and Ni from laterite ore with iron	Gluconic acid was the most carboxylic acid in the supernatant of these bacteria and fungi. After 3 h at 90 °C, <i>Pseudomonas putida</i> supernatant had the maximum recovery of Ni and Co. Chemical control was more successful on the dissolution rate of Ni and Co from the examined laterite sample than diffusion control	Hosseini Nasab et al. (2021)
<i>Salimivibrio kushneri</i> supernatant metabolites are used in a two-step bioleaching process to remove nickel and copper from iron-rich laterite	After 3 h of bioleaching at 90 °C, the supernatant <i>Salimivibrio kushneri</i> , a halophilic bacterium, recovered 58.4% Ni and 60.6% Co from the lateritic sample	Hosseini Nasab et al. (2021)
Direct and indirect bioleaching of Co and Ni from iron-rich laterite ore	The acidophilic bacteria (<i>Deiftia acidovorans</i> and <i>Acidithiobacillus ferrooxidans</i>) were used to explore the bioleaching of nickel and cobalt from an iron-rich laterite material. The bioleaching of this sample was aided by the metabolic products of acidophilous bacteria	Hosseini Nasab et al. (2021)

Fig. 9 The mode of action for **a** iron-oxidizing and acidophile bacteria, **b** iron-reducing bacteria, and **c** fungus, in the bioleaching process of the laterites



from microorganisms then removing impurities such as iron from bioleaching solution to increase the recovery of nickel and cobalt from laterites. *Aspergillus* and *Penicillium* species are the most effective fungi for dissolving laterites. Metabolites of the *Delftia* genus, which is one of the nickel tolerant heterotrophic species, well recovered Ni and Co from studied laterite sample. In addition, *At. ferrooxidans* and *At. thiooxidans* are acidophilic autotrophic bacteria that mainly were used in different research. In general, biological dissolution was more effective compared with chemical dissolution by mineral and organic acids. Iron dissolution simultaneously with nickel and cobalt dissolution in laterites is a serious problem that needs the complicated process of iron precipitation. Finally, bioleaching, due to many advantages in comparison to traditional methods was

introduced as a new and promising technology for recovering valuable elements from laterites. However, still there is a weakness in the industrial development of this technology.

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