ORIGINAL ARTICLE

Enhancement of nickel laterite ore bioleaching by *Burkholderia* sp. using a factorial design

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Abstract

Interest in low-grade Ni-laterite ores has increased in recent years; however, the laterite process has proven technically difficult and costly, and the development of alternative low-cost biotechnologies for Ni solubilization has been encouraged. In this context, for the first time, a sample of Brazilian Ni-laterite ore was subjected to microbial bioleaching using a heterotrophic *Burkholderia* sp. strain. Experiments were performed in a 2^3 two-level full factorial design by determining the influence of glucose concentration (5–15%, w/v), Ni-laterite ore concentration (0.25–0.75%, w/v), and cultivation period (14–42 days) on Ni solubilization. The variable more important for Ni-laterite bioleaching was the glucose concentration (x_1). Bioleaching batch experiments demonstrated that about 87% Ni (7.5 mg Ni/g ore) were solubilized by *Burkholderia* sp. after 42 days. This study's significance is that it has opened up an opportunity for the potential application of potassium-solubilizing bacterial strains to process low-grade Ni-laterite ores.

Keywords Nickel laterite · Bioleaching · *Burkholderia* sp. · Factorial design · Organic acids

Introduction

Nickel (Ni) is a metal that has many industrial applications, nowadays mainly in lithium–nickel–cobalt–aluminum (LiNiCoAl) and lithium–nickel–manganese–cobalt (LiNiMnCo) batteries. A high demand for Ni is expected in the next twenty years due the electric vehicles' boom. Within this sector alone, the forecast global demand to increase by 2.6Mt Ni to 2040, up from only 92kt Ni in 2020 with an average price of approximately US$15,521/t (Fraser et al. 2021).

Ni is found in deposits of primary sulfides and laterite deposits, the latter corresponding to rather 60–70% of the known reserves of this metal in the world (Butt and Cluzel 2013; Mudd and Jowitt 2014). All nickel ores have relatively low Ni content, the classification is as follows: high-grade (Ni > 1.8%), middle-grade (Ni 1.3–1.7%), low-grade (Ni 0.6–1.2%). Ni is continuously mined, even at these low percentages of ores, because it is in high demand. The sulfide

ores are generally treated by pyrometallurgical methods due the possibility of enriching the Ni concentration by flotation. The lateritic ores are also best suited for pyrometallurgical processes, where the beneficiation steps of saprolite ores (high magnesium laterites) have been involving drying, calcining/reduction and electric furnace smelting to produce ferro-nickel or nickel (Meshram et al. 2019).

Ni-laterite ores are currently classified as silicate or oxide ores, and the Ni content in different Ni-laterite ores varies as shown in Table 1. Saprolite-type ores (silicates) are characterized by high magnesia and silica contents and are generally treated in pyrometallurgical processes (Luo et al. 2010). However, with an ever-increasing demand for Ni and daily depletion of high-grade Ni ore reserves, bacterial bioleaching processes have been developed aiming at Ni recovery from low-grade saprolite Ni-laterite ores (Chaerun et al. 2017).

New hydrometallurgical strategies for the Ni recovery from the low-grade lateritic ores has gained attention over the last years once the conventional hydrometallurgical, and pyrometallurgical techniques have certain disadvantages, such as poor product recovery, high costs of operation, high energy consumption and environmental incompatibility due to high pollution rates (Rohwerder et al. 2003; Giese 2019). These new process routes include

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Table 1 Nickel content in silicates and oxides minerals (Meshram et al. 2019)

Type of ores	Ni (%)
Çaldag lateritic nickel ore	1.22
Laterite ore Tubay region, Mindanao, Philippines	1.28
Lateritic nickel ore from mid-Anatolia region of Turkey	1.37
Nickel silicate ore, Rudinici	1.29
Limonitic, Indonesia	1.22
Chromite overburden	0.87
Saprolite ore	2.7
Sulawesi saprolitic ore	1.76
Halmahera saprolitic ore	1.28
Low-grade haematitic laterite ore	0.73
Garnieritic type	4.23
Lizardite	4.33
South African nickel laterite	0.02

nitric acid leaching, sulfation-roasting leaching, bioleaching, and the use of microwave and ultrasonics in the laterite leaching (Meshram et al. 2019).

Biohydrometallurgy is an interesting alternative that can be used to recover metals from lateritic ores on a massive scale, and it has already been utilized to recover metals from sulfide deposits (Watling 2006; Giese 2021). The majority of current Ni-laterite microbial leaching research is focused on the utilization of heterotrophic fungi, such as *Aspergillus* sp. and *Penicillium* sp. (Valix et al. 2001; Le et al. 2006) as well as the chemolithotrophic bacteria *Acidithiobacillus* sp. (Simate and Ndlovu 2007, 2008).

Higher Ni extraction rates were described for pretreated Indian chromite ($FeCr_2O_4$) Ni-laterite bioleaching using *Aspergillus niger* strain. Behera et al. (2011) obtained 18% of Ni bioleaching at 2.5% pulp-density within 25 days in shake flasks, while Mohapatra et al. (2009) obtained 30% of Ni extraction with an ore pulp density of 8.8% after 37.5 days. *A. niger* mut. *schiemanni* SUK101, the most potent isolate, leached 54.1% Ni, 29.4% Cr, 22.1% Fe and 25.1% Co, whereas *Pseudomonas* sp. SUK201 leached only 15% Ni, 6.01% Cr, 5.29% Fe and 4% Co for the same Indian chromite (Gosh and Paul 2017). For Ni recovery from a Turkish Ni-laterite ore using different *A. niger* strain, bioleaching experiments resulted in 95% of Ni extraction (Ciftci et al., 2018).

Giese et al. (2019) reported Ni extraction rates of 8% from a Brazilian saprolite magnesium-rich Ni-laterite after 7 days using *Bacillus subtilis* bacterial strain. Coto et al. (2008) have described leaching of 7% Ni extraction from a Cuban serpentine ($Mg_3Si_2O_5(OH)_4$) Ni-laterite after 6 days using *Aspergillus niger* fungus strain. Chaerun et al. (2017) have found similar results to an Indonesian saprolite (mainly kaolinite, $Al_2Si_2O_5(OH)_4$) sample; however, only after 28 days of incubation.

Studies on modeling and optimization to increase the efficiency of Ni-laterite bioleaching processes is very important to increase the metal extraction yields improving an existing hydrometallurgical process. Therefore, the objective of this study was to determine the conditions that maximize the Ni recovery from saprolite magnesium-rich Ni-laterite by the action of heterotrophic bacteria *Burkholderia* sp. Among the heterotrophic bacteria, members of the genus *Burkholderia* have been found most effective in the leaching of potassium and phosphorus from rock minerals (Baghel et al. 2020), phosphorus from iron ore (Delvasto et al. 2009; Silva et al. 2022) and rare-earth elements from hydroxy apatite (Fathollahzadeh et al. 2018). These bacteria have been described as producing gluconic and acetic acids, siderophore, hydrogen cyanide, and exopolysacharides (Stephen and Jisha 2011; Fathollahzadeh et al. 2018), which are excreted into the culture medium and dissolve heavy metals and lanthanides by direct displacement of metal ions from the ore matrix by hydrogen ions and by the formation of soluble metal complexes and chelates (Boseckerk 1997).

Gluconic acid production has been described as the principal mechanism of mineral phosphate solubilization by *Burkholderia* sp. Although the mechanism of Ni-laterite bioleaching by biogenic organic acids is yet unknown, it appears to be a viable alternative to using a strong acid in the process. Chemical leaching using citric, oxalic, and acetic acids, and a mixture of these acids, was used to evaluate the Ni recovery from a Ni-laterite ore. The maximum dissolution yields detected were Ni 19%, Co 6%, and Fe 56% in the presence of citric acid 100 mM (Ciftci et al. 2018). Citric, lactic, and malic acids were evaluated in the solubilization of limonite and nontronite ores. The authors observed that the extraction of Ni and Co depended more on the concentration of acid used than the type of acid (Tang and Valix 2004). In another comparative study, citric acid favored the solubilization of saprolite ore. In contrast, oxalic acid favored the solubilization of limonite ore (Le et al. 2006), which was also described as more useful for the recovery of metallic nickel garnierite (Sahu et al. 2011). For Indonesian saprolite, citric acid was very effective for dissolving Ni from serpentine, but did not recover Ni from goethite (Astuti et al. 2016). Thereby, in this paper, a series of chemical leaching experiments was conducted complementary to evaluate whether organic acids produced in the metabolism of carbohydrate media by fermentation pathways were also capable of solubilizing this Ni-laterite sample.

Experimental

Mineral sample

The sample of Brazilian Ni-laterite used in the present study consisted of SiO_2 46.4%, MgO 32.6%, Fe_2O_3 7.9%, Al_2O_3 1.7%, and NiO 1.0%, determined by X-ray fluorescence [18]. After crushing, grinding, and homogenization procedures, fractions smaller than 150 mm were separated to perform agitated flasks experiments. Previous X-ray diffraction analyses revealed that the sample consisted of serpentine $(Mg_6(Si_4O_{10})(OH)_8 \rightarrow (Mg,Fe^{2+},Ni)^{6-x}$ $[Si^{4-x}(Fe^{3+},Al,Cr)_xO_{10}](OH)_8))$, kaolinite $(Al_2Si_2O_5(OH)_4)$, quartz $(SiO_2))$ and lizardite $(Mg, Al)_3((Si, Fe)_2O_5)(OH)_4$. Peaks of goethite (FeOOH) and magnetite were also observed (Giese et al. 2019).

Bioleaching experiments in shaken flasks

Burkholderia sp. screened from soil (Cara et al. 2016) and belonging to Centro de Tecnologia Mineral (CETEM/MCTI) was stored at TSA (tryptone soy broth (TSB), 30 g/L; and agar, 20 g/L) at 4 °C. Microorganism was transferred to Petri dishes containing TSA-YE medium (yeast extract, 5 g/L), which were incubated for 24 h in a bacteriological stove. After this period, a loopful from Petri dishes were used to inoculate 500 mL Erlenmeyer flasks containing 200 mL of liquid TSB-YE medium (yeast extract, 5 g/L; TSB, 30 g/L) during 48 h, 150 rpm at 30 °C.

The bioleaching experiments were conducted in 500 mL Erlenmeyer flasks containing 2 mL of the inoculum described above, 10 mL of 10% (w/v) glucose, Ni-laterite ore 0.25 or 0.75% (w/v) and 90 mL of the liquid medium containing KH_2PO_4, 0.5 g/L; $MgCl_26H_2O$, 0.5 g/L; $MgSO_4.7H_2O$, 0.2 g/L; KCl 0.2 g/L, and $(NH_4)_2SO_4$, 0.1 g/L; and adjust the pH to 7.0 with 1 M NaOH. The liquid medium containing the Ni-laterite ore and glucose solution was sterilized separately by autoclaving for 20 min at 1 atm. The cultures were kept under constant stirring at 100 rpm, 30 °C for 14 or 42 days. All experiments were performed in replicates of 3, and the results represent the mean value ± SD. All analyses were compared with a control test without the addition of inoculum (abiotic control). Determination of the final concentration of metal ions in solution was performed by inductively-coupled plasma atomic emission spectrometry (ICP-OES Perkin Elmer, OPTIMA3000, USA).

The Ni bioleaching extraction was taken as an index and calculated using the following Eq. 1:

$$Ni(\%) = \frac{C_2}{C_1} \times 100\% \tag{1}$$

where the Ni bioleaching extraction (%), C_1 is the Ni content in the Ni-laterite ore in initial solution (g/mL), and C_2 is the Ni concentration in leachate (g/mL).

Optimization for bioleaching conditions using factorial design

Bioleaching conditions for the Ni-laterite solubilization by *Burkholderia* sp. strain were studied as variables in a 2^3 two-level full factorial design. The independent variables were $x_1 =$ glucose concentration, $x_2 =$ Ni-laterite ore concentration and $x_3 =$ time of incubation. The level of these variables and the various levels for experimental studies on Ni extraction ($Y_1 =$ Ni %, w/v) are shown in Table 1. Analysis of variance (ANOVA) and multiple regression analyses were performed using STATISTICA 13.2.

Chemical leaching experiments

Organic acid leaching assays were conducted for comparison purposes. Leaching assays were conducted in 500 mL Erlenmeyer flasks containing 1 g of Ni-laterite ore in 100 mL of 50% acid solution (v/v) of acetic, citric, or lactic acids separately. The tests were performed duplicated under constant agitation at 100 rpm, 30 °C for 7 days. All experiments were performed in replicates of 3, and the results represent the mean value ± SD. Determination of the final concentration of metal ions in solution was performed by inductively-coupled plasma atomic emission spectrometry (ICP-OES Perkin Elmer, OPTIMA3000, USA).

Results and discussion

In the present study, a heterotrophic *Burkholderia* sp. bacterium was evaluated for the dissolution of Ni-laterite ore. This strain was recently evaluated as a potential bacterial strain as potassium solubilizer from verdete ore (Schueler et al. 2021).

Table 2 shows the Ni-laterite bioleaching results under not-optimized conditions. The flasks containing *Burkholderia* sp. cells presented amounts of 39.7% of Ni in the leachate solution, concomitantly to small amounts of Si, Al, Fe, and Cr. In previous studies, it was identified that initial pH, particle size, pulp density, and substrate type were statistically significant operating parameters, while microbial inoculum size was not statistically significant in the recovery of Ni from Ni-laterites (Simate and Ndlovu 2007, 2008). Furthermore, to study the effect of important factors: $x_1 =$ glucose concentration, $x_2 =$ Ni-laterite ore concentration and $x_3 =$ time of contact in Ni-laterite bioleaching by *Burkholderia* sp., and their interaction on Ni-laterite bioleaching, a 2^3 two-level, full factorial design was used. Through

Table 2 2^3 Two-level full factorial design table defining conditions for Ni-laterite bioleaching by *Burkholderia* sp

Run Nº	x_1	x_2	x_3	Ni extraction (%, w/v)
1	-1	-1	-1	9.67
2	-1	-1	$+1$	19.86
3	-1	$+1$	-1	4.41
4	-1	$+1$	$+1$	7.47
5	$+1$	-1	-1	62.63
6	$+1$	-1	$+1$	87.07
7	$+1$	$+1$	-1	46.67
8	$+1$	$+1$	$+1$	48.19
Factors				
	-1	$+1$		
x_1, glucose (%, w/v)	5	15		
x_2, Ni-laterite ore (%, w/v)	0.25	0.75		
x_3, time of contact (days)	14	42		

multiple regression analysis of the experimental data, a first-order polynomial equation was obtained (Eq. 2):

$$\widehat{Y}_1 = 35.74625 + 25.39375x_1 \tag{2}$$

Effect terms of the variables x_2 and x_3 and its interactions as well as the interactions between variable x_1 and variables x_2 and x_3 were discarded as being non-significant as showed in the analysis of variance (ANOVA) (Table 2). The variable more important for Ni-laterite bioleaching by *Burkholderia* sp. was the glucose concentration (x_1). ANOVA showed the lack-of-fit ($p > 0.05$) was not significant, indicating that the model was predictive. The experimental and predicted Ni extractions were in agreement.

The R-squared value implies 98.78% of the variability in the observed response values can be explained by the model, or by experimental factors and their interactions. The pure error was low, indicating good reproducibility of the experimental data. Mohapatra et al. (2009) has successfully employed statistical analysis by the response surface methodology to optimize the Ni extraction by *Aspergillus niger* from, and combined the effects of sucrose concentration, pulp density, temperature and duration of the bioleaching. The authors found significant interaction between temperature and duration as well as sucrose and pulp density. Like *Burkholderia* sp. in the present study, *A. niger* was found to be efficient in extracting nickel at high sugar concentration in the medium because it serves as the energy source in the ore as well as for the strain.

According to our experimental data, maximum Ni extraction yields (87%) were obtained using glucose 15% (w/v); Ni-laterite ore 0.25% (w/v) during 42 days of incubation. The analysis of 3-dimensional surface (Fig. 1) shows that

highest glucose concentrations (x_1) had a tendency to enhance the Ni extraction despite ore concentration (x_2, Fig. 1a) or time of incubation (x_3, Fig. 1b).

To our knowledge, this is the first article reporting the use of *Burkholderia* sp. for Ni-laterite bioleaching. The results obtained could suggest that the Ni extraction rates were strongly influenced by the levels of carbon source present in the bioleaching system, once these are important for organic acids production. Stephen and Jisha (2011) reported that gluconic acid production as the principal mechanism of mineral phosphate solubilization by *Burkholderia* sp. (MTCC 8369). In this case, low molecular weight organic acids, mainly gluconic and ketogluconic acids are responsible for tricalcium phosphate and rock phosphate solubilization (Kumari et al. 2008).

During the heterotrophic bioleaching process, metals' leaching depends on different metabolites produced by the microorganism during its growth, such as organic acids, amino acids, exopolysaccharides, proteins, e.g., Organic acids provide ligands and protons, important in oxide reduction and complexation reactions (Saidan et al. 2012). The chemical leaching of Ni-laterite ores employing a range of organic acids (viz. microorganism metabolites) and admixtures with sulfuric acid has been examined (Tzeferis 1994). In terms of types of minerals, it has been found that silicates, saprolites, and garnierite ($(Ni, Mg)SiO_3 \cdot nH_2O$) leach more easily than limonites, which constitute oxides of the type $(Fe, Ni)O(OH) \cdot nH_2O$ containing mainly goethite; silicate clays (nontronite) are the most difficult to disrupt (Simate et al. 2010) (Table 3).

In the present study, chemical leaching experiments showed that hydroxycarboxylic acids could dissolve Ni

Fig. 1 Response surface plot showing the effect of Ni extraction (Y_1) as a function of **a** glucose (x_1) and Ni-laterite ore (x_2), at a fixed time of contact ($x_3 = 14$ days); and **b** glucose (x_1) and time of contact (x_3), at fixed Ni-laterite ore concentration ($x_2 = 0.75\%$, w/v) according to a 2^3 two-level full factorial design
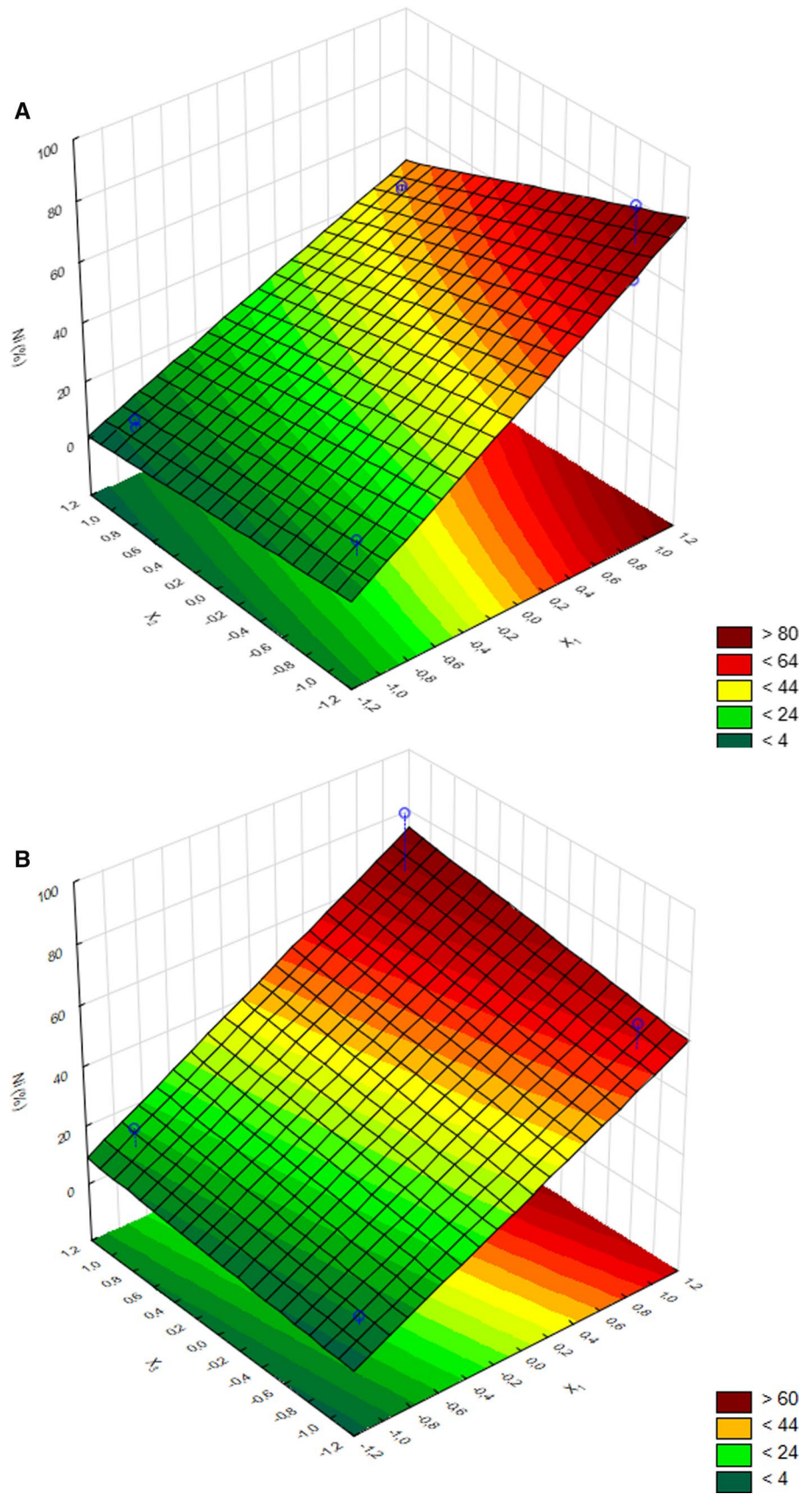
Table 3 Bioleaching experiments of Ni-laterite (0.25%, w/v) by *Burkholderia* sp. after 14 days

	Metal extraction (%)				
	Ni	Si	Al	Fe	Cr
% Total	39.71 ± 1.56	10.18 ± 0.23	6.94 ± 0.80	6.95 ± 0.47	1.85 ± 0.18
% Abiotic (control)	2.20	2.29	0	0.09	0
% Biotic (*Burkholderia* sp. presence)	37.52 ± 1.56	7.89 ± 0.23	6.94 ± 0.80	6.86 ± 0.47	1.85 ± 0.18

Table 4 ANOVA data for the Ni-laterite bioleaching by *Burkholderia* sp., and test of significance for the regression coefficients

Source of variation	Sum of squares	Degrees of freedom	F-test	p
Glucose, x_1	5158.740	1	165.5272	0.049383
Ni-laterite ore, x_2	656.850	1	21.0762	0.136538
Time of contact, x_3	192.178	1	6.1664	0.243720
$x_1 x_2$	172.887	1	5.5474	0.255610
$x_1 x_3$	20.193	1	0.6479	0.568534
$x_2 x_3$	112.875	1	3.6218	0.308000
Pure error	31.166	1		
Total	6344.889	7		

% of explained variance = 99.509

% of maximum explained variance = 96.562

from a Brazilian saprolite magnesium-rich Ni-laterite ore. According to Table 4, the Ni extraction (%) rates decreased according organic acid molecular weight: citric acid (68%) > lactic acid (23%) > acetic acid (10%). Ni-laterite chemical leaching kinetics were slow, especially at room temperatures requiring more than 20 days to reach equilibrium (Tzeferis 1994).

Citric acid, a well-established product of fungal metabolism, was the most effective organic solubilizer promoting the extraction of about Ni 68%, presumably facilitating Ni dissolution by chelation. The leaching rates in the experiments with acetic and lactic acids were much lower than citric acid under comparable conditions. It seems like higher molecular weight organic acids are responsible for silicate solubilization: citric ($C_6H_8O_7$, 192.1 g/mol) > lactic ($C_3H_6O_3$, 90.1 g/mol) > acetic (CH_3COOH, 60.1 g/mol), unlike what happens for rock phosphate solubilization. The same behavior was observed for the Greek "Kastoria" garnierite laterite ore chemical leaching, for which the Ni leaching efficiencies varying from 36% to as low as 1.5%, and the order of acid solubilization was acetic < lactic < citric acids (Tzeferis and Agatzini-Leonardou 1994).

Strength of organic acids used in chemical leaching is important in the solubilization of Ni-laterites (Kursunoglu and Kaya 2015). Strength of organic acids is generally based on several factors including relative stability of the conjugate base of the acid and other groups (–OH, –OS, enol, alcohol, thiol and phenol) and is determined by corresponding dissociation constant (pKa). The pKa is the pH in which acid and anion concentrations are equal, thus, the lower the pKa, the more strength acid (Theron 2011; Souza and Giese 2021, 2022). Acetic acid exhibited very low (< 10%) leaching efficiencies for the metals because of the acetic acid dissociation constant. The pKa values of the organic acids tested decrease in the order citric acid (pKa = 4.76) > lactic acid (pKa = 3.85) > citric acid (pKa = 3.15). The pKa values of the three acids tested indicate that citric acid is a stronger acid than lactic acid and acetic acid (Fig. 2).

Ni recovery from a Ni-laterite ore from Manisa (Turkey) was investigated through chemical leaching using citric, oxalic, and acetic acids and a mixture of these acids. The maximum dissolution yields detected were Ni 19%, Co 6%, and Fe 56% in the presence of citric acid 100 mM. Acetic acid resulted in the lowest leaching values, which corresponded to Ni 3%, Co 2%, and Fe 0.1% (Ciftci et al. 2018). Citric, lactic, and malic acids were evaluated in the solubilization of limonite and nontronite ores. The authors observed that the extraction of Ni and Co depended more on the concentration of acid used than the type of acid (Tang and Valix 2004). In another comparative study, citric acid favored the solubilization of saprolite ore. In contrast, oxalic acid favored the solubilization of limonite ore (Le et al. 2006), which was also described as more useful for the recovery of metallic nickel garnierite (Sahu et al. 2011). For Indonesian saprolite, citric acid was very effective for dissolving Ni from serpentine, but did not recover Ni from goethite (Astuti et al. 2016).

In comparison to chemical leaching, *Burkholderia* sp. strain showed more favorable Ni extraction from saprolite Ni-laterite (Table 2), which does not mimic to the organic acids leaching reported in Table 4. Furthermore, the metabolic bio-acid appears to solubilized Ni more effectively, which was demonstrated by the high bacterial selective capacity in recovery Ni (39.71%) than Fe (6.95%), Al (6.94%). For citric acid, e.g., higher amounts of Ni were extracted (67.64%), however, similar amounts of Fe (68.59%) were extracted during the process, which is not required (Table 5).

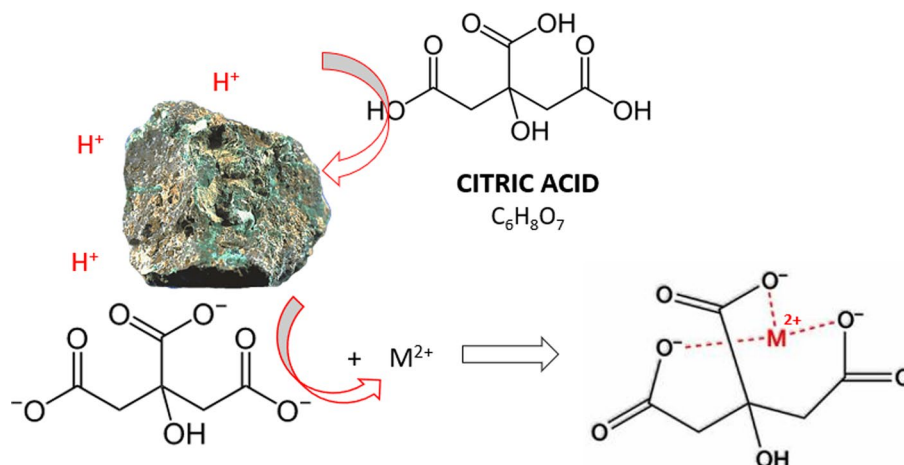**Fig. 2** Proposed mechanism for citric acid leaching and chelating reactions

Table 5 Chemical leaching of Ni-laterite (0.25%, w/v) under different acidic solutions (50%, v/v) during 7 days

Acids	Metal extraction (%)					
	Ni	Si	Al	Fe	Mg	Cr
$C_6H_8O_7$ (citric), pKa 3.13	67.64	20.59	26.01	68.59	14.83	4.50
$C_3H_6O_3$ (lactic), pKa 3.83	22.97	12,56	10.00	13.57	4.60	N.d
CH_3COOH (acetic), pKa 4.76	9.80	3.74	N.d	N.d	2.23	N.d

N.D. not detected

In conclusion, *Burkholderia* sp. was more selective for Ni solubilization than citric acid, dissolving Ni at higher rates than Fe, Si, Al and Cr. Despite the free hydrogen ion action and the formation of Ni-citrate soluble complexes; obtained results suggests that the Ni-laterite bioleaching process does not result simply from the chemical leaching from the metabolic acids secreted by *Burkholderia* sp., but that the bacteria appear to participate in the leaching process.

Availability of data and materials Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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