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ORIGINAL ARTICLE

Influence of organic acids on pentlandite bioleaching by *Acidithiobacillus ferrooxidans* LR

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Abstract
Bioleaching is a bio-hydrometallurgical process of solubilizing metals from low-grade sulfide ores by microbial action employing chemolithoautotrophic microorganisms capable of promoting redox reactions. Organic acids have been applied in bioleaching once can act in two different ways; providing hydrogen ions for mineral acidolysis and complexing metals due to their chelating capacity. This study investigates the synergy of different organic acids (acetic, ascorbic, citric, lactic, and oxalic) and *Acidithiobacillus ferrooxidans* LR pentlandite bioleaching. The addition of oxalic acid had a positive effect on the Ni extraction during pentlandite biological–chemical leaching after 15 days, and the yields observed were 45.6% (26.5 mg Ni/g ore). The yields for Co extraction were meager, and Co extraction values were found to only 2.8% (1.6 mg Co/g ore) in the presence of citric acid. A design of experiments with mixtures was used to evaluate the interaction of organic acids in bioleaching. According to simplex-centroid experiments, only citric acid presented a statistically significant effect and has contributed in a synergistic form in pentlandite biological–chemical leaching in the presence of *A. ferrooxidans.* This study provides a new synergistic bioleaching system to improve Ni and Co extraction from sulfide ores.

Keywords Bioleaching · Pentlandite · *Acidithiobacillus ferrooxidans* · Simplex-Centroid Design

Introduction

Cobalt is one of the most important heavy metal used in the production of lithium-ion batteries for portable advices (mobile phones, laptops, tablets, cutting tools), E-mobility (electric vehicles and hybrid electric vehicles, electric trains, electric bikes) and renewable energy power stations and home storage, ancillary services to the electrical grid. Nickel is also employed in lithium-nickel–cobalt-aluminum and lithium-nickel-manganese-cobalt batteries for the same purposes. With growing demand driven continuously by electric vehicles' boom, an annual increase in cobalt and nickel commodities is estimated (Castro et al. 2013; Giese, 2017a, b).

Nickel usually occurs in nature as sulphide or oxide forms, and its concentrates commonly contain different proportions of iron sulfides (pyrite and pyrrhotite) and Ni (pentlandite).

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The nickel extraction from low-grade ores has been conducted in the biohydrometallurgical process employing chemolithoautotrophic microorganisms capable of promoting redox reactions and solubilizing the metal of interest. The proposed reactions for pentlandite microbial dissolution are as follows (Eqs 1, 2, 3, 4) (Giese and Vaz, 2015):

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

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

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

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

Bioleaching of minerals is a process that allows the treatment of insoluble sulfides and insoluble oxides via hydrometallurgy. Bacterial leaching is based on the ability of acidophilic microorganisms, such as *Acidithiobacillus, Leptospirillum, Sulfobacillus, Sulfolobus*, and *Acidianus*, to oxidize ferrous ions (Fe^{2+}) with the regeneration of ferric ions (Fe^{3+}) and/or reduced sulfur species with the consequent extraction of the metal of interest (Watling 2008; Schippers et al. 2014). In the bioleaching of primary nickel ore, the leaching bacteria perform the dissolution of the pentlandite ($(NiFe)_9S_8$), making it possible to obtain metallic nickel (Giese and Vaz 2015; Giese 2017a, b).

In addition to the chemolithotrophic bacteria strains, heterotrophic species have also been used in bioleaching processes (Castro et al. 2000; Valix et al. 2001). These microorganisms produce organic acids that act to dissolve minerals such as silicates (Castro et al. 2000) and laterites (Giese et al. 2019), e.g., as described in Table 1. Organic acids have the advantage of being readily biodegradable, potentially reducing the environmental impact of processes using them. Organic acids act in two different ways; providing hydrogen ions for mineral acidolysis and complexing metals due to their chelating capacity (Zhiqing et al. 2019).

The use of heterotrophic microorganisms in sulfide bioleaching is not expected due to ore mineralogy, which favors the action of autotrophic microorganisms that depend on sulfur and iron as an energy source (Yin et al. 2020). In a comparative study, the recovery of nickel from a low-grade chromite overburden was attempted by employing two fungal strains and a mixed mesophilic acidophiles culture. It was found that the *A. ferrooxidans* culture solubilized nickel more effectively than fungal strains (Mohapatra et al. 2007).

Use both heterotrophic and acidophilic cultures in synergism is difficult because only a few cultures can grow in the presence of carbon sources. The use of synergy among different bacteria in sulfide bioleaching has been described in the literature for improving metal extraction yields. In copper bioleaching from chalcopyrite, e.g., the heterotrophic *Rhizobium phaseoli* have metabolized polysaccharides from *A. ferrooxidans*, and organic acids could also damage the mineral lattice to increase the copper leaching effect (Zheng and Li 2016). The synergistic effects between A. *ferrooxidans* and heterotrophic *Pseudomonas aeruginosa* could improve the leaching rate of heavy metal from contaminated sediments (Zhu and Zhang 2014).

Thiobacillus species have been described as growing on many organic acids, pentoses, hexoses, and α-linked disaccharides, but not on β-linked disaccharides galactosides (Wood and Kelly 1977). However, constant concentrations of organic acids could retard iron oxidation by the cells, negatively compromising the efficiency of bioleaching (Tuttle et al. 1977). Recently, some authors have been described that low-molecular organic acids such as formic, acetic, propionic, and butyric can inhibit the biological activity of *Acidithiobacillus* species (Song et al. 2016). The presence of organic acids as oxalic, malic, and citric appears to be more tolerable for *A. ferrooxidans* (Ren et al. 2019).

Few researchers have studied the assistance of microbial organic acid producers in the sulfide bioleaching process. In this paper, we report the influence of the addition of different organic acids (acetic, ascorbic, citric, lactic, and oxalic) during the pentlandite bioleaching by *A. ferrooxidans* LR. As highest Ni and Co extraction yields resulted from bioleaching under the addition of oxalic, citric, and lactic acids, we herein developed a mixture-design experiment using mixtures of these organic acids to evaluate the best condition to bioleaching process of pentlandite primary nickel ore.

Experimental

Mineral sample

A primary nickel ore, kindly provided by Mineração Serra da Fortaleza (Grupo Votorantim, Brazil), was studied. This ore sample is composed of (w w^{-1}) 0.29% Ni, 11.9% Fe and 0.002% Co presenting pentlandite [$(NiFe)_9S_8$] as the primary sulfide phase (Giese and Vaz 2015).

Table 1 Examples of heterotrophic microorganisms that produce organic acids used in the bioleaching process

Mineral type	Metal value	Microorganism	References
Silicates	Zn and Ni	*Aspergillus niger*	Castro et al. (2000)
Low-grade chromite overburden	Ni	*Aspergillus niger* and *Aspergillus fumigatus*	Mohapatra et al. (2007)
Chalcopyrite	Cu	*Rhizobium phaseoli* + *A. ferrooxidans*	Zheng and Li (2016)
Contaminated sediments	Zn, Mn, Cu, and Cd	*Pseudomonas aeruginosa* + *A. ferrooxidans*	Zhu and Zhang (2014)
Sulfide concentrate	Ni and Co	*Bacillus megaterium* QM B1551	Cui et al. (2018)
Laterite	Ni	*Bacillus subtilis*	Giese et al. (2019)
Monazite	Rare-earth elements	*Enterobacter aerogenes*	Fathollahzadeh et al. (2019)
Lepidolite	Li	*Aspergillus niger* and *Rhodotorula mucilaginosa*	Sedlakova-Kadukova et al. (2020)
Bauxite	Rare-earth elements	*Aspergillus* sp.	Barnett et al. (2020)

Microorganism and bioleaching experiments

Acidithiobacillus ferrooxidans strain LR was initially isolated from uranium mine effluents in Brazil (Garcia 1991). The bioleaching experiments were carried out in 500 mL Erlenmeyer flasks containing 180 mL of 4 × diluted MKM medium (Olson et al. 2003) containing (g L^{-1}) 0.08 $(NH_4)_2SO_4$, 0.08 $MgSO_4.7H_2O$ and 0.008 K_2HPO_4 at pH 1.8. The flasks were sterilized by autoclaving (20 min, 121 ºC) and inoculated with *A. ferrooxidans* LR (10%, v v^{-1}) after the addition of pentlandite (10%, w v^{-1}). Erlenmeyer flasks were incubated at 150 rpm and 30 ± 2 ºC for 15 days.

To evaluate the effect of the time-point of organic acids, at 0 and 7 days after inoculation, the following acids: acetic, ascorbic, citric, lactic, and oxalic were added to the growing cultures. To this end, 10 mL of 1 M organic acid solution was added to each test Erlenmeyer flask. The tests were compared with three control tests: i) MKM medium (abiotic control); ii) MKM medium + organic acids and (abiotic acid control) (iii) MKM medium + bacteria (inoculated control). The pH of all assay flasks was adjusted daily to 1.8 with the addition of 5 M H2SO4 solution drops. The evaporated volumes were controlled by the addition of new volumes of MKM medium autoclaved every 3 days. Analysis of dissolved Ni and Co was performed by atomic absorption spectrometry. The bioleaching experiments were carried out in duplicate, and the average results are presented.

The Ni and Co extraction were taken as an index and calculated using the following Eq. (5):

$$M(\%) = \frac{C_2}{C_1} \times 100\% \tag{5}$$

where the metal (M) bioleaching extraction (%), C_1 is the M content in the pentlandite in the initial solution (g mL^{-1}), and C_2 is the M concentration in leachate (g mL^{-1}).

Statistical experimental design

Conditions to optimize Ni and Co extraction from pentlandite in the presence of *A. ferrooxidans* LR were performed using a statistical mixture-design matrix with three components as organic acids in the formulation with seven experimental runs (Table 2). In a mixture experiment, the sum of the component fractions must be equal to unity, and their proportions must be non-negative. The restrictions on the levels of each factor are expressed as follows (Eq. 6):

$$\sum_{i=1}^{q} x_i = 1 \quad \text{(i.e., 100\%)} \tag{6}$$

where x_i represents the proportion of the ith component in the mixture, and q is the number of components. The independent variables in the mixture experiments for optimization of Ni extraction (Y_1, %) and Co extraction (Y_2, %) were: x_1 (oxalic acid, mol L^{-1}); x_2 (citric acid, mol L^{-1}), and x_3 (lactic acid, mol L^{-1}) as described in Table 1. Analysis of variance (ANOVA) and multiple regression analyses was performed using Statistica version 13.5 (StatSoft, Inc.).

Table 3 Physical and chemical characteristics of organic acids

	Formula	Molecular mass (g mol^{-1})	pKa_I	pKa_{II}	pKa_{III}
Citric acid	$C_6H_7O_8$	192.14	3.13	4.76	6.40
Ascorbic acid	$C_6H_8O_6$	176.09	4.17	11.6	
Lactic acid	$C_3H_6O_3$	90.08	3.83		
Oxalic acid	$C_2H_2O_4$	90.03	1.25	4.14	
Acetic acid	$C_2H_4O_2$	60.05	4.76		

Table 2 Statistical mixture-design matrix defining conditions for the addition of organic acids for Ni and Co extraction during pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR

Exp	Organic acid (M)			Ni extraction (Y_1) (%)	Co extraction (Y_2) (%)
	Oxalic acid (x_1)	Citric acid (x_2)	Lactic acid (x_3)		
1	0	0	1	77.2	4.2
2	1	0	0	38	7.8
3	0	1	0	107	6
4	0.5	0	0.5	53.5	2.9
5	0.5	0.5	0	55	3
6	0	0.5	0.5	31.4	1.7
7	0.33	0.33	0.33	43	2.2

Experimental conditions: inoculum (10%, v v^{-1}), pentlandite (10%, v v^{-1}), 150 rpm, 30 ºC, 15 days

Results and discussion

In the present paper, the influence of the addition of five organic acids (Table 3) on the bioleaching of pentlandite in shaken flasks in the presence of *A. ferrooxidans* LR bacterium was evaluated. There is wide-ranging variation in the optimal time-point at which inducing compounds should be added to the process to increase the bioextraction yields effectively. In our study, the time-point of organic acid addition resulted in essential differences in the amount of nickel (Fig. 1) and cobalt (Fig. 2) extracted during biological–chemical leaching experiments, once the addition of organic acids can be deleterious to Fe(II) oxidation and cell viability (Alexander et al. 1987).

According to Fig. 1a, it was observed that the addition of oxalic acid at the beginning of the bioleaching process, i.e., together with inoculation, positively influenced the solubilization of pentlandite, and Ni extraction values were found to 12.7% (7.4 mg Ni/g ore) and 45.6% (26.5 mg Ni/g ore) after 7 and 15 days of testing time, respectively. The addition of citric acid decreased the yield extraction at 2.4-fold, corresponding to 18.9% (10.9 mg Ni/ g ore) in 15 days of bioleaching. There was no influence of the addition of acetic, lactic, and ascorbic acids since the variation in the percentage of Ni extracted was quite similar between the treatments. On the other hand, as observed in Fig. 2a, the highest Ni extraction yields occurred to citric (38.7%, 22.5 mg Ni/g ore), acetic (31.1%, 18.0 mg Ni/g ore), and lactic (27.9%, 16.2 mg Ni/g ore) acids, only when acidic solutions were added to the bioleaching leachate at 7 days of the current

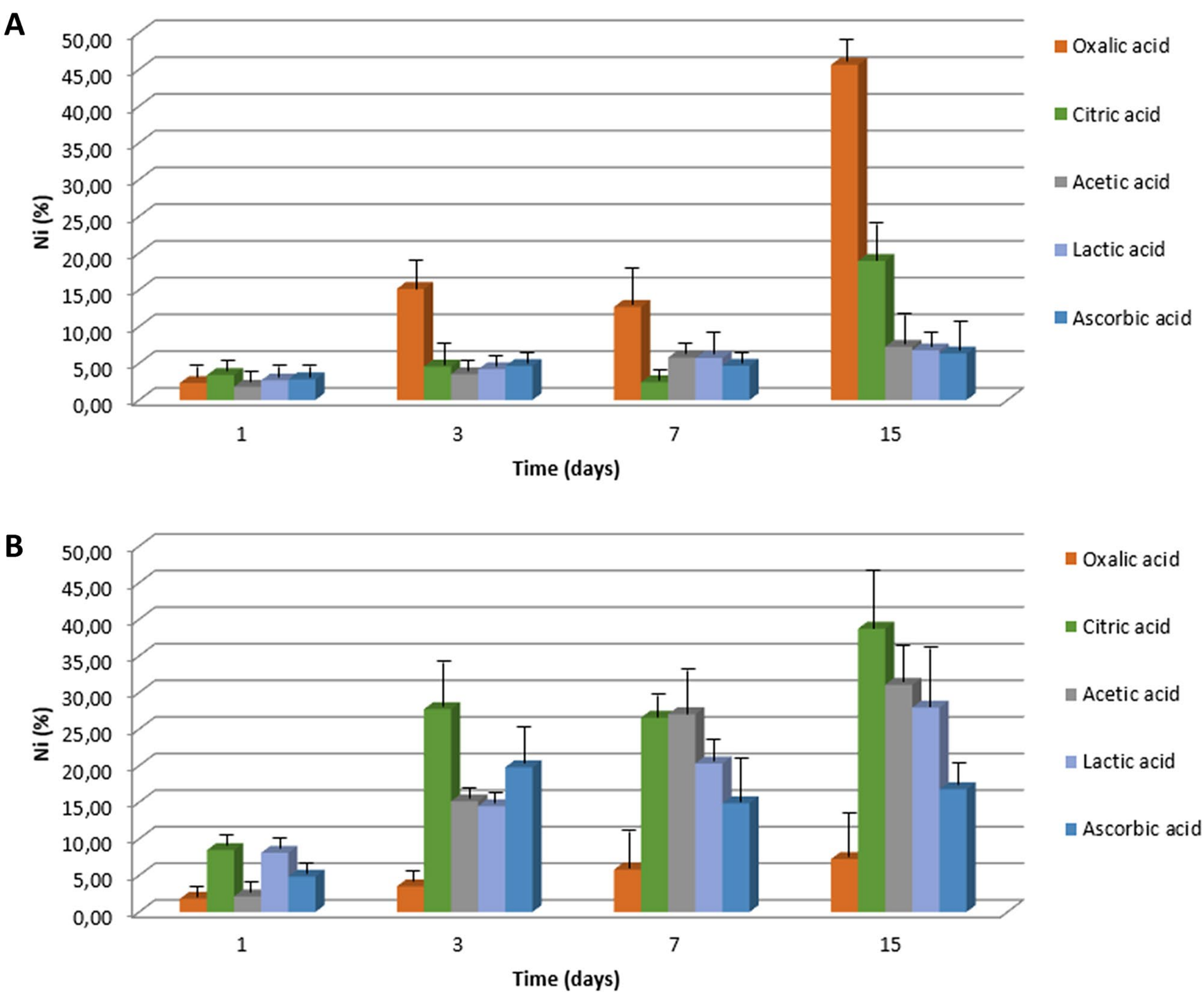

Fig. 1 Profile of Ni extraction from pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR in shaken flasks at time-point organic acids at 0 days (**a**) and 7 days (**b**). Experimental conditions: inoculum (10%, v v^{-1}), pentlandite (10%, v v^{-1}), organic acids (0.05 M) 150 rpm, 30 ºC

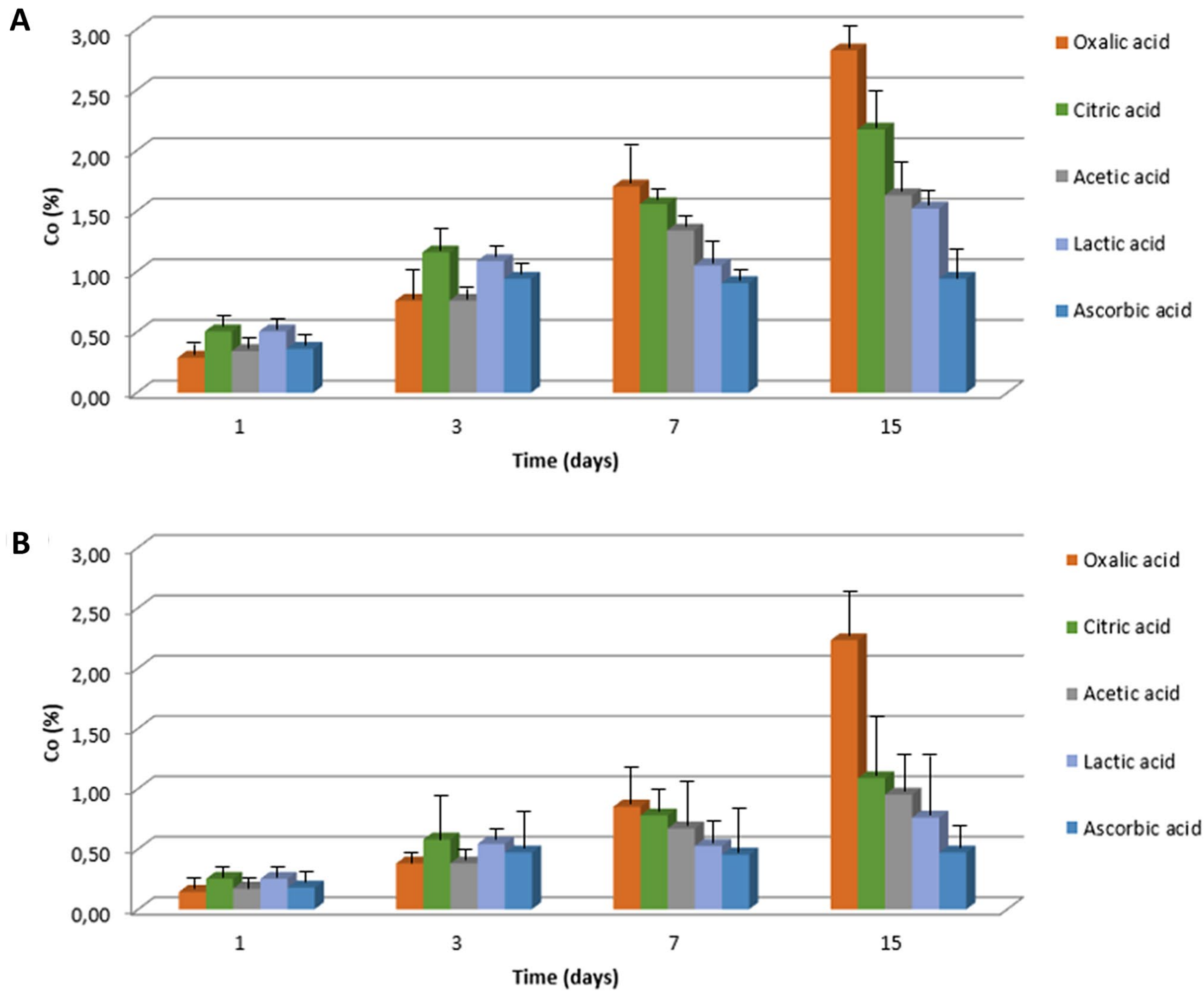

Fig. 2 Profile of Co extraction from pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR in shaken flasks at time-point of the addition of organic acids at 0 days (**a**) and 7 days (**b**). Experimental conditions: inoculum (10%, v v^{-1}), pentlandite (10%, v v^{-1}), organic acids (0.05 M) 150 rpm, 30 ºC

process. The yields for cobalt bioextraction were meager. According to Fig. 2a, it was observed that the addition of oxalic acid at the beginning of the bioleaching process also influenced the solubilization of pentlandite, and Co extraction values were found to 1.7% (0.9 mg Co/g ore) and 2.8% (1.6 mg Co/g ore) after 7 and 15 days of testing time, respectively. The control results showed in Table 4 indicated lower leaching yields of Ni and Co contents from pentlandite in the absence of synergistic action between *A. ferrooxidans* LR and organic acids.

A chelating effect was observed since the percentages of Ni extracted in the inoculated controls ranged from 45 to 55%. The organic acids are capable of forming complexes with metallic ions such as nickel (Zelenin 2007), so the amount of Ni ions in the leachate solution tends to decrease with the passage of contact time between the ore and the acid solution used in the process of bioleaching. Citric acid can react with Ni ions to form a soluble nickel-citrate ligand complex by chelating mechanism (Behera et al. 2010).

It is known that proton ions and ferric ions could promote the break and oxidation of Ni-S and Fe-S bonds in pentlandite, and it could be possible to solubilize pentlandite at a high pH value, inhibiting the dissolution of Fe^{3+} and Mg^{2+} from ore (Sun et al. 2020). Zhang and Fang (2005) have described a Ni extraction yields of 25% for pentlandite bioleaching at 30 ºC during 5 days using *A. ferrooxidans*, while only 8% of Ni was extracted under sulfuric acid leaching. Yang et al. (2008) observed an increase in Ni extractions yields from 15 to 65% when increased the temperature of

Table 4 Profile of Ni and Co extraction from control flasks experiments from pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR at time-point of the addition of organic acids at 0 days and 7 days

	Oxalic acid		Citric acid		Acetic acid		Lactic acid		Ascorbic acid	
	Ni (%)	Co (%)	Ni (%)	Co (%)	Ni (%)	Co (%)	Ni (%)	Co (%)	Ni (%)	Co (%)
Time-point acid addition of 0 days										
Control 1										
1 d	0.15	0.03	0.16	0.03	0.18	0.05	0.12	0.06	0.06	0.11
3 d	0.81	0.02	0.58	0.09	0.76	0.03	0.46	0.09	0.09	0.06
7 d	1.56	0.05	1.57	0.06	1.80	0.05	1.25	0.08	1.08	0.07
15 d	2.24	0.15	2.89	0.15	2.22	0.18	2.96	0.15	2.18	0.09
Control 2										
1 d	0.29	0.05	0.21	0.09	0.52	0.10	0.19	0.11	0.09	0.17
3 d	1.06	0.04	0.78	0.11	0.81	0.04	0.89	0.18	0.15	0.09
7 d	2.23	0.08	1.89	0.15	2.03	0.16	1.56	0.21	1.56	0.09
15 d	2.89	0.56	3.02	0.56	2.56	0.54	3.06	0.46	2.89	0.19
Control 3										
1 d	0.23	0.09	0.19	0.03	0.18	0.05	0.12	0.06	0.06	0.11
3 d	1.56	0.05	0.64	0.09	0.76	0.03	0.46	0.09	0.09	0.06
7 d	1.98	0.12	1.89	0.06	1.80	0.05	1.25	0.08	1.08	0.07
15 d	2.45	0.21	2.91	0.22	2.65	0.23	3.05	0.45	2.89	0.27
Time-point acid addition of 7 days										
Control 1										
1 d	0.17	0.05	0.16	0.07	0.23	0.11	0.18	0.28	0.34	0.35
3 d	0.89	0.06	0.58	0.18	1.21	0.09	0.87	0.29	0.65	0.19
7 d	1.51	0.09	1.57	0.11	2.21	0.14	1.83	0.27	1.98	0.27
15 d	2.15	0.22	3.06	0.56	2.89	0.84	3.19	0.68	2.89	0.14
Control 2										
1 d	0.78	0.45	0.21	0.23	0.98	0.14	0.23	0.22	0.25	0.22
3 d	1.06	0.04	0.78	0.11	0.81	0.04	0.89	0.18	0.15	0.15
7 d	2.23	0.08	1.89	0.15	2.03	0.16	1.56	0.21	1.56	0.18
15 d	2.89	0.56	3.02	0.56	2.56	0.54	3.06	0.46	2.89	0.22
Control 3										
1 d	0.56	0.10	0.14	0.05	0.73	0.08	0.22	0.11	0.42	0.22
3 d	1.89	0.08	0.75	0.08	0.45	0.04	0.86	0.18	0.15	0.15
7 d	2.02	0.19	1.86	0.08	1.91	0.08	1.35	0.16	1.65	0.19
15 d	2.91	0.28	2.98	0.27	2.88	0.24	3.11	0.21	3.23	0.35

30 to 70 ºC using biologically produced ferric sulfate as a leaching agent.

In this study, a simplex-centroid design technique was used to study the effects of organic acids and their mixtures on the Ni and Co extraction from pentlandite in a biological–chemical test. The response was obtained as a function of each component's proportions in the mixture (Table 2). Through multiple regression analysis of the experimental data, a first-order polynomial equation was obtained for Ni (Y_1) and Co (Y_2) extraction yields:

$$Y_1 = 93.29048x_2 \quad (7)$$

$$Y_2 = 5.224762x_2 \quad (8)$$

Effect terms of the variables x_1 (oxalic acid) and x_3 (lactic acid) were discarded as being non-significant, as showed in the analysis of variance (ANOVA). It can be observed that the effects of variable x_1 for Ni extraction were much higher than for Co extraction, indicating a more substantial influence of citric acid in Ni leaching. Table 5 summarizes the result obtained in the variance analysis (ANOVA) at the significance level of 95%. It was observed that the linear model presented the values of $F_{\text{calculated}} < F_{\text{tabled}}$. Thus, it can be stated that the models cannot be used for predictive purposes.

Figure 3 shows the Pareto diagram for the linear model for Ni (Fig. 3a) and Co (Fig. 3b) extraction from pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR. Pareto charts show the standardized effects, i.e., the effects divided by their respective standard

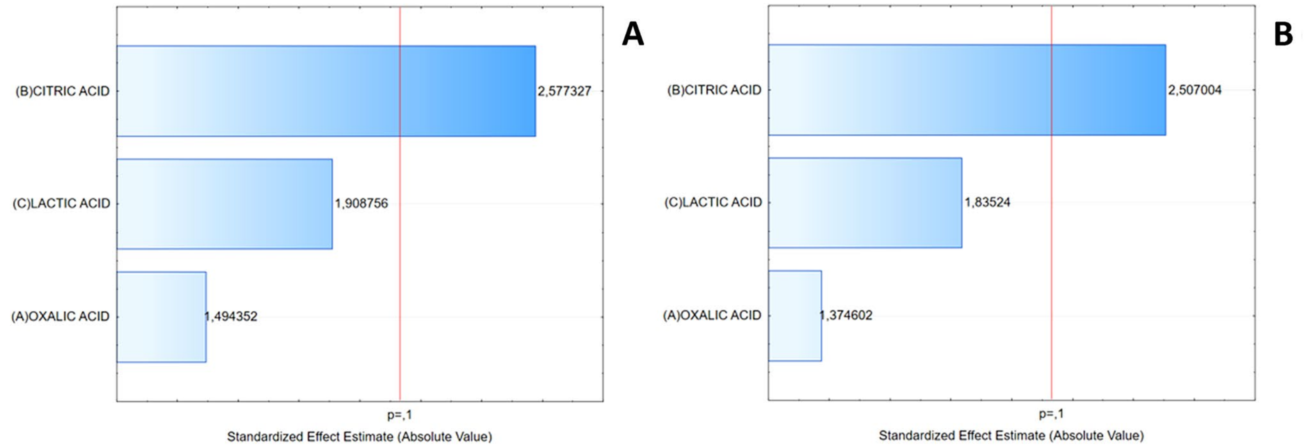

Fig. 3 Pareto charts of the estimated effects for Ni (**a**) and Co (**b**) extraction from pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR

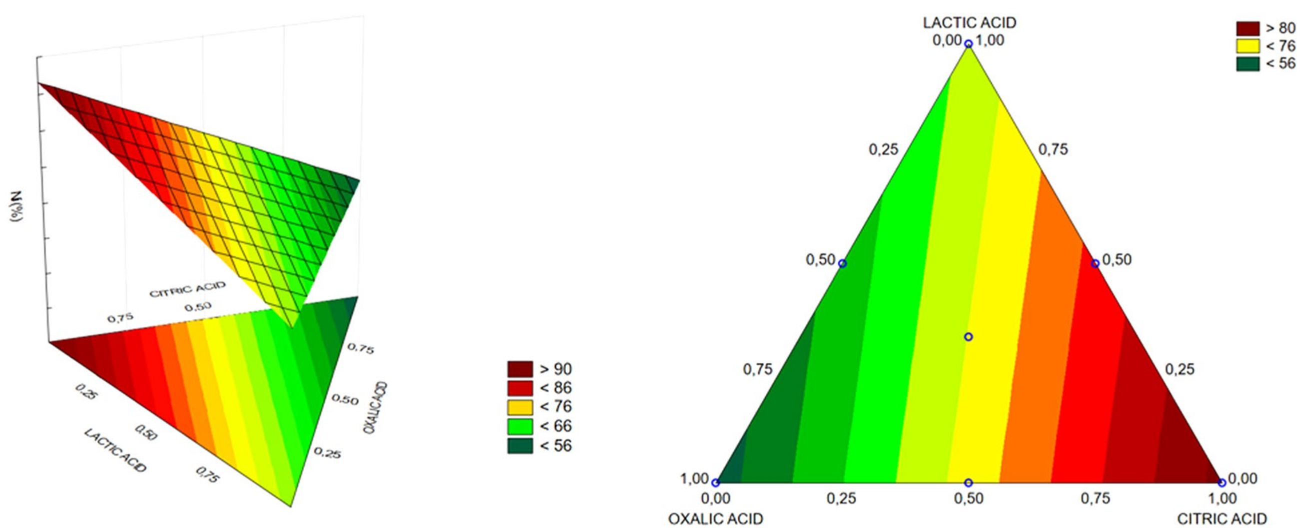

Fig. 4 Simplex-centroid surface design plot for Ni extraction from pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR. Experimental conditions: inoculum (10%, v v^{-1}), pentlandite (10%, v v^{-1}), 150 rpm, 30 ºC

deviation, enabling identifying the statistically significant effects and the most relevant factors. The vertical line ($p = 0.1$) indicates the minimum magnitude of statistically significant effects, considering the statistical significance of 90%. According to the response surface plot, for Ni (Fig. 4) and Co (Fig. 5) leaching, only citric acid presented a statistically significant effect. Among the organic acids studied, the citric acid is the second strongest acid, following the order oxalic (pKa = 1.25) > citric (pKa = 3.13) > lactic (pKa = 3.83) > ascorbic (pKa = 14.17) > acetic (pKa = 4.76) (Table 3).

The effectiveness of citric acid in dissolving Ni and Co is associated with this acid's strength or its ability to dissociate in solution (Tang and Valix 2004). The chelation of metal ions by citric acid through a combined method of acidolysis and complexolysis improves the recovery of Ni and Co from oxidized ores (Mehta et al. 2010). A generalized equilibrium reaction for the formation of soluble organometallic complexes by chelation can be described as (Tzeferis and Agatzini-Leonardou 1994):

$$M^{2+} + H_4Cit \rightarrow MH_{4-i}Cit^{2-i} + iH^{+} \quad (9)$$

where I = 1, 2, 3; H_4Cit = citric acid; M = the metal species; M^{2+} = metal^{2+}; $MH_{4\text{-}i}Cit^{2-I}$ = the metal–ligand complex.

It can be concluded that the citric acid contributes in a synergistic form in pentlandite biological–chemical leaching

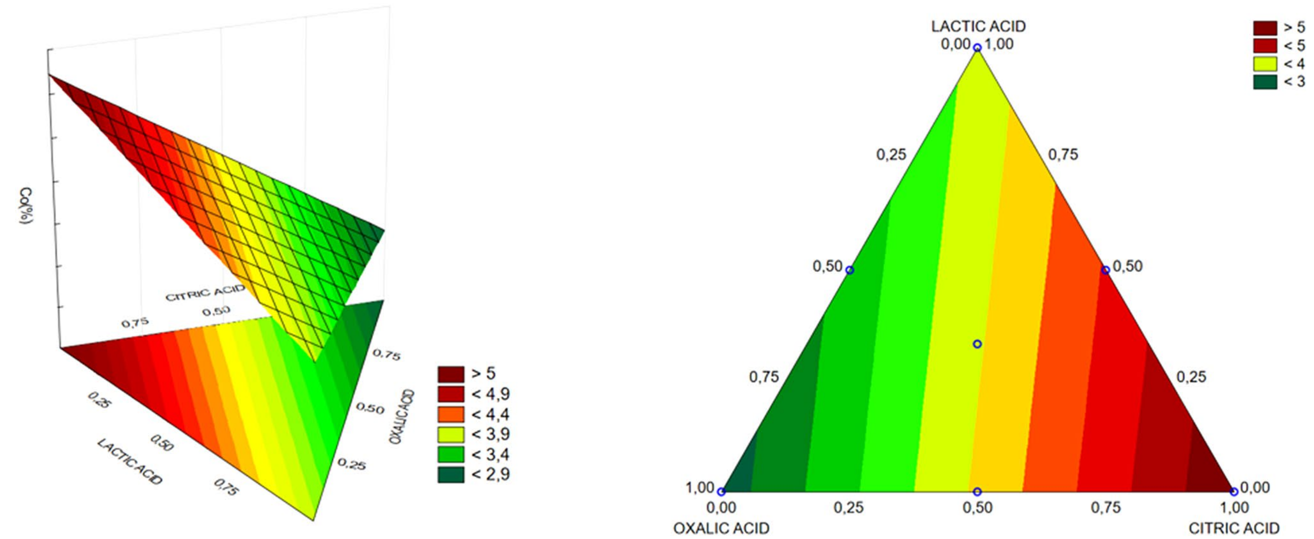

Fig. 5 Simplex-centroid surface design plot for Co extraction from pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR. Experimental conditions: inoculum (10%, v v^{-1}), pentlandite (10%, v v^{-1}), 150 rpm, 30 ºC

Table 5 ANOVA data for Ni and Co extraction during pentlandite biological–chemical leaching in the presence of *Acidithiobacillus ferrooxidans* LR

	SS	*df*	MS	*F*	*p*
Y_1	978.033	2	489.017	0.252381	0.788454
Y_2	3.52133	2	1.760667	0.274109	0.773459

SS quadratic sum; *df* degrees of freedom; *MS* quadratic mean; *F* calculated value for a significance level of 95%

in the presence of *Acidithiobacillus ferrooxidans* LR. Further studies are necessary to develop new systems of chemolithoautotrophic microorganisms in combination with organic acid producers to generate higher metal extraction yields and more comprehension of this bioprocess.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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