

Solubilization of a K-silicate rock by *Acidithiobacillus thiooxidans*

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ABSTRACT

Sulfur-oxidizing bacteria are used in bioleaching processes to recover metals such as gold and copper. The mechanisms for solubilizing metals used by the bacterium *Acidithiobacillus thiooxidans* involve sulfur oxidation reactions, with the consequent formation of acid. In this study, solubilization of a K-silicate rock (verdete rock, VR) was investigated in a biological trial using *A. thiooxidans* and elemental sulfur (S^0). Erlenmeyer flasks containing modified 9 K medium, bacterial cultures and VR were incubated at 30 °C for periods ranging from 0 to 49 days. pH, redox potential were determined, and chemical and mineralogical analyses were performed after each incubation period. In the presence of *A. thiooxidans*, the estimated initial pH of 4.2 was reduced to 0.57 after 49 days of incubation. Under acidic conditions, partial solubilization of VR was detected by increases in the concentrations of structural elements of rock minerals, such as K, Al, Fe and Si in the medium containing bacteria, which corresponded to 6.6, 5.8, 14.1 and 1.7% of the total content of these elements in VR, respectively. Mineralogical analysis showed similar changes in X-ray Diffraction spectra (XRD) for VR samples in the absence and presence of *A. thiooxidans* at 49 days. Our study shows that solubilization of VR occurs when *A. thiooxidans* and S^0 are included in the culture medium, suggesting potential use of a biological route to leach value metals from silicate minerals using low energy input, compared to conventional processes in which pure acids/bases or high temperatures are used.

1. Introduction

Sulfur-oxidizing bacteria play an important role in the biogeochemical cycling of sulfur (Bobadilla Fazzini et al., 2013). The ability of these microorganisms to oxidize compounds containing reduced inorganic sulfur is applied in biohydrometallurgical technologies such as bioleaching of metals (Mohapatra et al., 2008; Olson et al., 2003). Bioleaching technologies are used for the treatment of low-grade ores, which generally contain low metal concentrations. Although several minerals are potentially amenable to bioleaching, commercial-scale production has only been successful for gold and copper (Harvey et al., 2002).

Bacterial leaching of metals involves solubilization through oxidation of metal sulfides to sulfate and sulfuric acid, processes carried out by specialized bacteria such as species of *Acidithiobacillus* and *Leptospirillum* (Schippers and Sand, 1999). These genera are the dominant microbial communities in the bioleaching systems at Dexing Copper Mine (Jiangxi, China) (Niu et al., 2016). *Acidithiobacillus*

thiooxidans is a chemolithoautotrophic species which uses CO_2 from the atmosphere as C source, and acquires its energy from oxidation of reduced inorganic S compounds, including S^0 (Chan and Suzuki, 1994).

The mechanisms by which *A. thiooxidans* solubilizes metals involve S oxidation reactions, with the consequent formation of inorganic acids (protons). Sulfuric acid is one of the most important compounds used by the chemical and mining industries; it is used to make many substances, including phosphoric and fluorine acids, paints and fertilizers. It is the main acid found in leaching environments, where there is activity of *Acidithiobacillus*, and the presence of reduced sulfur forms (Brandl, 2008).

Bioleaching of non-sulfide ores is a challenge because they contain no energy source for microorganism growth. However, S^0 , which is produced predominantly by recovery from the oil and gas industry (Eow, 2002), is an important low-cost raw material in the fertilizer industry. It may be feasible to add S^0 into catalytic reactions to facilitate the recovery of valuable metals from non-sulfide ores, such as those of K-silicates.

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Agriculture worldwide is highly dependent on K supply, and few countries other than Canada and Russia have soluble mineral reserves to produce K-fertilizers. However, many other countries have large deposits of K-silicate minerals, such as micas and feldspars, but they have no economic interest. Brazil has a considerable deposit of a K-silicate rock, which is regionally known as verdete rock (VR). Geologically, it is found in the San Francisco Craton, in the Bambuí Group, along the Serra da Saudade formation. This material has a considerable content of K (5.8–11.7% w w⁻¹) (Toledo Piza et al., 2011).

Bioleaching is an interesting approach to recover K or other value metals from silicate minerals. In fact, the high bond energy that keeps K in silicate structures, such as mica or feldspars, requires high temperatures and a fluxing agent or pure acids and bases to leach it (Santos et al., 2015), in very expensive processes. Moreover, using S⁰ in biological processes to produce K-fertilizer has additional advantages. The presence of S-SO₄²⁻ from biological oxidation of S⁰, such as in K-complexing oxyanions, should promote the formation of K₂SO₄, a highly valuable K-fertilizer. In addition, the remaining SO₄²⁻-bound H⁺ in a highly acidic medium can be recovered as H₂SO₄, a highly demanded industrial acid.

Our goal is to develop a biological route for K-silicate rock solubilization, using an S-oxidizing bacterium. The specific objective of this research was to analyze the leaching trends of some structural elements in materials produced by incubation of VR in the absence and presence of *A. thiooxidans* in a medium containing S⁰, and to relate them to the pH of the medium, bacterial growth and mineralogical changes in VR during the incubation periods.

2. Materials and methods

2.1. Chemical and mineralogical characterization of verdete rock

Representative samples of VR we collected in the municipality of Cedro do Abaeté, Minas Gerais state, Brazil. The rock samples were taken from outcrops at UTM geographic coordinates x = 426090.0 and y = 7882653.2. Data from our research group revealed a high concentration of K in VR, which is present at these coordinates (Santos et al., 2015). Prior to conducting the chemical and mineralogical analysis and the biosolubilization assay, rock samples were ground and passed through a 75 µm sieve (200 mesh). The chemical composition of the VR is shown in Table 1.

2.2. Bacterial growth and adaptation

The *A. thiooxidans* isolate FG-01 was firstly obtained by Garcia Jr. (1991) from an acid effluent, which is produced by a uranium mine in Brazil. For this study, we used an isolate descending from the initial one

(Garcia Jr., 1991), that is deposited in the Instituto de Química of the Universidade Estadual Júlio de Mesquita Filho under the care of Dr. Denise Bevilacqua. For the trial, the *A. thiooxidans* isolate was initially grown for 10 generations in 250 mL Erlenmeyer flasks containing 100 mL of modified 9K medium (Silverman and Lundgren, 1959) adding 1.0 g of elementary sulfur, replacing Fe²⁺ as specific energy source for growth of *A. thiooxidans*. In addition, 1.0 g of VR was added to the medium aiming to adapt the bacteria to the presence of the rock. Erlenmeyer flasks were maintained at 30 °C in an orbital shaker at 150 rpm. When the pH of the medium changed from 2.5 (initial) to 1.0, the bacterial suspension was passed through filter paper to remove the solid materials (remaining VR and S⁰). The filtrate was then centrifuged for 15 min at 3000 g, and the supernatant was discarded. Then, the cells were transferred to another Erlenmeyer flask containing VR and 9 K culture medium for growth of the next generation. We followed this procedure until the 10th generation of the bacteria was obtained. Finally, a suspension of adapted bacteria was concentrated by centrifugation, and the number of cells counted by flow cytometry. The concentrated suspension of bacteria had 5 × 10⁹ cells ml⁻¹, and it was used in the biosolubilization assay.

2.3. Biosolubilization trial

The biosolubilization experiment was carried out in 250 mL Erlenmeyer flasks containing 50 mL of 9 K medium (pH 4.5), 1 g of VR (< 75 µm), 1 g S⁰ per week, totaling 7 g S⁰ for 49-day corresponding treatments (< 75 µm), and 300 µL of the suspension of adapted bacteria at 5 × 10⁹ cells mL⁻¹ (only for inoculated treatments). Experiments were performed in an orbital shaker at 150 rpm and 30 °C, using a 2 × 5 factorial scheme: treatments without and with bacteria (2), and incubated for 0, 7, 14, 21 and 49 days (5). The experiments were carried out in a completely randomized design, with three replicates.

The trial was run under sterile conditions. Thus, VR and 9 K culture medium were autoclaved separately for 20 min at 120 °C. The Erlenmeyer flasks were stoppered with sponges to avoid contamination and to allow admission of O₂ and CO₂. At each sampling time, the water lost during incubation was compensated by addition of sterile deionized water, with the pH adjusted to the acidity conditions of the culture medium at the time of collection.

For each incubation period, Erlenmeyer flasks were removed from the incubator and the bacterial cultures left to settle at room temperature for 1 h. Then, 1 mL of the supernatant was collected in order to count cells by flow cytometry. The material was re-suspended and filtered through slow quantitative filter paper (~28 µm pore size), and the filtrate used for measurement of pH and redox potential (Eh), and for chemical analysis as described above. The solid materials withheld on the filter papers were dried (at room temperature), ground and analyzed by X-ray diffraction (XRD).

2.4. Chemical and mineralogical analysis

The total K, Al, Fe and Si concentrations in the VR were measured by total X-ray fluorescence spectroscopy (TXRF; Bruker S8 Tiger). The concentrations of these elements in the filtered extracts were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES; Perkin Elmer 8300 DV, US). Hydrogen ion concentration was determined by acid–base titration.

Mineralogical analysis was performed on the VR samples before and after subjecting them to the treatments, by XRD analysis using a Shimadzu XRD-6000 diffractometer, from 5° to 80° 2θ range with Cu-Kα radiation (λ = 0.154 nm) at a rate of 1.2° 2θ min⁻¹. Powder mounts were prepared by packing ground (< 75 µm) samples into Al holders.

2.5. Statistical analysis

Statistical analysis was carried out by one-way analysis of variance

Table 1
Chemical composition of Verdete rock for some chemical elements.

Element	Concentration (g kg ⁻¹)
Si	280.8
K	85.8
Al	66.8
Fe	42.8
Mg	12.9
Ti	5.2
V	0.4
Rb	0.4
Mn	0.3
Zr	0.2
Zn	0.1
Ni	0.1
Cr	0.1

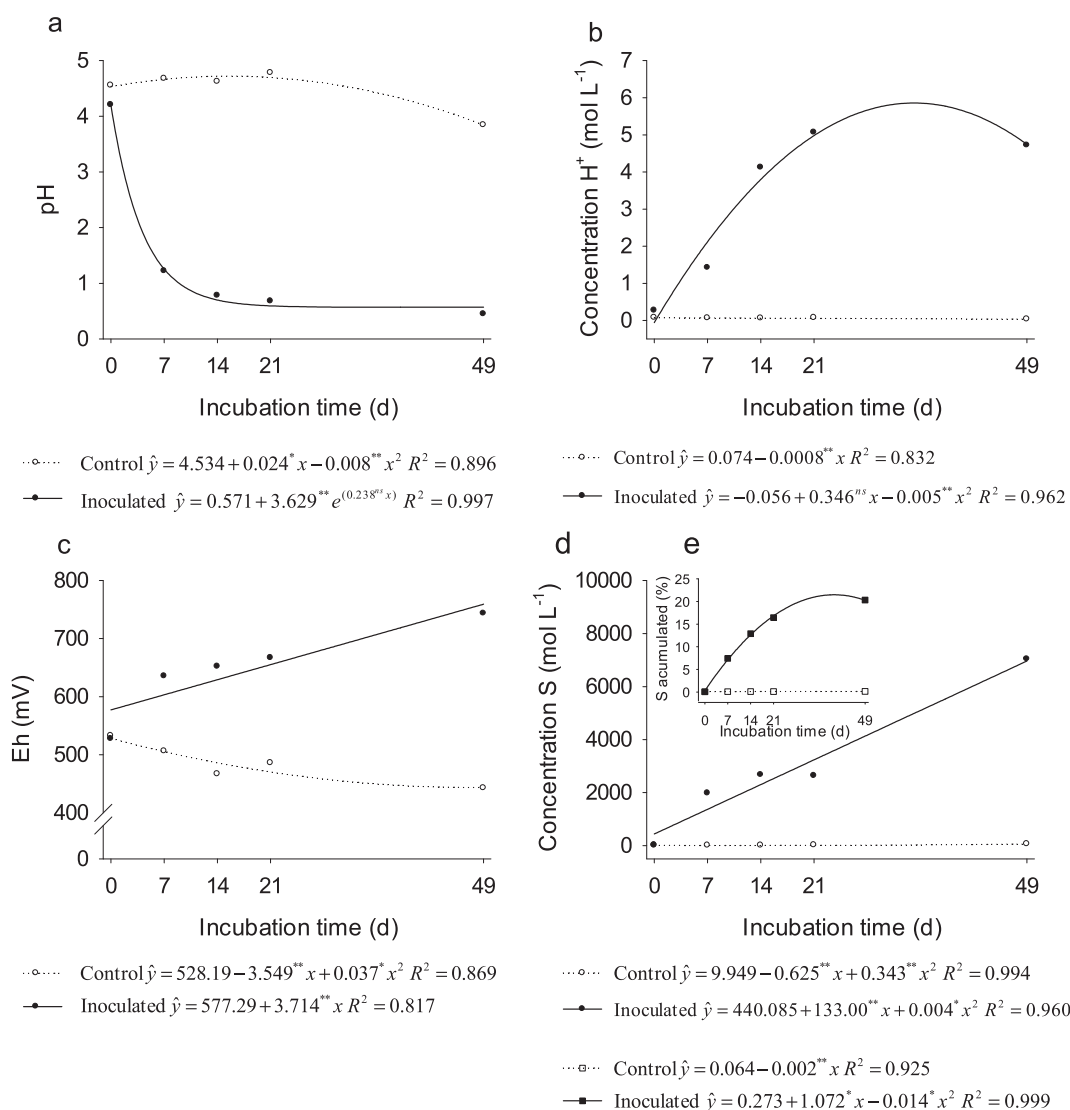


Fig. 1. Effects of incubation of a K-silicate rock (verdete rock) in the absence (control) and presence of *Acidithiobacillus thiooxidans* (inoculated) on pH (a), concentration of H^+ (b), redox potential (c), amount of S oxidized (d) and accumulated amount of S (e).

(ANOVA) followed by a general linear model using R software version 3.2.0. The differences among treatments with incubation time were analyzed by regression. Pearson's correlation analysis was performed between pH, redox potential (Eh) and the elements released during solubilization of VR.

3. Results

3.1. Acidity generation, redox potential and sulfur oxidation

The presence of *A. thiooxidans* in the medium caused a large increase in its acidity. Seven days after incubation (dai), the initial pH of 4.2 was reduced to 1.2, and it reached 0.57 at 49 dai (Fig. 1a). Over the same periods, the H concentration increased from 0.0 to 2.11 mol L⁻¹ and from 2.11 to 4.66 mol L⁻¹, respectively, reaching a maximum (4.96 mol L⁻¹) at 21 dai (Fig. 1b). In the control treatments, the pH and H content remained stable during the incubation. The redox potential in the medium containing VR, S⁰ and bacteria increased linearly over the incubation period, reaching 759.3 mV at 49 dai (Fig. 1c), in contrast to the non-inoculated medium, in which it was 443.6 mV.

A linear increase in the S concentration (soluble forms) as a function of incubation time was observed in the presence of *A. thiooxidans*. The S concentration in solution increased about 16-fold (from 440.1 to 6

966.1 mg L⁻¹ from 0 to 49 dai, respectively) (Fig. 1d), which corresponded to 20% of the S added during the incubation period (Fig. 1e). We expect S⁶⁺ to be the main S species in solution, because it is an end-product of oxidation of reduced S compounds.

3.2. Solubilization of VR

A. thiooxidans was effective in solubilizing VR, in terms of releasing elements into the medium. As shown in Fig. 2, in the presence of the bacterium, the medium was enriched with structural elements of VR, such as K, Al, Fe and Si. Leaching of K, Al, Fe and Si from the rock followed a similar trend of dissolution, with a linear increase in their concentrations in solution over the incubation period.

The soluble K content increased linearly during the incubation period. At 49 days, the medium containing bacteria had approximately 150 mg L⁻¹ K, while the control treatment contained 30 mg L⁻¹ K (Fig. 2a). The amount of K leached into the medium with bacteria corresponded to 6.6% of the total K present in VR (Fig. 2b).

Incubation of VR with bacterial cells promoted a marked increase in Al concentration in the culture medium within the first 7 dai (from 4.33 to 20.6 mg L⁻¹) (Fig. 2c), and it reached 118.5 mg L⁻¹ at 49 dai. At this point, the amount of Al released corresponded to 5.8% of the total Al content of VR, while in the control treatment the amount of Al released

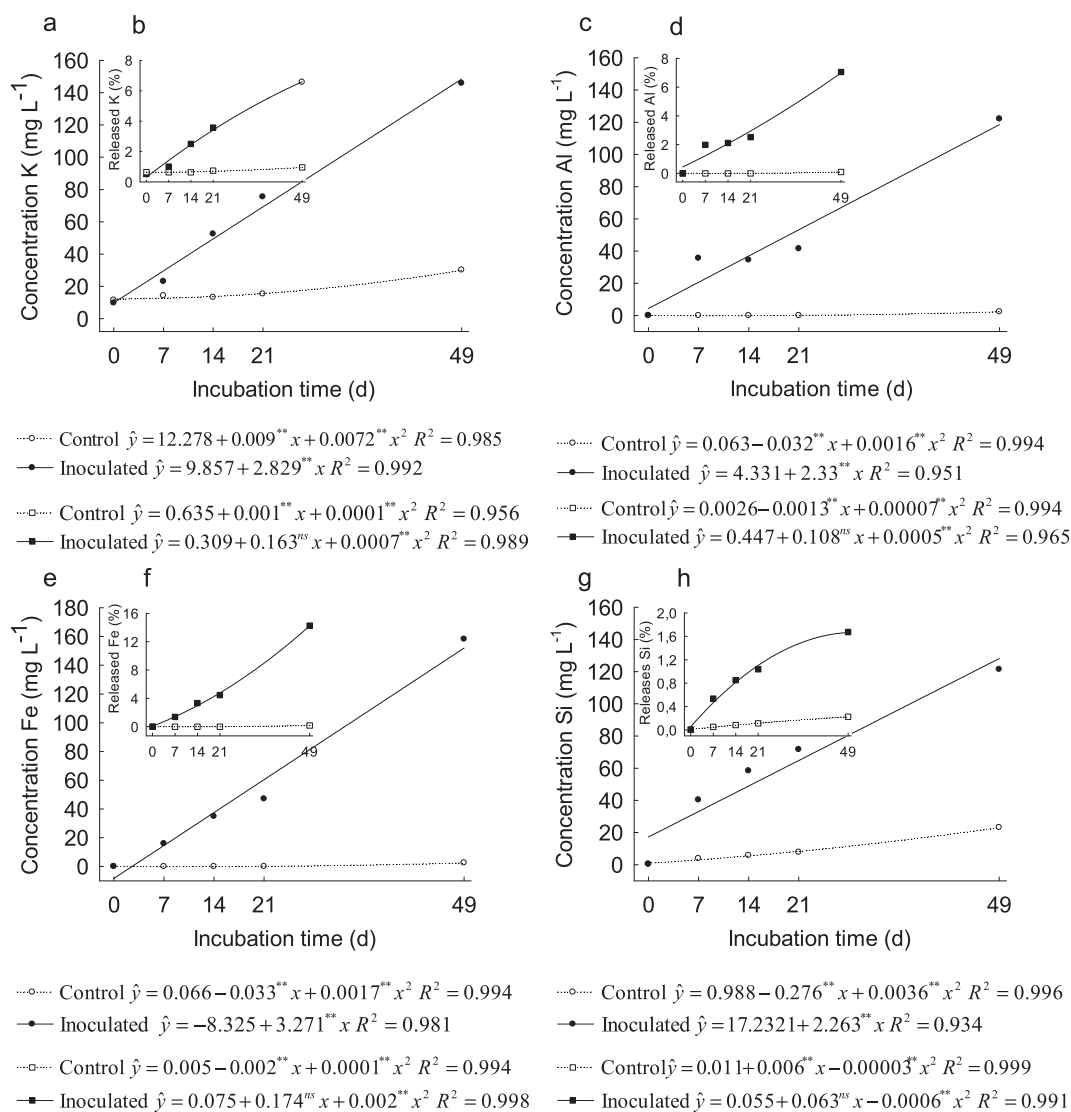


Fig. 2. Concentration and amount of K, Al, Fe and Si released during solubilization of a K-silicate rock (verdete rock) in the absence (control) and presence of *Acidithiobacillus thiooxidans* (inoculated). (a, c, e, g) Concentration of K, Al, Fe and Si in the culture medium. (b, d, f, g) Released K, Al, Fe and Si (%) represents the amount of these elements leached in relation to their total amount in the rock.

represented only 0.1% (Fig. 2d).

The amount of Fe in the culture medium (Fig. 2e) in the presence of *A. thiooxidans* increased from 14.6 (7 dai) to 60.4 mg L⁻¹ (21 dai). We observed a 2.5-fold increase in the amount of Fe released during the last time interval, corresponding to 14.1% of the total present in VR (Fig. 2f). For Si, the culture medium contained 23.2 and 128.1 mg L⁻¹ at 49 dai for control and inoculated treatments, respectively. The amount of Si released into the medium with bacteria after 49 dai was about 1.7% of the total contained in the rock (Fig. 2g, h).

3.3. Correlation among pH, Eh, acidity and elements released

There were significant correlations ($p < 0.01$) among pH, Eh and concentrations of H, K, Al, Fe, Si and S (Table 2). The correlations between pH and all other variables under study were negative. The correlations among H, K, Al, Fe, Si and S were all positive. During the incubation time, the release of Al, Fe and Si showed a trend similar to that of K (Fig. 2). The coefficients of simple linear correlations among the concentrations of these elements with K concentration were higher than 0.9.

Table 2

Pearson's correlation coefficients between pH, redox potential (Eh) and elements released during solubilization of a K-silicate rock (Verdete rock, VR) by *Acidithiobacillus thiooxidans*.

	pH	H	K	Al	Fe	Si	S
H	-0.86						
K	-0.75	0.76					
Al	-0.81	0.76	0.94				
Fe	-0.71	0.73	0.96	0.98			
Si	-0.90	0.84	0.95	0.96	0.93		
S	-0.83	0.82	0.94	0.99	0.97	0.96	
Eh	-0.88	0.74	0.75	0.83	0.76	0.85	0.82

All correlations were significant by *t* test ($p < 0.01$).

3.4. Bacterial growth

There was a marked reduction in bacterial population at 7 dai, due to bacterial adaptation to the acidic conditions of the culture medium. The pH of the medium was adjusted to 4.5 to avoid highly acidic conditions at the beginning of the experiment. Thus, after 7 days of incubation, due to the metabolic activity of the bacteria, the pH value of

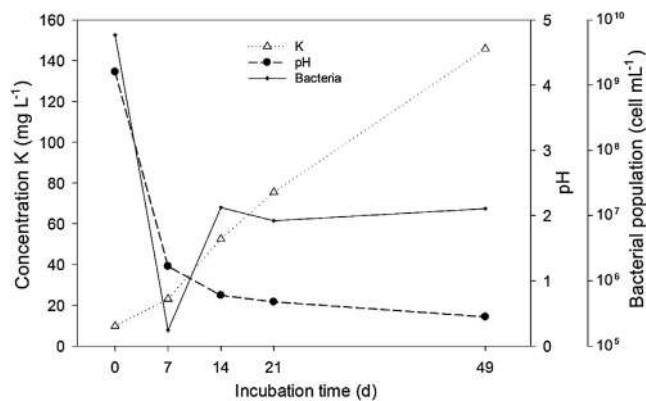


Fig. 3. Evolution of K concentration in leaching solution, pH and number of bacterial cells with incubation time of a K-silicate rock (verdete rock) with *Acidithiobacillus thiooxidans*.

the culture medium was reduced to an optimum range for bacterial growth, and exponential growth was observed later in the incubation (Fig. 3).

3.5. Mineralogical changes

Mineralogical analysis showed similar changes in XRD spectra for VR samples in the absence and presence of *A. thiooxidans* at 49 dai. All Bragg peaks of glauconite, related to its 001 'c' dimension, feldspar and quartz (from 5° to 35° 2θ) disappeared, except for the peaks at 0.334 nm for quartz and 0.257 nm for glauconite, which exhibited differences in intensity. In addition, ES peaks were detected; this was expected, because ES was supplied in the medium for those treatments to which *A. thiooxidans* was added (Fig. 4).

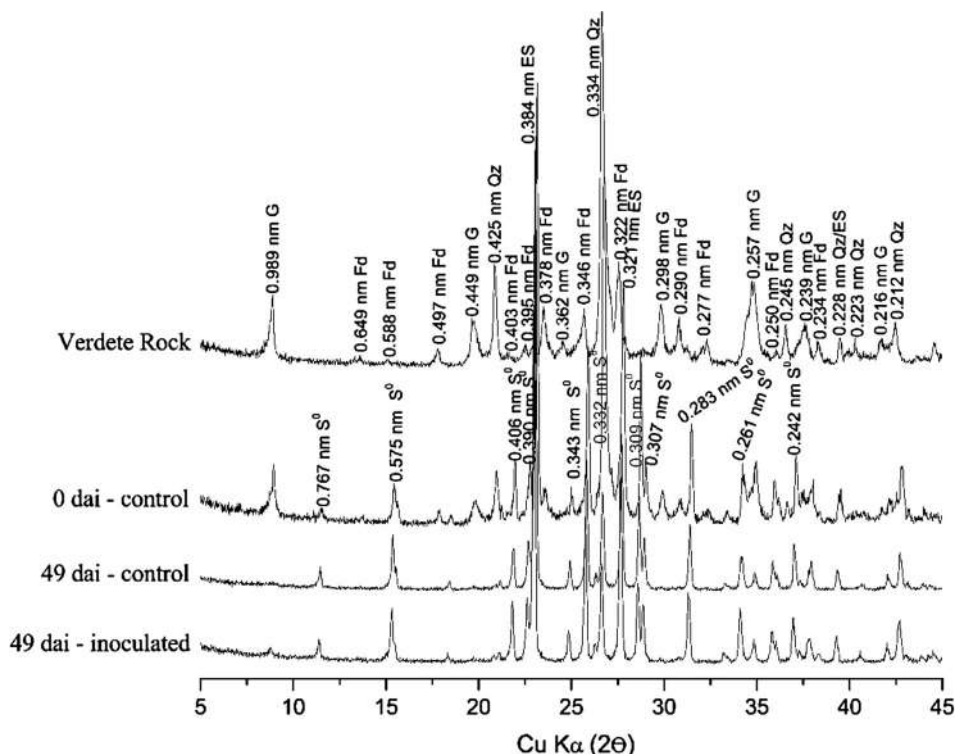
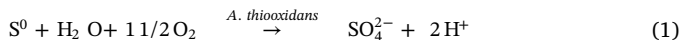


Fig. 4. X-ray diffraction (XRD) patterns of samples of a K-silicate rock (verdete rock) incubated in the absence (control treatment at 0 and 49 days after incubation, dai) and presence (49 dai) of *Acidithiobacillus thiooxidans*. Data were collected using Cu K_{α1} radiation (1.54 Å). G: glauconite, Qz: quartz; Fd: feldspar; S⁰: elemental sulfur.

4. Discussion

4.1. Effect of bacteria on acidification and sulfur oxidation

Elemental S is a fundamental substrate for S-oxidizing bacteria (Pokorna et al., 2007), such as species from the *Acidithiobacillus* genus. During the oxidation of reduced sulfur compounds, inorganic acids are produced (Watling, 2015). As observed in our study, the occurrence of this reaction, catalyzed by *A. thiooxidans*, promoted a decrease in pH as a consequence of proton production, and the release of soluble S forms into the medium. In these systems, it is possible to estimate sulfur oxidation as a function of decreasing pH (Janiczek et al., 1998) or sulfate concentration in the medium, following the stoichiometry in Eq. (1).



The soluble S-SO₄²⁻ concentration (7200 mg L⁻¹), pH (0.5) and acidity (5.6 mol L⁻¹) reached at 49 dai indicate the formation in the culture medium of molecular H₂SO₄, a very valuable acid for many industrial activities. We assume that the soluble S species is S-SO₄²⁻ because it is the end S species from oxidation of reduced S forms. In fact, only at pH ≥ 1.92 is the majority of H₂SO₄ ionized, according to its dissociation constants (pK_a = -3.9 and 1.92).

4.2. Solubilization of VR

The acidity produced from oxidation of S⁰ by *A. thiooxidans* promoted solubilization of the minerals that make up VR. Indeed, solubilization occurs when minerals are in unstable conditions, such as acidic conditions, causing leaching of their chemical elements. Solution conditions, including ion concentrations, acidity, redox potential and ionic strength, can generate both soluble and insoluble species (Terry, 1983). In our case, using Visual Minteq freeware for chemical equilibrium modeling, we observed that the medium did not produce any precipitated species of K, Al, Fe, Si or S, because of the right acidity and undersaturation of elements in the medium.

The increasing concentration of K, Al, Fe and Si in the culture

medium during the incubation period is evidence of partial solubilization of VR promoted by bacterial activity. Moreover, our results suggest that these elements did not come from crystalline minerals present in VR. XRD analysis of samples from treatments in the absence or presence of *A. thiooxidans* revealed similar changes in the XRD spectra. On the other hand, the content of soluble elements increased only in the presence of bacteria (see Fig. 3). Around 7% of the total K was leached from VR in presence of bacteria. This amount is low for an industrial procedure; however, the linear increase of K content in the medium up to 49 dai indicates the need to investigate more long-term incubation.

The main K mineral found in VR is glauconite (Santos et al., 2015; Toledo Piza et al., 2011), a mica that belongs to the illite group, which exhibits a considerable isomorphous substitution of Al^{3+} by Fe^{2+} in its octahedral structure (Fassbender, 1975). Such substitutions cause weakening of the silicate structure, increasing its susceptibility to acid attack (Terry, 1983). Several studies, including this one, have shown that the 001 'c' dimension of this mineral is most susceptible to thermal (Santos et al., 2017) or chemical attack (see Fig. 4). Rov (1949) reported that there is an expansion in the 'c' dimension of muscovite upon heating.

The concurrent release of other elements with K during the biosolubilization of VR has received little attention in previous studies. In this work, it has been clearly demonstrated that various elements, such as Al, Fe and Si, are released together with K (see Fig. 2), although in different proportions (see Fig. 2d, f and h). These results suggest that the acidic attack occurs throughout the structure of the K minerals present in VR, leaching elements present in the 2:1 layers, mainly K, as well as in those located in tetrahedral and octahedral structures.

The bond energy of K, Al and Fe with O (1.3, 7.2 and 3.5 MJ mol⁻¹, respectively) is significantly weaker than that between Si and O (13.2 MJ mol⁻¹) (Sun and Huggins, 1947). The greater bond energy of Si–O makes it less susceptible to acid attack, causing silicate groups to be removed intact from the mineral surface. Thereby, these groups can form active complexes with protons in solution (Crundwell, 2014). Also, the release of Si into an acid solution, like in our system, can favor silicon polymerization, which is stable at low pH. The isoelectric point of silicon is between pH 1.7 and 3.5, a range in which its polymerization is optimum (Terry, 1983). It is likely that this is the reason we found very low Si concentrations in leached solution.

The increase in redox potential, from 442.3 mV in the medium without *A. thiooxidans* to 743.4 mV in the presence of the bacterium, is attributed to an increase in the concentration of oxidized species of the elements. In our experiments, this increase was due to the oxidation of S^0 to S^{6+} catalyzed by *A. thiooxidans*, production of protons (H^+) and possibly the oxidation of Fe^{2+} to Fe^{3+} during the incubation period. Glauconite, the main mineral constituent of VR, is formed by slow sedimentation in a marine environment, under reducing conditions. During this process, loss of Al and silica, along with enrichment of Fe^{2+} and K minerals, occurs (Fassbender, 1975; Lima et al., 2007).

The biotechnological process presented here is an alternative method for recovering elements, such as K, Al, Fe and Si, from silicate minerals such as micas or feldspars. It may represent a strategic way to produce K-fertilizer in countries where there are insufficient reserves of soluble K minerals, with environmental and cost advantages. However, further studies using more sophisticated leaching bioreactors, aiming to recover greater amounts of elements and higher concentrations of soluble species, and to develop routes to recover sulfuric acid, are required.

5. Conclusions

Our data demonstrate that acidification of the culture medium, caused by catalytic activity of the bacterium *A. thiooxidans*, promotes the partial dissolution of VR, increasing the concentration of K, Al, Fe and Si in solution throughout the incubation period. Biotechnological studies aimed at separating and concentrating element species in the

leaching solution must be conducted in order to exploit this technology of low energy consumption.

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Conflict of interest

The authors declare that they have no conflict of interest.

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