PATRÍCIA CARDOSO MATIAS

BIOSOLUBILIZATION OF K-SILICATES IN PELITIC ROCK BY *Acidithiobacillus thiooxidans*

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de *Magister Scientiae.*

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APROVADA: 14 de fevereiro de 2017.

Víctor Hugo Alvarez V.
(Coorientador)

Walter Antônio Pereira Abrahão

Jorge Luis Badel Pacheco

Edson Marcio Mattiello
(Orientador)

Aos meus pais Sandra e Ronaldo.
Dedico

"Somos o que repetidamente fazemos.
A excelência, portanto, não é um feito, mas um hábito".
Aristóteles

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BIOGRAFIA

PATRÍCIA CARDOSO MATIAS, filha de Vicente Ronaldo Matias e de Sandra Cardoso da Silva Matias, nasceu no dia 05 de agosto de 1992 na cidade de Viçosa-MG. Iniciou a graduação em Agronomia na Universidade Federal de Viçosa em março de 2010, graduando-se em janeiro de 2015. Em março de 2015, iniciou o curso de Mestrado em Solos e Nutrição de Plantas pela Universidade Federal de Viçosa, concluindo-o em fevereiro de 2017.

SUMÁRIO

ABSTRACT

MATIAS, Patrícia Cardoso, M.Sc., Universidade Federal de Viçosa, February, 2017. **Biosolubilization of K-silicates in pelitic rock by *Acidithiobacillus thiooxidans*.** Adviser: Edson Marcio Mattiello. Co-advisers: Víctor Hugo Alvarez V. and José Rogério de Oliveira.

Bacteria that oxidize reduced forms of S are used in industry to leach metals, such as gold and copper. The generation of acidity, from the oxidative dissolution of S, is the main mechanism by which it is possible to solubilize minerals using the bacterium *Acidithiobacillus thiooxidans*. In this study, the solubilization of a potassium silicate rock, regionally known as Verdete rock (VR), was evaluated by means of a biological assay using *A. thiooxidans*. Erlenmeyer flasks containing 9 K medium, bacterial culture and VR were incubated at 30 °C at times ranging from 0 to 49 d. After the incubation times, the filtrate and solid phase were collected. The solid phases were subjected to X-Ray Diffraction (XRD) analysis for mineralogical characterization. In the filtered, pH, redox potential and soluble contents of some elements (K, Al, Fe, Si and S) were measured. In the presence of *A. thiooxidans* the estimated initial pH of 4.2 was reduced to 0.57 at 49 d after incubation (dai). Under acidic conditions, with the presence of the bacterium, there was partial solubilization of the VR, evidenced by a significant increase in the concentrations of the structural elements of rock minerals. The released amounts of K, Al, Fe and Si, at 49 d, in the culture medium corresponded to 6.6, 5.8, 14.1 and 1.7 % of their total contents in VR. Mineralogical analysis showed similar changes in DRX spectra for VR samples in absence and presence of *A. thiooxidans* at 49 dai. In addition, the study confirmed that the solubilization of VR, as indicated by leaching of rock elements (K, Al, Fe and Si), is due to the acidification of the medium promoted by the biological catalysis of *A. thiooxidans* in the oxidation of S^0, and suggests the potential use of this bacterium to solubilize silicate rocks rich in K, or other metals of interest, since it is a technology of low energy consumption compared to conventional processes. Further studies should be carried out into optimize the biosolubilization of K-silicate minerals, including the using of more sophisticated bioreactors, processes for recovering soluble elements and microbial associations.

RESUMO

MATIAS, Patrícia Cardoso, M.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Biosolubilização de silicatos de K em rocha pelítica por *Acidithiobacillus thiooxidans*.** Orientador: Edson Marcio Mattiello. Coorientadores: Víctor Hugo Alvarez V. e José Rogério de Oliveira.

Bactérias oxidantes de formas reduzidas de S são utilizadas na indústria para lixiviar metais, como ouro e cobre. A geração de acidez, a partir da dissolução oxidativa do S, é o principal mecanismo pelo qual consegue-se solubilizar minerais utilizando a bactéria *Acidithiobacillus thiooxidans*. Neste estudo, avaliou-se a solubilização de uma rocha silicatada potássica, regionalmente conhecida como rocha Verdete (RV), por meio de um ensaio biológico utilizando *A. thiooxidans*. Frascos erlenmeyers contendo meio 9 K, cultura bacteriana e RV foram incubados a 30 °C em tempos variando de 0 a 49 d. Após os tempos de incubação, coletaram-se o filtrado e a fase sólida. As fases sólidas foram submetidas a Difração de Raios X (DRX) para caracterizações mineralógicas. Já no filtrado, mediram-se pH, potencial redox e teores solúveis de alguns elementos (K, Al, Fe, Si e S). Na presença de *A. thiooxidans* o pH inicial estimado de 4,2 foi reduzido para 0,57 aos 49 d após a incubação (dai). Sob condições ácidas, promovida pela presença bacteriana, ocorreu solubilização parcial da RV, com expressivo aumento nas concentrações em solução de elementos estruturais de minerais da rocha. As quantidades liberadas de K, Al, Fe e Si, aos 49 d, no meio de cultura contendo bactérias corresponderam a 6,6; 5,8; 14,1 e 1,7 % dos seus teores totais na RV. A análise mineralógica da RV, aos 49 dai, revelou alterações semelhantes nos espectros de Difração de Raio-X para as amostras da RV, na ausência e presença de bactéria *A. thiooxidans*. No entanto, o estudo confirmou que a solubilização da RV, como indicado pela lixiviação dos elementos rochosos (K, Al, Fe e Si), é devida à acidificação do meio promovida pela catálise biológica da bactéria *A. thiooxidans* na oxidação do S^0, e sugere o uso potencial dessa bactéria para solubilizar rochas silicitadas ricas em K, ou em outros metais de interesse, uma vez que é uma tecnologia de baixo consumo de energia em relação aos processos convencionais. Estudos adicionais devem ser realizados para otimizar a biossolubilização de minerais de silicato de K, incluindo o uso de biorreatores mais sofisticados, processo de recuperação de elementos solúveis e associações microbianas.

Paper

Biosolubilization of K-silicates in pelitic rock by *Acidithiobacillus thiooxidans*

Biosolubilization of K-silicates in pelitic rock by *Acidithiobacillus thiooxidans* [1]

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Abstract

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

Keywords: bioleaching, elemental sulfur, potassium, glauconite, verdete

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[1] The manuscript has been prepared according to the journal's instructions to be submitted for publication in Applied Microbiology and Biotechnology Journal

Introduction

Sulfur-oxidizing bacteria play an important role in the biogeochemical cycling of sulfur (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 (Olson et al. 2003; Mohapatra et al. 2008). 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 processes through oxidation of metal sulfides to sulphate and sulfuric acid, carried out by specialized bacteria, such as species of *Acidithiobacillus* and *Leptospirillum* (Schippers and Sand 1999). These genera are the dominant microbial communities in bioleaching systems at Dexing Copper Mine (Jiangxi, China) (Niu et al. 2016). *A. thiooxidans* is a chemolithoautotrophic species, which uses CO_2 from the atmosphere as C source, and acquares its energy from the 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 the main inorganic acid found in leaching environments (Brandl 2008). These acids solubilize the primary minerals or complex Si ions of the mineral, releasing the elements to the medium (Basak and Biswas 2009).

Bioleaching of non-sulfide ores is a challenge because they contain no energy source for growth of microorganisms. 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 potassium silicates.

Agriculture worldwide is highly dependent on K supply and few countries have soluble mineral reserves to produce K-fertilizers, highlighting Canada and Russia. However, many other countries have large deposits of K-silicate minerals, such as micas and feldspars, but they have not been of economic interest until now. Brazil has a considerable reserve of a silicate-K rock, consisting of pelitic metassediments, 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}) (Piza et al. 2011). Biosolubilization is an interesting approach to produce K-fertilizer from silicate ores.

The objectives of the present work were to evaluate the solubilization of a K-silicate rock in absence and presence of *A. thiooxidans* in medium containing S^0 and to investigate changes in mineral phases and leached ions over the time of incubation.

Materials and Methods

Chemical characterization of Verdete rock

Representative samples of VR we collected in the municipally of Cedro do Abaeté, state of Minas Gerais, Brazil. The rock samples were taken from outcrops at Geographic Coordinates UTM, x=426090.0 y=7882653.2; 23 K. Data from our search group revealed high concentration of K for these coordinates (Santos et al. 2015). Prior to conducting the chemical and mineralogical analysis and the biosolubilization assay, the rock samples were ground and passed through a 75 µm sieve (200 mesh). The chemical composition of the VR is shown in table 1.

Bacterial growth and adaptation

Isolates of *A. thiooxidans* were initially grown for ten generations in 250 mL erlenmeyer flasks containing 100 mL of 9 K medium (Silverman and Ludgren 1959) and 1 g VR at 30 ºC 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 S^0). The filtrate was then centrifuged for 15 min at 3 000 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 obtaining the 10th generation of the bacteria. Finally, the suspension of adapted bacteria was concentrated by centrifugation and the number of cells counted by flow cytometry. The concentrated suspension of adapted bacteria had 5 x 10^9 cells ml^{-1} and it was used in the biosolubilization assay.

Table 1. Chemical composition of Verdete Rock for some 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

Biosolubilization assay

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) and 1 g S^0 per week (< 75 µm), and 300 µL of the suspension of adapted bacteria at 5 x 10^9 cells mL^{-1} (only inoculated treatments). Experiments were performed in an orbital shaker at 150 rpm and 30 ºC, using a 2×5 factorial scheme; treatments without and with bacteria incubated for 0, 7, 14, 21 and 49 d. The experiments were carried out in a completely randomized design with three replicates.

The trial was run under sterile conditions. Thus, the VR and the 9 K culture medium were autoclaved separately for 20 min at 120 ° C. The erlenmeyers flasks were stoppered with sponges avoiding contamination while allowing O_2 and CO_2 to enter. At each sampling time, the water lost during incubation was compensated by addition of sterile deionized water, with the pH adjusted corresponding the culture medium at the time of collection.

At the end of the incubation period, erlenmeyers 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 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 the redox potential (Eh), pH, and for chemical analysis as described below. The solid material on the filter paper was dried (at room temperature, ~ 25 ^oC), ground and analyzed by XRD as described below.

Chemical and mineralogical analysis

The total K, Al, Fe and Si concentration 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.

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

Statistical analysis

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

Results

The presence of *A. thiooxidans* to the medium caused a large increase in the acidity of the medium. In fact, the initial pH 4.2 was reduced to 1.26 at 7 d after inoculation (dai), reaching values of 0.57 at 49 dai (Fig. 1a). Over the same periods of time, the H concentration increased from 0.0 to 2.11 and from 2.11 to 4.66 mol L^{-1}, respectively, reaching its maximum concentration (4.96 mol L^{-1}) at 21 dai (Fig. 1b). In the control treatments, the pH and H content remained stable during the incubation time. The redox potential in the medium containing VR, S^0 and bacteria increased linearly over the incubation time, reaching 759.3 mV at 49 dai (Fig. 1c), in contrast to the non-inoculated medium, in which it was 443.6 mV.

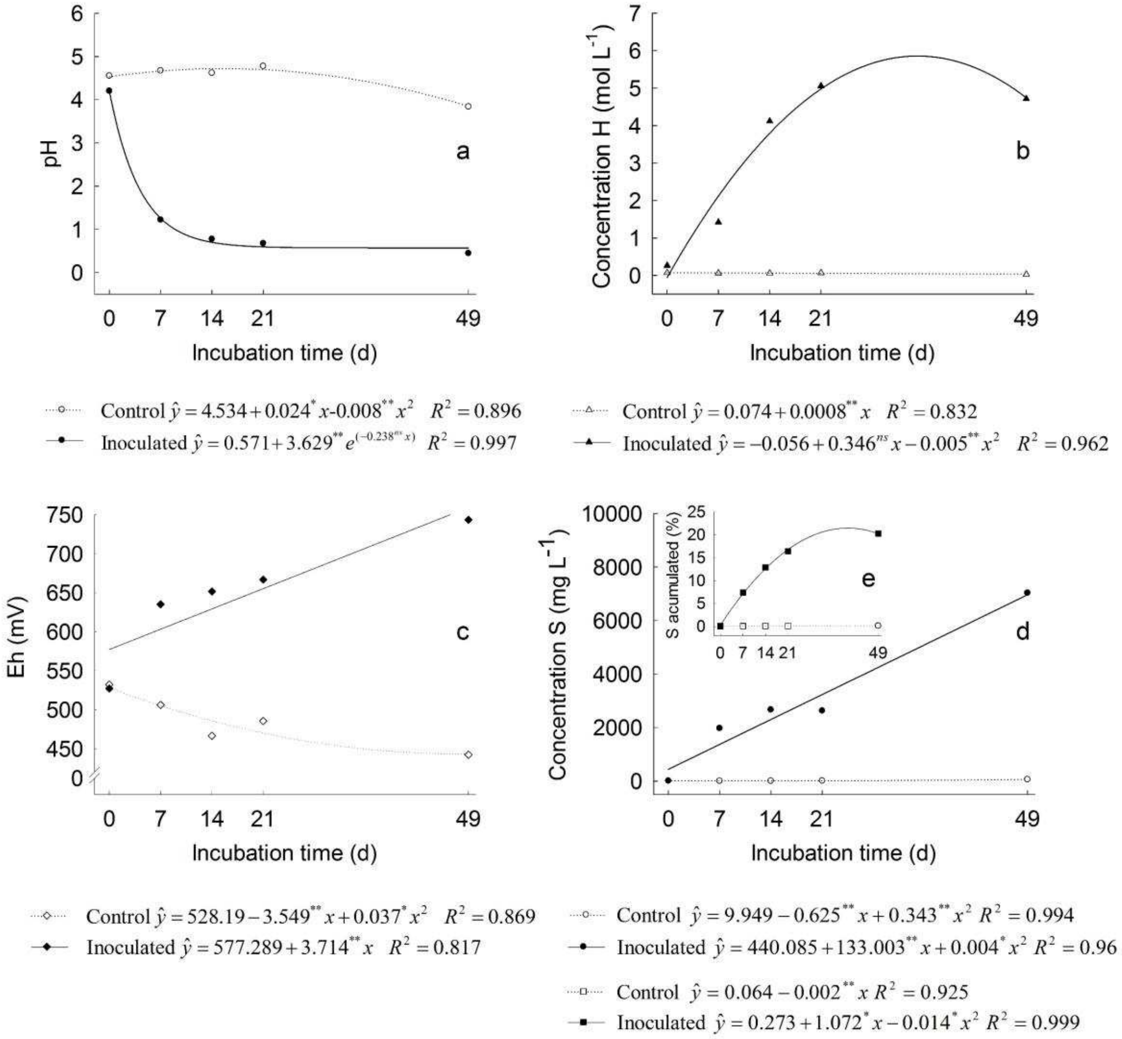

Figure 1 – Effects of incubation of a K-silicate rock (Verdete rock) in the absence (control) and presence of *Acidithiobacillus thiooxidans* (inoculated) on variation of pH (a), concentration of H (b), redox potential (c), and amount of S oxidized (d) and percentual accumulated (e).

It was observed a linear increase of the S concentration (soluble forms) as a function of incubation time 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} at 0 and 49 dai, respectively) (Fig. 1d), that corresponded to 20 % of the S added along the incubation period (Fig. 1e). We expect S^{6+} to be the main S species in solution, because it is an end-product from oxidation of reduced S compounds.

Acidithiobacillus thiooxidans was effective to solubilize the VR, in terms of releasing elements to the medium. As shown in fig. 2, in the presence of the bacterium, the medium was enriched in 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 contents increased linearly along the incubation time. At 49 d, the medium containing bacteria had approximately 150 mg L^{-1} K while the control treatment contained 30 mg L^{-1} K (Fig. 2b). The amount of K leached to the medium with bacteria corresponded to 6.6 % of the total K present in VR (Fig. 2a).

Incubation of VR with bacterial cells promoted a great increase in Al concentration in the culture medium within the first 7 dai (from 4.33 to 20.6 mg L^{-1}) (Fig. 2d), and reached 118.5 mg L^{-1} at 49 dai. At this time, the amount of released Al corresponded to 5.8 % of the total Al content of the VR, while in the control treatment the amount of Al released represented only 0.1 % (Fig. 2c).

The amount of Fe in the culture medium (Fig. 2f) 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 in the last time interval, corresponding to 14.1 % of the total present in the VR (Fig. 2e). For Si, the culture medium had 23.2 and 128.1 mg L^{-1} at 49 dai for control and inoculated treatments, respectively. The amount of released Si in the medium with bacteria after 49 dai was about of 1.7 % of the total contained in the rock (Figs. 2g, h).

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 concentrations of H, K, Al, Fe, Si and S were all positive, of which that between Al and S was the most significant. During the incubation time, the release of Al, Fe and Si showed a trend similar to that of K (Figs. 2). The coefficients of simple linear correlations among the concentrations of these elements with the K concentration were higher than 0.9.

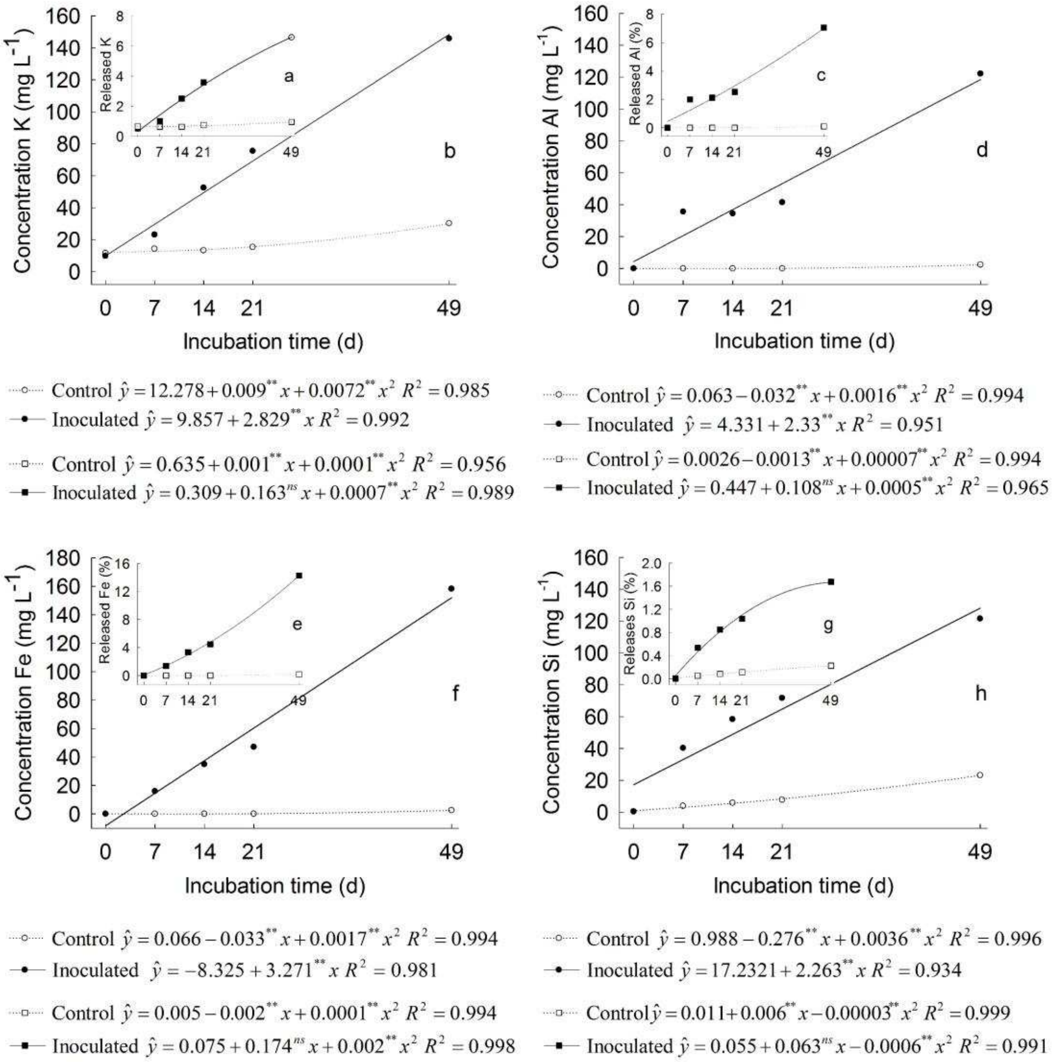

Figure 2 – Concentration and amount the Releasing of K, Al, Fe and Si during the solubilization of a K-silicate rock (Verdete rock) in the absence (control) and presence of *Acidithiobacillus thiooxidans* (inoculated). (b, d, f, g) Concentration of K, Al, Fe and Si in the culture medium. (a, c, e, g) K, Al, Fe and Si (%) represents the amount of leached theses elements in relation its total amount in the rock.

There was an expressive reduction of the bacterial population in 7 dai, due to the bacterial adaptation to the acid conditions of the culture medium. The pH of the medium was adjusted to the value of 4.5 so as not to have a high acidity condition of the medium at the beginning of the experiment. Thus, after 7 d of incubation, due to the metabolic activity of the bacteria, the pH value of the culture medium was reduced to an optimum range for bacterial growth, and an exponential growth was observed in the later incubation times (Fig. 3).

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* teste ($p < 0.01$)

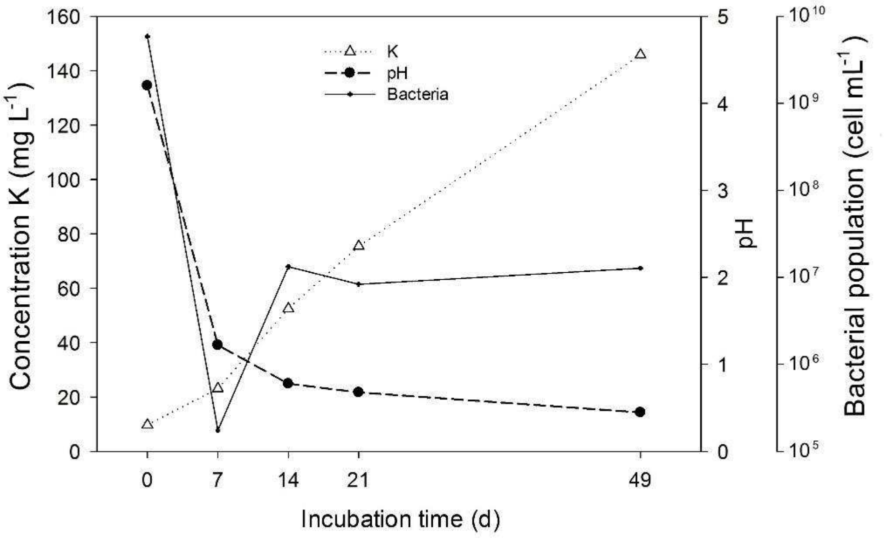

Figure 3 – Evolution of K content in leaching solution, pH and number of bacterial cells along the incubation time of a K-silicate rock (Verdete rock) with *Acidithiobacillus thiooxidans*.

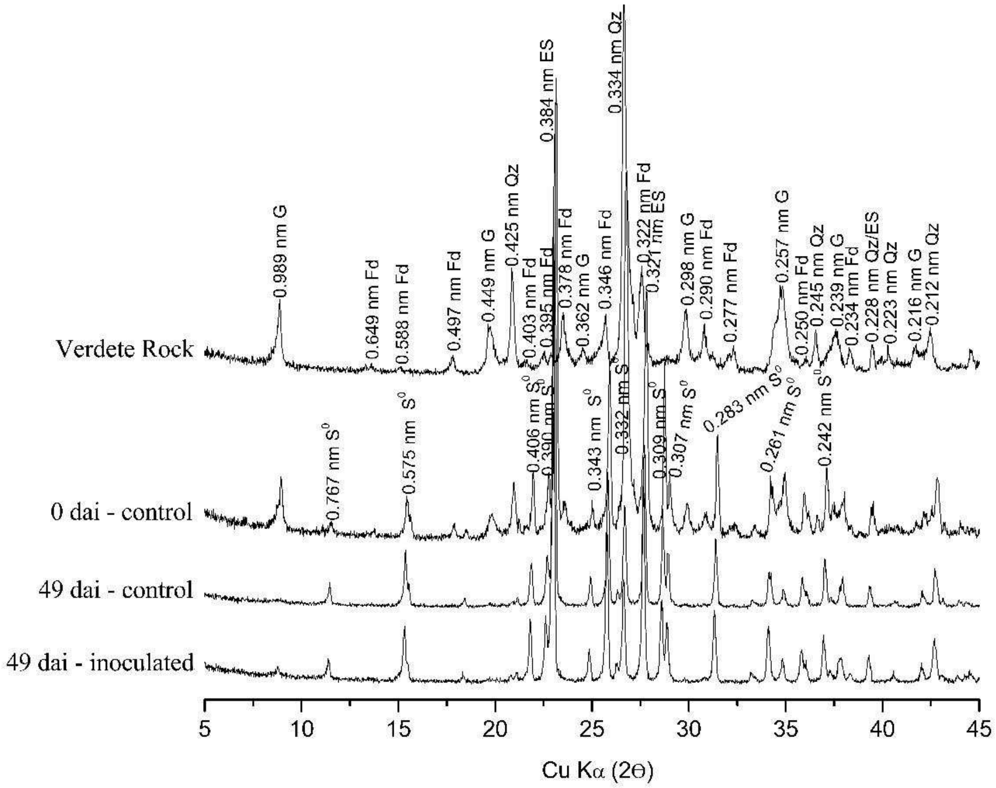

Figure 4 – X-Ray diffraction patterns (XRD) of samples of a K-silicate rock (verdete rock) incubated in the absence (control treatment at 0 and 49 d after incubation, dai) and presence (49 dai) of *Acidithiobacillus thiooxidans*. Data were collected using Cu $K_{\alpha 1}$ radiation (1.54 Å). G: glauconite, Qz: quartz; Fd: feldspar; S^0: elemental sulfur.

Mineralogical analysis showed similar changes in DRX spectra for VR samples in absence and presence of *A. thiooxidans* at 49 dai. All Bragg peaks of glauconite, feldspar and quartz, related to dimension 001c and located between 5-35 degree (2θ) disappeared, except for the peaks at 0.334 nm of quartz and 0.257 nm of glauconite that exhibited differences in their intensities. In addition, ES peaks were detected, which was expected since it was supplied in the medium (Fig. 4).

Discussion

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 acid is produced (Watling, 2015). In fact, as observed in our study, the occurrence of this reaction, catalyzed by *A. thiooxidans*, promoted a decrease of pH as a consequence of proton production, and the release of soluble S forms to the medium.

The acidity produced by the metabolic activity of acidophilic bacteria, like *A. thiooxidans,* has the capacity to attack mineral structures (Sand, 1996), promoting their decomposition. The collapse of minerals can cause leaching and generate both soluble or insoluble species of structural elements (Terry, 1983). In our case, using the Visual Minteq freeware for chemical equilibrium modeling, we observed that the ion concentrations in the medium did not produce an oversaturated solution that would promote precipitation of K, Al, Fe, Si or S minerals.

The increasing concentration of K, Al, Fe and Si in the culture medium over the incubation time evidences a partial solubilization of VR promoted by bacterial activity. Moreover, our results suggest that these elements come from less crystalline minerals and by partial dissolution of crystalline minerals present in VR. In fact, XRD analysis of samples from either treatments, 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 the bacteria (see Fig. 3). Our results show that K solubilization was around 7 % of the total K in VR subjected to bacterial catalytic activity. Considering the known dependence of microbial growth rate on temperature and aerobic conditions, and the effect of leaching on removal of dissolution products, the percentage of K solubilization from VR is expected to be much higher in bioleaching reactor systems.

The main K mineral found in VR is glauconite (Piza et al. 2010; Santos et al., 2015), a mica that belongs to the illite`s group, which exhibits a considerable isomorphic substitution of Al^{3+} by Fe^{2+} in an 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 been shown that the 001 (c) dimension of this mineral is the most susceptible to thermal (Santos et al., 2017) or chemical attack (see Fig. 5). 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. 3), although in different proportions (See Figs 3 c, e and g). 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 at tetrahedral and octahedral structures.

The bond energy of K, Al and Fe with O (1.3; 7.2; 3.5 MJ mol^{-1}, respectively) are significantly weaker than that between Si and O (13.2 MJ mol^{-1}) (Hugins and Sun, 1946). The higher Si-O bond energy makes it less susceptible to acid attack, causing the silicate groups to be removed intact from the mineral surface. Thereby, these groups can form active complexes with protons in solution (Crundwel, 2014). Also, the release of Si to 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 at which its polymerization is optimum (Terry, 1983). It is likely that this is a reason why we found very low Si concentration 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 the 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 time. Glauconite, the main mineral constituent of VR, is formed in a slow sedimentation marine environment, under reducing conditions. During this process, loss of Al and silica, along with enrichment of Fe and K minerals occurs (Fassbender 1975; Lima et al. 2007).

The biotechnological process presented here is an alternative to recover elements, such as K, Al, Fe and Si from silicate minerals, such as micas or feldspars. For K, it may represent a strategic way to produce K fertilizer in countries where there are not enough reserves of soluble K-minerals, with environmental and cost advantages. However, additional studies using leaching bioreactors aiming at recovering higher amounts of elements and higher concentrations of soluble species are required. Also, experiments involving other microorganisms potentially capable of promoting the dissolution of silicate minerals are necessary.

Conclusions

A join of our data demonstrates that acidification of the culture medium, caused by the catalytic activity of the bacterium *Acidithiobacillus thiooxidans,* promotes the partial dissolution of VR, increasing the concentration of K, Al, Fe and Si in solution throughout the incubation time. Biotechnological studies aiming at separating and concentrating the element species of the leaching solution must be conducted in order to exploit this technology of low energy consumption.

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