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Phosphorus recovery from high concentrations of low-grade phosphate rocks using the biogenic acid produced by the acidophilic bacteria *Acidithiobacillus thiooxidans*

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ABSTRACT

In this paper, we are presenting a two-step biological process to recover phosphorus solubilizing low-grade phosphate rocks. We start with a "growing" stage, where the biogenic acid produced by the acidophilic bacteria *Acidithiobacillus thiooxidans* endows the proper conditions for solubilization. It is followed by a "recovery" stage, where the phosphate rock is added to the culture once desired levels of acidity are reached. We varied the concentration of the phosphate rock and the bacterial growing time to elucidate optimal conditions and to get insight into the biological process. We found that 10% (w/v) of phosphate rock with 23 days of bacterial growth results in a recovery of 94%. FTIR spectrums and XRD-Rietveld quantification reveal that the high phosphorus solubilization is correlated with gypsum as the predominant phase, whereas, for less efficient conditions, a significant proportion of fluorapatite, predominant in the un-treated rock, is still observed. As a general trend, increasing the bacterial growing time results in higher amounts of phosphorus recovery. However, the biogenic acid is unable to efficiently solubilize rock concentrations higher than 20% due to an inhibition in the diffusion-controlled reaction. We present a one-to-one comparison with a chemical acidulation using sulfuric acid. The biogenic acid shows better performance and achieves higher percentages of recovery at all conditions, thereby suggesting that additional metabolites in the *A. thiooxidans* culture enhance the bioleaching. Finally, we delineated an economical viability that indicates that the chemical recovery is four times more expensive than the biogenic method. From a technological perspective, our process offers fertile grounds for non-conventional and efficient phosphorus recovery in tropical soils or for low-grade phosphate rock reservoirs.

1. Introduction

Phosphorus is an essential nutrient and major player during metabolism required for the synthesis of nucleic acids, proteins and adenosine triphosphate (ATP). The only useful biological state of phosphorus is its inorganic form found in phosphate minerals. In agriculture, soils must be constantly supplemented with phosphorus to ensure desirable yields and to compensate the intake from the livestock and crops (Chien and Menon, 1995; Scholz et al., 2013). This is especially crucial in tropical soils where phosphorus is not available due to complexation with aluminum and iron ions (Zapata and Roy, 2004). *Phosphate rocks* are one of the preferable sources of phosphorus to fulfill its deficiency; this imprecise term covers unprocessed rocks and beneficiated concentrates. Its global production reaches 160 millions of tons a year, from which 72% corresponds to non-renewable deposits in Morocco, China, the United States of America and Russia (Van Kauwenbergh, 2010). In Colombia, there are estimated reserves of 367 million metric tons with an average 21% content of P_2O_5; however, the majority of the phosphate fertilizers are imported since the local production is unable to satisfy its demand (Union Temporal GI. Georecursos, 2005). The phosphate rock from Colombia has high contents of impurities that could interfere with the acidulation process. Perhaps the most significant are the carbonates, and their effect can be estimated using the CaO/P_2O_5 ratio (Van Kauwenbergh, 2010). Phosphate rock from Huila, Colombia has a ratio of $CaO/P_2O_5 > 1.6$, and it is defined as low-grade rocks, thereby increasing costs during a conventional chemical phosphorus recovery. On the other hand, biotechnology offers novel pathways and avenues to achieve agriculture sustainability through non-conventional phosphorus resources and methodologies.

Some of the past studies have focused on *in-situ* solubilization of soil

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phosphorus using solubilizing microorganisms, typically bacteria or fungus that are usually isolated from the soil (De Oliveira Mendes et al., 2014; Delvasto et al., 2008; Kaur and Sudhakara Reddy, 2014; Reddy et al., 2002). This process enhances the assimilation of nutrients by plants but is not applied in the processing of the raw material. For the direct treatment of phosphate rocks as a material, previous efforts have been made to solubilize phosphate rocks using acidophilic bacteria; in these studies, the biogenic acid, produced by bacteria like *Acidithiobacillus ferrooxidans* (Chi et al., 2007, 2006, 2009), *A. thiooxidans* (Bhatti and Yawar, 2010; Konishi et al., 1995) or *Leptospirillum ferrooxidans* (Priha et al., 2014), was used to acidulate the phosphate rock. In the majority of these works, low amounts of phosphate rocks were used and the solubilization followed a conventional "one-step" process, where the solubilization is done simultaneously to the bacterial growth. Similar to past reports (Bhatti and Yawar, 2010), we have shown that the acidulation of phosphate rock is not possible with a one-step process when *A. thiooxidans* is used because the bacteria is not viable in the presence of the rock. Consequently, we have established that a "two-step" process, growing-then-recovery, is the alternative to achieve good performance and efficiency during phosphate rock solubilization.

In this work, we are presenting the adequate processing conditions to acidulate high concentrations of phosphate rocks using the biogenic acid produced by *Acidithiobacillus thiooxidans*. To build up the arguments behind our solubilization process, we expose the biological problems in the "conventional" one-step process and we describe how the two-step, growing-then-recovery, process allows a reliable phosphorus recovery. We include a consistency study where the growing bacterial time and the phosphate rock concentration are modified in order to elucidate optimal biotechnological conditions for high recovery. We compare the results from our two-step process with a conventional chemical acidulation and found that the biogenic acid shows a better performance and efficiency, suggesting that there are additional mechanisms promoted by the microorganisms. We use XRD Rietveld quantification and FTIR analysis to provide a detailed material mineralogical evolution. We found that the higher percentage of solubilized phosphorus was achieved when 10% (w/v) of phosphate rock was added to the biological solution after a bacterial growth time of 23 days. Finally, an economical viability reinforces the two-step biological process as the most suitable method when compared with the chemical acidulation.

2. Materials and methods

2.1. Phosphate rock

The phosphate rock was purchased from Fertipáez S.A. mine "La Juanita", located in Huila, Colombia. The rock is a pulverized phosphorite with a particle size less than 0.149 mm for 85% of the mineral. The chemical composition of the phosphate rock is: 19.9 wt% P_2O_5, 43.4 wt% CaO, 19.7 wt% SiO_2 and 17 wt% others, including Al_2O_3, Fe_2O_3, MgO, Na_2O, SO_3, F and H_2O. Notice that the CaO/P_2O_5 ratio is high, resulting in a low-grade rock (Van Kauwenbergh, 2010). The material was characterized by FTIR and XRD to determinate the mineral and chemical composition of the sample; the XRD is included in the Supplemental Information (SI).

2.2. Microbial growth

The *Acidithiobacillus thiooxidans* strain is the ATCC 19377; it was obtained from the American Type Culture Collection (ATCC), U.S.A. The modified medium 9 k (Silverman and Lundgren, 1959) is used as the growth medium with composition (per liter) of 3.0 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.1 KCl and 0.001 g $Ca(NO_3)_2{\cdot}5H_2O$. The media was modified, supplementing it with elemental sulfur and adjusted to an initial pH of 2.5 with H_2SO_4.

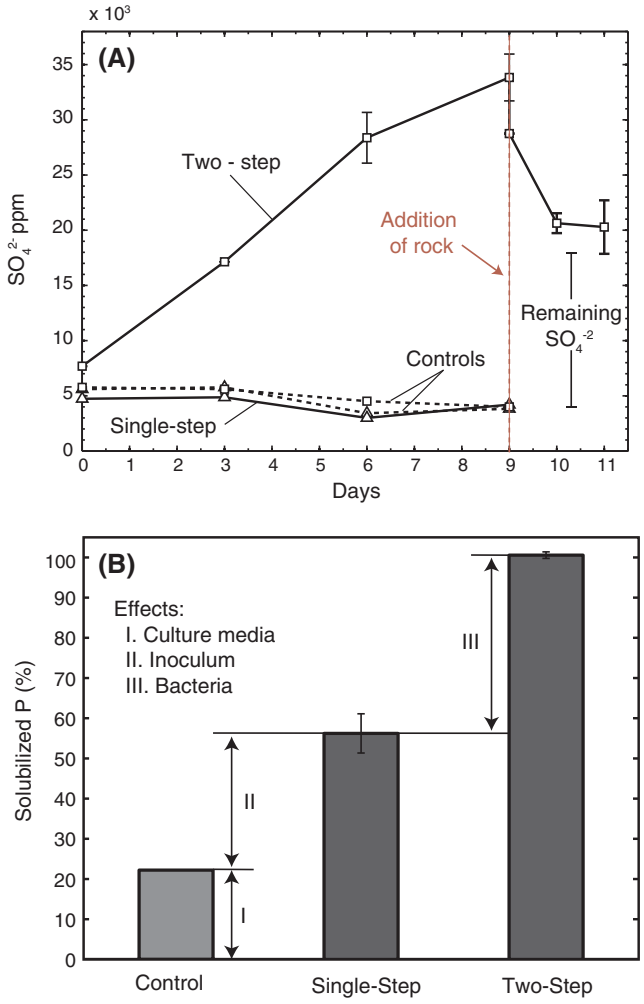

Fig. 1. Comparison between the single-step and the two-step phosphorus recovery processes. **(A)** Evolution of the concentration of sulfates (SO_4^{2-}) in solution during the single-step (growing-and-recovery) and a two-step (growing-then-recovery) process using for 1% (w/v) of phosphate rock. The rock is added after 9 days in the two-step process. **(B)** Percentage of solubilized phosphorus from the 1% (w/v) phosphate rock. Sterile controls are included for both methods.

2.3. Leaching experiments

The leaching experiments were carried out in quadruplicate, divided in two blocks (for statistical proposes), using a 500 mL Erlenmeyer flask containing 190 mL of modified 9 k medium, supplemented with 4% (w/v) of elemental sulfur and inoculated with 10% (v/v) of *Acidithiobacillus thiooxidans*. The strain was previously activated on elemental sulfur. The sterile controls had the same volume and conditions but without the inoculum. The flasks were incubated in a rotating orbital shaker at 180 rpm and 30 °C. The bacterial growing times were 17 (t_1), 23 (t_2) and 29 (t_3) days. After the growing stage, the phosphate rock was added in four different concentrations (w/v): 10%, 15%, 20% and 25%. The flasks were placed in the rotating orbital shaker during three days after the addition of the rock. For the analysis, aliquot samples of 5 ml were taken every three days and every day after the phosphate rock was added; they were analyzed for pH, free sulfuric acid and the content of soluble phosphorus. For the conventional chemical acidulation, sulfuric acid at the same acidity as the biogenic acid was used (from Fig. 3: t_1 starting at pH 0.32, t_2 starting at pH 0.25, t_3 starting at pH 0.21), and the experiments were carried out using the same phosphate rock concentrations with a residence time of one day.