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Recycling Influence in Phosphorus Biossolubilization from Rock Phosphate Concentrate in Air-Lift Bioreactor

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The nutrients availability for the plants is a determining factor for their development and growth. Phosphorus is an essential compound to plants, it is necessary to perform photosynthesis, respiration, gene transfer and reproduction. An alternative mechanism to the use of chemical fertilizers for increasing the cultures productivity on the soil phosphorus deficiency is the use of biotechnology by mainly biological processes occurring in the system soil/plant realized by phosphorus solubilizing microorganisms (PSM). Therefore, the objective of this study was to evaluate the phosphorus biossolubilization process from phosphate rock concentrate by *Trichoderma harzianum* in air-lift bioreactor employing reactive medium recycle. During 6 days was evaluated the phosphorus biossolubilization process by *Trichoderma harzianum* in air-lift reactor employing ascending air 0.5 vvm (volumetric flow per minute) and reaction medium recycle containing phosphate rock concentrated (5 g/L). Phosphorus biossolubilization by *Trichoderma harzianum* in air-lift reactor only with upward aeration (0.5 vvm) showed maximum solubilization equal to 212.19 ppm (27.23%), followed by 98,18% of glucose consumption and total acid concentration equal to 1.25 µmol/ml (0.5 µmol/ml of lactic acid, and 0.73 µmol/ml of acetic acid) in the fifth process day at 28 ± 0,5°C. By employing reaction medium recycle flow of 200 mL/min in air-lift reactor with aeration of 0.5 vvm at 28 ± 0.5°C, initial cell inoculum concentration equal to 3.5 ± 0.5 g/L and 5 g/L of phosphate rock concentrate and glucose was obtained on the third day of process 244.9 ppm (31.42% solubilization) of soluble phosphorus. By increasing the volumetric flow rate of recycle to 400 ml/min maintaining the same conditions, in the fourth day was reached 216.1 ppm (27.73% solubilization) of soluble phosphorus and 8.2 µmol/ml of organic acids (lactic and acetic). Comparing the results achieved between the three phosphorus biossolubilization process from phosphate rock concentrate in air-lift reactor by *Trichoderma harzianum* was observed that by employing recycle of reactive medium at 200 mL/min associated with compressed air flow at 0.5 vvm was obtained for a shorter period (3 days) a higher concentration of phosphorus soluble in a liquid medium (244.9 ppm). Indicative of the positive influence of the reactive medium recycle in the phosphorus availability process by *Trichoderma harizianum* from the production of organic acids using glucose as substrate.

1. Introduction

The cerrado biome is a large Brazilian ecosystem characterized by soils with low pH and low phosphorus concentration (P) (Marschner, 1995; Novais, Smyth, 1999; Hinsinger, 2001; Wakelin et al. 2004). One of the main problems related to nutrient absorption by the plants is due to the fact that a large portion of soluble inorganic phosphate applied to the soil as compost, is rapidly immobilized in the forms of insoluble phosphate.
The accumulated insoluble phosphorus occurs either in organic or inorganic forms, unavailable to plants. Phosphate anions can be immobilized by means of precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al3+. The organic phosphorus in the soil, usually represents about 50% of the total insoluble phosphorus present in soils with high organic matter content (Bayer et al. 2001; Gyaneshwar et al. 2002). An alternative mechanism to increase crop productivity in the face of soil phosphorus deficiency is the use of biotechnology,

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mainly the biological processes that occur in the soil / plant system performed by phosphorus solubilizing microorganisms (PSM) (Aquino, Correia, 2005). The production of inorganic and organic acids, and/or the decrease of pH by the phosphate solubilizing microorganisms stand out as the important mechanisms of solubilization (Gyaneshwar et al. 2002). Microorganisms must be constantly involved in environments suitable for growth as an air-lift bioreactor (Cerri et al. 2008).
Species of fungi of the genus *Scutellospora*, *Gigaspora* and *Entrophospora* (D'souza and Rodrigues 2013), *Paenibacillus polymyxa*, *Paenibacillus macerans* (Wang et al. 2012) have all presented phosphorusbiossolubilizationpotential. In this work, the originality andbiossolubilizationcapacity of the Trichoderma harzianum microorganism in the process of phosphorusbiossolubilizationfrom phosphate rock concentrate in an air-lift reactor is studied.The synthesis of products through biotechnological processes makes it possible to discover routes to obtain products with low environmental impact (Lunelli et al. 2011). Therefore, the main of this work was to evaluate thebiossolubilizationprocess of phosphorus from phosphate rock concentrate in air-lift bioreactor by *Trichoderma harzianum* using recycle of the reactive medium.

2. Materials and Methods

2.1 Phosphate rock concentrate

The phosphorus source used in this work was the phosphate rock concentrate (fluorapatite), containing 35.7% of P_2O_5, supplied by the Tapira Mining Complex (Vale Fertilizantes), Minas Gerais.

2.2 Microorganism and culture medium

The microorganism employed was isolated/collected at the Araxá Mining Complex (Vale Fertilizantes), Minas Gerais. The fungal isolate was identified as *Trichoderma harzianum* by biochemical tests of conventional taxonomy, by the André Tosello Foundation for Research and Technology (Campinas-SP). The solid selective synthetic medium used for *Trichoderma harzianum* culture on petri plates had the following composition (g/L): Dextrose (10); Agar (15); Yeast extract (0.5); Tricalcium phosphate (5); Magnesium sulphate (0.1); Ammonium sulphate (0.5); Ferrous sulphate (0.0001); Manganese sulfate (0.001) Potassium chloride (0.2) (Pikovskaya, 1948).

Figure 1.Air lift reactor

2.3 Phosphorus biossolubilization process

The influence of reaction medium recycle on the process of solubilization of phosphorus in an air-lift reactor (Figure 1) was analyzed using a volumetric flow rate of compressed air per minute equal to 0.5 vvm. The volumetric flows of recycle medium studied in air-lift reactor were equal to 200 and 400 mL/min. The reaction medium used in the volume of 2500 mL of air-lift reactor was composed of Glucose (5 g/L), yeast extract (0.5 g/L), phosphate rock concentrate (5 g/L), magnesium sulfate (0.1 g/L), ammonium sulfate (0.5 g/L), ferrous sulfate (0.001 g/L), manganese sulfate (0.001 g/L), potassium chloride g/L). The phosphorus biossolubilization process was evaluated in air-lift reactor with and without reaction medium recycle during the period of 6 days, followed by the concentration of metabolites and phosphorus soluble in air-lift reactor operating at 28 ± 0.5°C. The temperature was controlled by an Optherm HAAKE DC3 thermostatic bath and the recycle of the liquid medium by an Easy-load Masterfle Model 7518-00 peristaltic pump.

The soluble phosphorus was quantified in the liquid extracts (liquid medium) according to the procedure described in APHA - AWWA - WEF 4500 - P Phosphorus (1998). The concentration of organic acids were performed by HPLC (High Performance Liquid Chromatography), Shimadzu brand LC-20A Prominence, SUPELCOGEL C-610H column in which the components are separated and detected by light refraction. Phosphoric acid (0.1%) was used as the mobile phase, with flow rate of 0.5 mL / min, oven temperature 32°C and injection volume of 20 µL.

3. Results and Discussion

By studying the phosphorus biossolubilization from phosphate rock concentrate in air-lift bioreactor using only compressed air at 0.5 vvm of volumetric flow, the results of soluble phosphorus and organic acids concentration, presented in Figure 2, were obtained. Employing the reaction medium recycle of 200 and 400 mL/min, the results of soluble phosphorus and organic acids concentration obtained and are shown in Figures 3 and 4, respectively. The organic acids (lactic and acetic) presented maximum concentrations on the third day of process (3.84 and 4.4 µmol/mL, respectively), reaching 8.24 µmol/mL of organic acids (pH 5.08) and 131.70 ppm (16.90% of solubilization) of soluble phosphorus.

By employing there reaction medium recycle of 200 mL/min (Figure 3), the maximum soluble phosphorus concentration was equal to 244.9 ppm (33,78% phosphorus biosolubilization), on the third day of the process, followed by a maximum total concentration of organic acids (9,01 µmol/mL, pH 4.87). By increasing the reaction medium recycle to 400 mL/min (Figure 4) a total concentration of organic acids (acetic and lactic acid) equal to 8.2 µmol / mL and pH 5.08 was observed in Figure 2 on the third day of operation of the air bioreactor -lift followed by the concentration of 216.10 ppm of soluble phosphorus (biossolubilization of 27.73%).

The values found in this work were higher than the values found (13-31 ppm) by Wahid and Mehana, (2000) and Abd-alla and Omar, (2001) studying *Aspergillus niger* and phosphate rock. Comparing the presented results it can be noticed that the use of recycle of the reaction medium in air-lift reactor increased in 64.08% the biossolubilization of phosphorus from phosphate rock concentrate on the third day of operation of the bioreactor.

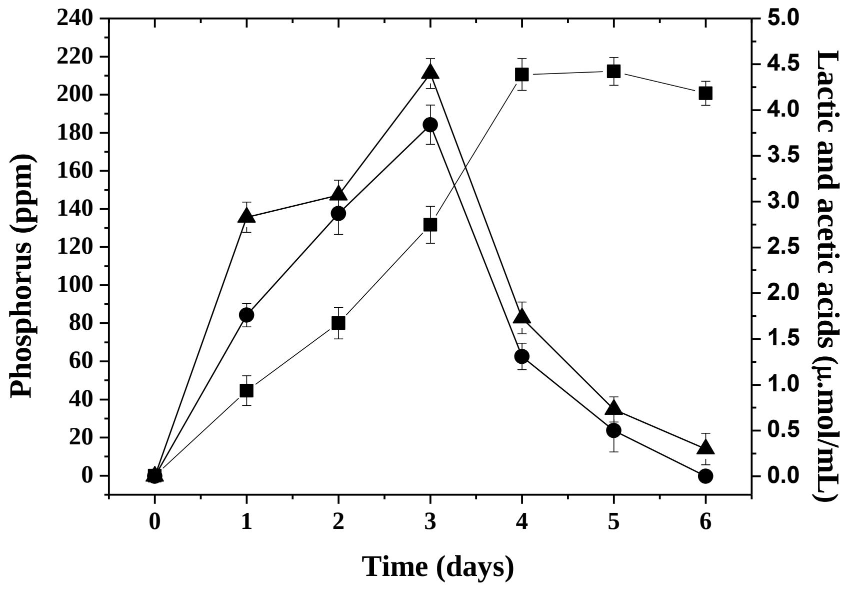

Figure 2. Concentration of phosphorus (■) and organic acids (lactic acid: acetic acid: ▲) in air-lift reactor at 28 ± 0.5°C (glucose concentration: 5 g/L, initial cell concentration: 3. 8 ± 0.5 g/L, phosphate rock concentrate: 5 g/L, 0.5 vvm)

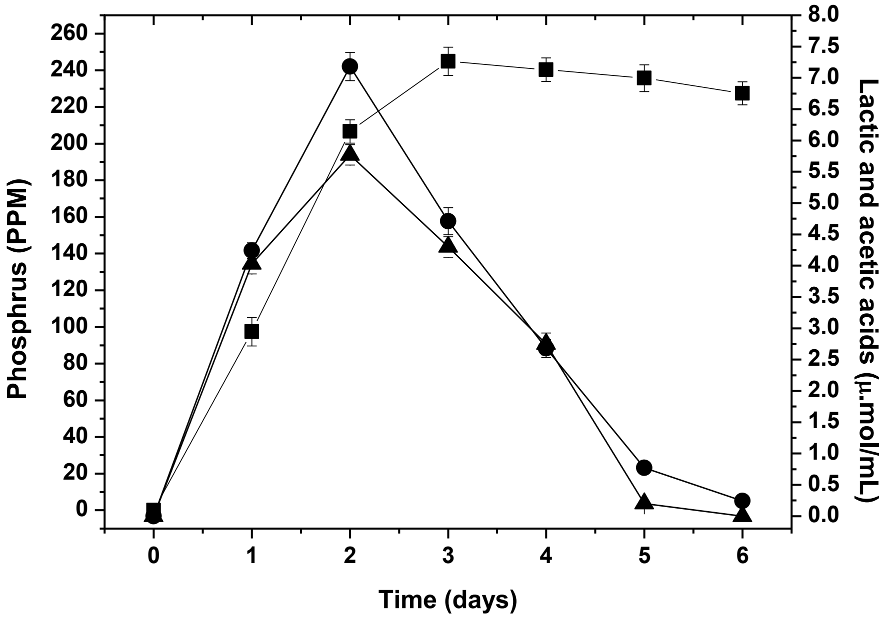

Figure 3. Concentration of phosphorus (■) and organic acids (lactic acid: ●, acetic acid: ▲) in air-lift reactor at 28 ± 0.5°C (glucose concentration: 5 g/L, phosphate rock concentrate: 5 g/L, initial cell concentration: 3.5 ± 0.5 g/L, 0.5 vvm, reaction medium recycle: 200 mL/min)

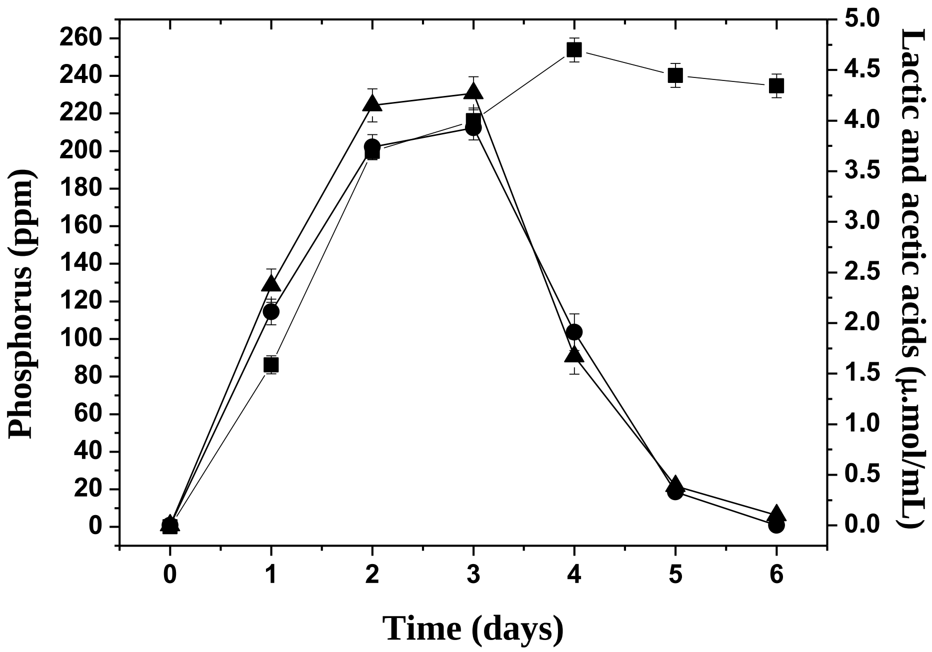

Figure 4. Concentration of phosphorus (■) and organic acids (lactic acid: ●, acetic acid: ▲) in air-lift reactor at 28 ± 0.5°C (glucose concentration: 5 g/L, phosphate rock concentrate: 5 g/L, initial cell concentration: 3.5 ± 0.5 g/L, 0.5 vvm, reaction medium recycle: 400 mL/min)

The phosphorus biossolubilization process can be verified by the evaluation of acid concentration in the reactive medium and the hydrogen ion potential. According to the work of Souchie et al. (2007) when evaluating phosphate solubilization by rhizospheric microorganisms in different soil classes, an inverse relationship was observed between the pH of the medium and the amount of soluble P. Some fungus isolates increased the soluble P content above 180 ppm with pH values below 2.0, while some bacteria, although some isolates showed high acidification potential, reduced pH to 3,0, solubilized the equivalent To only 1.8 and 9.0 ppm of P. Of these 15 fungal isolates identified, 14 belonged to the genus Aspergillus and one to the genus *Penicillium*.

The values found by Souchie et al. (2007) presented lower than those found in the present study when studying the recycle flow 400 mL/min (216.1 ppm) until the third day of operation of the air reactor, followed by organic acids concentration equal to 3.93 µmol/mL of lactic acid and 4.27 µmol/mL of acetic acid.

Working with different phosphate sources ($AlPO_4$, $Ca_3(PO_4)_2$ and Catalão and Araxá phosphate rock), Mendes et al. (2014) obtained expressive results with some fungal species.

Penicillium canescens (FS23) produced gluconic acid in all treatments and citric acid in treatments with AlPO4, Ca3(PO4)2 and Araxá phosphate rock. Concentrations between treatments varied from 25 to 1242 ppm of gluconic acid, and from 25 to 813 mg/L of citric acid. *Eupenicillium ludwigii* (FS27) and *Penicillium islandicum* (FS30) produced only gluconic acid. Gluconic acid production was partially inhibited by $FePO_4$ treatment for the two isolates. In the presence of $AlPO_4$, $Ca_3(PO_4)_2$ and the phosphate rocks, *E. ludwigii* (FS27) accumulated the highest amounts of this acid among all the isolates, reaching 3 g/L in the $Ca_3(PO_4)_2$ treatment. Mendes et al. (2014) found organic acids in considerable concentrations for different sources of phosphate using also different fungal species, different from this work, thereby justifying the different acids produced.

4. Conclusions

The biossolubilization process presents a promising alternative to the use of chemical fertilizers. By evaluating the biossolubilization process of phosphorus from phosphate rock concentrate by *Trichoderma harzianum* in an air lift reactor, a higher concentration of organic acids can be observed in the third day of the process. Followed by a higher phosphorus biossolubilization using a reaction medium recycling equal to 200 mL/min, showing that the recycling of the reaction medium could positively influence the process of phosphorus release.

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