ISSN: 2634 - 8845

Journal of Earth and Environmental Sciences Research

SCIENTIFIC Research and Community

Review Article

Open Access

Phosphorus Biosolubilization by Micro-Organisms

Glalber Luiz da Rocha Ferreira[1]*, José Daniel Gonçalves Vieira[2] and Nelson Roberto Antoniosi Filho[3]

[1]Department of Microbiology, Institute of Tropical Pathology and Public Health, Federal University of Goiás-UFG. Goiânia, Goiás / Brazil. Rua Delenda Resende de Melo Left /1. Avenida S/N Laboratory of Environmental Microbiology and Biotechnology, University Sector, CEP 74605-050.

[2]Department of Microbiology, Institute of Tropical Pathology and Public Health, Federal University of Goiás-UFG. Goiânia, Goiás / Brazil. Rua Delenda Resende de Melo Left /1. Avenida S/N Laboratory of Environmental Microbiology and Biotechnology, University Sector, CEP 74605-050.

[3]Institute of Chemistry, Laboratory of Extraction and Separation Methods. Federal University of Goiás-UFG. Goiânia, Goiás/Brazil. Campus Samambaia, CEP 74690-900.

ABSTRACT

Phosphorus (P) is one of the essential macronutrients for the growth and reproduction of plants, the main source of which is phosphate rocks (PRs). In view of the extraction process of this mineral, major environmental impacts are characterized, with the generation of solid residues that represent great economic value when using appropriate techniques for using minerals in solid residues, representing an extractive process with better efficiency and utilization of the extracted amount. There are many possible techniques applicable to a better yield in the extractive process, for example, in particular biosolubilization, which makes use of different microorganisms in the solubilization of the phosphorus element. In this review article, different microorganisms will be presented in the phosphorus solubilization, as well as the knowledge of the chemical and biological characteristics of the microorganisms in the biosolubization process.

***Corresponding author**

Glalber Luiz da Rocha Ferreira, Department of Microbiology, Institute of Tropical Pathology and Public Health, Federal University of Goiás-UFG. Goiânia, Goiás / Brazil. Rua Delenda Resende de Melo Left / 1. Avenida S / N Laboratory of Environmental Microbiology and Biotechnology, University Sector, CEP 74605-050. Tel: (62)99113-9140; E-mail: glalberocha@gmail.com

Received: November 19, 2020; **Accepted:** November 25, 2020; **Published:** November 28, 2020

Keywords: Biosolubilization, Phosphorus, Microorganisms, Solubilization, Phosphate Rocks.

Introduction

Inevitably, the challenges of the environmental issue permeate all activities related to industrial processes. And that is why it is possible to affirm that mining, added to agriculture, forestry, energy production, transportation, civil construction (urbanization, roads, etc.) and basic industries (chemical and metallurgical) are considered the causes of almost all the environmental impact on Earth [1].

In highlight, the mining activity in Brazil, asserts itself with great socio-economic prominence for the country, contributing decisively to the well-being and improving the quality of life of present and future generations, which is fundamental for the development of an egalitarian society, which is through social responsibility, always considering the precepts of sustainable development. It is important to recognize and keep under control the impacts that this activity causes on the environment, and thus, providing an adequate environment for future generations [2].

According to the Mineral Yearbook of Brazil, the main substances extracted in the Brazilian territory are: iron; limestone, titanium, phosphate / phosphate rock, aluminum (bauxite); gold; tin; copper; zirconium; niobium; kaolin; manganese; nickel and zinc [3]. Among the minerals mentioned above, phosphate rock stands out, data presented by the United States Geological Survey - USGS indicate that, in 2019, the global production of phosphate rock was 240 million tons, with China responsible for half of the production of 2019, worldwide [4].

As presented to USGS, referring to the years 1996 to 2019, corresponding to data from 24 years of phosphate rock extraction process, 129,950,000 tons of concentrated phosphate in the form of phosphorus pentoxide (P_2O_5) were produced in the mines of Brazil). As shown in Figure 1, it is possible to observe a progression in the increase in the production of P_2O_5 in mines in Brazil, and thus, accounting for a significant increase of 47.22% from 1996 to 2019, which is suggestive to an average of 5,414 , 6 103 tonnes of P_2O_5 produced in Brazil. In Figure 2, there is a clear verification of a fluctuation in the indices of P_2O_5 production capacity by reserves in Brazil, with a significant drop in the production capacity of P_2O_5 from 2016, reaching a drop of 48.48%, being this corresponding to the period from 1996 to 2019.

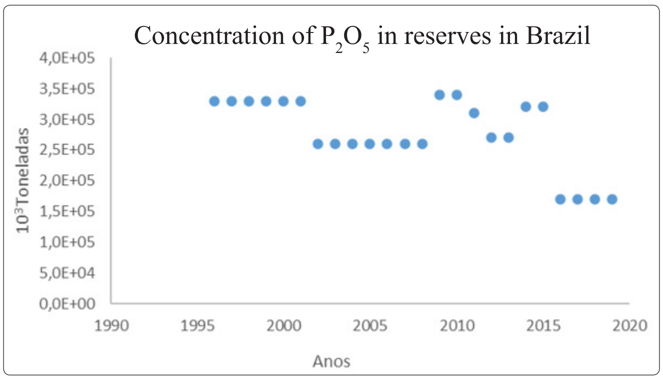

Figure 1: Graphical representation of the concentration of P_2O_5 in reserves in Brazil from the information generated from the USGS database (2020).

In Brazil, the largest deposits of phosphorus (P) are associated with alkaline-carbonatitic complexes, of igneous origin, and this is due to processes of weathering, as seen in Complexes de Salitre, in which they have a low content of P, when compared to deposits of sedimentary origin, such as marine phosphorites, and deposits associated with alkaline complexes, presenting a marked mineralogical complexity [5].

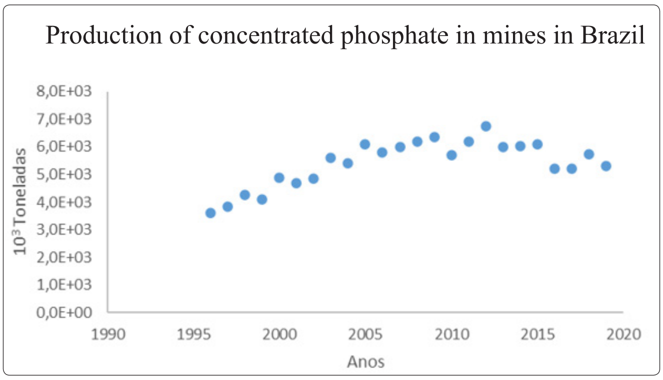

Figure 2: Graphical representation of the production of concentrated Phosphorus in mines in Brazil from information generated from the USGS database (2020).

In the various mining activities, large volumes and masses of materials are extracted and moved, causing great environmental impacts, as in the generation of solid waste, known as sterile and tailings. Sterile waste is waste generated by extraction or mining activities, in the stripping of the mine, considered as material with no economic value and is usually disposed of in piles. Waste, on the other hand, is waste resulting from the treatment and processing of mineral substances [6].

There is also another type of waste, consisting of a very diverse set of materials, such as the waste containment dam, from the operation of the extraction and processing plants for mineral substances. The amount of waste generated is always very large and depends, among other factors, on the process used to extract the ore and the location of the deposit in relation to the surface [7]. The mineral substances contained in solid waste have the potential for use in other industrial activities or in agriculture [8].

The P contained in phosphate is not considered a toxic element to the health of living beings, being, on the contrary, essential to the maintenance of life. The harmful presence of P in the environment is related to the excessive intake of water. In this case, the nutrient can trigger processes of eutrophication, or fertilization, causing, for example, the proliferation of algae and other higher aquatic plants, the macrophytes [9]. The increase in eutrophication levels may lead to a decrease in the quantity and quality of water and a loss of the system's sustainability capacity, with a consequent increase in the level of toxicity and deterioration of human health [10]. According to the analysis of current technological expectations, the mining sector has not stood out as a sector with high standards of innovation, even if it uses high technology equipment, and for this reason, the mining industry has not been able to achieve its goal. full use of the elements of nature, and this is due to fallible methods of the mining process [11]. In view of this situation, it is necessary for the mining companies to face the search for new challenges in the search for innovative behavior, given the market pressures, and thus, needing new technologies that are more productive, with less losses in the extraction process and productive and at the same time are less expensive than current technologies [12].

In view of this, several techniques, being physical, chemical and biological, have been developed for the solubility of toxic elements and mineral metals. Therefore, the solubility of phosphorus can be achieved by several physical-chemical processes, among them, by methods considered conventional, more that result in many disadvantages, such as high cost, low metal removal and high demand for reagents, and can still be ineffective, especially when dealing with large volumes and low concentrations [13,14].

As a solution to the problem itself, it is possible to mention the biosolubilization technique as a promising alternative, as it is a low-cost process, with high removal efficiency, capable of regeneration, high selectivity in relation to different metallic species, high recovery capacity metal, in addition to being less aggressive to the environment [15]. And as an advantage, the biosolubilization technique consists of the detoxification of the site, or even the removal of contaminating elements from the soil by biomass through active microorganisms, which have the ability to modify or decompose such toxic elements and mineral metals [16].

Highlighted, biosolubilization makes use of several microorganisms in their active function, such as bacteria, fungi, yeasts and cyanobacteria, which have specific underlying mechanisms necessary for biosolubilization, being dependent on several factors such as the chemical composition of the cell wall of the biosorbent , the physical-chemical conditions of the external environment, and finally, the chemical properties of the metal [15].

Match

Phosphorus (P) is an essential element for all living organisms, considered an essential macronutrient, being present in nucleic acids, phospholipids, lipopolysaccharides, as well as in several cytoplasmic solutes, as well as in the energy metabolism of animated beings, as in the cycle of ATP-ADP, storage, expression of genetic information (DNA and RNA), and bone structure, in addition to actively participating in the process of growth and bodily support of plants and animals. Comparing phosphorus with other chemical elements, it is considered irreplaceable [17].

Considering in world terms, P is found in the rocks of deposits of sedimentary, igneous and biogenetic origins. In terms of development and maintenance of plant growth, P is generally disposed in the form of P_2O_5, being largely responsible for generating energy for plant production [18]. Among the existing minerals, phosphorus is one of the most scarce resources in the world, besides being one of the main as a limiting factor for several cultures in different types of soils. The mineral phosphorus in its soluble form is considered highly reactive with the elements calcium, iron, aluminum, as well as clays, organic matter and carbonates, and thus, leading to the formation of complexes with high insolubility, that is, they become unavailable for plants [19].

The main source of phosphorus are the so-called phosphate rocks (PRs), which represents an imprecise term, because the phosphate rocks are available in unprocessed and concentrated forms, that is, in the benefited form, which is considered the main economically viable source. phosphorus, being expressed as P_2O_5. In Brazil, it is known that 80% of the phosphate deposits are of igneous origin with considerable content of P_2O_5, representing a percentage of about 17% worldwide [20,21].

Elemental phosphorus is not found available in nature, but it spontaneously and easily combines with the chemical element oxygen, forming phosphorus pentoxide (P_2O_5), and that when combined with water, phosphoric acid is formed. P_2O_5, occupies the eleventh place in the order of abundance of elements in the igneous rocks of the earth's crust [17].

In Brazil, most of the soil is classified as oxisols, which has high depth, highly degraded, highly drained and strongly acidic characteristics. In this type of soil there is a dominance of oxides, hydroxides and oxy-hydroxides of the elements iron (Fe) and aluminum (Al), which easily facilitate adsorption with phosphate, occurring the formation of iron and aluminum phosphates [22].

Table 1: Presentation of bacteria, fungi, intercropping between bacteria and intercropping between bacteria and fungi in the solubilization of the chemical element phosphorus

Microorganism	Genre	Amount of solubilized P	Efficiency (%)	Reference
Bactéria	*Pantoea. agglomerans* ZB	1247,78 mg L^{-1}	24,95	Li et al., 2020
	Enterobacter sakazakii	1728,00 mg L^{-1}	79,00	Mouradi et al., 2017
	Pseudomonas pseudomallei	1432,20 mg L^{-1}	77,00	
	Pseudomonas cepacia	1272,10 mg L^{-1}	60,00	
	Bacilus megaterium	2,757 mg L^{-1}	55,14	Carmo et al., 2019
	Bacilus licheniformis	2,730 mg L^{-1}	54,60	
	Pseudomonas fluorescens	1,395 mg L^{-1}	27,90	
Fungos	*Aspergillus niger*	75,79 mg L^{-1}	40,53	Xiao et al., 2011
	Aspergillus japonicus	66,18 mg L^{-1}	35,39	
	Aspergillus simplicissimum	85,43 mg L^{-1}	45,68	
	Fusarium moniliforme	11,54 mg L^{-1}	3,59	Abdel-Ghany et al., 2019
	Aspergillus chevalieri	24,40 mg L^{-1}	7,60	
	Trichoderma harzianum	28,40 mg L^{-1}	8,85	
	Trichoderma asperellum	11,92 mg L^{-1}	95,39	Kribel et al., 2019
Consórcio bacteriano	*Serratia liquefaciens*	TCP – $Ca_3(PO_4)_2$ – 715 µg mL^{-1} TRP – fosfato de rocha da Tunísia – 605 µg mL^{-1}	TCP – 71,60 TRP – 92,42	Zineb et al., 2020
	Pseudomonas spp			
	Pseudomonas koreensis			
	Pseudomonas corrugata			
	Bacillus safensis			
	Burkholderia spp			
	Pseudomonas frederiksbergensis			
	Bacillus circulans			
	Bacillus	299,0µg L^{-1}	29,0	Emami et al., 2020
	Pseudomonas			
	Staphylococcus			
	Paenibacillus			
	Stenotrophomonas			
	Sphingobacterium			
	Lysinibacillus			
	Advenella			
	Enterobacter			
	Variovorax			

	Plantibacter			
Consórcio entre bactéria e fungo	*Rhodospirillales*	153,00 mg L^{-1}	86,10	Xiao et al., 2017
	Rizobiales			
	Esfingebacteriana			
	Esfinge			
	Lactobacillales			
	Burkholderiales			
	Rhodocyclales			
	Flavobacterianos			
	Caldilineales			
	Xanthomonadales			
	Bacillales			
	Nitrosomonadales			
	Rodobacterales			
	Planctomycetales			
	Clostridiales			
	Saccharomycetales			
	Hypocreales			
	Ofiostomatales			
	Bacillus sp	370 µg mL^{-1}	18,53	Saxena et al., 2015
	Aspergillus niger			

Biosolubilization

Many studies show that microorganisms could solubilize chemical elements considered insoluble, and convert them into soluble form, and among the techniques mentioned by the bioremediation process, the biosolubilization technique arises, which can be explored through environmental biotechnology in the removal of contaminants of soils, sediments and industrial residues [21].

In the search for environmentally sound technologies for the mining industry, biological processes such as biosolubilization are seen as one of the most promising and certainly the most revolutionary solutions for solving different types of environmental problems, mainly applicable in mining industries [23].

Considering the biosolubilization process, it is seen as a biological treatment carried out by microorganisms, which, through their metabolic reactions, generate chemicals such as mineral acid, organic acids, polymers and enzymes. These chemical by-products attack the tailings contained in the ore, promoting its dissolution and producing its selective removal [24].

There are several microorganisms that have been used with considerable efficiency in the solubilization of various chemical elements, such as bacteria, fungi, or even when associated, as shown in Table 1, when bacteria, fungi, intercropping of bacteria and consortium of bacteria and fungi in phosphorus solubilization.

Bacteria

Under optimized culture conditions Li et al., carried out studies of phosphorus solubilization from the bacterium Pantoea [25]. agglomerans ZB, the same obtained from the rhizospheric soil of Araucaria that grows on the beach of the Qingshan River in the capital Wuhan, province of Hubei in China, and thus, reaching a maximum solubilization of 1247.78 mg L^{-1} of phosphorus, which corresponds at a 24.45% efficiency of biosolubilization.

According to studies by Mouradi et al., strains isolated from the El Halassa phosphate deposit identified as Enterobacter sakazakii, Pseudomonas pseudomallei and Pseudomonas cepacia, showed a maximum capacity to release soluble phosphate from $Ca_5 (PO_4)_3 (OH)$ in concentrations of 1728 ± 0, 5, 1432.2 ± 1 and 1272.1 ± 0.5 mg Pi L^{-1} respectively, corresponding to the efficiency values of 79%, 77% and 60% respectively in the applied phosphorus biosolubilization technique [26].

Carmo et al., in its phosphorus biosolubilization studies, proceeded to its solubilization tests in an air lift bioreactor from phosphate rocks, using three separate strains, Bacilus megaterium, Bacilus licheniformis and Pseudomonas fluorescens, who responded with a biosolubilization of 2.757 mg L^{-1}, 2.730 mg L^{-1} and 1.395 mg L^{-1}, respectively of phosphorus, corresponding to an efficiency of 55.14%, 54.60% and 27.90% of phosphorus solubilization [21].

Fungi

In the studies developed by Xiao et al., three fungal strains were used, Penicillium simplicissimum, Aspergillus niger and Aspergillus japonicus and, which were isolated from the rhizospheric soil of wheat, which strains demonstrated the phosphorus solubilization capacity of the order of 85.43 mg L^{-1}, 75.79 mg L^{-1} and 66.18 mg L^{-1}, respectively, on the seventh day of incubation, and thus, corresponding to an efficiency of 45.68%, 40.53% and 35.39% of phosphorus solubilization, respectively [27].

Fusarium moniliforme, Aspergillus chevalieri and Trichoderma harzianum, obtained in isolation from rhizospheric soil grown with Sorghum bicolor L., are fungi that were used by Abdel-Ghany et al., aiming at the solubilization of inorganic phosphorus, obtained a maximum biosolubilization of P of 11.54 mg L^{-1}, 24.40 mg L^{-1} and 28.40 mg L^{-1}, respectively, meaning in this order an efficiency of 3.59%, 7.60% and 8.85% in phosphorus solubilization [28].

Kribel et al., in his studies carried out the isolation of the Trichoderma fungus from the soil with roots adjacent to the Moroccan phosphate mines, which tested qualitatively in vitro as to its potential to solubilize phosphorus from phosphate rock, being positive the development in Modified Pikovskaya Agar (MPA) medium, containing the phosphorus analyte, with a good radial growth greater than 79.8 mm being observed in the period of 6 days, even if there was no clear zone around the colony, which is characterized as a potential phosphorus solubilizer [29]. The fungus demonstrated the ability to solubilize a maximum concentration of 11.92 mg L^{-1} of phosphorus, referring to an efficiency of 95.39% of P. biosolubilization.

Microbial consortium

There are many different types of phosphorus-solubilizing microorganisms, as mentioned in Table 1, as well as bacteria and fungi, make phosphorus insoluble in soluble, by the function called phosphorosolubilizer (PS), which can be easily absorbed by plants [30]. There are few studies that report the use of the microbial consortium in biosolubilization studies, therefore, little is known about its solubilization mechanisms, making it difficult to carry out comparative reports for new studies [31].

Bacterial consortium

In the studies developed by Zineb et al., the bacteria Serratia liquefaciens, Pseudomonas spp, Pseudomonas koreensis, Pseudomonas corrugata, Bacillus safensis, Burkholderia spp, Pseudomonas frederiksbergensis and Bacillus circulans, were used in consortium in the phosphate solubilization process in phosphate (TCP) samples Ca_3 (PO_4) 2, and Tunisia rock phosphate (TRP), containing 30% P_2O_5. The results presented by Zineb, demonstrate a solubilization of 715 µg mL^{-1} of phosphorus in the TCP sample, and 605 µg mL^{-1} of phosphorus in the TRP sample, equivalent to an efficiency of 71.60% and 92.42%, respectively [23].

The bacterial consortium of Bacillus, Pseudomonas, Staphylococcus, Paenibacillus, Stenotrophomonas, Sphingobacterium, Lysinibacillus, Advenella, Enterobacter, Variovorax and Plantibacter, target of Emami et al., revealed the solubilization of 299 µg mL^{-1} of phosphorus from soil samples of wheat root and rhizosphere (Triticum aestivum L.), indicating a 29% efficiency of phosphorus solubilization [32].

Consortium between bacteria and fungus

Xiao et al., in his study made use of a microbial consortium obtained through the isolation and further enrichment made from the activated sludge, collected from a municipal wastewater treatment plant in Edmonton, Canada [19]. The researchers used a microbial consortium composed of (Bacteria) Rhodospirillales (8.73%), Rizobiales (8.13%), Sphingebacterial (7.57%), Sphinx (6.70%), Lactobacillales (5.30%) , Burkholderiales (5.22%), Rhodocyclales (3.6%) 2%), Flavobacterials (3.10%), Caldilineales (2.79%), Xanthomonadales (2.71%), Bacillales (1.96%)), Nitrosomonadales (1.95%), Rodobacterales (1.95%), Planctomycetales (1.72%) and Clostridiales (1.56%), at the order level, and (Fungi) Saccharomycetales (39.46%) , Hypocreales (0.51%) and Ofiostomatales (0.18%) at the order level. The results obtained by Xiao's group obtained a solubilization of 153.00 mg L-1 of phosphorus, revealing an efficiency of 86.10%.

The scientists Saxena et al., the bacterial and fungal consortium worked on their study, reporting the use of Bacillus sp and Aspergillus niger in the solubilization of phosphorus, in function of the growth and promotion of the chickpea, which was reported a greater increase in the length of the shoot and the root of 43.73 and 13.96 cm in the plant, respectively, being also observed a maximum solubilization of phosphorus by the consortium around 370 µg mL^{-1}, corresponding to the efficiency of 18.53% solubilization of the chemical element phosphorus, after the second day of incubation [33].

Phosphorus biosolubilization

The solubilization of phosphorus (P) compounds by microorganisms, called P-solubilizers which are naturally abundant in soils, are effective in releasing inorganic P through solubilization by organic functions of the microorganism, driven by insoluble phosphorus mineralization. present in the soil and availability to the plant [25]. It is also known that the soil's microbial biomass contains a significant amount of immobilized phosphorus, which is potentially available to plants [28].

However, there are several theories proposed that explain the mechanisms of P solubilization by microorganisms, which are organized into three groups, the first referring to the theory proposed by Cunningham and Kuiack, based on the function of organic acids; the second contemplates the sink theory, defended by Halvorson, keynan and Kornberg; and finally the third contribution by Illmer and Schinner, which explains about the theory of acidification and excretion of H +. Of these, the organic acid theory is the one that best explains the phosphorus solubility mechanism and is the most accepted theory by the majority of the scientific community worldwide [27,34-36].

In the theory of organic acid, the insoluble sources of P are solubilized by bacteria and fungi P-solubilizers promoting the lowering of the pH, that is, increasing the acidity of the reaction medium. The decrease in pH in the reaction medium suggests the release of organic acids by microorganisms [37]. Such organic acids can directly dissolve the mineral phosphorus, this as a result of the anionic exchange of PO_4^{2-} by acid anion, or even, it can chelate the Fe and Al ions associated with P. In this synthesis, the discharge of organic acid by strains occurs microbials becoming acidic and the surrounding environment, which ultimately leads to the release of P ions from mineral P by replacing H + with Ca^{2+} [38]. Of the different organic acids involved in the solubilization of insoluble P, the most prominent are released by fungal strains, such as: succinic, citric, glophonic, α-acetogluconic and oxalic acids [39].

In the dissipation theory proposed by Halvorson, keynan and Kornberg, P-solubilizing microorganisms assimilate phosphorus from the liquid medium, activating the indirect dissolution of calcium phosphate compounds by consistent removal of phosphorus from the culture medium [35]. The dissipation theory generally elucidates solubilization in the form of mineralization of organic P compounds in which the P content in the microbial biomass is consistently correlated with the decomposition of organic substrates.

The ability of enzymes made available by microorganisms to perform a desired function in the rhizosphere is a crucial aspect for their effectiveness in plant nutrition. And these enzymes play an important role in the release of phosphorus through organic compounds in the soil, such as: i. nonspecific phosphatase from dephosphorylation of organophosphate or phosphoanhydride bonds of organic matter; ii. Phytases, responsible for specifically causing the release of P from phytic acid, and finally iii. phosphonatases and C-P lyases, and the latter causes the cleavage of the C-P bond of organophosphonates [37].

According to Rodriguez et al., the organic depletion of phosphorus by the plant in the rhizosphere has been compensated by the absorption of phosphorus by plants, through the efficiency of microbial phosphatases, which are released outside the cell and participate in the dissolution and mineralization of organic phosphorus compounds [40]. Among phosphatases, acid phosphatase stands out, which is commonly found in fungi, being detected in vacuoles, vesicles and other intracellular and extracellular fungi organs.

Another significant application of phosphorus-dissolving enzymes is the solubilization of organic phosphorus present in the soil through the degradation of phytate, which is mediated by the action of the phytase enzyme [39]. According to Richardson et al., it is reported in their studies that the ability of plants to obtain phosphorus directly from phytate is very limited, but that when phytate is supplied the plant shows a significant improvement in plant growth and nutrition, leading to an increase in phosphorus nutrition to such an extent that the growth and phosphorus content of the plant were equivalent to the control of plants supplied with inorganic phosphorus [41].

Final considerations

The study consisted of assessing different microorganisms such as bacteria, fungi, consortium of bacteria, and consortium of bacteria with fungi, applicable to the phosphorus solubilization technique, since it is insoluble in the soil, and thus, being solubilized by the biosolubilization process. As well as understanding the chemical and biological functions of microorganisms in the process of biosolubilization, to improve the technique, and aiming at higher yields and efficiency in the phosphorus biomineration process [42].

Thanks

The authors would like to thank the National Council for Scientific and Technological Development (CNPq) for their financial support.

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