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Phosphorus Microbial Solubilization as a Key for Phosphorus Recycling in Agriculture

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“We may be able to substitute nuclear power for coal, and plastics for wood, and yeast for meat, and friendliness for isolation—but for phosphorus, there is neither substitute nor replacement”.

Isaac Asimov, The Relativity of Wrong, 1975

Abstract

The chapter describes the phenomenon of microbial solubilization carried out both by bacteria and fungi in the natural environment. The mechanism of dissolving phosphorus compounds by microorganisms, the importance of phosphorus solubilization for its accessibility to plants, as well as the possibility of using microbial solubilization in the process of valorization of secondary raw materials were discussed in this chapter. It was underlined that by using the biofertilizers, we can be able to reduce the amount of artificial fertilizer needed for cropping. Moreover, few models were mentioned to describe this phenomenon to express the changes observed during the solubilization process.

Keywords: microbial solubilization, phosphorus fertilizers, biofertilizers, soil microorganism, *Bacillus*

1. Introduction

The need for mitigation of dependence on phosphate rock of fertilizer industry is one of the main issues to be solved in the next years. Current trends in agriculture are focused on enhancing the efficiency of fertilizer use since approximately 50% of applied mineral fertilizers are lost from the plant-soil system through gaseous emissions, runoff, erosion, and leaching [1]. Green revolution aims to enhance crop yields, improving soil fertility through better management practices, breeding crops with greater tolerance to edaphic stresses and by the development of new inputs based on optimization of the biological/microbiological process [2].

Fertilizer	Content of phosphorus (as P_2O_5) %, w/w	pH	Retrogradation
Single superphosphate (SSP)	18	Neutral	Fast
Granulated superphosphate	19	Neutral	Fast
Superphosphate "SuperMag"	14	Neutral	Fast
Triple superphosphate (TSP)	46	Neutral	Slow
Triple superphosphate granulated, boronated	44	Neutral	Slow
Phosphate rock flours	10–30	Neutral	Slow
Ammonium polyphosphate (APP)	34	Slightly acidic	Fast
Diammonium phosphate (DAP)	46	Slightly acidic	Fast

Table 1. Characteristic of standard phosphorus fertilizers.

Phosphorus is one of the main microelements in plants nutrition, applied to the soils in the form of phosphorus fertilizers that are mainly produced from phosphate rock. In **Table 1**, the most popular form/types of phosphorus fertilizers used nowadays were presented.

Many initiations were raised during past decade to increase the awareness of need for developing the recycling of phosphorus in the industrial scale. The European Union in 2014 (EU) has included phosphorite within the list of 20 critical raw materials [3] since the EU countries are largely dependent on their import. Moreover, recycling of phosphorus from sewage sludge and slaughter waste has been mandatory in Switzerland, Germany, and Austria. Since Switzerland banned the direct application of sewage sludge onto the soil in 2006, its regulation will result in technical recovery and recycling in the form of inorganic products [4].

There is a growing need for ecological engineering approaches that go beyond phosphorus retention to create pathways for phosphorus recovery and recycling, supporting both eutrophication control and food recovery and recycling with ecological engineering [5].

Phosphate rock plays the main source of phosphorus in the chemical industry and its resources are finite and only five countries mainly control its nonrenewable reserves: Morocco, China, Algeria Syria, and Jordan. Since Europe is highly dependent on phosphate rock import, PR was listed within the critical raw materials as it is assessed against its economic importance and its supply risk. At the first stage, phosphate rock is used to produce phosphoric acid, either by treatment with a furnace or with a sulfuric, phosphoric, or nitric acid to produce phosphoric acid. Obtained phosphoric acid is then used in the production of different kinds of fertilizer mainly by the reaction of phosphate rock with phosphoric acid. Since the phosphate rock is critical "spot" in fertilizer industry, many efforts have been done to find alternative sources of phosphorus or/and more sustainable methods of its production by the utilization of more environmentally friendly approach [6].

According to many specialists, its deposits are scarce and according to the most optimistic forecast, geographically, economically, and logistically available resources would be finished within the 150 years. At the same time, there are large amounts of phosphate available in waste streams

from, for example, agriculture, sewage treatment, and from industrial side streams, which pose an increasing problem. To avoid wasting these in, for example, landfills, and to counteract the depletion of natural phosphate sources, routes for reuse are explored [7]. In few European countries, the recycling of phosphorus starts to be obligatory (e.g., Austria and Germany). According to the document published on December 17, 2012, the “European Commission has no choice but to go for the transition to a resource-efficient and ultimately regenerative circular economy.”

Although the phosphate content of waste streams is usually lower than the typical P_2O_5 content of phosphate rock which is 30–40% (=13–17.5% P), it is necessary to start to apply the circular economy within the phosphorus fertilizers industry since the agriculture is the main recipient of phosphorus [7].

Phosphorus in the soil is present in organic forms and consists 20–80% of the total. Often, less than 10 μM of phosphorus in soil solution is available to plants, while the mineral fraction of phosphorus from soil can be available to plant, but via the desorption and solubilization process [8]. Integrated soil fertility management (ISFM) is a soil management approach that emphasizes the combined application of organic and mineral fertilizer inputs with the goal of improving yields and fertilizer use efficiency. Both organic and inorganic fertilizers were and are widely applied to sustain soil productivity [9].

2. Microbial solubilization

Unlike inorganic fertilizers, organic nutrients from the plant and animal-based residues are often not readily available to the plant and must be converted into plant available forms by microbes in the substrate.

One of the methods that could be efficiently used as a way to mitigate the phosphorus problem is the utilization of natural microorganisms that in the native environment are able to increase phosphorus availability for its cells as well as for root plants, which is called microbial solubilization. The natural ability of soil microorganism to produce organic acids, enzymes that released into the soil environment, results in increasing the availability of many nutrients that in the soil are present in the retrogradative form such as phosphorus, as well as many micronutrients such as zinc or selenium [10].

Plants can uptake the phosphorus or any other nutrients from the soil when is soluble/present in the soil solution. Phosphorus fertilizer used as a soil conditioner delivered phosphorus in available-to-plant form but dissolved in the soil solution and in the presence of Al(III) and Fe(II), it forms unavailable-to-plant compounds and becomes retrogradative [11]. According to many specialists, the amount of phosphorus retrograde in the soil is so abundant that there is no need to use fertilizers. The solution to cover the requirements of plants would be to “activate” retrogradative form of phosphorus into available, for example, by utilization of soil microorganism. Unavailable phosphorus present in the soil in organic as well as inorganic form is launched via solubilization of inorganic form and mineralization of organic forms of phosphorus [12]. Thus, the residual phosphate fertilizers in the soil can be well utilized and the external application should be optimized. Microbial solubilization is performed by the production of acids that by decreasing pH and attraction of cations from structure that effects with the release of phosphorus [13].

Many products are nowadays available on the market that delivers the soil microorganisms in concentrated forms such as lyophilisate or single bacterial strain or consortium. Such beneficial microorganism is so-called biofertilizers, which are classified as biostimulants. According to the definition proposed by Vessey [14], biofertilizers are substances which contain living microorganisms which, when applied to seed, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant, and promote growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers, regardless of its form, can be applied in three ways (**Figure 1**) that are related to the stage of growth of the plant to be inoculated. Biofertilizers are produced in liquid, powder, and granular forms and applied to soil, compost, seed, seedling, and plant leaves.

Many bioproducts which activity is based on soil microorganism are available on the market (**Table 2**). According to the industrystock.com database, 35 companies were listed as the ones that are related to the production of biofertilizers.

“Microbial consortia,” are in other words biopreparations of various microorganisms, are a beneficial microbial mixture that supports plant nutrition and health, becoming more popular. Especially when combined with organic materials, they can serve as organic fertilizers. Some of the effects of combinations of bioorganic fertilizers were discussed elsewhere [15]. The application of biofertilizers has illustrated that 50% of recommended NPK fertilizers can be reduced through inoculation with microbial consortia with no adverse effects on growth, nutrition, or yield [16]. The mechanisms by which these preparations enhance plant growth are numerous; the main mechanism of solubilization of inorganic phosphorus compounds is the activity of organic acids, siderophores, protons, hydroxyl ions, and CO_2 [17].

In this context, the reduced use of chemical fertilizers with increased application of organic fertilizers is considered a compulsory route to alleviate the pressure on the environment derived from agricultural practice [18]. Such an approach could mitigate the problem of depletion of phosphate rock. Microbial solubilization could be also utilized in the “activation”

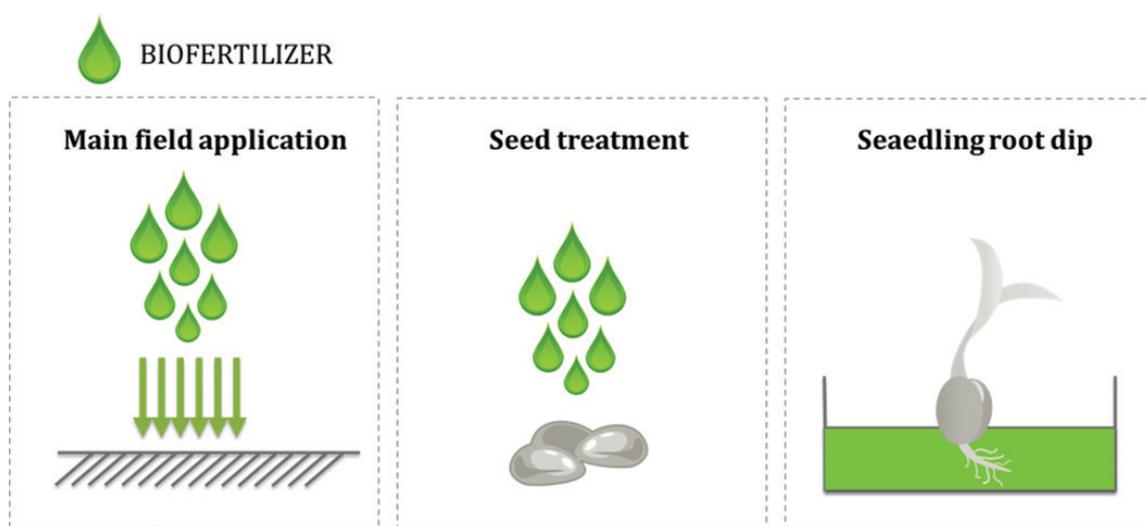


Figure 1. Application of biofertilizers.

No.	Product	Producer	Content	Effect	
1	Bactim receptor	Intermag, Poland	<i>Glomus coronatum</i> , <i>Glomus caledonium</i> , <i>Glomus mosseae</i> , <i>Glomus viscosum</i> , <i>Rhizophagus irregularis</i> , <i>Streptomyces</i> spp., <i>Streptomyces avernichilis</i> , <i>Pochonia chlamydosporia</i>	7.2×10^6 CFU/g	<ul style="list-style-type: none"> • It limits damage to the root system through nematodes. • Increases plant resistance to root system diseases. • Limits the accumulation of harmful substances in plants.
2	Bactim vigor	Intermag, Poland	<i>Glomus coronatum</i> , <i>Glomus caledonium</i> , <i>Glomus mosseae</i> , <i>Glomus viscosum</i> , <i>Rhizophagus irregularis</i> , <i>Bacillus subtilis</i> , <i>Streptomyces</i> spp., <i>Trichoderma harzianum</i> , <i>Trichoderma viride</i> , <i>Pichia pastoris</i>	10.2×10^7 CFU/g	<ul style="list-style-type: none"> • Increases the resistance of field vegetables and fruit trees and bushes to abiotic and biotic stresses. • It improves soil structure and increases the availability of nutrients from the soil. • It improves the quality and postharvest life of crops.
3	Bactim starter	Intermag, Poland	<i>Azospirillum brasilense</i> , <i>Azotobacter chroococcum</i> , <i>Bacillus subtilis</i> , <i>Bacillus megaterium</i> , <i>Pseudomonas fluorescens</i>	2.5×10^{10} CFU/g	<ul style="list-style-type: none"> • Supports seed germination, ensures faster and better plant emergence. • Improves the supply of nitrogen seedlings. • Provides better phosphorus uptake by the young root system.
4	Acetobacter spp. fertilizers	Jay Ambe Agro Products, India	<i>Gluconacetobacter diazotrophicus</i>	1×10^9 CFU/ml	<ul style="list-style-type: none"> • Produces growth promoting substances such as indole acetic acid (IAA) and gibberellins that promote root proliferation and increase the rootlet density and root branching which resulting in increased uptake of mineral and water which promotes cane growth and sugar recovery from the cane.
5	Azospirillum fertilizer	Jay Ambe Agro Products, India	<i>Azospirillum brasilense</i>	1×10^9 CFU/ml	<ul style="list-style-type: none"> • Actively fix atmospheric nitrogen through asymbiotic relation with the leguminous plants.

Table 2. Examples of bioproducts available on the market.

of phosphorus bound in the by-products originated from agriculture, food processing, or from ashes obtained from incineration of different types of wastes that are characterized by high content of phosphorus.

Several technologies for solubilization of phosphorus compounds have been developed that can be described as processes which transform phosphorus into bioavailable forms via a range of chemical reactions and biological interactions [19]. They include phosphate-solubilizing

microorganisms, phosphatase enzymes and enzyme activators, low-molecular-weight organic acids, humic acids, lignin, crop residues, biochar, and zeolites. The goal is to elaborate integrated procedure/P recovery techniques to treat the waste stream to generate a product more suitable as a soil amendment [20].

According to the data evaluated by Koppelaar and Weikard [21], applying phosphorus-solubilizing biofertilizing microorganisms results in 10% reduction of phosphorus fertilizer application. Zabihi et al. [22] demonstrated the possibility to reduce up to 50% of fertilizer application without crop yield reduction under controlled conditions. Unfortunately, results are contrasting between greenhouse and field experiments possibly due to plant-soil interaction knowledge gaps, differences between plant and soil types, and incorrect microorganism strain selection [23].

The efficiency of microbial solubilization can be enhanced by the presence of numerous compounds. Humic substances that comprise a major part of organic matter and their influence on soil properties are well known and could be used to improve microbial activity [24]. Phosphate-solubilizing microorganisms, selected for RP solubilization, combined with humic acid, positively stimulated root and shoot weight compared with noninoculated plants by 17 and 22%, respectively. Despite this biomass increase, no difference was observed in P concentration, indicating an increased P use efficiency. The application of both PSM and HS with RP may be a suitable method for reduction of soluble P fertilizer demands without compromising plant yields [25]. Results described in the literature show a promising use of humic substances to improve the benefit of phosphorus solubilizing microorganism [26]. Zeolites seem to be also one of the materials that have the potential to improve the results of solubilization since it was used as an activator of phosphate rock. The zeolite/rock phosphate combination possibly acted as an exchange fertilizer, with Ca exchanging onto the zeolite in response to plant uptake of nutrient cations enhancing the dissolution of the rock phosphate [27, 28]. Another modification of the solubilization process is its combination with composting what can provide many potential agronomic benefits (e.g., slow-release nutrients) [29].

3. Phosphorus by-products

Waste streams originating from the food industry (e.g., agricultural runoff, stormwater, animal manures, food and food-processing wastes, human urine and feces, municipal wastewater, biosolids) [5] seem to be one of the most interesting sources of phosphorus that could be valorized into valuable products such as fertilizers. Phosphorus in the wastes is present in unavailable-to-plants form and needs to be treated before applied. The methods that are described and successful consider the solubilization of phosphorus compounds present in the phosphate rock [30]. The advantage of this approach is the production of phosphorus fertilizer without the problematic side products such as phosphogypsum but it is not solving the problem with the depletion of phosphate rock which is still the most important raw material for phosphorus fertilizer industry. Another solution is the utilization of solubilization of secondary raw materials such as meat-bone meal [31], ashes of the waste sludge from wastewater treatment plant with the secondary stage of biological treatment process [32], bones and fish bones [33].

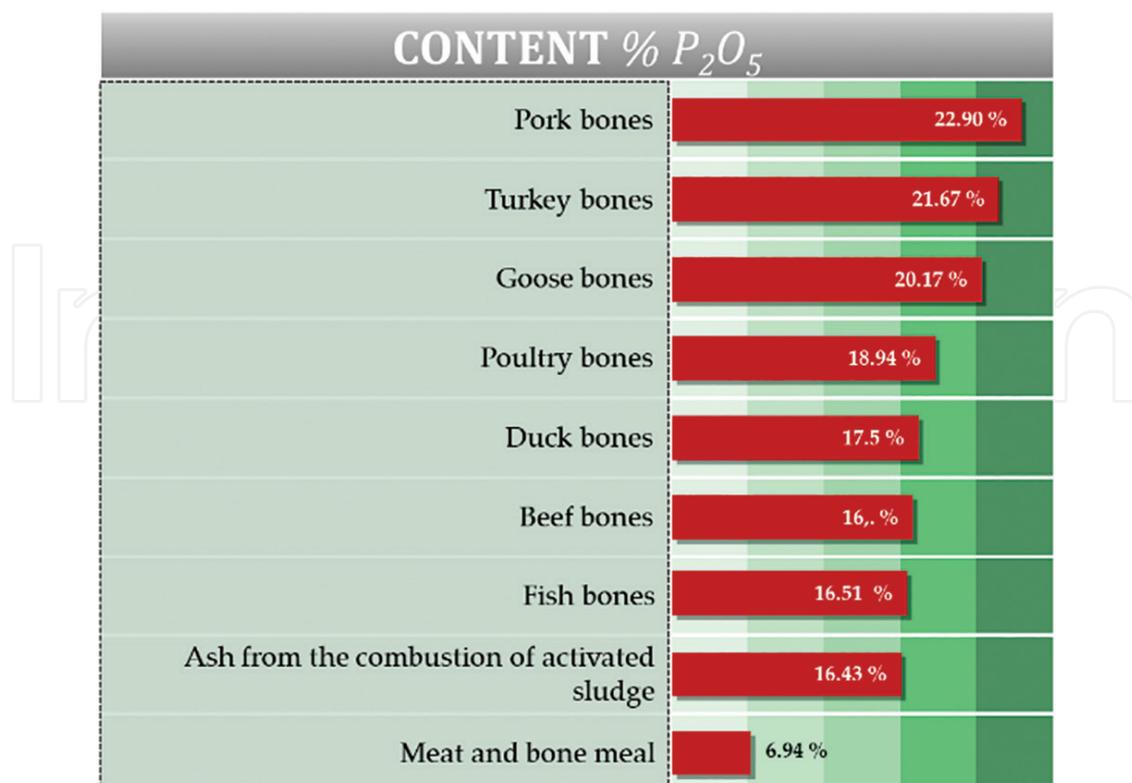


Figure 2. The content of P_2O_5 in P-bearing renewable raw materials.

Phosphorus from phosphate rock that has been utilized in the agriculture and fertilizer or chemical industry is present in the following forms:

- retrogradative form present in soil as a result of not proper fertilization;
- wastes from food production, such as bones and fish bones;
- ashes originated from incineration of activated sludge from wastewater treatment plant or wastes originated from the slaughterhouse.

Mentioned streams of phosphorus seem to be the most concentrated ones, thus have been chosen to be the secondary source of phosphorus in the circular economy strategy [7, 34].

Figure 2 presents the content of phosphorus expressed as P_2O_5 in few phosphorus renewable raw materials; its exact content can significantly vary and is strongly related to the origin, kind of animals, its nutritional status, diet, etc. In **Figure 2**, last bar—meat-bone meal—was added to compare the P_2O_5 content and to prove that it is better to use the original raw materials without treatment since it causes significant phosphorus losses.

The second sector, after the agriculture, that uses phosphorus obtained from phosphate rock is private households. It generates a significant amount of wastewaters; after the treatment in a wastewater treatment plant, almost all phosphorus is removed by chemical and biological treatment methods and is fixed in the sewage sludge. Since its direct application in agriculture is no longer accepted, incineration is undertaken that generated significant amounts of ashes with up to 21% P_2O_5 in the inorganic form [5].

4. Phosphorus biofertilizers based on renewable raw materials

Introduction of beneficial and natural microorganisms in the form of the consortium with confirmed properties of releasing crucial for plant growth nutrients from unavailable forms seems to be the best solution. Such an approach would result in higher yield, better quality of plants as an effect of delivery of nutrient in available forms, without utilization of phosphate rock mining products. In many cases, two, three, or more species of beneficial microorganism acts much more efficient than in the case of the single strain. Some of the sources of phosphorus are rich with organic compounds and when found in the growth medium can be utilized by bacterial cells as a source of nutrient and then the higher growth is observed, such as bones or fish bones. In the case of ashes that deliver the nutrients only in inorganic form since it is deprived of the organic matter, the growth is much lower when compared with bones and fish bones but at the same time ashes are known to have more than 20% of P_2O_5 . A good solution would be to mix organic and inorganic raw materials to ensure at the same time source of valuable nutrient to guarantee the best growth condition and deliver the high dose of phosphorus in the form of ashes. In this case, the enhanced growth of bacterial cells would produce more acids or enzymes that would solubilize the phosphorus from ashes. **Figure 3** shows the stages in the production of phosphorus biofertilizers [35–48].

Nine formulations were obtained based on the single bacterial strain, such as *B. megaterium* or *A. ferrooxidans* as shown in **Figure 2**. Three different raw materials were used as a source of

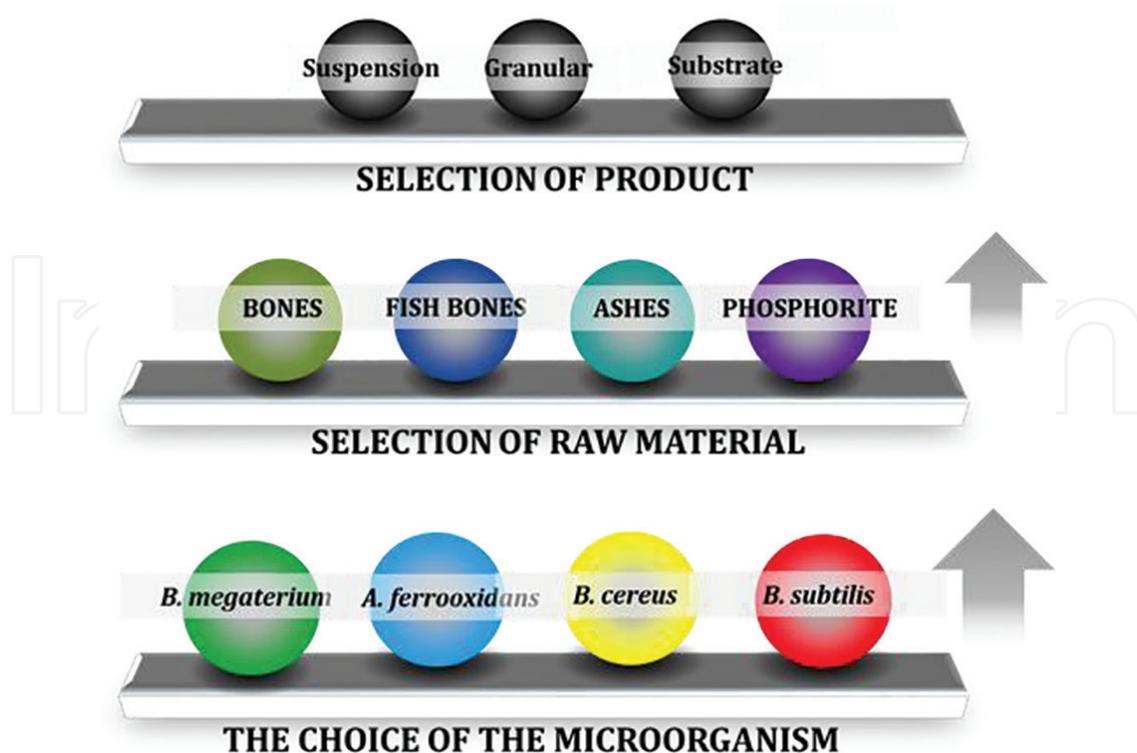


Figure 3. The general scheme of stages in the solubilization.

Fertilizer	Form	Raw material	Bacteria	P ₂ O ₅ , % mass
1	Suspension	Ash	<i>B. megaterium</i>	0.406
2	Suspension	Bones	<i>B. megaterium</i>	0.595
3	Suspension	Ash	<i>B. megaterium</i>	0.406
4	Granular	Ash	<i>A. ferrooxidans</i>	21.2
5	Granular	Ash + bones	<i>A. ferrooxidans</i>	17.2
6	Granular	Ash + bones	<i>B. megaterium</i>	13.5
7	Granular	Ash + blood	<i>B. megaterium</i>	21.9
8	Granular	Ash + blood	<i>B. megaterium</i>	11.3
9	Granular	Ash + blood	<i>B. megaterium</i>	11.3

Table 3. The content of P₂O₅ in biofertilizer formulation.

phosphorus: bones and ash but also blood meal, which was used as a source of nitrogen but at the same time played a role of binder in the granular formulations.

The content/concentration of P₂O₅ in obtained phosphorus biofertilizers based on the *Acidithiobacillus ferrooxidans* and *Bacillus megaterium* was presented in **Table 3**.

Obtained formulations in semitechnical scale were tested in the field test. More details concerning the production process [49] as well as the more detailed results from field tests [50] were described elsewhere.

In terms of the impact on the yield of test plants, phosphorus biofertilizers from renewable raw materials were comparable to commercial fertilizers: moreover, they did not affect adversely on the morphological and physiological features of the test plants; did not affect the degree of weed infestation, infection by fungal pathogens, and lodging of test plants; also, did not affect the humidity, temperature, salinity, and pH of the soil, the total number of heterotrophic bacteria and fungi in the soil, and the presence of earthworms; and did not change the elemental composition of plants and soil. Utilization of biofertilizers based on the *B. megaterium* did not increase the number of *Bacillus megaterium* in the soil, but they were conducive to the stabilization of this strain in the soil environment that is crucial since any pressure affected on the homeostasis of soil environment has an adverse effect [50].

5. Modeling of solubilization

The process of microbial solubilization can be defined as the transformation of insoluble compounds present in inorganic form to a soluble form, and thus available to plants. As a result of the activity of microorganisms, dissolved minerals are formed. The production of organic acids results in acidification of the microbial cell and its surroundings by decreasing the pH. The amount of soluble phosphate released depends on the strength and type of produced acids [51].

Parameters that express and characterize the solubilization are used to evaluate the effectiveness and are as follows: pH, μ (day^{-1}), and $C_{P_2O_5}$. To describe experimental data, a few models were evaluated to understand the changes in the process [43–45].

Acids produced by bacteria caused an increase in the concentration of hydrogen ions and higher release of P_2O_5 . Decreasing pH as a result of production of acids was described in Eq. (1):

$$pH = f(C_{P_2O_5}) = \frac{A + pH_{\min} \cdot C_{P_2O_5}}{C_{P_2O_5}} \quad (1)$$

where A (mg/L) is a constant describing the decay of curve. Evaluated value of pH_{\min} can be interpreted as the minimal value of pH. The P_2O_5 concentration is correlated with the pH of the liquid phase through the following model (**Figure 4**).

To describe the changes in P_2O_5 concentrations during solubilization, the proposed model that describes kinetics of releasing phosphorus (expressed as the P_2O_5) was used (Eq. (2)):

$$C_{P_2O_5} = f(t) = \frac{C_{P_2O_5}^{\max}}{1 + b \cdot \exp^{-kt}} \quad (2)$$

where the $C_{P_2O_5}^{\max}$ (mg/L) is the maximum concentration of P_2O_5 , b is the constant that expresses time when $C_{P_2O_5}$ is equal to $\frac{1}{2}$ of $C_{P_2O_5}^{\max}$, and k (1/day) constant is the variable slope, which is called the Hill slope. When k is greater, the curve changes more sharply and it means that solubilization process proceeds faster (**Figure 5**).

In case the solubilization is conducted in the columns in the *in situ* studies, the concentration of released phosphorus expressed as the amount of P_2O_5 is monitored versus the amount of remaining in the column that was strongly correlated with the pH of the liquid phase through Eq. (3), (**Figure 6**):

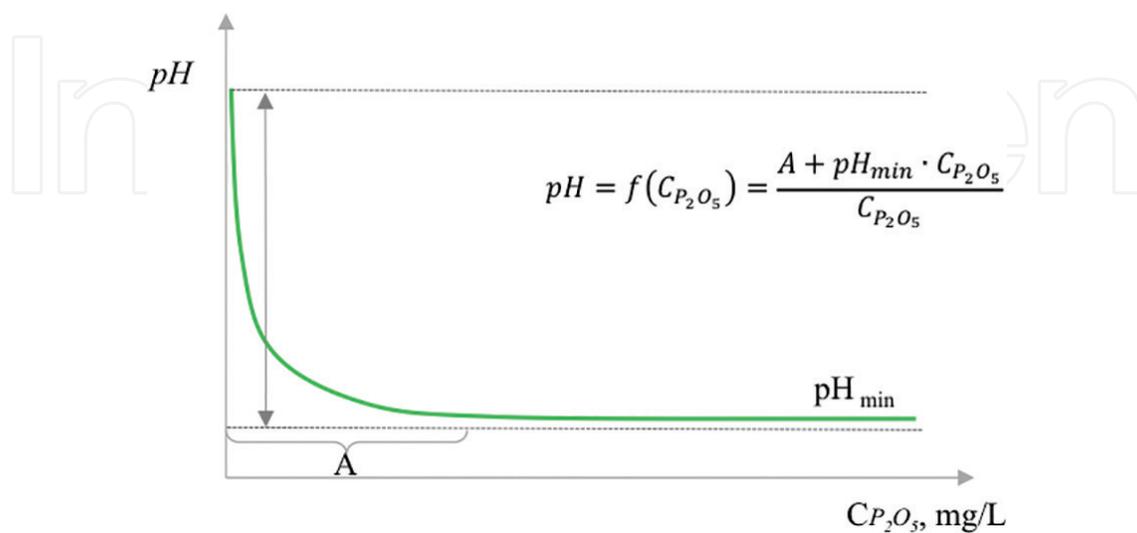


Figure 4. Description/visualization of proposed model $f(C_{P_2O_5}) = pH$.

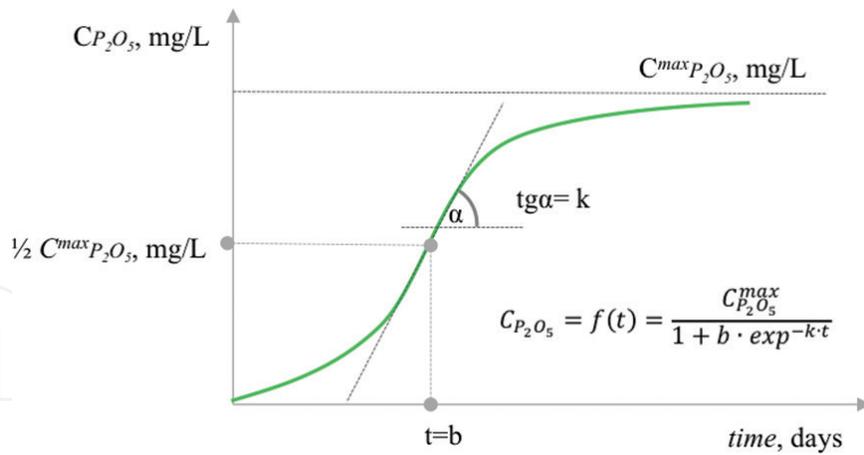


Figure 5. Description/visualization of proposed model $f(t) = C_{P_2O_5}$.

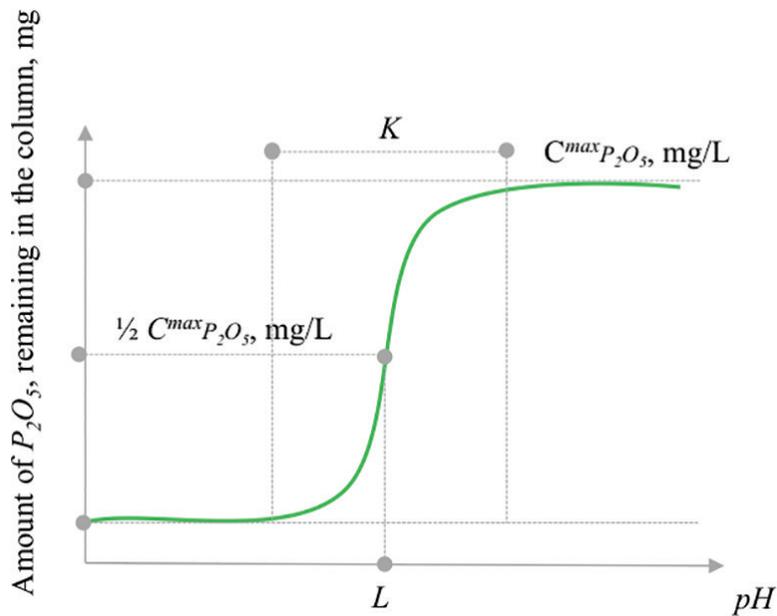


Figure 6. Description/visualization of proposed model $f(pH) = C_{P_2O_5}$.

$$C_{P_2O_5} = C_{P_2O_5}^{min} \frac{C_{P_2O_5}^{max} - C_{P_2O_5}^{min}}{1 + e^{-\frac{(pH-L)}{K}}} \quad (3)$$

This function has four parameters. $C_{P_2O_5}^{min}$ and $C_{P_2O_5}^{max}$ are two asymptotic values. The curve crosses over between these two asymptotic values in a region of pH whose approximate width is K and which is centered around L. $C_{P_2O_5}^{min}$ and $C_{P_2O_5}^{max}$ (mg/L) are constants describing the decay of curve. Evaluated value of L can be interpreted as value of pH that corresponds with the half of phosphorus (expressed as P_2O_5) that under considered condition could be released/solubilized [43].

As the solubilization progresses, the unavailable form of phosphorus becomes more available to plants and the same for bacterial cells that are responsible for phosphorus solubilization. That is why, measuring the concentration of P_2O_5 only in the solution as an evaluation of

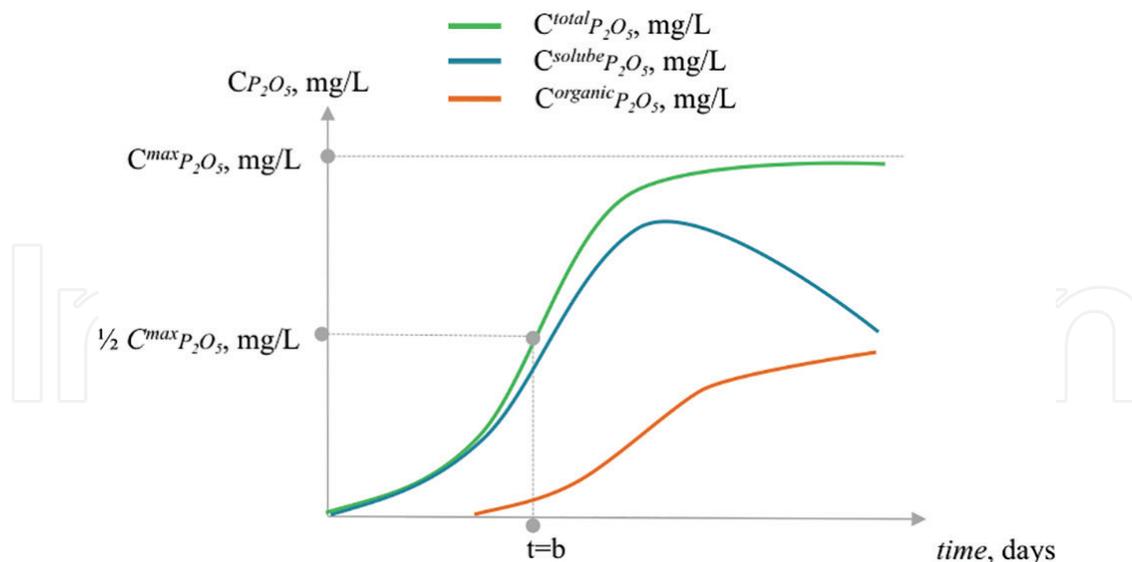


Figure 7. Kinetics of forming/liberating different forms of phosphorus during the microbial solubilization of inorganic forms of phosphorus.

solubilization effective is not so much accurate since some part of liberated phosphorus from unavailable forms was used by bacterial cells to maintain its own metabolism since phosphorus is one of the main macroelements not only for the plant. That is why, the following fraction of phosphorus should be distinguished according to **Figure 7**.

Phosphorus present in the biomass of bacterial cells in the organic form as a result of its immobilization can become available to plants in the mineralization process. To evaluate the efficiency of microbial solubilization, both organic and soluble forms of phosphorus should be concerned and included in the empirical model, for better understanding this phenomenon.

6. Conclusions

The natural ability of soil microbiota to solubilize nutrients present in the soil is the crucial link in the nutrient cycling in the natural environment. By the incorporation of this phenomenon with the processing of P-bearing renewable raw materials, it is possible to produce a source of available forms of phosphorus that could be applied in agriculture as a soil conditioner. Utilization of one of the components of the subsystem of saprotrophs to accelerate the circulation of phosphorus seems to be the easiest and the most beneficial for the natural environment. Since the by-products that carry a significant load of phosphorus are abundant and are produced in significant amount, every year, all over the world, they should be utilized or valorized into valuable products; such an attitude could serve as an efficient way of introduction of one of the most important nutrients in plant growth into the soil in available form, that could significantly limit the number of chemical fertilizers that should be used to cover the nutritional requirements of plants. Moreover, this proposal is within the circular economy approach that nowadays is in the main direction of developing new methods of treatment of by-products to lessen the amount of raw materials used in the production process.

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