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Production of phosphate biofertilizers from bones by phosphate-solubilizing bacteria *Bacillus megaterium*

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Abstract: In this paper, the production of phosphate biofertilizers from bones by phosphate-solubilizing bacteria *Bacillus megaterium* is presented. The biofertilizers used in this study contain phosphorus compounds that are in available form to plants as well as components of growth medium. The solubilization was performed under two conditions; with chlorides and with sulphates instead of chlorides. Three biofertilizer forms are proposed in relation to the doses of bones applied in the solubilization process (4, 10 or 20 g L\(^{-1}\)). The solubilization degree varied according to the bacterial medium formulation and the bones doses. The replacement of chlorides with sulphates yielded a lower growth rate, and resulted, in a lower solubilization. The specific growth rate of the cells of *B. megaterium* in a sulphate medium was lower than compared with the specific growth rate of cell culture in a medium of chlorides of about 22.4, 39 and 14%, for 4, 10 and 20 g L\(^{-1}\) of bones concentration, respectively. In the stationary phase, the solubilization factor (SF) was higher (61.7%) for the solubilization process conducted in a medium with chlorides – C\(_{\text{bone}}\) 4 g L\(^{-1}\) compared with the solubilization process conducted in the medium of sulphates (52.7%).

Keywords: *Bacillus megaterium*, solubilization, poultry bones, phosphorus fertilizer

1 Introduction

To achieve a high-yield in agriculture, it is necessary to apply phosphorus fertilizers that deliver the nutrients to plants [1]. However, the production of phosphorus fertilizer is a costly process that requires use of non-renewable phosphate resource (phosphorite), mineral acids (sulfuric acid) and generates many environmental hazardous byproducts [2,3].

Earlier experiments showed that it was possible to obtain liquid biofertilizer by utilizing the waste from phosphorus raw material such as bones and sewage sludge via a solubilization process performed by microorganisms that are naturally present in the soil. Labuda et al. [4] confirmed that the solubilization yield conducted with phosphorus by-products was higher (up to 80%) when compared with utilization of phosphorite as a source of phosphorus in the solubilization process (solubilization degree about 20–40%).

Living cells of microorganisms, naturally present in the soil can help crop plants to take up nutrients via their interactions in the rhizosphere when applied on the soil. Many accelerate certain microbial processes in the soil and mobilize nutrients from non-usable to usable form through biological processes [6]. Some bacteria produce acids or enzymes as metabolites and thus are able to solubilize phosphorus. The production of acids result in decrease of pH of the growth medium, affecting the solubilization of phosphorus [5]. With the release of phosphorus from the hydroxyapatite structure into the solution, that is present in available form to plants, and can be utilized in their metabolism. For example the Gram-positive soil bacterium *Bacillus megaterium*, has been used for many decades in industrial biotechnology. *B. megaterium* has a high secretion capacity directly into the surrounding medium as it is lacking an outer cell membrane [8]. Many reports of the usefulness of bacteria strain in the plant cultivation can be found in the literature. For example, the reduction of Se(IV) [9], the suppression of *S. sclerotiorum* on oilseed
rape [10], the phytostabilization of Ni contaminated soil [11] and the reduction of septoria tritici blotch [12].

It is possible to use meat and bone meal (MBM) as a renewable source of phosphates in the production of fertilizers according to Regulation (EC) No 999/2001 of the European Parliament and of the Council from May 22nd 2001 [13]. Additionally, bones, fish bones or ashes from the incineration of sludge from wastewater plants with III° stage of biological treatment can serve as a renewable source of phosphates in solubilization process. Developed/obtained liquid biofertilizers contain lower content of fertilizer nutrients than conventional fertilizers. Therefore preferred techniques of applying are drop-fertilization system or fertigation techniques constituting irrigation combined with fertilization.

Culture broth with phosphate raw material after solubilization process can be used as a liquid phosphate biofertilizer [14]. Beside the phosphate compounds in the form of soluble phosphates released from bones, bacterial medium after the solubilization process contains additional nutrients such as potassium, nitrogen, magnesium and sulfur, that when delivered to the soil in the form of fertilizer can be used by plants in the growth process [15]. However, beside the nutrients that are desired in the culture broth as well as in fertilizer, such compounds like chlorides (in the form of KCl and NaCl), are also present. Some crops such as potatoes and hops can be sensitive to chlorides [16]. Chlorine is vital for all plants, but for many plants a high dose and concentration is undesirable (potato, tobacco, hops, vegetables such as beans, cucumber, onion, lettuce, strawberries and blueberries). Chlorine is considered as an essential micronutrient and plays an important role in the process of photosynthesis and transpiration. For many plants such as sugar beet, fodder beet, cabbage, celery, spinach chloride promotes growth. The application of fertilizers containing chloride or sulphate ions depends on the plants.

It is possible to replace chlorine with sulphates and produce chloride-free fertilizer, and this would be considered because of the beneficial effect of sulphur on cultivation of vegetables [17,18]. Sulphur is the crucial macronutrient for all plants. This element plays an important role in amino acids and proteins synthesis [19,20]. Additionally, sulphur is utilized by many microorganisms in the soil that oxidize sulphur and thereby decreasing the pH of the soil by production of acids [7,20].

The production of chloride-free phosphorus biofertilizer with PSB (phosphate solubilizing bacteria) can play many roles: 1) supplementation of phosphorus to plants in available form, 2) supplementation of sulphur and other medium ingredients that can be used both by plants and by microorganisms that are present in the soil or delivered in fertilizer, 3) inoculation of bacteria into the soil which can additionally release phosphorus from phosphate source that is present in soil and in unavailable form to plants.

The aim of that work was to present the formation of two types of phosphate biofertilizers based on poultry bones that were obtained via solubilization process and performed in two different growth medium formulations, with and without chlorides in the bacterial growth medium. The effectiveness of the solubilization process, the growth of microorganism as well as utilitarian properties of obtained biofertilizers were evaluated to compare these two kinds of medium formulations.

2 Material and methods

2.1 Microorganism

Solubilizing bacteria Bacillus megaterium used in experiments was obtained from Polish Collection of Microorganisms located at the Institute of Immunology and Experimental Therapy in Wroclaw. The bacteria strain was cultivated into two kinds of medium. The first is composed of: 10 g of glucose; 0.5 g (NH₄)₂SO₄; 0.2 g NaCl; 0.1 g MgSO₄ · 7H₂O; 0.2 g KCl; 0.002 g MnSO₄ · H₂O; 0.002 g FeSO₄ · 7H₂O; 0.5 g yeast extract (per liter of distilled water) [14] prepared for B. megaterium with technical grade reagents (from POCh S.A. Gliwice, Poland). The second was prepared in the same way, but chlorides (NaCl and KCl) were replaced with sulphates (0.245 g Na₂SO₄ and 0.235 g K₂SO₄).

2.2 Solubilization experiment

Solubilization experiments were conducted for three different doses of bones (4, 10 and 20 g L⁻¹), in two formulation of growth medium, and with and without chlorides. Six Erlenmeyer’s flasks, containing 250 mL of Bacillus megaterium medium (three with mediums containing chlorides and three with mediums containing sulphates instead of chlorides) were incubated with crushed, poultry bones. The solutions were sterilized and then inoculated with bacteria Bacillus megaterium. During 14 days of cultivation/solubilization, culture media were shacked and incubated at 34°C (Thermoshake Gerhardt). The process was performed in batch culture
mode. Samples of microorganism suspension from both culture groups were collected at the same time and the biomass concentration of *B. megaterium* was measured spectrophotometrically. The permeates were used for the evaluation of pH and P\(_{2}\)O\(_{5}\) concentration, that was measured by colorimetric vanadomolybdophosphoric acid method. pH measurements were conducted with pH-meter Mettler-Toledo (Seven Multi) equipped with an electrode InLab413 with compensation of temperature.

### 2.3 Analytical methods

#### 2.3.1 Cell growth

The biomass concentration of *B. megaterium* was measured spectrophotometrically [4]. The cultures were sampled daily to determine optical density. The optical density was the absorbance of samples at 550 nm (OD\(_{550}\)) measured by a UV/Visible spectrophotometer (Varian Cary 50 Cone). Each sample was diluted to make an absorbance of less than 1.0 if the optical density was greater than 1.0. The concentration of *Bacillus megaterium* was estimated by the equation describing the relationship between the absorbance, \(A\(_{550}\)\), and the concentration of dry weight, Eq. 1:

\[
C = 0.00532 \times A_{550}, \quad R^2 = 0.922 \text{ mg L}^{-1} \tag{1}
\]

The biomass was dried at 60°C for three days (Manufacturing of medical and laboratory equipment, WAMED; Warsaw, Poland) and weighed.

Relative growth rate of bacteria was determined from the graphically depicted correlation of \(\ln C_s = f(t)\). The linear regression of specific growth rate was described by an Eq. 2 and used in the evaluation of \(\mu\), 1/day:

\[
\ln C_s^t = \mu \cdot t + \ln C_s^0 \tag{2}
\]

where: \(t\) – time period (in days), after which the culture concentration was measured (assuming \(t^0 = 0\)), \(C_s^t\) – the culture concentration after time \(t\) (mg L\(^{-1}\)), \(C_s^0\) – the initial concentration of the culture (mg L\(^{-1}\)) and parameter \(\mu\), 1/day is the slope.

#### 2.3.2 Colorimetric determination of concentration of phosphorus

The soluble P\(_{2}\)O\(_{5}\) concentration in culture medium was measured by colorimetric vanadomolybdophosphoric acid method with a Varian Cary 50 Cone UV-Visible Spectrophotometer at 420 nm. The method is based on the formation of yellow vanadomolybdophosphoric acid upon the addition of ammonium molybdate and vanadium to the ortho-phosphate solution. Ammonium molybdate reacts under acid conditions to form a heteropolyacid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed, the intensity of which indicates the concentration of orthophosphate present in the solution. The concentration of soluble P\(_{2}\)O\(_{5}\) \((C_{p2O5}, \text{ mg L}^{-1})\) was determined by means of Eq. 3 describing the relationship between the absorbance, \(A_{420}\), and the concentration of P\(_{2}\)O\(_{5}\):

\[
C_{p2O5} = 0.0233 \times A_{420} - 0.0542, \text{ mg L}^{-1}, \quad R^2 = 0.988 \tag{3}
\]

### 2.4 Calculations

The arithmetic mean values, standard deviations (SD) and \(t\) tests as well as the model parameters of equations describing the experimental data were determined using nonlinear estimation and multiple regression modules of *Statistica* software ver. 9.0. Correlation was considered statistically significant at \(\alpha < 0.05\).

Chi-square test (\(\chi^2\) test) was also used, which was calculated from Eq. 4, which accurately described the fit of the model to experimental data compared to the determination coefficient \(R^2\).

\[
\chi^2 = \frac{(\text{experimental value} - \text{model value})^2}{\text{model value}} \tag{4}
\]

### 3 Results and discussion

#### 3.1 Changes of pH

Among the many of extracellular metabolites released into growth medium by *B. megaterium*, organic acids can be found, and are responsible for lowering the pH and as a result solubilization of phosphorus [21]. The pH critically affects the solubilization of phosphates.

The initial pH in the solutions prepared for solubilization after addition of bones with bacterial medium composed of chlorides, were lower when compared with the mediums composed of sulphates. At the same time within the groups (chlorides and sulphates), a pH decrease as the bone doses decrease was observed. The pH drops significantly during solubilization. With an initial pH value above 6.5 that dropped below
in the majority of batch solubilization processes was observed.

The measurements showed decrease in pH up to eighth day of the experiment. The largest changes in pH in the cultures were observed for the lowest concentration of bones 4 g L\(^{-1}\) (ΔpH = 2.40 for chlorides and 2.75 for sulphates). While, in the mediums with concentration of bones 20 g L\(^{-1}\), ΔpH was 1.99 and 2.18 for chlorides and sulphates, respectively. The lower changes in the pH were found in the cultures with higher doses of bones. It may be explained by the fact that the metabolites of bacterial cells could interact and release various compounds from the bones, then in turn could neutralize bacterial acids [5] (less free hydrogen ions = higher pH) (Table 1).

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Acids produced by bacteria caused an increase in the concentration of hydrogen ions and higher release of P\(_2\)O\(_5\). The P\(_2\)O\(_5\) concentration is correlated with the pH of the liquid phase according to the following Eq. 5 (Fig. 1):

\[
pH = f(C_{P_2O_5}) = \frac{A + B C_{P_2O_5}}{C_{P_2O_5}}
\]

where: \(A, \text{mg L}^{-1}\) is a constant describing the reaching plateau. The evaluation parameter, \(B\), can be interpreted as the minimum value of pH. The parameters of the proposed model as well as the \(p\) values and errors that express the fit of the model to the experimental data are presented in the Table 2.
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### 3.2. Solubilization Factor

The effect of solubilization is expressed as the Solubilization Factor (SF, %) defined as the ratio (expressed as percentage) of soluble P\(_2\)O\(_5\) present in the solution and phosphorus (expressed as P\(_2\)O\(_5\)) introduced to solubilization medium in the solid form. At 4th day of solubilization process, at the end of logarithmic phase of bacterial growth, SF was higher in cultures of \(B.\ megaterium\) conducted in the medium with chlorides when compared with the

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**Table 1:** The equation describing the change in the pH during the solubilization process/growth of \(B.\ megaterium\).

| Group | Bone dose, \(g\ L^{-1}\) | \(pH=f(t)\) | \(R^2\) |
|-------|--------------------------|--------------|---------|
| Chlorides 4 | \(y = 5.571 \times 10^{-4} \times x - 9.0092 \times 10^{-6} \) | 0.907 |
| 10 | \(y = 4.89 \times 10^{-6} \times x + 2.897 \times 10^{-7} \) | 0.854 |
| 20 | \(y = 2.48 \times 10^{-6} \times x + 2.3193 \times 10^{-7} \) | 0.853 |
| Sulphates 4 | \(y = 5.85 \times 10^{-6} \times x - 9.4627 \times 10^{-7} \) | 0.797 |
| 10 | \(y = 4.10 \times 10^{-7} \times x + 1.3615 \times 10^{-7} \) | 0.911 |
| 20 | \(y = 2.72 \times 10^{-6} \times x + 3.193 \times 10^{-7} \) | 0.782 |

**Table 2:** The parameters of the model \(pH = f(S) = \frac{A + B C_{P_2O_5}}{C_{P_2O_5}}\) describing the change in the of pH and P\(_2\)O\(_5\) concentrations during the solubilization process.

| Medium Type | Bones dose, \(g\ L^{-1}\) | Parameter | Value | Standard error | \(p\) value | \(R^2\) | \(\chi^2\) |
|-------------|--------------------------|-----------|-------|----------------|-------------|--------|---------|
| Chlorides 4 |  | \(A, \text{mg L}^{-1}\) | 116 | 15 | 6.27 \times 10^{-5} | 0.787 | 0.132 |
|  |  | \(B\) | 4.12 | 0.12 | 4.93 \times 10^{-10} | 0.888 | 0.0921 |
| 10 |  | \(A, \text{mg L}^{-1}\) | 96.5 | 12.1 | 4.52 \times 10^{-9} | 0.722 | 0.159 |
|  |  | \(B\) | 4.26 | 0.11 | 1.56 \times 10^{-10} | 0.885 | 0.0921 |
| 20 |  | \(A, \text{mg L}^{-1}\) | 1.07 | 22.1 | 0.000186 | 0.722 | 0.159 |
|  |  | \(B\) | 4.51 | 0.136 | 7.58 \times 10^{-10} | 0.885 | 0.0921 |
| Sulphates 4 |  | \(A, \text{mg L}^{-1}\) | 64.0 | 8.2 | 5.32 \times 10^{-6} | 0.883 | 0.136 |
|  |  | \(B, \text{mg L}^{-1}\) | 3.91 | 0.14 | 2.44 \times 10^{-9} | 0.934 | 0.0622 |
| 10 |  | \(A, \text{mg L}^{-1}\) | 80.1 | 7.51 | 5.24 \times 10^{-6} | 0.979 | 0.0195 |
|  |  | \(B\) | 1.26 | 0.08 | 2.18 \times 10^{-10} | 0.934 | 0.0622 |
| 20 |  | \(A, \text{mg L}^{-1}\) | 116 | 6 | 4.75 \times 10^{-4} | 0.979 | 0.0195 |
|  |  | \(B\) | 4.44 | 0.04 | 1.16 \times 10^{-10} | 0.979 | 0.0195 |
medium with sulphates (Fig.2). The highest SF (52.4%) was found for medium with chlorides and bone concentration (4 g L\(^{-1}\)) and was five times higher when compared with the medium with sulphates (SF = 10.3%). In the stationary phase, the SF was also higher (61.7%) for chlorides group – C\(_{\text{Bone}}\) 4 g L\(^{-1}\), when compared with the sulphates group – 52.7%.

Attempts to describe the changes of Solubilization Factor in the time were undertaken. The following model was used (Eq. 6):

\[ SF = f(t) = \frac{SF_{\text{max}}}{1 + 10^{k(L-\gamma)}} \]  

where the \( SF_{\text{max}} \) is the maximum solubilization factor, \( L \) is the time when \( SF \) is equal to \( \frac{1}{2} \) of \( SF_{\text{max}} \) and \( k, \text{ 1/day}^{-1} \) 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 is performed faster. Table 3 collects all parameters of proposed model, as well as the \( p \)-value and errors that express the fit of the model to the experimental data.

### 3.3 The growth rate of B. megaterium cells

Based on the slope of \( \ln X = f(t) \), the specific growth rate, \( \mu \), for all six cultures of \( B. \) megaterium was determined (Table 4). The specific growth rate of culture cells of \( B. \) megaterium proceeding in the medium with sulphates

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**Table 3:** The parameters of the model \( SF = f(t) = \frac{SF_{\text{max}}}{1 + 10^{k(L-\gamma)}} \) describing the change in the Solubilization Factor during the solubilization process.

| Medium Type | Bones dose, g L\(^{-1}\) | Parameter          | Value  | Standard error | \( p \)-value | \( R^2 \)  | \( \chi^2 \) |
|-------------|---------------------------|---------------------|--------|----------------|--------------|----------|----------|
| Chloride    | 4                         | \( TF_{\text{max}} \) % | 64.2   | 1.37           | 4.9 \times 10\(^{-11}\) | 0.987    | 8.65     |
|             |                           | \( k, \text{ 1 day}^{-1} \) | 0.624  | 0.097          | 0.000209    |          |          |
|             |                           | \( L, \text{ day} \)  | 3.18   | 0.12           | 3.29 \times 10\(^{-9}\) |          |          |
|             | 10                        | \( TF_{\text{max}} \) % | 46.8   | 8.7            | 0.000661    | 0.907    | 19.9     |
|             |                           | \( k, \text{ 1 day}^{-1} \) | 0.144  | 0.047          | 0.0109      |          |          |
|             |                           | \( L, \text{ day} \)  | 7.00   | 1.47           | 0.00143     |          |          |
|             | 20                        | \( TF_{\text{max}} \) % | 92.5   | 24.1           | 0.00496     | 0.962    | 13.6     |
|             |                           | \( k, \text{ 1 day}^{-1} \) | 0.176  | 0.046          | 0.00502     |          |          |
|             |                           | \( L, \text{ day} \)  | 11.4   | 1.5            | 7.01 \times 10\(^{-5}\) |          |          |
| Sulphates   | 4                         | \( TF_{\text{max}} \) % | 61.4   | 7.9            | 5.28 \times 10\(^{-5}\) | 0.959    | 10.04    |
|             |                           | \( k, \text{ 1 day}^{-1} \) | 0.189  | 0.041          | 0.00183     |          |          |
|             |                           | \( L, \text{ day} \)  | 8.63   | 0.78           | 4.13 \times 10\(^{-4}\) |          |          |
|             | 10                        | \( TF_{\text{max}} \) % | 47.6   | 5.37           | 2.08 \times 10\(^{-5}\) | 0.961    | 11.6     |
|             |                           | \( k, \text{ 1 day}^{-1} \) | 0.164  | 0.0314         | 0.000810    |          |          |
|             |                           | \( L, \text{ day} \)  | 7.04   | 0.86           | 3.78 \times 10\(^{-5}\) |          |          |
|             | 20                        | \( TF_{\text{max}} \) % | 34.7   | 3.19           | 4.55 \times 10\(^{-6}\) | 0.963    | 5.26     |
|             |                           | \( k, \text{ 1 day}^{-1} \) | 0.222  | 0.045          | 0.00120     |          |          |
|             |                           | \( L, \text{ day} \)  | 7.37   | 0.57           | 1.28 \times 10\(^{-6}\) |          |          |
were lower when compared with the specific growth rate of cell culture conducted in the medium with the chlorides of about 22.4, 39 and 14%, for 4, 10 and 20 g L\(^{-1}\) of bones concentration, respectively (Table 4). The growth of bacteria in the medium with sulphates was limited by the presence of an ingredient that was not originally present in the growth medium for Bacillus megaterium. Using Monod equation, the rate of microorganisms growth in time (\(r_x \text{ mg L}^{-1} \text{ day}^{-1}\)) was determined (Fig. 3). A Lower \(r_x\) value was observed for cells of Bacillus megaterium conducted in the medium with sulphates in the comparison with the chlorides.

### 3.4 \(P_2O_5\) yield coefficient against biomass

As a result of a production of low molecule organic acids during the growth of cells of Bacillus megaterium by lowering the pH of the medium solution caused the solubilization, the concentration of the released phosphorus (expressed as a \(P_2O_5\)) from solids (bones) is increased. Table 5 presents the linear equation, with the \(R^2\) coefficient, that expresses the relationship between the \(C_{P2O5}\) and \(C_s\). \(P_2O_5\) yield coefficients against the biomass (\(Y_{P2O5/Cs}\)) according to the Eq. 7.

\[
Y_{P2O5/Cs} = \frac{\frac{dC_{P2O5}}{ds}}{r_{P2O5}/Cs} \tag{7}
\]

The optimal feeding medium requires a higher total sugar concentration (400 g L\(^{-1}\)) and a C/N molar ratio of 10 mol/mol [22]. Many proposals for the modification of the growth medium were made in the literature to induce the synthesis of valuable extracellular metabolites. For example, a higher level of an inorganic phosphate in a medium is potentially more favorable for the excretion of penicillin acylase by Bacillus megaterium [23]. The proposed modification did not change these process parameters. Nevertheless, the replacement of chlorides with sulphates yielded lower growth rate, and as a result lower solubilization.

Chloride ions in the soil behave as a nitrification inhibitor, in other words fertilization with chloride ions will lower the utilization of nitrogen fertilizers and result in a lower yield of agriculture production. The uptake of

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**Table 4:** The equation describing the specific growth rate of Bacillus megaterium in two different medium compositions and three different doses of bones.

| Group     | Bone dose, g L\(^{-1}\) | \(\ln C_i = f(t)\) | \(R^2\) | \(\mu, \text{ 1 day}^{-1}\) |
|-----------|------------------------|--------------------|--------|----------------------|
| Chlorides | 4                      | \(y = 1.30x + 1.86\) | 0.932  | 1.301                |
|           | 10                     | \(y = 1.41x + 1.98\) | 0.957  | 1.410                |
|           | 20                     | \(y = 1.26x + 2.53\) | 0.922  | 1.256                |
| Sulphates | 4                      | \(y = 1.01x + 2.93\) | 0.935  | 1.009                |
|           | 10                     | \(y = 0.85x + 3.48\) | 0.871  | 0.857                |
|           | 20                     | \(y = 1.08x + 3.08\) | 0.780  | 1.081                |

**Table 5:** Biomass yield coefficient of \(P_2O_5\) \(Y_{P2O5/Cs}\).

| Group     | Bone dose, g L\(^{-1}\) | \(\ln C_{P2O5} = f(C_s)\) | \(Y_{P2O5/Cs}\) | \(R^2\) |
|-----------|------------------------|-----------------------------|-----------------|--------|
| Chlorides | 4                      | \(y = 0.385x + 36.4\)       | 0.385           | 0.845  |
|           | 10                     | \(y = 0.250x + 12.9\)       | 0.249           | 0.819  |
|           | 20                     | \(y = 0.213x + 51.7\)       | 0.213           | 0.984  |
| Sulphates | 4                      | \(y = 0.051x + 28.9\)       | 0.051           | 0.756  |
|           | 10                     | \(y = 0.391x - 18.4\)       | 0.391           | 0.674  |
|           | 20                     | \(y = 0.256x + 12.1\)       | 0.256           | 0.900  |
large amounts of chloride may lead to a reduced in the organic acids content and other valuable compounds such as: sugar, starch, protein and result in a decrease in the storage or processing properties as an enhance degree of hydration of plant tissues [24,25]. The elaboration the chloride free a fertilizer formulation is a method to meet the expectations and needs of agriculture. To avoid symptoms of chloride excess, such as: curling of the leaf margins, marginal leaf scorch, leaf necrosis or leaf drop, there are a few products available with low content of chlorides, such as KALISOP® or Patentkali®. The above mentioned symptoms of chloride toxicity can be avoided [26].

It has been shown that changes in anions (sulphates with chlorides), with the corresponding cations changes (Na⁺ and K⁺) in the preparation of the growth medium of B. megaterium, effects the microbiological solubilization process. The sulphates were chosen because this anion was already present in the medium; the introduction of other compound could have bigger effect on the metabolism of the cells and as a result of this is a strong influence on the solubilization.

4 Conclusions

The aim of that work was to see whether changing the composition of medium will affect the solubilization effectiveness performed by Bacillus megaterium strain, which is used in the production of phosphorus fertilizers. It was found that substituting the chlorides by sulphates in the growth medium, influenced the growth of microorganism and as a result of this a lower biomass concentration was observed, which turn yielded a lower of solubilization process. These changes were not statistically significant. It is necessary to evaluate the utilitarian properties of such fertilizer formulations in germination tests to find the influence of the substitution of chlorides with sulphates on the yield parameters and the content of elements in plants. The second stage for this work will allow a comparison of the application results of both formulations. These experiments should be long term to have a possibility to observe potential effect of toxicity of chloride and the effect of a lower amount of phosphorus available to plants, as a result of a lower solubilization (an effect of substituting chlorides with sulphates).

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