BIODECOMPOSITION OF JORDAN PHOSPHORITE BY PHOSPHATE-SOLUBILIZING FUNGI

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Abstract - The bio-solubilization of Jordan phosphorite by the phosphate-solubilizing fungus Aspergillus niger has been investigated. The effect of the phosphate concentration in the liquid medium, the duration of biodecomposition, titratable acidity and the effect of preliminary mechanical activation on the process of dissolution have been studied. The investigations indicate that almost complete extraction of P\textsubscript{2}O\textsubscript{5} from Jordan Phosphorite in a form utilizable by plants can be achieved. A maximum degree of P\textsubscript{2}O\textsubscript{5} extraction 99.10% was obtained on the 15\textsuperscript{th} day in a medium containing 0.5% w/v non-activated Jordan phosphorite. The preliminary mechanical activation of the phosphate facilitates the dissolution until a definite period of the biocconversion. Investigations with mechanically-activated Jordan phosphorite showed that a maximum extent of 92.40% of phosphate solubilization was observed on the 10\textsuperscript{th} day at a phosphorite concentration of 0.5% w/v.

Keywords: Rock phosphate; Solubilization; Biodecomposition; Aspergillus niger.

INTRODUCTION

Phosphorus is second only to nitrogen in mineral nutrients that most commonly limit the growth of crops. A deficiency in soluble P for many agricultural soils is one of the major factors hampering crop production worldwide. Only 1 to 5% of the total soil P is in a soluble plant–available form (Arcand and Schneider, 2006). Usually it is introduced through the traditional phosphoric fertilizers- the super phosphates. It is known that the utilization of phosphorus from these fertilizers is about 15-20%, because a large portion of soluble inorganic phosphate applied to soil is rapidly immobilized soon after application and becomes unavailable to plants (Bojinova et al., 1997; Rodriguez and Fraga, 1999; Kang et al., 2007; Kang et al., 2008). This phenomenon occurs as a result of complex chemical and biochemical processes in the soil, resulting in fixation and precipitation of P in the soil. This generally depends on pH and the soil type. The fixed forms of P in acidic soils are aluminum and iron phosphates, while in alkaline soils they are calcium phosphates (Rfaki et al., 2014). According to Lindsay (1979) super phosphate contains a sufficient amount of calcium for the precipitation of its own P as dicalcium phosphate (CaHPO\textsubscript{4}) or dicalcium phosphate dihydrate (CaHPO\textsubscript{4}.2H\textsubscript{2}O).

The second major component of soil P is organic matter (nucleic acids, phospholipids, phosphotriesters, etc.). The organic forms of P may constitute up to 30-50% of the total phosphorus in most soils. Many of these P compounds are materials with high molecular mass. They can be assimilated by the cell (Goldstein, 1994) after their bioconversion to either soluble ionic phosphate (Pi, HPO\textsubscript{4}\textsuperscript{2-}, H\textsubscript{2}PO\textsubscript{4}\textsuperscript{-}), or low molecular-mass organic phosphate. These Ca-P compounds are generally resistant to chemical hydrolysis and biodegradation, but recently several reports documented microbial release from these sources (Rodriguez and Fraga, 1999).

Based on the current rate of use, it is expected that the worlds, known reserves of high quality rock
phosphate (RP) will be depleted within the current century. Consequently, the production of phosphate-based fertilizers will require the processing of low-grade rock phosphates at significantly higher cost and the production costs of P-fertilizers will rise (Mendes et al., 2013).

The development of new nonacid methods, applicable to both high-quality and low-quality raw materials, is important for solving technological and ecological effectiveness of phosphorus fertilizers production. One of the available non-acid methods of rock phosphate processing is direct application of phosphates as a source of phosphorous in the soil. The phosphorus released from directly applied ground phosphate rock is often too low to provide sufficient P for crop uptake (Vassilev et al., 2001). The direct application of the phosphate as a fertilizer is limited due to its structure, resulting in low solubility. The improvement of phosphate structure through mechanical activation changes the phosphate chemistry and increases its solubility (Ibrahim et al., 2010).

The usage of phosphate-solubilizing microorganisms (PSM) as a biotechnological alternative for producing soluble P fertilizers from rock phosphate (RP) is the other alternative. Microorganisms are an important component in the soil. The ability of PSM to mobilize P from sparingly soluble sources can be a useful tool in P fertilization management. Some studies have shown that the product obtained after the treatment of RP with PSM or even the direct application of PSM to soil can improve plant growth and P uptake (Mendes et al., 2013). Therefore, an efficient process including microbial mediated ones able to exploit lower-grade RP and/or after native P sources (Vassilev, et al., 2013) at low cost has to be developed in the near future.

Soil bacteria and fungi mediate soil process such as decomposition, nutrient mobilization and mineralization, storage release of nutrients and water (Rashid et al., 2004). Several reports have indicated that some microorganisms are able to solubilize rock phosphates and to release soluble P (Sharma et al., 2012; Sanjotha et al., 2011; Yadav et al., 2011; Deepa et al., 2010; Pradhan and Sukla, 2005; Kang et al., 2002). A wide range of microorganisms able to solubilize inorganic P have been cultivated from soil, including bacteria (e.g. Actinomycetes, Pseudomonas and Bacillus spp.) and fungi (e.g. Aspergillus and Penicillus spp.). It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms. The production of organic acids such as citric, oxalic, gluconic, malonic, succinic, etc. by phosphate-solubilizing microorganisms has been well documented and seems to be most frequent agent for mineral phosphate solubilization (Khan et al., 2007). Such organic acids can either directly dissolve the mineral phosphate as a result of anion exchange of PO$_4^{3-}$ by acid anion or can chelate both iron and aluminum ions associated with phosphate (Omar, 1998). Some elements that may be released during RP solubilization could affect these mechanisms by promoting changes in microbial metabolism (Gadd, 1993). The amount of P solubilized is dependent on the form of inorganic P precipitate used (including various sources of RP, pure Ca, iron and aluminum phosphates) along with culture and sampling procedures (Whitelaw et al. 1999; Barroso et al., 2006; Richardson and Simpson, 2011). In general, phosphate-solubilizing fungi produce more acids and consequently exhibit greater phosphate-solubilizing activity than bacteria in both liquid and solid media (Venkateswarlu et al., 1984). The fact that all identified P-solubilizing fungi belong to the Aspergillus or Penicillium genus agrees with reports of several authors (Nahas et al. 1994; Ghosh and Banik, 1998; Rashid et al. 2004; Seshadri et al., 2004; Deepa et al., 2010). Fungal diversity affects soil agglomeration, thereby increasing the soil quality and fertility (Tallapragada and Seshachala, 2012). Species of fungi, particularly Aspergillus, are capable to produce citric acid and form non-ionizable association with calcium. Aspergillus niger, used in the industrial production of citric acid, has been reported as one of the most effective organisms for rock phosphate solubilization (Arcand and Schneider, 2006).

The dissolution of different types of P-contained resources (including Ca, iron and aluminum phosphates and various sources of rock phosphate) by Aspergillus niger has been demonstrated earlier (Vassilev et al. 2005; Nahas, 1996; Bojinova et al., 1997; Goenadi et al. 2000; Barroso et al., 2006; Bojinova et al., 2008; Mendes et al., 2013). The amount of P solubilized in culture is also depend on the composition of the medium (carbon and nitrogen composition), medium volume, pulp density, particle size, initial pH of the medium, temperature, inoculum concentration, along with culture and sampling procedures for the solubilization of the phosphates.

Solubilization of inorganic phosphate by microorganisms involves a wide range of processes concerning the secretion of organic acids, lowering of the pH as a result of acid production, ion chelating and exchange reactions which are a part of the phosphorus cycle (Akuntokin et al., 2007).

The aim of the present study is to investigate the biodecomposition of Jordan phosphorite using the phosphate-solubilizing fungus Aspergillus niger. We
use in our investigation Jordan phosphorite, which has not been studied with this objective. Jordan phosphorite is imported into Bulgaria for the phosphoric fertilizer industry. The effects of the phosphate concentration in the liquid medium, the duration of biodecomposition, the concentration of citric acid generated from the fungus and results with/without preliminary mechanical activation of the phosphorite, were studied.

MATERIALS AND METHODS

Natural Phosphate

The chemical composition of the initial Jordan phosphorite (JP) is shown in Table 1. The fraction below 0.2 mm was used. The total content of phosphorus was determined by dissolving in 25% HCl, the citric-soluble and water-soluble phosphorus was determined after extracting with 2% citric acid or water followed by spectrophotometric analysis as a vanadate-molybdate complex (Jackson, 1967). Ca was determined complexometrically, Si by weight and the other elements using Atomic Absorption Spectrophotometry (AAS).

The mechanical activation was performed for 4 hour using a “Pulverisite 5” planetary mill. Metal balls of 20 mm diameter were used and the rotation applied was 320 rpm. The weight ratio of phosphorite to milling bodies was 1:20.

The phosphorus content was determined in the non-activated Jordan phosphorite (NAJP) as well as after its mechanical activation (MAJP). It was analyzed as total P$_2$O$_5$ (P$_2$O$_5$ t.), citric-soluble (P$_2$O$_5$ c.s.) and water-soluble (P$_2$O$_5$ w.s.). The phosphate P$_2$O$_5$ t. value was 35.37% for the both examined phosphate types (NAJP and MAJP). The values of P$_2$O$_5$ c.s. were 11.75% (NAJP) and 16.99% (MAJP) and those of P$_2$O$_5$ w.s. were 0.01% and 0.03%, respectively.

Microorganisms and Nutritive Medium

Investigations were performed using the Aspergillus niger strain obtained from the Institute for Microbiology, Bulgarian Academy of Sciences. The bioconversion was studied through deep incubation of the microorganisms in a liquid nutritive medium containing (in g/L): Glucose - 120; (NH$_4$)$_2$SO$_4$ - 3; KH$_2$PO$_4$ - 1; K$_2$HPO$_4$ - 1; MgSO$_4$·10H$_2$O - 0.5; MnSO$_4$·5H$_2$O - 0.02; FeCl$_3$·6H$_2$O - 0.01.

The initial pH value of the nutritive medium was 6.8.

Experimental Methods

Incubation with Aspergillus niger was carried out in 300 ml Erlenmeyer flasks containing 100 ml of sterilized nutritive medium. After cooling to 30 °C, 1 mL inoculums with a concentration of spores of 1×10$^7$/mL and JP (MAJP and NAJP) were introduced into the reaction medium. The flasks were incubated in a shaking water bath at 31±1 °C for different period of time with a rotational speed of 150 rpm. The concentrations of NAJP and MAJP in the nutrient medium were 0.5, 1 and 2% w/v. After different time intervals, the samples were filtered and pH, sugar content (Bernfeld, 1959), titratable acidity through titration with 0.1 N NaOH and the content of water-soluble P$_2$O$_5$ w.s. (c$_1$) in this first filtrate were determined. The precipitate (biomass and remaining mineral mass) was treated for 2 hours with 2% citric acid at room temperature. After filtration the solution obtained was analyzed for citrate-soluble P$_2$O$_5$ c.s. (c$_2$). The precipitate, which contained residual mineral mass and biomass was dried to a constant weight at 60 °C and was ashed to a constant weight at 500 °C. The loss of weight during heating is equal to the biomass produced during cultivation. The P$_2$O$_5$ content in the residue of mineral mass after thermal treatment was also determined - P$_2$O$_5$ m.m. The P$_2$O$_5$ content in the biomass (P$_2$O$_5$ b.) was determined using material balance for P$_2$O$_5$ based on the quantity of P$_2$O$_5$ input in the system (with phosphorite and nutritive medium) and, at the exit, after biodecomposition, (P$_2$O$_5$ w.s., P$_2$O$_5$ c.s. and P$_2$O$_5$ m.m).

The process was analyzed by using two parallel samples at various times of incubation and the results obtained were averaged.

Investigations of solubilization of Jordan phosphorite in the nutritive medium without microorganisms were made for the longest incubation period of 15 days at the studied concentrations of Jordan phosphorite. The obtained α value for 0.5, 1.0 and 2.0% JP was nearly 2%.

| Table 1: Chemical composition of Jordan phosphorite. |
|-----------------|-----|---|---|---|---|---|---|---|---|---|---|
|                  | Al$_2$O$_3$ | CaO | Fe$_2$O$_3$ | SiO$_2$ | TiO$_2$ | MgO | P$_2$O$_5$ | Pb | Cu | Zn | Cd | Ni | Ag | As | Mo |
| mg/kg            | 0.26 | 53.69 | 0.27 | 2.60 | 0.02 | 0.26 | 35.37 | 18 | 32 | 183 | 4 | 10 | 1 | 7 | 4 |
Calculation of the Conversion of Jordan Phospho-
rite

On the basis of the results obtained, the extent of
the JP solubilization and conversion of $P_2O_5$ to wa-
ter-soluble ($\alpha_1$), citrate-soluble ($\alpha_2$) forms and $P_2O_5$
in biomass ($\alpha_3$) were determined and expressed as
follows:

$$\alpha_1 = \frac{P_2O_{5w.s.}}{P_2O_{5t.}} \times 100 \text{, } \% \text{ w/w}$$

(1)

$$\alpha_2 = \frac{P_2O_{5c.s.}}{P_2O_{5t.}} \times 100 \text{, } \% \text{ w/w}$$

(2)

$$\alpha_3 = \frac{P_2O_{5b.}}{P_2O_{5t.}} \times 100 \text{, } \% \text{ w/w}$$

(3)

The total degree of extraction is:

$$\alpha = \alpha_1 + \alpha_2 + \alpha_3 \text{, } \% \text{ w/w}$$

(4)

where:

$P_2O_{5w.s.}$ – $P_2O_5$ content in the first filtrate (g)

$P_2O_{5c.s.}$ – $P_2O_5$ content in the second filtrate (g)

$P_2O_{5b.}$ – $P_2O_5$ content in the dry biomass (g)

$P_2O_{5t.}$ – total $P_2O_5$ content in the system, with the
phosphorite and nutritive medium (g).

A simple correlation was run to determine corre-
lution coefficients ($r$) by the method of Ordinary
Least Squares (OLS).

RESULTS AND DISCUSSION

Figure 1 presents the change in the concentration
of glucose and citric acid (A) and the extents of $P_2O_5$ ex-
traction, $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha$ (B), after 3, 5, 9, 12 and 15 days
of incubation. The values are the average ± SD ($p <$
0.05) from duplicate experiments.

When 0.5% w/v MAJP was added to the nutritive
medium the titratable acidity increased and reached a
value of 10.66 $\mu$E.mL$^{-1}$ on the 12th day (Figure 2A).
The extents of $P_2O_5$ extraction are insignificantly
lower than those in the investigation with 0.5% w/v
NAJP, but these values were achieved for a shorter
period of incubation (Figure 2B). For example, using
0.5% NAJP the $\Sigma (\alpha_1 + \alpha_2)$ and $\alpha$ reached values of
82% and 92.7% on the 12th day. If MAJP was used
under the same conditions the value for the $\Sigma (\alpha_1 + \alpha_2)$
and $\alpha$ were 84% and 92.4% on the 10th day. $\alpha_2$ de-
creased in the range of 23.4 to 21.25 at the end of the
incubation period. The quantity of $P_2O_5$, separated
with biomass ($\alpha_3$) increased from the first
day up to the 15th day for (NAJP) and 12th day for
(MAJP) with the values in the range from 4.7 to
13.2% and from 1.29 to 10%, respectively.

Figure 1: Change in the concentration of glucose
and the titratable acidity (A) in the culture medium
containing 0.5% w/v NAJP, and extents of $P_2O_5$ ex-
traction, $\alpha_1$, $\alpha_2$, $\alpha_3$, $\alpha$ (B), after 3, 5, 9, 12 and 15 days
of incubation. The values are the average ± SD ($p <$
0.05) from duplicate experiments.
With increasing concentration of JP (NAJP and MAJP) in the nutritive medium the extents of P2O5 extraction fall (Figure 3 and Figure 4). The result shows that the higher rate of phosphate dissolution was achieved in the investigations with 1% w/v NAJP - α reached a maximum of 80.80% on the 15th day (Figure 3B).

The maximum of α in nutritive medium containing 1% w/v MAJP was 69.29% on the 12th day (Figure 4B). If we compare the results for Σ (α1 + α2) and α, the same tendency can be seen. In the investigations with NAJP Σ (α1 + α2) had a value of 56% on the 9th day, but for MAJP this value was 59% on the 8th day. In the same conditions, the α values were 61.5% and 64%. The reason is the higher values of α3 for MAJP compared with those for NAJP. The titratable acidity is higher in the experiments with MAJP - 13.88 µE.mL⁻¹ on the 10th day (Figure 4A) compared with 11 µE.mL⁻¹ on the 12th day for NAJP (Figure 3A). The results show that there is no correlation between citric acid produced and the phosphate solubilization after the 12th day (NAJP) and 10th day (MAJP).

Increasing the JP concentration to 2% w/v in liquid medium the titratable acidity increases (Figure 5 and Figure 6). In the experiment with 2% w/v NAJP it reached a maximum of 13 µE.mL⁻¹ on the 12th day and slowly decreased to the end of the period when its value was 11.2 µE.mL⁻¹ (Figure 5A). In the nutritive medium containing 2% w/v MAJP the titratable acidity achieved a value of 15.7 µE.mL⁻¹ on the 12th day (Figure 6A).
Figure 4: Change in the concentration of the glucose and the titratable acidity (A) in the culture medium containing 1.0% w/v MAJP, and extents of P2O5 extraction, α1, α2, α3, α (B), after 2, 4, 6, 8, 10 and 12 days of incubation. The values are the average ± SD (p < 0.05) from duplicate experiments.

Figure 5: Change in the concentration of the glucose and the titratable acidity (A) in the culture medium containing 2.0% w/v NAJP, and extents of P2O5 extraction, α1, α2, α3, α (B), after 3, 5, 9, 12 and 15 days of incubation. The values are the average ± SD (p < 0.05) from duplicate experiments.

Figure 6: Change in the concentration of glucose and the titratable acidity (A) in the culture medium containing 2.0% w/v MAJP, and extents of P2O5 extraction, α1, α2, α3, α (B), after 2, 4, 6, 8, 10 and 12 days of incubation. The values are the average ± SD (p < 0.05) from duplicate experiments.
There is no significant difference between the extent of P$_2$O$_5$ extraction in both experiments (2% w/v NAJP and 2% w/v MAJP). The total degree of phosphate solubilization ($\alpha$) had a maximum value of 46.02% in the experiments with NAJP on the 12$^{th}$ day (Figure 5B) and 49.735% in the investigations with MAJP on the 10$^{th}$ day (Figure 6B), respectively, and these values were preserved to the end of the incubation period. The same tendency was observed for the lower concentrations connected with higher $\alpha$ value and shorter time: for the study with 2% NAJP, $\alpha$ had a value of 33.4% on the 9$^{th}$ day, but for MAJP a value of 39.5% was obtained on the 8$^{th}$ day.

The culture pH and the concentration of P$_2$O$_5$ extracted from the phosphate detected in the first filtrate, the second filtrate and in biomass expressed as P$_2$O$_5$ (C$_1$, C$_2$, C$_3$) and their sum (C) in the investigation with NAJP in the culture are presented in Table 2.

### Table 2: Change in culture pH and P concentrations (C, g/L) in different forms, after 3, 5, 9, 12 and 15 days of incubation in nutritive medium containing 0.5, 1.0 and 2.0% NAJP.

<table>
<thead>
<tr>
<th>Day</th>
<th>pH</th>
<th>Concentration of P$_2$O$_5$ (g/L)</th>
<th>C$_1$</th>
<th>C$_2$</th>
<th>C$_3$</th>
<th>C</th>
</tr>
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<tbody>
<tr>
<td>0.5% NAJP</td>
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<td></td>
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<td>3</td>
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<td>1.06</td>
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* c$_1$ - P$_2$O$_5$ w.s.; c$_2$ - P$_2$O$_5$ c.s.; c$_3$ - P$_2$O$_5$ b.; c - $\Sigma$(c$_1$+c$_2$+c$_3$)

The values are the average ± SD (p < 0.05) from duplicate experiments.

The results from the present as well as earlier (Ivanova et al., 2006; Bojinova et al., 2008a) studies indicate that the lower the quantity of phosphate applicable, the greater is the conversion percentage, independent of the type of JP used (NAJP or MAJP). These results are in conformity with those reported by others (Nahas et al., 1990; Ghosh and Banic, 1998; Reddy et al., 2002).

In our earlier study we also documented that preliminary mechanical activation facilitated phosphate dissolution during bioconversion of Morocco phosphorite (MP) by Aspergillus niger up to the 3$^{rd}$ day, when the total P$_2$O$_5$ extraction had higher values using AMP (Bojinova et al., 2008b). In conformity with these investigations a positive correlation between the solubility of nonactivated (NMP) and activated Morocco phosphorite (AMP) and titratable acidity was observed to the 12$^{th}$ day of the incubation period with 1% w/v in the liquid culture. When the incubation time was prolonged to the 15$^{th}$ day under the same conditions this correlation was negative. If...
the concentration of NMP and AMP was 2% the total P2O5 extraction increased with increasing production of citric acid for all investigated incubation periods of time. According to the present studies the data showed that in the investigations with NAJP (0.5%, 1% and 2%) the tendency is similar. The titratable acidity achieved a maximum on the 12th day of incubation and decreased to the 15th day (Figure 1, Figure 3 and Figure 5), but the extent of P2O5 extraction into water-soluble forms (αt) and the total degree of extraction (α) increased for all periods of time investigated. The decrease of the titratable acidity after the 12th day of incubation (Figure 3), 10th day (Figure 4) and 12th day (Figure 5), respectively, can be explained by the partial neutralization as Ca-citrate of the citric acid produced with the Ca2+ ions liberated as a result of decomposition of the phosphate structure. As seen from the data (Figure 2, Figure 4 and Figure 6) for experiments with MAJP, αt and α increased together with increasing titratable acidity, independent of the concentration of MAJP used. The correlation coefficients (r) between the quantities of extracted P (C, g/L) and the titratable acidity for different concentrations of NAJP were: (0.64 at 0.5% w/v; 0.92 at 1% w/v and 1.0 at 2% w/v; p < 0.001), and: (1.0 at 0.5% w/v; 0.96 at 1% w/v and 0.98 at 2% w/v; p < 0.001) for MAJP. This is in accordance with the results obtained by others (Nahas et al., 1996; Goldstein, 2000).

The results show a negative correlation between the final pH value and titratable acidity. However, the results with MAJP showed values for r (-0.96; -0.96; -0.90, p < 0.001) distinct from the results with NAJP (r = -0.001; -0.60; -0.60, p < 0.001), respectively for 0.5, 1.0 and 2% w/v RP.

The result obtained by Rashid et al. (Rashid et al., 2004; Deepa et al., 2010) show a positive correlation between organic acid excretion and P solubilization and a negative correlation between pH and P solubilization. Our results indicate a negative correlation between the quantity of P extracted from the phosphate (C, g/L) and the change of pH. The values of the correlation coefficients (r) were (-0.74; -0.85 and -0.65, p < 0.001) for NAJP and (-0.96; -0.97 and -0.94, p < 0.001) for MAJP at similar conditions.

Many of the calcium phosphates, including rock phosphate ores (fluorapatite, francolite), are insoluble in soil with respect to the release of inorganic P at rates necessary to support agronomic levels of plant growth (Goldstein, 2000). Gerretsen (1948) first showed that pure cultures of soil bacteria could increase the P nutrition of plants through increased solubility of Ca phosphates. Their solubility increases with a decrease of soil pH. Phosphate solubilization is the result of the combined effect of pH decrease and organic acid production (Fankem et al., 2006). Obviously, various chemical elements contained in phosphates are liberated concurrently with P during microbial solubilization. The fungus Aspergillus niger used in our investigations produced mainly citric acid and low concentrations of other organic acids. The dynamic variations of the medium conditions due to changes in Aspergillus niger metabolism and in chemical equilibria are probably the reason for the variations in the solubilized P concentrations (P2O5 Ca, and P2O5 Al) observed throughout the incubation (Mendez et al., 2013). Vassilev et al. (1995) observed that decreases in soluble P in the fermentation medium were accompanied by decreases in titratable acidity and suggested that this resulted from the consumption of organic acid by fungus under conditions of C depletion. The data obtained in our work support this hypothesis since the decreases in titratable acidity apparently occurred after the 12th day of incubation (Figures 1, 2 and 3) in response to the beginning of a new growth cycle, when the fungus may have used part of the organic acid in the metabolism. The fact that the concentration of biomass increased constantly from the 1st to the 15th day (NAJP) or from the 1st to the 12th day (MAJP) may be a proof for this. At the same time the organic acids form complexes with metal ions like Ca, Fe and Al, liberating soluble phosphate. This fact is the other reason for the increasing titratable acidity.

The extent of P2O5 extraction in citric soluble form (αt) decreased from 20.8% to 12.2% (NAJP) and from 23.4% to 20.5% (MAJP), from the 3rd to the 15th day and from the 2nd to the 12th day, respectively, when the concentration was 0.5% (Figure 1 and Figure 2). The same tendency can be seen for the other concentrations of RP used in the study (Figure 3 – Figure 6). Obviously, the bioconversion was performed in a complex heterogeneous system with simultaneously occurring biosynthetic and chemical reactions, with different velocities depending on the continuously changing concentrations of macro- and microelements, diffusion velocity, etc. Phosphorus-containing compounds and other ions (Ca2+, Mg2+, Fe3+, Al3+, K+, Na+, etc.) move to the liquid medium face after a defined period of bioconversion. As a result of the phosphate solubilization and a high Ca2+ ion concentration, a process of partial reprecipitation of P as slowly soluble and insoluble phosphates in citric acid together with biomass may possibly occur.

Gyaneshwar et al. (2002) suggested that the organic acid secreted can either directly dissolve the mineral phosphate as a result of anion exchange of PO4 3- by the acid anion or can chelate both Fe3+ and Al3+ ions associated with phosphate. Complexing of
cations is an important mechanism in P solubilization if the organic acid structure favors complexation (Fox et al., 1990). Organic acids may form soluble complexes with metal ions associated with insoluble P and thus P is released (Rashid et al., 2004; Kim et al., 2005).

Some authors indicate that acid production is not the only reason for phosphate release into the medium (Pradhan and Sukla, 2005; Gyaneshwar et al., 2002). In certain cases phosphate solubilization is induced by phosphate starvation (Gyaneshwar et al., 1999). Buffering capacity of the medium reduces the effectiveness of PSB in releasing P from tricalcium phosphates (Stephen and Jisha, 2009).

CONCLUSIONS

The production of chemical phosphoric fertilizers is a highly energy-intensive process. On the other hand most of these fertilizers are transformed into insoluble compounds in the soil, unavailable for plants. Thus, the dependence of fertilizer production on fossil energy, and the prospects of diminishing availability of costly input material for fertilizer production in years to come have obviously brought the subject of mineral phosphate solubilization to the forefront (Khan et al., 2007). It is well known that the high-grade rock phosphate reserves deplete continuously. Hence, the exploration of an alternative phosphate source like low-grade phosphate at low cost should be developed in the near future. These are important reasons to use phosphate–solubilizing organisms in agronomic practice as advocated by several researchers.

According to our investigations and the arguments mentioned above, the study with MAJP can be considered to be an alternative for intensification of P-utilization. The investigations indicate that almost complete extraction of P2O5 from Jordan Phosphorite in a form utilizable by plant can be achieved by using the phosphorus-solubilizing fungus Aspergillus niger. The results obtained can be employed to reduce the fertilizer used if a half dose of P-fertilizer mixed with biofertilizer is introduced into the soil. It is possible to achieve a double effect: the production cost is minimized and the net return maximized.

NOMENCLATURE

C total concentration of soluble P2O5 (g/L)

C1 concentration of soluble P2O5 in the first filtrate (g/L)

C2 concentration of soluble P2O5 in the second filtrate (g/L)

C3 concentration of soluble P2O5 in the biomass (g/L)

P2O5b content of P2O5 in the dry biomass (g/L)

P2O5c.s. content of citric-soluble P2O5 (g)

P2O5m.m. content of P2O5 in the residue of mineral mass (g)

P2O5t. content of total P2O5 (g)

P2O5w.s. content of water-soluble P2O5 (g)

rpm rotational speed (revolutions per minute)

Greek Letters

α total extent of P2O5 extraction (% w/w)

α1 extent of P2O5 extraction in water-soluble forms (% w/w)

α2 extent of P2O5 extraction in citric-soluble forms (% w/w)

α3 extent of P2O5 extraction in biomass (% w/w)

Abbreviations

JP Jordan Phosphorite

MAJP Mechanically Activated Jordan Phosphorite

NAJP Non-Activated Jordan Phosphorite

OLS Ordinary Least Squares

RP Rock Phosphate

PSM Phosphate-Solubilizing Microorganisms

PSB Phosphorus-Solubilizing Bacteria

REFERENCES


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