EVALUATION AND OPTIMIZATION OF ROCK PHOSPHATE AND TRICALCULUM PHOSPHATE SOLUBLIZATION BY SOME SOIL FUNGI

ABSTRACT:
Nine fungal species were isolated from Egyptian soil. They were identified as Aspergillus niger, Aspergillus fumigatus, Aspergillus terreus, Aspergillus flavus, Trichoderma spp., Fusarium spp., Pencillium spp., Mucor spp. and Alternaria spp. The efficiency of the isolated fungi for phosphate ore (Rock phosphate and Tricalcium phosphate) solubilization was studied. A. niger and A. fumigatus showed the highest solubilization value. The optimum growth parameters for phosphate ore solubilization by both fungi were, 28°C, 10 days incubation period, 1% ore concentration, initial pH 6.5-7, sucrose as a carbon source, NaNO₃ as a nitrogen source for A. niger and NH₄Cl for A. fumigatus. The amount of solubilized phosphate from ores (Rock phosphate and Tricalcium phosphate) under optimum conditions reached to approximately 78% and 74% for A. niger, respectively whereas, it was 56% and 54% for A. fumigatus, respectively. Total amount of organic acids produced by both fungi in the growth medium containing rock phosphate was higher than in the control medium. The presence of rock phosphate in the growth medium was highly stimulated the production of oxalic and acetic acid by A. niger. While, it stimulated oxalic and malic acid by A. fumigatus. Whereas, formic and citric acid was high in the control medium of both fungi and only little amounts was detected in rock phosphate containing medium, this may refer to the consumption of both acids in the solubilization process.

KEY WORDS: Phosphate solubilization, Organic acids, Soil fungi.

INTRODUCTION:
Phosphorous is second only to nitrogen as an essential macronutrient for plant growth and development (Scheffer et al., 1998). Phosphates are essential ingredients in the fertilizers used to supply food and feed for mankind and animals. Application of phosphate fertilizers can enhance agricultural production in soils with low phosphate availability, especially in the tropical and subtropical region. However, phosphate application in excess of plant requirements often results in contamination of aquatic systems. Chemical processing of insoluble rock phosphate ore results in almost complete dissolution of the ore, as a result, undesirable ore contaminants are released. These contaminants then must be dealt with as potential air and water environmental pollutants (Goldstein, 2002). However, bioconversion process of rock phosphate ore occurs at a low temperature and is more selective to phosphate extraction than chemical conventional process.

It is generally accepted that the major mechanism of mineral phosphate solubilization is due to the action of organic acids synthesized by soil microorganisms. Several authors have studied the ability of fungi, mainly of genus Aspergillus and Penicillium, to solubilize phosphates under in vitro conditions (Omar, 1998; Seshadri et al., 2004; Wakelin et al., 2004). Aspergillus and Penicillium genera were the most well studied fungi used in bioleaching studies as they were found to be able to liberate considerable amounts of organic acids such as citric, oxalic and tartaric acids which were thought to be the main phosphate solubilizing tool (Aung and Ting, 2004). The inoculation of P-solubilizing microorganisms is a promising technique because it can increase P availability in soils fertilized with rock phosphates (Reyes et al., 2002). Clover growth was stimulated by the presence of PSF and arbuscular mycorrhizal fungi (AM) in
soil amended with aluminum phosphate. It was also found that there is a synergism between microorganisms utilized to improve plant nutrition, (Souchie et al., 2006). The phosphate solubilizing microorganisms (PSM) have been considered as plant growth promoters as the inoculation of soil with these microorganisms increase the crop yield. Inoculation of soil with PSM yields crop similar to those obtained by addition of soluble phosphate (Peix et al., 2001). The AM fungus and Glomus intraradices were able to increase plant available phosphate due to its phosphate solubilization ability (Duponnois et al., 2005). Inoculation of soil with rock phosphate enriched composts (RP-compost) with A. awamori increased total phosphate, water soluble phosphate, organic phosphate and alkaline phosphatase activity (Biswas and Narayanasamy, 2006).

**MATERIAL AND METHODS:**

Rock phosphate samples were collected in sterile plastic bags from phosphate mines present in Safaga and Elkosir on the red sea cost in Egyptian eastern desert. Insoluble Ca$_3$(PO$_4$)$_2$ samples were obtained from Talkha Fertilizers Company, El-Dakahlia governorate Egypt. Chemical composition of the studied phosphate samples was determined using inductively coupled plasma emission spectroscopy (ICP) at the Egyptian Geological Survey laboratories, Cairo, Egypt.

**Isolation of fungal species:**

The common fungal species were isolated from cultivated soil according to the serial dilution technique as described by (Johnson et al., 1959). 0.1 ml of each dilution was placed on the surface of sterile agar plate of Czapek's agar medium containing rose Bengal. After 4 days of incubation at 28°C the developed fungal colonies were purified and identified according to Gilman (1957) and Ellis (1971).

**Culture media:**

Four different types of culture media were used throughout the practical study of this work, which are:

**Czapek's Dox medium:**

It contains (g/l): NaNO$_3$, 2; KH$_2$PO$_4$, 1; MgSO$_4$·7H$_2$O, 0.5; KCl, 0.5; FeCl$_3$. 0.02 and 1 liter distilled water. pH 6.6 up to 7.2.

**NM7 medium (Illmer and Schinner, 1992):**

It contains (g/l): NaCl, 0.1; NH$_4$Cl, 0.4; KNO$_3$, 0.78; MgSO$_4$·7H$_2$O, 0.5; KH$_2$PO$_4$, 1; CaCl$_2$.2H$_2$O; dextrose, 10 and 1 liter distilled water. KH$_2$PO$_4$ was replaced with 1% of insoluble phosphate samples.

The above media were solidified by adding 15g agar per liter. They were autoclaved for 20 min at 1.5 Atm. Pressure. After cooling, the medium was poured in sterile Petri plates for use.

**Testing phosphate solublization by different fungal species:**

**Alizarin red test:**

The isolated fungal species were tested for phosphate solubilization and organic acids production according to the method described by Cunningham and Kuiack (1992). One drop of spore suspension of each fungus was placed in the center of each Czpek's Dox agar plate supplemented with 0.2g of CaHPO$_4$ powder and 1ml of 1% (w/v) alizarin red (Sigma), both were added to the molten medium at 50°C. Triplicate sets of plates were prepared for each fungal species. The solubilization activity was detected by the presence of clear zone around the fungal colony. Medium acidification was visualized by the presence of yellow zone. The diameter of yellow zone around the fungal culture is proportional to organic acid production by the fungus.

**Liquid culture medium test:**

Each of the isolated fungal species were grown on Czapek's Dox liquid medium supplemented with 1% rock phosphate and incubated at 28°C. After the incubation period the fungal filtrate was filtrated several times using Whatman No1 filter paper and centrifuged at 6000 rpm for 20 min. The amount of soluble phosphate in the culture filtrate was determined colorimetrically according to the method described by Olsen et al. (1954).

**Procedures:**

25 ml of culture filtrate was transferred to 50 ml Pyrex graduate flask, 5 ml of molybdate reagent (12.5 g of sodium molybdate (NaMoO$_4$·2H$_2$O) was dissolved in 5M sulphuric acid and was completed to 500 ml with 5M sulphuric acid) was added followed by 2 ml of hydrazinium sulphate reagent (1.5g of hydrazinium sulphate was dissolved in 1 liter of deionised distilled water) and completed to 50 ml with distilled water and then mixed well. The flask was placed in boiling water bath for 10 minutes.
After cooling the absorbance was measured at 830 nm against reagent blank. Phosphate content in each sample was calculated using a standard curve of potassium dihydrogen phosphate.

**Effect of different growth parameter on phosphate solubilization:**

Aspergillus niger and Aspergillus fumigatus were grown in 250 ml Erlenmeyer flasks containing 50 ml lots of Czapek's Dox. Sucrose ammonium, Richard's and NM7 media, separately supplemented with 1% (w/v) of each phosphate samples (rock phosphate and tricalcium phosphate). Triplicates set of flasks were prepared for each phosphate source, different types of culture medium and organisms. Each flask was inoculated with 0.5 ml of spore suspension (10^6 spore/ml) and incubated at 28°C. The amount of soluble phosphate in the culture filtrate was determined. The previous steps were conducted on Czapek's Dox medium supplemented with insoluble phosphate samples for each fungal species at different incubation periods, incubation temperatures, ore concentrations, initial pH, carbon, and nitrogen sources.

**Organic acids production by A. niger and A. fumigatus:**

A. niger and A. fumigatus were grown on Czapek's Dox liquid medium supplemented separately with 1% (w/v) of insoluble phosphate samples as main phosphorus source. Triplicate sets of flasks of each sample were incubated for 10 days at 28°C. The culture filtrate was filtrated several times using whatman No1 filter paper and centrifuged at 6000 rpm for 20 min. Organic acids were determined in the culture filtrate in the presence of 1% (w/v) insoluble phosphate and in its absence (control) using HPLC (GBC) LC -1445 Agricultural research centre, Cairo, Egypt. Organic acids were identified by using authentic samples of the produced acids under the same condition of sample determination.

**RESULTS:**

**Chemical composition of rock phosphate and tricalcium phosphate samples:**

Chemical analysis of the two studied insoluble phosphate samples (rock phosphate and tri-calcium phosphate), revealed the presence of high content of calcium in both samples reached to approximately 32.5% of rock phosphate sample and 36.25% of tri-Ca –P, respectively. Phosphate contents of both samples exhibited also high amounts, reached approximately to 30% of RP and 33.75% of tri-Ca-P. Other elements such as K⁺, Mn⁴⁺, and Na⁺ were found in small quantities ranging from 0.25% to 5% in both insoluble phosphate samples. Whereas Fe²⁺ or Fe³⁺ was not detected in both sample (Table 1).

Table 1. Chemical analysis of insoluble phosphate ores. Data are expressed as mg/g ore.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Insoluble phosphate samples.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rock phosphate (RP)</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>300 (30%)</td>
</tr>
<tr>
<td>CaO</td>
<td>500 (32.25%)</td>
</tr>
<tr>
<td>K₂O</td>
<td>11,250 (1.125%)</td>
</tr>
<tr>
<td>MnO</td>
<td>4,750 (0.475%)</td>
</tr>
<tr>
<td>Na₂O</td>
<td>16,250 (5%)</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: Not detected

**Detection of phosphate solubilization by alizarin red as a pH indicator:**

Production of organic acids by soil fungal species and it's diffusion into the medium were recorded by color change of alizarin from red to yellow, the radius of the yellow zone around the fungal colony reflects the organic acids diffusion into the medium. However the clear zone around the fungal colony refers to the solubilized CaHPO₄ by fungal species. The obtained results indicated that A. fumigatus and A. niger exhibited high value of organic acids production and consequently high phosphate solubilization in the agar medium while, A. terrus showed a little phosphate solubilization ability. A feeble phosphate solubilization activity was also found with penicillium spp. On the other hand phosphate solubilization activity by Fusarium spp. Mucor spp., Alternaria spp. and A. flavus was not detected (Table 2).

Table 2. Solubilization of CaHPO₄ by different fungal species using alizarin as indicator. Data are expressed as the diameter of the clear zone and yellow zone (cm).

<table>
<thead>
<tr>
<th>Fungal spp.</th>
<th>Clear zone (solubilized CaHPO₄)</th>
<th>Yellow zone (acid zone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>4.2 ±0.2</td>
<td>4.7 ±0.2</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>5.3 ± 0.5</td>
<td>5.5 ±0.2</td>
</tr>
<tr>
<td>Aspergillus terrus</td>
<td>2.35 ±0.21</td>
<td>1.97±0.1</td>
</tr>
<tr>
<td>Trichoderma spp.</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>0.5 ±0.2</td>
<td>0.62 ±0.1</td>
</tr>
<tr>
<td>Mucor spp.</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Alternaria spp.</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: Not detected

**Rock phosphate solubilization by different fungal species:**

All fungal isolates could grow well when the phosphorus source of Czapek's Dox liquid medium (KH₂PO₄) was replaced by 1%
Trichoderma spp. also showed a high pH value. Growth which may consume phosphate and Richard's medium, this might be due to high amount of soluble phosphate was low with A. niger medium (5.8) in case of rock phosphate and Dox medium (3.5) and high with Richard's pH, since the final pH was low with Czapek's (Fig. 1). The results were monitored with final results were achieved with both organisms occurred with Richard's liquid medium. These while, the minimum phosphate solublization represented 76.87% and 54.89%, respectively of the total phosphate in the growth medium. Both fungi exhibited also high growth in the presence of rock phosphate.

Table 3. Rock phosphate solublization by various fungal species grown on Czapek's Dox liquid medium supplemented with 1% rock phosphate.

<table>
<thead>
<tr>
<th>Fungal spp.</th>
<th>Final pH</th>
<th>Solublized P (mg/ml)</th>
<th>Dry weight (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger</td>
<td>3.35 ± 0.01</td>
<td>2.306 ± 0.2(76.87%)</td>
<td>10.64±1.2</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>4.72 ± 0.2</td>
<td>1.647 ± 0.02(54.89%)</td>
<td>8.08±0.8</td>
</tr>
<tr>
<td>A. terrus</td>
<td>5.73 ± 0.05</td>
<td>0.309 ± 0.02(10.3%)</td>
<td>2.78±0.3</td>
</tr>
<tr>
<td>A. flavus</td>
<td>4.98 ± 0.01</td>
<td>0.117 ± 0.05(3.72%)</td>
<td>1.13±0.2</td>
</tr>
<tr>
<td>Trichoderma spp.</td>
<td>6.62 ± 0.13</td>
<td>0.102 ± 0.1(13.1%)</td>
<td>1.81±0.2</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>4.98 ± 0.2</td>
<td>0.1558 ± 0.02(5.19%)</td>
<td>2.11±0.2</td>
</tr>
<tr>
<td>Pencillium spp.</td>
<td>3.55 ± 0.003</td>
<td>0.096 ± 0.003(3.2%)</td>
<td>3.54±0.5</td>
</tr>
<tr>
<td>Mucor spp.</td>
<td>6.52 ± 0.04</td>
<td>2.55 ± 0.2(11.7%)</td>
<td>1.45±0.1</td>
</tr>
<tr>
<td>Alternaria spp.</td>
<td>5.23 ± 0.14</td>
<td>0.135 ± 0.01(0.09%)</td>
<td>0.12±0.42</td>
</tr>
</tbody>
</table>

* Data are expressed as mg P/ml culture medium, dry weight (mg/ml culture medium). (±) SE of three determinations.

Effect of different liquid media on phosphate solublization by A. niger and A. fumigatus:

Four different types of liquid media were used each contained 1% w/v insoluble phosphate ore as a main source of phosphorus. The results revealed that maximum phosphate solublization was obtained with Czapek's Dox liquid medium while, the minimum phosphate solublization occurred with Richard's liquid medium. These results were achieved with both organisms (Fig. 1). The results were monitored with final pH, since the final pH was low with Czapek's Dox medium (3.5) and high with Richard's medium (5.8) in case of rock phosphate and A. niger. Whereas, it was quite similar with both media for A. fumigatus while, the amount of soluble phosphate was low with Richard's medium, this might be due to high growth which may consume phosphate and also high pH value.

**Effect of different incubation periods:**

Phosphate solublization by A. niger and A. fumigatus was found to be markedly affected by incubation period as it regularly increased as incubation period increased till a maximum value reached at 10 days of incubation period and represented 75.48% and 63.3% of phosphate in rock phosphate, and 77.35% and 53.54% of phosphate of Tri-Ca-P, respectively, then a slight decrease in phosphate solublization had occurred at 15 days incubation period after which any further increase of incubation period did not affect on the phosphate solublization by both organisms and seemed to be nearly stable (Fig. 1). It was also observed that the dry weight of both fungi increased with increasing incubation period up to 10 days and slightly decreased above this period. On the other hand, the final pH continues to decrease till 10 days of incubation period and little changes had occurred above this period. Therefore, there are no significant changes in phosphate solublization with increasing incubation period above 10 days.

**Effect of different incubation temperatures:**

The growth and rock phosphate solublization by A. niger and A. fumigatus were increased with increasing incubation temperature up to 28°C and then decreased above this temperature. Phosphate solublization by A. niger and A. fumigatus reached to approximately 79% and 59%, respectively at 28°C (Fig. 1). Similar observation was also obtained when A. niger and A. fumigatus were grown in the presence of...
Effect of bulk density:

A. niger and A. fumigatus grew well in the presence of different concentrations of phosphate ore in the growth medium up to 8% (Fig. 2). The optimum growth and best phosphate solubilization occurred at a concentration of 1% of the rock phosphate ore and decreased above this concentration. It was also observed that the final pH value at a concentration of 1% ore was the lowest pH value; this may be due to the production of high amounts of organic acids. At a concentration of 1% ore A. niger and A. fumigatus could solubilize approximately 70% and 53% of phosphate content of the ore, respectively. At this concentration the amount of soluble phosphate were 14.7 and 4.04 times, respectively higher than at a concentration of 0.5% ore.

On the other hand, the growth of A. niger and A. fumigatus increased with increasing tri-calcium phosphate concentration in the growth medium up to 4% and decreased above this concentration. The final pH value also decreased till a concentration of 2% of tri-Ca-P. However, it increased above this concentration (Fig. 2). The best amount of soluble phosphate in the culture filtrate was obtained at a concentration of 2% of tri-calcium phosphate for both organisms. Whereas, the highest percentage of phosphate solubilization was obtained at a concentration of 1% of tri-Ca-P, it represented approximately 72 and 59.5% for A. niger and A. fumigatus, respectively.

Effect of different initial pH:

The maximum growth of A. niger and A. fumigatus on a medium containing rock phosphate was observed at initial pH 7. At this pH value phosphate solubilization exhibited high amounts it represented 77% and 65% for A. niger and A. fumigatus, respectively. It was also observed that phosphate solubilization at pH 8 was sharply decreased for both organisms. While, in case of tricalcium phosphate the optimum phosphate solubilization was obtained at pH 7 for both organisms. Moreover, the best growth was also observed at this pH value (Fig. 2).

Effect of different carbon sources:

The results revealed that A. niger and A. fumigatus could grow well on Czapek’s Dox liquid medium containing different carbon sources and supplemented with 1% rock phosphate or tricalcium phosphate as a main source of phosphorus. Whereas, high amounts of soluble phosphate was detected only in the culture filtrate of A. niger and A. fumigatus with sucrose and glucose followed by fructose. While, lactose, maltose, and starch exhibited low amounts of soluble phosphate. The fungal growth of A. niger and A. fumigatus exhibited remarkable variation according to the utilized carbon source, the best fungal growth reached when glucose was utilized as a carbon source while, the minimum growth reached when lactose was utilized as a carbon source (Fig. 3).
Effect of different nitrogen sources:

*A. niger* could solubilize high amount of phosphorus from rock phosphate ore with all tested nitrogen sources. NaNO₃ was found to be the best nitrogen source utilized by *A. niger* for maximum phosphate solubilization followed by NH₄Cl and it was represented by 79.35% and 77.74% of phosphorus in rock phosphate and tri-Ca-P ores, respectively. Whereas, with *A. fumigatus* phosphate solubilization represented high value with NaNO₃ and NH₄Cl. While, with NH₄NO₃ and urea it exhibited low amounts (Fig. 3). It was also observed that the growth of both fungi at different nitrogen sources did not reflect the solubilization activity.

**Optimum cultural conditions for phosphate solubilization:**

Application of the optimum conditions for phosphate solubilization revealed that *A. niger* could solublize approximately 78.8% and 74.8% while, *A. fumigatus* solublized 56.5% and 54.9% of the phosphate found in rock phosphate and tri-Ca phosphate, respectively (Table 4).

**Table 4.** Solubilization of rock phosphate (RP) and tricalcium phosphate (Tri-Ca-P) by *A. niger* and *A. fumigatus* under optimum cultural conditions.

<table>
<thead>
<tr>
<th>Fungal spp.</th>
<th>Insoluble phosphate ores.</th>
<th>RP</th>
<th>TRI-Ca-P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td>Sol. P (mg/ml) 2.365±0.03 (78.84%)</td>
<td>2.525±0.063 (74.81%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D.Wt. (mg/ml) 11.902±5.2</td>
<td>11.04±3.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F. pH 3.41±0.1</td>
<td>3.39±0.2</td>
<td></td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Sol. P (mg/ml) 1.696±0.2 (56.54%)</td>
<td>1.854±0.1 (54.94%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D.Wt. (mg/ml) 8.9±0.2</td>
<td>9.46±1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F. pH 4.73±0.1</td>
<td>5.21±0.1</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Sol. P (mg/ml) 0.271±0.1 (9.85%)</td>
<td>0.33±0.02 (9.85%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F. pH 7.2±0.05</td>
<td>7.47±0.03</td>
<td></td>
</tr>
</tbody>
</table>


**Organic acids production:**

*A. niger* and *A. fumigatus* were able to produce organic acids such as oxalic, tartaric, citric, malic, lactic, and acetic acids, in the growth medium as secondary metabolites. Utilization of insoluble phosphate as main phosphorus source highly increased the production of some organic acids by both organisms. When *A. niger* grown on Dox liquid medium supplemented with 1% RP, the amount of oxalic acid highly increased to proximally 3.7 times higher than in the control. Tartaric acid is not detected in the control medium while, it was produced in small amount in the presence of RP in *A. niger* growth medium. The production of formic acid and citric acid was high in the control medium while, they highly decreased in the presence of RP, and this may be attributed to the consumption of these acids in the solubilization process. Acetic acid was not detected in the control medium while it was produced in the presence of 1% RP in the growth medium. Almost similar observation to some extent was also found with *A. fumigatus*. Oxalic and malic acids were the major detected organic acids that were highly increased in the presence of RP in the growth medium. Formic and citric acids were produced in high quantities in the control medium and not detected or detected with little amount in the presence of RP. On the other hand tartaric and acetic acids were...
not detected in the control medium or in the presence of RP alone (Table 5 & Fig. 4).

### Table 5. Organic acids production (mg/ml) in culture filtrate of A. niger and A. fumigatus grown on Czapek’s Dox liquid medium supplemented with 1% rock phosphate (RP), incubated for 10 days at 28±2°C.

<table>
<thead>
<tr>
<th>Organic acids</th>
<th>Control</th>
<th>RP</th>
<th>Control</th>
<th>RP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalic acid</td>
<td>8.7</td>
<td>32.3</td>
<td>5.4</td>
<td>35.6</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>ND</td>
<td>3.3</td>
<td>ND</td>
<td>13.0</td>
</tr>
<tr>
<td>Formic acid</td>
<td>12.09</td>
<td>0.6</td>
<td>14.1</td>
<td>ND</td>
</tr>
<tr>
<td>Citric acid</td>
<td>17.3</td>
<td>0.07</td>
<td>15.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>ND</td>
<td>7.9</td>
<td>ND</td>
<td>40.7</td>
</tr>
<tr>
<td>Malic acid</td>
<td>ND</td>
<td>ND</td>
<td>40.7</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td>38.09</td>
<td>44.17</td>
<td>34.9</td>
<td>76.33</td>
</tr>
</tbody>
</table>

*Data are expressed as (mg/ml culture filtrate).

### DISCUSSION:

Chemical and elemental analysis of the two insoluble phosphate samples utilized throughout this study (Rock phosphate and Tricalcium phosphate) demonstrated that, total phosphate content of both samples reached to approximately 30% and 33.75%, respectively. They also contain considerable amounts of Ca, Na, K, and Mn. In another study it was found that some Pakistani rock phosphate ores contain about 33.6% P₂O₅, 0.012 U₂O₅, 40% CaO, and significant amounts of Na₂O and K₂O. Other trace elements such as Si₂O₅, Fe₂O₅, Al₂O₃, and MnO₂ were also found (Saeed et al., 2002).

Microbial solubilization of phosphate materials has been studied as a means of removing phosphate contents from iron ore, and as an alternative process for producing solution phosphate from fluorapatite (Cunningham and Kuiack, 1992). The most intensively studied aspects of microbial phosphate solubilization (MPS), have been the provision of phosphate for plant uptake by the solubilization of phosphates in rhizosphere environment (Bolan et al., 1995).

In our study 9 fungal species were isolated from agricultural soil. They were tested for their phosphate solubilization activity. A. niger and A. fumigatus were found to be the most efficient organisms that are able to release high amounts of phosphate from insoluble phosphate ores. The inoculation of soil with *pencillium spp* may cause increasing of plant phosphate uptake as it is able to release phosphate from unlabeled sources leading to increasing of soil available phosphate, it was also proved that Phosphate solubilization is less effective in poor soil under field conditions (Asea et al., 1987). About 40.5% from Indian rock phosphate was solubilized by a mutant strain of *A. niger* under proved optimum leaching parameters such as RP concentration of 0.1%, initial pH 4, 160 ml fermentation medium and 8 days incubation period, comparing with 10% to 15% solubilized phosphate by parent strain of *A. niger* (Ghosh and Banik, 1998).

The optimum phosphate solubilization activity of *A. niger* and *A. fumigatus* as well as fungal growth were obtained at a concentration of 1% of rock phosphate where, it decreased with increasing phosphate ore concentration in the growth medium, that may be attributed to toxic effect of some metal ions which may be released into the culture medium such as Mn⁺⁺, Ca ++ and Na + ions. These results found to be almost similar to that obtained by (Hefnawy et al., 2002) where the optimal uranium solubilization obtained at 1% of ore for *A. terrus* and *Penicillium spinulosum*. Similar observations was also found in removing of uranium from coal by a filamentous cyanobacteria, where the optimal yield of biologically extracted uranium reached 96% at 1% bulk density (Lorenz and Krumein, 1985). Whereas, *A. niger* showed maximal bioleaching of copper (60%) at a concentration of 5% of mining residue (Maini et al., 2000). These observations refer to the fact that the applied ore concentration varied not only with the ore type but also with the organism used in the bioleaching process.

It was also observed that the highest extraction of metal values from the spent catalyst (54.5% Al, 58.2% Ni and 82.3% Mo) for particle size < 37 μm were obtained in bioleaching by *A. niger* at 1% w/v pulp density in 60 days. Further, oxalic acid was the major lixiviant among the metabolites produced by the fungus (Deenan and Ting, 2005). In recent bioleaching study, it was found that recovery of nickel from low-grade chromate over burden was attempted by employing two fungal species Aspergillus niger and Aspergillus fumigatus, and Acidithiobacillus ferrooxidans. *A. niger* showed maximum recovery of 34% nickel with roasted chromate over burden, at 2% pulp density, while 32% nickel was solubilized by *A. fumigatus* (Mohapatra et al., 2007).

In the present study the best solubilization activity of both tested fungi was obtained at initial pH 7 for *A. niger* and *A. fumigates* with consequent lowering in the final pH. This was in agreement with the result obtained by Achal et al., (2007) who suggested that tri-calcium phosphate and rock phosphate solubilization by phenotypic mutants of *Aspergillus tubingensis* is due to lowering of the final pH of the culture filtrate and also the activity of acid phosphatase and phytase. Also similar result was obtained by
Pradhan, and Sukla, (2006) who proved that Phosphate solubilization by Aspergillus fumigatus and Penicillium sp. was accompanied by a decrease in the pH of the medium by both strains. Mehat et al., (1979) they found that the solubilization of aluminum from its rocks by P. simplicissimum was found to be optimum at initial pH 7 after 30 days of incubation. Whereas, Hefnawy et al., (2002) found that A. terrus was able to solublize (88%) of uranium from its ores at initial pH 4. Additionally Ghosh and Banik, (1998) revealed that a mutant of A. niger was able to solublize 40% of RP at initial pH 4. However, A. japonicus and A. foetidus were found to solublize phosphate from five types of Indian phosphate ores in the alkaline reaction at initial pH 8 and 9.

Nutritional constituent of the culture medium may play an important role in phosphate solubilization from its ores. In our study carbon and nitrogen sources are crucial in this process, sucrose, represented the best carbon source utilized by A. niger and A. fumigatus during phosphate solubilization process. It may be used in the production of certain organic acids that involved in solubilization process. On the other hand the best solubilization of phosphate by A. niger occurred in the presence of sodium nitrate as sole nitrogen source while, NH₄Cl was best N-source utilized by A. fumigatus. It was reported that solubilization of tricalcium phosphate by strains of Sacharomyces cervisiae and Rhodotula minuta was at maximum value when sodium nitrate was utilized as N-source (Narsion and Patel, 1995). Variation of medium composition markedly altered the phosphate mobilization by phosphate solubilizing microbes (Illmer and Schinner, 1992). Hefnawy et al., (2002) proved that sucrose was the best C-source utilized by A. terrus, for maximum uranium solubilization to be reached, while NH₄Cl was the best utilized N-source.

The optimum incubation temperature for best phosphate solubilization activity by A. niger and A. fumigatus was approximately at 28°C. Quite Similar results were obtained by Hefnawy et al., (2002) who reported that A. terrus was able to solublize 75% of uranium content of the ore at 30°C. Optimum phosphorus solubilization by pseudomonas sp. and Pencillium spp. were at 25°C and 30°C (Illmer and Schinner, 1992). An incubation temperature of 25°C was found to be optimum for AlPO₄ and Calcium phosphate solubilization by P. simplicissimum (Illmer et al., 1995).

The optimum incubation period for best phosphate solubilization by A. niger and A. fumigatus was 10 days which may be in agreement with results obtained by Asea, (1987) who mentioned that maximum solubilization for RP by P. bilaii and P. fucsem was reached after 12 days of incubation period. Whereas, in a closed incubation system the dissolution of rock phosphate by A. niger reached a maximum value (40 %) within 28 days (Bolan et al., 1995). Quite similar observation was obtained by Darmwal et al., (1991) who proved that A. niger was found to be the best phosphate solubilizer among several tested fungi and bacteria and that the maximum amount solubilized from P₂O₅ was reached after 7 to 10 days of incubation period. Slightly different results were obtained by Hefnawy et al., (2002) who mentioned that the maximum uranium released by A. terrus was reached after 6 to 8 days.

Much work has been done on the role of organic acids in metal solubilization and mobilization. Citric, malic, tartaric and acetic acids produced by A. niger were believed to have a great effect on the release of rare earth elements (Shan et al., 2002). It was reported that citric, oxalic and gluconic acids produced by A. niger were found to be an enhancing factor which improve fungal bioleaching and metal extraction from municipal solid waste incinerator fly ash (Hung and Ting, 2005).

Phosphorus solubilizing microorganisms are reported to dissolve insoluble phosphates by the production of inorganic or organic acids and/or by the decrease of the pH (Whitelaw, 2000). Phosphate solubilization has been attributed to the release of organic acids in culture solution (Illmer et al., 1995). Amounts of citric and oxalic acids produced into culture media was found to be a limiting factor for the efficiency of CaHPO₄ solubilization by P. bilaiii (Cunningham and Kuicak, 1992).

The obtained results in our study are in agreement with all previous literatures where, A. niger and A. fumigatus were able to produce oxalic, citric, malic and tartaric acids in the growth medium and their concentrations were increased in the presence of 1% RP or Tri-Ca-P in the growth medium.

CONCLUSION:

A. niger and A. fumigatus were the best isolated fungal species for phosphate solubilization from its ores. The optimum conditions for solubilization were organism’s incubation at temperature 28°C, initial pH 7, 1% bulk density, and sucrose as a carbon source and sodium nitrate as a nitrogen source. Both fungi were able to produce considerable amounts of organic acids in the culture medium with subsequent increase in the presence of phosphate ores.
REFERENCES:


Pradhan N, Sukla LB. 2006. Solubilization of inorganic phosphates by fungi isolated...


