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Evaluation and Optimization of Abu Tartur Egyptian Phosphate Ore Dissolution

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ABSTRACT:

Phosphorus is the most required nutrient of plants, but its availability in Egypt is relatively low. The use of phosphate solubilizing microorganism to dissolve phosphate content of phosphate ore instead of conventionally methods is an ecologically safe and economically reasonable. In this study dissolution of phosphorus from Abu Tarture Egyptian ore which has low solubility level was applied by two different fungal isolates *penicillium chrysogenum* and *Aspergillus niger* to increase availability of phosphorous. The results of this study indicated that *p. chrysogenum* gave the highest value with 95% maximum dissolution of 0.5 % phosphate ore. The objective of the present work, study all factors affecting on phosphate dissolution of Abu Tartur phosphate ore. The optimum conditions for dissolution of phosphate ore by *p. chrysogenum* were 0.25 g ore per 50 ml of broth PVK medium, inoculated with spore suspension $5.1x10^6$ cfu/ml incubated for 4 days at 30° C**,** glucose and yeast were the most effective carbon and nitrogen source. By apply this optimum conditions, maximum dissolution of phosphate content of ore reaches to 95%. Whereas, *A. niger* isolate optimum dissolution conditions were 0.25 g ore for 50 ml of PVK broth medium inoculated with spore suspension $5.2x10^6$ cfu/ml and incubated for 4 days at 30 ° C**,** sucrose and tryptone were the most effective carbon and nitrogen source. By applying this optimum conditions maximum dissolution of phosphate content of ore reaches to 85.5%.

Key words:

Abu Tartur phosphate ore, *penicillium chrysogenum*, *Aspergillus niger*, Phosphate dissolution

INTRODUCTION:

Phosphorus deficiency in agriculture soils is a factor that limits plant productivity (Oberson *et al*., 2006; Cramer, 2010). So, it is important to apply soluble fertilizers to reach the sufficient concentrations of phosphorous in agriculture soil solution (Narsian & Patel, 2000; Vassilev *et al*., 2012).

To overcome the specific phosphorous deficiency, different forms of phosphate, varying from processed rock phosphates (phosphate -fertilizers) to ground phosphate rocks are applied. The use of commercial Phosphate fertilizers is not cost effective. Among the alternative phosphate sources, the most important are locally available Rock Phosphate resources (Rajan *et al*., 1996)

Filamentous fungi are widely used as producers of organic acids and in particular *Aspergillus niger* and some species of *Penicillium* have been tested in fermentation system or inoculated directly into soil in order to solubilize rock phosphate (Vassilev *et al*., 1996)

Reddy *et al*., 2002 found that all the isolates of *A. niger* isolated from rhizospheric soils were found to be capable of solubilizing all the natural forms of rock phosphates. Showed that this fungus might serve as an excellent rock phosphate solubilizer when inoculated into soils where rock phosphate is used as Phosphate fertilizer.

One problem of intensive agriculture is that the fertilizers are expensive and their efficiency is very low (Vassilev & Vassileva, 2006), making it unsustainable. Because of this, there is a pressing need to identify sustainable alternatives that may allow for a more efficient use of P for cultivated plants (Consensus Statement Declaration, 2011).

Rock phosphate is a locally available and cheap alternative, but its reactivity is low due to its slow dissolution and release of Phosphorous (Yusdar *et al*., 2007; Hamdali *et al*., 2010). On the other hand, it is known that the dissolution of rock phosphate improves when microorganisms capable of dissolving it are added to the system. This is the case with phosphate solubilizing microorganisms (Galindo *et al*., 2011; Paiva-Coutinho *et al*., 2012).

There have been various reports on the dissolution of Rock Phosphate with individual inoculates of just one species, but little is known about the effectiveness of microbial consortia (Singh and Reddy, 2011). Additionally, existing reports on the subject have focused on one particular type of rock phosphate, which has limited the results to very specific effects (Xuan Yu *et al.*, 2012).

Roos and Luckner 1984 observed that an isolate of *Penicillium cyclopium* employed an NH_4^+ /H^+ exchange mechanism in presence of NH_4Cl and glucose in the medium resulting in the generation of inorganic acid which caused reduction of medium pH.

Phosphate rock is the major source of phosphorus in nature and is being used as the raw-material for manufacturing commercial phosphate fertilizers (90%) and elemental phosphorus (10%) used in the chemical and food industries. The rock phosphate consists of insoluble calcium phosphate, generally known as apatite. The general formula of apatite is $Ca_5 (PO_4)_3$ (OH, F, Cl) and depending on the end member, apatite can be named as hydroxyl apatite, fluorapatite or chloroapatite , (Tilak, K.V.B.R. *et al*. (2005)).

The dissolution of rock phosphate can be increased through microbial mediation. *Penicillium bilaii* produces citric and oxalic acids to solubilize calcium phosphate (Cuuingham & Kuiack, 1992), others *Penicillium* sp, also produces gluconic acid (Vassilev *et al*., 1996).

In this work, the factors affecting on phosphate dissolution of Abu Tartur phosphate ore by using two different fungal isolates were approved for maximum dissolution of P_2O_5.

MATERIAL AND METHODS:

Egyptian Phosphate Ore Collection:

Abu Tartur phosphate deposit is one of the largest phosphates mining area (1000 million tons and 200 million tons proved) in the Middle East Rock phosphate sample is collected in plastic bags from phosphate mine present in Safaga and Elkosir on the red sea coast in Egyptian eastern desert. Chemical composition of the studied phosphate sample is determined by using XRD analysis.

Isolation of Phosphate Solubilizing Fungi:

Two fungal isolates were isolated and characterized from post infection of dry meat. phosphate solubilizing activity were done on Pikovskaya agar Composition: Ingredients g/ l Yeast extract 0.5, Dextrose 10, Calcium phosphate 5, Ammonium sulphate 0.5, Potassium chloride 0.2, Magnesium sulphate 0.1, Ferrous sulphate 0.0001 Agar 15. Formula adjusted, standardized to suit performance parameters.

Different culture media used in optimization of phosphate dissolution by two fungal isolates

Different types of culture media are used in the practical study of this work, which are:

Czapek's Dox medium (Thom C. and Church M.B., 1926**):**

It contains (g\l): $NaNO_3$, 2; $Ca_3(PO_4)_2$, 1; $MgSO_4.7H_2O$, 0.5; KCl, 0.5; $FeSO_4.5H_2O$ traces; sucrose, 30 and 1 liter distilled water.

Glucose-yeast extract medium(Wickerham, J. 1939)**:**

It contains (g\l): Glucose, 10 g; Peptone, 5.0 g; Yeast extract, 3.0g; Agar, 20.0 g, Distilled water, 1.0 l and adjust pH to 6.8 before autoclaving, autoclave at 121°C for 15 min.

Pikovskaya's medium, (Sundara Rao and Sinha (1963)**:**

It contains (g/l): 0.5 g/l Yeast extract, 10 g/l Dextrose, 5 g/l Tri calcium phosphate, 0.5 g/l Ammonium sulphate, 0.2 g/l Potassium chloride, 0.1 g/l Magnesium sulphate, 0.0001 g/l Manganese sulphate and 0.0001 g/l Ferrous sulphate. Suspend 16.3 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Dispense as desired.

The above media are solidified by adding 15g agar per liter. They are autoclaved for 20 min at 1.5 Atm. Pressure.

Characterization of phosphate solubilizing fungal isolates:

DNA isolation and PCR condition:

DNA extraction was done by use protocol of Gene Jet Plant genomic DNA purification Kit (Thermo). PCR by using Maxima Hot Start PCR Master Mix (Thermo) #K0221and Primer of ITS1 5- TCCGTAGGTGAACCTGCGG-3 and ITS4 5- TCCTCCGCTTATTGATATGC-3 (White et al. 1990). Initial denaturation. 95^0 C for 10 min Denaturation. 95^0 C for 30 sec. annealing 55^0 C for 1 min Extension 72^0 C for 1min Final extension 72^0 C for 15min Number of cycles 35. PCR clean up for the PCR product using GeneJET™ PCR Purification Kit (Thermo)

Sequencing is done to the PCR product on GATC Company by use ABI 3730xl DNA sequence by using forward and reverse primers.

Optimization of phosphate dissolution:

Different types of media, ore amount, inoculum size, incubation period, different carbon and nitrogen source and different incubation temperature were evaluated for increase phosphate dissolution by two different fungal isolates

Organic acid production screening:

Two fungal species isolated is subjected for organic acid production. One disk of fungal isolate is inoculated on petri plates containing mineral agar organic acid indicator (Sunstornsuk *et al*., 1994) and incubated for five days for the formation of yellow zone around the mycelial growth. The medium used is Czapek's agar medium with 1% bromocresol green as organic acid indicator.

The HPLC analysis

The HPLC analysis is performed on clarity chromatography data system, the HPLC system consisted of two pressure pumps (Sykam S1122 delivery system), the injection port with a 2ml loop (Sykam S 5111 Injector valve bracket), a UV detector (Jasko – UV – 2070 Plus, Intelkigent UV/Visible detector, Japan). For chromatographic separation, C-18 column (Thermo Hypersil Keystone, 5 µm, 250×4.6 mm) was used. Potassium

dihydrogen phosphate buffer (pH 2.8) was used as mobile phase and flow rate was adjusted to 1 ml /min. Sample volume (20 µl) was injected with the help of a micro syringe, the run time was adjusted to 10 min and UV absorbance was determined at 214 nm. Autochrom 3000 software was the data acquisition system. Authentic sample of oxalic and fumaric acids are used (Sigma). The results obtained from HPLC analysis of the samples are monitored using the above mentioned authentic samples.

RESULTS AND DISCUSSION:

Chemical Composition of Abu Tartur Phosphate Ore:

Abu Tartur phosphate ore is characterized by XRD (Figure1) which shows the presence of insoluble P_2O_5 is 24.5 % and the other element presence in this ore is Ca 39.5 %, L.O.I. 12.32 %, SiO_2 with 7%, SO 5.07%, Fe_2O_3 with 6.6%, Al_2O_3 2.02%and other traces elements (Table 1).

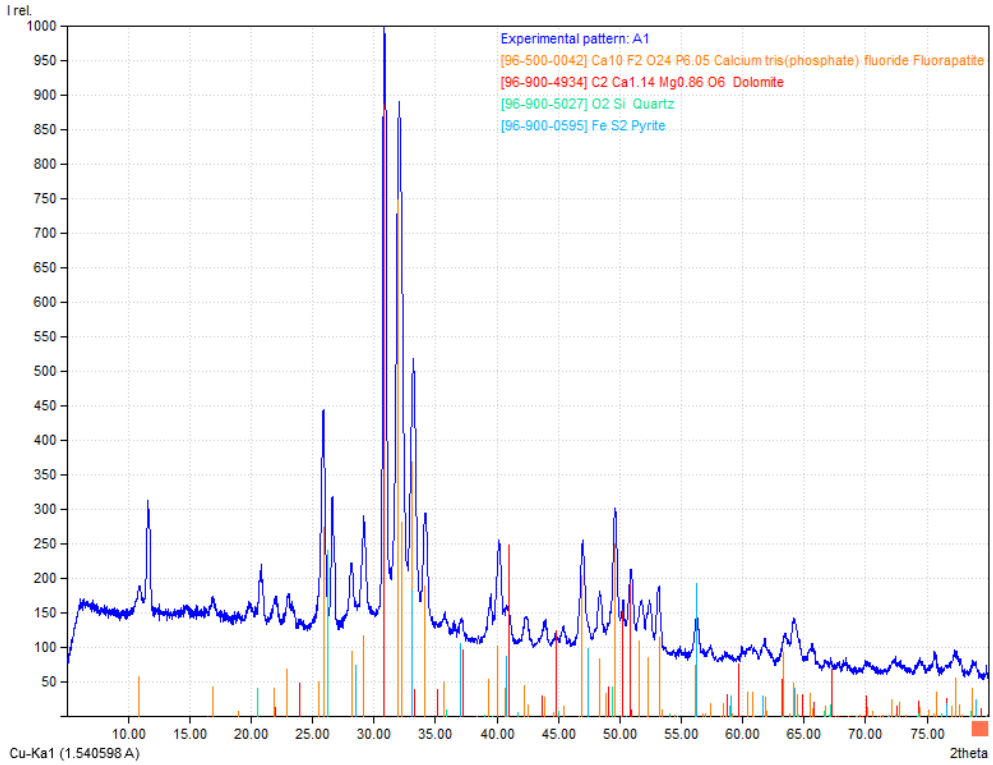

Figure (1): XRD Analysis of Abu Tartur Phosphate ore.

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Table (1): Components of Abu Tartur Phosphate ore

Elements	Percentage %	Elements	Percentage %
P_2O_5	24.5	Na_2O	0.194
Fe_2O_3	6.6	Al_2O_3	2.025
Ca	39.5	MgO	1.750
SiO_2	7.0	MnO	0.503
SO_2	5.072	K_2O	0.254
Cl	0.064	Cr_2O_3	0.052
L.O.I	12.32	F	0.157

Molecular identification of two fungal isolates:

Molecular identification of the selected isolate ITS1 and ITS 2 sequencing is a powerful tool for rapid identification and phylogenetic analysis of fungal species. Nucleotide sequence was compared with available nucleotides ribosomal sequences in the NCBI database using BLASTN.. The first isolate has been enrolled into a cluster containing *Penicillin* sp. and was found to be closely related to *penicillium chrysogenum.* The second isolate has been enrolled into a cluster containing *Aspergillus* sp. and was found to be closely related to *Aspergillus niger*, Figure(2).

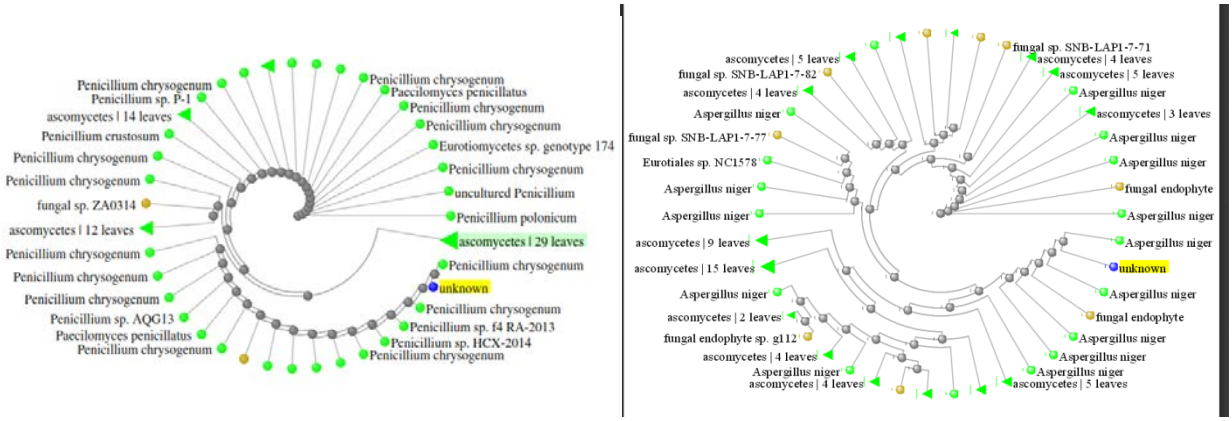


Figure (2): Phylogenetic Tree of *Penicillium and Aspergillus* Isolate Respectively.

Optimization of phosphate dissolution by *penicillium chrysogenum* isolate:
Effect of different growth factors on phosphate ore dissolution:

P. chrysogenum and *A. niger* isolates were grown in Erlenmeyer Flask 100 ml contains 50 ml of Pikovskaya broth at 28°C and shaking incubator 150 rpm is studied with different growth factors on phosphate ore dissolution. The amount of soluble phosphate, pH and redox potential in culture filtrate is determined. The previous conditions on

pikovskaya broth media are studied with different culture media, incubation period, ore amount, inoculums size, carbon and nitrogen sources.

Effect of different incubation period on phosphate dissolution by *penicillium chrysogenum* :

Whereas, Using PVK medium in presence 0. 5 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with spore suspension $5.2x10^6$ CFU/ml of *penicillium chrysogenum* and incubated at 28°C, 150 rpm and measuring P_2O_5 each 2 days and also pH and redox potential.

As the results obtained from above conditions, the effect of different incubation period was studied to evaluate phosphate ore dissolution by *penicillium chrysogenum.* The results show that phosphate dissolution markedly affected by incubation period as it regularly increased till a maximum value reached after 4 day with 18.9 % phosphate dissolution, Figure(3). In addition the final pH sharply decreased and highly increased in redox potential after 4 day of incubation and then increases in pH and decreases in redox potential have occurred till 8 day of incubation period. This correlated with growth curve of *penicillium chrysogenum* that begins the log phase from second day until fourth day from growth then enter in stationary phase up to 13^{th} day of growth that produce all acids in these phase which may be in agreement with results obtained by Pradhan and Sukla, (2005) who proved that *pencillium* were found to be the best phosphate solublizer among several tested fungi and bacteria. This agrees with Maisa, 2013 who found that Increasing of the incubation period was accompanied by gradual increase in the uranium leaching efficiency which reached its maximum value with the fourth day.

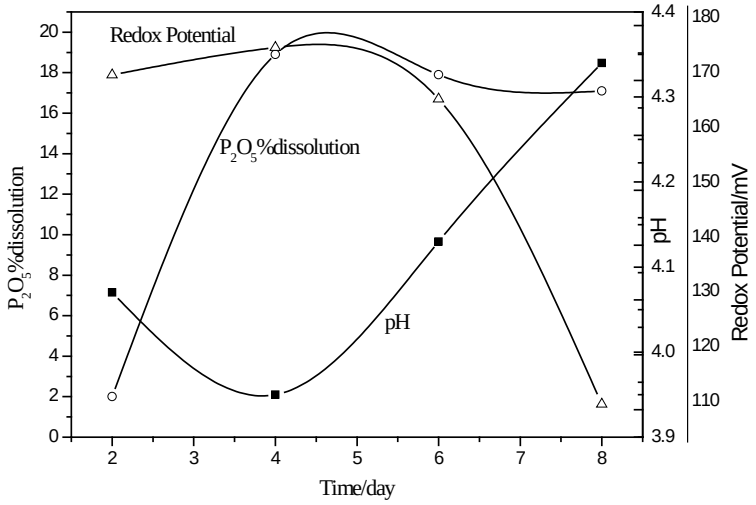


Figure (3): Effect of Different Incubation Time Period on Phosphate Dissolution by *Penicillium chrysogenum*

Effect of different incubation period on phosphate dissolution by *A. niger* isolate:

Using PVK medium in presence 0.25 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with spore suspension $5.2x10^6$ CFU/ml spores and measuring P_2O_5 each 2 days and also pH and redox potential.

As the results obtained from above conditions, the effect of different incubation period was studied to evaluate phosphate ore dissolution by *A. niger* isolated. The results show that phosphate dissolution markedly affected by incubation period as it regularly increased till a maximum value reached after 4 day with 47.8 % phosphate dissolution, Figure(4). In addition the final pH sharply decreased and highly increased in redox potential after 4 day of incubation and then slightly changes in pH and redox potential have occurred till 8 day of incubation period. This correlated with growth curve of *A.niger* isolated that begins the log phase from third day until fifth day from growth then enter in stationary phase up to 12^{th} day of growth that produce all acids in these phase which may be in agreement with results obtained by Pradhan and Sukla 2005 who proved that *A. niger* was found to be the best phosphate solublizer among several tested fungi *Aspergillus niger* solubilized 480g/ml of phosphorus, from 0.5% tricalcium phosphate after 4 days of growth respectively.

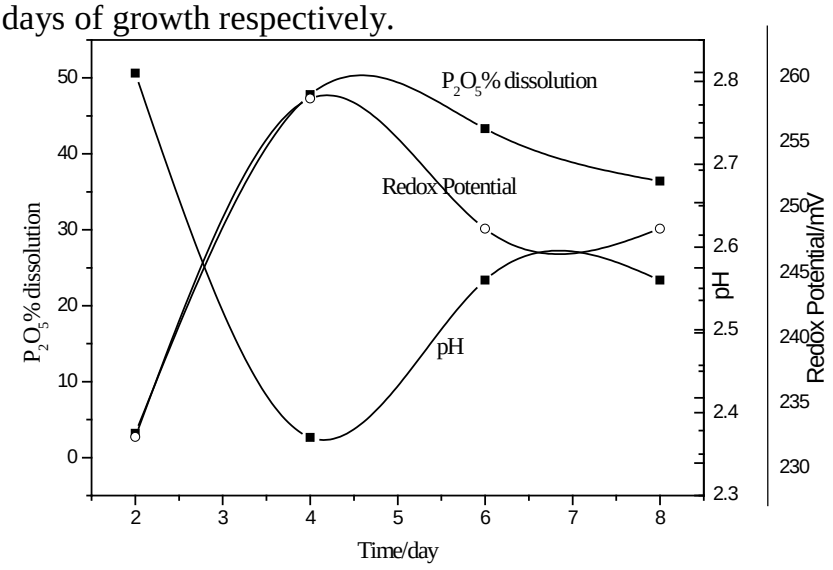


Figure (4): Effect of Different Incubation Time Period on Phosphate Dissolution by *A. Niger* Isolated.

Effect of different media on phosphate dissolution by *penicillium chrysogenum*:

Three different types of liquid media are used, Pikovskaya with initial pH 6, Czapex's Dox with initial pH 6 and glucose yeast extract medium with initial pH 6, each contained

0.5 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with spore suspension 5.1x10^6 CFU/ml of *penicillium chrysogenum* isolate and incubated at 28 °C and 160 rpm and measuring P_2O_5 each 2day and also pH and redox potential.

The results reveal that Pikovskaya broth media gave maximum insoluble phosphate ore dissolution 18.9% after 4 day whereas Czapex's Dox broth media with *penicillium chrysogenum* isolate gave 9.4 % phosphate ore dissolution after 6 day. On the other hand glucose yeast extract medium gave very low phosphate dissolution with 7 % (Figure 5) and this refers to best growth of *penicillium chrysogenum* isolate on components of PVK medium and produces acids to leach the phosphate content of ore.

This agrees with Pradhan and Sukla (2005) who evaluated two P solubilizing fungi isolated from rice filed soil, using different media to select P solubilizing. They confirmed the impact of media type on phospherous solubilization ability. Similarly, Son *et al.* (2006) affirmed the effect of the concentrations of some elements of the media as salts and nitrogen source on solubilization of the inorganic phosphates.

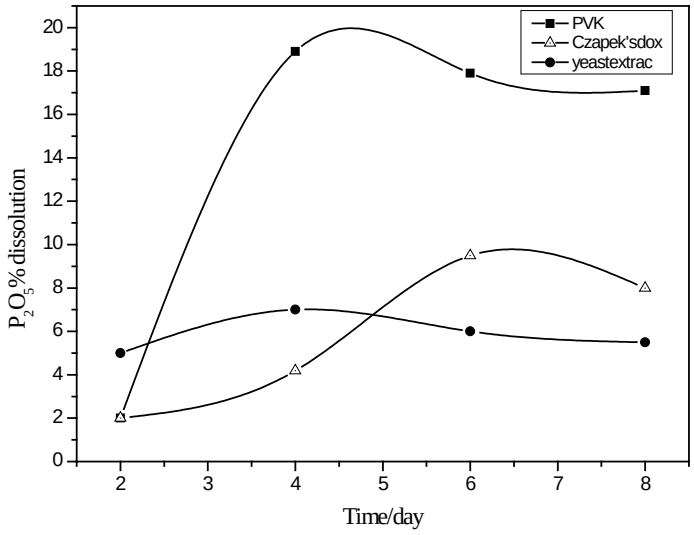


Figure (5): The Effect Of Type Of Medium On Dissolution Of P_2O_5 Content of Ore by *penicillium chrysogenum* isolate .

Effect of different media on phosphate dissolution by *A. niger* isolated:

Three different types of media are used in this study Pikovskaya with initial pH 6, Czapex's Dox with initial pH 6 and glucose yeast extract with initial pH 6. each contained 0.25 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with 5.2x10^6 spores of *A. niger* isolated and incubated at 30°c and 160 rpm and measuring P_2O_5 each 2day and also pH and redox potential.

The results reveal that Pikovskaya broth media gave maximum insoluble phosphate ore dissolution 47.8% after 4 day with final pH 2 whereas Czapex's Dox broth media with *A. niger* isolated gave 39 % phosphate ore dissolution after 6 day with final pH 2. On the other hand yeast dextrose gave very low phosphate dissolution with 5 % with no change in pH value (Figure 6) and this refers to best growth of *A. niger* isolated on components of PVK medium and produces acids to leach the phosphate content of ore.

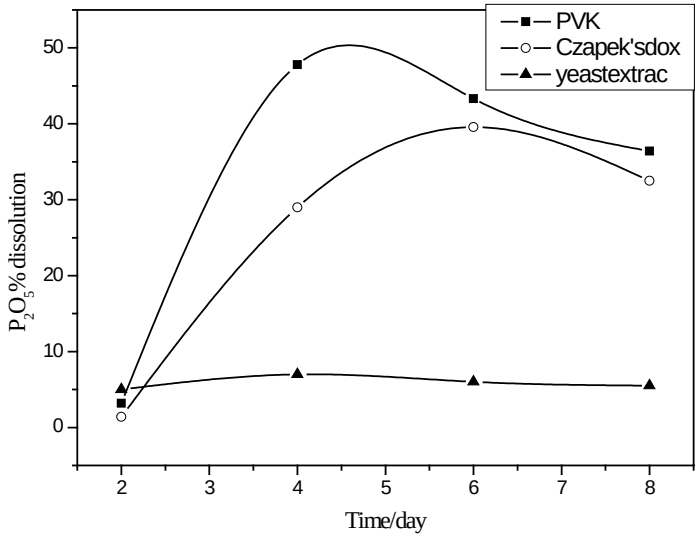


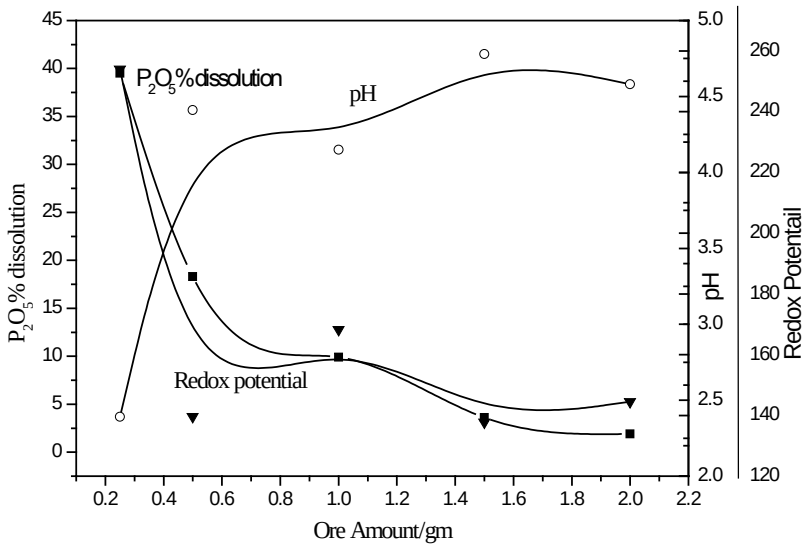
Figure (6): The Effect of Type of Medium on Dissolution of P_2O_5 Content of Ore.

Effect of different ore amount on phosphate dissolution by *penicillium chrysogenum* isolate:

Different phosphate ore amount per 50 ml of broth Pikovskaya medium as (0.25, 0.5, 1, 1.5, 2) gm are used to determine the ability of *penicillium chrysogenum* isolate to dissolve large amount of Abu Tartur phosphate ore incubation for 4 day at 28° C and 155 rpm. The results show that maximum phosphate solubilization reaches to 39.5% phosphate dissolution for 0.25 g ore with highest redox potential and lowest pH value then dissolution of phosphate content of ore begins to decreases with increasing concentration of ore (Figure 7).

The dissolution of phosphate decreases 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^{+2} and Na^{+1}, Ca^{+2} ions and these ions can react with soluble phosphate and form insoluble phosphate so decrease total soluble phosphate, these results found to be almost similar to that obtained by (Hefnawy *et al*., 2002). Also, it may be due to inhibitory effect on further phosphate solubilization

(Narsian et al., 1995), the negative effect of soluble P on microbial acid productivity (Rohr et al., 1983) might also be responsible for final soluble P concentration Another explanation for this might be formation of an organo-P compound induced by organic metabolites released, which in turn, reduces the amount of available P (Illmer and Schinner, 1992).



Figure(7): Effect of Different Ore Amount on Phosphate Dissolution by *Penicillium Chrysogenum* Isolate**.**

Effect of different ore amount on phosphate dissolution by *A. niger* isolated:

Different phosphate ore amount per 50 ml of broth Pikovskaya medium as (0.25, 0.5, 1, 1.5, 2) g are used to determine the ability of *A. niger* isolated to dissolve large amount of Abu Tartur phosphate ore which inoculated with $2x10^6$ spore forming unit and incubation for 4 day at 30° C and 155 rpm. The results show that maximum phosphate solubilization reaches to 48 % phosphate dissolution for 0.25 g ore and begins to decreases with increasing concentration of ore (Figure 8).

The dissolution of phosphate decreases 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^{+2} and Na^{+1}, Ca^{+2} ions and these ions can react with soluble phosphate and form insoluble phosphate so decrease total soluble phosphate, these results found to be almost similar to that obtained by (Hefnawy *et al*., 2002). Also, it may be due to inhibitory effect on further phosphate solubilization

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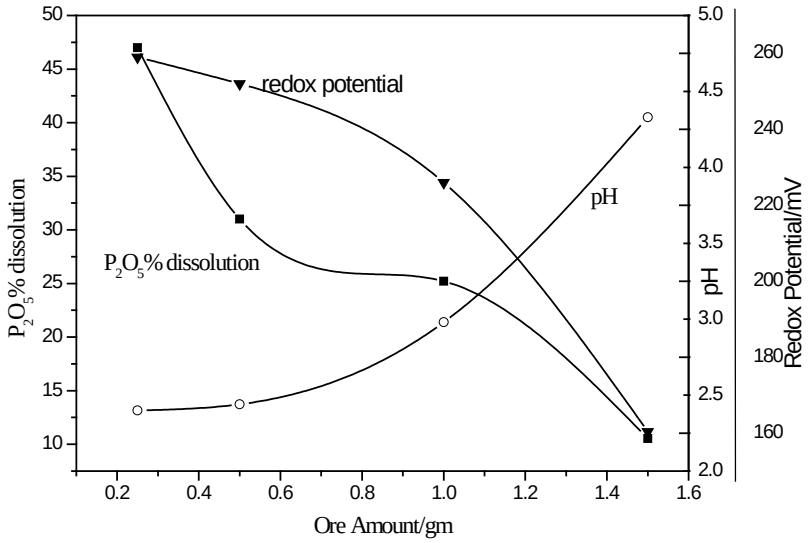


Figure(8): Effect Of Different Ore Amount on Phosphate Dissolution by *A. niger* Isolated.

Effect of different inoculums size on phosphate dissolution by *penicillium chrysogenum* isolate:

Different inoculums size are used from *penicillium chrysogenum* isolate ($5.1x10^6$, $10.2x10^6$, $15.3 x10^6$ and $20.4 x10^6$) SFU of fungal growth supplied with 0.25 g phosphate ore in 50 ml PVK medium then incubated for 4 days at 28°C and 155 rpm. The results reveal that at a concentration of $5.1x10^6$ spores, *penicillium chrysogenum* isolate can solublize approximately 39.5% of phosphate content of the ore after 4 days of incubation (Figure 9).

Concentration of fungus affects on dissolution of phosphate content of the ore. The best phosphate solublization occurs at a concentration of $5.1x10^6$ spores of fungus and decreases at high concentration of fungus with increase pH value and decrease redox potential and this may be due to competition factor between spores themselves, decreases the aeration and also high growth which may consume phosphate.

This agrees with Laura Osorno (2014) who found that maximum capacity of *Mortierella* sp. was detected with lowest population level ($1x10^7$ CFU) suggested that this fungus is effective using nutrients and producing the required acidity to dissolve rock phosphate and by increasing the population density of *Mortierella* sp. their effectiveness decreases, which may be due to intraspecific competition.

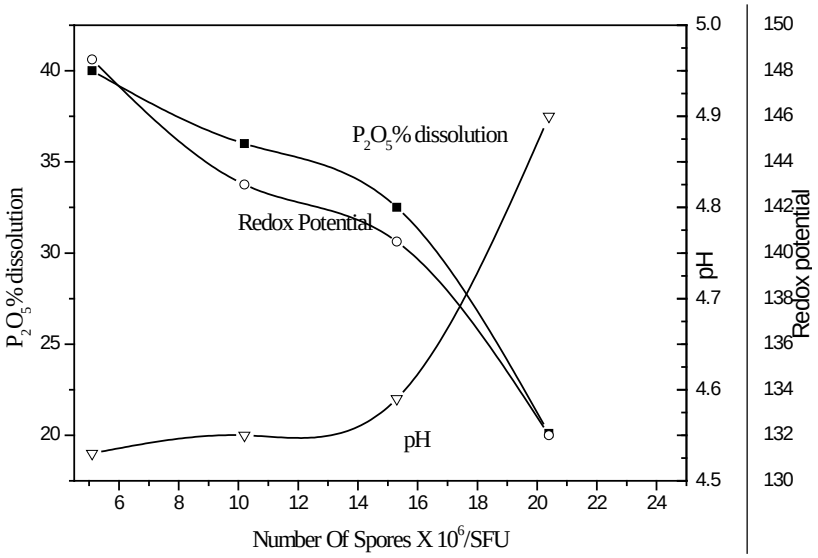


Figure (9): The Effect of *Penicillium Chrysogenum* Isolate Amount on Dissolution of P_2O_5 Content of Ore.

Effect of different inoculums size on phosphate dissolution by *A. niger* isolated:

Different inoculums size are used from *A. niger* isolated ($2x10^6$, $4 x10^6$, $6 x10^6$ and $8 x10^6$) SFU of fungal growth supplied with 0.25 g phosphate ore for 4 days of incubation. The results reveal that at a concentration of $2x10^6$ spores, *A. niger* can solublize approximately 47% of phosphate content of the ore after 4 days of incubation (Figure 10).

Concentration of fungus affects on dissolution of phosphate content of the ore. The best phosphate solublization occurs at a concentration of $2 x10^6$ spores of *A. niger* isolated and decreases at high concentration of fungus with increase pH value and decrease redox potential and this may be due to competition factor between spores themselves, decreases the aeration and also high growth which may consume phosphate.

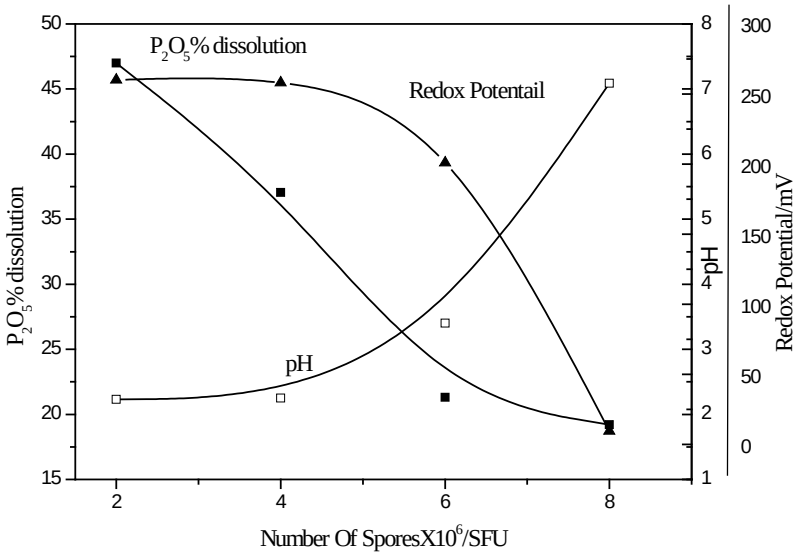


Figure (10): The Effect of *A.Niger* Isolated Amount on Dissolution of P_2O_5 Content of Ore.

Effect of different incubation temperature on phosphate dissolution by *Penicillium Chrysogenum* Isolate:

It is studied by using three different temperature(20, 30, 40 °c) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of PVK medium and inoculated with $5.1x10^6$ spores of *penicillium chrysogenum* isolate and incubated for four days then measuring P_2O_5, pH and redox potential. The growth and rock phosphate solublization by *penicillium chrysogenum* isolate is increased with increasing incubation temperature up to 30°C and then decreased above this temperature. Phosphate solublization by *penicillium chrysogenum* isolate reaches to approximately 45 % at 30°C after 4 days of incubation (Figure 11).

The optimum growth of fungus occurs at 30°C which refers to high production of acids lead to increase phosphate content solublization in the ore at this incubation temperature. It is also observed that the final pH is the lowest one at this temperature and increase redox potential and this associated with organic acids production.

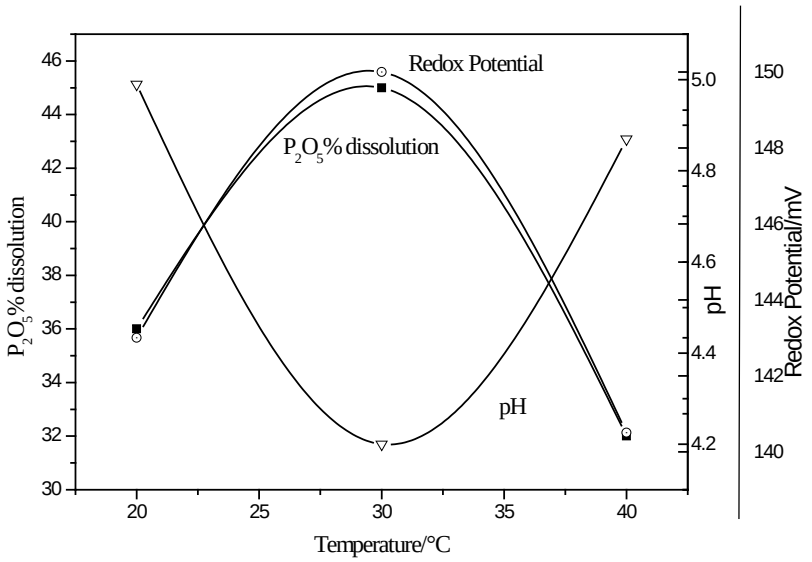


Figure (11): The effect of Temperature on Dissolution of P_2O_5 Content of Ore.

Effect of different incubation temperature on phosphate dissolution by *A. niger* isolated:

It is studied by using three different temperature(20, 30, 40 °c) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of PVK medium and inoculated with $2x10^6$ spores of *A. niger* isolated and incubated at 30 °c and 160 rpm and measuring P_2O_5 . The growth and rock phosphate solublization by *A. niger* isolated is increased with increasing incubation temperature up to 30°C and then decreased above this temperature. Phosphate solublization by *A. niger* isolated reaches to approximately 50 % at 30°C after 4 days of incubation (Figure 12). The optimum growth of *A. niger* isolated occurs at 30ºC which refers to high production of organic acids lead to increase phosphate content solublization in the ore at this incubation temperature. It is also observed that the final pH is the lowest one at this temperature and increase redox potential and this associated with organic acids production. 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.

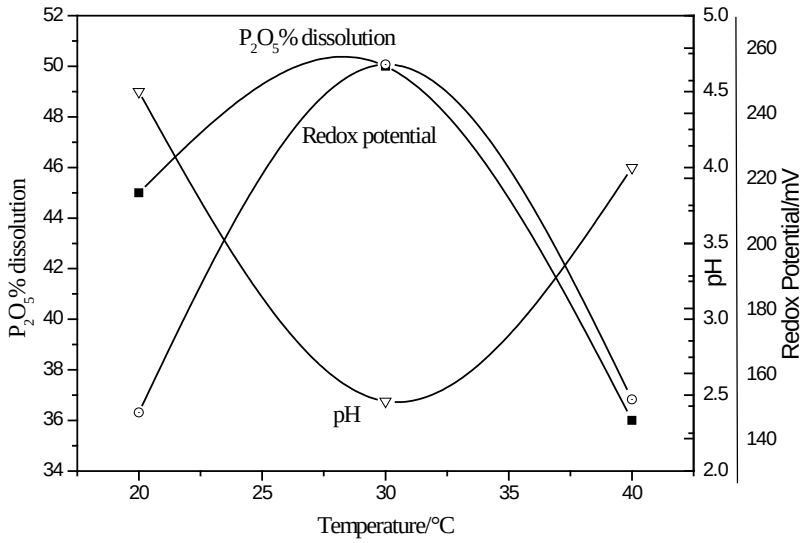


Figure (12): The Effect of Temperature on Dissolution of P_2O_5 Content of Ore

Effect of different carbon source on phosphate dissolution by *Penicillium Chrysogenum* Isolate:

It is studied by using five different carbon sources of (glucose, sucrose, dextrose, lactose, starch) for 50 ml of PVK medium in presence 0.25 g Abu Tartur phosphate ore, inoculated with $5.1x10^6$ spores of *penicillium chrysogenum* isolate, incubated at 30 °c and 160 rpm and measuring P_2O_5**.**

Nutritional constituent of the culture medium plays an important role in phosphate solublization from its ores. The results reveal that *penicillium chrysogenum* isolate grows well on PVK medium containing different carbon sources. Whereas, the maximum amount of soluble phosphate is detected only in the culture filtrate of *penicillium chrysogenum* isolate with glucose that reaches approximately to 80% and starch followed by sucrose and dextrose, while lactose exhibits low amount of soluble phosphate (Figure 13). glucose is represented the best carbon source utilized by *penicillium chrysogenum* isolate during phosphate solublization process which may be used in the production of certain organic acids that involved in solublization process. According to Cerezine *et al.* (1988), glucose is the most frequent and abundant sugar detected in plant exudates that possibly affect the microbial population which solubilizes insoluble phosphates. This finding may be related to the complexity of sucrose and the preference of glucose.

This observation is consistent with Son *et al.* (2006) and Song *et al.* (2008) who have shown that the dissolved P in glucose medium was at less 2 times more than that of sucrose and galactose medium. Moreover, Sagervanshi *et al.* (2012) found that PVK with glucose gave maximum P solubilization followed by galactose.

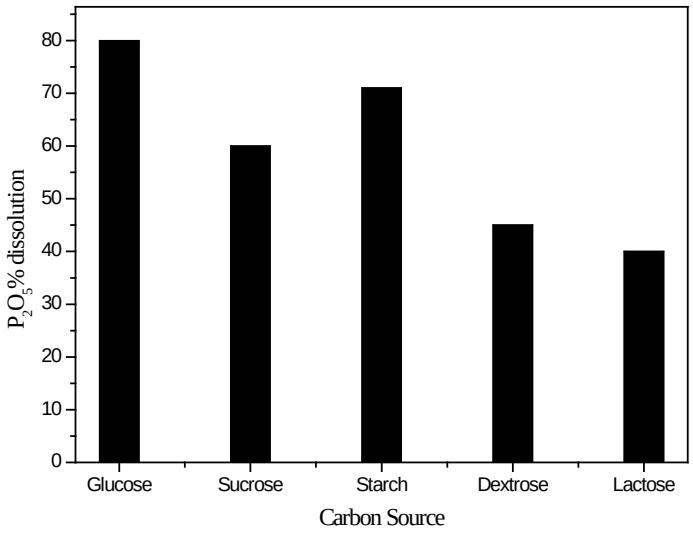


Figure (13): The Effect of Carbon Source on Dissolution of P_2O_5 Content of Ore by *Penicillium Chrysogenum* Isolate.

Effect of different carbon source on phosphate dissolution by *A. niger* isolated:

It is studied by using five different carbon sources of (glucose, dextrose, lactose, sucrose, starch and mannitol) for 50 ml of PVK medium in presence 0.25 g Abu Tartur phosphate ore, inoculated with $2x10^6$ spores of *A. niger* isolated, incubated at 30 °c and 160 rpm and measuring P_2O_5**.**

Nutritional constituent of the culture medium plays an important role in phosphate solublization from its ores. The results reveal that *A. niger* isolated grows well on PVK medium containing different carbon sources. Whereas, the maximum amount of soluble phosphate is detected only in the culture filtrate of *A. niger* isolated with sucrose and starch followed by mannitol, glucose and dextrose, while lactose exhibits low amount of soluble phosphate (Figure 14). sucrose is represented the best carbon source utilized by *A. niger* isolated during phosphate solublization process which may be used in the production of certain organic acids that involved in solublization process and this agrees with Gilberto de Oliveira Mendes *et al* (2015) and also agrees with Oktay Bayat *et al* (2011) which Sucrose was provided as the sole carbon source for *A. niger* and was hydrolysed to glucose and fructose and metabolized to organic acids used in leaching of phosphate content of ore.

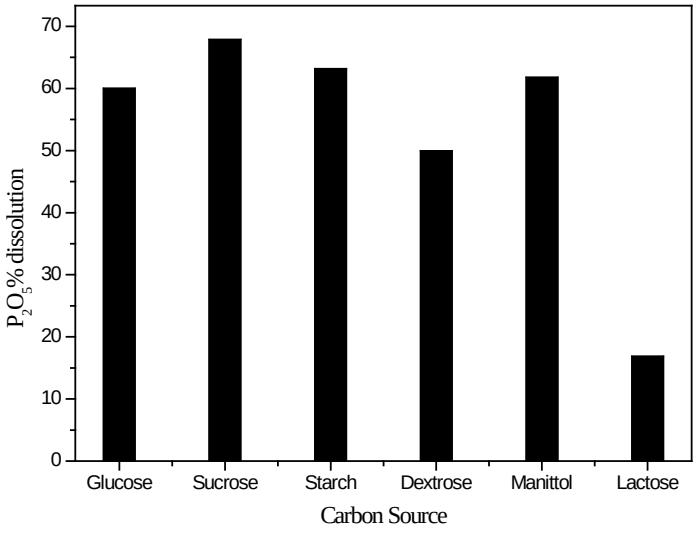


Figure (14): The Effect of Carbon Source on Dissolution of P_2O_5 Content of Ore.

Effect of different nitrogen source on phosphate dissolution by *Penicillium Chrysogenum* Isolate:

It is studied by using five different nitrogen sources of (asparagine, peptone, yeast extract, tryptone, ammonium sulfate, ammonium oxalate) for 50 ml of PVK medium containing glucose as carbon source in presence 0.25 g Abu Tartur phosphate ore, inoculated with $5.1x10^6$ spores of *penicillium chrysogenum* isolate, incubated at 30 °c and 160 rpm for four days then measuring P_2O_5 dissolution from ore**.**

penicillium chrysogenum isolate can solublize high amount of phosphorus from rock phosphate ore with all tested nitrogen sources. Yeast extract is found to be the best nitrogen source utilized by *penicillium chrysogenum* isolate for maximum phosphate solublization which reaches to 90% after 4 days of incubation followed by tryptone and the lowest dissolution of phosphate content of the ore at using ammonium chloride as nitrogen source, Figure (15). Variation of medium composition markedly altered the phosphate mobilization by phosphate solublizing microbes (Illmer and Schinner, 1992).

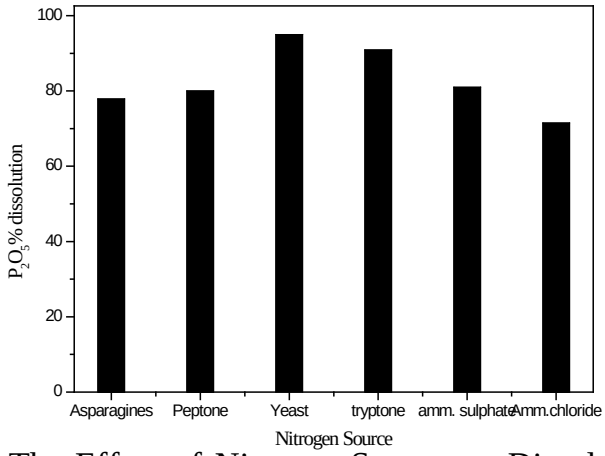


Figure (15): The Effect of Nitrogen Source on Dissolution of P_2O_5 Content of Ore by *Penicillium Chrysogenum* Isolate:

Effect of different nitrogen source on phosphate dissolution by *A. niger* isolated:

It is studied by using five different nitrogen sources of (asparagine, peptone, yeast extract and tryptone) for 50 ml of PVK medium containing sucrose as carbon source in presence 0.25 g Abu Tartur phosphate ore, inoculated with $2x10^6$ spores of *A. niger* isolated, incubated at 30 °c and 160 rpm then measuring P_2O_5 dissolution from ore**.**

A.niger isolated can solublize high amount of phosphorus from rock phosphate ore with all tested nitrogen sources. tryptone is found to be the best nitrogen source utilized by *A.niger* isolated for maximum phosphate solublization which reaches to 85.8% after 4 days of incubation followed by asparagine and the lowest dissolution of phosphate content of the ore at using ammonium sulphate as nitrogen source, Figure (16). Variation of medium composition markedly altered the phosphate mobilization by phosphate solublizing microbes (Illmer and Schinner, 1992).

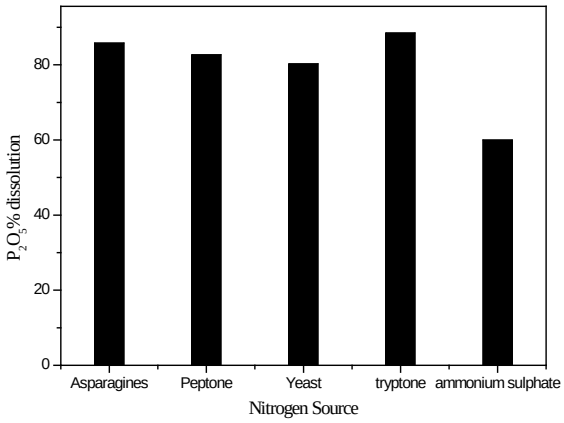


Figure (16): The Effect of Nitrogen Source on Dissolution of P_2O_5 Content of Ore.

Organic acids production:

1- In case of Penicillium chrysogenum isolate

penicillium chrysogenum isolate is able to produce organic acids such as oxalic and fumaric acids in the growth medium as secondary metabolites resulting from utilization of glucose. Utilization of insoluble phosphate as main phosphorus source highly increased the production of some organic acids by organism. Detection of acids present in filterate of bioleaching experiment by HPLC which found that presence of higher amount of oxalic acid and fumeric acids in flasks containing of *penicillium chrysogenum* isolate and 0.25gm Abu Tartur phosphate ore in 50 ml of glucose-yeast extract medium(Figure 18) than control flask without ore(Figure 17)that containing fumaric acid only and this lead to decrease of pH of medium and increase of redox potential and improve the dissolution of phosphate content in Abu Tartur ore to reach to 90%, these organic acids are produced from utilization of glucose by *penicillium chrysogenum* isolate.

Many studies have investigated mechanisms in which P-solubilizing fungi enhance the mobility of P to plants (reviewed by Whitelaw (2000)). Most accepted P-solubilization mechanisms used by these fungi are: 1) acidification by exuded H^+ and organic acids, and through the production of carbon dioxide (CO_2), 2) complexation of cation partners of P by the exuded organic acids, and 3) ligand exchange (i.e., exchange of P) by the exuded organic acids (reviewed by Gulden and Vessey (2000)).

Previous studies have shown consistent pH reduction in the solid and liquid culture of *P. bilaiae* (Asea et al., 1988; Cunningham and Kuiack, 1992). The larger pH reduction was related to assimilation of ammonium by *P. bilaiae*
rather than nitrate. Also, citrate and oxalate were detected as the major acidic metabolites of *P. bilaiae*(Cunningham and Kuiack, 1992).

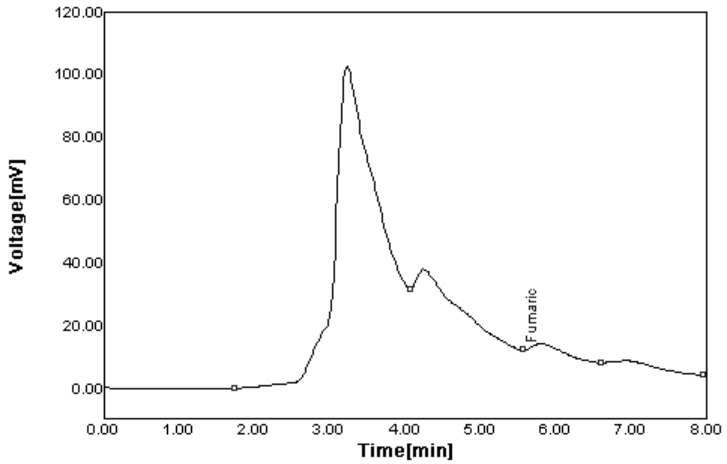


Figure (17): HPLC Analysis of Filterate of Control Flask Containing Medium with *Penicillium* and without Ore.

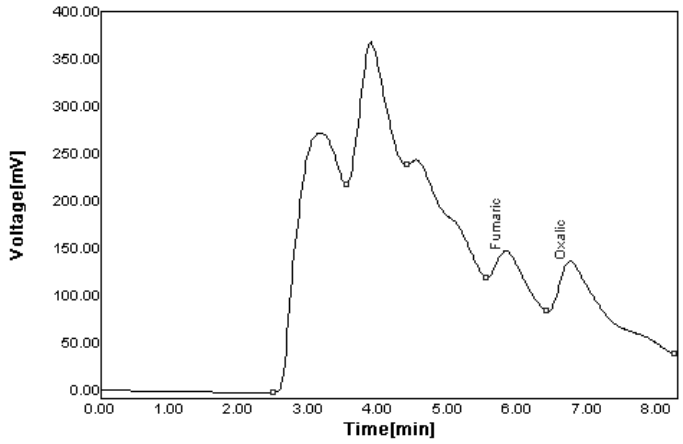


Figure (18): HPLC Analysis of Filterate of Sample Flask Containing Medium with *Penicillium* and Ore.

2- In case of *A.niger* isolate

A.niger isolated is able to produce organic acids such as oxalic and fumaric acids in the growth medium as secondary metabolites resulting from utilization of sucrose. Utilization of insoluble phosphate as main phosphorus source highly increased the production of some organic acids by organism. Detection of acids present in filterate of bioleaching experiment by HPLC which found that presence of higher amount of oxalic acid and fumaric acids in flasks containing of *A.niger* isolated and 0.25gm Abu Tartur phosphate ore in 50 ml of glucose-yeast extract medium(Figure 20) than control flask without ore(Figure 19)that containing fumaric acid only and this lead to decrease of pH of medium from 6 to 3 and increase of redox potential and improve the dissolution of phosphate content in Abu Tartur ore to reach to 85.8%, these organic acids are produced from utilization of sucrose by *A.niger*, and this agree with Hefnawy *et al*. (2009), and also this accepts with Hung and Ting, (2005) who 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.

To ensure production of organic acid by *A. niger* isolated by using bromo cresol green as indicator (1%) supplemented in czapek's agar on plate at pH6 which the color changes from blue to yallow and the spore suspension of the isolate is spread on the surface of the medium plates from third day of cultivation, Figure(21) and this refers to production of organic acid and this agree with Helen Shnada Auta *et al*, (2014).

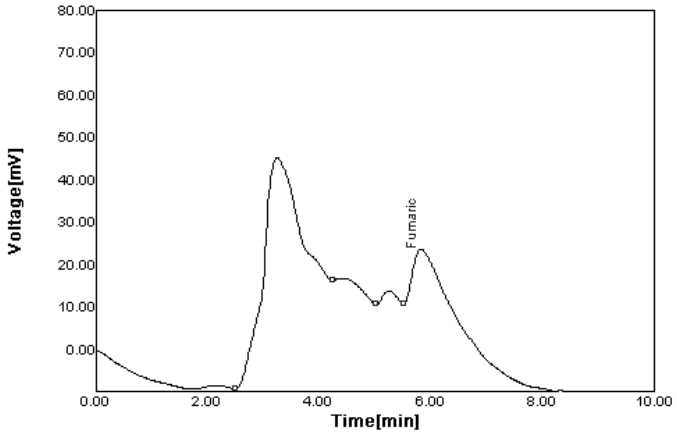


Figure (19): HPLC Analysis of Filterate of Control Flask Containing Medium with *A.Nger* Isolated and without Ore.

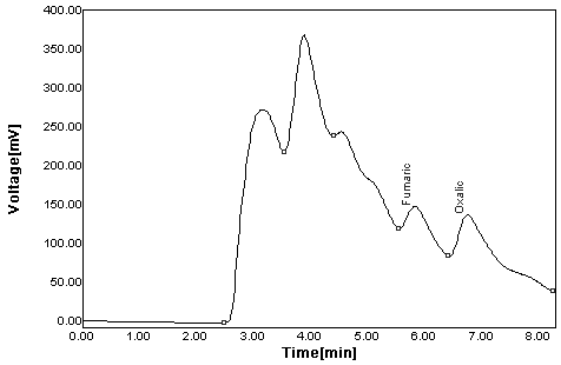
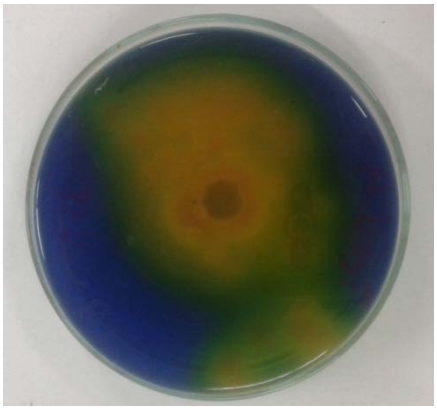


Figure (20): HPLC Analysis of Filterate of Sample Flask Containing Medium with *A.Niger* Isolated and Ore.



Figure(21) : Cultivation of *A. Niger* Isolated on Czapek's Dox Medium Supplemented with 1% Bromocresol Green as Acid Indictor for 4 Days.

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