IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 3 Issue 11, November 2016
ISSN (Online) 2348 – 7968 | Impact Factor (2015) - 4.332
www.ijiset.com

Studies on The Efficiency of Dissolution of Phosphate Content of Abu Tartur Phosphate Ore using Nocardiopsis Dassenvillei

T.A. Elbarbary[a], M.A.El-Badry[b], , I.A. Ibrahim[a], S.A. Abd EL-Halim,[c], H.M. Sharada [c], Y. M. Abdel-Fatah[a]

a:Central Metallurgical Research and Development Institute, Egypt

b: Botany and Microbiology Department, Faculty of Science, Al-Azher University, Egypt

c: Biochemistry Department, Faculty of Science, Helwan University, Egypt

Abstract

The acidulation of rock phosphate with small amounts of H_2SO_4 or H_3PO_4 is one traditional method used as phosphate dissolution, but this is uneconomical and environmentally nonviable so using of bioleaching technique is preferred. The objective of the present study the factors affecting on dissolution of phosphate content in Abu Tartur phosphate ore by using microorganism

The serial dilution method was performed only one bacterial isolate that have ability to dissolve phosphate ore were identified by 16 sRNA as *Nocardiopsis dassenvillei*. The optimum conditions of bioleaching of Abu Tartur phosphate ore was 1 days incubation period at pikovskaya's medium with 6.5 pH supplemented with tricalcium phosphate medium, $2X10^{32}$ CFU and 0.25 gm ore for each 50 ml medium incubated at 30 °C and different carbon and nitrogen sources were evaluated. Ammonium oxalate was the best nitrogen source, lactose was the best carbon source. initial pH 5 at 150 rpm. The effeicency of *N. dassenvillei* for phosphate content dissolution of Abu Tartur phosphate ore by applying all optimum conditions was 69.1%.

Key words: Abu Tartur phosphate ore, *Nocardiopsis dassenvillei*, Phosphate dissolution.

Corresponding Author: Mohamed Ali El-Badry Hafez Amin, Botany and Microbiology Department, Faculty of Science, Al-Azhar University E-mail: mohamed.helwanuni@yahoo.com

Introduction:

Phosphorus is one of the most important elements for crop production. 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. 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 consists of insoluble calcium phosphate, generally known as apatite (Rogers, *et al*, 1991).

Natural rock phosphate is a complex raw material and is mainly used in the manufacture of phosphate fertilizer (Rodriguez & Fraga, 1999). Almost 80% of rock phosphate all over the world is low-grade and not suitable for direct application to soils as a phosphate fertilizer because of its low phosphorus content and poor solubility (Rajan, 1996). Conventionally, rock phosphate is chemically processed with sulfuric acid or phosphoric acid into phosphate fertilizer. This process makes the fertilizer more expensive and contributes to environmental pollution (Vassilev, (2006).

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. However, bioconversion process of rock phosphate ore occurs at a low temperature and is more selective to phosphate extraction than chemical conventional process (Gharabaghi, *et al*, 2010).

Some microorganisms, including bacteria and fungi, are known to be involved in the solubilization of rock phosphate These phosphate-solubilizing microorganisms used for industrial production of phosphate fertilizer lower the production cost. Their activity may also be exploited when an insoluble mineral phosphate is applied directly to soils (Antoun & Babana 2006; Hamdali et *al* 2008 and Xiao *et al* 2009). 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).

In this work, the factors effect on dissolution of phosphate content in Abu Tartur phosphate ore were evaluated by using *Nocardiopsis dassenvillei* to reach to maximum dissolution of P_2O_5 from ore were evaluated.

Material and methods:

Rock phosphate sample was collected in plastic bags from phosphate mine present in Safaga and Elkosir on the red sea cost in Egyptian eastern desert. Chemical composition of the studied phosphate sample is determined by using XRD analysis.

Isolation of bacterial species:

The bacterial species isolated from Abu Tartur phosphate ore according to the serial dilution technique as described by (Johnson *et al*., 1959). 100µl of each dilution is placed on the surface of sterile agar plate of pikoveskey's agar medium. After 2 day of incubation at 30°C only one bacterium isolated.

Culture media:

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

Modified 9 k medium:

It contains (g\l): solution A; $(NH_4)_2SO_4$, 3; $MgSO_4.7H_2O$, 0.5; K_2HPO_4, 0.5; KCl, 0.1; $Ca(NO_3)_2$, 0.01; glucose, 1; yeast extract, 0.3. These components are dissolved in 700 ml bidistilled water and are sterilized by autoclave at 121^oc for 15 min. Solution B; 44.2 g/l $FeSO_4.7H_2O$ is dissolved in 10 ml (1N) H_2SO_4 and 290 ml bidistilled water then sterilized by filtration system and is added to solution A after cooled it

Initial pH value is adjusted to 2.5 with about 50% H_2SO_4.

9 k medium (Silverman M P & Lundgren D G, (1959)**:**

It contains (g\l): solution A; 3 g/l $(NH_4)_2SO_4$, 0.5 g/l $MgSO_4.7H_2O$, 0.5 g/l K_2HPO_4, 0.1g/l KCl, 0.01 g/l $Ca(NO_3)_2$. These components are dissolved in 700 ml bidistilled water and are sterilized by autoclave at 121^oc for 15 min. Solution B; 44.2 g/l $FeSO_4.7H_2O$ is dissolved in 10 ml (1N) H_2SO_4 and 290 ml bidistilled water then sterilized by filtration system and is added to solution A after cooled it

Initial pH value is adjusted to 2.5 with about 50% H_2SO_4.

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. This medium is solidified by adding 15 g agar per liter.

Modified Pikovskaya's medium:

It contains (g/l): 0.5 g/l Yeast extract, 10 g/l Dextrose, 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. This medium is solidified by adding 15 g agar per liter.

Nutrient medium (Lapage S.,*et al* (1970)**:**

It contains (g/l): 5g/l peptone, 3 g/l beef extract, 5g/l sodium chloride. Heat if necessary to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. This medium is solidified by adding 15 g agar per liter.

Modified nutrient medium:

It contains (g/l): 5g/l peptone, 3 g/l beef extract, 5g/l sodium chloride, 1 g/l methionine. Heat if necessary to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. This medium is solidified by adding 15 g agar per liter.

Phosphate solublization evalution by isolated bacterium:

Pikovskaya's Agar Medium:

Bacterium isolate was evaluated for its ability to phosphate solublization. 100µl of bacterial isolate was placed in the center of Pikovskaya's medium agar plate and incubated at 30°C for 24 h. The solublization activity was detected by the presence of clear zone around the bacterial colony.

Characterization of Phosphate solubilizing *bacterium* isolate:

DNA isolation and PCR condition:

DNA extraction by use protocol of GeneJet genomic DNA purification Kit (Thermo K0721) as following., then PCR by using Maxima Hot Start PCR Master Mix (Thermo K1051) with 61 S universal primer Forward primer :-5- AGA GTT TGA TCC TGG CTC AG-3 and Reverse R:- 5- GGT TAC CTT GTT ACG ACT T-3. The PCR cycle was done as the following Initial denaturation 95° C for 10 min one cycle (Denaturation 95° C for 30 sec Annealing 65° C for 1 min Extension 72° C for 1.30 min) 35 cycle Final Extension72° C for 10 min one cycle. PCR clean up to the PCR product using GeneJET™ PCR Purification Kit (Thermo K0701). Finally sequencing to the PCR product was done on GATC Company by use ABI 3730xl DNA sequencer by using forward and reverse primers.

Only by combining the traditional Sanger technology with the new 454 technology, can genomes now be sequenced and analyzed in half the usual project time, with a considerable reduction in the number of coatings and gaps. In addition, considerable cost advantages now make genome sequencing with the 454 technology accessible to the research community.

Sequence data were aligned and compared with available sequences of bacterial lineage in the National Center for Biotechnology Information Gen Bank (http://www.ncbi.nlm.nih.gov/) using BLAST.

Experiment method:

Sterilized PVK broth medium 50 ml in 100 ml conical flask 0.25 gm sterilized Abu Tartur phosphate ore inoculated by 100 µl of bacterial isolate, compare with control then incubated in shaking incubator at 30 °C and 160 rpm, Five ml from filtrate of sample with bacterium and control were taken. The amount of soluble phosphate in the culture filtrate was evaluated every 24 h and determined calorimetrically according to the method described by (Olsen *et al*. 1954).

Effect of different growth parameter on phosphate solublization:

Different culture media, 9k, modified 9K, Pikovskaya's, modified Pikovskaya's, nutrient broth and modified nutrient broth, separately supplemented with 0.25g of Abu Tartur phosphate for 50 ml medium. Each flask was inoculated with $1x10^{32}$ CFU of bacterium and incubated at 30 °C. The amount of soluble phosphate in the culture filtrate was determined. The previous conditions were conducted on pikovskaya's medium supplemented with Abu Tartur rock phosphate for *bacterium* at different incubation periods, incubation temperatures, ore concentrations, bacterial concentration, carbon, and nitrogen sources, initial pH, number of shaking flask, addition of medium, bacterial and both of them during experiment, diameter of conical flask base.

Detection organic acid production:

Preparation of Pikovskaya's agar medium supplemented with 1% of bromocresol green as indicator at pH 6 then is inoculated with *bacterium* at the center of plate then leave for one day (Sunstornsuk *et al*., 1994).

Results and discussion:

Chemical composition of Abu Tartur phosphate ore:

Chemical analysis of Abu Tartur phosphate ore,

Abu Tartur phosphate ore was characterized by XRD (Figure1) which showed that the presence of insoluble P_2O_5 was 24.5 % and the other element presence in this ore was 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).

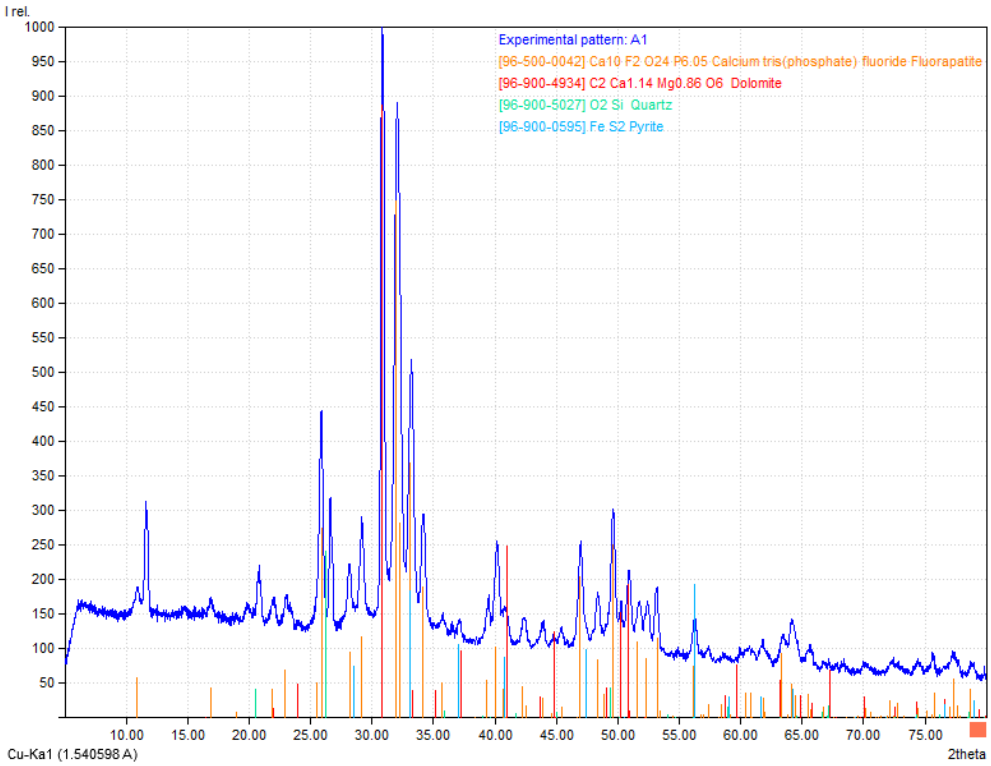


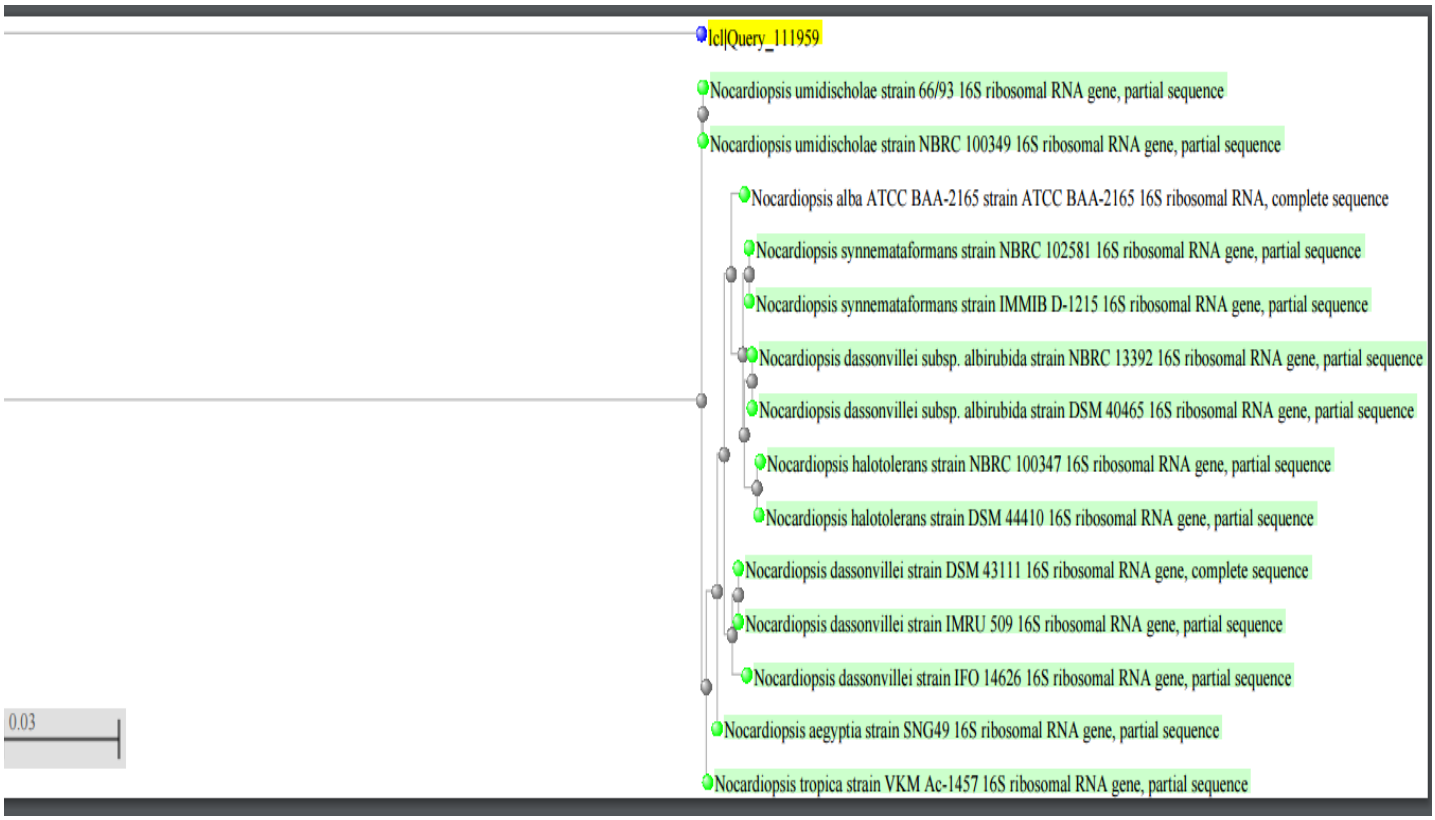
Figure (1): XRD Analysis of Abu Tartur Phosphate Ore.

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

Identification of *bacterial* isolate:

Molecular identification of the selected isolate 16S rRNA sequencing was a powerful tool for rapid identification and phylogenetic analysis of bacterial species. The obtained -1200 nucleotide sequence was compared with available 16S ribosomal sequences in the NCBI database using BLASTN. The *Nocardiopsis* isolate had been enrolled into a cluster containing *Nocardiopsis* species and was found to be closely related to *Nocardiopsis* dassenvillei strain(Figure 2).



Figure(2): Identification of Bacterial Isolated from Abu Tartur Phosphate Ore.

Detection of the ability of *Nocardiopsis dassenvillei on* phosphate solublization by using Pikovaskay's agar medium:

Production of organic acids by *Nocardiopsis dassenvillei* isolate and it's diffusion into the medium were recorded by formation clear zone around the bacterial colony on pikovaskay's agar medium and this refers to the solublization of $Ca_3 (PO_4)_2$ by bacterium. The obtained result indicated that which may be related to the production of exopolysaccharides or enzymes by *Nocardiopsis dassenvillei* isolate since these

compounds produced on solid media remain concentrated locally and may function to solubilize phosphates in conjunction with organic acids (Yi et al., 2008), Figure(3).

Figure (3): Solubilization of Tricalcium Phosphate of PVK Medium by *Nocardiopsis Dassenvillei***.**

Effect of incubation period on phosphate solublization by *Nocardiopsis dassenvillei*:

It was studied by using PVK medium in presence 0.25 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with $1x10^{32}$ CFU of *Nocardiopsis dassenvillei* and measuring P_2O_5 every 24 h and also pH and redox potential. The results revealed that maximum phosphate solublization was recovered after 24 h which reached to 29% with decreasing pH value and increasing redox potential value then P_2O_5 dissolution begins to decrease above this period as in Figure (4) and this correlated with growth curve of *Nocardiopsis dassenvillei* isolate that stationary phase ended at 21 hour that produced all acids and enzymes which began to dissolve phosphate content of ore. Solubilization of rock phosphate depended on its structural complexity, particle size and metabolites of microorganism (Pradhan & Shukla 2005).

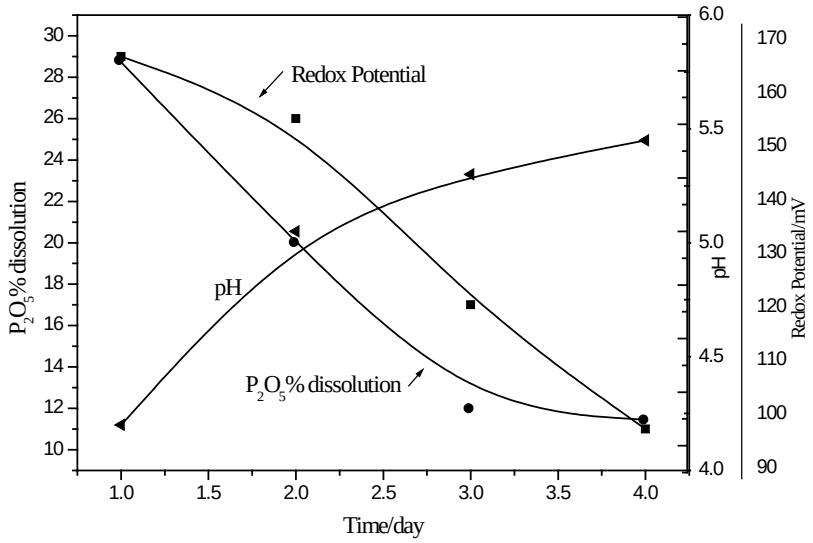


Figure (4): Effect of Incubation Period on Dissolution of Phosphate Content of Ore.

Effect of different media on phosphate solublization by *Nocardiopsis dassenvillei*:

It was studied by using six different types of media (9 k, modified 9 k, nutrient broth, modified nutrient broth, PVK, modified PVK) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with $1x10^{32}$ CFU *Nocardiopsis dassenvillei* isolate and incubated at 30 °C, 160 rpm and measuring P_2O_5, also pH and redox potential each 24h.

The results revealed that maximum phosphate solublization was obtained with pikovasky's medium reaching to 29% while, the minimum phosphate solublization occurred with modified general liquid medium, Figure (5). The results were monitored with final pH, since the final pH was low with pikovasky's medium (4.2) and high with modified general nutrient medium (6.3).

pH in modified 9k medium was lower than pikovasky's medium and this do not correlate with phosphate content dissolution (Sperber, 1958)

The solubilized phosphate may react with calcium or magnesium present in rock phosphate (equation 1) as soon as the pH of the growth medium increases and form insoluble phosphate. As the dissolved phosphate concentration increases, the solution may become saturated and the re-crystallization of the mineral-phosphate species such as brushite can occur (Delvasto *et al*., (2006)).

$$Ca^{+2} + HPO_4^{-2} + H_2O \longrightarrow CaHPO_4.2H_2O \qquad (1)$$

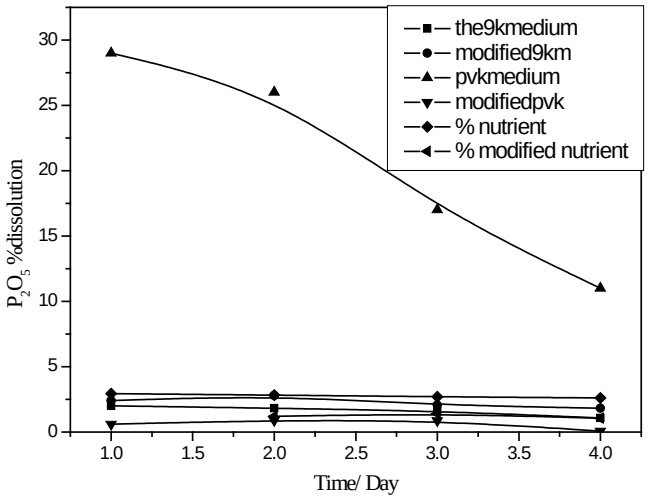


Figure (5): Effect of Type of Medium on Dissolution of Phosphate Content of Ore.

Effect of different incubation temperatures:

It was studied by using four different temperatures (20, 30, 40, 50 °C) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of PVK medium and inoculated with $1x10^{32}$ CFU of *Nocardiopsis dassenvillei* and incubated at 30 °C and 160 rpm and measuring P_2O_5, pH and redox potential each 24h.

The dissolution of phosphate content of ore increased with increasing the temperature up to 30 °C then begins decrease (Figure 6), accomplishment that maximum decreasing of pH and increasing of redox potential occuring at 30°C so the optimum incubation temperature by *Nocardiopsis dassenvillei* was 30 °C at which dissolution of phosphate content of ore reaches to 32.2% which occurred optimum growth for *Nocardiopsis dassenvillei* and adapted to their indigenous environment so their metabolic activites were linked to the temperature of the environment (Sadaf Shahab, 2008, Varsha 2002). The growth of *Nocardiopsis dassenvillei* at 30°C refered to mesophilic bacterium which grows best in moderate temperature, neither too hot nor too cold, (Willey *et al* , 2008).

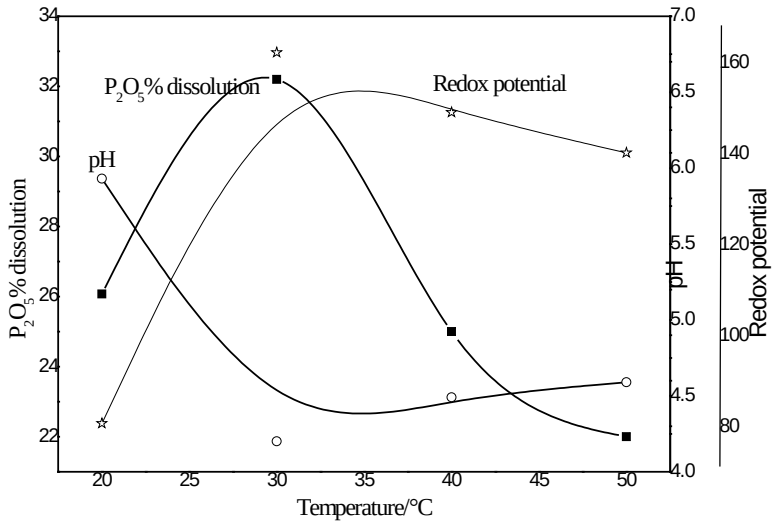


Figure (6): Effect of Temperature on Dissolution of Phosphate Content of Abu Tartur Phosphate Ore.

Effect of bulk density

Different weights of Abu Tartur phosphate ore (0.25, 0.5, 1, 1.5)gm were evaluated with previous optimum conditions then measuring P_2O_5, pH value and redox potential after 24h of incubation.

Nocardiopsis dassenvillei had varied growth in the presence of different weight of phosphate ore in the growth medium up to 1.5 gm for 50 ml of medium (Figure 7). The optimum growth and best phosphate solublization occurred at a concentration of 0.5% of the Abu Tartur phosphate ore concentration and decreased above this concentration. It is also observed that the final pH value at weight of 0.25gm ore for 50 ml of medium was the lowest pH value; this may be due to the production of organic acids and acidic phosphatase enzymes. At a weight of 0.25 gm ore, *Nocardiopsis dassenvillei* can solublize approximately 32.4% of phosphate content of the ore.

The dissolution of phosphate 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^{+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 & Schinner, 1992).

The adverse effect of increasing pulp density could be attributed to the inhibitory effect of increasing concentrations of ferric iron, the limited availability of nutrients and, O_2 and CO_2 with increasing pulp density and the mechanical damage to bacterial cells by solids, (Venkateshwarlu *et al*, (1984).

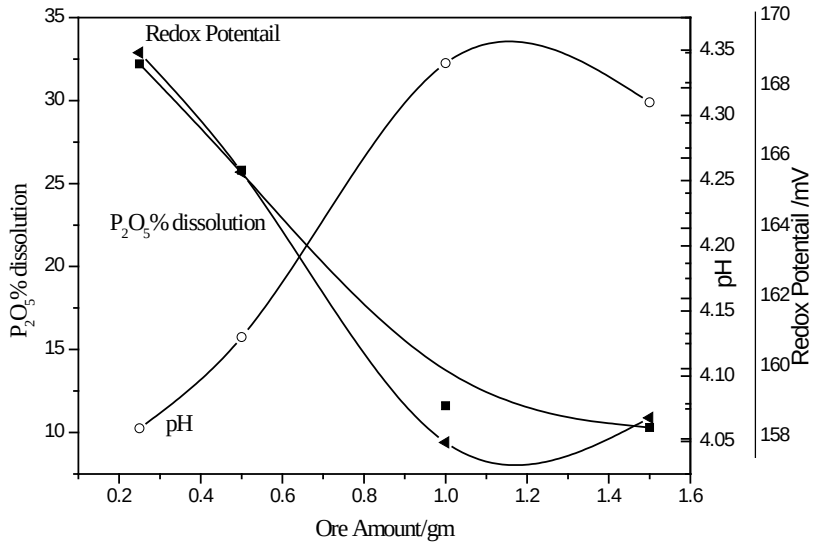


Figure (7): Effect of Bulk Density on Bioleaching of Abu Tartur Phosphate Ore.

Effect of *Nocardiopsis dassenvillei* inoculum size:

It was studied by using four different inoculums size of *Nocardiopsis dassenvillei* ($1x10^{32}$, $2x10^{32}$, $3x10^{32}$, $4x10^{32}$) CFU were evaluated with previous optimum conditions then measuring P_2O_5, pH value and redox potential after 24h of incubation.

Inoculums size affected on dissolution of phosphate content of the ore. The most potent inoculums size was $2x10^{32}$CFU of *Nocardiopsis dassenvillei* and decrease at high inoculums size with no significant change in final pH value and this may be due to competition factor between bacterial calls, decrease the aeration and also high growth which may consume phosphate. At inoculums size $2x10^{32}$ CFU, *Nocardiopsis dassenvillei* can solublize approximately 35.4% of phosphate content of the ore, Figure (8).

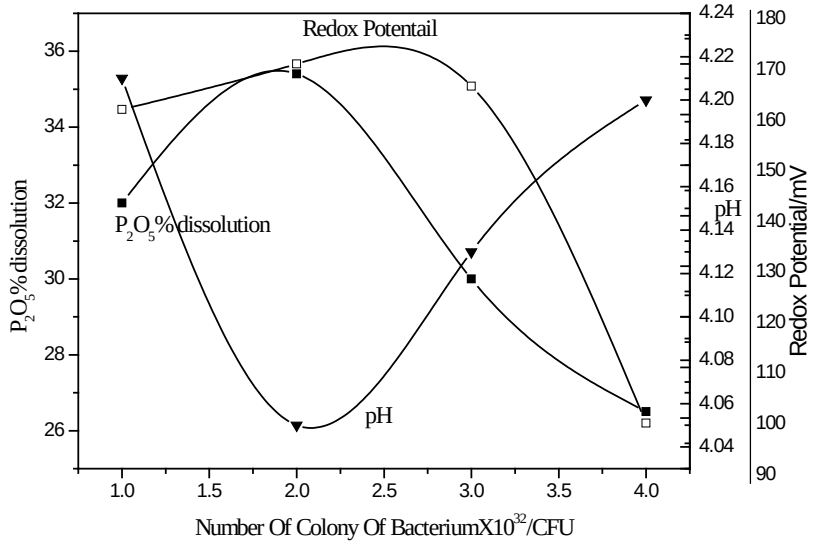


Figure (8): Effect of Inoculum Concentration of *Nocardiopsis Dassenvillei* on Bioleaching of Abu Tartur Phosphate Ore.

Effect of different carbon sources:

Different carbon sources (glucose, starch, dextrose, lactose, sucrose) were evaluated with previous optimum conditions then measuring P_2O_5, pH value and redox potential after 24h of incubation.

The results revealed that *Nocardiopsis dassenvillei* grow well on PVK liquid medium containing different carbon. Whereas, high amounts of soluble phosphate was detected only in the culture filtrate of *Nocardiopsis dassenvillei* with lactose followed by sucrose and dextrose, while starch exhibited low amount of soluble phosphate. Dissolution of phosphate content of ore reaches to 46.5% at using lactose as carbon source. The bacterial growth exhibits remarkable variation according to the utilized carbon source, the best bacterial growth to produce enzyme and organic acids reached when lactose is utilized as a carbon source while, the minimum growth reached when starch was utilized as a carbon source (Figure 9).

The maximum amount of phosphorus solubilized corresponds to the highest value of the organic acid produced. The sugar consumption and organic acid liberation are seen to be most active up to 1 day. A partial neutralization of the acids with ions, liberated due to the decomposition of the phosphate, is the reason for maximum of organic acid production on the 1th day as in equation (2, 3).

$$Lactose + H_2O \xrightarrow{\beta-\text{galsctosidase}} \beta - D - glucose \quad (2)$$

$$\beta - D - glucose \xrightarrow{Glucose\ oxidase} \text{Gluconic acid} + H_2O_2 \quad (3)$$

This agrees with Usharani Krishnaswamy *et al*, (2009) which found that lactose was observed to yield maximum phosphate removal (63.4%) and growth by the *consortium*.

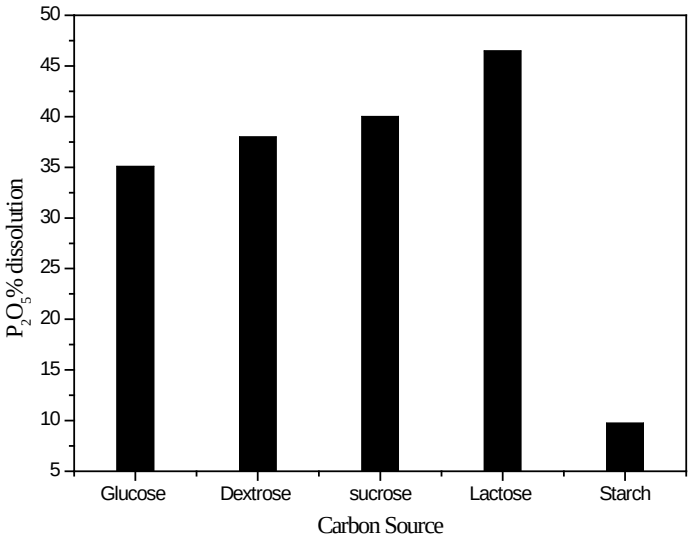


Figure (9): Effect Of Carbon Source On Bioleaching Of Abu Tartur Phosphate Ore.

Effect of different nitrogen sources:

Different nitrogen sources of (ammonium sulphate, ammonium chloride, ammonium oxalate, asparagine, glycine) were evaluated with previous optimum conditions then measuring P_2O_5, pH value and redox potential after 24h of incubation.

Nocardiopsis dassenvillei solublized high amount of phosphorus from rock phosphate ore with all tested nitrogen sources, Figure (10). Ammonium oxalate was found to be the best nitrogen source utilized by *Nocardiopsis dassenvillei* isolate for maximum phosphate solublization that reached to 53.5% followed by ammonium sulphate and lowest dissolution of phosphate content of the ore at using glycine as nitrogen source.

As a nitrogen source, ammonium oxalate was found to give maximum soluble P. Oxalate ions had the ability to form stable complexes with calcium, iron and aluminum to liberate phosphates (Saghir et al., 2009) and are known to extract P from soils (Narteh & Sahrawat, 1999). The phenomenon of P solubilization was correlated with the assimilation of both ammonium and chelation by oxalate ions in the culture medium and

this observation may be attributed to the release of protons from the cytoplasm to the outer surface leading to dissolution of phosphate content of ore (Illmer & Schinner, 1995).

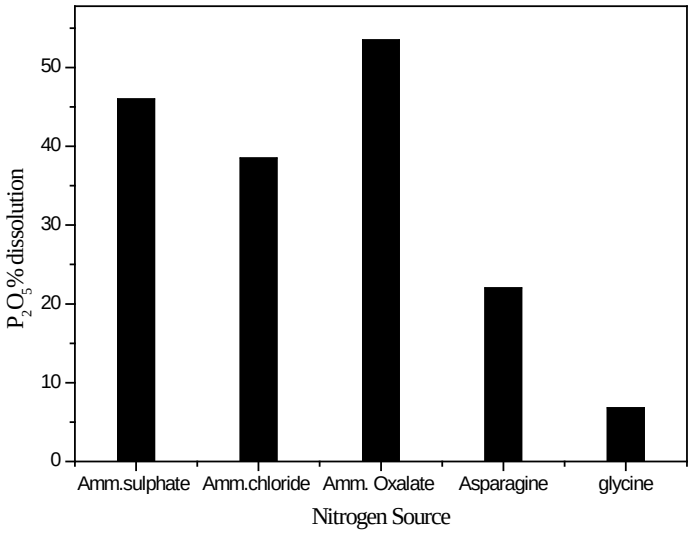


Figure (10): Effect of Nitrogen Source on Bioleaching of Abu Tartur phosphate Ore.

Effect of Diameter of conical flask base:

Different diameter of conical flask base (4.5, 5.5, 6, 8)cm were evaluated with previous optimum conditions then measuring P_2O_5, pH value and redox potential after 24h of incubation.

The growth of *Nocardiopsis dassenvillei* affected with change of diameter of conical flask base from 4.5 to 8 cm, Figure (11). Increasing the diameter of conical flask base, increase the dissolution phosphate content of the ore and decrease pH value and increasing redox potential reflect to high increase the dissolution phosphate content of the ore and this up to 5.5cm at which dissolution of phosphate content of ore reaches to 53.5% then begins to decrease.

Increase the diameter of conical flask up to 5.5 cm may lead to increase the aeration that necessary for growth of *Nocardiopsis dassenvillei* isolate and oxidation of β-D glucose and more increase for production of acids and enzymes.

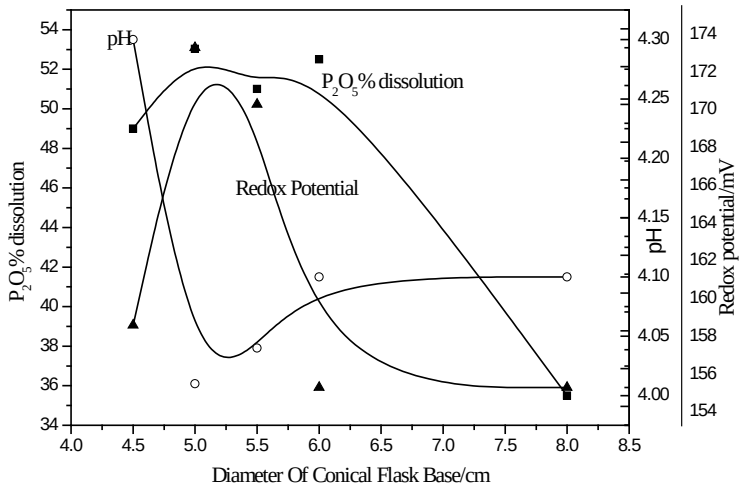


Figure (11): Effect of Diameter of Conical Flask Base.

Addition of supplement media during incubation:

It was studied by addition of medium, inoculants of *Nocardiopsis dassenvillei* and both of them during experiment was carried out with previous optimum conditions ten measuring P_2O_5, pH value and redox potential after 24h of incubation.

The addition of inoculants of bacterium during experiment of bioleaching didn't have significant effect in dissolution of phosphate content of ore and this may be due to consumption on nutrients by the first addition of inoculants, also addition of medium during experiment that was carried out didn't have significant effect on dissolution of phosphate and also addition of both of *Nocardiopsis dassenvillei* and medium which dissolution of phosphate content of ore reached to 53.5% , Figure (12).

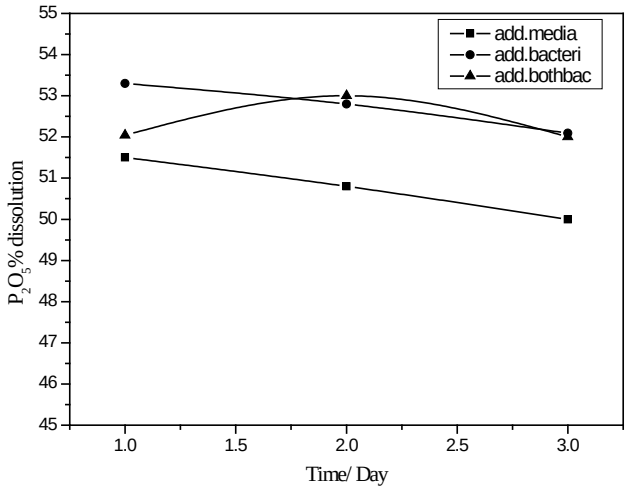


Figure (12): Effect of Addition of medium, inoculants of bacterium and Both of Them during Experiment Is Carried out.

Effecting of initial pH:

Different initial pH(4, 5, 6, 7, 8) were evaluated with previous optimum conditions then measuring P_2O_5 after 24h of incubation.

The growth of *Nocardiopsis dassenvillei* isolate was affected with the initial pH of the PVK medium, Figure (13). The maximum growth of *Nocardiopsis dassenvillei* isolate on a medium containing rock phosphate was observed at initial pH 5. At this pH value phosphate solublization exhibited high amounts it represented 69.1%. It is also observed that phosphate solublization at pH 5 was sharply increased.

The pH of the culture medium directly influences the growth of microorganisms and the biochemical processes they performed. In many cases, acidification is the main mechanism involved in phosphate solubilization (Halder *et al.*, 1990; Jha *et al.*, 2009; Marra *et al.*, 2012; Whitelaw, 2000). However, several studies had shown a lack of correlation between solubilized phosphorus and pH of the medium (Chaiharn & Lumyong, 2009; Xie, 2009). Therefore, a better understanding of the behavior of phosphate-solubilizing bacteria inoculated into culture media at different initial pH values may contribute to the production and management of inoculants that improve phosphate solubilization.

Several authors had suggested that a decrease in pH due to the production of organic acids and the release of protons is a basic principle of phosphate solubilization, (Chen *et al.*, 2006; Sperber, 1958; Whitelaw, 2000). There were several solubilization mechanisms were involved at the pH of the medium varies. These mechanisms can be: proton exclusion (via cellular respiration and ammonium absorption as N source) (Illmer P and Schinner F, 1992), siderophores (Hamdali *et al.*, 2008) and exopolisaccharide (EPS) production (Yi *et al.*, 2008). The production of EPS could act synergistically with acid production as suggested by Yi *et al.* (2008).

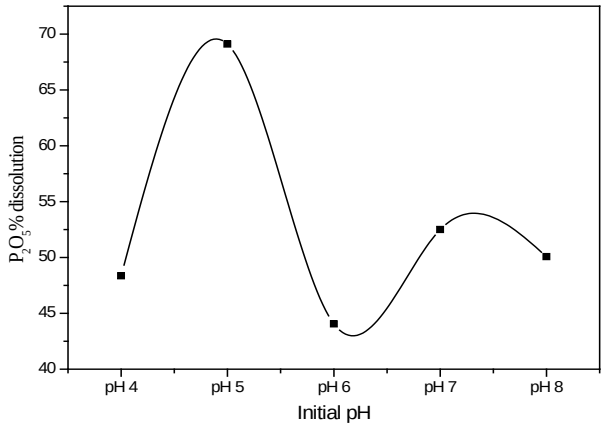


Figure (13): Effect of Initial pH on Dissolution of Phosphate Content of Abu Tartur Phosphate Ore.

Effect of aeration on phosphate content of Abu Tartur phosphate ore dissolution :

Different shaking speed of flask(50, 100, 150, 200)rpm were evaluated with previous optimum conditions then measuring P_2O_5 after 24h of incubation.

The growth of *Nocardiopsis dassenvillei* was varied with change of shaking of flask that studied from 50 to 200 rpm which found that amount of dissolution of phosphate content of Abu Tartur ore increase with increase of shaking speed from 50 to 150 rpm which reaches to 69.1 but began to decrease at high speed, Figure(14).

This due to shear stress produced from strong shaking reduce the growth of bacterium and this is accepted with (Xiao *et al*, 2008).

Under these optimum conditions led to optimum the growth of bacterium led to maximum dissolution of phosphate content in Abu Tartur phosphate ore reached to 69.1%

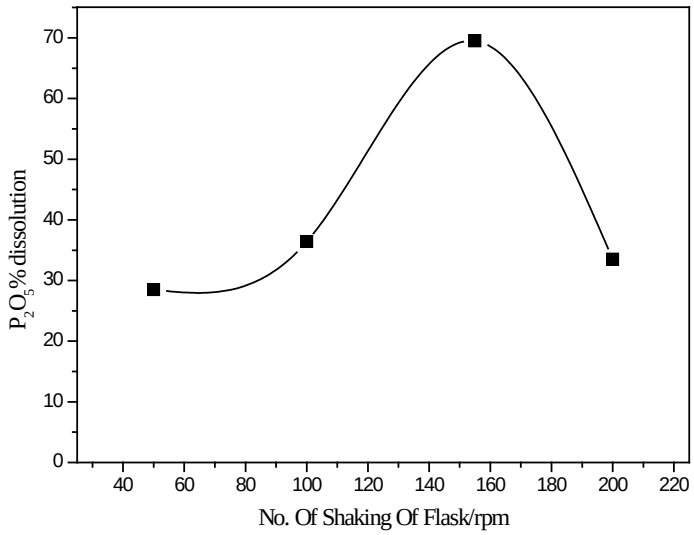
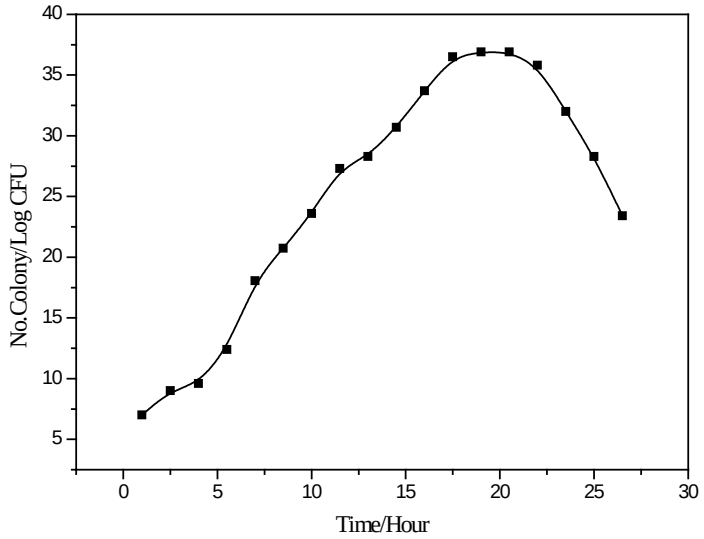


Figure (14): Effect of Shaking Flask on Dissolution of Phosphate Content of Abu Tartur Phosphate Ore.

Growth curve of *Nocardiopsis Dassenvillei*:

The growth curve of bacterium was carried out by serial dilution and standard plate method (Waksman 1927), *Nocardiopsis dassenvillei* began to grow from first hour and this refered to lag phase up to four hours then enter log phase up to 17.5 hours and produce primary metabolites represented in enzymes which reach to optimum growth then the growth remains constant to 22 hours and this refered to stationary phase of its

growth and at this phase the *Nocardiopsis dassenvillei* produced all the acids then its growth began to decrease and this is decline phase (Figure 15).



Figure(15): Growth Curve of *Nocardiopsis Dassenvillei*.

Mechanism of dissolution of phosphate content of Abu Tartur phosphate ore:

Organic acid production:

It was generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by microorganisms [Halder AK. *et al* 1990). Production of organic acids resulted in acidification of the microbial cell and its surroundings. Consequently, Pi may be released from a mineral phosphate by proton substitution for Ca^{+2} (Goldstein AH, 1994).

The production of organic acids by phosphate solubilizing bacteria had been well documented. Among them, gluconic acid seems to be the most frequent agent of mineral phosphate solubilization. It was reported as the principal organic acid produced by phosphate solubilizing bacteria such as *Pseudomonas* sp. (Illmer P, Schinner F, 1992) and *Bacillus amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric,and acetic acids. Other organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among phosphate solubilizers (Banik S, Dey BK., 1982).

Aliphatic acids were found to be comparatively more effective in phosphate solubilization than phenolic acids and citric acid and fumaric acid had highest P solubilizing ability. As indicated by Pohlman and McColl (1986), several factors were

important in determining the degree or rate of dissolution of insoluble phosphate by organic acids. These were rate of diffusion of organics from bulk solution and diffusion of products from the site of reactivity, contact time between the organic acids and mineral surface, degree of dissociation of organic acids, type and position of functional groups, and chemical affinities of chelating agents for the metals.

Most of the reports on solubilization of insoluble phosphate by microorganisms suggested that organic acid metabolites excreated by microorganisms were solely responsible for the process (Sperber 1957, Bajpai and Sundara Rao 1971, Banik and Dey 1982).Organic acids, e.g., 2- ketogluconic acid, act as good chelators of divalent cations besides their acidifying effects

Chelating substances had also an important role in solubilization of insoluble phosphates. The acids chelate Ca^{++} ions and the chelation depends on the hydroxyl ions of the acid. The Ca^{+2} is chelated to a small extent with a-hydroxy aliphatic monobasic acid like lactic acid, more strongly with dibasic acids like malic and tartaric and more strongly with tribasic like citric acid. Among the dibasic aliphatic acids, hydroxy derivatives like malic acid form strongest complexes and a-substitutions by hydroxyl group of an aliphatic acid exhibit greater effect than b-substitutions of the same acid. Dibasic aromatic acids also chelate Ca ions but not the monobasic aromatic acids. Under acidic pH conditions the phosphate ions are precipitated by Fe^{3+} and Al^{3+}. The organic acids prevent such precipitation by chelation, forming metallo organic molecules e. g. Ferric citrate by citric acid. Dibasic acids also form chelates hydroxy phosphates and form hydroxy salts thereby releasing the phosphate ions. Some bacteria produce 2-ketogluconic acid which was a strong chelator of Ca. It can also solubilize insoluble phosphates like hydroxyapatite, fluorapatite.

So *Nocardiopsis Dassenvillei* may consume lactose to produce lactic acid that is used in dissolution of phosphate content of Abu Tartur phosphate ore.

To ensure production of organic acid by *Nocardiopsis dassenvillei* by using bromo cresol green as indicator (1%) supplemented in PVK agar on plate at pH 6 which the colour changed from blue to yallow resulting from growth of colony of bacterium at the center of plate and production of organic acids, Figure (17) and this agree with Fankem, H., *et al*, (2006).

Figure (17): Production of Organic Acids by *Nocardiopsis Dassenvillei* which The Colour PVK Agar Medium Supplemented with 1% Bromo Cresol Green Changes from Blue to Yallow in The Center of Plate.

Reference:

Banik S, Dey BK, 1982. Available phosphate content of an alluvial soil is influenced by inoculation of some isolated phosphate-solubilizing microorganisms. Plant Soil; 69:353–64.

Chaiharn M, Lumyong S (2009) Phosphate solubilization potential and stress tolerance of rhizobacteria from rice soil in Northern Thailand. World J Microbiol Biotechnol 25:305–314.

Chen YP, Rekha PD, Arun AB *et al.* (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41.

Fankem, H., D. Nwaga, A. Deubel, L. Dieng, W. Merbach and F. X. Etoa. 2006. Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (Elaeis guineensis) rhizosphere in Cameroon. African J. Biotech. 5:2450-2460.

Goldstein AH, 1994. Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. In: Torriani-Gorini A, Yagil E, Silver, S, editors. Phosphate in microorganisms: Cellular and Molecular Biology. Washington, DC: ASM Press. pp. 197–203.

Halder AK, Mishra AK, Bhattacharyya P *et al.* (1990) Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium* J Gen Appl Microbiol 36:81–92.

Hamdali H, Bouizgarne B, Hafidi M *et al.* (2008) Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines. Appl Soil Ecol 38:12–19.

Illmer P, Schinner F, 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soil.Soil Biol Biochem;24:389–95.

Jha BK, Pragash MG, Cletus G *et al.* (2009) Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent pseudomonad strains, *Pseudomonas aeruginosa*, *P. plecoglossicida* and *P. mosselii*. World J Microbiol Biotechnol 25:573–581.

Jones DL (1998) Organic acids in the rhizosphere: a critical rewiew. Plant Soil 205:25–44.

L.T. Narteh, K.L. Sahrawat, (1999). Oxalate and EDTA extractable soil phosphorus and iron in relation to P availability in lowland rice soils of West Africa Ghana J. Agric. Sci., 32, pp. 189–198.

Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic
Press, London.

Liu TS, Lee LY, Tai CY, Hung CH, Chang YS, Wolfram JH, Rogers R, Goldstein AH, 1992. Cloning of an Erwinia herbicola gene necessary for gluconic acid production and enhanced mineral phosphate solubilization in Escherichia coli HB101: Nucleotide sequence and probable involvement in biosynthesis of the coenzyme pyrroloquinoline quinone. J Bacteriol; 174:5814–9.

M.K. Saghir, A. Zaidi, P.A. Wani, (2009).Role of phosphate solubilizing microorganisms in sustainable agriculture—a review Sust. Agric., 5, pp. 551–570

Marra LM, Soares CRFS, Oliveira SM *et al.* (2012) Biological nitrogen fixation and phosphate solubilization by bacteria isolated from tropical soils. Plant Soil 357:289–307.

P. Delvasto, A. Valverde, A. Ballester , J.M. Igual, J.A. Munoz, F. Gonzalez, M.L. Blazquez, C. Garcıa, (2006). Characterization of brushite as a re-crystallization product formed during bacterial solubilization of hydroxyapatite in batch cultures. Soil Biology & Biochemistry 38 (2006) 2645–2654.

P. Illmer, F. Schinner, (1995). Solubilization of hardly soluble $AlPO_4$ with P-solubilizing microorganisms. Soil Biol. Biochem., 27, pp. 265–270.

Pradhan N, Shukla LB (2005). Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr. J. Biotechnol. 5: 850-854.

Rajan S S S, Watkinson J H, Sinclair A G, (1996). Phosphate rocks for direct application to soils [J]. Adv Agron, 57: 77-159.

Rodriguez H, Fraga R, (1999). Phosphate solubilizing bacteria and their role in plant growth promotion [J]. Biotechnol Adv, 17: 319-359.

Rogers, R.D., Wolfram, J.H., 1991. Biological separation of phosphate from ore. In: Smith,R.W., Misra, M. (Eds.), Mineral Processing. Minerals, Metals and Materials Society, Nevada, USA, pp. 219–232.

Sadaf Shahab and Nuzhat Ahmed, (2008). Effect of various parameters on the efficiency of zinc phosphate solubilization by indigenous bacterial isolates African Journal of Biotechnology Vol. 7 (10), pp. 1543-1549, 16 May

Silverman M P & Lundgren D G, (1959). Studies on the chemoautotrophic bacterium *Ferrobacillus ferrooxidans*, I. An improved medium and harvesting procedure for securing high cell yields, J Bacteriol, 77, 642-647.

Sonam Sharma*, Vijay Kumar and Ram Babu Tripathi, (2011). Isolation of Phosphate Solubilizing Microorganism (PSMs) From Soil. J. Microbiol. Biotech. Res., 1 (2): 90-95

Sperber, J.I. 1958. The incidence of apatite solubilizing organisms in the rhizosphere and soil. Australian Journal of Agricultural Research 9, 778-781.

Vassilev N, Medina A, Azcon R, Vassileva M, (2006). Microbial solubilization of rock phosphate on media containing agro-industrial waste and effect of the resulting products on plant growth and P uptake [J]. Plant Soil, 287: 77-84.

Venkateshwarlu B., Rao A.V., Raina P, (1984). Evaluation of phosphorus solubilization by microorganisms isolated from Arid soils. J Ind Soc Soil Sci.;32:273–277.

Usharani Krishnaswamy, Muthukumar Muthusamy and Lakshmanaperumalsamy Perumalsamy, 2009. Studies on the efficiency of the removal of phosphate using bacterial *Consortium* for the biotreatment of phosphate wastewater. European Journal of Applied Sciences 1 (1): 06-15.

Waksman SA (1927). Principles of soil microbiology, Batimore; W illiam & W ilkins co.

Whitelaw MA (2000) Growth promotion of plant inoculated with phosphate-solubilizing fungi. Adv Agron 69:99–151.

Willey, Joanne M., Linda Sherwood, Christopher J. Woolverton, and Lansing M. Prescott, (2008). Prescott, Harley, and Klein's Microbiology. New York: McGraw-Hill Higher Education.

Xiao, C.Q., R.A. Chi, X.H. Huang, W.X. Zhang, G.Z. Qiu and D.Z. Wang, 2008. Optimization for rock phosphate solubilization by phosphate-solubilizing fungi isolated from phosphate mines. Ecol. Eng., 33: 187-193.

Xie J, Knight JD, Leggett ME (2009) Comparison of media used to evaluate *Rhizobium leguminosarum* bivar *viciae* for phosphate solubilizing ability. Can J Microbiol 55:910–915.