

Original Research Article

Azotobacter vinelandii Evaluation and Optimization of Abu Tartur Egyptian Phosphate Ore Dissolution

M.A. El-Badry¹, T.A. Elbarbary², I.A. Ibrahim², Y. M. Abdel-Fatah²¹Botany and Microbiology Department, Faculty of Science, Al-Azher University, Egypt²Central Metallurgical Research and Development Institute, Egypt***Corresponding Author:**

M.A. El-Badry

Email: mohamed.helwanuni@yahoo.com

Abstract: In order to minimize the use of expensive chemical fertilizers, this study investigate the potential of phosphate solubilization by bacterium isolated from soil which the most of the bacteria isolated from the soil have the ability to dissolve rock phosphates both in the soil and in the culture medium is well known. The microorganisms produce low molecular mass organic acids, which attack the phosphate structure and transform phosphorus from non-utilizable to the utilizable for the plants form. The objective of the present study is to study the factors affecting on dissolution of phosphate content in Abu Tartur phosphate ore by using bacterium isolated from soil. Serial dilution method was performed to inoculum solutions to achieve microorganism's isolation which obtaining one bacterium that has ability to dissolve phosphate ore and molecular identification by 16 sRNA which suggest it called *Azotobacter vinelandii*. Optimum conditions of bioleaching of Abu Tartur phosphate ore are 3 days incubation period, modified PVK medium is the best medium for dissolution of Abu Tartur phosphate ore, 0.1×10^{29} colony forming unit of *Azotobacter vinelandii* for 50 ml medium, 0.5% Abu Tartur phosphate ore concentration, 30°C, ammonium oxalate as nitrogen source, glucose as carbon source, no significant effect of addition factor, also there is decreasing in pH and increases in electric potential, initial pH 7, which the leaching efficiency of phosphate content in Abu Tartur phosphate ore reaches to 52.6%.

Keywords: Abu Tartur phosphate ore, *Azotobacter vinelandii*, Phosphate dissolution.

INTRODUCTION

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.

Natural rock phosphate is a complex raw material and is mainly used in the manufacture of phosphate fertilizer [1]. 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 [2]. 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 [3].

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.

The role of microorganisms in phosphate solubilization or enhanced phosphate availability has been related to production of organic acids [4], H₂S [5], mineral acids [6] and to H⁺ protonation [7]. Organic acids exuded by microorganisms form stable complexes with phosphorus adsorbents (aluminium, iron and calcium) and thus increase phosphate solubilization. Illmer and Schimmer [7] hypothesized that the release of protons accompanying respiration or ammonium assimilation were related to phosphate solubilization by microorganisms that are not producing organic acids.

Soil microorganisms play a very significant role in mobilizing P for the use of plants by bringing about changes in pH of the soil microenvironment and

producing chelating substances which lead to native as well as added insoluble phosphates [8].

Some microorganisms, including bacteria and fungi, are known to be involved in the solubilization of rock phosphate [9], Hamdali H *et al* [10], Xiao C Q *et al.* [11]. 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.

The inoculation of P-solubilizing microorganisms is a promising technique because it can increase P availability in soils fertilized with rock phosphates [12].

In this work, studying the factors affect on dissolution of phosphate content in Abu Tartur phosphate ore by using bacterium isolated from soil to reach to maximum dissolution of P₂O₅ from ore.

MATERIAL AND METHODS

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 bacterial species from soil

The common bacterial species are isolated from soil according to the serial dilution technique as described by Johnson *et al.*, [13], 0.1 ml of each dilution is placed on the surface of sterile agar plate of Ashyb's medium. After 3 day of incubation at 30°C the developed one type bacterium colonies which screen its phosphate dissolution activity by pikoveskey's agar medium

Culture media

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

Pikovskaya's medium (PVK medum)

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 [14].

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 [14].

Nutrient medium

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 [15].

Ashyb's medium

It contains (g/l): 20 g/l mannitol, 0.2 g/l dipotassium phosphate, 0.2 g/l magnesium sulphate, 0.2g/l sodium chloride, 0.1 g/l potassium sulphate, 5 g/l Calcium carbonate, 15 g/l Agar. Final pH (at 25°C) 7.4±0.2 [16].

Testing phosphate solublization by isolated bacterium

Pikovskaya's Agar Medium

The isolated bacterium is tested for phosphate solubilization. Loopful sample of bacterium is placed in the center of Pikovskaya's medium agar plate and put in incubator at 30°C. The solubilization activity is 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 Gene Jet 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 Extension 72° C for 10 min one cycle. PCR clean up to the PCR product using Gene JET™ 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.

Experiment method

Prepare 50 ml of modified PVK broth medium in 100 ml conical flask and sterilized in autoclave for 15 min. at 121 °c and 1.5 atm. pressure and weigh 0.25 gm sterilized Abu Tartur phosphate ore then put 0.25 gm sterilized Abu Tartur phosphate ore on 50 ml of sterilized PVK medium and add inoculants of bactrium and also prepare another sample without addition *bacterium* which represented control then leave the flasks in shaking incubator at 30 °c and 160 rpm through the incubation period, take 5 ml from filtrate then centrifuged at 9000 rpm for 10 minuts . The amount of soluble phosphate in the culture filtrate is determined calorimetrically according to the method described by [17].

Effect of different growth parameter on phosphate solublization:

Azotobacter vinelandii isolate is grown in 100 ml Erlenmeyer flasks containing 50 ml lots of PVK medium, modified PVK medium, Ashby’s medium and nutrient medium separately supplemented with 0.25g of Abu Tartur phosphate for 50 ml medium. Each flask is inoculated with 0.1×10^{29} colony forming unit of *Azotobacter vinelandii* isolate and incubated at 30 °C. The amount of soluble phosphate in the culture filtrate

is determined. The previous steps are conducted on modified pikovskaya’s medium supplemented with Abu Tartur rock phosphate for *Azotobacter vinelandii* isolate at different incubation periods, incubation temperatures, ore concentrations, bacterial concentration, carbon, and nitrogen sources, initial pH, addition of medium, bacterial and both of them during experiment, diameter of conical flask base.

Detection organic acid produced by *Bacterium*

Preparation of Pikovskaya’s agar medium supplemented with 0.1% of bromocresol green as indicator at pH 6 then is inoculated with *Azotobacter vinelandii* isolate at the center of plate then leave up to 3 days.

RESULTS AND DISCUSSION

Chemical composition of Abu Tartur phosphate ore

Chemical analysis 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).

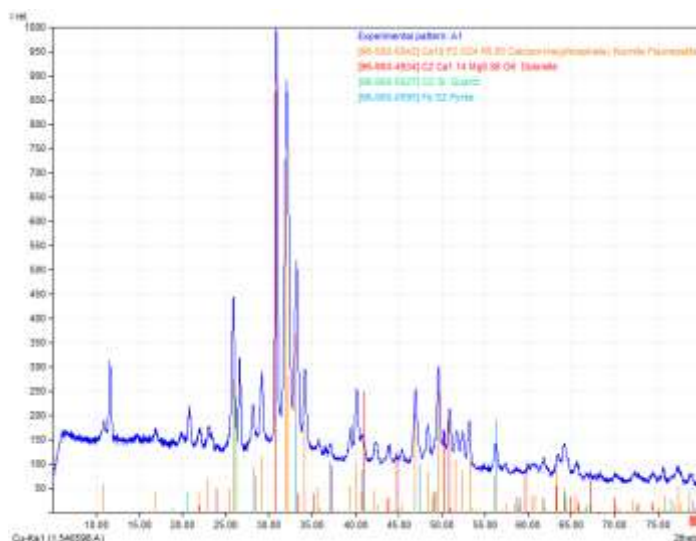


Fig-1: XRD Analysis Of Abu Tartur Phosphate Ore

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 *bacterium* isolate

Molecular identification of the selected isolate 16S rDNA sequencing is 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 *Azotobacter* isolate has been enrolled into a cluster containing

Azotobacter species and was found to be closely related to *Azotobacter vinelandii* strain (Figure 2).



Fig-2: Identification Of Bacterial isolated From Soil

Detection of phosphate solubilization by *Azotobacter vinelandii* isolate on Pikovaskay’s agar medium

Azotobacter vinelandii isolate has ability to dissolve phosphate which form clear zone around the bacterial colony on pikovaskay’s agar medium and this refers to the solubilization of $Ca_3(PO_4)_2$ by bacterium and this may be due diffusion of organic acids and enzymes into the medium are recorded by formation clear zone around the bacterial colony on pikovaskay’s agar medium (Figure 3). This

agrees with Kumar *et al.* [18], Garg *et al.* [19] and Farajzadeh *et al.* [20], the *Azotobacter* is isolated was able to dissolve inorganic and organic phosphate compounds. As previously reported by the phosphate solubilizing index results obtained in the solid medium was significantly higher than other observations and corroborated with Farajzadeh *et al.* [20], indicating that indigenous strains of *Azotobacter* isolated from alkaline soils are effective P_i solubilizers.



Fig-3: *Azotobacter vinelandii* clear zone around the bacterial colony on pikovaskay’s agar medium

Effect of incubation period on phosphate solubilization by *Azotobacter vinelandii* isolate

Using PVK medium in presence 0.25 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with 0.5×10^{29} colony forming unit of *Azotobacter vinelandii* isolate and measuring P_2O_5 each 1day and also pH and redox potential. The results revealed that maximum phosphate solubilization is obtained after 3day which reaches to 25.3% with decreasing pH value and increasing redox potential value then P_2O_5 dissolution begins to decrease above this period (Figure 4). Solubilization of rock phosphate depends on its structural complexity, particle size and metabolites of microorganism [21].

This agrees with Rahim Nosrati *et al.* [22] that show *A. vinelandii* O6 delayed for 72 h and arrived at stationary phase and maximum phosphate solubilizing index ($\sim 230\text{mg/l}$) after 72 h and also noticeable reduction in phosphate solubilizing index during stationary phase of growth found in all cases supports the dependence of phosphate solubilizing index on bacterial metabolism. It was also shown that the phosphatase activity of bacterial strain could synergistically enhance the release of P_i in the acidified medium. The advantage of bacteria capable of phosphate solubilizing with simultaneous secretion of organic acids and phosphatase activity on production and yield were shown in both green house and field trials [23].

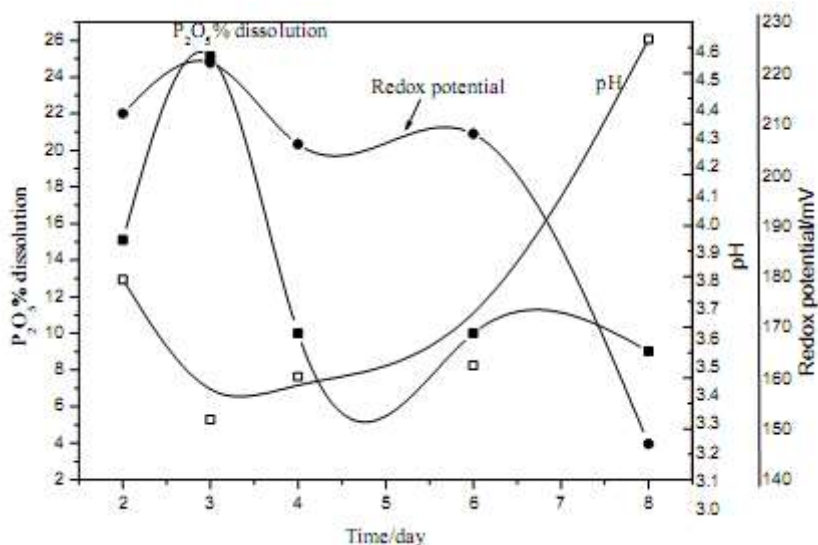


Fig-4: Effect Of Incubation Period On Dissolution Of Phosphate Content Of Abu Tartur phosphate Ore

Effect of different liquid media on phosphate solubilization by *Azotobacter vinelandii* isolate

It is studied by using four different types of liquid media (PVK, ashyb's, modified PVK, nutrient medium) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of medium and inoculated with 0.5×10^{29} colony forming unit of *Azotobacter vinelandii* isolate and incubated at 30 °c and 160 rpm and measuring P₂O₅ each 1day and also pH and redox potential. The results revealed that maximum phosphate solubilization is obtained with modified pikovasky's medium reaching to 25.3% while, the minimum phosphate solubilization occurred with general liquid medium (Figure 5). The

results are monitored with final pH, since the final pH is low with modified pikovasky's medium (3.2) and high with ashyb's medium and nutrient broth (above 7).

The solubilized phosphate may react with calcium or magnesium present in rock phosphate as soon as the pH of the growth medium increases and form insoluble phosphate (equation 1). 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 [24].

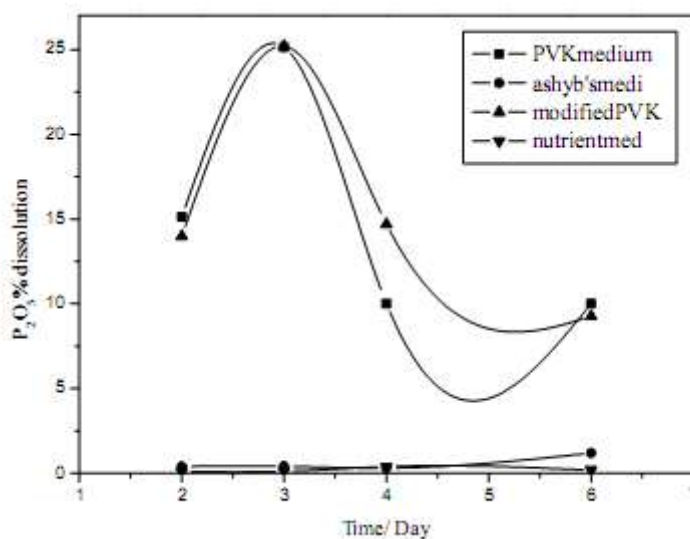
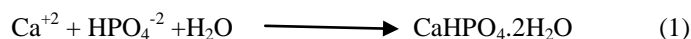


Fig-5: Effect Of Type Of Medium On Dissolution Of Phosphate Content Of Abu Tartur Phosphate Ore

Effect of initial pH

It is studied by using four different initial pH (4, 5, 6, 7, 8) of 50 ml of modified PVK medium in presence 0.25 g Abu Tartur phosphate ore and inoculated with 0.5×10^{29} colony forming unit of *Azotobacter vinelandii* isolate in 100 ml flask and incubated at 30 °c and 160 rpm and measuring P_2O_5 after 3 day of incubation. The growth of *Azotobacter vinelandii* isolate is affected with the initial pH of the modified PVK medium as in the Figure (6).

The maximum growth of *bacterium* on a medium containing rock phosphate is observed at initial pH 7. At this pH value phosphate solubilization exhibited high amounts it represented 34.7%. It is also observed that phosphate solubilization at pH 5 was sharply decreased and this agree with Tejera N *et al.* [25] showed that a lower number of isolates grew on N-free media at pH value as high as 8.7.

The pH of the culture medium directly influences the growth of microorganisms and the

biochemical processes they perform. In many cases, acidification is the main mechanism involved in phosphate solubilization [8, 26, 27]. However, several studies have shown a lack of correlation between solubilized phosphorus and pH of the medium [28, 29]. 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 have 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 [30, 27]. There are several solubilization mechanisms are involved at the pH of the medium varies. These mechanisms can be: proton exclusion (via cellular respiration and ammonium absorption as N source) [31], siderophores [10] and exopolysaccharide (EPS) production [32]. The production of EPS that could act synergistically with acid production as suggested by Yi *et al.* [32].

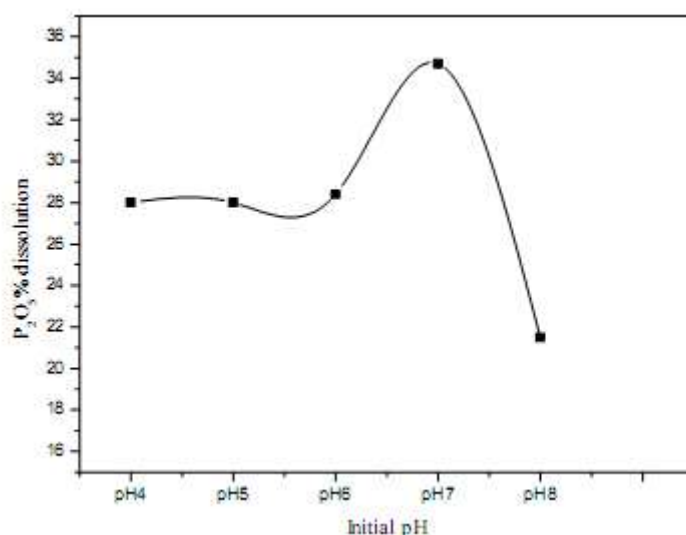


Fig-6: Effect Of Initial pH On Dissolution Of Phosphate Content Of Abu Tartur Phosphate Ore

Effect of *Azotobacter vinelandii* isolate inoculum size

It is studied by using four different concentration of bacterium (0.1×10^{29} , 0.5×10^{29} , 1×10^{29} , 3×10^{29} , 5×10^{29}) colony forming unit of *Azotobacter vinelandii* isolate for 50 ml of PVK medium with initial pH7 in presence 0.25 g Abu Tartur phosphate ore and incubated at 30 °c and 160 rpm and measuring P_2O_5 after 3day of incubation.

Concentration of *Azotobacter vinelandii* isolate effect on dissolution of phosphate content of the ore which study in the growth medium. The best phosphate

solubilization occurred at a concentration of 0.1×10^{29} colony forming unit of *Azotobacter vinelandii* isolate and decrease at high concentration of *Azotobacter vinelandii* isolate with no highly change in final pH value and this may be due to competition between bacterial cells themselves, decrease the aeration and also high growth which may consume phosphate. At a concentration of 0.1×10^{29} colony forming unit of *Azotobacter vinelandii* isolate, *Azotobacter vinelandii* isolate can solubilize approximately 43% of phosphate content of the ore, Figure (7).

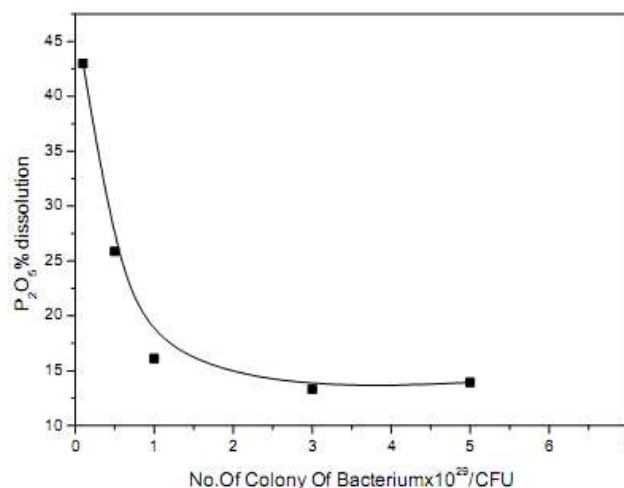


Fig-7: Effect Of Bacterial Inoculum Size On Dissolution Of Phosphate Content Of Abu Tartur Phosphate Ore

Effect of bulk density

It is studied by using four different weights of ore (0.25, 0.5, 1, 2) gm for 50 ml of modified PVK medium with initial pH 7 in presence 0.25 g Abu Tartur phosphate ore and inoculated with 0.1x 10²⁹ colony forming unit of *Azotobacter vinelandii* isolate and incubated at 30 °c and 160 rpm and measuring P₂O₅ after 3day of incubation.

Azotobacter vinelandii isolate has varied growth in the presence of different concentrations of phosphate ore in the growth medium up to 4% (Figure 8). The optimum growth and best phosphate solubilization occurred at a concentration of 0.5% of the Abu Tartur phosphate ore concentration and decreased above this concentration. It is also observed decrease pH value and no change highly between various concentration of ores; this may be due to the production of organic acids and acidic phosphatase enzymes. At a concentration of 0.5% ore, *Azotobacter vinelandii* isolate can solubilize approximately 43% of phosphate content of the ore.

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 [33]. Also, it may be due to inhibitory effect on further phosphate solubilization [34], the negative effect of soluble P on microbial acid productivity [34] 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 [31].

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, O₂ and CO₂ with increasing pulp density and the mechanical damage to bacterial cells by solids, [36].

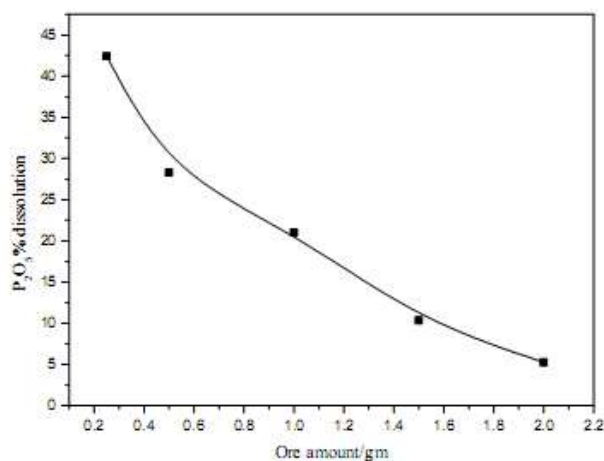


Fig-8: Effect Of Bulk Density On Dissolution Of Phosphate Content Of Abu Tartur Phosphate Ore

Effect of different incubation temperatures

It is studied by using four different temperature (20, 30, 40, 50 °c) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of modified PVK medium with initial pH 7 in presence 0.25 g Abu Tartur phosphate ore and inoculated with 0.1×10^{29} colony forming unit of *Azotobacter vinelandii* isolate and incubated at 30 °c and 160 rpm and measuring P_2O_5 after 3day of incubation.

The dissolution of phosphate content of ore increase with increase the temperature of incubation up to 30 °c then begins decrease (Figure 9) and dissolution of phosphate content of ore reach to 46.8% so the optimum incubation temperature for best phosphate

solubilization activity by *Azotobacter vinelandii* isolate is 30 °c at which optimum growth for *Azotobacter vinelandii* isolate and adapt to their indigenous environment so their metabolic activities are linked to the temperature of the environment [37, 38].

This agrees with Rahim Nosrati *et al* [22] which bacterial growth and consequently PSI of bacteria were reduced at both high and low temperatures and also agrees with Neeru Narula *et al* [39]. The growth of Bacterium at 30°C refers to mesophilic bacterium which grows best in moderate temperature, neither too hot nor too cold [40].

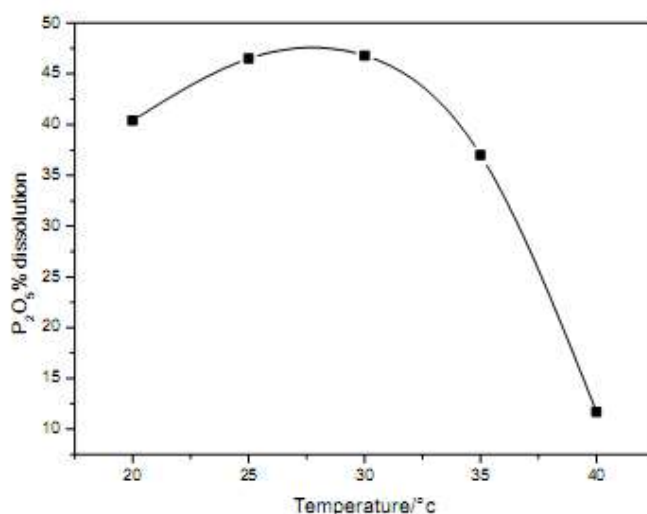


Fig-9: Effect Of Incubation Temperature On Dissolution Of Phosphate Content Of Abu Tartur Phosphate Ore

Effect of different nitrogen sources

It is studied by using five different nitrogen source of (ammonium sulphate, ammonium chloride, ammonium oxalate, asparagine, glycine) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of modified PVK medium with initial pH 7 in presence 0.25 g Abu Tartur phosphate ore and inoculated with 0.1×10^{29} colony forming unit of *Azotobacter vinelandii* isolate and incubated at 30 °c and 160 rpm and measuring P_2O_5 after 3day of incubation.

Azotobacter vinelandii isolate can solubilize high amount of phosphorus from rock phosphate ore with all tested nitrogen sources, Figure (10). Ammonium oxalate is found to be the best nitrogen source utilized by *Azotobacter vinelandii* isolate for maximum

phosphate solubilization which reaches to 50.8% followed by asparagine and lowest dissolution of phosphate content of the ore at using ammonium chloride as nitrogen source.

As a nitrogen source, ammonium oxalate was found to give maximum soluble P. Oxalate ions have the ability to form stable complexes with calcium, iron and aluminum to liberate phosphates [41] and are known to extract P from soils [42]. The phenomenon of P solubilization is 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 [31].

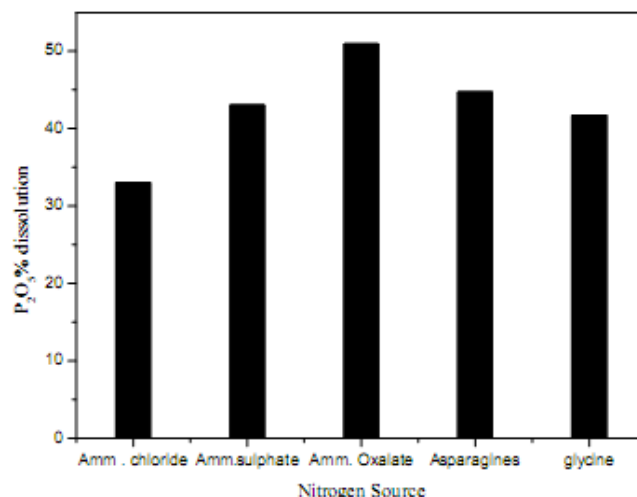


Fig-10: Effect Of Nitrogen Source On Dissolution Of Phosphate Content Of Abu Tartur Phosphate Ore

Effect of different carbon sources

It is studied by using four different carbon source of (glucose, starch, dextrose, sucrose) in presence 0.25 g Abu Tartur phosphate ore for 50 ml of modified PVK medium containing ammonium oxalate as nitrogen source with initial pH 7, inoculated with 0.1×10^{29} colony forming unit of *Azotobacter vinelandii* isolate and incubated at 30 °c and 160 rpm and measuring P₂O₅ after 3day of incubation.

The results revealed that *Azotobacter vinelandii* isolate grows well on modified PVK liquid medium containing different carbon sources. Whereas, high amounts of soluble phosphate is detected only in the culture filtrate of *bacterium* with glucose which reaches to 52.8% then dextrose with low pH value, while starch and sucrose exhibited low amount of soluble phosphate with high pH value. The bacterial growth exhibited remarkable variation according to the utilized carbon source, the best bacterial growth to produce enzyme and organic acids reached when glucose is utilized as a carbon source (Figure 11). The maximum amount of phosphorus solubilized corresponded to the highest value of the organic acid

produced. The sugar consumption and organic acid liberation are seen to be most active up to 3 day. It is generally accepted that the release of insoluble and fixed forms of P carried out by the action of phosphate-solubilizing bacteria (PSB) via the secretion of low molecular weight organic acids mainly gluconic and keto-gluconic acids and phosphatases. These acids are produced in the periplasm of many Gram-negative bacteria through a direct oxidation pathway of glucose (DOPG, non-phosphorylating oxidation), consequently, the organic acids diffuse freely outside the cells and may release high amounts of soluble P from mineral phosphates, by supplying both protons and metal complex organic acid anions [43].

Also this result agrees with M. Sridevi, K. V. Mallaiah, [44] that how glucose was the best carbon source for phosphate solubilization. The maximum decrease in pH was recorded in glucose-containing medium. In other carbon sources little decrease in pH and no correlation between acidic pH and quantity of P₂O₅ liberated were observed. And also this result reported earlier in *Bradyrhizobium* species isolated from *Cicer arietinum* [45].

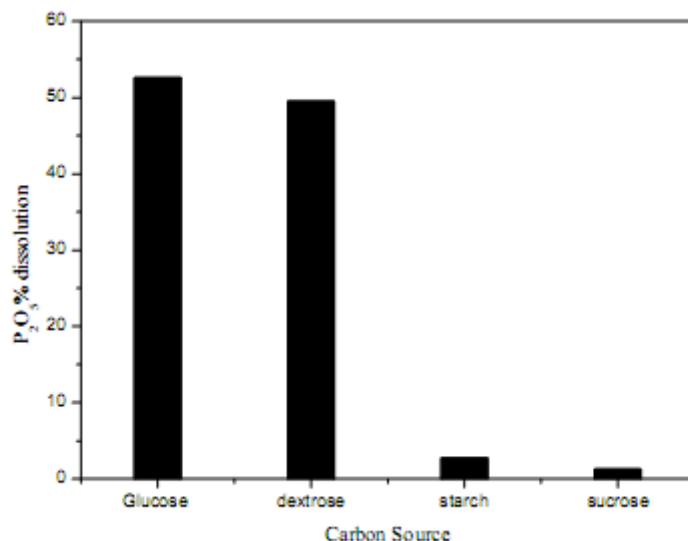


Fig-11: Effect Of Carbon Source On Dissolution Of Phosphate Content Of Abu Tartur Phosphate Ore

Addition factor during experiment

It is studied by addition of medium, inoculants of *Azotobacter vinelandii* isolate and both of them during experiment is carried out in presence 0.25 g Abu Tartur phosphate ore for 50 ml of modified PVK medium containing ammonium oxalate as nitrogen source and glucose as carbon source with initial pH 7, inoculated with 0.1x 10²⁹ colony forming unit of *Azotobacter vinelandii* isolate and incubated at 30 °c and 160 rpm and measuring P₂O₅ after 3day of incubation.

The addition of inoculants of *Azotobacter vinelandii* isolate during experiment of bioleaching don't have significant effect in dissolution of phosphate content of ore and this may be due to consumption of nutrient by the first addition of inoculants, also addition of medium during experiment that is carried out don't have significant effect on dissolution of phosphate and also addition of both of *Azotobacter vinelandii* isolate and medium, Figure (12).

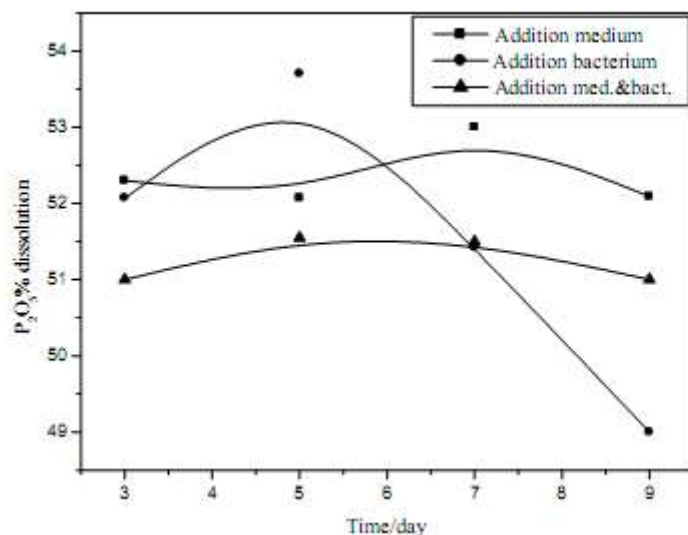


Fig-12: Effect Of addition Of Medium, Azotobacter Vinelandii Isolate, Both Of Medium And Azotobacter Vinelandii Isolate On Dissolution Of Phosphate Content Of Abu Tartur Phosphate Ore

Growth curve of Azotobacter Vinelandii Isolate

The growth curve of *Azotobacter vinelandii* isolate is carried out by serial dilution and standard plate method [46], *Azotobacter vinelandii* isolate begins to grow from first hour and this refer to lag phase up to four hours then enter log phase up to 18 hours and

produce primary metabolites represented in enzymes which reach to optimum growth then the growth remains constant to 24 hours and this refer to stationary phase of its growth and at this phase the *Azotobacter vinelandii* isolate produced all the acids then its growth begins to decrease and this is decline phase (Figure 13).

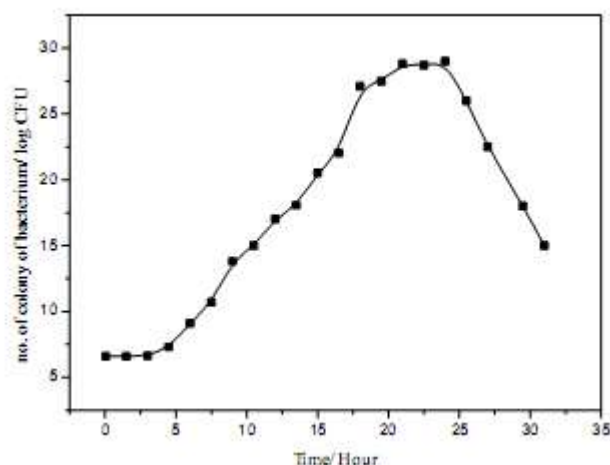


Fig-13: Growth Curve Of *Azotobacter Vinelandii* Isolate

Mechanism of biodissolution of phosphate content of Abu Tartur phosphate ore

Organic acid production

It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms [8]. Production of organic acids results in acidification of the microbial cell and its surroundings. Consequently, P_i may be released from a mineral phosphate by proton substitution for Ca^{+2} [47].

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 [48], several factors are important in determining the degree or rate of dissolution of soils in organic acids. These are 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 suggest that organic acid metabolites excreted by microorganisms are solely responsible for the process [14, 30, 49]. Organic acids, e.g., 2- ketogluconic acid, act as good chelators of divalent cations besides their acidifying effects

Chelating substances have 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 α -substitutions by hydroxyl group of an aliphatic acid exhibit greater effect than β -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 is a strong chelator of Ca. It can also solubilize insoluble phosphates like hydroxyapatite, fluorapatite.

To ensure production of organic acid by *Azotobacter* by using bromo cresol green as indicator(1%) supplemented in PVK agar on plate at pH 6 which the colour change from blue to yellow and the colony of bacterium grows on the surface of the medium plates, Figure (14) and this refer to production of citric acid and this agree with Fankem *et al.*, [60].



Fig-14: Change Of The Colour Of PVK Agar Medium Supplemented With 1% Bromo Cresol Green As Indicator From Blue To Yellow At The Center Of Plate

Enzyme action

Another mechanism of bioleaching of Phosphate by enzyme action which some bacteria produce phosphatases that are collective name for enzymes that cleave phosphate from organic compounds like phospholipids nucleic acids etc. Depending on the optima of enzymes they have been classified as alkaline and acid phosphatases. Phosphatase activity is widespread in soil with larger percentage in rhizosphere. Agree with Amal M. *et al.* [51]. Also agree with Sashidhar B, Podile AR [43].

Role of exopolysaccharides substances (EPS) in P solubilization

Recently the role of **polysaccharides** in the microbial mediated solubilization of P was assessed by Yi *et al.* [32]. Microbial exopolysaccharides (EPSs) are polymers consisting mainly of carbohydrates excreted by some bacteria and fungi onto the outside of their cell walls. Their composition and structures are very varied; they may be homo- or hetero-polysaccharides and may also contain a number of different organic and inorganic substituents [52]. Four bacterial strains of *Enterobacter sp.* (EnHy-401), *Arthrobacter sp.* (ArHy-505), *Azotobacter sp.* (AzHy-510) and *Enterobacter sp.* (EnHy-402), possessing the ability to solubilize TCP (tri calcium phosphate), were used to assess the role of exopolysaccharide (EPS) in the solubilization of P by Yi *et al.* [32]. These Phosphate Solubilizing bacteria produced a significant amount of EPS and demonstrated a strong ability for P-solubilization.

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