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Separation of high quality phosphate by Rhisobium rhizogenes of Egyptian Sebaiya west low-grade phosphate ore

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Abstract

The current study was conducted to explore the potential of a phosphate solubilizing for the preparation of high quality phosphate fertilizer by bioleaching from low grade Egyptian ore. *Bacillus megaterium*, *Rhizobium rhizogenes* and *Thiobacillus thiooxidans* were evaluated for their ability to phosphate dissolution from Egyptian Sebaiya west low-grade phosphate ore by studying optimization conditions of incubation temperature, incubation time and inoculum size, pH and different carbon and nitrogen as energy sources. Results revealed that dissolution of P_2O_5 reached to 19.9, 68.4 and 32.2 for *B. megaterium*, *R. rhizogenes* and *T. thiooxidans* respectively. A model has been suggested which assumes that the phosphate radical was attacked by the reducing bacteria to produce unstable soluble phosphorus ions that readily oxidized to P_2O_5 with water oxygen. Kinetically, the leaching process was a zero-order reaction. In this work applied of microorganism in industrial processes was practically feasible with low cost and friendly environmental process.

Key words: phosphate beneficiation, bioleaching, phosphate upgrading, phosphate ore

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1. Introduction

Phosphorus is an essential substance of ATP (Adenosine Triphosphate) phospholipids and nucleic acids includes in the regulation of different metabolic pathways in plants [1]. Plants directly use soluble phosphate from soil by root system; soluble phosphate in rhizosphere is limited and found in small levels of availability [2]. Therefore, it is urgent need for phosphate addition to soil as chemical fertilizers, but the regular use of it affects the soil nature and its microbial community, which there is a need for alternative sources insert of natural phosphate bio fertilizers [3]. The organic acids produced from soil microbial communities chelates the ions of insoluble metal phosphates and other sources containing phosphorus such as phosphate ores so that the phosphorus could be dissolute and solubilized. The estimation of organic acids production by microorganisms on phosphorus ore in soil fertilizer exists obviously and the activating ratio increases with plant growth [4].

In addition, the production of several organic acids, like citric, gluconic, oxalic and formic, which contribute to phosphorus dissolution has been achieved using fungus genus as Aspergillus, Streptomyces and Penicillium [5]. Inorganic acids, however, are stronger than these organic acids, thereby making acidophilic species more suitable for industrial phosphorus solubilisation. For instance, bacteria from the genus *Acidithiobacillus*, like *A. ferrooxidans*, *A. thiooxidans*, oxidize elemental sulphur, reduce S-compounds and sulfide minerals to produce sulfuric acid and soluble metal sulfates, resulting in an acidic bioleaching environment. These bacterial strains had been used in the past to bioleach phosphorus: *A. ferrooxidans*, to acidulate phosphate rock and pyrite [6].

Phosphate ore consider the most important sources of phosphorus to cover its deficiency. Phosphate rock global production reaches 160 million of tons a year, from which 72% corresponds to non-renewable deposits in Morocco, China, the United States of America and Russia [7]. In our previous study, we found that *Azotobacter vinelandii* used for phosphate impurities dissolution from oasis and Aswan iron ore by 73.4 % [8]. The factors affecting on dissolution of Abu Tartur phosphate ore by *Azotobacter vinelandii* with leaching efficiency of phosphate content in Abu Tartur phosphate ore Maximized to 52.6% [9]. The aim of this study was to obtain a good quality phosphate from Egyptian phosphate low grade ore by bioleaching technique. Parameters affecting the efficiency of the process and the quality of the end products have been investigated.

2. Materials and methods

Phosphate rock and sulfur-mud

The phosphate rock sample used in this study was obtained from Nile Valley – Sebaiya west a run of mine with composition: 24.88% P_2O_5, 43.52% CaO, 12.18% SiO_2, 1.52% MgO, 1.83% $A1_2O_3$, 2.27% Fe_2O_3, 1.62% Na_2O, 0.12% K_2O, 1.1% F, and 9.52% loss of Ignition. The sample contained the following minerals composition: fluorapatite (Ca_5 (PO_4HF). calcite ($CaCO_3$. trigonal). vaterite ($CaCO_3$, hexagonal) and lime (CaO) as the main minerals.

Microorganisms and growth media

Three microorganisms as *Bacillus megaterium*, *Rhizobium rhizogenes*, *Thiobacillus thiooxidans* obtained from were purchased from Microbial Wealth Center - Faculty of Agriculture - Ain Shams University used to evaluate their phosphate bioleaching efficacy of phosphate rock.

Culture media: Different types of culture media are used for microbial growth and dissolution activity assay 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 [10].

Modified 9 k medium: as described in El Barbary et al. [11].

Ashyb's medium as described by El Badry et al., [9].

Experiment method: as described in El Badry et al., [9].

3. Results and discussion

Effect of initial pH on phosphate dissolution

A series of experiment was carried out under the following condition (ore amount 0.25, temperature of 30°C, peptone as nitrogen source and beef extract as carbon source, cell count is 0.1×1029 cfu) the pH studied from 4 up to 8) after the incubation time the P2O5 for the three microorganism were measured to define each of the are the more suitable for dissolution. Figure 1 described the effect of pH on dissolution of P2O5. It was found that 13, 45 and 21 % P2O5 recovery by using *Bacillus megaterium*, *Rhizobium rhizogenes* and *thiobacillus thiooxidans* respectively with same PH value equal 7 for all microorganisms

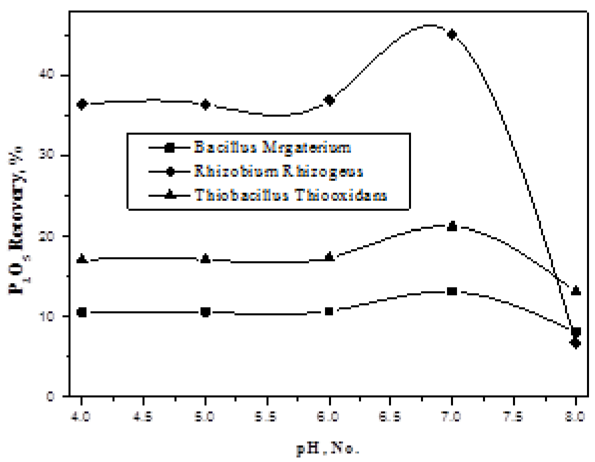

Fig (1): Effect of initial pH on P2O5 dissolution from Sebaiya West ore.

R. rhizogenes with 45% Phosphate dissolution was the most potent organisms by this results it was the foirst time to evaluate R. rhizogenes in phosphate ore dissolution rather than Bacillus megaterium and thiobacillus thiooxidans which ordinary used in phosphate dissolution. Kang et al[12] evaluated phosphate dissolution ability of *B. megaterium* which enhanced by optimal culture conditions at pH 7.0 and 35 °C which might be due to the presence of malic in the culture medium wherease For many years T. ferrooxidans was considered to be the most important micro-organism in commercial bioleaching and biooxidation plants that operate at 40 0 C or less [13].

Effect of ore amount on Phosphate dissolution

The effect of the ore amount of phosphate gram on the extent of bioleached phosphate is graphically demonstrated in Fig 2. It was seen that the extent of leached phosphate decreases with the increase in the weight of phosphate ore, and increases with time attaining an optimum at 30 h. It is seen that the extent of leaching directly depends on the weight ratio of the selected microorganism to the weight of the phosphate ore subject to leaching as given in Figure 2. The results revealed that obvious changes in phosphate dissolution by 16, 55 and 26 % using *Bacillus megaterium, Rhizobium rhizogenes* and *thiobacillus* thiooxidans respectively. The dissolution of phosphate ore 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.[14]. Also, it may be due to inhibitory effect on further phosphate solubilization, the negative effect of soluble P on microbial acid productivity [15], might also be responsible for final soluble P concentration. Another explanation for this might be formation of an organo- Phosphorus compound induced by organic metabolites released, which in turn, reduces the amount of available phosphorus [16].

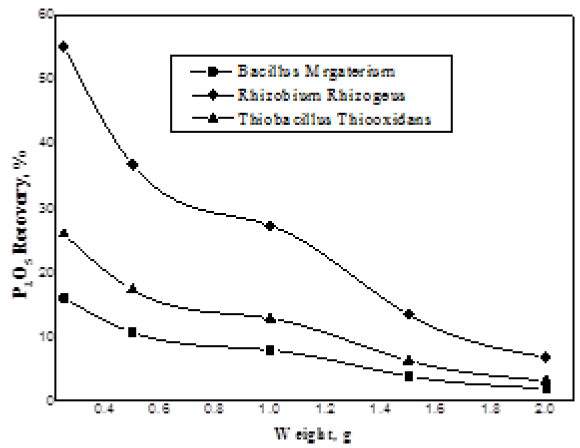

Fig (2): Effect of ore amount on P2O5 dissolution from Sebaiya West phosphate ore.

Effect of inoculum size on phosphate dissolution

The Effect of inoculum size on the phosphate P_2O_5 dissolution from Sebaiya west Phosphte ore from 0.5 x10^{29} up to 3 x 10^{29} plotted in figure 3. The results revealed that the dissolution of P_2O_5 from phosphate ore reached to 16, 55 and 26 % with *Bacillus megaterium, Rhizobium* *rhizogenes* and *thiobacillus thiooxidans* respectively.

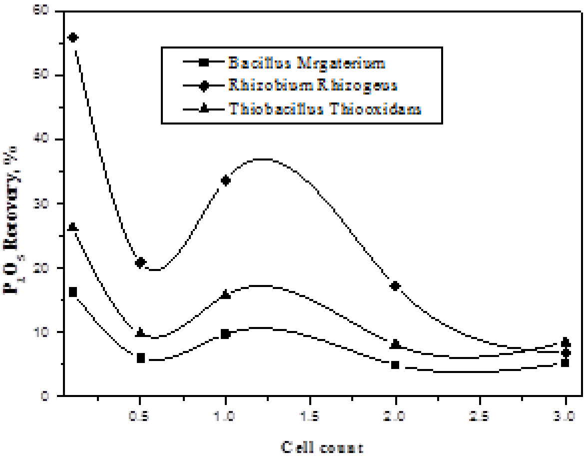

Fig (3): The effect of cell count on the P_2O_5 dissolution from Sebaiya West phosphate ore.

Effect of temperature on the phosphate dissolution

The effect of temperature on the dissolution of P_2O_5 of phosphate ore was evaluated after 30 hr of reaction. It is seen that the extent of bioleaching is favored at temperatures 30°C after 30h in the incubator and represented in Figure 4.

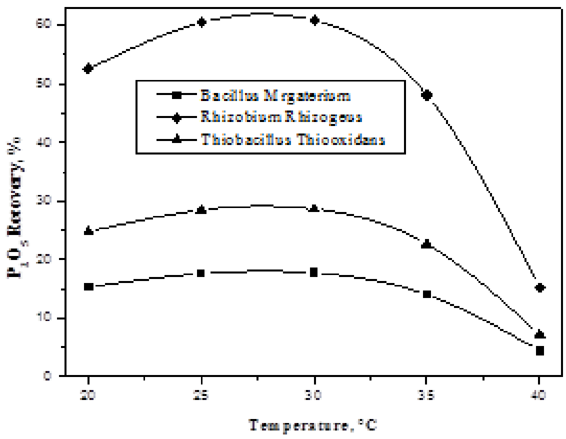

Fig (4): Effect of temperature on the extent of bioleaching the phosphate ore

From Figure 4 it was found that the three bacteria dissolve P_2O_5 from the ore as 17, 60 and 28 % with *Bacillus megaterium, Rhizobium rhizogenes* and *thiobacillus thiooxidans* respectively. The growth of Bacterium at 30°c refers to mesophilic bacterium which grows best in moderate temperature, neither too hot nor too cold [16].

Effect of different Nitrogen as Energy source on the phosphate bioleaching

The effects of nitrogen as energy source on the dissolution of P_2O_5 of phosphate ore, 5 nitrogen sources are used (Ammonium chloride, Ammonium sulphate, Ammonium oxalate, Asparagine and Glycine) to study the effect after 30 h of reaction. It is seen that the extent of bioleaching is favored with ammonium oxalate as nitrogen energy source. This result agrees with El badry et., [9]. From the plot it was found that the best nitrogen energy source with the three microbe is ammonium oxalate where it gives 19, 66 and 31 % with *Bacillus megaterium, Rhizobium rhizogenes* and *thiobacillus thiooxidans,* respectively.

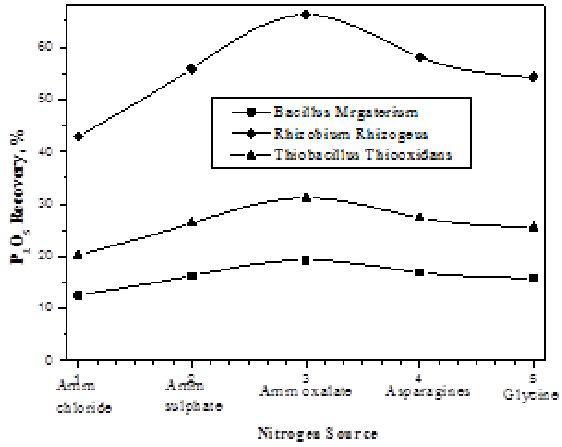

Fig (5): Effect of nitrogen as energy source on bioleaching of phosphate ore.

Effect of different Carbon as Energy source on the phosphate dissolution

The effect of different carbon as energy source on the P2O5 dissolution from Sebaiya West phosphate ore four sources of Carbon Sources are used Glucose, Starch, Sucrose and dextrose. The results are plotted in figure 6.

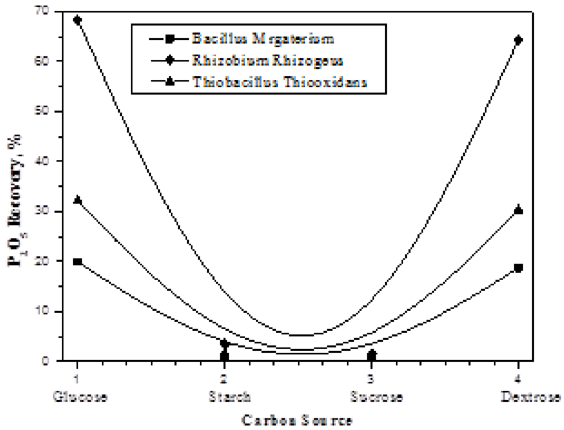

Fig (6): Effect of carbon as energy source on the phosphate bioleaching from phosphate ore.

Results revealed that Glucose is the most suitable carbon Energy source with the three microbe is where it gives 19, 68 and 32 with *Bacillus megaterium, Rhizobium rhizogenes* and *thiobacillus thiooxidans* respectively which agree with [8&9].

4. Conclusion

It is concluded that bioleaching of P_2O_5 depend on different influential factors in the premise that Rhizobium rhizogenens is most suitable microorganisms for dissolution of P_2O_5 from Sebaiya West Phosphate ore. It helps to convert the phosphate present in the low-grade ore to an intermediate hydrogen phosphate salt. Hydrogen phosphate readily oxidizes with atmospheric oxygen to the end product insoluble.

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