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Phosphate by Bioleaching of Egyptian Low Grade Phosphate Ore

Tarek A. A. El-Barbary

***Abstract* –The present work was done to evaluate the ability of some bacterial solubilizing phosphate to synthesis high quality phosphate fertilizer by bioleaching from low grade Egyptian ore. *Bacillus megaterium*, *Rhizobium rhizogenes* and *Thiobacillu sthiooxidans* 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.**

***Keywords* – phosphate beneficiation, bioleaching, phosphate upgrading, phosphate ore**

I. INTRODUCTION

Phosphorus is an essential substance of ATP (Adenosine Triphosphate) phospholipids and nucleic acidsincludes in the regulation of different metabolic pathways in plants[1]. Plants directly usesoluble phosphate from soil by root system; soluble phosphate in rhizosphereis limited and found in small levels of availability[2].Therefore, it is urgent need forphosphate 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 acid production by microorganisms on phosphorus ore in soil fertilizer exists obviously and the activating ratio increases with plant growth [4], also, synthesis of many organic acids as, gluconic, citric, formic and oxalic help to dissolve phosphate had achieved by *Aspergillus, Streptomyces* and *Penicillium* [5].

However organic acids are weaker than Inorganic acids, so acidophilic microbial species are more excellent for industrial solubilisation of phosphate, as, *Acidithiobacillus*, such as *A. ferrooxidans, A. thiooxidans*, oxidize elemental sulfur, 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 considers the most important sources of phosphorus to cover its deficiency. Global production o f Phosphate rock arrived 160 millions of tons per 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 *Azotobactervinelandii* 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 *Azotobactervinelandii* with a 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. The Parameteres affecting the efficiency of the process and the quality of the end products have been investigated.

II. MATERIALS AND METHODS

Phosphate rock and sulfur-mud

The samples of phosphate ore rock used in this work 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 rock composed o f the following:

lime (CaO) as the main minerals.

fluorapatite ($Ca_5(PO_4HF)$. calcite ($CaCO_3$. trigonal). vaterite ($CaCO_3$, hexagonal) and

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Microorganisms and growth media

Three microorganisms as*Bacillus megaterium*, *Rhizobium rhizogenes*, *Thiobacillusthiooxidans* obtained from were purchased from Microbial Wealth Center - Faculty of Agriculture - Ain Shams Universityused 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/lYeast 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 etal.[11]. **Ashyb's medium**as described byEl Badry et al., [9].

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

III. 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 0.1×10^{29}cfu) the pH studied from 4 up to 8) after the incubation time the P_2O_5 for the three microorganism were measured to define each of the are the more suitable for dissolution. Table 1 described the effect of pH on dissolution of P_2O_5. It was found that 13, 45 and 21 % P_2O_5 recovery by using *Bacillus megaterium*, *Rhizobium rhizogenes* and *thiobacillusthiooxidans* respectively with same PH value equal 7 for all microorganisms.

Table 1:
Effect of pH on Dissolution of Phosphate from Sebaiya West Phosphate Ore

Bacteria Type / pH	*Bacillus Megaterium*	*Rhizobium Rhizogenes*	*Thiobacillus Thiooxidans*
4	10.6158	36.441	17.1617
5	10.612	36.428	17.1556
6	10.764	36.9484	17.4007
7	13.151	45.1447	21.2607
8	8.1485	6.84326	13.1731

R. rhizogenes with 45% Phosphate dissolution was the most potent organisms by this results it was the first time to evaluate *R. rhizogenes* in phosphate ore dissolution rather than *Bacillus megaterium* and *thiobacillusthiooxidans* which ordinary used in phosphate dissolution. Kang et al[12] evaluated phosphate dissolution ability of *B. megaterium* which induced by optimize microbial conditions at pH 7.0 and 35 °C which due to the synthesis of malic in the broth medium whereas *T. ferrooxidans* was presented as the most vital organisms in industrial bioleaching and biooxidation plants that operate at 40° C or less for many years [13].

Effect of ore amount on Phosphate dissolution

The effect of the ore amount of phosphate gram on the extent of bioleached phosphate is Tabulated in Table 2. It was found that the extent of leached phosphate decrease 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 asgiven in Figure2.The results revealed that obvious changes in phosphate dissolution by 16, 55 and 26 % using *Bacillus megaterium* ,*Rhizobium rhizogenes* and *thiobacillusthiooxidans* respectively.

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The biosolving of phosphate rock ore minimize by maximize phosphate ore concentration in broth medium, that leades to be to toxic infulence of many metal ions that released into the broth medium such as Mn^{+2} and Na^{+1}, Ca^{+2} ions that 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].

Table 2:
Effect of Ore amount on Dissolution of Phosphate from Sebaiya West Phosphate Ore

Bacteria Type / Ore, g	*Bacillus Megaterium*	*Rhizobium Rhizogenes*	*Thiobacillus Thiooxidans*
0.25	16.08476	55.21444	26.003
0.5	10.7257	36.8183	17.339
1	7.97037	27.36003	12.88
1.5	3.91886	13.45234	6.33532
2	1.99354	6.84326	3.22802

Effect of inoculum size on phosphate dissolution

The Effect of inoculum size on the phosphate P_2O_5 dissolution from Sebaiya west Phosphate ore from 0.5 $x10^{29}$ up to 3 x 10^{29} written in Table 3.

Table 3:
Effect of Inculum Size on Dissolution of Phosphate from Sebaiya West Phosphate Ore

Bacteria Type / Inoculum Size, 10^{29}	*Bacillus Megaterium*	*Rhizobium Rhizogenes*	*Thiobacillus Thiooxidans*
0.1	16.297	55.943	26.3461
0.5	6.1019	20.946	9.8645
1	9.816	33.6959	15.869
2	5.041	17.303	8.1489
3	5.268	6.84326	8.5165

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.

Effect of temperature on the phosphate dissolution

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

Table 4:
Effect of Temperature on Dissolution of Phosphate from Sebaiya West Phosphate Ore

Bacteria Type / Temperature, °C	*Bacillus Megaterium*	*Rhizobium Rhizogenes*	*Thiobacillus Thiooxidans*
20	16.297	55.943	26.3461
25	6.1019	20.946	9.8645
30	9.816	33.6959	15.869
35	5.041	17.303	8.1489
40	5.268	6.84326	8.5165

From Table 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&17].

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 source are used (Ammonium chloride, Ammonium sulfate, Ammonium oxalate, Asparagine and Glycine) to study the effect after 30h of reaction the results are summarized in Table 5. It is seen that the extent of bioleaching is favored with ammonium oxalate as nitrogen energy source. This results agree with El badry et al[9]. From the Table 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.

Table 5:
Effect of Nitrogen Source on Dissolution of Phosphate from Sebaiya West Phosphate Ore

Bacteria Type / Nitrogen source	*Bacillus Megaterium*	*Rhizobium Rhizogenes*	*Thiobacillus Thiooxidans*
Ammonium sulphate	16.297	55.943	26.3461
Ammonium chloride	6.1019	20.946	9.8645
Ammonium oxalate	9.816	33.6959	15.869
Asparagines	5.041	17.303	8.1489
Glycine	5.268	6.84326	8.5165

Effect of different Carbon as Energy source on the phosphate dissolution

The effect of different carbon as energy source on the P_2O_5dissolution from Sebaiya West phosphate ore four sources of Carbon Sources are used Glucose, Starch, Sucrose and dextrose. The results are plotted in Figure 1.

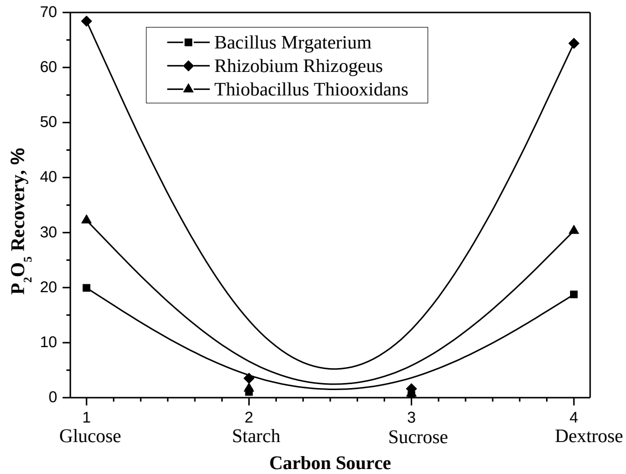

Figure 1:Effect of carbon as energy source on the phosphate bioleaching from phosphate ore.

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 *thiobacillusthiooxidans* respectively which agree with [8&9].

IV. 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. Ithelp 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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