Role of *Pseudomonas songnenensis*-Apatite Interaction on Bio-Flotation of Calcareous Phosphate Ore

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Abstract: Phosphate mineral ores are non-renewable resources. It is the main source of fertilizers and phosphorous chemicals. The enrichment process is essential to meet the industrial specifications through the separation of undesirable gangues. The biological processes are more attracted to mineral processing due to their lower operating costs and easy mineral ores applications. Bacteria can bring out surface modification leading to the separation of minerals by bio-flotation. Through this study, *Pseudomonas songnenensis* is isolated from phosphate ore surface and identified by 16SrRNA. The zeta potential and adhesion measurements showed that *the Pseudomonas songnenensis* has a higher affinity to apatite than calcite, which is confirmed by FTIR measurements. A concentration of 32.7% P_2O_5 and 6.8% $CaCO_3$ has been obtained from a binary mixture with 25% P_2O_5 and 20% $CaCO_3$%. By applying the optimum conditions on natural phosphate ore, a concentrate with 31.5% P_2O_5 has been obtained from a feed containing 21.2% P_2O_5 in the presence of 4×10^7 cells/ml (*Pseudomonas songnenensis*) at 25°C and pH 6.5.

Keywords: bio-flotation; bio-beneficiation; phosphate; apatite; calcite; *Pseudomonas songnenensis*.

1. Introduction

The phosphate mineral ore is the main source of phosphate fertilizers. It has other applications such as animal feed, insecticides, vitamins, drugs, soaps, detergents, beverages, ceramics, optical glass, toothpaste, and dental cements [1]. The enrichment process is essential to meet industrial specifications by separating undesirable gangues such as sand, clay, and/or calcareous minerals by froth flotation. Many studies had been made to improve the strategy for phosphate flotation [2-4].

Although fine grinding is required to reach liberation, the fine particles' treatment presents a complex problem. The fines generally range from 1 to 100 µm in size, can substantially impact production, even at low concentrations, and can cause potential environmental problems if released to the environment. Consequently, the situation is more complicated and difficult if the selective separation of fines is needed [5]. Because of the fines' relatively high surface area, various problems affect their separation by flotation technology, including high reagent consumption, excess froth stability, low adhesion probability, higher dissolution, rapid oxidation, and nonspecific collector adsorption [6-8]. During the past twenty years, innovations and improvements have been made in the beneficiation of phosphate ore,

but few innovations have considered an activation technique to enhance the ores' flotation response [9-12].

Bio-beneficiation refers to the selective removal of undesirable mineral constituents from ore by applying microorganisms as surface modifiers to enhance one mineral's separation from another either by flotation or flocculation [13].

Thus, it is enriched with respect to the desired valuable minerals [14]. Recently, the biological processes are becoming more attractive in mineral processing due to bacteria can act as surface modifiers, depressants, collectors, or dispersing agents [15]. Such interactions of microbes and their agents with minerals can be indirect, through biological products acting as surface-active agents, or directly due to microbial adhesion or attachment to the particles bringing out surface modification. These interactions can change the hydrophobicity, and thus, flocculation or dispersion of mineral suspension can occur. Bacterial attachment to the mineral surface is an important step in both processes. This attachment is affected mainly by both bacteria and mineral's surface properties, including surface charge, surface composition, and hydrophobicity [8-10]. Bacteria can selectively depress a mineral through oxidizing [16].

Selective adsorption of *T. ferrooxidans* onto the pyrite mineral has been reported [17]. *Mycobacterium phlei* has been used for flocculation and flotation of several minerals, including hematite, apatite, and dolomite [18-21]. The selective adsorption of *M. phlei* on to mineral surface has also been reported. The difference in the minerals' surface properties, such as hydrophobicity or surface charge, leads to a selective attachment of microorganisms to reach a selective flotation or flocculation [22-23].

This work investigates apatite and calcite minerals' physicochemical behavior in the presence of *Pseudomonas songnenensis* as a surface modifier to enhance apatite floatability and thus concentration in its ore.

2. Materials and Methods

2.1. Materials.

Pure apatite and calcite mineral samples were supplied by the Egyptian Mineral Resources Authority (EMRA) for flotation experiments and surface behavior investigation. Both samples of particle sizes below 150 µm and more than 99% purity. Freshly prepared nutrient broth/ agar was used for the cultivation of bacterial strains. A representative sample of natural phosphate ore from the Nile Vally deposit was wet ground to less than 150 µm particle size using a rod mill to reach the liberation.

2.2. Methods.

2.2.1. Bacterial isolation.

Pseudomonas songnenensis is isolated from phosphate ore surface and identified by 16SrRNA. It purified by streaking on nutrient agar plates, then transferred to nutrient agar slopes stored at 4°C and sub-cultured monthly. The nutrient agar is used for the cultivation of bacterial strains. A hemocytometer is used for bacteria cell counting [24].

2.2.2. Measurements.

Complete chemical analysis of the calcite and apatite minerals and phosphate ore was carried out using X-ray fluorescence "Rigaku Super Mini 200".

The zeta-potential was measured using Malvern laser zeta-meter, model zeta-sizer-2000. The measurement was carried out by mixing 0.01 g of pure apatite or calcite minerals

with 50 ml of pure water in the presence of bacteria isolates. Sodium chloride solution was added to keep ionic strength at 2×10^{-2} M, and then it was conditioned for 30 min at desired pH [25].

The morphology was determined using a scanning electron microscope (SEM) model JEOLJSM-5400, Japan. The FTIR spectrum was obtained using a Spectrum 2000 Perkin Elmer spectrometer of 4000 and 400 cm^{-1} range. The spectrum is obtained with KBr pellets prepared with solid sample and analytical grade KBr from Merck [26].

2.2.3. Adhesion measurements.

The adhesion of bacterial isolates on the mineral surface was determined by adding 1g dry sample of calcium carbonate or apatite to the bacterial solutions (50 cm^3) in a 100 cm^3 volumetric flask and mixing for 15 min in a shaker (Model Janke & Kunkel VX10). The pH was adjusted to the desired values using 0.1 M HCl and NaOH solutions, after which the samples were filtered through filter paper. Then, a spectrophotometer was used to measure the adsorption of the bacterial isolate to a mineral surface [14].

2.2.4. Flotation experiments.

The flotation was carried out in a glass flotation column of 100 ml capacity. One gram of the mineral was mixed with bacteria for 15 min at desired pH. Both floated and sank fractions were collected, dried, weighted, and chemically analyzed. The experiment was repeated triplets and averaged as a confirmed result [14].

3. Results and Discussion

3.1. Surface characteristic of pure phosphate and calcite minerals.

3.1.1. Zeta potential.

Figure 1 illustrates the zeta potential of the *Pseudomonas songnenensis* in which the values of zeta potential are varied from (−10 mV to +40 mV) over the entire range of pH (2-12). The iso-electric point (IEP) of bacteria is about a pH of 8.6. It originates due to the bacterial cell charge resulting from the dissociation or protonation of carboxyl and amino groups, so it depends on pH. The surface turns negative charged at higher pH due to proton dissociation [27]. The zeta potential curve of apatite intersection at pH 4.7, while calcite showed positive values of zeta potential all over the pH range with no IEP.

The apatite surface is strongly affected due to bacterial interaction, and its IEP became 6.5 rather than 4.7. On the other hand, Figure 2 shows no significant change in the zeta potential of calcite mineral after treatment. The adhesion of negative bacteria to the negative mineral surface at pH 6 is due to surface heterogeneity with a polysaccharide envelope. The released ions from the mineral surface affect the surface charge of bacteria and thus, the repulsion is reduced [28]. The chemical and hydrogen bonds play significant roles in bacterial adhesion [29]. These results are in agreement with that of bacterial interaction with single minerals in which *Pseudomonas songnenensis*, more or less, has a hydrophobic character which causes some degree of aggregation for the treated apatite mineral [30].
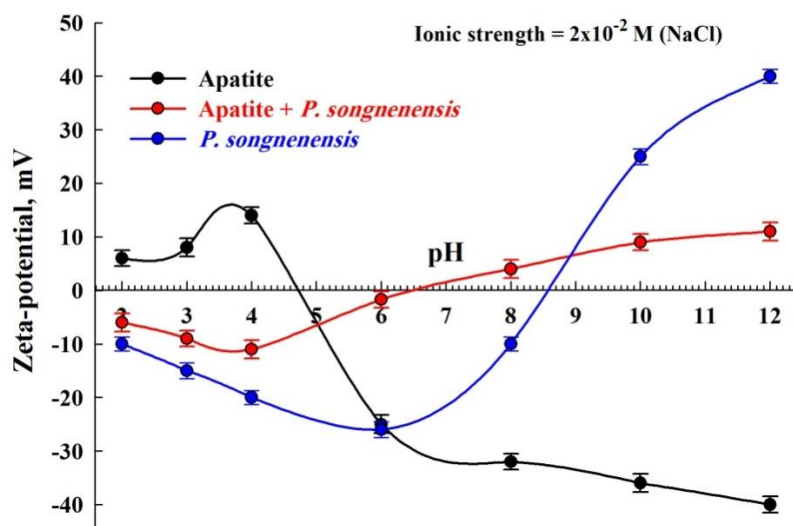
Figure 1. Zeta potential of *P. songnenensis*, apatite mineral, and treated apatite with *P. songnenensis*.
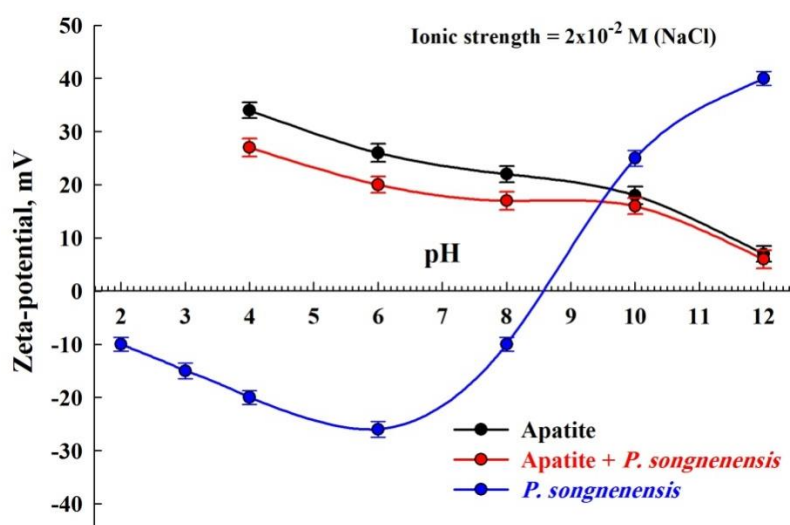

Figure 2. Zeta potential of *P. songnenensis*, calcite mineral, and treated calcite with *P. songnenensis*.

3.1.2. FTIR of apatite, calcite, and *Pseudomonas songnenensis*.

FTIR spectra of *Pseudomonas songnenensis*, pure minerals, and treated minerals are shown in Figure 3. The main characteristic peaks for *Pseudomonas songnenensis* are from 456-896 cm^{-1}, belonging to alkene C-H. The peaks at 1050-1150 cm^{-1} and 1100-1350 cm^{-1} are related to C=O of alkoxy, acyl, and phenyl groups. The narrow band at 1639.1 cm^{-1} belongs to C=O saturated carbonyl group from both carboxylic acid and amide. Weak peaks from 2993-3718 cm^{-1} appear due to the presence of C-H, aldehyde H-C=O and stretching vibration of NH and OH of both alcohol and carboxylic acid [31].

FTIR spectrum of treated apatite with *Pseudomonas songnenensis* showed new bands at 419-596 cm^{-1}, 689.5 cm^{-1}, and 744.1 cm^{-1} due to the interaction between C-H and mineral surface. Also, the two bands at 1047.1 cm^{-1} and 1431.8 cm^{-1} are emergences as a broadband due to the interaction with C-O of the acyl group of bacteria. The band shift from 1644 cm^{-1} to 1638.2 cm^{-1} due to the hydrogen bond formed with saturated C=O of amides and N-H bending of amide rather than amines. The band shift from 2357.5 cm^{-1} to 2352.7 cm^{-1} and from 3012-3741 cm^{-1} to 3027-3769 cm^{-1} due to the hydrogen bond formed with saturated C=O of amides and N-H bending of amide rather than amines [32, 33]. The presence of new surface species proved that the adsorption type is mainly chemical adsorption [34].
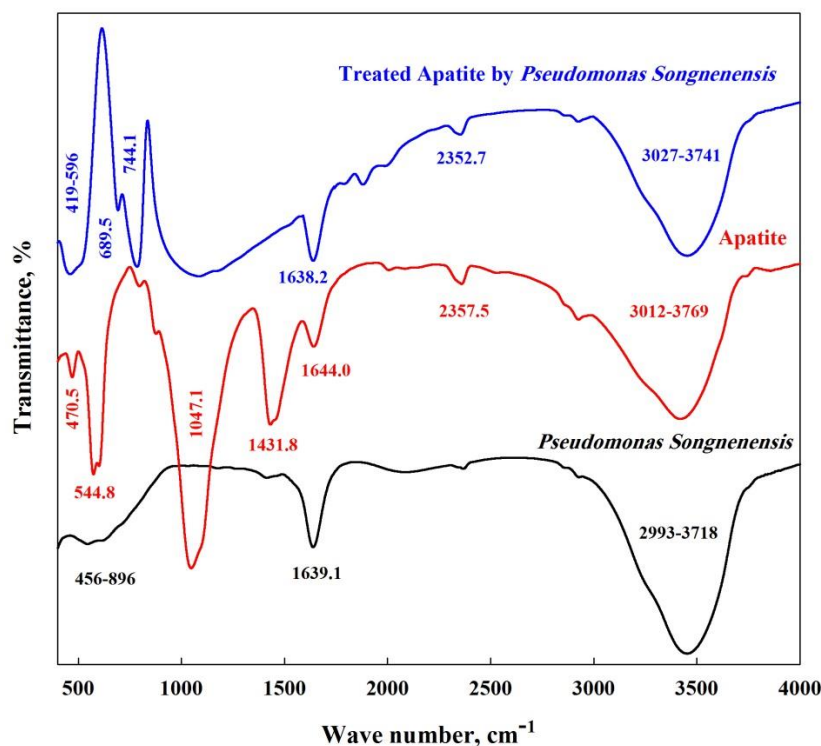
Figure 3. FTIR spectra of *P. songnenensis*, apatite mineral, and treated apatite with *P. songnenensis*.

Figure 4 shows that the FTIR spectrum of calcite mineral. It is characterized by a strong band at 1438.4 cm^{-1}, concerning the C-O stretching mode of carbonate and a peak at 874 cm^{-1} of the bending mode. The symmetric bending is represented by a peak at 713 cm^{-1}. As a result of *Pseudomonas songnenensis* interaction, there is no significant change in the calcite spectrum. It is believed that there is no interaction between *P. songnenensis* and calcite surface. The stretching vibrations of the H_2O molecules in the calcite are located at 2841.5 cm^{-1} [35].
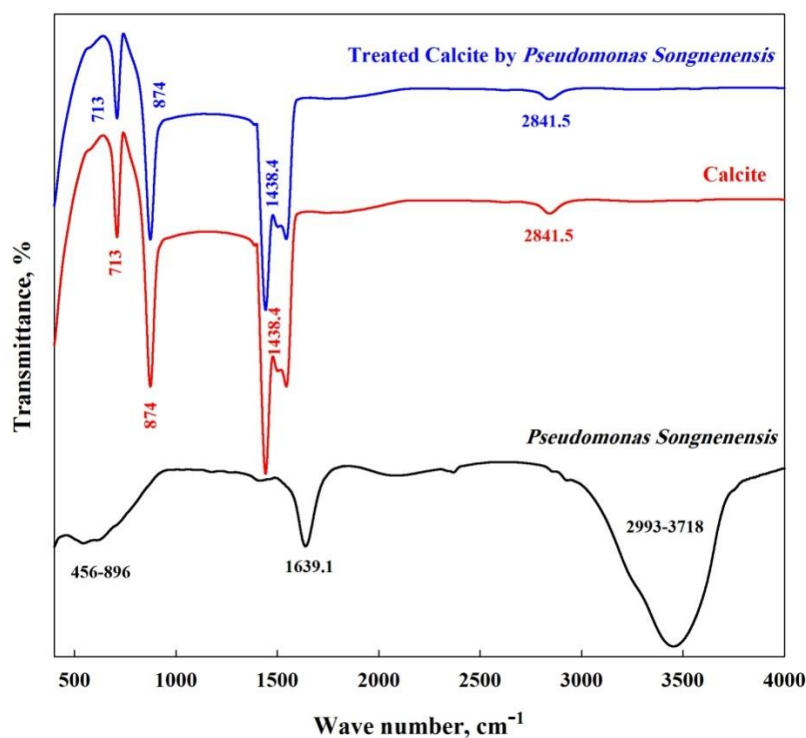

Figure 4. FTIR spectra of *P. songnenensis*, calcite mineral, and treated calcite with *P. songnenensis*.

3.1.3. SEM images of apatite and calcite.

Figure 5 shows significant adsorption of *Pseudomonas songnenensis* on the apatite surface, leading to a degree of aggregation of apatite particles. This is due to the biofilm formation of bacteria onto the apatite surface. It is proved the significant change in apatite surface behavior, such as in the zeta-potential measurements and thus its floatability. The aggregation proved that the apatite surface became more hydrophobic, in turn, enhances its separation from calcite. This is in agreement with the literature [36]. On the other hand, Figure 6 indicated no significant change of calcite particles after interaction with bacteria. Thus, it has been decided to use *P. songnenensis* as a surface modifier and/or sole flotation agent in this study.
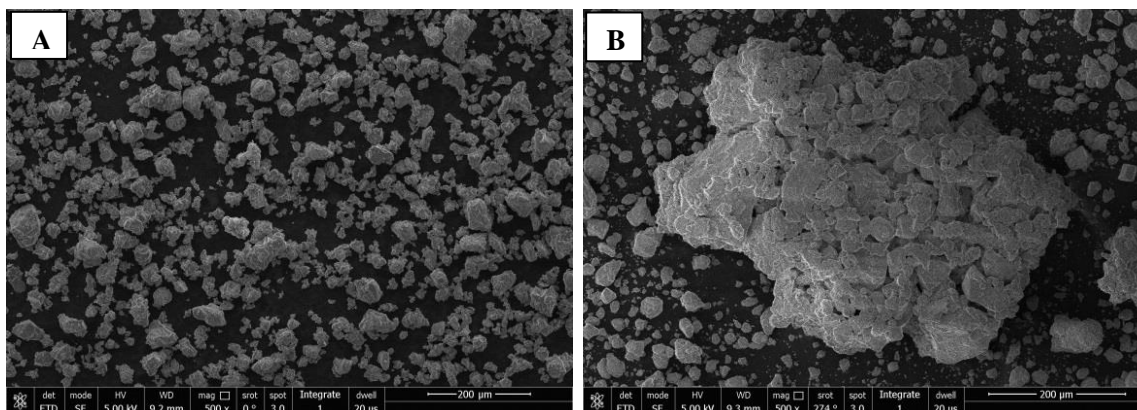

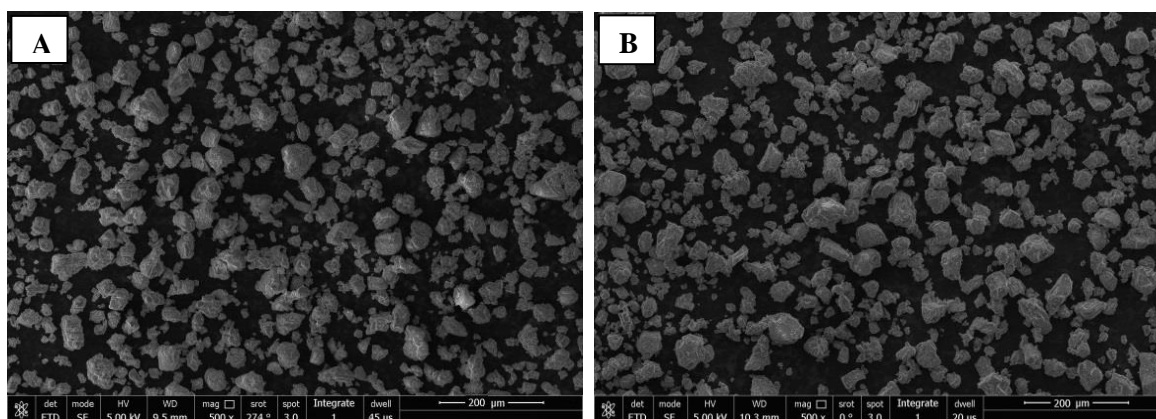**Figure 5.** SEM images of apatite mineral and after treatment with *P. songnenensis*.

Figure 6. SEM images of calcite mineral and after treatment with *P. songnenensis*.

3.1.4. Adhesion measurement.

The adhesion of *P. songnenensis* on the apatite and calcite single minerals was carried out by conditioning a single mineral with 1×10^7 cells/ml of bacteria strain. It was found that the adsorption *P. songnenensis* is pH-dependent. Figure 7 shows that the adsorption stability occurs at a pH 6.5 for apatite, while the adhesion on calcite decreases with increasing the pH. A higher affinity to apatite minerals may be due to surface charges on both cells and microorganisms. The interface region of the mineral substrate and biofilm is modified by the presence of microorganisms and their metabolic products. Charge separation can occur by dissociating ionizable groups, such as -OH, -COOH, -NH, and -SO, -H [37].
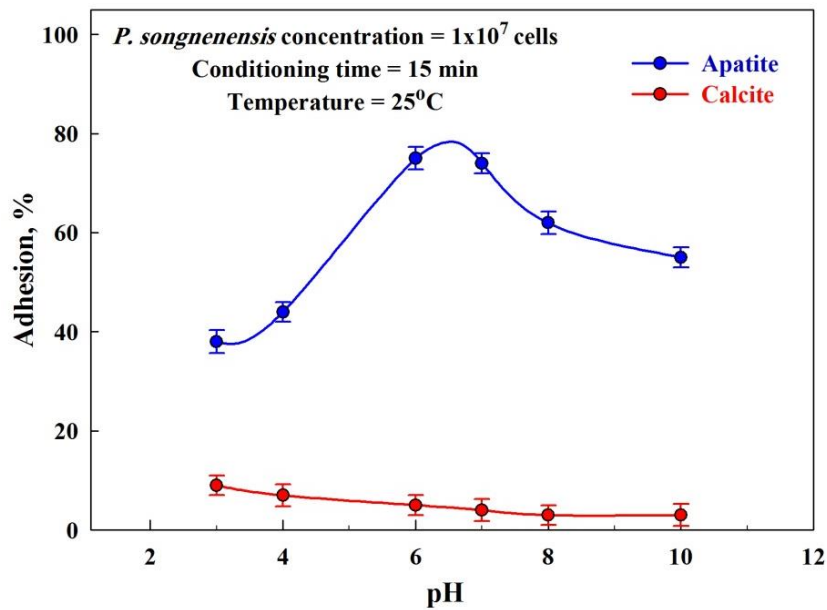
Figure 7. Effect of pH on the adhesion of *Pseudomonas songnenensis* onto single minerals.

Figure 8 shows the adhesion behavior on the minerals as a function of *P. songnenensis* concentration at pH 6.5. The adhesion percent of the apatite mineral increases with increasing *P. songnenensis* concentration to reach 88% in the presence of 4×10^7 cells/ml then it is almost constant. The higher adhesion on the apatite compared with calcite is related to high selectivity. It may be due to the presence of surface appendages, cell surfaces, and exopolymers. The appendages are mostly protein and may bind to specific mineral surface molecules [38]. The electrostatic forces, hydrogen bonding, and chemical interaction play significant roles in bacterial interaction with minerals [39]. The adsorption of exopolysaccharides (biofilm) can occur on a hydrophilic surface by strong hydrogen bonding between amino or carboxyl groups, peptide units, or other polar groups on biological and mineral surfaces [40].
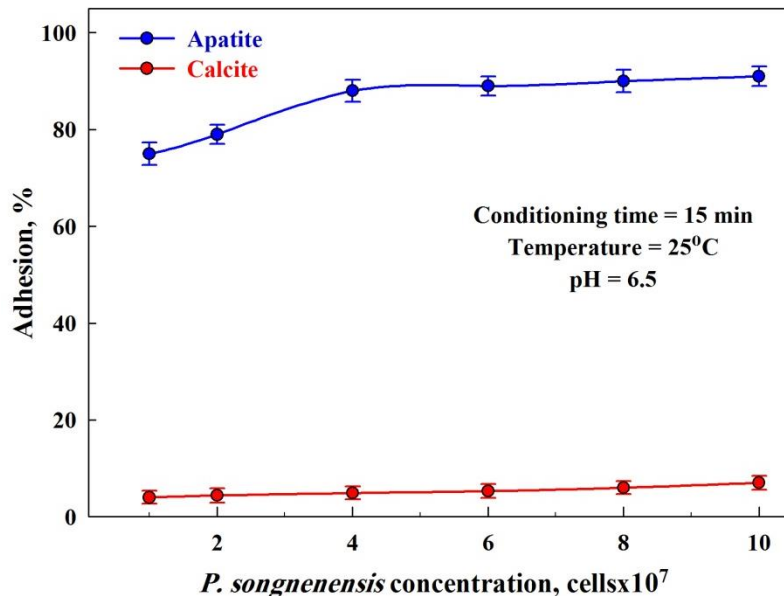

Figure 8. Effect of *Pseudomonas songnenensis* concentration on its adhesion onto single minerals.

3.2. Bio-flotation.

3.2.1. Bio-flotation of a single mineral.

Figure 9 shows that the floatability of single minerals as a function of pH in the presence of 4×10^7 cells/ml of bacteria strains. The floatability of apatite increases with increasing pH to

reach about 95% at pH 6 - 7, and then it is decreased to 74% at pH 10. These results agree on the zeta potential measurement, which showed that the IEP became 6.5 as a result of bacteria treatment. On the other hand, calcite's floatability is poor (4 - 7%) over the pH range. The interface region of the mineral substrate and biofilm is modified by the presence of microorganisms and their metabolic products. Charge separation can occur by dissociating ionizable groups, such as -OH, -COOH, -NH, and -SO, -H [37].

Figure 10 shows that the floatability of apatite mineral is increased to reach 98% in the presence of 4×10^7 cells/ml, and then it slightly decreased to 90% at 10×10^7 cells/ml. Thus, the adsorption of bacteria on the apatite mineral surface-enhanced its flotation, which provides a more hydrophobic surface and stable agglomeration [31]. The lower recovery at a higher bacteria concentration may be due to crowds of adsorbed bacteria on the apatite surface, reducing hydrophobicity. This is in agreement with the literature [37-40].
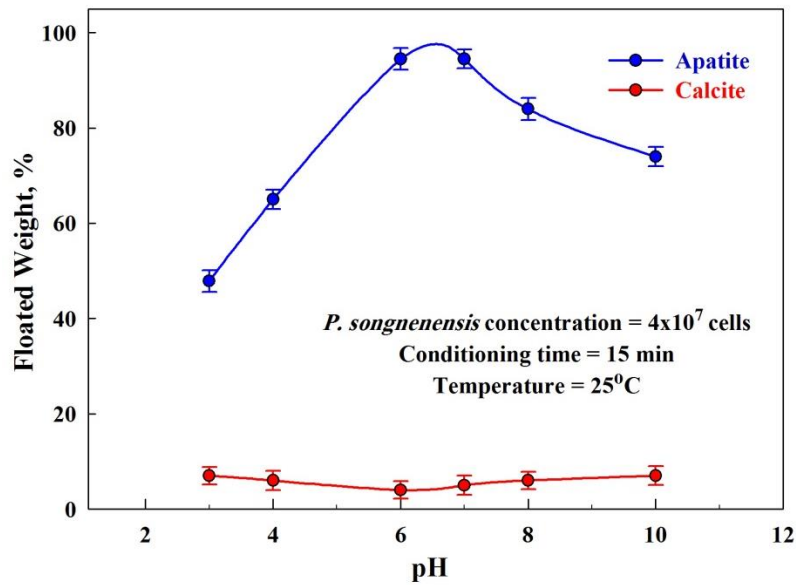

Figure 9. Effect of pH on the floatability of single minerals in the presence of *Pseudomonas songnenensis*.
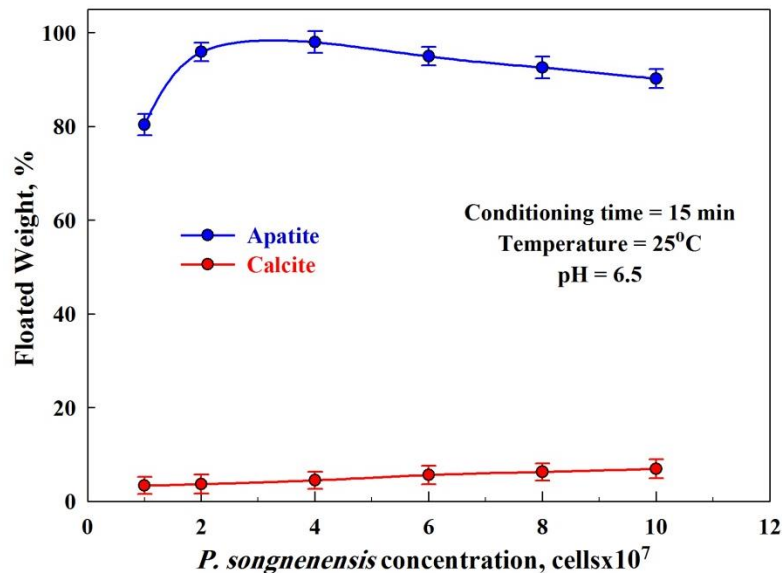

Figure 10. Effect of *Pseudomonas songnenensis* concentration on the floatability of single minerals.

3.2.2. Bio-flotation of a binary mixture.

The flotation study of binary mixture aims to determine the effect of interaction between apatite and calcite minerals, which may be occurred during their conditioning together

rather than individual conditioning. A synthetic binary mixture contains 25% P_2O_5 and 20% $CaCO_3$ ($\approx$ 8.8% ignition loss) is employed for this investigation.

The P_2O_5 and ignition loss contents were determined in the floated fraction as a function of *Pseudomonas songnenensis* concentration at 25°C and pH 6.5. Fig.11 shows that the P_2O_5 content is increased from 26.8 to 32.7% with increasing the bacteria concentration up to 4×10^7 cells/ml then it is decreased again to 30.1%. The ignition loss is slightly increased from 2.3 to 4.7%, increasing the bacteria concentration up to 1×10^8 cells/ml. The higher grade of 32.7% P_2O_5 and 3% ignition loss is produced in the presence of 4×10^7 cells/ml *Pseudomonas songnenensis*.
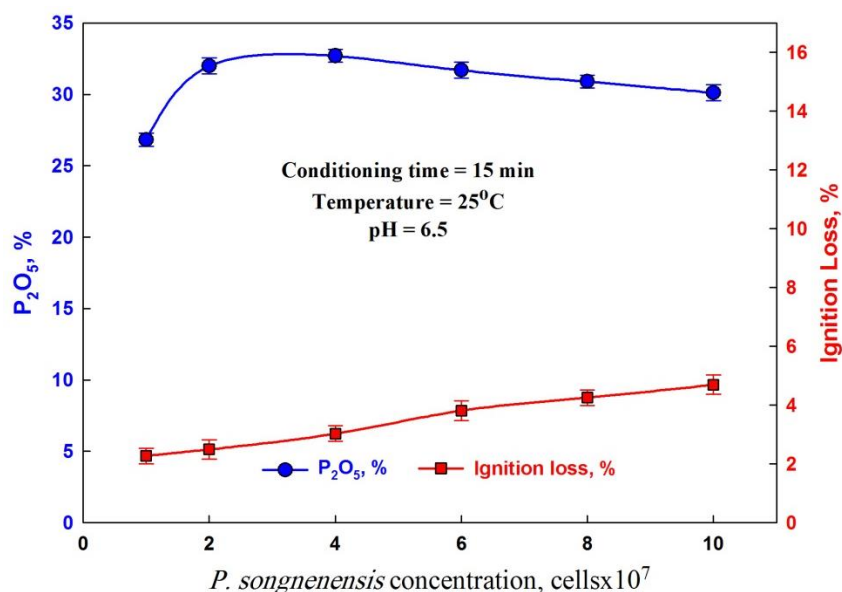

Figure 11. Effect of *P. songnenensis* concentration on the P_2O_5 and $CaCO_3$ content in the binary mixture.

3.2.3. Bio-flotation of natural phosphate ore.

The flotation of the natural phosphate ore aims to determine the effect of liberation between apatite and calcite minerals compared with single minerals. The natural ore contains 21.2% P_2O_5 and 25.6% $CaCO_3$ ($\approx$ 11.3% ignition loss) is employed for this investigation. Fig.12 shows that the P_2O_5 content is increased from 25.8 to 31.5% with increasing the bacteria concentration up to 4×10^7 cells/ml then it is decreased again to 29%. The ignition loss is slightly increased from 3.7 to 6% by increasing the bacteria concentration up to 1×10^8 cells/ml.
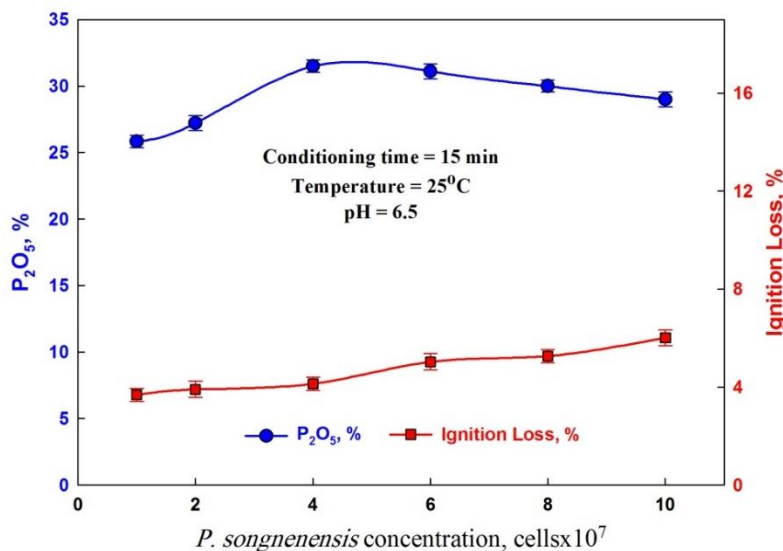

Figure 12. Effect of *P. songnenensis* concentration on the P_2O_5 and $CaCO_3$ content in the natural ore.

The higher grade of 31.5% P_2O_5 and 4.1% ignition loss are produced in the presence of 4×10^7 cells/ml *Pseudomonas songnenensis*. The slightly decreasing of the ore product may be related to the incomplete liberation between the phosphate and calcite minerals in the natural ore. The conventional flotation using oleic acid could increase the P_2O_5 content to 29.5% P_2O_5 [41]. So, these results recommend biotechnology as an alternative technique to upgrade Nile Valley phosphate ore, besides its advantages as low cost and eco-friendly process.

4. Conclusions

Pseudomonas songnenensis could be used as a surface modifier to enhance the apatite mineral flotation in phosphate ore. The apatite surface is strongly affected by bacterial interaction, and its IEP increased while there is no significant change of the calcite mineral.

FTIR spectra proved that the selective adsorption of *P. songnenensis* on apatite through chemical adsorption while there is no interaction with the calcite surface. The adhesion of *P. songnenensis* on the apatite is pH-dependent, and the adsorption stability is occurring at a pH 6.5. It leads to a degree of aggregation of apatite particles by increasing the surface hydrophobicity.

The maximum floatability of apatite mineral is achieved at pH 6.5, while calcite's floatability is poor. A binary mixture's flotation contains 25% P_2O_5, and 20% $CaCO_3$ yielded a concentrate contains 32.7% P_2O_5 and 6.8% $CaCO_3$ (3% ignition loss) in the presence of 4×10^7 cells/ml at 25°C and pH 6.5. The natural phosphate ore flotation contains 21.2% P_2O_5, and 25.6% $CaCO_3$ yielded a concentrate contains 31.5% P_2O_5 and 9.1% $CaCO_3$ (4.1% ignition loss).

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Conflicts of Interest

The authors declare no conflict of interest.

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