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Bioprocessing of natural phosphate ore with Staphylococcus aureus bacteria

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Original scientific paper

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Abstract

Phosphate ores are in high demand around the world because they are the primary raw materials used in the manufacturing of phosphatic fertilizers and other chemicals. Since the grade of the ore is gradually declining, it is becoming economically viable to mine and beneficiate numerous lower-grade deposits, and a significant number of precious minerals are discarded due to the inadequacy of new technological advances. Thus, biological processes are becoming more appealing in mineral processing due to their lower operating costs and potential applications to beneficiate low-grade complex ores through the interaction of bacteria and mineral surfaces, resulting in surface modification and mineral separation via bio-flotation. Staphylococcus aureus was supplied by the mineral bioprocessing lab, CMRDI. Bacterial adhesion measurements revealed a higher affinity for apatite than quartz. A binary mixture containing 12.5% P_2O_5 and 42.5% SiO_2 yielded a concentrate containing 20.15% P_2O_5 and 33.5% SiO_2. Using the bio-flotation process with Staphylococcus aureus and the optimal conditions on natural phosphate ore, a concentrate with 30.25% P_2O_5 was obtained from a feed containing 21.89% P_2O_5.

Keywords:

Staphylococcus aureus; phosphate; bio-flotation; adsorption; mineral, apatite; quartz

1. Introduction

In 2011, approximately 191 Mt of phosphate was produced. The global phosphate production is expected to be increased to 228 Mt in 2025. The majority of phosphate rock is consumed in the production of fertilizers and animal feed supplements (95%). The remainder of the output is used to produce elemental phosphorus (**Vlahović et al., 2005**). In recent years, the mining industry has faced several challenges, including the depletion of high-grade ores and environmental regulations. The mining industry is compelled to process low-grade ores, fine mineral particles, and flotation tailings to produce material suitable for the global market (**Jasinski, 2011; Abdel-Khalek et al., 2014**). Phosphate deposits form ore bodies in a single thick bed or several successive beds intercalated with non-phosphatic material (**Collinson and Thompson, 1989**). Phosphate ores differ greatly in composition from gangue materials (**Abouzeid and El-Jallad, 1980**). Because of the rising global phosphate demand, the P_2O_5 content of the ore is gradually decreasing, and it is becoming economically viable to mine and beneficiate many low-grade deposits for the production of phosphate fertilizers in addition to phosphate fines (**Ibrahim et al., 2010**).

There are a lot of instances in processing systems when the ore has to be discarded since the conventional approach is insufficient in the sub-sieve range (**Subrahmanyam and Forssberg, 1990; Rao, 2004; Miettinen et al., 2010; Farrokhpay et al., 2021**). Since today's technology is insufficient to process these large amounts of valuable minerals economically, they are discarded as fines and ultra-fines. The treatment of fine particles is a difficult problem in the chemical industry and raw material processing that must be solved (**Corin et al., 2021**).

Biological processes have recently gained popularity in mineral processing due to their lower operating costs and potential applications in treating difficult-to-beneficiate low-grade complex ores (**Larsen and Chilingar, 1979**). By definition, bio-beneficiation is the selective removal of undesirable mineral constituents from ore by using bacteria as surface modifiers to enhance the separation of one mineral from another via flotation or flocculation, thus enriching it with the desired valuable minerals (**Chipakwe et al., 2022**). The use of biological processes involving microbes in mineral processing has been reported by several researchers (**Somasundaran et al., 1998; Donati and Sand, 2007; Ikumapayi, 2010;**

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Mishra et al., 2022). Biological processes are enticing because microbes, microbial fat, and secreted metabolites can have specific interactions with minerals. Such interactions of microbes and their agents with minerals can be indirect, with biological products acting as surface-active agents, or direct, with microbial adhesion or attachment to particles causing surface modification. Both types of interactions can alter mineral hydrophobicity and, in some cases, cause flocculation or dispersion of a mineral suspension (**Altschul et al., 1997; Zhang et al., 1997**).

In this study, a bio-beneficiation of the phosphate rock processing technique will be investigated for the treatment and upgrading of low-grade and fine phosphate ores to increase their grade to about 29-30% P_2O_5 to be suitable for phosphoric acid production and fertilizers.

2. Materials and Methods

Samples of single minerals of quartz (SiO_2) and apatite $Ca_5(PO_4)_3(OH, F, Cl)$ were delivered from 'Wards' Company, USA. The purity (99.9 %) of the samples was confirmed using XRF. The –200 mesh fractions were used in adsorption and flotation studies. Analytical grade HCL and NaOH, from Aldrich, were used for pH regulations. Natural phosphate ore was collected from New Valley, Egypt. S. aureus was supplied by the mineral bio-processing lab, CMRDI. The methods include sample collection and characterization, measuring the selectivity of S. aureus to mineral surface, the measurements of Zeta potential, adsorption measurements and the analysis of Fourier Transform Infrared Spectrometer (FTIR).

2.1. Sample collection and characterization

Apatite samples were recovered from the Nile Valley using a 'Denver' Jones riffle and stored in sealed bags weighing 5 kg. One of these batches was ground using rod mills to achieve the liberation size required for the bio-beneficiation process. The ore sample was subjected to routine chemical analysis using standard methods. Phosphorus content was determined using the spectroscopic technique. Quartz was determined by the gravimetric method.

2.2. Measuring the selectivity of S. aureus to mineral surface

To measure the size of the mineral after bacterial treatment, a fixed volume of 10 ml containing 106 cfu/ml bacteria was conditioned with 1 g of each mineral for one hour before recording the change in size distribution (**Cao et al., 2011**).

2.3. Zeta potential measurements

For zeta potential measurements, a 'Malvern Instruments Model Zeta Sizer 2000' laser Zeta meter was used. 0.05 g of ground sample was placed in 50 ml of double distilled water containing a known concentration of bacteria (106 cfu/ml) at a constant ionic strength of 2 X 10-2 M NaCl. pH was adjusted using NaOH and HCL. The pH of the suspension was adjusted after conditioning for 60 minutes. The equilibrium pH was measured after shaking. It was then allowed to settle for 3 minutes before transferring 10 ml of the supernatant into a standard cuvette for zeta potential measurement. The temperature of the solution was kept constant at 25°C ±2°C. The average of five measurements was reported as the measured zeta potential (**Natarajan and Das, 2003**).

2.4. Measurements of adsorption

By adding 1 g dry sample of quartz or apatite to the bacterial solutions (50 ml containing 106 cfu/ml) in a 100 ml volumetric flask, the adsorption of bacterial isolate on the mineral surface was determined. An orbit shaker was used to shake the mixture for 15 minutes (Model JANKE & KUNKEL Type VX10). HCl and NaOH were used to adjust the pH to the desired levels. The adsorption of the bacterial isolate to the mineral surface was measured using a spectrophotometer (**Shaw, 1992**).

2.5. FTIR analysis

Using a Fourier Transform Infrared Spectrometer (Model FT/IR 6300), apatite, quartz and bacteria absorption spectra were recorded before and after interactions. Following bacteria interaction, the mineral samples were thoroughly washed with double distilled water and then vacuum dried. The spectra were recorded using the KBr pellet technique **(Abdel-Khalek et al., 2014; Elbendary et al., 2019; Yehia et al., 2019; Hellal et al., 2019; Kaba et al., 2021; Abdallah et al., 2021; Pavón et al., 2022**).

2.6. Bio flotation experiments

A glass flotation column has been employed in a variety of bench-scale flotation tests. S. aureus has been applied as a surface modifier and/or the sole flotation agent. Starches have been used to prepare quartz and apatite ore samples at specific concentrations and pH levels on a mechanical shaker for a specific treatment period before being delivered to a glass flotation column. A 30 cm^3/min airflow adjustment was made. During the flotation experiment, a magnetic stirrer has been adopted to keep the particulates from accumulating.

3. Results and discussion

The findings are presented and discussed in the following sections (from 3.1. to 3.6.).

3.1. Sample collection and evaluation

The XRD analysis of natural phosphate rock revealed that it is primarily composed of francolite. The main associated gangue minerals are quartz and calcite, as shown in **Figure 1**.

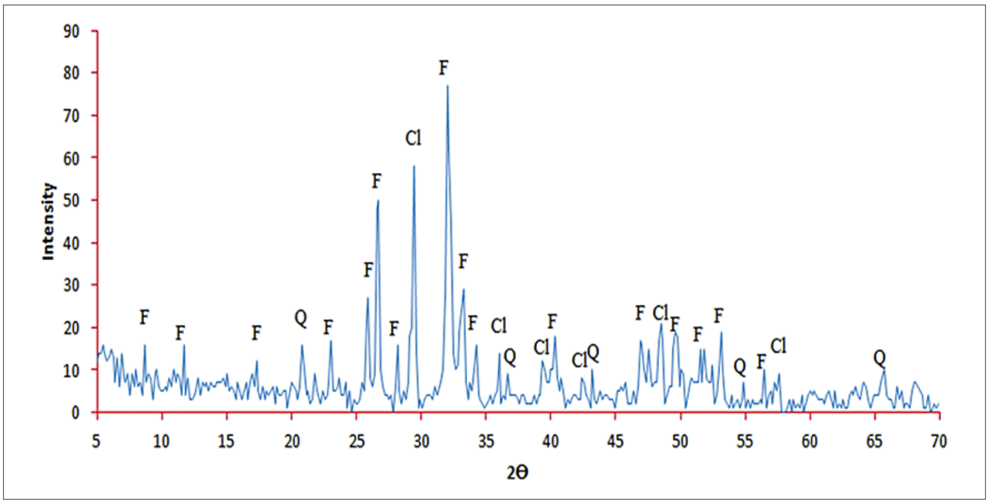

Figure 1: XRD of natural phosphate rock

The chemical analysis of the natural ore sample indicated that it contained about 21.27% P_2O_5, 48.62% CaO and a lower amount of quartz, i.e. 11.81% SiO_2. It had also about 2.78% Fe_2O_3 and 0.99% Al_2O_3. The sample contained a trace amount of MgO, Na_2O, Cr_2O_3, ZnO, BaO and PbO.

3.2. Measuring the selectivity of S. aureus to mineral surface

The particle size distribution of single mineral samples, apatite and quartz, after treatment with S. aureus, was used to indicate bacterial selectivity. The successful adsorption of the bacterial isolate caused mineral particles to aggregate or disperse, resulting in a change in their size distribution. The results in **Figure 2** and **Figure 3** show the changes in the particle size distribution of samples after bacteria treatment. S. aureus caused some apatite surface aggregation, as d50 changes from 10.9 µm to 20.9 µm and d90 changes from 55.7 µm to 87.1 m. **Table 1** also shows that there is no significant change in either d50 or d90 for quartz minerals after interaction with bacterial strain, it is evident from the val-

Table 1. Changes in the mean particle size of single minerals following bacterial isolate treatment

Staphylococcus aureus	Apatite		Quartz	
	Before Treatment µm	After Treatment µm	Before Treatment µm	After Treatment µm
(d90)	55.7	87.1	30	35.7
(d50)	10.9	20.9	9.5	9.75

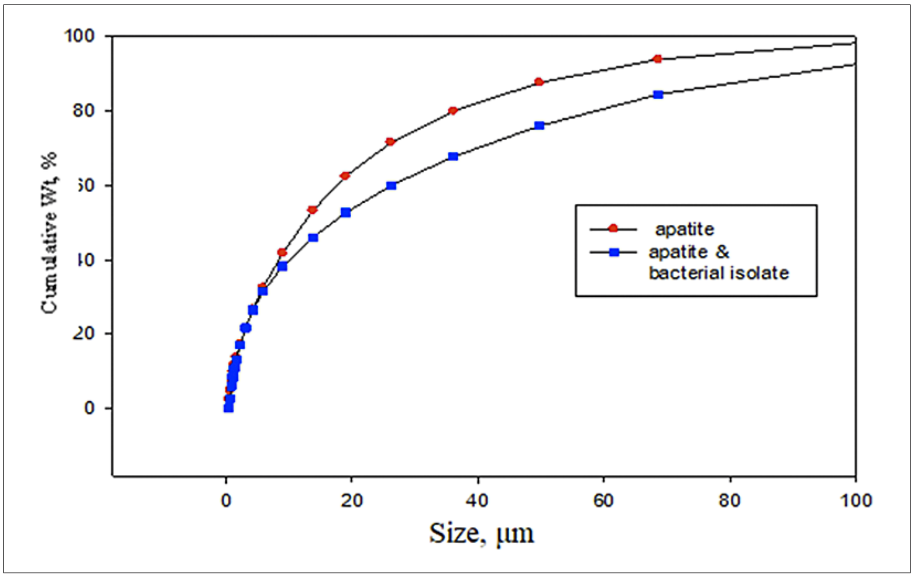

Figure 2: Apatite particle size distribution in association with S. aureus

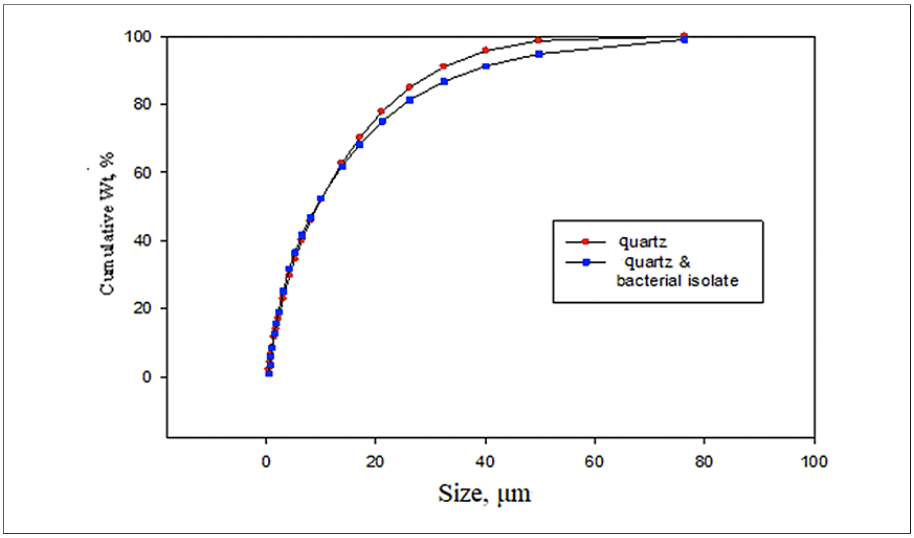

Figure 3: Quartz particle size distribution in the presence of S. aureus

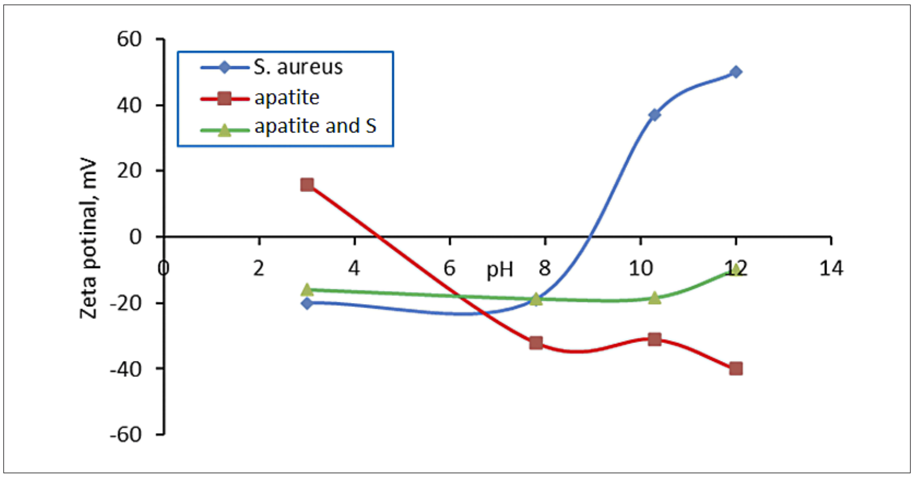

Figure 4: Zeta potential of S. aureus, apatite and apatite treated with S. aureus

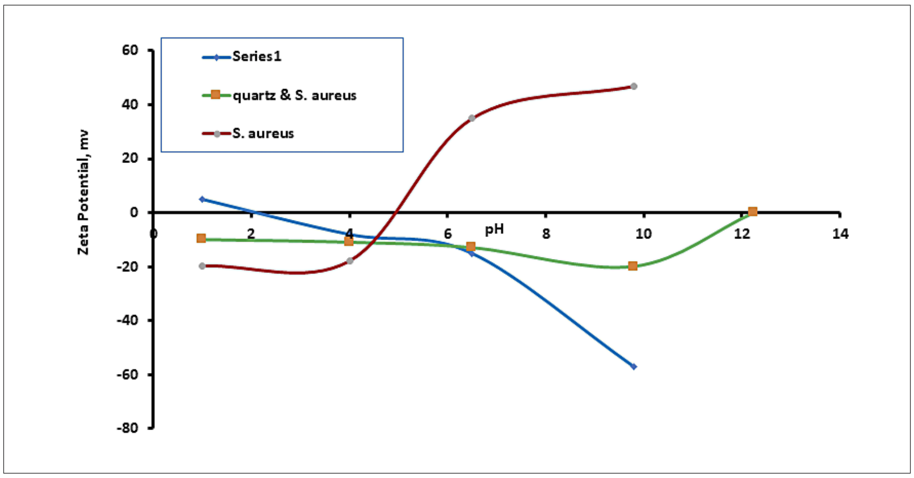

Figure 5: Zeta potential of S. aureus, quartz and quartz treated with S. aureus

ues in **Table 1** that bacteria have no discernible impact on quartz mineral. In this study, S. aureus can be used as a surface modifier and/or sole flotation agent

3.3. Apatite, quartz, and S. aureus surface properties

Figure 4 and **Figure 5** show the zeta potential of S. aureus alone and in combination with single minerals of apatite and quartz. **Figure 4** depicts the zeta potential of S. aureus, with zeta potential values ranging from -20 to +50 mV over the entire pH range (3 to 12). The bacteria's iso-electric point (IEP) is at pH 8.7. It can be shown that S. aureus has a hydrophobic effect on zeta potential values in the pH range of 6.5 to 12. When quartz and apatite are exposed to bacteria, as seen in **Figures 4** and **5**, their negative zeta potentials become less negative.

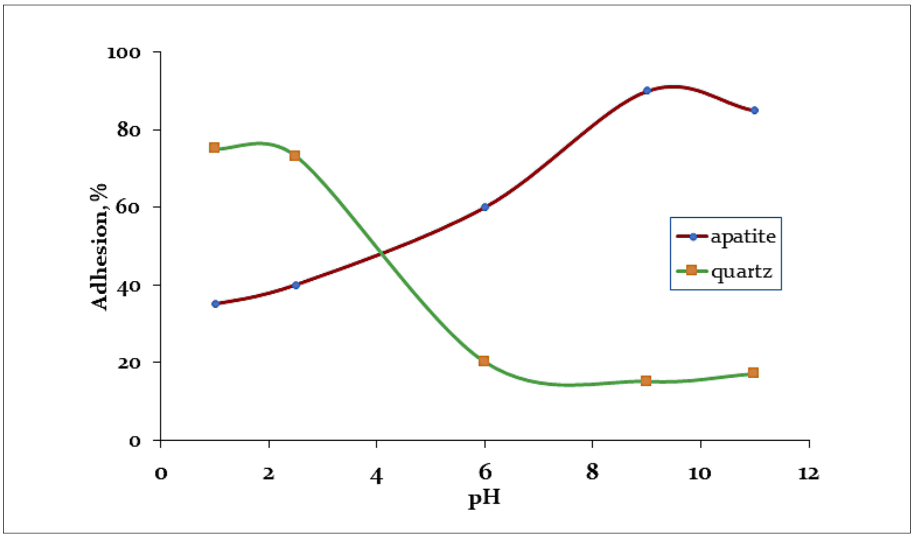

Figure 6: The influence of conditioning time on staphylococcus aureus adhesion to single minerals

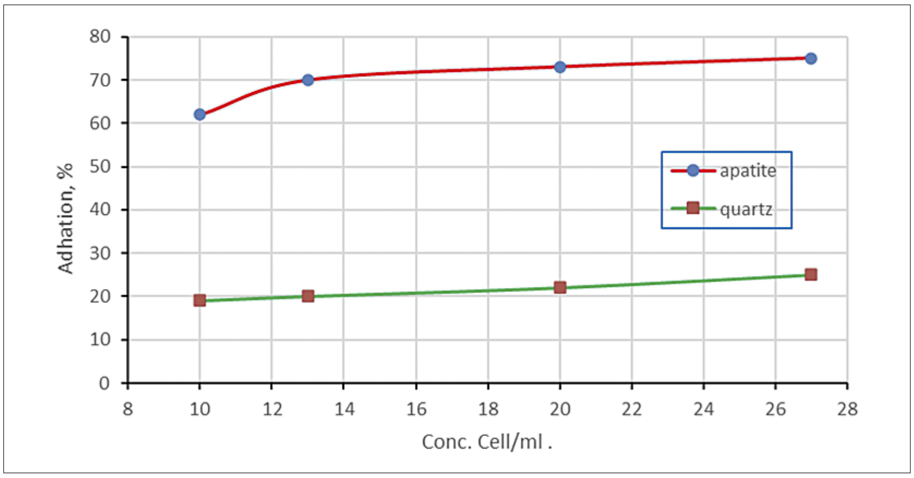

Figure 7: The effect of bacterial concentration on the adhesion of Staphylococcus aureus to single minerals

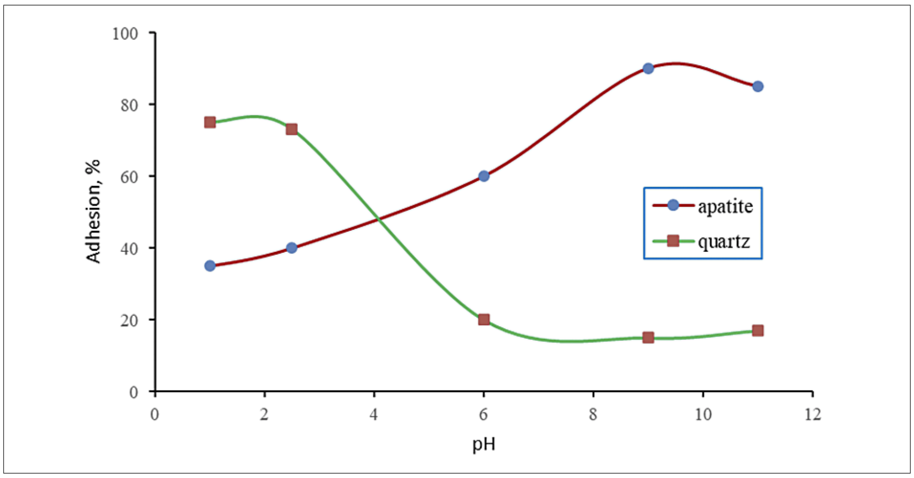

Figure 8: The effect of pH on the adhesion of Staphylococcus aureus to single minerals

After interacting with S. aureus bacteria, the IEP for both apatite and quartz vanished.

3.4. Bacterial adhesion testing – a single mineral

The results showed that the adhesion of S. aureus to the apatite surface increases with conditioning time, pH and bacterial concentration. It is evident from **Figures 6**, **7**, and **8** that bacteria adhere to quartz to a lesser extent than they do to apatite, and the order was derived from these results.

Quartz < Apatite

It is obvious that apatite has a higher bacterial affinity than quartz as shown in **Figures 6** and **7**. Electrostatic forces are responsible for the increased adsorption tendency of the bacterial strain onto apatite at pH 6. As shown in **Figure 8**, as the pH level increases from 2 to 6, quartz's adhesion percentage drops from 75% to cca 20%. In contrast, apatite's adhesion percentage rises from 40% to 80% as the pH level increases from cca 2.5 to 8.0. Hydrogen bonding and chemical interaction, in addition to electrostatic forces, play important roles in bacterial interaction with single minerals.

3.5. FTIR spectrum of bacterial isolate Staphylococcus aureus, apatite and quartz

Figure 9 depicts the FTIR spectrum of the bacterial isolate S. aureus and the apatite mineral with which it interacted. The results showed that the main characteristic peaks for Staphylococcus aureus and apatite (red curve) are between 500 and 800 cm^{-1}, which belong to the alkene C-H, and at 1000-1200 cm^{-1} and 1150-1400 cm^{-1} corresponding to the C=O bond of alkoxy, acyl and phenyl groups. The sharp band at 1639 cm^{-1} is a C=O saturated carbonyl group derived from carboxylic acid and amide. Due to the presence of C-H, there are weak peaks from 2500-3500 cm^{-1}. The presence of an aldehyde's H-C=O group was confirmed at 2800-2900 cm^{-1}, with broadband at 3000-3400 cm^{-1} due to the presence of an NH stretching vibration and OH of both alcohol and carboxylic acid (**Tamura et al., 2013**). Because of the interaction between C-H and the mineral surface, new bands appeared at 572 and 600 cm^{-1}.

Because of the interaction with the C-O of the acyl group of bacteria, a new band at 1431 cm^{-1} was formed. The hydrogen bond formed with saturated C=O of amides and N-H of amides causes a decrease in the intensity of the band at 1641 cm^{-1}. The broadness of the new peak at 2400 cm^{-1} is due to the hydrogen bond formed with the O-H group of carboxylic acid. The new band at 3735 cm^{-1} confirms the formation of a hydrogen bond after S. aureus treatment with O-H carboxylic acid and N-H amide (**Selim, 2006; Selim et al., 2020**).

Figure 10 depicts the characteristic bands of quartz before and after interaction with a bacterial isolate of S. aureus. At 2326 cm^{-1}, a new band is formed that is related to the O-H of carboxylic acid secreted by bacteria. At the same time, bands at 1080 cm^{-1} and 784 cm^{-1} were observed as a result of interactions with the C=O stretch of the ether group and the C=C stretch of the phenyl group, respectively. The decrease in the intensity of the peak at 1639 cm^{-1} is due to the amino group's partial interaction with N-H as a result of the formation of silanol groups on the quartz surface. There was also a partial interaction with the alcoholic (O-H) group, which resulted in a decrease in band broadness at 3200-3400 cm^{-1}.

3.6. Bio-flotation of binary mixture

At natural pH 6-7, 10 minutes and 10 × 10^7 cell / ml of bacterial strain, a concentrate containing 20.15% P_2O_5 and 33.5% SiO_2 was obtained from a feed containing 12% P_2O_5 and 42% SiO_2, with a recovery of approximately 94%. When the same optimum conditions were applied to a natural phosphate ore, a concentrate containing 30.25% P_2O_5 was obtained from a feed containing 21.89% P_2O_5.

Due to their lower operating expenses and potential uses for treating complex low-grade ores that are challenging to beneficiate, biological techniques are drawing increased attention in the field of mineral processing. Therefore, the employment of microorganisms as bioregents, or surface modifiers has seen a significant increase in research. This investigation shows that the bioflotation studies have made it quite evident that this particular type of bacteria (Staphylococcus aureus) is capable of improving raw phosphate ore of New Valley, Egypt, which contains a significant amount of silica (SiO_2) for use.

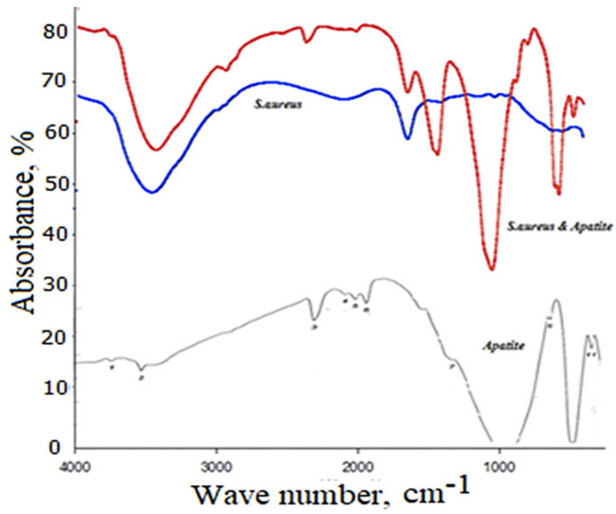

Figure 9: FTIR spectrum of apatite, S. aureus, and apatite treated with S. aureus

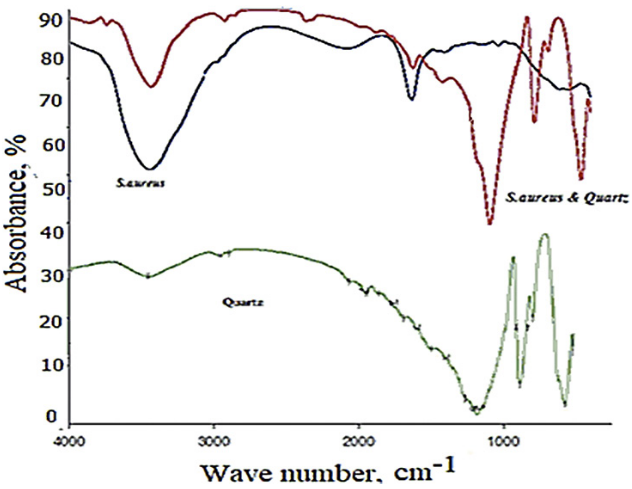

Figure 10: FTIR spectrum of quartz, S. aureus and quartz with S. aureus

4. Conclusions

Bio beneficiation is a new processing technique, one of the most important recent technologies where microorganisms can replace conventional flotation reagents and/or act as a single modifier to enhance the selectivity of separation. Biological processes are becoming more attractive in mineral processing due to their lower operating costs and their possible applications to beneficiate low-grade complex ores. In this study, a new bio-beneficiation of phosphate rock processing technique was investigated for the treatment and upgrading of low-grade and fine phosphate ores to increase their grade to about 29-30% P_2O_5 to be suitable for phosphoric acid production and fertilizers. The main conclusions are summarized below:

1. The findings revealed a strong interaction between S. aureus bacteria and mineral surfaces, particularly apatite.
2. S. aureus had a higher affinity for the apatite mineral surface based on adhesion, adsorption, and zeta potential measurements.
3. In flotation tests, apatite flotation was found to be selective against quartz. The results demonstrate the feasibility of using S. aureus bacteria as the sole flotation reagent, with a concentrate containing 20.15% P_2O_5 obtained from a mixture containing 12%.
4. When the same conditions (pH 6-7, 10 minutes, and 10×10^7 cell/ ml of bacterial strain) were applied to the flotation of natural phosphate ore, the concentrate contained 30.25% P_2O_5.

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SAŽETAK

Bioprocesiranje prirodne fosfatne rude bakterijom *Staphylococcus aureus*

Diljem svijeta velika je potražnja za fosfatnom rudom s obzirom na to da je riječ o primarnoj sirovini koja se koristi u proizvodnji fosfatnih gnojiva i drugih kemikalija. Budući da se koncentracija rude postupno smanjuje, ekonomski je sve isplativije eksploatirati i oplemenjivati brojna ležišta niže koncentracije s obzirom na to da se znatne količine korisne mineralne sirovine nedovoljno iskorištavaju zbog neadekvatnosti novih tehnologija. Stoga biološki procesi postaju sve zanimljiviji u oplemenjivanju SiO_2 mineralnih sirovina zbog nižih operativnih troškova i potencijalne primjene za obogaćivanje kompleksnih ruda niskoga stupnja koncentracije, i to interakcijom bakterija i površine minerala, što rezultira površinskom modifikacijom i odvajanjem minerala putem bioflotacije. Bakteriju *Staphylococcus aureus* isporučio je laboratorij za bioprocesiranje minerala, CMRDI. Mjerenja bakterijske adhezije pokazala su veći afinitet za apatit nego za kvarc. Binarna smjesa koja je sadržavala 12,5 % P_2O_5 i 42,5 % SiO_2 dala je koncentrat koji je sadržavao 20,15 % P_2O_5 i 33,5 % SiO_2. Postupkom bioflotacije bakterijom *Staphylococcus aureus* pri optimalnim uvjetima na prirodnoj fosfatnoj rudi dobiven je koncentrat s 30,25 % P_2O_5 iz sirovine koja je sadržavala 21,89 % P_2O_5.

Ključne riječi:
Staphylococcus aureus, fosfat, bioflotacija, adsorpcija, mineral, apatit, kvarc

Author's contribution

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