Recycling and Sustainable Development

www.rsd.tfbor.bg.ac.rs

Online ISSN 2560-3132
Print ISSN 1820-7480

Selective Bio-Flocculation of Hematite Mineral Using *Bacillus subtilis* for Phosphorous Removal from Iron Ores

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ARTICLE INFO

Received 15 September 2025
Accepted 15 November 2025

Scientific article

Keywords:
Bacillus Subtilis
Phosphorous
Hematite
Apatite
Bio-flocculation
Minerals

Extended and revised version of a conference article - presented on XVI International Mineral Processing and Recycling Conference (IMPRC 2025)

ABSTRACT

Bacillus subtilis is used as a bio-surface modifier to flocculate hematite mineral (Fe_2O_3). The effect of bacterial interaction on the behavior of hematite and apatite minerals, as the main source of phosphorous in iron ore, was investigated using zeta-potential, SEM, and FTIR measurements. The effects of pH, contact time, and *Bacillus subtilis* concentration on the flocculation of the two minerals were studied. The zeta potential of hematite is strongly affected by interaction with *Bacillus subtilis*, and maximum flocculation efficiency was achieved at pH 6. Hematite could be separated from its mixture with apatite in the presence of 8 - 12×10^5 cells of *Bacillus subtilis* at pH 6 and 35 °C for a 10-minute contact time.

1. Introduction

Iron ores have become more complex in their mineralogical composition, with phosphorus occurring more widely. Phosphorus is harmful in the steel-making process, as it increases hardness and brittleness and decreases ductility. Phosphorus may occur as apatite [$Ca_5(PO_4)(F,Cl,OH)$], while wavellite [$Al_3(PO_4)_2(OH)_3.5(H_2O)$] is the second phosphate present in high-phosphorus iron deposits (Ofoegbu, 2019). Phosphorus can be removed from iron ores by different processes depending on the mineralogy and cost. It is important to conduct fundamental studies to determine the most effective separation process. Physical and biological processes are the most promising techniques, as they require low amounts of reagents, low power, minimal facilities and equipment, and produce less pollution (Buis, 1995; Bao et al., 2021; Wu et al., 2021; Abdallah et al., 2025). Acid leaching has been used to treat the fine-particles using acid or basic media. Although, alkaline leaching is ineffective for widespread iron matrix, it is effective for apatite and silicates. Acid leaching (sulfuric acid) is the most effective for this purpose. Nitric acid was used due to its low reactivity with the magnetite but it has disadvantage as the formation toxic gases (Wen-tang et al., 2011). Bio-leaching was used through acid-producing microorganisms, including filamentous fungi and iron-oxidizing bacteria (Buis, 1995). Selective agglomeration was used in the presence of oleic acid as a collector (Xu

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doi: 10.5937/ror2501067A

et al., 2012; Zhu et al., 2013; Abdallah et al., 2025). A fatty acid as collector and sodium silicate as a depressant was used in anionic flotation for apatite separation from iron oxides. Also, dodecyl amine was used for wavellite flotation (Nunes et al., 2012; Abdallah et al., 2025). Thermal processes such as sintering and reductive calcination were used for phosphorus removal (Bao et al., 2021).

Nowadays, biological processing routes are sought to solve the problems associated with lean grade ores and where the traditional methods fail to separate the minerals from complex ores. The Bio-beneficiation refers to selective removal of undesirable mineral constituents from an ore by utilizing microorganisms as surface modifiers, depressants, collectors, or dispersing agents to enhance the separation of one mineral from another (Geoghegan et al., 2008; Abdel-Khalek et al., 2009; Kuyumcu et al., 2009; Hori and Matsumoto, 2010; Rao et al., 2010; El-Sayed et al., 2021). The behavior of bacterial cell on the mineral surface is the basis for a successful of the bio-beneficiation processes. The selective bacterial adhesion on mineral surface is important for selective surface modification which leads to the efficient flotation or flocculation processes (Abdel-Khalek and El-Maidany, 2013; Elmahdy et al., 2013; Abdallah et al., 2025).

Bio-beneficiation processes are relatively new and are under intense investigation in recent years (Abdallah et al., 2021; El-Sayed et al., 2021; Farghaly et al., 2021). Since the bacteria adhere to a mineral surface within a few minutes and alter the surface properties that are essential in mineral beneficiation techniques, the microorganisms have numerous applications in flotation and flocculation processes (Abdel-Khalek and Elmaidany, 2013a; Abdel-Khalek and Elmaidany, 2013b; Elmahdy et al., 2013a; El-Sayed et al., 2021; Abdallah et al., 2025).

2. Experimental

2.1. Materials

Two samples of hematite and apatite minerals of high purity were supplied by the Egyptian Mineral Resources Authority (EMRA). Each sample was ground to less than 0.105 mm. *B. subtilis* strain was supplied by the mineral bio-processing Lab., CMRDI. All chemicals used in this work were of analytical grade. NaOH and HCl of Sigma-Aldrich (USA) were used for preparation of 0.1 M solutions for pH regulation. Nutrient broth/ agar from Difco (France) was used for cultivation of bacterial strains.

2.2. Methods

2.2.1. Bacterial cultivation

For preparation of bacterial inoculum, *B. Subtilis* was picked up from a stock dish and cultivated in nutrient broth medium consisting of 5 g/L peptone, 3 g/L beef extract, and 5 g/L NaCl at pH 7 and incubated at 30 °C on a rotary shaker at 180 rpm/min. Bacterial cells were sub-cultured monthly by inoculating 10 ml of pure strain in 90 ml nutrient broth medium in 250 ml flask. The bacterial population was estimated by colony counting after plating (Tsezos, 2007).

2.3. Measurements

2.3.1. Minerals

The minerals phases were identified by X-ray diffraction (XRD) using "Philips type 1710 XRD unit" with Ni filter Cu radiation (Kα = 1.5446 Å) at 40 kV and 20 mA at scanning speed of one theta degree per min. The chemical composition of each mineral was determined using "Rigakusuper Mini 200" X-ray fluorescence. The sample was mixed with Boreox BM-0008 binder and the mixture was subjected to 15-ton press using "Fluxana PR-25N" for 20 s to obtain a pellet.

2.3.2. Zeta potential measurements

A laser Zeta Meter [Malvern Instruments], Model Zeta Sizer NAno ZS' was employed for zeta potential measurements. A 0.05 g of solid sample is placed in 50 ml of 0.01 M KCl solution and interacted with known bacterial concentrations, conditioned for 10 min at desired pH at room temperature. Measurements were performed as a function of pH.

2.3.3. FTIR measurements

The solid mineral sample after interaction with bacterial cells was filtered, air dried, and mixed analytical grade KBr from Merck to prepare KBr pellet then subjected to FTIR for recording the spectrum. The FTIR spectrum was obtained with a Spectrum 2,000 Perkin Elmer spectrometer was obtained between 4,000 and 400 cm^{-1}.

2.3.4. SEM analysis

The morphology and structural formation was determined using scanning electron microscope (SEM) model JEOLJSM-5400, Japan.

2.3.5. Flocculation Experiments

A bench-scale flocculation experiment was carried out mixing the mineral with bacteria solution followed by decantation of the un-flocculated mineral. One gram of hematite or apatite minerals was conditioned in the bacteria solution of definite concentration for desired time at constant pH. The flocculation was carried out by decantation. Each flocculated and dispersed fractions were collected, dried, and weighted. For binary minerals

mixture, each flocculated and dispersed fractions were collected, dried, weighted and chemically analyzed.

3. Results and discussion

3.1. Chemical Composition and XRD of Pure Minerals

The chemical analysis of apatite mineral which has a chemical formula $[Ca_{10}(PO_4CO_3)_6(OH,F,Cl)]_2$] confirmed its high purity. Table 1, showed that it composed of 56.97 % CaO, 34.58 % P_2O_5, 4.49 % CO_2 and 3.89 % fluoride with total impurities of about 0.055% such as Fe_2O_3, MgO, SiO_2, Al_2O_3, K_2O, and Na_2O. It indicated that its purity is more than 99.9 %. On the other hand, the hematite mineral contains 99.87 % Fe_2O_3. These results were confirmed by the X-ray diffraction investigation. The pattern of each mineral showed only its characteristic peaks without significant peaks of impurities, Figure 1, confirmed the higher purity of both minerals (Soejoko and Tjia, 2003).

Table 1
Chemical composition of pure hematite and phosphate minerals (Abdallah et al., 2025)

Item	Weight %	
	Hematite	Apatite
Fe_2O_3	99.87	0.001
P_2O_5	0.001	34.58
CaO	0.005	56.97
MgO	0.007	0.005
SiO_2	0.018	0.003
Al_2O_3	0.015	0.016
K_2O	0.013	0.012
Na_2O	0.016	0.018
CO_2	0.054	4.498
F	0.001	3.897
Total	100,0	100,0

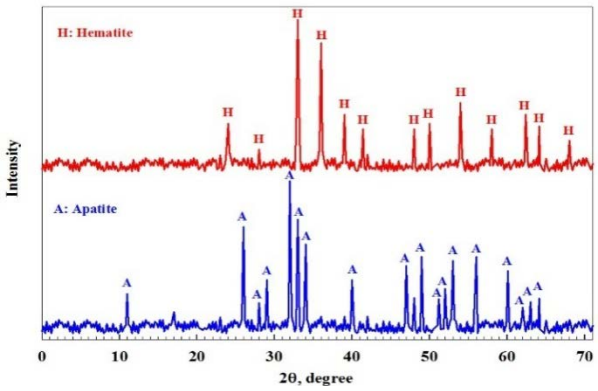

Figure 1. X-Ray diffraction patterns of pure hematite and apatite minerals

3.2. Mineral Bacteria Interaction

3.2.1. Zeta Potential Measurement

The presence of various ions alters the zeta potential of both bacteria and minerals. Hydrophobicity originated from the hydrogen bonding energy of cohesion of water molecules (van Oss, 1992). Similarly altering the surface hydrophobicity can bring about a difference in adsorption. Thus by changing one or more of the properties of the interacting mineral surfaces, adsorption can be made more selective (Shashikala and Raichur, 2002; Abdallah et al., 2025).

Zeta-potential of bacterial isolate alone were performed. Bacterial cell charge originates from dissociation or protonation of carboxyl and amino groups and consequently depends on pH. At higher pH it becomes progressively negatively charged due to proton dissociation (Rong et al., 2008; El-Sayed et al., 2021; Laible et al., 2021). Thus, the zeta potential and isoelectric point were measured carefully in standardized conditions to ensure comparability. Figure 2, shows that Bacillus subtilis is positively charged at less than pH 3.25 (its isoelectric point).

The hematite mineral has positive charge at pH less than 5.3 (its isoelectric point), while it turned negative charge at the pH range higher 5.3. It indicates that both H^+ and OH^- are potential determining ions. Generally, the zeta potential and isoelectric point of minerals are strongly influenced by the adsorption of ionic species, as ionic surfactants and polyelectrolytes. The results showed that the negativity of zeta potential increases sharply with increasing the pH. The hematite surface is strongly affected as a result of bacterial interaction. The results showed that the negativity of zeta potential increases sharply up to pH 7 then it is slightly increased. The IEP of the treated hematite mineral was not determined (Natarajan and Deo, 2001; Abdallah et al., 2025).

A neutral macromolecule can be adsorbed on charged or uncharged mineral surfaces. If the surface is charged, then adsorption of a biomolecule can cause a redistribution of the counter ion charge. This would lead to shifts in zeta potentials. (El-Ghammaz et al., 2021) The adhesion of negative bacteria on to the negative mineral surfaces is based on the surface heterogeneity of the bacteria with a polysaccharide envelope. The adsorption includes also, hydroxyl, hydrophobic and ionic moieties. The flexible fimbriae regulate the surface charge. The dissolving ions from the mineral alter the surface charge of the bacteria to reduce repulsion. The hydrogen bonding and chemical interaction may also play significant roles in bacterial interaction with single minerals. It can occur by bonding between amino or carboxyl groups, peptide units or ether as well as other polar groups on biological and mineral surfaces. (El-Ghammaz et al., 2021) It causes redistribution of charges (Kosmulski, 2020; Farghaly et al., 2021).

On the other hand, the apatite mineral has positive charge at pH less than 4.75 (its isoelectric point), while it turned negative charge at the pH range higher 4.75. The apatite mineral surface is slightly affected as a result of

bacterial interaction up to pH 6. The isoelectric point (IEP) became 4.55 rather than 4.75 before treatment. Figure 3, showed that the negativity of zeta potential is significant increased at pH range higher than 6. (Abdallah et al., 2025) It has been reported that *Bacillus subtilis* could solubilize the phosphate at pH range of 6 - 12 led to release of organic acids by activity of phosphatase enzyme (Ahmad et al., 2018; Mohamed et al., 2018; Ahmad et al., 2021).

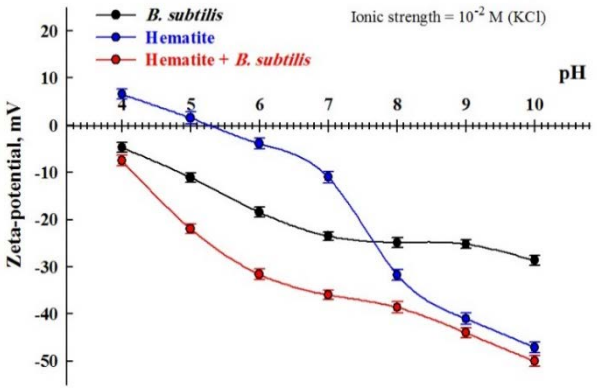

Figure 2. Effect of pH on zeta potential of treated hematite mineral with 8×10^5 cells/ml of *B. subtilis*

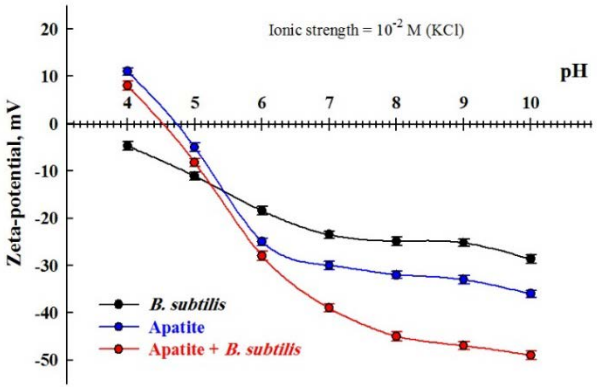

Figure 3. Effect of pH on zeta potential of treated phosphate mineral with 8×10^5 cells/ml of *B. subtilis*

3.2.2. FTIR Investigation

The FTIR of bacteria, usually includes the O-H, C-C, CH_2, C-O, C-N and C=O bands for polysaccharides and lipids (protein) (Hussein et al., 2020; El-Sayed et al., 2021). The main characteristic peaks for *Bacillus subtilis* are located at 1641 cm^{-1} which belongs to C=O of amide group and of O–C=O carboxylic groups. (El-Ghammaz et al., 2021) The broad band located in the range 2993 - 3592 cm^{-1} are attributed to stretch C–H, C–H_2 and C–H_3 of alkyl groups and stretching vibration of O–H and N–H, (Figure 4) (Wang et al., 2020; Abdallah et al., 2025).

Figure 4, shows the typical FTIR spectrum of iron oxide. It exhibits various well-defined peaks at 459; 541; 991; 1,485, 1,637; 2,843, 2,919 and 3,429 cm^{-1} (Hwang et al., 2014). The appearance of two well-defined peaks at 459, 541 and 991 cm^{-1} are due to the presence of iron–oxygen (Fe–O). Moreover, the peaks positioned at 1,485, 1,637; 2,843; 2,919 and 3,429 cm^{-1} are due to the bending vibration of absorbed water and surface hydroxyl and O–H stretching mode, respectively (Alangari et al., 2022). The observed FTIR results confirmed the iron oxide without any significant impurity.

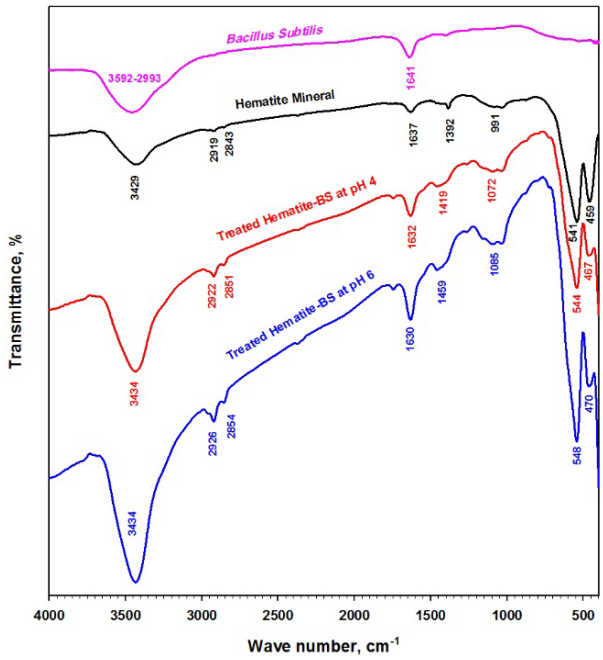

Figure 4. FTIR spectra of Hematite mineral before and after treatment with *B. subtilis* at different pHs

After interacting with *Bacillus subtilis* the band at 3,429 cm^{-1} became more intense and shifted 3,424 and 3,434 cm^{-1} for treatment at pH 4 and 6 respectively. Also, the peaks at 2,919; 2,843 and 1,637 became more intense and shifted to 2,922-2,926 cm^{-1}, 2,851-2,854 cm^{-1} and 1,632-1,631 cm^{-1}, for treatment at pH 4 and 6, respectively. The peaks at 1,392 and 991 cm^{-1} were disappeared and new bands were appeared at 1,419-1,459 cm^{-1} and 1,072-1,085 cm^{-1}, respectively. In addition, more intense and little shift was noted for the peaks from 541 to 544-548 cm^{-1} and from 459 to 467-470 cm^{-1}. These results suggest the higher adsorption affinity of *B. subtilis* on to hematite surface. It agrees with higher change in the surface charge which obtained by zeta-potential measurements. (Abdallah et al., 2025)

Figure 5, shows the FTIR spectrum of the apatite mineral. The spectrum includes the characteristic bands of the apatite mineral. The strongest peak at 1,046 cm^{-1} is attributed to PO_4^{3-} (Benbow and Bridgwater, 1993). The peaks at 792 and 582 cm^{-1} are assigned to P–O mode (Halcomb and Young, 1993). The band located at 473 cm^{-1} is resulted from the v2 phosphate mode (Kandori et al., 1997). In addition, the bands at 875; 1,425 and 1,646

cm^{-1} are related to CO_3^{2-} ions which are present. The bands appearing at 2,915 and 3,436 $cm^{-1}$are assigned to stretching vibrations of adsorbed water molecules (Kandori et al., 1997). Although, a little shift of some peaks but there is no significant change of the apatite mineral spectrum after interacting with *B. subtilis*, at different contact pH. These results suggest the lower adsorption affinity of *B. subtilis* to its surface. It agrees with little change in the surface charge which obtained by zeta-potential measurements. (Abdallah et al., 2025)

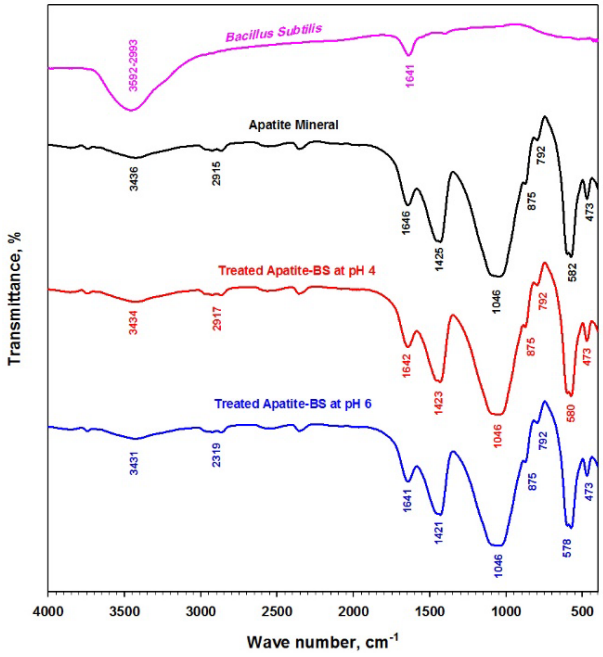

Figure 5. FTIR spectra of apatite mineral before and after treatment with *B. subtilis* at different pHs (Abdallah et al., 2025)

3.2.3. SEM Investigation images of Pure Minerals

Figure 6, shows the images of Scanning Electron Microscope (SEM) for the hematite (A) and after treatment with *Bacillus subtilis* (B). The hematite surface is covered with condensed bio-film of both bacterial cells and biological metabolite. The results also showed the presence of nitrogen, oxygen and carbon elements on its surface. This is due to bio-film formation of bacteria onto the hematite surface. It is proved the significant change in its surface behavior such as in the zeta-potential measurements and thus its flocculation. (El-Sayed et al., 2021)

On the other hand, Figure 7, shows the images of Scanning Electron Microscope (SEM) for the apatite (A) and after treatment with *Bacillus subtilis* (B). It is clear that there is no significant adsorbed *Bacillus subtilis* on the apatite surface. However, there is no significant change in hydrophobicity of the apatite mineral after interaction with *Bacillus subtilis*. The SEM images proved the selective adhesion of *Bacillus subtilis* cells on the hematite surface rather than the apatite surface. These results agree with the zeta potential and FTIR measurements which showed the little effect of bacterial interaction with apatite.

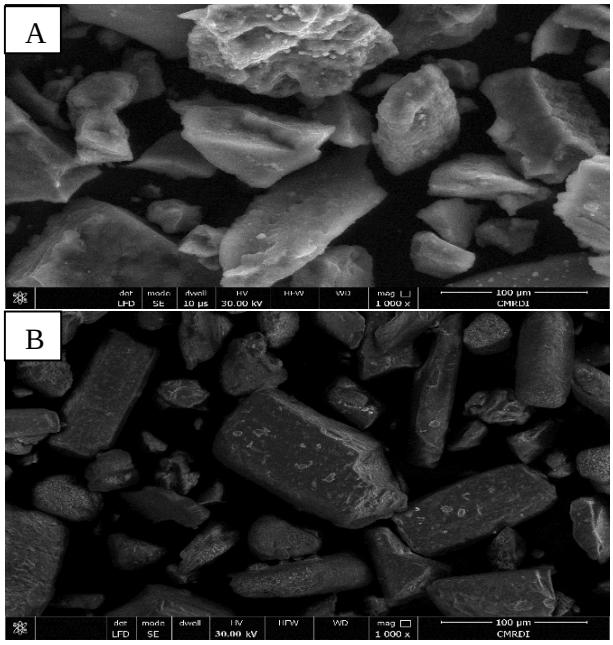

Figure 6. SEM images of hematite mineral (A) and after treatment with *Bacillus subtilis* (B)

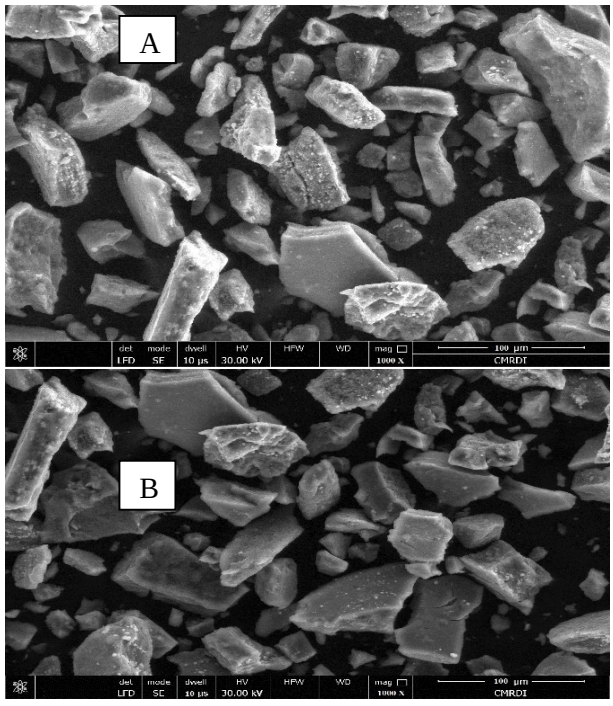

Figure 7. SEM images of apatite mineral (A) and after treatment with *Bacillus subtilis* (B)

3.3. Flocculation of Pure Hematite and Apatite Minerals

3.3.1. Effect of pH

The pH is essential parameter which alters the zeta

potential and hydrophobicity based on the hydrogen bonding and cohesion with water molecules (van Oss, 1992). The effect of pH on the flocculation of hematite and apatite in the presence of 8×10^5 cells of *Bacillus subtilis* for 10 min is presented in Figure 8. The flocculation of hematite mineral was increased with increasing pH to reach its maximum value (99 %) at pH 6 and then it decreased again. While the increasing of pH increases the apatite flocculation to 17 % at pH 10. The maximum flocculation difference (95 %) was achieved at pH 6. These results confirm the zeta potential measurements. The maximum change of hematite surface was achieved at pH 6 at which there is no significant change of the apatite surface. (Abdallah et al., 2025)

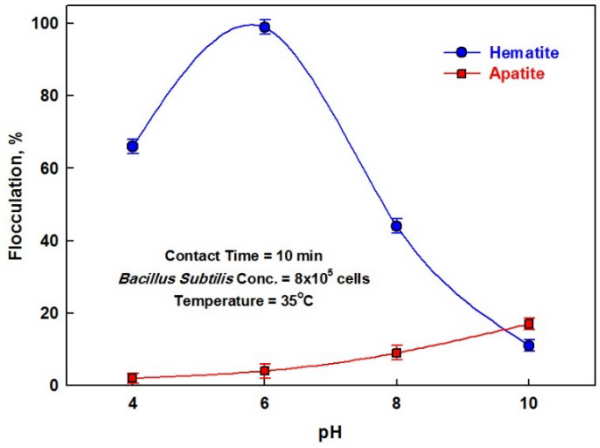

Figure 8. Effect of pH on the flocculation of pure hematite and apatite minerals

3.3.2. Effect of Contact Time

Figure 9 shows the effect of contact time of 8×10^5 cells of *Bacillus subtilis* with each mineral on their flocculation at pH 6 and 35 °C.

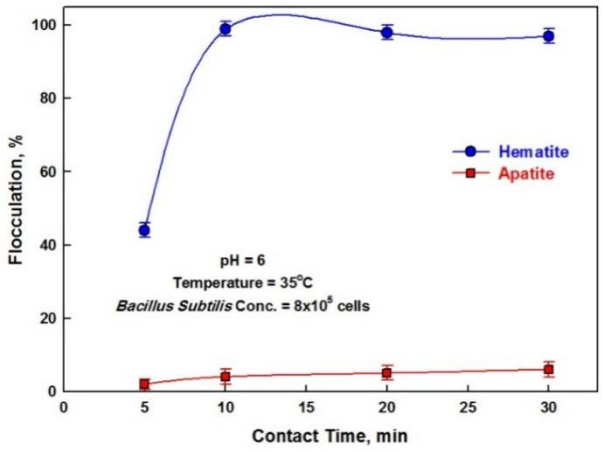

Figure 9. Effect of contact time on the flocculation of pure hematite and apatite minerals (Abdallah et al., 2025)

The flocculation of hematite was increased sharply to reach its maximum value (99 %) after 10 min and then it slightly decreased. While the increasing of contact time increases the apatite flocculation to 6 % after 30 min. The maximum flocculation difference (95 %) was achieved after 10 min contact time. The particle-particle attachment needs time (induction time). Induction time is associated with the properties of the thin water film around the particle. For a hydrophobic surface, induction time is short, a few milliseconds while it is very large for hydrophilic particle (Ramírez-Aldaba et al., 2017; Li et al., 2018).

3.3.3. Effect of Bacillus Subtilis

Figure 10 shows the effect of *Bacillus subtilis* concentration (cell count) on the flocculation of the treated minerals with *Bacillus subtilis* for 10 min at pH 6 and 35 °C. The flocculation of hematite was increased sharply to reach its maximum value (99 %) in the presence of 8×10^5 cells of *Bacillus subtilis* and then it reached to 100 % with 16×10^5 cells. While the increasing of cell count slightly increases the apatite flocculation to 4 % in the presence of 8×10^5 cells of *Bacillus subtilis* and then it is increased with higher rate to reach 16 % with 16×10^5 cells.

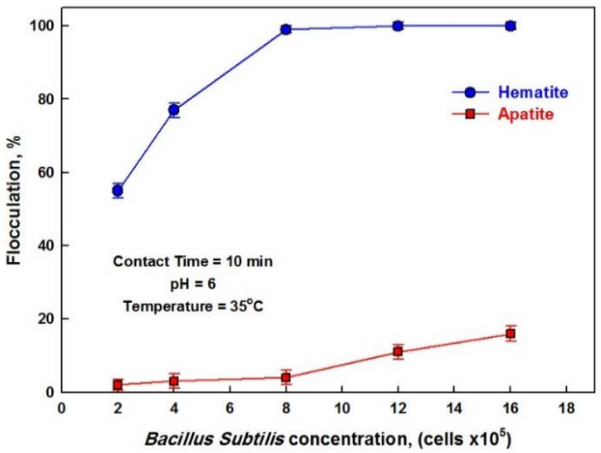

Figure 10. Effect of *Bacillus subtilis* on the flocculation of pure hematite and apatite minerals

It is suggested that the attachment and agglomeration of bacterial cells to solid surfaces provides a stable growth environment for the cells and enhance catalytic functions through the localization of cells into biofilms. The excretion of an extracellular polymeric substance composed of macro-molecules as polysaccharides, proteins and lipids promotes the development of the biofilms on mineral surfaces. It is suggested that hydrogen bond is formed between bacteria and mineral surface as a result of the presence of (OH^-) group of polysaccharide of metabolite. The charge and spacing between them control the biomolecules interaction with the solid surfaces (Ramírez-Aldaba et al., 2002; El-Sayed et al., 2021).

The adsorption of bacteria on the hematite surface provides more hydrophobic surface and a stable agglomeration. The exopolymers, or metabolites interact with the organism and the minerals in a variety of ways. The metabolites of *Bacillus Subtilis*, such as the polysaccharides, proteins and organic acids are responsible for the surface modification. (Farghaly et al., 2021) Many of the interacting mechanisms are still unknown and need an explanation of how the bacteria adhere to the solid surface (Feng et al., 2012).

3.3.4. Binary mixture flotation

The flotation study of binary mixture aims to determine the effect interaction between the two minerals which may be occurred during their contact together rather than individual contact. One gram of synthetic binary mixture composed of 95 % of hematite with 5 % of apatite (≈ 1.729 % P_2O_5) was employed. A concentrate of about 99.5 % Fe_2O_3 with 95 % recovery was obtained using 8×10^5 - 12×10^5 cells of *Bacillus subtilis* at pH 6. The apatite content was reduced from 5 % (≈ 1.729 % P_2O_5) to about 0.1 % (≈ 0.0346 % P_2O_5), Figure 11. (Abdallah et al., 2025)

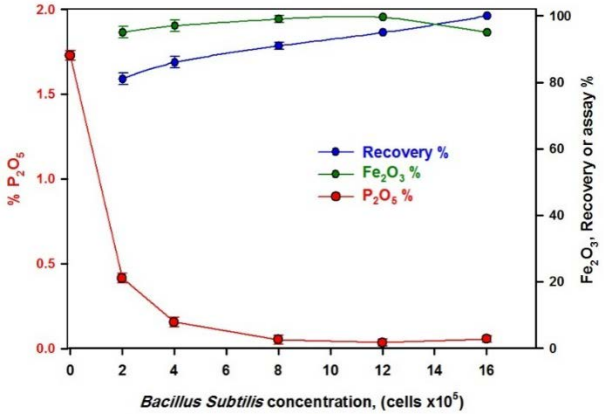

Figure 11. Effect of *Bacillus subtilis* on the flocculation of hematite from its binary mixture with apatite at pH 6 and 35°C

4. Conclusion

Bacillus subtilis was used as a surface modifier to flocculate hematite mineral selectively from its mixture with apatite mineral. The hematite surface was strongly affected by bacterial interaction while the apatite was not affected. The FTIR and SEM analyses confirmed the selective adhesion of *Bacillus subtilis* on the hematite mineral surface.

Maximum flocculation of hematite mineral was achieved with 8×10^5 cells of *Bacillus subtilis* at pH 6 and 35 °C for a 10-minute contact time.

A binary mixture containing 95 % hematite and 5 % apatite produced a concentrate of 99 % hematite with 95 % recovery in the presence of 8×10^5 cells of *Bacillus subtilis* at pH 6 and 35 °C for a 10-minute contact time.

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Selektivna bioflokulacija minerala hematita korišćenjem *Bacillus subtilis* za uklanjanje fosfora iz gvozdenih ruda

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INFORMACIJE O RADU

Primljen 15 septembar 2025
Prihvaćen 14 novembar 2025

Naučni rad

Ključne reči:
Bacillus subtilis
Fosfor
Hematit
Apatit
Bioflokulacija
Minerali

Proširena i recenzirana verzija rada - rad prezentovan na simpozijumu XVI Međunarodna konferencija pripreme mineralnih sirovina i reciklaže (IMPRC 2025)

I Z V O D

Bacillus subtilis korišćena je kao biološki modifikator površine za flokulaciju minerala hematita (Fe_2O_3). Ispitivan je uticaj interakcije bakterija na ponašanje minerala hematita i apatita, koji predstavlja glavni izvor fosfora u gvozdenim rudama, primenom merenja zeta-potencijala, skenirajuće elektronske mikroskopije (SEM) i infracrvene spektroskopije sa Furijeovom transformacijom (FTIR). Analizirani su efekti pH vrednosti, vremena kontakta i koncentracije *Bacillus subtilis* na proces flokulacije ova dva minerala.
Rezultati pokazuju da zeta-potencijal hematita značajno zavisi od interakcije sa *Bacillus subtilis*, pri čemu je najveća efikasnost flokulacije postignuta pri pH 6. Mineral hematita mogao je biti uspešno odvojen iz smeše sa apatitom u prisustvu $(8 - 12)\times10^5$ ćelija *Bacillus subtilis* pri pH 6 i temperaturi od 35 °C tokom vremena kontakta od 10 minuta.