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SELECTIVE BIO-FLOCCULATION OF HEMATITE MINERAL USING *BACILLUS SUBTILIS* FOR PHOSPHOROUS REMOVAL FROM IRON ORES

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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 the hematite and apatite minerals as the main source of phosphorous in the iron ore was investigated using zeta-potential, and FTIR measurements. The effect of pH on the flocculation of the two minerals was investigated. The zeta-potential of hematite is strongly affected by *Bacillus subtilis* interaction while the maximum flocculation efficiency was achieved at pH 6. The hematite mineral could be separated from its mixture with apatite mineral in the presence of *Bacillus subtilis* at pH 6.

Keywords: *Bacillus Subtilis*, Phosphorous, Hematite, Apatite, Bio-Flocculation, Minerals.

INTRODUCTION

Iron ores became more complex in their mineralogical composition which carrying phosphorus more widespread. Phosphorus is harmful in the steel-making process which increases hardness and brittleness and decreases ductility. Phosphorus may occur as apatite [$Ca_5(PO_4)(F,Cl,OH)$], whereas wavellite [$Al_3(PO_4)_2(OH)_3.5(H_2O)$] is the second phosphate present in high phosphorus content iron deposits [1]. Phosphorus can be removed from iron ores by different processes depending on the mineralogy and cost. It is important to investigate fundamental studies to get the most effective separation process. The physical and biological processes are most likely techniques. They require low reagents, power requirement, facilities, equipment, and pollution [2-4]. Bio-leaching was used through acid-producing microorganisms, including filamentous fungi and iron-oxidizing bacteria [5]. Selective agglomeration was used in the presence of oleic acid as a collector [6, 7]. 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 [8]. 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

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separation of one mineral from another [8-13]. 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 [14-15].

Bio-beneficiation processes are relatively new and are under intense investigation in recent years [16]. 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 [17].

EXPERIMENTAL

Materials

Two samples of hematite and apatite minerals of high purity were supplied by the Egyptian Mineral Resources Authority (EMRA). *B. subtilis* strain was supplied by the mineral bio-processing Lab., CMRDI. All chemicals used in this work were of analytical grade.

Methods

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.

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 2000 Perkin Elmer spectrometer was obtained between 4000 and 400 cm^{-1}.

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.

RESULTS AND DISCUSSION

Chemical Composition 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.

Table 1 Chemical composition of pure hematite and phosphate minerals.

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**	**100**

Mineral Bacteria Interaction

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. 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. Figure 1, shows that *Bacillus subtilis* is positively charged at less than pH 3.25 (its isoelectric point).

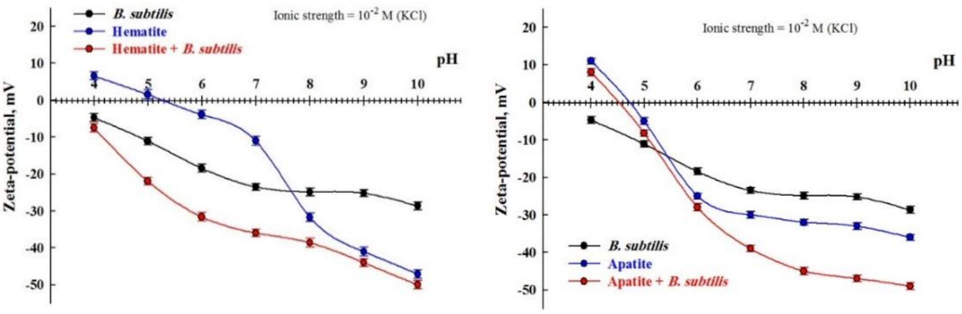

Figure 1 Effect of pH on zeta potential of treated hematite and treated phosphate with *B. subtilis.*

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. 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. 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 1, showed that the negativity of zeta potential is significant increased at pH range higher than 6.

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). 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. 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 2, which shows the typical FTIR spectrum of iron oxide. It exhibits various well-defined peaks at 459, 541, 991, 1485, 1637, 2843, 2919 and 3429 cm^{-1} [35]. The appearance of two well-defined peaks at 459, 541 and 991 cm^{-1} are due to the presence of iron–oxygen (Fe–O). The observed FTIR results confirmed the iron oxide without any significant impurity.

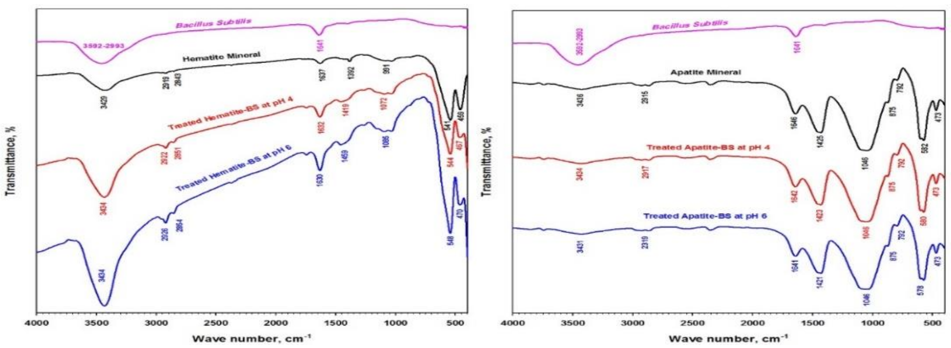

Figure 2 FTIR spectra of Hematite and Apatite before and after treatment with *B. subtilis* at different pHs

After interacting with *Bacillus subtilis* the band at 3429 cm^{-1} became more intense and shifted 3424 and 3434 cm^{-1} for treatment at pH 4 and 6 respectively. Also, the peaks at 2919, 2843 and 1637 became more intense and shifted to 2922-2926 cm^{-1}, 2851-2854 cm^{-1} and 1632-1631 cm^{-1}, for treatment at pH 4 and 6, respectively. The peaks at 1392 and 991 cm^{-1} were disappeared and new bands were appeared at 1419-1459 cm^{-1} and 1072-1085 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 agree with higher change in the surface charge which obtained by zeta-potential measurements.

Figure 2, shows the FTIR spectrum of the apatite mineral. 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 agree with little change in the surface charge which obtained by zeta-potential measurements.

Flocculation of Pure Hematite and Apatite Minerals

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. 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 3. 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.

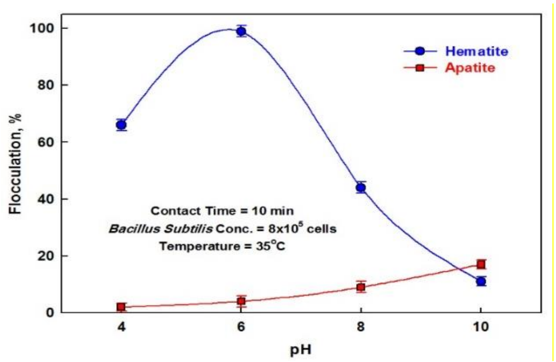

Figure 3 Effect of pH on the flocculation of pure hematite and apatite minerals

Binary mixture flocculation

The flocculation 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 *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).

CONCLUSION

Bacillus subtilis was used to as a surface modifier to flocculate hematite mineral selectively from its mixture with apatite mineral. The hematite surface is strongly affected as a result of bacterial interaction while the apatite didn't affect. The FTIR proved the selective adhesion of *Bacillus subtilis* on the hematite mineral surface.

The maximum flocculation of hematite mineral was achieved at pH 6. A binary mixture of 95% hematite with 5% apatite produced a concentrate of 99% hematite with 95% recovery in the presence of *Bacillus subtilis* at pH 6.

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