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Bio-flotation of Eastern Desert Iron Ores Using *Bacillus subtilis*

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ABSTRACT

With increasing demand for steel and depletion of high-grade iron ore deposits, more research efforts are being directed toward extending the life of existing ore reserves and developing technology to treat low-grade iron ore resources. Interaction between Bacillus subtilis and iron ore minerals such iron oxide and, quartz brought about significant surface chemical changes. Quartz was rendered more hydrophilic, while iron oxide became more hydrophobic mineral after bacterial interaction. The surface properties were studied using zeta potential, SEM, adhesion of strains to the minerals' surface and contact angle. In this work Bacillus subtilis was isolated from Egyptian iron ore surface. The results revealed that a strong interaction was occurred. A concentrate contains 90.88% Fe_2O_3 [64 % Fe (total)] and 4.46% SiO_2 was obtained through bio-flotation from a feed contains 70.04% Fe_2O_3 [49.33% Fe (total)] and 21.18% SiO_2.

Introduction

Iron ores are present in different locations in Egypt such as at East Aswan, at Bahariya Oasis, in the Western Desert, and in several localities of the Eastern Desert near the Red Sea coast. The iron-ore deposits of these localities vary greatly in their mineralogical and chemical composition as well as in the nature of their associated rocks, as also regarding the assemblage of trace elements **[**1**].** The formation of various iron oxides under the earth's crust are due to both biological and abiotic reaction mechanisms [2-6]. Presently available physic-chemical methods are not environment friendly. Bio-beneficiation is considered as eco-friendly, promising and revolutionary solutions to these problems and is gaining more importance due to depletion of high grade ores and enforcement of strict anti-pollution laws. Utility of microorganisms in iron ore beneficiation was understood and developed only in the last decade [7-10]. Smith and coworkers have reported extensive studies on the relevance and promise of microorganisms in mineral bio-processing [11-15]. Many types of microorganisms including autotrophic and heterotrophic bacteria, fungi, yeast and algae which inhabit iron ore deposits find use in iron ore beneficiation [16-18]. Bio-flotation is a biotechnology by employing microbes, in particular bacteria, involving the adhesion of the bacteria on the minerals that meets the industrial needs more selective and environmentally friendly mineral separation [19-22]. By adhering the surface of minerals, the bacteria essentially change the surface characteristics of the minerals as a result of interaction of microbes and mineral surfaces. The presence of functional non-polar groups (hydrocarbon chains) and polar groups (carboxyl, hydroxyl, phosphates) at the microbial cellular surfaces or metabolic products give the microbial culture characteristics similar to those of surfactant molecules [23]. Therefore, the microorganisms can modify the mineral surfaces, either directly or indirectly. The direct mechanism involves the adhesion of cells to mineral particles while the indirect mechanism refers to the biological reagents such as excreted metabolites acting as surface-active reagents [24] or as soluble fractions of the microorganisms derived from their rupture [25,26].

Materials and Methods

Materials

Sample of single mineral of hematite (Fe_2O_3), and Quartz was 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. Nature iron ore was collected from Eastern Desert Locality, Egypt.

Characterization

A Philips PW 1730 powder X-ray diffractometer with Fe-filtered Co (K-alpha) run at 30 kV and 20 mA was used to examine single minerals. Selected samples were observed on fractured surface under a JSM-6400 scanning electron microscope (SEM) to examine the morphology of single mineral.

Bacterial Growing and Isolation

A suspension containing 0.5 gm of mineral sample in 10 ml distilled water was prepared. After that, 1 ml of suspension was taken and sprayed onto a nutrient agar plate surface then incubated for 24-48 hr. at 30^{o}C. The developed colonies were picked up and streaked on nutrient agar plates and incubated at 30^{o}C for 24-48 hr. The final step was repeated several times until pure colonies have been obtained. Separate colonies were picked up, streaked on nutrient agar slopes, stored at 4^{o}C and sub-cultured monthly [27-29].

Growth Media

Two forms of nutrient media were used, solid form (nutrient agar) and liquid form (nutrient broth). Nutrient agar (NA) including peptone, 5gm; beef extract, 3gm; sodium chloride, 8gm; agar, 12gm and distilled water, 1000ml. The constituents were dissolved with heating, adjusted to pH 6.8-7.0 and sterilized at 120^{0}C for 20 min.

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Graphical Expression of Bacterial Growth

Bacterial growth was measured through inoculating the preserved bacterium into 100 ml nutrient broth and incubated overnight in 250 ml measuring flask after that optical density was measured to determine the start point. Then 1 ml of the previous culture was taken in 100 ml nutrient broth followed by measuring OD550 along time intervals of 30 min. OD550 was measured using "Perkin-Elmer" Spectrophotometer" model Lambda 3B. Growth curve was obtained by plotting the logarithm of OD550 versus time, [27-29].

Preparation of Inoculum

A liquate of 350 ml was dispensed into 1 litre flask, then sterilized at 120^0C for 20 min. and after that inoculated with a loop full of the bacterial strain under test and incubated at 30^oC for 48 hr according to growth curve of three strains of bacteria under test [27-29].

The Gram Stain Technique

A loopful of tap water was placed on a slide; using a sterile cool loop transfer a small sample of the colony to the drop, and emulsify. The film was allowed to be air dried. The dried film was fixed by passing it briefly through the Bunsen flame two or three times without exposing the dried film directly to the flame. The slide should not be so hot as to be uncomfortable to the touch. The slide with crystal violet solution was flooded for up to one minute and was washed off briefly with tap water (not over 5 seconds) and drained. After that, the slide was flooded with Gram's Iodine solution, and be allowed to act (as a mordant) for about one minute followed by washing off with tap water and drainage. Excess water was removed from slide and blot, so that alcohol used for decolourization is not diluted. The slide was flooded with 95% alcohol for 10 seconds and washed off with tap water. (Smears that are excessively thick may require longer decolourization). This is the most sensitive and variable step of the procedure, and requires experience to know just how much to decolorize). The slide after that was drained, flooded with safranin solution and be allowed to counterstained for 30 seconds. Finally, the slide was washed off with tap water, drained and blotted dry with bibulous paper and didn't be rubbed. All slides of bacteria must be examined under the oil immersion lens [27-29].

BIOLOG Microbial Identification System

Bacteria identification was done using the BIOLOG GEN III Micro-plate microbial identification system. A pure culture was grown on BIOLOG recommended agar media and incubated at 30° C. Inoculums were prepared where the cell density was in the range of 90-98%T. precisely 100 µl of the cell suspension was transferred by multichannel pipette into the wells of BIOLOG micro-plate. The plates were incubated for 36 hours at 30° C into the Omni-Log incubator/reader. The BIOLOG micro-plate tests the ability of an organism to utilize or oxidize a pre-selected panel of 95 different carbon sources. The dye tetrazolium violet is used to indicate utilization of substrates. A panel of 95 different substrates gives a very distinctive and repeatable pattern of purple wells for each organism in which the manufacturers literature terms a "Metabolic Fingerprint". Finally; micro plate was read using BIOLOG's Microbial Identification Systems software through biology reader

Zeta Potential Measurements

A laser Zeta Meter 'Malvern Instruments Model Zeta Sizer 2000' was used for zeta potential measurements. 0.05 g of ground sample was placed in 50 ml double distilled water with definite concentration of the bacterial isolate at fixed ionic strength of 10^{-2} M NaCl. NaOH and HCL were used as pH modifiers. The suspension was conditioned for 60 minutes during which the pH was adjusted. After shaking, the equilibrium pH was recorded. It was then allowed to settle for 3 min, after which 10 ml of the supernatant was transferred into a standard cuvette for zeta potential measurement. Solution temperature was maintained at (25^oC ± 2). Five measurements were taken and the average was reported as the measured zeta potential [27-29].

Adhesion measurements

Adhesion of the bacterial isolate on the mineral surfaces was determined by dry weight difference before and after conditioning with the mineral particles. 0.5 gram of the ground mineral (-200 mesh) was added to 80 ml of the 48 hour bacterial suspension with a fixed initial concentration of the bacterial isolate $2x10^8$cell/ml, and conditioned for 60 minutes after adjusting the pH values. An additional time of 20 min. was allowed for settling of the mineral particles, after which 20 ml of the supernatant was collected in a porcelain crucible and dried on a hot plate at 40 – 45°C. Adhesion studies were performed as a function of difference in weight before and after drying.

Surface Tension Measurement

The surface tension measurements were performed using t he ring method in a Kruss K9 digital TENSIOMETER with an accuracy of ±0.1 mN m^{-1}. The surface tension of the bacterial suspensions was set at different bacterial concentrations and i n function of the pH suspension, the pH suspensions were regu lated with diluted HCl and NaOH solutions [27-29]

Contact Angle Measurements

The contact angle measurement were carried out through a GONIOMETER RAMÉ.HART using the captive bubble method. The mineral crystal was carefully cut (dimensions 0.5*0.5*1) cm^3 and polished with a diamond paste. The samples were cleaned with jets of distilled water to remove any particles sticking to the polished surface. The contact angle measurements were taken before and after interaction with the bacterial isolates [30-31].

Adsorption experiments

The adsorption density of bacterial isolate on the mineral surface was determined by adding 1 g dry sample of kaolin or anatase to the bacterial suspension in a 100 cm3 volumetric flask with a definite concentration of bacterial cells. The mixture was shacked for 15 minutes using a shaker at 150 rpm (Model JANKE & KUNKEL Type VX10). The pH was adjusted to the desired values using HCl and NaOH. A potentiometer (Orion Mod. 720A) equipped with a combined electrode was used to monitor the pH. The potentiometer was calibrated before each test by using buffer solutions of pH 4, 7 and 10. The solution was shaken at 150 rpm for 1h at controlled temperature of 25 ± 2°C. Then, the samples were centrifuged at 15000 rpm for 20 min at room temperature to separate supernatant from the settled fraction. The total content of organic carbon, (residual concentration), in 40 ml of supernatant was determined using a 'Phoenix 8000' Total Carbon Analyzer" instrument. The average of three readings was taken as a measure for the residual concentration of organic carbon. The adsorbed amount was then calculated as the difference between initial and residual concentrations [32].

Flotation Experiments

A series of bench-scale flotation experiments were conducted using a modified Halimond tube with 150 ml capacity.

In carrying out these flotation experiments one gram (of single minerals or their binary mixture as well as natural ore) were conditioned at the pre-determined optimum conditions of pH 3, concentration of bacterial isolate of **10 ml (10^6 cells mL^{-1})** and conditioning time 10 minutes using a horizontal shaker. The pH was adjusted with dilute solutions of NaOH and HCl. The flotation was conducted for 5 minutes at air follow rate of 0.7 cm^{-3}/min. Both floated and sink fractions were collected, dried, weighted, and analyzed [33].

Results and Discussions

Sample Characterization

Chemical analysis of a representative sample in Table 1 indicated that the sample contains a total Fe% of about 49.33%. In addition, the analysis shows that the sample has high content of SiO_2 (21.18%) which is found as impurities. The contents of MgO, MnO, K_2O, Na_2O, Cl, ZnO, TiO_2, P and Al_2O_3 are minor, while (Al_2O_3) is slightly high due to the presence of a clayey fraction. On the other hand, the XRD pattern, Figure 1 illustrated the characteristic peaks for iron oxide in the form of hematite, Magnetite, while Calcite and quartz as gangue minerals and the presence of minor amounts of kaolin and smectite.

Table 1.Chemical Analysis of iron Ore Sample

Oxide	%
(Fe total)	(49.33)
SiO_2	21.18
CaO	3.12
Al_2O_3	1.97
MgO	0.55
Na_2O	0.111
P_2O_5	0.95
SO_3	0.39
TiO_3	0.146
K_2O	0.019
MnO	0.086
Co_3O_4	0.126
ZnO	0.007
SrO	0.014
Cl	0.05

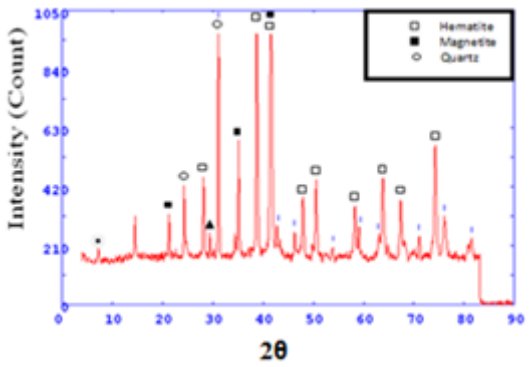


Figure 1. XRD of the origin Sample.

Surface Properties of Single Minerals and Bacillus subtilis

Figure 2 depicts that this bacterial isolate is, more or less, hydrophobic in nature where zeta potential values are varied fr om about +2 to 40 mv over the entire range of pH (pH 2- 12), and its isoelectric point (iep) is about 2.5 and this in agreement with the fact that iep of these types of microorganisms are less than 4. This may be due to that the cell wall of a gram-positive bacterium is composed by a porous three-dimensional macromolecular network containing peptidoglycan teichuronic acid, protein, lipids and polysaccharides [34-38]. Figures 3 and 4 showed the zeta potential values of single minerals of iron oxide and quartz before and after interaction with *Bacillus subtilis.* The results clearly indicate that the two curves of zeta potential conducted at pH ~ 7 for iron oxide and at pH~2.7 for quartz at ionic strength 10^{-2} M NaCl in the absence of bacterial isolate. These values are considered as iso-electric point (iep) for iron oxide and quartz respectively which are in agreement with literature. After interaction with bacterial isolate of *Bacillus subtilis*, the values of zeta potentials are shifted to lower values of ~ 5.8 and 2.2 for iron oxide and quartz respectively which confirmed the role of the bacterial strains on changing the surface properties of single minerals. This is attributed to the presence of anionic groups on the bacterial cell wall that dominate over the cationic groups [39-41]

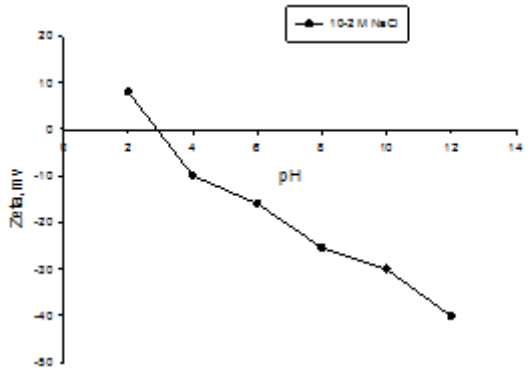


Figure 2. Zeta potential of Bacillus subtilis.

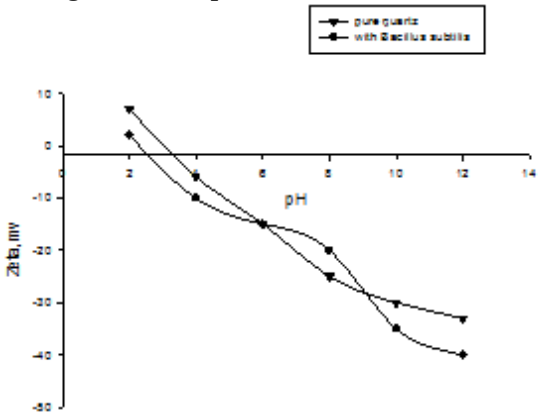


Figure 3. Zeta potential of quartz before and after interaction with *Bacillus subtilis*

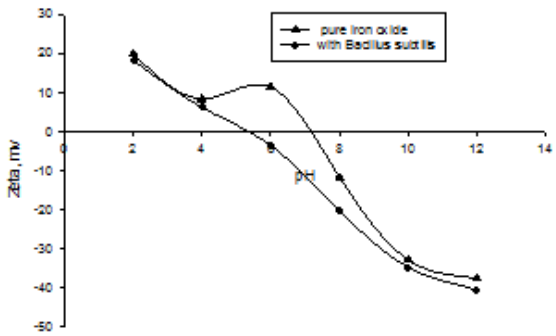


Figure 4. Zeta potential of iron oxide before and after interaction with Bacillus subtilis

Bacillus subtilis Adhesion onto Minerals' Surfaces

The adhesion results showed that the bacterial isolate of *Bacillus subtilis* could be adhered to both single minerals' surfaces with higher affinity to the iron oxide than that of quartz. This is due to the specific adsorption of functional

groups on the bacterial cell wall on the available adsorption sites of iron oxide surface, specially at pH range 5.5 – 7 and followed by a gradual decrease till reaching pH 12 [42-43], Figure 5. The adsorption results are in agreement with the above results of adhesion, Figure 6, which indicated the higher affinity of Bacillus subtilis to be adsorbed onto iron oxide surface more than that onto quartz surface. The maximum adsorption density has been reached for iron oxide at pH 6.

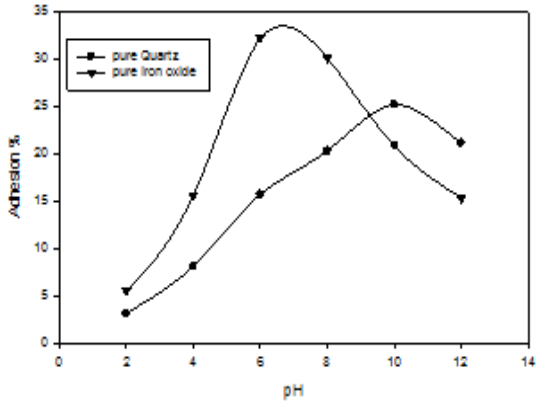


Figure 5. Adhesion of *Bacillus subtilis* onto single minerals surface.

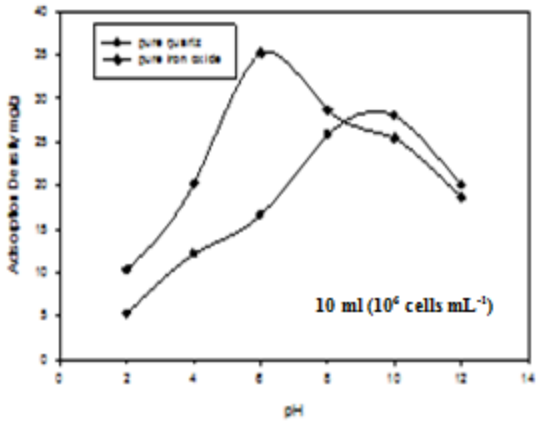
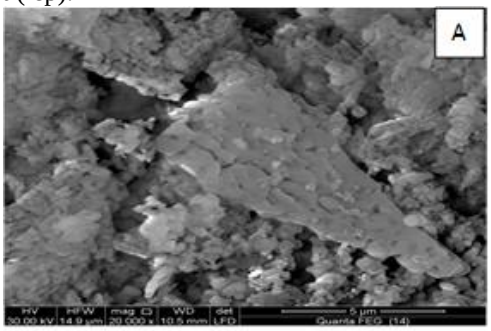


Figure 6. Adsorption densities of *Bacillus subtilis* onto single minerals' surfaces.

The above results were confirmed by SEM of single minerals' surface before and after treatment with *Bacillus subtilis*, Figures 7A, 7B, 8A and 8B for iron oxide and quartz respectively. The results stated that *Bacillus subtilis* bacteria could be adhered onto both minerals'surfaces but with higher affinity towards iron oxide. This revealed the network formation of cells with aggregation on minerals' particles surfaces forming a bio-film that leading to hydrophobicity of iron oxide surface due to the displacement of its isoelectric point (iep).



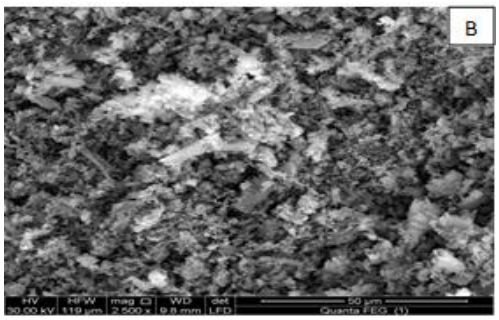


Figure 7. SEM of single iron oxide before and after interaction with Bacillus subtilis.

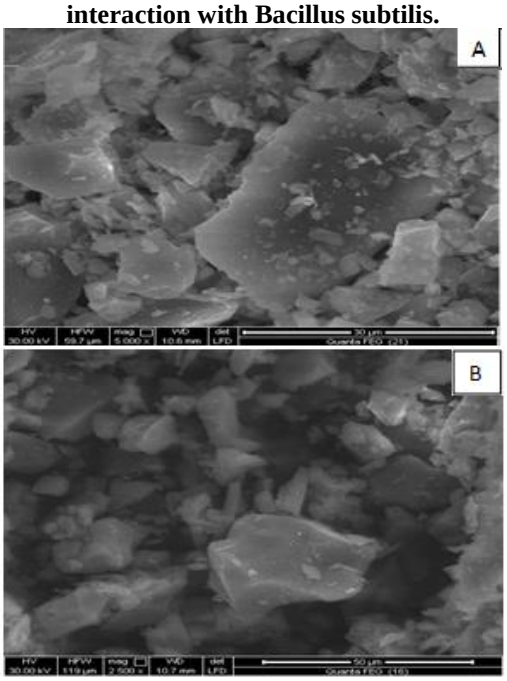


Figure 8. SEM of single quartz before and after interaction with Bacillus subtilis.

Surface Tension and Contact Angle

The surface tension of *Bacillus subtilis* bacteria was conducted at air-water interface at room temperature, $25^{O}C\pm2$ and pH 6, Figure 9.

The results confirmed a gradual decrease of surface tensio n values (80-20 mN/m) by increasing the biomass concentration (2-10 mL) till reaching a stable state (10 mL). On the other hand, the lowest values of surface tension of biomass suspensions of *Bacillus subtilis* were achieved at pH 6. As shown in Figure 10, it was expected that the bacterial suspension could form stable high-density foam at these pH range while the foam that formed at high pH would be less stable [44].

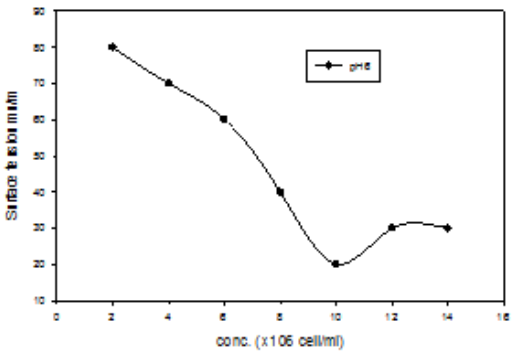


Figure 9. Effect of concentration on the surface tension of Bacillus subtilis

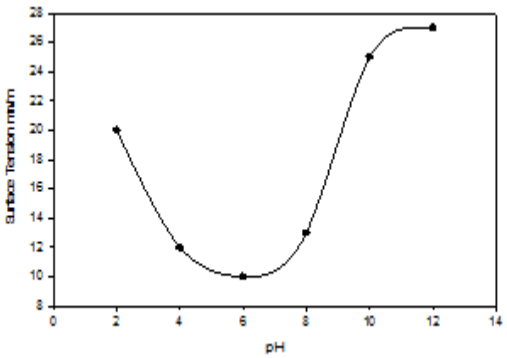


Figure 10. Effect of pH on the surface tension of Bacillus subtilis.

Contact angle is a measure of static hydrophobicity. Resul ts obtained for both single minerals iron oxide and quartz before and after interaction with *Bacillus subtilis* bacterial isolate confirmed its hydrophobic effect onto mineral surface. As noticed in Figure 11, the contact angle values as a function of pH are very small, (~17^O for iron oxide and ~14^O for quartz) which indicated the hydrophilic nature of single minerals' surfaces before treatment with bacteria. After interaction, a significant increase in contact angle values for single minerals of iron oxide and quartz at pH 6, (~40^O for iron oxide and ~25^O for quartz). This confirmed the higher affinity of *Bacillus subtilis* for iron oxide rather than quartz. This will increase the selectivity of collector adsorption that could be attributed to the electrostatic interaction between the protonated amine group (NH_3) of the collector and silanol groups ($Si–OH_2$) on the quartz surface. On the other hand, as a result of bacterial cell adsorption on the iron oxide surface and formation H-bonding between Fe^{3+}- OH^- and hydrocarbon chains of the polysaccharides and carboxylic acids, the iron oxide surface became more hydrophobic due to the formation of a bio-film [45-51].

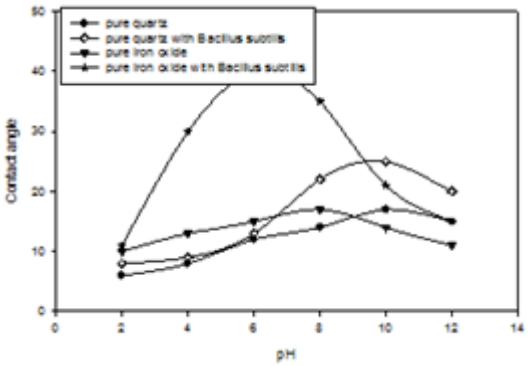


Figure 11. Contact angles of polished minerals crystal sections before and after interaction with *Bacillus subtilis.*

Bio-Flotation

The amenability of applying ***Bacillus subtilis***, to be used as the sole flotation reagent, to selectively separate iron oxide and quartz from their binary mixtures was studied. The results obtained at optimum operating conditions indicate that on using the bacterial isolate for flotation a mixture containing 90 % by weight iron oxide and 10% by weight quartz, a high floatability (~ 90% floated) for iron oxide was reached while low flow floatability (~ 27% floated) for quartz was obtained specially at pH 6, Figure 12.

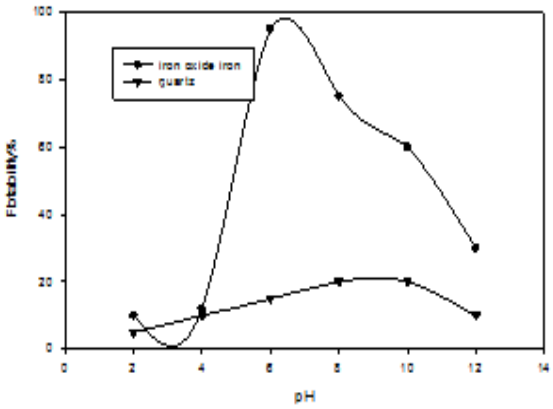


Figure 12. The Floatability of Single Mineral with *Bacillus subtilis.*

On applying the same conditions on bio-flotation of the natural iron ore of Eastern Desert, A concentrate contains 90.88% Fe_2O_3 [64 % Fe (total)] and 4.46% SiO_2 was obtained from a feed contains 70.04% Fe_2O_3 [49.33% Fe (total)] and 21.18% SiO_2, Table 2.

Table 2. Chemical Analysis of Final Concentrate of Natural Iron Ore.

oxide	%
Fe(Total)	64.00
SiO_2	4.46
CaO	0.92
Al_2O_3	1.59
MgO	0.31
Na_2O	0.06
P_2O_5	0.28
SO3	0.26
TiO_2	0.06
K_2O	0.01
MnO	0.03
Cr_2O_3	0.02
ZnO	0.01

Conclusions

- The results showed a strong interaction between ***Bacillus subtilis*** bacteria and minerals' surfaces, especially with iron oxide.
- Adhesion, adsorption and zeta potential measurements showed a better affinity of ***Bacillus subtilis to*** iron oxide mineral surface rather than quartz.
- The results of zeta potential showed that the iso-electric points (IEP) for both iron oxide (~ pH 7) and quartz (~ pH 2.7) are significantly displaced to lower values (~ 5.8 and 2.2 respectively) after interaction with the ***Bacillus subtilis.***
- Higher bacterial affinity to hematite in comparison to quartz is readily evident from the results of adhesion and adsorption of ***Bacillus subtilis*** minerals' surfaces where higher values for adhesion and adsorption with hematite surface are noticed.
- The selectivity of hematite flotation against quartz was observed in the micro-flotation tests through measurement of floatability of the two single minerals in the presence of Bacillus subtilis.
- The results show the potentiality for using ***Bacillus subtilis*** bacteria as a sole flotation reagent where a concentrate contains 90.88% Fe_2O_3 [64 % Fe (total)] and 4.46% SiO_2 was obtained from a feed contains 70.04% Fe_2O_3 [49.33% Fe (total)] and 21.18% SiO_2.

References

1. Khaled .E.Yassin "Flotation of Iron Ores using Microorganisms as a Surface Modifier" Ph.D. Helwan University, Faculty of Science, Chemistry Department (2012).
2. A, Arakaki, H, Nakazawa, Nemoto, M, Mori, T and T. Matsunaga, "Formation of magnetite by bacteria and its application", J.R.Soc.Interface, 5, 977-999.(2008)
3. S,Bonneville, Van Cappellen, P and T., Behrends, , "Microbial reduction of iron (III) oxyhydroxides; Effect of mineral solubility and availability", Chemical Geology, 212, 255- 268.(2004)
4. D Fortin, and S.,Langley,. "Formation and occurrence of biogenic iron-rich minerals", Earth-Science Reviews, 72, 1-19.(2005)
5. P U P A Gilbert, M, Abrecht, and B H.,. Frazer, "The organic-mineral interface in biominerals" Rev. Mineralogy and Geochemistry, 59, 157-185. (2005)
6. P J Williams, and T. Cloete, E., June, "Microbial community study of the iron ore concentrate of the sishen iron mine", South Africa, World J. Microbiology & Biotechnology, (on-line publication) (2008)
7. N Deo, and K. A, Natarajan, , "Interaction of Bacillus polymyxa with some oxide minerals with reference to mineral beneficiation and environmental control", Minerals Engineering, 10:1339-1354.(1997)
8. N. Deo, and K. A.,. Natarajan, "Studies on interaction of Paenibacillus polymyxa with iron ore minerals in relation to beneficiation", Int. J. Miner. Process, 55:41-60.(1998)
9. K. A., Natarajan and, Partha Patra "Microbially induced flocculation and flotation for separation of chalcopyrite from quartz and calcite", Int. J. Miner. Process, 74:143-155.(2004)
10. K. A Natarajan, and Partha Patra. "Surface chemical studies on selective separation of pyrite and galena in the presence of bacterial cells and metabolic products of Paenibacillus polymyxa," J. Colloid and Int Sci, 298:720-729. (2006)
11. J, Dubel, R. W, Smith, M Misra, and S., Chen. "Microorganisms as chemical reagents: the hematite system" Minerals Engineering, 5, 547-556.(1992)
12. M., Misra, S,Chen, Smith. R.W, Raichur, A.M. "Mycobacterium phlei as a flotation collector for hematite". Minerals and Metallurgical Processing 10, 170–175.(1993)
13. R. W, Smith, M. Misra, and S., Chen, "Adsorption of a hydrophobic bacterium onto hematite": Implications in the froth flotation of hematite, 11, 63-67.(1993)
14. I. A. H, Scheider, M Misra, and R. W., Smith, "Bioflocculation of fine hematite suspensions with products from yeast cell rupture", Chemical Engineering and Mineral Processing, 4, 248-252.((1994)
15. R. W., Smith, and M., Miettinen, "Microorganisms in flotation and flocculation: Future technology or laboratory curiocity", Minerals Engineering, 19, 548-553. (2006)
16. M, Madigan, J. Martinko, Brock Biology of Microorganisms, 11th ed., Prentice Hall, New York.1,2,3,4,5.(2005)
17. ET Pecina-Treviño, GT,Ramos-Escobedo PM Gallegos-Acevedo, FJ López-Saucedo, E.Orrantia-Borunda "Bio-flotation of sulfide minerals with Acidithiobacillusferrooxidans in relation to copper activation and surface oxidation". Canadian Journal of Microbiolology;58:1073–83. (2012)
18. KH Rao, S.Subramanian "Bio-flotation and bio-flocculation of relevance to mineral bio-processing". In: Donati ER, Sand W, editors. Microbial processing of metal sulfides. Netherlands: Springer .p. 267–286. (2007)
19. Wei YH, Lai CC, Chang JS. "Using Taguchi experimental design methods to optimize trace element composition for enhanced surface in by Bacillus subtilis ATCC 21332.Process" Biochemistry.42:40–5. (2007).
20. MH, Fazaelipoor H, M. Khoshdast Ranjbar "Coal flotation using a bio-surfactant from Pseudomonas aeruginosa as a frother". Korean Journal of Chemical Engineering;27:1527–31.(2010)
21. H, Khoshdast A, Sam Z. Manafi. "The use of rhamno lipidbio surfactants as a frothing agent and a sample copper ore response". Minerals Engineering 26:41–9.(2012)
22. H. Khoshdast, A. Sam, Z. Manafi "The use of rhamnolipid bio-surfactants as a frothing agent and a sample copper ore response" Minerals Engineering, 26 pp. 41–49. (2012),
23. A. Vilinska, K. Hanumantha Rao "Leptosririllum ferrooxidans-sulfide mineral interactions with reference to bio-flotation and bio-flocculation Trans. Non ferrous". Met.Soc. China 18,1430 – 1409. (2008).
24. P.K., Sharma and K., Hanumantha Rao, "Role of a heterotrophic Paenibacillus polymyxa bacteria in the bio-flotation of some sulfide minerals," Minerals & Metallurgical Processing, Vol. 16, pp. 35-41. (1999).
25. I.A.H., Schneider, M., Mistra, and R.W., Smith, "Bioflocculation of fine suspensions by Candida parasilosis and its sonication productions," Reagents for Better Metallurgy, P.S. Mulukutla, ed., TMS, Warrendale, pp. 197-208. (1994)
26. A.M., Raichur, M., Misra, S.A., Davis, and R.W.Smith "Flocculation of fine coal using synthetic and biologically derived flocculants," Minerals & Metallurgical Processing, Vol. 14, No. 1, pp. 22-26. (1997).
27. N. A. Abdel-Khalek, K. A. Selim, Samah S. Abdallah, K. E. Yassin, "Bioflotation of low Grade Egyptian Iron Ore using *Brevundimonas diminuta* Bacteria: Phosphorus removal" 2013, Elixir Bio Technology, 63, 18666-18760.
28. N. A. Abdel-Khalek, Samah M. El-Sayed, K. A. Selim, H. H. El-Hendawy, Reham M. Elbaz, "Interaction between kaolinite and *Staphylococcus gallinarum*bacteria", Elixir Bio Technology, 67,2014, 21825-21830.
29. N. A. Abdel-Khalek, K. A. Selim K. E. Yassin, ,Samah S. Abdallah''Bio flotation of Egyptian Phosphate using *Desulfovibrio desulfuricans* Bacteria"., Journal of Mining World Express Volume 4 Issue 1, 2015
30. Nagui A. Abdel-Khalek, K. A. Selim and Samah S. Abdallah, ''Characterization of *Bacillus cereus* Isolated From Egyptian Iron Ore Surface". Elixir Bio Technology, 81, 2015, 32011-32015
31. E.; Dziubakiewicz K.; Hrynkiewiczb, M. Walczykc, B. Buszewskia "Study of charge distribution on the surface of bio-colloids". Colloids Surf. B: Biointerfaces, 104, 122-12. (2013)
32. W., Achouak F., Thomas, T., Heulin, "physico-chemical surface properties of rhizobacteria and their adhesion to rice roots". Colloides Surf. B Bio-interfaces 3, 131-137. (1994)
33. Nagui A. Abdel-Khalek, Khaled A. Selim, Ahmed Amir, Mohsen M. Farahat, Hoda H. El-Hendawy and Reham M. Elbaz, "Enhancement of Selective Bioflocculation of (Quartz-Pyrolusite-Hematite) System Using Paenibacillus polymyxa Bacteria", Elixir Bio Technology, 89, 2015, 36712-36717.

34. L.M.S. Mesquita, F.F. de.; Lins, M.L. Torem, " Interaction of a hydrophobic bacterium strain in a hematite– quartz flotation system". Int. J. Miner. Process., 71, 31-44. (2003)
35. T.J. Silhavy, D Kahne, S. Walker, "The bacterial cell envelope. Cold Spring Harb Perspect" Biol., doi: 10.1101/cshperspect.a000414, abr. (2010).
36. Voei, 0., Voei, J G. Bio-chemistry 2 ed., New York, John Willey & sons, Inc. Yang. (1995)
37. K.A. Natarajan Deo Namita "Studies on interaction of Paenbacillus polymyxa with iron ore minerals in relation to beneficiation" Int.J.Mineral Process 55, 41-60. (1998)
38. Dobias "Surfactant adsorption on minerals related to flotation and bonding series" vol 56, verlage Berlin, p9. (1984)
39. S.K. Mishra, "The Electrokinetics of Apatite and Calcite in Inorganic Electrolyte Environment", Int. J. Miner. Process., 5,69-83.(1978)
40. J. Rubio J. A..Kitchener, "The mechanism of adsorption of polyethylene oxide flocculent on silica", J. Colloid Interface Sci., 57, (1), 132–142.(1976)
41. A.G Merma , M.L., Torem .J.V., Morán, M.B.M Monte,. "On the fundamental aspects of apatite and quartz flotation using a Gram positive strain as a bio-reagent". Miner. Eng., 48, 61-67. (2013)
42. A.E. Botero, M.L. Torem, , L.M.S. Mesquita "Surface chemistry fundamentals of biosorption of Rhodococcus opacus and its effect in calcite and magnesite flotation". Miner. Eng., 21, 83-92. (2008)
43. N. Deo,. K.A Natarajan,. "Interaction of Bacillus polymyxa with some oxides minerals with reference to mineral beneficiation and environmental control". Miner. Eng., 10, 1339–1354.(1997)
44. W.D. Lambert, L. Du, Y. Ma, V. Loha, Burapatana, V. Pro kop, A. Tanner R.D., N.B., Pamment
"The effect of pH on the foam fractionation of β-glucosidase a nd cellulose" Bio-resource Technology, 87, pp. 247–
253, 2003
45. R. M. F., Lima, P. R. G Brandao,. and A. E. C. Peres, "The infrared spectra of amine collectors used in the flotation of iron ores", Miner. Eng., 18, 267–273.(2005)
46. S. Montes, and G. Atenas,." Hematite floatability mechanism utilizing tetradecylammonium chloride collector", Miner. Eng., 18, 1032–1036. (2005)
47. M. B., Geraldo, A., Batisteli, E. C. Antonio, and B.,Peres, "Residual amine in iron ore flotation", Miner. Eng., 21, 873–876.(2008)
48. D., Mowla, G Karimi and K.. Ostadnezhad, "Removal of hematite from silica sand ore by reverse flotation technique", Sep. Purif. Technol., 58, 419–423.(2008)
49. J. S., Laskowski Q.Liu, and N. J.Bolin, "Polysaccharides in flotation of sulphides, Part I. Adsorption of polysaccharides onto mineral surface", Int. J. Miner. Process., 33, 223–234. (1991)
50. J. S., Laskowski, Q.Liu, and C. T.O'Connor, "Current understanding of mechanism of polysaccharides adsorption at the mineral aqueous solution interface", Int. J. Miner. Process., 89, 59–68. (2007)
51. O., Liu, Y. Zhang, and J. S.. Laskowski, "The adsorption of polysaccharides onto mineral surface: an acid/base interaction", Int. J. Miner. Process., 60, 229–245. (2000).