Interaction of Microorganisms in a Hematite-Quartz Flotation System

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ABSTRACT

The increasing world demand for mineral raw materials has led to the exploitation of low-grade ores. This fact associated with more rigorous specification of concentrates, hard environmental legislation and a necessity to achieve lower operating costs has led to numerous investigations aimed at finding better processing techniques and more effective flotation reagents. Recent literature has showed microorganisms can be used as flotation reagents.

In the present study, a microorganism has been investigated as a flotation reagent for a hematite - quartz system. Such microorganisms were isolated from the surface of iron ore. The study was based on zeta potential, adhesion and adsorption measurements as well as micro-flotation experiments in absence and presence of microorganisms. Microbe-mineral interactions resulted in significant changes on mineral surfaces. The changes in zeta potential, adsorption, adhesion and flotation behavior of hematite and quartz particles after microbial interaction are discussed.

Key words: bio-flotation, microorganisms, hematite, quartz.

INTRODUCTION

Bio-processing techniques possess attractive features for treating complex ores. Additionally, compared to conventional inorganic reagents, biologically derived secretions are non-toxic and environmentally safe. Using microorganisms in mineral processing is due to special interaction of these microorganisms with the minerals. Interaction of microorganisms with minerals such as hematite, quartz…etc brought about significant surface chemical changes on minerals surfaces where rendered some more hydrophobic and others more hydrophilic after biotreatment (Abdel-Khalek and Farrah, 2004; Deo and Natarjan 1998). The separation of minerals by bio-flotation is governed by selective adhesion of microbial cells or it's by products onto minerals surfaces. In general bacterial adhesion can be explained by surface thermodynamics and extended DLVO theory (Sharma and Rao 1999). Bio-flotation process may be defined as one in which microorganism act as collectors or modifiers, making possible the selective separation of minerals. This concept of using microbes as flotation reagent is recent (Smith and Misra, 1993; Rao et al., 2010). Microorganism can modify the mineral surface either directly or

indirectly. The direct mechanism involves adhesion of cells to mineral particles, while indirect mechanism refers to the biological reagents such as extracted metabolites acting as surface active reagents (Sharma and Rao 1999) or as soluble fractions of microorganism derived from their rupture (Schneider et al, 1994, Raichur et al 1997). Presence of functional non polar groups (hydrocarbon chains) and polar groups (carboxyl, hydroxyl, phosphate ….etc.) at the microbial cellular or metabolic products lend the microbial culture similar characteristics of surfactant molecules, so it is possible to use microbial species as flotation reagents in selective separation for several systems such as pyrite – coal (Raichur et al 1996), hematite – quartz and corundum – quartz (Deo and Natarajan 1998), apatite – dolomite (Abdel-Khalek et al., 2009a, 2009b, 2007; El-Mahdy et al., 2009, 2007; Zheng et al 1998), and chalcopyrite – pyrite (Sharma et al 2000). Recently, these applications in addition to other microbial-mineral systems have been reviewed by Rao and co-workers (2010). Invariably, these researches have observed that the interaction between microorganism and mineral particle led to significant changes in the chemistry of the surface mineral. Some types of bacteria such as paenibacillus polymyxa secrets exopolysaccharides, protein and several organic acids such as acetic, formic and oxalic acids. The structure of bacterial cell wall plays a significant role in bacterial adhesion to mineral substrates, peptidoglycan accounting to 40 – 90% of the dry weight of the cell wall is covalently linked to other macromolecules constituent which include several types of polysaccharides and polyphosphates polymers known as teichoic acid (Stanier et al 1993).Also, the cell wall of a bacterium can be considered to be a layer of micro porous ion exchanger, having the additional attribute of containing non-ionic functional groups capable of complexing with metal cations. Recently, development in biotechnology has paved the way for development of alternative mineral beneficiation techniques. Microbe- mineral interactions yield results that are of relevance to various applications:

1- Adhesion of microorganisms to mineral substrates resulting in bio film formation
2- Bio-catalyzed oxidation, reduction, complexation and precipitation reactions,
3- Reactions of bacterial cells and metabolic products with different mineral constituents in an ore matrix.

The result of such biological processes is formation and conversion of various mineral forms, surface modification, and selective dissolution of mineral constituents and bio-accumulation of dissolved metal ions. Mineral surface hydrophobicity itself can be brought about by controlled microbe mineral interactions metabolic products as well as the bacterial cell components. It is well established from previous study that either paenibacillus polymyxa or its metabolite renders the hematite and corundum surface hydrophilic (Deo and Natarajan 1997). However, similar interactions make quartz surface hydrophobic. Such difference in surface properties has been utilized to efficiently separate quartz from hematite and alumina (Deo and Natarajan 1997b). As mentioned above, the mechanisms responsible for bio-mineral beneficiation are not fully known. Further, it will be helpful to identify various physicochemical and biochemical parameters that can affect microbial induced beneficiation processes. In this work interaction between microorganisms and hematite or quartz was investigated.

EXPERIMENTAL TECHNIQUES

Materials

Samples of single minerals of hematite (Fe_2O_3), quartz (SiO_2) 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

Methods

Microorganism Growing and Isolation

28 gm of bacterial medium consisting of peptone, beef-extracted, NaCl and mycological Agar was dissolved in 1L of bi-distilled water. The solution was autoclaved at 120^{o}C, coiled and poured in Petri dish for solidification. After that, about 0.5 gm from the autoclaved solid was suspended in 100 ml of bi-distilled water, 1 ml of solution was poured on agar plate and then incubated at a temperature of 37^{o}C for 24 – 48 hours. Appearing different colored spots indicated the presence of different types of microorganisms. The microorganisms were grown in a bacterial medium without agar and incubated for 24 hr at 37°C.

The bacterial population can be determined by measuring the turbidity or the optical density of the bacterial suspension using a UV visible spectrophotometer (Lambda 3B, Perkin-Elmer). Because the turbidity is directly proportional to the number of cells, this property was used as an indicator for bacterial concentration. The cells suspended in the suspension interrupt the passage of light, allowing less light to reach the photoelectric cell and the amount of light transmitted through the suspension is measured as percentage transmission (Sharma, 2001). The turbidity for cell suspension is measured at a wavelength of 550 μm against clear water as reference, at which the 0.01 reading is equivalent to 10^6 cells mL^{-1}. Five micro-liters of bacteria was centrifuged at 15 000 rev min^{-1} and rinsed three times with double distilled water and thereafter, diluted in 100 mL double distilled water.

Four different types of microorganisms were isolated, grown, counted and their efficiency was screened using a laser particle sizer analyzer. Based on the later test, one of these four types of microorganisms (which showed the best efficiency) has been selected to conduct this study. Microbiological investigations indicated that this type of bacterium (identified as Corynebacterium-diphtheriae-intermedius) has 5 X 10^7 cells mL^{-1} (Abdel-Khalek and Farrah, 2004; Elmahdy, 2004). These values were used in calculating the concentration of bacteria in the experiments.

Measuring Selectivity of Microorganisms to Mineral Surface

A laser particle size analyzer (FRITSCH Model Analyst 22) was employed for measuring size analysis of single minerals before and after treatment with microorganism. Fixed

volume 10 ml of each microorganism was conditioned with one gram of each mineral for 60 minutes before recording the change in size distribution.

Adhesion Measurements

Adhesion of the microorganism 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 cellular suspension with a fixed initial concentration of the microorganism, 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.

Chemical Analysis

Routine chemical analysis of samples was conducted using standard methods. Iron oxide was determined by atomic absorption technique using "Perkin- Elmer" Atomic Absorption model "A Analyst 200". Silica content was determined gravimetrically. Meanwhile complete chemical analysis of the samples was conducted using "Philips" X-ray fluorescence (XRF).

Zeta Potential Measurements:

A laser Zeta Meter 'Malvern Instruments Model Zeta Sizer 2000' was used for zeta potential measurements. 0.01 g of ground sample was placed in 50 ml double distilled water with definite concentration of the microorganism at fixed ionic strength of $2x10^{-2}$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^{o}C. Five measurements were taken and the average was reported as the measured zeta potential.

Adsorption Measurements

The adsorption density of microorganism on the mineral surface was determined by adding 1 g dry sample of hematite or quartz to the microorganism solutions (50 cm^3) in a 100 cm^3 volumetric flask. The mixture was shacked for 60 minutes using a shaker (Model JANKE & KUNKEL Type Vx10). The pH was adjusted to the desired values using HCl and NaOH, after which the samples were centrifuged at 15000 rpm for 15 min to separate supernatant from the settled fraction. The total organic carbon content

(residual concentration) in the supernatant was determined using a 'Phoenix 8000' Total Carbon Analyzer". The average of three readings was taken as a measure for the residual concentration of organic carbon. All the experiments were done at room temperature (~ 25 ^{o}C).

FTIR Measurements

Infrared absorption spectra were recorded for hematite; quartz and microorganisms before and after interactions using Fourier transform infrared spectrometer (Model FT/IR 6300). After interaction with microorganism, the mineral samples were thoroughly washed using double distilled water and vacuum dried. The KBr pellet technique was used to record the spectra. The difference spectrum was obtained by subtracting the spectrum of untreated minerals from that of interacted minerals.

Flotation Experiments

A series of bench-scale flotation experiments were conducted using a modified Halimond tube with 150 mL capacity. Samples of hematite and quartz were conditioned with certain concentration of the microorganism at different pH values on a horizontal shaker for certain conditioning time. In carrying out experiments 1 gram (of single minerals or their binary mixture as well as natural ore) was first conditioned with 135 mL solution for 1h with certain concentration of microorganism at different pH values, adjusted with dilute solutions of NaOH and HCl. The flotation was conducted for 5 minutes under an air follow rate of 0.7 cm^{-3}/min. Both of the float and sink fractions were collected, dried, weighted, and analyzed.

RESULTS AND DISCUSSION

Selectivity of Microorganism to Mineral Surface

The change in size distribution of single mineral samples, hematite or quartz, after its treatment with the microorganism was taken as a measure for the selectivity of adsorption. Successful adsorption of the microorganism will cause, therefore, a degree of aggregation (or dispersion) for mineral particles leading to a change in their size distribution. The larger the change in size distribution, the more selective the microorganism to the mineral surface. This techniques was successfully used to screen different microorganisms for selective adhesion onto apatite or dolomite surfaces (El-Mahdy, 2004; Abdel-Khalek and Farrah, 2004; Boice, 2000). The change in size distribution of each single mineral of hematite and quartz after treatment with the microorganism (M.O.) was recorded, the results of which are depicted in Figures (1-2). These results show different degrees of variation in the size distribution of samples after their treatment with the microorganism. The microorganism showed, interestingly, the largest degree of selectivity for hematite. However, a slight degree of dispersion for

quartz particles was noticed. Based on these analyses, it has been decided to use this microorganism in this study.

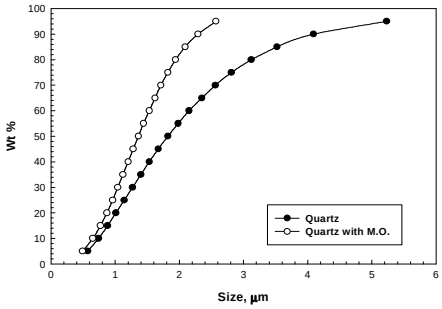


Figure (1) Size distribution of quartz with M.O.

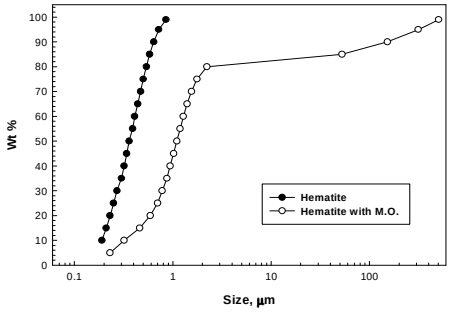


Figure (2) Size distribution of hematite with M.O.

Surface Properties of Single Minerals and Microorganisms

In a trial to understand the effect of microorganisms in enhancing bio-flotation of single minerals, zeta potential of each single mineral–microorganism system was studied. The zeta potential measurements of the microorganisms alone as well as for each single mineral (hematite – quartz) in absence and presence of these microorganisms have been conducted. These measurements were performed at constant ionic strength of 2.0 $x10^{-2}$ M NaCl. Figure (3) illustrates the zeta potential of single minerals of hematite and quartz while Figure (4) shows the zeta potential of the microorganism alone. The latter depicts that such microorganism is, more or less, hydrophobic in nature where its zeta potential values showed a minor change (from -2 mv to -6 mv) over the entire range of pH (from pH 2 to pH 12) .

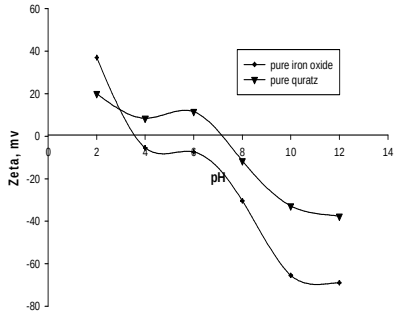


Fig.3: Zeta potential of hematite and quartz

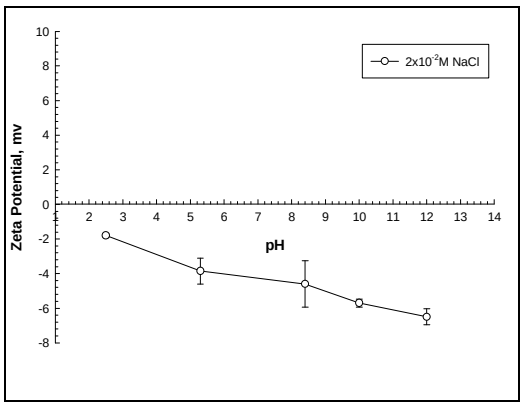


Fig. 4: zeta potential of MO

On the other hand, Figures 5 and 6 show the effect of fixed concentration of microorganism (1.0 X 10^8 cells) on the zeta potential of single minerals. It can be seen that the iso-electric points for both minerals disappeared after their treatment with the microorganism. The surface of minerals became more negative with increasing the concentration of the microorganism (results not shown). The surface of hematite becomes more negative in the acidic medium till pH ~ 8.0, after which the zeta potential starts to become less negative to be close from that of the microorganism itself. On the contrary, a little change in zeta potential of quartz was noticed after treatment with microorganism. These results clearly indicate that the nature of surface chemical changes brought about by bacterial interaction could be different with quartz, in comparison to hematite.

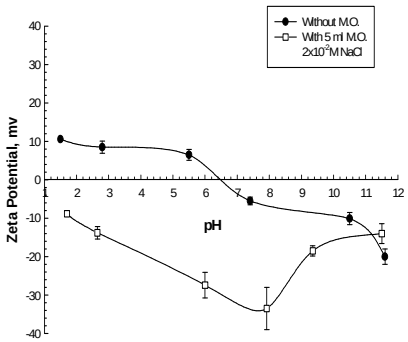


Figure (5). . Zeta potential of hematite in the absence and . presence of M.O

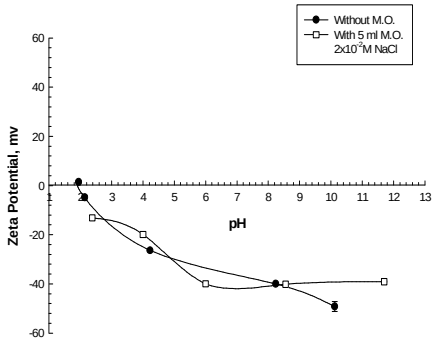


Figure (6). Zeta potential of quartz in the absence and presence of M.O

Adsorption Isotherms of Microorganisms

The adsorption isotherm of the microorganism onto each single mineral was also studied, the results of which are shown in Figure (7). The experiments are performed at pH 6.0. These results indicate that the adsorption density onto hematite and quartz is generally increases with increasing the concentration of the microorganism. Also, the adsorption density at higher concentration of the microorganism for hematite is more than quartz. Such higher bacterial affinity to hematite in comparison to quartz is readily evident. The increased adsorption tendency of bacterial cells onto hematite at pH 6.0 can be attributed to electrostatic forces. Besides electrostatic forces, hydrogen bonding and chemical interaction also play significant roles in bacterial interaction with these minerals. FTIR studies (Deo and Natarajan, 1998) on bacterial cells and minerals before and after interaction have strongly indicated the role of hydrogen bonding and chemical interaction. Such interactions between mineral surface and microorganism are seen to

result in significant surface chemical changes, not only on the cell surfaces but also on the interacted minerals (Deo and Natarajan, 1998).

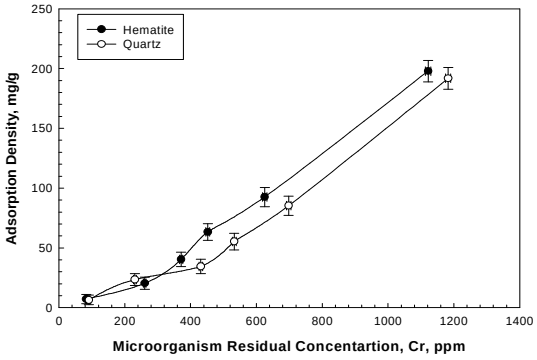


Figure (7) Adsorption isotherms of M.O. onto minerals surfaces

To understand the influence of such bio-modification in enhancing the selectivity of the bio-flotation process and the role of interaction between mineral surface and microorganism, FTIR measurements were conducted for both the microorganism and single minerals. Figure (8), showed the existence of O-H, C-C, CH_2, C-O, C-N and C=O bands in decreasing order in the FTIR of microorganism. These bands reflect the general organic structure of microorganisms which are mainly composed of polysaccharides and lipids (protein). Polysaccharides are defined as high molecular weight carbohydrates containing many of monomeric units connected to one another by a type of covalent bonds referred as a glycosidic bond (Brock et al., 1994).

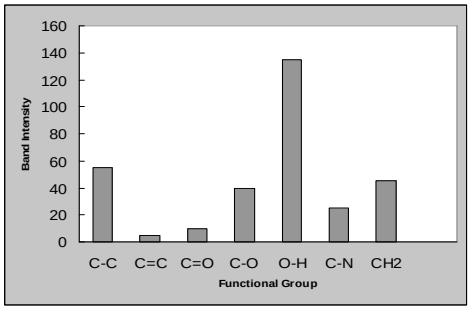
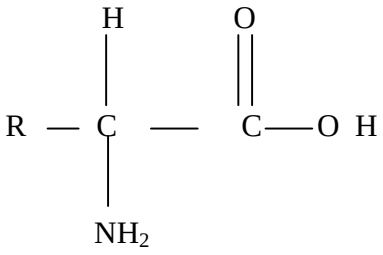


Fig.(8). FTIR of microorganisms

Meanwhile, amino acids are the monomeric units of protein. Most amino acids consist only of carbon, hydrogen, oxygen, and nitrogen. Protein contains two important functional groups, a carboxylic acid group (-COOH) and an amino group ($-NH_2$) as shown in its structure (Brock et al., 1994):



Structure of protein

These carboxylic (-COOH) and amino (-NH_2) groups may explain the presence of C-N, C=O, and OH bonds shown in the FTIR of the microorganism. The concentrations of O-H and C-C are predominant in Figure 8 since these groups represent major constituents in the structure of both polysaccharides and protein. Adsorption of microorganisms onto hematite surfaces can take place first onto their positive site of Fe^{3+} through the OH (of the polysaccharides part) and/or the COOH of both the polysaccharides or the protein fractions of the microorganisms. This is confirmed from the FTIR, shown in Figures (9) and (10), where a band at 3675 cm^{-1} indicated the formation of hydrogen bond after treatment of hematite with microorganisms. Such occupation of the bacteria to some of the positively adsorption sites of hematite leads to a reduction in the zeta potential of its surfaces to be close from that of the microorganism itself. The surface of hematite became therefore, more or less, hydrophobic in nature.

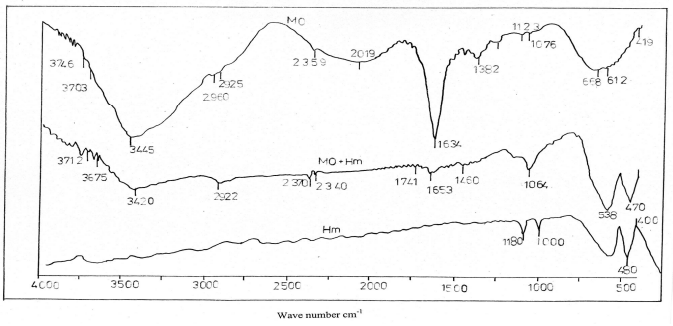


Figure (9): FTIR for microorganism, hematite and its treated surface with MO

In the mean time, the highly negatively charged quartz particles due to the formation of silanol groups might hinder the adsorption of bacteria. Instead, the adsorption of bacteria in such a case can be proceeded through their positively amino (-NH_2) groups that exist in the protein fraction, leaving the other negatively functional groups to be directed toward the medium. This also is confirmed from the FTIR, shown in Figure (10), which showed a band for hydrogen bond formation at 3675 cm^{-1} after adsorption of the microorganism onto quartz surface.

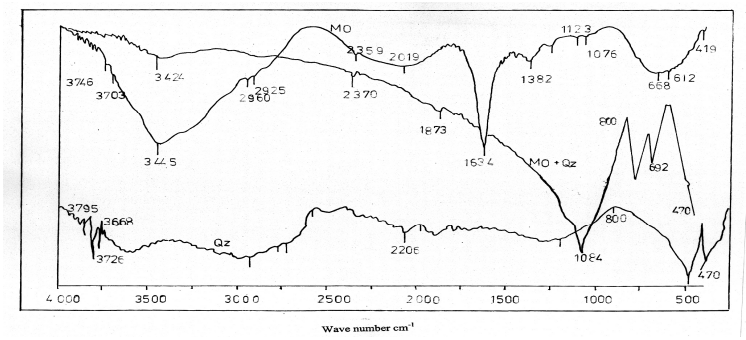


Figure (10)**:** FTIR for microorganism, quartz and its treated surface with MO

Microorganism Adhesion onto Minerals Surfaces

Figure 11 shows the adhesion of microorganism onto the surface of single minerals at different pH. The results confirmed that the adhesion of microorganism onto hematite surface is higher than that onto quartz surface all over the pH range. Other authors indicated that the adhesion of mycobacterium onto hematite surface increases with increasing the pH till reaches the maximum at pH 8 and after which it starts to decrease (Shashikala and Raichur 2002).

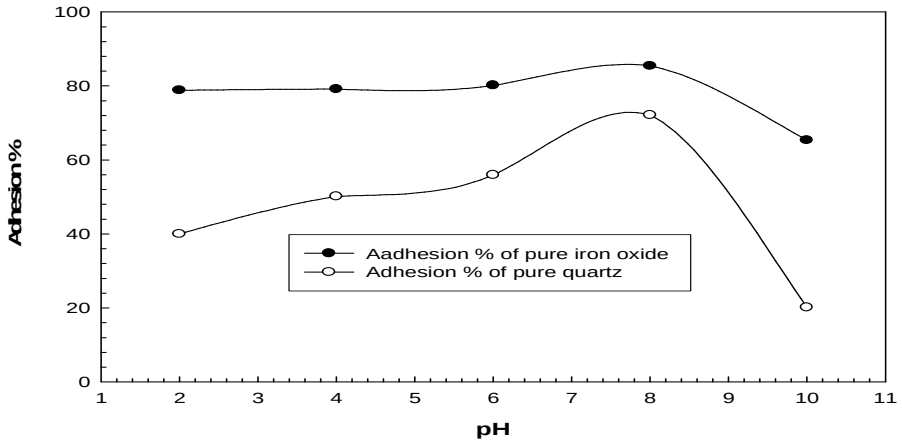


Fig.11: Adhesion of MO onto single minerals

Flotation of Hematite -Quartz System with the Microorganisms

The floatability of hematite and quartz as a function of pH is presented in Figure 12. It can be observed that, for both minerals, there is an increase in floatability with the increase in pH. Figure (12) illustrates a good floatability for hematite, about 30% floated at pH 8 in comparison to hematite. The best floatability of quartz (~ 12 % floated) was obtained in the pH range 8 - 12. These results are in accordance with zeta potential (Figure 5) and adhesion (Figure 11) measurements shown before which depicted different response of interaction between microorganism and each single minerals, Figure (12).

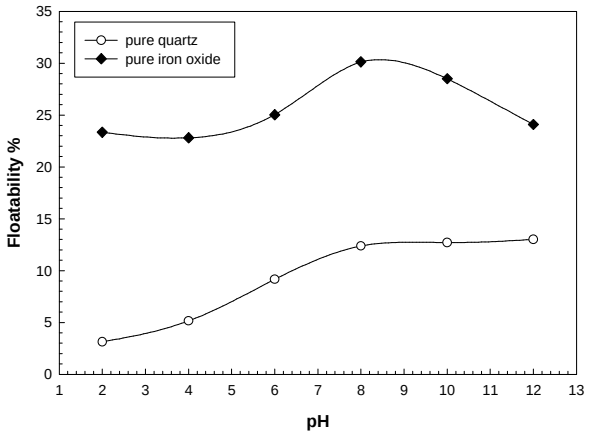


Figure (12) Effect of microorganism on the Floatability of pure minerals

Separation of a binary mixture containing about 89.5 % Fe_2O_3 and 10.5 % SiO_2 gave a concentrate assaying ~ 97.1 % Fe_2O_3 and 4.4 % $SiO_2$with a recovery of ~61 %. Applying the same conditions (1.0 $x10^8$ cells at pH 8) on a natural iron ore ground to 53 microns (containing ~ 55.8 % Fe_2O_3 and 40 % SiO_2) gave a concentrate of high grade (95.8 % Fe_2O_3 and 2.0 % SiO_2 with ~ 50.4 % recovery). These results indicate that application of such bio-beneficiation process represents promising techniques for treatment of fine iron ores.

Conclusions

- The results showed a strong interaction between microorganisms and mineral particles, especially with hematite. Adhesion, adsorption, FTIR and zeta potential measurements showed the presence of microorganisms have better affinity to hematite mineral surfaces. These results show different degrees of variation in the size distribution of single minerals after their treatment with the microorganism. The microorganism showed, interestingly, the largest degree of selectivity for

hematite. Also, the results of zeta potential showed that the iso-electric points for both minerals disappeared after their treatment with the microorganism. However, the nature of surface chemical changes brought about by bacterial interaction could be different with quartz, in comparison to hematite. Higher bacterial affinity to hematite in comparison to quartz is readily evident from the results of adhesion of microorganism onto mineral surface where higher values for adhesion with hematite surface are noticed, all over the pH range, in comparison to quartz surface .

- At constant ionic strength, the change in the surface properties of mineral showed a high influence for pH on the adhesion process, and both physical and chemical adsorption would be involved on particle–cell interactions.

- The selectivity of hematite flotation against quartz was observed in the microflotation tests of a synthetic mineral mix. The results show the potentiality for using microorganisms as a collector at pH 8 in flotation systems where concentrates of high Fe_2O_3 % and low % SiO_2 can be obtained from low grade iron ores.

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