Bioflotation of low Grade Egyptian Iron ore using *Brevundimonas diminuta* Bacteria: Phosphorus removal

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**ABSTRACT**

The bio-sorption process concern the mineral response to the bacterium presence, which is essentially interplay between microorganism and the physicochemical properties of the mineral surface. The adhesion of microorganisms to minerals results in alteration of surface chemistry of minerals relevant to beneficiation process due to a consequence of the formation of a biofilm on the surface or bio-catalyzed surface oxidation or reduction products. Low grade of finely disseminated iron ores have become the main sources of raw iron ores in many countries with the depletion of high grade deposits. In this paper, the amenability of utilization of *Brevundimonas diminuta* isolated and adapted on surfaces of iron and phosphate ores, as flotation reagents for separating the harmful impurities such as phosphorus in the bio-flotation of iron oxide-apatite minerals system is studied. The effect of micro-organism on the surface properties of the two single minerals has been studied through zeta potential and adhesion measurements as well as micro-flotation tests. The effect of pH of the medium on the surface properties and flotation behaviour of each single mineral is determined. Flotation of binary mixtures of haematite-apatite as well as natural iron ore has also been performed at different operating parameters.

**Keywords**


**Introduction**

The new millennium rightly belongs to biotechnology, and rapid progress in minerals processing based on biological principles is just around the corner. Minerals exist in nature abundantly in the earth's crust in the form of ore bodies, i.e., in association with other minerals. Conventionally, physicochemical methods are used in mineral processing, but recently, biotechnological 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 biological processes are becoming more attractive in mineral processing due to their lower operating costs and their possible applications to treat difficult to beneficite low grade complex ores, [Solonenken *et al.*, 1976; Somasundaran *et al.*, 998; Rao *et al.*, 2010]. Egyptian iron ores present in Aswan locality are associated with different gangue minerals. The main gangue minerals are silica in addition to minor amounts of phosphorus in the form of apatite. These impurities are finely disseminated in the matrix that need fine grinding to achieve a considerable degree of liberation. The recovery of valuable mineral from such low grade ores using present technology is prohibitively expensive due to high energy and capital costs. It is imperative to develop novel economically more efficient and environmentally benign methods of flotation and waste processing, exploiting the intriguing and exciting ability of bacteria to selectively modify the surface properties of solids. Several recent investigations revealed that adapted bacteria associated with ore deposits can selectively be attached to minerals, thereby essentially modifying the surface properties relevant to bio-flotation, [Patra and Natarajan, 2008; Pradhan *et al.*, 2006].

Several studies have been carried out on the use of microorganisms act as bio-reagents and may induce hydrophobic properties once they can adhere selectively on mineral surface, [Sharma and Hanumantha, 2003]. Physico-chemical properties of microbial cell surface influence their adhesion behaviour, therefore the physico-chemical characterization of microbial cell is essential in order to fully understand and control the bio-mineral beneficiation process. There is an urgent need for developing basic knowledge that would underpin biotechnological innovations in the natural resource processing technologies that deliver competitive solutions. Microbes or microbial fat and secreted metabolites can have specific interactions with minerals. Such interactions of microbes and their agents with minerals can be indirect, with biological products acting as surface-active agents, or direct due to microbial adhesion or attachment to particles bringing out surface modification. Both types of interactions can lead to alteration of mineral hydrophobicity. The two major factors, which contribute to selectivity in bio-flotation process, are selective adhesion of microbial cells on the mineral surface, which forms a bio-film and causes alteration on the mineral surface, and secondly, selective interaction of attached microbial cells with the added chemicals. Thus, the bacterial adhesion plays a critical role in both bio-leaching and bio-beneficiation processes. The bio-modification of mineral surfaces involves the complex action of microorganism on the mineral surface. There are three different mechanisms by means of which the bio-modification can occur, [Botero *et al.*, 2007]:

1. Attachment of microbial cells to the solid substrate.
2. Oxidation reactions.
3. Adsorption and/or chemical reaction with the metabolite products.
Application of bio-reagents as collectors involves several fundamental aspects, such as surface charge, presence of specific hydrophobic groups, and polymers compounds which deeply affect their adsorption to the mineral [Pearse, 2005; Smith and Miettinen, 2006]. Therefore, this paper aims at studying the role of bacteria in bio-beneficiation of iron oxide-apatite system in relation to Egyptian iron ore. The role of bacteria on the surface properties of the two single minerals has been studied through zeta potential, adsorption and adhesion measurements as well as micro-flotation tests. Flotation of binary mixtures of haematiteapatite as well as natural iron ore has also been performed.

**Experimental Techniques**

**Materials**

Samples of single minerals of haematite (Fe₂O₃), apatite (P₂O₅) were delivered from 'Wards' Company, USA. The purity (99.9 %) of the samples was confirmed using XRF. The –200 mesh fractions were used in adsorption and flotation studies. Analytical grade HCl and NaOH, from Aldrich, were used for pH regulations. Natural iron ore was collected from Aswan Locality, Egypt.

**Methods**

**Bacterial Growing and Isolation**

28 gm of bacterial medium consisting of peptone, beef-extract, NaCl and mycological Agar was dissolved in 1L of bi-distilled water. The solution was autoclaved at 120°C, cooled and poured in Petri dish for solidification. After that, about 0.5 gm of sample 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°C for 24 – 48 hours. Appearing different coloured spots indicated the presence of different types of bacteria. The bacterial isolates were grown in a nutrient broth 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 photoreceiver and the amount of light transmitted through the suspension is measured as percentage transmission. The bacterial concentration. The cells suspended in the suspension interrupt the passage of light, allowing less light to reach the photoreceiver and the amount of light transmitted through the suspension is measured as percentage transmission. 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 106 cells mL⁻¹.

**Measuring Selectivity of Brevundimonas diminuta 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 bacteria. Fixed volume 10 ml of *Brevundimonas diminuta* was conditioned with one gram of each mineral for 60 minutes before recording the change in size distribution.

**Adhesion Measurements**

Adhesion of *Brevundimonas diminuta* 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 bacterial isolate, 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". Phosphorus content was determined using spectroscopic technique. Meanwhile complete chemical analysis of 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. 0.05 g of ground sample was placed in 50 ml double distilled water with definite concentration of the bacterial isolate at fixed ionic strength of 2 X 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°C ± 2). Five measurements were taken and the average was reported as the measured zeta potential.

**Adsorption Measurements**

The adsorption density of bacterial isolate on the mineral surface was determined by adding 1 g dry sample of haematite or apatite to the bacterial solutions (50 cm³) in a 100 cm³ volumetric flask. The mixture was shaken for 15 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 12000 rpm for 20 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°C ± 2).

**FTIR Measurements**

Infrared absorption spectra were recorded for haematite, apatite and bacteria before and after interactions using Fourier Transform Infrared Spectrometer (Model FT/IR 6300). After interaction with bacteria, the mineral samples were thoroughly washed using double distilled water and vacuum dried. The KBr pellet technique was used to record the spectra.

**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 (9 x 10¹⁰ 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 flow rate of 0.7 cm³/min. Both floated and sink fractions were collected, dried, weighted, and analyzed.

**Results and discussion**

**Selectivity of Microorganism to Minerals’ Surfaces**

The change in size distribution of single pure mineral samples, iron oxide and apatite after its treatment with *Brevundimonas diminuta* was taken as a measure for the
selectivity. Successful adsorption of the bacterial isolate will cause, therefore, a degree of aggregation (or dispersion) for mineral particles leading to a change in their size distribution. This technique was successfully used to screen different microorganisms for selective adhesion onto apatite or iron oxide surfaces, [Abdel-Khalek et al., 2009a; Abdel-Khalek et al., 2009b; Abdel-Khalek and Farrah, 2004]. The results obtained in Figures 1 and 2 show different degrees of variation in the size distribution of samples after their treatment with bacteria which indicates the largest degree of selectivity of *Brevundimonas diminuta* for iron oxide more than apatite. However, a slight degree of dispersion for apatite particles was noticed.

**Surface Properties of Single Minerals and Brevundimonas diminuta bacteria**

Zeta potential of each single mineral–bacteria system was studied. Measurement of zeta potential of *Brevundimonas diminuta* alone as well as for each single mineral, iron oxide and apatite, in absence and presence of bacteria has been performed, Figures 4-6. These measurements were done at constant ionic strength of 2 ×10^{-2} M NaCl. These results indicate that NaCl acts as indifferent electrolyte for iron oxide and apatite. Generally, the value and sign of zeta potential depend upon the pH of the medium, indicating that both H^{+} and OH^{-} are potential determining ions for each mineral. The results, also, showed that the electronegativity of zeta potential increases gradually with increasing the pH value. It can, also, be noticed that the zeta potential increases in magnitude with decreasing ionic strength of counter ions (NaCl), due to the increase in thickness of the diffuse layer, due to columbic interactions which is a dominant role in adsorption process, [Somasundaran and Moudgil, 1988]. Figure 4 illustrates the zeta potential the *Brevundimonas diminuta* in which the values of zeta potential are varied from (+5 to -20 mv) over the entire range of pH (2.0-12). This means that such type of bacterial isolate is hydrophobic in nature with iso-electric point (IEP) corresponding to pH of 2. These results are in agreement with literature [Deo and Natarajan, 1998; Deo and Natarajan, 1997].

![Fig.1. Size distribution of iron oxide before and after treatment with *Brevundimonas Diminuta*](image1)

![Fig.2. Size distribution of apatite before and after treatment with *Brevundimonas Diminuta*](image2)

**Bacterial Adhesion onto Minerals’ Surfaces**

Figure 3 shows the adhesion of bacteria onto the surface of single minerals over a wide range of pH (1-12). The results showed that, the *Brevundimonas diminuta* can be adhered onto both minerals’ surfaces with a higher affinity to iron oxide surface. Also, it is noticed that the highest values for adhesion for both iron oxide and apatite are obtained at pH from 1-3 while a gradual decrease in adhesion values is occurred starting from pH 4 till reaching pH 11.

![Fig.3. Adhesion of *Brevundimonas diminuta* onto Minerals’ Surfaces](image3)

![Fig.4. Zeta potential of *Brevundimonas diminuta*](image4)

Meanwhile, Figure 5 depicts the zeta potential of apatite as a function of pH before and after interaction with bacteria. These results show that the iso-electric point (IEP) of apatite corresponds to pH of about 4.8. However, conditioning of apatite with bacteria resulted in a displacement for the IEP of apatite to about 2.6, i.e., close to that of *Brevundimonas diminuta* itself. Moreover, the values of zeta potential of apatite after interaction with bacteria became less negative over the entire pH range. The hydrophobic effect of *Brevundimonas diminuta* is appeared at pH range (4.5-8)

![Fig.5. Zeta potential of apatite before and after interaction with *Brevundimonas diminuta*](image5)
On the other hand, Figure 6 depicts the zeta potential of iron oxide as a function of pH before and after interaction with bacteria. The iso-electric point (IEP) of iron oxide corresponds to pH of about 6.3. Conditioning of iron oxide with bacteria resulted in a significant displacement for the IEP of iron oxide to that of bacteria, i.e., at about pH 1.9. Interestingly, the values of zeta potential were seen to be shifted to lower values in the entire pH range (1-11), i.e., the surface of iron oxide became more hydrophobic and close to those of bacterial isolate of *Brevundimonas diminuta* from +5mv to -20mv as shown before in Fig. 4.

**Fig.6. Zeta potential of iron oxide before and after interaction with *Brevundimonas diminuta***

**Adsorption Density of *Brevundimonas diminuta* onto Minerals’ surfaces**

The adsorption density of bacteria onto each single mineral was also studied, the results of which are shown in Figure 7. The experiments are performed at pH range (3-4). These results indicate that the adsorption density is generally increases with increasing the concentration of bacteria over the entire range of concentration. Moreover, at fixed concentration of *Brevundimonas diminuta*, the bacteria adsorption onto iron oxide is generally greater than that occurred onto apatite mineral. Such higher bacterial affinity to iron oxide mineral surface in comparison to that of apatite is readily evident. The increased adsorption tendency of bacterial isolates of *Brevundimonas diminuta* onto iron oxide can be attributed to electrostatic forces besides hydrogen bonding and chemical interaction. FTIR studies, [Deo and Natarajan, 1996] on bacterial cells and minerals before and after interaction have strongly indicated the role of hydrogen bonding and chemical interaction. Therefore, the shift in iso-electric points of hematite and apatite can be explained based on these surface interactions. Such interactions between mineral surface and microorganisms are seen to result in significant surface chemical changes, not only on the cell surfaces but also on the interacted minerals.

**FTIR Measurements:**

To understand the role of interaction between mineral surface and *Brevundimonas diminuta*, FTIR measurements were conducted for the bacteria and single minerals. FTIR of bacteria showed the existence of O-H, C-C, CH2, C-O, C-N and C=O bands in decreasing order, Figures 8 and 9. These bands reflect the general organic structure of bacteria which are mainly composed of polysaccharides and lipids (protein). Polysaccharides are defined from their hydroxyl bands at 3600-3200 cm\(^{-1}\) and carboxyl group bands at 1210-1740 cm\(^{-1}\) whereas the protein is characterized by its amino group bands at 3460 – 3150 cm\(^{-1}\) and 1650 – 1500 cm\(^{-1}\) respectively.

**Fig.7. Adsorption of *Brevundimonas diminuta* onto surfaces of apatite and iron oxide minerals**

**Bioflotation of Binary Mixtures**

The amenability of applying *Brevundimonas diminuta*, to be used as the sole flotation reagent, to selectively separate iron oxide and apatite from their binary mixtures was studied. The net results obtained at optimum operating conditions are shown in Tables 1 and 2. The results indicate clearly that on using the studied bacterial isolate for flotation a mixture containing 90 %
by weight iron oxide and 10% by weight apatite gave a concentrate containing 96.4% Fe$_2$O$_3$ and only 0.61% P$_2$O$_5$ with a good recovery of 87.7%. The experiments are performed at pH 3.0 using 9.0 x 10$^{-5}$ cell/ml of bacterial isolate, Table 1.

Table (1) Separation of hematite from apatite in a binary mixture

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Fe$_2$O$_3$ %</th>
<th>Apatite %</th>
<th>% Recovery Fe$_2$O$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed (Binary Mixture)</td>
<td>90</td>
<td>10</td>
<td>87.5</td>
</tr>
</tbody>
</table>

On applying the same conditions on flotation of the natural iron ore, a concentrate containing only 0.28% P$_2$O$_5$ and 95.6% Fe$_2$O$_3$ with 77.8% recovery was obtained, Table 2.

Table (2) Separation of hematite from apatite in natural iron ore sample

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Fe$_2$O$_3$ %</th>
<th>P$_2$O$_5$ %</th>
<th>% Recovery Fe$_2$O$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ml (9.0 x 10$^{-5}$ cell/ml)</td>
<td>95.5</td>
<td>0.28</td>
<td>77.8</td>
</tr>
</tbody>
</table>

| Natural Iron Ore | 70.19 | 1.6 | |

Conclusions

1. The results showed a strong interaction between Brevundimonas diminuta bacteria and minerals’ surfaces, especially with hematite. Adhesion, adsorption, FTIR and zeta potential measurements showed a better affinity of Brevundimonas diminuta to hematite mineral surface rather than apatite.

2. The results of zeta potential showed that the iso-electric points (IEP) for both iron oxide (at pH 6.3) and apatite minerals (at pH 4.8) are significantly displaced to lower values (at pH 1.9 and 2.6 respectively) after their treatment with the bacterial isolates, i.e., the IEP became very close to that of Brevundimonas diminuta.

3. Higher bacterial affinity to hematite in comparison to apatite is readily evident from the results of adsorption and flotation of Brevundimonas diminuta onto mineral surface where higher values for adsorption and flotation with hematite surface are noticed, all over the pH range, in comparison to apatite surface.

4. The selectivity of hematite flotation against apatite was observed in the micro-flotation tests. The results show the potentiality for using Brevundimonas diminuta bacteria as a sole flotation reagent where a concentrate containing 96.4% Fe$_2$O$_3$ and only 0.61% P$_2$O$_5$ with a good recovery of 87.7% could be obtained from a mixture containing 90% by weight iron oxide and 10% by weight apatite.

5. Applying the same conditions on flotation of the natural iron ore yielded a concentrate containing only 0.28% P$_2$O$_5$ and 95.6% Fe$_2$O$_3$ with a recovery of 77.8%.

References