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Role of Interaction between Rhodcoccus Erythropolis Bacteria and (Hematite-Quartz System)

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ABSTRACT

In a world of diminishing resources, current research efforts are often directed to extending the life of existing resources and developing technology to treat resources deemed uneconomic. To this end, biotechnology has been explored as a potential low cost, environmentally benign alternative to many of the current mineral processing techniques. Microorganisms and their metabolites have been successfully applied in the leaching of metals from medium and low grade sulfide minerals for many years. Recent fundamental studies have shown that selected bacteria may also assist in the beneficiation of these minerals through bio-flotation. Interaction between ***Rhodococcus erythropolis*** with minerals such as hematite, and quartz brought about significant surface chemical changes of the minerals surfaces. Quartz was rendered more hydrophilic, while hematite became more hydrophobic after bio-treatment. Different characterization techniques for single minerals and bacteria before and after interaction as scanning electron microscope (SEM), Fourier transform infrared spectrophotometry (FTIR), zeta potential have been done.

1. Introduction

Reserves of high-grade ores are diminishing all over the world at an alarming rate as a result of rapid increase in demands for metals. The recovery of mineral value from the low-grade ores using conventional technologies is prohibitively expensive due to high energy and capital costs. Presently available physico-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. Bio-beneficiation is a process that utilizes microorganisms as surface modifiers to enhance the separation of one mineral from another by flotation or flocculation **(Deo et al., 2001; Natarajan and Deo, 2001; Patra and Natarajan, 2008; Sharma et al., 2003)** Attachment or adsorption of microorganisms to the mineral surfaces is a prerequisite step for most biological activities like bio-corrosion, bioleaching and bio-beneficiation. The attachment of microorganisms to mineral surfaces depends on both the solution conditions (e.g., pH and ionic strength) and the surface properties of the mineral and the microorganism (e.g., zeta potential and hydrophobicity). Although the use of microorganisms in ore leaching is well established, the mechanism of bio-beneficiation is not adequately understood. The micro-organism cell surface is confirmed by functional groups like polymers, peptides, proteins and micolic acids (**Van der Wal et al., 1997)**. These groups must adhere to the mineral surface directly and utilize cell surface associated or extracellular biopolymers to catalyze chemical reactions on the mineral surface (**Chandaphara et al., 2006**). Like traditional reagents, bacteria interact with the mineral surface and gives amphoteric characteristics to it **(Mesquita et al., 2003).**

On the other hand, the presence of functional non-polar groups (hydrocarbon chains) and polar groups (carboxyl, hydroxyl, phosphates) at the microbial cellular surface or metabolic products lend the microbial culture similar characteristics of surfactant molecules. Therefore, the microorganisms can modify the mineral surfaces, either directly or indirectly. The direct mechanism involves adhesion of cells to mineral particles while the indirect mechanism refers to the biological reagents such as excreted metabolites acting as surface-active reagents (**Sharma and Rao, 2003)** or as soluble fractions of the microorganisms derived from their rupture. There are many microorganisms whose cell walls display varying degrees of negative charge and hydrophobicity, such microorganisms may be used as hematite collectors due to they have properties resembling conventional surfactants, If bacterial cells can be adsorbed to the mineral surface similar to a chemical collector, the electrical properties and hydrophobicity of the mineral surface will be correspondingly changed by the microorganisms (**Huief et al. 2013).** ***R. erythropolis***, a nonpathogenic microorganisms found widely in nature, has been widely used for bioremediation of oil-contaminated water and soil (**Carla, 2012**). Oil pollutants were removed and recovered by ***Rhodococcus erythropolis*** through formation of oil bio-floccules (**Chang et al., 2009 and Carla, 2012**).

This paper includes complete characterization of ***Rhodococcus erythropolis*** bacteria to be used in upgrading of difficult to treat Egyptian ores. Also, studying the interaction between single minerals of hematite and quartz and bacteria before and after interaction has been tested.

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2. Materials & Methods

2.1 Mineral preparation

Samples of single minerals of hematite (Fe_2O_3) and silica (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 studies.

2.2. Bacterial strain and growth conditions

Rhodococcus erythropolis bacteria were delivered from the Agricultural Culture Collection of China (ACCC, serial number ACCC10188). It was used as a flotation collector after culturing in medium containing hematite. The composition of this medium was (in g/L water): beef extract 3, peptone 10, NaCl 15 and pure hematite 0.01, pH 7.2. Cultures were grown in 250 ml flasks with continuous shaking at 180 rpm in a water bath held at 30 °C. After 28 h of incubation, the cultures were centrifuged and washed four times with deionized water. The cells were resuspended in 0.01 M NaCl solution, and then stored in a refrigerator at 4°C for direct use in the experiments. The cell concentration was quantified by measuring dry weight, (**Huief et al. 2013).**

2.3. Chemical Analysis

Routine chemical analysis of samples was conducted using standard methods. Iron oxide content was determined using spectroscopic technique. Quartz was determined by Gravimetric method. Meanwhile complete chemical analysis of samples was conducted using "Philips" X-ray fluorescence (XRF) (**Abdel-Khalek *et al.*, 2013 and 2014)**.

2.4. Zeta-potential Measurements

Zeta-potential measurements for ***Rhodococcus erythropolis*** strain, hematite and quartz were carried out on a micro-electrophoresis apparatus Zeta Meter System; NaCl 0.01 M was used as an indifferent electrolyte. The iso-electric point (IEP) of pure hematite and quartz minerals and *R.* erythropolis suspension were evaluated. The evaluation of zeta-potential profiles hematite and quartz were also carried out in the absence and presence of *R.* erythropolis. **(Abdel-Khalek *et al.*., 2013 and 2014)**.

2.5. Adhesion Measurements

Based on previous studies, a suspension of ***Rhodococcus erythropolis*** was separately added to 100 mg dm^{-3} of hematite and quartz suspensions. The adhesion tests were done on rotary shaker for 30 min. and the solution was centrifuged at 2000 rpm for selected ***Rhodococcus erythropolis*** concentration and different pH values. The bacterial concentration was measured in relation to supernatant suspension and compared with the dry weight curve. The assessment was done before and after to the mineral interaction. (**Mesquita et al., 2003).**

2.6. Scanning Electron Microscopy

Scanning electron microscopy (SEM) studies were carried out with the aim to observe the bacterial attachment onto the mineral particles. After washing and drying, the minerals of the adsorption tests were gold coated under vacuum in a BAL-TEC sputter coater. Secondary electron images were acquired in a Carl Zeiss – DSM 960 scanning electron microscope.

3. Results and Discussions

3.1. Surface Characterizations

Zeta potentials for ***Rhodococcus erythropolis*** and single minerals of hematite and quartz are shown in Figures 1-2. As shown in Figure 1, the surface of ***Rhodococcus erythropolis*** was negatively charged between pH 2 and 10. The surfaces of the two single minerals varied from positively charged to negatively with increasing pH.

The iso-electric points (IEPs) corresponded to pH values of approximately pH 6.7 for hematite, pH 2.0 for quartz. Bacterial cells with negative charges will be most likely to interact electrostatically with positively charged minerals, especially at pH values lower than mineral IEPs. Such electrostatic attraction is un-likely between bacteria and hematite, quartz, at acidic pH values because of negative mineral surface charges. Microbe-mineral interactions resulted in significant surface charge changes on mineral surfaces. Figure 3 represents zeta potential values single minerals after interaction with ***Rhodococcus erythropolis.*** As seen, the IEP of hematite decreased from pH 6.7 to pH 6.1, while there is no significant effect on quartz surface. This indicated that affinity of bacterial hematite interaction.

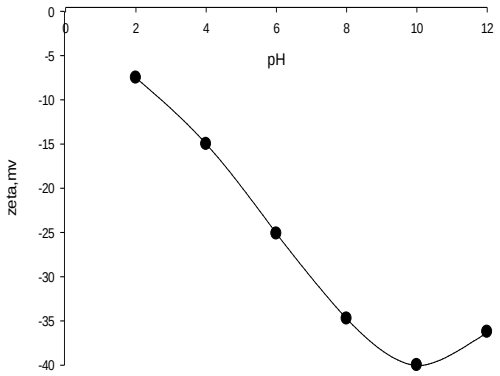

Fig.1. Zeta potential of *R. erythropolis* bacteria.

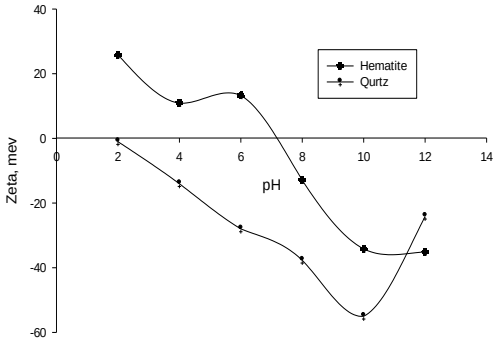

Fig.2. Zeta potential of hematite &quartz.

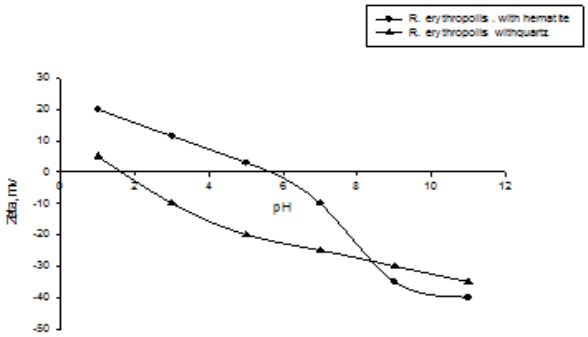

Fig.3. Zeta potential of single minerals after interaction with *Rhodococcus erythropolis.*

3.2. Bacteria Adhesion onto Minerals' Surfaces

Figure 4 shows the adhesion of ***Rhodococcus erythropolis*** bacteria onto the surface of the two single minerals over a wide range of pH (1-11). The results indicated that ***Rhodococcus erythropolis*** could be adhered onto both minerals' surfaces with a higher affinity to hematite surface rather than quartz surface.

At the same time, the highest values for adhesion for hematite were obtained at pH from 3-5 followed by a gradual decrease in adhesion values till reaching pH 11 in agreement with zeta potential measurements where the electrophoresis behavior of the minerals, before and after bacterial interaction.

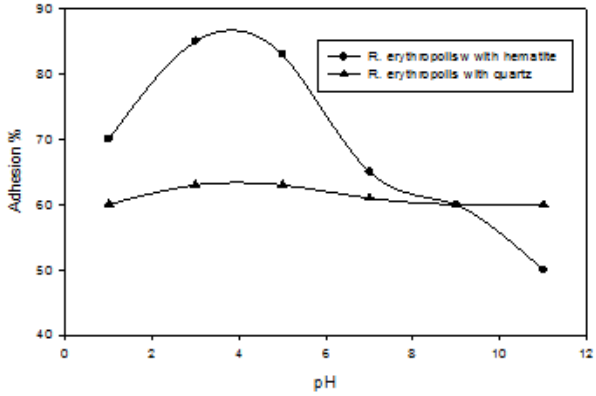

Fig.4. Effect of pH on the adhesion of *Rhodococcus erythropolis* onto minerals' surfaces.

3.3. SEM micrograph and FTIR spectra of *Rhodococcus erythropolis* bacteria and single minerals before and after bacterial interaction

SEM micrograph of ***Rhodococcus erythropolis*** is depicted in Figure 5 to illustrate its morphological characteristic. The strain was a rod-shaped with a cell size of 0.5 lm (2–4) lm. FTIR spectra for ***Rhodococcus erythropolis*** are shown in Figure 6.

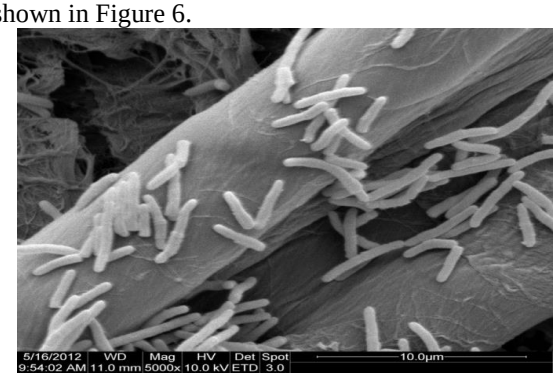

Fig.5. SEM of *R. erythropolis* bacteria.

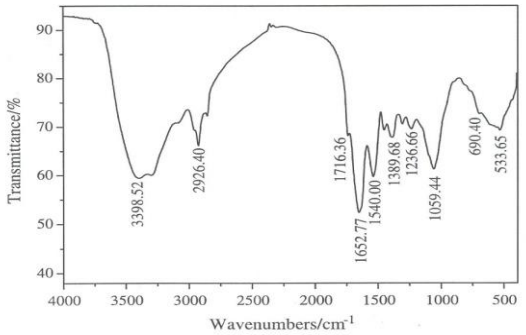

Fig.6. FTIR of the *R. erythropolis* bacteria.

The band at 3398 cm^{-1} can be assigned to stretching vibrations of the hydroxyl groups (a combination of OH and NH bands). The band at 2926 cm^{-1} can be assigned to C-H stretching of the CH2 and CH3 groups. The band at 1716 cm^{-1} can be assigned to COO^{-1} bending of carboxylic groups. The band at 1652 cm^{-1} is characteristic of C=O stretching of amide in the protein. The band at 1540 cm^{-1} is characteristic of NH bending vibrations of amide II in the protein. The band at 1389 cm^{-1} can be assigned to bending vibrations of CH_2 and CH_3. The band at 1236.66 cm^{-1} can be assigned to complex vibrations (C-O-C) of polysaccharides.

The band at 1054.44 cm^{-1} can be assigned to asymmetric stretching of phosphate groups in teichoic acid and complex vibration modes of polysaccharides. The band at 690.40 cm^{-1} can be assigned to C=O bending or COO- bending of the carboxylic group. The peak at 533 cm^{-1} is due to CH_2 rocking vibrations. The phosphate groups and carboxylic can make surface of the cell hydrophilic. Obviously, the surface groups on the ***R. erythropolis*** are similar to the groups for fatty acids, (**Huifen et. al, 2013).** On the other hand, Figures 7 and 8 illustrated SEM of single minerals before and after treatment with ***R. erythropolis*** bacteria. SEM micro-images indicated that bacterial isolate could be adhered onto both minerals but with higher affinity towards hematite as the amount adhered onto hematite surface is higher than that adhered onto quartz surface.

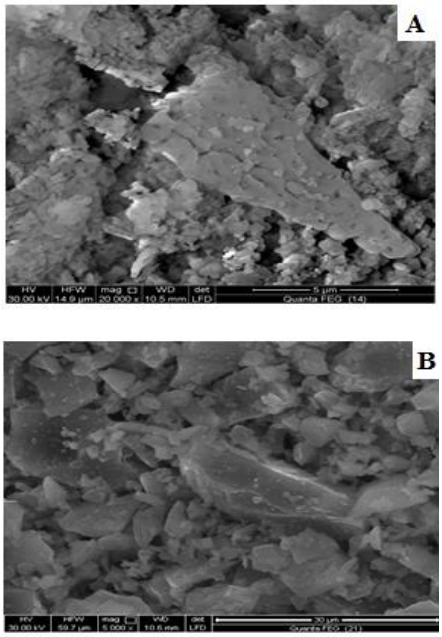

Fig.7. SEM of hematite (A) and quartz (B) before treatment with *R. erythropolis.*

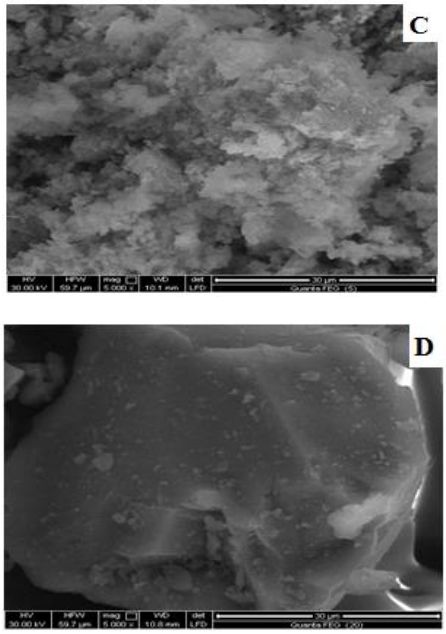

Fig.8. SEM of hematite (C) and quartz (D) after treatment with *R. erythropolis* bacteria.

According to the FTIR results, adsorption of ***R. erythropolis*** bacteria onto hematite surface can take place first onto their positive site of Fe^{3+} through the OH and/or the COOH groups (polysaccharides) secreted from bacteria.

This could be confirmed from the band at 3675 cm^{-1} which indicated the formation of hydrogen bond after bacterial interaction with hematite. Such occupation of the ***R. erythropolis*** to some of the positively adsorption sites of hematite lead to a reduction in the zeta potential of its surfaces. The surface of hematite treated with bacteria became therefore, more or less, hydrophobic in nature. In the meantime, the highly negative charged quartz particles, due to the formation of silanol groups, might hinder the adsorption of ***R. erythropolis***. Instead, adsorption in such a case can be proceeded through their positively amino ($-NH_2$) groups of protein fraction, leaving the outer surface with high negatively charges that causes the hydrophilic character of quartz surface. This also is confirmed from Figure 10 in which there is a band for hydrogen bond formation at 3675 cm^{-1} after adsorption of the ***R. erythropolis*** onto quartz surface. On the other hand, the N-H band of protein of ***R. erythropolis*** at about 1650 cm^{-1} disappeared after interaction with quartz **(Abdel-Khalek *et al.*., 2013 and 2014)**.

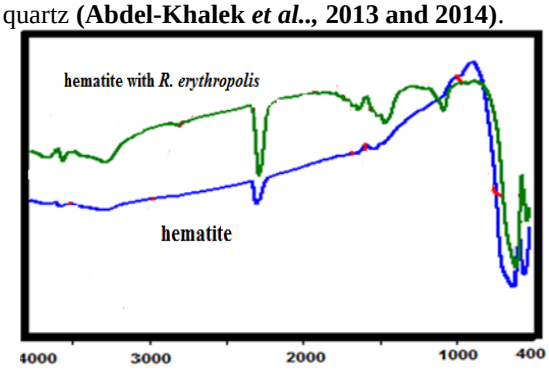

Fig.9. FTIR of the hematite treated with *R. erythropolis* bacteria.

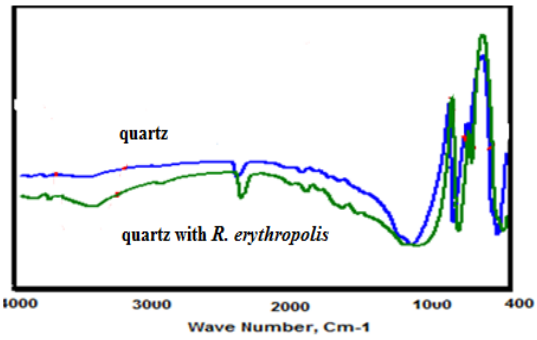

Fig.10. FTIR of the quartz treated with *R. erythropolis bacteria* .

3.4 Bacteria Adhesion onto Minerals' Surfaces

Figure 11 shows the adhesion of ***R. erythropolis*** bacteria onto the surface of the two single minerals over a wide range of pH (1-11). The results showed that ***R. erythropolis*** bacteria could be adhered onto hematite minerals surface with a higher affinity.

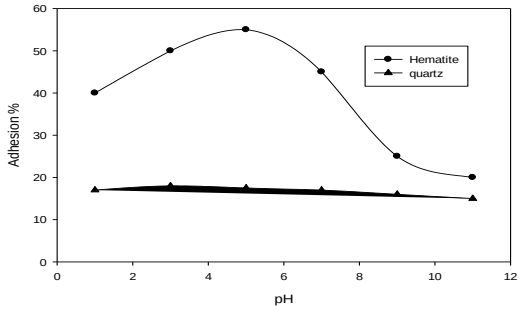

Fig.11. Adhesion of *R. erythropolis* onto minerals' surfaces.

On the other hand, the quartz surface has not been affected.The highest values for adhesion for hematite was obtained at pH from 3-5 followed by a gradual decrease in adhesion values till reaching pH 11.

3.5 Floatability of minerals using *R. erythropolis* bacteria

Figure 12 shows the effect of ***R. erythropolis*** bacteria onto the floatability of the two single minerals at different concentrations of bacterial isolate (ml@10^6 cell) at pH 3. The high floatability was achieved at concentration of about 5ml ($5x10^6$cell/ml) with higher affinity for hematite where the floatability % was 63.16% and 29.18% for hematite and quartz respectively. While the increase of the bacterial dose leads to a gradual decrease in the floatability. This may be due to formation of more than one layer of the bio-film.

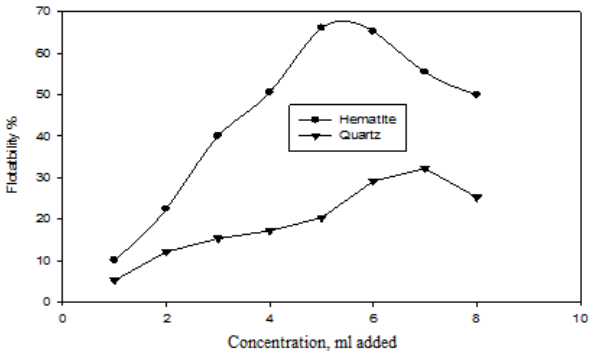

Fig. 12. Effect of bacterial cell concentration on the floatability of single minerals.

4. Conclusions

- There is a strong interaction between ***R. erythropolis*** bacterial isolate and minerals' surfaces, especially with hematite.
- Adhesion, adsorption, zeta potential measurements showed a better affinity of ***R. erythropolis*** to hematite mineral surface rather than quartz surface.
- The results of zeta potential showed that the iso-electric points (IEP) for hematite mineral (at pH 6.7) is displaced to lower values (at pH 6.1) after interaction with the bacterial isolates.
- Higher bacterial affinity to hematite mineral surface in comparison to quartz surface is readily evident from the results of adhesion, adsorption, and floatability %.

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