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Enhancement of Selective Bioflocculation of (Quartz- Pyrolusite-Hematite) System using *Paenibacillus polymyxa* Bacteria

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ABSTRACT

Paenibacillus polymyxa was used in pretreatment of hematite to facilitate the flocculation removal of quartz and pyrolusite minerals. The adsorption results showed that the affinity of *P. polymyxa* to the three minerals according to the order: pyrolusite> quartz >hematite all over the pH range. On applying *P. polymyxa* bacterial strain, to be used as sole flocculating reagent, to selectively separate hematite from its mixture with pyrolusite at pH 6.5 and $5x10^9$ cell/ml succeeded in the removal of 73.5 % of MnO_2 as a concentrate containing about 2.65% MnO_2 was obtained from a feed containing about 9.97 % MnO_2 with 77 % Wt. % flocculated. Applying the same conditions for flocculation of a natural iron ore yielded a concentrate containing 2.54% MnO_2, 0.25% SiO_2 and 74.40% Fe_2O_3 with a recovery of 75% from a feed containing 8.79% MnO_2 , 0.49% SiO_2 and 67.90% Fe_2O_3. In this paper, the role of *Paenibacillus polymyxa* on the surface properties of the three single minerals has been studied through zeta potential measurements as well as the adsorption experiments. Complete characterization of both single minerals and bacteria strain have been done using XRF, SEM, and FTIR.

Introduction

Bio-surface modification of natural minerals became a promise technique in mineral processing filed. In this technique, microorganisms are used as surface modifiers to enhance the beneficiation of difficult to separate minerals either by flotation (microorganisms are used as depressants or collectors) or flocculation (microorganisms are used as flocculants or dispersants). Bio-beneficiation is a branch of mineral bio-processing in which microorganisms or bio-reagents (vitamins, enzymes or proteins) are used as surface modifiers [1-6]. Bio-surface modification differs from bioleaching; bioleaching means dissolution of a specific constituent in the ore matrix with the aid of microorganism. due to geochemical properties of minerals [7], environmental sound and cost effectiveness. The iron ore in Egypt present in different locations as in East Aswan, in the Eastern Desert, 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 [8, 9]. These ores suffer from harmful elements as silica, Mn, Ba, carbonates or chlorides where Aswan has silica and phosphorus. In addition, manganese recovery required due to its needs toward its industry as ferromanganese industry. Microorganisms play an important role in previous studies to develop the bioleaching of manganese from its ores particularly low grade ores [10, 11]. *Paenibacillus polymyxa was* utilized to separate silica from iron ore and flocculate silica from some sulphide minerals [12]. Due to the similarity of their chemical and physical properties, Fe and Mn are always associated in the Fe and Mn ore deposits throughout the geological record of different settings and origins. Also, due to silicon oxide acts as general gangue impurities in all ores. These explain the importance to find a green surface modifier to these oxide minerals.

In this work, the ability of using *Paenibacillus polymyxa* strain as a surface modifier to hematite, pyrolusite and quartz was investigated. Adsorption experiments of *P. polymyxa* to the studied minerals were conducted at different conditions of time, pH and cell concentration. Flocculation behavior of the three oxides was studied in presence and absence of *P. polymyxa*.

Material and methods

Materials

Samples of single minerals of hematite (Fe_2O_3) and pyrolusite (MnO_2) were delivered from 'PROLAB' Company, Poland. The purity 99% of the samples was confirmed using XRF. The –200 mesh fractions were used in adsorption. Analytical grade HCl and NaOH, from Aldrich, were used for pH regulations. A pure bacterial strain (*Bacillus polymyxa* DSM-292, CCM-1609) was delivered from Microbiological Resources Center-Cairo MIRCEN- Ain Shams University.

Methods

Cultivation of Bacterial Strain and Harvesting

The Pure bacterial strain was activated upon modified Difco™ nutrient medium [3 g/L beef (yeast extract was replaced by beef) and 5 g/L peptone dissolved in 1 liter deionized water at neutral pH] and was overnight incubated at 200 rpm and 30° C in 250 ml round-flask. A 10 % (V/V) of active inoculum containing at least $l0^9$ cells/ml was added to new medium as sub-culturing and, the initial inoculum concentration was 5×10^7 cells/mL which used to study the growth curve. Cell number over interval time was microscopically measured using a Petroff-Hausser counter and the change in pH values was also measured during the experiment. Cell harvest collected through

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centrifugation of bacterial slurry at 10,000 rpm for 10 minutes to obtain the cell pellets. These pellets were washed several times by re-suspending in neutral, deionized water and centrifuging in order to remove the metabolic products from the cell surface [13].

Zeta Potential Measurements

A Laser Zetameter "Malvern Instruments" model "ZetaSizer 2000", was employed for zeta potential measurements for surface characterization of both mineral oxides and bacterial cells before and after treatments. 0.01 gram of ground sample was placed in 50 ml double distilled water with a definite bacterial isolate concentration at ionic strength of $2X10^{-2}$ M NaCl. The pH was then adjusted to the required value. The sample was shacked for15 minutes. After shaking, the equilibrium pH was recorded and the zeta potential of the mineral particles was measured [14, 15].

Adsorption Experiments

Adsorption of *P. polymyxa* strain on the studied minerals was performed in a 250 mL round flask containing 0.5 g of mineral sample in 50 mL of 10^{-2} M NaCl. A certain concentration of bacterial cells was added into the flask to achieve the initial target cell concentration at the required pH. The slurry was conditioned by GFL rotatory shaker at 30° C and 180 rpm under different conditions of pH, conditioning times and initial cell concentration. The number of adsorbed cells onto the mineral surfaces was microscopically calculated using a Petroff-Hausser counter and the number of adsorbed cells was calculated from the difference between the initial and final cell numbers [14, 15].

FTIR Measurements

The adsorption density of the bacterial isolate under test on the mineral surface was measured using "PYE Unicam" Model "SP 1200 infrared spectra". A 0.2 gram of each single mineral was conditioned with selected bacterial isolate at pH 8 for 30 min. The samples were centrifuged at 15,000 rpm for 15 min. The supernatant was separated from the residual solid which was rinsed several times before drying at 30°C [14-16].

Flocculation Experiments

Flocculation experiments were utilized for measuring the settling rate of 3 oxides in absence and presence of bacterial cultured broth at 2 level; single minerals and binary system. Prior the measurement, the conditioning process for 1 g of solid particles (single oxide or binary mixture) with certain concentration of Bacterial cultured broth in 100 ml liquid (distilled water with additives like cultured broth and dispersing agents) at required pH, room temperature and for 10 minutes as conditioning time using intensive stirring [15].

Results and discussions

Morphological and characteristics of the bacterial strain

Paenibacillus polymyxa is a spore forming, Gram-positive, heterotrophic facultative neutrophil, aerobic bacterium associated with oxide mineral deposits and uses organic sugar as energy source [17]. It is motile with peritrichous flagella and occurs in rods that vary in size from 0.5 to 1µm in width and 2 to 8 µm in length. It secretes exopolysaccharides, several proteins, enzymes and organic acids like acetic, formic, and oxalic acid. The extracellular polysaccharide (ECP) aids in biological uptake of metal ions necessary for metabolism and growth. Scanning electron micrograph showed that *Paenibacillus polymyxa* is rod shaped with a length of around 2.5 µm and diameter of 0.6 to 0.8 µm, Figure1 [18].

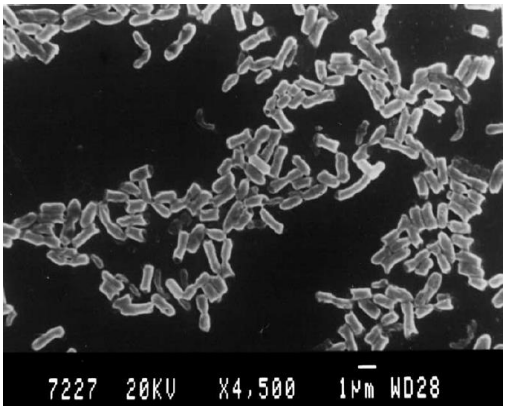

Figure 1. SEM of *P. polymyxa*

Growth Curve of Paenibacillus polymyxa

A typical Growth curve of *P. polymyxa* is given in Figure 2. It consists of four phases:

1) Lag phase
2) Exponential growth phase
3) Stationary phase
4) Death phase.

Firstly, when fresh media is inoculated, the adaptation of bacterium towards the new environment is required before growing. In which, there is no significant increase in cell number in this phase that is called lag phase. In the case of *Bacillus polymyxa*, the lag phase is less than 30 minutes. After the lag phase, cells start to grow exponentially within a logarithmic phase. In which, the bacterium undergoes a binary fission to produce a duplicate of itself. Log phase spends about 9 hours. In which the cell number increase from 10^7 to 10^9 cells per ml. After that, cells growth is very slow and reaches stationary phase in which, the cell count remains almost constant at its maximum value. The growth of bacterial population is limited either by the exhaustion of available nutrients or by the accumulation of toxic products of metabolism. As a result the rate of growth declines and growth eventually stops. The cell growth is going with decrease in pH of the culture. After 25 hours of inoculation the pH decrease and reach saturation value due to the production of organic acids like formic, succinic, acetic and lactic acids. The optimum pH is 4-7. When the pH of the culture decreases below 4, the growth of the cells hindered to death phase where the cell number decreases. Death results from a number of factors due to depletion of the cellular reserves of energy. Based on previous observations, this strain shows maximum cell count within 10 hours upon Difco™ medium and its modification obtained within this study agrees with Campbell, 1969 [19]. *P. polymyxa* also can show another trend within its growth upon Bromfield medium where the maximum growth of this strain was obtained within 16 hours [20].

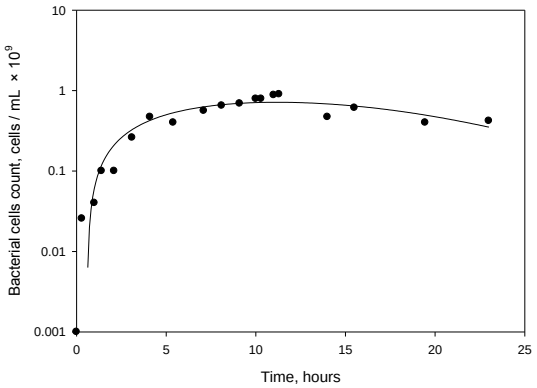

Figure 2. Growth Curve of *P. polymyxa*

Physico-Chemical Properties of Single Minerals after Treatment with *Paenibacillus polymyxa*

For iron oxide, the measured zeta potentials were seen to be shifted in a more negative direction after interaction with *P. polymyxa*, Figure 3 except in the pH range (6-10) where the values tend to go the positive direction indicating the hydrophobic character of mineral surface after treatment at this pH range. The iso-electric point (IEP) of hematite is shifted from its initial (before interaction) value of about 2.2 to 2.6. This is in an agreement with trend reported in other works [21]. On the other hand, the effect of interaction of *P. polymyxa* with Pyrolusite was slightly different in that the measured zeta potentials were observed to be in the negative direction but with values higher than that obtained in case of hematite. The isoelectric point (IEP) of Pyrolusite is shifted from its initial (before interaction) value of about 2.6 to 3.2 as shown in Figure 4.

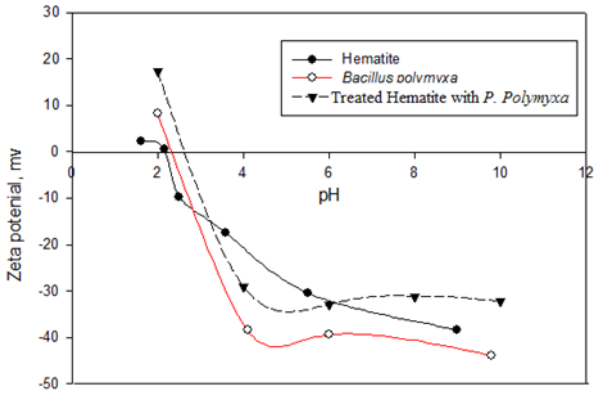

Figure 3. Zeta Potential of iron oxide treated with *P. polymyxa*

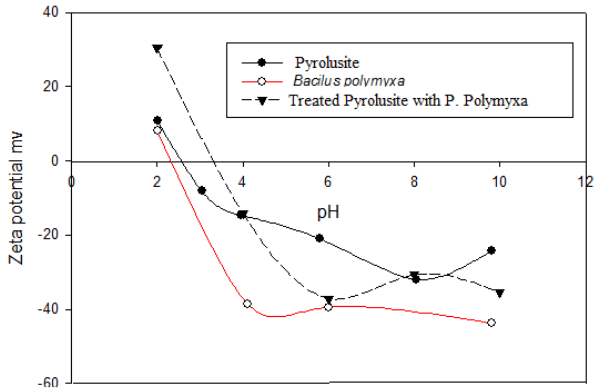

Figure 4. Zeta Potential of Pyrolusite treated with *P. polymyxa*

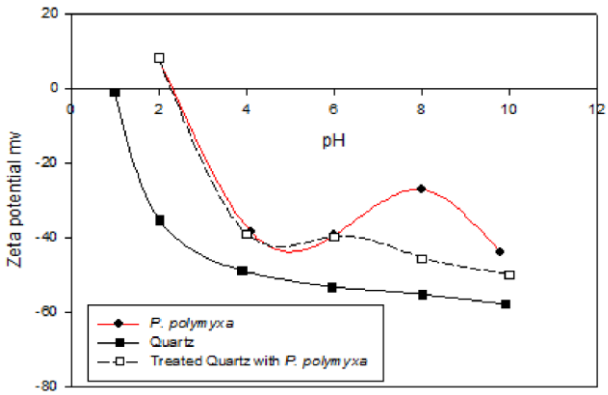

Figure 5. Zeta Potential of Quartz treated with *P. polymyxa*

The effect of interaction of *P. polymyxa* with quartz is shown in Fig. 3.8. The results indicated that measured zeta potentials were very close to that of bacterial strain specially at pH ranging from 2.4 to 6. After that, the values observed to be in the negative direction but with values lower than that obtained in case of untreated quartz. The isoelectric point (IEP) of quartz is shifted from its initial (before interaction) value of about 1 to 2.4 (IEP of *P. polymyxa* itself).

Effect of Changing pH on Adsorption of Paenibacillus polymyxa onto Single Minerals

The effect of changing pH of the medium on adsorption of the *P. polymyxa* on the surfaces of single minerals was also studied. The experiments are performed using a concentration of $5x10^8$ cell/mL of bacterial strain. It can be seen that the adsorption characteristics of the *P. polymyxa* and its adsorption densities are pH dependent.

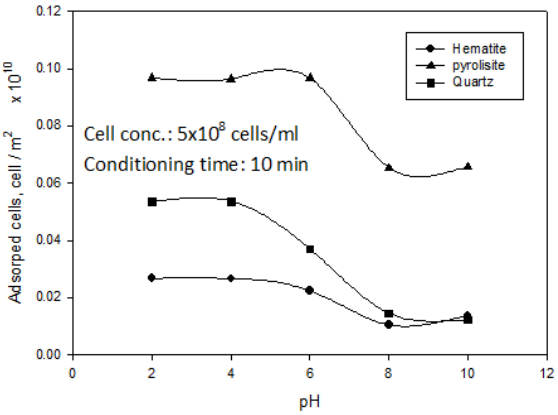

Figure 6. Effect of pH on Adsorption of *P. polymyxa* onto single minerals

Figure 6 shows the effect of pH on adsorption of *P. polymyxa* onto the three single minerals' surfaces in which, there is a stability in the adsorption behviour at pH from 2 – 6 for the three single minerals with higher affinity to pyrolusite followed by a gradual decrease from pH 6-10. The amount adsorbed onto quartz was ~ 0.025×10^{10} cells per square meter (4.2×10^{10} cells per gram) where the amount of adsorbed cells onto hematite and pyrolusite was 0.015×10^{10} cells per square meter and 0.04×10^{10} cells per square meter (3.7×10^{10} cells per gram and 4×10^{10} cells per gram) respectively.

Adsorption Isotherm of Paenibacillus polymyxa – Single Minerals Systems

The adsorption isotherm of bacterial cells were also studied. The results of which are shown in Figure 7. The experiments are performed at natural pH of about 5.5-6.5 according to the results obtained from the effect of pH onto the adsorption of the selected bacterial strain on different minerals' surfaces. The results indicated that the adsorption density onto hematite, pyrolusite and quartz generally increases with increasing the concentration of *P. polymyxa*. The results show that the adsorption behavior at higher concentration of *P. polymyxa* has the following order:

Pyrolusite > Quartz > hematite

FTIR of single minerals after treatment with Paenibacillus polymyxa

As shown in Figure 8, FTIR spectra of the pyrolusite sample, obtained before the *P. polymyxa* pretreatment, showed the characteristics bands for pyrolusite (absorbance bands of 3445 cm^{-1} assigned to hydroxyl groups, 1091 cm^{-1} assigned to Si-O. The FTIR spectra of pyrolusite, after *P. polymyxa* pretreatment, showed similar characteristics bands as the untreated one.

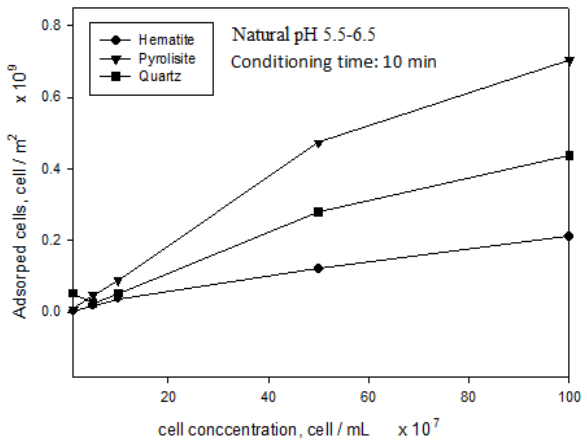

Figure 7. Adsorption Isotherm of *P. polymyxa* onto minerals' surfaces

This indicated that absorbance bands of 3422 cm^{-1} and 1091 cm^{-1} might not associate with functional groups presented on the *P. polymyxa* cell wall which could interact with the sample surfaces. The absorbance band of hydroxyl group was broadened and the wave number 3445 cm^{-1} move lower, 3422 cm^{-1}, due to the role of hydrogen bonding. The absorbance band of 1091 cm^{-1} was widened, which may be subject to interference from other bands [22].

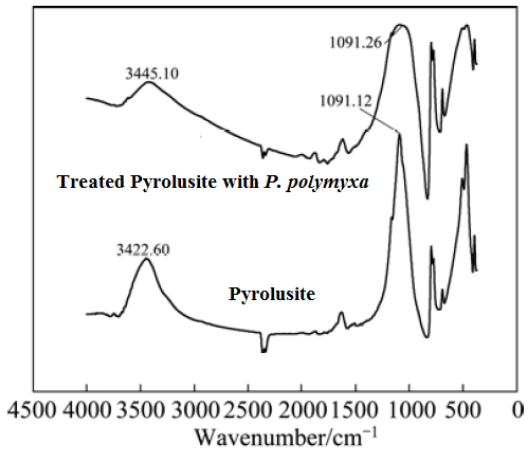

Figure 8. FTIR spectra of Pyrolusite after treatment with *P. polymyxa*

Figure 9 shows the FTIR spectra of quartz after treatment with bacterial cells, *P. polymyxa*. The difference spectrum illustrates new peaks that appear due to interaction with bacterial cells. A sharp peak near 3430 cm^{-1} is assignable to the free asymmetric and symmetric N-H stretching modes, while small peak at around 2927 cm^{-1} is due to C-H stretching mode of the CH_2 groups. The small peak at 1880 cm^{-1} is due to C=O group. The large peak around 1650 cm^{-1} is due to an amide group while the 1540 cm^{-1} peak is assignable to NH bending of the secondary amide group -CONH. The broad peak, 1400-1350 cm^{-1}, is due to the vibration of CH_3 and CH_2 groups. The sharp peak at 1050 cm^{-1} is due to primary alcoholic group (CH_2OH) stretching. The strong sharp peak at around 775 cm^{-1} may be as a result of the shift of silanol groups owing to some chemical bonding with bacterial cells or its secreted products. The peak at around 696 cm^{-1} C=O bending while that at 465 cm^{-1} is due to the CH_2 rocking vibrations. The above results clearly suggest the presence of amino groups of proteinaceous compounds on the quartz surface after interaction with bacterial cells. The difference spectrum of cells treated hematite is given in Figure 10. New peaks that have appeared on the difference spectrum are due to the interaction of cells with minerals. While the 1640 cm^{-1} peak is attributed to the presence of carboxylate anion, another peak at 1460 cm^{-1} is that of CH_2 rocking and OH bending modes or the C-OH group of free polysaccharides. The peak at 1118.97 cm^{-1} is due to C-OH stretching vibrations. The peak at 1041 cm^{-1} is of primary alcoholic group of CH_2OH and another peak at 674 cm^{-1} may be due to CH_2 rocking vibrations, [3].

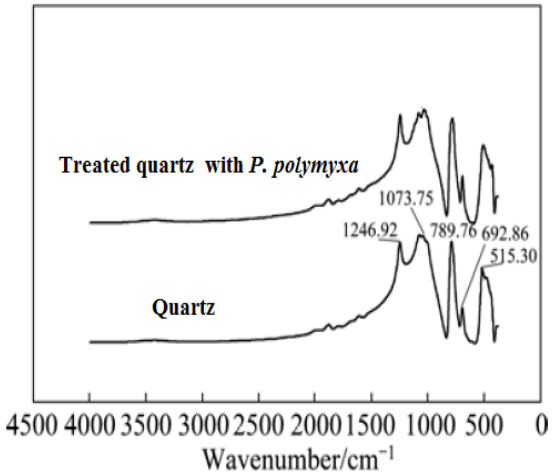

Figure 9. FTIR spectra of quartz after treatment with *P. polymyxa*

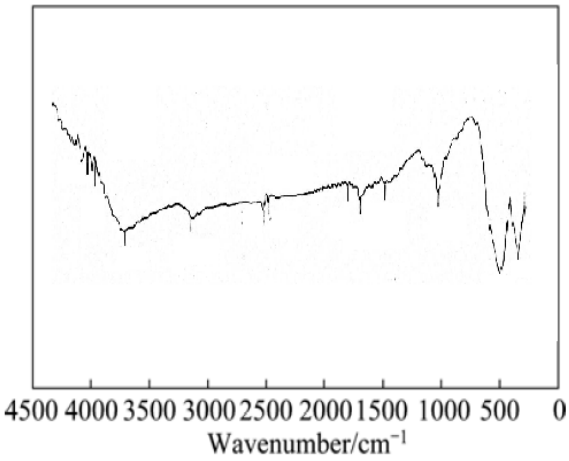

Figure 10. FTIR spectra of hematite after treatment with *P. polymyxa*

Bio-Flocculation of Single Minerals

Effect of pH on Flocculation of Single Minerals in the Presence of Bacterial Strain

Figure 11 showed that effect of changing pH of the medium on flocculation of single minerals using $5x10^9$ cell/ml of *P. polymyxa*. The results indicated that similar behavior for bacterial strain on flocculation of single minerals. For the three single minerals, the flocculation power decreased gradually till pH 6.5. The minimum flocculation occurred after that till reaching pH 12. The results showed that the flocculation power of *P. polymyxa* all over the pH values has the following order:

Pyrolusite > Quartz > hematite

Effect of Concentration of Bacterial Strain on Flocculation of Single Minerals

Figure 12 showed that the effect of changing the concentration of *P. polymyxa* bacterial strain on the flocculation efficiency of single minerals. The experiments were performed at pH 6.5. The results showed that the best selectivity occurred where maximum separation between the three minerals can be obtained at concentration of $5x10^9$ cell/ml of *P. polymyxa.*

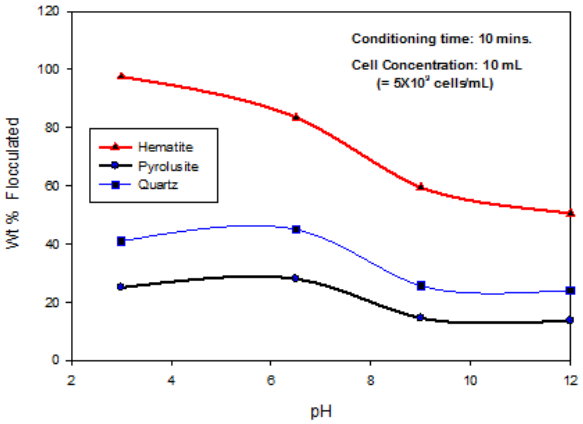

Figure 11. Flocculation power of single minerals using 10 ml @ (5x10^9 cells/ml) *P. polymyxa* at different pH

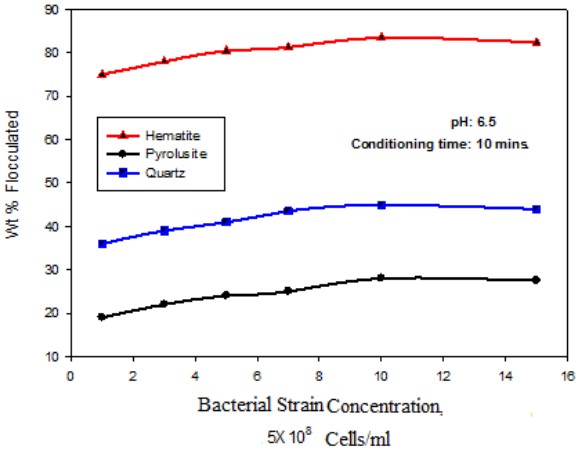

Figure 12. Flocculation of Single Minerals as a Function of Concentration of *P. polymyxa* at pH 6.5

Bio-Flocculation of Binary Mixtures and Natural Iron Ore

The amenability of applying *P. polymyxa* bacterial strain, to be used as sole flocculating reagent, to selectively separate hematite from its mixture with pyrolusite was studied. The experiments were performed at pH 6.5 and 5x10^9 cell/ml of *P. polymyxa*. The results indicated clearly that the using of bacterial strain of *P. polymyxa* for flocculation a mixture containing 90% by weight hematite and 10% by weight pyrolusite succeeded in the removal of 73.5 % of MnO_2 as a concentrate containing about 2.65% MnO_2 was obtained from a feed containing about 9.97 % MnO_2 with 77 % Wt. % flocculated. Applying the same conditions for flocculation of a natural iron ore yielded a concentrate containing 2.54% MnO_2, 0.25% SiO_2 and 74.40% Fe_2O_3 with a recovery of 75% from a feed containing 8.79% MnO_2 , 0.49% SiO_2 and 67.90% Fe_2O_3 These results illustrated clearly the amenability of upgrading El Gedida iron ores, Bahariya Oasis of high pyrolusite using *P. polymyxa* bacterial strain which gave a good potential for utilization of Egyptian iron ores.

Conclusions

- A successful adsorption of the *Paenibacillus polymyxa* bacterial strain onto (hematite-quartz-pyrolusite) surfaces caused a degree of aggregation for minerals particles leading to a change in their size distribution which indicates the largest degree of selectivity for pyrolusite mineral.
- The values of zeta potential of *P. polymyxa* are varied from (+5 to -40 mv) over the entire range of pH (1.0-12) and iso-electric point (IEP) corresponding to pH of 2.4.
- Conditioning of the three single minerals (hematite-quartz-pyrolusite) with bacterial strain leads to a displacement for the IEP of them to about 2.6, 2.4 and 3.2 respectively.
- Applying *P. polymyxa* bacterial strain as a flocculating reagent at pH 6.5 and 5x10^9 cell/ml succeeded in the removal of 73.5 % of MnO_2 as a concentrate.
- Applying the same conditions for flocculation of a natural iron ore yielded a concentrate containing 2.54% MnO_2, 0.25% SiO_2 and 74.40% Fe_2O_3 with a recovery of 75% from a feed containing 8.79% MnO_2 , 0.49% SiO_2 and 67.90% Fe_2O_3.

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