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Bioflocculation of Egyptian High Manganese Iron Ore Using Paenibacillus polymyxa Bacteria

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Abstract -*Paenibacillus polymyxa was used in pretreatment of hematite to facilitate the flocculation removal ofpyrolusite mineral. The adsorption results showed that the affinity of P. polymyxa to the two minerals according to the order: hematite>pyrolusite 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 5x10⁹ 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% Fe2O3 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 two single minerals has been studied through zeta potential measurements as well as the adsorption experiments.Complete characterization of both single minerals and bacterial strain have been done using XRD, XRF and FTIR.*

Key Words: Bioflocculation, Hematite, pyrolusite, *P. polymyxa and adsorption*

1.INTRODUCTION

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, and also in the assemblage of trace elements [1, 2]. These ores suffer from harmful elements as silica, Mn, Ba, carbonates or chlorides.

Although manganese is added into steel for its deoxidizing and desulfurizing properties [3], the occurrence of Mn in the iron ore raw materials causes harm to the reduction process of iron oxides in the blast furnace. Mn forms strong oxides, which are partially reduced in the blast furnace with parts entering the slag. This is why the reduction behaviour of Mn in the blast furnace was studied extensively before, trying to avoid the harmful effect of Mn in the ironmaking and steel industries [4, 5, 6]. At the same time, due to the similarity of their chemical and physical properties, Fe and Mn are always associated in the Fe and Mn deposits throughout the geological record of different settings and origins [7, 8, 9].

Separation of MnO_2, from Egyptian iron ores by conventional methods of beneficiation is difficult due to the extremely complex nature of these ores. For this reason, the use of these iron ores is limited in Egyptian iron and steel industry. Recently, the biological processes are becoming more attracting in mineral processing due to their lower operating costs and their possible applications to treat difficult to beneficiate low grade complex ores. Microorganisms play an important role in previous studies to develop the bioleaching of manganese from its ores particularly low grade ores [10, 11, 12]. Paenibacillus polymyxa was utilized to separate silica from iron ore and flocculate silica from some sulphide minerals [13].

This paper aims to study the role of interaction between isolated Paenibacillus polymyxaon and the surface properties of hematite and pyrolusite single minerals through the zeta potential and adsorption experiments. Flocculation behavior of the two oxides was studied in presence and absence of P. polymyxain. The separation of MnO_2 from high manganese iron ores will lead to improvements in reduction of these ores and to manganese recovery which required in its industry as ferromanganese industry.

1. MATERIAL AND METHODS

2.1 Materials

Samples of single minerals of hematite and pyrolusite were delivered from 'Wards' Company, USA. The purity 99% of these samples was confirmed using XRF. The –200 mesh fractions were used in adsorption. HCl and NaOH of analytical grade from Aldrich were used for pH regulations.

2.2 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. Infrared vibrational spectra were recorded on a Nicolet Magna 750 Fourier-transform spectrometer. For each sample, 28 scans were accumulated over the 4000-400 cm^{-1} spectral range employing the transmittance mode and a resolution of 4 cm^{-1}.Selected samples were observed on fractured surface under a JSM-6400 scanning electron microscope (SEM) to examine the morphology of single minerals.

2.3 Isolation and Growing of Bacteria

Bacterial strain was isolated from surface of iron ore through vigorous agitation of iron sample with 0.4% sodium chloride, NaCl, solution for 30 min on a rotary shaker at 30°C, and allowed to settle. The supernatant obtained was serially diluted with sterile water and spread on the surface of nutrient agar plates which were incubated at 30 ºC. Selected bacterial isolates was isolated, purified by streaking on nutrient agar plates, then transferred to nutrient agar slopes stored at 4°C and subcultured monthly. The efficiency of these isolates was screened using a laser particle size analyzer [14, 15]. Based on the later test, the most promising bacterial isolate has been selected to conduct this study.

2.4 Bacterial Identification

Bio-Chemical Identification

The selected bacterial isolate was identified using the BIOLOG GEN III Micro-plate microbial identification system. A pure culture was grown on biolog recommended agar mediaand 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 36hours at 30 °C into the Omni-Log incubator/reader. The biology micro-plate tests the ability of an organism to utilize or oxidize apre-selected panel of 95 different carbon sources. The dyetetrazolium violet is used to indicate utilization of substrates[16, 1]. 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 [16, 15]. Also, Microscopic examination and gram staining of the selected bacterial isolate were carried out.

2.5 Measuring Selectivity of Paenibacillus polymyxato 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 Paenibacillus polymyxa was conditioned with one gram of each mineral for 60 minutes before recording the change in size distribution[1].

2.6 Zeta Potential Measurements

A laser Zeta Meter 'Malvern Instruments Model Zeta Sizer2000' 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 bacterial isolate at fixed ionic strength of 2×10^{-2} M NaCl. NaOH and HCL were used as pH modifiers. The suspension was conditioned for 30 minutes during which the pH was adjusted. After shaking, the equilibrium pH was recorded. It was then allowed to settle for 3min, after which 10 ml of the supernatant was transferred into a standard cuvette for zeta potential measurement. Solution temperature was maintained at ($25^{o}C \pm 2$). Five measurements were taken and the average was reported as the measured zeta potential [17].

2.7 Adhesion Measurements

Adhesion of the bacterial isolate on the mineral surfaces was determined by dry weight difference before and after conditioning the mineral particles with bacteria. 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 [15].

2.8 Flocculation Experiments

Flocculation experiments were utilized for measuring the settling rate of 2 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 [17, 18].

2. RESULTS AND DISCUSSION

3.1 X-ray Diffraction Analysis of Samples

XRD analysis indicated the presence of Mn-bearing minerals in the high-Mn iron ores from the Bahariya Oasis (El Gedida area), Table 1. Pyrolusite (MnO_2) was identified in the XRD pattern by its characteristic peak at 3.12 Å in the Ni-filtered Cu- Kα, Fe-filtered Co-Kα, and non-filtered Fe-Kα. Mn-bearing minerals in these samples are dominated by a mixture of Mn oxides and hydroxide minerals. In all samples, the Fe-bearing minerals occur as hematite and goethite. Traces of quartz were identified in the XRD patterns of the studied samples.

Table 1. XRD data of high – Mn-iron Ore

Area	Ni-filtered Cu-Kα		Fe-filtered Co-Kα	
El Gedida	2θ°	Mineral identification	2θ°	Mineral identification
	24.2	Goethite	24.8	Goethite
	28.5	Pyrolusite	28.2	Hematite
	33.1	Hematite	33.5	Pyrolusite
	35.7	Hematite	39	Hematite
	37.2	Pyrolusite	41.7	Pyrolusite
	40.8	Hematite	43	Goethite
	49.5	Hematite	43.8	Pyrolusite
	54	Hematite	48.2	Pyrolusite
	56.3	Hematite	50	Pyrolusite
	62.4	Hematite	54	Pyrolusite
	64	Hematite	58.3	Hematite

3.2 Chemical Analysis of Samples

Data of XRF analyses of selected sample of El Gedida iron ores, Bahariya Oasis are shown in Table 2. MnO_2 content is 8.79 %. MnO_2 shows strong negative correlation with the Fe_2O_3 contents. Fe_2O_3 content in the analyzed samples is 67.9 %. SiO_2 and Al_2O_3 contents are generally low, 0.49 and 1.49 % respectively. The average contents of TiO_2, CaO, MgO, Na_2O, K_2O, and P_2O_5 are relatively low in analyzed sample (averages of 0.04, 0.2, 0.81, 0.54, 0.07, and 0.07 wt. %. The Loss of ignition is about 19.6%. On the other hand, chemical analysis of single minerals, hematite and pyrolusite showed their high purity (99.9%).

Table 2. Chemical analysis of the iron ore sample

Main Constituents	Concentration (Wt %)
Na_2O	0.54
MnO_2	8.79
MgO	0.81
Al_2O_3	1.49
SiO_2	0.49
P_2O_5	0.07
K_2O	0.07
CaO	0.20
TiO_2	0.04
Fe_2O_3	67.9
L.O.I	19.6

3.3 Size Analysis of the Studied Samples

The size distribution of the single minerals (hematite and pyrolusite) is shown in Fig. 1. Samples of single minerals have very fine size distribution with 90 % below 60 μm, 31 μm forhematite and pyrolusite with d_{50} of 25.6 μm, and 6.4 μm respectively. The samples of natural iron ore are ground to 100 % below 200 mesh (74 μm).

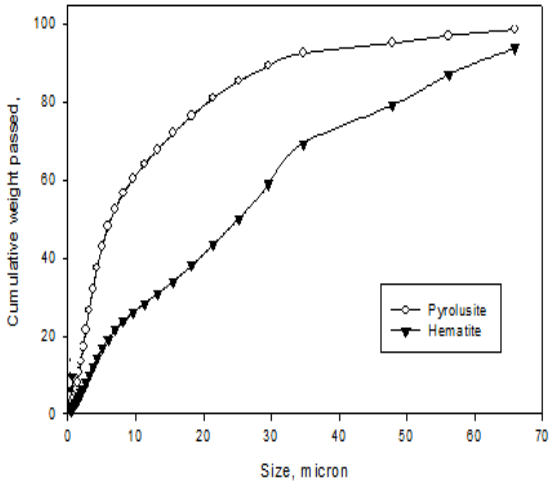

Fig. 1. Size distribution of single minerals

3.4 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 [19]. 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, Fig. 2[20].

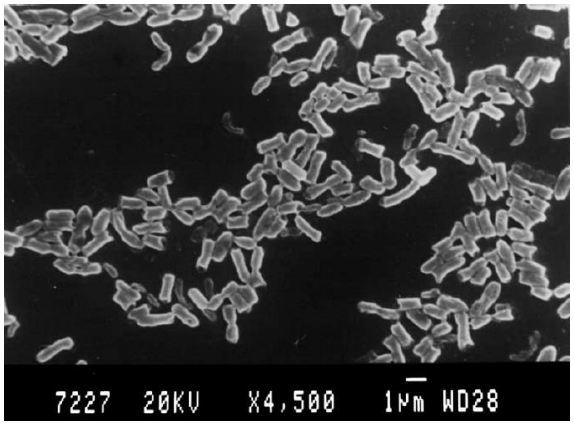

Fig. 2.SEM of P. polymyxa

3.5 Selectivity of Microorganism to Minerals' Surfaces

The change in size distribution of single minerals of pyrolusite and hematite, after their treatment with bacteria (Paenibacillus polymyxa) was taken as a measure for its selectivity. Successful adsorption of such bacteria 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 ore selective the bacteria to the mineral surface [1]. The results show different degrees of variation in the size distribution of samples after their treatment with bacteria. Paenibacillus polymyxa showed, interestingly, the largest degree of aggregation for hematite, Figs. 3 and 4. In these figures MO means minerals after treatment with microorganism.

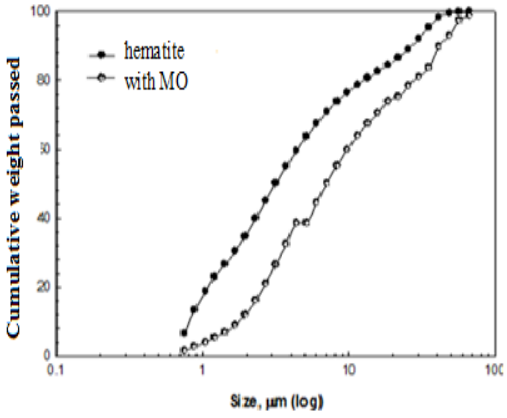

Fig. 3. Effect of P. polymyxa on Size Distribution of Hematite

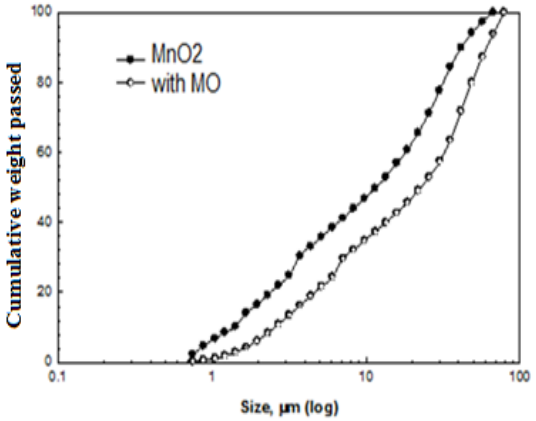

Fig. 4. Effect of P. polymyxa on Size Distribution of pyrolusite

3.6 Physico-Chemical Properties of Single Minerals after Treatment with P. polymyxa

For iron oxide (hematite), the measured zeta potentials were seen to be shifted in a more negative direction after interaction with P. polymyxa, Fig. 5 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, 22, 23]. 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 Fig. 6.

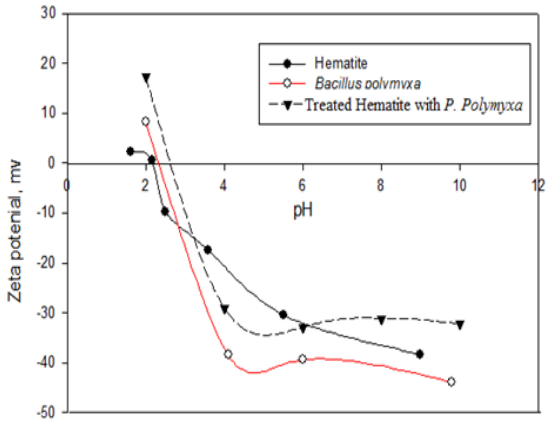

Fig.5. Zeta Potential of hematite treated with P. polymyxa

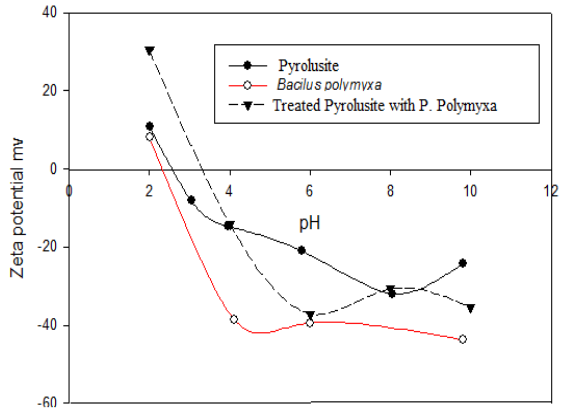

Fig. 6. Zeta Potential of Pyrolusite treated with P. polymyxa

3.7 Effect of Changing pH on Adsorption of*P. polymyxa* onto Single Minerals

In this context, 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. As shown in Fig. 7, there is stability in the adsorption behavior at pH from 2 – 6 for both single minerals with higher affinity to pyrolusite followed by a gradual decrease from pH 6-10. The amount 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.

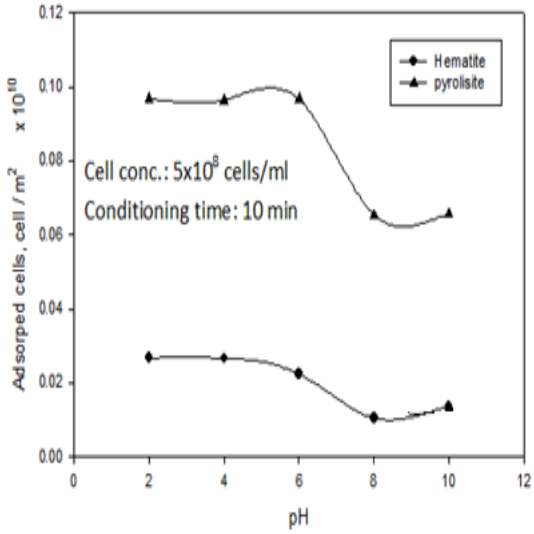

Fig. 7. Effect of pH on Adsorption of P. polymyxa onto single minerals

3.8 Adsorption Isotherm of *P.polymyxa* – Single Minerals Systems

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 and pyrolusite 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 > hematite, Fig. 8.

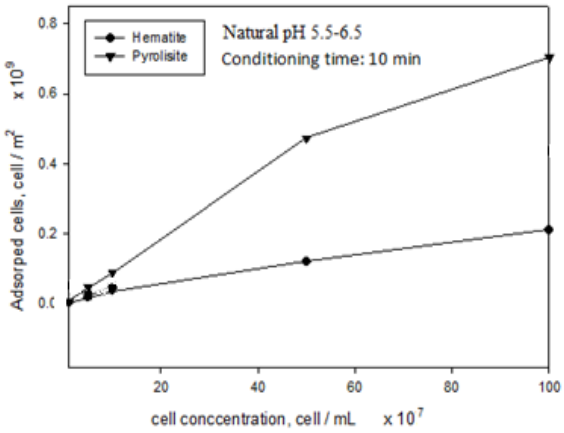

Fig. 8. Adsorption Isotherm of P. polymyxa onto minerals' surfaces

3.9 FTIR of single minerals after treatment with P. polymyxa

As shown in Fig. 9, FTIR spectra of the pyrolusite sample, obtained before the P. polymyxatreatment, 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 treatment, showed similar characteristics bands as the untreated one. 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 [24].

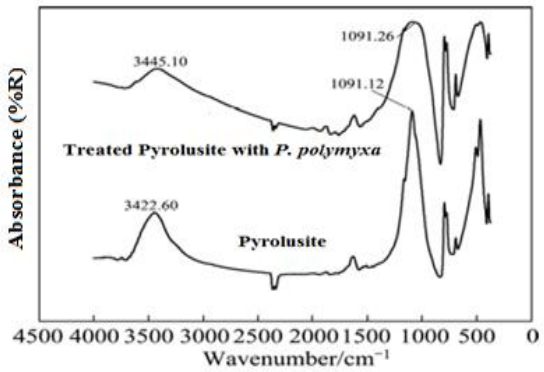

Fig. 9. FTIR spectra of Pyrolusite after treatment with P. polymyxa

FTIR spectra of hematite before and after treatment with P. polymyxa are given in Figure 10. New peaks have appeared these peaks due to the interaction of P. polymyxa cells with hematite mineral. While the 1640 cm-1 peak is attributed to the presence of carboxylate anion, another peak at 1460 cm-1 is that of CH2 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 CH2OH and another peak at 674 cm-1 may be due to CH2 rocking vibrations, [21].

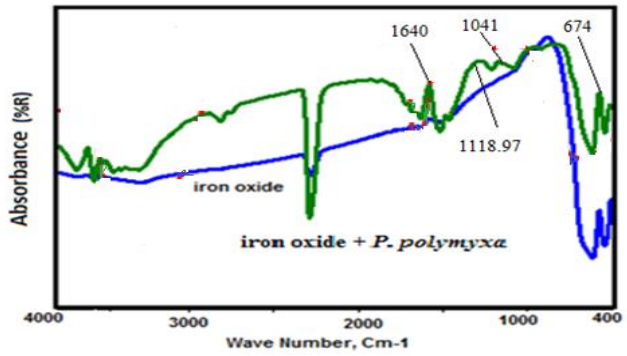

Fig. 10. FTIR spectra of hematite after treatment with P. polymyxa

3.10 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 action of bacterial strain on flocculation of single minerals. For both single minerals, the flocculation power decreased gradually till pH 6.5. The minimum flocculation occurred at pH 12.The results showed that the flocculation power of *P. polymyxa* all over the pH values has the following order: Hematite>Pyrolusite

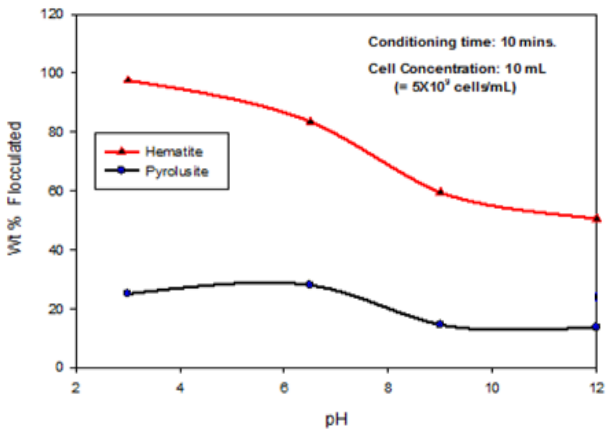

Fig. 11. Flocculation power of single minerals using 10 ml (5x109 cells/ml) P. polymyxa at different pH values

Effect of Bacterial Strain Concentration 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 two minerals can be obtained at concentration of 10 ml (5x10^9 cell/ml) of P. polymyxa.

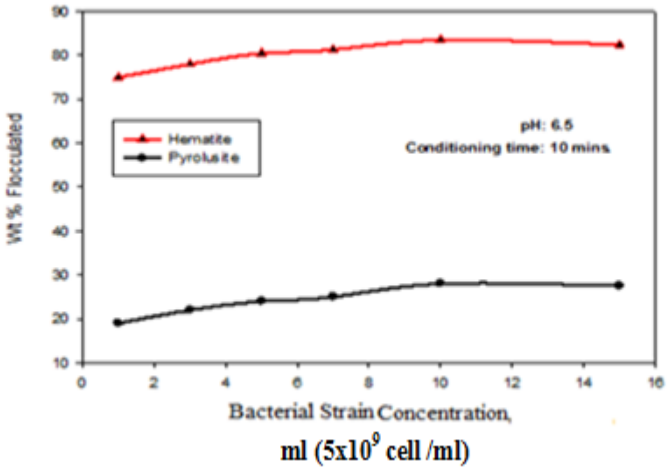

Fig. 12. Flocculation of Single Minerals as a Function of Concentration of P. polymyxa at pH6.5

3.11 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 of 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. This was obtained from a feed containing about 9.97 % MnO_2 with 77 % flocculated by wt. 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 hematite 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 these Egyptian iron ores.

3. CONCLUSIONS

- A successful adsorption of the Paenibacillus polymyxa bacterial strain onto (hematite-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 hematite 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 two single minerals (hematite-pyrolusite) with bacterial strain leads to a displacement for the IEP of them to about 2.6 and 3.2 respectively.
- Applying P. polymyxa bacterial strain as a flocculating reagent at pH 6.5 and at concentration of $5x10^9$ 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 hematite 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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