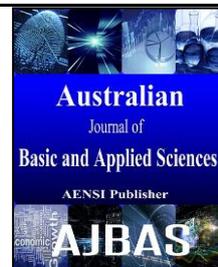




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Studying the adsorption behaviour of polyacrylamide and bacillus psychrodurans onto apatite and silica surfaces

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ABSTRACT

The processing of fine-grained ores frequently results in the production of ultra-fine particles, which respond poorly to conventional physical separation techniques. These ultra-fine particles are often rejected from circuits following comminution. They represent one of the major losses of valuable minerals that are mined but not recovered during primary processing. For example, phosphate rock is a strategic raw material that is indispensable in a wide array of industries and is always associated with gangue minerals such as silica. When these impurities are present as finely disseminated particles in the matrix, their separation process become extremely difficult by the physical separation methods. In this case, fine grinding of these ores is a must to reach a reasonable degree of liberation. In this paper, the amenability of improving the separation efficiency of the system P_2O_5 - SiO_2 was studied through applying the concept of site blocking agent using low molecular weight polymer and bio-reagents (metabolites) from *bacillus psychrodurans* bacteria which is considered as a promising new approach based on integral green chemistry methods that could be used to separate the system of P_2O_5 - SiO_2 . The effect of some operating parameters such as concentration of dispersing agent, anionic PAM dosage, and pH of the medium were investigated. The role of modification of the surfaces of both minerals using low molecular weight polymers was highlighted. Zeta potential and adsorption measurements were also conducted. The best grade (~35.68 P_2O_5 % and 7.42 % silica content), was obtained using 6.0×10^{-5} M SHMP as a dispersing agent and 80 ppm polyacrylamide (PAM) as a flocculant for apatite from a binary mixture of apatite and quartz (consisting of ~ 33.6 % P_2O_5 and 8.27 % SiO_2), in the absence of surface modifier. However, On using the concept of site blocking agent, a concentrate assaying 36.07 % P_2O_5 and 5.89 % silica with total recovery of about 72.64 % has been obtained. On the other hand, application of bioprocessing using *bacillus psychrodurans* bacteria succeeded in obtaining a concentrate assaying 37.5 % P_2O_5 with a recovery of about 89.28 and 4.75% SiO_2 after only a rougher stage at pH 9.0 @ 2.0×10^8 cells of bacteria. This means that there is a strong interaction between *bacillus psychrodurans* bacteria and minerals' surfaces, especially with apatite. Adhesion, adsorption, and zeta potential measurements showed a better affinity of *bacillus psychrodurans* bacteria to apatite mineral surface rather than silica.

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INTRODUCTION

The efficiency of selective adsorption process for solid-solid separation of minerals suspensions in the fine and ultrafine size range is of critical importance to the mineral industry. The process depends, among other things, on applying a suitable selective polymer that can flocculate only one mineral and not the other minerals present in the suspension, (Mathur *et al.*, 2000). The process needs to be understood in terms of mineral surface

interactions, adsorption of used reagents and other operating parameters. The competition between different surfaces for the flocculants has to be controlled to achieve adsorption on the targeted components. Hetero-flocculation is the most technical barrier that affects the selectivity of the process and in turn leads to lower grade. The concept of site blocking agents (SBA) has been developed to minimize such hetero-flocculation. These site blocking agents (SBA) control the surface properties

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of the mineral and/or polymer adsorption through increasing the selectivity of the flocculant onto only one mineral of the mixture, (Moudgil and Prakash, 1998; Mathur *et al.*, 2000).

The new millennium rightly belongs to biotechnology, and rapid progress in minerals processing based on biological principles is just around the corner. 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 attracting in mineral processing due to their lower operating costs and their possible applications to treat difficult to beneficiate low grade complex ores, (Abdel-Khalek *et al* 2013 and Rao *et al.*, 2010).

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]:

- Attachment of microbial cells to the solid substrate.
- Oxidation reactions.
- Adsorption and/or chemical reaction with the metabolite products

Application of bio-reagents as flocculants involves several aspect, surface charge, presence of specific hydrophobic groups and polymers compounds which deeply affect their adhesion to the mineral [Pearse, 2005 ; Smith and Miettinen, 2006].

This study is very important as phosphate rocks are vital nonrenewable resources and are essential components in agricultural fertilizers. About 85% of the phosphate produced is consumed in the fertilizer industry. In the recent years, mining industry faced several problems such as the depletion of high-grade ores and environmental regulations. Thus, it has become very important to develop appropriate and environmentally friendly technologies able to complement the conventional techniques used at the mineral concentration. In this context, bio-beneficiation is increasing its role in mineral processing. The main purpose of this procedure is to remove undesirable mineral constituents from an ore, through interaction with microorganisms and/or their metabolic products.

Therefore, in this study, the amenability of applying the technique of selective flocculation for ($P_2O_5 - SiO_2$) system was studied. The effect of operating parameters on the efficiency of selective separation of a binary mixture (apatite – silica system) was investigated. The role of surface modification, through applying the concept of site blocking, to enhance the selectivity of separation for both minerals was also highlighted. The amenability of utilization of *Bacillus psychrodurans* isolated and adapted on surfaces of two single minerals as a

flocculation reagent for separating the harmful impurities such as silica in the bio-flocculation of apatite-silica minerals system was studied. The adsorption of the bacterial isolate on the surface properties of the two single minerals has been studied through zeta potential and adhesion measurements as well as bio-flocculation tests.

Experimental:

Materials:

Samples of pure minerals of apatite and quartz were delivered from "Wards" Company, USA. The samples were dry ground in a porcelain mill. The - 200 mesh fractions were used in adsorption. The purity (99.9 %) of samples was confirmed using X-ray diffraction (XRD) and chemical analyses. Sodium hexametaphosphate (SHMP), from BDH Company, was used as a dispersing agent. Anionic polyacrylamide (PAM) with Molecular Weights of (7,000,000 -10,000,000), and (700,000-1,000,000) Magnafloc Co, were tested as flocculants. Analytical grade NaOH and HCl were used for pH regulation. Freshly prepared solutions of each SHMP and polymers were used as dispersing and flocculating agents.

Methods:

Isolation and Growing of Bacteria:

0.5 gm of minerals was serially diluted with sterile water and spread on the surface of nutrient agar plates which were incubated at 30 °C from 1 to 3 days at 30°C (Shashikala, 2001).

Morphological and Gram Staining Identification:

Microscopic examination and gram staining of the selected bacterial isolate were carried out.

Bio-Chemical Identification:

The selected bacterial isolate was identified using the BIOLOG GEN III Micro-plate microbial identification system. A pure freshly grown culture was isolated on Biolog recommended agar media and incubates at 33°C. An inoculum was prepared at cell density in the range of 90-98%T. Biolog micro-plate wells were inoculated with 100 µl of cell suspension. The micro-plate was incubated at 30°C then it was Read Micro-Plates using Biolog's Microbial Identification Systems software (e.g. OmniLog® Data Collection).

Zeta Potential Measurements:

A Laser Zetameter "Malvern Instruments" model "Zeta Sizer 2000" was used for zeta potential measurements. 0.05 gram of sample was placed in 50 ml double distilled water with definite dispersant or flocculant concentration at ionic strength of 2×10^{-2} M NaCl. The pH was then adjusted to the required value. The suspension was shaken for 1 hour. After shaking, the equilibrium pH was recorded, allowed to settle for 3 min, and about 10 ml of the supernatant

was transferred into a standard cuvette for zeta potential measurement. Solution temperature was maintained at 25°C (Abdel-Khalek *et al* 2013).

Measuring Selectivity of *Bacillus Psychrodurans* 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 *Bacillus Psychrodurans* was conditioned with one gram of each mineral for 60 minutes before recording the change in size distribution.

Adhesion Measurements:

Adhesion of *Bacillus Psychrodurans* 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 –

$$q_e = \frac{(C_o - C_e)V}{W \times \sigma}$$

45°C. Adhesion studies were performed as a function of difference in weight before and after drying.

Adsorption Measurements:

In experiments of studying adsorption, 0.5 gram dry sample of both apatite and quartz was added to 50 ml double distilled water at the required

concentration of dispersing agent or polymer. The pH was adjusted using HCl and NaOH. A potentiometer (Orion Mod. 720A) equipped with a combined electrode was used to monitor the pH. The potentiometer was calibrated before each test by using buffer solutions of pH 4, 7 and 10. After conditioning the pulp for the equilibrium time, the samples were centrifuged at 15000 rpm for 15 minutes to separate the supernatant from the settled fraction. The samples were centrifuged at 15,000 rpm for 15 minutes to separate supernatant from the settled fraction. The total organic carbon (TOC) content (residual concentration) in the 40 ml of the supernatant was determined using a "Phoenix 8000" Total Carbon Analyzer. The average of 3 readings was taken as a measure for the residual concentration of organic carbon (Abdel-Khalek *et al* 2013). The adsorption density at equilibrium q_e (g/m²) was calculated according to the following equation:

Where C_0 and C_e are the initial and equilibrium solution concentrations (g/m³), respectively, V the volume of the solutions (m³) and W the weight of sample used (g). σ is the specific surface area of the solids (m²/g).

RESULTS AND DISCUSSIONS

Bacillus Psychrodurans Description:

Morphologically, *Bacillus Psychrodurans* bacterial colonies are circular, sigmoid; brighten with a yellow brownish color as shown in Figure 1. While microscopic examination showed that cells of the bacterium are gram positive, rod shaped, Figure 2. Also, it is growing optimally at temperatures 34-37°C. It is motile by peritrichous flagellum. Optimum pH is from 6-7, (Abdel-Khalek *et al* 2013).



Fig. 1: Bacterial Colonies

Growth at 1% NaCl is positive and no growth at 4% NaCl or 8% NaCl. Acid production from L-glucose, D-arabinose, L-xylose and L-mannitol is negative. No hydrolysis of cellulose. No hydrolysis of pectin. No hydrolysis of gelatin. No hydrolysis of casein. No hydrolysis of aesculin. Hydrolysis of Tween 80 positive. No hydrolysis of urea. No hydrolysis of 4-methylumbelliferone glucuronide. No

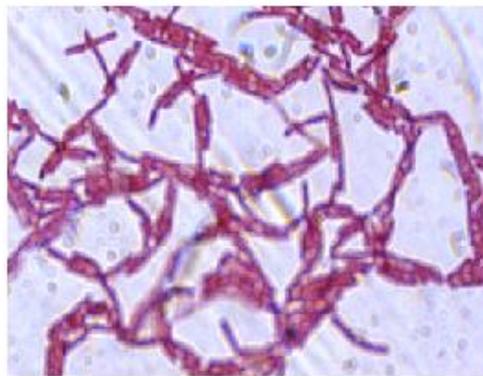


Fig. 2: Gram Positive Rods

citrate hydrolysis. Nitrate is reduced to nitrite. Indole-negative.

The growth curve of the selected bacterial isolate is shown in Figure 3. The lag phases for bacteria are fairly short after which exponential growth takes place. The absorbance was taken as a measurement of the maximum number of cells from the curve shown that the maximum number was

obtained after about 17 for *Bacillus Psychrodurans*. All adsorption experiments and adhesion measurements were performed at the above mentioned growth time for *Bacillus Psychrodurans*

bacteria in which maximum numbers of cells and large amounts of products were obtained. At these points, best results were obtained in comparison with those obtained before this time (Samah Saleh, 2013).

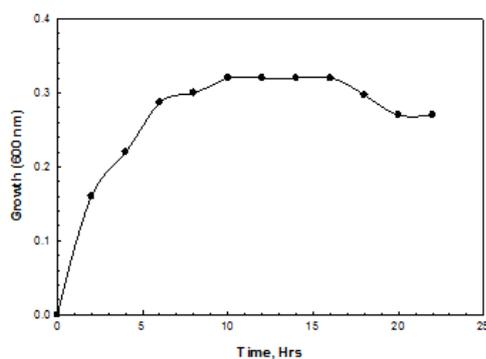


Fig. 3: Growth Curve of *Bacillus psychrodurans*

The Adsorption Isotherm of SHMP onto Minerals:

The adsorption isotherm of sodium hexametaphosphate (SHMP) onto apatite and quartz minerals at room temperature (25°C) and pH 6 is

shown in Figure 4. It is noticed that with an increase in dispersant concentration from 5×10^{-4} M to 3×10^{-2} M, the amount of SHMP adsorbed also increases.

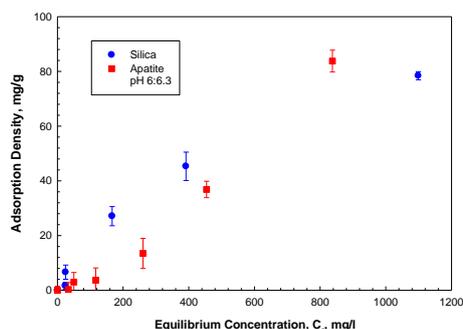


Fig. 4: Adsorption Isotherm of SHMP onto Apatite and Silica

This is also confirmed by increasing the dispersion (in terms of turbidity), of each mineral suspension, with increasing the dosage of SHMP up to a certain concentration and above which a plateau is noticed, Figure 5. Increasing the concentration of SHMP is also accompanied by a gradual increase in the negativity of their surfaces as shown in Figure 6. Leja (1982) concluded that polyphosphate ions in

addition to their electrostatic adsorption onto quartz, they can form covalent bonds with a number of metallic cations. Therefore, they can become either incorporated in the electrical double layer or can react covalently through one or two of their ionized oxygen, leaving all other un-reacted groups to charge the surface with excess negative charges (Selim *et al*, 2009).

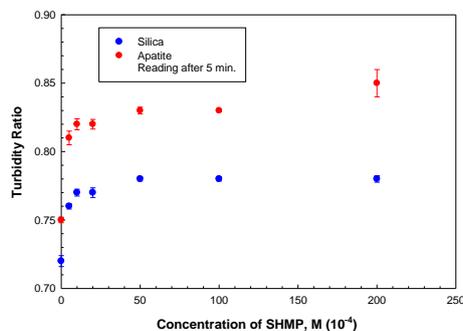


Fig. 5: Effect of SHMP Concentration on Turbidity of Single Minerals

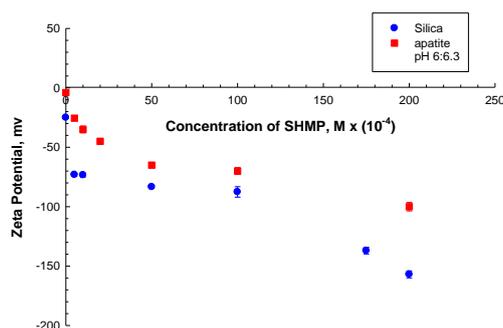


Fig. 6: Effect of SHMP Concentration on Zeta Potential of Single Minerals

The Adsorption Isotherm of PAM onto Apatite Mineral:

The adsorption isotherm of anionic polyacrylamide (PAM) with molecular weight of (7,000,000 -10,000,000) onto apatite mineral at room temperature (25°C) and pH 6 is shown in Figure 7. It is noticed that there is a gradual increase in the adsorption density of PAM onto apatite surface from ~ 0.95 mg/g to 3.38 mg/g with increasing the concentration of PAM from 10 to 40 mg/l and thereafter a plateau is obtained. The results also show that addition of about 10 mg/l can flocculate about 94 % by weight of the sample indicating the higher efficiency of this type of anionic polymer towards

apatite. The adsorption of PAM onto apatite surface at pH 6 is expected to be high since at such pH (6), the zeta potential is low which favor the adsorption process. The higher flocculation power at lower concentration of PAM suggests also that the process proceeds through bridging mechanism. Further addition of PAM above 40 mg/l adversely affected the flocculation power where wt % settled decreased although the noticeable increase in adsorption. This might be correlated to a reduction in the bridging process between the particles as a result of the expected increase in repulsive forces between the negatively charged adsorbed polymer molecules (Abdel-Khalek *et al* 2015).

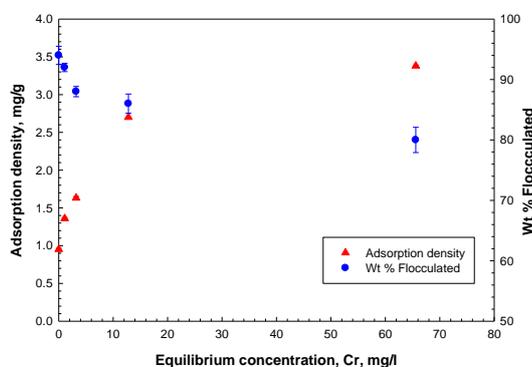


Fig. 7: Adsorption isotherm of polyacrylamide onto Apatite (Effect of Polymer Concentration)

It has been reported that polyacrylamides adsorb weakly on quartz because of electrostatic repulsion. This phenomenon could also be explained in view of the probable role of polymer conformation that might occur at higher polymer concentration thereby preventing the polymer from lying flat on the surface. Reduced stretching of polymer at higher polymer concentration is due to crowding of the polymer on the surface and in turn decreasing the chances of bridging between the particles (Abdel-Khalek *et al* 2015, Samah Saleh, 2013 and Somasundaran *et al.*, 1992).

The Effect of pH on Adsorption:

The effect of initial pH, within range of 2 to 11, on the equilibrium adsorption at 20 mg/l initial PAM concentration at 25°C are shown in Figure 8. It is well-known that the solution pH plays a major role in determining the amount of collector adsorbed onto minerals surfaces. The variation of surface species with pH affects the zeta potential and mineral surface properties. Solution pH also influences both the solid surface and its active sites and the solution chemistry. Higher adsorption with the maximum flocculation power obtained at lower pH values may be due to the electrostatic attractions between negatively charged polymer anions and positively

charged solid surface. In the acidic medium the polymer has two types of hydrogen donating groups via $-\text{COOH}$ and $-\text{CO-NH}_2$. However, the hydrogen bond via $-\text{COOH}$ is stronger than via $-\text{CO-NH}_2$, due to the greater electronegativity of the oxygen atoms (Khangaokar and Subraman, 1993). It was suggested

that flocculation at highly alkaline conditions might result in formation of more open and weak flocs on account of the minimal PAM adsorption due to the repulsive forces between the highly negative charged apatite surface and the anionic polymer (Samah Saleh, 2013 and Bersa *et al*, 2002).

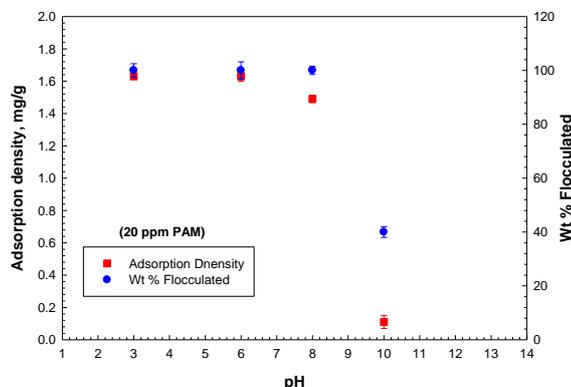


Fig. 8: Effect of Changing pH on Adsorption and Flocculation of Apatite

Role of Site Blocking Agent, (SBA):

In order to illustrate the role of surface modifier (SBA), low molecular weight PAM (700,000-1,000,000), its adsorption on both apatite and quartz is performed, under similar operating conditions, Figure 9. The results indicate that the modifier can adsorb onto both minerals with different degrees where it has a higher affinity to quartz surface. This

means that the modifier has strong affinity to act as site blocking for quartz rather than apatite under similar operating conditions and in turn improves the selectivity of the adsorption where concentrates of better grade (in terms of higher percentage of P_2O_5 with lower % silica) can be obtained (Van de Ven *et al*. 2004)

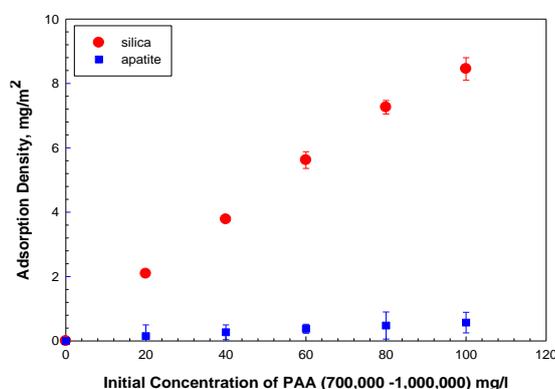


Fig. 9: Adsorption of SBA onto Apatite and Quartz

For a binary mixture of apatite and quartz (consisting of ~ 33.6 % P_2O_5 and 8.27 % SiO_2), in the absence of surface modifier, the best grade (~35.68 P_2O_5 % and 7.42 % silica content), was obtained using 6.0×10^{-5} M SHMP as a dispersing agent and 80 ppm polyacrylamide (PAM) as a

flocculant for apatite. However, repeating the experiment at the same conditions but in presence of 60 ppm of surface modifier yielded a concentrate assaying 36.07 % P_2O_5 and 5.89 % silica with total recovery of about 72.64 %, Table 1.

Table 1: Selective flocculation of apatite - quartz mixture in absence and presence of surface modifier

SBA (PAM ,700,000-1,000,000), ppm	Concentrate		
	P_2O_5 %	SiO_2 %	% P_2O_5 Recovery
0	35.68	7.42	92.85
60	36.06	5.89	72.64
Feed	33.60	8.27	

Physico-Chemical Properties of *Bacillus Psychrodurans* Bacteria:

Physico-chemical properties of the selected bacterial isolate was studied by different techniques

including zeta potential measurements, adsorption of microorganisms onto minerals' surfaces and adhesion measurements for the two single minerals of apatite and silica. The zeta potential of the isolated microorganism was performed at constant ionic strength of 10^{-2} M NaCl over a wide range of pH, the

results of which are shown in Figure 10. The results clearly indicate that the curve conducted at the definite ionic strength of electrolyte, intersect with pH axis at $\text{pH} \sim 3.5$ for *Bacillus psychrodurans*. This value is considered the isoelectric point (iep) for this microorganism.

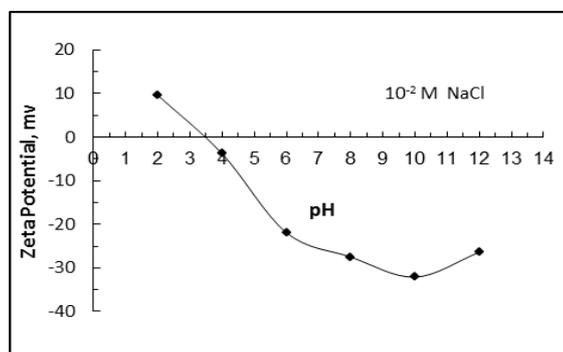


Fig. 10: Zeta Potential of *Bacillus Psychrodurans*

Measurement of Size Distribution:

The change in size distribution of single mineral samples, of apatite and quartz, after their treatment with *Bacillus Psychrodurans* was taken as a measure for the selectivity for adsorption. Successful adsorption of such microorganism will cause, therefore, a degree of aggregation (or dispersion) for mineral particles leading to a change in their size

distribution. The larger change in size distribution for mineral means the more selectivity of microorganism to the mineral surface, (Samah Saleh, 2013, Abdel-Khalek & Farrah, 2004 and Abdel-Khalek et al., 2015). The change in size distribution of apatite and silica single minerals was depicted in Figures 11 and 12.

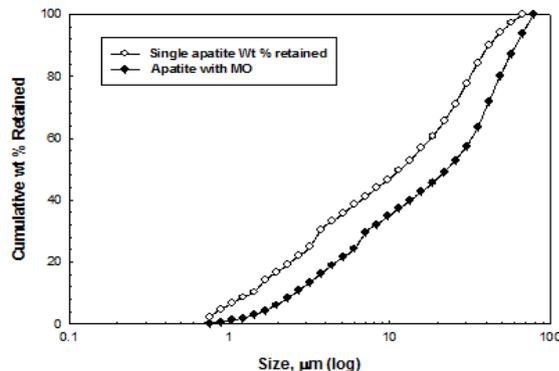


Fig. 11: Size Distribution of Apatite with *Bacillus Psychrodurans*

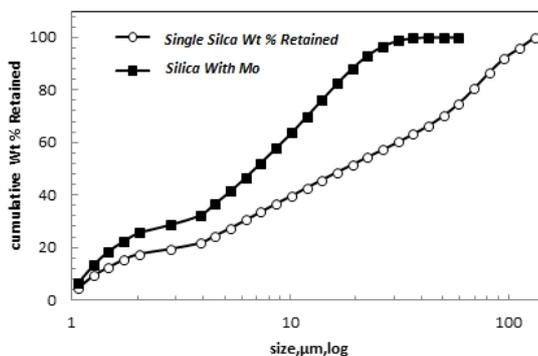


Fig. 12: Size Distribution of Silica with *Bacillus Psychrodurans*

These results show different degrees of variation in the size distribution of samples after their treatment with *Bacillus Psychrodurans* bacteria. It is clear that such treatment caused a degree of aggregation for apatite mineral leaving the silica particles more dispersing in the pulp. Based on these analyses, it has been decided to use this type of bacteria as surface modifiers (or as a flocculation reagent) in separation of silica from apatite.

Measurement of Adhesion Properties:

Figure 13 shows the adhesion of *Bacillus Psychrodurans* onto the surfaces of single minerals

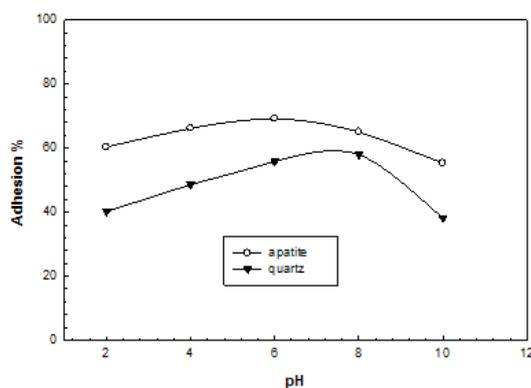


Fig. 13: Adhesion of *Bacillus Psychrodurans* onto Single Minerals

Adsorption Isotherm of *Bacillus Psychrodurans* Bacteria/(Apatite–Quartz System):

The adsorption isotherm of *Bacillus Psychrodurans* bacteria was performed at pH 9. The results indicated that the adsorption density onto apatite and quartz generally increased with increasing the concentration of bacteria, Figure 14. The results showed that the adsorption density at higher concentration of bacterial isolates has the

following order: apatite > quartz. Such higher bacterial affinity to apatite in comparison to quartz is readily evident. The increased adsorption tendency of bacterial cells onto apatite at the mentioned pH can be attributed to electrostatic forces. Besides electrostatic forces, hydrogen bonding and chemical interaction also play significant roles in bacterial interaction with these minerals (Dwyer *et al.*, 2012).

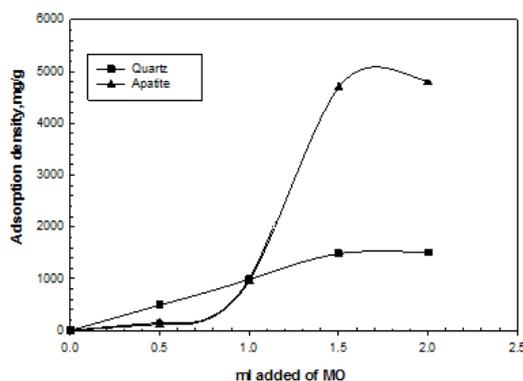


Fig. 14: Adsorption Isotherm of *Bacillus Psychrodurans* onto Apatite and Quartz

Bio-Flocculation of Binary Mixture (Apatite – Silica):

The results in Table 2 showed that it is possible to use *Bacillus Psychrodurans* bacteria as a flocculating agent to separate the valuable mineral of apatite from its associated silica particles. After only

a rougher stage at pH 9.0 while using about 2.0×10^8 cells of bacteria yielded a concentrate assaying 37.5 % P_2O_5 with a recovery of about 89.28 and 4.75% SiO_2 from a binary mixture of apatite and quartz (consisting of ~ 33.6 % P_2O_5 and 8.27 % SiO_2). Such grade can be further improved upon cleaning stages.

Table 2: Selective Bio-flocculation of apatite - quartz mixture

Conditions		Concentrate		
Micro-organism Conc., (cells)	pH	P ₂ O ₅ %	SiO ₂ %	% P ₂ O ₅ Recovery
2.0 X10 ⁸	9.0	37.5	4.75	89.28
Feed		33.60	8.27	

Conclusions:

- Adsorption of sodium hexametaphosphate (SHMP) causes a change in the surface charge density where the zeta potential of the particles of both minerals becomes more negative.

- pH plays an important role in the adsorption characteristics of PAM and its flocculation power.

- The selectivity of flocculation process can be greatly maximized by applying a suitable surface modifier prior the addition of the flocculant.

- The modifier adsorbs onto both minerals with different degrees where it has a strong affinity to act as site blocking for quartz rather than apatite under similar operating conditions.

- For a binary mixture of apatite and quartz (consisting of ~ 33.6 % P₂O₅ and 8.27 % SiO₂), in the absence of surface modifier, the best grade (~35.68 P₂O₅ % and 7.42 % silica content), was obtained using 6.0 x10⁻⁵ M SHMP as a dispersing agent and 80 ppm polyacrylamide (PAM) as a flocculant for apatite. However, repeating the experiment at the same conditions but in presence of 60 ppm of surface modifier yielded a concentrate assaying 36.07 % P₂O₅ and 5.89 % silica with total recovery of about 72.64 %.

- 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 and this method is characterized by its environmental sound and cost effectiveness

- The results of zeta potential clearly indicate that the isoelectric point (iep) for *Bacillus psychrodurans* is at pH~3.5.

- The results of size distribution after their treatment with *Bacillus Psychrodurans* bacteria showed that there is a degree of aggregation for apatite mineral leaving the silica particles more dispersing in the pulp.

- The adsorption results showed that the adsorption density at higher concentration of bacterial isolates has the following order: apatite > quartz

- On using *Bacillus Psychrodurans* bacteria as a flocculating agent, at pH 9.0 and at 2.0 X 10⁸ cells of bacteria, a concentrate assaying 37.5 % P₂O₅

with a recovery of about 89.28 and 4.75% SiO₂ from a binary mixture of apatite and quartz (consisting of ~ 33.6 % P₂O₅ and 8.27 % SiO₂) was obtained.

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