

Surface Characteristics of *Bacillus megaterium* and Its Adsorption Behavior onto Dolomite

Mohsen Farahat, Tsuyoshi Hirajima

Abstract—Surface characteristics of *Bacillus megaterium* strain were investigated; zeta potential, FTIR and contact angle were measured. Surface energy components including Lifshitz-van der Waals, Hamaker constant, and acid/base components (Lewis acid/Lewis base) were calculated from the contact angle data. The results showed that the microbial cells were negatively charged over all pH regions with high values at alkaline region. A hydrophilic nature for the strain was confirmed by contact angle and free energy of adhesion between microbial cells. Adsorption affinity of the strain toward dolomite was studied at different pH values. The results showed that the cells had a high affinity to dolomite at acid pH comparing to neutral and alkaline pH. Extended DLVO theory was applied to calculate interaction energy between *B. megaterium* cells and dolomite particles. The adsorption results were in agreement with the results of Extended DLVO approach. Surface changes occurred on dolomite surface after the bio-treatment were monitored; contact angle decreased from 69° to 38° and the mineral's floatability decreased from 95% to 25% after the treatment.

Keywords—*Bacillus megaterium*, surface modification, flotation, dolomite, adhesion energy.

I. INTRODUCTION

UTILIZATION of microorganisms as surface modifiers has become one of the interesting research topics in mineral processing field, extensive studies have been conducted in this regard; some microbial strains were found to act as flotation collectors, quartz and corundum were floated by using *E. coli* as a collector [1], [2], *Rhodococcus erythropolis* and *Rhodococcus ruber* strains were found to act as powerful collectors for hematite mineral [3], [4]; on the other hand, some strains were found to act as depressants; pyrite was preferably depressed from galena mixture by *Paenibacillus polymyxa* [5], arsenopyrite was separated from pyrite mixture by using *Acidithiobacillus ferrooxidans* as a depressant [6], floatability of pyrite decreased dramatically after being treated with *Ferropalsma acidiphilum* archaeon [7], [8]. Moreover, some microbial strains were successfully used as bio-flocculants; quartz was flocculated by modified *E. coli* at acidic pH region [9], pyrite was selectively flocculated from galena mixture by *Paenibacillus polymyxa* [5] and from arsenopyrite mixture by *Acidithiobacillus ferrooxidans* [6].

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Microbe surface characteristics play a crucial role in its utilization as a surface modifier. Attachment of a microbe to a mineral surface is a prerequisite for most of biological activities including bio-surface modification; microbe-mineral attachment is influenced by several parameters like surface characteristics of microbe and mineral, microbe morphology and the chemistry of the solution in which microbe and mineral meet. Several methods have been reported for microbial cell physicochemical characterization and its relevance in microbial adhesion [10]. Zeta potentials have been extensively used to understand changes in the surface charges of cells and mineral particles and to explain the adsorption behavior at different pH regions [11]. FTIR has been used to determine the functional groups present on the outer surface of microbial cells and [12]. Contact angle of microbial cells and minerals has been extensively studied as a measure of hydrophobicity and to calculate surface energy parameters and free energy of adhesion between microbes and minerals [13].

Bacillus megaterium is a heterotrophic, neutrophilic, mesophilic bacterium. It is characterized with its big size; it has an approximate volume 100 folds more than that of *E. coli*. The strain is widely found in soil, sediment, honey, fish, and dried food [14]. *B. megaterium* as a Gram positive bacterium, its outer surface is rich in peptidoglycan, teichoic and teichuronic acids [15], [16]. The microbe has distinctive features like secretion of exoenzymes and expression of many metabolic genes, these features made the microbe has a potential in agriculture and medical fields [14].

Few studies were conducted to utilize *B. megaterium* in mineral processing filed [17], [18] and unfortunately, these studies focused only on utilization of cellular/extracellular/adapted cells in sulfide mineral flotation.

In this research work, surface characteristics of vegetative wild cells of *B. megaterium* strain were investigated and its adhesion behavior to dolomite has been studied as well. Extended DLVO approach was used to estimate the microbe-mineral adhesion energy. Subsequent changes on the mineral surface after the biotreatment were followed by measuring contact angle and mineral floatability.

II. MATERIALS AND EXPERIMENTAL METHODS

A. Mineral Sample

Hand-picked natural pure dolomite sample was received from British geological survey. The sample was received as lumps and it was crushed with a hammer and ground in a planetary ball mill (Fritch pulverisette 6). The ground samples were sieved to obtain the -105 μm + 78 μm size fraction for

flotation experiments and the $-38\ \mu\text{m} + 5\ \mu\text{m}$ size fraction for adsorption and electrokinetic experiments. For contact angle measurements, massive sample was prepared as follow: the sample was cut as a flat shape and then it was exposed for polishing from #800 to #4000 emery paper and Texmet (Buehler, USA) perforated non-woven pat and DP-Nap (Struers, Germany) fine polishing cloth mounted on a plate using $3\ \mu\text{m}$ and $1\ \mu\text{m}$ diamond spray.

B. Cultivation of *Bacillus Megaterium*

A pure culture of *B. megaterium* strain was received from Japan Collection of Microorganisms under JCM2506 number. The lyophilized strain was rehydrated by nutrient broth consisting of peptone (5.0 g/l), beef extract (3.0 g/l) then the suspension was spread over solid medium plates consisting of peptone (10.0 g/l), beef extract (10.0 g/l), NaCl (5.0 g/l) and Agar (15.0 g/l) with pH 7.0-7.2. The plates were incubated in a static incubator at $28\ ^\circ\text{C}$ for 24 hours. After incubation, a single colony was picked up and a subculture was established in a fresh liquid medium in shake flasks at 130 rpm and at $28\ ^\circ\text{C}$. The cell concentration was determined by measuring the optical density at 660 nm on a spectrophotometer. Cells were harvested by centrifugation (Tomy SRX-201) at 10000 g for 20 min. The harvested cell pellets were washed three times with 2 mM Tris buffer and then stored in a refrigerator at $4\ ^\circ\text{C}$ until being used in adsorption and flotation experiments.

C. Zeta Potential

After conditioning at the required pH values, zeta potentials of *B. megaterium* cells and dolomite were measured using the ZEECOM ZC-2000 system equipped with a video recorder. Zeta potential of bacterial cells was measured at a bacterial concentration of 10^7 cells/ml. All measurements were conducted at the same ionic strength (10^{-3} M KNO_3).

D. FTIR

A freshly prepared cell suspension of *B. megaterium* strain was centrifuged and washed well with distilled water. The washed cells were dried under vacuum using a freeze dryer (EYELA FD-5N). The dried cells were used for FTIR measurements.

Infrared spectra were measured using a JASCO 670 plus spectrometer. Samples were prepared by dispersing it in KBr. Radiation was measured against nonadsorbing KBr, which serves as a reference. Measurement time was approximately 1 min for 100 scans at a resolution of $16\ \text{cm}^{-1}$.

E. Contact Angle Measurements

Contact angles of *B. megaterium* lawn were measured according to procedures followed in previous studies [19]-[21]. Bacterial lawn was prepared as follow, a cell suspension was filtered on a cellulose triacetate filter membrane (pore size $0.4\ \mu\text{m}$) by applying negative pressure, and the bacterial lawns were deposited to a density of about 700-900 layers. The bacteria-containing filter was placed in a petri dish on a surface of a layer of 1% (w/v) agar in water containing 10% (v/v) glycerol to establish constant moisture content. The filter with the bacterial lawns was cut into strips of appropriate size

and fixed with double-sided adhesive tape onto a sample holder. Contact angles of different liquid drops positioned on the bacterial lawns were measured using a goniometer (Dropmaster 300, Kyowa Interface Science Co., Ltd.). Drops of water, chloroform, and 1-bromonaphthalene were used.

F. Adhesion Experiments

Adsorption experiments were run according to this protocol: a 300-ml Erlenmeyer flask containing 0.5 g dolomite ($-38\ \mu\text{m}$) and 50 ml of 10^{-3} M KCl was conditioned for 5 min before adjusting the pH to a desired value, then, a specific concentration of the cell suspension was added to the flask to achieve the preselected initial concentration of cells, and the slurry was conditioned on a mechanical shaker at 120 rpm and $25\ ^\circ\text{C}$ for further 5 minutes. The bacterial concentration in the slurry after adsorption was measured microscopically using the Petroff-Hausser counter, and the difference between the number of cells that remained after adsorption and the initial one equals the number of adsorbed cells. The effect of pH was examined and the average of three runs was reported here.

G. Flotation Experiments

Microflotation tests were carried out using a micro flotation column. In each experiment, 0.5 g of dolomite sample (size $-105\ \mu\text{m} + 75\ \mu\text{m}$) was conditioned in 130 ml of 10^{-3} M KNO_3 for 1 min. The pH was then adjusted to a desired value, and sodium oleate was added as a collector. Then the slurry was conditioned on a mechanical shaker for 5 min. The entire solution was transferred into the flotation column and MIBC was added as a frother, and flotation was initiated by passing nitrogen gas at a flow rate of 20 ml/min for 1 min. When *B. megaterium* was used, the mineral sample was immersed in a predetermined concentration of cells in 100 ml of 10^{-3} M KNO_3 at a desired pH and conditioned for 5 min. The same procedures that were used in the absence of the microorganism were followed. The floated and tailing fractions were collected separately and dried and weighed for calculations.

III. RESULTS AND DISCUSSION

A. Growth Characteristics

The cells showed a typical growth curve and distinct phases could be observed as shown in Fig. 1; lag phase lasted for 2 hours, log phase ranged from 2- 13 hours, and then stationary phase was commenced. Vegetative cells were collected after 90% of the time of the log curve i.e. after 8 hours of the growth.

B. FTIR Analysis

Fig. 2 shows the FTIR spectra of *B. megaterium*. The spectra show similar absorption patterns of the main constituents of Gram positive cell wall; peptidoglycan which represents 40-80% of the dry weight of the cell wall, its function groups' bands; carboxyl, amide, and hydroxyl groups were obtained. In addition to peptidoglycan, the function groups of teichoic and teichuronic acids were observed. The absorbance bands were assigned according to previous reports [16], [22]-[24]. The intense sharp band at $1655\ \text{cm}^{-1}$ indicates

the presence of an amide group (amide I band). The band at 1230 indicates the presence of phosphate group and 1057 assigned for carbohydrate. The band at 1545 cm^{-1} characterizes $-\text{NH}$ bending of the secondary amide group ($-\text{CONH}$). The band at 1400 cm^{-1} is assigned to COO^- groups. The band at 1236 cm^{-1} is due to $-\text{CH}_3$ wagging modes. Thus, the obtained FTIR spectra showed that peptidoglycan and teichoic acids are the major constituents of *B. megaterium* outer surface.

C. Zeta Potential

Fig. 3 depicts zeta potential curves for the microbial cells and dolomite sample at different pH values using 10^{-3} M KNO_3 as the basic electrolyte. In the case of *B. megaterium* cells, the net surface charge of the cells was always negative over the studied pH range, and the isoelectric point (IEP) could not be determined under the experimental conditions. The surface charge of *B. megaterium* can be explained on the basis of the composition of its outer surface structure that was explained in FTIR section, *B. megaterium* outer surface is rich in teichoic and teichuronic acids. Thus, the outer surface of the cell has mainly PO_4^{3-} , COO^- and OH^- groups, which

render the surface negative charges over the entire pH range. These results are in agreement with those of FTIR.

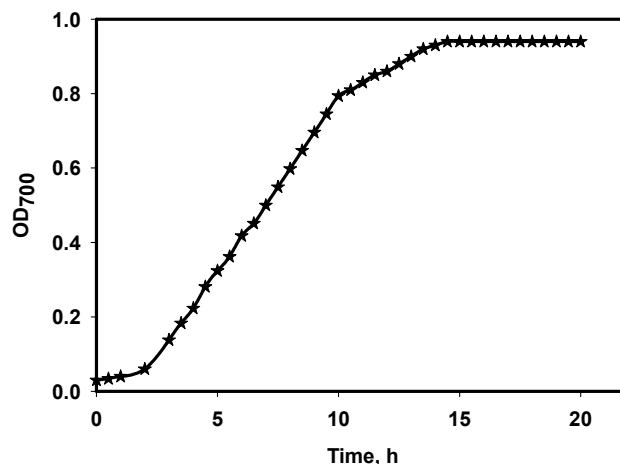


Fig. 1 Growth curve of *Bacillus megaterium* strain

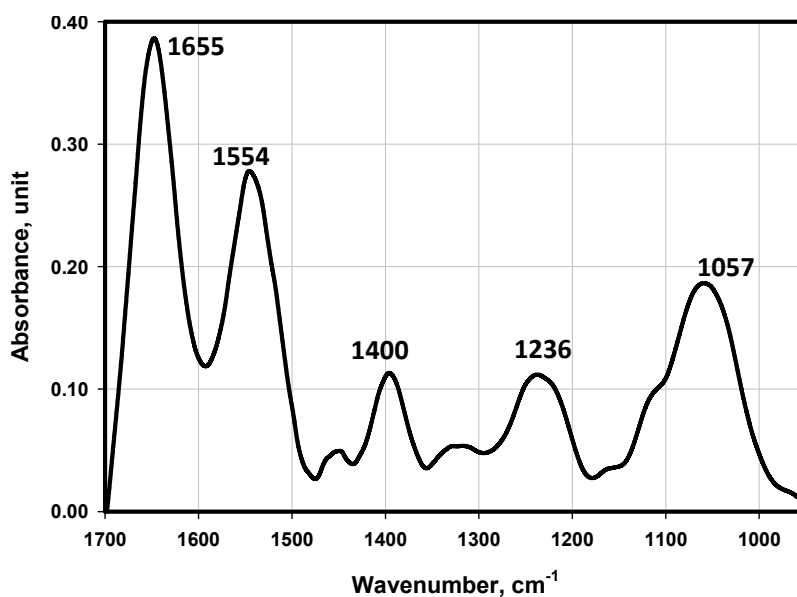


Fig. 2 FTIR pattern of *B. megaterium* vegetative cells

In case of dolomite, the figure shows that the surface charge of dolomite was correlated with the pH and the IEP was obtained at pH 5.2. This can be explained as follows, net surface charge of dolomite is based on the concentration of its positive and negative species; below the IEP, zeta potential of dolomite is positive due to high concentration of the species positively charged ($[\text{Mg}^{2+}] + [\text{Ca}^{2+}] + [\text{MgOH}^+] + [\text{CaOH}^+] > [\text{HCO}_3^-]$). And above the IEP, ($[\text{Mg}^{2+}] + [\text{Ca}^{2+}] + [\text{MgOH}^+] + [\text{CaOH}^+] < [\text{HCO}_3^-]$), and therefore, zeta potential becomes negative [25]-[27]. Zeta potential of dolomite showed a similar trend same as that reported in other literatures [28].

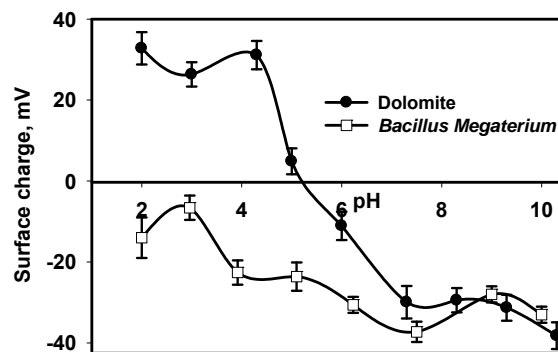


Fig. 3 Zeta potential curves for *B. megaterium* and dolomite, 10^{-3} M KNO_3 .

D. Hydrophobicity of *B. megaterium*

Hydrophobicity of *B. megaterium* was evaluated through calculating the free energy of adhesion between two microbial cells immersed in water [13], [29] ΔG_{bwb} using the following equation [30], [31]:

$$\Delta G_{bwb} = -2 \left(\sqrt{\gamma_b^{LW}} - \sqrt{\gamma_w^{LW}} \right)^2 - 4 \left(\sqrt{\gamma_b^+ \gamma_b^-} + \sqrt{\gamma_w^+ \gamma_w^-} - \sqrt{\gamma_b^+ \gamma_w^-} - \sqrt{\gamma_b^- \gamma_w^+} \right) \quad (1)$$

where γ^{LW} , γ^+ and γ^- refers to Lifshitz-van der Waals surface tension component, electron acceptor and electron donor component respectively, while b and w stand for bacteria and water respectively.

Surface tensions components for water are known from literatures and those for *B. megaterium* were calculated from contact angle data of different three liquid droplets (water, formamide and 1-bromonaphthalene) on the bacteria lawn according to Young-Dupre equation:

$$\frac{1}{2}(1 + \cos\theta)\gamma_L = \sqrt{\gamma_b^{LW} \gamma_L^{LW}} + \sqrt{\gamma_b^+ \gamma_L^-} + \sqrt{\gamma_b^- \gamma_L^+} \quad (2)$$

where γ_L is the liquid surface tension and θ is the contact angle.

Table I shows contact angle results; these data were used as in (2) to calculate surface energy components. Calculated surface energy parameter are shown in Table II.

By using the data in Table II, the ΔG_{bwb} value between two cells of *B. megaterium* immersed in water was positive (+25.80 mJ/m²), this means the cells prefer to be dispersed in water than being aggregated, the result confirms a hydrophilic property for the strain. This is may be attributed to the presence of hydrophilic groups (phosphate, hydroxyl, carboxyl) in the outer surface of the strain, this result coincides with zeta potential and FTIR data.

TABLE I
CONTACT ANGLE DATA FOR DOLOMITE AND BACTERIAL CELLS

	Contact angles		
	Water	Formamide	1-Bromonaphthalene
Dolomite	41.8°	57.0°	28.1°
<i>B. megaterium</i>	20.8°	23.9°	25.7°

TABLE II
SURFACE FREE ENERGY COMPONENTS (MJ/M²) OF *B. MEGATERIUM* AND DOLOMITE

	γ	γ^{LW}	γ^+	γ^-	γ^{AB}
Water*	72.80	21.80	25.50	25.50	51.00
Formamide*	58.00	39.00	2.30	39.60	19.09
1-Bromonaphthalene*	44.40	44.40	0.00	0.00	0.00
<i>B. megaterium</i>	51.36	40.15	0.66	47.61	11.21
Dolomite	41.80	39.35	0.07	23.19	2.47

*These data are taken from literature [33]

E. Adsorption Experiment

Effect of pH on adsorbed cell percent onto dolomite is depicted in Fig. 4. These experiments were carried out at an initial cell concentration of 5×10^8 cells/ml and 5 min conditioning time. The adsorption was high at acidic pH regions comparing to alkaline ones; maximum adsorption was noticed at pH 2-6 where 55-60% of the initial cells were adsorbed. When the pH increased over pH 6, the adsorbed cell percent decreased to 35%. These results coincide with those of the zeta potential measurements (Fig. 3) where at acidic pH values (2-5), attractive forces are present between the cells and dolomite particles (the cells are slightly negative charged while dolomite particles are slightly positive charged), and these promote the adsorption process. However, at high pH values, both cells and dolomite are highly negatively charged, which leads to repulsive forces between them, and these decrease the adsorption density. The adsorption of cells onto dolomite surface at neutral (pH 6) may be attributed to other forces (acid-base or Lifshitz) which will be explained in the next section.

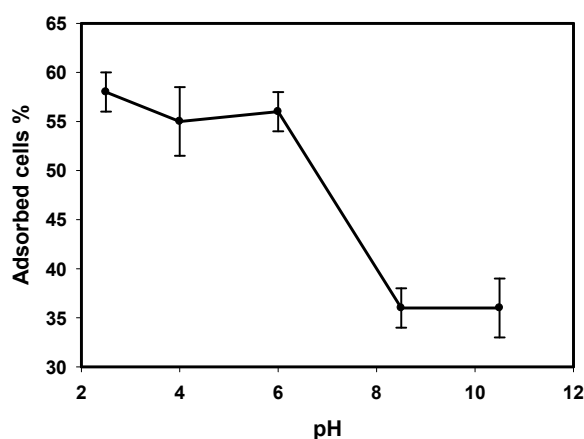


Fig. 4 Effect of pH on adsorption behavior of *B. megaterium* cells onto dolomite

F. Estimation of Interaction Energies by Extended DLVO Theory

Interaction energy between *B. megaterium* and dolomite was calculated using the extended DLVO theory. The classical DLVO theory as described by [32] and [20] includes attractive or repulsive electrostatic forces and Lifshitz-van der Waals attractive forces (LW). The acid-base interaction component, which is based on electron-donating and electron-accepting interactions between polar moieties in aqueous solution, was added by van Oss to formulate the extended DLVO theory (XDLVO).

According to the extended DLVO theory, the total interaction energy (G^{Total}) is determined by:

$$G^{Total} = G^{EL} + G^{AB} + G^{LW} \quad (3)$$

where G^{EL} is the repulsion potential due to the formation of an electrical double layer around particles and cells, and G^{AB} is the acid-base interaction energy and G^{LW} is the Lifshitz-van der Waals attractive energy.

1. Electrostatic Interaction Energy G^{EL}

The electrostatic repulsive force between *B. megaterium* cell dolomite particles can be expressed by the following equation for the sphere-sphere system:

$$G^{EL} = \pi\alpha\epsilon_1 \left(\zeta_1^2 + \zeta_2^2 \right) \left[\frac{2\zeta_1\zeta_2}{\zeta_1^2 + \zeta_2^2} \ln \frac{1 + \exp(-\kappa H)}{1 - \exp(-\kappa H)} + \ln \{1 - \exp(-2\kappa H)\} \right] \quad (4)$$

where ϵ is the dielectric constant of the aqueous medium and is equal to $8.84 \times 10^{-12} \text{ Jm}^{-1}\text{V}^{-2}$; a_1 represents the radius of the *B. megaterium* cell; ζ_1 and ζ_2 are the surface charges of the mineral and cell, respectively; H is the separation distance between the cell and particle; and κ^{-1} is the double layer thickness.

2. Acid-Base Interaction Energy G^{AB}

The acid-base interaction energy for the particle-cell system can be calculated according to the following equation (sphere-sphere system)

$$G^{AB} = \pi a \lambda (\Delta G_{adh}^{AB}) e^{[(d_0 - H)/\lambda]} \quad (5)$$

where a is the radius of the solid particle, λ is the correlation length of the molecules in the liquid ($\sim 6 \text{ \AA}$), d_0 is the minimum separation distance between two surfaces (1.57 \AA), and H is the separation distance. ΔG_{adh}^{AB} can be calculated as follows from the Lifshitz-van der Waals acid-base approach

$$\begin{aligned} \Delta G_{adh}^{AB} = & 2(\sqrt{\gamma_b^+} - \sqrt{\gamma_m^+})(\sqrt{\gamma_b^-} - \sqrt{\gamma_m^-}) \\ & - 2(\sqrt{\gamma_b^+} - \sqrt{\gamma_w^+})(\sqrt{\gamma_b^-} - \sqrt{\gamma_w^-}) \\ & - 2(\sqrt{\gamma_m^+} - \sqrt{\gamma_w^+})(\sqrt{\gamma_m^-} - \sqrt{\gamma_w^-}) \end{aligned} \quad (6)$$

In these equations, γ^+ and γ^- refer to electron acceptor and electron donor parameters, respectively, and b , m , and w represent bacteria, mineral, and water, respectively. These parameters were calculated as shown in Table II.

3. Lifshitz-van der Waals Interaction Energy

For a sphere-sphere interaction, the Lifshitz-van der Waals attractive energy can be obtained from:

$$G^{LW} = \frac{-a_1 A_{bwm}}{6H} \quad (7)$$

where a_1 represents the radius of the *B. megaterium* cell and A_{bwm} is the effective Hamaker constant for the bacteria-water-mineral system.

The effective Hamaker constant A_{bwm} was calculated by

$$A_{bwm} = -12 \pi d^2 \Delta G_{adh}^{LW} \quad (8)$$

where d is the minimum separation distance and ΔG_{adh}^{LW} is the Lifshitz free energy of adhesion and can be evaluated by

$$\Delta G_{adh}^{LW} = -2 \left(\sqrt{\gamma_b^{LW}} - \sqrt{\gamma_w^{LW}} \right) \left(\sqrt{\gamma_m^{LW}} - \sqrt{\gamma_w^{LW}} \right) \quad (9)$$

where γ^{LW} is the Lifshitz-van der Waals energy component and b , m , w stand for bacteria, mineral and water respectively

The total interaction energies between *B. megaterium* cells and dolomite particles according to the extended DLVO theory were calculated at different pH values as a function of the separation distance between the cell and the particle in 10^{-3} M KNO_3 . The results are shown in Fig. 5. At all pH values, it can be observed that the primary contribution to the total energy comes from the electrostatic energy G^{EL} , followed by the acid-base energy G^{AB} and the Lifshitz-van der Waals energy G^{LW} . The effect of the acid-base interaction G^{AB} is much higher than that of the electrostatic and Lifshitz-van der Waals interactions. However, the acid-base interactions are relatively short-range, and the interacting surfaces must be close (less than 5 nm) before these forces become operative. At pHs less than 6, the total extended interaction energy is negative, primary minimums can be observed, which indicate that the adhesion between *B. megaterium* cells and dolomite particles at these pH values is thermodynamically preferred (irreversible adhesion). The magnitude of G^{Total} decreases with the increase in the pH and moved to be positive at pH higher than 6 with high potential barriers which means theoretically, adhesion of the *B. megaterium* cells to dolomite mineral is not favored. The results of extended DLVO approach interpreted those of adsorption experiments over all pH regions.

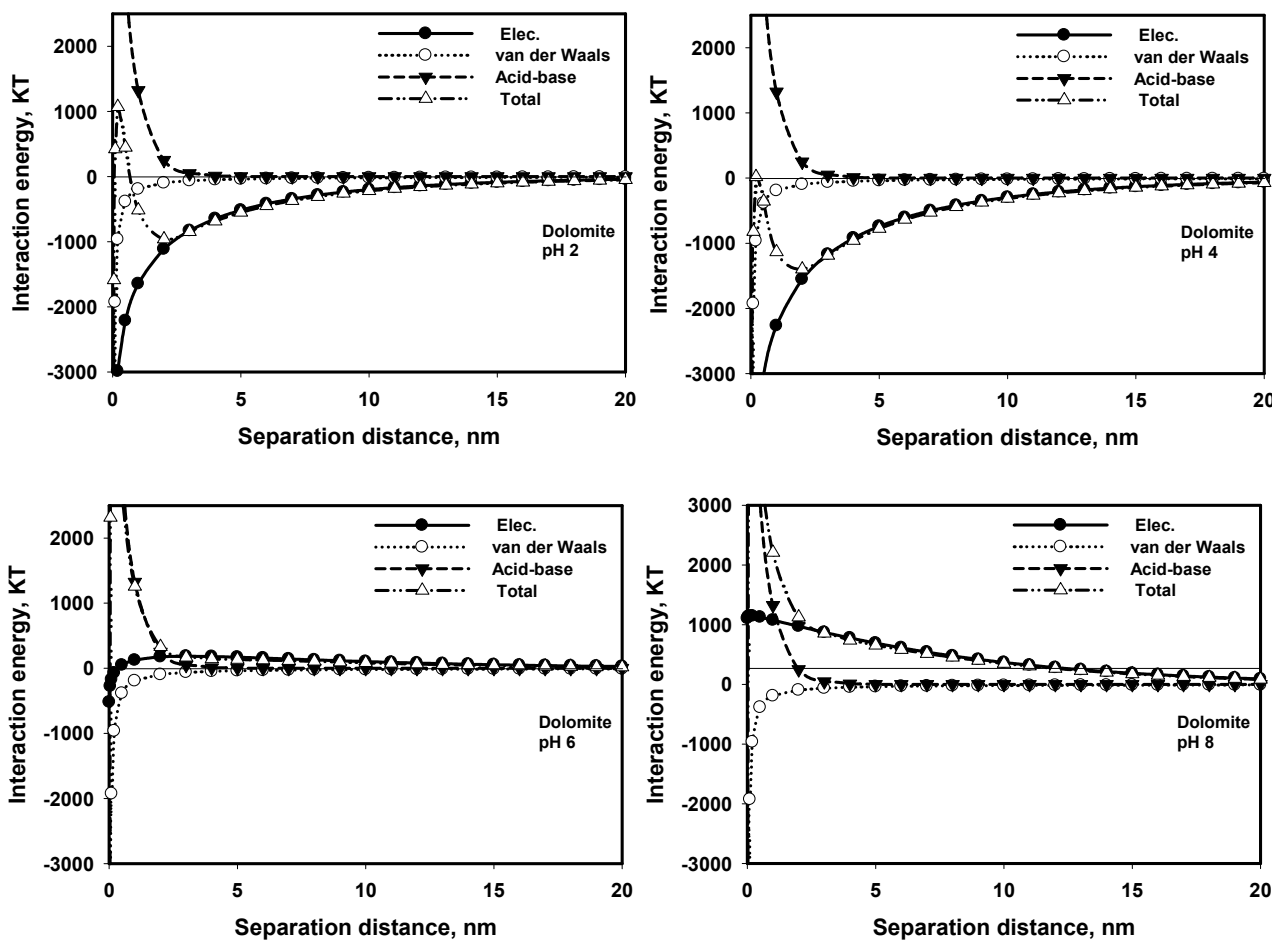


Fig. 5 Interaction energy components for *B. megaterium* cell-dolomite as a function of the separation distance under different pH values

G. Effect of *B. megaterium* on Dolomite Floatability

Fig. 6 depicts floatability of fresh and bio-treated dolomite at different pH values, sodium oleate was used as a collector with a concentration of 2.5×10^{-5} M. It is obvious that for the untreated mineral, over 95% of the feed weight was recovered as a float fraction over all pH regions. When dolomite was pretreated with *B. megaterium*, its flotation recovery decreased dramatically from 95% to about 25-30% at pH regions below pH 6, then a slight effect on dolomite floatability was observed when the bio-treatment was made at alkaline regions. These results match well with those of extended DLVO and adsorption experiments where the number of cells adsorbed (thermodynamically and experimentally) onto dolomite at pH values below pH 6 was higher than that at other pHs. The decrease in the flotation recovery may be attributed to that the adhesion of *B. megaterium* onto dolomite renders dolomite a hydrophilic nature and hinders the formation of hydrophobic compounds (Ca/Mg oleate) on the dolomite surface.

This hypothesis was confirmed by measuring contact angles of dolomite after oleate treatment in presence and absence of *B. megaterium* using captive bubble method. The results are shown in Fig. 7, it can be noticed that contact angle of untreated dolomite increased from 41° to 69° (became hydrophobic) after being treated with oleate. But when the

dolomite was pretreated with *B. megaterium*, almost there was no noticeable change to the contact angle after oleate interaction.

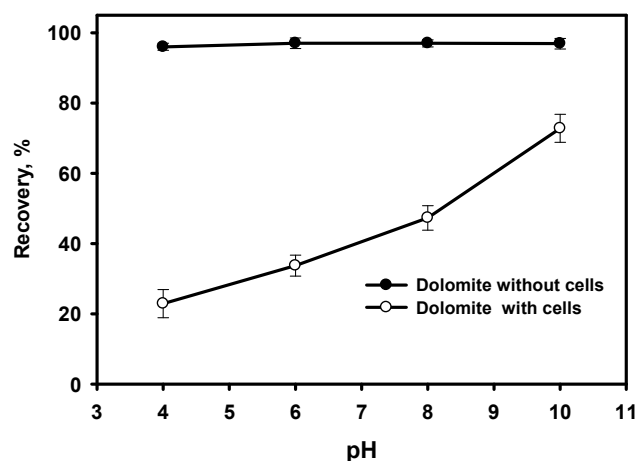


Fig. 6 Floatability of dolomite by oleate in presence and absence of *B. megaterium* at different pH

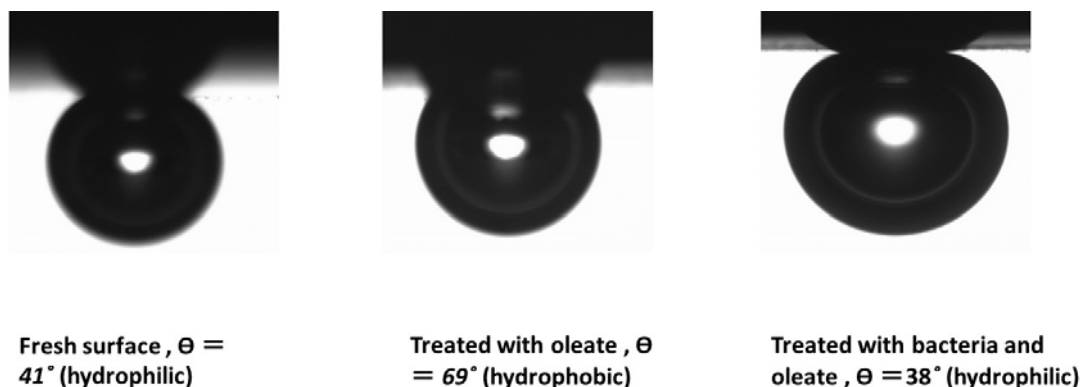


Fig. 7 Contact angle of dolomite under different conditions

IV. CONCLUSION

Surface characteristic experiments confirmed that *B. megaterium* strain has a hydrophilic surface. This is due to the nature of the microbe outer surface, where the outer surface of *B. megaterium* is rich in hydrophilic functional groups like PO_4^{3-} , COO^- and OH^- groups as confirmed by FTIR data. The presence of these functional groups made the microbe negatively charged over all pH regions. The hydrophilicity of *B. megaterium* strain was confirmed from calculated surface energy parameters. The adhesion of *B. megaterium* onto dolomite showed that the microbe has high affinity to dolomite at pH regions below pH 6. Adhesion experiment results coincided with the results of *B. megaterium*-dolomite interaction energy calculated by the extended DLVO theory. Biotreatment of dolomite with *B. megaterium* significantly changed the surface properties of dolomite, contact angle was decreased from 69° to 38° and as a consequence its floatability decreased from 95% to 25% after the treatment.

ACKNOWLEDGMENT

The authors would like to thank Japan Society for the Promotion of Science (JSPS) for financial support to conduct this research work.

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