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The Role of Ionic Strength and Mineral Size to Zeta Potential for the Adhesion of *P. putida* to Mineral Surfaces

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Abstract**—**Electrostatic interaction energy (ΔE^{EDL}) is a part of the Extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory, which, together with van der Waals (ΔE^{VDW}) and acid base (ΔE^{AB}) interaction energies, has been extensively used to investigate the initial adhesion of bacteria to surfaces. Electrostatic or electrical double layer interaction energy is considerably affected by surface potential; however it cannot be determined experimentally and is usually replaced by zeta (ζ) potential via electrophoretic mobility. This paper focusses on the effect of ionic concentration as a function of pH and the effect of mineral grain size on ζ potential. It was found that both ionic strength and mineral grain size play a major role in determining the value of ζ potential for the adhesion of *P. putida* to hematite and quartz surfaces. Higher ζ potential values lead to higher electrostatic interaction energies and eventually to higher total XDLVO interaction energy resulting in bacterial repulsion.

Keywords**—**XDLVO, Electrostatic interaction energy, zeta potential, *P. putida*, mineral.

I. Introduction

BIOFILMS can be beneficial, where their properties are utilized for environmental protection, bioreactor technology, wastewater treatment, waste air purification, soil remediation, and solid waste decomposition. However, biofilms can also have hazardous consequences and detrimental effects especially in equipment damage, product contamination, energy losses and medical infections. Metal corrosion and microbial induced weathering of mineral materials such as stone or cement, resulting in damage (e.g., to oil tanks and pipelines and to concrete sewers) has led to substantial pollution of soil, groundwater and surface waters. In water distribution systems biofilms may cause contamination of drinking water leading to discolouration, off-odours and pathogenic infections such as *Legionella sp*. and *Pseudomonas aeruginosa*. [1]-[6]

In the food industry, the formation of biofilms on food processing equipment is known to cause spoilage and disease [7]. On ship hulls, biofilms are responsible for increased fuel consumption. In the medical field, the formation of biofilms on devices such as catheters and orthopaedic implants frequently constitutes a reason for infection, device failure and removal [8].

The formation and maturation of biofilms is not a well understood process, nor is the mechanism regulating bacterial colony size and species. Biofilms are generally more resistant to antibiotics, disinfectants and cleaning fluids than planktonic microorganisms. Conventional methods of killing bacteria (such as antibiotics, and disinfection) are often ineffective with biofilm bacteria. The large doses of antimicrobials required to get rid systems of biofilm bacteria are environmentally undesirable and are often not allowed by environmental regulations. It is also medically impractical since what it would take to kill the biofilm bacteria would also kill the patient [9]. Conversely, microbial processes at surfaces also offer opportunities for positive industrial and environmental effects, such as bioremediation of hazardous waste sites, bio-filtration of industrial water, and forming bio-barriers to protect soil and groundwater from contamination. Therefore, new strategies based on a better understanding of how bacteria attach, grow and detach are urgently needed by many industries.

The initial stage of biofilm formation is the approach and attachment of microorganisms to the substratum where the free living planktonic bacteria first adhere to the surface of the solid substrate. The types of bacteria that will adhere to a particular surface very much depend upon the surface characteristics of the substratum including its surface chemistry, charge and hydrophobicity [10]. Attachment may occur via either specific or non-specific interactions depending on the stereo-chemistry of the bacterial-surface interaction. This is the three-dimensional configuration of the atoms that make up a molecule at the bacterial-substrate surfaces and the ways in which this arrangement affects the physical and chemical properties of the surfaces [11], [12].

Both specific and non-specific interactions originate from fundamental physico-chemical forces. These consist of van der Waals, electrostatic, and acid-base interactions [13]. Specific interactions operate when molecular recognition takes place between ligand, receptor molecules, and stereo-chemical regions on the bacterium and a receptor on the substratum, which is established by interactions between acid-base, electron accepting and electron donating groups at close approach distances (<1.5 nm) [14].

Attachment via nonspecific interactions is thought to be influenced by a number of physicochemical properties possessed by the substratum and cell, such as charge and

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hydrophobicity, and the suspension medium, such as the ionic concentration [11], [13]-[15]. Other physicochemical factors that influence bacterial adhesion are surface roughness, material, bacterial shell functional groups, biopolymers, and residence time [2], [16].

The Derjaguin-Landau-Verwey-Overbeek (DVLO) theory which consists of van der Waals and electrical double layer interaction energy was developed by [17], and [18], to describe physicochemical interactions of non-biological colloidal particles as they approach a surface.

The surface potential, φ_o is the electric potential at the position on the ionisable surface, where the potential drops across the mobile part of the double layer, which is responsible for electrokinetic phenomena, such as electrophoresis and streaming potential. According to Boltzmann's law, the degree of the ionization depends on the electric potential, because the local proton activity is a function of the local electric potential.

Concentration of electrolytes, such as KCl and NaCl, and pH in solutions influence bacterial adhesion [10]. The effect of ionic strength on bacteria/mineral interactions can be studied by the streaming potential technique (ζ determined by measuring the potential created as a fluid moves past a stationary charged macroscopic surface). Therefore the focus of this paper lies in investigating how ionic strength of salt solution and the size of mineral grain affect ζ potential values.

II. Zeta Potential Theory

Surface potential (ϕ) is the electric charge present at an interface. However, as it cannot be determined experimentally, it is usually replaced by ζ potential. ζ potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to a particle. The ζ potential is located at the slipping plane (stern layer) in the electrical double layer. In the stern layer, the negatively charged surface adsorbs positive ions from the fluid in the immediate vicinity of the surface. The ζ potential is used to quantify the magnitude of the electrical charge at the double layer and it is a measure of the charges carried by particles suspended in an electrolyte solution. Colloids with high ζ potential are electrically stable and the effect of ζ potential on their stability is shown in Table I.

TABLE I
Stability of Suspensions with Relation to Zeta Potential (Reproduced from [24])

Zeta Potential (mV)	Stability Behaviour of the colloid
0 to +3	Maximum agglomeration and precipitation
+5 to -5	Strong agglomeration and precipitation
-10 to -15	Threshold of agglomeration
-16 to -30	Threshold of delicate dispersion
-31 to -40	Moderate stability
-41 to -60	Good stability
>-60	Excellent stability

Electrophoresis and streaming potential techniques are used to determined ζ potential. In electrophoresis technique, the net charge density of the surface, including the stern layer, equals the diffuse double layer charge. Based on this charge density, the measurement of the ζ potential is presented as a function of ionic strength. For a particle in high ionic strength liquid, the electrophoretic mobility (EPM) is related to the ζ potential by the Helmholtz-Von Smoluchowski equation:

$$\mu = \frac{\varepsilon}{\eta}\zeta \quad (1)$$

where ε is the dielectric permittivity and η is the bulk viscosity of the suspending solution.

In streaming potential technique, ζ potential is quantified by measuring the potential created as a fluid moves past a stationary charged macrosocopic surface. For the calculation of electrical double layer (ΔE^{EDL}) interaction energy, [19] gives the expression for the ΔE^{EDL} for a spherical cell (bacteria) and a flat surface (mineral) at constant surface potential as with:

$$\Delta E^{EDL} = 64\pi\varepsilon_0\varepsilon_w a_{c,m}\left(\frac{kT}{Ze}\right)^2 \gamma_c\gamma_m \exp(-\kappa H) \quad (2)$$

$$\gamma_{c,m} = tanh\left(\frac{Ze\Psi_{c,m}}{4kT}\right) \quad (3)$$

$$k^2 = \frac{(2eN_AC)}{\varepsilon_0\varepsilon_w kT} \quad (4)$$

where ε_w is the dielectric constant of water, ε_0 is the permittivity of free space (C/Vm), $a_{c,m}$ is the radius of cell/mineral (m), k is the Boltzmann constant (JK-1), T is temperature (K), Z is the electrolyte valency, e is the electron charge (C), $\gamma_{c,m}$ is a dimensionless function of surface potential, κ is the layer thickness (m), H is separation distance (m), $\Psi_{c,m}$ is the surface potential (V) which is usually replaced by ζ potential since it cannot be determined experimentally, NA is the Avogadro's constant and finally C is the concentration of electrolyte (M).

For sphere-sphere interaction, an equation proposed by [20] was applied:

$$\Delta E^{EDL} = \frac{\pi\varepsilon r_1 r_2(\zeta_1^2+\zeta_2^2)}{r_1+r_2}\left[\frac{2\zeta_1\zeta_2}{(\zeta_1^2+\zeta_2^2)} ln\left(\frac{1+e^{-\kappa H}}{1-e^{-\kappa H}}\right) + ln(1-e^{-2\kappa H})\right] \quad (5)$$

where ζ_1 and ζ_2 are electrical ζ potentials for bacteria and mineral respectively and κ is the inverse of the electrical double layer thickness with the value of $0.328x10^{10}(l)^{1/2}m^{-1}$. Thus, by measuring zeta potential we can determine the electrical double layer interaction energy between cell and minerals.

III. Experimental

A. Preparation: Bacteria

Pseudomonas Putida sp. strain ATCC 11172 from Kroto Research Institute UK was used for this study. The cells were grown in LB broth containing 1% peptone 140, 0.05% yeast extract and 0.5% sodium chloride at temperature 30^OC. Cells were harvested at the final stage of growth (after 24 hours).

Then the cells were centrifuged for 15mins at 4000g using Centrifuge 5804, Eppendorf Hamburg Germany.

The growth pallet was then washed in 10mM NaCl solution, suspended using a vortex vibrator, and centrifuged. Centrifugation and rinsing steps were repeated twice with fresh electrolyte solution to ensure the total separation of bacterium. Besides that, the electrolytes solution used were prepared with ultra-pure water (PureLAB-Ultra, ELGA) and reagent grade NaCl (Fisher Scientific, SRG UK) with no pH adjustment (~pH6) in microbiological safety cabinet.

B. Preparation: Mineral

Hematite (Rock shop, Huddersfield UK) and quartz (Geo supplies, Sheffield UK) were used in this experiment. These minerals were altered into grain form. These minerals were ground and crushed using a percussion mortar and sieved to <10µm mineral powder, 50-250µm, and 500-1000µm. The grains were then washed using UHQ water, dried in air at room temperature, and stored in separate labelled sample bag.

C. Surface Characterization: Zeta Potential

Two techniques of ζ potential characterization were used in this study. Electrophoresis technique is used for fine particles (10µm) and the streaming potential technique is used for larger particles.

In the electrophoresis technique, ζ potential values of *P. putida* with hematite and quartz samples were measured as a function of ionic strength at different pH values using a microelectrophoresis analyser (ZetaPALS, Brookhaven Instruments Corporation, Holtsville, NY 11742).

For each experiment a bacterial pellet of *P. putida* from the last rinse was resuspended in the desired NaCl solution and the optical density (OD) of the suspension was adjusted to an OD of 0.1 (≈10^8 cells/ml) using a Biowave spectrophotometer (S2100Diode Array). Cells were diluted 1000 times in a 5ml solution of the desired ionic strength and pH value. The EPM measurement of the sample in 4ml micro-cuvette was conducted using an electric field of 2.5Vcm-1 at a frequency of 2.0 Hz. The reported values for each EPM represent an average of 10 successive runs of 20 cycles each. The EPM was determined using freshly harvested cells suspended in NaCl solutions of 0.01mM, 0.1mM, and 0.3 mM and pH 3, 6, 9 and 11 at 25^{O}C and was repeated at least three times using freshly rinsed cells for each experiment. Precautions were taken during the experiment to avoid the bacterial sample splashing onto the environment while putting the electrode into the cuvette and any excess liquid on the outside of the cuvette was wiped before being put into the sample holder.

Streaming potential measurements were carried out using an Anton Paar Electrokinetic Analyzer (EKA) attached to an Anton Paar Titrator. Two electrodes are inserted into the ends of the flow cell and connected to an electrometer. As the streaming solution is forced to pass through the grains, the accumulation of ions around the grains set up the electric field. The potential of this field is the streaming potential detected by the electrodes. The potential readings are automatically stored and displayed for data processing.

Initially, 1gram of >500µm mineral grains was placed in the cylindrical cell (1cm inside diameter and 15cm length). The cell was flushed and rinsed under 20mbar with 70% ethanol and ultrapure water several times and finally rinsed with 0.01, NaCl electrolyte only for titration from pH 11 to pH 2 at a constant pressure difference of 500mbar. The experiments under the same conditions were then run for bacteria. 10ml bacterial suspension was placed in 600ml of 0.01M NaCl and automatically titrated as before as the streaming potential was measured. The same procedure was repeated for both mineral types and sizes at three different electrolyte concentrations (0.01M, 0.05M, 0.1M) with and without bacterial suspensions.

IV. Results and Discussions

A. Effect of Ionic Strength in Terms of pH on Zeta Potential

Figs. 1 and 2 show similar trends for different hematite sizes. H50 symbolizes small grain size (50-250µm) while H500 symbolizes large grain size (500-1000µm). Hematite-*P. putida* in 0.01M NaCl has the highest ζ potential value (most negative) at -45mv for H50 and -15mv for H500, both at pH 11. The large gap between the maximum values in the two graphs gives an early indication that mineral grain size has a marked effect on ζ potential. Both figures show the data for hematite-*P. putida* has more negative values (higher ζ potential) compared to hematite alone.

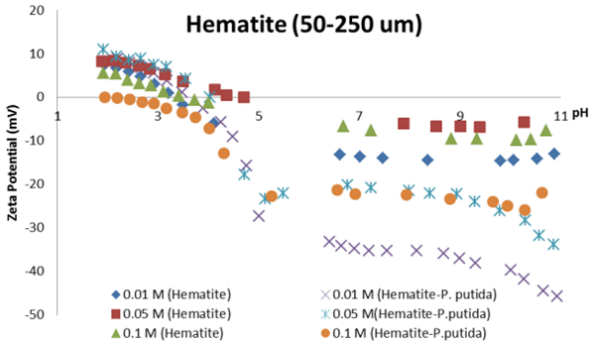

Fig. 1 The zeta potential of hematite grains (50-250µm) with and without *P. putida* suspension in various electrolyte concentrations

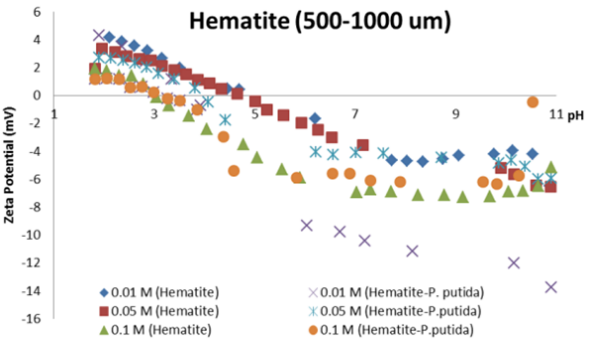

Fig. 2 The zeta potential of hematite grains (500-1000µm) with and without *P. putida* in various electrolyte concentrations

Comparing different ionic strength of electrolyte for hematite-*P. putida* (Fig. 1), 0.01M gives a highest ζ potential value of -45mv, indicating good stability expressed as repulsive behavior towards bacterial adhesion. At 0.1M (Table I) ζ potential is in the threshold range for delicate dispersion. Any higher ζ potential will result in stability of the surface and any lower will lead to agglomeration between mineral and bacteria.

Quartz (Figs. 3 and 4) shows similar trends to that of hematite. Q50 (Quartz 50-250μm) has a highest ζ potential of -55mv at 0.01M quartz- *P. putida* while Q500 (Quartz 500-1000μm) is highest at -20mv in 0.01M electrolyte for quartz only. The highest ζ potentials are at pH11 (most alkaline).

At pH lower than the Point of Zero Charge (PZC - the pH value where ζ=0mv), all the concentration curves have positive values with small gaps between each curve. At pH greater than PZC, all curves have negative values with large gaps between them. The surface of bacteria and minerals carry positive proton charge at pH values below the PZC and a negative proton charge at pH above PZC. This results in electrostatic interaction or repulsion of all ions other than protons. A change in pH by one or two units can increase or decrease the uptake of ions by an order of magnitude [21]. Therefore the affinity of hematite and quartz to anions and cations is highly dependent on pH.

The highest ζ potential is found for the mineral-*P. putida* runs at the lowest ionic concentration (0.01M) and higher ionic concentrations result in lower ζ potentials. As the concentration of ion increases, they are no longer adsorbed to the surface but build up on and compress the electrical double layer (EDL) [22]. This causes the EDL to become thinner and ζ potential value lower and the bacterial cell surface may thus increase adherence.

According to Bunt [23] when a complex protein (to which *P. putida* can be compared) is bound to hydrophobic ligands such as found on minerals in aqueous solution, the binding will increase when the surface tension of water is increased by the addition of salts, thereby increasing the value of ζ potential. As the charge potential increases at higher ionic strength the adhesion becomes stronger [23].

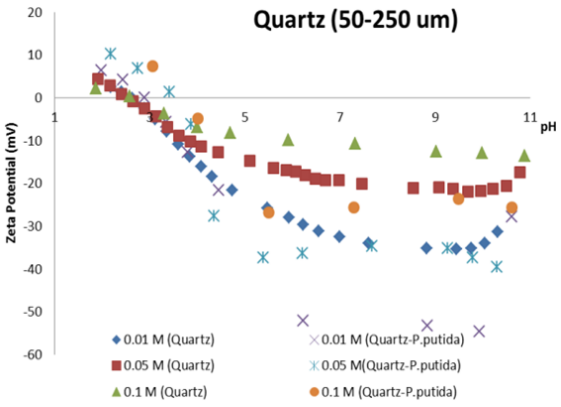

Fig. 3 The zeta potential of quartz grains (50-250) with and without *P. putida* in various electrolyte concentrations

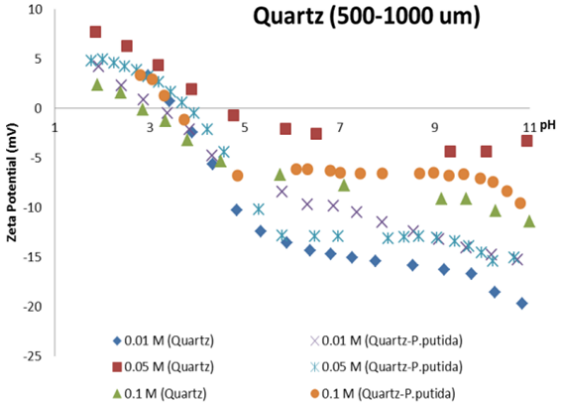

Fig. 4 The zeta potential of quartz grains (500-1000) with and without *P. putida* in various electrolyte solutions

B. Effect of Mineral Grain Size on Zeta Potential

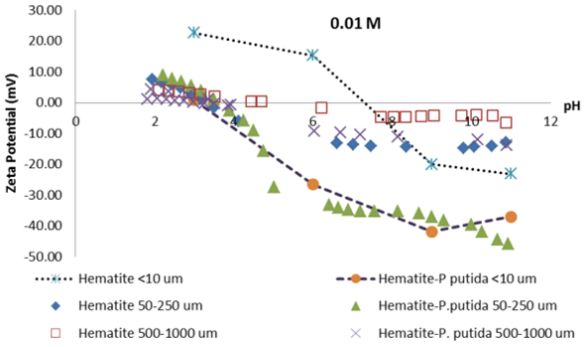

Fig. 5 Effect of mineral grain size to the surface electrokinetic of hematite and *P. putida* interaction in 0.01M NaCl

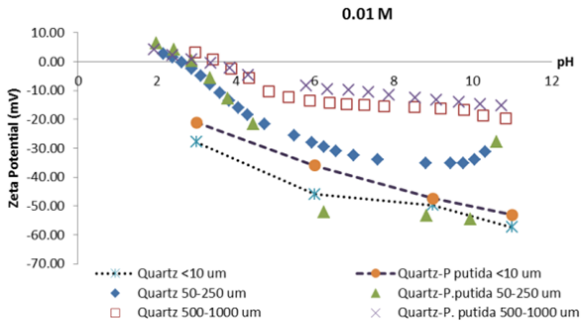

Fig. 6 Effect of mineral grain size to the surface electrokinetic of quartz and *P. putida* interaction at 0.01M NaCl

In relation to grain size, hematite and quartz show similar trends (Figs. 5 and 6). Both minerals exhibit low ζ potentials at the largest grain size and the curve is close to zero, while the ζ potential curves of small (50-250μm) and fine grains (<10nm) are higher and close to each other. For the hematite-*P. putida* system (Fig. 5), the maximum ζ potential value of the large grain curve at pH 11 is -15mv, while for fine grain it is -40mv. At this mineral size, the electron cloud of the fine grains overlap causing the charge density, and therefore ζ potential, to increase. The ζ potential for the large grain size is

in the range of agglomeration (Table I) while for the fine grain size it results in stability of the mineral and repulsive behavior towards bacterial adhesion.

C. EDL Interaction Energy

Fig. 7 shows the interaction energy of the electric double layer. For a separation distance lower than 5nm, there is a large difference in interaction energy between the four systems. Hematite with grain size of 500-1000µm has very low repulsion ($0.6x10^{-17}$J). Quartz (50-250µm) has the highest repulsion ($3.6x10^{-17}$J) which decayed at larger separation distances. The Interaction energy (ΔE^{EDL}) between the smallest grain size of hematite and the bigger quartz grain size are close to each other and reach the same order level at a separation distance of 5nm.

Both the data sets for quartz have higher ΔE^{EDL} than hematite and this indicates that quartz exhibits greater repulsive behaviour towards *P. putida*. Comparing both hematite curves, the ΔE^{EDL} curve of hematite 250µm is much higher than hematite 500µm. The smaller grain size results in higher ζ potential and ΔE^{EDL} and ζ potential and ΔE^{EDL} have a proportional relationship.

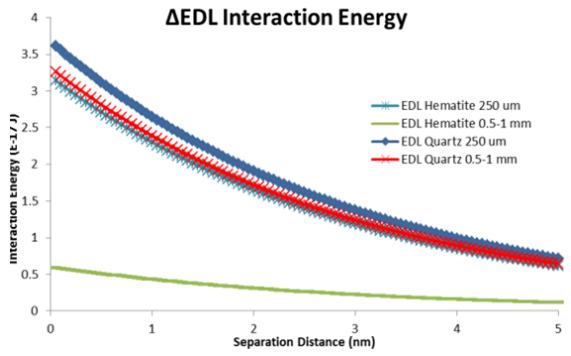

Fig. 7 Electric double layer interaction energy for hematite and quartz at pH6 in 0.01M NaCl

V. Conclusion

In this study, ionic strength and mineral grain size were found to have strong effects on ζ potential values for interactions between *P. putida* and both hematite and quartz. Hematite has lower negatively charged ζ potential due to its surface physicochemical properties. This provides better bacterial adhesion compared to quartz, which is more negatively charged and, due to this stability, tends to resist approaching bacteria.

Higher strength electrolyte solutions reduce the stability of the mineral surface which enhances bacterial adhesion. Based on the XDLVO theory, at a short distance, lower electrostatic repulsion energies provide better attraction between the surfaces in low ionic strength solutions. Higher ΔE^{EDL} increases the total interaction energy and a higher overall interaction energy causes repulsion therefore bacteria attachment is inhibited. The greater attraction of *P. putida* to hematite with larger grain sizes due to the less electronegative surface of the bacteria as shown by the repulsion interaction energy from the XDLVO theory.

Therefore, through this study, by measuring the zeta potential of materials at certain conditions, the repulsion interaction energy could be determined in order to characterize the attachment behavior of materials surface to environment.

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