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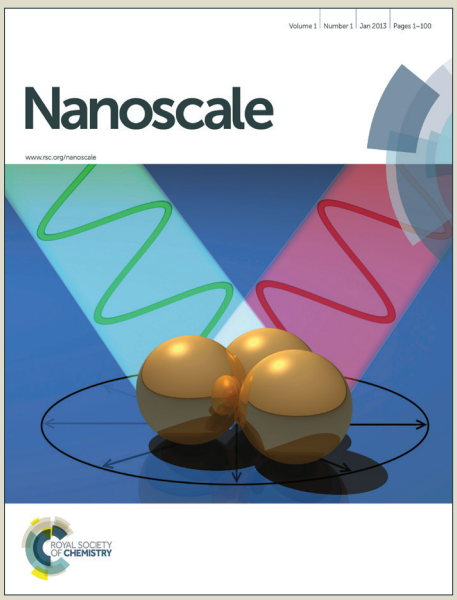

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PAPER

The Role of Particle-to-Cell Interactions in Dictating Nanoparticle Aided Magnetophoretic Separation of Microalgal Cell

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Successful application of magnetophoretic separation technique for harvesting biological cells often relies on the need to tag the cell with magnetic nanoparticles. This study investigates the underlying principle behind the attachment of iron oxide nanoparticles (IONPs) onto microalgal cells, *Chlorella* sp. and *Nannochloropsis* sp., in both freshwater and seawater, by taken into account the contributions of various colloidal forces involved. Complex interplay between van der Waals (vdW), electrostatic (ES) and also Lewis acid-base interactions (AB) in dictating IONPs attachment was studied under the framework of Extended Derjaguin-Landau-Vewey-Overbeek (XDLVO) analysis. Our results showed that ES interaction plays an important role in determining the net interaction between the *Chlorella* sp. cells and IONPs in freshwater condition while the AB and vdW interactions play a more dominant role in dictating the net particle-to-cell interaction in high ionic strength media (≥ 100mM NaCl), such as seawater. XDLVO predicted effective attachment between cells and surface functionalized IONPs (SF-IONPs) with an estimated secondary minimum of -3.12kT in freshwater. This prediction is in accordance to the experimental observation in which 98.89 % of cells can be magnetophoretically separated out from freshwater with SF-IONPs. We have observed successful magnetophoretic separation of microalgal cells from freshwater and/or seawater for all cases as long as XDLVO analysis predicts particles attachment. For both conditions, no pH adjustment is needed for particle-to-cell attachment.

1 Introduction

Given the current stage of energy crisis, the recovery of the microalgae biomass is crucial as it could serve as a sustainable alternative for the production of biofuel.[1,2] In this regard, magnetophoretic separation has been developed as an effective downstream separation technique to harvest microalgal biomass from aqueous environment.[3-7] The origin of this idea can be traced all the way back to 1970s, in which this method was applied for environmental engineering application in removing microalgae that plague the fresh water lake and causing eutrophication.[8,9] Comparing to the more conventional separation technology, magnetophoretic separation shows remarkable potential for harvest microalgal cells as this technique has (1) high throughput, (2) low operational cost, (3) less energy intensive, (4) high separation efficiency, and (5) flexible for implementation and scalability.[3,10,11]

The underlying working principle for magnetophoretic separation technique is pretty straightforward. It centered on the need of tagging the non-magnetic microalgal cells with iron oxide nanoparticles (IONPs).[5,7,12,13] Later, the tagged biomass will be exposed to an externally applied magnetic field to promote their separation from surrounding media. Hence, the ability to predict the success or failure of magnetic nanomaterial attachment onto the microalgal cell surface is crucial for the implementation of this technology. From the seminal work of Xu and coworkers, adsorption isotherm analysis has been employed to make accurate yet simple estimation on the binding affinity and also the adsorption capacity of IONPs onto microalgae.[5] In order to verify the nature of interactions involved between the IONP and microalgal cell it is necessary to further identified the main driven factor behind this adsorption process.

So far the attachment of IONPs onto microalgal cell could be promoted through the present of macromolecules as binding agent. Numerous macromolecules, such as chitosan, polyethylenimine, poly(diallyldimethylammonium chloride) and etc,[3,11,13,14] have been employed and shown remarkable ability to bind the IONPs onto the negatively charged microalgal cell. For freshwater species, the nature of interactions between the nanoparticles and microalgae is very likely dominated by electrostatic interaction.[13,14] As for marine species is concerned, the magnetophoretic technique works equally well with the separation efficiency of more than 90%.[4] Supposedly, the ionic stress induced by high salt concentration in seawater would cause retardation of Debye screening length of the IONPs and inhibit the electrostatic (ES) attraction between the cells and IONPs.[15] Under this circumstance, bridging flocculation can be important, but

in the absence of extracellular polymeric substances on microalgae surface there present some existing experimental evidences that against this possibility.[16] By excluding both the ES and bridging effects, van der Waals (vdW) interaction should play a more pronounced role on promoting the IONPs attachment onto marine microalgal cells. Moreover, since microalgae are considered as hydrophilic bio-colloids due to its natural surface properties,[17] it might need to account for Lewis acid-based interactions into the entire adsorption mechanism.[11] It is the aim of this study to investigate the complex interplay of aforementioned interactions in dictating the attachment of IONPs on microalgal cells.

The present study is dedicated to identify the colloidal interactions involved in determine the successful attachment of IONPs onto microalgal cells. Experimentally we verified the attachment of IONPs by checking the magnetophoretic responsiveness of the tagged cells and direct visualization through optical microscope. Electrophoretic mobility measurement is used to provide information on surface charge of IONP before and after its surface functionalization and also for microalgal cell. By employing *Chlorella* sp. as a modeled system, we investigate the attachment efficiency of IONPs onto this cell line within both freshwater and seawater. Extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory, by taken into account the contribution of vdW, ES and the Lewis acid-base (AB) interaction, is employed to rationalize the colloidal interactions involved for particle-microalgae interactions. Here XDLVO analysis can be a clear-cut method to provide guidance in predicting the success or failure of IONPs attachment onto microalgal cells which further leading to the effective implementation of magnetophoretic separation. A marine species, *Nannochloropsis* sp. is employed to further confirm the reliability of the XDLVO prediction.

2 Experimental section

2.1 Materials

Bare iron oxide magnetic nanoparticles (bare-IONPs) with diameter at around 20 – 30 nm were obtained from Nanostructured & Amorphous Materials, Inc. The 35 wt.% very low molecular weight poly(diallyldimethylammonium chloride) (PDDA) in water with molecular weight, Mw < 100,000 g mol^{-1} was supplied by Sigma-Aldrich, Inc. Hydrochloric acid was purchased from PC Laboratory Reagent. The sodium hydroxide pellet and sodium chloride were supplied by MERCK & Co., Inc. Deionised water was obtained by reverse osmosis and further treated by the Milli-Q Plus system (Lillipore) to 18 MΩcm resistivity.

2.2 Culture, Preparation and Characterization of *Chlorella* sp. and *Nannochloropsis* sp. cells

The *Chlorella* sp. and *Nannochloropsis* sp. strain were obtained from School of Biological Sciences, Universiti Sains Malaysia. The *Chlorella* sp. was cultivated in 250 ml Bold's Basal Medium (BBM) while the *Nannochloropsis* sp. was cultivated in 250 ml Conway medium. Both cultures were maintained under continuous illumination at 2000 lux and 25°C. The medium and flask were autoclaved at temperature 121°C for 15 minutes before cell cultivation. Continuous aeration was provided for the culture medium throughout the cultivation period. In this study, cell density of *Chlorella* sp. was maintained at around $3x10^7$ cells/ml (after 10 culturing days) for all experiment. For subsequent experiments, the cells were collected through centrifugation and redispersed in deionized water multiple times to exclude the present of growth media. Later, the concentrated biomass was then redispersed into desired medium (with different pH and ionic strength) as planned for experiment. For *Nannochloropsis* sp., the removal experiment was conducted right after 7 culturing days to ensure the same cell density has been achieved similar to *Chlorella* sp. Cell numbers were determined by using the heamocytometer while the size of cells was measured microscopically through the Image Analysis Software. Average size of cells was taken from 500 measurements.[18] The pH was measured by Eutech CyberScan pH 1500. The zeta potential of the microalgae is calculated based upon Helmholtz-Smoluchowski limit by using the Malvern Zetasizer.

2.3 Preparation and Characterization of Surface Functionalized Iron Oxide Nanoparticles (SF-IONPs)

"Attached-to" approach was employed to form attachment between the IONPs and cells.[14] The bare-IONPs were dispersed into deionized water and sonicated to form well dispersion at 1 g L^{-1}. PDDA was dispersed in deionized water (17 g L^{-1}) and stirred (500 rpm) for one day to achieve complete dissolution. Then the bare-IONPs dispersion was added into the macromolecule solution and the entire mixture was sonicated in a low power bath sonicator (40 KHz) to avoid degradation of the molecular structure.[19] This solution was then left on an end-to-end rotating mixer at 40 rpm overnight. After this surface modification step, a permanent magnet was used to collect the surface functionalized IONPs (SF-IONPs). The supernatant was discarded while the SF-IONPs were again dispersed in deionized water. The electrophoretic mobility of bare-IONPs and SF-IONPs in different pH and ionic strength medium were measured by using the Malvern Instruments Nanosizer. Unless otherwise stated, zeta potential of the bare-IONPs and SF-IONPs is then calculated based upon Helmholtz-Smoluchowski limit.

2.4 Low Gradient Magnetic Separation (LGMS)

For every separation study, 300 mg L^{-1} of bare-IONPs/SF-IONPs were added into cell medium to ensure excess supply of particles. A total of 2 ml of 1.5 g L^{-1} particles, either bare-IONPs or SF-IONPs, was added into 8 ml cell medium followed with simple mixing for 30 seconds to ensure uniform mixing and dispersion. The mixture was left for another 30 seconds before used for magnetophoretic separation study. Magnetophoretic separation of *Chlorella* sp. was carried under low gradient magnetic separation (LGMS) for 6 minutes. A N50-graded NdFeB permanent magnet was used to induce inhomogeneous magnetic field with field gradient $\nabla B < 80$ T m^{-1}.[3] The absorbance of the cell was measured spectrophotometrically by UVmini-1240 Shimadzu at the wavelength of 660 nm (measured by Agilent Technologies Carry 60 UV-Vis). The cell separation efficiency was determined as

$$\text{Cell separation efficiency } (\%) = \frac{I_0 - I(t)}{I_0 - I_{centrifuged}} \times 100\% \qquad (1)$$

where the I_0 represents initial absorbance intensity of microalgae suspension after diluted with 2 mL deionized water, I(t) represents the absorbance intensity of microalgae suspension during magnetophoretic separation at time t, and the $I_{centrifuged}$ represents the clear sample after centrifugation with same dilution factor.

2.5 Contact Angle Measurement

The surface free energy of the microalgal cells and the bare-IONPs/SF-IONPs were determined by contact angle measurements. These contact angles were measured by using a contact angle gionometer (Rame-Hart Instrument Co.). Microalgal cells were pre-concentrated by centrifugation at 2500×g for 4 minutes. Flat layer of cells were deposited on agar to stabilize the cell moisture content.[20]

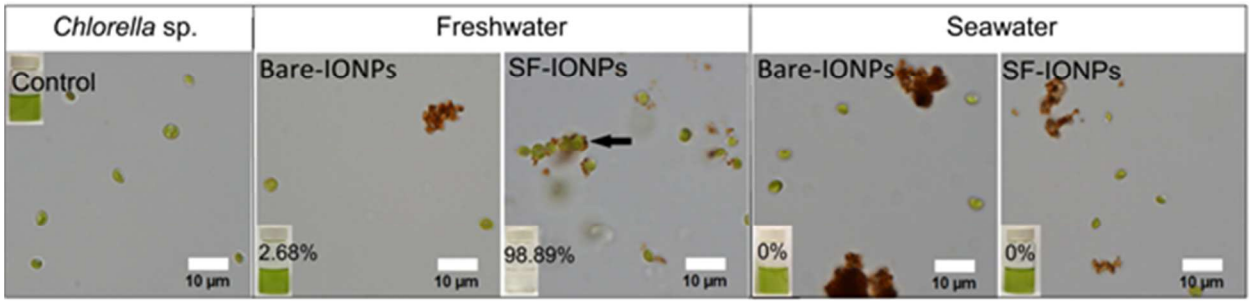

Figure 1. Optical micrographs showing the degree of attachment for both bare- and SF-IONPs onto *Chlorella* sp. cell in freshwater and seawater. For bare-IONPs, under both aqueous environments, the particles aggregated extensively to form large clusters with no hint of attachment (darken ring around the cell) on the cell. Whereas for the case of SF-IONPs, inserted black arrow pointing toward the darken area indicate the presence of particles on cell surface in freshwater. However, there is no clear indication showing the attachment of SF-IONPs onto microalgal cells in seawater. On bottom left corner of each micrograph is accompanying with a photo depicting the microalgae suspension after going through magnetophoretic separation. Bare-IONPs recorded minimum magnetophoretic separation efficiency at 2.68% and 0% for freshwater and seawater, respectively. There is also no clear cell separation for the case of SF-IONPs in seawater but this particle is proven to be an effective tagging agent to promote magnetophoretic separation of microalgal cells in freshwater with 98.89% of separation efficiency.

The flat surface of bare-IONPs was obtained by mechanically compressed the powder into pellet.[21] The concentrated solutions of SF-IONPs were deposited onto glass slide to form completely covered flat surface and dry at room temperature. Measurements were performed with two polar liquids (water and glycerol) and an apolar liquid (1-bromonaphthalene). The contact angle measurements were carried out by sessile drop technique and the readings were averaged from three replicate of measurements.[18]

3 Results and discussion

3.1 Particles-microalgae Interaction in Freshwater and Seawater

Magnetophoretic separation of microalgae from freshwater medium has proven to be feasible in previous studies.[3,14] We hypothesized that the effective attachment of iron oxide nanoparticles (IONPs) onto the surface of *Chlorella* sp. is mediated by the electrostatic attraction (ES).[12,13] The similar model system is employed in this study to further identify the actual contribution of the interaction involved. Figure 1 shows that the bare iron oxide nanoparticles (bare-IONPs) do not adhered onto *Chlorella* sp. cells in freshwater. There is about 2.68% of cells were being removed magnetophoretically with most of the cells were entrapped within the flocculated nanoparticle matrix (see Supporting Information, Figure S1). On the other hand, the surface functionalized iron oxide nanoparticles (SF-IONPs) are very likely attached on cell surface in freshwater condition by judging from the fact that a darken ring has formed around most of the microalgal cell (arrow pointed area in Figure 1). Cell separation efficiency as high as 98.89% was recorded at the particle dosage of 0.84 g per one gram of dry biomass. This observation has also served as an indirect confirmation on our speculation about the successful magnetic seeding of microalgal cells by SF-IONPs. By referring to Table 1, the surface charge of cells and bare-IONPs are similar while the SF-IONPs are in opposite charge to the cells. These measurements have verified that the electrostatic interaction (ES) cause the cells and SF-IONPs attract together while causes the cells and bare-IONPs repel with each other.

In seawater, both the bare-IONPs and SF-IONPs do not attach onto the cells (Figure 1). As a consequence, there is no cell collection can be detected optically. Table 1 shows that the surface charge of the cells and nanoparticles are suppresed to very low value when compared to freshwater condition. Typical seawater contains various type of salt dissolved in it, mainly the Na^+ and Cl^- ions. The ionic strength of seawater is approximately 700 mM.[22] Hence, we

Table 1. Detail on zeta-potential of each surface when dispersed in different medium condition together with pH.

Conditions		Surface	Zeta-potential (mV)
Fresh Medium	pH		
Freshwater	6.58	*Chlorella* sp.	-27.8 ± 5.5
		Bare-IONPs	-30.4 ± 4.9
		SF-IOPNs	25.2 ± 4.7
Seawater	6.32	*Chlorella* sp.	-9.1 ± 0.3
		Bare-IONPs	3.84 ± 1.0
		SF-IOPNs	4.95 ± 0.6

hypothesized that the high ionic strength seawater has causing strong Deybe screening and weaken the electrostatic-repulsion between the particles. The particles aggregate extensively before their attachment onto the cell. Large aggregate found in seawater, as illustrated in Figure 1, strongly support our hypothesis.

In classical DLVO analysis, the total interacting potential (U_{DLVO}) between two colloidal particles is described as a balance between the van der Waals (U_{vdW}) and ES (U_{ES}) interactions

$$U_{DLVO} = U_{vdW} + U_{ES} \quad (2)$$

in which the extent of ES interaction is depending on the surface charge/zeta potential of the interacting particles, it can be attractive or repulsive.

In this study, due to the huge size mismatch between the IONPs and the microalgal cell (see Supporting Information, Figure S2), where the mean size of *Chlorella* sp. cells is 3.45 µm and is far larger than the IONPs with diameter at around 25 nm, hence the surface of the cell is assumed to be flat with respect to the spherical IONPs.. Under this scenario, the surface curvature of cell is negligible with respect to high curvature of nano-sized IONPs. Lifshitz-van der Waals equation for sphere-plane configuration is applied,[23] as a function of separation distance, d:

$$U_{vdW} = -\frac{A_{eff}R}{6}\left[\frac{1}{d} + \frac{1}{d+2R}\right] - \frac{A_{eff}}{6}\ln\left[\frac{d}{d+2R}\right] \quad (3)$$

Here d is the surface-to-surface separation distance between the cell and the IONP (Figure 2); A_{eff} represents the effective Hamaker constant; and R is the radius of IONP. The Hamaker constant of each surface was estimated by contact angle measurement (see Supporting

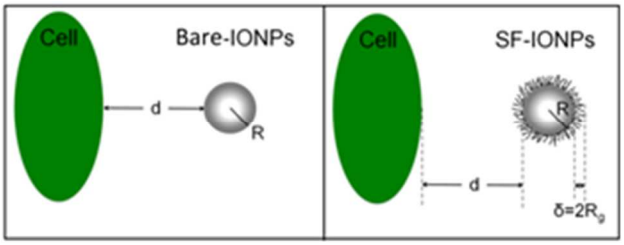

Figure 2. Schematic illustration of a microalgal cell interacting with either a bare-IONPs (left) and SF-IONPs (right). Due to the large size mismatch between these two entities, we simplified our analysis to sphere-plane interaction. In this diagram, d represent the surface-to-surface separation distance between the cell and particle, R is the particle radius, δ is the the thickness of the polymer adlayer that coated on IONP surface. All the symbol are accordance to the parameters used in our analysis.

Information, Table S1) with the use of at least three different liquids (polar or apolar), which two must be polar liquid (see Supporting Information, Table S2).[24,25] The Hamaker constant of material i, A_i is proportional to the vdW component of the material surface tension, γ_i^{LW} and can be defined as follow:[24]

$$A_i = 24\pi d_0^2 \gamma_i^{LW} \quad (4)$$

where d_0 is the minimum equilibrium distance between two condensed-phase surfaces (d_0 = 0.657 ± 0.01 nm).[26] The γ_i^{LW} can then be determined by using the Young's equation (Table 2):[27]

$$(1+\cos\theta)\gamma_L = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{\oplus}\gamma_L^{\ominus}} + \sqrt{\gamma_S^{\ominus}\gamma_L^{\oplus}}\right) \quad (5)$$

where γ is surface tension, mJ m^{-2}; $\gamma^{\oplus}$ is the electron-acceptor parameter of the polar surface tension component while the $\gamma^{\ominus}$ is the electron-donor parameter of the polar surface tension component; and the subscripts S and L stand for the solid and liquid respectively.

The effective Hamaker constant, A_{eff} is the combining relation between different materials that contributed to the vdW body-body interaction. A_{eff} of interaction between material 1 and 3 separated by medium 2 can be defined as:[28]

$$A_{eff} = A_{123} = \left(\sqrt{A_{11}} - \sqrt{A_{22}}\right)\left(\sqrt{A_{33}} - \sqrt{A_{22}}\right) \quad (6)$$

According to this definition, for two colloidal particles suspended in liquid, the vdW interaction can be either attractive or repulsive.[29] The calculated A_{eff} between the *Chlorella* sp. cell and the bare-IONPs and SF-IONPs are 2.86x10^{-21} J and 2.81x10^{-21} J respectively.

ES interaction between charged plane and charged sphere is modeled as:[23]

$$U_{ES} = \pi\epsilon R'(\xi_1^2 + \xi_2^2)\left[\frac{2\xi_1\xi_2}{\xi_1^2+\xi_2^2}\ln\frac{1+e^{-\kappa d}}{1-e^{-\kappa d}} + \ln\left(1-e^{-\kappa d}\right)\right] \quad (7)$$

where ξ is the zeta potential; κ is the Debye-Hückel parameter; and the ϵ represents the permittivity of the medium, where $\epsilon = \epsilon_r\epsilon_0$. Permitivity of free space, ϵ_0 is 8.854x10^{-12} F m^{-1}. In the case of SF-IONPs, R' = R+δ where δ is the adlayer thickness which is determined at 4.85 nm.[15]

According to the classical DLVO analysis under freshwater condition (Figure 3a), the ES interaction between different charged cells and SF-IONPs enable effective particle-to-cell linkage as evident from the net attractive interaction. Interestingly, we observed particle internalization into the cells for the case of SF-IONPs.[12] This process is most probably happened through the membrane deformation caused by reorganization of lipid bilayer when the nanoparticles successfully attach on cell surface (see Supporting Information, Figure S3). This observation is in accordance with the optical micrograph shown in Figure 1. While for the bare-IONPs, the energy barrier with the *Chlorella* sp. cell is more than 10kT and prohibits the attachment of particles. In seawater, the classical DLVO theory (Figure 3b) predicts the bare-IONPs and SF-IONPs are always readily to attach on cell surface promoted by the attractive vdW force. However, we observed no separation of microalgal cell for both species of nanoparticles (Figure 1). Even though the rapid aggregation of particles might be the dominant factor causing poor separation efficiency but from the optical microscopy observation we have also failed to detect any hint of particle attachment. This in turn has indirectly suggested that the classical DLVO analysis is insufficient to provide useful prediction related to particles attachment within our experimental condition.

3.2 XDLVO Considerations

From existing literatures, there are a number of paper emphasized on the need of including polar interactions into the analysis involved biological cell.[11,18,23,27] Since microalgal cell is also a hydrophilic biocolloid due to its natural surface properties, where the outer cell membrane is made up of the hydrophilic end of the phospholipid layer,[17,27] the polar interaction based on electron acceptor-electron donor (Lewis acid-base, AB) interactions may be important. Under this context, the AB interaction can be up to two orders of magnitude higher than those commonly encountered among the components of traditional DLVO energy balance.[24] In addition complexity may arise as the changes in ionic strength of culturing media would suppress the electric double layer and therefore retards the ES interaction. This condition will become more pronounced in seawater culture with complex ionic environment. As such, a more physically sound analysis by taking into account the AB interaction might be necessary and this new physicochemical approach, by taking into account the contribution other than vdW and ES interaction, is known as extended DLVO (XDLVO) analysis.

In this study, van Oss-Chaudhury-Good (OCG) thermodynamic approach is used to determine the AB interaction.[24] Table 2 shows the *Chlorella* sp., bare-IONPs and SF-IONPs are strongly hydrophilic since their $\gamma^{\ominus}$ are larger than that of the water while $\gamma^{\oplus}$ are far less than that of water, where $\gamma_w^{\oplus} = \gamma_w^{\ominus} = 25.5$ mJ m^{-2} for water. Larger $\gamma^{\ominus}$ surface exhibits strong affinity to water molecules via the formation of hydrogen bond. The *Chlorella* sp., bare-IONPs and SF-IONPs tend to bind water strongly with free energies in

Table 2. Surface energy and Interfacial energy involved for the calculation of Lewis acid base interaction. Subscript *iw* implied the interfacial energy of surface *i* in water and *iwi* implied the interfacial energy of surface *i* to surface *i* in water.

Surface, i	Surface Energy (mJ m^{-2})					Interfacial Energy (mJ m^{-2})	
	γ^{tot}	γ^{LW}	γ^{AB}	$\gamma^{\oplus}$	$\gamma^{\ominus}$	ΔG_{iw}	ΔG_{iwi}^{AB}
Chlorella sp.	75.0	22.2	52.8	4.8	145.1	-188	80
Bare-IONPs	48.1	44.0	4.2	0.06	74.2	-151	69
SF-IONPs	45.31	43.5	1.81	0.01	74.4	-150	71
Nannochloropsis sp.	40.0	26.2	13.8	0.65	73.3	-142	60

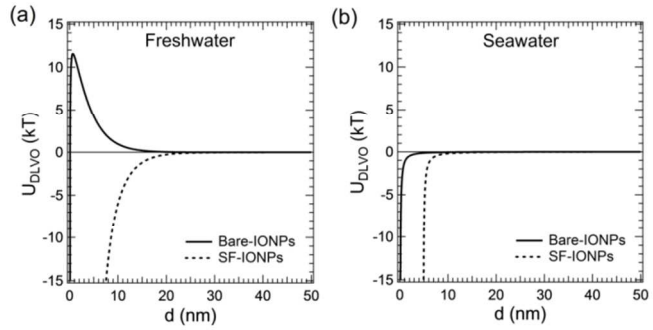

Figure 3. Classical DLVO profile on the interaction between *Chlorella* sp. cells and bare-IONPs/SF-IONPs in (a) freshwater and (b) seawater with the overall interacting potential U_{DLVO} is plotted for both species of IONPs used.

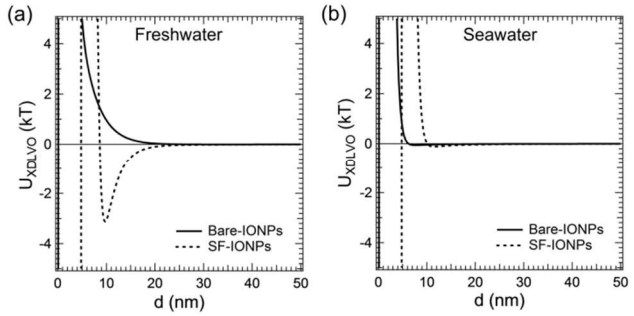

Figure 4. XDLVO profile on the interaction between *Chlorella* sp. cells and bare-IONPs/SF-IONPs in (a) freshwater and (b) seawater with the overall interacting potential U_{XDLVO} is plotted for both species of IONPs used.

range of -140 to -190 mJ m^{-2}, which are stronger than the hydrogen-bonding energy of water (-102 mJ m^{-2}).[30] When these hydrophilic cells/particles disperse in water, they experienced hydrophilic repulsion with AB interfacial interaction energy greater than zero, ($\Delta G_{iwi}^{AB} > 0$). This interfacial interaction energy is defined as:

$$\Delta G_{iwi}^{AB} = 4\left(\sqrt{\gamma_i^{\oplus}\gamma_w^{\ominus}} + \sqrt{\gamma_w^{\oplus}\gamma_i^{\ominus}} - \sqrt{\gamma_i^{\oplus}\gamma_i^{\ominus}} - \sqrt{\gamma_w^{\oplus}\gamma_w^{\ominus}}\right) \quad (8)$$

ΔG_{iwi}^{AB} decreases in the order of *Chlorella* sp. > SF-IONPs > bare-IONPs, with 80, 71 and 69 mJ m^{-2} respectively.[25,27] After cells and bare-IONPs/SF-IONPs are mixed in water, both surfaces would experience hydrophilic repulsion with respect to each other. The ΔG_{iwj}^{AB} for the configuration of *Chlorella* sp. cells and bare-IONPs is 87.7 mJ m^{-2} and for cells interact with SF-IONPs is 89.6 mJ m^{-2}.

$$\Delta G_{iwj}^{AB} = 2\left(\sqrt{\gamma_i^{\oplus}\gamma_w^{\ominus}} + \sqrt{\gamma_j^{\oplus}\gamma_w^{\ominus}} + \sqrt{\gamma_w^{\oplus}\gamma_i^{\ominus}} + \sqrt{\gamma_w^{\oplus}\gamma_j^{\ominus}} - 2\sqrt{\gamma_w^{\oplus}\gamma_w^{\ominus}} - \sqrt{\gamma_i^{\oplus}\gamma_j^{\ominus}} - \sqrt{\gamma_j^{\oplus}\gamma_i^{\ominus}}\right) \quad (9)$$

From above point of view, AB interaction should be included in XDLVO analaysis:

$$U_{XDLVO} = U_{vdW} + U_{ES} + U_{AB} \quad (10)$$

The AB interaction between plane and sphere, as a function of distance, can be predicted as:[23,31]

$$U_{AB} = 2\pi a\lambda\Delta G_{d_0}^{AB}\exp\left[\frac{d_0 - d}{\lambda}\right] \quad (11)$$

where λ is the correlation length of molecule in a liquid medium. For hydrophilic repulsion, λ=0.6 nm; for hydrophobic attraction, λ=13 nm.[30]

In freshwater, the XDLVO analysis showed in Figure 4a predicts the high energy barrier between cells and bare-IONPs at around > 1000 kT, which strongly prohibits the effective particle-to-cell attachment. For the case of SF-IONPs, a secondary minimum (-3.12kT) is observed once the AB interaction is considered. At this point, the attachment of cells with SF-IONPs is effective.[32] The major difference between DLVO and XDLVO analysis for interaction between *Chlorella sp.* and SF-IONPs is that the DLVO predict net attraction, whereas, XDLVO predict the present of a secondary minimum follow with strong hydrophilic repulsion (due to U_{AB}) as the particle-cell separation distance approaching the layer thickness of PDDA coating (see Supporting Information, Figure S4a). Nevertheless, both analyses predicted successful attachment of the SF-IONPs onto *Chlorella sp.*

In seawater, as shown in Figure 4b, the XDLVO analysis predicts net repulsion between the cells and the bare-IONPs/SF-IONPs. The available repulsive AB interaction has strongly prohibited the effective attachment for both cases in which the energy barrier is much higher than 100 kT (see Supporting Information, Figure S4b). This prediction is in accordance with the observation delineated in Figure 1. Therefore, from a theoretical perspective, the AB interaction plays a crucial role in determining the net interaction between the nanoparticles and microalgal cell under seawater condition.

3.3 Variation of Ionic Strength verified the important role of AB interaction

Our XDLVO analysis confirms that the ES and AB interactions are both important in govern the net interaction of cells and nanoparticles in different ionic strength condition. An interacting system between the cells and SF-IONPs is monitored to investigate the inter-relationship of the ES and AB as ionic strength increased from 1 to 700 mM. Since the dissolved mineral substance of seawater mainly made up of Na^+ and Cl^- ions, about 30.6 % and 55% respectively, hence the sodium chloride (NaCl) salt is used for the

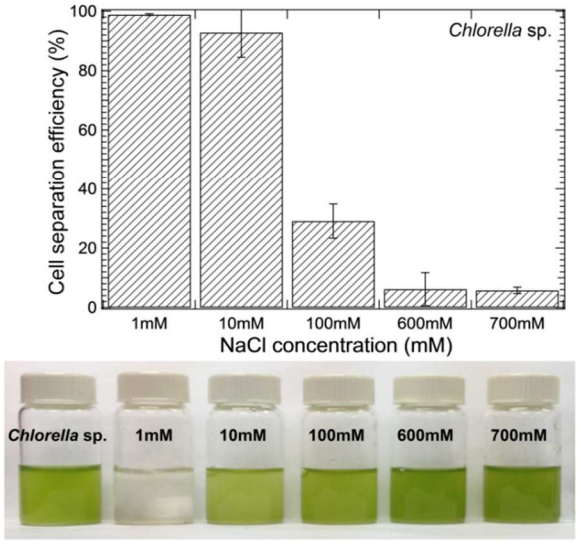

Figure 5. Separation efficiency of *Chlorella* sp. cell as a function of NaCl concentration up to 700mM, which is equivalent to the ionic strength of seawater. Here, 300 mg L^{-1} of SF-IONPs is added into the cell suspension correspond to 1.27 g nanoparticles per g dry biomass. All separation process was conducted at low field gradient for 6 minutes. Bottom image showing how the final suspension looks like after going through the separation.

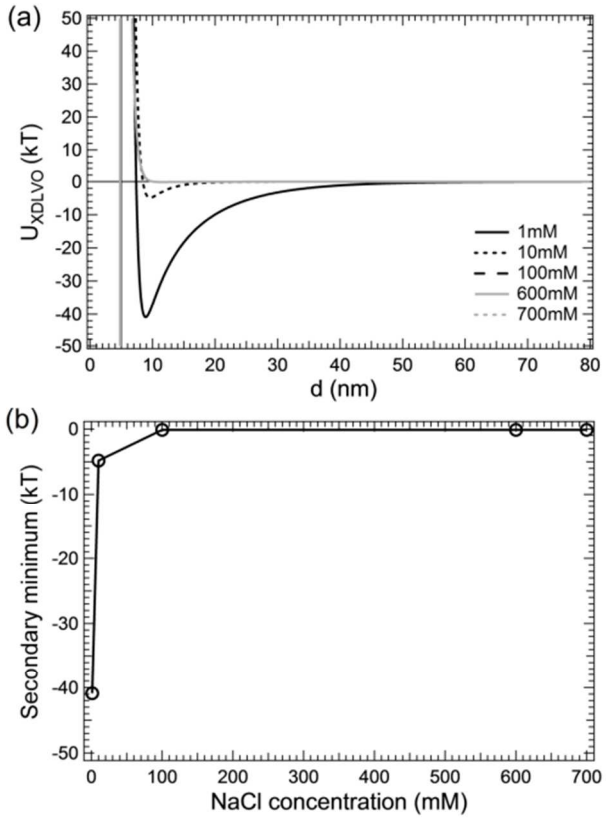

Figure 6. (a) XDLVO profile showing net interaction between the *Chlorella* sp. cells and SF-IONPs at different NaCl concentration. (b) Well depth of the secondary minimum predicted by the XDLVO as a function of NaCl concentration from part (a).

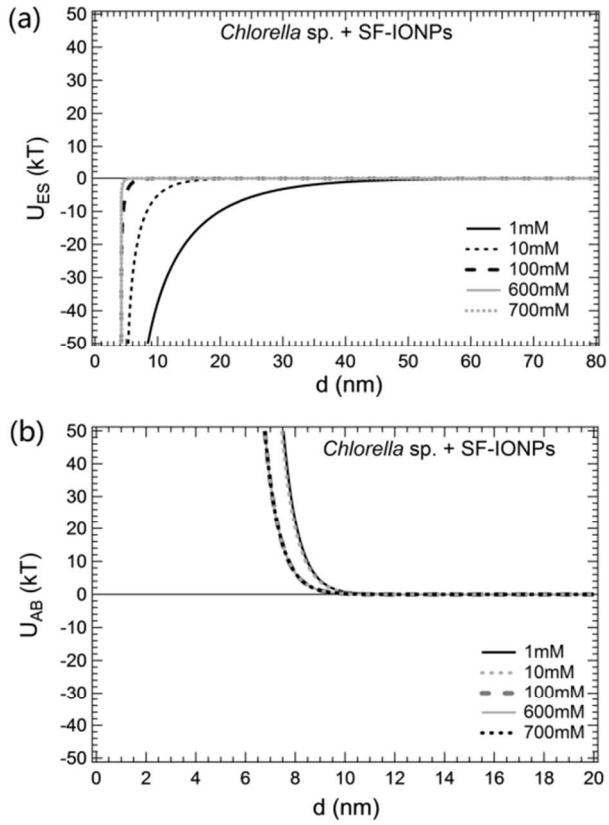

Figure 7. Normalized interacting potential of (a) electrostatic and (b) Lewis acid base interaction with respect to the separation distance, d between *Chlorella* sp. and SF-IONPs at difference NaCl concentration. Regardless of ionic strength, U_{ES} is always negative (attractive) and U_{AB} is always positive (repulsive) within the investigated concentration range of NaCl.

ionic strength adjustment.[33]

Figure 5 shows the cell separation efficiency is the highest in 1 mM ionic strength medium by using SF-IONPs. It gains a crystal clear medium at the end of separation recording an overall separation efficiency of 98.82 ± 0.31 %. Then, followed by 10 mM medium that is 92.84 ± 8.32 %. When ionic strength topped up to more than 100 mM, cell separation efficiency become much weaker and only 5.75 ± 1.09 % of cells are being removed in 700 mM medium. This experimental observation is in accordance with the XDLVO model prediction as shown in Figure 6. The well depth of secondary minimum sharply decreases from -41 kT to -5 kT when ionic strength increases from 1 mM to 10 mM. While the net repulsive interaction predicted from XDLVO without secondary minimum find in 100 mM medium and above indicate that no effective attachment between the SF-IONPs and cells. They tend to repel each other and is in good agreement with our previous observation in which no SF-IONPs attachment is observed in seawater.

By referring to Figure 7a and 7b both the effective length scale for ES and AB interaction between SF-IONPs and *Chlorella* sp. decreased with the increment of ionic strength. For the former case, the reduction in term of the effective interaction range is due to Debye screening effect as discussed previously (see Supporting Information, Figure S5). Moreover, it should be noticed that the extent of reduction in the effective interaction range is much more pronounced for ES interaction compared to AB interaction. In fact, this slight reduction of interaction range for AB case is very likely due to the complex interplay of salting out effect and conformational changes in the polyelectrolyte layer.[34,35] Suppression of the physical barrier formed due to polyelectrolyte layer adsorption, which subsequently reduces the absolute surface-to-surface separation distances, should be the main reason for the decreasing range of AB interaction.

This study shows the ES interaction is crucial to facilitate effective particle-to-cell attachment in freshwater while the AB interaction is more dominant in high ionic strength condition (≥ 100mM). Having this kind of information, especially by knowing the extent of interaction involved, has provided us a guideline to design an effective strategy to promote particle detachment. For an example, since ES interaction is most influential for the particle-to-cell attachment in freshwater, hence, suppression of this interaction by introducing ionic stress could disrupt the entire interaction scheme which subsequently causing particle detachment (see Supporting Information, Figure S6). This investigation is important for the recovery of SF-IONPs from magnetophoresis without the need of pH adjustment for effective implementation of magnetophoretic separation of microalgae without taking the risk of causing cell lysis.[36,37]

3.4 Domination of ES Interaction with respect to pH

Throughout the entire time course of the cultivation process, the pH of the culture media shifted to more alkaline condition (see Supporting Information, Figure S7). In addition, the ES interaction is sensitive toward the pH changes of the surrounding media by influencing the surface charge. These two important phenomena motivated our work in this section focuses on the effect of pH upon the interaction between microalgal cells with bare-IONPs/SF-IONPs.

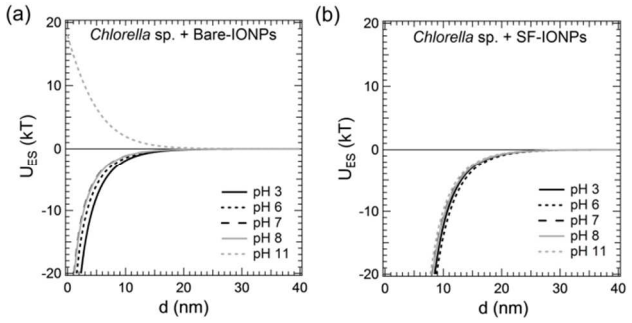

Figure 8. U_{ES} curve as a function of distance between *Chlorella* sp. and (a) bare-IONPs and (b) SF-IONPs at different pH.

In this study, the bare-IONP have an isoelectric point at about pH 8 while both *Chlorella* sp. cells and SF-IONP didn't show any charge reversal behavior within the pH range of 3 to 11 (see Supporting Information, Figure S8: cells will always carry net negative charge and the SF-IONPs will always in positive charge). Result in Figure 8 shows that the U_{ES} in the case of cells with bare-IONPs have changed from attraction (negative zone) to repulsion (positive zone) when pH change across the isoelectric point of bare-IONPs. Associated to this change is the obvious diminishing of secondary minimum with a well depth of -7.68 kT at pH 3 to net repulsion at pH 11 (Figure 9a). Our experimental result (Figure 9b) has proven the reliability and versatility of the XDLVO analysis where the attractive interaction binds the cells and bare-IONPs together and forming cells-particles cluster at pH 3 as shown in Figure 9c. For the case of SF-IONPs, the results in Figure 8b illustrated that the U_{ES} always maintain attraction between the negative charged cells and positive charged SF-IONPs. The secondary minimum predicted by XDLVO theory (Figure 9a) is nicely developed (> 9kT in magnitude) and promote the SF-IONPs to attach on microalgal cell surface. This observation is in consistent with the experimental result (Figure 9b) with cell separation efficiency more than 90% in all pH. It should be noted that in our case the ES interaction is slightly long range compares to AB interaction (see Supporting Information, Figure S9), so the ES has dominated the net interaction energy, U_{XDLVO}, with respect to pH. Our optical microscopy observation (Figure 9d) clearly shows the effective attachment of particles-to-cell for the case of SF-IONPs. This study has confirmed the important role of ES and AB interactions under freshwater condition. Therefore, these two interactions need to be considered in order to predict the successful of particle-to-cell attachment by XDLVO analysis.

3.5 Attachment of nanoparticles on marine species

In order to further verify the feasibility of the XDLVO theory in predicting the particle-to-cell interaction in seawater, another marine species of microalgae, namely *Nannochloropsis* sp., was employed for particle-to-cell attachment study.

In general, *Nannochloropsis* sp. cell is having a simple spherical structure with diameter at around 3-5 µm resembles the size and shape of *Chlorella* sp. Table 2 shows that the AB interfacial interaction energy (per unit contact area) between the surfaces of *Nannochloropsis* sp. is weaker than the *Chlorella* sp. The *Nannochloropsis* sp. experiences weaker AB repulsive interaction when in contact with the bare-IONPs and SF-IONPs, with its surface energy at 64.0 mJ m^{-2} and 65.1 mJ m^{-2} compared to that of the *Chlorella* sp., at 87.7 mJ m^{-2} and 89.6 mJ m^{-2}, respectively. Our XDLVO analysis predicts a secondary minimum well depth at -0.90 kT for cell/bare-IONPs interaction and -1.94 kT for cell/SF-IONPs

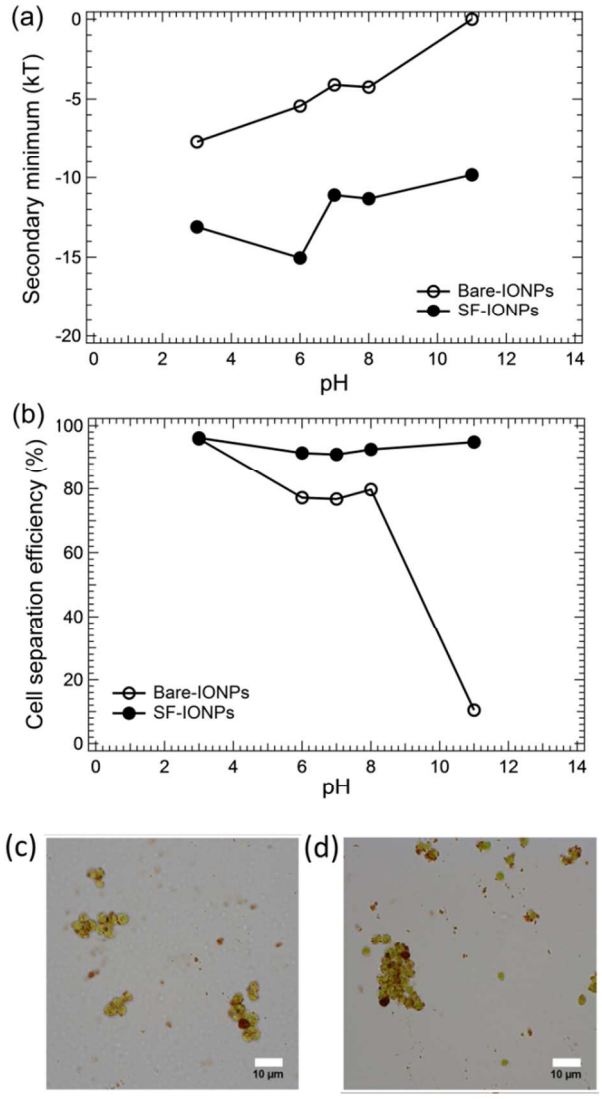

Figure 9. Diagrams showed the (a) well depth of secondary minimum predicted by the XDLVO analysis and the (b) cell separation efficiency from experiment observation when the *Chlorella* sp. cells are interacting with the bare-IONPs and SF-IONPs at different pH. Cell separation is performed by a NdFeB cylindrical magnet with total collection time of 6 minutes at dosage of 1.27 g nanoparticles per g dry biomass. Microscopy images showing the effective attachment between cells and (c) bare-IONPs in pH 3 and (d) SF-IONPs in pH 11. Darken rings and spots detected over most of the cell surface indicated the successfully attachment of particles.

interaction (see Figure 10). With low particles-to-cell ratio at 0.42 g g^{-1}, there is no obvious attachment of bare-IONPs onto cells and almost all of the particles have aggregated to form large clusters (see Figure S10 in supporting information). However, for the case of SF-IONPs, we observed particle attachment even at very low particles-to-cell ratio of 0.21 g g^{-1} (Figure S10). Together with the results presented in Figure 10, our microscopy observation supported our hypothesis in which magnetic separation would definitely occur as long as there is particles attachment. Figure 10b shows that the magnetophoretic separation efficiency achieved are 97.90 ± 0.27 % and 4.94 ± 0.50 % for SF-IONPs and bare-IONPs, respectively when in particles-to-cell ratio of 0.42 g g^{-1} (see Supporting Information, Figure S10). The recorded separation efficiency has indirectly confirmed that particles-to-cells aggregation is weak and insignificant for well depths less than ~ 1.5 kT.[38] In thermal

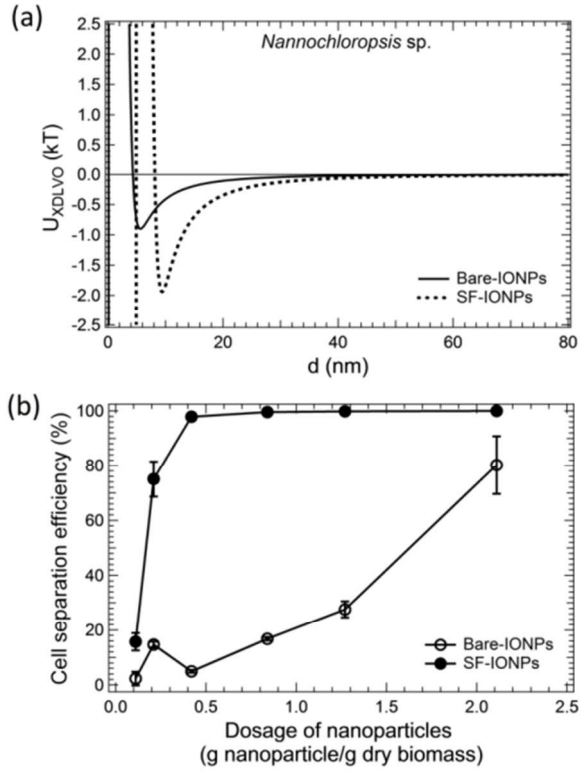

Figure 10. (a) XDLVO analysis for the interaction between the marine species *Nannochloropsis* sp. with bare-IONPs and SF-IONPs. The well depth of secondary minimum predicted for each case is -0.90 kT (bare-IONPs) and -1.94 kT (SF-IONPs). (b) Separation efficiency of *Nannochloropsis* sp. under LGMS at different dosage.

equilibrium, according to the equipartition theorem, a particle has the same average energy associated with each independent degree of freedom of their motion. Hence the translational kinetic energy possessed by a free particle is equivalent to 3/2 kT ($<E_{kinetic}>$ = 1.5 kT). The overall attractive interaction between the IONPs and microalgal cells need to overcome this energy for binding to happen. Results shown in Figure 10a and 10b are nicely aligned with this classical prediction and provided a proper guideline for magnetic seeding of microalgal cell. Under this principle, particle attachment could only be happened if the particle-to-cell interaction achieving a well-depth of secondary minimum beyond 1.5 kT under XDLVO analysis. Even though the magnitude of attractive interaction is weak for the case of bare-IONPs on *Nannochloropsis* sp., however, magnetophoretic separation of this species of microalgae can still be implemented by increasing the particles concentration. Since the electrostatic interaction within seawater is relatively weak, as suggested by our previous XDLVO analysis, we hypothesized that the vdW and AB interactions should play a major role here. Moreover, the high concentration of particles enables the cells and bare-IONPs to collide more frequently, hence increase the probability of particles attachment. Figure 10b shows separation efficiency of *Nannochloropsis* sp. up to 80.22 ± 10.57 % can only be achieved with particles-cell ratio of 2.11 g g^{-1} (see Supporting Information, Figure S10). By taking the density of iron oxide at 4.95 g cm^{-3} and mean diameter of the particles as 25 nm we estimated that there are around 1.74×10^{12} particles per cell.

By referring back to the case of *Chlorella* sp. with bare-IONPs, the net repulsive interaction predicted by XDLVO theory has suggested that under all circumstances, even at high particles concentration, there should be no particles attachment hence no microalgae separation. Our results has confirmed this observation (Supporting Information, Figure S11) and verified the critical need to take into account AB interaction in predicting the particle-to-cell interaction. Moreover, the vdW and AB interactions should be taken into account to predict the effectiveness of particle-to-cell attachment in seawater without the need to consider the ES interaction due to Debye screening effect.[15] From all the data presented, magnetophoretic separation can be implemented successfully as long as the particles attached. This finding makes the prediction on particles attachment, such as discussed in this work, crucial for the implementation of magnetophoretic separation of microalgal cells.

4 Conclusions

XDLVO analysis can be employed as a very useful mathematical analysis to predict the successful IONPs attachment onto the microalgal cells, either in freshwater or seawater. In freshwater, the effectiveness of the particle-to-cell attachment is always governed by ES interaction, in which this interaction is strongly affected by the surface zeta-potential. The AB interaction between the *Chlorella* sp. and bare-IONPs/SF-IONPs has a shorter interacting distance than ES interaction. XDLVO analysis has predicted a net repulsion between the *Chlorella* sp. cells and bare-IONPs while predicted effective attachment of SF-IONPs onto cell surface with a secondary minimum of -3.12 kT, which are in accordance with our experimental observation. In seawater, the AB interaction plays a pivotal role in determining the net interaction between the cells and IONPs. High ionic strength has suppressed the Debye screening length of the charged particles and induces a relatively short length ES interaction, between cells and IONPs. This observation is beneficial for the design of an effective protocol to promote particles detachment from cell surface which would greatly improve the reusability of the IONPs for subsequent separation cycle. The XDLVO theory has predicted the present of secondary minimum with well depth of -1.94 kT, which is mainly dominated by the vdW interaction, and followed by a strong hydrophilic repulsion (U_{AB}) for the case of marine species *Nannochloropsis* sp. cells interact with SF-IONPs in seawater. Under this scenario, 97.90 ± 0.27 % of cell was magnetohporetically separated out at particle dosage of 0.42 g particles per g dry biomass. This prediction is strongly related to the less hydrophilic surface of *Nannochloropsis* sp. We anticipated that the XDLVO analysis presented here can be employed to aid the process of (1) selecting appropriate molecular binder for surface functionalization of IONPs which subsequently promote the particle attachment onto microalgal cell, and more importantly, (2) predicting the successful implementation of magnetophoretic separation without the need to conduct tedious and lengthy experiments.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Table S1: Contact angle measurements. Table S2: Surface energy components of the liquids. Figure S1: Microscopy images of *Chlorella* sp. cells trapped inside the bare-IONPs flocculated matrix. Figure S2-S3: TEM micrograph shows the relative size of IONPs to *Chlorella* sp. cell and the internalization of SF-IONPs into *Chlorella* sp. cell after effective attachment. Figure S4: XDLVO diagram of the interaction between *Chlorella* sp. cells and SF-IONPs in freshwater and seawater. Figure S5: Zeta potential of *Chlorella* sp. and SF-IONPs with respect to NaCl concentration. Figure S6: Detachment efficiency of *Chlorella* sp. Cells from SF-IONPs-attached-cells biomass in different concentration of NaCl. Figure S7: pH of the *Chlorella* sp. culture medium in function of day. Figure S8: Zeta potential of *Chlorella* sp., bare-IONPs and SF-IONPs in function of pH. Figure S9: XDLVO diagram of *Chlorella* sp. interact with SF-IONPs in pH 11 of 1mM NaCl medium. Figure S10: Microscopy images showed the attachment of particles to marine species *Nannochloropsis* sp. Figure S11: Cell separation efficiency of *Chlorella* sp. in different dosage of bare-IONPs.]. See DOI: 10.1039/b000000x/

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