**Adhesion of algal cells to surfaces**

Altan Ozkan\textsuperscript{a} and Halil Berberoglu\textsuperscript{b}\*  

\textsuperscript{aCivil, Architectural and Environmental Engineering Department, Cockrell School of Engineering, The University of Texas at Austin, Austin, TX, USA; \textsuperscript{b}Mechanical Engineering Department, Cockrell School of Engineering, The University of Texas at Austin, Austin, TX, USA}

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This paper reports the cell–substratum interactions of planktonic (Chlorella vulgaris) and benthic (Botryococcus sudeticus) freshwater green algae with hydrophilic (glass) and hydrophobic (indium tin oxide) substrata to determine the critical parameters controlling the adhesion of algal cells to surfaces. The surface properties of the algae and substrata were quantified by measuring contact angle, electrophoretic mobility, and streaming potential. Using these data, the cell–substratum interactions were modeled using thermodynamic, DLVO, and XDLVO approaches. Finally, the rate of attachment and the strength of adhesion of the algal cells were quantified using a parallel-plate flow chamber. The results indicated that (1) acid–base interactions played a critical role in the adhesion of algae, (2) the hydrophobic alga attached at a higher density and with a higher strength of adhesion on both substrata, and (3) the XDLVO model was the most accurate in predicting the density of cells and their strength of adhesion. These results can be used to select substrata to promote/inhibit the adhesion of algal cells to surfaces.

**Keywords:** algae; adhesion; biofilm; biofouling; benthic; XDVLO

**Introduction**

Microalgae are a diverse group of unicellular or multicellular photosynthetic microorganisms with a size range of 2–140 μm (Madigan & Martinko 2006; Gupta et al. 2009) which are capable of forming highly productive biofilms over surfaces (Callow 2000). The unintended formation of algal biofilms is undesirable; on ship hulls they increase the drag and decrease the fuel efficiency (Schultz 2007; Briand 2009; Schultz et al. 2011; Zargiel et al. 2011), on cooling–heating systems they increase the pressure drop and decrease the thermal efficiency (Sekar et al. 2004), and in photobioreactors they lower performance (Borowitzka 1999; Chisti 2007). Moreover, algal biofilms are also associated with the biocorrosion of surfaces, e.g., stainless steel immersed in marine and freshwater environments (Landoulsi et al. 2011). Conversely, algal biofilms are complex biological systems where optimized cultivation offers unique advantages in wastewater treatment and biofuel production technologies (Craggs et al. 1996; Pizarro et al. 2002; Schumacher & Sekoulov 2003; Mulbry et al. 2008; Ozkan & Berberoglu 2012). In wastewater treatment, algal biofilms impart higher quality effluent compared to planktonic systems, since the effluent is free of algal cells (Craggs et al. 1996). In biofuel production, the cultivation of algae as biofilms reduces both the volume of water and the associated power required for pumping, as well as producing a concentrated biomass thus minimizing the need for energy intensive and costly harvesting and dewatering technologies (Ozkan & Berberoglu 2012). Understanding the processes involved in the formation and control of algal biofilms has significant implications for all of these applications. This study investigates the parameters that control the first step of algal biofilm formation, the initial adhesion of cells to substrata.

The studies published on the interactions between algal cells and substrata focus only on the surface energy of the substrata excluding the effects that the surface properties on the algae have on cell adhesion. No comprehensive study takes into account the surface properties, including zeta potential and surface free energy, of both the algae and the substrata. This paper addresses this gap in the literature by (1) reporting a complete set of physico-chemical properties for the surface of algae and substrata; (2) modeling surface interactions through thermodynamic, Derjaguin, Landau, Verwey and Overbeek (DLVO) and extended DLVO (XDLVO) models; and (3) validating the predictions of the models with experimental data. To identify critical parameters controlling algal adhesion, glass and indium tin oxide (ITO) were chosen as substrata with high and low surface energies, respectively. *Chlorella vulgaris* and *Botryococcus sudeticus* were chosen as representative planktonic and benthic algal species, respectively, so that (1) the physico-chemical surface properties of the two algal species could be

\*Corresponding author. Email: berberoglu@mail.utexas.edu

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quantified and compared and (2) the influence of these surface properties on the initial adhesion could be determined.

Models of initial cell adhesion

Thermodynamic approach

This approach determines the change in the total interfacial free energy ($\Delta G_{\text{adh}}$) of a substratum, microorganism, and liquid system before and after the adhesion of the microorganism to the substratum. Formation and removal of interfaces are compared in terms of their interfacial free energy defined according to Bos et al. (1999):

$$\Delta G_{\text{adh}} = \gamma_{\text{ms}} - \gamma_{\text{ml}} - \gamma_{\text{sl}}$$  \hspace{1cm} (1)

where $\gamma_{\text{ms}}$ is the microorganism-substratum, $\gamma_{\text{ml}}$ is the microorganism-liquid, and $\gamma_{\text{sl}}$ is the substratum-liquid interfacial free energy in J m$^{-2}$ (Facchini et al. 1988). For this study, the interfacial free energy is calculated using the Lifshitz-van der Waals-acid base (LW-AB) approach with the surface free energy properties of the algal cells and adhesion substrata being quantified according to the extended Young’s equation (Bos et al. 1999; van Oss 2008). This model suggests that the attachment of the cell is thermodynamically favorable if the total interfacial energy of the system decreases after adhesion, i.e., if $\Delta G_{\text{adh}}$ is less than zero (Facchini et al. 1988). The total free energy of adhesion can also be considered as the sum of the LW and AB components where the AB component is calculated as in Bos et al. (1999):

$$\Delta G_{\text{adh}}^{\text{AB}} = 2\sqrt{\gamma_{\text{m}}^2 - \gamma_{\text{l}}^2}(\sqrt{\gamma_{\text{m}}^2} - \gamma_{\text{l}}^2) - 2(\sqrt{\gamma_{\text{m}}^2} - \gamma_{\text{l}}^2) \times (\sqrt{\gamma_{\text{s}}^2} - \gamma_{\text{l}}^2)$$  \hspace{1cm} (2)

where $\gamma_{\text{m}}^2$ and $\gamma_{\text{l}}^2$ are the electron donor and acceptor parameters, respectively, while subscripts m, s, and l refer to the microorganism, substratum, and liquid medium. In addition, the LW component is calculated as in Bos et al. (1999):

$$\Delta G_{\text{adh}}^{\text{LW}} = -2\sqrt{\gamma_{\text{m}}^2 \gamma_{\text{l}}^2 - \gamma_{\text{LW}}^2}$$  \hspace{1cm} (3)

where $\gamma_{\text{LW}}$ refers to van der Waals component of the surface free energy.

Derjaguin, Landau, Verwey, Overbeek (DLVO) approach

In the DLVO approach, microbial adhesion is described as a balance between van der Waals (LW) and electrostatic interactions (EL) (Bos et al. 1999). LW forces originate from the instantaneous asymmetrical distribution of electrons in molecules and it is usually attractive (Erbil 2006). Electrostatic interactions are the result of Coulomb interactions between the cell and the substratum. The latter is usually repulsive as both the algal cells and substrata normally carry a negative charge (Hermansson 1999). The total interaction energy $G_{\text{TOT}}$ which is a function of the distance between the cells and the substrata is defined as:

$$G_{\text{TOT}}(d) = G_{\text{LW}}^*(d) + G_{\text{EL}}^*(d)$$  \hspace{1cm} (4)

where $G_{\text{LW}}^*$ and $G_{\text{EL}}^*$ are the Lifshitz-van der Waals and the electrostatic interaction energy, respectively. While a negative $G_{\text{TOT}}^*$ indicates adhesion, a positive sign indicates a repulsive interaction between the cell and the substratum. The magnitude of the total interaction and the associated separation distance determines the extent of reversible adhesion. An interaction energy scale of $kT$ in joules, where $k$ is the Boltzmann constant and $T$ is the temperature of the cell and the medium, is commonly used for comparison of the interaction energy between microorganism-substratum with that of the thermal energy of the microorganism (Bos et al. 1999). The Lifshitz-van der Waals component of free energy ($G_{\text{LW}}^*$) is defined as:

$$G_{\text{LW}}^*(d) = -\frac{A}{6} \left[ \frac{a}{d} + \frac{a}{d+2a} + \ln \left( \frac{d}{d+2a} \right) \right]$$  \hspace{1cm} (5)

where $a$ is the radius of the algal cell in m, $d$ is the separation distance in m, and $A$ is the Hamaker constant (Bos et al. 1999):

$$A = -12\pi d_0^2 \Delta G_{\text{adh}}^{\text{LW}}$$  \hspace{1cm} (6)

where $d_0$ is the minimum separation distance between two surfaces, equal to $1.57 \times 10^{-10}$ m (Bos et al. 1999). Finally, the electrostatic component of free energy, $G_{\text{EL}}^*$, between the cell and the surface is defined by Bos et al. (1999):

$$G_{\text{EL}}^*(d) = \frac{\pi a}{2} (\psi_m^2 + \psi_s^2) \left[ \frac{2\psi_m^2 \psi_s}{\psi_m^2 + \psi_s^2} \ln \frac{1 + e^{-kd}}{1 - e^{-kd}} + \ln(1-e^{-2kd}) \right]$$  \hspace{1cm} (7)

where $\varepsilon$ is the permittivity of water, equal to $6.88 \times 10^{-10}$ F m$^{-1}$, $\psi_m$ and $\psi_s$ are the surface potentials of the algal cells and substrata in V, respectively, and $k^{-1}$ is the double layer thickness in m which is given by Bos et al. (1999):
where \( e \) is the electron charge equal to 1.6022 \( \times 10^{-19} \) C, \( z_i \) is the charge number for ions of species \( i \), and \( n_i \) is the concentration of ions of species \( i \) in the solution as \# of ions m\(^{-3}\). To quantify the surface potential, the following relation is used:

\[
\psi = \zeta \left( 1 + \frac{\nu}{a} \right) e^\nu \tag{9}
\]

where \( \nu \) is the thickness of the hydration layer associated with the algal cells in m. The value of \( \nu \) changes inversely with the ionic strength of the medium and varies from \( 3 \times 10^{-11} \) m–5 to \( 5 \times 10^{-11} \) m (Sharma & Rao 2003). A value of \( 5 \times 10^{-11} \) m was used in this study based on the ionic strength of phosphate buffered saline (PBS).

**XDLVO approach**

The XDLVO approach considers the contribution of the AB interactions in addition to those of electrostatic and van der Waals forces in the DLVO approach. AB forces originate from electron transfer interactions between polar components of the cell and the surface. AB interactions can be attractive (hydrophobic attraction) or repulsive (hydrophilic repulsion) based on the hydrophobicity of the interacting surfaces (van Oss 2003). The total interaction energy \( G_{TOT} \) is given as:

\[
G_{TOT}(d) = G^{AB}(d) + G^{EL}(d) + G^{W}(d) \tag{10}
\]

where \( G^{AB} \) is the AB interaction energy (Bos et al. 1999):

\[
G^{AB}(d) = 2\pi a\lambda \Delta G^{AB}_{adh} e^{(d_0/d)\nu} \tag{11}
\]

where \( \Delta G^{AB}_{adh} \) is the polar free energy change in the system given by Equation (2) and \( \lambda \) is the correlation length, also known as the gyration radius of water molecules, in a solution. In this study, \( \lambda \) was equal to 6 \( \times 10^{-10} \) m for hydrophilic and 1.5 \( \times 10^{-9} \) m for hydrophobic interactions as water molecules have a larger gyration radius around hydrophobic surfaces (van Oss 1994; Bos et al. 1999; Erbil 2006). Based on this model, the main driving force for hydrophobic attraction is the cohesion energy of water molecules (due to hydrogen bonding), which is present around the interacting surfaces (van Oss 1997, 2003). This attractive free energy is always present when surfaces are immersed in water, even if the interacting surfaces are hydrophilic. Thus, for net repulsion to exist between surfaces, the magnitude of the polar attraction between the hydrophilic surfaces and water molecules (\( \gamma^m \) and \( \gamma^e \) binding to \( \gamma^w \) of water molecules) has to exceed the hydrogen bonding free energy of cohesion of water molecules (van Oss 2007).

**Materials and methods**

**Cultivation of algae**

*Chlorella vulgaris* (UTEX 2714) and *Botryococcus sudeticus* (UTEX B 2629) were obtained from the Culture Collection of Microalgae at the University of Texas at Austin, UTEX. The algae were grown as batch cultures in BG-11 nutrient medium (Andersen 2005) supplied with 2.5 vol.\% CO\(_2\) under continuous irradiation of 125 \( \mu \)Em\(^{-2}\)s\(^{-1}\) (26.25 Wm\(^{-2}\)) of photosynthetically active radiation (PAR) using fluorescent light bulbs. In this study, *C. vulgaris* and *B. sudeticus* are referred to as planktonic and benthic species, respectively, based on their growth characteristics in the natural environment (Latala et al. 2009). For adhesion experiments, log phase cultures were harvested by centrifuging at 3,000 rpm (1,962 x g) for 5 min. Pellets were washed twice and re-suspended in PBS containing 1.62 mM KH\(_2\)PO\(_4\), 6.49 mM K\(_2\)HPO\(_4\), and 1.35 mM NaCl. PBS had a pH of 7.559 ± 0.019 (Accumet AB15 Plus, Fisher Scientific, USA), a conductivity of 1.777 ± 0.026 mS cm\(^{-1}\) (Con 2700, Oakton, USA), and an ionic strength of 19.60 mM. The PBS was prepared to match the pH and ionic strength of BG-11.

**Characterization of the morphological properties of algal cells**

The morphological characteristics of algal cells (Supplementary information, Figure S1) were quantified from images obtained with an inverted microscope (Nikon Ti-E, Nikon, USA) using ImageJ image analysis software (Rasband 1997–2011). [Supplementary material is available via a multimedia link on the online article webpage.] In this analysis, the cells were approximated as ellipsoids with the associated major and minor diameters along their major and minor axes, respectively. In addition, the equivalent spherical diameters were determined such that the projected area of an equivalent sphere was the same as that of the ellipsoidal cell with the specified major and minor diameters. Also, the circularity of cells was determined according to:

\[
\text{Circularity} = \frac{4\pi A_{cell}}{P_{cell}^2} \tag{12}
\]

where \( A_{cell} \) and \( P_{cell} \) are the imaged area and the perimeter of the cell, respectively (Rasband 1997–2011). A circularity of one indicates that the cell is perfectly spherical.
Preparation of surfaces for adhesion tests

Glass (Fisher Scientific, USA) and ITO coated glass (CG-40IN-1115, Delta Technologies, USA) were selected as hydrophilic and hydrophobic surfaces, respectively. ITO was selected as it has been successfully used in the literature to cultivate healthy algal biofilms for microbial fuel cell applications (McMorrow et al. 2010; Sailey et al. 2012). Microscope glass slides were cleaned in 5 M HCl for 5 h, rinsed with deionized (DI) water and then sonicated successively in 1% Alconox solution (Alconox Inc., Alconox, NY), ethanol and acetone (10 min each), before being rinsed with DI water and air-dried. ITO coated glass surfaces were cleaned in an ultrasonic bath with 1% Alconox solution for 10 min, rinsed with DI water, and air dried.

Measurement of the contact angle of algal cells and substrata

The surface energy of the algal cells and the substrata was quantified based on contact angle measurements made using the sessile drop technique with DI water, diiodomethane, and formamide as the reference liquids (Busscher et al. 1984). These probe liquids have been used successfully to measure the contact angle of a number of different microorganisms including algae, yeasts, and bacteria and no degrading effects on the cells or cell walls have been reported (Branicky et al. 2004; Barberousse et al. 2007; Hwang et al. 2012; Montag et al. 2012; Wu et al. 2012). The contact angles of all the probe liquids were measured using a goniometer (Model 190 CA, Rame-Hart, USA). The surface free energy of algal cells was quantified based on the sessile drop technique described by Busscher (Busscher et al. 1984; van der Mei et al. 1998). Algal mats were prepared on 0.45 μm mixed cellulose acetate filters (Nalge Nunc International, USA) by filtering suspensions of cells which had been washed twice with DI water. In order to prevent the algal mats from drying, they were placed over 1% agar (w/v) prepared with 10% (v/v) glycerol in water until contact angle measurements could be made. For the measurements, the mats were removed from the agar plates and placed over a metal sample holder, then air-dried. To determine the extent of the drying, 5 μl droplets of DI water were placed over the mats at different locations every 5 minutes and their contact angles were measured. As drying progressed, the contact angle of water increased. After 20 and 45 min of drying for B. sudeticus and C. vulgaris mats, respectively, the water contact angles stabilized, indicating completion of excess water evaporation from the mats. At this point, the contact angle measurements with the other probe liquids began. The stabilization time for the probe liquids on the mats was 0.2 to 0.3 s and the contact angle was recorded within 2 s. These times are similar to those reported by Sharma and Rao (2002). Thus, in all experiments algal cells were exposed to diiodomethane and formamide for a maximum of 2 s before the contact angle measurement was recorded. The results presented are the average of at least 14 measurements for each probe liquid, which resulted in a standard deviation of <9%. The thickness of the mats generated on the filters in this study was approximately 200 μm after the evaporation of excess water. Algal mats were imaged from the side using the goniometer setup and the thickness was measured using the ImageJ software. The surface energy of the substrata was also determined using the sessile drop technique with the same probe liquids.

Measurement of zeta potential

Suspensions of algae with cell concentrations of 2.5 × 10^10 m^-3 were used for the measurement of zeta potential. Electrophoretic mobility measurements of cells were conducted with a Zeta meter (ZetaCompact, CAD, France) at a voltage of 80 V. The zeta potential (ξ) of cells was determined using Smoluchowski’s equation (Shaw 1980). To quantify the zeta potential of glass and ITO-coated glass slides, streaming potential measurements were made using an electrokinetic analyzer (SurPASS, Anton Paar GmbH, Austria). Streaming current measurements for ITO were determined to ensure that the surface conductivity of the substratum was taken into account when quantifying zeta potential (Luxbacher 2006). The Fairbrother-Mastin approach was used to calculate the zeta potential of the substrata based on the streaming potential and current measurements performed (Fairbrother & Mastin 1924; Kirby & Hasselbrink Jr. 2004).

Measurement of adhesion density and adhesion strength

A parallel-plate flow chamber was constructed to measure the density and the strength of adhesion of cells attached to different substrata. Figure 1 shows the flow chamber in side and top view. A 0.75 mm thick spacer of polydimethylsiloxane (PDMS) (Sylgard 184 Silicone Elastomer Kit, Dow Corning, USA) was sandwiched between a transparent polycarbonate sheet (top) and the test surface (bottom) to create the flow chamber. The length, width, and height of the chamber were 50.8, 26.0, and 0.75 mm, respectively. Two fluidic adapters were placed at the inlet and outlet of the flow chamber to control the flow of the algal suspension. The fluid introduced at the inlet was slowly expanded at an angle of 15° to ensure laminar flow.

Figure 2 illustrates the setup used for the adhesion tests. A syringe pump (NE-1000, New Era, USA) delivered the algal suspensions from the reservoir to the flow chamber by pulse-free suction. The algal suspension...
inside the reservoir was stirred continually at 120 rpm (Isotemp, Fisher Scientific, USA). Algal suspensions (9.47 ± 0.12 /C2 1012 cells m/C0 3) were delivered to the chamber at a rate of 0.450 ± 0.05 ml min/C0 1 for 5 min. The required cell densities were obtained by measuring the optical density at 750 nm (Genesys 20, Thermo Scientific, USA), which had been calibrated by counting the number of cells using a hemocytometer (Bright-line, Hausser Scientific, USA). After operating for 5 min, the pump was stopped and the cells were left undisturbed for 2 h. During the period of flow the Reynolds number, Re, and γ, over the test section were 0.97 ± 0.01 and 10.13 ± 0.10 s/C0 1, respectively. The temperature was 20 ± 1 °C. The surface with attached cells was imaged with an inverted microscope (Nikon Ti-E, Nikon, USA) equipped with a digital camera (DS-Q11, Nikon, Japan) and a 10x objective (Nikon Fluor 10X, Nikon, USA). A surface area of 0.541 mm² was imaged every minute during the period of flow. Images were processed using ImageJ software to quantify the number of attached cells as a function of time (Rasband 1997–2011). The flow, imposing a wall shear rate of 10 s/C0 1 was switched on for 5 min every 2 h. Cells that remained on the surface were considered to be adhered and the experiment was continued until the density of cells on the surface reached saturation. Results are presented as the average of at least duplicate experiments.

In order to quantify the adhesion strength, the flow rate inside the chamber was increased stepwise from 4.50 to 31.50 ml min/C0 1 with increments of 2.25 ml min/C0 1 which corresponds to Re and γ ranging from 9.71 to 67.99 and from 101.27 to 708.86 s/C0 1, respectively. In this study the net force due to lift and drag forces acting on cells, was calculated based on the equations of Buscher and van der Mei (2006). Before the desorption experiments, the algal suspension in the reservoir was replaced with PBS to avoid the effects of suspended cell – attached cell collisions on desorption kinetics. Each of the flow rates were maintained for a duration of 1 min and the area of the substratum with adhered cells was imaged at the end of each flow period.

Results and discussion

Physico-chemical properties of the algal species and substrata

Table 1 shows the dimensions of the two algae (see Supplementary information, Figures S1 and S2 for images of live cells of C. vulgaris and B. sudeticus, respectively). Both species have a circularity close to one, 0.93 for C. vulgaris and 0.94 for B. sudeticus. Thus, both organisms can be approximated as spheres, with equivalent spherical diameters of 5.34 and 4.48 μm for C. vulgaris and B. sudeticus, respectively. These equivalent spherical diameters were used as the size parameters of cells in the subsequent modeling studies.

Table 2 summarizes the empirical surface properties of glass and ITO as well as the surfaces covered with cells of C. vulgaris and B. sudeticus, including the mean contact angles measured with water, formamide, and diiodomethane, the total surface free energy (γ), the free energy of cohesion (ΔGcoh), and the surface potential. These results are comparable to the surface properties of
other bacterial and algal species reported in the literature (van der Mei et al. 1998; Sharma & Rao 2002; Barberosse et al. 2007). It should be noted that contact angle measurements should be made ideally over continuous and flat surfaces. However, due to the size and shape of the cells, it is not possible to create an ideal surface. Instead, contact angles were measured over algal mats according to Busscher et al. (1984). Although these mats were flat and homogeneously covered the filters, they had a surface roughness similar to the size of the cells, i.e., in the micrometer range. As the size and shape parameters of the two algal species were similar, both being close to perfect spheres that varied in diameter by only about 20%, the magnitude of the roughnesses of the algal mat and the effect this would have on the contact angle data is expected to be similar. Bachmann et al. (2000) studied the influence of soil grain size on contact angle and showed that the measured contact angles were within 15% of a standard deviation even though particle sizes varied by a factor of 5 (~20–100 μm), resulting in large changes in both surface roughness and porosity.

As indicated by the contact angle data, the surface free energy of B. sudeticus is smaller than that of C. vulgaris and the surface free energy of ITO is smaller than that of glass. According to van Oss (2008) while a negative ΔGcoh indicates hydrophobicity where surface–surface interactions are stronger than surface–water interactions, a positive value indicates hydrophilicity (Azeredo et al. 1999). Based on the free energy of cohesion, the data in Table 2 indicate that glass and C. vulgaris have hydrophilic surfaces, while ITO and B. sudeticus have hydrophobic surfaces. The reason for the different surface properties of the 2 algae can be explained by the surface groups present in their cell walls. Cell walls of species of the genus Botryococcus contain various hydrocarbons with long carbon chains (Banerjee et al. 2002). The hydrocarbons contain hydrophobic groups such as methyl and methylidene and, to a lesser extent, hydrophilic groups such as hydroxyl and carboxyl (Banerjee et al. 2002; Erbil 2006). Because the number ratio of hydrophobic to hydrophilic groups is lesser extent, hydrophilic groups such as hydroxyl and carboxyl (Banerjee et al. 2002). The hydrocarbons contain hydrophobic groups such as methyl and methylidene and, to a lesser extent, hydrophilic groups such as hydroxyl and carboxyl (Banerjee et al. 2002; Erbil 2006). Because the number ratio of hydrophobic to hydrophilic groups is high, the surface of these algae is expected to be hydrophobic (Banerjee et al. 2002; Erbil 2006). Conversely, the cell wall of C. vulgaris consists of uronic acids, neutral sugars, glucosamine, and protein expressing hydrophilic surface groups such as carboxyl, hydroxyl, and amine (Blumreisinger et al. 1983; Erbil 2006; Hadjoudja et al. 2010). The contact angles, the surface free energy, and the free energy of cohesion quantified for both species are in agreement with these expectations. All hydrophilic surfaces have a larger surface free energy cf. hydrophobic surfaces. The LW component of the surface free energy of glass is larger than that of ITO indicating larger LW interaction. Bayoudh et al. (2006) reported similar values for the surface energy properties of ITO.

All surfaces analyzed in this study had negative surface potentials. The surface potentials of B. sudeticus and C. vulgaris were calculated according to Equation (9) using the zeta potentials quantified experimentally. The results indicated that B. sudeticus had a larger surface potential cf. C. vulgaris. Moreover, ITO had a surface potential close to zero. The pH of the PBS used for the experiments was 7.6, and thus this result is in agreement with the data reported in the literature where the isoelectric point of ITO, the pH at which the surface potential is zero, is between pH 7 and 8 (Chen et al. 2008). Furthermore, glass had a much larger zeta potential than that of ITO indicating that a much larger repulsive cell–substratum interaction would be predicted.

### Thermodynamic model

Table 3 shows the free energy of adhesion and the associated AB and LW components based on the thermodynamic model. The results indicate that greater adhesion strength is expected for cells of B. sudeticus on both glass and ITO, and for cells of C. vulgaris on ITO. Adhesion is not expected for cells of C. vulgaris on

| Interacting pair | ΔG<sub>LW</sub> (mJ m<sup>-2</sup>) | ΔG<sub>AB</sub> (mJ m<sup>-2</sup>) | ΔG<sub>adh</sub> (mJ m<sup>-2</sup>) |
|------------------|-------------------------------|-------------------------------|-----------------------------|
| C. vulgaris-glass| -6.5                          | 33.9                          | 27.4                        |
| C. vulgaris-ITO  | -3.5                          | -14.0                         | -17.5                       |
| B. sudeticus-glass| -2.9                          | -6.3                          | -9.2                        |
| B. sudeticus-ITO | -1.5                          | -64.4                         | -65.9                       |
glass as both surfaces are hydrophilic. The largest attractive energy is observed for *B. sudeticus* and ITO due to the hydrophobicity of both surfaces resulting in a large AB attraction. An attractive LW interaction is expected for all systems, with those associated with cells of *C. vulgaris* being larger in magnitude than those for cells of *B. sudeticus*.

**DLVO model**

Figure 3 shows the EL, LW, and total energy of *C. vulgaris* interacting with glass and ITO. Figure 3a indicates that for the *C. vulgaris*-glass pairing, the LW component of interaction has a larger range cf. to EL repulsion. Moreover, while the magnitude of LW attraction is larger than that of EL repulsion at separation distances >9 nm resulting in attractive total interaction, at smaller separation distances the total interaction is repulsive as EL repulsion is dominant. This results in a local energy minimum with a magnitude of ~39.4 kT at a separation distance of 13.6 nm (see inset graph). When cells of *C. vulgaris* interact with glass they can adhere to the substrate at this energy minimum. This type of adhesion is called secondary adhesion, or adhesion at the secondary minimum, and such adhesion is considered to be

![Figure 3. The interaction energy (G(kT)) of C. vulgaris with (a) glass and (b) ITO as predicted by the DLVO model. The inset graph shows an expanded view of the interaction energies at ~10 nm to better illustrate the magnitude and separation distance of the secondary energy minimum for the C. vulgaris-glass system.](image)

![Figure 4. The interaction energy (G(kT)) of B. sudeticus with (a) glass and (b) ITO as predicted by the DLVO model. The inset graph shows an expanded view of the interaction energies at ~10 nm to better illustrate the magnitude and separation distance of the secondary energy minimum for the B. sudeticus-glass system](image)
weak as (1) the magnitude of attractive interaction is small and (2) the cells are kept at a distance from the surface (Hermansson 1999). Moreover, Figure 3b indicates that for the *C. vulgaris*-ITO pairing, EL repulsion is negligible due to the small surface potential of ITO and the LW interaction is attractive. The sum of these 2 energies, i.e. the total interaction energy, is attractive at all separation distances, and there is a global energy minimum at the substratum surface with a magnitude of \( \frac{1}{3868} kT \). Cell adhesion based on this type of cell–substratum interaction is called adhesion at the primary minimum or primary adhesion. Primary adhesion is considered to be stronger than secondary adhesion due to the location of the interaction and the larger magnitude of the attractive energy involved (Hermansson 1999). Thus, based on the DLVO theory the strength and the rate of adhesion are expected to be larger for the *C. vulgaris*-ITO pairing compared to those parameters for *C. vulgaris*-glass. For the *C. vulgaris*-glass pairing, the domination of LW interaction over EL interaction at large separation distances is due to the high ionic strength of the PBS. High ionic strengths compress the electrical double layers present around the cells and the substrata, lowering the range and magnitude of EL repulsion. At higher ionic strengths, the double layers can be compressed further and instead of adhesion at a secondary minimum, adhesion at a primary minimum is expected (Hermansson 1999).

Figure 5. The interaction energy \((G(kT))\) of *C. vulgaris* with (a) glass and (b) ITO as predicted by the XDLVO model. The inset graph shows an expanded view of the interaction energies at \( \sim 10 \) nm to better illustrate the magnitude and separation distance of the secondary energy minimum for *C. vulgaris*-glass system.

Figure 6. The interaction energy \((G(kT))\) of *B. sudeticus* with (a) glass and (b) ITO as predicted by the XDLVO model.
Figure 4a and b present the EL, LW, and total energy of interaction for the B. sudeticus-glass and B. sudeticus-ITO systems, respectively. As for the results with C. vulgaris, adhesion at the secondary and primary minima are expected for the interaction of cells of B. sudeticus with glass and ITO, respectively. Based on this approach, the adhesion of cells of B. sudeticus to ITO is predicted to occur preferentially to glass. Figure 4a shows that the magnitude of the total interaction energy at the secondary minimum for the B. sudeticus-glass pairing is equal to $-11.4 \ kT$ at a separation distance of 17 nm (see inset graph), which is smaller than the total interaction energy of C. vulgaris with the same surface at the secondary energy minimum. This is attributed to (1) the larger surface potential of B. sudeticus giving rise to larger EL repulsion and (2) the smaller LW component for this species resulting in weaker LW attraction. The lower secondary energy minimum between C. vulgaris and glass indicates a larger attractive interaction cf. that occurring between B. sudeticus and that surface. Finally, as presented in Figure 4b, the total energy at the primary minimum is equal to $-3982 \ kT$ for the B. sudeticus-glass pairing indicating a strong attractive interaction.

**XDLVO approach**

Figure 5 illustrates the EL, LW, AB, and total interaction energy for the C. vulgaris-glass and C. vulgaris-ITO systems as a function of separation distance. Based on the AB components, hydrophilic repulsion and hydrophobic attraction are predicted for C. vulgaris-glass and C. vulgaris-ITO, respectively. Compared to the DLVO model, the addition of the AB component does not change the mode of adhesion for C. vulgaris on either of the surfaces; adhesion at the secondary and at the primary minima are expected for glass and ITO, respectively. However, the magnitude of the attractive interaction energy at the primary minimum increases to $-89,716 \ kT$ for C. vulgaris-ITO. Due to the increased magnitude of attractive energy, a larger strength and rate of adhesion are predicted for this pairing by the XDLVO model. The introduction of this attractive energy is due to the hydrophobicity of the ITO. Conversely, the magnitude of the secondary minimum is unaltered for the C. vulgaris-glass pairing as AB repulsion decays rapidly, and is negligible for separation distances $>6$ nm. However, the magnitude of the repulsive energy between glass and C. vulgaris, ie the energy barrier between the cell and the substratum, is larger due to the AB repulsion.

Similarly, Figure 6 shows the EL, LW, AB, and total interaction energy for B. sudeticus. Adhesion at the primary minimum is expected for cells of B. sudeticus interacting with the surface of either of the substrata. Compared to the DLVO model, the introduction of the AB interaction changes the mode of adhesion for cells of B. sudeticus on glass from adhesion at the secondary minimum to adhesion at the primary minimum (Figure 6a). Moreover, compared to the DLVO model, a larger attractive interaction energy is expected on contact between cells of B. sudeticus and the ITO substratum. Thus, based on these results greater adhesion strength and rate of cell attachment are predicted for both systems by the XDLVO model. Moreover, the XDLVO model predicts that the strength of adhesion will increase in the following order (smallest to largest): C. vulgaris-glass, B. sudeticus-glass, C. vulgaris-ITO, and B. sudeticus-ITO. Finally, the adhesion of cells of B. sudeticus to glass at the primary minimum is due to the attractive AB interactions resulting from the hydrophobicity of this species. Thus, the repulsive energy between B. sudeticus and glass predicted by the DLVO model no longer exists. The total interaction energy for glass and ITO systems are equal to $-28,634 \ kT$ and $-322,630 \ kT$, respectively.

**Parallel-plate flow experiments**

Figure 7 shows the density of adhered cells ($\rho$) of C. vulgaris on the glass substratum as a function of time. The results indicate that the rate of attachment of C. vulgaris to glass can be approximated into 3 linear parts: in the first 2 h the density increased with a rate of $\sim$56 cells mm$^{-2}$h$^{-1}$, this rate decreased to $\sim$30 cells mm$^{-2}$h$^{-1}$ during the subsequent 4 h, and finally in the last 4 h the rate decreased further to $\sim$5 cells mm$^{-2}$h$^{-1}$ resulting in a final density of adhered cells of $\sim$250 cells mm$^{-2}$.

![Figure 7](image.png)
The adhesion of *C. vulgaris* to ITO was very different from its adhesion to glass. At the end of the first 2 h without shear, all of the cells on the surface of ITO were attached, which resulted in a density of $8504 \pm 498$ mm$^{-2}$. Moreover, none of the adhered cells desorbed after flow was applied for 5 min with a shear rate of $10$ s$^{-1}$. Similarly, none of the cells of *B. sudeticus* that had adhered to ITO were removed by the same flow conditions resulting in an adhesion density of $7960 \pm 11$ cells mm$^{-2}$.

Figure 8 shows the percentage of cells remaining on each substratum after the application of flow for 5 min at a wall shear rate of $10$ s$^{-1}$. The results indicate that $3.37 \pm 0.13\%$ of cells of *C. vulgaris* remained on the glass substratum after the application of flow; for *B. sudeticus* $87.85 \pm 0.81\%$ of cells remained on this surface. No cells of either species were removed from ITO using these flow parameters. Based on these findings, it can be concluded that for an intermittent shear rate of $10$ s$^{-1}$ (1) the rate of attachment for *C. vulgaris* on glass was $\sim 3.5\%$ and $2.9\%$ of the rate of attachment of *B. sudeticus* on glass and of *C. vulgaris* on ITO, respectively; (2) the rate of cell attachment of both species was greater on ITO than on glass; and (3) similar rates of cell attachment can be expected for both species interacting with the ITO.

For all the systems studied, with the exception of the *C. vulgaris*-glass pairing, cells covered the entire surface of the substratum at the end of the first 2 h of the experiment. When surfaces were saturated with cells attachment experiments were terminated. Desorption experiments were started subsequently to quantify the adhesion strength of the cells on the respective substrata. During the desorption experiments, the flow rate was increased incrementally (steps of $2.25$ ml min$^{-1}$) from $4.5$ to $31.5$ ml min$^{-1}$ resulting in wall shear rates of $100$–$700$ s$^{-1}$ (increments of $50$ s$^{-1}$) calculated as per Busscher and van der Mei (2006). Each flow rate was maintained until the number of cells remaining on the substratum reached a steady value. At these flow rates, the cells experienced both lift (in the direction normal to the flow) and drag (in the direction of the flow). These forces were quantified according to Busscher and van der Mei (2006). Based on the flow rates introduced into the parallel-plate flow chamber, the lift forces ranged from $8.16 \times 10^{-13}$ N to $2.9 \times 10^{-11}$ N with drag forces of between $1.49 \times 10^{-11}$ N and $1.0 \times 10^{-10}$ N being generated. The resultant net
force on the cells ranged from $1.49 \times 10^{-11}$ N to $1.62 \times 10^{-10}$ N. Thus, drag force is expected to dominate the desorption phenomena for the algae studied.

Figure 9 shows the percentage of the initial number of cells remaining attached to glass and ITO as a function of wall shear rate (Figure 9a) and net force (Figure 9b). Based on these results, the adhesion strength of _C. vulgaris_ to glass was weakest, with adhesion strength increasing in the following order: _B. sudeticus_ to glass, _C. vulgaris_ to ITO, and _B. sudeticus_ to ITO. The results showed that all cells of _C. vulgaris_ that were adhered to the glass substratum were removed by a wall shear rate of 100 s$^{-1}$, which corresponds to a net force of $2.28 \times 10^{-11}$ N acting on the cells. This indicates that the adhesion strength of cells of _C. vulgaris_ to glass was smaller than $2.28 \times 10^{-11}$ N. The results indicated that the desorption characteristics of cells from ITO followed an S-curve relationship. While 99.8% and 87% of the cells remained attached with shear rates of 100 and 200 s$^{-1}$, respectively, the cells desorbed exponentially as shear rates increased from 200 to 700 s$^{-1}$. Only 5% of the cells remained attached after being subjected to a shear rate of $\sim 700$ s$^{-1}$. These results indicate that the adhesion strength of _C. vulgaris_ on ITO was $<1.62 \times 10^{-10}$ N for 95% of the cells. The results further indicate that cells of _B. sudeticus_ had the strongest adhesion to ITO. At the largest shear rate of 700 s$^{-1}$, only 8% of the cells were desorbed, which indicates that 92% of the cells had an adhesion strength $>10.55 \times 10^{-11}$ N. The number of cells of _B. sudeticus_ remaining on ITO decreased linearly with increasing shear rates and net force, whereas removal from glass occurred in an exponential fashion. A shear rate of 100 s$^{-1}$, corresponding to a net force of $1.49 \times 10^{-11}$ N, resulted in desorption of 56% of the cells from glass. At a shear rate of $700$ s$^{-1} \sim 9$% of the cells remained adhered indicating that 91% of the cells had an adhesion strength to the glass substratum of $<10.55 \times 10^{-11}$ N. The results also show that, due to the larger size of the cells of _C. vulgaris_, the net force acting on this species was greater than that for cells of _B. sudeticus._

**Comparison of the experimental results with the adhesion models**

Based on the experimental data obtained in the parallel-plate flow chamber experiments, the thermodynamic model was successful in predicting the attachment density of cells of _C. vulgaris_ to ITO and cells of _B. sudeticus_ to both substrata. This model predicts that cells of _C. vulgaris_ should only adhere to ITO due to the hydrophobicity of this surface. However, the experimental results showed that the alga also adhered to the hydrophilic glass surface. Thus, the thermodynamic model was not successful in predicting the adhesion of cells of _C. vulgaris_ to the hydrophilic surface. Figure 10 shows the attractive interfacial energy predicted by the thermodynamic model, $\Delta G$, vs the percentage of cells remaining on the respective substrata after application of net forces of 50–100 pN, for all the alga-substratum systems studied. The figure indicates that the percentage of cells remaining on the surface, i.e., the adhesion strength, increased with increasing attractive interfacial energy in

![Figure 10](image1)

**Figure 10.** The percentage of cells remaining adhered (after a net force ranging from 50–100 pN) for algal-substratum systems as a function of the attractive interfacial energy predicted by the thermodynamic model.

![Figure 11](image2)

**Figure 11.** The percentage of cells remaining adhered (after a net force ranging from 50–100 pN) for algal-substratum systems as a function of the attractive free energy predicted by the DLVO model.
all cases, corroborating the success of the thermodynamic model. Moreover, for a net force of 80 pN acting on the cells, the figure shows that the percentage of cells remaining adhered over the respective substrata increased linearly with interfacial energy. However, for other net forces acting on the cells, a linear correlation did not exist.

For both species of algae, the DLVO theory successfully predicted the density of cells adhered to both substrata. Moreover, the model was successful in predicting that the relative strength of adhesion of cells of *B. sudeticus* and *C. vulgaris* should be stronger on ITO compared to that on glass; predicting adhesion at a primary minimum for both strains on ITO whereas adhesion at a secondary minimum was expected for glass. However, this model failed to predict the stronger interaction of cells of *B. sudeticus* with glass cf. the weaker interaction of cells of *C. vulgaris* with that substratum. The model predicted a stronger adhesive interaction between *C. vulgaris* and glass. To illustrate the deviations between adhesion strength and the predicted attractive free interaction energy, Figure 11 shows the interaction energy predicted by the DLVO model at the primary and secondary minima vs the percentage of cells remaining adhered after application of net forces of 50–100 pN for all the alga-substratum systems studied. No meaningful correlation existed between adhesion strength (percentage of cells remaining adhered) and attractive free energy predicted by the DLVO model. Moreover, the predictions of the model were not confirmed by the experimental results. Specifically, the model predicted that, (1) the attractive energy of the *C. vulgaris*-glass pairing was larger than that for *B. sudeticus* with that substratum, but experimental results showed that adhesion strength of the latter was larger; and (2) a similar magnitude of attractive energy was predicted for both algal species with ITO, yet adhesion strength was demonstrated as being much larger for the latter.

Finally, the attachment of cells as well as the adhesion strength of all the systems were successfully predicted by the XDLVO model. This model takes into account the AB interactions between cells and substrata, whereas the DLVO model does not. For cells of *C. vulgaris*, a weak and reversible adhesion to glass was predicted at the secondary minimum, due to the hydrophilicity of both surfaces resulting in acid–base repulsion. Adhesion at the primary minima was predicted for cells of *C. vulgaris* on ITO and cells of *B. sudeticus* on both glass and ITO with total interaction energies at the primary minima of −89,716 kT, −28,634 kT, and −322,630 kT, respectively. The adhesion strengths derived from the parallel-plate flow experiments are in agreement with these interaction energies. The total interaction energy of cells of *B. sudeticus* to ITO was ~4 times greater than that of cells of *C. vulgaris* to that substratum. While only 8% of cells of *B. sudeticus* were desorbed from the surface of ITO when subjected to a net force of 10.55 × 10⁻¹¹ N, 90% of cells of *C. vulgaris* desorbed from that surface when the same net force was applied. Moreover, while all the cells of *C. vulgaris* were desorbed from glass when subjected to a net force of 2 × 10⁻¹¹ N, 44% of cells of *B. sudeticus* remained adhered to glass under these conditions.

Figure 12 presents the attractive free energy predicted at the secondary or primary minima by the XDLVO model vs the percentage of cells remaining adhered after the application of net forces from 50 to 100 pN for all alga-substratum systems studied. In accordance with the results of the thermodynamic model, Figure 12 indicates that the percentage of cells remaining on the surface increased with increasing attractive free energy for all cases; a linear relation existed at a net force of 80 pN acting on the cells. These results suggest that the AB component is critical for explaining cell–substrata interactions considering that the *B. sudeticus*-glass system had (1) larger EL repulsion, (2) smaller LW attraction, and (3) 26 times larger adhesion density cf. the *C. vulgaris*-glass system. Based on the XDLVO model, the reason for the existence of acid–base attraction between 2 hydrophobic or 1 hydrophilic and 1 hydrophobic surface is the hydrogen bonding component of the cohesion energy of water molecules that surround these surfaces (van Oss 2007). Conversely, for hydrophilic repulsion to develop (1) the AB interaction between the hydrophilic surface and water molecules, ie the electron donor of the surfaces attracting the electron acceptor of water, has to be stronger than the attractive polar interaction between the water molecules; and
(2) the electron acceptor parameter of the hydrophilic surfaces have to be smaller than that of water. This is usually the case as polar surfaces mostly have a negligible electron acceptor parameter (van Oss et al. 1997, 1998; van der Mei et al. 1998; van Oss 2003).

Conclusions
A comprehensive study has been conducted (1) to experimentally quantify the physico-chemical surface properties of 2 algal species with opposing tendencies for adhesion and (2) to determine the influence of these properties on their adhesion characteristics. The results showed that the hydrophobic cells of the benthic species B. sudeticus, had a faster rate of attachment and greater strength of adhesion to the substrata studied than cells of the planktonic species C. vulgaris, which has a hydrophilic surface. Cells of B. sudeticus displayed the fastest rate of cell attachment and strongest adhesion strength to the hydrophobic ITO substratum. In addition, the lower electrostatic repulsion and larger AB attraction of ITO resulted in a 30-fold increase in the density of adhered cells of C. vulgaris when compared to glass. It was shown that AB interactions between the alga and the substratum were critical for the rate of cell attachment and the strength of adhesion. Finally, the XDLVO model was the most successful model in predicting the rate of cell attachment and the strength of adhesion of the algae to ITO and glass. The methods used, models validated, and experimental data obtained in this study can be used to select and/or design surfaces to promote or inhibit the adhesion of algal cells, with applications in wastewater treatment, biofuel feedstock production, and prevention of biofouling.

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