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# The influence of surface energy on the wetting behaviour of the spore adhesive of the marine alga *Ulva linza* (synonym *Enteromorpha linza*)

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The environmental scanning electron microscope has been used to image the adhesive pads secreted by zoospores of the marine alga *Ulva linza* as they settle on a range of self-assembled and grafted monolayers of different wettability, under natural, hydrated conditions. Results reveal that the diameter of the adhesive pad is strongly influenced by surface wettability, the adhesive spreading more (i.e. wetting the surface better) on the more hydrophilic surfaces. This is in direct contrast to previous observations on the spreading of marine bioadhesives and is in apparent contradiction to the predictions of the Young–Dupre equation for three-phase systems. In this paper, we attempt an explanation based upon thermodynamic analysis of the wetting properties of hydrophilic proteins.

**Keywords: adhesion; wetting theory; biofouling; *Ulva*; environmental scanning electron microscopy; self-assembled monolayers**

## 1. INTRODUCTION

Representatives of all the phyla living in the sea, from bacteria, through lower plants (algae) to invertebrate animals, use sticky materials with permanent or temporary adhesive capabilities at some point in their life histories. Larvae of invertebrates and spores of algae need to find and bind quickly to a surface in order to complete their life history. This adhesion process takes place within minutes, under water, to a wide range of substrates, over a wide range of temperatures, salinities and conditions of turbulence. In certain cases the adhesion is effectively permanent, in other cases adhesion needs to be reversible as the organism moves around on a surface to find the most appropriate settlement site. The interaction between an adhesive material and the substrate involves (i) wetting of the substrate by the adhesive which must be in a liquid state at the beginning of the adhesion process in order to form a bond with the surface and (ii) curing of the adhesive which determines the microstructure of the solid film, thus influencing both the mechanical properties and the cohesive strength. The wetting process determines the actual area of contact between the adhesive and the substrate and has an important role in determining the interaction force between the adhesive and the substrate. Understanding wetting is therefore important to understanding adhesion.

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Green algae of the genus *Ulva* (syn. *Enteromorpha*) are common, green macroalgae found throughout the world in the upper intertidal zone of seashores and as a fouling organism on a variety of man-made structures including ships' hulls (Callow 1996). The genus *Enteromorpha* has recently been incorporated into the genus *Ulva* and the latter name is used forthwith (Hayden *et al.* 2003). Dispersal is achieved mainly through asexual zoospores; quadriflagellate, pear-shaped cells, 5–7 μm in length. Colonization of substrata involves the transition from a free-swimming spore to an adhered non-motile spore (Callow *et al.* 1997), adhesion being achieved via the exocytotic secretion of an adhesive which is present in spores in highly condensed form within vesicles (Evans et al. 1970; Stanley *et al.* 1999). The adhesive is a polydisperse glycoprotein, the molecular mass of the main component being 110 kDa under denaturing conditions (Stanley *et al.* 1999). Under native conditions the protein forms extensive aggregates of molecular mass greater than 1000 kDa (Callow *et al.* 2000*c*). The adhesive is initially liquid and displays a hydrogel-like behaviour on release, swelling rapidly to approximately 300 times its original volume through the absorption of water (Callow *et al.* 2000*b*, 2003). It then starts to undergo cross-linking with a corresponding increase in adhesion strength (Finlay *et al.* 2002*b*).

In previous papers (Callow *et al.* 2000*a*; Finlay *et al.* 2002*a*), we used patterned self-assembled monolayers

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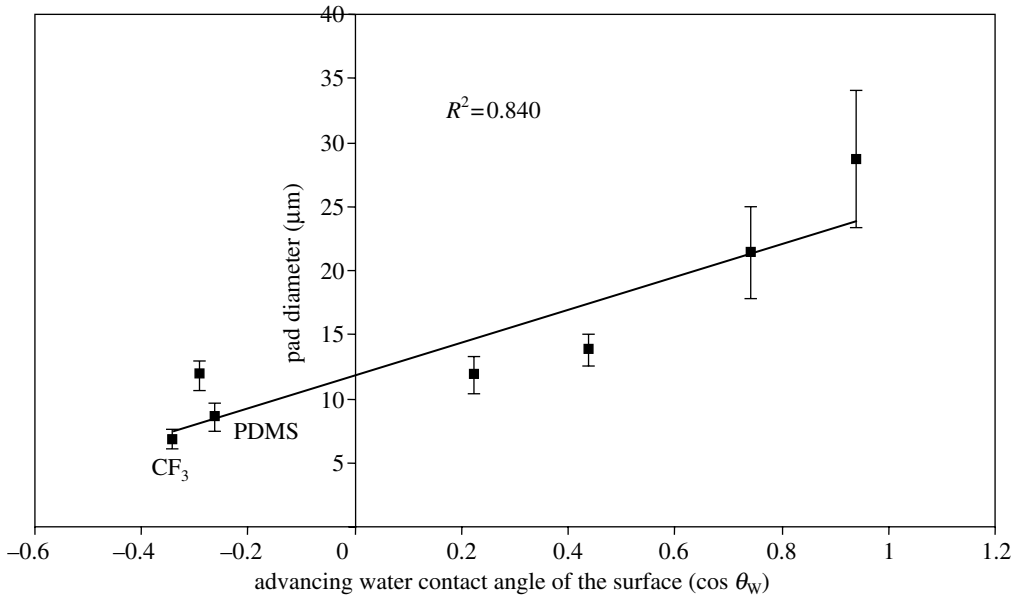


Figure 1. Adhesive pad diameters on surfaces formed from a mixed $OH/CH_3$ alkane thiol SAM series, a self-assembled $CF_3$ monolayer and a PDMS polymer brush, plotted against the cosine of the advancing water contact angle of the surface in air as a measure of wettability. (Note: it is the internal contact angle that the water droplet forms with the surface in air that is measured, i.e. hydrophobic surfaces have a large $\theta_W$ and therefore cos $\theta_W$ is small.)

(SAMs) formed from alkanethiols terminated with methyl ($CH_3$) or hydroxyl (OH) groups, or mixtures of the two, to examine the effect of surface wettability on adhesion properties of settled (adhered) spores of *Ulva*. It was shown that primary adhesion, as measured by the transition from a motile spore to a settled, sessile organism, is strongly promoted by the hydrophobic surfaces. On the other hand, adhesion strength of the settled spores, as measured by resistance to detachment in a turbulent flow cell, was greatest on a hydrophilic surface (Finlay *et al.* 2002*a*). In the present paper, we seek to understand the basis of these effects of surface wettability on adhesion, by examining wetting properties of the adhesive on a range of chemically defined alkanethiol monolayers of different wettability. Monolayers of polydimethyl siloxane (PDMS) and $CF_3$-terminated fluorocarbons were also used as models for low-surface-energy materials with anti-fouling properties.

## 2. MATERIAL AND METHODS

### *2.1. Surfaces*

SAMs of ω-substituted alkane thiolates on gold were prepared and characterized by advancing water contact angle and X-ray photoelectron spectroscopy at the University of New Mexico, as described previously (Callow *et al.* 2000*a*; Finlay *et al.* 2002*a*). The substrate used was gold-coated glass cover-slips (22×50×0.25 mm) prepared as described by Callow *et al.* (2000*a*). To create the $CH_3$/OH SAMs, cover-slips were submerged in 1 mM ethanolic solutions of dodecanethiol (hereafter referred to as $CH_3$-thiol), mercaptoundecanol (OH-thiol) or a mixture of $CH_3$- and OH-thiols. The samples were immersed in thiol solution overnight at 4 °C. The cover-slips were rinsed in ethanol and shipped to the University of Birmingham by courier service in sealed jars filled with ultrapure, degassed water. Thin PDMS brushes and SAMs of fluorocarbon ($CF_3$-terminated) were prepared on glass slides according to the methods published previously (Newby & Chaudhury 1997; She *et al.* 1998). The PDMS chains were grafted to glass slides according to the following method. The glass slides were cleaned in hot piranha solution (($H_2SO_4/H_2O_2$) 7:3, v/v), rinsed in distilled and deionized (DDI) water, and then dried by blowing nitrogen over them. After they were cleaned further in an oxygen plasma for 45 s, they were reacted with $CH=CH(CH_2)_9SiCl_3$ to form the olefin-terminated SAMs using vapour-phase adsorption. These silanized glass slides were then reacted with hydrido functional PDMS ($((CH_3)_2(OSi(CH_3)_2)n(CH_2)_3CH_3$, Mn = 8.5 kg mol$^{-1}$) in the presence of a Pt catalyst. A mixture of 0.5 g of polymer and 0.05 g of platinum catalyst was deposited on to the monolayer-coated silicon wafer surface as thin films. After overnight grafting, the sample was washed with chloroform in a Soxhlet extractor for several hours. The thickness of the PDMS layer on silicon wafer, prepared using an identical method, is estimated to be 80 Å by ellipsometric analysis. The $CF_3$ terminated monolayer was prepared by reacting the pirhana/plasma cleaned glass slides with the vapour of $CF_3(CF_2)_7(CH_2CH_2)SiCl_3$ according to the methods previously described (Newby *et al.* 1997). Advancing water contact angles in air ($\theta_W$) were measured immediately before shipping as described previously (Callow *et al.* 2000*a*). (zNote: by convention these contact angles are measured as the *internal* contact angle and are expressed in figure 1 as their cosines.) On receipt in Birmingham, static water contact angles were measured to check integrity of all surfaces. In all cases, contact angles on receipt were within a few degrees of those measured before shipping.

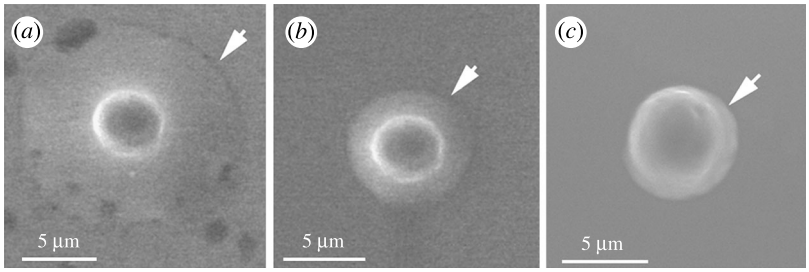


Figure 2. ESEM images of representative settled spores of *Ulva* on: (*a*) OH-terminated alkanethiol SAMs; (*b*) $CH_3$-terminated alkane thiol SAMs; (*c*) $CF_3$-terminated monolayers. The arrows indicate the margins of the adhesive pads.

### *2.2. Zoospore settlement*

Fertile plants of *Ulva linza* were collected from Wembury beach, UK (50° 18′ N; 4° 02′ W). Zoospores were released and prepared for adhesion experiments as described previously (Callow *et al.* 1997). Zoospores were settled on pre-scored cover-slips with the appropriate surface monolayer in individual 2.5 cm polystyrene Petri dishes (the cover-slips were lightly scored with a diamond pen on the reverse, uncoated face to allow for subsequent easy fragmentation at the environmental scanning electron microscope (ESEM) stage). Two millilitres of zoospore suspension (typically $1–2\times10^6$ spores $ml^{-1}$) were added to each dish and incubated in the dark at approximately 20 °C for 30 min. The cover-slips were then gently washed in filter-sterilized artificial sea-water (ASW, Instant Ocean) to remove zoospores that had not properly attached before imaging settled spores in the ESEM.

### *2.3. ESEM*

The spore adhesive pad of *Ulva* is not visible by optical microscopy and therefore observations on settled spores were conducted by ESEM using an FEI XL30 FEG-ESEM with a gaseous secondary electron detector, as described by Callow *et al.* (2003). Fragments of the pre-scored cover-slips (*ca.* 5 $mm^2$) were secured to specimen stubs with aqueous colloidal graphite and mounted on a Peltier cooling stage set at 2 °C. Specimens were examined with a low accelerating voltage of 7.5 kV to minimize beam damage. Since the adhesive pad of *Ulva* is hygroscopic and subject to swelling/contraction, comparison of pad diameters required a consistent protocol and viewing conditions. Specimens were introduced to the microscope chamber at 5.2 Torr (representing 100% relative humidity (RH) at 2 °C). The RH was then reduced to 4.6 Torr at which point the spores surrounded by their adhesive pads became evident. The RH was then raised back to 5.2 Torr which caused a slight swelling of the adhesive pads. Images of radially symmetrical spores plus adhesive were captured and the diameters of the spore body and the adhesive pads were directly measured using the image analysis functions of the ESEM software. For each surface, 10 individual spores were measured.

## 3. RESULTS

Settled spores imaged in the specimen chamber of the ESEM at 5.2 Torr at 2 °C (equivalent to a RH of 100%) showed characteristic profiles, as previously reported (Callow *et al.* 2003), of spherical spore bodies, approximately 4–5 µm in diameter, surrounded by an annulus or pad of secreted adhesive material. The size of the pad (taken as the diameter of the whole structure on the assumption that there is adhesive beneath the spore body) was strongly influenced by the surface properties of the alkanethiol SAMs. On the 100% OH-alkanethiol monolayers, the adhesive had spread over the surface to form pads of diameter $28.7\pm2.7$ µm (mean ± s.e.; figure 2*a*), whereas on the hydrophobic 100% $CH_3$-alkanethiol, the pads were more discrete at $11.6\pm0.6$ µm (figure 2*b*). Adhesive pad diameters on grafted monolayers of PDMS and fluorocarbon ($CF_3$) were also small, the pads on the highly hydrophobic fluorinated monolayers being hardly larger than the diameter of the spore body itself (figure 2*c*). A plot of pad diameter against the cosine of the advancing *internal* water contact angle ($\cos\theta_W$) for all the test surfaces gave a straight line relationship with an $R^2$ of 0.840 (figure 1).

## 4. DISCUSSION

The range of marine surfaces available for colonization by spores of *Ulva* is vast, and most seem to be utilised by this opportunistic genus. The different characteristics of the surfaces will affect the physical and chemical interactions with the adhesive that will probably be reflected in bonding strength. By examining the adhesion processes to surfaces of known chemical and physical properties, greater insight into the mechanics of adhesion can be gained. The surface property most frequently correlated with adhesion is surface-free energy, a measure of the capacity of a surface to interact spontaneously with other materials by forming new bonds, often expressed as the related parameter, surface tension ($\gamma_c$), a measure of surface wettability (Baier 1973; Becka & Loeb 1984). The effect of surface energy on adhesion has often been studied by employing widely different materials, ranging from urethanes and epoxies (high energy), to silicones and fluorinated materials (low energy). Young & Crisp (1981), in their studies on surface wetting by mussel byssus plaques, used even more diverse materials including slate, glass, paraffin wax and polytetrafluoroethylene (PTFE). Such diverse materials differ in more than just surface energy, with widely different physico-chemical properties including polarity, roughness and modulus, and more recently, those interested in fundamental adsorption and adhesion phenomena

have used model surfaces formed from SAMs on gold-coated glass or some other common substrate (Sigal *et al.* 1998; Callow *et al.* 2000*a*; Ostuni *et al.* 2001; Ista *et al.* 2004). SAM surfaces are chemically defined and uniform with respect to surface topography and modulus, and in previous studies (Callow *et al.* 2000*a*; Finlay *et al.* 2002*a*) we used SAMs formed from alkanethiols terminated with methyl ($CH_3$) or hydroxyl (OH) groups, or mixtures of the two, to examine the effect of surface wettability on adhesion properties of adhered spores of *Ulva*, independent of any effects caused by surface charge since the SAM surfaces would be uncharged at the pH of sea-water (pH 8.1). It was shown that adhesion strength of the settled spores, as measured by resistance to detachment in a turbulent flow cell, was greatest on a hydrophilic surface. For an adhesive to bind to other surfaces, it has to 'wet' that surface, and whether it does so will depend on the competition between the adhesive and water interacting with the surface. The interaction is complex, involving the displacement of water from the adhesive and the surface, formation of contact between the adhesive and surface, as well as the formation of the cohesive contacts of the water molecules that were displaced from the adhesive and the surface. The main aim of the present paper was to examine whether the effect of substratum surface energy in controlling adhesion was correlated with differential wetting of the surface by the adhesive.

In a study of the byssus pad of the blue mussel, Young & Crisp (1981) and Crisp *et al.* (1985) showed that the surface area of contact of the byssus pad was greatest on the low energy surfaces, which were also non-polar (PTFE, paraffin wax) and lowest on the high energy surfaces, which were also polar (slate and glass). These authors then used conventional wetting theory and the Young–Dupre equation describing the consequences of competing interfacial tensions on the contact angle of a fluid adhesive, to explain how an initially liquid adhesive would have to compete with sea-water in wetting or spreading over the solid surface. It was suggested that this would be more readily achieved on a hydrophobic surface than on a hydrophilic surface. In the case of marine adhesion processes under water, the wetting system consists of a solid (S), a liquid (sea-water, L), and the adhesive (A) that must be released from the organism initially as a fluid in order to spread on the surface. According to the Dupré equation (4.1), wetting systems are characterized by the contact angle that the liquid makes with a solid surface in the presence of a fluid. Thus, in a solid–liquid–fluid adhesive system, by convention it is $\theta_{LA}$, the angle between the liquid (sea-water) and the solid (figure 3):

$$\cos\theta_{LA} = (\gamma_{SA} - \gamma_{SL})/\gamma_{LA}, \tag{4.1}$$

where $\gamma_{SA}$, $\gamma_{SL}$, $\gamma_{LA}$ are the interfacial tensions of the solid–adhesive, solid–liquid and liquid–adhesive interfaces, respectively. Young & Crisp (1981) further developed this equation to the equivalent of

$$W_{SL} - W_{SA} = \gamma_L - \gamma_A + \gamma_{LA}\cos\theta_{LA}. \tag{4.2}$$

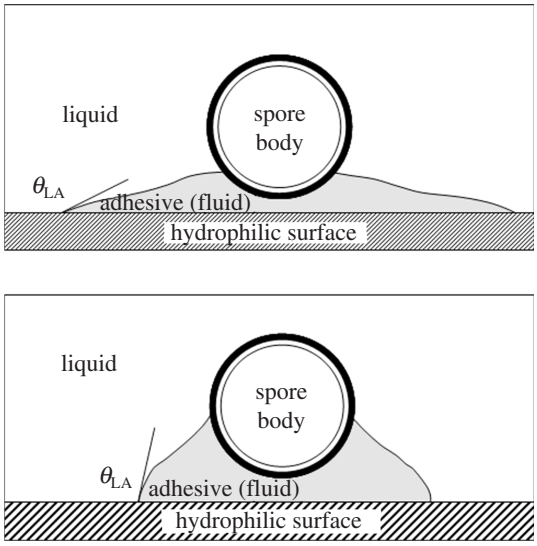


Figure 3. Interpretation of the consequences of different degrees of adhesive (fluid) spreading on surfaces of different wettability, for the contact angle between the solid and liquid. The diagram shows the same volume of fluid adhesive (released by the attached spore) spreading to different degrees on different solid surfaces. (Note: in order to be consistent with the convention used by Young & Crisp (1981), we take the contact angle ($\theta_{LA}$) at the adhesive, solid and liquid contact line to be the external contact angle, i.e. measured from the water side.)

They explained that since $\gamma_L$, $\gamma_A$ and $\gamma_{LA}$ (the surface tensions of the liquid, the adhesive and the interfacial tension between the liquid and the adhesive, respectively) will be the same for a variety of solid surfaces, $\theta_{LA}$ is effectively controlled by $W_{SL} - W_{SA}$ (the work of adhesion of the solid to sea-water less the energy of adhesion of the solid to the adhesive). If $W_{SL} - W_{SA}$ is large, which will be the case for a high energy surface readily wet by water, $\cos\theta_{LA}$ will be large and $\theta_{LA}$ will be small, i.e. the contact area of the adhesive with the surface will be small. Conversely, a low energy surface which is not easily wet, would give a large $\theta_{LA}$, with a larger adhesive spreading. This indeed was the behaviour observed by Clint & Wicks (2001) for a variety of inert probe liquids.

The results presented in the present paper show that the diameter of the adhesive pads produced by *Ulva* spores is strongly influenced by the energy of the surface, but in this case, the adhesive apparently spreads more on a high energy, hydrophilic surface than on a hydrophobic, low-energy surface. It is important to emphasize that within the short timescale of the observations, what is being witnessed in making these measurements, is the fate of a finite amount of pre-formed adhesive that is laid down in vesicles within spores before they are released from the parent plant (Evans & Christie 1970). These vesicles and their contents are then completely discharged from the spore during the primary adhesion process.

So how can we reconcile the data on *Ulva*, with the apparently different results reported for blue mussel by Young & Crisp (1981) and Crisp *et al.* (1985)? The simplest interpretation of the observations on adhesive spreading in *Ulva* is that a finite amount of

adhesive physically spreads on the different surfaces to different extents. If this interpretation is correct then differences in spreading, i.e. wetting, should be associated with differences in contact angle with the liquid–solid contact angle being greater on the hydrophilic surface than the hydrophobic surface (figure 3). Unfortunately, the transparency of the spore adhesive pads by optical microscopy precludes any simple approach to the measurement of actual contact angles that the adhesive pads form with the surface. While ESEM has been applied to the measurement of contact angles of water droplets on model substrates (Stelmashenko *et al.* 2001), its application to a labile, less well-controlled and characterized biological system presented by the adhesive pads of *Ulva* would be both technically and theoretically daunting, and well beyond the scope of the present investigation. However, thermodynamic equations can be derived that might explain the data for *Ulva*, or at least, be used to predict what conditions must prevail in order that the results be harmonized with the considerations of thermodynamics.

Assuming that the adhesive released by the spore is a continuous medium, the thermodynamic work of adhesion between the protein (A) and the surface (S) through water (L) can be expressed according to the well-known equation of Hamaker (see the reviews Chaudhury (1996) and Van Oss *et al.* (1988)) as follows:

$$W_{\mathrm{ALS}} = W_{\mathrm{AS}} + W_{\mathrm{LL}} - W_{\mathrm{AL}} - W_{\mathrm{SL}}. \tag{4.3}$$

where $W_{ij}$ ($i$, $j \in$ [A, S, L]) is the work of adhesion between phases $i$ and $j$ through vacuum.

$W_{\mathrm{SL}}$ can be obtained from the contact angle ($\theta_{\mathrm{W}}$) of water on the substrate (measured on the 'water side'), according to the standard convention using the Young–Dupre equation as follows:

$$W_{\mathrm{SL}} = \gamma_{\mathrm{L}}(1 + \cos\theta_{\mathrm{W}}). \tag{4.4}$$

By combining equations (4.3) and (4.4), and using the definition of surface tension that $\gamma_{\mathrm{L}} = W_{\mathrm{LL}}/2$, we have:

$$W_{\mathrm{ALS}} = W_{\mathrm{AS}} - W_{\mathrm{AL}} + \gamma_{\mathrm{L}}(1 - \cos\theta_{\mathrm{W}}). \tag{4.5}$$

Further decomposition of the terms $W_{\mathrm{AS}}$ and $W_{\mathrm{AL}}$ into dispersion and polar (H-bonding interactions) and using the geometric mean combining rules for the dispersion interaction (Chaudhury 1996; Van Oss *et al.* 1988), one obtains:

$$\begin{aligned} W_{\mathrm{ALS}} = {} & 2(\gamma_{\mathrm{A}}^{\mathrm{d}})^{1/2}((\gamma_{\mathrm{S}}^{\mathrm{d}})^{1/2} - (\gamma_{\mathrm{L}}^{\mathrm{d}})^{1/2}) \\ & + (W_{\mathrm{AS}}^{\mathrm{p}} - W_{\mathrm{AL}}^{\mathrm{p}}) + \gamma_{\mathrm{L}}(1 - \cos\theta_{\mathrm{W}}). \end{aligned} \tag{4.6}$$

Equation (4.6) is the generalized equation of interaction in three condensed phases. According to the Young–Dupre equation, the contact angle of the adhesive (measured from the water side) on the substrate under water can be expressed as:

$$\gamma_{\mathrm{LA}}(1 - \cos\theta_{\mathrm{LA}}) = W_{\mathrm{ALS}}. \tag{4.7}$$

Equation (4.6) together with equation (4.7) is essentially the same as that used by Young & Crisp to interpret the spreading of adhesive on surfaces under water, except that equation (4.6) expresses the interaction in terms of surface tension components. For adhesive with a given volume, as $W_{\mathrm{ALS}}$ increases, $\theta_{\mathrm{LA}}$ increases, i.e. the adhesive spreads more. Now we consider the following limits: let us consider that the substrate is composed of methyl groups, which has very similar dispersive interaction as water. In this case, $\gamma_{\mathrm{S}}^{\mathrm{d}} \sim \gamma_{\mathrm{L}}^{\mathrm{d}}$ and $W_{\mathrm{AS}}^{\mathrm{p}} \sim 0$. We thus have from equation (4.6):

$$W_{\mathrm{ALS}}(\mathrm{CH_3}) \approx -W_{\mathrm{AL}}^{\mathrm{p}} + \gamma_{\mathrm{L}}(1 - \cos\theta_{\mathrm{W}}^{\mathrm{CH_3}}). \tag{4.8}$$

On such a surface, as $\theta_{\mathrm{W}} \sim 110°$, the term $\gamma_{\mathrm{L}}(1 - \cos\theta_{\mathrm{W}}^{\mathrm{CH_3}})$ is about 97 mJ m$^{-2}$. However, this positive work of adhesion has to be greater than the energy needed to dehydrate the protein $W_{\mathrm{AL}}^{\mathrm{p}}$. It is plausible that for very hydrophilic protein, $W_{\mathrm{AL}}^{\mathrm{p}}$ is larger than 97 mJ m$^{-2}$, in which case the adhesive would not spread on the hydrophobic surface. The situation on the hydrophilic surface, as is the case with alcohol functional surfaces is somewhat different. Here, as before, we may set $\gamma_{\mathrm{S}}^{\mathrm{d}} \sim \gamma_{\mathrm{L}}^{\mathrm{d}}$, but $W_{\mathrm{AS}}^{\mathrm{p}} \neq 0$. We thus have from equation (4.6):

$$W_{\mathrm{ALS}}(\mathrm{OH}) \approx (W_{\mathrm{AS}}^{\mathrm{p}} - W_{\mathrm{AL}}^{\mathrm{p}}) + \gamma_{\mathrm{L}}(1 - \cos\theta_{\mathrm{W}}^{\mathrm{OH}}). \tag{4.9}$$

On the hydroxyl surface, as $\theta \sim 20°$, the term $\gamma_{\mathrm{L}}(1 - \cos\theta_{\mathrm{W}}^{\mathrm{CH}})$ is approximately 4 mJ m$^{-2}$. Now, if the large negative $W_{\mathrm{AL}}^{\mathrm{p}}$ is somewhat compensated by the positive value of $W_{\mathrm{AL}}^{\mathrm{p}}$, i.e. the adhesive engages in stronger hydrogen bonding interaction with the substrate than it does with water, a positive value of $W_{\mathrm{ASL}}$ may well result. Now, in the absence of many parameters required to estimate the work of adhesion using equations (4.7) and (4.8), we may examine, as an extreme example, if it is plausible for the term $W_{\mathrm{ALS}}(\mathrm{OH})$ to be larger than $W_{\mathrm{ALS}}(\mathrm{CH_3})$. This can be accomplished by subtracting equation (4.8) from (4.9) to obtain:

$$W_{\mathrm{ALS}}(\mathrm{OH}) - W_{\mathrm{ASL}}(\mathrm{CH_3}) = W_{\mathrm{AS}}^{\mathrm{p}} - 97\,(\mathrm{mJ\,m^{-2}}). \tag{4.10}$$

For $W_{\mathrm{ALS}}(\mathrm{OH})$ to be larger than $W_{\mathrm{ASL}}(\mathrm{CH_3})$, $W_{\mathrm{AS}}^{\mathrm{p}}$ needs to be larger than 97 mJ m$^{-2}$. For a highly polar glycoprotein adhesive it is not implausible for the above condition to be satisfied resulting in $W_{\mathrm{ALS}}(\mathrm{OH}) > W_{\mathrm{ALS}}(\mathrm{CH_3})$. Although a detailed molecular characterization of the *Ulva* spore adhesive is still lacking, we know that it is extremely hydrophilic in character (Callow et al. 2003). Thus, the current observation that the adhesive spreads to a great degree on the hydroxyl containing surfaces than on the methyl functional surfaces could be quite consistent with the Young–Dupre equation provided that we satisfy the condition that $W_{\mathrm{AS}}^{\mathrm{p}}$ is at least larger than 97 mJ m$^{-2}$. In a general situation, in order to determine whether $W_{\mathrm{ALS}}$ is positive and an increasing function of the surface energy of the solid, we need to decompose the above equations further in terms of molecular interactions. This decomposition is very complex and can easily lead to serious error if appropriate molecular interaction models are not properly identified. However, as the polarity of the surface increases, both $W_{\mathrm{AS}}$ and $W_{\mathrm{SL}}$ would increase; but based on the previous discussions, $W_{\mathrm{AS}}$ must be greater than $W_{\mathrm{SL}}$. If, for the sake of argument, we assume that $W_{\mathrm{AS}}$ and $W_{\mathrm{SL}}$ form a constant ratio $\beta$ ($\beta > 1$), we can rewrite equations (4.3)

and (4.4) as follows:

$$W_{\mathrm{ALS}} = (\beta - 1)\gamma_{\mathrm{L}}(1 + \cos\theta_{\mathrm{W}}) + (W_{\mathrm{LL}} - W_{\mathrm{AL}}). \quad (4.11)$$

Since $W_{\mathrm{LL}} - W_{\mathrm{AL}}$ is a constant, we can state that $W_{\mathrm{ALS}}$ should increase overall with $\cos\theta_{\mathrm{W}}$. This, in conjunction with equation (4.7), leads us to conclude that $\theta_{\mathrm{LA}}$, and therefore adhesive pad diameter, increases with $\cos\theta_{\mathrm{W}}$, as was observed experimentally.

In reality, the constraints on the adsorption and adhesion processes on both the hydrophilic and hydrophobic surfaces can be relaxed if we recognize the fact that almost all proteins are amphoteric surfactants, i.e. the protein has both hydrophobic and hydrophilic functionalities, as well as acidic and basic amino acids. When such a protein comes into contact with a surface, it can undergo a conformational change, thereby exposing the functionalities that would optimize adsorption/adhesion with both the substrate and the adhesive either by hydrophobic or electrostatic interactions. The role played by the electrostatic interactions is not surprising for inorganic substrates, which have ionizable functional groups. It is not obvious that electrostatic interactions could prevail for non-ionizable substrates used in the current studies. However, several recent studies (Marinova *et al.* 1996; Kreuzer *et al.* 2003) have demonstrated that non-ionizable hydrophobic surfaces could acquire electric charge in water owing to the preferential adsorption of hydroxyl ions. In high salt concentration, as in sea-water, the resultant electrostatic interaction is expected to be screened beyond the double layer screening length. Nevertheless, as the potential drop occurs over a very short distance (i.e. the Debye length), the electric field gradient should be substantial at the substrate/sea-water interface, which could play an additional role (i.e. adding a positive component to $W_{\mathrm{AS}}^{\mathrm{p}}$) in the local restructuring and the adhesion of proteins on surfaces.

Finally, the spreading of the adhesive and the ensuing crosslinking reactions may not be entirely governed by the forces at equilibrium. Enhancement of spreading by other forces, e.g. by the Marangoni effect, may take effect if the *Ulva* spore adhesive has components of various surface tensions. In this case, selective adsorption of one of the components near the contact line region may create a surface tension gradient thus inducing a Marangoni flow. This effect is well known in the context of surfactant-enhanced spreading of water drops on surfaces, which is otherwise known as superwetting (Stoeb *et al.* 1996).

Irrespective of the mechanism involved, the consequence would appear to be that settled spores have a higher surface area of contact with a hydrophilic substratum, thus a lower contact angle than a hydrophobic substratum, and this has implications for the analysis of detachment mechanisms by increasing the area of contact, as well as suppressing the crack-tip singularity. These are the subjects of future investigations.

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