The performance of aminoalkyl/fluorocarbon/hydrocarbon-modified xerogel coatings against the marine alga Ectocarpus crouaniorum: relative roles of surface energy and charge

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The effect of a series of xerogel coatings modified with aminoalkyl/fluorocarbon/hydrocarbon groups on the adhesion of a new test species, the filamentous brown alga Ectocarpus crouaniorum, has been explored, and compared with the green alga Ulva linza. The results showed that E. crouaniorum adhered weakly to the less polar, low wettability coatings in the series, but stronger adhesion was shown on polar, higher surface energy coatings containing aminoalkyl groups. The results from a separate series of coatings tuned to have similar surface energies and polarities after immersion in artificial seawater (ASW), but widely different surface charges, demonstrated that surface charge was more important than surface energy and polarity in determining the adhesion strength of both E. crouaniorum and U. linza on xerogel coatings. No correlation was found between adhesion and contact angle hysteresis. X-ray photoelectron spectroscopy analysis of samples after immersion in ASW confirmed the presence of charged ammonium groups on the surface of the aminoalkylated coatings.

Keywords: marine biofouling; xerogels; brown alga; Ectocarpus crouaniorum; Ulva linza; fouling-release; surface energy; charge

Introduction

Marine surfaces are rapidly colonised by an accumulation of diverse marine organisms such as bacteria, algae and barnacles. Marine biofouling, which has been an economic problem for centuries, leads to an increase in the drag on ships’ hulls with environmental and economic penalties (Schultz 2007; Schultz et al. 2011). Over the past two to three decades, antifouling (AF) research has focused on the development of new environmentally benign technologies, in particular ‘fouling-release coatings’ (FR) that facilitate the removal of biofouling by water flow or cleaning (Finnie & Williams 2010; Tribou & Swain 2010).

Studies on FR coatings have focused on silicone elastomers where the combination of low modulus and low-surface energy favour the release of adhered fouling organisms. The high relative cost of silicones, their susceptibility to mechanical damage and their propensity to foul with certain classes of fouling organism, notably diatoms, has led to extensive research to improve silicones, or to find alternatives (Callow & Callow 2011). Organosilica-based xerogels prepared by the sol-gel process have been considered as candidates for certain applications since they are inexpensive and physically robust. Xerogel surfaces have uniform surface roughness and can be tuned to provide surfaces with a wide range of wettability, total surface energy and charge (Tang et al. 2005; Bennett et al. 2010; Finlay et al. 2010). A particular feature of these coatings is that they can vary not only in total surface energy, but also in the relative proportion of polar and dispersive components, allowing a more detailed analysis of the role of surface energy in bioadhesion. Xerogel coatings have been shown to have both AF and FR properties in laboratory assays against several fouling organisms including barnacles (Balanus amphitrite), the green macroalga Ulva linza and the diatom Navicula perminuta (Tang et al. 2005; McMasters et al. 2009; Bennett et al. 2010; Finlay et al. 2010, Sokolova et al. 2012). The efficacy of these coatings in field tests has been demonstrated using Aquafast®, an organically modified, hybrid xerogel (Selvaggio et al. 2009; Gunari et al. 2011).

In the case of the laboratory studies with U. linza and N. perminuta, xerogel films were prepared from aminopropylsilyl-, fluorocarbonsilyl-, and hydrocarbonsilyl-containing precursors. The xerogel coatings gave reduced settlement of zoospores of U. linza compared to a poly(dimethylsiloxane) elastomer (PDMSE) standard and reduced settlement correlated with increased water wettability and increased polar contribution to the surface free energy (Bennett et al. 2010). The strength of attachment of sporelings (young plants) of U. linza on...
several of the xerogels was similar to that on PDMSE although no overall correlation was observed with either wettability or the percentage polar contribution across the whole series of coatings. However, there was a correlation between wettability/total surface energy on the xerogels modified with fluorocarbon/hydrocarbon groups. The aminooalkylated xerogels did not follow this trend, sporelings adhering more strongly to the aminopropylated xerogel than to either the methyaminopropylated or dimethylaminopropylated materials, despite these three coatings having similar surface energies. However, somewhat different results were obtained with the diatom *N. perminuta* on the same xerogel coatings (Finlay et al. 2010). In this case, the adhesion strength of attached cells was correlated with wettability/surface energy, adhesion being weakest on the more hydrophilic, more polar materials, including the mono-, di-, and trimethylaminopropyl-substituted materials.

The algae are a diverse, polyphyletic assemblage of eukaryotic organisms. Of the algae previously explored in experimental studies of xerogels, the green macroalga *U. linza* is a member of the phylum Chlorophyta whereas the unicellular *N. perminuta* is a member of the Bacillariophyta (diatoms) in the phylum Heterokonta (Cavalier-Smith 2004). Within the Heterokonta, there is further evolutionary diversity and a range of life forms, from microscopic, single-celled organisms like diatoms, to complex multicellular brown macroalgae (Phaeophyta). In view of the different results with *U. linza* and *N. perminuta*, it was of interest to test similar xerogel materials against another fouling alga. The ectocarpoid algae are macroscopic, filamentous brown algae (Phaeophyta: Heterokonta) and species of *Ectocarpus* have been known to colonise ships’ hulls for many years (Fletcher 1980; Mineur et al. 2007). Recently, a new adhesion bioassay, based on the colonisation of surfaces by multicellular filaments of ectocarpoid algae, has been developed in order to widen the range of fouling algae that are used in the testing of novel, experimental AF and FR materials (Evariste et al. 2012). In the present study, the initial attachment and adhesion strength of *E. crouaniorum* were tested on two series of xerogel coatings with a range of surface properties. An initial set of coatings (SET 1) had a wide range of surface wettabilities and had been previously tested against *U. linza* (Bennett et al. 2010) and diatoms (Finlay et al. 2010). In this paper, additional information is presented on the surface properties of these coatings after immersion in seawater. The initial results with *E. crouaniorum* indicated that surface charge might be an important factor in controlling adhesion rather than wettability. To test this hypothesis, a second series of coatings (SET 2) was evaluated with a smaller range of surface wettabilities after immersion, but different surface charge properties.

**Materials and methods**

**Composition of xerogel coatings**

Two sets of xerogel coatings were prepared by the general method described in Bennett et al. (2010). For the purposes of this paper, only brief compositional details are provided, but surface (contact angle analysis, surface energies and XPS data) and bulk properties (surface roughness and modulus) are described in detail.

The coatings were prepared by mixing two cross-linkers, tetraethylorthosilicate (TEOS) and alkyltrialkoxyxilane with different modified silanes: 3-aminopropyltriethoxysilane (AP), 3-methyaminopropyltrimethoxysilane (MAP), 3-dimethylaminopropyltrimethoxysilane (DMAP), 3,3,3-trifluoropropyltrimethoxysilane (TFP), phenyltriethoxysilane (PH), *n*-octadecyltrimethoxysilane (C18), *n*-octyliothriethoxysilane (C8) and tridecafluorooctyltriethoxysilane (TDF). The final sol/xerogel composition was designed in terms of the molar ratio of Si-containing precursors. Thus, a 1:1 PH/TEOS composition contains 50 mol% PH and 50 mol% TEOS. Ethanol and water were used as solvents and hydrochloric acid as the catalyst.

Xerogel films were formed as described by Bennett et al. (2010) by spin-coating 400 µl of the sol precursor onto precleaned 25 mm × 75 mm glass microscope slides. Slides were cleaned by soaking in ‘piranha solution’ for 24 h, rinsed with copious quantities of deionised (DI) water, soaked in isopropanol for 10 min, air dried and stored at ambient temperature until use. A model P6700 spin coater was used at 100 rpm for 10 s to deliver the sol and at 3000 rpm for 30 s to coat. All coated surfaces were dried at ambient temperature for at least 1 day and up to 30 days prior to analysis of the surface properties. Properties are independent of storage time in this interval.

The SET 1 coatings consisted of 1:9 DMAP/TEOS, 1:9 MAP/TEOS, 1:1 PH/TEOS, 1:1 TFP/TEOS 1:1 C8/TEOS, 5:45:50 C18/C8/TEOS and 1:1 TDF/TEOS xerogel surfaces where the numbers refer to the molar ratios of the trialkoxyxilane precursors. The SET 2 coatings consisted of 1:9 AP/TEOS, 1:9 MAP/TEOS, 1:9 DMAP/TEOS, 1:4 TFP/TEOS and 1:4 PH/TEOS xerogel coatings. While there is some overlap of formulations in SET 1 and SET 2, all formulations were characterised individually.

**Characteristics of xerogel coatings: contact angles and surface energies pre- and post-immersion**

Xerogel films were stored in air prior to characterisation. Following determination of static contact angles in air, the coatings were immersed in artificial seawater (ASW) (Reef Crystals®, pH 7.8, at a specific gravity of 1.025) for 24 h followed by 1 h in DI water to remove salts. The coatings were then dried in air under ambient conditions for 2–3 h, before contact angles were remeasured. Static water contact angles (θ_WS) and static diiodometh-
ane contact angles $[\theta(CH_3)]$ were measured on a 15-μl drop of fluid on the xerogel surface with a contact angle goniometer [Rame-Hart, Model NRL 100]; both sides of the droplet profile being measured. Values of $\theta_{WS}$ after air drying were stable, taking 2–3 weeks to return to pre-immersion values. Prolonged immersion in ASW beyond the standard 24 h (up to 5 days) also did not change values of $\theta_{WS}$.

Contact angles measured with water and diiodomethane were treated as described by Owens and Wendt (1969) to give total surface energy ($\gamma_S$) and its dispersive and polar components (Baier & Meyer 1992). Advanced and receded water contact angles were also measured by the sessile drop technique. A 15 μl drop of water was placed on the sample surface and the angle of contact ($\theta$) between the droplet and the surface was measured as described above. Another droplet of water was placed on top of the first droplet (ie the fluid is advanced across the surface), and the measurements were repeated. Receded contact angles were measured by removal of fluid from the ‘advanced’ droplet. The values of advanced ($\theta_{WS}$) and receded water contact angles and the resulting contact angle hysteresis are contained in Supplementary Tables S1 and S2 (Supplementary material is available via a multimedia link on the online article webpage.).

**Characteristics of xerogel coatings: X-ray photoelectron spectroscopy pre- and post-immersion**

Since contact angle analysis showed some differences in surface properties for the SET 2 coatings before and after immersion in ASW, these coatings were also examined by X-ray photoelectron spectroscopy (XPS), pre- and post-immersion in ASW, using techniques previously described (Tang et al. 2005; McMaster et al. 2009). Each coated slide was cut into duplicate samples using a glass cutter, then samples were either stored in air or immersed in ASW for 24 h. Immersed samples were rinsed with DI water to remove salts and air dried for 8 h to allow bulk water to evaporate prior to introduction to the high vacuum chamber of the instrument.

While the air drying step in this pretreatment may reverse changes to the coatings caused by the immersion, dry coatings are required for the high vacuum conditions of XPS. The surface composition of these ‘dry but previously immersed’ films was expected to correspond to a kinetically trapped condition rather than the thermodynamic equilibrium state when in actual contact with water since the ‘recovery’ from immersion appears to be quite slow for these coatings. A similar observation was made by Martinelli et al. (2008) for block copolymer-based coatings.

After air drying, samples were held in the introduction chamber, under vacuum, for 15–16 h before the spectra were taken. The XPS spectra were recorded using Physical Electronics Laboratories (PHI) Model 5000 VersaProbe equipped with an Al source, a hemispherical analyser and a sixteen channel detector. A monochromatic Al Ka$^2$ source (1486.6 eV) was operated at 100 μm 25 W 15 kV. The operating pressure in the main chamber did not exceed $5.0 \times 10^{-6}$ Pa. A pass energy of 117.4 eV was used to obtain the survey scan spectra and 25.30 eV was used for high-resolution, multiregion scans. Spectra were obtained at 45° take-off angles. Data manipulation was performed using PHI MultiPak™ Software version 8.

On pristine glass slides stored in air, the background levels of the C(1s), N(1s), and F(1s) signals were determined as described in Tang et al. (2005) and McMaster et al. (2009). Background ratios of C(1s)/Si(2p3) were 0.2 and background levels of N(1s)/Si(2p3) and F(1s)/Si (2p3) were <0.01. The values for these ratios for the xerogel of this study were significantly larger than the background ratios ($p < 0.05$, pairwise comparisons, Student’s t-test) for samples stored in air and samples immersed in ASW.

**Characterisation of SET 1 and SET 2 xerogel surfaces by profilometry and AFM**

Film thicknesses were determined at SUNY Buffalo using a Tencor Instruments, alpha-step 500 profilometer with a diamond-point stylus. Measurements on each surface were performed in triplicate and indicated that the xerogel films were 1.0 ± 0.1 μm thick.

Values for the $R_{rms}$ roughness and Young’s modulus of 1:9 MAP/TEOS, 5:45:50 C18/C8/TEOS, 1:1 TDF/TEOS, 1:4 TFP/TEOS and 1:4 PH/TEOS xerogels were determined by AFM using techniques described in Bennett et al. (2010) and Sokolova et al. (2012) after immersion in ASW for 24 h, followed by 1 h in DI water, followed by drying in air for 2–3 h. These conditions captured the films in a ‘kinetic state’ of surface reorganisation (ie reorganisation back to the ‘dry’ state is slow) rather than in the ‘thermodynamic state’ of being in contact with ASW (Martinelli et al. 2008). The values of the root-mean-square roughness ($R_{rms}$) were calculated on six 5-μm × 5-μm images (512 × 512 arrays) for each sample, where $R_{rms}$ is defined as the root-mean-square average of the topographic deviations ($t$) as shown in Equation (1):

$$R_{rms} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (t_i - \bar{t})^2} \quad (1)$$

Previously determined values for surface roughness and modulus of 1:9 AP/TEOS, 1:9 DMAP/TEOS, 1:1 PH/TEOS, 1:1 TFP/TEOS and 1:1 C8/TEOS (Bennett et al. 2010) are also recorded in Tables 1 and 2 for completeness. It should be noted that values of $R_{rms}$ and
modulus reported in Bennett et al. (2010) were measured while immersed in ASW, while values reported herein were measured on surfaces that were air dried for 2–3 h following immersion in ASW for 24 h before AFM measurement. However, as pointed out above the surface properties of these coatings appeared to be relatively stable after drying since values of $h_{WS}$ took 2–3 weeks to return to pre-immersion values.

### Cultivation of E. crouaniorum

*E. crouaniorum* CCAP 1310/300, a sporophytic isolate obtained from the Culture Collection for Algae and Protozoa, Dunstaffnage, Oban, Scotland was grown as non-axenic, unialgal cultures in 650 ml flasks (Cellstar filter cap suspension culture flask, Greiner Bio-One) containing 200 ml of half-strength enriched Provasoli medium pH 8.1 (Starr & Zeikus 1987) prepared using autoclaved artificial seawater (ASW, 33 ppt Tropic Marin® pH 8.1, specific gravity 1.026). Cultures were grown statically, without aeration at 15 °C with a 12:12 h light:dark cycle (light intensity 20 μE m$^{-2}$ s$^{-1}$) and subcultured every 2–3 months.

### Preparation of algal inoculum

Algal inoculum was prepared following the method of Evariste et al. (2012). In brief, approximately 2 g (wt) of cultured alga were blended with a hand-held kitchen homogeniser for 1 min in 100 ml of ½-strength Provasoli medium. The blended alga was filtered through a nylon cell strainer (mesh size 100 μm, BD Falcon). Large fragments were retained on the filter, which was discarded. The filtrate, consisting of small filaments averaging 7.3 ± 1.3 cells (mean of 50 observations ±2 × SE), was used as the inoculum. The biomass

Table 1. Properties of xerogel coatings (SET 1).

| Composition/name | 1:9 DMAP/TEOS | 1:9 MAP/TEOS | 1:1 PH/TEOS | 1:1 TFP/TEOS | 1:1 CS/TEOS | 5:45:50 C18/TEOS | 1:1 TDF/TEOS |
|------------------|---------------|--------------|-------------|--------------|-------------|-----------------|-------------|
| Stored in air    |               |              |             |              |             |                 |             |
| Static water contact angle ($\theta_{WS}$) | 50 ± 3 | 49 ± 1 | 80.4 ± 0.6 | 83 ± 1 | 96 ± 6 | 107.9 ± 0.7 | 105 ± 3 |
| Total surface energy $\gamma_S$ (mN m$^{-1}$) | 53 ± 2 | 53.5 ± 0.8 | 38.2 ± 0.3 | 25.7 ± 0.7 | 25 ± 1 | 22.0 ± 0.4 | 12.4 ± 0.8 |
| Immersed 24 h in ASW |               |              |             |              |             |                 |             |
| Static water contact angle ($\theta_{WS}$) | 31 ± 3 | 32.6 ± 0.8 | 54 ± 2 | 64.2 ± 0.2 | 80 ± 5 | 91 ± 3 | 83 ± 2 |
| Total surface energy $\gamma_S$ (mN m$^{-1}$) | 64 ± 2 | 63.6 ± 0.3 | 53 ± 1 | 39.3 ± 0.3 | 35 ± 2 | 27 ± 1 | 27 ± 2 |
| Dispersive surface energy $\gamma_D$ (mN m$^{-1}$) | 29.7 ± 0.1 | 30.5 ± 0.1 | 30.5 ± 0.1 | 19.5 ± 0.1 | 24.1 ± 0.0 | 19.5 ± 0.1 | 16.1 ± 0.5 |
| Polar surface energy $\gamma_P$ (mN m$^{-1}$) | 34.1 ± 0.2 | 33.1 ± 0.1 | 22.0 ± 0.2 | 19.8 ± 0.1 | 11.1 ± 0.5 | 7.5 ± 0.2 | 10.9 ± 0.4 |
| Percentage of polar surface energy | 53.4 ± 0.2 | 52 ± 1 | 41.8 ± 0.2 | 50.5 ± 0.1 | 32 ± 1 | 27.6 ± 0.2 | 40.5 ± 0.4 |
| Surface roughness $R_{rms}$ (nm) | 0.6 ± 0.1 $^{a}$ | 0.5 ± 0.1 | 0.39 ± 0.0$^{a}$ | 0.55 ± 0.1$^{a}$ | 0.81 ± 0.1$^{a}$ | 0.20 ± 0.1 | 0.7 ± 0.1 |
| Modulus (GPa) | 17 ± 2$^{a}$ | 8.5 ± 0.2 | 0.07 ± 0.01$^{a}$ | 1.2 ± 0.2$^{a}$ | 0.06 ± 0.01$^{a}$ | 0.2 ± 0.1 | 1.2 ± 0.2 |

Note: Values are the average of 3–5 replicate runs; error limits are ± one SD.

*Values of $R_{rms}$ and Young’s modulus from Bennett et al. (2010).

Table 2. Properties of SET 2 xerogel coatings with amine-/non-amine-containing surfaces.

| Composition/name | 1:9 AP/TEOS | 1:9 MAP/TEOS | 1:9 DMAP/TEOS | 1:4 TFP/TEOS | 1:4 PH/TEOS |
|------------------|--------------|--------------|---------------|--------------|-------------|
| Stored in air    |               |              |               |              |             |
| Static water contact angle ($\theta_{WS}$) | 56 ± 1 | 47 ± 2 | 48 ± 3 | 83 ± 1 | 81 ± 1 |
| Total surface energy $\gamma_S$ (mN m$^{-1}$) | 52 ± 1 | 54 ± 1 | 53 ± 2 | 25.9 ± 0.9 | 36.8 ± 0.2 |
| Immersed 24 h in ASW water |               |              |             |              |             |
| Static water contact angle ($\theta_{WS}$) | 39 ± 2 | 33 ± 3 | 24 ± 6 | 44 ± 9 | 59 ± 2 |
| Total surface energy $\gamma_S$ (mN m$^{-1}$) | 62.2 ± 0.4 | 66 ± 2 | 69 ± 3 | 55 ± 6 | 50 ± 1 |
| Dispersive surface energy $\gamma_D$ (mN m$^{-1}$) | 35.5 ± 0.1 | 35.6 ± 0.2 | 33.1 ± 0.1 | 23.9 ± 0.1 | 34.8 ± 0.1 |
| Polar surface energy $\gamma_P$ (mN m$^{-1}$) | 26.6 ± 0.1 | 29.9 ± 0.1 | 36.1 ± 0.2 | 31.1 ± 0.6 | 15.1 ± 0.1 |
| % Polar surface energy | 46.4 ± 0.1 | 47.8 ± 0.1 | 51.1 ± 0.2 | 53 ± 1 | 40 ± 1 |
| Surface roughness $R_{rms}$ (nm) | 1.60 ± 0.05 | 0.5 ± 0.1 | 0.60 ± 0.03 | 0.20 ± 0.09 | 0.23 ± 0.09 |
| Modulus (GPa) | 23.1 ± 3.2 | 8.5 ± 0.2 | 17 ± 2 | 6.2 ± 0.4 | 1.5 ± 0.5 |

Notes: Modulus and roughness data for AP/TEOS and DMAP/TEOS xerogels from Bennett et al. (2010). Values are the average of 3–5 replicate runs; error limits are ± one SD.
of filaments in the inoculum was quantified as the concentration of chlorophyll \( a \) (chl \( a \)) after extraction with dimethylsulfoxide (DMSO) (Shoaf & Lium 1976) using the formula for diatoms (Jeffrey & Humphrey 1975). The filtrate was diluted with ½-strength Provasoli medium to a chl \( a \) concentration of \( \sim 0.4 \mu \text{g ml}^{-1} \). This biomass value gave sufficient growth of filaments on the surfaces for quantification.

### Adhesion bioassay for E. crouaniorum

The protocol (Evariste et al. 2012) enables two measurements of adhesion performance on different surfaces: (a) a measure of initial attachment, assessed by estimating the biomass of unattached filaments after incubation of the test slides with inoculum for 8 days and (b) a measure of adhesion strength of the attached filaments after exposure to shear stress. The latter was assessed by measuring the proportion of attached filaments removed after exposure to a shear stress in a water channel.

Test surfaces were pretreated in the same manner as for the assays described in Bennett et al. (2010), ie immersed in DI water for 24 h, transferred into ASW (Tropic Marin®) for 2 h prior to the start of the assay. Surfaces (nine replicates), each contained in individual compartments of Quadriperm polystyrene culture dishes (Greiner Bio-One), were inoculated with 15 ml aliquots of filaments and incubated under the same conditions as those used for algal cultivation. After 8 days, non-adhered filaments were removed by gently rinsing each slide in ASW. The rinsed slides were put back into the Quadriperm dishes and 15 ml fresh enriched Provasoli culture medium (see above) were added; the dishes were incubated for a further 6 days, ie 14 days in total. The unattached biomass in each test compartment after 8 days was retained and the biomass of unattached filaments estimated through determination of the chl \( a \) content as described above (three replicates).

After the total incubation period of 14 days, test slides were gently rinsed in seawater to remove any remaining unattached filaments. The attached biomass on each test slide was quantified by measuring the fluorescence of chlorophyll in a plate reader (excitation = 430 nm, emission = 670 nm, TECAN GeniosPlus) (Finlay et al. 2008). Fluorescence was measured as relative fluorescence units (RFU). The RFU value for each slide was the mean of 168-point fluorescence readings taken within an area \( 6.0 \times 1.9 \) cm along the central axis of the slide. Uninoculated test surfaces that had been hydrated in ASW were used to blank the plate reader.

To measure the adhesion strength of attached filaments, the nine replicates of each test surface were exposed to a wall shear stress of 8 Pa for 5 min in a calibrated water channel producing fully developed turbulent flow (Schultz et al. 2000, 2003). The biomass that remained after exposure in the water channel was quantified as RFU as described above. The percentage removal was determined by comparison of the biomass (RFU) before and after exposure to shear stress.

### Comparative assays with sporelings of the green alga U. linza

In order to discuss the data obtained for \( E. \) crouaniorum in a broader fouling context, the FR performance of the SET 2 coatings with respect to \( U. \) linza was assessed. Fronds of \( U. \) linza were collected from Llantwit Major, Wales (51°40’N; 3°48’W) and a suspension of zoospores (\( 1.0 \times 10^6 \) spores ml\(^{-1}\)) was prepared by the method described in Cooper et al. (2011). Six-replicate slides of each treatment were randomly placed in Quadriperm dishes (Greiner Bio-One) and 10 ml of zoospore suspension were added. After 45 min in darkness, the slides were washed in ASW to remove unsettled (swimming) spores and replaced in Quadriperm dishes. The spores that attached to the surface were cultured in supplemented seawater medium in an illuminated incubator at 18°C with a 16h:8h light:dark cycle, for 6 days. The culture medium (Starr & Zeikus 1987) was changed every 2 days. Sporeling biomass was measured in situ by measuring the fluorescence of chlorophyll in a plate reader, as described above.

To assess the adhesion strength of the sporelings, the slides were exposed to a wall shear stress of 33 Pa for 5 min in a water channel (a higher shear stress was used for \( U. \) linza because this alga adhered more strongly to the test coatings compared with \( E. \) crouaniorum). The percentage removal was determined from RFU values before and after exposure to shear, as described for ectocarpoid algae (see above).

### Statistical analysis of bioassay data

Generalised linear modelling (GZLM, SPSS) with pairwise comparisons was used to assess coating performance as measured by each individual biomass metric (8 days unattached biomass, 14 days attached biomass, percentage biomass removal). GZLM gives flexibility over a classical ANOVA to account for a non-normal distribution of the response variable (biomass metric). Different statistical models of the relationship between the predictor (coating) and response variables were modelled by changing the link function, and the best model for the data was determined by maximum likelihood estimation (ie the model with the lowest Akaike’s information criterion (AIC value, Quinn & Keough 2002). Pairwise comparisons between coatings were performed using the resulting models. GZLM was also used to analyse the data for \( U. \) linza.
Results

Characterisation of surfaces: contact angles and surface energies

Immersion of surfaces can lead to changes in surface properties relative to samples stored in air. Static water contact angles, $\theta^*_w$, and static diiodomethane contact angles, $\theta^*_D(CD_2I_2)$, were measured for all xerogel surfaces described in this study pre- and post-immersion in ASW (Tables 1 and 2). Advanced ($\theta^*_w$) and receded ($\theta^*_w$) values of $\theta^*_w$ were also measured for all of the surfaces and are compiled in Supplementary Tables S1 and S2 [Supplementary material is available via a multimedia link on the online article webpage].

Prior to immersion in ASW, the SET 1 xerogels gave a range of values of $\theta^*_w$ from 49° to 107.9°. Following immersion in ASW, values of $\theta^*_w$ decreased by 15°–25° for each coating to give a lower range of values (31°–91°; Table 1). Pairwise comparisons (Student’s $t$-test) showed all changes to be statistically significant ($p<0.05$).

Contact angles measured with water and diiodomethane were treated as described by Owens and Wendt (1969) to give total surface energy ($\gamma_S$) (Baier & Meyer 1992) for the SET1 xerogels pre- and post-immersion in ASW (Table 1). Prior to immersion in ASW, $\gamma_S$ values ranged from the very low energy, hydrophobic 1:1 TDF/TEOS xerogel ($\gamma_S$ of 12.4 mN m⁻¹), through several xerogels (1:1 C8/TEOS, 5:45:50 C18/C8/TEOS and 1:1 TFP/TEOS) near the Baier minimum for surface energy (22–25.7 mN m⁻¹), to the higher energy 1:1 PH/TEOS xerogel (38 mN m⁻¹), and to the high energy 1:9 DMAP/TEOS and 1:9 MAP/TEOS xerogels (53 mN m⁻¹ and 53.5 mN m⁻¹, respectively). Following immersion in ASW, $\gamma_S$ values increased by statistically significant amounts (pairwise comparisons with Student’s $t$-test, $p<0.05$) for all coatings, by 5–15 mN m⁻¹. The 1:9 DMAP/TEOS and 1:9 MAP/TEOS xerogels gave an increase in ~20% in the surface energy following immersion, as did the 5:45:50 C18/C8/TEOS xerogel. The 1:1 PH/TEOS and 1:1 C8/TEOS xerogels gave an increase in ~40% in the surface energy following immersion. The two fluorinated xerogels (1:1 TFP/TEOS and 1:1 TDF/TEOS) gave the largest percentage increases in $\gamma_S$ following immersion (53 and 118%, respectively).

Based on the biological results described below, indicating that surface charge rather than wettability might be an important factor in controlling adhesion, a second set of coatings (SET 2) was designed to have similar chemistries to SET 1, but their composition was adjusted so their wettabilities and surface energies were more similar, following immersion in ASW. Three of the coatings incorporated the aminoalkyl silanes, AP, MAP and DMAP: new sets of 1:9 DMAP/TEOS and 1:9 MAP/TEOS coatings were prepared for SET 2.

Figure 1. Changes in surface energy ($\gamma_S$) between SET 2 xerogel samples stored in air for 14 days (●) and xerogel samples stored in air for 14 days then soaked for 24 h in ASW followed by 1 h in DI water and 2–3 h drying (○). Error bars represent ± one SD from the mean for three independent measurements pre- and post-immersion. Coatings (post-immersion in ASW) that share a letter have values of $\gamma_S$ that are not significantly different from one another.

The SET 2 coatings behaved similarly to the SET 1 coatings. Prior to immersion in ASW, the SET 2 xerogels gave a range of values of $\theta^*_w$ from 47° to 56° for the three amine-containing surfaces, and 81° and 83° for the 1:4 PH/TEOS and 1:4 TFP/TEOS surfaces, respectively. Following immersion in ASW, values of $\theta^*_w$ decreased 14°–24° for the amine-containing coatings to give a range of $\theta^*_w$ of 24°–39° (Table 2). Following immersion in ASW, values of $\theta^*_w$ for the 1:4 PH/TEOS and 1:4 TFP/TEOS surfaces were 59° and 44°, respectively. Pairwise comparisons showed all changes to be statistically significant (Student’s $t$ test, $p<0.05$). Surface energies for the SET 2 xerogel surfaces were also determined pre- and post-immersion in ASW (Table 2). The values of $\gamma_S$, pre- and post-immersion in ASW, are compared graphically in Figure 1. The surface energies of all SET 2 coatings significantly increased after immersion in ASW (pairwise comparisons by Student’s $t$-test, $p<0.01$). Prior to immersion in ASW, values of $\gamma_S$ for the SET 2 xerogels were between 25.9 mN m⁻¹ and 54 mN m⁻¹. The SET 2 xerogel surfaces following immersion in ASW for 48 h gave values of $\gamma_S$ between 50 and 69 mN m⁻¹. As was observed for the two fluorinated surfaces in the SET 1 coatings, the fluorinated 1:4 TFP/TEOS xerogel gave the largest change in $\gamma_S$ (change of 29 mN m⁻¹ or 112%) following immersion in ASW (Table 2). While all of the SET 2 xerogels post-immersion in ASW had measured values of $\gamma_S \geq 50$ mN m⁻¹, there were still significant differences in $\gamma_S$ among the SET 2 test coatings (ANOVA $p<0.001$) as summarised in Figure 1.
**Characterisation of SET 1 and SET 2 xerogel surfaces by AFM**

Surface roughness ($R_{\text{rms}}$) was low (~1 nm or less) for SET 1 coatings and values for Young’s modulus by AFM indentation ranged from 0.06 to 17 GPa. The surface roughnesses of the SET 2 coatings after immersion (0.2–1.6 nm) were similar to those of SET 1 and the elastic modulus values were also in the GigaPascal range (1.5–23.1 GPa). In comparison to silicones, these are relatively hard and topographically smooth coatings. The softest (0.06 GPa) is well above the range (by an order of magnitude) where modulus affects release of sporelings of *U. linza* (Chaudhury et al. 2005), and there is no evidence that a surface roughness on the scale of 1 nm or less can affect the adhesion properties of macroalgae. It is highly unlikely therefore that these differences in xerogel modulus and roughness would influence the adhesion strength of *E. crouaniorum*.

**Characterisation of SET 2 xerogel surfaces by XPS**

XPS spectra of the SET 2 xerogel films were recorded at a take-off angle of 45° to determine the atomic composition at the surface of the xerogel coating/film following storage in air and after treatment of the surface with ASW for a total of 24 h and a DI water rinse. The ratios of C(1s)/Si(2p3), N(1s)/Si(2p3) and F(1s)/Si(2p3) are compiled in Table 3. For the 1:9 AP/TEOS, 1:9 MAP/TEOS and 1:9 DMAP/TEOS surfaces, the C(1s)/Si(2p3) ratio increased significantly in the ASW-immersed surfaces relative to the non-immersed surfaces ($p < 0.005$ for pairwise comparisons using Student’s $t$-test). While the trend in the N(1s)/Si(2p3) ratios showed an increase in nitrogen at the surface following immersion in ASW, the differences were not significant ($p > 0.10$ for pairwise comparisons using Student’s $t$-test). For the 1:4 TFP/TEOS and 1:4 PH/TEOS coatings, there were no significant differences ($p > 0.10$) in C(1s)/Si(2p3) and F(1s)/Si(2p3) ratios in the surfaces stored in air or after immersion in ASW.

High-resolution XPS spectra showed two types of nitrogen in the 1:9 AP/TEOS, 1:9 MAP/TEOS and 1:9 DMAP/TEOS surfaces in both the air stored and ASW-immersed surfaces, with N(1s) peaks at ~401 eV and 399 eV (Table 4). Following immersion in ASW, the ratio of the 401–399 eV band decreased for the 1:9 AP/TEOS surface from 64:36 (Figure 2(a)) to 49:51 (Figure 2(b)). The 1:9 MAP/TEOS surface behaved similarly with the 401 eV/399 eV N(1s) ratio decreasing from 60:40 (Figure S1) [Supplementary material is available via a multimedia link on the online article webpage] to 42:58 (Figure 2(c)), following immersion in ASW. The 1:9 DMAP/TEOS surface behaved somewhat differently with the 401 eV/399 eV N(1s) ratio nearly identical both in air stored (62:38) and ASW-immersed (67:33) surfaces (Figure 2(d) and Figure S2).

**Adhesion strength of *E. crouaniorum* on SET 1 coatings**

Eight days after inoculation, unattached filaments were removed and quantified through chl a determination. The biomass of unattached filaments in dishes containing TFP/TEOS slides was significantly greater than for all the other coatings (Table 5; $p \leq 0.05$; GZLM, Pairwise comparison). However, no significant difference was observed for most of the other coatings. Although this suggests lower initial attachment to the TFP/TEOS

### Table 3. Element ratios determined by XPS for SET 2 coatings pre- and post-immersion in ASW.

| Coating         | Before soaking in ASW | After soaking in ASW |
|-----------------|-----------------------|----------------------|
|                 | C(1s)/Si(2p)  | N(1s)/Si(2p)  | F(1s)/Si(2p)  | C(1s)/Si(2p)  | N(1s)/Si(2p)  | F(1s)/Si(2p)  |
| 1:9 DMAP/TEOS   | 1.12 ± 0.10          | 0.09 ± 0.03         | –             | 1.46 ± 0.03   | 0.13 ± 0.02   | –             |
| 1:9 MAP/TEOS    | 0.91 ± 0.02          | 0.08 ± 0.03         | –             | 1.22 ± 0.03   | 0.12 ± 0.04   | –             |
| 1:9 AP/TEOS     | 0.86 ± 0.05          | 0.10 ± 0.01         | –             | 1.44 ± 0.05   | 0.14 ± 0.03   | –             |
| 1:4 PH/TEOS     | 2.24 ± 0.72          | –                  | 0.72 ± 0.03   | 1.57 ± 0.02   | –              | 0.70 ± 0.02   |
| 1:4 TFP/TEOS    | 1.39 ± 0.47          | –                  | –             | –              | –              | –             |

Note: Errors are ± one SD; values are the average of triplicate runs.

### Table 4. Component peaks of high-resolution XPS studies of the N(1s) region before and after soaking in ASW and the ratios of the component peaks.

| Coating         | Before soaking | After soaking |
|-----------------|----------------|--------------|
|                 | N(1s) Peak 1 (eV) | N(1s) Peak 2 (eV) | Peak 1/Peak 2 | N(1s) peak 1 (eV) | N(1s) peak 2 (eV) | Peak 1/Peak 2 |
| 1:9 DMAP/TEOS   | 401.3          | 398.6         | 62:38         | 401.5           | 398.9           | 67:33         |
| 1:9 MAP/TEOS    | 401.1          | 397.8         | 60:40         | 401.3           | 399.2           | 42:58         |
| 1:9 AP/TEOS     | 401.3          | 398.6         | 64:36         | 401.0           | 399.0           | 49:51         |
coatings, the greater unattached biomass could also be due to differences in the growth rates of unattached filaments between coatings. It is therefore important to interpret these results with caution, knowing they might not indicate differences in initial adhesion per se.

Before exposure to shear stress, attached filaments were incubated for further 6 days (ie 14 days in total after inoculation). The amount of biomass of attached filaments on the different surfaces was measured as fluorescence of chlorophyll and significant differences were found both before and after exposure to a shear stress of 8 Pa (Figure 3(a) and (b); \( p < 0.05 \), GZLM, Pairwise comparison). The most notable feature of the results is that adhesion was greatest (ie % removal lowest) on the aminoalkylated DMAP/TEOS and MAP/TEOS coatings (Figure 3(b)). Weak adhesion (80–95% removal) was shown on all the other coatings, with relatively minor differences between them. No systematic influence of total surface energy or the proportion of polar to total surface energy could be detected across the series (Figure 3(d) and (e)).

**Adhesion strength of E. crouaniorum on SET 2 coatings**

The quantity of unattached filaments, measured by chl \( a \), after growth for 8 days (Table 6) revealed marked differences between the amount of unattached filaments in dishes containing the different coatings. The dishes containing the surfaces prepared with aminoalkyl silanes (AP/TEOS, DMAP/TEOS and MAP/TEOS) had significantly less unattached biomass than the other two coatings (by a factor of between tenfold and twentyfold), suggesting stronger initial adhesion of the inoculated filaments to these three coatings (Table 6; \( p < 0.05 \), GZLM, Pairwise comparison).

Significant differences were observed in the biomass of attached filaments after growth for 14 days (Figure 4(a)). The three amine-containing coatings MAP/TEOS, DMAP/TEOS and AP/TEOS with positively charged organic side chains had significantly higher biomass than the two coatings (TFP/TEOS and PH/TEOS) prepared using uncharged side chains (\( p < 0.05 \), GZLM, Pairwise comparison). Coating 1:4 PH/TEOS had an intermediate amount of biomass attached to the slides while the coating 1:4 TFP/TEOS had the lowest biomass (\( p < 0.05 \), GZLM, Pairwise comparison).

| Composition       | Unattached biomass as chl \( a \) (\( \mu g \)) |
|-------------------|-----------------------------------------------|
| 1:9 DMAP/TEOS     | 0.21 ± 0.08 \( a \)                           |
| 1:9 MAP/TEOS      | 0.2 ± 0 \( b \)                              |
| 1:1 PH/TEOS       | 0.34 ± 0.03 \( b \)                           |
| 1:1 TFP/TEOS      | 0.52 ± 0.11 \( a \)                           |
| 1:1 TDF/TEOS      | 0.28 ± 01 \( ab \)                            |
| 5:45:50 C18/C8/TEOS | 0.23 ± 01 \( ab \)                           |
| 1:1 C8/TEOS       | 0.22 ± 0.1 \( ab \)                           |

Notes: Means of 3 replicates ±2 \( \times \) SE. Values that are significantly different at \( p \leq 0.05 \) in GZL test are indicated by different superscript letters.

Table 5. Unattached biomass measured as chl \( a \) after 8 days incubation of SET 1 xerogel coatings with an inoculum of filaments.

Figure 2. High-resolution XPS spectra for the N(1s) region for (a) the 1:9 AP/TEOS surface stored in air and (b) immersed in ASW for 24 h; (c) the 1:9 MAP/TEOS surface immersed in ASW for 24 h and (d) the 1:9 DMAP/TEOS surface immersed in ASW for 24 h.
Table 6. Unattached biomass after growth of *E. crouaniorum* for 8 days on charged/uncharged SET 2 xerogel surfaces, as measured by chl *a*.

| Composition       | Unattached biomass as chl *a* (μg) |
|-------------------|-----------------------------------|
| 1:9 AP/TEOS       | 0.03 ± 0.02<sup>a</sup>           |
| 1:9 DMAP/TEOS     | 0.04 ± 0.02<sup>a</sup>           |
| 1:9 MAP/TEOS      | 0.02 ± 0.01<sup>b</sup>           |
| 1:4 TFP/TEOS      | 0.41 ± 0.06<sup>b</sup>           |
| 1:4 PH/TEOS       | 0.23 ± 0.09<sup>b</sup>           |

Notes: Means of 3 replicates ±2 × SE. Values that are significantly different at *p* ≤ 0.05 in GZLM test are indicated by different superscript letters.

Figure 3. Adhesion assays on SET 1 coatings with *E. crouaniorum*. (a) Biomass of attached alga before and after exposure to a wall shear stress of 8 Pa after incubation for 14 days at 15 °C. Biomass was measured as RFUs. (b) Percentage removal after exposure to a shear stress of 8 Pa, calculated from data presented in (a). Means from 9 replicates ±2 × SE. Values that are significantly different at *p* ≤ 0.05 in GZLM test are indicated by different letters above the bars.

Figure 4. Adhesion assays with *E. crouaniorum* on SET 2 coatings. (a) Biomass of attached alga before and after exposure to a wall shear stress of 8 Pa on xerogel surfaces, after incubation for 14 days at 15 °C. Biomass was measured as RFUs. (b) Percentage removal after exposure to a shear stress of 8 Pa, calculated from data presented in (a). Means from 9 replicates ±2 × SE. Values that are significantly different at *p* ≤ 0.05 in GZLM test are indicated by different letters above the bars. (c), (d) and (e) show the relationship between percentage removal and the surface properties of coatings after immersion in ASW: static water contact angle (c), total surface energy (d) and percentage of polar surface energy (e). Means of nine replicates ±2 × SE. In (c), (d) and (e), the letters associated with data points refer to coating codes: A = DMAP/TEOS; B = MAP/TEOS; C = PH/TEOS; D = TFP/TEOS; E = TDF/TEOS; F = C18/C8/TEOS and G = C8/TEOS.
The adhesion strength data (ie the percentage removal after exposure to shear stress, Figures 4(b) and 5) showed that *E. crouaniorum* adhered significantly more weakly to TFP/TEOS and PH/TEOS, compared to the three aminoalkyl-containing coatings (*p* ≤ 0.05, GZLM, Pairwise comparison). There were also significant differences between TFP/TEOS and PH/TEOS, and between DMAP/TEOS and the other two aminoalkyl-containing coatings.

**Adhesion strength of sporelings of *U. linza* on SET 2 coatings**

The amount of biomass that develops broadly reflects the density of spores that settle on the test surfaces. After cultivation for 6 days, all surfaces were covered by a green lawn of sporelings (young plants) and the biomass on the coatings was not significantly different except between DMAP/TEOS and MAP/TEOS (Figure 6(a); *p* ≤ 0.05, GZLM, Pairwise comparison). The adhesion strength of sporelings growing on the xerogels was generally relatively high (the maximum percentage removal was 41% for 1:4 TFP/TEOS at a wall shear stress of 33 Pa). However, significant differences in the percentage removal of sporelings were observed between the xerogel surfaces with positively charged aminopropyl side chains and uncharged organic side chains. Sporelings on the surfaces with uncharged side chains (1:4 TFP/TEOS and 1:4 PH/TEOS) had a lower adhesion strength compared to the other surfaces. There was also a significant difference in performance between AP/TEOS and DMAP/TEOS (Figure 6(b); *p* ≤ 0.05, GZLM, Pairwise comparison).

**Discussion**

Previous studies using similar xerogel coatings to those in SET 1, with a wide range of surface energy values, showed various responses regarding the attachment and adhesion strengths of different fouling algae, viz. *U. linza* and *N. perminuta*. For sporelings of *U. linza*, there was a correlation between the adhesion and the wettability/total surface energy of the xerogels modified with fluorocarbon/hydrocarbon groups (Bennett et al. 2010). The aminooalkylated xerogels did not follow this trend, sporelings adhering more strongly to the aminopropylated xerogel than to either the methyl- or dimethylaminopropylated materials, despite these three coatings having similar surface energies. However, the diatom *N. perminuta* adhered less strongly to the more polar, high surface energy coatings, including those containing aminooalkyl groups (Finlay et al. 2010). It should be noted that in these two previous publications, surface analyses were performed on pristine coatings. The present study has shown that the wettability and the surface energy properties of dry surfaces change following immersion for 24 h in ASW, with both increasing. If the values for surface energy determined on coatings after immersion are considered against the adhesion data for both algae as reported in Bennett et al. (2010) and Finlay et al. (2010), the general conclusions and trends outlined in these papers do not change. Since *U. linza* and *N. perminuta* clearly respond differently it was therefore of interest to evaluate the same xerogel coatings with *Ectocarpus*, a fouling alga with a different phylogeny to *U. linza*, and a different adhesion biology to diatoms. A further point of practical interest is that ectocarpoid algae have been observed on non-biocidal FR coatings during recent field experiments by International Paint (D. Williams personal communication).

The starting hypothesis for this work was that there would be a correlation between the wettability/surface...
energy and the adhesion strength of *E. crouaniorum*. The results presented in this paper on SET 1 coatings showed that there was high removal of attached filaments of *E. crouaniorum* from five of the seven coatings tested (PH/TEOS, TFP/TEOS, C18/C8/TEOS, TDF/TEOS and C8/TEOS), which included low-surface energy (C18/C8/TEOS and TDF/TEOS), intermediate surface energy (TFP/TEOS and C8/TEOS) and high-surface energy (PH/TEOS) surfaces. However, adhesion strength was high on MAP/TEOS and DMAP/TEOS, two coatings containing aminoalkyl silanes and with the highest total surface energies.

However, before examining the biological data in detail, it is important to understand the properties of the coatings (their similarities and differences) before and after immersion in ASW. SET 1 coatings had very similar, low values (<1 nm, Table 1) of surface roughness, which removes *Rms* as a variable. Water contact angle hysteresis (CAH) has been correlated directly (Ucar et al. 2010) and inversely (Schmidt et al. 2004) with ease of removal of fouling. However, in the SET 1 coatings, surfaces both with low values of CAH (TFP/TEOS and C18/C8/TEOS, 6° and 8°, respectively, Table S1) and with high values of CAH (TFP/TEOS and C8/TEOS, 28° and 29°, respectively, Table S1) [Supplementary material is available via a multimedia link on the online article webpage] gave high removal of attached filaments of *E. crouaniorum*, while the MAP/TEOS and DMAP/TEOS surfaces with high values of CAH (>24°, Table S1) gave the highest adhesion strengths. Among the four coatings with the highest values of CAH, the strongest adhesion of *E. crouaniorum* came from the two surfaces with amine-containing side chains, while high removal was observed from the two coatings with neutral side chains.

The methylaminopropyl group of MAP and the dimethylaminopropyl group of DMAP have *pKaq* values in solution of 10–11 (Hall 1957; McMaster et al. 2009). At the pH of the seawater-based medium used in the algal adhesion experiments (pH 8.1), a large percentage of the amino groups will be protonated and therefore it was hypothesised that the strong adhesion shown to these two coatings was perhaps a consequence of charge rather than surface energy/polarity per se. The SET 2 coatings were designed to give materials with similar surface topographies/roughnesses and more similar surface energies following immersion in ASW, but with xerogel side chains that either were neutral or were amine-containing, which could be protonated in ASW to provide a positive charge.

Among the SET 2 xerogels, the AP/TEOS coating had a value of *Rms* of 1.6 nm while all other SET 2 surfaces had values of *Rms*<1 nm. All of these surfaces are still ‘smooth’ among the xerogel surfaces in this and other studies (Bennett et al. 2010) and there is no evidence that roughness at this scale has any influence on adhesion of macroalgae. All of the SET 2 xerogels also had high values of Young’s modulus (1.5 to 23 GPa) and were thin coatings (1 µm) so modulus effects on adhesion of these ‘soft-fouling’ organisms should not be relevant (Chaudhury et al. 2005).

The xerogel coatings described here were produced by the sol-gel process involving the acid-catalysed condensation of an organotrialkoxysilane with TEOS (Brinker & Scherer 1990) to give a sol solution with a molecular weight on the order of 1400 Da to >5000 Da (Bennett et al. 2010). After the sol is coated, further condensation driven by evaporative loss of alcohol and water gives the final xerogel surface. However, the condensation is incomplete and free silanol (=SiOH) functionality will be present on the xerogel surface. Therefore, the condensation is incomplete and free silanol (=SiOH) functionality will be present on the xerogel surface (Brinker & Scherer 1990; Pandey et al. 2000). The silanol group (*pKa* ≈ 3) is very similar in acidity to carboxylic acid functionality (*pKa* ≈ 4) and will be ionised in seawater (pH 8.1 in these experiments) resulting in a net negative charge (from =SiO−) on the xerogel films that do not have aminopropyl functionality. For the SET 2 coatings, the trifluoropropyl group of the 1:4 TFP/TEOS xerogel and the phenyl group of the 1:4 PH/TEOS xerogel are neutral side chains under all aqueous conditions and the xerogel surfaces will have a net negative charge due to the conjugate base (=SiO−) of the weak silanol acid.

The MAP/TEOS, DMAP/TEOS and AP/TEOS xerogels in SET 2 had aminopropyl functionality whose protonated forms have *pKaq* values of the order of 10–11 (Hall 1957; McMaster et al. 2009). High-resolution XPS studies of the aminopropyl-containing xerogels in SET 2 show both positive (protonated) and neutral nitrogen atoms in the N(1s) region at 401 and 399 eV, respectively, both pre- and post-immersion in ASW (Figures 1, S1 and S2, Table 4) [Supplementary material is available via a multimedia link on the online article webpage] indicating that positively charged ammonium groups were present on the surface. A more detailed discussion of the interpretation of XPS data is found in the Supplementary information.

The DMAP/TEOS coating behaved somewhat differently relative to the AP/TEOS and MAP/TEOS coatings with respect to the XPS data. This difference may be relevant to differences in adhesion observed between *E. crouaniorum* and each of the three amine-containing surfaces. Immersion in ASW gave very little change in the peak1/peak 2 ratio in the high-resolution XPS data for the N(1s) region for the DMAP/TEOS coating. While the electrostatic attraction of the positively charged amine group with the negatively charged deprotonated silanol gives most of the enthalpic contribution to the interaction, the entropic contribution will vary significantly from protonated AP to MAP to DMAP as a
proton donor to the silanol conjugate base. The protonated AP group has three equivalent protons that can be donated to the $\equiv$SiO$^-$ groups and all can be accessed by three equivalent rotations about a single bond (Figure 7). The protonated MAP group has two equivalent protons and two orientations. DMAP, in contrast, has only one favourable interaction and one orientation. The entropic cost will reduce the number of protonated DMAP groups interacting with the $\equiv$SiO$^-$ groups, which is reflected in the relatively small difference in the peak 1/peak 2 ratios in the high-resolution XPS pre- (62:38) and post-immersion (67:33). In an aqueous environment rather than the high vacuum of the sampling chamber, the close association of protonated amines and the $\equiv$SiO$^-$ groups will be diminished by solvation/hydration – ie the XPS peak 1/peak 2 ratios need not hold in ASW.

The results shown in Figures 4 and 6 support the revised hypothesis that the presence of positively charged groups promotes adhesion of the test algae, with stronger adhesion to all three xerogels bearing positively charged amino functionality, compared to the amine-free xerogels where the negative charge from the conjugate base of the weakly acidic free silanol groups will be the only contribution to a charged surface. The results obtained were comparable to those previously obtained for U. linza with similar xerogels, where strong adhesion of sporelings to the aminopropylated AP/TEOS xerogel was found (Bennett et al. 2010). This was confirmed by the results of tests with U. linza on the SET 2 coatings, which showed significantly stronger adhesion of sporelings to the positively charged surfaces.

Among the three xerogels bearing positively charged amino functionality, E. crouaniorum showed significantly weaker adhesion (greater removal) from the DMAP/TEOS surface compared to the AP/TEOS and MAP/TEOS surfaces (Figure 4). As shown in Figure 7, secondary hydrogen bonding in addition to electrostatic interactions alone can contribute to adhesion driven by charge. The entropic penalty from the DMAP/TEOS surface would reduce adhesion relative to AP/TEOS and MAP/TEOS surfaces. The extent of protonation of the amino groups could also be influenced by the chemical changes in the local environment caused by the presence of the alga, for example, changes in the pH of the microenvironment due to the secretion of ions and metabolites and the depletion of CO$_2$ concentrations due to photosynthesis.

The effect of surface charge on the settlement/adhesion strength of fouling organisms has not been extensively studied. Previous studies have suggested that surface charge rather than surface energy per se may determine the surface selection and settlement of cyprids of two barnacle species (Aldred et al. 2011; Petrone, Di Fino et al. 2011). Moreover, it has been shown that the settlement of zoospores of U. linza was higher on surfaces with positively charged functionality (arginine-rich peptide SAMS) (Edeth et al. 2008, 2009) and quaternised copolymers (Park et al. 2010), while the adhesion of sporelings was also high on the latter surface.

The physico-chemical basis of the stronger adhesion of filaments of E. crouaniorum and sporelings of U. linza to surfaces with positively charged functionality may lie in electrostatic interactions between the positively charged amine-containing functionality on surfaces and the biological polymers on the surface that may play a role in adhesion. Previous research on adhesives used by brown macroalgae has mainly focused on the extracellular matrix of zygotes of fucoids where anionic sulphated fucans and alginates appear to play an important role in adhesion to the substratum (see Potin & Leblanc 2006 for a review). More generally, the cell wall or extracellular matrix of brown algae consists of a relatively small proportion of
crystalline cellulose microfibrils embedded in an amorphous matrix of anionic polysaccharides, chiefly alginates and fucans (Kloareg & Quatrano 1988). Recently, it has been shown that adhesive secreted by the spores of another brown alga, Undaria pinnatifida was composed of anionic polysaccharides (Petrone, Easingwood et al. 2011). While there have been only a few biochemical studies on the cell wall composition of ectocarpoid algae, the recently released genome sequence for Ectocarpus siliculosus supports the existence of the appropriate genes and enzymes for synthesis and modification of sulphated fucans and alginates (Michel et al. 2010). It is also known that the cell wall of Ulva linza is mainly composed of ulvan, a sulphated glucuronorhamnoglycan, i.e. it is negatively charged (Ray & Lahaye 1995). While it has not been established that such molecules have adhesive functionality in Ulva linza, their location in the cell wall could provide the basis for non-specific, electrostatic adhesion.

In conclusion, the results obtained demonstrating strong adhesion of Ulva linza and E. crouaniorum on xerogel surfaces with positively charged amino groups can potentially be explained through the presence of anionic polysaccharides in the cell walls of these algae. While the primary function of these polysaccharides may not be in specific adhesion processes, their location at the cell surface would contribute to strong, non-specific, electrostatic adhesion to positively charged functional groups. Whilst it has been suggested that xerogel coatings (Selvaggio et al. 2009) the results of this paper suggest that only alkyl modifications incapable of generating positive charge should be considered.

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Note

Emmanuelle Evariste and Caitlyn M. Gatley contributed equally to this paper.

References

Aldred N, Ekbald T, Andersson O, Liedberg B, Clare AS. 2011. Real-time quantification of microscale bioadhesion events in situ using imaging surface plasmon resonance (iSPR). ACS Appl Mater Interfaces. 3:2085–2091.

Baier RE, Meyer AE. 1992. Surface analysis of fouling-resistant marine coatings. Biofouling. 6:165–180.

Bennett SM, Finlay JA, Gunari N, Wells DD, Meyer AE, Walker GC, Callow ME, Callow JA, Bright TV, Denny MR. 2010. The role of surface energy and water wettability in aminoalkyl/fluorocarbon/hydrocarbon-modified xerogel surfaces in the control of marine biofouling. Biofouling. 26:235–246.

Brinker C, Scherer G. 1990. Sol-gel science: the physics and chemistry of sol-gel processing. New York (NY): Academic Press.

Callow JA, Callow ME. 2011. Trends in the development of environmentally friendly fouling-resistant marine coatings. Nature Comms. 2:10.1038/ncomms1251.

Cavalier-Smith T. 2004. Only six kingdoms of life. Proc R Soc London, Ser B. 271:1251–1262.

Chaudhury MK, Finlay JA, Chung JY, Callow ME, Callow JA. 2005. The influence of elastic modulus and thickness on the release of the soft-fouling green alga Ulva linza linza (syn. Enteromorpha linza) from poly(dimethylsiloxane) (PDMS) model networks. Biofouling. 21:41–48.

Cooper SP, Finlay JA, Cone G, Callow ME, Callow JA, Brennan AB. 2011. Engineered antifouling microtopographies: kinetic analysis of the attachment of zoospores of the green alga Ulva linza to silicone elastomers. Biofouling. 27:881–892.

Ederth T, Nygren P, Pettitt ME, Ostblom M, Du CX, Broo K, Callow ME, Callow J, Liedberg B. 2008. Anomalous settlement behavior of Ulva linza zoospores on cationic oligopeptide surfaces. Biofouling. 24:303–312.

Ederth T, Pettitt ME, Nygren P, Du CX, Ekbald T, Zhou Y, Falk M, Callow ME, Callow JA, Liedberg B. 2009. Interactions of zoospores of Ulva linza with arginine-rich oligopeptide monolayers. Langmuir. 25:9375–9383.

Evariste E, Gachon CMM, Callow ME, Callow JA. 2012. Development and characteristics of an adhesion bioassay for ectocarpoid algae. Biofouling. 28:15–27.

Finlay JA, Bennett SM, Brewer LH, Sokolova A, Clay G, Gunari N, Meyer AE, Walker GC, Wendt DE, Callow ME, et al. 2010. Barnacle settlement and the adhesion of protein and diatom microfouling to xerogel films with varying surface energy and water wettability. Biofouling. 26:657–666.

Finlay JA, Fletcher BR, Callow ME, Callow JA. 2008. Effect of background colour on growth and adhesion strength of Ulva linza sporelings. Biofouling. 24:219–225.

Finnie AA, Williams DN. 2010. Paint and coatings technology for the control of marine fouling. In: Durr S, Thomason J, editors. Biofouling. Oxford: Wiley-Blackwell; p. 185–206.

Fletcher RL. 1980. Catalogue of main marine fouling organisms. Vol. 6 Algae. Brussels: Office d’études marines et atmosphériques.

Gunari N, Brewer LH, Bennett SM, Sokolova A, Kraut ND, Finlay JA, Meyer AE, Walker GC, Wendt DE, Callow ME, et al. 2011. The control of marine biofouling on xerogel surfaces with nanometer-scale topography. Biofouling. 27:137–149.

Hall HK. 1957. Correlation of the base strengths of amines. J Am Chem Soc. 79:5441–5444.

Jeffrey SW, Humphrey GF. 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pfl. 167:191–194.
Kloareg B, Quatrano R. 1988. Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. Oceanogr Mar Biol Annu Rev. 26: 259–315.

Martelli E, Agostini S, Galli G, Chiellini E, Gisenti A, Pettit ME, Callow ME, Callow JA, Graf K, Bartels FW. 2008. The surface-segregate nanostructure of fluorinatated copolymer-poly(dimethylsiloxane) blend films. Langmuir. 24:13138–13147.

McMaster DM, Bennett SM, Tang Y, Finlay JA, Kowalke GL, Nedved B, Bright FV, Callow ME, Callow JA, Wendt DE, et al. 2009. Antifouling character of 'active' hybrid xerogel coatings with sequestered catalysts for the activation of hydrogen peroxide. Biofouling. 25:21–33.

Michel G, Tonon T, Scornet D, Cock JM, Kloareg B. 2010. The cell wall polysaccharide metabolism of the brown alga Ectocarpus siliculosus. Insights into the evolution of extra-cellular matrix polysaccharides in Eukaryotes. New Phytol. 188:82–97.

Mineur F, Johnson MP, Maggs CA, Stengenga H. 2007. Hull fouling on commercial ships as a vector of macroalgal cellular matrix polysaccharides in Eukaryotes. New Phytol. 173:97–114.

Owens DK, Wendt RC. 1969. Estimation of the surface free energy of polymers. J Appl Polym Sci. 13:1741–1747.

Pandey S, Baker GA, Kane MA, Bonzaghi NJ, Bright FV. 2000. On the microenvironments surrounding dansyl sequestered within class I and II xerogels. Chem Mater. 12:3547–3551.

Park D, Finlay JA, Ward RJ, Weinman CJ, Krishnan S, Paik M, Sohn KE, Callow ME, Callow JA, Handlin DL, et al. 2010. Antimicrobial behavior of semifluorinated-quatem-ized triblock copolymers against airborne and marine microorganisms. ACS Appl Mater Interfaces. 2:703–711.

Petrone L, Di Fino A, Aldred N, Sukkaew P, Ederth T, Clare AS, Liedberg B. 2011. Effects of surface charge and Gibbs surface energy on the settlement behaviour of barnacle cyprids (Balanus amphitrite). Biofouling. 27:1043–1055.

Potin P, Leblanc C. 2006. Phenolic-based adhesives of marine brown algae. In: Smith AM, Callow JA, editors. Biological adhesives. Heidelberg: Springer. p. 105–124.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. Cambridge: Cambridge University Press.

Ray B, Lahaye M. 1995. Cell-wall polysaccharides from the marine green alga U. linza ‘rigida’ (Ulva linzales, Chlorophyta). Extraction and chemical composition. Carbohydr Res. 274:251–261.

Schmidt DL, Brady RF, Lam K, Schmidt DC, Chaudhury MK. 2004. Contact angle hysteresis, adhesion, and marine biofouling. Langmuir. 20:2830–2836.

Schultz MP. 2007. Effects of coating roughness and biofouling on ship resistance and powering. Biofouling. 23:331–341.

Schultz MP, Bendick JA, Holm ER, Hertel WM. 2011. Economic impact of biofouling on a naval surface ship. Biofouling. 27:87–98.

Schultz MP, Finlay JA, Callow ME, Callow JA. 2000. A turbulent channel flow apparatus for the determination of the adhesion strength of microfouling organisms. Biofouling. 15:243–251.

Selvaggio P, Tusa S, Detty MR, Bright FV, Ciriminna R, Pagliaro M. 2009. Ecofriendly protection from biofouling of the monitoring system at Pantelleria’s Cala Gadir underwater archaeological site. Sicily Int J Naut Archaeol. 38:417–421.

Shoaf WT, Lium BW. 1976. Improved extraction of chlorophyll a and b from algae using dimethyl sulfoxide. Limnol Oceanogr. 21:926–928.

Sokolova A, Bailey JJ, Waltz GT, Brewer LH, Finlay JA, Fornalik J, Wendt DE, Callow ME, Callow JA, Bright FV, Detty MR. 2012. Spontaneous multiscale phase separation within fluorinated xerogel coatings for fouling-release surfaces, Biofouling. 28:143–157.

Starr R, Zekius J. 1987. UTEX – The culture collection of algae at the University of Texas at Austin. J Phycol. 23: 1–47.

Tang Y, Finlay JA, Kowalke GL, Meyer AE, Bright FV, Callow ME, Callow JA, Wendt DE, Detty MR. 2005. Hybrid xerogel films as novel coatings for antifouling and fouling release. Biofouling. 21:59–71.

Tribou M, Swain G. 2010. The use of proactive in-water grooming to improve the performance of ship hull antifouling coatings. Biofouling. 26:47–56.

Ucar IO, Cansoy E, Erbil HY, Pettitt ME, Callow ME, Callow JA. 2010. Effect of contact angle hysteresis on the removal of the sporelings of the green alga Ulva from the fouling-release coatings synthesized from polyolefin polymers. Biointerphases. 5:75–84.