Hybrid xerogel films as novel coatings for antifouling and fouling release

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Abstract
Hybrid sol-gel-derived xerogel films prepared from 45/55 (mol ratio) *n*-propyltrimethoxysilane (C3-TMOS)/tetramethylorthosilane (TMOS), 2/98 (mol ratio) bis[3-(trimethoxysilyl)propyl]-ethylenediamine (enTMOS)/tetraethylorthosilane (TEOS), 50/50 (mol ratio) *n*-octyltriethoxysilane (C8-TEOS)/TMOS, and 50/50 (mol ratio) 3,3,3-trifluoropropyltrimethoxysilane (TFP-TMOS)/TMOS were found to inhibit settlement of zoospores of the marine fouling alga *Ulva* (syn. *Enteromorpha*) relative to settlement on acid-washed glass and give greater release of settled zoospores relative to glass upon exposure to pressure from a water jet. The more hydrophobic 50/50 C8-TEOS/TMOS xerogel films had the lowest critical surface tension by comprehensive contact angle analysis and gave significantly greater release of 8-day *Ulva* sporeling biomass after exposure to turbulent flow generated by a flow channel than the other xerogel surfaces or glass. The 50/50 C8-TEOS/TMOS xerogel was also a fouling release surface for juveniles of the tropical barnacle *Balanus amphitrite*. X-ray photon electron data indicated that the alkylsilyl residues of the C3-TMOS-, C8-TEOS-, and TFP-TMOS-containing xerogels were located on the surface of the xerogel films (in a vacuum), which contributes to the film hydrophobicity. Similarly, the amine-containing silyl residues of the enTMOS/TEOS films were located at the surface of the xerogel films, which contributes to the more hydrophilic character and increased critical surface tension of these films.

Keywords: *Xerogels, biofouling, algae,* Ulva, *barnacles,* Balanus amphitrite, *fouling release*

Introduction

Marine biofouling is a worldwide problem costing billions of dollars per year in transportation costs due to increased fuel consumption (Townsin, 2003). Within seconds of immersion, surfaces acquire a proteinaceous conditioning film prior to organism attachment. Within a few hours of immersion, bacteria, unicellular algae, and cyanobacteria (blue-green algae) can colonize a clean surface to form a biofilm, which on unprotected surfaces will rapidly become overgrown by macrofoulers such as barnacles, tubeworms, and macroalgae (Callow, 2000). The barnacle *Balanus amphitrite* is a common member of fouling communities in coastal environments and it is often found in great abundance on ships' hulls and pier pilings and it has an extremely wide geographical distribution. As adult barnacles are sessile (non-motile), dispersal is by movement of adults attached to moving objects (e.g., ships' hulls, floating debris) or by transport of planktonic larval stages via ocean currents. Barnacles typically have six naupliar feeding stages, followed by metamorphosis to a final larval stage, the cyprid, which is non-feeding and specialized for attachment to hard substrata. *Ulva* (syn. *Enteromorpha* [Hayden et al. 2003]) is a key macroalga that fouls ships and is tolerant of a wide range of environmental conditions and surface coating types including biocidal antifouling paints (Callow, 1996). Dispersal of *Ulva* is mainly through motile, quadriflagellate zoospores (approximately $7-8$ μm in length), which are released in large numbers and which respond to a large number of settlement cues (Callow & Callow, 2000).

One settlement cue shared by *Ulva* zoospores (Callow et al. 2000) and the settlement stage of a

number of invertebrate larvae is surface wettability (Rittschof & Costlow, 1989; Rittschof et al. 1998; Gerhart et al. 1992). For *Ulva*, zoospore settlement on uncharged (HO-, CH_3 and mixed HO-CH_3) self-assembled monolayers (SAMs) is positively correlated with low surface wettability (Callow et al. 2000). However, once settled, spores are more easily removed from hydrophobic SAMs (Finlay et al. 2002a). Spore settlement involves a change from a motile to a permanently adhered spore that germinates and grows into a new plant (Callow et al. 1997). The ease of removal of spores and sporelings can be measured by application of hydrodynamic forces using a water-jet apparatus (Finlay et al. 2002b) or a turbulent flow channel (Schultz et al. 2000; 2003). Similarly, the ease of removal of barnacles from surfaces can be determined from the application of a shear force parallel to the surface to the barnacle base plate (Anonymous, 1997).

The development of an inexpensive, environmentally friendly, robust 'coating' that can minimize settlement and adhesion of marine fouling organisms on to the coated surface would be an important contribution toward solving the marine fouling problem. Sol-gel processed xerogel materials are readily prepared by the hydrolysis of metal or semi-metal alkoxides with tunable surface characteristics, such as surface composition, surface area, and surface wettability (Brinker & Scherer, 1990; Avnir, 1995; Dave et al. 1995; Ingersoll & Bright, 1997). These materials are easily processed near ambient conditions and are economically and environmentally friendly. Furthermore, sol-gel-derived xerogel films can be applied to surfaces by a variety of means including spraying, brushing, dip coating and spin coating. The composite xerogel surfaces of this study are model systems for comparison of anti-fouling and fouling-release properties. The sols were spin cast onto glass microscope slides to provide thin xerogel films of reproducible thickness and uniformity (Jordan et al. 1998; Pandey et al. 2000).

This investigation examines the performance of hybrid xerogel films (including films with low surface energy) with respect to (i) the settlement of *Ulva* zoospores and cyprids of *B. amphitrite* and (ii) the adhesion characteristics of *Ulva* spores, *Ulva* sporelings and juvenile barnacles of *B. amphitrite*.

Materials and methods

Chemical reagents

All reagents were used as received. Deionized water was prepared to a specific resistivity of at least 18 MΩ using a Barnstead NANOpure II system. Tetramethoxysilane (TMOS) was purchased from Aldrich Chemical Company. Tetraethoxysilane (TEOS), *n*-propyltrimethoxysilane (C3-TMOS), *n*-octyltriethoxysilane (C8-TMOS), 3,3,3-trifluoropropyltrimethoxysilane (TFP-TMOS), and bis[(3-trimethoxysilyl)propyl]ethylenediamine (enTMOS) were obtained from Gelest, Inc. Hydrochloric acid was obtained from Fisher Scientific Co. Ethanol was a product of Quantum Chemical Corp. Borosilicate glass microscope slides were obtained from Fisher Scientific, Inc.

Preparation of glass control slides

Plain glass slides were cleaned by soaking in 1 N HCl for 24 h and were then rinsed with copious amounts of deionized water and EtOH. These treated slides were dried under ambient conditions and used within one day.

Sol preparation

The sol/xerogel composition is designated in terms of the mole-% of the Si-containing precursors. Thus, a 20/80 C3-TMOS/TMOS composition contains 20 mole-% C3-TMOS and 80 mole-% TMOS.

45/55 C3-TMOS/TMOS and 50/50 C8-TEOS/TMOS

C3-TMOS (1.92 g, 11.7 mmol) and TMOS (2.17 g, 14.3 mmol) were mixed and EtOH (5.0 ml, 87 mmol) and HCl (1.6 ml of 0.1 N HCl, which contains 0.16 mmol HCl and 89 mmol H_2O) were added. The resulting solution was capped and magnetically stirred under ambient conditions for 2 h. For 50/50 C8-TEOS/TMOS, sols were prepared as described above for 45/55 C3-TMOS/TMOS with total silane of 0.026 mol. Sols for 50/50 C8-TEOS/TMOS were also prepared with stirring for 30 min, 1 h and 2 h, respectively.

50/50 TFP-TMOS/TMOS

TFP-TMOS (1.50 ml, 7.8 mmol) and TMOS (1.15 ml, 7.8 mmol) were mixed and deionized H_2O (0.563 ml, 31 mmol) and EtOH (3.5 ml, 60 mmol) were added. The resulting solution was capped and sonicated under ambient conditions for 30 min.

2/98 enTMOS/TEOS

The individual precursors were hydrolyzed separately in different vials and then mixed together at 1:1 (v/v). Ethanol (3.0 ml, 52.2 mmol), deionized H_2O (40 μl, 2.2 mmol) and HCl (10 μl of 12 N HCl, 0.12 mmol) were mixed and enTMOS (100 μl, 0.16 mmol) was then added. The sol was mixed with sonication for 10 min and then was mixed at 1:1 (v/

The liquid/vapor surface tension for each diagnostic fluid used in the analyses reported here was determined using data obtained with a ring tensiometer (Cenco-duNuoy). Raw data are obtained for a single liquid several times, and then input to an equation that takes liquid density, air pressure (height above sea level at the location where the liquids are used), and temperature into account. The ring tensiometer measurements are made when the liquids are first prepared for comprehensive contact angle measurements. After a volume of each liquid is prepared and characterized, and found to have a satisfactory liquid/vapor surface tension (i.e., as high as possible in comparison with theoretical values for that liquid), the liquid is placed in an acid-cleaned low-actinic-glass bottle, with inverted cap, for storage. These bottles reduce the likelihood of chemical contamination and photolytic breakdown of the liquids.

Subsequent characterization of the liquids so-prepared is accomplished by measurement of contact angles on laboratory reference materials. After the liquids are first prepared (see above), several contact angle measurements of each liquid on the reference materials (Type C PTFE film; primary reference LDPE film) are made; average and standard deviation values are calculated. These measurements are repeated on a regular basis, and compared to the values obtained after the liquids were first prepared. If the average contact angle of a liquid on the reference substrata significantly deviates from the original average contact angle, the volume of liquid is discarded, the bottle cleaned, and a new volume of liquid is prepared as described above.

Table I. Critical surface tensions (γ_C) and XPS results for xerogel films.

Sample	γ_C,[a] mN m^{-1}	C(1s)/Si(2p3)[a]	F(1s)/Si(2p3) or N(1s)/ Si(2p3)[b]
Glass	33.5 ± 0.5	0.2 ± 0.1	
45/55 C3-TMOS/TMOS	21.2 ± 0.3	1.37 ± 0.12	
2/98 enTMOS/TEOS	35.7 ± 0.5	1.1 ± 0.1	0.05 ± 0.01
50/50 TFP-TMOS/TMOS	19.9 ± 0.3	1.70 ± 006	1.43 ± 0.04
50/50 C8-TEOS/TMOS (2 h)[c]	19.9 ± 0.3	3.87 ± 0.19	
50/50 C8-TEOS/TMOS (1 h)[c]	–	3.52 ± 0.36	
50/50 C8-TEOS/TMOS (30 min)[c]	–	3.70 ± 0.08	

[a]95% confidence limits; [b]mean of three independent measurements for coatings stored in air prior to measurement; imprecision is reported as ± 1 SD from three independent measurements; [c] sol processing time in parentheses.

The technique of 'advanced angle' analysis was used, wherein a sessile drop of liquid is placed on the sample surface and the angle of contact (θ) between the liquid and the solid is measured with a contact angle goniometer (Rame-Hart, Model NRL 100); both sides of the droplet profile are measured. Another droplet of the same fluid is placed on top of the first droplet (the fluid is advanced across the surface), and the measurements are repeated. If the contact angles for the first droplet are $\leqslant 20^\circ$, no further measurements are taken for that liquid on the sample; fluids having contact angles of $\leqslant 20^\circ$ use a relatively large amount of the limited sample surface area. Zisman plots were constructed by plotting the cosine of the average angle measured for each liquid against the liquid/vapor surface tension of the diagnostic liquid (Zisman, 1964; Baier & Meyer, 1992). A linear least squares analysis is performed to determine the critical surface tension (γ_C) of the sample at the cos $\theta = 1$ axis. In cases of large data scatter (non linearity), the data for the spreading liquid ($\theta = 0$) with the greatest liquid/vapor surface tension and for those liquids closest to, but greater than, in surface tension to the first spreading liquid are used to determine γ_C.

Settlement and strength of attachment assay for Ulva *zoospores*

Fertile plants of *Ulva linza* were collected from Wembury Beach, England (50°18' N; 4°02' W). Zoospores were released and prepared for attachment as previously described (Callow et al. 1997). Ten ml aliquots (1.5×10^6 spore ml^{-1}) were pipetted into individual compartments of polystyrene 'quadriperm' culture dishes (Fisher), each containing a glass microscope slide coated with a test xerogel surface for a total of six replicates of each test surface. After 1 h incubation in darkness at ~20°C, all slides were gently washed in seawater to remove zoospores that had not attached. All slides were then incubated in the light at ~20°C for a further 3 h (total of 4 h). The density of zoospores attached to the surface was counted on each of three replicate slides using an image analysis system attached to a fluorescent microscope. Spores were visualized by chlorophyll autofluorescence and counted as previously described (Callow et al. 2002). Thirty counts were taken at 1mm intervals along the middle of the long axis of each of the three replicate slides (each view was 0.064 mm^2). Means ($x = 90$) and 95% confidence limits were calculated and expressed as mean number of attached (settled) spores mm^{-2}.

The remaining three replicate slides settled with 4-h zoospores were exposed to an automated water

jet (Finlay et al. 2002b; Pettitt et al. 2004) that produced a pressure at the surface of 64 kPa. A 5 cm^2 area of the sample was subjected to the compressive and shear stresses induced by the water jet. The number of spores remaining attached was counted as described above. The mean number of spores remaining attached to the slide surface after exposure to the water jet was compared with the mean number on the controls, i.e., slides not subjected to the water jet. Results are expressed as percentage removal; 95% confidence limits were calculated from arcsine transformed data. Significant differences in removal were identified using one-way variance supported by Tukey tests.

Leaching assays for toxic compounds during spore settlement

Spore motility was used to indicate whether toxic compounds were leaching from the xerogel coatings. At the washing stage (1-h post addition of zoospores), the seawater and unsettled zoospores were poured into 20-ml glass vials and placed in the light. If toxic materials had leached from the coatings, the swimming zoospores would have lost their ability to swim (zoospores are negatively phototactic and quickly move to the shaded side of a container) and hence they would have remained in suspension (Callow et al. 1997; 2000).

Growth and strength of attachment assays for Ulva *sporelings*

Zoospores were settled for 1 h as described above, with four replicates per test surface. After washing, the slides were replaced in 'quadriperm' dishes and 10 ml of enriched seawater medium (Starr & Zeichus, 1987) were added. Sporelings were cultured under a 16h:8h light:dark cycle at 18°C. The medium was refreshed every two days and the sporelings cultured for eight days. Biomass was estimated by extraction of chlorophyll *a* in dimethylsulfoxide (Shoaf & Lium, 1976) using the equations of Jeffrey and Humphrey (1975). Biomass was harvested by using a razor blade to scrape off half of each slide. Results are expressed as mean weight of chlorophyll *a*/unit area of test surface. Error bars reflect the standard error of the mean.

To evaluate the sporeling attachment strength to the surfaces, slides were placed in a flow channel (Finlay et al. 2002a) and exposed to fully developed turbulent flow for 5 min at 55Pa wall shear stress as described in Shultz et al. (2000). The slides were positioned such that the area from which the biomass had been removed was at the leading edge with respect to water flow. Biomass was harvested from the remaining half of each slide and the chlorophyll extracted. Percentage removal was calculated as for the spores. Error bars represent the standard error of the mean from arcsine-transformed data.

Cyprid settlement assays using B. amphitrite

Thirty to 50 larvae were 'drop assayed' onto each of the replicate surfaces. Assays lasted approximately 48 h; although the exact duration depended on the time it took 50% of the larvae to settle in the control standard – an uncoated glass slide. Stopping the assays after approximately 50% of the larvae have settled on the control surfaces provides information on antifouling and inductive characteristics of the experimental coatings. At the end of the initial assay period the numbers of larvae that successfully attached and metamorphosed were counted. Larvae that did not settle by the end of the 24 h period were observed for signs of abnormal behavior to assess any compromise to normal physiological function. Fouling resistance was estimated by determining the percentage of individuals settling on each coating. Settlement of larvae was ignored on portions of the xerogel surfaces with coating defects.

Laboratory rearing of juvenile barnacles

Newly metamorphosed juveniles on their respective coatings were transferred to growth chambers where they were fed the unicellular green alga *Dunaliella* sp. and the diatom *Skeletonema costratum* for two weeks and then a mixture of *Dunaliella* sp., *S. costratum*, and naupliar larvae of *Artemia* sp. Juveniles were maintained in a constant temperature incubator at 25°C on a 12 h:12 h light:dark cycle for 6 – 8 weeks, which is the time it took the juvenile barnacles to reach a basal plate diameter of 3 – 5 mm, the minimum size necessary to conduct force gauge tests according to ASTM D 5618.

Critical removal stress for barnacles in shear

The procedures for critical removal stress followed from ASTM D 5618 with the following modifications: (i) The force measuring device was operated by a motorized stand, thus insuring a constant application of force during dislodgement; and (ii) Barnacle dislodgement studies from coatings were performed under water. The apparatus consists of an IMADA AXT 70 digital force gauge (4.4lb) mounted on an IMADA SV-5 motorized stand. The slides are clamped into a custom-built Plexiglas chamber that allows their complete submersion during dislodgement tests.

Juvenile barnacles were selected for testing based on healthy appearance and minimum size requirements. Only barnacles occurring at least 5 mm from

the edges of the slide were tested. Other barnacles in close proximity to the test subject were removed if they could potentially interfere with measurements. The barnacle base dimensions were measured with calipers. The base diameter was measured in two directions perpendicular to one another. Basal area (A) was then estimated using the formula $A = \pi(0.5d_1)(0.5d_2)$, which is the area of an ellipse. After size measurements were taken the slide was clamped into the Plexiglas chamber. The force gauge mounted on the motorized stand was used to apply a shear force to the base of the barnacles at a rate of approximately 4.5 N s^{-1} (1 lb s^{-1}) until the organism was detached. Force was applied parallel to the film surface. The force required for detachment was noted and observations were made as to the mode of failure. If any portion of the base of the organism was left attached to the substratum, the test was deemed void. The surfaces were examined visually for damage to the xerogel film caused by barnacle removal and by stereomicroscope if there were any ambiguity. The critical removal stress was calculated by dividing the force (F, Newtons) required to remove the test subject by the area of attachment (A, mm^2).

Results

Characterization of xerogel surfaces

Profilometry showed (32 replicates) that the xerogel films were 1.0 ± 0.1 μm thick. While films this thin would not be 'practical' surfaces for marine deployment, the uniformity offered by the spin casting method provides model surfaces that can be reproduced lot to lot.

Figure 1 presents three typical SEM images of xerogel films derived from pure TMOS, 45/55 C3-TMOS/TMOS, and 50/50 C8-TEOS/TMOS. The pure TMOS film was highly cracked and poorly adherent. The hybrid xerogel film surfaces were more uniform and uncracked (Tang et al. 2003). The incorporation of the organic functional groups in the hybrid xerogels reduces the available crosslinking in the silicate structure from $Si(OSi\equiv)_4$ in the pure TMOS or TEOS to $RSi(OSi\equiv)_3$ in the hybrid xerogels leading to a more flexible, less friable surface.

The organic residues within the C3-TMOS, C8-TEOS, TFP-TMOS, and enTMOS silanes are not involved with crosslinking in the silicate and could organize randomly in the sol particles/xerogels, could lie on the surface of the sol particles/xerogels, or could organize within the sol. To address this issue the XPS spectra of the xerogel films at a take-off angle of 45° were recorded to determine the atomic composition at the surface of each xerogel coating/film. The results are compiled in Table I. In glass, the ratio of the C(1s)/Si(2p3) signals was 0.2, which suggests a relatively carbon-free surface. The fact that there is any carbon found at the ostensibly SiO_2 surface arises from adventitious/adsorbed carbon-containing species from the atmosphere. The C/Si for each of the xerogel surfaces was much higher in comparison to the glass standard, ranging from 1.1 for the 2/98 enTMOS/TEOS surface to 3.9 for the 50/50 C8-TEOS/TMOS surface with a 2-h hydrolysis time. These results suggest that the carbon-bearing functionality is on the hybrid film surfaces. Further support for this conclusion comes from the

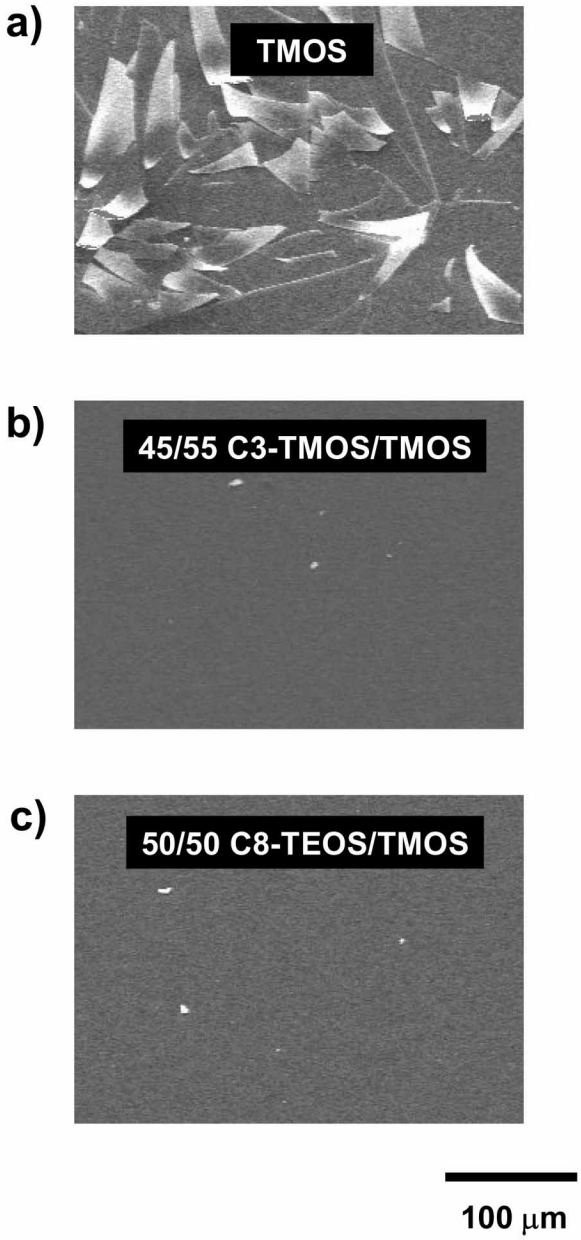

Figure 1. SEM images of (a) pure TMOS, (b) 45/55 C3-TMOS/TMOS, and (c) 50/50 C8-TEOS/TMOS xerogel films.

N/Si ratio for the 2/98 enTMOS/TEOS surface of 0.5 and the F/Si ratio of 1.4 for the 50/50 TFP-TMOS/TMOS indicating that the functional groups to which these two heteroatoms are attached are also on the hybrid xerogel film surface (see Table I).

The xerogel surfaces and the glass standard were also characterized by comprehensive advanced contact angle analyses with up to 12 different diagnostic fluids in addition to water (Zisman, 1964; Baier & Meyer, 1992). Zisman plots were constructed by plotting cos θ, where θ is the advanced contact angle, against the liquid/vapor surface tension of the diagnostic liquid. The intercept of a linear least-squares analysis of the data with the cos $\theta = 1$ axis defines the critical surface tension of the surface, γC. Values of γC for each of the four xerogel surfaces and the glass standard are compiled in Table I and range from approximately 20 mN m^{-1} for the 45/55 C3-TMOS/TMOS, 50/50 C8-TEOS/TMOS, and 50/50 TFP-TMOS/TMOS xerogel films to approximately 35 mN m^{-1} for the 2/98 enTMOS/TMOS xerogel film and the glass standard.

Settlement and adhesion studies on Ulva *zoospores*

The xerogel surfaces were incubated in stirred seawater at $\sim 20^{\circ}$C for 24 h prior to the zoospore assay. None of the xerogel films changed visually.

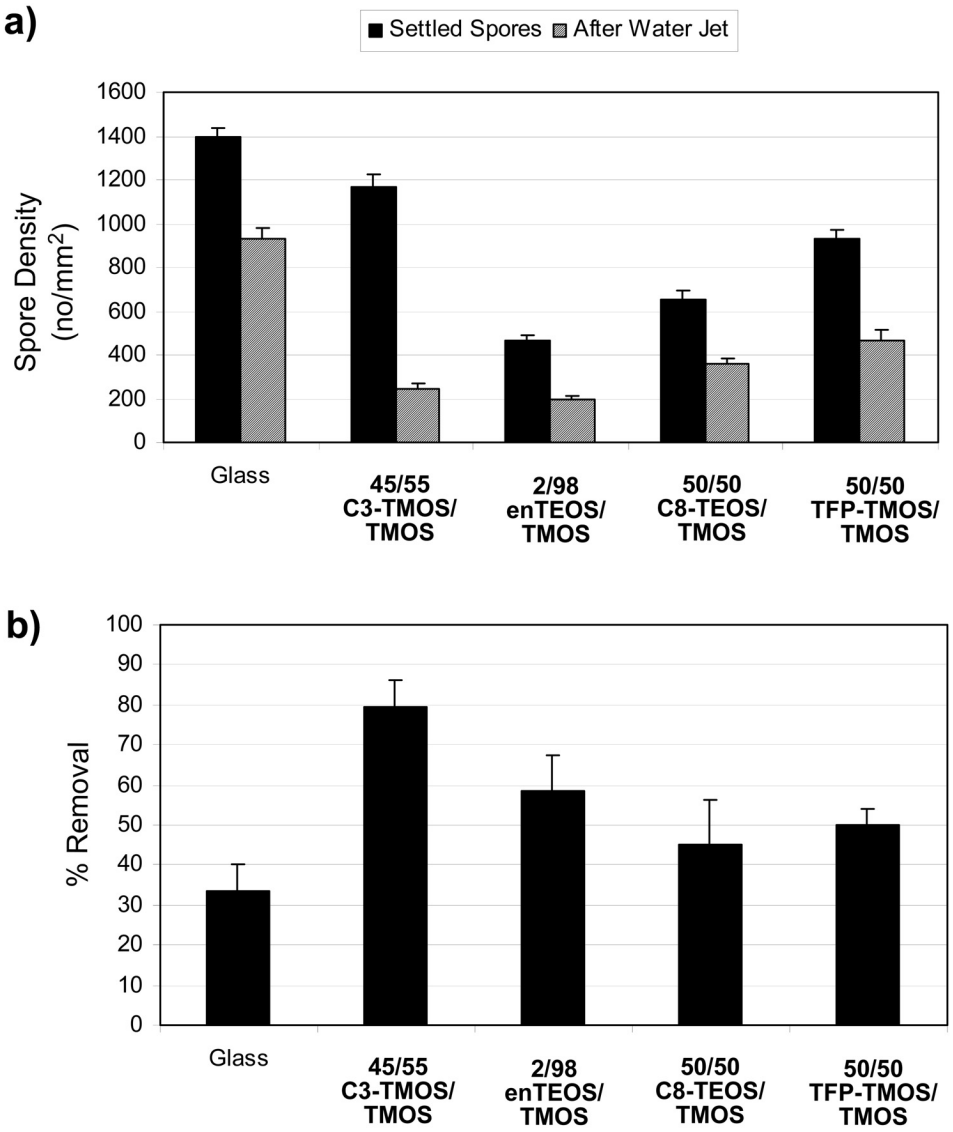

Figure 2a. The settlement of *Ulva* zoospores on sol-gel coatings and the number of spores remaining after exposure to a water-jet surface pressure of 64 kPa. Each histogram bar is the mean from 90 counts, 30 from each of three replicate slides. Error bars show 95% confidence limits. Figure 2b. The percentage removal of zoospores following exposure to the water jet. Each histogram bar represents the mean percentage removal of zoospores from 90 observations of controls (30 from each of three replicate slides) and 90 observations of treatments (30 from each of three replicate slides). Error bars represent 95% confidence limits from arcsine transformed data.

The results of settlement and detachment studies of *Ulva* zoospores are summarized in Figure 2.

As shown in Figure 2a, the number of spores that settled on all four xerogel films was lower in comparison to the glass standard. One-way analysis of variance showed that the settlement density on all four xerogel films was significantly less than the settlement density on the glass control (F4, 445 = 293 $p < 0.05$). None of the leachates from the xerogel films were toxic to *Ulva* zoospores.

The strength of attachment of 4-h zoospores to each surface was measured by exposure to a surface stress of 64 kPa water pressure, delivered by a water jet. Removal of zoospores was greater from all of the xerogel film surfaces relative to the glass standard. As shown in Figure 2b, ~33% of the settled spores were removed by the water jet from glass and 45–79% from the xerogel surfaces. Removal of zoospores from the 45/55 C3-TMOS/TMOS (2-h sol processing time) xerogel coating was significantly greater than from the other three xerogel surfaces or from the glass standard (one-way analysis on arcsine transformed data F4, 445 = 61 $p < 0.05$).

Settlement of B. amphitrite *cypris larvae*

The settlement of *B. amphitrite* cypris larvae on the 45/55 C3-TMOS/TMOS, 50/50 C8-TEOS/TMOS, and 2/98 enTMOS/TMOS hybrid xerogels showed a trend toward reduced settlement on these films relative to the glass standard (see Figure 3). However, only settlement on the 50/50 C8-TEOS/TMOS film was significantly less than the glass standard ($p < 0.05$). This reduced settlement did not appear to be because of any adverse physiological effect upon the barnacle larvae; cyprids were active and behaving normally on all coatings for the duration of the test. Settlement on the 50/50 TFP-TMOS/TMOS xerogel films was greater than on the glass standard (Figure 3).

Growth and adhesion strength of Ulva *sporelings*

The eight-day sporeling growth and biomass removal studies are summarized in Figure 4. The growth of sporelings in terms of chlorophyll *a* per unit area was substantially reduced on the hybrid xerogel film surfaces relative to the glass control (one-way analysis of variance and Tukey test (F 4, 15 = 13.5 $p < 0.05$).

The detachment of sporelings in terms of chlorophyll *a* per unit area showed that there were significant differences in the strength of adhesion within the data set at the 5% level. On the glass standard, only 20% of the sporeling biomass was removed in the flow cell while 38–67% of the sporeling biomass was removed from the hybrid xerogel film surfaces (see Figure 4b). The highest percentage of biomass removed was found in the 50/50 (mol ratio) C8-TEOS/TMOS film (2-h sol processing time), which was the only treatment significantly different from the glass control (one-way analysis of variance on arcsine transformed data F4, 14 = 3.61 $p < 0.05$). The 45/55 (mol ratio) C3-TMOS/TMOS, 2/98 (mol ratio) enTMOS/TMOS, and 50/50 (mol ratio) TFP-TMOS/TMOS hybrid xerogels were similar to one another in terms of percent sporeling biomass removal and were not

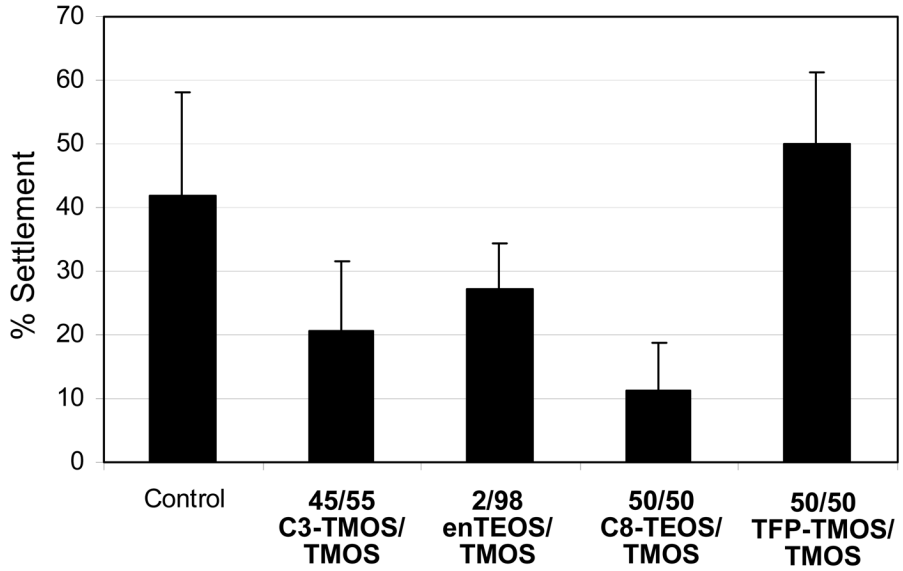

Figure 3. Percent settlement of *B. amphitrite* on xerogel coatings and a glass standard surface. Each histogram bar represents the mean of five replicate trials for each condition. Error bars are equal to one SE of the mean.

significantly different in comparison to the glass standard.

Removal of juvenile barnacles

The juvenile barnacle strength of attachment to all four hybrid xerogel films was measured via force gauge measurements to forces applied in shear. Only the 50/50 C8-TEOS/TMOS xerogel films performed as fouling-release surfaces. Barnacles on all other coatings broke when force was applied to them in shear, and left a base plate attached to the surface (see Figure 5). Critical removal force could not be evaluated for these barnacles, because force gauge measurements reflected the barnacle cohesive strength itself, not the barnacle adhesion strength to the surface. The 50/50 C8-TEOS/TMOS hybrid xerogel (2-h sol processing time) released 100% of the adhered barnacles with a mean critical removal force of 0.097 ± 0.025 N mm^{-2} (14.1 ± 3.6 psi) (see Table II).

Influence of hydrolysis time on fouling-resistance/fouling release

Sol hydrolysis time (sol processing time) can influence the properties of the resulting xerogel by impacting the porosity of the xerogel and the extent

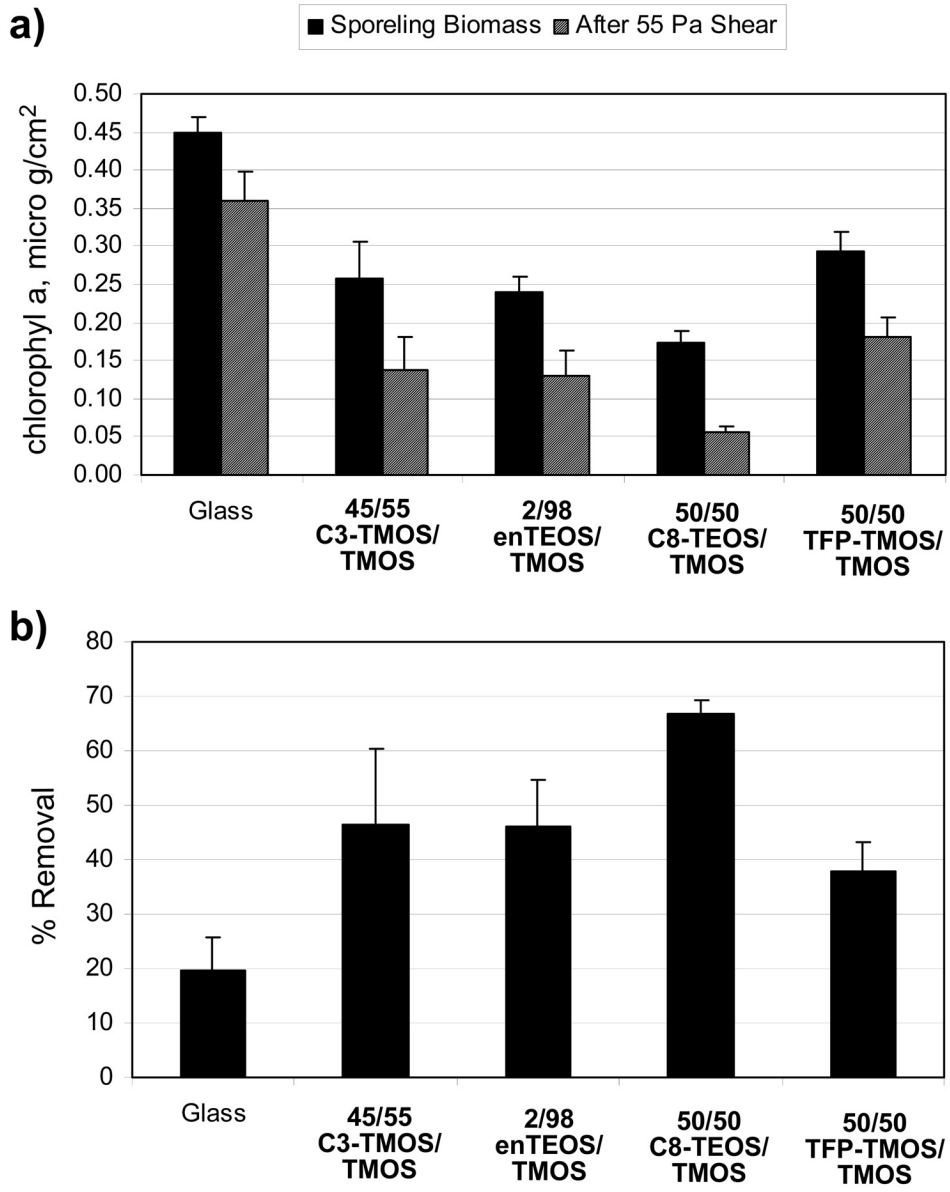

Figure 4a. The biomass of *Ulva* sporelings after eight days' growth and the biomass remaining after exposure to shear stress of 55 Pa, measured as chlorophyll *a* (μg cm^{-2}), and Figure 4b. The percent removal of sporeling biomass. Each histogram bar is the mean of four replicate slides. Error bars represent one SE of the mean.

Figure 5. An example of two base plates of *B. amphitrite* on a coating after shear removal tests. The applied shear force removed the animals from their base plates. Each base plate is about 5 mm in diameter.

of crosslinking (Brinker & Scherer, 1990; Avnir, 1995; Dave et al. 1995; Ingersoll & Bright, 1997). The 50/50 C8-TEOS/TMOS hybrid xerogel behaved as both a fouling-resistant and a fouling-release surface. The settlement of *Ulva* zoospores and the cypris larvae of *B. amphitrite* was examined on 50/50 C8-TEOS/TMOS hybrid xerogels with hydrolysis times of 0.5, 1, and 2 h. These data are summarized in Figure 6.

All three surfaces were similar to one another with respect to settlement of either *Ulva* zoospores or barnacle cyprids. Settlement of *Ulva* spores on all three hybrid xerogel surfaces was significantly reduced relative to the glass standard (one-way analysis of variance, $F_{3,359} = 166$ $p < 0.05$) but there was no significant difference among the three xerogels with different processing times. Settlement of barnacle cypris larvae on the 50/50 C8-TEOS/TMOS hybrid xerogel with a 1-h hydrolysis time was significantly less compared to the glass standard ($p < 0.05$) although the differences among the three xerogel films were not significant ($p > 0.05$).

Hydrolysis times had no significant impact on the critical removal force for juvenile barnacles as shown in Table II. Approximately 50% of the barnacles were removed intact from the 50/50 C8-TEOS/TMOS hybrid xerogels with 0.5-h and 1-h hydrolysis times with 13 and 11 total barnacles, respectively, removed from each surface. Although 100% of the barnacles were removed from the 50/50 C8-TEOS/TMOS xerogel with a 2-h hydrolysis time, the total number of barnacles removed was only four, which obfuscates any statistical comparison of these surfaces.

Discussion

The four xerogel surfaces used in this study consisted of:

(1) 45/55 (mol ratio) *n*-propyltrimethoxysilane (C3-TMOS)/tetramethylorthosilane (TMOS);
(2) 2/98 (mol ratio) bis[3-(trimethoxysilyl)propyl]ethylenediamine (enTMOS)/TEOS;
(3) 50/50 (mol ratio) *n*-octyltriethoxysilane (C8-TEOS)/TMOS; and
(4) 50/50 (mol ratio) 3,3,3-trifluoropropyltrimethoxysilane (TFP-TMOS)/TMOS.

These particular materials were selected to provide a range of hydrophobic (45/55 C3-TMOS/TMOS, 50/50 C8-TEOS/TMOS, and TFP-TMOS/TMOS xerogel films) and hydrophilic (2/98 enTMOS/TEOS) surfaces. Results from these xerogel coatings were compared to those from cleaned uncoated glass slides as a standard surface with respect to settlement and removal of *Ulva* zoospores, growth and removal of *Ulva* sporelings, the settlement of cypris larvae of *B. amphitrite*, and the removal of juvenile *B. amphitrite* barnacles

Sol processing time can influence the properties of the resulting xerogel by impacting the porosity of the xerogel and the extent of crosslinking (Brinker & Scherer, 1990; Avnir, 1995; Dave et al. 1995; Ingersoll & Bright, 1997). The 45/55 C3-TMOS/TMOS and 50/50 C8-TEOS/TMOS xerogel films were formed after the sol solutions had hydrolyzed for 2 h while the 2/98 enTMOS/TEOS xerogel films were formed after the TEOS sol solutions had hydrolyzed for 6 h. The 50/50 TFP-TMOS/TMOS xerogels were coated following sol processing for 30 min. As described below, the performance of the 50/50 C8-TEOS/TMOS coating in settlement and removal studies surpassed those of the other xerogels. The 50/50 C8-TEOS/TMOS xerogel was also coated after the sol solution was allowed to hydrolyze for only 0.5 and 1 h to examine the effect, if any, of hydrolysis time (i.e., sol-processing time) on observed film performance.

For the 50/50 C8-TEOS/TMOS hybrid xerogel coatings, sol hydrolysis times between 0.5 and 2 h do not appear to be critical to xerogel performance.

On many surfaces, high settlement is associated with a high water contact angle (Callow et al. 2000). However, recent studies with polystyrene-based, surface-active block copolymers have shown that surface wettability alone cannot predict settlement in some systems (Youngblood et al. 2003). For *Ulva* zoospores and for cypris larvae of *B. amphitrite*, both hydrophilic and hydrophobic xerogel films displayed reduced settlement relative to a glass standard, which suggests that settlement

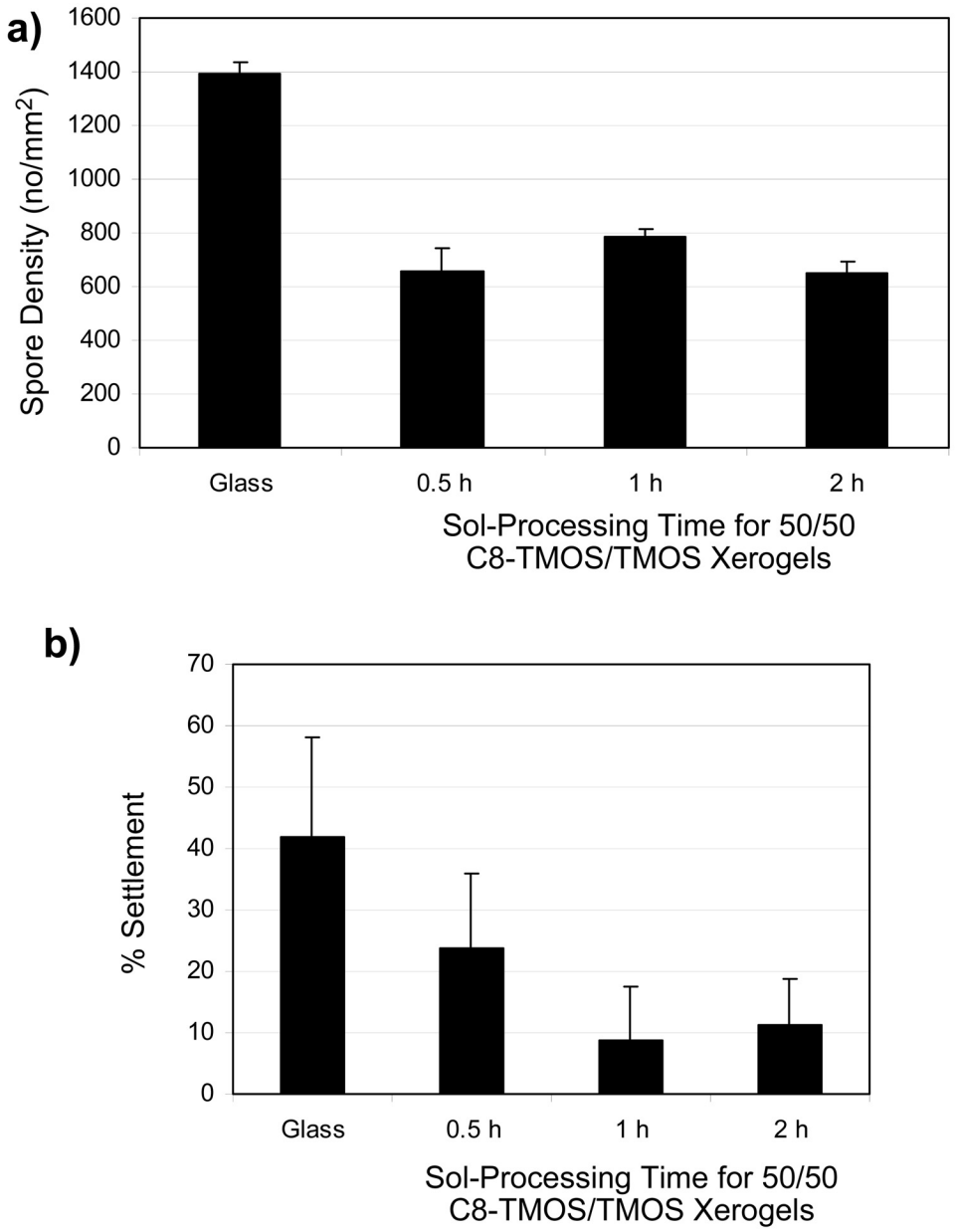

Figure 6. The effects of sol-processing time on the fouling-resistance performance of 50/50 C8-TEOS/TMOS xerogel surfaces relative to a glass standard for a) settlement of *Ulva* zoospores and b) settlement of *B. amphitrite* cypris larvae. Error bars represent one SE of the mean.

must be determined by some factor other than wettability alone.

Comprehensive contact angle analysis characterizes the first 4–5 Å of a surface and the critical surface tension, γC, of surfaces measured by such analysis empirically and reproducibly characterizes the surface (Zisman, 1964; Baier & Meyer, 1992). There is a zone of minimal bioadhesion for surfaces with values of γC between 20 and 30 mN m^{-1} where weak boundary layers are formed, which allows biofouling to be removed by shear forces (Baier et al. 1968; Baier, 1984; Baier & Meyer, 1992). The 45/55 C3-TMOS/TMOS, 50/50 C8-TEOS/TMOS, and 50/50 TFP-TMOS/TMOS xerogels of this study had values of γC of 21, 20, and 20 mN m^{-1}, respectively (Table I), which fall within this range. The 45/55 C3-TMOS/TMOS xerogel gave significantly greater removal of *Ulva* zoospores relative to the other xerogel coatings and glass controls. The 50/50 C8-TEOS/TMOS xerogel films gave significantly greater removal of *Ulva* sporeling biomass relative to the other xerogel films and glass controls. The 50/50 C8-TEOS/TMOS xerogel film also performed as a fouling-release surface for juvenile barnacles.

The performance of the 50/50 C8-TEOS/TMOS hybrid xerogel films is comparable to the perfor-

Table II. Mean critical removal force for 50/50 C8-TEOS/TMOS xerogel coatings with different sol-processing times.

Sol-processing time	Number of barnacles removed	Percentage barnacles removed intact	Mean critical removal force, N mm^{-2}
2 h	4	100	0.097 ± 0.025
1 h	13	54	0.107 ± 0.025
0.5 h	11	45	0.078 ± 0.025
Gelest PDE[a]	31	87	0.069 ± 0.030

[a]Gelest PDE is provided as a reference fouling-release coating. Data were collected at a different time and using a different batch of barnacles.

mance of a polydimethylsiloxane elastomer (Gelest PDE, Wendt et al. personal communication) as shown in Table II with respect to mean critical removal force for juvenile barnacles as well as the percentage of barnacles removed intact. A total of 31 barnacles were removed from the Gelest PDE surfaces with 87% removed intact while 28 barnacles were removed from the combined 50/50 C8-TEOS/TMOS surfaces with 57% removed intact. The mean critical removal force of 0.069 ± 0.030 N mm^{-2} for the Gelest coating is not significantly different than the average removal force of 0.092 ± 0.015 N mm^{-2} for the barnacles removed intact from the combined 50/50 C8-TEOS/TMOS surfaces ($p > 0.05$).

Overall, the results show that surfaces based on sol-gel-derived xerogel materials offer a novel pathway to the development of marine antifouling coatings. The xerogel films can be tuned (Cho et al. 2002) to provide surfaces of different wettability and critical surface tension. Reduced *Ulva* zoospore settlement, increased removal of zoospores, increased removal of *Ulva* biomass, and fouling release of juvenile barnacles of *B. amphitrite* have been achieved with xerogel surfaces of low wettability and low critical surface tension. Other xerogel surfaces as well as active xerogel surfaces that might improve fouling resistance are being explored.

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