Final Scientific/Technical Report

Environmentally Benign and Permanent Modifications to Prevent Biofouling on Marine and Hydrokinetic Devices

Semprus BioSciences

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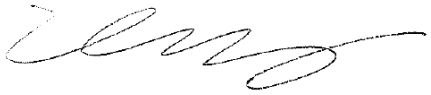


Zheng Zhang

April 17, 2012

Executive Summary

Semprus Biosciences is developing environmentally benign and permanent modifications to prevent biofouling on Marine and Hydrokinetic (MHK) devices. Biofouling, including growth on external surfaces by bacteria, algae, barnacles, mussels, and other marine organisms, accumulate quickly on MHK devices, causing mechanical wear and changes in performance. Biofouling on crucial components of hydrokinetic devices, such as rotors, generators, and turbines, imposes substantial mass and hydrodynamic loading with associated efficiency loss and maintenance costs. Most antifouling coatings leach toxic ingredients, such as copper and tributyltin, through an eroding process, but increasingly stringent regulation of biocides has led to interest in the development of non-biocidal technologies to control fouling. Semprus Biosciences' research team is developing modifications to prevent fouling from a broad spectrum of organisms on devices of all shapes, sizes, and materials for the life of the product.

The research team designed and developed betaine-based polymers as novel underwater coatings to resist the attachment of marine organisms. Different betaine-based monomers and polymers were synthesized and incorporated within various coating formulations. The formulations and application methods were developed on aluminum panels with required adhesion strength and mechanical properties. The coating polymers were chemically stable under UV, hydrolytic and oxidative environments. The sulfobetaine formulations are applicable as nonleaching and stable underwater coatings.

For the first time, coating formulations modified with highly packed sulfobetaine polymers were prepared and demonstrated resistance to a broad spectrum of marine organisms. Assays for comparing nonfouling performance were developed to evaluate protein adsorption and bacteria attachment. Barnacle settlement and removal were evaluated and a 60-day field test was performed. Silicone substrates including a commercial fouling release coating were used for comparison. Compared with the unmodified silicone substrates, the sulfobetaine-modified formulations were able to exhibit a 98% reduction in fibrinogen adsorption, 97.0% (*E. coli*), 99.6% (*S. aureus*), and 99.5% (*C. lytica*) reduction in bacteria attachment, and 100% reduction in barnacles cyprid attachment. In addition to the significant improvement in fouling resistance of various organisms, the 60-day field test also showed an evident efficacy from visual assessment, foul rating, and fouling removal test.

The research confirmed that the novel antifouling mechanism of betaine polymers provides a new avenue for marine coating development. The developed coatings out-performed currently used nontoxic underwater coatings in a broad spectrum of fouling resistance. By further developing formulations and processing methods for specific devices, the technology is ready for the next stage of development with demonstration in MHK systems.

Contents

1. Project Overview

The research performed by Semprus BioSciences was an effort to develop benign and permanent modifications to prevent biofouling on marine and hydrokinetic (MHK) devices. These modifications will improve efficiency, maintenance requirements, and environmental impact of MHK systems by reducing marine biofouling.

Biofouling (growth on external surfaces by bacteria, algae, barnacles, mussels, and other marine organisms) accumulates fast on MHK devices, causing mechanical wear and reducing performance. Biofouling on crucial components of hydrokinetic devices such as rotors, generators, and turbines imposes substantial mass and hydrodynamic loading with associated efficiency and maintenance penalties. Most anti-fouling approaches rely on non-permanent coatings, which leach active ingredients such as copper and tributyltin (TBT) through an eroding or self-polishing process. Increasingly stringent regulation of biocides has led to interest in the development of non-biocidal technologies to control fouling [1]. Non-toxic foul-release coatings are developed based on silicones and fluoropolymers with low surface energy [2]. Nearly all silicone foul-release coatings are augmented with an oil additive to decrease macrofouling attachment strength [3]. However, these coatings cannot resist biofouling and the foulants have to be removed on a floating platform or onshore for a static device. The betaine technology is a long lasting polymer surface that harnesses water and mimics biological entities, preventing the adhesion of bacteria, barnacles, and other marine organisms (Figure 1)[4-6].

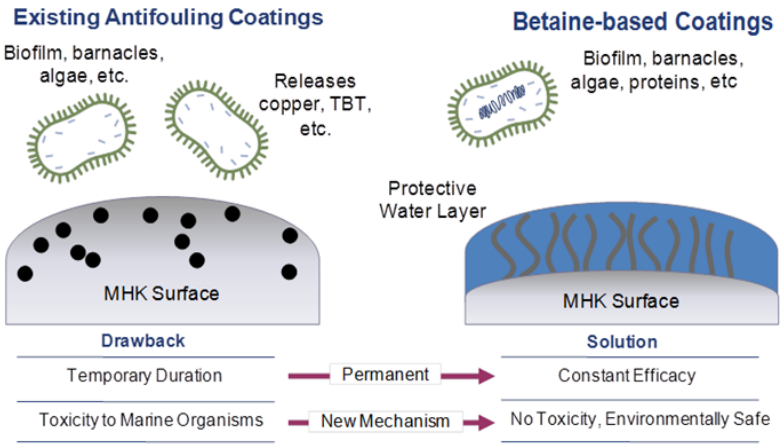


Figure 1. Existing anti-fouling coatings vs. proposed betaine-based coating

Through the span of this project, betaine-based monomers and copolymer combinations were investigated first on polydimethylsiloxane (PDMS), a model substrate to apply marine coating. Then the selected formulations were followed by the optimization of adhesion and performance of silicone coated aluminum samples. Final formulations were tested in house for protein adsorption, biofilm adhesion, and stability, and sent to external testing centers for barnacle adhesion assays and field testing. The project calendar is displayed in Figure 2.

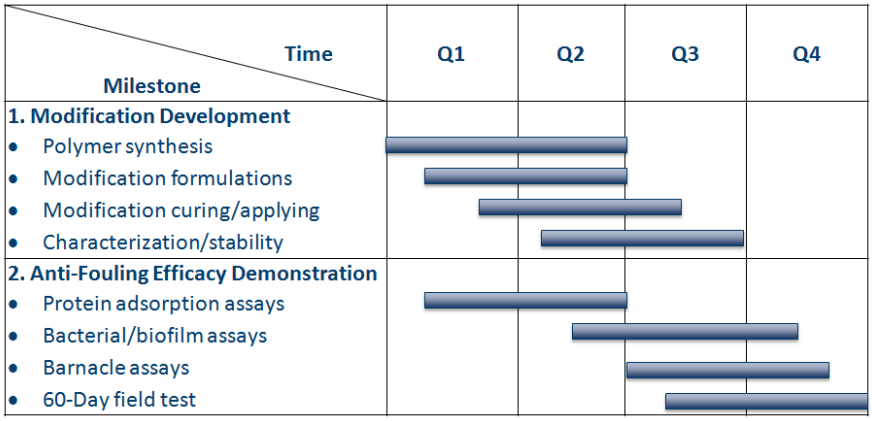


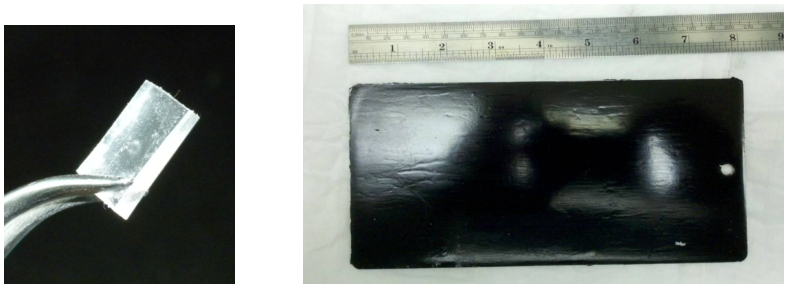
Figure 2. Project Calendar for year 2011

The proposed research included two milestones. The first milestone was to design modification systems and synthesize polymers applicable for permanent anti-fouling modifications. The second milestone was to demonstrate a broad spectrum of anti-biofouling performance, long-term efficacy, and biocompatibility. Through a year of research, both of the proposed milestones were achieved. The research team confirms that betaine-based polymers can be designed as novel antifouling underwater coatings. The developed betaine-based formulations are able to be applied on underwater substrates with long-term stability and nontoxic characteristics. The coatings present a broad spectrum of anti-fouling performance in protein resistance, bacteria attachment, barnacle settlement, which outperformed commercial antifouling coatings. The developed technology is ready for further demonstration in MHK systems.

2. Coating formulations and Application Methods

2.1 PDMS films

Initial work was developed on thin films of PDMS to prove the ability to modify silicone with poly-sulfobetaines. Creating and modifying PDMS films allowed for a high throughput of proof of concept data without raising issues unrelated to the sulfobetaine methacrylate (SBMA, N-(3-sulfopropyl)-N-methacryloxyethyl-N,N-dimethylammonium betaine) and sulfobetaine methacrylamide (SBMAam, [3-(methacryloylamino)-dimethyl(3-sulfopropyl)ammonium hydroxide) modification (i.e. adhesion of silicones to primary substrates). Sylgard 184 (Dow Corning) is a two-component silicone curing kit that is specified to be mixed in a 10 : 1 (A : B) ratio for optimum performance. Formulation blends of were mixed until homogeneous and then poured into a PTFE dish until the film was 2 mm thick. The dish was placed into a 37 ^{o}C oven for 48 hours to ensure full cure prior to use. Cured films were then cut into 5 x 10 mm rectangles. These were strung on stainless steel wire to keep substrates from touching each other while reacting (Figure 3a).



(a) (b)

Figure 3. (a) A Sylgard 184 PDMS film (10 x 5 mm), and (b) Intersleek 425-coated aluminum panel (4 X 8"). Both substrates were modified with a sulfobetaine-based coating.

2.2. Silicone-Coated Aluminum Substrates

2.2.1. Silanization

For optimum adhesion to aluminum, the aluminum substrate was first rinsed with acetone and DI water to remove residual chemicals, then ammonized by rinsing in 2 % (w/v) APTMS ((3-aminopropyl)-trimethoxysilane, Sigma-Aldrich) in toluene for 10 minutes. Excess APTMS and toluene was rinsed with acetone and DI, and then the sample was dried.

2.2.2. Epoxy Primer

For a marine coating formualation, it is important to prevent rust and other corrosion underwater. Intergard 264 (International), a universal anticorrosive epoxy, prepared in a 4 : 1 volume ratio was then applied to the surface of the silanized aluminum using a paint roller so that the entire sample was coated with a thin conformal grey coating. The epoxy was allowed to cure at least 12 hours so that curing was complete before coating it further. In some cases a tie-layer, an adhesive layer that binds the epoxy primer and a topcoat, was used to coat the epoxy, followed by the application of topcoat.

2.2.3. Sylgard 184

As a model surface for silicone coating, curing Sylgard 184 on aluminum was attempted. When mixed at the specified 10 : 1 ratio and used to dip coat aluminum, it would cure with very poor adherence. The addition of GPTMS (3- glycidoxypropyltrimethoxysilane, Sigma-Aldrich) sometimes was used to increase adherence to amino groups. When concentrations as low as 1 % GPTMS were added to a Sylgard 184 mixture, curing could not occur after six days at 37 ^{o}C (usually curing in two days). To further develop a model silicone coating on aluminum, an industrial silicone rubber, Elastosil M4514, was applied on aluminum.

2.2.4. Intersleek 425

Intersleek 425 (Azko Nobel) was a three-component industrial marine coating system. No tie layer was specified to be used with Intersleek 425, so the top coat was applied directly to the epoxy layer. The optimum ratio for curing pure Intersleek 425 was 15:4:1 (wt). During the preparation and testing of Intersleek 425, a film applicator was used to spread the topcoat over the epoxy layer. At some point after the first set of barnacle tests, Intersleek 425 was discontinued and an updated version of the silicone coating, Intersleek 757, took its place.

2.2.5. Intersleek 757

Intersleek 757 (Azko Nobel) is also a three component industrial marine coating system. Adherence of the Intersleek 757 was optimum when a tie layer, Intersleek 731 (Azko Nobel) is used, which is suggested by Azko Nobel. The Intersleek 731 was prepared in a 1 : 1 (A : B by volume) ratio and applied to the epoxy surface with a paint brush so that a thin, conformal pink coating exists on the surface. This was allowed to cure for 5 hours at room temperature. The top coat Intersleek 757 was then prepared in a 15 : 4 : 1 (A : B : C by volume) ratio and applied by paint brush to the cured tie layer. The properties of Intersleek 757 allow the coating to be thicker than that of the epoxy and tie layer, and creates a smooth surface ready to be modified.

2.2.6. Elastosil M4514

Elastosil M4514 (Wacker) is a two component system industrial silicone rubber that is most often used in mold making, and cures with a condensation reaction. Primer G, a mixture of primarily Naptha, was applied to the surface of the epoxy with a paint brush so a thin shiny layer exists to assist the adherence of the Elastosil silicone and allowed to dry for 3 hours. Elastosil M4514, a highly white viscous liquid rubber, was poured and then T Catalyst 121 Blue was added for 10 % wt mixture. Then the components were mixed until entire mixture was completely homogeneous light blue. A spatula or bar was used to coat the samples evenly, making sure to keep the edges coated and entire surface smooth, and the top coat was allowed to cure for 12 hours before performing any further chemistry.

2.3. Sulfobetaine Modification

For samples prepared on aluminum substrates for barnacle settlement and field test, only one side was coated with silicone. The other side was covered with laboratory tape after treating with APTMS, and the tape was removed after the topcoat was applied. During any reaction, the side previously taped would not have any initiator present, and would therefore not graft any polymer to the surface. For some samples with aluminum substrate for fibrinogen adsorption or bacteria attachment, both side need to be coated. In this case, the coating formulations were dip-coated on aluminum substrates, and all the surfaces of the samples were modified.

Typically samples produced (including those for barnacle testing) were performed on 2.5 x 7.5 cm aluminum slides. To perform modification on these samples, two slides were placed back to back in 50 ml reaction tubes (diameter of 2.5cm), solution was poured, bubbles were removed, and the tubes were placed in an oven at 37 ^{o}C on a shaker at 120 rpm for 18 hours. Samples produced for field testing were performed on 4 x 8” aluminum panels (Figure 5b). Reaction was performed on up to 7 panels at a time in a cylindrical 5L jacketed reactor. A stir bar kept flow throughout the reactor, and a nitrogen head kept oxygen from inhibiting the reaction. The jacket remained at 37 ^{o}C for 18 hours. When removed from reactors, all samples were fully washed with 150 mM saline until all excess entangled homopolymer was removed.

For zone of inhibition, microbiology, and protein resistance testing, thin films of the top coats were cast and reaction was applied on all sides. These procedures involved only mixing the top coat to the aforementioned ratios of components A, B, C, and the initiator and pouring the mixture into a PTFE dish or glass petri dish until 2-5mm thick, and then cutting into smaller pieces 5 x 10 mm before grafting sulfobetaine polymer in a reactor by the procedure mentioned for Sylgard 184.

3. Characterization

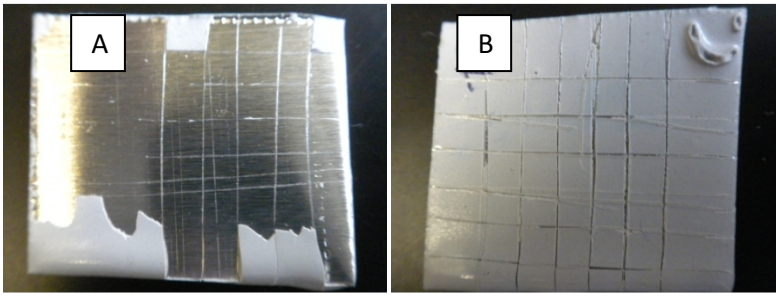
Throughout the whole research, the applied sulfobetaine polymers were characterized by various methods. At first, it was pertinent for the sulfobetaine layer, silicone layer, and the primer layer to remain adhered to the aluminum substrate with stability, which was tested with a tape test per ASTM standards. The modification was then analyzed via ATR FT-IR, X-ray Photoelectron Spectroscopy (XPS), Energy-dispersive X-ray Spectroscopy (EDS) to confirm the chemical structure and the elemental composition. The wettability of the surfaces was analyzed by both static and dynamic contact angle measurement. A surface staining method combined with optical microscopy was developed to check the homogeneity of the surface modification. Laser confocal microscopy and Scanning Electron Microscope (SEM) were used to examine the morphology of the surface and the cross-section of the coating.

3.1. Adhesion Strength

A tape test was conducted per ASTM D 3359-09. This test measures the mechanical adhesion of the coating on the substrate which it is bound to. The surface was washed and dried, and a $1cm^2$ location free of blemishes and minor surface imperfections was chosen to perform the test. Parallel cuts 2 mm apart were made with a new razor blade for a total of six cuts. Six cuts perpendicular to these and 2 mm apart created a 5 x 5 grid within the location chosen. Tape was applied to the surface and then peeled off. The adhesion strength was rated based on the amount of silicone that is removed from the surface.

This tape test was used to ensure that the Intergard 264 epoxy coating and subsequent tie-layers/top coats were adhered well enough to the surface under curing conditions before measuring effects of adding sulfobetaine polymers to the silicone surface. If poor adhesion was exhibited, chemical constituents of the mixtures were slightly altered until optimum adhesion was achieved. For control samples of industrial silicones and epoxies, a mixture ratio was provided by the manufacturing corporation and was kept consistent.

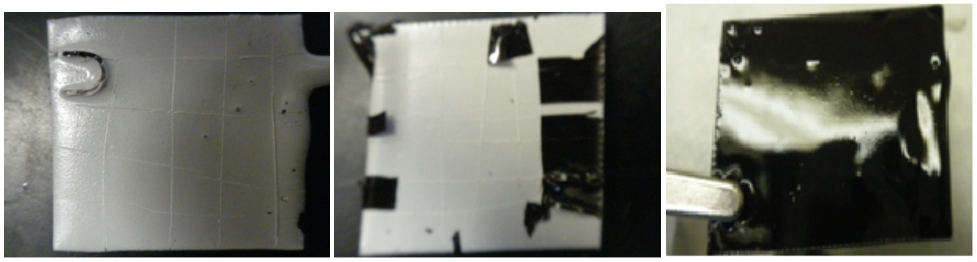
Figure 4 shows the improvement of adhesion of Intergard 264 epoxy coating on an aluminum substrate using the tape test. At first, the Intergard 264 epoxy cured on aluminum at designated mixture ratio yielded poor tape test results. It was found that treating the aluminum with APTMS to remove hydroxyl groups and add amino groups to the aluminum surface was sufficient to allow total adherence of epoxy after the challenge assay.



(a) (b)

Figure 4. a) Epoxy coating before silane treatment, and b)Epoxy coating after silane treatment.

Figure 5 shows an example of a tape test performed on a sample of Intersleek 731 on aluminum with Intergard 264 epoxy. When a grid was cut in the sample, the Intersleek coating was removed before the tape challenge was issued, and after that 100% of the top coat was removed. To increase adhesion of Intersleek 425, APTMS was added to the top coat to increase adhesion between it and the epoxy. The Intersleek 757 top coat was developed to adhere to a tie layer Instersleek 731, and did so without the need of additional silanes or other additives.



(a) (b) (c)

Figure 5. Intersleek 425 through tape test procedure with improved adhesion: (a) Intersleek 425, (b) Intersleek with 0.5% of AATMS, and (c) Intersleek with 2% APTMS.

3.2 Infrared Spectroscopy

Modification analysis was performed using ATR FT-IR. Spectra were taken using a Thermo Scientific iZ10 with Omnic software. The samples were placed on the diamond IRE of the Smart ATR attachment and clamped down to the point where the silicone was completely pressed against the diamond, but not breaking in half. Samples were scanned 32 times, and backgrounds were taken every 20 minutes to ensure accurate measurements.

Sulfobetaine compounds contain sulfonate groups (SO_3^-), which exhibits a characteristic peak at 1036 cm^{-1}. The presence/absence of this peak allows for qualification of modification presence on the silicone. As shown in the figure below, when modified, spectra of the modified surface exhibit peaks seen both in the control substrate spectrum and the homopolymer spectrum. This is due to the thickness of the modification not exceeding the depth of penetration of the evanescent wave from the spectrometer, which is approximately 8 µm (Figure 6 and Figure 7).

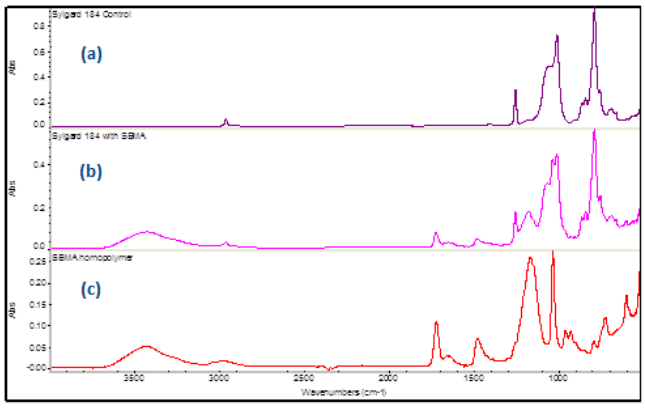


Figure 6. ATR-FTIR spectra of: a) control PDMS, b) SBMA modified PDMS, and c) SBMA homopolymer.

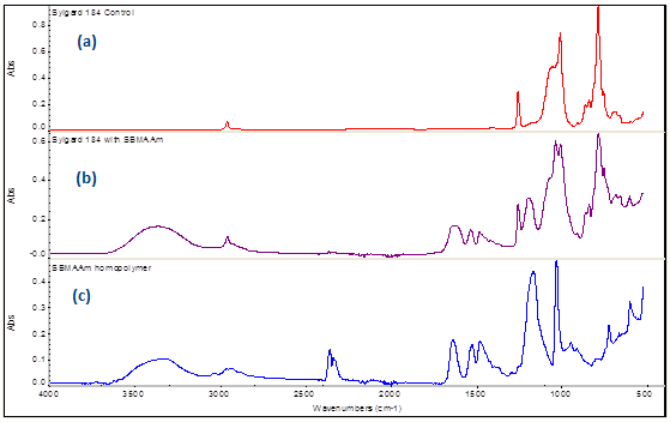


Figure 7. ATR-FTIR spectra of: a) control PDMS, b) SBMAam modified PDMS, and c) SBMAam homopolymer.

It was also possible to determine a relative modification thickness using FAD-IR (Fixed Angle Depth by Infrared). FAD-IR determines the thickness of a polymeric surface modification layered on a polymeric substrate by comparing the closest statistical-distance match between an ATR FT-IR spectrum and a library of spectra spanning the thicknesses of 0 to 5000 nm in 1 nm increments. FAD-IR assumes that the individual polymer layers are pure components and have no interaction with each other. The depth and utility of FAD-IR is restricted to the depth of penetration of the ATR evanescent wave. For a typical polymer surface modification with a refractive index of nD≈1.5, using a single bounce, diamond (nD = 2.41) internal reflection element with an incident angle of 45°, the depth of penetration of the evanescent wave ranges from ~ 5000 nm to ~ 500 nm across the mid-infrared range of 400 cm^{-1} to 4000 cm^{-1}. Displayed in Figure 8 is an example of four Sylgard 184 samples modified with SBMA. It can be noted that the modification developed by FAD-IR directly correlates to the strengths of the characteristic peaks of SBMA homopolymer (that are not characteristic of Sylgard 184), namely the sulfate peak at 1036 cm^{-1}.

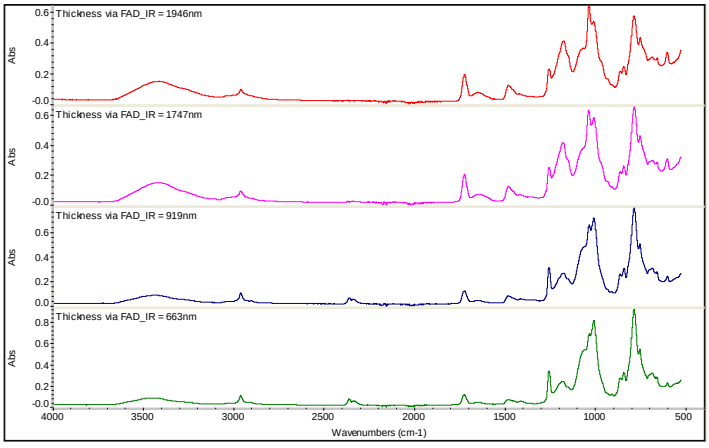


Figure 8. ATR-FTIR spectra of SBMA coating with different thickness.

3.3 Contact Angle Measurement

Goniometry was used to measure the wettablity of coating surfaces by contact angle measurement, which directly correlates to surface energy in water. The static contact angle was measured using a sessile drop method, where a small droplet of water was released on the surface in air at ambient conditions. The drop sticks to the surface and stabilizes within one to four seconds. At this point, the drop starts to evaporate slowly, lowering the contact angle over time. The point at which the slope changes drastically is the point at which contact angle was recorded. This is demonstrated in Figure 9a, where the contact angle recorded is the intersection of the black and red lines drawn on the graph. Dynamic sessile drop method was also used to determine advancing and receding contact angles on Sylgard 184 samples. This method was performed by growing a water droplet on the surface until the contact angle reaches equilibrium and then drawing the droplet back into the syringe and measuring the receding angle. The droplet added was 1µl in volume (Figure 9b).

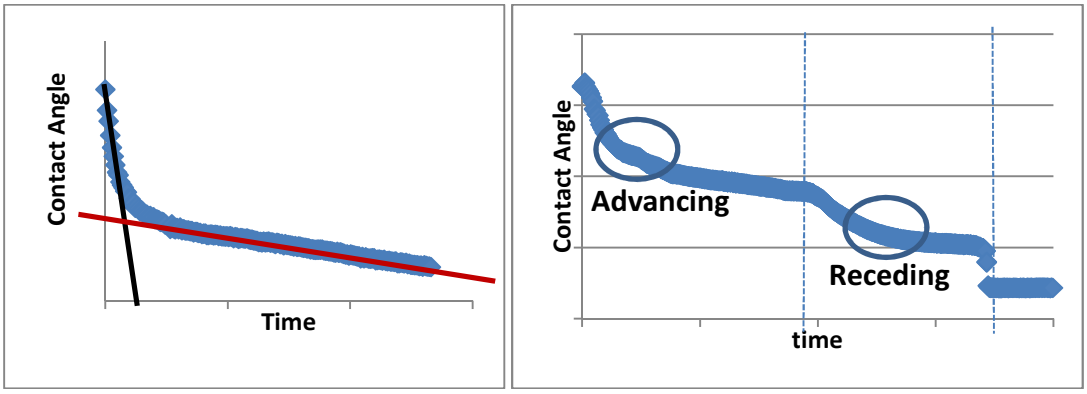


Figure 9. Measurement of (a) static contact angle and (b) dynamic contact angle over time using sessile drop method.

The static sessile drop method is a quick method of determining how hydrophilic/hydrophobic a material is. Silicones are by nature very hydrophobic with low surface energy. Unmodified silicones usually have static contact angles of about 116°, whereas silicone samples modified with SBMA typically have contact angles of 21° and SBMAam as low as 23°. Dynamic contact angles also show significant difference (Figure 10). The super hydrophilicity is a characteristic of sulfobetaine polymers. By strongly harnessing water molecules on the surfaces, the sulfubetaine-modified surfaces resist the attachment of various biomolecules and organisms.

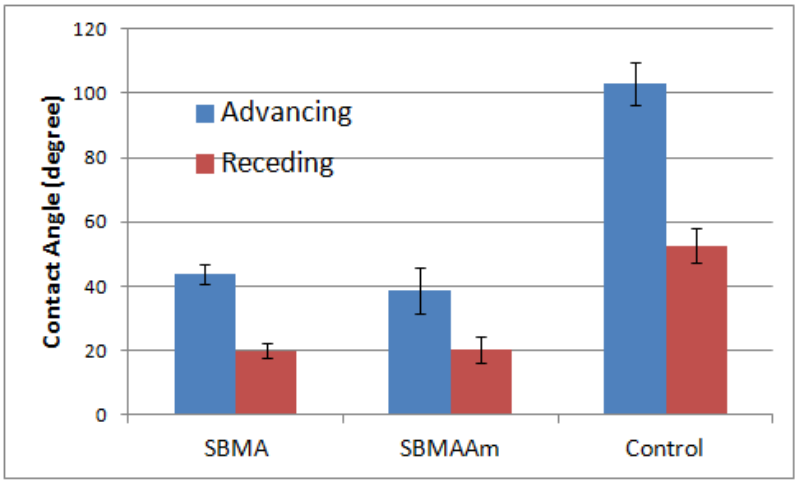


Figure 10. Dynamic contact angle of SBMA and SBMAam modified silicone substrate (For comparison: static contact angle: SBMA: 21 ± 2°, SBMAam: 23 ± 3°, control silicone: 116 ± 3°).

3.4 Laser Confocal Microscopy

Images of the silicone samples were taken with an Olympus LEXT OLS4000, a 3-dimensional laser microscope for nanometer level imaging, 3D profiling, and roughness measurements. The LEXT was used to view and compare the surface of samples before and after modification. Figure 11 shows the differences in surface morphology of Intersleek 757 and Elastosil M4514 after modifying with SBMA. There are clear increases of roughness on the surface of the modified samples, and very different morphologies depending on the topcoat grafted from.

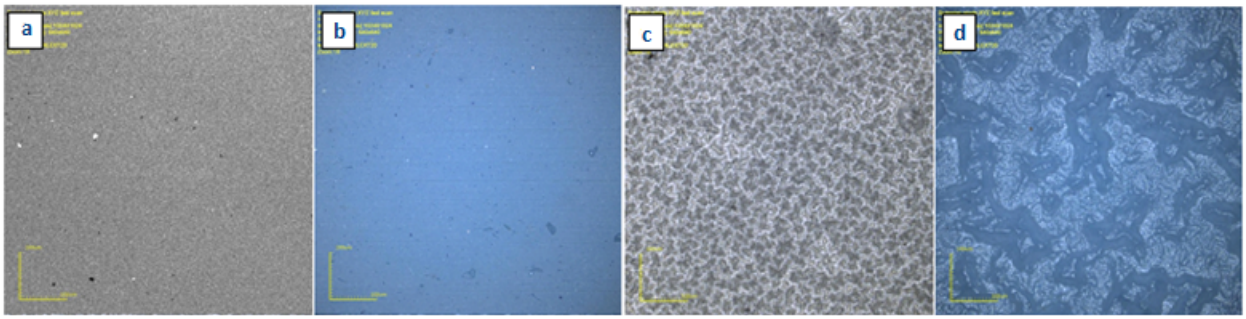


Figure 11. LEXT images of a) Intersleek 757 unmodified, b) Elastosil M4514 unmodified, c) Intersleek 757 modified, and d) Elastosil M4514 modified (Scale bar: 100 µm).

3.5. X-ray Photoelectron Spectroscopy

While ATR-FTIR allows the determination of chemical structures that occupy the surface modification, XPS obtains a very sensitive measurement of atomic composition of < 100 nm of the exposed surface. Figure displays the elemental composition on the surface of an unmodified PDMS sample, and samples modified with SBMA and SBMAAm. The presence of sulfur and nitrogen indicates that the polySBMA and polySBMAam were grafted on the surfaces. The N/S ration is 1 for SBMA and 2 for SBMAam, which correlated well with the XPS results.

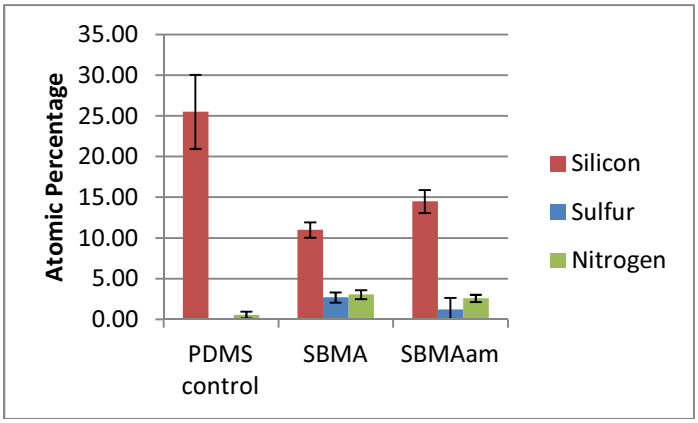


Figure 12. Elemental analysis of Sylgard 184 surface with different sulfobetaine modifications using XPS.

3.6. Scanning Electron Microscopy and Elemental Analysis

Compared with a less than 1000 x magnification of optical microscopy , a scanning electron microscope (SEM) can be used to obtain images up to 20,000 x with great clarity to determine substrate or surface morphology (relative roughness, phase separation, etc.). To view silicone samples with an SEM, they are placed on aluminum sample stubs with double sided conductive carbon tape, and then gold sputter coated with a Denton Vacuum Desk V sputter coater to allow the surface to conduct electrons. Using an SEM equipped with an energy dispersive spectrometer (EDS) also allows the ability to perform elemental analysis on the sample. This can be performed either on the entire area in the image being taken (averages elements across entire area) or in a vector (elements are graphed along the vector) on smoothly cut cross sections. This tool is very useful for determining surface morphologies by looking at concentrations of sulfur (for the sulfobetaine) and silicon (silicone substrate), which explains how the sulfobetaine is integrated into the surface at the interface.

Figure 14 and figure 15 show SEM images of the cross-section of PDMS samples coated with polySBMA and polySBMAam. Across the cross-section (as indicated by red lines on the images), there is a clear interfacial region where the concentration of sulfur and nitrogen decrease and the concentration of silicone decrease. The region is about 1-2 um away from the surface, matching with the SEM images very well. From the structure of SBMA and SBMAam, the atom ratio of nitrogen to sulfur is 1 and 2 respectively, which reflect well on the EDS concentration. The SEM-EDS results indicate that the coatings are mostly pure sulfobetaine layer, which were grafted on top of the existing topcoat.

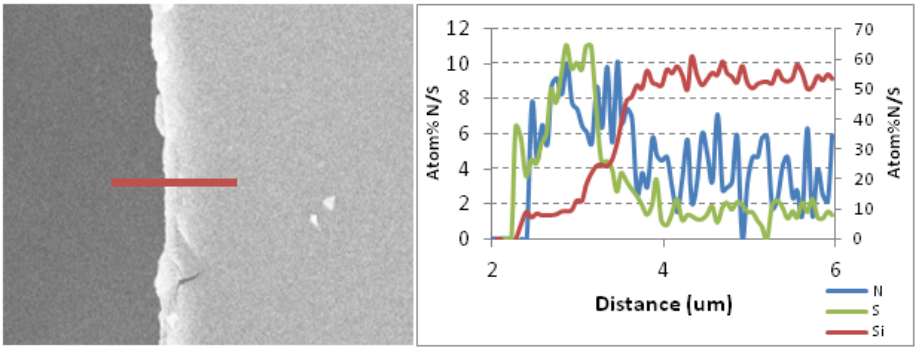


Figure 2. SEM-EDS image of a cross-section of SBMA modified PDMS.

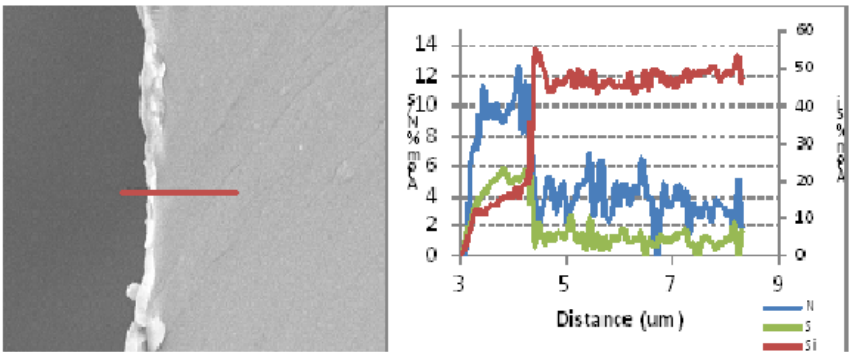


Figure 3. Cross SEM-EDS image of a cross-section of SBMA modified PDMS.

4. Aging/Stability Test

4.1. Homopolymer Hydrolytic and Oxidative Stability

To test the hydrolytic and oxidative stability of polySBMA and polySBMAam, homopolymers of both monomers were synthesized and tested for their chemical structure and molecular weight. The polymers were prepared by polymerizing SBMA or SBMAam in methanol, initiated with AIBN at reflux condition (ca. 65 ^{o}C) under nitrogen for 16 hours. The resultant polymer was washed with methanol and dried under vacuum.

The polymers were dissolved in different aging solutions (0.5 g polymer in 2 mL solution): PBS, 3% hydrogen peroxide and Fenton's Reagent (an oxidative solution of hydrogen peroxide and iron gluconate). Solutions were incubated at 37 ^{o}C up to 30 days. Samples were then removed and tested by 1H-NMR to compare to the original homopolymer spectra. Under these conditions, no evident difference was found from the spectra. Figure 15 shows three examples of polySBMA after aging in PBS solution, 3% hydrogen peroxide solution and Fenton's reagents, resulting in the same spectra as the original samples.

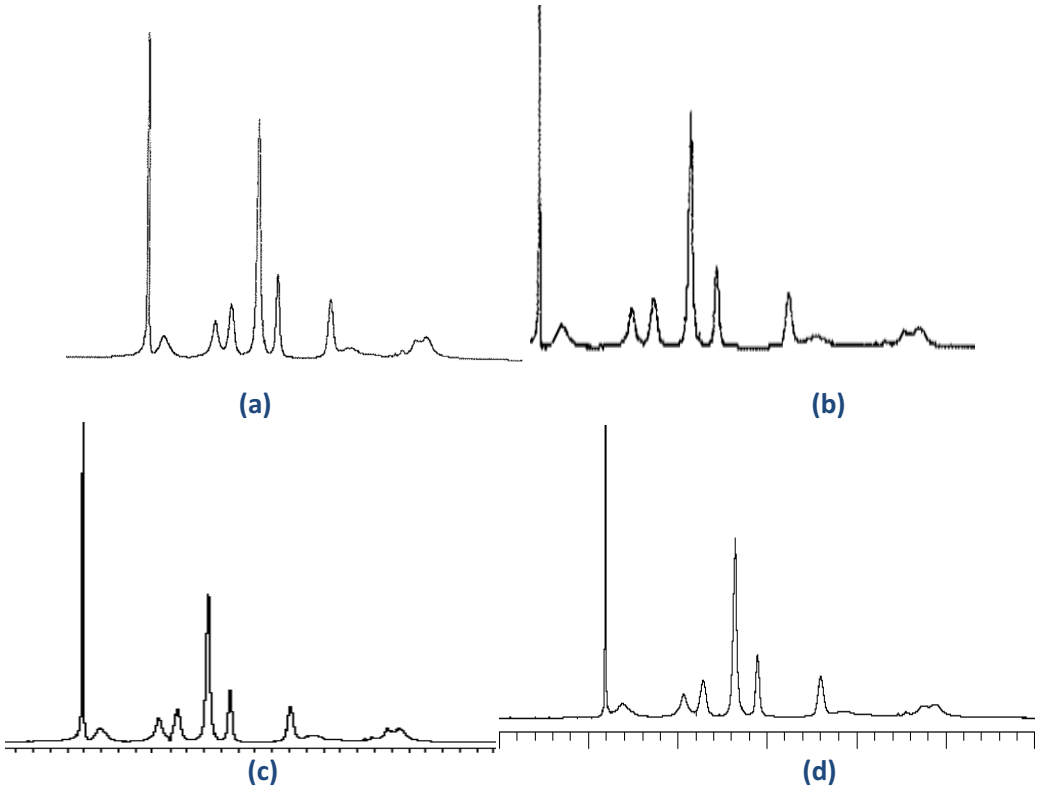


Figure 15.1H- NMR spectra of a) polySBMA , and the same polymer after aging at 37 C in b) PBS solutions for 12 days, c) 3% hydrogen peroxide for 27 days, and d) Fenton's reagents for 30 days.

The polymer samples were also measured for molecular weight and molecular weight distribution by Gel Permeation Chromatography (GPC) analysis. The samples were analyzed in a mobile phase of water with 0.1 M $NaNO_3$ at 30 °C (Malvern Viscotek SEC system). The samples after aging in PBS, 3% hydrogen peroxide and Fenton's Reagent (an oxidative solution of hydrogen peroxide and iron gluconate) at 37 ^{o}C exhibited no degradation. Figure 18 shows an example of GPC analysis of polySBMA and the same polymer aged at 37 ^{o}C in 3% hydrogen peroxide for 24 hours. From the aging test, no evident differences were found between these two homopolymers in terms of hydrolytic and oxidative stability. The further formulations for other substrates were mainly based on polySBMA.

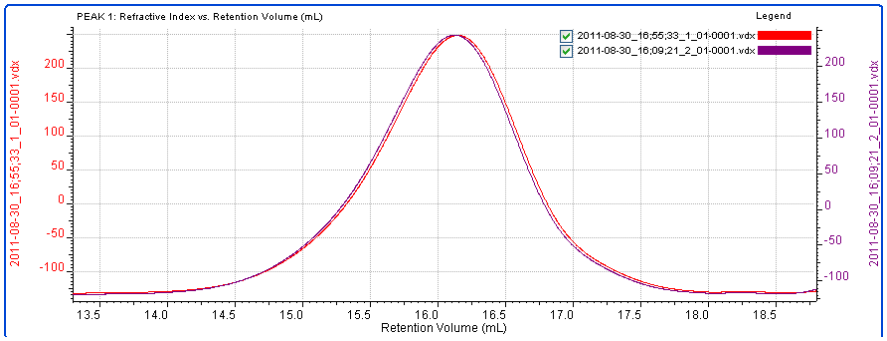


Figure 16. GPC analysis of polySBMA and the same polymer after aging at 37 ^{o}C in 3% hydrogen peroxide for 24 hours.

4.2. Interfacial Stability on Silicones

Modified Sylgard 184 samples were produced and the ATR-FTIR in conjunction with FAD-IR software was used to measure modification thickness prior to exposure to harsh environments. The samples were then incubated in 3 % hydrogen peroxide and Fenton's Reagent for seven days, then re-examined by IR. Modification thickness differences were statistically insignificant and, thicknesses breached the upper limit of detection of the IR. Stability of the PDMS was also tested in NaOCl (bleaching solution with 10-15% chlorine), 2 M NaOH, and adult human serum, all of which did not affect the modification. The final test was for samples to be exposed to artificial seawater (AWS) and placed in an ultraviolet incubator for up to seven days. Protein resistance was tested at the end of the duration, and resistances up to 98% were still exhibited.

PolySBMA-modified Intersleek 425 samples were also examined for stability to ensure a commercial antifouling coating would show the same results. When submerged in 3% hydrogen peroxide, Fenton's Reagent, bleach, or AWS for 30 days, no significant change in modification thickness was seen (Figure 19). Samples were also exposed to AWS in conjunction with sunlight for seven days; once again, the modification proved to be stable. Stability was confirmed by calculating modification thicknesses with FAD-IR and comparing initial thicknesses to those after one and seven days of exposure. No significant differences were seen.

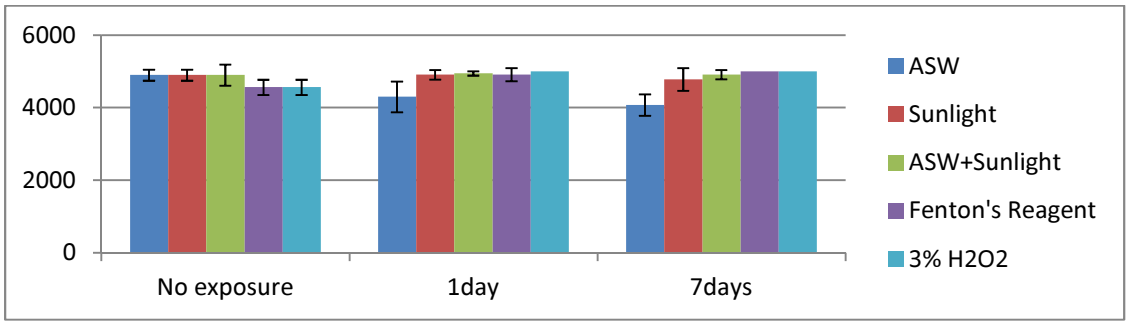


Figure 17. Interfacial stability of SBMA modified Intersleek coating for up to seven days.

5. Protein Adsorption Assays

Protein adsorption is usually the first stage of biofouling and is highly related to the attachment of microorganisms. For example, the attachment of algal spores, barnacle cyprids, and mussels starts from adhesion of proteinaceous adhesive on the substrate[7, 8]. Fibrinogen adsorption serves as a very good indicator for protein fouling in general, because it is highly adhesive to most artificial substrates. When grafted under controlled conditions, SBMA brushes have imparted superior protein resistance to existing nonfouling surface treatments on various substrates and especially in complex media [9].

5.1. Assay Development

A protein adsorption assay was developed to analyze samples using Iodine-125 labeled fibrinogen based on a previously reported method[9]. Substrates with a size of 10 x 5 mm were used in this assay,

with eight unmodified pieces of the same substrate as controls. First, the wells to be used in a 96 well plate were filled with 500 µl of 20 mg/mL BSA in 1 x PBS and stored at 4 °C for at least one hour. To remove this solution (and any other solutions in the well plate for the entire procedure), a vacuum was used to collect all solution in a vacuum flask and not contaminate other wells. Each well was then rinsed three times with PBS. Hot and cold solutions were defrosted and gently mixed to create the test solution. Three 400 µL samples of test solution were pipetted into separate analysis tubes (using a new pipette tip for each) and analyzed with the gamma counter. These readings were used to ensure proper radioactivity. 400 µL of test solution were added into each well with samples, incubating at 37 °C on a shaker revolving at 150 rpm for one hour.

After incubation, test solution was removed with the vacuum, and each well was rinsed four times with 10 mM sodium iodide in PBS, letting the solution rinse the each well for at least one minute before removing. One final rinse was performed with PBS, which also remained in each well for at least one minute. Samples were then transferred to plastic analysis tubes and read with the gamma counter. A Perkin Elmer Gamma Counter 2470 with WIZARD^2 Software Version 1.0 was used.

Laboratory coats, safety glasses, and two pairs of gloves (latex over nitrile) were worn. Hand and body dosimeters were worn to track exposure to radioactive materials. All liquid waste generated was bottled to make only solid waste and collected/recorded in a 55 gallon radioactive waste drum. Wipe tests were performed after conducting this assay to ensure minimal exposure to radioactive isotopes.

5.2. Protein resistance data

In this research, most formulations were tested for their protein adsorption as an initial evaluation of their nonfouling performance. Protein resistance could be calculated in two ways: percent reduction from control by cpm, or ng/cm^2 adsorbed on sample surfaces. The first is a simple ratio of cpm on each test sample over the average of all control samples of the same substrate. The second is adsorption of fibrinogen per area calculated from cpm and surface area. Table 2 lists some typical formulations on different substrates. On Sylgard 184, both SBMA and SBMAAm had very strong protein resistance. Very strong resistance was also seen on both Intersleek 757 and Elastosil M4514 when higher initiator was used (F2 and F3). From the fibrinogen resistance data, it is apparent that Intersleek 757 (F1) causes uneven coatings (based on large standard deviation with low reduction). The formulation of F1 F2, and F3 are developed marine coatings for field test that was listed in Table 2.

Table 1. Fibrinogen reduction data for optimized formulations.

Sample Description	Exposed Surface Area (cm^2)	Unmodified		Modified		Reduction in Protein
		Average Counts	Counts/ cm^2	Average Counts	Counts/ cm^2	
SBMA-Sylgard 184	1	9358	9358	877	877	91±3%
SBMAam-Sylgard 184	1	9358	9358	171	171	98±0%
F1 (SBMA-Intersleek)	1.6	5496	3435	2267	1417	59±28%
F2 (SBMA-Intersleek)	1.6	5496	3435	512	320	91±1%
F3 (SBMA-Elastosil)	2.5	4192	1677	370	148	91±3%

Table 2. Formulation and polySBMA thickness for field test panels.

Group #	Substrate	Color	pSBMA Average Thickness (nm)
PVC	PVC panels (negative control)	Black	-
C1	Al/silane/Intergard264/Intersleek 731/Intersleek 757 Control	Grey	-
F1	Al/silane/Intergard264/Intersleek 731/Intersleek 757/pSBMA	Grey	1977±1728
F2	Al/silane/Intergard264/Intersleek 731/Intersleek 757/pSBMA	Grey	2937±239
C2	Al/silane/Primer G/Elastosil M4514 Control	Blue	-
F3	Al/silane/Primer G/Elastosil M4514/pSBMA	Blue	1986±384

6. Bacterial/Biofilm Assays

Bacterial attachment and biofilm formation are the first stages of underwater biofouling and are highly related to biofouling of other macro-organisms such as algae, diatoms, barnacles, and tubeworms[10, 11]. Some attached organisms, such as tubeworms, use biofilm as a food source. Nonfouling surfaces were examined for bacterial attachment and biofilm formation. Considering the modifications are designed for both static and dynamic components of a MHK system, coated substrates will be tested under both conditions. These methods were based on industrial methods for antimicrobial materials and modified by Semprus specifically for non-leaching nonfouling materials. In this research, three bacterial species were evaluated: *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative), and *Cellulophaga lytica* (a marine bacteria), which are commonly used for evaluating anti-microbial materials including marine coatings by industrial companies.

6.1. Nutrient Controlled Assay Development

The microbiology assay performed for these samples was referred to as the N.C. (Nutrient Controlled) assay. On the first day of the assay, each bacteria to be examined was cultured (in a media specific to its species) in a round bottom centrifuge tube at 37 ^{o}C set at an angle on a shaker at 240rpm for 16-19 hours (C. lytica is cultured in artificial seawater at 30 ^{o}C for 24 hours). This allowed about 1 x 10^{9} cfu/ml to grow.

On the second day, 90 minutes before the culturing was complete, samples were sterilized in 70 % ethanol in RO water for one hour on the shaker at 120rpm, followed by 30 minutes of 1x PBS. Each culture was then diluted four times by a factor of 10 to reduce the concentration to about 1x10^{5} cfu/ml (deemed inoculum). The overnight culture and inoculums were tested to verify the correct amount of bacteria was grown and diluted to appropriate concentration. Each sample was placed in a 15 mL conical tube, and 10.8 mL of inoculums was added to the tube. One tube with inoculums and no tube was also made as a control (to show bacteria levels are the same whether exposed to a sample or not). Each sample was incubated at 37 ^{o}C set at an angle on a shaker at 120 rpm for 26 hours.

On the final day, each sample was removed from the inoculums and rinsed in 7mL 1x PBS at 37 oC (30 oC for *C. lytica*) on a shaker at 120rpm for five minutes. Random planktonic solutions (inoculums after removing silicone samples) for each test group and the control inoculums were then tested to once again ensure that inoculums have the correct amount of bacteria. Samples were then placed in 3ml of D/E neutralizing broth in a round centrifuge tube. Each tube was sonicated at 7 watt output four times for three seconds (rinsing probe in 1x PBS each time). The sonicates (D/E after sonication) was then plated and incubated upside-down at 37 ^{o}C for 24 hours (*C. lytica* at 30 ^{o}C for 27 hours). Colonies were counted after incubation and modified sample numbers were compared to controls.

6.2. High Through-put Biofilm Assay Development

A high through-put assay was used to screen different sulfobetaine-based formulations based on previous literature. In this assay, bacteria solution and samples (10 X 10 mm) were inoculated in a 24-well plate. The wells were rinsed after incubation and biofilm cells were stained with a solution of crystal violet. After a washing procedure, the crystal violet on bacteria was dissolved in ethanol and absorbance was determined by measuring at 600 nm. However, it was found that the polysulfobetaine could adsorb crystal violet, which made this method not suitable for analyzing betaine-based coating (data not shown).

6.3. Bacterial Attachment Results

6.3.1 SBMA modifications on different substrates

Figure 18 lists fouling resistant performance of three different types of sulfobetaine coatings: copolymer (primer-topcoat as shown in scheme 2, with 70 mol. % sulfobetaine in the topcoat by NMR), polySBMA on PDMS (Sylgard 184), and a polySBMA formulation on aluminum substrate coated with a anticorrosive primer and a fouling-release topcoat. Fouling resistance was tested using radio-labeled fibrinogen as a model protein and bacterial resistance was tested using *S. aureus* and *E.coli* as model strains applied on modified and unmodified samples. The copolymer-coated samples were prepared using a formulation optimized by their protein resistant performance and adhesion properties, presenting a 93 % fibrinogen reduction. Their resistance to S. aureus and E. coli was 70 % and 55 % respectively. The sufobetainne-grafted PDMS showed a better antifouling performance with 98% reduction for fibrinogen, 98 % reduction for *S. aureus*, and 88 % reduction for *E. coli*. Since the sulfobetaine is the only active moieties to resist fouling, a surface grafting formulation with 100% sulfobetaine, can present a better fouling resistance to a copolymer formulation with 70 % sulfobetaine. Based on these results, the surface-grafting chemistry was chosen to develop the modification for marine coating.

A model marine coating was made from aluminum substrates with a Intergard 264 anti-corrosive coating and an Intersleek 425 fouling release coating, both are commercial marine coatings. The Intersleek 425 is a three-component silicone based coating and its formulation was modified to adapt to the sulfobetaine modification. While Intersleek 425 is regarded as an anti-fouling coating, it does not present resistance to fibrinogen and bacteria fouling in the research due to its hydrophobic characteristic. After the sulfobetaine modification, the fouling on the Intersleek 425 was evidently reduced (76% fibrinogen, 83% *S. aureus*, and 61% *E. coli*). This research confirmed that the sulfobetaine coatings have a much better resistance than the currently used fouling resistant coating.

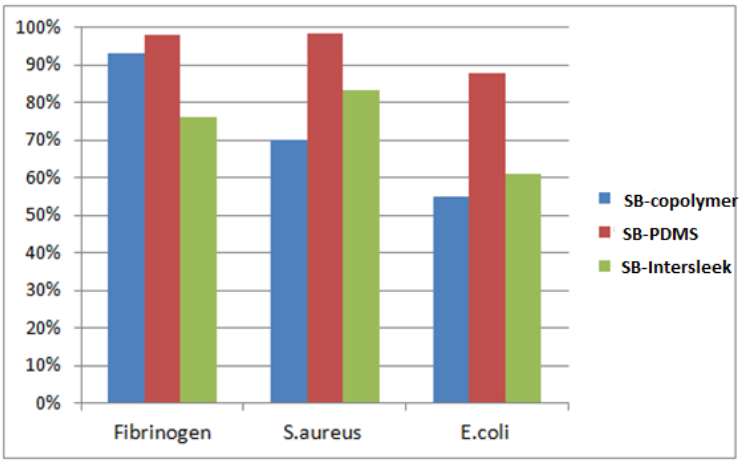


Figure 18. Protein and bacteria resistance on different substrates modified with sulfobetaine (n=4)

6.3.2 SBMA and SBMAam on PDMS

The NC assay was performed on PDMS (Sylgard 184) with either SBMA or SBMAAm grafted on the surfaces. Both surfaces exhibited significant resistance to *E. coli* , *S. aureus* and *C. lytica* at a reduction of 96.4 -98.8%. No evident difference was seen between polySBMA and polySBMAam in terms of their antifouling performance. The pendent groups, i.e., sufobetaine, function as the moieties to resist bacteria attachment. The backbones of the polymers, i.e., acrylamide and methacrylate, have less effect on the nonfouling performance.

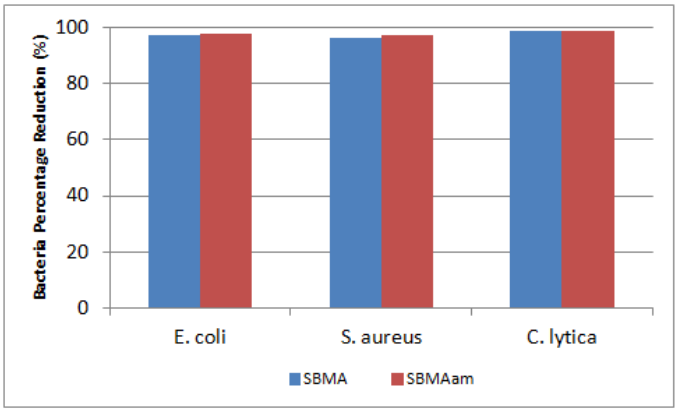


Figure 19. Bacteria reduction on sulfobetaine-modified Sylgard 184 (n=6).

6.3.3 SBMA Modifications on Formulations for Field Test

As shown in Table 2, two formulations F2 and F3 are sulfobetaine coatings on Intersleek 757 (a three-component fouling release coating) and Elastosil M4514 (a two component silicone rubble) respectively test were evaluated for their bacteria resistance. These formulations were prepared on cast topcoat only. Sulfobetaine formulation on PDMS Sylgard 184 was also been tested. The three silicone formulations were then tested with three strains. An average reduction of 97.7%, 73.1%, and 98.8%

reduction for *E coli*, *S. aureus*, and *C. lytica* respectively was found on modified Sylgard 184 coatings. These results are constant with previous Sylgard results except a little decrease of *E. coli* performance. For *E coli*, *S. aureus*, and *C. lytica*, 90.7%, 88.5%, and 93.4% reduction were found on modified Intersleek 757 coatings, and 97.0%, 99.6%, and 99.5% reduction was found on Elastosil coating. Due to the high bacterial resistance of these two formulations, these two formulations were applied on the aluminum substrates for the barnacle settlement test and the 60-day field test.

Intersleek 757 is a currently used marine coating formulation. When applied on the substrate, the surface releases foulants due to the low surface energy of their surfaces. The results confirmed this coating could not resist the attachment of a model Gram negative bacteria *E coli*, a model Gram positive bacteria *S. aureus*, and a model marine bacteria *C. lytica*. A coating with sulfobetaine could significantly reduce the biofilm formation of these strains.

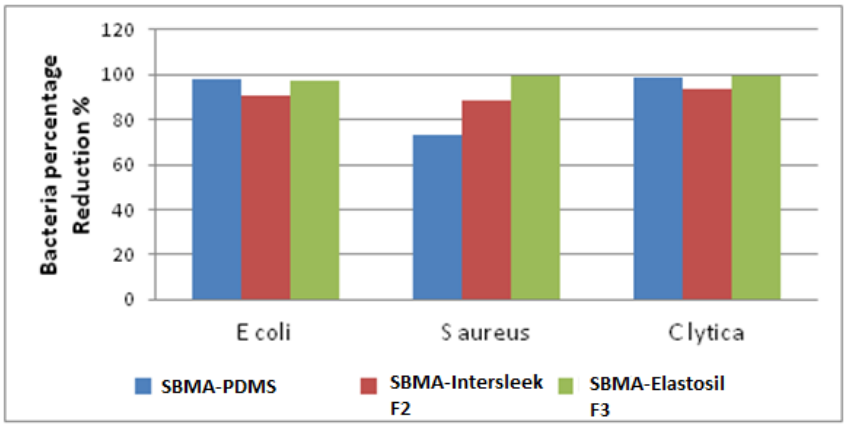


Figure 20. Bacteria reduction for sulfobetaine modified substrates (Sylgard 184, Intersleek, and Elastosil) (n=3-7).

7. Biocompatibility/Leachables

Different from current biocidal anti-fouling coatings, sulfobetaine-based surface coatings are intended to be non-leaching and non-toxic. To verify that our final formulations were environmentally benign and nonleaching, the formulations were tested with zone of inhibition (ZOI) test. The assay consists of coating plates with agar and a bacterial strain, then placing a sample of the desired formulation in the center of the plate. If harmful leachables exist, a ring will slowly form around the sample where the bacteria are dying; the larger the ring, the more leaching has occurred. The commercial silicones in this study claim to be non-toxic, and the polysulfobetaine surfaces have been demonstrated non-toxic in previous studies [4].

For the final formulations sent for barnacle assays and field testing, films of equivalent formulations were prepared and tested with the zone of inhibition test with the same bacterial strains used for the NC assay. The size of each zone is shown in Table 3. The zones exhibited were deemed insignificant. Elastosil control presented a small ZOI of 0.22 mm on S. aureus, which was not seen on coated samples, probably due to the residue of the solvents. All the unmodified and modified samples from cast films, showed no signs of toxicity to these bacteria.

Table 3. Zone of inhibition data for films and silicone coated aluminum

Sample	Strain	Avg Zone (mm) for cast film samples
C1 (Intersleek 757 control)	C. lytica	0.00
	SA25923	0.00
	EC700928	0.00
F1 (SBMA-Intersleek)	C. lytica	0.00
	SA25923	0.00
	EC700928	0.00
F2 (SBMA-Intersleek)	C. lytica	0.00
	SA25923	0.00
	EC700928	0.00
C2 (Elastosil M4514 control)	C. lytica	0.00
	SA25923	0.22
	EC700928	0.00
F3 (SBMA-Elastosil)	C. lytica	0.06
	25923	0.00
	700928	0.00

8. Resistance to Barnacle Settlement

8.1. Barnacle Settlement Assay

The objective of this assay was to determine the effect of the sulfobetaine modification on the adherence of barnacle cyprids, larval stage crustaceans whose role during the barnacle life-cycle is to find a suitable spot to settle. A barnacle cyprid attaches head first using its antennules and secretes glycoproteins to aid in adherence. These organisms are known to access surfaces based on texture, chemistry, wettability, color, and biofilm presence/composition[12, 13].

Two types of substrates were prepared for the test. The first substrate was cast PDMS (Sylgard 184) film with a size of 75 X 25 X 1 mm. The second type of substrate was aluminum (75 X 25 X 1 mm) slides coated with a layered coating of marine coating formulations including primers, tie layers, and topcoat, as described previously. The samples were prepared and submitted to Prof. Anthony S. Clare's lab at Newcastle University, UK for the barnacle test. In this test, Barnacle cyprids (*Balanus amphitrite*) were batch cultured and the cyprids were then introduced to polystyrene Petri dishes containing artificial seawater (ASW) and samples. For each formulation, 12 replicates were tested.

8.2. Barnacle Settlement on Modified PDMS

Initial testing on barnacle settlement was performed on Sylgard 184 and Intersleek 425 silicones, both having one control group and one test formulation. The Sylgard 184 samples were prepared unattached to a solid substrate. All samples were placed dry in Quadriperm dishes in a dark room at 20 oC for twelve days, then samples were moved to an incubator with circulating fan at 28 °C for fourteen additional days to attempt to evaporate any residual solvents. Three days prior to the test, thirty cyprids (per test sample) were stored at 6 °C. Each sample was placed in its own well with 1.5ml of 0.2 µl-filtered artificial seawater (AWS) with ten cyprids. In-house standards T2 (silicone elastomer-coated glass microscope slides) and IWAKI (empty 24-well polystyrene plate, which had 10 cyprids in 2 mL AWS in 6 replicate wells) were used to ensure settlement and mortality in the lab standard assay met expectations. Samples were placed in their own well with thirty cyprids and kept in the dark at 28 °C for 24 and 48 hours prior to recording settlement and mortality.

Settlement and mortality in the lab standard assay met expectations (Figure 21), i.e. the cyprids were healthy and displayed normal settlement behavior. Of the test samples, cyprids only settled on the PDMS (Sylgard 184). The PDMS control group showed very little (perhaps negligible) mortality after 48 hours, and less settlement than the lab standard. The Sylgard 184 modified with SBMA showed no settlement, and little mortality after 24 and 48 hours. The results shows that the surface modified with sulfobetaine polymers could reduce barnacles 100% on the surface with good biocompatibility.

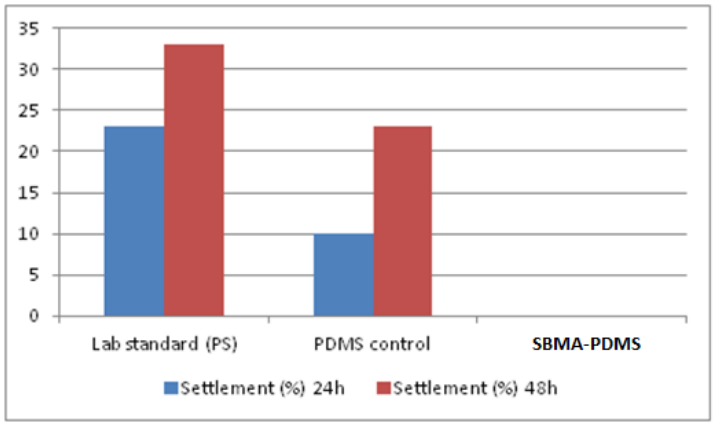


Figure 21. Settlement of barnacle on PS, PDMS, and sulfobetaine-modified PDMS (Sample size: 25 X 75 mm, n=12).

8.3. Barnacle Settlement on Marine Formulations

Marine formulations were coated on aluminum slides as described previously and their barnacle settlement was evaluated on the surface of the substrates. To evaluate the biocompatibility of the surfaces, the mortality of the cyprids was also measured. The samples were soaked with ASW for seven days before the barnacle settlement test. Twenty cyprids (that had been maintained at 6 °C for 3 days prior to use) were added to each test slide (as well as an in-house standard T2 coated microscope slides) in 1.5 mL of 0.2 µm-filtered artificial seawater (ASW). An in-house standard, 24-well polystyrene plate (IWAKI) was set up with 2ml of ASW containing 10 cyprids placed in 12 replicate wells. Samples were kept in the dark at 28 °C and settlement was enumerated at 24 and 48 h.

Figure 22 shows the attachment of barnacle cyprids after 24 hours and 48 hours. F1and F2 coatings are sulfobetaine coatings on the top of Intersleek silicone coatings, with different average thickness of ca. 2 µm and 3 µm respectively. It was shown that after 24 hours, cyprids have zero settlement on F1formulation and 13% on the F2 surfaces, compared with control silicone surface has a settlement percent of 45%. After 48 hours, the settlement on all surfaces increased to 21% and 36% on the modified, compared with 73% reduction on the control silicone surfaces.

While zero settlement was found on F1 after 24 hours, the sulfobetaine coating on commercial marine coating-based surfaces does not perform all zero attachment on modified Sylgard 184 substrates as shown in the previous section. Considering the marine coatings are similar to the Sylgard 184 in chemical structure, both formulations and process methods have room to improve the nonfouling performance for barnacle attachment.

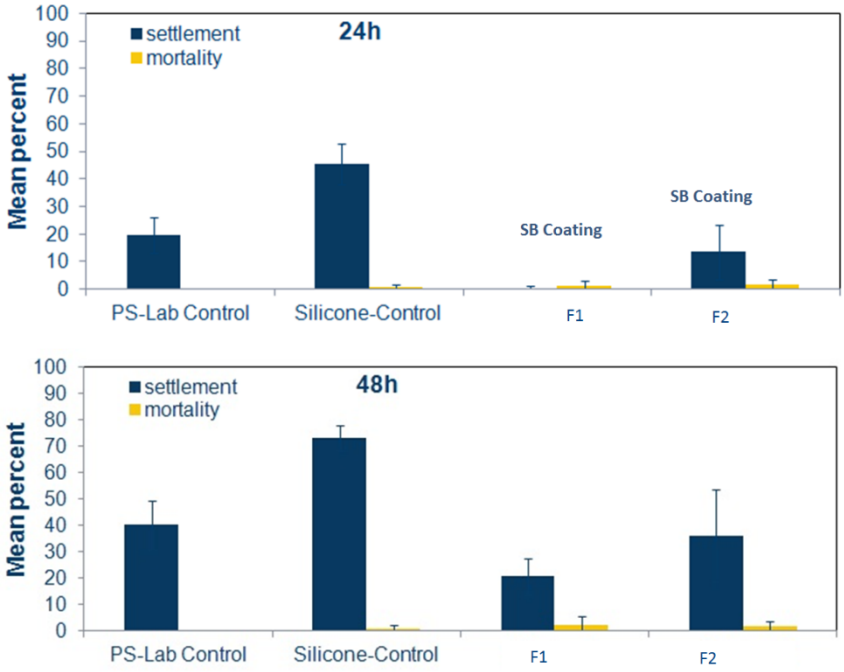


Figure 22. Settlement and mortality of cyprids on test coatings and internal lab standards (mean ±95% CI) after a) 24h and b) 48h (Sample size: 25 X 75 mm, n=12).

The research also shows high mortality aluminum slides with aluminum substrate coated with both anticorrosive primer Intersleek 264, tie layer Intersleek731 and Intersleek 757 coatings (77% after 48 hours), even after a 7-day soaking in ASW. While no toxicity was found on the casted Intersleek 757 formulations from the ZOI test on three strains of bacteria, the toxicity from the control substrate may be attributed to solvents or leachables within the anticorrosive coatings or tie layers. Interestingly, after a modification with sulfobetaine, the mortality rates of F1 and F2 are negligible. It seems that either the sulfobetaine coating process removed the leachable substances from the substrate, or the sulfobetaine coatings prevent the biocidal effect on the Intersleek surfaces. More experiments are needed to investigate the effect of underneath coating on the mortality of the cyprid.

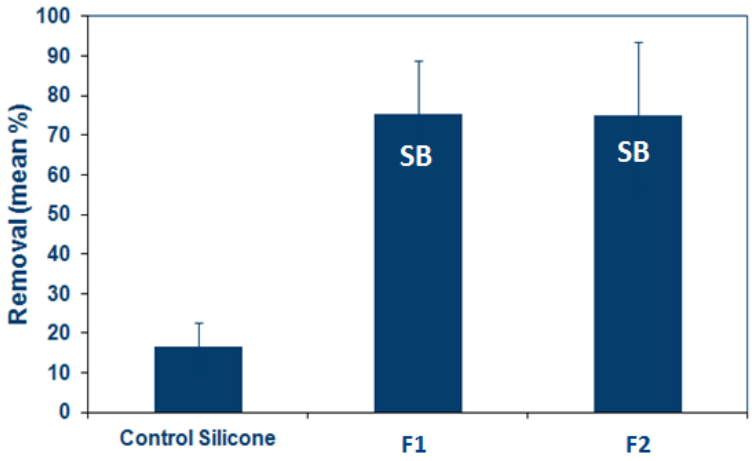


Figure 23. Ease of removal of newly metamorphosed individuals from two test surfaces and a control silicone internal lab standard (mean ±95% CI, Sample size: 25 X 75 mm, n=12).

Cyprids settled on surfaces and subsequently metamorphosed were counted and their positions noted. The surfaces were then exposed to a jet of water with an impact pressure of 45kPa delivered from a fully automated waterjet apparatus. Immediately after jetting, the number of individuals cleanly removed (leaving no visible adhesive) were enumerated. Significantly higher removal of newly metamorphosed individuals was obtained for the two sulfobetaine modified surfaces compared to the control silicone (Figure 23). The test coatings did not differ significantly from each other with respect to ease of removal of metamorphosed barnacles. In addition to low settlement rate on the sulfobeatine on the surface, the research indicates the barnacle removal on the test is easier than PDMS surfaces, indicating the barnacle resistance of sulfobetaine surfaces are effective in both static and dynamic environments.

9. Field Test Results and Analysis

9.1. Sample Preparations and Characterization

The developed coatings were applied on 4 X 8" aluminum panels and the formulations are listed on Table 2. PVC panels were used as a negative control. Two types of positive control panels are formulated from commercial silicones: a marine coating formulation based on Intersleek 757 as a topcoat, an epoxy Intergard 264 as a primer coat, and a tie coat Intersleek 731 to bind them together. Another silicone topcoat M4514 was applied on aluminum panels with silane and a primer layer. The F1 formulations were coated on the Intersleek substrate, with an average polySBMA thickness of 1977 nm. The substrate was inhomogenously coated with sulfobetaine coatings on the surface. This formulation was to test the performance of combination of fouling release silicone and hydrophilic polySBMA. F2 formulation was thicker and more homogenous polySBMA coating on top of Intersleek coatings, with a polySBMA thickness of 2937 nm. The fibrinogen reduction on the casted topcoat with and without SBMA is shown in Figure 26. Both F2 and F3 resulted in a fibrinogen reduction higher than 90% compared with their unmodified substrate. F1, due to its inhomgenous characteristics, resulted in an average reduction of 59% but with a high standard deviation.

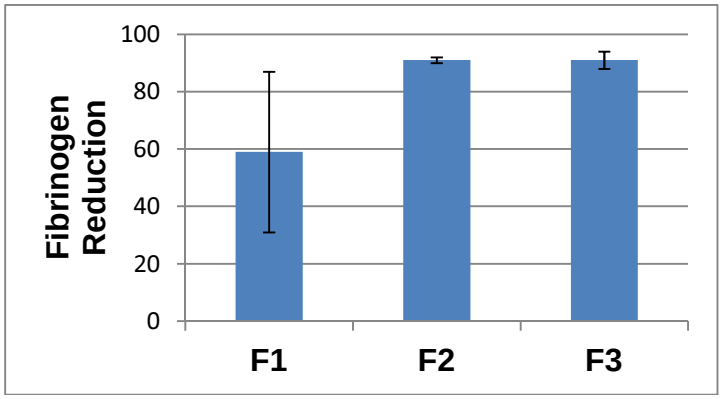


Figure 24. Fibrinogen reduction on three formulations (F1; sulfobetaine partially modified Intersleek®; F2: sulfobetiane modified Intersleek®; F3: sulfobetaine modified Elastosil®(Table 2)).

9.2. Field Test

Fifty seven panels were immersed at the Florida Institute of Technology test site at Indian River Lagoon near Sebastian on September 23, 2011[14]. All panels were caged before the immersion. Panels were placed back to back on two frames, one for 12-day panels and one for 27-day and 60-day panels. Panels were randomized on the frames. Panels were removed from the water October 5, 2011 (after 12-day immersion), photographed and waterjetted. The 12-day panels were removed from the water and returned to Semprus. On October 20, 2011 (after 27-day immersion), panels were removed from the water, photographed and visual assessment was performed. The 27-day panels were removed and returned to Semprus. On November 22, 2011 (after 60-day immersion), panels were removed from the water, photographed and visual assessment was performed. On November 28, 2011 (after 66-day immersion), panels were removed from the water and hard fouling adhesion was performed. Panels were then returned to Semprus for further analysis.

The waterjet method for the field test samples was performed using an apparatus consisting of one SCUBA tank containing compressed air with a pressure range of 0 - 240 psi attached to a SCUBA tank containing water, with a blow gun with a 1.6 mm diameter nozzle to apply the water jet. The regulator is set to 20 psi and an area of 50 x 50 mm (2 x 2 in) is sprayed until the maximum amount of fouling can be removed at that pressure setting. The pressure is then increased by 20 psi and the process is repeated until all the fouling is removed; the pressure which removed the last remnant of the fouling is recorded.

9.3. Visual Assessment of Immersed Panels

After 12 days, 27 days, and 60 days of being immersed in the lagoon, all samples were removed to be evaluated. Figure 25 shows the photos of one test frame as an example of the panel layout and visual evaluation. Panels were randomized on frames and no panels with the same formulations were placed next to each other. Fouling was moderate and consisted primarily of biofilms, algae, hydroids, encrusting and arborescent bryozoans, barnacles, tube worms and sponges.

After immersion for 12 days (Figure 25b), The PVC control panels had significantly higher percent coverage than all modified samples and C2. C1 (Intersleek coating) visually showed more fouling than other coatings except PVC. Sulfobetaine-based coatings (F2 and F3) presented a leaner surface compared with Intersleek coatings (C1) and silicone coatings (C2). After immersion for 27 days (Figure 25c), all samples including PVC got more foulants on the surfaces. F3. F2 accumulated less foulants compared with C1, C2, and F1. Some amples were removed after 27 days and the layout of the panel was changed so only typical panels of each formulation was shown in Figure 25d. The 60-day immersion made more foulant attachment on most surfaces and some visual defects were observed on some formulations. Sulfobetaine modified silicone (F3) shows a cleaner surface compared with unmodified silicones (C2), the latter was covered with foulants on the originally blue silicones. It was found that foulant from Intersleek coating (C1) decreased after 60-day immersion. Some foulant on the 27-day samples were removed from previously fouled surfaces, and the mark of previous foulant could be observed on the surface. A partially modified Intersleek coating (F1) presented the strongest fouling compared with either pure sulfobetaine coating or pure Intersleek coating. After 60-day immersion, some defects were found on the sulfobetaine-modified surfaces. Samples from F3 had some “blisters” and the F2 had some white spots. Delamination of one F3 panel was found after 60-day immersion.

Based on the previous results, sufobetaine-coated panels had better fouling resistance than Intersleek coatings on protein adsorption, bacteria attachment, and barnacle settlement. As a result, sulfobetaine coatings should have better fouling resistance than Intersleek coatings. However, some foulants on the 27-day panels of Intersleek coatings disappeared after 60-day immersion, and the mark of previous foulant could be observed on the surface. The releasing of foulants can be attributed to the low surface energy that make the foulants fall from the surface by the accumulated weight of the foulants. Both Intersleek marine coating C1 and Elastosil blue silicone coating C2 are silicone-based coatings with similar contact angle or surface energy. However, only Intersleek marine coatings present a fouling release efficacy on the surface, which is due to other additives in the formulation such as releasing agents. Silicone oil is a common releasing agent added in the coating formuation, which is gradually released from the surface to reduce the adhesion strength of the foulants. Nearly all silicone foul release coatings are augmented with an oil additive to decrease macrofouling attachment strength [3]. The antifouling mechanism of sulfobetaine is mainly due to the protein resistance characteristic of the zwitterionic moieties. That explains why much less accumulated fouling was found in the first 12 days of immersion. Applying a sulfobetaine coating may remove the additives from the silicones, or block them from releasing to the surface, which make partially coated sulfobetaine coatings on Insterleek coating perform worse than both sulfobeatine coatings and Intersleek coatings. The serious fouling on F1 also indicates a combination of hydrophilicity and hydrophobicity may not have a favorable synergetic effect on fouling performance.

In summary, sulfobetaine modified Elastosil coatings (F3) showed significant improvement on the surface compared with unmodified surfaces through the 12-day, 27-day, and 60-day tests. The fouling increased on the surfaces with immersion time, and some mechanical defects were observed after immersion. A better formulation to increase the adhesion strength and thickness would be expected to get better results. Sulfobetaine modified Intersleek coatings (F2) showed visual improvement on the surface for the first 12 and 27 days compared with unmodified samples. The 60 day-results shows a comparable performance of Intersleek and sulfobetaine-modified Intersleek. Considering control Intersleek reduces foulants through depleting silicone oil, comparison after a longer immersion time (> 2 months) would be expected to get a better nonfouling performance of sulfobetaine polymers.

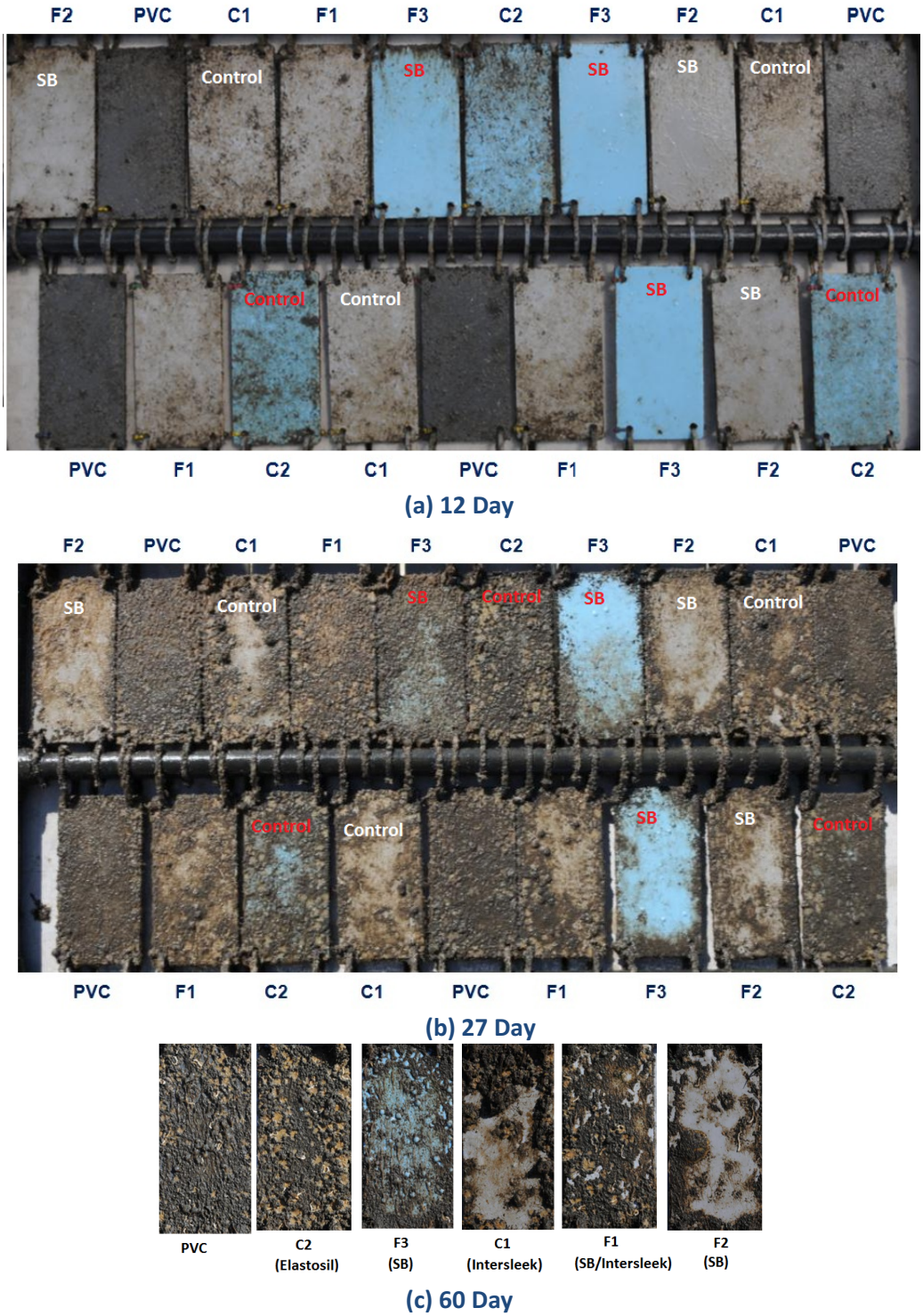


Figure 25. Frame photographs (Frame 57N) showing the visual appearance of panels immersed with different time. (PVC: negative control; C1: Intersleek® antifouling coating, grey, positive control 1; C2: Elasosil®silicone coating, blue, positive control 2; F2: sulfobetiane modified Intersleek®; F3: sulfobetaine modified Elastosil®; F1; sulfobetaine partially modified Intersleek® (Table 2))

It should be stated that both F2 and F3 have a sulfobetaine thickness of 2-3 µm, less than 1% of the thickness of a normal antifouling coating. The evident efficacy from the visual assessment confirmed the proposed antifouling mechanism of sulfobetaine as a new avenue to develop underwater coatings, and indicated that there could be enough room for further improving coating efficacy by optimizing formulations and application methods.

9.4. Foulant Removal through Waterjet

The attachment strength of the foulants was measured after a 12-day immersion (Figure 26). There were significant differences in biofilm adhesion on PVC, Intersleek (C1), and silicone coating (C2). Sulfobetaine-modified coatings, F2 and F3, required lower water pressures to remove biofilms than F1 and C2. PVC was not waterjetted as it had no release properties and was a negative fouling control. Applied as a sulfobetaine coating on C2, F3 exhibited an evident improvement on removal pressure, indicating sulfobetaine have a better biofilm removal rate compared with silicone rubber. Similar results were also found by previous barnacle removal tests. As a commercial marine coating, C1 differentiate C2 with different formulations, which may include silicone oil that keeps migrating on the surfaces. A total coverage of the C1 with sulfobetaine, F2 also shows low biofilm removal pressure, which is comparable to C1. However, partially coated sulfobetaine-Intersleek panels (F1), have a high removal pressure than control Intersleek or F2. The waterjet results show that the sulfobetaine-based coating could reduce the adhesion strength of marine foulant. The results indicate that betaine-based coatings are effective in both static and dynamic environment of MHK devices.

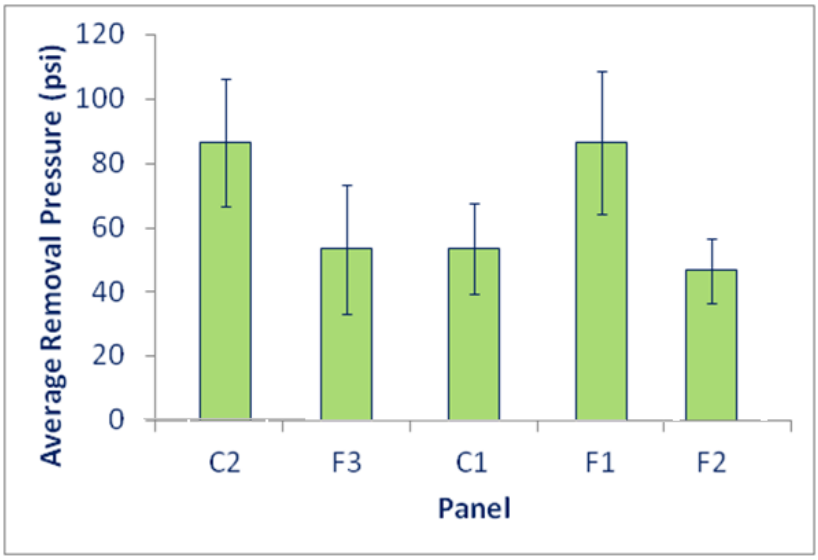


Figure 26. Average biofilm removal pressure after 12 day immersion. Both sulfobetiane-coated formulations (F3 and F2) have lower removal pressure compared with Elasosil® coatings (C2), and have comparable removal pressure compared with fouling-release Intersleek® coating (C1: Intersleek® antifouling coating, grey, positive control 1; C2: Elasosil® silicone coating, blue, positive control 2; F2: sulfobetiane modified Intersleek®; F3: sulfobetaine modified Elastosil®; F1; sulfobetaine partially modified Intersleek® (Table 2))

10. Summary

This research designed and developed sulfobetaine-based polymers as novel underwater coatings to resist the attachment of marine organisms. Betaine-based monomers and polymers were synthesized and incorporated within various coating formulations. The formulations and application methods were developed on aluminum panels with the required adhesion strength and mechanical properties. The coating polymers were chemically stable under UV, hydrolytic and oxidative environments. The sulfobetaine formulations are applicable as nonleaching and stable underwater coatings.

For the first time, surfaces modified with highly packed sulfobetaine polymers were prepared and demonstrated resistance to a broad spectrum of marine organisms. Assays for comparing nonfouling performance were developed to evaluate protein adsorption and bacteria attachment. Barnacle settlement and removal were evaluated and a 60-day field test was performed. Silicone substrates including a commercial fouling release coating (Intersleek 757) were used for comparison. Compared with the unmodified silicone substrates, the sulfobetaine-modified formulations were able to exhibit a 98% reduction in fibrinogen adsorption, 97.0% (*E. coli*), 99.6% (*S. aureus*), and 99.5% (*C. lytica*) reduction in bacteria attachment, and 100% reduction in barnacles cyprid attachment. In addition to the significant improvement in fouling resistance of various organisms, the 60-day field test also showed an evident efficacy from visual assessment, foul rating, and fouling removal test.

The research confirmed that the novel antifouling mechanism of betaine polymers provides a new avenue for marine coating development. The developed coatings out-performed currently used nontoxic underwater coatings in a broad spectrum of fouling resistance. By further developing formulations and processing methods for specific devices, the technology is ready for the next stage of development with demonstration in MHK systems.

11. Presentation and Publication

11.1. Presentation

Z. Zhang, C. Huval, and C. Loose, “Betaine-Based Polymers as Environmentally Benign and Biofouling-Resistant Underwater Surface Modification” 241st National ACS Meeting, Division of Environmental Chemistry, Anaheim, CA (Oral presentation, 2:00-2:25 PM, March 29, 2011).

12.2 Publication

Z. Zhang, and C. Loose, *Developing Antifouling Marine Coatings Using Protein-Resistant Betaine-Based Polymers*. It's All in the Water: Studies of Materials and Conditions in Fresh and Salt Water Bodies, ed. M.A. Benvenuto, et al. Vol. 1086, Chapter 7. 2011: The ACS Symposium Series

Z. Zhang et al. *Betaine-Modified Silicone Surfaces with Long-Term Antimicrobial Performance*, in preparation

Z. Zhang et al. A Betaine-Based Underwater Coating that Resist the Attachment of Biofilm and Marine Organisms, In preparation

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