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Bioinspired synthetic macroalgae: Examples from nature for antifouling applications

James Chapman [a,*], Claire Hellio [c], Timothy Sullivan [b], Robert Brown [b], Sonia Russell [b], Eolann Kiterringham [b], Laurianne Le Nor [b], Fiona Regan [b,**]

[a] School of Medical & Applied Sciences, Central Queensland University, Queensland, Australia
[b] Marine and Environmental Sensing Technology Hub, National Centre for Sensor Research, School of Chemical Sciences, Dublin City University, Dublin, Ireland[1]
[c] School of Biological Sciences, University of Portsmouth, King Henry Building, Portsmouth PO1 2DY, UK

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ABSTRACT

The demand to develop a novel, environmentally friendly antifouling (AF) material is ever increasing. An attractive option in developing such a material is biomimicry—learning from nature's own designs and solutions and transferring them to solve problems that affect human day-to-day living. In order to achieve this goal the actual mechanisms and strategies that evolution has produced need to be elucidated from the subject species. The work presented herein investigated the role of surface topography and chemistry combined in a single material – a property that naturally exists in some common macroalgae. *Saccharina latissima* (sugar kelp) and *Fucus guiryi* (Guiry's wrack) were selected as a platform for "bioinspiration." Here, the surfaces of the samples were characterised and then replicated using simple polymeric reproduction methods. Furthermore, a pre-extracted brominated furanone was doped into this matrix (0.05 μg ml^{-1}). The replicated macroalgae samples containing the brominated furanone compound were compared in a 7-day marine study to investigate the effects of biofouling. The bioinspired samples directly demonstrated that combinatory approaches (using topography and chemistry) exhibited lower levels of biofouling. Here it is reported that both chemistry and topography demonstrated 40% less biofouling when compared to blanks in all of the pre-designed biochemical biofouling assays. This represents an attractive nontoxic alternative to the current state of the art.

1. Introduction

The management of marine biofouling on artificial structures is essential in maintaining good, operational effectiveness of marine structures. Marine biofouling organisms, as previously described by Maréchal and Hellio (2009), can be divided into three categories: (a) microfoulers, i.e., bacteria, diatoms, fungi, and microalgal biofilms, all of which are responsible for a 1–2% augmentation in frictional drag; (b) soft macrofoulers (macroalgae), which account for 10% of ownership costs; and (c) hard macrofoulers—barnacles, mussels, tubeworms, and bryozoans—accounting for up to 40% increase in cost of ownership of any deployed marine structure. It has been reported that maritime transportation accounts for 90% of global exchange (Hellio et al., 2004). Thus the sailing and movement of ships presents a constant confrontation with significant biofouling challenges, requiring hull protection all year round. The effects of biofouling on ship hulls do not only manifest as increased ownership costs (transport delays, loss manoeuvrability, biocorrosion-related hull repairs, cleaning, dry-docking and paint application), but also as environmental pollution (increases in carbon dioxide, carbon monoxide, and sulphur dioxide) and more laterally cross-contamination of alien species all of which are worth approximately €120 billion per year to remedy.

During the 1960s chemical companies developed the organotin compounds tributyltin (TBT) and triphenyltin (TPT) as effective self-polishing materials. However, these chemicals were later found to be highly toxic for many aquatic organisms and have been shown to be bioaccumulative and to contaminate the food chain.

* Corresponding author.
** Corresponding author.
E-mail addresses: j.chapman@cqu.edu.au (J. Chapman), fiona.regan@dcu.ie (F. Regan).
[1] Tel.: +61 (0)1749306493.

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New biocide formulations have shifted towards using high volumes of copper and herbicides such as Irgarol 1051® and Zineb®. However, evidence is accumulating that these paints (and their included biocide matrices, although initially reported to be environmentally friendly) show significant build-up in marinas and harbours. Moreover, evidence is also amassing that bacteria, when in contact with these AF formulations, are rapidly developing resistance to these biocides, especially in estuaries (Berlinck et al., 2004).

There is a large need for the development of novel, non-toxic alternatives to limit severe fouling organisms in the marine environment, such as algae, barnacles, mussels, and bryozoans (Maraldo and Dahllof, 2004). Increasingly, natural products are seen as a new approach in AF capabilities for use in marine coatings and paints (Chambers et al., 2006), offering an alternative solution for this age-old problem. Attempting to mimic natural AF solutions from nature is known as biomimicry and, in the broader sense, "bioinspiration." Indeed, most marine organisms are prone to biofouling, where colonisation and proliferation of fouling organisms causes significant stresses to the species being colonised. Organisms that settle on the surface or body of another organism are called epibionts, where the host organism is known as the basibiont. A range of chemical, physical, and mechanical defence mechanisms have evolved as natural AF strategies for these relationships. All of these mechanisms have been highlighted as potential routes in devising novel AF developments (Ralston and Swain, 2009). This has brought about new and exciting research avenues in the microtexturing of surfaces, marine natural product use and novel foul release coating design.

A bioinspired approach provides a method to incorporate nature's own defence mechanisms, and indeed for this example, AF solution to solve its own biofouling issues. Recently, attention has been paid to the physical defence mechanisms of marine organisms of molluscan shells, coralline algae, and shark skin (Schumacher et al., 2007). Research teams have subsequently tried to perfect replication methods to reproduce these microtextured surfaces. These methods include laser ablation (Scholz et al., 2011), photolithography (Scardino et al., 2009), nanoparticles, and moulds and casting. Furthermore, their testing for AF efficacy has also shown promise in the laboratory and the field. For instance, Scardino and de Nys (2011) have highlighted the fact that fouling organisms (during the attachment phase) vary significantly in size and shape (bacteria, 1 μm; diatoms, 3–15 μm; algal spores, 5–10 μm; and larval macroorganisms, 120–500 μm), which affects the surface they attempt to adhere to (Scardino and de Nys, 2011). They have also shown that attachment points are crucial for the success of settlement correlated to the microtexturing (size and surface features).

In addition to microtexturing defence, many marine organisms also produce natural compounds that exhibit AF properties. Some organisms do not seem to present mechanical or physical defence mechanisms against primary colonising organisms but are able to resist overgrowth of epibionts (Marris, 2006). This ability has been linked to the production of secondary metabolites that are involved in the chemical defence (Steinberg et al., 2002) category and has been well-documented (Scardino and de Nys, 2004). Biofilm-forming microorganisms tend to produce slimes that are protective against environmental fluctuations that would usually disrupt the overall living capacity of the organisms contained within. The production of slime, known formally as exopolymeric substances (EPS), can be measured and quantified on the surface of a substrate. Typically, EPS contain ranges of biochemical signatures such as proteins, carbohydrates, uronic acids, and deoxyribonucleic acid (DNA), to name a few. Biofilm formation can be divided into three distinct stages, from the initial attachment of bacteria to the surface to the formation of the mature biofilm with a characteristic three-dimensional architecture (Costerton et al., 1995; Stoodley et al., 1999). Microbial functions, such as motility, adhesion, stress response, metabolic pathways, and transport along with gene expression (some of which is involved in exopolysaccharide, liposaccharide and the development of mature biofilm architecture) are required at each stage of biofouling development (Stewart et al., 1995).

One AF approach is well established by some macroalgae, in particular macroalgae that live in the photic zone region of seas and oceans. These macroalgae exhibit low epibiont coverage, and are known to possess effective defence mechanisms. Chemicals known as furanones, in particular halogenated furanones, are released from the macroalgae. These halogenated furanone compounds have the ability to inhibit N-acyl-L-homoserine lactones (AHLs), which are used to control the synthesis of products that facilitate interactions with the surrounding environment for many microorganisms. It has been suggested that AHL-dependent gene expression constitutes the way in which a microorganism alters its behaviour or density of population; this process is commonly referred to as quorum sensing. The literature concentrates on the isolation and subsequent synthesis of natural products from these macroalgae as a chemical defence (Hellio et al., 2009; Plouguerné et al., 2010; Thabard et al., 2011) with little to no mention of the influence of topography contributing to enhanced biofouling protection. It has already been shown by Sullivan and Regan (2011) that surface topography can actually greatly influence the overall epibiosis of a given surface – in particular shark skin has been replicated and then tested for its AF efficacy in a marine environment. The authors found that a general reduction in epibiosis occurred in the scale-replicated surfaces. Although vast volumes of research has been carried out in investigating macroalgae, investigating surface topography and chemistry as a synergistic, viable antifouling resource has not. Therefore, the authors recognise that this is possibly the first time this has ever been investigated.

The aim of this work was to create an AF material with a combined bioinspired defence mechanism taken directly from macroalga. The macroalgae used in this study were chosen because they show no sign of biofouling on their surfaces during high and low fouling seasons. Two common macroalgae native to Irish shores in Dublin, Ireland, were isolated and replicated in a low-surface-energy polymer. The polymers were then doped with a brominated furanone, previously extracted from *Ulva rigida* (known to cause inhibitory effects in the quorum-sensing pathway of some microorganisms). In this study it was hypothesised that an AF effect could be induced through the replication of a synthetic surface where both the combination of surface features and chemistry would enhance the AF ability of these replicated surfaces. This idea was formulated on the basis that certain macroalgae have this natural ability to produce AF activity, thus the replication and biomimicry of such a surface would also perform in this manner when introduced into a marine environment.

2. Materials and methods

A series of material replication steps were performed in order to isolate the topography of the macroalgae followed by doping. The replicated materials were then exposed to a marine water body in Dublin Bay, Ireland, for 7 days. The materials were subsequently analysed for biochemical responses for biofouling in its initial stages and characterised with a series of biochemical assays. The epoxy matrix selected for this study had previously demonstrated significant reductions in microbial adhesion (Kharchenko et al., 2012) and represented an excellent starting point for this work.

2.1. Chemicals and reagents

A liquid polymer polydimethyl siloxane (PDMS) kit (Sylgard 182 elastomer) was purchased from Dow Corning, Ireland. Protein standard chemicals (bovine serum albumin (BSA), Lowry reagent

powder, Folin–Ciocalteu Phenol, glutaraldehyde, D-(+)glucose, and D-glucuronic acid) were purchased from Sigma Aldrich, Ireland, and used without further purification. Epoxy resin (Araldite CY212) was purchased from Agar Scientific, Ireland. AF (benchmark) paints—Cruiser Uno International and Waterways International—were purchased from Kehoe Marine Ltd, Ireland.

2.2. Artificial macroalgae replication

Parts of two macroalgae species native to Ireland, *Saccharina latissima* (blade of sugar kelp), and *Fucus guiryi* (receptacle of Guiry's wrack, Fig. 1) were prepared and characterised for replication. Specific regions of the thallus were copied.

2.2.1. Macroalgae preparation

In the first step the macroalgae were removed from their fixed points on the shore and were fixed on site using 2.5% glutaraldehyde in artificial seawater. The samples were placed in ice and taken back immediately to the laboratory for preparation. In a typical preparation, a 10-mm × 10-mm sample of the macroalga was cut using a sterile scalpel. A piece of scotch tape was then firmly placed on the surface, and quickly removed. This was repeated twice or better still optimised for the piece of alga using SEM to visualise this detail. This sample was then placed in a 50-mm × 50-mm square-shaped weigh boat. The sample was then pressed to be as flat as possible to the weigh boat bottom surface. Alongside, a PDMS polymer solution was prepared by mixing well with a glass rod 10:1 elastomer:curing agent (both supplied in the kit), instructions for the preparation of PDMS are also comprehensively described in the manufacturer's instructions. This solution was then placed in a vacuum to degas for 10 min. The PDMS solution was then poured over the macroalga sample to a depth of 4–5 mm and cured in an incubating oven at 50 °C overnight with degassing. The macroalgal sample was removed with sterile tweezers and a scalpel. The mould was then washed with Milli-Q water three times, and allowed to air-dry. Epoxy resin was then prepared by mixing 20 ml (23 g) Araldite CY212 (M) with 22 ml (22 g) dodecenyl succinic anhydride (DDSA) and finally 1.1 ml (1.2 g) benzyl-N,N-dimethylamine (BDMA) (C.3%). This mixture was degassed thoroughly, poured in to the mould with some agitation, and then allowed to cure at 60 °C.

2.2.2. Artificial chemistry of replicates

An extracted furanone, 3-bromo-5-(diphenylene)-2(5H)-furanone from *U. rigida*, was taken. To prepare the artificially doped replicates, the furanone component was directly doped into the epoxy mixture (0.05 μg ml^{-1}); this concentration was selected as previous literature demonstrated 0.02 μg ml^{-1} was an effective concentration for a 50% reduction of microorganism growth (Chambers et al., 2006). The epoxy replicates were allowed to air-cool before removal from the mould. This process was repeated for each of the macroalgae samples and for an experimental mean of $n = 9$. The overall casting and replication process is shown in Fig. 2.

2.3. Marine deployment and test site

In order to test the materials, the replicated macroalgae samples were attached to glass slides in rows of three. Five glass slides containing a total of 15 samples per replicated macroalga sample were then deployed in engineered test racks suitable for a marine grade deployment, as previously described in Chapman and Regan (2012) for a 7-day period. The site was situated in brackish water in Dublin, Ireland, at Poolbeg Marina; the temperature of the water was 13.7 °C during the month of August 2012.

2.4. Protein and carbohydrate adsorption assay

Protein adsorption was measured and carried out using a modified Lowry assay. Three reagents were prepared: (1) The Lowry reagent powder was dissolved in 40 ml Milli-Q water; (2) Folin–Ciocalteu's phenol reagent was dissolved with a 1:5 ratio in Milli-Q water; and (3) BSA was prepared using 2 mg dissolved in 5 ml Milli-Q to achieve a 400 μg ml^{-1} protein concentration. A calibration curve was established. In a typical assay, Lowry reagent, Folin–Ciocalteu's reagent, and 0.5 ml of sample was mixed and then incubated for 30 min at room temperature. This solution was then measured at 750 nm on a UV–visible spectrophotometer.

Carbohydrate adsorption was determined by taking a 0.5-ml aliquot of sample. This was transferred into a 15-ml centrifuge tube, into which 0.5 ml phenol solution and 2.5 ml of concentrated sulphuric acid were added immediately. The samples were then incubated for 10 min at room temperature and then at 30 °C for 15 min. After a 5-min resting time, the samples were then measured at 480 and 490 nm using UV–visible spectrophotometry.

2.5. Deoxyribonucleic acid adsorption assay

In a typical assay DNA adsorption on the artificial macroalgae surfaces was determined using a commercial Quant-IT™ PicoGreen® dsRNA Reagent Kit (Invitrogen, Molecular Probes). A series of low and high calibration curves were derived using the following solution setup. Solution 1: 20 × TE buffer (200 mM Tris–HCl, 20 mM EDTA, pH 7.5) was diluted 20-fold in water for molecular biology. Solution 2: PicoGreen reagent was diluted 200-fold in 1 × TE buffer. Lambda-DNA stock solution was prepared thus: A 2 μg ml^{-1} (high range) stock solution was prepared by diluting the 100 μm ml^{-1} Lambda-DNA standard 50-fold in 1 × TE buffer. A

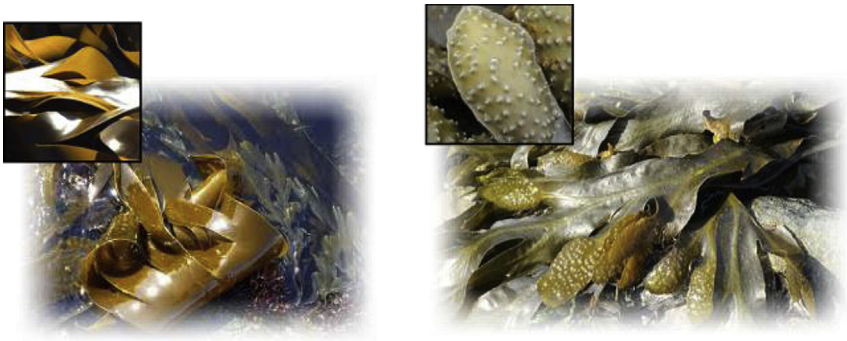

Fig. 1. Photograph detailing, left: *Saccharina latissima* and right: *Fucus guiryi*, with inset images highlighting region selected for replication.

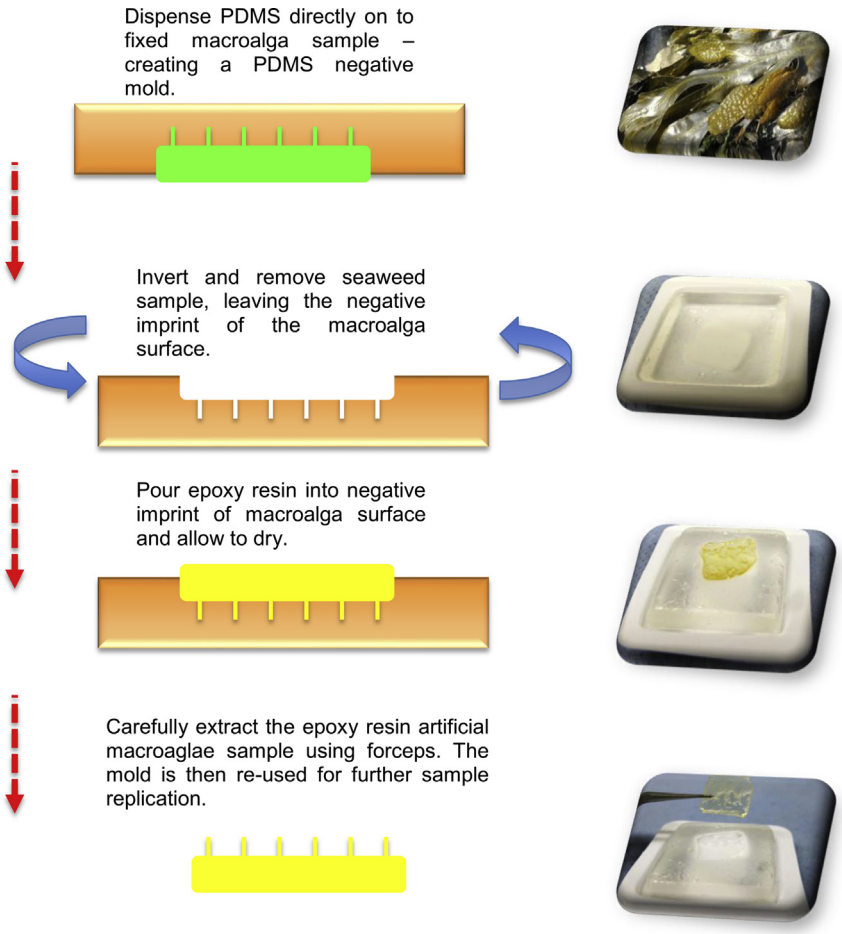

Fig. 2. Replication procedure for synthetic macroalgae used in this study.

50 ng ml^{-1} (low range) stock solution was prepared by diluting the 100 μg ml^{-1} Lambda-DNA standard 2000-fold in 1 × TE buffer. A series of low and high calibration curves were then derived from the calibration standards in the kit instructions. If necessary, samples were diluted in 1 × TE buffer to reach DNA concentrations within the range of one of the calibrations. One millilitre of standards or samples was transferred into a 1-cm disposable plastic cuvette, where 1 ml of solution 2 was added and incubated for 2 min in the dark at room temperature. Relative fluorescence of all the solutions was measured at the excitation wavelength of 480 nm and the emission wavelength of 520 nm.

2.6. Biomass and slime analysis

Each replicated macroalgae substrate was weighed before and after immersion into Poolbeg Marina to ascertain mass fluctuation across the surface of each of the samples. Samples, following exposure, were rinsed with Milli-Q water to remove any unadhered material and then allowed to air-dry in order to eliminate water from the biomass result. A deduction of weight of each sample gave a result pertaining to overall adhered content. On the same samples, slime (glycocalyx) was evaluated through a series of fixation and staining steps. Slime production was measured on the test material that was attached to the surface of the replicate macroalgae only. The samples, once washed, were reacted with Carnoy's solution (6:3:1, absolute ethanol: chloroform: glacial acetic acid) for 30 min. The solution was then decanted and toluidine blue 0.1% (v/v) (Avonchem Ltd., UK) was added to stain the biofilm present on the polymers for 1 h. The excess stain was decanted off, and the polymers were rinsed twice with Milli-Q water (3 ml). NaOH (0.2 M) was added and the solution was heated to 85 °C for 1 h. Each sample was then left to cool to room temperature and the optical density was measured at 590 nm (Cary 50 UV–visible spectrophotometer).

2.7. Physical characterisation of the macroalgae replicates

The sample had been previously placed into a 2.5% glutaraldehyde solution in seawater to fix and preserve any material on the surface. Surface analysis of adhered microorganisms and cells of the washed artificial macroalgae samples was carried out using a Hitachi S3400 scanning electron microscope (SEM). Accelerating voltages of 5–15 keV with secondary electron (SE) detection was used; varying the working distances from 5 to 10 mm. Nine replicate samples were prepared. The artificial seaweed sample of dimensions 1 cm × 1 cm was placed onto a carbon stub. The samples were sputter-coated with gold for 2 min, at which point an approximate deposition of a 65-nm gold layer was achieved.

2.8. Statistical analysis

All experiments were performed in triplicate. Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate differences between groups. A p-value of $^{*}<0.05$ and $^{**}<0.01$ was considered statistically significant. Student's t-test was

also used to identify statistically significant differences at a 95% confidence level.

3. Results and discussion

3.1. Surface characterisation

Characterisation of the macroalgae samples was assessed using SEM. Both the natural and artificial samples were characterised in this manner to demonstrate an artificial and experimentally viable replica of the original sample prior to the immersion tests (to be described). The macroalgae selected in this study all contain cellulose in their inner cell wall, with the outer cell wall consisting of an amorphous bedding matrix. In particular, in Phaeophyceae this matrix is predominantly alginic acid with a small quantity of sulfanated fucoidan (Davis et al., 2003); in Rhodophyceae this is comprised of xylans and several sulfanated galactans, including agar and carrageenan, while in Chlorophyceae, the consistency results from both xylans and mannans (Davis et al., 2003). Generally, a mucilage and sheath are both present on both prokaryotic and eukaryotic algae, demonstrating a gelatinous covering, which in turn does not generally enable SEM to resolve any "true" detail of the surface topographical features. In this case, a series of mucilage removal steps were integrated into the sample preparation in order to remove the layer. The latter, in turn, reveals the surface topography for each of the macroalgae used (Fig. 3).

It is clear from the electron micrographs presented in Fig. 3 that the artificial epoxy samples are similar in surface morphology to the actual macroalgae sample, highlighting PDMS as an effective surface setting polymer for this type of application. It was found that in measuring surface features of the *F. guiryi* scotch tape samples for $n = 10$ surface features selected, the mean surface feature length was 9.9 ± 1.5 μm, and for the reproduced sample, 10.3 ± 2.01 μm. For the *S. latissima*, scotch tape method surface feature analysis revealed spicules of sizes 3.3 ± 1.322 μm in the real sample and 3.1 ± 0.88 μm for the replicated. Both these results show statistical similarities when a one-way ANOVA was applied. This has also been repeated for other types of topographical replication and similarly enables simple and effective low-cost surface topography replication. To the best of the authors' knowledge this is the first time this type of surface topographical replication using macroalgae has been achieved, and to this level of reproducibility. Similar surface features have been observed using SEM by Andrade et al. (2004). However, the authors made no attempt to remove the outer layer of mucilaginous matrix that may have revealed further details using enhanced characterisation and surface preparatory steps. Sullivan and Regan (2011) achieved comparable results using soft polymer replication of fish scales. Here the authors successfully replicated a slow-swimming shark's dermal denticles using a similar PDMS method. The authors found that significant differences in biofouling were observed on denticles of various dimensions under specific field conditions. This strategy used with marine organisms has been widely reported, with low-drag performance of shark skin surfaces having been seen producing drag reduction of some 5–10%. For instance, in the Sharklet technology, surface features of 2–15 μm have been found to effectively inhibit some specific bacterial attachment. Similarly, pilot whale skin has shown to be antimicrobial owing to the presence of nano-pores and ridges in combination with an enzymatic gel capable of denaturing proteins and carbohydrates (Baum et al., 2002).

Surface topographical engineering that has evolved in nature can influence a system immensely, where hierarchical changes in the micro and nanoscales broadly change equilibria and dissipation and cause varied stability in the system. Frequently, a change in surface topography will lead to capillary effects (Nosonovsky and Bhushan, 2009) that invoke functionality of a surface. An example of this is the shell of the blue mussel *Mytilus edulis*, where the shell possesses multiple defence mechanisms (ripple-like microtopography and AF compound production (Salta et al., 2010)). This

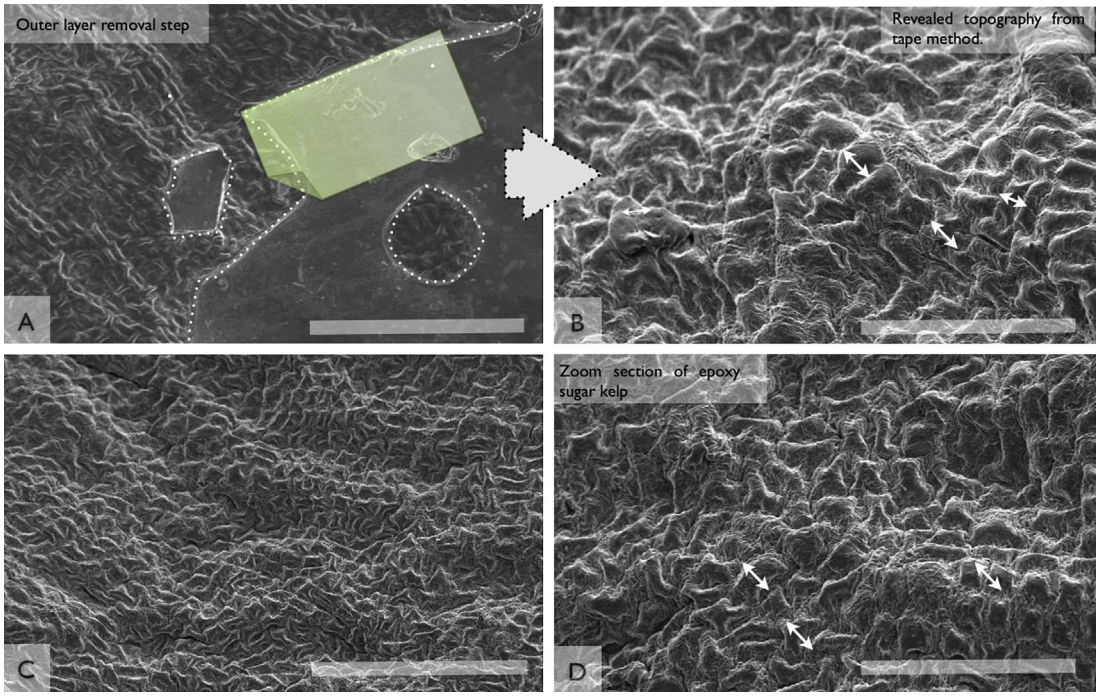

Fig. 3. Scanning electron micrograph images of macroalgae replication procedure: (A) *Saccarina latissima* surface, scale bar 100 μm with scotch tape removal method where the dotted lines indicate the mucous layer; (B) revealed topography after scotch tape method applied, scale bar 50 μm; (C) epoxy replicate of *Saccarina latissima*, scale bar 100 μm; and (D) epoxy replicate, scale bar 50 μm.

is a very similar approach in designing the 'synthetic' macroalga reported in this work (where both topography and chemistry interact synergistically). The work carried out shows a macroalga with smooth morphology compared to a rougher one.

3.2. Biofouling characterisation

The samples were evaluated using a series of characterisation assays determining the overall antifouling efficacy of each material. The composition of a biofilm is fundamentally comprised of a range of biochemicals, such as deoxyribonucleic acid (DNA), protein, carbohydrate, and uronic acids. The assays in this work were designed to analyse some of these components and thus enable the overall AF capacity can be determined per material.

This was achieved through a series of assays developed for marine biofouling testing to detect biochemical components that exist within a biofilm and indeed in microbial attachment and proliferation upon any surface. Measurements for slime, mass, protein, carbohydrate, and DNA were all performed and presented herein.

3.2.1. Protein adsorption

Following a 7-day deployment of the artificial macroalgae samples, protein adsorption measurements were carried out to ascertain the level of biofilm development for each of the samples (Fig. 4).

Total protein adsorption on each of the replicated macroalgae surfaces was analysed using a modified Lowry reaction. It was found that following the 7-day sample deployment, all combinations of chemical doped replicated topography samples demonstrated less protein adsorption when compared to the topography replicates alone and the epoxy blank in this study. The epoxy blank demonstrated most protein adsorption (36.43 ± 1.34 μg ml^{-1}), in contrast to the furanone-doped Guiry's wrack (7.01 ± 1.22 μg ml^{-1}), which showed five times less protein attachment. Statistical analysis using a one-way ANOVA assessing the combined AF mechanisms coupled with topography showed a significant difference ($p < 0.001$) between the furanone-doped material and surface topography.

3.2.2. Carbohydrate adsorption

Fig. 5 depicts the total level of carbohydrate adsorption following the 7-day immersion assay in the marine environment.

In a similar trend to that observed with the protein adsorption results, it was found that the combination of topography and doped furanone had shown the least carbohydrate adsorption. An analysis of variance also showed that there was statistical difference ($p < 0.05$) between the combined topography and furanone samples and the single topography samples for carbohydrate adsorption. Again, these results reflect that by doping a brominated furanone into the epoxy topographically active matrix, biofilm development and proliferation is repressed in these sample types.

3.2.3. DNA determination

Another key biochemical present in a biofilm is in the form of signature DNA which is readily produced in biofouling organisms, shown in Fig. 6 as DNA adsorption to the samples.

Total DNA for each of the furanone-doped and surface topographically active artificial macroalgae samples have all shown less DNA adsorption compared to the undoped samples. Both the blank epoxy sample (0.954 ± 0.076 μg ml^{-1}) and the furanone-doped blank epoxy (0.943 ± 0.084 μg ml^{-1}) showed most DNA adsorption. The furanone-doped blank epoxy showed, as corroborated by protein and carbohydrate adsorption results, most biochemical adsorption in all assays thus indicating the lowest level of AF effectiveness. However, the furanone-doped samples showed reduced DNA adsorption when compared to the undoped, highlighting that the compound is delivering an active AF effect. The level of DNA on the surface of the substrate is directly linked to microbial and biofilm-forming species and can be quantified using this commercially available quantification method (Black et al., 2004). Doping the brominated furanone in to epoxy demonstrated added AF response to the topographically active substrates in all assays used within this work. It is known that brominated furanone compounds from brown algae have the capacity to interrupt the complex communication system used by some microorganisms (de Araujo et al., 2010). Microorganisms behave as single organisms at lower cell densities; however, they may shift their behaviour to the multicellular type by sensing that the AHL pathway is interrupted, resulting in a state of confusion, and thus interrupting settlement and the overall biofouling/biofilm process. Since EPS plays an important role in biofilm formation, interrupting the quorum-sensing pathway known as quorum quenching (QQ) poses a new opportunity in developing an effective AF material.

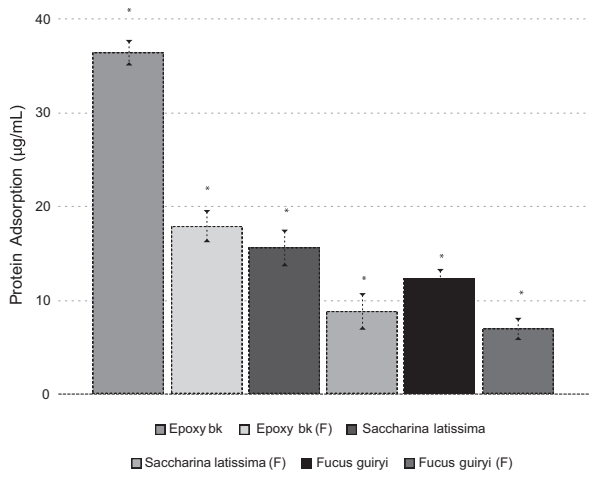

Fig. 4. Protein adsorption for synthetic macroalgae following a 7-day study in the marine environment. F = denotes furanone added ($n = 3 \pm 1$ SD).

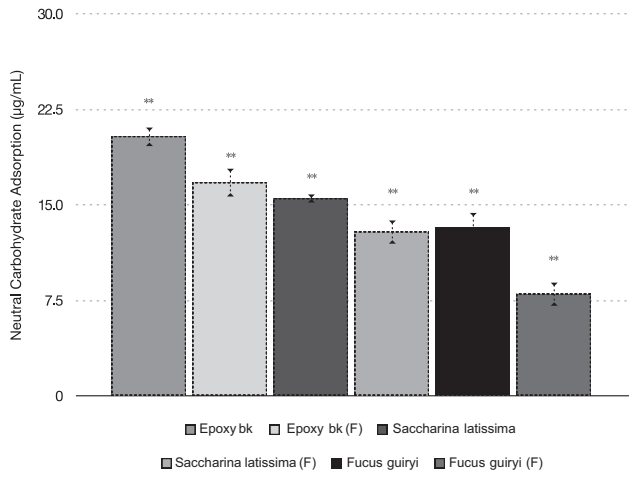

Fig. 5. Neutral carbohydrate adsorption for synthetic macroalgae following a 7 d study in the marine environment F = denotes furanone added. ($n = 3 \pm 1$ SD.)

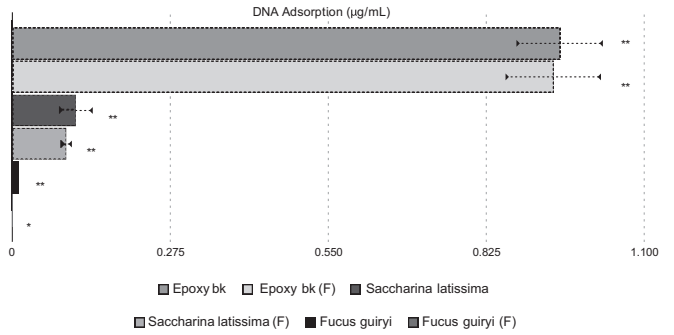

Fig. 6. Total DNA adsorption of each of the replicated synthetic macroalgae samples following a 7-day marine exposure study. F = denotes furanone added. ($n = 3 \pm 1$ SD.)

3.2.4. Slime and mass adsorption assay

A further screening method in determining the AF property of the substrates was run in tandem analysing slime and overall mass (Fig. 7). Slime was used to quantify the level of glycocalyx (inclusive of excreted EPS) adsorbed to each of the materials. In other publications this has proved to be an effective assay; however, only when run in conjunction with concrete quantitative biochemical assays, where the slime test can be used as a confirmatory screening assay, as demonstrated (Tsai et al., 1988).

Mass adsorption was found to be lower on each of the topographical epoxy macroalgae samples (as shown in the radar plots) and also on the epoxy blank (0.043 ± 0.09%) where this demonstrated elevated slime adsorption (0.21 ± 0.012 AU). The best performing samples in this assay were found to be a combination of topographical and furanone-doped chemically active replicates, and these are results that have been fully confirmed throughout the entire suite of biofouling assays used within this work.

In similar attempts to resolve the effectiveness of a brominated furanone, Baveja et al. (2004) physio-sorbed a furanone derivative on to a polymer used for medical devices. The resulting surfaces were shown to reduce production of biofilm by *Staphylococcus epidermis* on all materials tested (Baveja et al., 2004). In another article, Al-Bataineh et al. (2006) performed a covalent immobilisation of a furanone compound onto a functional silane monolayer. In this paper, the direct doping of a furanone into the epoxy resin matrix allowed an active slow diffusion and AF effect through the polymer matrix. Measurements to characterise the release of furanone from the matrix were not performed in this study. However, other researchers found epoxy resins gave excellent release activity when doped with metals during the epoxidation process (Arnold et al., 2006). This is true of the furanone that was doped in the interpenetrating network of the epoxy resin used, where the furanone potentially leached through the epoxy matrix, thus improving the AF activity. In principle, the furanone allows for improved AF effectiveness when used in combination with the epoxy with macroalgae topography. Epoxy-based marine coatings have been widely applied to combat the effects of corrosion of certain materials owing to their low cost and efficient behaviours in aggressive media, such as the marine environment (Brady, 1999). Epoxy materials have also been used for immobilisation of cells, secondary metabolites and enzymes alike presenting a retained surface activity over long periods of time. The retention of surface activity is a desirable AF property, especially in high fouling seasons where prolonged activity is crucial to the success of the material. If the epoxy matrix affords a longer lifetime in a marine system deployment, this reduces the need for cleaning and indeed, time – reducing the overall cost of ownership of the deployed system. The results reported herein show significant potential as a novel approach to an environmentally friendly alternative material.

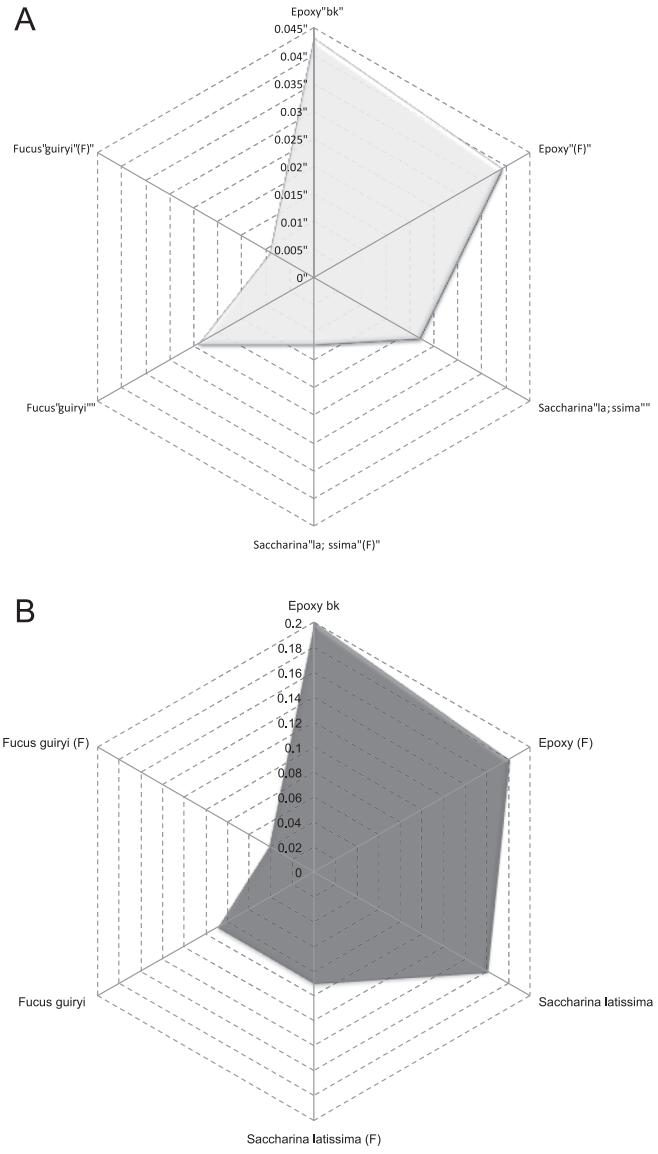

Fig. 7. Radar plots showing (A) mass and (B) slime adhesion of the replicated macroalgae samples following a 7-d marine exposure assay. F = denotes furanone added. ($n = 3 \pm 1$ SD.).

The effect of combined chemistry and topography has been similarly demonstrated in Díaz et al. (2012), where the authors also describe the synergy between surface chemistry and surface topography as presented in this work. All the biofouling assays point toward the use of combinatory approaches rather than single endeavours. The combined *F. guiryi* topography and doped furanone have exhibited the lowest levels of biofouling in all of the assays. Protein adsorption was 35% lower when both approaches were compared, carbohydrate adsorption was 43% lower, and DNA was found to be in minimal concentrations.

Clearly, this work demonstrates that furanone-doped materials with replicated surface topography have shown encouraging results in preventing biofouling dominance on each of the chemically doped replicates as there was an almost 45% improved AF response for each of the assays used. Unfortunately, natural and synthetic brominated furanones reported to date vary in the types of regioselectivity of the substituents; thus the actual mode of action remains unclear (Rice et al., 2005). Thus, information for the mode of action on furanone compounds is critically important for developing biofilm control

strategies at the molecular level for future technologies. Interestingly, Hahn and Padmanabhan (1995) have reported on the use of furanone for the inhibition of biofilm forming *Escherichia coli*.

To the best of the authors' knowledge this is the first report of topographical and chemical antifouling mechanisms being replicated in simple polymers – and then tested as a viable AF material. Hopefully these results can be studied in the near future and used as a basis for research on additional models taken from nature – a bioinspired route.

4. Conclusions

The field of bioinspiration allows researchers to examine dynamic problems solved through nature (such as marine epibiosis) and harness them for their own needs, such as the field of biofouling and AF materials. The potential of the synthetic macroalgae samples has been demonstrated showing excellent AF activity when surface topography and chemistry are combined. Most authors present single-approach AF results, and indeed these are sometimes effective and excellent results have been shown. However, the results presented in this work demonstrate conclusively that a combined approach using bio-inspired solutions enhances the AF system considerably.

In nature, multiple AF systems exist and have been counteracting the effects of epibiosis and biofouling for millennia. Bioinspiration and bioinspired design of materials to counteract biofouling represent a plausible research route for the creation of such a material. This route may also offer answers to many other problems for different applications that have been addressed and solved in nature.

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