Bubbles *vs* biofilms: A novel method for the removal of marine biofilms attached on antifouling coatings using an ultrasonically activated water stream.

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ABSTRACT

The accumulation of marine organisms on a range of manmade surfaces, termed biofouling, has proven to be the Achilles's heel of the shipping industry. Current antifouling coatings, such as Foul Release Coatings (FRCs), only partially inhibit biofouling, since biofilms remain a major issue. Mechanical ship hull cleaning is commonly employed to remove biofilms, but these methods tend to damage the antifouling coating and often do not result in full removal. Here, we report the effectiveness of biofilm removal from FRCs through a novel cleaning device that uses an Ultrasonically Activated Stream (UAS). In this device, ultrasound enhances the cleaning properties of microbubbles in a freely flowing stream of water. The UAS was applied on two types of commercial FRCs which were covered with biofilm growth following twelve days immersion in the marine environment. Biofilm removal was quantified in terms of reduction in biovolume and surface roughness, both measured using an optical profilometer, which were then compared with similar measurements after cleaning with a non-ultrasonically activated water stream. It was found that the UAS significantly improves the cleaning capabilities of a water flow, up to the point where no detectable biofilm remained on the coating surfaces. Overall biofilm surface coverage was significantly lower on the FRC coatings cleaned with the UAS system when compared to the coatings cleaned with water or not cleaned at all. When biofilm biomass removal was investigated, the UAS system resulted in significantly lower biovolume values even when compared to the water cleaning treatment with biovolume values close to zero. Remarkably, the surface roughness of the coatings after cleaning with the UAS was found to be comparable to that of the blank, non-immersed coatings, illustrating that the UAS did not damage the coatings in the process.

Keywords: Biofilms, Adhesion, Ultrasonically Activated Stream, Antifouling, Coatings, Cleaning

INTRODUCTION

The unwanted growth of marine organisms on the hulls of ships, known as biofouling, is a well-known phenomenon with serious economic consequences. The attachment of plants and animals such as seaweed (algae) and barnacles causes a great increase in frictional drag, resulting in increased fuel consumption with correspondingly higher emissions of carbon and sulphur dioxides (IMO, 2001). The significant impact of biofilms on a ship hull's surface roughness, and therefore drag, has been repeatedly reported (e.g. Schultz, 2004; Schultz, 2007). Recent findings by Schultz et al. (2015) demonstrated that biofilms were responsible for an up to 70% increase in skin-friction when they tested FRC systems with biofilm thickness up to ~500 µm. As the global oceangoing fleet was calculated to consume between 200 and 290 million tonnes of fuel in the year 2000 (Eyring et al., 2010), this level of drag results in the annual wastage of fuel costing billions of dollars.

Throughout history various poisonous compounds have been applied to ships below the waterline to kill and deter biofouling organisms. These have often had deleterious effects on the environment. From the 1970s onwards many ships were coated with antifouling paint containing organotin (primarily tributyltin) compounds. These extremely toxic compounds were very successful at preventing biofouling but also killed many non-target species in harbours and coastal waters resulting in a total ban on their use by the International Marine Organisation (IMO) in 2003 (IMO, 2009).

Over the past couple of decades a new technology for marine antifouling called Foul Release Coatings (FRCs) has been developed. FRCs are non-toxic and reduce biofouling by having 'non-stick' surfaces with low surface energy. These coatings are mainly based on silicones but may also contain fluoropolymers or hydrogels (Chambers et al., 2006). FRCs are effective against macrofouling organisms such as algae and barnacles, as these large organisms cannot attach themselves strongly to the surface and are therefore removed by hydrodynamic forces when the ship is underway at moderate speed in the region of 10 knots or more (Swain, 1999). The low energy, hydrophobic surfaces of FRCs are however easily colonised by biofilms (Schultz et al., 2011), which consist largely of bacteria and microalgae (diatoms) with pennate diatoms dominating (Dobretsov and Thomason, 2011; Salta et al., 2013; Hunsucker et al., 2014; Shultz et al. 2015). These biofilms are thin and flat, and are therefore subject to low shear forces by flowing water as they may not protrude far beyond the boundary layer. They remain attached to the hull of a ship even at higher speeds and accumulated slime must be physically removed at intervals.

The device of choice for removing biofouling during dry-docking is the power washer (also known as the 'pressure washer'). This method is effective as the high rate of flow induces pressure and shear onto the surface to be cleaned (Hagan et al., 2014). However, high pressure washers may damage the coating, especially FRCs that are particularly sensitive (Bureau of Reclamation, 2013). Other drawbacks include the production of backsplash, aerosol and spray, which can carry and redistribute contaminant onto the user (and which can be breathed in) and nearby articles (Leighton et al., 2013a). The importance of such redistribution depends on the application, and would differ depending on whether the contaminant contained sewage, bacteria, radionucleotides, petrochemicals or the chemical compounds within marine antifouling coatings.

Outside of dry-docking, the usual way of removing slime coverage is to send divers down with brushes and scrapers whilst the ship is in the water (Hagan et al., 2014). Increasingly this is not carried out in harbours owing to the problems associated with the large amounts of biofilm and potential alien species released into such confined bodies of water (Minchin and Gollasch, 2003). Deploying divers at sea brings problems of logistics and safety. Hull cleaning devices used by divers are normally hydraulically powered units with rotating heads bearing anything from polypropylene bristles to metal scrapers, depending on the degree of biofouling encountered. Mechanical hull cleaning inevitably causes some abrasion, especially to the relatively soft, elastomeric surfaces of FRCs. Damage to antifouling resulting from cleaning by divers using brushes has been estimated to cost US$6.00 per square meter (ANON, Ship Repair and Conversion Technology, 2015). Cleaning systems incorporating cavitation effects in the water flow, induced by the shape of the orifice, were tested almost two decades ago (Kalamuck et al., 1997) but have not been widely adopted.

We propose to use a newly developed non-abrasive cleaning technology to remove accumulated biofilm from a ship's hull: an Ultrasonically Activated Stream (UAS) technology. The UAS system was invented and developed at the University of Southampton (Leighton et al., 2016) and is being commercialised under licence by Ultrawave Ltd. The UAS system uses ultrasound to excite endogenous bubbles in a moderately flowing stream of water (1-2 L/min). This allows the removal of persistent contaminants without the necessity of chemical reagents that would otherwise be used to enhance cleaning. The ultrasonically activated bubbles are attracted and particularly effective at penetrating into cracks and crevices and lifting contaminants from the surface topography (Offin et al., 2014; Birkin et al., 2015a,b; Birkin et al., 2016).

Ultrasonic cleaning has been used by a range of industries for many decades, though always with the requirement that the target be immersed. Ultrasonic methods to prevent or remove marine biofouling require the target be immersed (Mazue et al., 2011; Legg et al., 2015). The most common way of inducing ultrasonic cleaning is in the form of the ultrasonic cleaning bath, although unlike UAS such baths could never be used for cleaning marine biofouling from a ship vessel hull because cleaning baths require immersion of the target to be cleaned. However the ability of ultrasonic cleaning baths (and related laboratory equipment, the sonochemical reaction vessel and the ultrasonically-equipped bioreactor) to remove biofouling from targets small enough to fit in baths has been tested (Zips et al., 1990; Wang et al., 2009).
Ultrasonic cleaning by UAS has properties, and provides useful facilities, that are wholly distinct from the form of ultrasonic cleaning that has been conducted for decades, for example, in ultrasonic cleaning baths (Leighton et al., 2005; Rivas and Verhaagen. 2016). Perhaps most obviously, UAS does not require immersion of the target to be cleaned, which restricts bath cleaning to components small enough to be contained within the bath. Indeed, such immersion can disturb the acoustic field that is set up in a bath, and reduce its cleaning ability (Leighton et al., 2013a) because slight changes to the container and volume in it can disturb a tuned field and reduce its amplitude (Birkin et al., 2003). UAS is immune to this drawback (Goodes et al., 2016). Moreover, UAS does not expose cleaned items to the ‘soup’ of contaminants that can be present in a used ultrasonic bath (Leighton 2015), which could have implications for secondary contamination. Furthermore, UAS has been found to cause no damage to delicate surfaces (e.g. Baby salad leaves) that would suffer when placed in an ultrasonic cleaning bath: this is because UAS cleans using only shear forces, and does not generate cleaning by stimulating inertial cavitation on the surface to be cleaned (the mechanism

by which all prior ultrasonic cleaning techniques), and so avoids blast waves, bubble jetting, and the production of free radicals. Further details are given in the next section.
The device has been successfully tested against biofilm formed of the medically important bacterium S. epidermidis (Birkin et al., 2015a) in which the UAS removed ~97% of the biomass, which was about 3 times the amount removed by the flow of water alone. The technology also proved effective at removing dental biofilms (plaque) composed of three different species of bacterium (Howlin et al., 2015), achieving 99.9% removal of S. mutans in ten seconds of exposure. Therefore it was of interest to test whether an UAS could be used to remove marine biofilms formed on commercial FRCs during exposure in the sea, and whether the UAS would cause any damage to the soft surface of such coating. The aim of the current work is to evaluate the effectiveness of this new cleaning method for the removal of biofilms formed on FRCs.

MATERIALS AND METHODS

Experimental rig and coatings

Two types of commercial FRCs were tested, referred to as FRC 1 and FRC 2. FRC1 is a modified silicone polymer coating, the hydrophobic surface of which develops a hydrophilic character on immersion in water. FRC2 is a fluoropolymer-based coating also with a moderately hydrophilic surface. Both coatings are smooth, relatively soft and elastomeric. These were applied on standard laboratory glass slides (Fisher Scientific) by the coating manufacturer. The coated slides were then mounted on custom-built experimental rosettes which each accommodate up to eight glass-slide-shaped surfaces (Meier et al., 2013). The design of the experimental rosette can be seen in Figure 1. In total, five duplicate coatings for each FRC were immersed in the sea at 1 m depth in June 2014 for twelve days, at a pontoon located at the National Oceanography Centre, Southampton, UK. Southampton is characterised by a temperate climate and June is a biofouling-intense month where increased biological activity is normally expected. For blanks, non-immersed FRC1 and FRC2 coatings (three duplicates per FRC) were used.

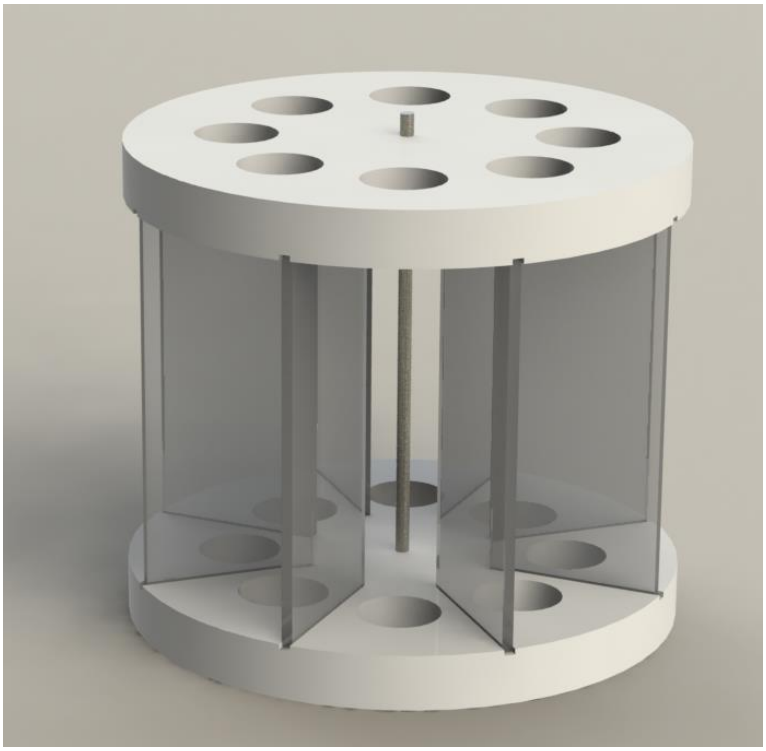

Figure 1. SolidWorks version 2013 (Dassault Systèmes, Vélizy-Villacoublay, France) model of the experimental rosette with eight glass slides mounted. The holes in the two holders (top and bottom) facilitate flow through the rosette.

Cleaning system and experimental procedure

The cleaning system that was assessed uses an Ultrasonically Activated Stream (UAS). In the UAS prototype that was used for this study, the water running through the device is ultrasonically stimulated before leaving its 1 cm diameter nozzle (the system is fully described in Birkin et al., 2015a). Rather than pressurising the stream (the prototype exerts pressures of less than 100 Pa on the surface to be cleaned, compared to 5 – 200 MPa for pressure washers), the ultrasonic stimulation causes surface waves to propagate on the bubbles-of-opportunity that are naturally present in the water supply (Maksimov and Leighton, 2001; Maksimov and Leighton, 2011). This is a phenomenon that occurs in presence of an acoustic field with the correct combination of frequency and amplitude (Birkin et al., 2002). The ultrasonically activated bubbles cause local convection and shear forces in the water surrounding the bubbles (Watson et al., 2003), mechanically enhancing the capability of the water stream to remove contaminants (Leighton, 2004; Howlin et al., 2015; Birkin et al., 2015a,b; Birkin et al., 2016). The ultrasonic activation furthermore forces the bubbles into any cracks and crevices that may exist in the surface that is to be cleaned, performing even within contours that are difficult to reach with conventional cleaning devices such as wipes and brushes (Leighton, 2015).

Although UAS systems can be made to work with chemicals such as surfactants, the prototype was here used without any additives. The device normally operates directly from mains water supply, but because the experiment was not executed in the vicinity of the mains water supply, the system was configured to run from a recirculating additive-free water stream originating from the mains. As the

rippling stimulated on the surface of the bubbles is of a far lower energy level than the inertial cavitation that can be generated from intense ultrasound, phenomena associated with such cavitation such as the generation of free radicals, luminescence and shock waves do not occur (Leighton et al., 1988, 2008, Birkin et al., 2005a,b; Turangan et al., 2008). The regime of non-inertial cavitation that UAS produces at the surface to be cleaned is one where the bubble is driven by the acoustic field to undergo small amplitude pulsations, corresponding to the zeroth order spherical harmonic perturbation. In UAS this pulsation in turn also excites the high order spherical harmonic perturbations, generating the surface ripples on the bubble wall and the shear and convection associated with UAS cleaning.

In this regime of non-inertial cavitation, the position and motion of the bubble wall are determined by the usual balance between inertia and stiffness found in an oscillator undergoing small amplitude oscillation. In a bubble, the inertia is associated with the dense liquid and the stiffness with the compressible gas. This balance determines the pulsation resonance for small amplitude oscillations, but it is a balance that breaks during the large amplitude bubble pulsations that characterise inertial cavitation (the phenomenon used by all other ultrasonic cleaning systems). During inertial cavitation, the bubble expands to many times its original size, and then as the bubble contracts the inertial forces associated with the liquid dominate the motion so substantially that the stiffness of the gas/vapour liquid in the bubbles plays no significant role in determining the motion of the bubble wall (Leighton et al., 2000). In cleaning processes, the bubble loses sphericity, the bubble can involute to form a jet, and the collapse ends in a liquid/liquid impact which releases a blast wave into the liquid (Leighton et al., 2013b). The compressed gas momentarily achieves high temperatures (which can generate free radical, Birkin et al., 2001), but the absolute amount of energy contained in the gas in tiny. The jetting and blast wave can generate physical damage (Birkin et al., 2004), particularly as crevices in the target to be cleaned can focus stress and become enlarged (Jamaluddin et al., 2011).

Although the use of UAS has in common parlance been likened to ‘placing an ultrasonic cleaning bath at the end of a water stream’, it differs in two important aspects, the first being the manner of the bubble activity as described. The second is the fact that traditional ultrasonic cleaning baths limit the size of the object to be cleaned, as it has to be able to fit into the bath; this does not occur with UAS which in such circumstances outclasses the ultrasonic cleaning bath in performance (Goodes et al., 2016). Goodes et al. (2016) furthermore demonstrated that UAS can clean a contaminant that an ultrasonic cleaning bath failed to remove, even though UAS has also cleaned delicate surfaces that would be severely damaged in an ultrasonic cleaning bath without damaging them, due to the fact that that the mechanism by which UAS operates is very different from traditional ultrasonic cleaning.

In order to quantify the cleaning performance of the UAS prototype on fouled FRC coatings, the coatings were exposed to the UAS as well as a similar water flow (WTR) in absence of ultrasonic activation. The control flow originates from the same device, the only difference being that the acoustic field is turned off. In both cases, the coatings were exposed in the laboratory to a flow of 2 L/min for 30 seconds at a 5 mm distance from the nozzle targeting a single spot for each cleaning area (25 cm x 25 cm), as seen in Figure 3. In the same Figure (3) it can be seen that each coating was separated in three parts, where each was either cleaned with the UAS system, WTR or non-cleaned (25 cm x 25 cm each area).The edges of the slides were avoided from the analysis to avoid bias and

artefacts. A total of five replicated coatings were tested for each FRC, where one side was cleaned using UAS and the other side of the same coating was tested with non-activated water (see Figure 3).

Surface roughness, biovolume removal measurements and biofilm surface coverage

The control and UAS-cleaned FRC surfaces were analysed using an Alicona InfiniteFocus G4 focus variation optical profilometer. Objective lenses were selected for analysis of a suitable sample area size, and roughness was assessed in those areas, according to ISO25178 recommendations. This generates a fully focussed optical image in the sample area, as well as determines the axial location of features, allowing derivation of 2D or 3D roughness data. Magnifications were selected from a range of 10x, 20x and 50x objective lenses as appropriate for the height of features in the selected area, as directed by the ISO and manufacturer guidelines. For the purpose of this study, 3D roughness parameters (*Sa*, the arithmetic average of the 3D roughness) were assessed as befits the random, heterogeneous and non-directional nature of the fouling present on the surface. The results of the biovolume measurements are expressed in terms of their relative layer thickness (in $\mu m^3/\mu m^2$), which is calculated by dividing the total biovolume (μm^3) by the area over which it is divided (μm^2). For each of the two parameters measured (surface roughness and biovolume), 9 images were acquired (N = 9 per coating, per cleaning treatment).

To quantify biofilm presence/absence at the macroscale, ImageJ (MacBiophotonics ImageJ, USA) images with the entire slides were measured for percentage of biofilm surface coverage. Images from cleaned (WTR or UAS) and non-cleaned (before) sections from the slide (approximately 25 x 25 cm each) converted into a binary format (i.e. pixel value was either 0 or 255) and covered areas (i.e. surface areas with biofilm) were quantified (N =18 measurements per treatment i.e. 18 for water, 18 for UAS, 18 before for each FRC type). To test for differences between the coatings following cleaning treatments, a Mann-Whitney statistical test was utilised using Matlab version R2015a (MathWorks, Natick, MA, USA).

RESULTS

Coating performance

Following twelve days of continuous immersion, the two FRC systems have been colonised with both biofilms (slime) and macrofouling (colonial hydrozoans and tubeworms), as seen in Figure 2. Figure 3 illustrates an example of post-cleaning with WTR and UAS for both FRCs, where there is a clear visual difference in cleaning efficiency with UAS resulting in clean surfaces.

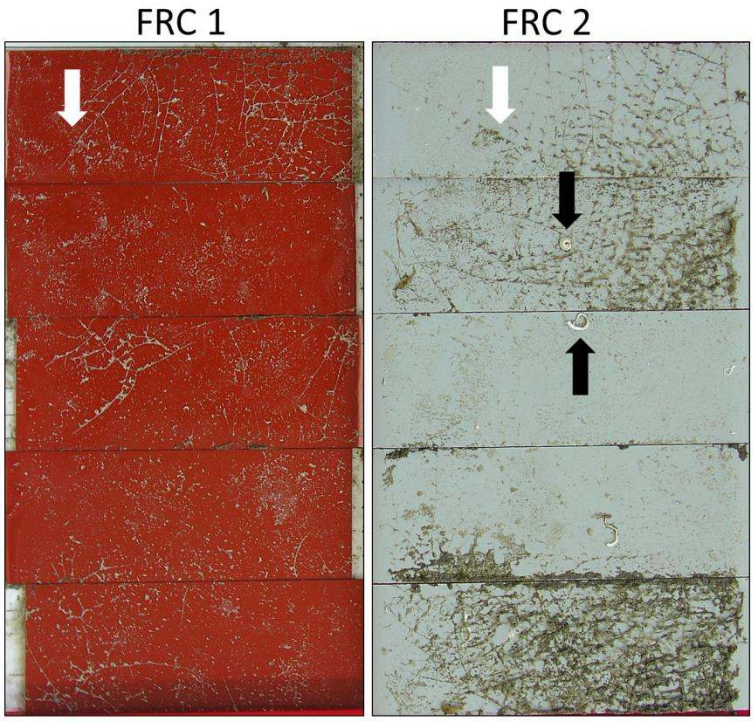

Figure 2. FRC coatings biofouled with initial biofilms, colonial hydrozoan species (white arrows) and tubeworms (black arrows) following immersion for twelve days in June.

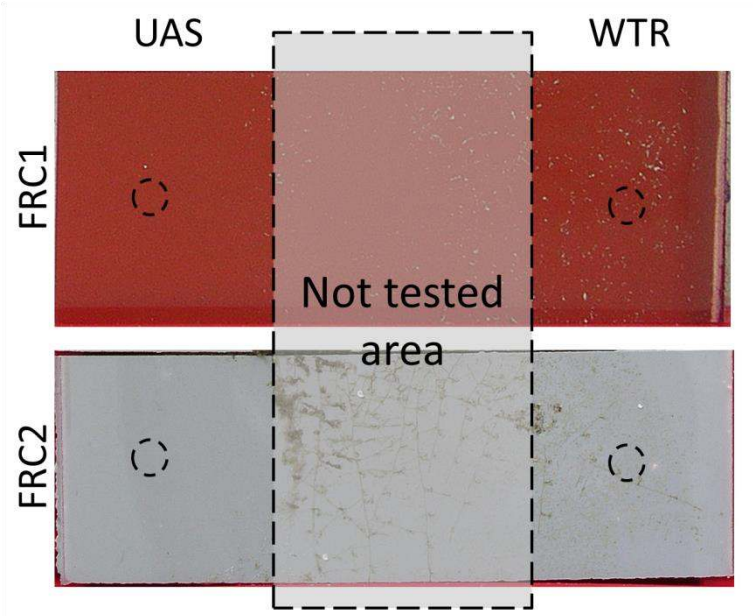

Figure 3. Example of FRCs exposed to either UAS or water (WTR) streams. The circles indicate roughly the position that UAS or WTR streams were applied (not to scale) on each coating. The middle part of the coating was not tested to allow sufficient distance between the two different streams, and to serve as non-cleaned control.

When biofilm formation on the FRCs was measured in terms of overall biofilm surface coverage, it was found that the area where the UAS was applied (approximately 24 cm x 25 cm) had almost no apparent biofilm left when compared to the non-cleaned areas (Before) and the areas cleaned with

water (WTR) (Figure 4). Specifically, following statistical analysis, biofilm surface coverage was found to be significantly lower on the FRC1 coatings cleaned with water (WTR) and the UAS system when compared to the non-cleaned (Before) area, with $p < 0.010$ and $p < 0.001$, respectively. When looking into biofilm surface coverage on coating cleaned with the UAS and WTR, it was found that the UAS part had almost non biofilm present ($p < 0.001$). The exact same was evident for the FRC2 system when biofilm surface coverage on the non-cleaned (Before) area of the coating had significantly higher coverage when compared to the WTR and UAS cleaned areas ($p < 0.001$ for both). Yet again, when comparing the WTR cleaned with the UAS cleaned areas, there is significantly less (almost non-existent) biofilm present on the coating area cleaned with the UAS ($p < 0.001$).

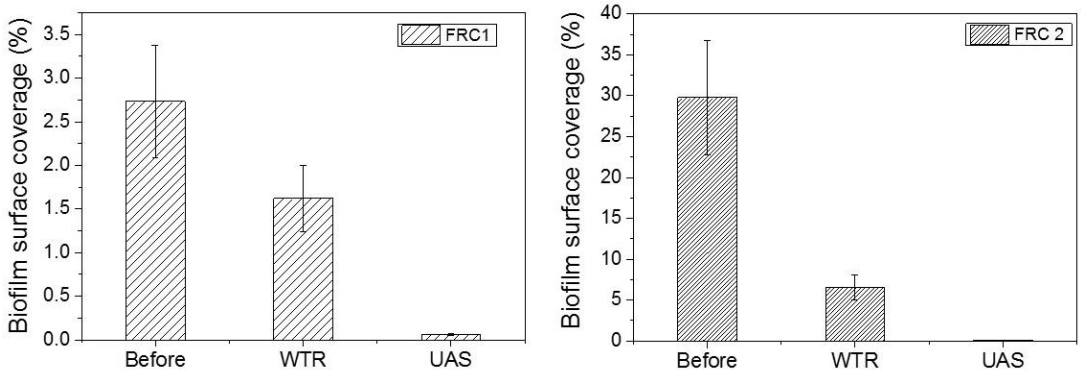

Figure 4. Surface coverage of biofilms (%) on the FRCs where Before = untreated samples (not cleaned), WTR = cleaned with water, UAS = cleaned with the Ultrasonically Activated Stream, for FRC1 (left) and for FRC2 (right); Error bar s= ± SEM.

Figures 5 and 6 illustrate the surface topography of FRC1 and FRC2, respectively; a substantial biofilm is formed on both FRC systems. However, FRC2 (Figure 6a) is characterised by higher biological activity that includes a substantially grown and complex macrofouling layer that reaches 700 µm in thickness, as opposed to 40 µm for the FRC1. The water treatment shows a more profound effect for FRC2 with peak thickness being reduced down to approximately 16 µm (Figure 6b), while the effect of the same treatment is less obvious on FRC1 (Figure 5b). The obvious removal of biofilm with the UAS system can be clearly seen for both FRCs since in both cases the peak thickness is reduced down ~3-4 µm (Figures 5c, 6c).

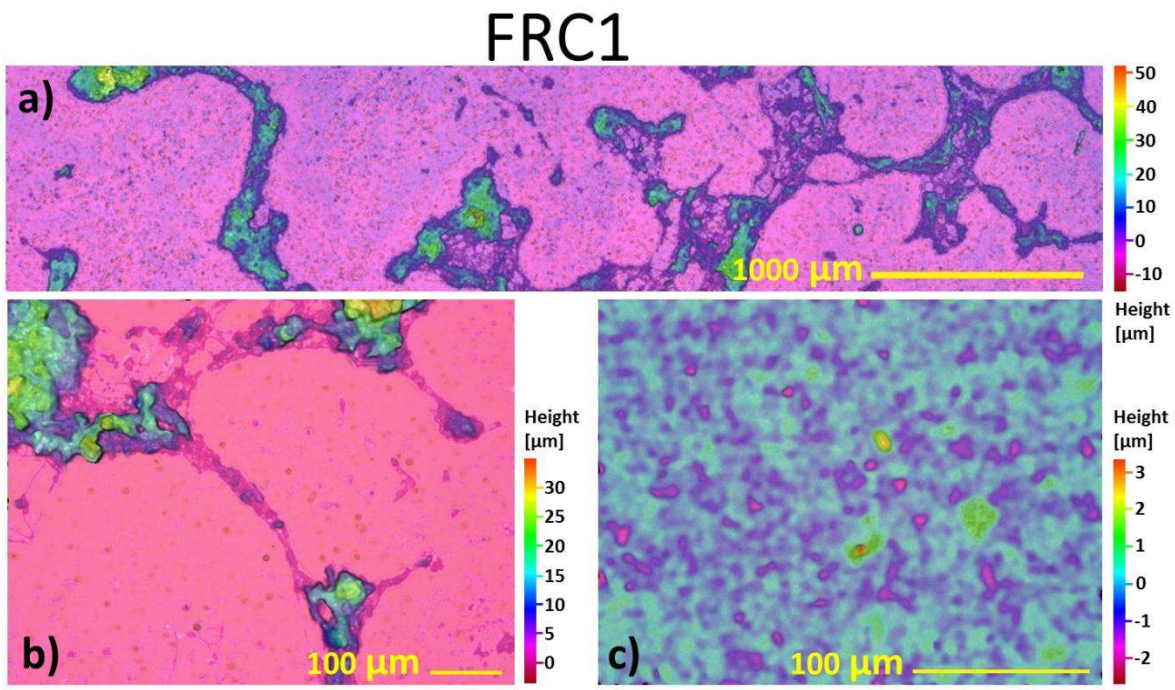

Figure 5. Alicona InfiniteFocus microscope images (artificial colours) illustrating surface topography where a) natural biofilm formed on FRC1 that was immersed for twelve days in Southampton Water, UK; b) FRC1 following water (WTR) treatment; c) FRC1 following UAS treatment. Colour bars illustrate the surface height map (µm).

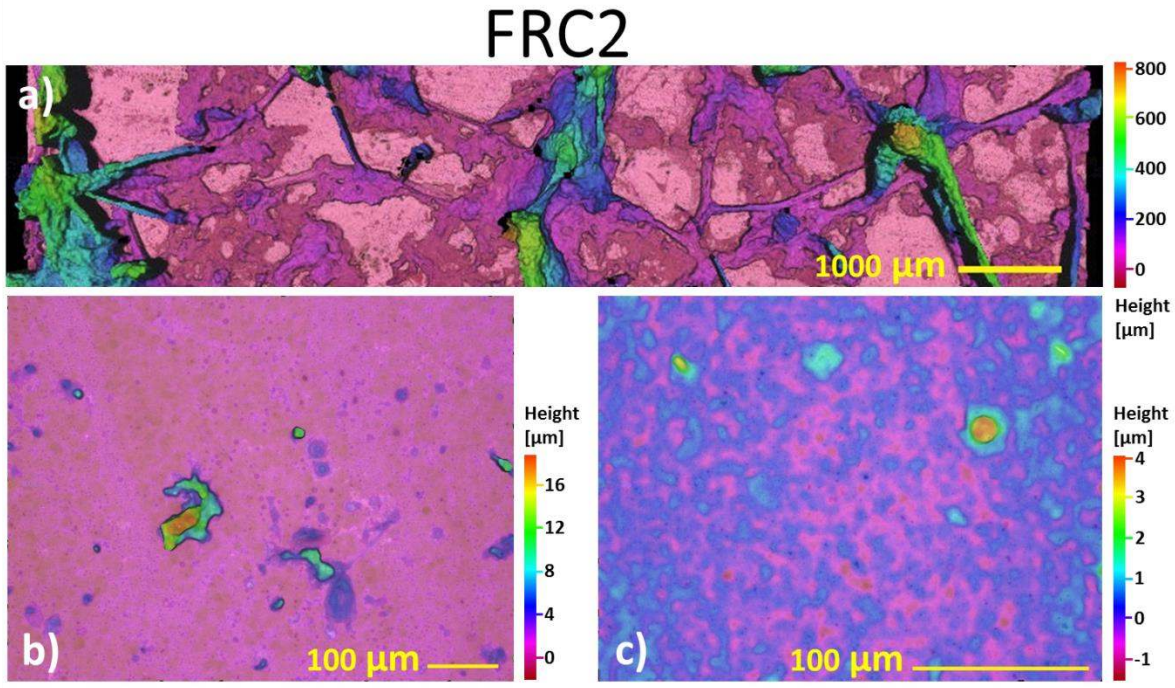

Figure 6. Alicona InfiniteFocus microscope images (artificial colours) illustrating surface topography where a) natural biofilm formed on FRC2 that was immersed for twelve days in Southampton Water, UK; b) FRC2 following water (WTR) treatment; c) FRC2 following UAS treatment. Colour bars illustrate the surface height map (µm).

Surface roughness and biomass removal by UAS

The effect of the UAS system on biofilm removal can be seen in Figure 7. In good agreement with the surface topography images, the water (WTR) cleaning method resulted in significant biofilm removal for both FRCs (Figure 7, Before *vs* WTR: FRC1, $p < 0.01$; FRC2, $p < 0.01$), however still leaving traces of thin biofilms on the coatings. Remarkably, the UAS system resulted in significantly lower biovolume values even when compared to the WTR cleaning treatment (Figure 7, WTR *vs* UAS: $p < 0.01$ for FRC1 and FRC2) with biovolume values close to zero (average biovolume for FRC1 = 0.001 µm and for FRC2 = 0.010 µm).

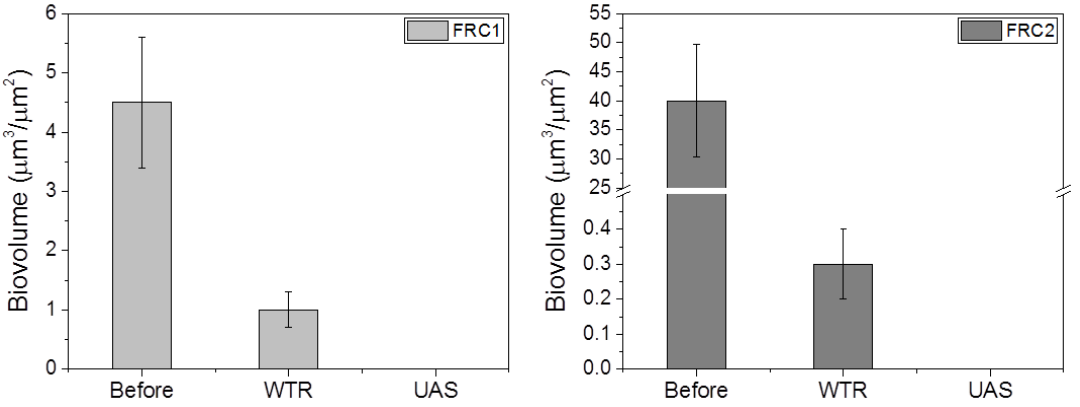

Figure 7. Surface biovolume (removal) of biofilms measured on the FRCs where Before = untreated samples (not cleaned), WTR = cleaned with water, UAS = cleaned with the Ultrasonically Activated Stream, for FRC1 (left) and for FRC2 (right); Error bar s= ± SEM.

Equally, surface roughness was significantly decreased when WTR cleaning treatment was used on both FRCs (Figure 8, Before vs WTR: $p < 0.01$ for FRC1 and FRC2). For FRC1, the surface roughness after UAS cleaning was even further reduced when compared to the WTR cleaning (Figure 8a, $p < 0.01$). Importantly, when blank FRCs (i.e. non-immersed) were compared with the fouled FRCs after both WTR and UAS treatments, there was no significant difference between the UAS and the blanks (FRC1, $p = 1.00$; FRC2, $p = 0.24$) although there was a significant difference between the WTR and the blanks ($p < 0.01$ for FRC1 and FRC2). This illustrates that only following cleaning with UAS, the surface roughness of the FRCs was reduced to levels similar as those prior immersions in the sea.

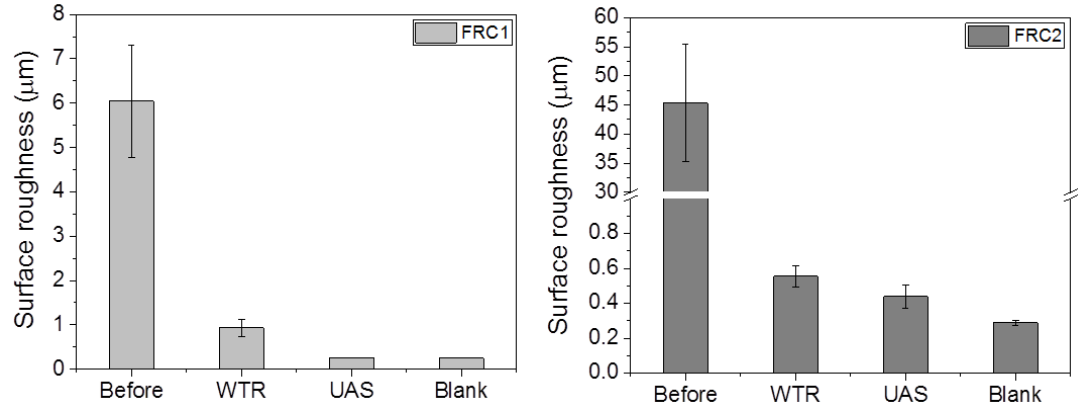

Figure 8. Surface roughness of FRCs where, Before = untreated samples (not cleaned), WTR =cleaned with water, UAS = cleaned with the Ultrasonically Activated Stream; for FRC1 (left) and for FRC2

(right); Blank indicates samples the two FRCs systems that have not been exposed to the sea. Error bars ± = SEM.

DISCUSSION

In the current work, for the first time, we report that the UAS system completely removed detectable marine biofilms from two types of FRCs. Measurements showed that the surface roughness of the fouled coatings was reduced from 6.05 µm to 0.25 µm for FRC1 and from 45.32 µm to 0.44 µm for FRC2 following UAS cleaning. Importantly, the surface roughness values after the UAS cleaning proved to be directly comparable to those of the blank, non-fouled coatings (0.25 µm for FRC1 and 0.29 µm for FRC2). This indicates first of all the high effectiveness of the UAS system against natural biofilms, but also shows that the cleaning is achieved without damaging the coating itself. The indication that the UAS cleaning is non-damaging to the FRCs is an important improvement to conventional high-pressure water blast cleaning methods for ship hulls (typically at a pressure of 20 - 60 MPa). In a study where FRCs were tested against a high-pressure cleaning system, it was found that the pressure from water jetting gives rise to intense pressure fluctuations from cavitation, leading to either small hole formation on the coatings or the complete removal of the coating in large pieces (Bureau of Reclamation, 2013).

In previous studies, increase in ship hull drag due to biofouling was measured over a period of two years and it was found to have increased by 35% by the end of that period (Kane, 2012). Interestingly, following a conventional full hull brush cleaning, the drag showed only a 10% decrease with pre-cleaning conditions. On top of that, silicone based coatings like FRCs are fragile and therefore intrusive cleaning methods, such as the traditional stiff rotating brushes, may damage these soft coatings (Baier et al., 1997; Christiaen, 1998; Holm, 2003) and impede their overall performance (Baier et al., 1997). Similar conclusions could be valid for other types of antifouling coatings like those that are loaded with booster biocides, such as copper, which are particularly toxic when released into the environment in an uncontrolled manner. Copper, in concentrations as low as a few µg/L, may impede photosynthesis in algae and interfere with enzyme function in both algae and animals (Yruela, 2005), and aggressive cleaning methods may lead to abrasion with elevated release of booster biocides (Valkirs et al., 1994; Bohlander and Montemarano, 1997). Although the UAS system was not tested against biocide-containing coatings here, potentially successful results of this non-abrasive cleaning method would eliminate the issue of biocide release into the environment.

The biovolume results also prove the UAS system to be particularly successful; before cleaning with the UAS the measured biovolume was found to be 4.52 $\mu m^3/\mu m^2$ and 39.00 $\mu m^3/\mu m^2$ for FRC1 and FRC2, respectively. Following cleaning with the UAS system, these values were markedly reduced to 0.00 $\mu m^3/\mu m^2$ for FRC1 and 0.01 $\mu m^3/\mu m^2$ for FRC2. When the FRCs were cleaned with the non-ultrasonically activated water stream, the biovolume was reduced to a significantly less extent reaching 1.01 $\mu m^3/\mu m^2$ and 0.30 $\mu m^3/\mu m^2$ for FRC1 and FRC2, respectively. Surface cleaning through the use of water hosing or jetting to remove biofilms is a typically used process which has been often problematic and cost effective. The degree of biofilm removal of *Pseudomonas aeruginosa* using a range of water pressures, water temperatures (cold vs hot) and chemical (surfactant) addition has been previously assessed and it was found that despite the combination of all three parameters,

biofilm reduction was partially observed, however removal was not achieved (Gibson et al., 1999; Burfoot and Middleton, 2009). The proposed cleaning method via the UAS system is thus very promising for a number of industries were cleaning with water alone is of importance.

Recent efforts towards the development of environmentally friendly antifouling coatings have focused on bio-inspired approaches with the most common being the reproduction of complex surface topographies often encountered on the skins or shells of marine organisms (Bers and Wahl, 2004; Bers et al., 2010; Salta et al., 2010; Scardino et al., 2011). These bio-inspired coatings are primarily made of soft materials such as silicones (polydimethylsiloxane), which are prone to damage, like the FRC systems, and therefore would greatly benefit from a non-abrasive cleaning method such as the UAS. Furthermore, these topographies often supply niches for biofilm forming species to anchor on (Scardino et al., 2006), which are difficult to reach with conventional cleaning methods. The acoustic field of the UAS method causes its bubbles to infiltrate and effectively clean micro structures that normal water streams would fail to reach (Leighton, 1994; Offin et al., 2014). In that respect, topographically enhanced coatings would benefit from the UAS cleaning technique on multiple levels, making the bio-inspired antifouling approaches especially efficacious.

Despite enhanced efforts to discover an environmentally friendly and cost effective coating that will have a permanent antifouling effect, no such coating is currently available and biofilm accumulation on marine industrial settings such as ship hulls but also water pipes, hydro-acoustic systems and reverse osmosis membranes (for water desalination) remains an on-going issue. Therefore, a cleaning method that will allow complete biofilm removal is desirable and indeed crucial. The current work has demonstrated some very promising results, with naturally occurring detectable biofilms being fully removed from commercially available FRCs using the UAS system. Future experiments will focus on testing the UAS cleaning efficiency on different types of antifouling coatings, such as ones containing booster biocides and topographically enhanced surfaces. If the promising results of the current work are sustained, cleaning using ultrasonically activated bubbles may become a standard method in marine biofilm removal.

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