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Standards for Testing and Reporting MHK Materials Performance

DOE-EERE-WPTO Seedling Effort

January 2023

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Abstract

To enhance research in the development and evaluation of materials for the construction of marine energy (ME) systems, this effort examined the need for standards, existing standard methods, gaps in available standards, and identified new methodologies that could become standards. The scope included methods covering corrosion prevention and anti-fouling performance. Developers of ME systems need reliable data to enable the selection of optimal materials of construction and coatings to protect structural features, sensors, mooring, and other elements of their devices. Test and evaluation data need to be relevant to ME, easy to interpret, quantitative, and standardized to allow comparisons of materials and techniques between different tests. The ability to extrapolate data to areas of operation (via models or comparison to reference materials tests) would further improve the usefulness of the data. As materials and coatings research continues in the US and abroad, a set of standards is needed to ensure that future tests have maximum utility for the industry. The search for and review of existing standards found that these do not adequately cover aspects such as prolonged immersion, the use of dissimilar materials, or quantitative measurement of fouling. Working in parallel with the WPTO-ME Multi-lab Advanced Materials program, a set of new methods was developed to address existing gaps in materials and coatings analysis. Draft manuscripts that detail each of these methods are included in this report. Following publication, these methods will also be proposed as new standards through the International Electrotechnical Committee (IEC), ASTM International, or other standards organization.

Acronyms and Abbreviations

AMPP	Association for Materials Protection and Performance
ASME	American Society of Mechanical Engineers
ASTM	ASTM International, formerly known as American Society for Testing and Materials
BGI	Biological Growth Index
CFRP	Carbon Fiber Reinforced Polymer
EIMR	Environmental Interactions of Marine Renewables
ESEM	Environmental Scanning Microscopy
ET	Elevated Temperature
FR	Fouling Rating (also “Fouling Resistance” in some publications)
GFRP	Glass Fiber Reinforced Polymer
ICMCF	International Congress on Marine Corrosion and Fouling
IEC	International Electrotechnical Committee
KHP	Potassium Hydrogen Phthalate
ME	Marine Energy
METS	Marine Energy Technology Symposium
MHK	Marine and Hydrokinetic Energy (see ME)
NACE	National Association of Corrosion Engineers (now part of AMPP)
NPOC	Non-purgable Organic Carbon
PDR	Physical Deterioration Rating
PVDF	Polyvinylidene Fluoride
RT	Room Temperature
SEM	Scanning Microscopy
SR	Softness Rating
SS	Stainless Steel
TC 114	Technical Committee 114 of the IEC
TOC	Total Organic Carbon
VARTM	Vacuum Assisted Resin Transfer Molding
ZRA	Zero Resistance Ammeter

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1.0 Introduction

To enhance research in the development and evaluation of materials for the construction of marine energy (ME) systems, this effort examined the need for standards, existing standard methods, gaps in available standards, and identified new methodologies that could become standards. The scope included methods covering corrosion prevention and anti-fouling performance. Developers of ME systems need reliable data to enable the selection of optimal materials of construction and coatings to protect structural features, sensors, mooring, and other elements of their devices. Test and evaluation data need to be relevant to ME, easy to interpret, quantitative, and standardized to allow comparisons of materials and techniques between different tests. The ability to extrapolate data to areas of operation (via models or comparison to reference materials tests) would further improve the usefulness of the data. As materials and coatings research continues in the US and abroad, a set of standards is needed to ensure that future tests have maximum utility for the industry. The search for and review of existing standards found that these do not adequately cover aspects such as prolonged immersion, the use of dissimilar materials, or quantitative measurement of fouling. Working in parallel with the WPTO-ME Multi-lab Advanced Materials program, a set of new methods was developed to address existing gaps in materials and coatings analysis. Draft manuscripts of these methods are included in this report.

1.1 Background

Marine energy is a relatively new industry when compared with wind, hydroelectric, fossil fuels, or nuclear power. Compared with other maritime industries (e.g., shipping and oil exploration), ME systems entail unique geometries, multiple materials of construction, unique performance demands, and unique operating conditions. Many ME systems are modular in design to allow for upgrading, maintenance, or repositioning of components during operations. Materials of construction include various metal alloys, composites, thermoplastics, and concrete. Structures may include both stationary and moving parts: for example, tidal systems typically entail moving turbines and wave energy converters may include floats or paddles coupled to a power takeoff system.

Existing ASTM standards for biofouling, which were developed primarily for the shipping industry, are subjective, non-quantitative, and often inappropriate for ME requirements (Table 1.1). For example, tests do not account for ME-relevant surface geometries, the wide variety of materials used to construct ME devices, or the location of ME devices within the water column. Critically, tests rarely account for relative water velocity across test surfaces, which impacts paint ablation rates, settling and growth rates for organisms, and the ability of organisms to be washed off of foul-release paint surfaces. Furthermore, paint companies and coatings researchers conduct tests using conditions that are often selected to optimize the performance results for their product. The lack of consistency results in product brochures and scientific publications that are often difficult to interpret, making comparisons and materials selection difficult. Table 1.1 identifies existing standards and additional methods published in technical journals and provides a critique for each.

Similarly, strength and corrosion tests for composites and joined composite-metal materials, while reasonably established for terrestrial applications, have not been standardized for marine applications or the demands of ME systems. For example, standard test methods are needed for assessing water intrusion into composites and the potential effects on shape and strength, or to examine the potential for galvanic corrosion when dissimilar materials are joined. Past efforts to develop guidelines for marine renewables include the “Guidelines for Manufacturing, Assembly and Testing of Marine Energy Conversion Systems” (EMEC 2009a) as well as the “Guidelines for Design Basis of Marine Energy Conversion Systems” (EMEC 2009b) provide simple considerations for the construction and testing of complete devices based on standards developed for shipping and petroleum industries. These references are based on steel and concrete and simply recommend testing against an undefined standard. The references do not encompass composites, materials or coatings test methods, nor standards for reporting data.

Currently, researchers in the field are left to develop their own methodologies for conducting exposure tests, measuring/analyzing effects, and reporting findings. This need for standardized methods and reporting was identified during industry workshops (e.g., at METS and with the MEC and EMEC), at the 2018 International Congress on Marine Corrosion and Fouling (19th ICMCF), and at the 2018 Environmental Interactions of Marine Renewables (EIMR). During the course of the EERE-funded Advanced Materials Program, team members from PNNL, SNL, NREL, FAU, and MSU evaluated and modified published methods in order to test materials and coatings for ME applications. Many new methods were also developed to produce useful data. A key outcome of the program has been an internet repository of data on materials and coatings performance that can be used by the ME and associated industries to assist with the design and construction of new systems (<http://energy.sandia.gov/energy/renewable-energy/water-power/technology-development/advanced-materials/ME-materials-database/>).

Through experiments funded under the DOE-WPTO-ME Advanced Materials and other programs, the project team conducted tests and experiments leading to the identification and early development of new, quantitative methods that fill some of the identified gaps. Under this seedling effort, the experimental methods were evaluated to identify candidates that could form the basis of new standards. These methods were written as draft manuscripts for publication and the pre-print drafts included as subsequent chapters. Each manuscript will be completed and it is anticipated that these will be submitted for publication in FY23, pending the need for and outcomes of any additional experiments deemed necessary for completion. Additionally, the team was successful in working with the International Electrotechnical Commission (IEC) Technical Committee 114 (TC 114) to promote a proposal to develop standards for fouling testing. This proposal was accepted by the international community in 2022 and a process was started for developing the first formal standard for quantitative analysis, based in-part upon the methods highlighted in this report.

Chapter 2.0 describes a new method for quantifying all of the organic matter (biomolecules, microorganisms, and multicellular organisms) that have accumulated on

a surface by extracting the biomass and quantifying the total organic carbon and nitrogen present. This approach entailed the identification of optimal solutions and procedures for removing all of the organic matter without damaging underlying paints, coatings, and substrate materials. The resulting method is suitable for quantifying biofouling accumulation on flat test coupons and highly irregular surfaces with complex geometries, and is ideal for conducting precise comparisons and evaluations of antifouling paints and materials.

Chapter 3.0 describes a method for testing the ‘non-stick’ properties of coatings and materials designed to prevent marine organisms from adhering to a surface. Rather than rely upon toxic chemistry in an attempt to prevent the growth of fouling organisms, a new strategy found in modern paints is to allow fouling to occur, but to diminish the ability of organisms to adhere, allowing the flow of water over the surface to wash away the organisms. This strategy works reasonably well for the surfaces of fast moving (greater than 10 knots or 5 m/s) ships that remain in motion. However, for stationary marine energy devices that experience relatively low velocities (less than 3 knots or 1.5 m/s), from tidal current or waves, most coatings do not reduce adhesion sufficiently. Prior to this report, no method existed to quantify bioadhesion on surfaces and to enable comparisons between coatings. The draft manuscript describes tests leading to a new method for accurately measuring bioadhesion with high resolution, precision, and accuracy. The approach entails allowing mussels to adhere to test or control surfaces by means of their byssal threads and plaques (Qureshi 2022), separating the mussel from the threads, and then measuring the force required to pull a thread and plaque off of the surface.

Chapter 4.0 describes a refinement of a PNNL patented method for photographing fouled surfaces after the application of a mixture of biological stains. An image processing approach quantifies the accumulation of the stain (observed as color intensity) to calculate the amount of organic matter on the surface. The new method examined the use of fluorescent stains for use on very dark or black background materials and compared the results with the use of the total organic carbon and nitrogen method described in Chapter 2.0. The paper demonstrates that the staining approach could be used to accurately measure fouling on any colored surface.

Chapter 5.0 describes a method for measuring galvanic corrosion of metal materials when the metal is in contact with a conductive material, such as carbon fiber. The marine energy sector is examining the use of strong, lightweight carbon fiber composites for construction; however, a key concern is how to join components made of these materials with other components given the high potential for corrosion of metal fasteners (bolts) or metal components. The draft manuscript presents a method for accelerated testing to assess corrosion potential and to identify suitable means of insulating joints to prevent corrosion.

1.2 References

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Table 1.1 Comparison and Analysis of Methods for Measuring Biofouling

Title	Author or Publisher	Year	Description of Method	Challenges and Deficiencies
D3623- 78a Standard Test Method for Testing Antifouling Panels in Shallow Submergence	ASTM	2012	Specifies a standard panel size and standard coating for comparison with test coatings	Fouling assessment uses the subjective Fouling Resistance (FR) system approach described below
D 5618-94 Standard Test Method for Measurement of Barnacle Adhesion Strength in Shear	ASTM	2011	Use of a hand-held probe to push laterally onto a barnacle	Error prone due to use of hands and potential to crush the shell of the barnacle; relies upon natural occurrence of barnacle growth on surfaces, so the coating may have been altered prior to testing
D6990-05 Standard Practice for Evaluating Biofouling Resistance and Physical Performance of Marine Coating Systems	ASTM	2011	FR system to estimate percentage of surface covered by selected organisms	Subjective visual estimate of coverage; time consuming and dependent upon expertise and judgement of the inspector to recognize species and life stages; approximate number of adult macroorganisms, but not fouling mass, average thickness, or volume; charts do not generate a useful numerical value with high resolution, precision, or accuracy
D4938- 89 Standard Test Method for Erosion Testing of Antifouling Paints Using High Velocity Water	ASTM	2007	Use of a recirculating pumped water system to assess how resistant coatings are to erosion or to determine a rate of ablation	Useful for studying the contribution of water flow to the erosion of a coating, but does not include sediment, thermal effects, or fouling. Fouling and corrosion are not measured
D4939-89 Standard Test Method for Subjecting Marine Antifouling Coating to Biofouling and Fluid Shear Forces in Natural Seawater	ASTM	2007	Describes an environmental exposure protocol entailing mounting coupons onto a rotating drum that is submerged in order to introduce a relative shear velocity across the test surface	Does not provide a quantitative approach to measure outcomes (fouling or corrosion); samples are limited to curved panels; significant risk of effects from neighboring panels or blanks
D 5479- 94 Standard Practice for Testing Biofouling Resistance of Marine Coatings Partially Immersed	ASTM	2007	Describes a method for exposing test panels at the waterline (partial submersion)	Does not provide a quantitative approach to measure outcomes (fouling or corrosion)

D6442-06, Standard Test Method for Determination of Copper Release Rate From Antifouling Coatings in Substitute Ocean Water	ASTM	2020	Describes a method to quantify how much copper is released from a coating in artificial seawater	Does not reflect the effects of velocity, paint age and ablation, changing temperature, or other environmental effects
D6903-07, Standard Test Method for Determination of Organic Biocide Release Rate From Antifouling Coatings in Substitute Ocean Water	ASTM	2020	Describes a method to quantify how much organic biocide is released from a coating in artificial seawater	Does not reflect the effects of velocity, paint age and ablation, changing temperature, or other environmental effects
D5108-90 Standard Test Method for Organotin Release Rates of Antifouling Coating Systems in Sea Water	ASTM	2016	Describes a method to quantify how much organotin biocide is released from a coating in artificial seawater (note, withdrawn in 2016)	Does not reflect the effects of velocity, paint age and ablation, changing temperature, or other environmental effects
D6132-13 Standard Test method for Nondestructive Measurement of Dry Film Thickness of Applied Organic Coatings Using an Ultrasonic Coating Thickness Gage	ASTM	2017	Describes a process for using an ultrasonic film thickness gage to determine the dry film thickness of a coating; useful for understanding the initial thickness of a coating prior to conducting exposure studies	Does not provide a quantitative approach to measure fouling or corrosion
ISO 13073-1:2012 Ships and marine technology - Risk assessment on anti-fouling systems on ships — Part 1: Marine environmental risk assessment method of biocidally active substances used for anti-fouling systems on ships	ISO	2012	Describes a risk assessment method to determine whether and to what extent biocidal coatings applied onto a ship might impact the marine environment	Does not provide a quantitative approach to measure fouling or corrosion
BS EN ISO 21716-3 Ships and marine technology - Bioassay methods for screening anti-fouling paints, part3: mussels	ISO	2020	Describes a method for measuring settlement of mussels onto test surfaces in a flow-through system	Single-species test for fouling; does not quantify adhesion; the settlement assay lacks environmental relevance—it uses young adult mussels, not the free-swimming larvae or villagers that actually colonize surfaces
BS ISO 21716-2:2020, Ships and marine technology - Bioassay methods for screening anti-fouling paints, barnacles	ISO	2020	Describes a method for measuring settlement of barnacle cyprid larvae onto test surfaces in a flow through system	Single-species test for fouling; does not quantify adhesion; it is difficult to acquire barnacle cyprids and to synchronize the age and settling behavior of a population, resulting in significant statistical variability (large error bars)

ISO/DIS 4768 Measurement method of anti-biofilm activity on plastic and other non-porous surfaces	ISO	draft	Describes a method for measuring biofilm accumulation on surfaces	Uses a single bacterial species and culture media to accelerate growth; measurement uses crystal violet staining and absorbance to quantify the biofilm, though it does not stain all of the biomolecules present in the encasing extracellular polymeric substance
SP21421-2017 Pictorial Standard for underwater Evaluation of Fouling Degree on Ship Hulls	AMPP (NACE)	2017	Provides a set of reference photos to assist with describing the extent fouling observed	Subjective and non-quantitative approach
AMPP SP21487-2021, Inspecting and Reporting Biofouling and Antifouling Systems' Condition during Underwater Inspections on Ships	AMPP (NACE)	2021	Provides guidelines for conducting underwater inspections of ship hulls to assess the condition of the antifouling coatings and extent of fouling; provides guidelines for reporting the results	Subjective and non-quantitative approach
NACE SP0616-2022, Standard for Hull Roughness Measurements on Ship Hulls in Dry Dock	AMPP (NACE)	2022	Provides guidelines for using a hand-held gauge to assess the thickness of accumulated corrosion, hard fouling, cracked paint, or other surface imperfections	No standardized gauge, but provides recommendations for a uniform strategy. Does not directly measure fouling or corrosion.
Biofilm Detection by a Fiber-Tip Ball Resonator Optical Fiber Sensor	A. Rakhimbekova	2022	Ball resonator optical fiber sensor with optical backscatter reflectometer detection; change in refractive index (an indicator of biofilm thickness) is related to the change in intensity and the shift of the wavelength	Useful for early stage biofilms, but not with hard fouling or macrobiota. Unclear how well this could be used to take measurements over a large surface to generate a reasonable average; meant as a means of detecting formation rather than quantifying
Parallelized microfluidic diatom accumulation assay to test fouling-release coatings	Nolte, K.	2018	diatom accumulation assay based upon fluorescence microscopy detection of settling by a model diatom introduced into a microfluidic platform	Examines early stage settling of microbial species; meant for use with a single species as a means of screening coatings; not suitable for measuring fouling or corrosion

2.0 Total Organic Carbon and Nitrogen Quantification

A methodology was developed to quantify marine biofouling on a wide variety of surfaces using non-purgeable organic carbon (NPOC) analysis. A variety of strategies including sonication and lyophilization and non-carbon-based solvents were tested for their ability to clean surfaces; it was determined that 3% H₂O₂ in combination with gentle sonication provided the best results, showing maximal removal of fouling material, minimal sample loss, short processing times with high sample throughput, precision, and reproducibility. Results from the NPOC analysis allowed us to precisely measure the accumulation of biomass and organic matter on a surface and to quantitatively compare the antifouling performance of one surface type to another. A key achievement was the ability to remove organic material from a wide variety of surface types without causing obvious damage to delicate coatings. As demonstrated herein, this methodology generates precise and reproducible measurements of soft-fouling accumulation, thus providing an objective alternative to the highly interpretive visual analyses currently employed for fouling evaluation.

2.1 Introduction

Biofilms may form at any interface of a solid, fluid, or air. At fluid-solid interfaces, the process begins with the accumulation of organic molecules on the solid surface to form the conditioning film, which is followed by cellular colonization, colony growth, and the formation of a contiguous biofilm consisting of cells and a protective organic matrix known as extracellular polymeric substances (EPS). In aquatic settings, biofilm formation may facilitate the settling of juvenile macrobiota, leading to the final phase of the biofouling process: outgrowth of visible multicellular species. Herein, *biofouling* will be used to refer to molecular and organismal fouling at any or all stages of development, including biofilms, *aquatic biofouling* to specify fouling in fresh or seawater environments, and *biofilm* will be used to specify fouling limited to conditioning film and biofilm formation in any environment (e.g., aquatic, biomedical, industrial).

Biofouling processes are difficult to inhibit and occur on virtually all surface types. Consequently, biofilms and aquatic biofouling are very costly problems that impact ecological and human health, infrastructure, carbon emissions, and mechanical performance. For example, estimates attribute over 80% of all infections, including 65% of nosocomial infections in the developed world to biofilms (1, 2). Aquatic biofouling is a leading cause of global transfer of aquatic invasive species (Williams 2013), contributing, for example, to 74% of the aquatic invasive species in Hawaii (3). A biofilm only 10 to 50 µm thick can increase drag on a ship by 22% (4) and a 250 µm thick biofilm may reduce heat transfer in a heat exchanger by as much as 50%.

In response to these and other consequences, billions of dollars are spent annually by impacted industries and governments on preventative maintenance and on research to develop new means of slowing or limiting biofouling accumulation, including antifouling coatings (5-9). However, a key challenge for both researchers and consumers of antifouling products has been the lack of

accurate, precise, and reproducible means to evaluate performance. Reports of antifouling material performance can be difficult to compare when the experiments are conducted by different laboratories or at different times. While one challenge in comparing studies is the combined effect of both engineered and uncontrollable variation between different test exposures, a more fundamental problem is the lack of uniform and broadly applicable quantitative methods to measure exactly how much biofouling accumulates on a surface. Without a means for taking high quality, quantitative measurements, researchers have a limited ability to compare and evaluate the performance of different materials in their own experiments, or to relate their results to the work of others. Similarly, consumers of antifouling coatings and paints lack access to useful performance specifications and reliable comparisons. Furthermore, the lack of high quality data limits the ability to monitor and forecast a material's continued performance.

Measuring biofouling growth on surfaces is very challenging and several reasons have contributed to the lack of robust methods development. The diversity of biomolecules, living or dead species, and life stages (e.g., spore, larvae, adult) found on biofouled surfaces makes traditional visual identification and counting very challenging. Molecular techniques must accommodate all kingdoms and domains/phyla. The complexity of biofilm and aquatic biofouling structures, including species distributions through the vertical profile of a fouled surface, requires thorough removal of the entire fouling community for accurate analysis; however, variability in the adhesive strength of the cells and organic matter on different surface types, can lead to inconsistencies with sample removal. Differences in the durability and chemical reactivity of different surfaces may also present challenges for removing cells without damaging the substrate. Continued reliance on traditional hand-count methods and subjective visual analysis inevitably produces results that are imprecise. Indeed, several of the methods in use by industry and the military, including the published ASTM methods (see for example ASTM standards D4939 – 89, D6990-05 and D3623), rely upon observations and the indeterminable skill of the observer (10-12). An alternative approach is required that may be used with both highly controlled studies using monocultures of test organisms or with exposures to mixed species in natural settings.

Herein, we report on a novel highly quantitative method to rapidly measure the accumulation of organic matter (i.e., conditioning film, biofilm, soft biofouling) on surfaces using by determining the amount of non-purgeable organic carbon (NPOC). Measuring NPOC is a direct approach to calculating the total organic carbon (TOC) content of a sample. Briefly, a sample is acidified to convert any inorganic carbon to CO₂, which is released unmeasured, and then the remaining carbon is oxidized and measured (the non-purgeable portion). This methodology provides greater accuracy and precision when examining fouling processes and comparing material performance.

To overcome these limitations, we developed collection and testing methodologies to quantify biofouling in the marine environment using NPOC analysis. Our approach applies the use of a cleaning method to gently but thoroughly remove fouling from test materials, followed by homogenization of the fouling material to prepare a suspension that may be used for NPOC analysis on a typical analyzer. The approach is suitable for analysis of a variety of natural and engineered surfaces, including aquatic biofouling, biomedical biofilms, analysis of sediments, and experimental evaluation of protective coatings and materials.

Determination of organic matter in water and sediment samples using NPOC is well established (e.g., by EPA SW-846 Method 9060)(13); however, continued evaluations and improvements are being made to identify new cleaning solutions and methods (14-16). NPOC analysis has not been widely used with environmental biofilm or biofouling analysis in part due to difficulties efficiently extracting the organic matter from a surface without destroying the surface (i.e., artificially adding to the carbon content of the test fluid) or converting a significant proportion of the organic matter to carbon dioxide prior to testing (14-16).

Our results show that a solvent solution of 3% hydrogen peroxide followed by heating and gentle sonication effectively removed deposited organic material from a fouled surface, and that subsequent NPOC analysis can effectively quantify the organic carbon concentration. In this paper we report how the method was applied to the study and monitoring of marine biofilm growth on a variety of bare and painted substrates to demonstrate the antifouling performance of these surfaces.

2.2 Experimental

Marine Test Conditions

Coupons were placed in a continuous flow 500-gallon water tank. Seawater was pumped directly from Sequim Bay (Sequim, WA, USA) into test tanks at a rate of ~2.5 gal/min. Tanks were exposed to a diurnal fluorescent light cycle of 12 hours on and off. Tank salinity was 32 ppt, water temperature was 9.8°C $\pm$ 0.2°C.

Sample Materials

One-inch square coupons were used in this study to support the growth of biofilms. Coupons were made of Teflon, FR4 fiberglass, 316L stainless steel, copper, and 316L stainless steel painted with Islands77 *plus* marine paint (Sea Hawk, FL USA) per manufacture's specifications. Islands77 *plus* is a self-polishing marine paint engineered to release the biocide zinc pyrithione as a method to mitigate biofouling.

Cleaning of Organics from Sample Surface

Following exposure to seawater, each coupon was placed in a clean, sterile 50 mL polypropylene disposable centrifuge tube. Coupons were then stored at -80°C until they were processed for organic carbon removal.

One set of coupons was subjected to lyophilization: drying cells and biomolecules results in significant contraction of the dried matter, and the hope was that

contraction of a fouling layer located on top of a hard surface would result in the fouling material flaking off of the surface. After drying, the coupons were then soaked in 18 MΩ water and sonicated in an attempt to remove any additional fouling material adhering to the surface and to help dissolve or create a uniform suspension of the organic matter.

Other sets of coupons were exposed to one of several different cleaning solutions. A solution of 3% hydrogen peroxide was used to recover biomass accumulation from the coupon's surface. We used an ultrapure hydrogen peroxide because lower quality hydrogen peroxide solutions can have varying concentrations of dissolved carbon. The 3% hydrogen peroxide solution was made by diluting 30% ULTREX™ II hydrogen peroxide (JT Baker, Central Valley, PA) with 18 MΩ (Milli-Q) water (1:10). Both the water and the stock solution of hydrogen peroxide were tested to ensure that these had carbon concentrations of less than 2-3ppm. Low-level carbon reagents are required to accurately quantify biofilm formation on surfaces. The 0.1 M solutions of NaOH and Na₂S₂O₈ were made by dissolving molecular biology grade NaOH (Fisher Scientific, USA) and Na₂S₂O₈ into 18 MΩ (Milli-Q) water. A 0.1 N solution of HCl was made by diluting TraceMetal™ Grade 12N HCl (Fisher Scientific, USA) stock into 18 MΩ (Milli-Q) water. The 3% NaOCl was made by diluting 14.5% NaOCl (Alfa Aesar, USA).

In the 50 mL tube containing the test coupon, 30 mL of the solvent solution to be tested was added and then vortexed for 10 seconds. Next the tube was placed in a 55°C water bath for 30 minutes. After 30 minutes tubes were removed and vortexed for an additional 10 seconds, then placed back into the 55°C water bath for another 30-minute incubation.

Next, the 50 mL tubes were fastened to a VialTweeter ultrasound unit (Hielscher USA, Inc., Ringwood, NJ). The tubes were secured to the ultrasound horn using two large rubber bands and aligned such that the flat surface of the coupon was facing the end of the horn. Sonication was performed for one minute (100% amplitude, 0.75 duty cycle), and then the tube was rotated 180° to position the opposite side of the coupon towards the horn's face and then sonication was performed for an additional minute. This step ensures that both sides of a coupon directly face the VialTweeter horn during the cleaning process.

Staining to Compare and Evaluate Biofilm Removal

To visually assess the performance of the different solvents utilized in the cleaning process, a coupon staining procedure was developed that highlighted the presence of any remaining surface adhered biofilms. After cleaning using the method and solvents described above, coupons were placed in a tray at a 45-degree angle. Next, Coomassie Brilliant Blue G-250 and Erythrosin B stains were applied to the coupons surface. Coomassie Brilliant Blue G-250 is an anionic molecule that non-covalently interacts with proteins and free amino acids to form visually identifiable protein-dye complexes; Coomassie Brilliant Blue G-250 will allow visualization of all proteinaceous biomass attached to the coupons surface. Erythrosin B is an acidic eosin vital dye, the dye will stain the basic (non-acidic) components of the cell such as: the cytoplasm and collagen fibers (prevalent in animals and cyanobacteria). 500 µL of the stain Erythrosin B was applied to the left side of each coupon and 500 µL of the stain Coomassie Brilliant Blue G-250 was applied to the right side of each coupon. Stain remained on coupon's surface for 1 minute, then the coupon was dipped gently into filtered seawater to remove excess stain. Seawater was used as an osmotically neutral rinse solution.

Coupons were photographed after completing the cleaning and staining procedure to compare their relative effectiveness.

Non-purgeable Organic Carbon Analysis (NPOC)

Test coupons used for NPOC analysis were not stained during testing. NPOC analysis was performed using a Shimadzu TOC-L (Shimadzu Scientific Instruments, Nakagyo-ku, Kyoto). A 30 mL aliquot of solvent used in the process of cleaning test coupons was removed from the sterile disposable centrifuge tube and placed in a 40 mL certified clean NPOC test vial (Fisher Scientific, Pittsburgh Pennsylvania) for NPOC testing. Separate 50 ppm and 5 ppm potassium hydrogen phthalate solutions made in 18M Ω (Milli-Q) water were used as test controls. Additional controls included clean coupons that were not exposed to seawater, but which underwent the same cleaning process to ascertain background organic carbon levels from the solvents and substrates and the sensitivity of the paint to the solvents. Similarly, samples of the all of the cleaning solutions tested were placed in the centrifuge tubes and processed (without coupons) to ensure that the cleaning steps did not extract organic carbon from the disposable plastic tubes. All NPOC vials were loaded into a Shimadzu ASI-L auto sampler. NPOC settings were as follows: sample sparge time was 2 minutes, acid addition 1.5% HCl trace grade (Fisher Scientific, Pittsburgh, PA), 3 of 5 injections within 2% coefficient of variation per test sample. To subtract any carbon input from the solvents, test samples of the solvent cleaning solutions and Milli-Q water were taken and tested in the NPOC analysis as blank controls.

Contact Angle Measurements

Contact angles were measured using a model 590 Adv. Automated Goniometer/Tensiometer (rame'-hart, USA). A 10 μ L drop of 18M Ω (Milli-Q) water was placed on the surface of the test coupon for contact angle measurement. Three separate contact angle measurements were made for each of the tested coupons. Individual contact angle results are the mean of contact angle measurements taken from opposing sides of the dispensed seawater droplet on the surface of the coupon. Final determination of the contact angle for each coupon was the average of 3 separate measurements using 3 droplets, each on a randomly selected location on the coupon surface.

2.3 Results and Discussion

Conditioning films, biofilms, and biofouling form as organic matter accumulates on a surface through passive and cell-directed activity. Although these layers may also trap inorganic materials (e.g., sand) or result in the growth of inorganic carbonates (shells), measurement of the organic carbon content would provide a direct quantification of the "soft" fouling material found on a surface. Instruments for measuring total organic carbon have sensitivity to sub-ppb (<1ng/mL). However, the ability to accurately measure the organic carbon content of a fouled surface depends upon the ability to get all of the fouling material off of the surface and into a suspension without damaging the surface (potentially inflating the carbon measurement) or mineralizing the organic carbon (converting it to CO₂ and diminishing the measurement) during the cleaning process and prior to measurement.

This report investigates the use of NPOC analysis as a novel means to quantify biofilms and marine biofouling on a surface. Of specific interest was the

development of a process that could work during multiple phases of biofilm and biofouling formation to effectively remove conditioning film, cells, and EPS without extracting carbon from the substrate or protective coatings/paints. An additional requirement was for the methods to be rapid and with minimal labor requirements to enable processing of the 10's to 100's of samples typically generated during protective materials and coatings development studies.

Selection of Cleaning Agents

A necessary first step was to develop effective methods to remove biomolecules and cellular material from a variety of surface types without damaging the surface. A list was prepared that identified and compared attributes of solutions used to extract organic matter in other fields of study, for example: TOC analysis of soils or sediments, for cleaning surfaces used in trace organics analysis (e.g., cleaning sample plates used in mass spectrometry), or for separating viable cells from tissues or monolayers. Both solvents and detergents were considered; however, many of the potentially most effective reagents were carbon based, contained high loads of background carbon, or were deemed to be so reactive that they could damage the substrate or significantly mineralize the organic matter prior to the NPOC analysis. These factors were used in selecting solvents for this study. The following detergents and surfactants were considered, but ultimately excluded from this study due to their high carbon content (>5 ppm): Tween 20, Tween 80, Triton X-100, sodium dodecyl sulfate (SDS), sodium lauryl ether sulfate (SLES), magnesium lauryl sulfate, sodium dodecylbenzenesulfonate and sodium myreth sulfate.

Sodium hypochlorite and disodium peroxodisulfate, two strong oxidizers used to remove organic matter in soil and sediment studies (17-19) were selected for testing in this study. Using these strong oxidizers to clean surfaces and in the subsequent measurement of NPOC raised three significant concerns. First was the ability to obtain ultrapure solutions with low carbon background. Second, it was critical not to damage surface coatings (e.g., paints) during cleaning; these powerful oxidizers have the potential to be reactive to many coating/surface types. Third, these oxidizers are also highly reactive with carbon-based molecules. Mineralization of the organic carbon found in environmental samples may preclude the ability to get accurate NPOC analysis of the attached surface biofilm. Ultimately, the concentrations of these two cleaning solvents that were chosen for the test matrix were based upon finding carbon-free (<5 ppm) based solvents, minimizing the mineralization of organic carbon, and low potential for damaging surface coatings. A sodium hypochlorite concentration of 3% and disodium peroxodisulfate of 0.1 M were selected to mitigate these effects.

Ultimately, a list of candidate reagents was assembled that included hydrogen peroxide, sodium hydroxide, hydrochloric acid, sodium hypochlorite, disodium peroxodisulfate, and 18 MΩ (Milli-Q) ultrapure water. To achieve the goals of minimal substrate damage and mineralization, solvent solutions of each type were used at low concentrations: 3% hydrogen peroxide, 0.1 M sodium hydroxide, 0.1 N hydrochloric acid, 3% sodium hypochlorite, 0.1 M disodium peroxodisulfate, and 18 MΩ (Milli-Q) water. Diluting each stock with ultrapure water had a further benefit of reducing the background carbon concentration of the cleaning solution.

Solvent Testing

The selected solvents were tested and compared to one another to ascertain their cleaning effectiveness, damage to the surface, and mineralization of organic carbon. Hydrochloric acid (0.1 N HCl), sodium hydroxide (0.1 M NaCl), Milli-Q water, hydrogen peroxide (3% H₂O₂), sodium hypochlorite (0.1 M NaOCl), disodium peroxodisulfate (0.1 M Na₂S₂O₈), and lyophilization were all tested; each treatment was followed by sonication to further remove fouled material from the surface. To assess the cleaning effectiveness of each cleaning method, coupons were stained with Erythrosin B (20, 21) and Coomassie Brilliant Blue G-250 (22) for 1 minute following the cleaning protocol, and then rinsed filtered seawater. The stains visually highlighted areas where fouling (organic matter) remained after the cleaning process (figure 2.1). The results from these tests provided a rough indication of how each method performed, and visual inspection showed that a coupon placed in the 3% hydrogen peroxide solvent for 1 hr at 55°C, followed by gently sonication was the most efficient at removing material from a hard non-coated, non-painted coupon surface. This analysis was used to down-select solvents and methods for further consideration as described below.

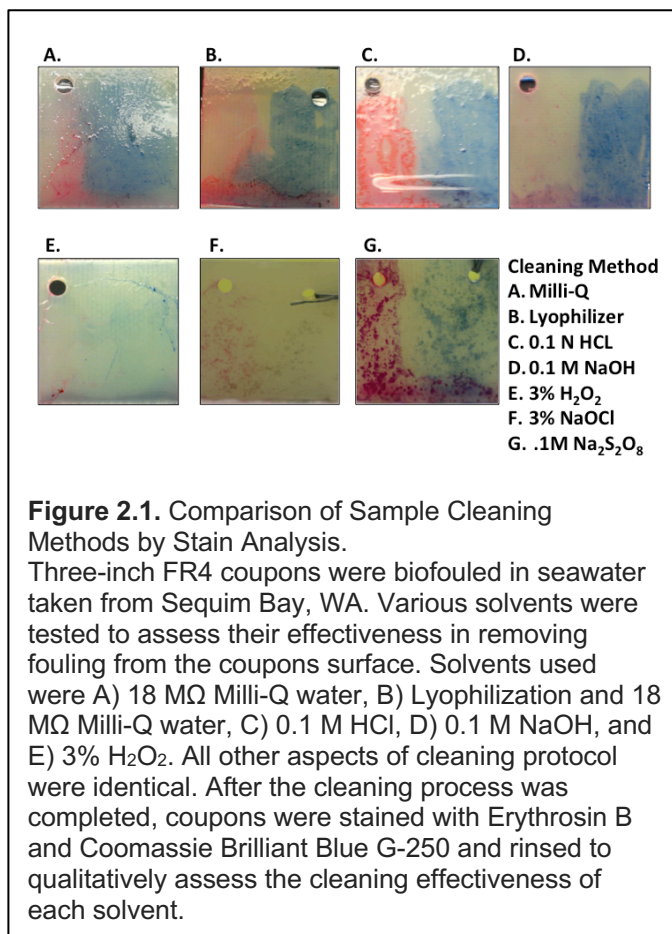


Figure 2.1. Comparison of Sample Cleaning Methods by Stain Analysis.

Three-inch FR4 coupons were biofouled in seawater taken from Sequim Bay, WA. Various solvents were tested to assess their effectiveness in removing fouling from the coupons surface. Solvents used were A) 18 MΩ Milli-Q water, B) Lyophilization and 18 MΩ Milli-Q water, C) 0.1 M HCl, D) 0.1 M NaOH, and E) 3% H₂O₂. All other aspects of cleaning protocol were identical. After the cleaning process was completed, coupons were stained with Erythrosin B and Coomassie Brilliant Blue G-250 and rinsed to qualitatively assess the cleaning effectiveness of each solvent.

Next, the solvents were tested on two very different classes of coatings: a silicone-based superhydrophobic coating, NeverWet™ (Rustoleum, Vernon Hills, IL) and a hard ablative marine paint, Islands77, (Sea Hawk, Clearwater, FL) to determine if any of the solvents were destructive to either of these coatings. NeverWet™ was selected as a representative of silicone-based coatings whose properties rely upon (nanoscale structures and fluoropolymer chemistry) and Islands77 was selected as a representative of polymer-based antifouling coatings. Painted surfaces were rinsed briefly with diH₂O before any of the analyses to remove any unincorporated solvents or uncured coating chemistry.

Results from cleaning the surface of NeverWet coated coupons are shown in **figure 2.2**. The performance of

coatings such as NeverWet™ rely upon the ability to repel water and other fluids from their surface. This ability can be assessed directly by measuring the contact angle a liquid (e.g., water) forms on the surface. To assess for potential damage, contact angles were measured on coated coupons following cleaning with each of the solvents. These measurements were compared with a control coupon that was not cleaned and with a coupon cleaned with MilliQ water (to assess for potential damage by the sonication step).

The surface of coupons cleaned with 1%, 2%, and 3% H₂O₂, 0.1N HCl, and 0.1M Na₂S₂O₈ solvents remained superhydrophobic after the cleaning treatment; conversely, 0.1M NaOH and 3% NaOCl solvents damaged the superhydrophobic coating. **Figure 2.3** shows the measured contact angles after cleaning with each solvent. The measured contact angle for 1%, 2% and 3% H₂O₂ was 155.5°, 150.9°, and 143.0° respectively. Treatment with HCl yielded a contact angle of 146.8°, Na₂S₂O₈ was 147.7°, and the MilliQ water control was 153.6°. No contact angle for NaOH and NaOCl was measured as water placed on the coupon immediately wetted the surface, indicating that the coating had been highly damaged.

NeverWet Applied Coupons After Cleaning Treatment in Different Solvents

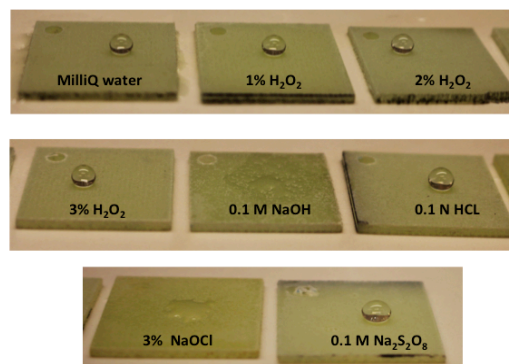


Figure 2.2. Analyses of NeverWet Coupons After Cleaning with Solvents.

The above pictures show NeverWet coated coupons after solvent treatment and cleaning. A drop of water was placed on the surface of the coupons to determine whether the surface was still superhydrophobic after cleaning. Coupons cleaned with Milli-Q water, hydrogen peroxide, HCl and Na₂S₂O₈ solvents were not damaged in the cleaning process and still maintained a superhydrophobic surface afterwards. Coupons treated with a NaOH or NaOCl solvent resulted in a damaged surface that

Next, the cleaning solvents and sonication procedure were tested for the presence of NPOC pre- and post- cleaning to determine if the solvent stripped carbon from the surface coating. MilliQ water was used, also with the sonication procedure, as a control solvent. An increase in NPOC in a solvent following the cleaning process was seen as one indication of damage to the NeverWet™ coating; results are provided in [figure 2.4](#).

NPOC analysis of the Milli-Q solvent post cleaning showed an increase in NPOC of 2.23 mg/L. The 1%, 2%, and 3% hydrogen peroxide solutions had an increased NPOC reading post cleaning of 2.44, 2.38 and 2.43 mg/L respectively. Interestingly, disodium peroxodisulfate showed an increase in NPOC of only 0.10 mg/L significantly lower than the Milli-Q control. Conversely, the highest measured increased concentrations of NPOC after coupon cleaning were detected in 3% NaOCl and 0.1 M NaOH. The 3% NaOCl solution measured 5.75 mg/L post cleaning and 0.1 M NaOH 8.56 mg/L. These results concur with the contact angle analysis; it is apparent that both the sodium hydroxide and sodium hypochlorite solutions were destructive to the NeverWet surface.

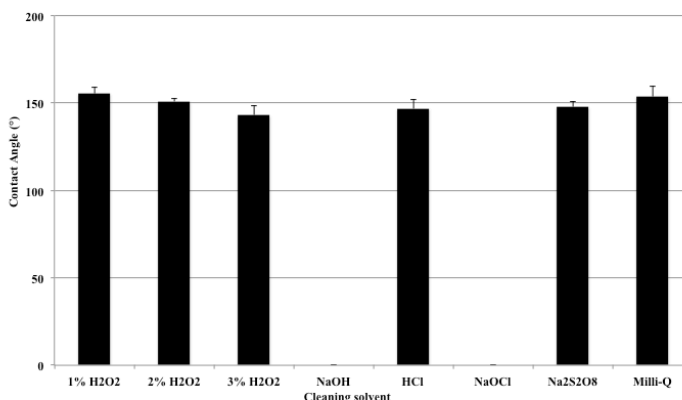


Figure 2.3 Measurement of Contact Angle Post- Cleaning: NeverWet Surface.

Graph above shows the measured contact angle values after solvent cleaning on a NeverWet coated coupon. The H₂O₂, HCl, Na₂S₂O₈, and Milli-Q solvent still remained hydrophobic after the solvent cleaning. However, cleaning with NaOH and NaOCl solvents stripped the NeverWet coating from the surface that resulted in the coupon no longer having hydrophobic properties. No contact angle measurements of NaOH and NaOCl could be completed, as coupon surface would immediately wet and not maintain a beaded water droplet for measurement.

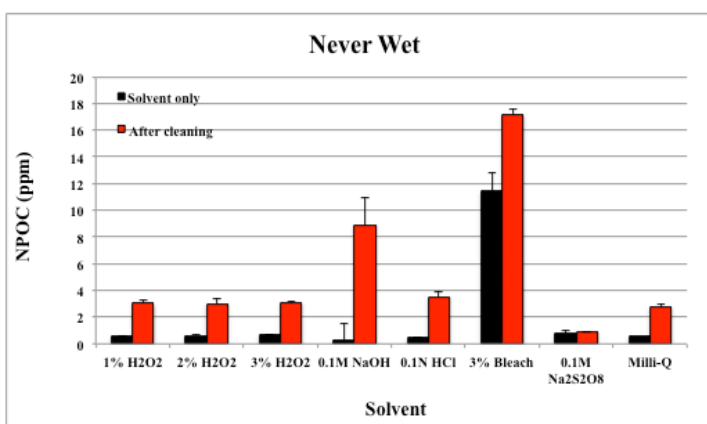


Figure 2.4. Measurement of NPOC in Solvent Pre- and Post-Cleaning: NeverWet.

NPOC results confirm all solvents, excluding the 3% bleach (NaOCl) solution had an initial low carbon background of approximately 1 ppm. After completion of the cleaning process, solvents were collected and tested for NPOC again. Hydrogen peroxide, hydrogen chloride, sodium persulfate, and Milli-Q had NPOC reading of ≤5 ppm after cleaning. Conversely, the sodium hydroxide and bleach solutions showed the highest levels of NPOC in their respective solutions.

Next, we tested the solvents on Islands77 marine paint. As with the NeverWet coatings, NaOH damaged the surface of the marine paint; this is illustrated in [figure 2.5](#) where the solvent has turned blue; furthermore, a substantial increase in NPOC was also measured in the NaOH solvent after the cleaning process (see [figure 2.6](#)). The NaOH solvent had a NPOC reading of 449.33 mg/L post cleaning. In comparison, the Milli-Q control had a NPOC measurement of 4.44 mg/L. The 3% NaOCl solution also had a similarly elevated NPOC measurement of 480.01 mg/L, although there was no visible effect (i.e., blue coloration of the solvent). The lowest NPOC measurement, 3.63 mg/L, was observed with $\text{Na}_2\text{S}_2\text{O}_8$. The 1%, 2%, and 3% H_2O_2 solvent generated NPOC results of 6.70, 7.73, and 7.49 mg/L, respectively; and treatment with the HCL solvent resulted in 38.24 mg/L NPOC after cleaning. These results show that NaOH and NaOCl are also destructive to Islands77 marine paint. The HCl solvent also caused damage to Islands77, but to a lesser extent. Conversely, washing with H_2O_2 resulted in only ~2-3 mg/L more NPOC than the Milli-Q control solvent. Similar to the NeverWet results, $\text{Na}_2\text{S}_2\text{O}_8$ had the lowest amount of NPOC; however, the $\text{Na}_2\text{S}_2\text{O}_8$ solvent cleaned the fouled coupons poorly (see [figure 2.1](#)).

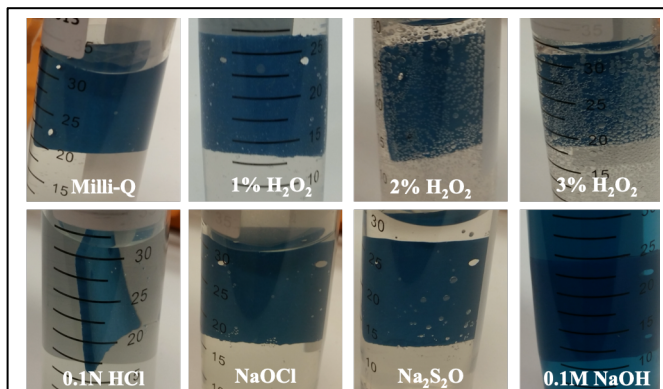


Figure 2.5. Analyses of Islands77 painted coupons after cleaning. Photos of painted coupons in cleaning solvents taken at the completion of the cleaning procedure. The NaOH solution caused the painted surface to dissolve in solution (note the transfer of blue pigment to the solution) while the other solvents showed no visible destruction to the surfaces. TOC results from the NaOH solution in the picture revealed elevated levels of carbon (see Figure 6). Treatment with NaOCL also resulted in elevated levels of carbon in the solvent after cleaning, even though, there was no color change to the solvent solution.

One challenge in comparing the effectiveness of each cleaning solvent on fouled coupons is that there is no direct means to assure that all coupons have the same amount of fouling material. Although sets of coupons were exposed simultaneously to identical conditions, any variability in the quantity or adhesion of the fouling community could skew comparisons. To address this

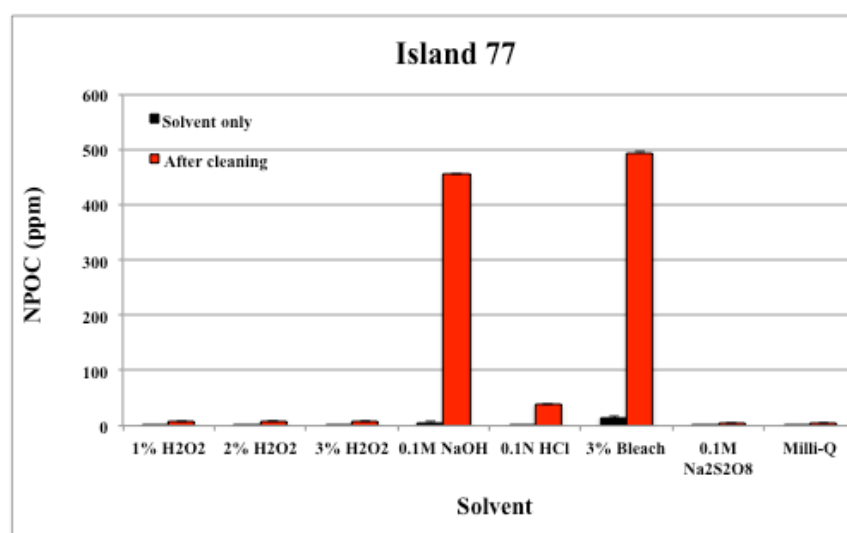


Figure 2.6. Measurement of NPOC in Solvent Pre- and Post- Cleaning: Islands77.

variability, an experiment was designed to compare pairs of solvents on the same coupons. The comparisons were performed with H_2O_2 , NaOH , and $\text{Na}_2\text{S}_2\text{O}_8$. Biofouled fiberglass coupons were first cleaned with one solvent, and then subjected to a second round of cleaning with an alternate solvent to determine if the second solvent could remove significant fouling left behind by the first solvent. Solvents from the first and second rounds of cleaning were measured for NPOC and results were used to compare how much more carbon could be removed from the surface with a different solvent. $\text{Na}_2\text{S}_2\text{O}_8$ removed an additional 6.49 mg/L from the surface of a H_2O_2 cleaned coupon. Compared to the NPOC results from the 1st round of cleaning, an additional 26% of material was removed. NaOH removed an additional 18.26 mg/L of carbon from the surface of a H_2O_2 cleaned coupon, this equates to 50% of the initial removed carbon during the first cleaning step. In comparison, H_2O_2 cleaned an additional 23.34 and 23.44 mg/L of NPOC off of a NaOH and $\text{Na}_2\text{S}_2\text{O}_8$ cleaned coupons. This equates to an additional 73% and 87% of removed material. These results demonstrate that NaOH and $\text{Na}_2\text{S}_2\text{O}_8$ leave behind more organic material during cleaning than H_2O_2 .

The amount of carbon left behind was surprising and suggests that further refinement of the wash protocol is needed to achieve an ideal balance of removing fouling relative to surface damage. While H_2O_2 performs better than other standard solutions, the results indicate that the absolute NPOC measurements still entail significant errors. Additional studies may identify a better washing protocol, but the current approach still provides a significant improvement over existing methods and provides a means to make relative comparisons of how well different surface coatings prevent fouling.

Taking into account cleaning performance and the ability to minimize potential damage to a surface coating, a single wash with a solution of 3% hydrogen peroxide was selected as the cleaning solvent for removing biofouling from all seawater-exposed test coupons in this study. NaOH was less effective at removing carbon from a fouled surface and it caused significant damage to NeverWet and Island77 coatings, and thus it was deemed unacceptable for this purpose. NaOCl also damaged surface coatings and this solvent had a higher initial carbon background. HCl caused slightly more damage to surfaces than H_2O_2 and didn't clean as well as the H_2O_2 . $\text{Na}_2\text{S}_2\text{O}_8$ and the lyophilization treatment were the least effective at cleaning fouled surfaces.

To further investigate the use of H_2O_2 and its effectiveness in cleaning, a variety of substrates were fouled and analyzed over a time course interval. Additionally, the potential for carbon loss due to mineralization of organic carbon by the oxidizing effects of H_2O_2 was examined. Finally, the potential for carbon contamination of the wash solutions resulting from carbon compounds leaching from the coatings (e.g., solvents, VOCs) as opposed to the result of damage by the wash solution.

Effects of H_2O_2 on NPOC Analysis

We wanted to determine and quantify potential mineralization of organic carbon by hydrogen peroxide. It has been reported in soil and sediment studies that the use of a strong oxidizer, such as >5% hydrogen peroxide, in the analysis of NPOC can convert a portion of the organic carbon contained in the sample into CO_2 (17, 23). This effect under-represents the amount of NPOC present in a sample. To determine whether a 1%, 2%, 3% or a 5% H_2O_2 cleaning solution causes significant loss of available organic carbon, control samples of potassium

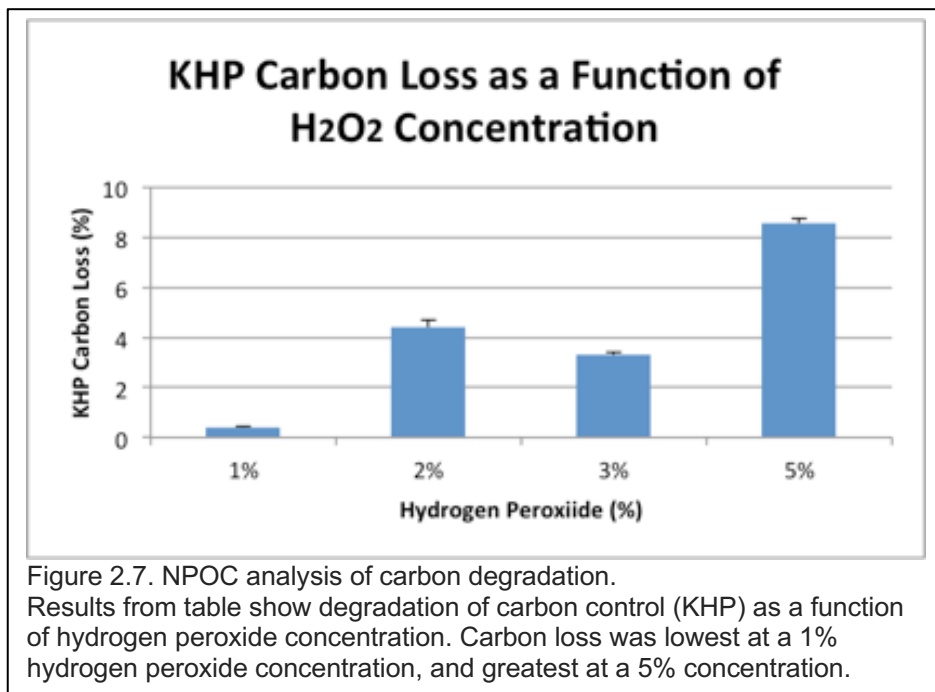
hydrogen phthalate (KHP) were used to quantify potential carbon destruction by the coupon cleaning process. KHP was selected because it is an easily quantified organic carbon molecule and is Shimadzu's recommended carbon source for instrument calibration.

XX (g or Molar) of KHP was added to 1%, 2%, 3%, and 5% hydrogen peroxide solutions or to 18 MΩ Milli-Q ultrapure water as a control. Each solution was subjected to the

sonication cleaning procedure, and each test set was performed in triplicate. As a control, the KHP carbon source was added to the 18 MΩ Milli-Q water for a comparison as a non-destructive solvent and loaded into the Shimadzu ASI-L for NPOC

analysis. Results from the tests are shown in [figure 2.7](#) and in [table 2.1](#). The 1%

solution of hydrogen peroxide had minimal effect on the KHP; NPOC analysis showed that there was only a 0.399% reduction in carbon. The 2% and 3% hydrogen peroxide samples were similar in effect, resulting in a 4.389 and 3.310 percent loss of carbon in the test sample. Nott unexpectedly, the 5% hydrogen peroxide had the largest loss of carbon at 8.560%, roughly twice the loss resulting from the 2% or 3% solutions.



An optimal cleaning solution will remove organic carbon from a surface without damaging the surface or mineralizing the carbon. The 3% H₂O₂ solution resulted in a loss of 3.3% of the available carbon, no more than was lost by using 2% H₂O₂, but it provided better cleaning than either the 1% or 2% solutions and caused no increase in damage to the surfaces tested.

Table 2.1. NPOC analysis of organic carbon control samples.

Solvent	KHP measured by TOC	Percent loss (normalized to MilliQ)
Milli-Q	3.258 mg/L σ 0.151	N/A
1% H ₂ O ₂	3.245 mg/L σ 0.026	-0.399%
2% H ₂ O ₂	3.115 mg/L σ 0.318	-4.389%
3% H ₂ O ₂	3.150 mg/L σ 0.119	-3.310%
5% H ₂ O ₂	2.979 mg/L σ 0.216	-8.560%

The table above shows the results from NPOC analysis to determine whether the use of H₂O₂ results in significant loss of organic carbon due to mineralization. 3 mL of KHP organic carbon standard (X mg/L) was added to either 27 mL of 18 M Ω Milli-Q water or a 1%, 2%, 3% or 5% hydrogen peroxide solution to create a solution with an initial known carbon concentration of Z mg/L. Results show little difference in carbon lost with 2% or 3% H₂O₂, but twice as much carbon lost when using 5% H₂O₂. 1% H₂O₂ resulted in less than 0.5% loss but was also ineffective as a cleaning agent.

Quantifying Marine Biofouling on Selected Surfaces

An experiment was performed to determine whether NPOC analysis is a suitable method to establish a quantifiable comparison of the accumulation of marine biofouling. A 20-week exposure experiment was set up to assess organic carbon accumulation on a coupons surface. Three sets of 1" coupons, FR4 fiberglass, Teflon, copper, stainless steel, and stainless steel painted with Islands77 *plus* marine paint were placed in a test tank with flowing, unfiltered seawater pumped from Sequim Bay, WA and removed at 10-week and 20-week time intervals. At each time point, three coupons of each material type were removed from the test tank for NPOC analysis and a sample of the test tank seawater was also taken at the same time and analyzed to determine the total amount of NPOC present: dissolved and suspended particulate material or cells. Results are shown in [Table 2.2](#).

Table 2.2 Quantification of biofouling by NPOC analysis.

Coupon	Time 0	10 week	20 week
FR4 fiberglass	0.03 mg	1.12 mg	1.65 mg
Teflon	0.05 mg	1.18 mg	1.27 mg
Copper	0.03 mg	0.20 mg	0.08 mg
Stainless Steel	0.04 mg	0.97 mg	1.14 mg
Islands77 <i>plus</i>	0.51 mg	2.34 mg	2.12 mg

The table above shows the results from NPOC analysis of biofouled 1" coupons. Results from the table show the total amount of organic carbon removed from the coupon at 0-, 10- and 20-week seawater exposures. Each coupon type was tested in triplicate at each time point. Seawater tank samples were removed at the ten- and twenty-week time points and analyzed by NPOC.

The copper coupons in our test tanks showed no visible signs of biological growth on their surfaces; this was confirmed by NPOC analysis that detected only trace amounts of carbon, likely attributable to the formation of a biomolecular conditioning film on the copper surface and/or seawater carryover. This was expected as copper has well-known biocidal activity against many microorganisms and is used in many antifouling marine paints to prevent surface colonization (24). After a 10-week exposure, the total mass of organic carbon removed from the copper coupon was only 0.20 mg and at 20-weeks the total mass of organic carbon was 0.08 mg. Most likely the majority of this carbon came from test tank carryover. This is supported by the fact that the concentration of organic carbon in the test tank seawater was higher at the 10-week exposure time point, 28.10 mg/L, compared to the 20-week time point, 9.75 mg/L.

Teflon, FR4 fiberglass, and stainless steel all had increasing amounts of fouling measured on their surfaces as a function of time. These coupons had two things in common: they are non-toxic and had a smooth surface initially. We hypothesize that once the fouling process initiated, it proceeded unimpeded. Lastly, we tested the ablative antifouling paint Islands77 *plus*. NPOC analysis of the Islands coupons after 10- and 20-week seawater exposures revealed approximately the same amount of bioaccumulation regardless of exposure time. This most likely is due the ablative properties of the Islands77 *plus* paint, that both sheds accumulated biofoulant material and paint continuously.

The effects of carbon leaching from marine paints will be addressed in the next section. The results from these coupon-fouling experiments show that NPOC analysis is a useful tool in assessing the antifouling performance of a surface. In this set of experiments, we selected coupons that had both known antifouling properties and coupons that were expected to foul by the conclusion of this experiment. Upon the completion of this experiment, NPOC analysis corroborated visual fouling of the coupons surface. There was one exception; Islands77 *plus* had the highest NPOC reading after 10 weeks, which we will show in the next section is caused by the ablative properties of the paint and is easily corrected for by proper test controls.

Carbon Leaching from Marine Antifouling Paints

Paints contain significant quantities of organic carbon in the form of solvents and other volatile organic carbon (VOC) sources that may leach into surrounding seawater even after the paint has cured. This leaching process could influence NPOC measurements and would lead to elevated organic carbon readings not related to biofouling. To examine this potential, a set of one-inch coupons painted with Islands77 *plus* marine paint was exposed to sterile artificial seawater over a 20-week period. Samples were kept in the dark and sterile artificial seawater was used to prevent biological growth from occurring over the course of this leachate experiment. At each time point, 0, 10 & 20 weeks, one sample was sacrificed: the water from the sample was removed and used for NPOC analysis and the coupon was also subjected to NPOC analysis using the H₂O₂ cleaning procedure.

The NPOC analysis of the sterile seawater samples showed that the carbon concentration increased with the length of time that the painted coupons were submerged in the water. The 10-week water sample had 21.66 mg/L more carbon than the seawater only control; the 20-week water sample had 32.34

mg/L more carbon than the seawater only control. This demonstrated that measureable amounts of carbon are released by the coating.

Carbon was also detectable by washing the coupons, and the amount of carbon washed from the coupon surface did not change with time during the 20-week interval. The time zero coupon had 0.51 mg of carbon removed after the cleaning process, the 10-week soak coupon had 0.50 mg of recovered carbon, and the 20-week soaked coupon had 0.55 mg of recovered carbon. Thus, when working with paints, there will always be an amount of carbon that is released from the surface coating that will contribute to the overall carbon content detected by NPOC analysis. However, this can easily be accounted for by the effective use of control samples: painted coupons that are not exposed to the fouling conditions, only to sterile seawater. Once a carbon release rate has been determined for a particular coating type, this can be subtracted from the fouling measurement. For example, the results for the Islands77 plus shown in Table 2 should be corrected by subtracting the NPOC values obtained from the control samples. This would lead to the Time 0 values being 0 mg and in greater agreement with the NPOC values for the other surface types. The amount of carbon leached from the paint (0.5 mg and 0.55 mg) amounted to approximately 21% and 26% of the values obtained for weeks 10 and 20, respectively.

2.4 Conclusions

In this study we successfully used NPOC analysis to quantify marine biofouling on a variety of surface types. We conclude that NPOC analysis is an effective methodology to quantify fouling on a surface, allowing for direct quantitative comparison of antifouling surfaces and coatings to one another. Further, we determined that 3% H₂O₂ provided effective removal of fouling from surfaces without damaging coatings or causing significant mineralization of available organic carbon (Table 2.3) in comparison to other solutions. This methodology removes the subjective nature of assessing biofouling formation/build-up that is inherent when using current ASTM standards and protocols; variability is removed by replacing the subjective interpretation of the technician/engineer with quantitative NPOC analysis that results in data that is highly reproducible. However, there are a few factors that must be considered when using this methodology, such as the effective use of controls to account for surface coating ablation and carbon destruction by hydrogen peroxide. Still, upon taking these factors into account this methodology proves effective at comparing multiple surface types under the same environmental conditions (e.g., exposure time, salinity, water flow,

light). With a defined set of environmental conditions and sample controls, one can set up an experiment to quantitatively determine the effectiveness of a surface, determine the conditions it performs best, and define areas where it needs to improve. We conclude that NPOC analysis is an effective tool to understand antifouling surface performance.

Table 2.3 Solvent Characterization Table.

Solvent	Cleaning Effectiveness	Damaging to Coatings	Solvent Carbon Contamination
H ₂ O ₂	●	○	○
NaOH	●	●	○
Na ₂ S ₂ O ₈	○	○	○
HCl	◐	◐	○
NaOCl	●	●	●

High = ● Medium = ◐ Low = ○
 The solvent table illustrates how effective each tested solvent was at cleaning a surface, not damaging the surface coating and whether the solvent had a high level of background carbon present before cleaning. Result are denoted as high, medium and low.

2.5 Acknowledgements

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3.0 Mussel Adhesion

3.1 Abstract

Herein we propose a standardized method for measuring tensile adhesion of the mussel plaque on a per-thread basis, allowing for meaningful comparisons between low-energy foul-release surfaces. This method provides greater accuracy, repeatability, and a finer resolution than push-based shear methods without the difficulties involved in comparing mussel size, thread attachment angle, or harming the mussels. *This method reduces the test to a single-thread normal force measurement.* Our method showed significant differentiation between commercial foul release coatings that could not be resolved using other methods.

3.2 Introduction

Biocides are commonly used to eliminate hard fouling organisms. However, as their deleterious effects on the marine environment have become increasingly apparent, foul-release coatings that reduce or eliminate fouling without harming organisms have become an increasingly popular solution. These coatings use surface chemistry and microstructure to create low-energy surfaces to which hard-fouling organisms cannot strongly adhere. Of particular interest is the efficacy of different foul-release coatings on mussel species, due to the alarming and costly invasion of the zebra and quagga mussels into North American waterways. Fouling release coatings provide a biocide-free approach to antifouling methods. The coating minimizes the adhesion between the substrate and fouling organism, allowing the fouling organisms to be naturally removed by hydrodynamic stress. This self-cleaning attribute is wholly dependent on the velocity at which the vessel is moving, as the relative motion of water across the vessel's surface provides enough force to remove objects from the coated surface. Furthermore, the smoothness of foul-release coatings allows for decreased drag of the vessel, reducing greenhouse gas emissions and fuel consumption [19].

Both fresh and saltwater mussels utilize their byssal complex to attach themselves to underwater surfaces. The byssal complex, also known as the byssus, is a proteinaceous holdfast composed of multiple fibrous threads that connect the interior of the mussel to an adhesive plaque. This plaque anchors the byssal threads to a substrate, as shown in [Figure 3.1](#). These threads are produced using the byssus gland in the mussel's foot and are secreted in a radial pattern from a central stem.

Mussels generally attach with between 50-100 byssal threads around the organism. Once attached, adult mussels rarely move, though they possess the capability to eject their threads if movement is necessary. The chemical and mechanical characteristics of the byssus are well-researched; this understanding combined with the threat invasive mussels pose makes the mussel an ideal candidate for fouling studies [1-10]. The most commonly studied mussel adhesion protein, Mefp-1, has adhesive properties similar to synthetic cyanoacrylate and epoxy resins, which are popular due to their quick polymerization, high bond strength, and ability to bond to multiple substrates [9].

Additionally, adult mussels are relatively easy to maintain and will attempt to attach to any class of material [11]. This makes standardizing their behavior easier.

Mussel Adhesion Standards

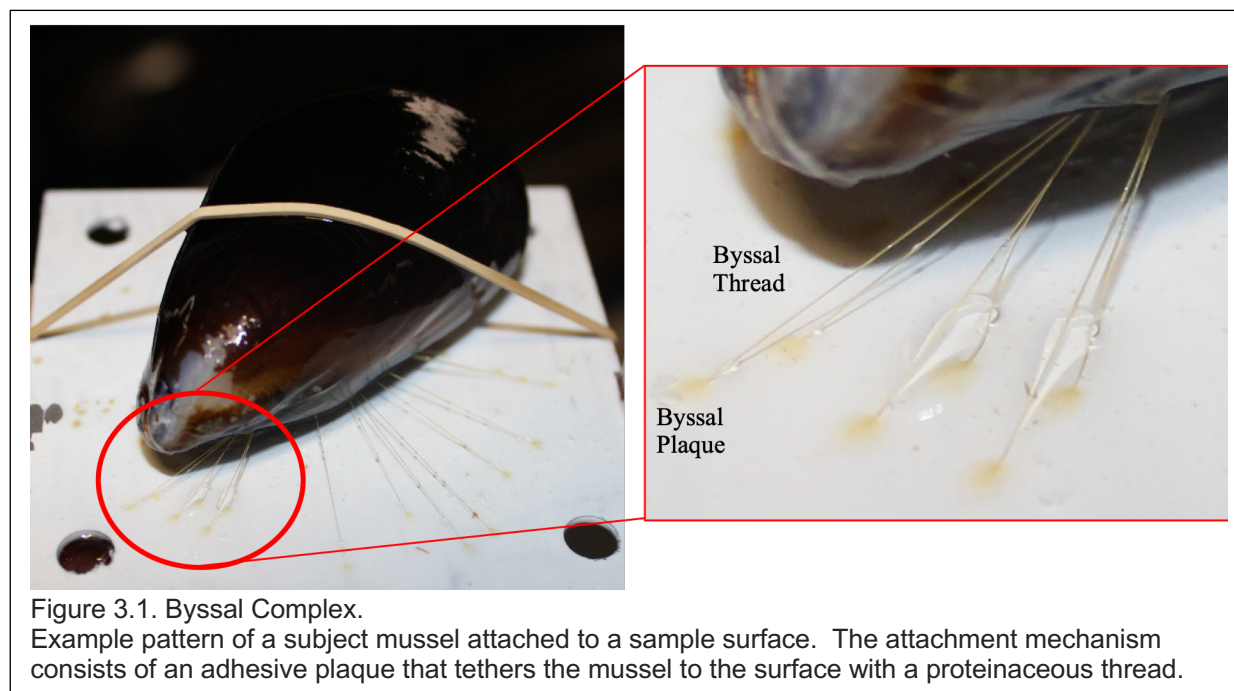


Figure 3.1. Byssal Complex.

Example pattern of a subject mussel attached to a sample surface. The attachment mechanism consists of an adhesive plaque that tethers the mussel to the surface with a proteinaceous thread.

An effective foul-release coating should both deter mussels from attaching through surface heterogeneity and make attachments easily removable through low-energy surface chemistry and omniphobicity. Although the quantity of material's surface energy can be used to make general characterizations of its efficacy at preventing adhesion, they are not absolute. A low friction, low surface energy material will outperform one with higher surface energy and roughness but may behave quite differently from other materials with similar surface energy. The best method for comparing foul release behavior is a straightforward live mussel adhesion test.

While ASTM offers several standards for assessing marine coatings, there are no standards for the attachment of marine organisms. Instead, foul control is measured through simple counts [12, 13]. The closest standard for measuring the attachment strength of a fouling organism is the now withdrawn ASTM D5618-94 (2011), "Standard Test Method for Measurement of Barnacle Adhesion Strength in Shear" [14]. This and other shear-based methods [1, 11, 14-17] that mimic scraping or current flow used to clear mussels and other hard fouling organisms from marine structures provide a robust method to gauge their level of attachment to a surface. However, these methods are too coarse to provide useful information for the comparison of low-energy foul-release coatings. When testing these specialized surfaces, the adhesion of the fouling organism is much lower than when attached to a standard coating or material. This weaker attachment causes mistrials and marginal readings to be common when testing with whole-mussel shear tests, leading to a dearth of meaningful data. Tensile methods [4, 6, 7] are more applicable, though the camera equipment needed to accurately

analyze the angle of each byssal thread adds an unnecessary complication to the testing process. We propose a standard method that measures tensile adhesion of the mussel plaque on a per-thread basis, allowing for meaningful comparisons between low-energy foul-release surfaces. This method eliminates the dependencies on thread angle, mussel size, and number of threads shown in whole mussel testing.

3.3 Materials and Methods

Shear Tests. Adult mussels of *Mytilus galloprovincialis*., ranging in size between 55 and 75 mm, were maintained in tanks of artificial seawater (Artificial Ocean) at conditions of 32-34‰ salinity and 12-16°C. The mussels were fed phytoplankton (Seachem) at a regular interval of 3 days to maintain consistent byssal thread production. To test adhesion, a single adult mussel was placed on a 76 mm x 76 mm coupon of the sample material. To reduce the chance of the mussel moving away from the sample surface, a rubber band was secured around the mussel and sample, and both were placed on a 76 mm cylinder immersed in the aquarium (figure 3.2).

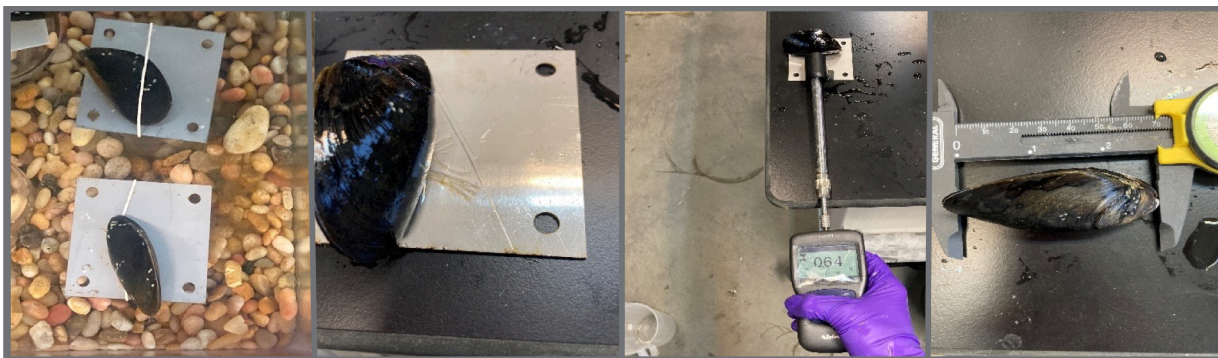


Figure 3.2. ASTM Shear method.

a) Test subjects during the attachment phase b) Attachment of a mussel to a stainless-steel coupon c) Using the force gauge to manually detach the mussel d) measure the mussel dimensions and return it to the tank.

After 24 hours, the mussels and samples were removed from the tank. The number of threads was counted, and a Shimpo 10 N handheld force gauge was used to push the mussel parallel to the sample surface until all threads were broken or removed from the sample surface. The peak force from the gauge was then measured and recorded. Because all threads were tested within 24 – 36 hours of their extrusion, the aging and curing of each thread and plaque did not differ [18].

This method was initially used to measure mussel adhesion but was ultimately found to be too coarse a method to differentiate between foul-release surfaces. The handheld force gauge introduces uneven force across all threads, which can cause twisting of the mussel on a consistent surface. This is due to the irregularly curved surface of the mussel, as well as the varying radial thread patterns and total thread count of each respective mussel, which are largely dependent on the mussel's size. [18]. Therefore, the method does not provide a fair comparison between trials as the number of threads and pattern of threads produced by the mussel can greatly impact individual test results, resulting in high error. For instance, over the course of 14 trials on a sanded steel

coupon, the attaching mussel produced as few as 2 threads and as many as 34 threads in a 24-hour period. The ASTM method makes no distinction between these results however, resulting incomplete data. Similarly, the placement of the plaques influences whole mussel testing. To accurately assess the strength of attachment, the relative angle of each thread to the direction of force, be it tensile or shear, must be known; the engagement of each thread (slack or taut) must be recorded and factored into any analysis as well [4]. Gathering this information is an unnecessary burden that can be avoided through the proposed single-thread test method.

Hardware. In contrast to the manually operated ASTM shear method, the proposed tensile method utilizes a fully motorized straining system. In these experiments, a Mark-10 ESM303 motorized stand in vertical orientation equipped with an M7-05 force gauge (test range 2.5 ± 0.005 N) was used to test the tensile strength of singular byssal threads (Figure 3.3). The gauge was pulled upward at a speed of 50.5 mm/min. This motorized system reduces operator influence on test results by providing a constant and definite strain rate and strain direction. The vertical orientation minimizes the impact of peeling and torque on adhesion force, allowing for accurate comparisons between individual plaques.

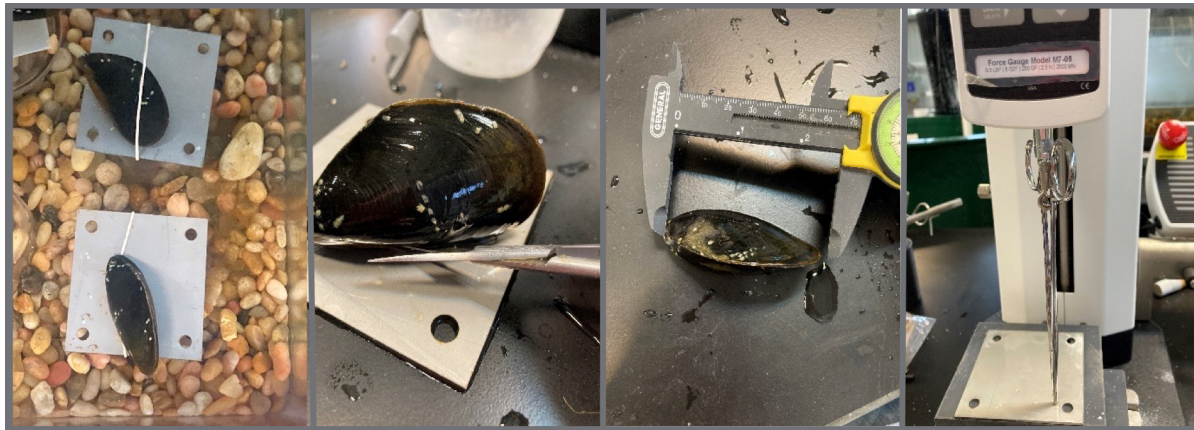


Figure 3.3. Method for tensile testing.

a) Test subjects during the attachment phase b) Sever byssal threads at their point of emergence from the shell c) measure mussel dimensions d) grip each thread with forceps and pull to detachment.

3.4 Single-thread Tensile Test

Adult mussels of *Mytilus galloprovincialis*, ranging in size between 55 and 75 mm, were acquired from commercial shellfish companies in the Puget Sound region of Washington state and maintained in tanks of artificial seawater (Instant Ocean) at conditions of 32-34 salinity and 12-16°C. The mussels were fed phytoplankton (Seachem) at a regular interval of three days to maintain consistent byssal thread production. To test adhesion, a single adult mussel was placed on a 76 x 76 mm coupon of the sample material. To reduce the chance of the mussel moving away from the sample surface, a rubber band was secured around the mussel and sample, and both were placed on a PP cylinder with diameter and height both of 76 mm.

Materials selected

To characterize the influence of different test materials on results, multiple surfaces representative (of marine structures) were selected for testing. For non-foul release materials, steel (polished and sanded), fiberglass, polypropylene (PP), stone, and a commercially- available epoxy-based bottom paint were chosen due to their universal presence in marine structures (Table 3.1). These standard surfaces also present a range of material properties such as hardness, surface roughness, and surface energy that could impact mussel adhesion or test results. Six foul release coatings (two commercially available, four developmental) were used to compare the effects of each test on the quality of results. The commercially available coatings utilize fluoropolymer paint to create a low-energy surface, while the developmental coatings present a liquid-like surface through lubrication. Again, comparing different surfaces is key to understanding the quality of test results and exploring the scope of a given test.

Table 3.1: Sample materials used for mussel attachment

Material	Description	Surface Energy (mJ/m ²)
Sanded Steel		51
Fiberglass		52
Stone	Locally sourced	undetermined
Marine Paint	Epoxy marine paint	23
Polypropylene		30
FR1	SLIPS foul release paint	21
FR2	Fluoropolymer foul release paint	16
FR3	Fluoropolymer foul release paint	23
FR4	SLIPS foul release paint	23
FR5	SLIPS foul release paint	23

After 24 hours, the samples and mussels were removed from the aquarium. Byssal threads attached to the sample surface were cut using fine scissors or a blade at their point of emergence from the mussel shell. The outer dimensions of the mussel were measured, and the mussel was returned to the aquarium. The sample was then clamped to the base of the motorized force measurement platform, as seen in Figure 3.4. Each thread was gripped with medical forceps and pulled normal to the sample surface at a rate of 50.5 mm/min with a Mark-10 ESM303 motorized test stand equipped with an M7-05 force gauge until the byssal plaque was removed from the surface or the byssal thread was broken.

It was assumed that the plaques and threads produced by each mussel were mechanically similar such that the sample surface was the driving force for difference in measured detachment force. It was also observed that each byssal thread emerged from the approximate center of each plaque such that peeling action was avoided when removing the plaque from the sample surface.

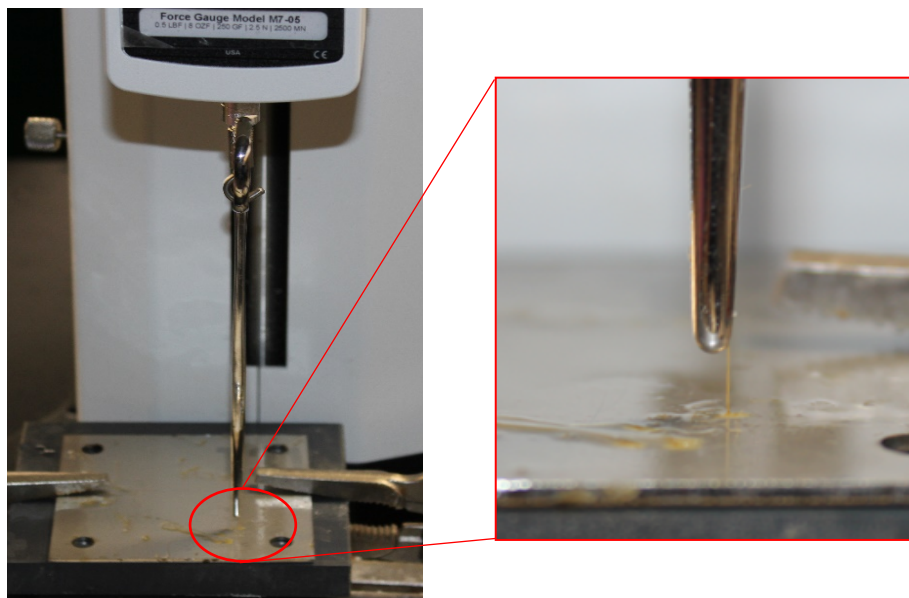
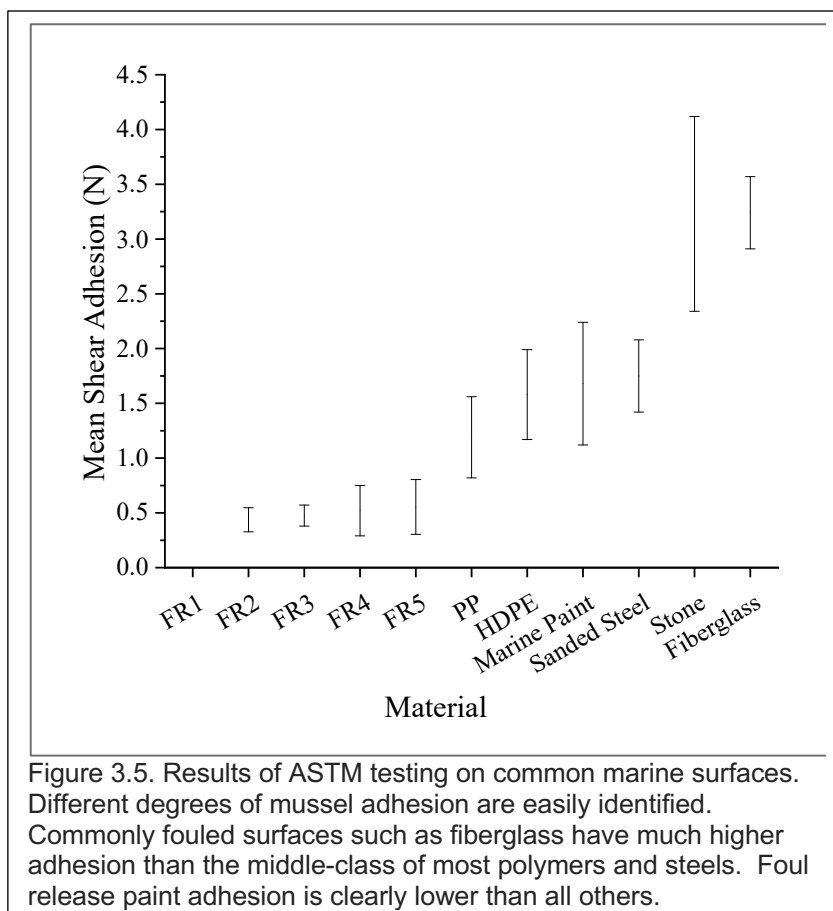


Figure 3.4. Testing apparatus in use for tensile methods. Medical forceps grip the byssal thread and are pulled normal to the sample surface with a M7-05 Force Gauge equipped with a 2.5 N load cell.

3.5 Results

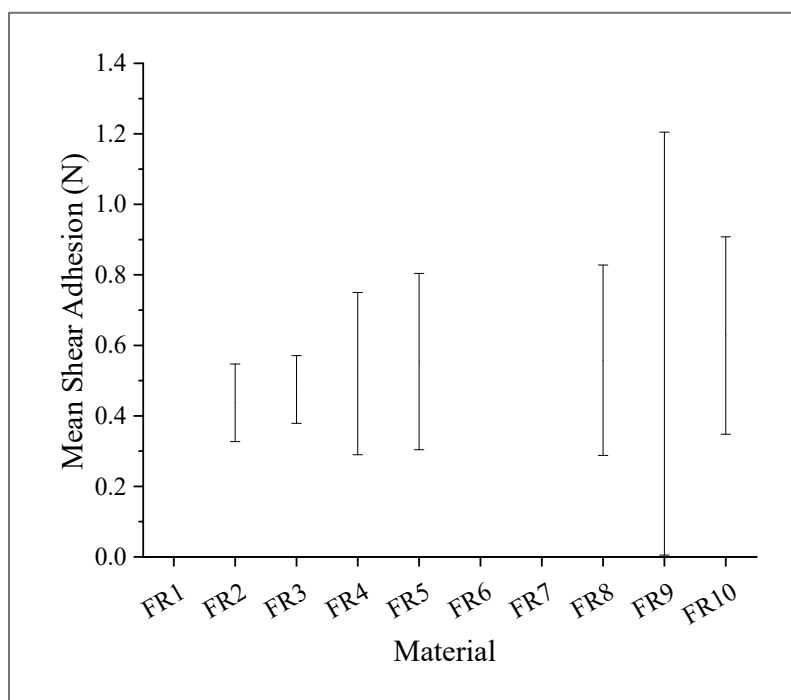
Shear

Results of testing following the ASTM method on standard marine surfaces are shown in **Figures 3.5 and 3.6** and in **Tables 3.2 and 3.3**. On these materials, which possess little to no foul-release behavior, the method works well as the mussels attach strongly enough to allow for easy differentiation between the surfaces.



Material	Mean Shear Adhesion (N)	Standard Error	Mean Tensile Adhesion (N)	Standard Error
FR1	--	--	0.078	0.008
FR2	0.437	0.11	0.091	0.017
FR3	0.475	0.096	0.075	0.010
FR4	0.52	0.23	0.143	0.012
FR5	0.554	0.25	0.121	0.009
PP	1.19	0.37	0.216	0.026
HDPE	1.58	0.41	0.259	0.047
Marine Paint	1.68	0.56	0.276	0.015
Sanded Steel	1.75	0.33	0.359	0.027
Stone	3.23	0.89	0.544	0.041
Fiberglass	3.24	0.33	0.442	0.023
FR = Foul Release coating --indicates no reading recorded on force gauge				
Table 3.2: Results of ASTM testing on common marine surfaces. Different degrees of mussel adhesion are easily identified. Commonly fouled surfaces such as fiberglass have much higher adhesion than the middle-class of most polymers and steels. Foul release paints are clearly lower than all others.				

However, when using the same method to compare between different foul-release coatings, the results are not so easily analyzed. Since the ASTM test is performed manually, error tends to be higher as the operator will not push at the same rate or angle each test. Additionally, the size of the mussel can influence test results at such low adhesion strengths. Figure 3.4 shows the results of this test.



FR = Foul Release coating

Figure 3.6. Results of ASTM testing on Foul Release Surfaces.

Seven of the ten tested coatings are indistinguishable with error bars, three of the ten did not register a reading on the force gauge used.

Material	Mean Shear Adhesion (N)	Standard Error
FR1	--	--
FR2	0.437	0.11
FR3	0.475	0.096
FR4	0.520	0.23
FR5	0.554	0.25
FR6	--	--
FR7	--	--
FR8	0.558	0.27
FR9	0.605	0.66
FR10	0.628	0.28

FR = Foul Release coating

--indicates no reading was recorded on force gauge

Table 3.3: Results of ASTM testing on Foul Release Surfaces.

Seven of the ten tested coatings are indistinguishable with error bars, three of the ten did not register a reading on the force gauge used.

The high error bars make distinguishing the foul release coatings impossible, except for those that performed well enough to not cause a reading on the force gauge.

Tensile. When the same materials are tested with the standard single-thread tensile method proposed here, the results have much higher resolution ([Figure 3.7](#) and [Table 3.4](#)). Additionally, materials that yielded no results in the ASTM standard can be compared.

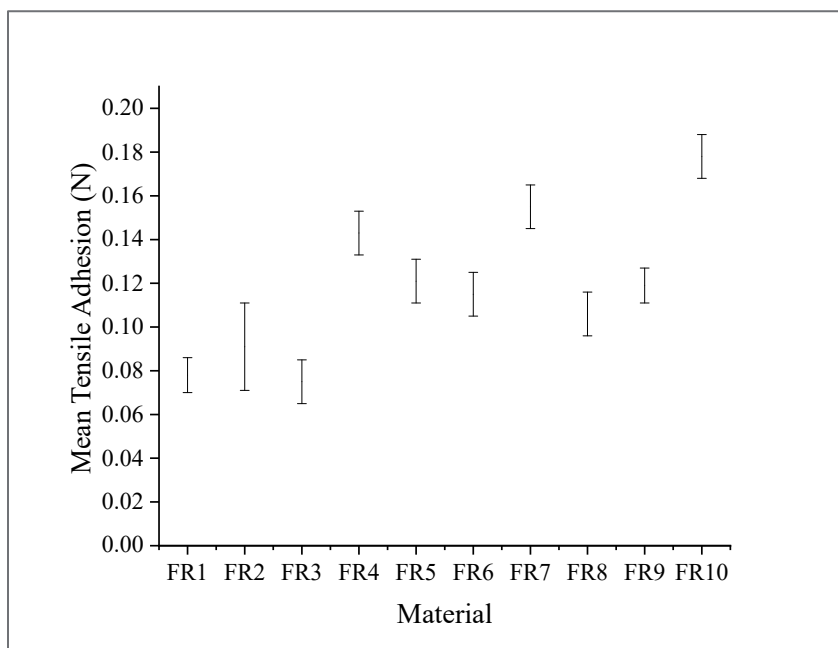


Figure 3.7. Results of single-thread tensile testing of foul-release materials.
FR = Foul Release coating

Material	Mean Tensile Adhesion (N)	Standard Error
FR1	0.078	0.008
FR2	0.091	0.02
FR3	0.075	0.01
FR4	0.143	0.01
FR5	0.121	0.01
FR6	0.115	0.01
FR7	0.155	0.01
FR8	0.106	0.01
FR9	0.119	0.008
FR10	0.178	0.01
FR = Foul Release coating		
Table 3.4: Results of single-thread tensile testing of foul-release materials.		

3.6 Discussion

In contrast to the ASTM standard testing results, the foul-release coatings can be compared. The error associated with this test is much lower, and all coatings tested yielded a result. Figure 6 elucidates this difference by comparing the percent error between methods directly.

These results indicate a standardized, single-thread tensile adhesion test method offers numerous benefits over existing standards while utilizing ubiquitous equipment. The measurement of a single thread per trial as opposed to the full mussel eliminates the confounding influence of thread position and number of [18]threads. The mass of the mussel and its shape are also eliminated as potential sources of error. This leads to a much more fundamental measurement and allows for caveat-free comparisons between test results as the test dynamics are replicated for every trial. Other sources of error such as the tensile strength of individual threads and the position of the thread-plaque juncture can be accounted for through repetition. Because a single mussel will likely produce many threads in one test period, obtaining a significant number of trials is much easier to achieve with this single-thread method than with a whole-mussel method.

Although this method makes no measurement of shear adhesion, a similar single-thread test for shear adhesion could easily be set up in a similar manner by gripping the sample substrate parallel to the gauge movement direction. Testing both tensile and shear adhesion would yield a better picture of the mussel's overall adhesion without the need for camera equipment to measure the angle between the thread and the sample substrate.

Similarly, this method could be improved by measuring the diameter of the plaque before detachment to provide an estimate of the tensile stress required for removal in addition to the tensile force.

Limitations

- This approach does not force mussels to attach, so if a coating is very effective, it can be hard to collect data
- Only applicable to mussels currently due to nature of thread attachment

3.7 Conclusion

Adoption of this standard test method allows for simple, direct comparisons between live-mussel testing of foul release coatings with minimal equipment. By providing a direct, standard context from which to view foul release coatings, industry and academia researchers can better monitor the quality and performance of existing and new coatings.

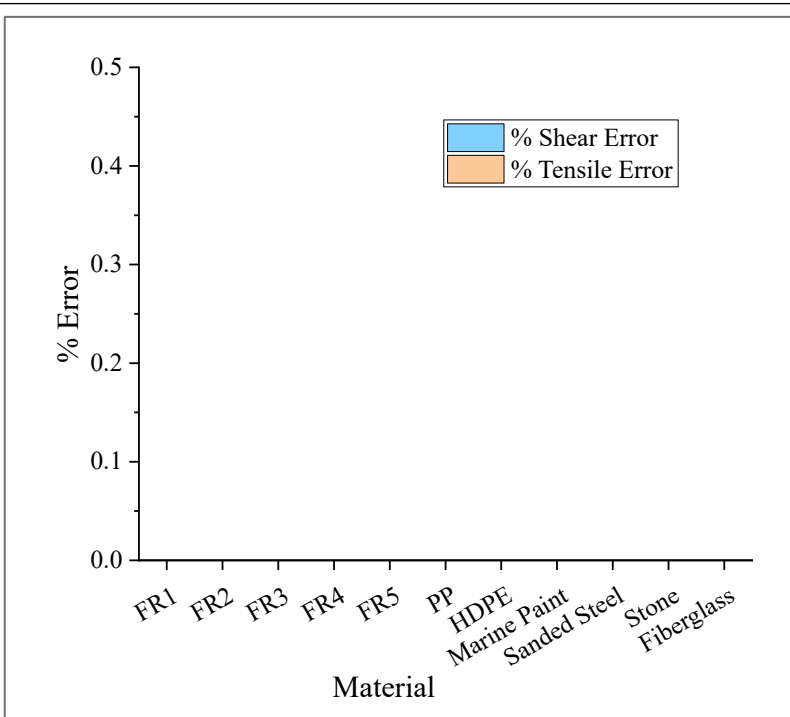


Figure 3.8. Comparison of percent error between ASTM Shear Method and proposed Tensile Method. Dashed lines indicate average percent error for each method. Samples FR1, FR4, FR5, and FR11 did not contribute to the shear error because they did not yield any results. The proposed tensile method (orange) offers a 4x reduction in error on average as compared to the ASTM standard (blue). FR = Foul Release coating

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4.0 Rapid, Quantitative Measurement of Marine Biofouling Using Staining and Digital Image Analysis

4.1 Abstract

Our research shows that the biofilm growth intensity (BGI) staining and visual photography methodology can be utilized as an inexpensive, objective, reproducible means to measure marine surface fouling. Environmental fouling of surfaces becomes problematic for surfaces deployed for any extended period of time. Quantitative measurement of the degree of biofouling, particularly in the early stages, is challenging to accurately measure using current, standard techniques. Herein we report a rapid quantitative measurement method for assessing marine biofouling using staining of the surface biofilm and subsequent digital image analysis of the sample.

Multiple samples, of variable material composition, surface roughness, and colors, were exposed to the marine environment for 1-28 weeks. Subsequently, the amount of fouling on each surface was determined by total organic carbon (TOC) and a novel method using staining and digital image analysis. The new approach, which harnesses digital averaging over large areas of the sample surface, provides repeatable results with relatively low variance. We found that the new method showed a consistent, positive correlation to TOC. Both TOC and the new method showed biofilms increasing as expected with exposure time, surface roughness, and nutrient levels. The impact on surface film biomass during a seasonal marine algal bloom was observed.

4.2 Introduction

Biofouling is a major problem associated with material usage in aquatic environments. Biofouling of surfaces increases microbial induced corrosion (MIC) [1, 2], degrades sensor performance [3, 4], increases the transfer of non-native organisms across vast distances [5], increases surface roughness of ships and other structures thereby increasing drag [6], structural stress and fuel consumption fuel [7].

Research on environmentally benign surfaces that are resistant to marine biofouling has intensified since the 2008 worldwide ban on TBT in antifouling paints. The ability to quantify surface fouling is key to guiding the development of better antifouling surface coatings as well as environmental studies of biofilms, biomedical studies [8, 9] and desalination membranes [10]. Advanced stages of surface fouling with macrobiota, such as: barnacle or tubeworm formation are easily observed, visually, however harder to see microbiota surface fouling can be detrimental to surfaces as well. Quantitative studies of young biofilms (without macrobiota) is much more challenging due to the limited mass and lack of clear observables but still very significant since the initial biofilm formation can create issues and may also seed subsequent macrofouling of the surface [11, 12]. These initial thin, soft biofilms layers can destroy sensor responses and have large costly impacts on big structures. For example, a soft, slime biofilm layer on a marine vessel results in a 10% increase in fuel consumption due to an increase in frictional drag. Effective methods to measure the early phase biofilms, also referred to as “soft-fouling” or slime layers, are needed. The method(s) need to be reproducible,

operator unbiased and quantifiable means to determine the extent of fouling on a surface.

The current, standard methods for biofilm and antifouling assessment is the ASTM D6990: Standard Practice for Evaluating Biofouling Resistance and Physical Performance of Marine Coating Systems when evaluating fouled surfaces and ASTM D3623 - 78a: Standard Test Method for Testing Antifouling Panels in Shallow Submergence [13] and . This ASTM standard explicitly states that its tools are designed for relative assessment and cannot be utilized as absolute indicators for antifouling materials. The ASTM standard determines a fouling rating (FR), physical deterioration rating (PDR) and a softness rating (SR) for each antifouling coating material. The FR is provided on a scale of 0-100 with 100 being a completely clean, fresh surface and 0 being completely fouled. While it is known that microfouling slime is a major contributor to increased drag for ocean ships, a 1% and 100% slime fouled surface would get the same FR 99 rating with the FR rating approaching 0 dependent upon the extent of calcareous macrofouling that is observed. The PDR follows the same 0-100 scale as the FR, but ignores all biofouling and looks at the percentage physical deterioration of the material surface where fouling does not occur. Additionally, the PDR incorporates the SR, which is a qualitative test based upon trace, slight or greater amounts of pigment removal of the surface by a cotton swab and can be hindered by operator bias. While the ASTM standard does not attempt to measure nor recognize the detrimental effects of micro/soft-fouling, the United States Navy technical manual standard (NSTM) for underwater inspection fouling devotes 40% of their fouling rating scale (FRS) to soft fouling [14, 15]. It has been shown that soft fouling can increase the resistance of a hull through the water from 9 to 20% and as such significantly increases the amount of fuel consumed to maintain a constant pace [15].

Both the ASTM and Naval standards are qualitative assessments of biofouling. As such, the testing leaves much room for subjective interpretation from the observer during both assessment and comparative analysis to positive/negative control samples. Potential biases during manual interpretation are compounded by the variability of testing controls due to external variables such as biomass loading. The fouling rates of samples may vary upon season, geographical location, conditions at test site and relative position/orientation of the samples therefore singular sample controls cannot be run for multiple sample sets when performing the qualitative testing standards rather than a quantitative test methodology. Additionally, relying on human subjects to assess fouling means that the fouling must be clearly visible and therefore current standards severely lack the ability to identify and quantify fouling in its early stages of formation.

Herein we investigate the utility of TOC (recently demonstrated for marine biofouling^{ref}) and a new method using staining of the surface biofilm and subsequent digital image analysis of the sample to provide rapid, quantitative measurements of early-stage, soft marine biofilms^{REF}. The new approach using digital image analysis of a stained surface produces a biofilm growth intensity (BGI) value as an objective and quantitative means to measure the extent of biofouling on a surface. The development of this new method based upon staining of the surface biofilm and subsequent digital image analysis to

generate a BGI value for the sample has been reported recently.^{ref} This BGI methodology is a test method that can be performed with little to no manual, subjective interpretation of results and can be utilized to rapidly quantify the biofouling of materials in varying conditions. A general review of the BGI method as applied to marine samples, including the digital image analysis method and specific stains selected for marine fouling is provided subsequently as part of the research discussion. Using this new method, as well as a more conventional TOC approach, we explore marine biofilm formation as a function of exposure time, material composition, and surface roughness.

4.3 Experimental Methods and materials

4.3.1 Sample Substrate Materials

Sample coupons were made of FR4 (fiberglass) or polypropylene material with dimension 3" square with 4-1/4" holes. Samples had a nominal surface area of 58.74 cm² or 9.104 in² after accounting for holes and edges. Tan (McMaster-Carr, Santa Fe Springs, CA), black, green and yellow-green (ePlastics, San Diego, CA) FR4 colored substrates and clear polypropylene (ePlastics) all with factory glossy and 180 grit (laboratory) sanded finishes were exposed during this study.

4.3.2 Sample Exposure and Handling

Sample coupons were placed in a continuous flow 500-gallon water tank. Seawater was pumped directly from Sequim Bay (Sequim, WA, USA) into test tanks at a rate of ~2.5 gal/min. Tanks were exposed to a diurnal fluorescent light cycle of 12 hours on and 12 hours off. Tank salinity was 32 parts per thousand (ppt), water temperature was 9.8°C or 0.2°C. Sample coupons were suspended ~12" below the surface of the water.

At the end of the predetermined exposure time, sample coupons were removed for staining or TOC analysis. Both testing methods are essentially destructive, as TOC testing required the fouling material to be removed from the coupon, while during the staining procedure an undeterminable amount of stain is retained by each sample coupon, preventing accurate TOC determination post-staining of the sample. Coupons were gently removed from the tanks with stain-directed coupons lifted and dipped in the sample tank 3 times to remove loosely associated fouling while TOC-directed coupons were lifted and dipped 3 times in 450 mL of Milli-Q water in a 600-mL beaker to remove and collect loosely associated fouling material and subsequently placed in an empty 600-mL beaker. All beakers utilized for TOC analysis were acid washed (10% HCl), rinsed 7 times using ddH₂O and ashed (overnight at 1000 °F).

4.3.3 Staining and Photographing Procedures

Trimix staining mixture (300 µL) was applied to the samples with a 1-mL micropipette in a dropwise fashion. The stain contained 0.1 g of erythrosine B (Thermo Fisher Scientific, Waltham, MA), 0.2 g KeyAcid Rhodamine (Keystone Analine Corporation, Chicago, IL), and 0.3 g Coomassie Brilliant Blue G-250 (Thermo Fisher Scientific). All were added to 100 mL phosphate buffered saline (PBS) at pH 7.4 and 0.1 M concentration. PBS 10X concentration is comprised of NaCl (80 g L⁻¹), KCl (2.0 g L⁻¹),

Na_2HPO_4 (14.4 g L⁻¹), and KH_2PO_4 (2.4 g L⁻¹) with each component purchased from ThermoFisher Scientific. Stain was allowed to sit on the coupon for 1 minute and then the samples were gently dipped in 0.45 μm -filtered seawater to remove excess stain. Samples were then imaged utilizing a Canon EOS 70D camera with Canon 100 mm L macro lens on a tripod, with 2 LimoStudio 500-watt equivalent, 5500K temperature LED lights operated at ~50% output. Images were shot under manual mode with $f/8.0$, 1/125 s exposure and ISO 320. Stained images were subsequently processed utilizing the digital fouling analysis program (described briefly below with full details in reference)[16]. Background blank non-fouled control coupons were stained in the same manner as the fouled coupons. After the photographs were taken coupons were discarded.

4.3.4 Digital Image Analysis

Digital image analysis was used to quantify fouling on each sample coupon. A previously reported algorithm was used to enhance contrast of major fouling and reduce quantitative information in each image to a single value that is representative of overall biofilm growth intensity (BGI) [16].

4.3.5 Stripping of organics from sample surfaces for TOC Analysis

A solution of 3% hydrogen peroxide (400 mL) was added to 600-mL beaker, with the fouled sample, to strip the fouled biomass from the sample surface. The 3% hydrogen peroxide solution was made by diluting 30% hydrogen peroxide BAKER ANALYZED® A.C.S. Reagent, (Stabilized), Item # JT2186-1 (VWR, Radnor, PA) with Milli-Q water (1:10). The final 3% hydrogen peroxide solution was tested and showed non-purgeable organic carbon (NPOC) concentration less than 2-3ppm. Low-level carbon reagents are required to accurately quantitate biofilm formation on surfaces using the Shimadzu TOC-L as a high background will limit the sensitivity of the assay. The fouled coupon in 3% hydrogen peroxide was incubated for 1 hour in 55°C water bath while the loose fouling samples proceeded directly to sonication. After 1-hour the samples were sonicated using a GE50 Ultrasonic Processor (Markson) with tapered 5mm probe for 6 pulses of 6 seconds each (3 on each side of coupon) at 100% amplitude. The sonicated samples were then aliquoted (30 mL samples) into 40-mL certified clean NPOC test vials (ThermoFisher Scientific) for TOC quantification.

4.3.6 TOC Measurement of Non-purgeable Organic Carbon Analysis (NPOC)

TOC quantification as a measure of NPOC was performed using the Shimadzu TOC-L (Shimadzu Scientific Instruments, Inc., Kyoto, Japan) with high salt Pt sample combustion tube kit and suspended sample tubing configuration. 50 ppm and 5 ppm potassium hydrogen phthalate, 3% H_2O_2 working stock, and Milli-Q were used as test controls to ensure proper functioning of the system and baseline subtraction of solvent organic carbon from the tested samples. All NPOC vials were loaded into the Shimadzu ASI-L auto sampler. NPOC settings were as follows: Acid Addition 1.5% HCl (Trace Metal Grade, ThermoFisher Scientific), Sparge time 2 minutes (Ultra-Pure Zero Grade Compressed Air, Central Welding Supply, Marysville, WA), 3 of 5 injections in multi-

injection mode with Coefficient of Variance Max of 2%. The resultant ppm value for each sample was corrected through subtraction of the control Milli-Q and H₂O₂ samples, and then divided by the sample volume (450 mL for loosely associated material and 400 mL for attached biofilm) to yield a solution ppm value. This value has also been converted to a $\mu\text{g}/\text{cm}^2$ value by dividing the ppm value by the sample coupon surface area (58.74 cm^2).

4.3.7 Sample Surface Roughness Measurements

Roughness was measured using a white light interferometric optical profiler (Contour Elite GT-I, Bruker Nano, Billerica, MA) in vertical scanning mode with a 2.5x objective lens. The profiler captured 3D images from three 1.9 by 2.5 mm areas. Root mean squared roughness (R_q) for each image was calculated using the profiler's accompanying Vision64 software. Surface roughness values were determined from triplicate measurements of samples.

4.4 Biofilm Growth Intensity Analysis Method

4.4.1 Quantified Biofouling with Digital Image Analysis of Stained Surfaces

The analytical process for determining the level of biofouling on a surface with digital image analysis of stained samples is shown in Figure 4.1.

After sample exposure the first step is to treat the surface with selected dyes. Dyes can be chosen to provide specific biological information or, as is used for this biofouling case a general indication of biological material presence. For determination of marine biofouling on surfaces we identified a mixture of stains, which provided the best results in quantitatively determining the percent coverage of a given substrate with biofilm. The stain mixture was a combination of Coomassie Brilliant Blue G-250 and Erythrosin B and KeyAcid™ Rhodamine WT.

- Coomassie G-250 is an anionic molecule that non-covalently interacts with proteins and free amino acids (mainly lysine and arginine) to form an easily visualized protein-dye complex. This stain allows visualization of proteinaceous biomass in biofilms.
- Erythrosin B is an acidic eosin vital dye in that it will only stain the interior of cells that have lost membrane integrity. If membrane integrity has been lost, the dye will stain the basic (non-acidic) components of the cell such as: the cytoplasm

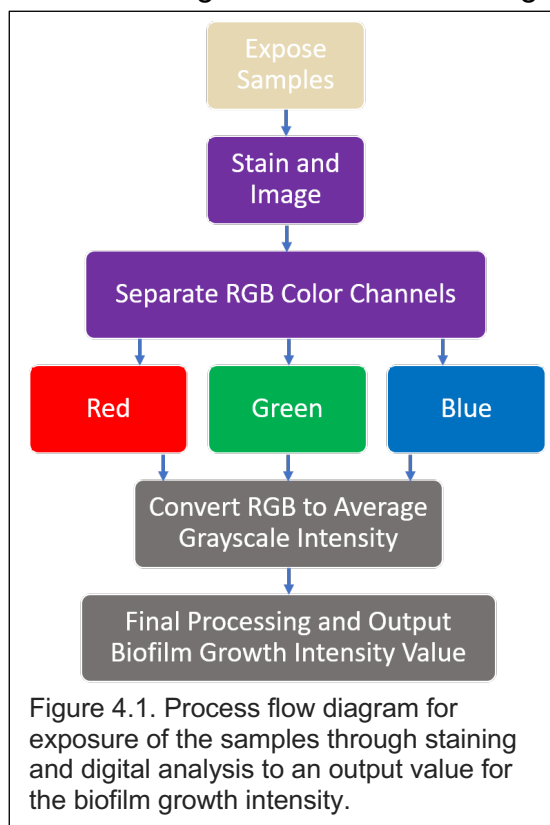


Figure 4.1. Process flow diagram for exposure of the samples through staining and digital analysis to an output value for the biofilm growth intensity.

and collagen fibers (prevalent in animals and cyanobacteria).

- Rhodamine WT is a xanthene-based, water tracer dye. Rhodamine is commonly used in microbiology as a dye complex with Auramine O for acid-fast bacilli identification and DNA staining. It has low binding to most inorganic and organic materials. As biofilms are 95% water, it is utilized in this mixture without Auramine O to primarily dye the biofilm-associated water.

By staining with the mixture of these three dyes we can quantitatively and generally determine the biofilm coverage of the sample due to fouling processes across a variety of surfaces more definitively than any of the 3 singly or in other combinations.

A picture of these stains separately on a marine biofilm is shown in [Figure 4.2](#). Pictures of the triple mixture of the 3 stains, as used for quantifying marine biofilms can be seen in [Figure 4.3](#).

Any digital image captured of a fouled surfaced could work for BGI analysis, however there are variables that could interfere with analysis which are:

- Lighting, we used multiple light sources to provide even illumination across the sample surfaces. Non-uniform lighting may introduce shadows or other artifacts that could alter the BGI value by mimicking dense biofouling;
- Lens, a high-quality macro lens was used for a life-size 1:1 reproduction ratio thereby minimizing subject magnification aberrations;
- Depth of field and stand-off distance, a macro lens typically has a short depth of field, but it is essential to have all sample fouling in focus so we used a large stand-off distance to both increase our depth of field and linearize light vectors from the sample thereby also reducing edge vignetting issues.
- Color accuracy; as the BGI toolkit uses RGB/grayscale conversion to determine a sample BGI value a blue square was included in each image for color calibration.



Figure 4.2. Image showing each of the stain components applied individually to strips of a fouled sample coupon. The three stains (From left to right, Erythrosin B [stains basic structures], Coomassie G-250 [stains proteins and amino acids] and Rhodamine WT [stains biofilm retained water]) each stain various different components of the biofilm, but can also each be used to determine a BGI value. The combination of the three allow greater sample surface variability (e.g., color variations) while maintaining quantifiability of the extent of fouling.

Other photography systems may be used for imaging, but care is needed to ensure production of an image that can be used for quantification. Typical images these days from cell phones, action cameras or point & shoot cameras use a high field of view or curvilinear lens, which unless special care is taken will yield an image in which the sample will have edge stretching effects (barrel or pincushion distortion) that may induce quantification variation. These curvilinear distortion issues can be mostly eliminated by switching the lens out for a rectilinear lens.

Analysis of the images of the stained sample surfaces proceeds according to a previously reported method [16]. This approach uses a biofilm growth intensity (BGI) algorithm that was developed to reduce the subjectivity in manual determination of biofilm coverage and implemented as a MATLAB® algorithm and determined to be effective for quantitative biofilm measurement. The BGI algorithm quantifies biofouling by algorithmically enhancing histograms of intensity for each color. After removing background, the histogram is stretched and divided into 10 threshold levels. The software splits the red, green, and blue intensity values from each RGB pixel into separate data sets. A fourth grayscale data set is also generated by taking a weighted average of the RGB channels. To preserve color contrast without perceptual information loss weighting was performed as by Sowmya.[17]. After a selected area is chosen manually the analysis program applies a multi-level threshold[18] with 10 thresholds (resulting in 11 bins). In essence, each of the pixel intensity values in the image is replaced by a value between zero and 10. The end result of image analysis is a single number for each color channel of each sample that is representative of total biomass. This value was named the Biofilm Growth Intensity (BGI) and is measured on a scale from 0 to 100. Note that BGI does not measure the percent of area covered by biofilm. A BGI value of 100 represents an image with 100% of pixel intensity values in the highest decile. Likewise, BGI of zero would be made up of pixels only in the lowest decile.

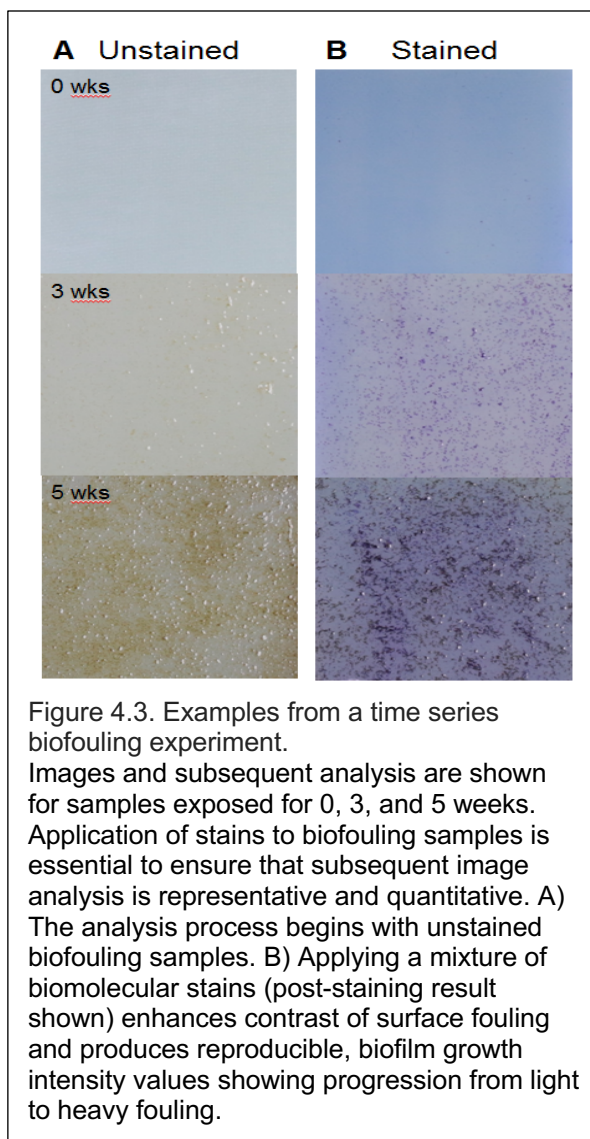


Figure 4.3. Examples from a time series biofouling experiment. Images and subsequent analysis are shown for samples exposed for 0, 3, and 5 weeks. Application of stains to biofouling samples is essential to ensure that subsequent image analysis is representative and quantitative. A) The analysis process begins with unstained biofouling samples. B) Applying a mixture of biomolecular stains (post-staining result shown) enhances contrast of surface fouling and produces reproducible, biofilm growth intensity values showing progression from light to heavy fouling.

4.5 Results and Discussion

In **Table 4.1**, the average BGI values for several samples are shown. Analysis of unstained coupons provided unrealistic results suggesting that surface fouling declined over time even though the initial sample is a time zero blank. The stained samples, however, trend with expected surface biomass loading with BGI values increasing with time. This trend is as expected for biofilms growing on newly exposed surfaces in a marine environment. The BGI value progression, over the exposure timeline, indicates that we can quantitatively determine low levels of soft fouling on a surface.

4.5.1 Comparative TOC and Digital Measurement for Marine Biofouling

One method recently demonstrated for quantifying early biofilm formation on a surface is total organic carbon (TOC) or Non-purgeable Organic Carbon Analysis (NPOC). The TOC method extract the organics from the surface of the sample and subsequently measure the amount of carbon released during heating at 680 °C. TOC analysis is an established quantitative, analytical method for measuring organic carbon in soils. Herein it was investigated A) how well results from the new staining and digital image analysis correlated to TOC measurements of marine biofilms and B) utility of TOC measurements as a means to calibrate the BGI output.

Table 4.1. Average grayscale biofilm growth intensity (BGI).

BGI values for samples shown in Figure 4.1 at selected times (0, 3 and 5 weeks). The unstained samples yielded equivalent (within 1 std. dev.) BGI values at each time point, while the stained samples showed a logical increase in biofilm intensity (ala growth) on the surface over time.

Marine Exposure Time (weeks)	Unstained Sample	Stained Sample
	Average $\pm$ std. dev.	Average $\pm$ std. dev.
0	50 $\pm$ 1.5	15 $\pm$ 2.0
3	50 $\pm$ 10	27 $\pm$ 5.0
5	43 $\pm$ 2.3	44 $\pm$ 4.4

To test the new BGI toolkit ability to measure biofouling, comparative test suite of samples were exposed to seawater for a period of up to 199 days. Samples were exposed in sets of 6 with 2 coupons undergoing BGI analysis (front and back for 4 total sides) and 4 undergoing TOC analyses. TOC analysis is destructive and the stain application for BGI analysis to samples inherently adds an unknown and variable quantity of carbon, as such the sample pathways were exclusive, and analyses could not be performed on identical samples, but rather samples exposed and tested in parallel.

Both the TOC and the BGI methods show biofilm growth on the surface progressing over the ~28 weeks of exposure. Biofilm formation followed an expected path of settling followed by an initial rapid growth rate that slows as the film matures and full coverage

of the surface is achieved [19, 20]. As can be easily observed in [Figure 4.4](#) the two analytical methods clearly correlate very well.

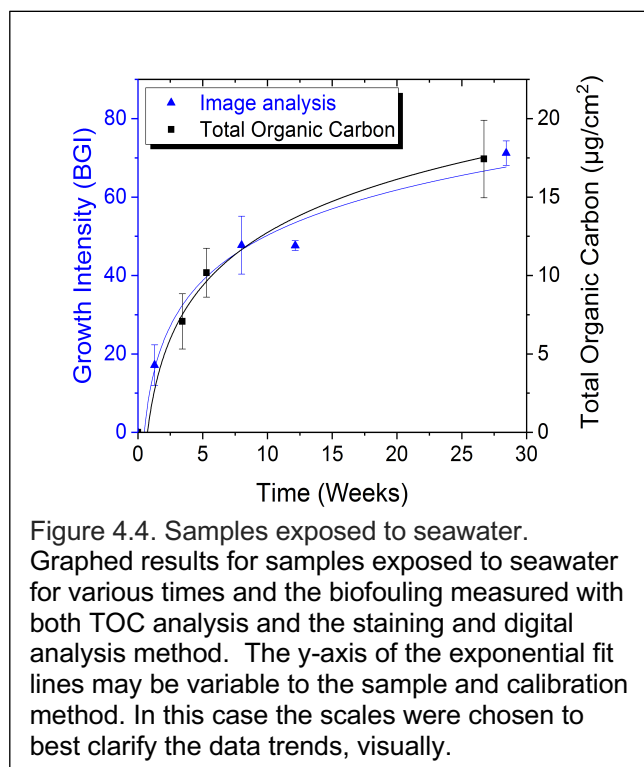
The data in [Figure 4.4](#) clearly show that early biofilm formation can be measured with TOC or staining with digital analysis of stained samples and the 2 methods provide highly correlated results. The stain digital assay BGI method can rapidly and simply provide very sensitive quantitative biofilm mass loadings from the BGI parameter if the system is calibrated against a quantifiable method such as TOC.

4.5.2 Impact of Surface Roughness on Marine Biofouling as Measured by BGI

Shown to be a useful means to both quantitate and visual surface fouling, the stain and digital assay methodology utility was further tested to assess marine biofouling on substrates with different surface roughness values. Sample surfaces were roughened with different grit sandpaper (80-220), optically measured to determine surface roughness ([Table 4.2](#)) and directly compared to each other and the factory finish (hereafter noted as 0-grit as the factory glossy or “smooth” finish). These factory finish and roughened samples were exposed in the low flow velocity tanks for 6 weeks.

After six-weeks of exposure, biofouling had visually occurred on each of the samples ([Figure 4.5](#)). The 120- and 180-grit coupons qualitatively had the most surface fouling, while the 0-, 80- and 220-grit samples had subjectively similar degrees of fouling. This qualitative assessment of the surface fouling mostly agrees to the quantitative values determined using the staining and digital assay analysis ([Table 4.2](#)) except for the 220-grit sample, which actually had a BGI value similar (~2% different) to that of the 180-grit sample.

The six-week coupon fouling results indicated a relatively low amount of surface fouling from ~2-9 on the BGI scale, with the 180- and 220-grit sanded surfaces showing ~300% more fouling than the glossy (0-grit), and 80-grit sanded surfaces, and ~80% more fouling than the 120-grit surface (RGB values, grayscale BGI 220-grit was similar to 180-grit). To further understand the biofouling results, the surface roughness of the sample, prior to exposure, are compared to the BGI results ([Table 4.2](#)). Results indicate that the smoothest surface was the factory finish, glossy samples while the roughest surface was that of the 80-grit sandpaper modified sample. The sample with the greatest fouling was the 180-grit treated sample, but it was the second smoothest



sample tested during this research. It has previously been indicated that biofouling is greatest on materials on which the surface features (or R_q) is most closely aligned with the physical size of the settling organism [21, 22]. Experiments showed reduced surface fouling of the samples at R_q values above $\sim 2.0 \mu\text{m}$, which may indicate primary surface fouling by bacteria and a dependence upon those micro-organisms for settling of larger organisms, such as an alga. As the 180-grit sanded sample surfaces showed the greatest fouling when compared to the other surface treatments at 6-weeks, the 180-grit treatment was applied to all other biofouling samples.

Impact of substrate color

The staining and digital assay method is based on color intensity assay of the stained biofilm and impact of substrate color on the analytical results was explored. To assess the robustness of the method to substrate color the digital assay multiple color samples (white, tan, green, black) were exposed over a 2-month period. Each sample set (4 for TOC analysis and 2 for BGI analysis-with front and back measured for BGI) were exposed and then analyzed and the results shown in Figure 8. In general, the stain and digital assay method generated BGI values similar in relative intensity to the TOC numbers for lighter colors (tan, white, and to a lesser extent green) as seen in previous tests (Figure 4?). This did not hold true for the black sample, for which the BGI value indicated significant biofouling with a value of ~ 90 , but the TOC concentration was closer to that of the other samples (which had BGI values of ~ 20). As the BGI algorithm is designed to detect/enhance the intensity of color on a surface and the darker (black) backgrounds preclude accurate measurement of color intensity measurement of the stained biofilm and prevents accurate determination of a BGI values. In summary, the stain and digital assay method can be used on light and moderately colored substrates to enable effective determination of color density of the stained biofilms and as such quantify biofouling. The methods, as

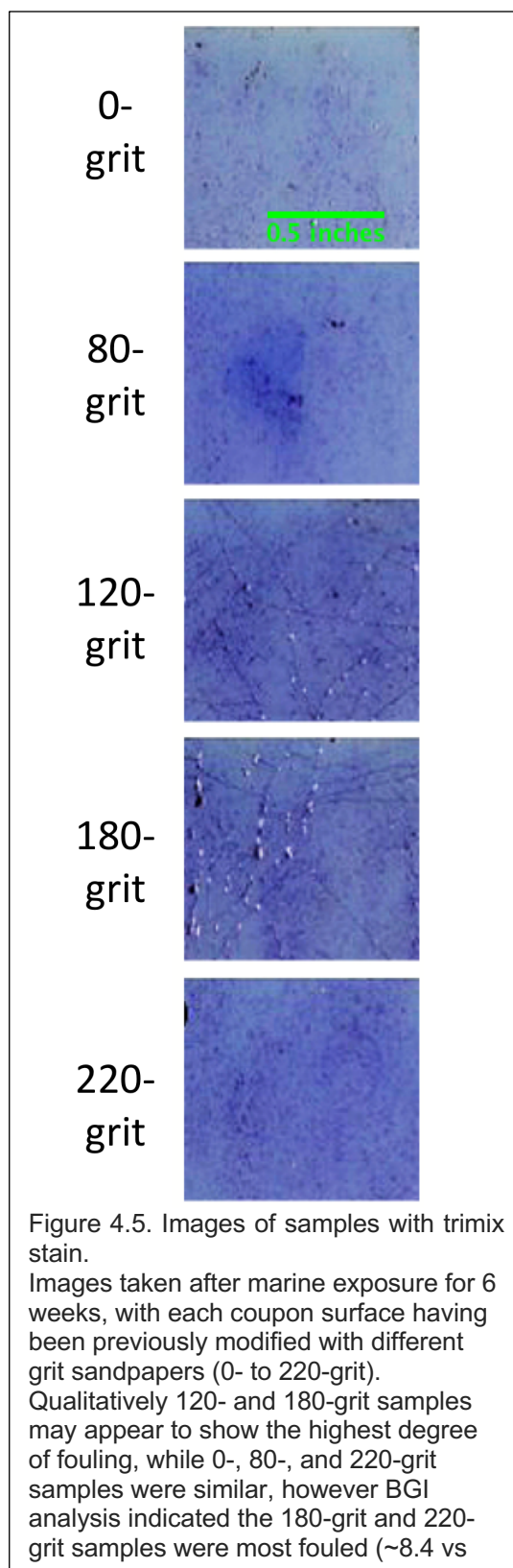


Figure 4.5. Images of samples with trimix stain.

Images taken after marine exposure for 6 weeks, with each coupon surface having been previously modified with different grit sandpapers (0- to 220-grit). Qualitatively 120- and 180-grit samples may appear to show the highest degree of fouling, while 0-, 80-, and 220-grit samples were similar, however BGI analysis indicated the 180-grit and 220-grit samples were most fouled (~ 8.4 vs

reported here become inaccurate on very dark or black substrates. Anticipating the problems with dark background issues the Trimix incorporates the fluorescent dye, Rhodamine WT, which will be tested in follow-on work for its ability to more accurately assess fouling on dark surfaces (using fluorescence) and expand the applicability of the method.

4.5.3 Measuring Impact of Marine Conditions on Surface Biofilm Loading

Samples coupons, with roughened surfaces (180-grit sandpaper) to encourage biofouling were immersed in seawater to explore long term biofilm growth. The unexpected results of biofilm loading as over time are shown in [Figure 4.7](#). The initial fouling of the samples followed the same trend previously observed ([Figures 4.3, 4.4 and 4.5](#)) with slow initial growth (BGI values ~2-10) in the first weeks of exposure. After this initial conditioning and colonization phase, the surface mass of the biofouling was observed to rapidly increase, as expected, around the 5-week exposure time. Unexpectedly, after 8 weeks of exposure, the samples showed an 81% reduction in the surface biofouling with values dropping to those similar to week-1 measurements. This sudden drop in surface biofouling is hypothesized to be a result of an algal bloom that is known to have occurred between weeks 4-6 of the experiment shown in [Figure 4.7](#). Clearly the algal bloom event impacted the biofilm. Algal blooms have previously been shown to affect the local marine environment by using available carbon/nitrogen resources, depleting the dissolved oxygen, increasing turbidity resulting in a decrease in light penetration, and production of harmful acidic compounds (e.g., domoic acid). The bloom effectively stopped the exponential growth of the biofilm and ultimately reduced the surface biomass. Any one or a combination of the above algal bloom effects could have caused the reduction in biofouling, by physical/chemical removal of the fouling or by causing planktonic release of the attached organisms in search for an environment more conducive to growth and propagation. Clearly, the stain assay and digital BGI analysis provides a convenient method to quantitatively measure biofilm response to large scale marine events such as an algal bloom. This indicates the toolkit could prove useful during investigations into events associated with oceanic change such as pollution plumes, sea water warming and acidification, changes in salinity, and other effects.

4.6 Conclusions

Biofouling of surfaces becomes problematic for surfaces deployed for any extended period of time and accurate measurement is needed for; assessment of industrial systems and installations (e.g., shipping and offshore platforms), guidance in the development of new antifouling coatings, and environmental research and monitoring. Quantitative measurement of the degree of biofouling, particularly in the early stages is challenging to accurately measure utilizing current standard techniques. In this work, we have shown that a rationally selected set of stains combined with simple photography of the sample and subsequent digital image analysis can provide an effective, rapid means to quantitatively assay the early stages marine biofouling. The method is relatively inexpensive, independent of user bias, fast, and reproducible. Our

research shows that the staining and digital analysis methodology can be utilized as an inexpensive, objective, reproducible means to measure marine surface fouling. This new BGI method was shown to correlate well with the more conventional TOC method. This stain and digital analysis methods works largely independent of the substrate background color except cases with very dark or black materials. Future work will explore using fluorescent imaging to acquire BGI values for very dark samples. The new method was shown to be effective on surfaces of various roughness with increased marine biofouling increasing with surface roughness as hypothesized. Finally, the impact of macroscale marine event, an algal bloom, and its impact on biofilm mass, was observed for an established biofilm with a steep reduction (81%) in surface fouling observed during and after the bloom. Future work on with this analytical technique will include optimization of the analysis algorithms, application of the method to assess antifouling coatings, using fluorescent stains to enable accurate biofilm measurement on dark substrates and development of customized stain mixtures for specialized applications.

4.7 Acknowledgments

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5.0 Crevice Corrosion In Seawater Using CFRP As Part Of The Crevice Former

5.1 Abstract

Marine Hydrokinetic (MHK) devices for renewable energy power generation are fully or partially immersed in seawater during service. Advances in MHK designs are being made to incorporate composites and a variety of alloys. Composites of interest include carbon fiber reinforced polymer (CFRP) composites, fiber glass reinforced polymer (GFRP) composites and hybrid composites with both fiber types. In some cases, the composites are fastened using metallic hardware. These fastened composites can cause tight occluded regions that later could become crevice corrosion on the fastened metal regardless of the type of composite. To understand the performance of these fastened systems, a modified crevice former was used to investigate crevice corrosion for fastened samples immersed in seawater using CFRP and hybrid plates. Three alloys were investigated: 316 stainless steel, monel and Titanium alloy. Fastened set specimens were immersed in seawater at room temperature and others were immerse in elevated temperature seawater. Selected samples were removed and dis-assembled for visual examination after more than 270 days (up to 740 days). The immersion time lasted longer than what is typically reported. Crevice corrosion was found on the stainless steel and monel hardware, and corrosion extent increased with time. Full details will be discussed. Appropriate joining technologies are required with respect to mechanical behavior and corrosion resistance.

5.2 Introduction

Fiber reinforced polymeric composites are being considered for marine renewable energy devices, also known as marine hydrokinetic devices. It is expected that composites will be joined through various methods that include adhesives and metallic interconnects (fasteners). Environmental effects on these features must be better understood. Galvanic corrosion on the alloy used in the interconnect could occur when carbon fiber reinforced polymer composites are used. Galvanic coupling is typically not a problem for glass fiber polymer composites.

Metal-to-composite joints are of interest with regards to marine hydrokinetic devices and other marine devices. In the case where the joints are bolted (fastened), localized corrosion could develop in the occluded areas via pitting and/or crevice corrosion, regardless of the type of composite. Carbon fiber reinforced polymer composites might enhance crevice corrosion as it is nobler than most metal alloys. The solution within the crevice gap (i.e., the space between the composite and bolted hardware) can change with time (e.g., as time passes the solution can become depleted of oxygen, become more concentrated and with pH changes) and as a function of the distance of the crevice mouth. After the incubation time, corrosion can initiate and propagate. Once corrosion has propagated for some time (with significant section loss), mechanical degradation could also occur. A better understanding of the conditions that could cause localized corrosion of bolted metal/composite joints is desirable. This study used a modified crevice former in which the composite was the plate.

Many alloys corrode when exposed in seawater, with some of these alloys still used but coated and with cathodic protection employed. In other cases, more corrosion resistant alloys are selected. Some of these more corrosion resistant alloys sometimes suffer localized corrosion (pitting, crevice corrosion).

5.2.1 Composite coupled to metals

Early studies [references] of graphite epoxy composites coupled to a variety of alloys dates the to 1970s. Most of these studies investigated the galvanic coupling of carbon fiber reinforced polymer composites to a variety of alloys, and recognized that galvanic corrosion of less corrosion resistant alloys occurs. Later, several investigations were carried out during the 1990s, looking at galvanic coupling of carbon fiber composites and metals for marine ship applications. [1-5] Blisters were found in some situations and it was found that the interconnection accelerated the corrosion of some alloys. A study sponsored by the NAVY [Ref] found that calcareous deposits formed when CF composited were coupled with steel alloys, for samples immersed in seawater. In some of the past studies, a sodium chloride solution was used instead of seawater, and some of the studies used a small solution volume.

Miller and Lee investigated the galvanic coupling of four different graphite-epoxy composites materials to 23 alloys immersed in 3.5% NaCl. The galvanic current was monitored over time using a zero resistance ammeter (ZRA). Ranking was based on 24 hour monitoring of the galvanic current and of coupled potential. Ti alloys and Ni based alloys were found to be compatible. Miller and Lee also included a review of the earlier studies investigating galvanic coupling of graphite-epoxy composites to alloys. Tucker and collaborators [Ref] have previously reported studies in which CFRP were coupled to different alloys. In some of the studies they found blisters on the composites (epoxy matrix) as a result of the galvanic coupling, for composites coupled to alloys with potentials more negative than -0.75 V_{sce} when immersed in seawater.

5.2.2 Crevice Corrosion

There are monographs and many journal publications that describe what crevice corrosion is, the reader is referred to NACE monograph as one example [1]. A recent review by Iannuzzi [2] describes crevice corrosion and the different methodologies that are used to investigate it, including most common standards. The reader is referred to this publication [Ref] and [Ref Scully Chapter 19 ASTM Manual20] for more details. Iannuzzi [2] notes that "... crevice corrosion resistance is not a property of a material. In this regard, the response of a particular alloy system to a given environment determines the crevice corrosion susceptibility." In addition, the geometry of the occluded region dictates if crevice corrosion initiates and if it remains active. The materials in contact (i.e., another metal, polymer) with the alloy, also influences crevice corrosion susceptibility. The gap (opening) have been found to range between 0.1 and 100 μ m. In a real crevice, the gap is not a fixed value. Iannuzzi [2] indicates that: "the gap has to be wide enough to allow the ingress of the solution, but sufficiently tight to promote the formation of an occluded environment within the cavity." Ross [3] indicates that galvanic

and crevice corrosion are critical conditions that need to be considered when selecting fastener alloys for seawater immersion.

5.2.3 Crevice corrosion in seawater

The CREVCORR program [Ref] developed a methodology to investigate crevice corrosion of stainless steel (and other alloys) when immersed in seawater. There were several findings. For example, it proposes the application of a constant pressure (force) by spring loading on the materials to be used as crevice former instead of using a certain torque. The study also recommends polyvinylidene fluoride (PVDF) as the material for the crevice former (considered the most versatile material). Other methods have been reviewed by Oldfield [1] and Kain[2].

5.2.4 Crevice corrosion and composites

The polymeric matrix in a composite is a known material that can cause crevice corrosion. Prince [Ref] concluded from salt spray (5% NaCl) tests conducted on fasteners in contact with graphite-epoxy composites that Ti-6Al-4V and multiphase MP35N were highly resistant to attack in the tested environment, other tested alloys were found to corrode.

CFRP composites are being explored for automobile applications [Ref]. Over the last decade several studies have used overlapping plates (composite and metal) with various interconnect modes and material for fastening the samples. Crevice corrosion was found on a number of cases.

In one of Tucker's studies [Ref], slots were machined into the composite to accommodate the metal samples, these samples were then immersed in seawater. An interference fit was machined, as to ensure good contact with the metal. This geometry resulted in crevice corrosion on monel, stainless steel and un-coated Al-2014 coupled composites. Un-coated Al-2024 caused blisters in the CFRP.

As indicated in the introduction, one of the methods being considered is to fasten metal alloy hardware and composites. Hence, fastener selection extends to cases in which a composite is involved as part of the structure. Ross includes a table to guide fastener selection when the plate is graphite composite (carbon fiber reinforced polymer composite). This galvanic combination may be harmful to corrosion-resistant nickel-base alloys, such as alloys 625, 725, C-276, 59, 686, and MP35N. This might be the reason that Ross [Ref] gave a precautionary rating to Ni-Cr-Mo alloys. Note that considerable caution is advised when using any fastener alloy with graphite composites due to lack of reported experience with this system. Ross proposed to consider XX, XX, XX, but that additional testing is needed to verify good performance.

5.3 EXPERIMENTAL SECTION

5.3.1 Carbon Fiber Reinforced Plastic Composite

T700 carbon fiber with FOE sizing (supplied by Toray Inc.) was used as the fiber reinforcement. Two types of vinyl ester resins were used; Derakane 8084 (VE8084) and Derakane 510A-40 (VE510A) (supplied by Ashland Inc.). The VE8084 resin was formulated with 1.5 phr of methylethenate peroxide (MEKP), 0.18 phr of cobalt naphthenate -10% (CoNap) and 0.05 phr of dimethylaniline (DMA), and VE 510A was formulated with 1.0 phr of MEKP, 0.20 phr of CoNap -10 %. These formulations allow 50 to 60 minutes of gel time. The properties of these materials provided by the manufacturers are listed in Table 5.1.

Table 5.1 Manufacturer's specifications for fiber and matrix

Specifications	Fiber	VE8084	VE510A
Density (g/cm ³)	1.8	1.02	1.23
Tensile Modulus(GPa)	230	2.9	3.4
Tensile Strength, MPa	4900	76	86
Strain to Failure, %	2.1	-	-
Flexural Modulus, GPa	-	3.3	3.6
Flexural Strength, MPa	-	130	150

Unidirectional composite panels, consisting of 6-8 plies, were prepared from T700/VE8084 and T700/VE510A using vacuum assisted resin transfer molding (VARTM). Each panel was cured at room temperature for 24 hours before removing the cover. The dimension of the panel was 80 cm x 80 cm, with 1.8 mm thickness. The panels were prepared in 2009 and were not post cured, but continue to cure at room temperature. Specimens 7.5 cm x 7.5 cm were cut off from the panels using a wet saw with a diamond blade during the summer of 2018, 36 specimens were prepared. A 9.4 mm diameter hole was drilled at the center of the specimen. A carbide brad-point drill bit was used to make the hole. This type of bit helps to protect the drilled area from fraying or delaminating of this type of material and also eliminates splintering on both sides of the hole. Through this hole the bolt was fastened, snug fit i.e., the interface fit tight.

5.3.2 Hybrid composite reinforced plastic composite

A hybrid composite sample containing carbon fiber and glass fiber was provided [David Report]. The hybrid composite sample was 0.3" thick, 5.8" long and 1.375" wide. Specimens 1.375" x 1.375" were obtained from the sample using the wet saw. A 9.4 hole was drilled at the center on each specimen using the procedure described above. Four specimens were obtained.

5.3.3 Fastener set materials

Commercially available bolts, nuts and washers were used. Nominal 316 SS, Monel (400?) and commercial Ti alloy were used to prepare the samples. All fastener materials

were acquired from a specialty hardware store. The alloys are commercial grade. The fasteners were used as is without any modification, except for rinsing with water the different components. Three types of metal alloys were selected: titanium, Monel and 316 stainless steel. The specifications of them are as follows:

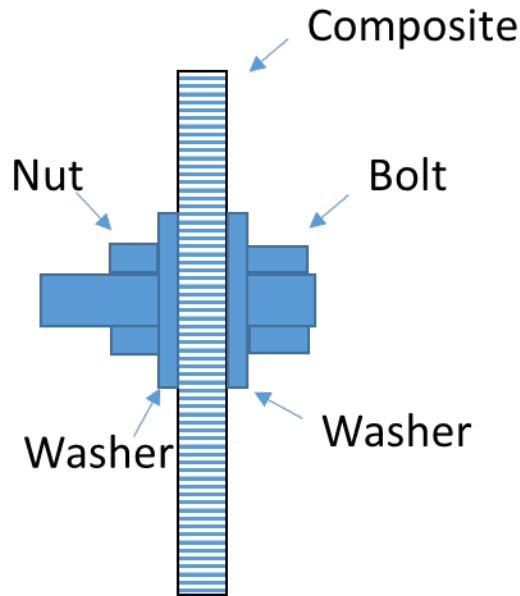
- 316 Stainless Steel fastener set: square-head, cup-point, set bolts, (ASME B18.6.2). Thread spacing: coarse. Case hardened. Thread size: 3/8"-16, 1" long, with a matching 316 stainless steel thin hex nut (width: 9/16", height: 7/32"). Standard 316 stainless steel type A flat washers: ID: 0.375", OD: 0.875", thickness: 0.07". The fastener set, as received, comes with light protective oil which was not removed.
- Nickel alloy fastener set: hex head screws, Thread size: 3/8"-16, 1" long. Thread spacing: coarse. Material: 400 nickel (Monel), with a matching Monel hex nut (width: 9/16", height: 21/64"). Monel flat washer: ID: 0.39", OD: 1", thickness: 0.06".
- Titanium fastener set: grade 5 titanium hex head screws, Thread Size: 3/8"-16, 1" long. Thread spacing: coarse. 40% lighter than medium-strength steel screws, with a matching hex nut, Grade 2 Titanium (width: 9/16", Height: 21/64"). Titanium flat washer: ID: 0.406", OD: 1", thickness 0.06".

5.3.4 Specimens assembly

A modified crevice former was designed. The composite plate was sandwiched between two washers, a nut and a bolt. The metallic components were fastened on each composite specimen through the drilled hole, as shown in Figure 5.1. The composite smooth surface was always placed on the bolt side, with the nut placed on the rough composite surface. When the fasteners were assembled attention was not always paid to use the washer in the right direction, however in most instances the sharper edge side faced the surface to be clamped against the composite. The rounded side typically faced the nut or bolt. All fastenings were performed at a torque of 20 lb-in (2.26 N.m) (2.825N.m=25 lb-in). Assembled specimens were immersed in a quiescent seawater (Atlantic Ocean, Dania Beach, FL, USA). A set of samples was immersed in seawater at room temperature and another set was immersed in a tank at 100 °F. Table 5.2 lists the number of specimens immersed on each tank and fastener set type. (RT identifies the specimens immersed in seawater at room temperature, ET identifies the specimens immersed in seawater at elevated temperature).

Table 5.2 Number of specimens prepared indicating immersion tank

	CF/VE RT	CF/VE ET	Hybrid ET
Stainless Steel	6	6	2
Monel	6	6	1
Ti alloy	6	6	1



Crevice assembly using composite as the plate

Figure 5.1 Diagram illustrating an assembled specimen.

5.3.5 Location where crevice corrosion could develop

The specimen as shown in Figure 1 can lead to the following crevice geometries:

- C1: Between the washer/smooth side of the carbon fiber
- C2: Between the washer/rough side of the carbon fiber
- C3: Between the nut and the washer
- C4: Between the screw head and the washer
- C5: Between the threads of the screw and the matching threads of the nut.

These tight occluded regions are sites prone for crevice corrosion to occur. In the results section the focus is on C1 and C2 geometries, as it corresponds to the composite to metal contact.

5.3.6 Seawater

Two seawater tanks were set up to hold the samples. Both tanks were filled with seawater. One tank contains a heater and a controller, the temperature was set to 100F, whereas the seawater in the other tank remained at room temperature (RT). (add the dimension of the tanks)

5.3.7 Periodic visual inspection.

The specimens were momentarily removed from the immersion tanks after 55, 98, 197 and 273 days of immersion. The samples were inspected, images taken, and then returned to immersion in seawater. Selected images after 56 days and 273 days are

included in the results section. Images were taken on the remaining samples after 600+ days of immersion.

Visual inspection took place at 55, 98, 197, 273, and 600+ days. The samples were briefly removed from immersion, the pictures taken and then return for immersion.

5.3.8 Forensic analysis.

Selected samples were removed from immersion and dis-assembled for visual inspection starting at day 273 and the most recent terminated sample was immersed for 740 days. In most instances a couple of wrenches were needed to remove the nut. In a couple of instances it was possible to unscrew the nut by hand. Table 5.3 lists the number of specimens dis-assembled and Table 5.4 lists the immersion time in days at which selected specimens were dis-assembled. The remaining samples continue exposure immersed in the seawater solution. No special cleaning was done on the metal fastener set materials after dis-assembling.

Pictures were taken upon disassembling each dis-assembled specimen. Additional images were taken using a stereo microscope. A few of the washers were observed using an E-SEM. The surface observed on the E-SEM was the side in contact with the composite surface.

Table 5.3 Number of samples dis-assembled

	CF/VE RT	CF/VE ET	Hybrid ET
Stainless Steel	2	4	2
Monel	2	4	1
Ti alloy	2	2	1

Table 5.4 Number of days at which samples were removed from immersion and dis-assembled

	CF/VE RT	CF/VE ET	Hybrid ET
Stainless Steel	302, 365	273, 323, 652, 729	316, 708
Monel	309, 365	288, 330, 659, 739	704
Ti alloy	330, 448	316, 448	713

Pictures were taken upon disassembling each terminated sample. Additional images were taken using a stereo microscope. A few of the washers were observed using an E-SEM. The surface chosen was the side in contact with the composite surface. No special cleaning was done on the metal hardware used to create the fastener set with the composite sandwiched in between.

5.3.9 SEM

Selected samples were inspected using an environmental SEM Model Quanta XXX.

5.3.10 Visual Inspection prior to dis-assembly

This section describes period visual inspection that took place. The specimens were removed from immersion and pictures were taken. Figure 5.2 shows images of three specimens with stainless steel fastener set after 55 days of immersion, and Figure 5.3 shows images of the same three specimens after 273 days of immersion in seawater at room temperature. After 55 days, the stainless steel hardware of the three specimens show corrosion products leaching from the washer, there is some rust on the washer and bolt surfaces in contact with the seawater. After 273 days, rust corrosion products stained a larger area of the composite. Figure 5.4 show two images of the remaining specimens after 645 days of immersion. The corrosion products show a modest build-up with time when compared to those observed after 273 days.

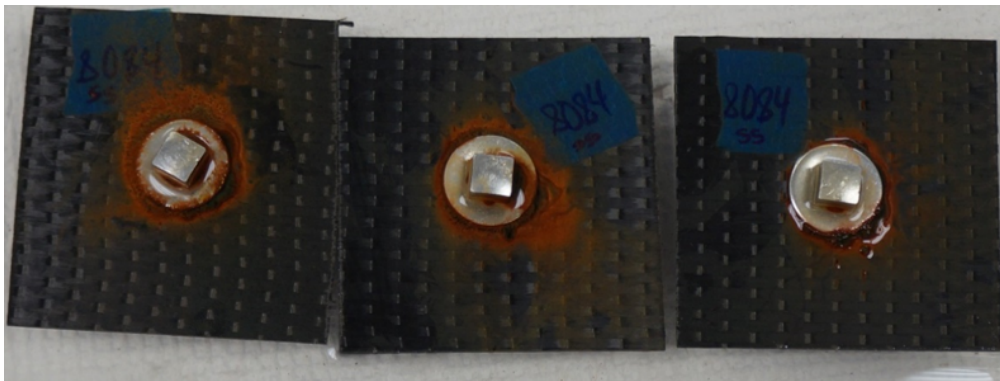


Figure 5.2 Room temperature exposed SS specimens after 55 days of immersion.

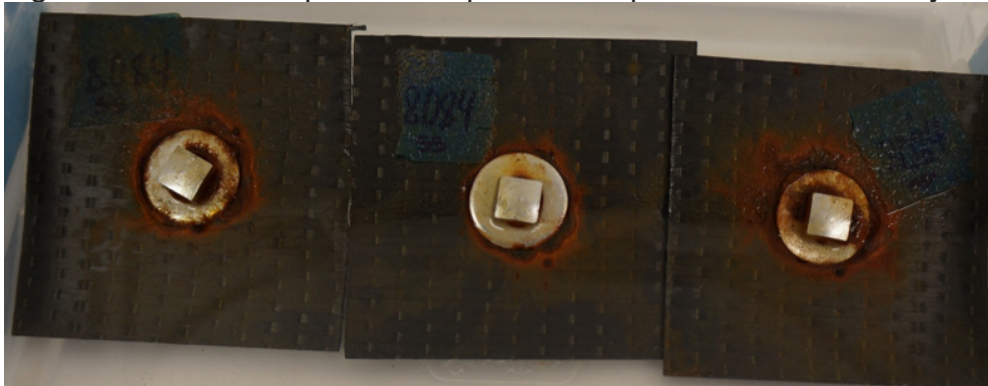


Figure 5.3 Room temperature exposed SS specimens after 273 days of immersion.

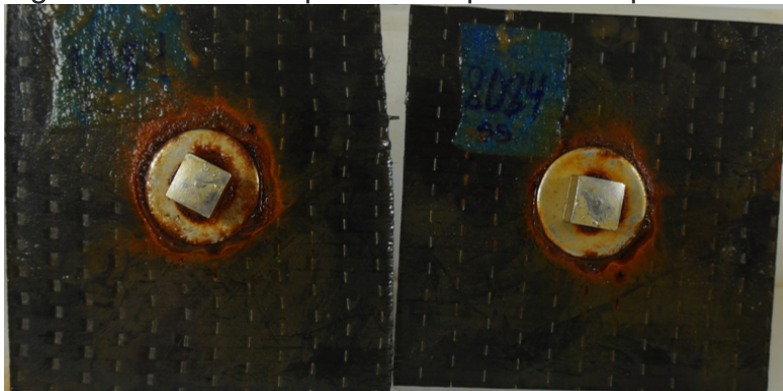


Figure 5.4 Room temperature exposed SS specimens after 645 days of immersion.

Figure 5.5 shows the surface condition after 273 for three specimens with stainless steel fastener set for the bolt side. Note that a large portion of the composite surface is covered with calcareous deposits. The calcareous deposits also formed on the specimens with Monel fasteners immersed in seawater at elevated temperature (Figure 5.6). The calcareous deposits were not visible after 55 days (not shown in here).



Figure 5.5 Elevated temperature exposed SS/CF specimens after 273 days of immersion



Figure 5.6 Elevated temperature exposed Monel/CF specimens after 273 days of immersion

5.3.11 Washers Visual Inspection (after dis-assembling)

Room Temperature Specimens. This section presents images of the washers after dis-assembling specimens exposed immersed in the room temperature seawater tank. Many of the images were taken several months after taking apart the specimens. Figure 5.7 presents the washers exterior surface for the specimens prepared with stainless steel fastener set. The images on the top row show indentations left by the bolt. The images in the bottom row show the washer exterior surface in contact with the nut, the nut produced minor markings. Some red-rust staining is observed in all four washers.

Note that the images present two washer flat surface sides and two washer round surface sides. Figure 5.8 shows images of the stainless steel washer surfaces in contact with the composite. The images in the top row show washers with significant crevice corrosion. The image on the top left shows that about 25 to 30 percent of the original surface remains, the non-corroding area forms an outer ring and a thin inner ring is also observed. This observation might suggest that crevice corrosion initiated a certain distance from the inner and outer radii; and then extended. The top right picture shows that there is almost no original surface left (except for an arch section on the bottom). The two images on the bottom row show a different corrosion morphology. The image on the bottom-left shows three corrosion spots but they represent a small portion of the total surface. The bottom right image shows one large corrosion site. The interior side (surface in contact with the composite) of the four washers experienced crevice corrosion.



Figure 5.7 SS-RT washers exterior surface



Figure 5.8 Washers SS-RT interior surface

Washers close-up SS-RT. Figure 5.9 shows two close-up images that correspond to two washers of a SS-RT specimen terminated September 2019. The image on the left is a close-up of the washer surface in contact with the smooth composite surface. Close to the outer radius there is an area with no section loss, with most of the washer showing crevice corrosion (cross section loss). The figure on the right shows a large corrosion spot for the washer in contact with the composite rough surface. There are regions of various depths (i.e., not uniform corrosion) on the corrosion spot. Both images suggest that most of the corrosion products leached out, except for a few red-rust or black spots.



Figure 5.9. RT-SS Washer surface in contact with composite: left smooth side and right rougher side.

Figure 5.10 presents four Monel washer images, the top two correspond to the external washer surface in contact with bolt, and the bottom two images correspond to the washer surfaces in contact with the nut. Both the bolt and the nut left markings on the washers, the markings left by the nuts were more pronounced. All four washers show green (different tonalities) corrosion products, but only the washer image on the left bottom showed significant amounts. All images were taken several months after disassembling the specimens.

Figure 5.11 shows the Monel washer surfaces (interior) in contact with the composite surface. The two columns on the left correspond to images taken shortly after disassembling the specimens, whereas the two columns on the right show images taken recently (several months later) on the same washers. The images on the top row show that similar to what observed on the stainless steel washers, the interior side experienced cross section loss (no much of the original surface remains). Moreover, the area subjected to crevice corrosion is greater on the Monel specimens, compared to those shown on Figure 5.9 for the stainless steel washers. The bottom row images show the interior side of the washer in contact with the rougher composite surface side. Note that on the left two columns (bottom row) each picture shows an outer ring of brown corrosion products with green corrosion products. These products upon drying and handling the washer flaked away, as can be observed on the two pictures to the

right. The images on the right two columns show there are regions with green corrosion products that remain, but not much of the brown corrosion products is left.



Figure 5.10. Washer Monel/CF RT exterior surface

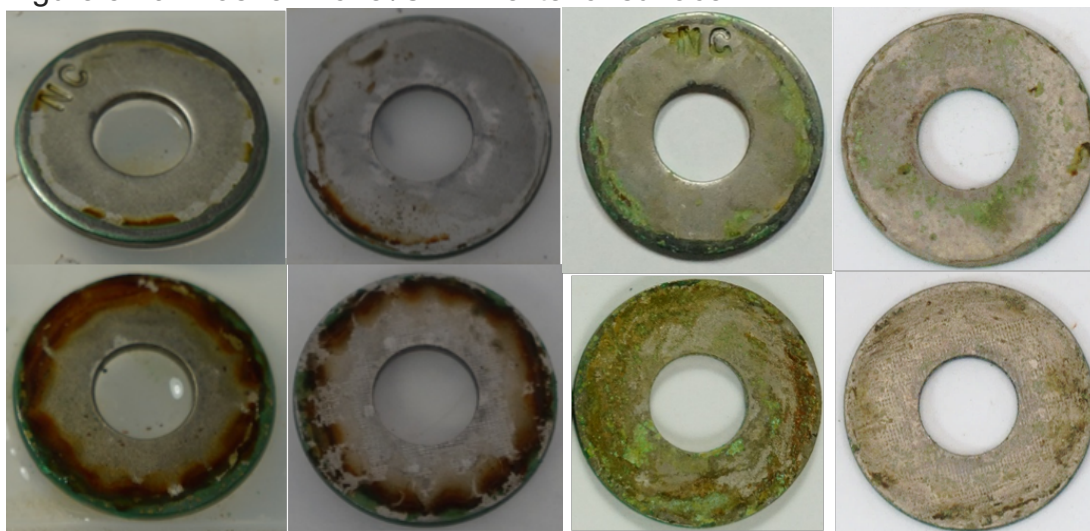


Figure 5.11. Washer Monel RT interior surfaces

Washers close-up Monel-RT. Three images are shown on Figure 5.12 for washers disassembled from RT Monel-CF/VE specimens. The image on the left shows a section of a washer in contact with the smooth surface. Close to the left edge there is a region where cross section loss has occurred. The image in the middle corresponds to a washer in contact with the rough composite surface, note the indentation marking close to the inner radius, the region close to the outer radius shows a number of brown (dark and light) and some green, these appear to be corrosion products. The figure on the

right is for a external surface of a washer in contact with a nut. Note the multiple grainy spots. These spots look like pits on the region that was below the nut.

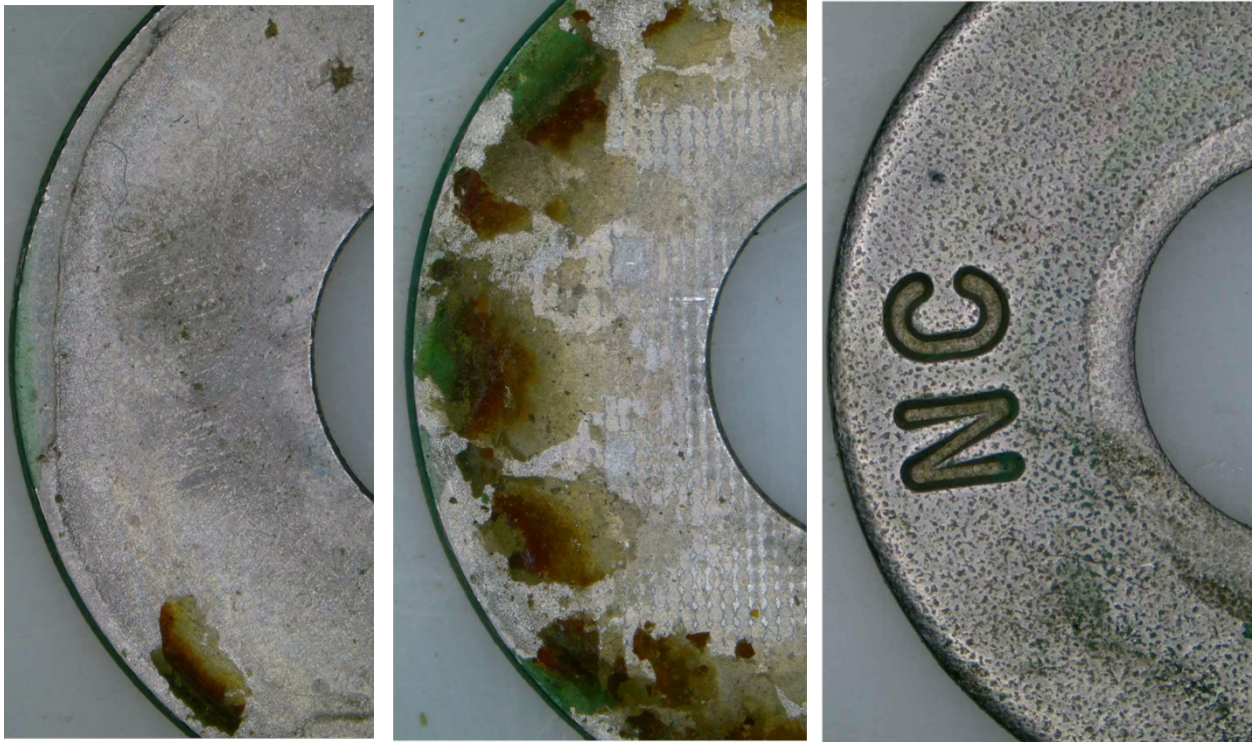


Figure 5.12. Washer Monel RT close-up images

Figure 5.13 and Figure 5.14 shows images of the Ti alloy washers after immersion in the RT tank. Figure 5.13 shows the washer (exterior) surfaces in contact with the bolt and nut, showing that all washers had minor indentations (compared to the Stainless steel and Monel washers). Some of the Ti washer showed staining. No crevice corrosion, nor pitting corrosion is observed on these images. Figure 5.14 shows the surfaces in contact with the composite, there are only three images, there is no image for one of the washers (this washer was grinded and polished for composition determination). No corrosion is observed on these washer surfaces.



Figure 5.13. Ti alloy RT exterior



Figure 5.14. Ti alloy RT interior

5.3.12 Elevated Temperature Specimens

Four specimens were removed for the specimens exposed immerse in seawater in the ET tank. Crevice corrosion was observed clearly on the interior surface of all stainless steel washers for specimens immersed in the elevated temperature seawater tank. Figure 5.15 shows images of the washer exterior surfaces. The top row shows images of the washers on the bolt side and the bottom row images of the washer surface in contact with the nut. Indentations due to the square shape of the bolt are observed on the top row images. The image on the left shows the least amount of red rust. There are two of the washers that show almost full coverage of red-rust products, both were part of specimens exposed for more than 650 days. All washers in contact with the nut show various degrees of crevice corrosion (metal-metal crevice).

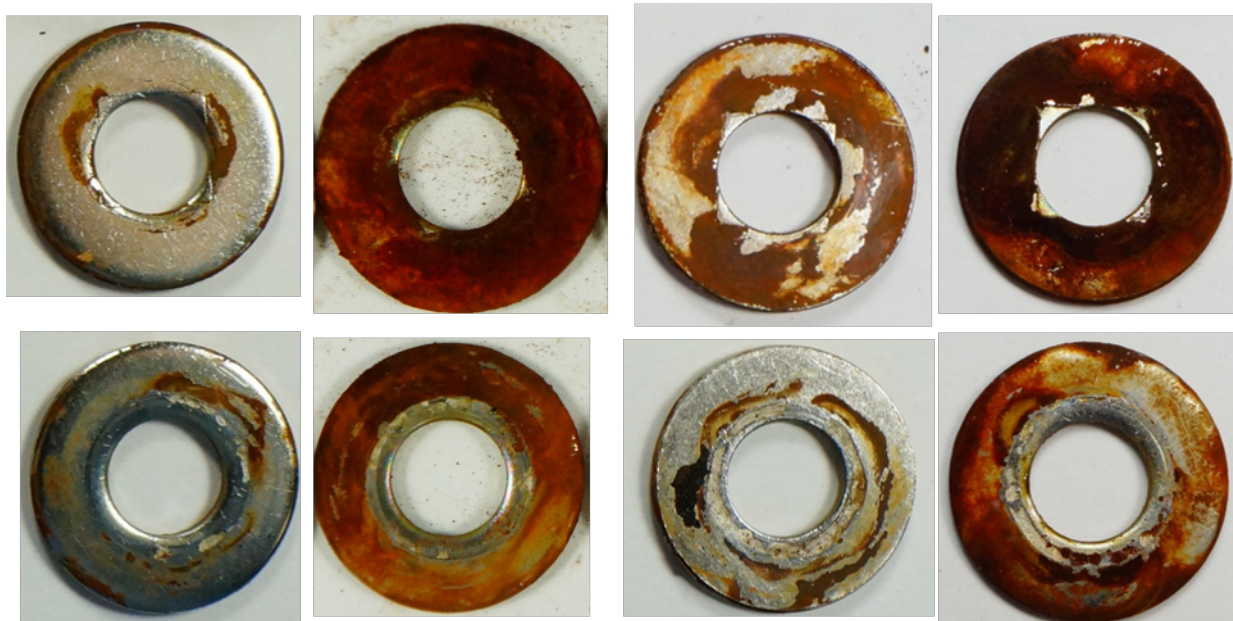


Figure 5.15. Exterior washer surface condition from specimens SS-CF immersed in ET tank

Figure 5.16 shows images of the SS washer surfaces in contact with the composite. The top row show images of the washers that were in contact with the smooth composite side, the two washers on the left show very thin inner and outer ring of the original surface. The two images on the top right show round washer side, which might explain why there are thicker ring areas with no corrosion, thicker rings are observed close to the outside diameter. The bottom row shows images of the SS washer in contact with the rough composite surface. Each images shows a number of corrosion spots. Those on the second and fourth column correspond to the specimens exposed immersed in ET seawater for more than 600 days. The image on the farther right bottom row show several dark black spots, suggesting that not all corrosion products leached out, whereas the washers on the first and third column do not show any dark corrosion spots.



Figure 5.16. Exterior washer surface condition from specimens SS-CF immersed in ET tank

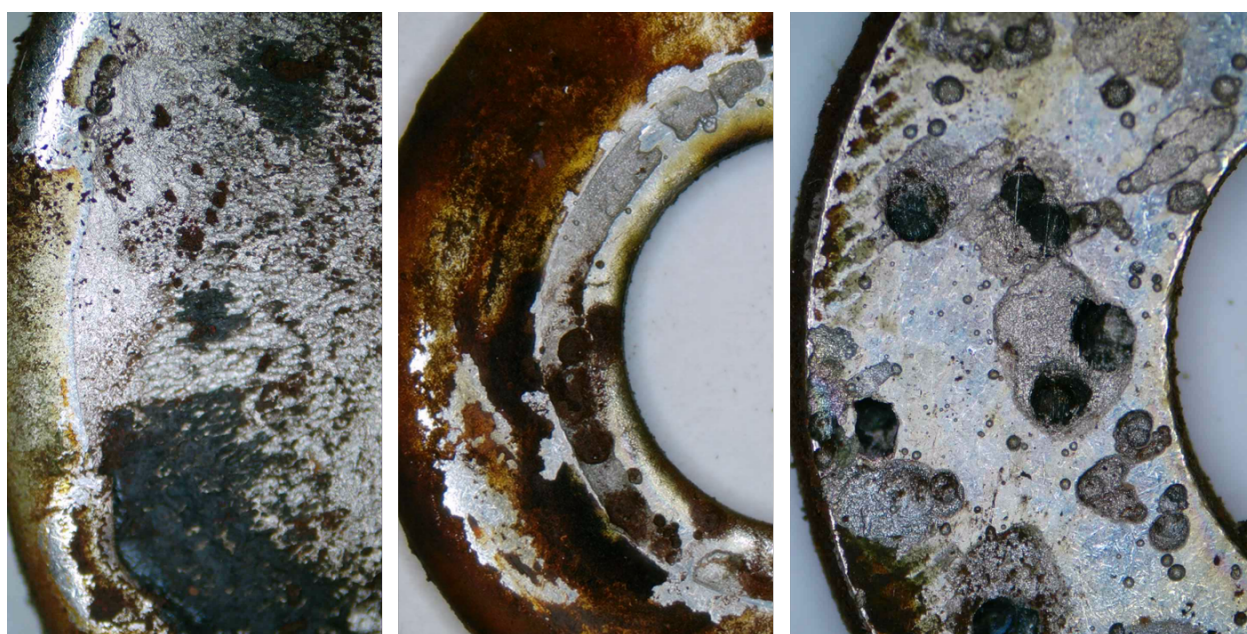


Figure 5.17. close-up SS washer surfaces immersed in ET tank

Figure 5.17 shows three images that correspond to washers of the specimen disassembled during August 2020 (ET SS). The image on the left is a close-up of the washer surface in contact with the smooth composite surface. The round side of the washer was in contact with the composite. Note that the region that experienced crevice corrosion had several black areas, whereas other regions are shiny (corrosion products leached out). The image in the center shows several crevice corrosion spots of the washer surface in contact with the nut, close to the inner diameter. The image on

the right shows multiple crevice corrosion spots for the washer surface in contact with the composite rough surface. The image shows a variety of corrosion spots sizes and depths. Some of the sites show regions with black corrosion products. Figure 5.18 shows four images displaying the various morphologies of the corrosion sites observed on the washer-surface in contact with the rough composite surface for the specimen dis-assembled after 729 days of immersion in seawater in the ET tank. There are numerous small round spots, and other spots where multiple small spots have coalesced. All images show blurriness due to some of the spots having different depths and not being possible to focus the different depths at the same time. Corrosion spots took place both close to the inner and outer diameter.

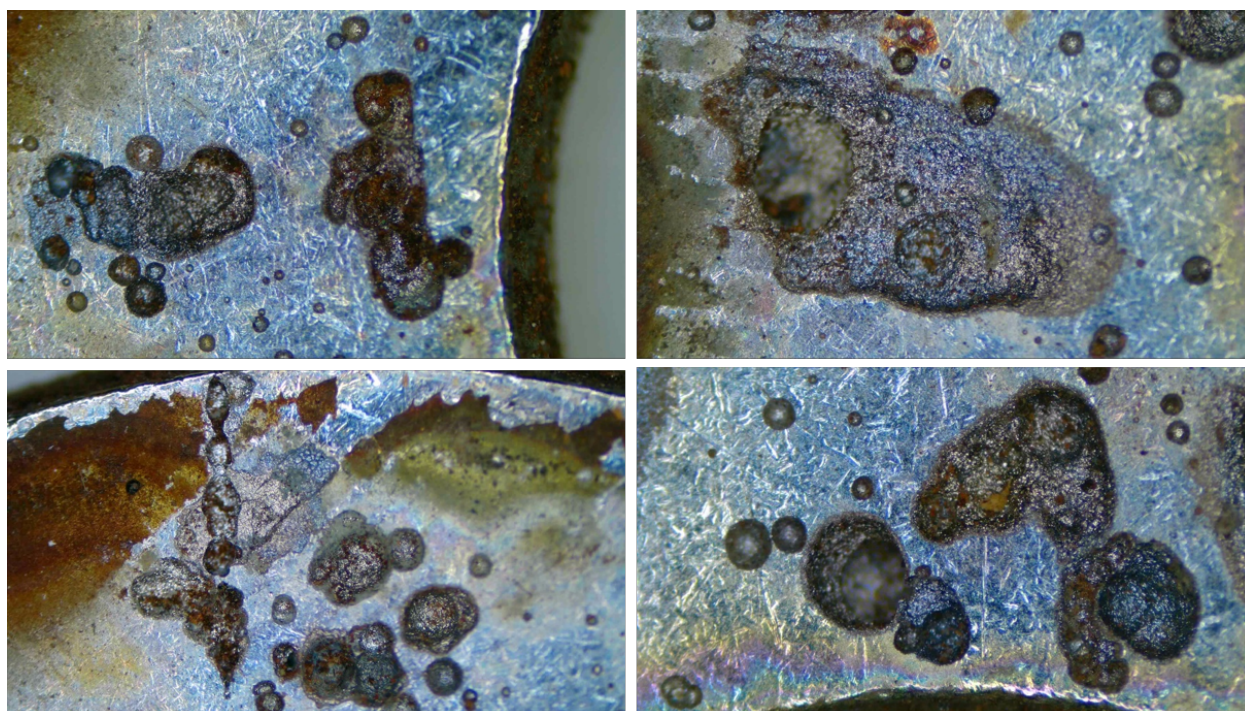


Figure 5.18. Close-up of corrosion spots for SS washer dis-assembled after 729 days of immersion.

Figure 5.19 shows images of the washers after dis-assembling the Monel-CF specimens immersed in the ET tank. The images in the top row show the exterior washer surface in contact with the bolt. These surfaces showed significant amount of green corrosion products and some section loss (visible on those in which corrosion products were removed). The images in the bottom row show the exterior washer surface in contact with the nut. Nut markings are visible in all four specimens. The two most recently terminated specimens show a larger areas of green corrosion products. One of the washers (column two bottom row) showed some lighter-brown corrosion products for the area in contact with the nut (crevice corrosion metal-metal surface). The other three washers in the bottom row show markings left by the nut. Figure 5.20 show images of the interior washer side in contact with the composite surface. The top row shows images of the interior washer surface (bolt side) in contact with the smooth composite surface. Two of the washers (column 1 and column 4) showed an outer ring

where corrosion took place and cross section loss took place. The pictures of washer on column 4 were taken the same day that the specimen was disassembled. Note the dark brown corrosion products on part of the outer ring. The images in the bottom row show the exterior washer surface in contact with the rough composite surface. There is evidence of corrosion products in all of them, but not significant cross section loss can be inferred from these images. All washers on the bottom row had brown corrosion products and some green corrosion products upon disassembling them. The products were flaky and most of it came loose upon handling. The image in the bottom row far right was taken shortly after dis-assembling and the dark corrosion products can be observed.



Figure 5.19. Exterior surface condition Monel-CF



Figure 5.20. Interior surface condition Monel-CF

Figure 5.21 show close-up images of the interior surface (i.e., in contact with the composite) for the specimen dis-assembled after 739 days immerse in seawater in the ET tank. Full view was shown on the farther right column in Figure 5.20. Note that in the close-up it is easier to observe some of the areas that suffered cross-section loss. The corrosion products have either leach out during exposure or flake-out during handling.

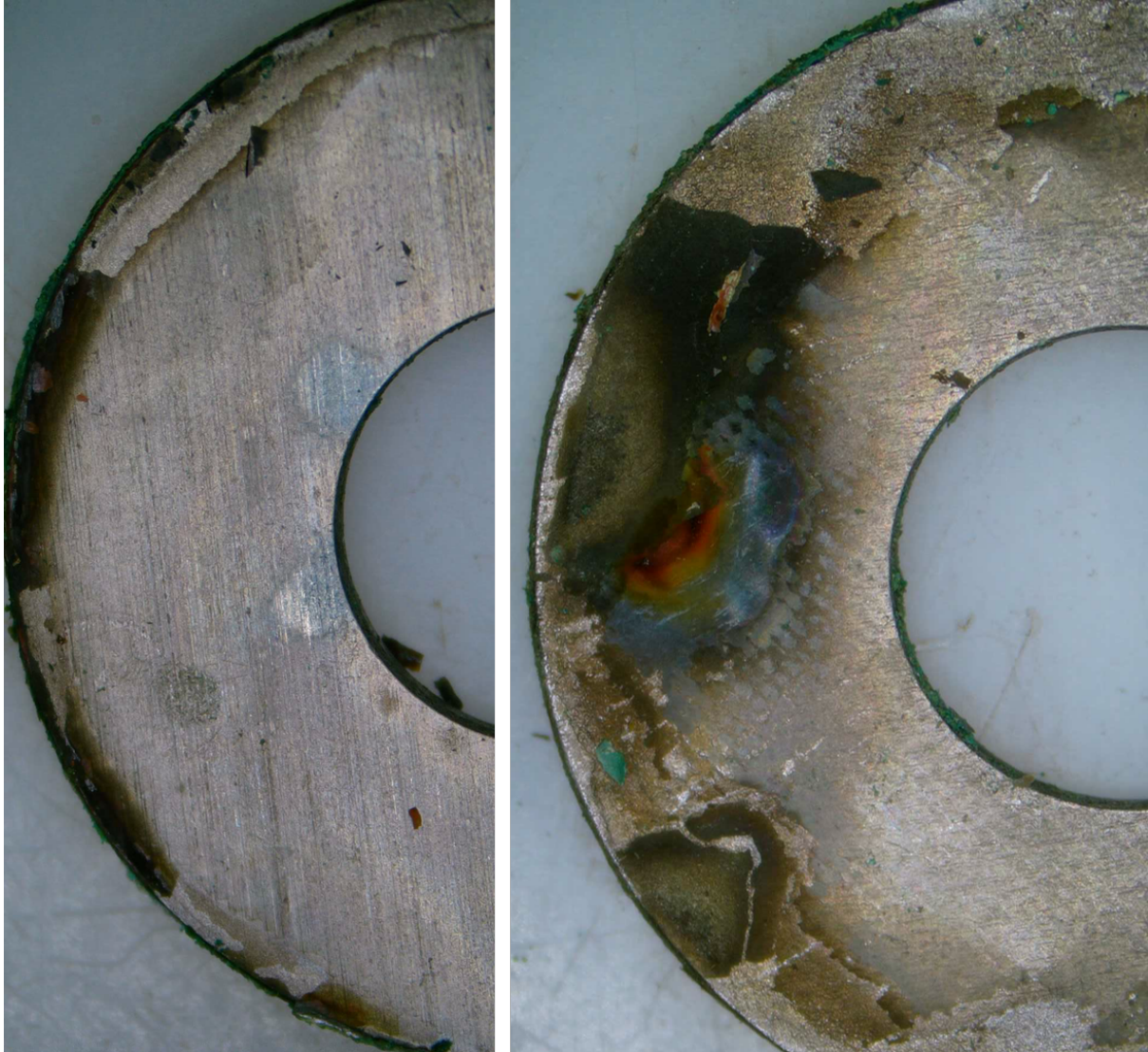


Figure 5.21. ET-Monel dis-assembled after 739 days immersed in seawater at ET

The Monel specimen terminated during August 2020: Images were taken before and after removing the corrosion products (particularly for the interior side of the washers). The bolt thread shows one crack (black line) close to the end (opposite to the bolt head). It might be good to take a closer look using the stereo microscope, and have a higher magnification picture taken. Verify that this is a crack.

For the images before and after, there are several close-ups that could be used to present the type of cross section loss that was experienced. It is somewhat difficult to say if it was significant, probably not as the image appears to be on focus.

The Ti washer exterior surfaces are shown in Figure 5.22 (ET tank). All four washers show indentation markings caused by the bolt (top row images) or the nut (bottom row images). The black stain is (left top) due to carbon tape (this washer was observed on the SEM). This washer also shows a brown stain. (Tucker [] and others have reported staining on Ti hardware exposed immersed seawater). Figure 5.23 shows the washers surfaces in contact with the composites. The top row images show that both washers had colorful stains in some areas. The washer on the left shows a Cu tape used to locate the area to be observed with the SEM. No corrosion was observed on any of the Ti washer surfaces.



Figure 5.22. Exterior surface condition Ti alloy – CF



Figure 5.23. Interior surface condition Ti alloy - CF

5.3.13 Hybrid specimens immersed in the ET tank

Two hybrid-stainless steel fastener sets specimens were exposed in the ET tank. Figure 5.24 shows images of these washers. On the left are washer images of the specimen dis-assembled after 316 days and on the right are washer-images of the specimen dis-assembled after 708 days. The top row images show that the bolt left square markings, the image on the left show a modest amount of red rust, whereas the image on the right shows almost all the surface covered with red rust. The bottom row shows images of the washers in contact with nuts, both show C4 (metal-metal crevice corrosion) crevice geometry. The image of the washer on the bottom left show two regions where corrosion and cross section loss took place, whereas the image on the right shows two small areas that suffered corrosion under the nut.



Figure 5.24. Exterior surface condition SS – Hybrid composite (immerse in seawater ET)



Figure 5.25. Interior surface condition SS – Hybrid composite (immersed in seawater ET)

Figure 5.25 shows the opposite surface for the washers just described, i.e., the surfaces in contact with the hybrid composite. The top row images show that the interior surface of both washers experienced crevice corrosion with most of the surface experiencing cross section loss. The images on the bottom row show dark markings, likely left by the hybrid composite surface. No cross-section loss appears to have occurred on the bottom-left washer. The washer on the bottom right shows that part of the interior surface suffered cross section loss (darker coloring area).

Figure 5.26 shows images of the Monel washer surfaces used with the hybrid composite. Images of the exterior surface are displayed on the left-column, these images show markings due to indentations caused by the bolt (top) and nut (bottom). Note that Cu redeposited in some areas in contact with the bolt, whereas small brown spots are observed on the area under the nut. The images on the right column show on the top row the surface in contact with the smooth composite surface, there is an outer ring with dark green corrosion products and inner ring area with no corrosion (pristine surface). On the right bottom image, the washer surface shows a different corrosion morphology, there is an outer ring with brown corrosion products and other areas where the products have flake-out, there is an inner ring (surrounding the inner diameter) where no corrosion took place (pristine surface is observed).



Figure 5.26. Exterior (left) and Interior (right) surface condition Monel alloy – Hybrid composite

Figure 5.27 shows images of the Ti washers used with the hybrid composite. The exterior surfaces are displayed on the left-column images, these images show markings due to indentations caused by the bolt (top) and nut (bottom). The top left image also shows a stained surface on the surface in direct contact with the seawater. The images on the right column show on the top row the washer surface in contact with the smooth composite surface, there is a lighter inner ring area. On the right bottom image, the washer shows black markings left by the composite rougher surface, these markings are observed on the region close to the inner diameter.



Figure 5.27. Exterior (left) and Interior (right) surface condition Ti – Hybrid composite SEM

Figure 5.28 and Figure 5.29 show SEM images for two different locations and various magnifications of a washer interior surface in contact with the rough composite surface. This washer was part of a specimen that was immersed in seawater in the elevated temperature tank for 323 days prior to removal and dis-assembly. Figure 5.28 shows on the left a low magnification image 26X, the red box indicates the area shown on the image in the middle. The image on the left shows a piece of Cu tape on the left, three small corrosion spots and two larger corrosion spots. The corrosion spot marked with the red box displays different levels of cross-section loss within that corrosion site. This is more evident in the middle image. The middle image and the image on the right allow to observe the grain boundaries. Figure 5.29 show two views of an almost perfect half sphere pit. The grain boundaries can be more easily identified on the image on the right. The image on the right also shows at the bottom of the pit what could be corrosion products or salt deposits. It appears that most corrosion products leached out.

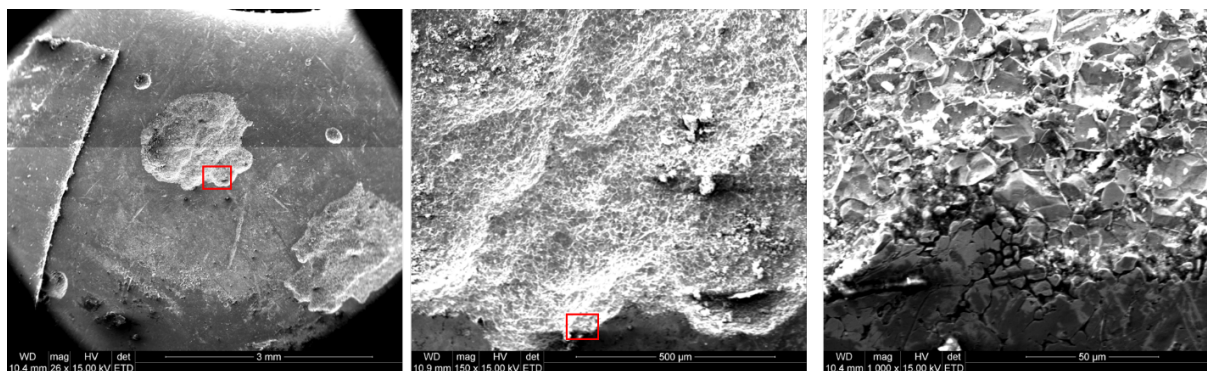


Figure 5.28. SEM images on the interior SS washer surface (specimen immersed in seawater at ET for 323 days)

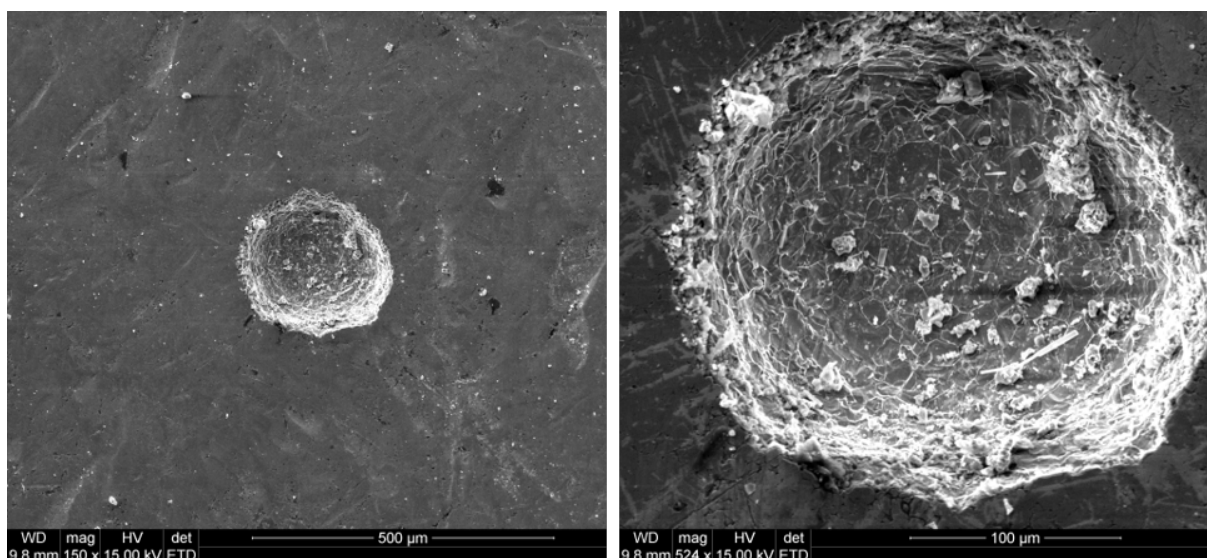


Figure 5.29. SEM images – location 2 for SS washer surface (specimen immersed in seawater at ET for 323 days)

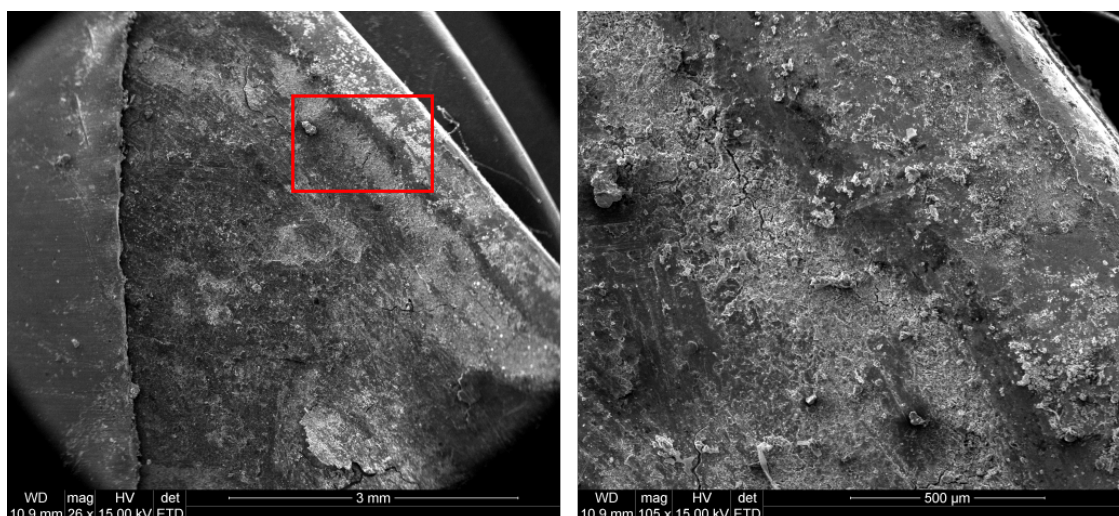


Figure 5.30. SEM images – location 1 for Monel washer surface (specimen immersed in seawater at ET for 288 days)

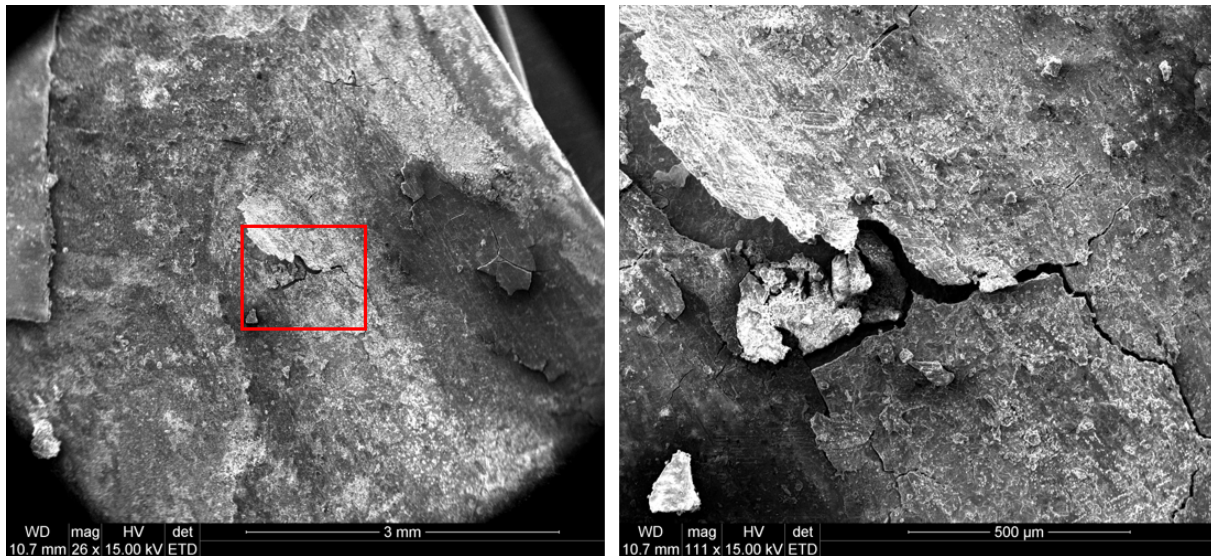


Figure 5.31. SEM images – location 2 for Monel washer surface(specimen immersed in seawater at ET for 288 days)

Images shown in Figure 5.30 and Figure 5.31 are of a Monel washer surface in contact with the rough composite surface (nut side). The specimen was immersed in the elevated temperature tank for 288 days prior to dis-assembly. Figure 5.30 shows on the left image a 26X magnification view, the red box shows the area that is at a higher magnification (105X) on the image on the right. The image on the right shows on the top right the inner radius of the washer, close to the edge there is an area that shows cross section loss. Figure 5.31 shows images of a different area of the same washer. The products that flake out can be observed in both images, these products are more clearly observed on the image on the right (111X).

5.4 Towards a standard

The methodology described in this document can be used as a first step towards developing a standard to assess fastener materials for use with composites in MHK devices, with respect to crevice corrosion. One advantage of the developed method is the use of the relevant composite material as part of the crevice former. Additional studies could look at GFRP composites and hybrid composites. A possible improvement to the test, is to use disc springs so that a constant force is applied onto the metal washers. This improvement is based on the findings from the CREVCOR program. Another possible modification is to continue using the as received materials, but implement a cleaning procedure. Something else to consider is to compare as received washer surface with modified washer surface. The modified washer surface preparation could be to abrade both sides of the washer, for example to 120 grit. Instead of using the prolonged exposure used in this study, identify a shorter exposure time say 60 days or 100 days of immersion. (And this might still be considered to be too long period of time).

5.4.1 Discussion

The developed methodology allowed to identify crevice corrosion on two of the three alloys tested. It was found that the temperature affected the corrosion morphology and allowed large coverage with calcareous deposits on specimens immersed in seawater at elevated temperature. The surface finish of composite also caused the corrosion morphology to vary, for example a larger washer area experienced crevice corrosion on the washer surface in contact with the smooth composite side, whereas the washer surface in contact with the rough composite side showed multiple corrosion spots (those terminated early had some small pits and other larger spots).

For the specimens with stainless steel hardware immersed in seawater at elevated temperature; most of the corrosion products leached out from below the washers. Whereas for Monel specimens, the washers in contact with the rough composite side, had corrosion products that flaked away upon dis-assembly and handling.

It was found that corrosion took place also on the surfaces in contact with the seawater for the stainless steel and the monel hardware. Fastener components terminated at a later time showed significantly more coverage of corrosion products. In this study, the fastener components were not acid cleaned.

For composites containing carbon fibers, the size of the plate appears to influence corrosion morphology, but in here the smaller coupon were made of hybrid composite. Testing with multiple plate size for cases with CFRP composites of the same time is desirable, plate size used in here (7.5 7.5 cm) appear to enhance corrosion more pronounced on the specimens immersed in seawater at elevated temperature.

5.4.2 Preventing Crevice Corrosion on Fastened Composites

Corrosion prevention and/or deterioration monitoring while in service need to be included in the design phase. Appropriate joining technologies are required with respect to mechanical behavior and corrosion resistance. One approach is to use a corrosion resistance fastener material for marine applications (those mentioned by Ross[Ref] and others) that is not prone to crevice corrosion, this is expensive. The Ti alloy used in here performed well within the monitored period of time.

The designer should consider methods that have been proposed over the years to minimize galvanic coupling of CFRP composites and alloys. Plastic sleeves and plastic washers have been suggested as a way to separate a carbon fiber composite and a metallic fastener. It has been reported [Ref] that in some cases fasteners are encapsulated into weld-able PU disc, combined with ultrasonic welding into the fabric layers. This allows to maintain an air and watertight seal.

Sloan and Talbot [Ref] describe an approach to prevent coupling damage in composite. Electrically isolate CF composites by applying fiber glass or organic-fiber plies (e.g., polyethylene) and sealer coatings to isolate from metallic fasteners. Sloan and Talbot note that even if these coatings are applied, the insulating properties degrade with time due to wear, chemical breakdown, or electrolyte absorption. It is recommended to

establish periodic monitoring. (Corrosion Journal paper). The polymer/coating deteriorates after prolonged exposure immersed in seawater (hence replacement, reapplication will be periodically needed, in conjunction with health monitoring (from a corrosion perspective)).

5.5 Summary and Conclusions

The immersion time lasted longer than what is typically done, this allowed to observe different corrosion stages (how corrosion progressed). It was found that crevice corrosion occurred on two of the alloys on washer surfaces in contact with the composite.

Different crevice corrosion morphologies were observed on Monel washer surface in contact with the smooth composite surface when compared to the stainless steel washer surface in contact with the smooth composite side. A ring with cross section loss was observed on the outer radius on the Monel washers, whereas a large area with wide region with cross section loss was observed on the stainless steel washers.

The surface finish of the composite also caused the corrosion morphology to vary, for example a larger washer area experienced crevice corrosion on the washer surface in contact with the smooth composite side (SS immersed in seawater at ET), whereas the washer surface in contact with the rough composite side showed multiple corrosion spots (those terminated early had some small pits and other larger spots).

Crevice corrosion also took place on metal/metal locations. For example, crevice corrosion was found on the washer and nut surface on fastener Monel sets disassembled after 600 days of immersion in seawater at elevated temperature. In some washers, the areas under the nut showed Cu re-deposition.

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