NDSU NORTH DAKOTA STATE UNIVERSITY

20 March 2020

Defense Technical Information Center
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Please find enclosed the Final Technical Report and SF298 for grant N00014-17-1-2153 "Amphiphilic, Siloxane-Based Fouling-Release Coatings for Oil Boom Applications and Comprehensive, Biological Laboratory Efficacy Testing."

Sincerely,

Dean C. Webster
Professor and Chair

COATINGS AND POLYMERIC MATERIALS
NDSU Dept 2760 | PO Box 6050 | Fargo ND 58108-6050 | 701.231.7633 | www.ndsu.edu/cpm

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Dean C. Webster, Shane J. Stafslien

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North Dakota State University
PO Box 6050, Dept 4000
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14. ABSTRACT
Objectives: 1. Explore lower cost alternatives to the synthesis of amphiphilic silicone fouling-release (FR) coatings; 2. Explore methods to improve the adhesion of FR coatings to oil boom components; 3. Provide testing to ONR researchers using laboratory assays of FR coating properties. The project focused on objectives 2 and 3. A screening experiment was carried out to explore different surface treatments on the adhesion of coatings subjected to a high pressure water jet. Based on the screening, several systems were selected for field trials in Florida. NDSU completed comprehensive biological laboratory efficacy assessments for 6 ONR PIs and 3 discretionary testing collaborators, comprising 428 unique coatings.

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Report Information

Name: Dean C. Webster
Organization: North Dakota State University
Email: dean.webster@ndsu.edu

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Program Officer: J. Paul Armistead
PI: Dean C. Webster
Co-PI: Shane J. Stafslien

Abstract

Objectives: 1. Explore lower cost alternatives to the synthesis of amphiphilic silicone fouling-release (FR) coatings; 2. Explore methods to improve the adhesion of FR coatings to oil boom components; 3. Provide testing to ONR researchers using laboratory assays of FR coating properties. The project focused on objectives 2 and 3. A screening experiment was carried out to explore different surface treatments on the adhesion of coatings subjected to a high pressure water jet. Based on the screening, several systems were selected for field trials in Florida. NDSU completed comprehensive biological laboratory efficacy assessments for 6 ONR PIs and 3 discretionary testing collaborators, comprising 428 unique coatings.

Technical Section

See succeeding pages.

Refereed Journal Articles

- A. Vena, S. Kolle, S. Stafslien, J. Aizenberg, P. Kim, "Self-stratifying porous silicones with enhanced liquid infusion and protective skin layer for biofouling prevention," Adv. Mat. Inter., 2020, submitted for publication.
- B. Eslami, P. Irajizad, P. Jafari, M. Nazari, A. Masoudi, V. Kashyap, S. Stafslien, H. Ghasemi, "Stress-localized durable anti-biofouling surfaces," Soft Materials, 2019, 15, 6014-6026.
- B.K. Ngo, K. Lim, S. Stafslien, M. Grunlan, "Stability of silicones modified with PEO-silane amphiphiles: Impact of structure and concentration," Polymer Degradation and Stability, 2019, 163, 136-142.
- B. Rasulev, F. Jabeen, S. Stafslien, B.J. Chisholm, J. Bahr, M. Ossowski, Philip Boudjouk, "Polymer coating materials and their fouling release activity: A cheminformatics approach to predict properties," ACS Applied Materials & Interfaces, 2017, 9, 1781-1792.

- R. Sachan, P. Jaipan, J.Y. Zhang, S. Degan, D. Erdmann, J. Tedesco, L. Vanderwal, S.J. Stafslien, I. Negut, A. Visan, G. Dorcioman, G. Socol, R. Cristescu, D.B. Chrisey, R.J Narayan, "Printing amphotericin B on microneedles using matrix-assisted pulsed laser evaporation," International Journal of Bioprinting, 2017, 3(2).

- M.L. Hawkins, S.S. Schott, B. Grigoryan, M.A. Rufin, B.K.D Ngo, L. Vanderwal, S.J. Stafslien, M. Grunlan, "Anti-protein and anti-bacterial behavior of amphiphilic silicones," Polymer Chemistry, 2017, 8, 5239-5251.

- M.A. Rufin, B.K.D. Ngo, M.E. Barry, V.M. Page, M.L. Hawkins, S.J. Stafslien, M.A. Grunlan, "Anti-fouling silicones based on surface-modifying additive (SMA) amphiphiles," Green Materials, 2017, 5(1), 1-10.

- C. Kuliasha, J. Finlay, S. Franco, A. Clare, S. Stafslien, A. Brennan, "Marine antifouling efficacy of amphiphilic poly(coacrylate) grafted PDMSe: effect of graft molecular weight," Biofouling, 2017, 33(3), 252-267.

Books and Chapters

- None

Technical Reports

- None

Contributed Presentations

- None

Patents

- None

Honors

- Prof. Webster named Fellow of the Polymeric Materials:Science and Engineering Division of the American Chemical Society in 2019

Related Sponsored Work

- None

ONR Statistics

Grad Students(total):	1
PI/Co-PI Women:	0
PI/Co-PI Minority:	0

Grad Students Women:	0
Grad Students Minority:	0
Post Docs Students:	3
Post Doc Women:	1
Post Doc Minority:	0
Under Grad Students(total):	1
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Under Grad Students Minority:	0
Degrees Granted:	0
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Title of Research	Amphiphilic, Siloxane-Based Fouling-Release Coatings for Oil Boom Applications and Comprehensive, Biological Laboratory Efficacy Testing
Principal Investigator	Dean C. Webster
Organization	North Dakota State University
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Technical Section

I. Amphiphilic Polysiloxanes (AmphiSil)

1.1 Technical Objectives

The objectives of this work are to identify lower-cost approaches to the design of amphiphilic siloxane elastomer fouling release coatings as well as to explore some new approaches to polymers having amphiphilic nature.

1.2 Accomplishments

It was decided to focus our efforts on the oil boom component of the program (Section II), so no work was done to address this objective.

Progress

II. Antifouling and Fouling-Release Coatings for Oil Booms

II.1 Technical Objectives

To improve the adhesion of fouling-release (FR) coatings on polymeric oil containment boom fabrics and floats, using surface treatments and/or adhesion promoters.

II.2 Technical Approach

The technical approach has involved obtaining samples of fabrics and floats from commercial suppliers and determining their composition and surface properties. Samples of commercial marine fouling-release coatings were obtained from International Paint (IP) AkzoNobel and Hempel. A siloxane-polyurethane formulation (A4-20) will also be used. Methods for improving adhesion to the substrates include sandblasting, use of adhesion promotors and corona treatment. A waterjet apparatus was constructed to test the adhesion since conventional adhesion testing methods are often not suitable for use with low surface energy coatings. After evaluating waterjet adhesion values of these systems, promising candidates were chosen for larger sample field testing at Florida Institute of Technology.

II.3 Accomplishments

Substrates— Effect of Solvents and Characterization

Commercially available oil containment fabrics and floats were received from DESMI Inc. (Virginia) and Elastec (Illinois). Figure II.1 shows details of the different substrates included in the study. The fabrics from both DESMI and Elastec were made from polyvinyl chloride (PVC) and polyurethane (PU), while the floats from both the manufacturers were made from polyethylene (PE). Prior to application of the FR formulations, the substrates were cleaned using common solvents, a solvent blend of acetone and hexane (Ac: H = 1:1 w/w), 1, 2-dichloroethane (DCE), dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and water, to remove impurities.

After cleaning, the substrates were analyzed using ATR-FTIR to check the effect of solvent cleaning on surface composition of the substrates. The spectra of substrates cleaned using the organic solvents were compared to the spectra of samples cleaned using water to identify surface changes caused by cleaning solvents. Figure II.2 shows results obtained from ATR-FTIR for the different samples. Overall, the sample surfaces remained almost unaltered when cleaned using Ac: H and water. But the PVC fabrics dissolved in DCE, while the PU fabrics dissolved in both hot DMSO and DMF. On the other hand, the PE floats remained mostly unaltered after cleaning with organic solvents. Based on the results obtained, Ac: H blend was identified as the best solvent for substrate cleaning.

DESMI ITEMS	ITEM DESCRIPTION Packing List 21623	MATERIAL	SAMPLE NAME
	FLOAT GB 8" BLACK WITH FOAM ITEM # 3013-05	Polyethylene	DF
	100OZ OF FABRIC SLIT TO 24" ITEM # 2002-24	Polyvinyl chloride	DB
	UHW 100OZ USN SPEC 24" Wide ITEM # 2028-24	Polyurethane	DO
	UHW 100OZ USN SPEC 24" Wide ITEM # 2028-24	Polyurethane	DO-T
ELASTEC ITEMS	**ITEM DESCRIPTION Packing List 21623**	**MATERIAL**	**SAMPLE NAME**
	Spare PermaFence(set) W/Hardware ITEM # 0502041 A	Polyethylene	EF
	Belting PVC 24" Black ITEM # 2005115	Polyvinyl chloride	EB
	Belting Ure 24" 100oz. Orange ITEM # 2005724	Polyurethane	EO

Figure II.1. Fabrics and floats included in the study.

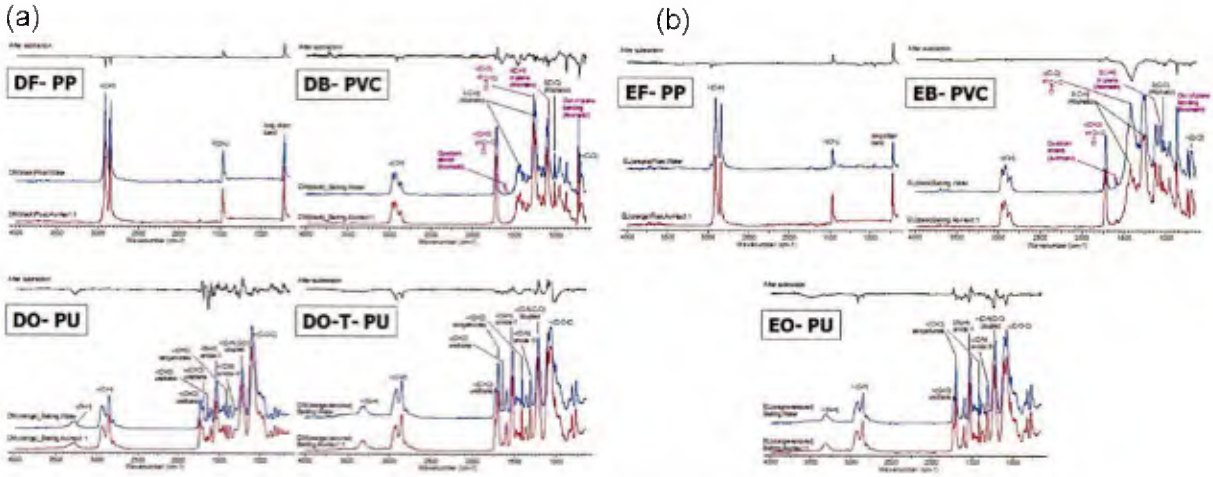

Figure II.2. ATR-FTIR spectra for (a) DESMI and (b) Elastec substrates.

To determine changes in "wettability" of the as-received substrates and Ac: H cleaned samples, the samples were characterized using water contact angle (WCA) and surface energy (SE) measurements. Table II.1 shows results obtained from contact angle experiment. The results obtained from the experiment showed that SE of most samples increased after solvent cleaning, except samples DO and EB that showed slight decrease in SE. WCA values decreased for DF, DO-T, and EO after solvent cleaning, while the values increased for DB, DO, and EB. EO showed biggest drop in WCA values from 92° to 71°; DO showed highest increase in WCA from 64° to 71°.

Table II.1. Changes in WCA and SE of the fabrics and floats after solvent cleaning.

Sample label	Before solvent cleaning			After solvent cleaning		
	WCA (°)	MICA (°)	SE (mN/m)	WCA (°)	MICA (°)	SE (mN/m)
DF	87	56	31.9	84	49	35.8
DB	80	52	35.4	83.7	24	46.4
DO	64	13	53.1	71.5	7	51.7
DO-T	110	75	20.2	102	45	38
EF	81	64	30.3	76	57	34.9
EB	71	36	44.7	74	39	42.6
EO	92	68	25.2	71	51	39.3

Surface Preparation and Formulations

Fabrics were cut into approximately 2" x 3" rectangles prior to coating application. In this study, surface treatments, sandblasting (SB) and corona (C), were used to increase roughness and

therefore, the "wettability" of the polymeric samples. Additionally, samples were made by applying coatings directly onto the substrates without any surface treatment (none).

In the first experiment, two commercial FR tie-coats, Hempasil Nexus and International Paint 730 (IP) tie-coats and the siloxane-polyurethane (SiPU) A4-20 formulation (no additional tie-coats) were selected to coat the treated and the untreated fabric samples. Since the commercial FR coatings require tie-coats that provide adhesion of the topcoats to the substrates, initial experiments with only the tie-coats were expected to provide insights into adhesive failure of the tie-coats from the polymeric substrates.

Apart from surface treatments, adhesion promoters (AP) were incorporated into the formulations to improve adhesion of coatings on the polymeric substrates. Four different Eastman chlorinated polyolefins (164, 343, 512, 730)— 10% resin solids loading levels— were used as APs. The APs were first dissolved in toluene to make 25% solid solutions. Two approaches were explored to incorporate the APs— as an additive and as a primer between the substrate and the coatings. Figure II.3 shows a schematic of the experimental plan for the study. According to the plan, total number of samples prepared in the study will be— 7 substrates (fabrics + floats) x 3 coatings x 5 (no AP + 4 APs) x 3 surface treatments (none, SB, C) x 6 replicates = 1890 test samples. Figure II.4 shows matrices of experimental samples included in the study. Till date, all samples with no surface treatment and SB, with and without APs, have been coated with FR coatings and are ready for adhesion tests.

The tie-coat formulations were made by mixing the binder resin and crosslinker components in plastic cups using tongue depressors. A4-20 formulation was made by mixing aminopropyl terminated PDMS (APT-PDMS), acrylic polyol (50% in toluene), and pentanedione pot-life extender overnight.[1] The next day, IPDI trimer and catalyst dibutyltin diacetate (DBTDAc) were added and the formulation was allowed to mix for another hour.[1] All formulations (two tie-coats and A4-20) were applied using disposable foam brushes. All the formulations were cured under ambient laboratory conditions for 1 week. After curing, the samples were stored in plastic bags.

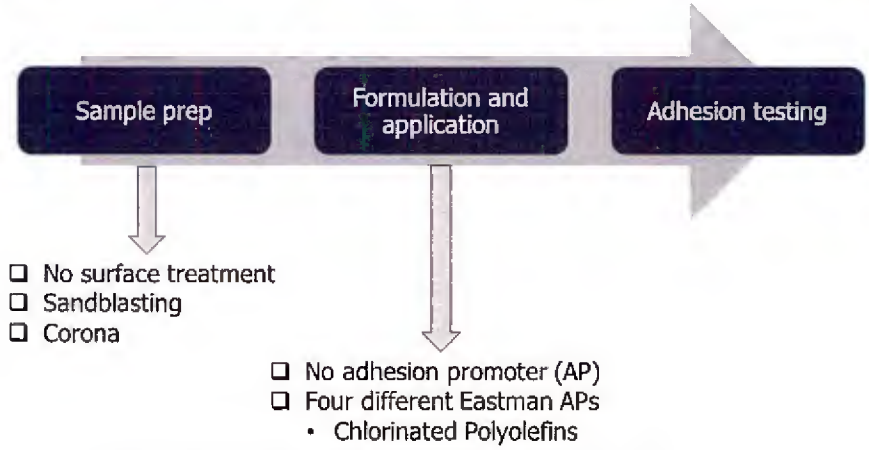

Figure II.3. Experimental plan for the study.

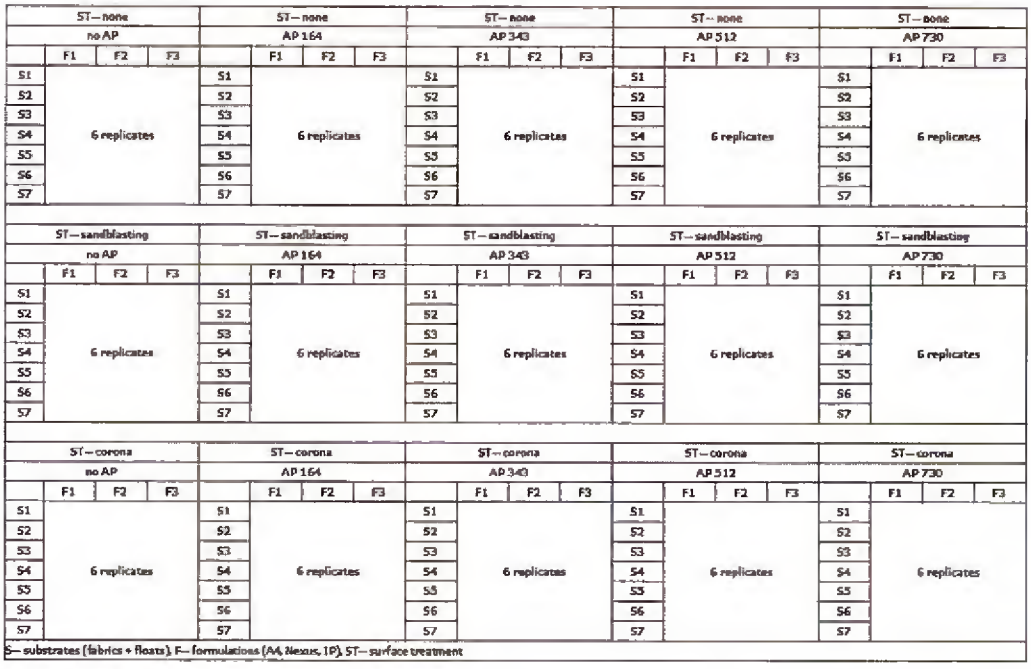

Figure II.4. Matrices of samples, formulations, APs, and surface treatments included in the study.

Coating Appearance of No Surface Treatment and Sandblasted Samples

A4-20 formulation was very easy to apply onto the substrates using foam brushes, independent of the surface treatment. Presence of significant amount of toluene in the formulations (50%) did not damage or dissolve the fabrics. Similarly, the elastomeric Nexus tie-coat formulation could be conveniently applied onto the substrates, although thicker tie-coats were needed to completely cover the substrates. Non-wetting nature of the IP tie-coat made application of coatings extremely difficult. For example, even after 2-3 coats, sample EB could not be uniformly coated.

Formulations applied onto substrates with no surface treatment and SB appeared similar visually. But, on closer inspection of the samples, it was observed that Nexus tie-coat formulations on substrates with no surface treatment showed formation of small cracks, which propagated when the samples were bent. Moreover, delamination was observed in samples coated using A4-20 formulation, especially on EO and DO-T fabrics (fabrics with surface "textures") in the absence of any surface treatment (Figure II.5). Sandblasting the substrates improved the adhesion slightly.

Use of adhesion promoters as a primer layer significantly improved the wetting nature of the substrates. Elastomeric formulations (Nexus, IP) could cover the entire area of the substrates and it was comparatively easier to apply multiple coats uniformly. No delamination was observed with A4-20 formulation on any substrate with incorporation of a priming layer of the adhesion promoters. Upon curing, all coatings showed smooth and uniform appearance. Conversely, adding adhesion promoters as additives directly to the tie-coats and A4-20

formulations resulted in formation of phase separated domains on the surfaces of the cured coatings. Viscosity of the IP and Nexus tie-coats increased rapidly as soon as the APs were added to the formulation cups and stirred, indicating acceleration of curing reactions in the two tie-coats. The rise in viscosity significantly affected the ability of the formulations to form smooth and uniform coatings on the substrates. Therefore, using APs as primers was identified as the optimum method to incorporate APs with the FR formulations.

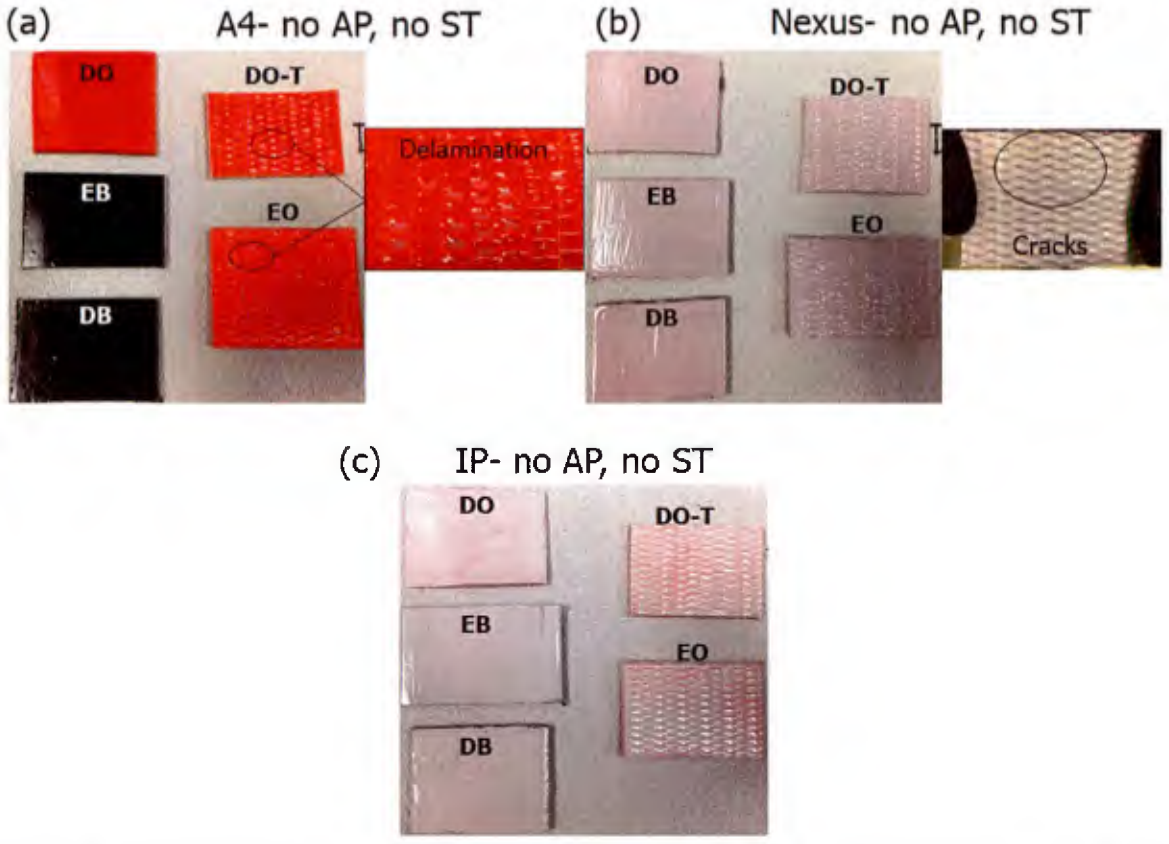

Figure II.5. Images of fabric samples with no surface treatments and no APs, coated with (a) A4-20, (b) Nexus, and (c) IP. Abbreviation ST indicates surface treatment.

Tantec Corona Treatment

A LabTEC corona surface treatment instrument, seen below in **Figure II.6**, was rented through Tantec and had a generator with a max power output of 200 watts.

Figure II.6[2]: Tantec LabTEC housing with applicator, which would be hooked up to a generator and controller unit

Previous test samples were given to Tantec representatives to determine if the substrates used in this experiment showed any difference in "wettability" after corona treatment. As seen in **Table II.2**, the selected substrates could indeed be modified to increase "wettability", with reductions in surface energy throughout all substrates analyzed.

Table II.2. WCA changes after corona treatment.

SAMPLE LABELS	WCA BEFORE CORONA	WCA AFTER CORONA
DB	115°	40°
DF	90°	60°
DO	80°	40°
EF	95°	60°

To treat substrates with the LabTEC corona instrument, the single point metal electrode was used. This electrode was attached to the generator and 6 uncoated samples at a time were placed on the sample platform. After turning the generator on, the controller was used to adjust power output to 100 watts. Neoprene gloves were always to be worn with rubber soled shoes, and great care was taken to ensure that the electrode and its wire did not touch any metal part of the frame to prevent shorting out and hazardous shock. Next, the electrode was passed over substrate samples ~1 inch from the surface until the entire surface was covered. This was performed for all required samples before coating with adhesion promoters, tie-coats, or A4-20.

Coating Application on Corona Treated Substrates and Water Ageing

Adhesion promoters were dissolved in toluene to make 10 wt.% solids solutions and then applied via 3" foam brush to substrates which required them. Next, IP and Nexus tie-coats, as well as A4-20 were prepared and applied to the required substrates with a 3" foam brush after letting adhesion promoter coats dry overnight. Coatings were inspected and it was noted that IP tie-coat produced smooth, uniform films over all substrates, whilst Nexus tie-coats produced the same small cracks seen in previous applications. A4-20 also produced smooth and uniform films over all substrates but did show some delamination when EO or DO-T substrates were slightly flexed. Final coated substrates were dried for at least one week and then placed in circulating water tanks for periods of 30, 60, 90, 120, 150, and 180 days.

Figure II.7: All 1350 samples placed in circulating water tanks

At the end of each time period, samples for each substrate, with each coating type, adhesion promoter, and surface treatment were removed. These samples were wiped with paper towels and dried before waterjet adhesion testing.

Adhesion Testing

To assess the potential differences in adhesion for sandblasted substrates, corona treated substrates, adhesion promoters' substrates, or combinations thereof, a semi-automated coating adhesion testing apparatus was constructed at NDSU and shown in **Figure II.8** and **II.9**.

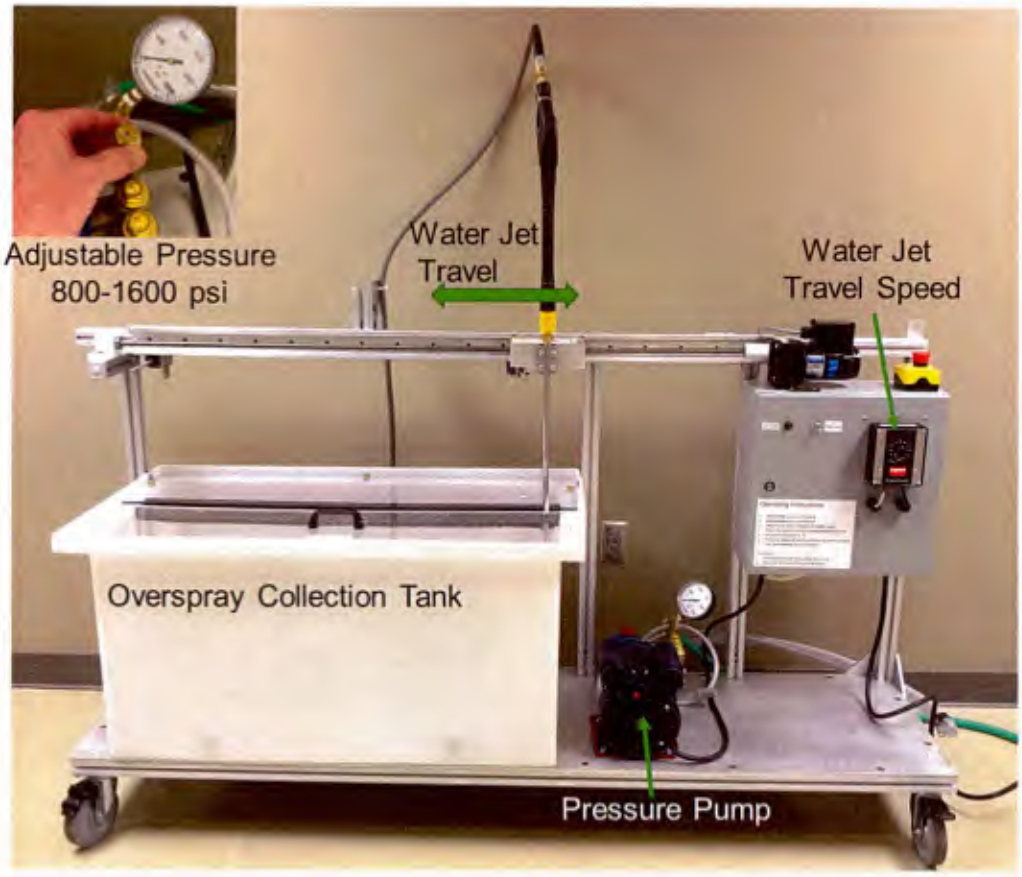

Figure II.8: Custom adhesion testing apparatus showing max pressure, direction of travel and variable speed control (photo credits – James Bahr)

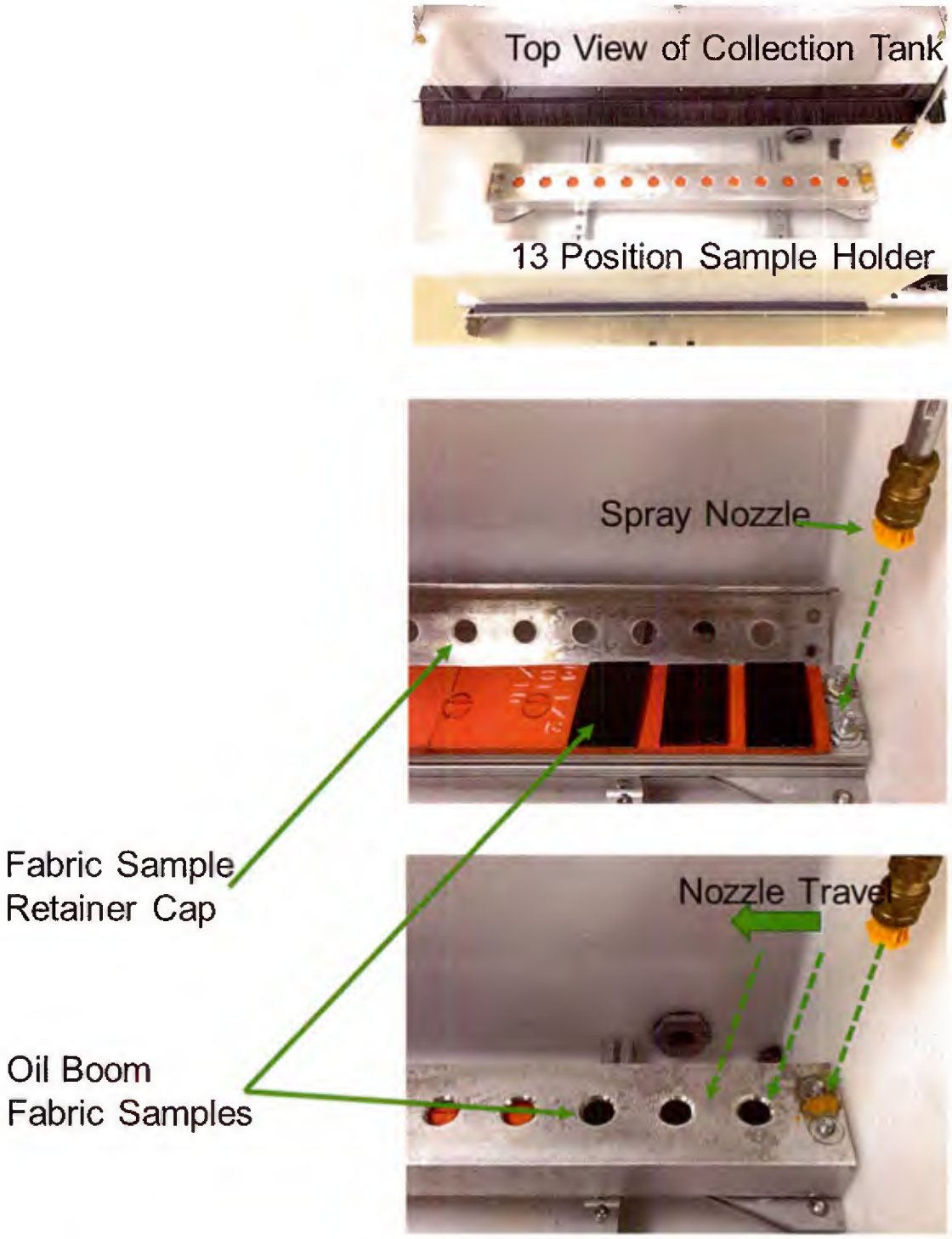

Figure II.9: Pictures showing the distance of nozzle from the substrates, 13-sample holder itself, and direction of nozzle travel (photo credits – James Bahr)

Before performing adhesion testing on coated samples, a method was determined to best evaluate coating adhesion. First, samples of the five different substrates coated with IP tie-coat, Nexus tie-coat, A4-20, with no adhesion promoters or other surface treatments, were prepared.

These samples were placed in the sample holder and the lid was placed over the tank. The goal of adhesion testing with this apparatus was to induce adhesive failure in each of the treatment groups by using a focused stream of water at variable psi. To start, a "fan" nozzle was attached with psi set to ~100, and the speed controller was turned and set to 6. A run is classified as the nozzle passing over the substrates twice, going forward and then returning to the original start position. After each run at increasing pressures, the coating appearance was assessed. If no damage is seen, it is classified as a 0F, a 1F if slight damage to the coatings surface is seen, 2F if the substrate can be seen with minor surrounding delamination, and a 3F if the coating is completely delaminated from the sample surface. Runs with the "fan" nozzle did not damage any coating even at 1600 psi. Therefore, a single stream nozzle was tested for the prepared samples. Using the single stream nozzle, one could see variations in failure classification at varying pressure values. As such, for analyzing the samples taken out of circulating water tanks, the single stream nozzle was used at pressures of 100, 200, 300, 400, 600, 800, 1000, and 1200 psi. Pictures of failure classifications can be seen in **Figure II.10**.

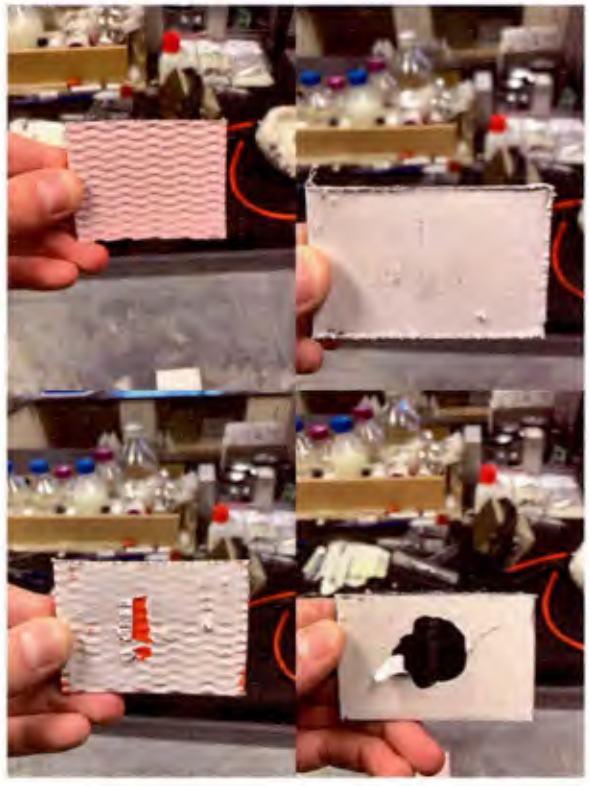

Figure II.10: Top left to right: 0F, 1F failure classification. Bottom left to right: 2F, 3F failure classification

When carrying out the adhesion testing method, samples from circulating water tanks were placed in the sample holder, the tank covered with a clear lid, and pressure started at 100 psi. After each run, samples were inspected for damage/adhesion loss and if there was any, pictures were taken at the first sign of failure taking note of the pressure the water was spraying at. Runs were completed for increasing pressures until either all coatings showed 3F failure, or

1200 psi was reached. Pictures of all failure classifications of the three coatings are shown below in **Figure II.11**, **II.12**, and **II.13**.

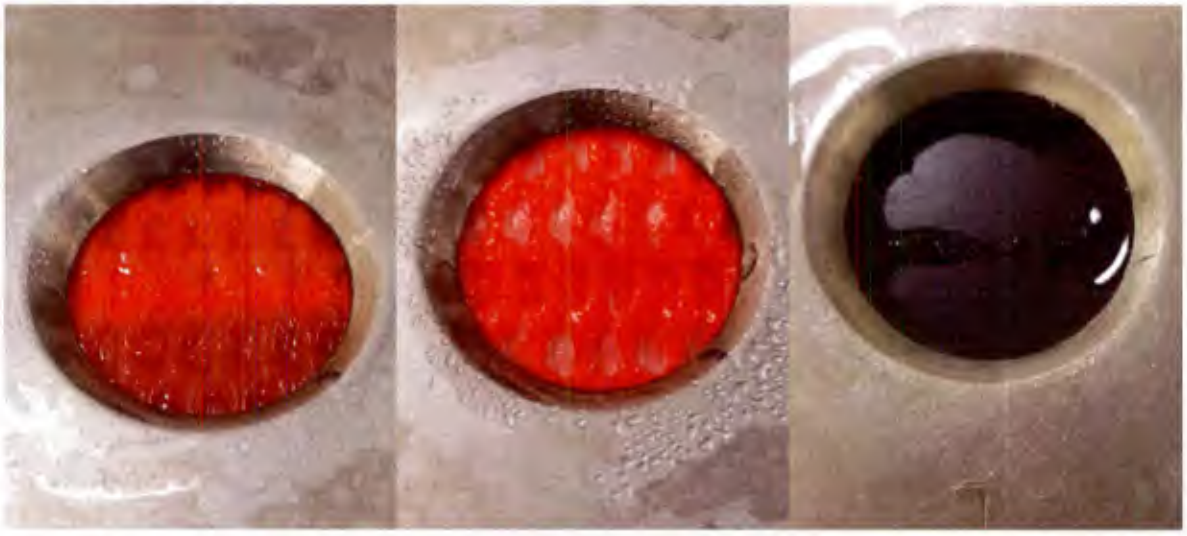

Figure II.11: From left to right: A4-20 1F, A4-20 2F, A4-20 3F failure classifications

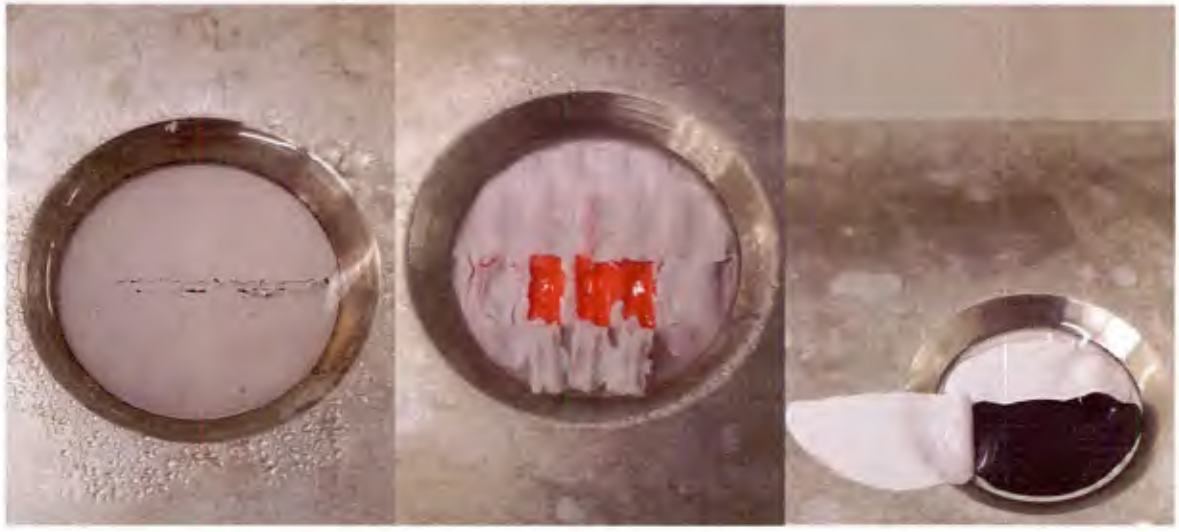

Figure II.12: From left to right: IP 1F, IP 2F, IP 3F failure classifications

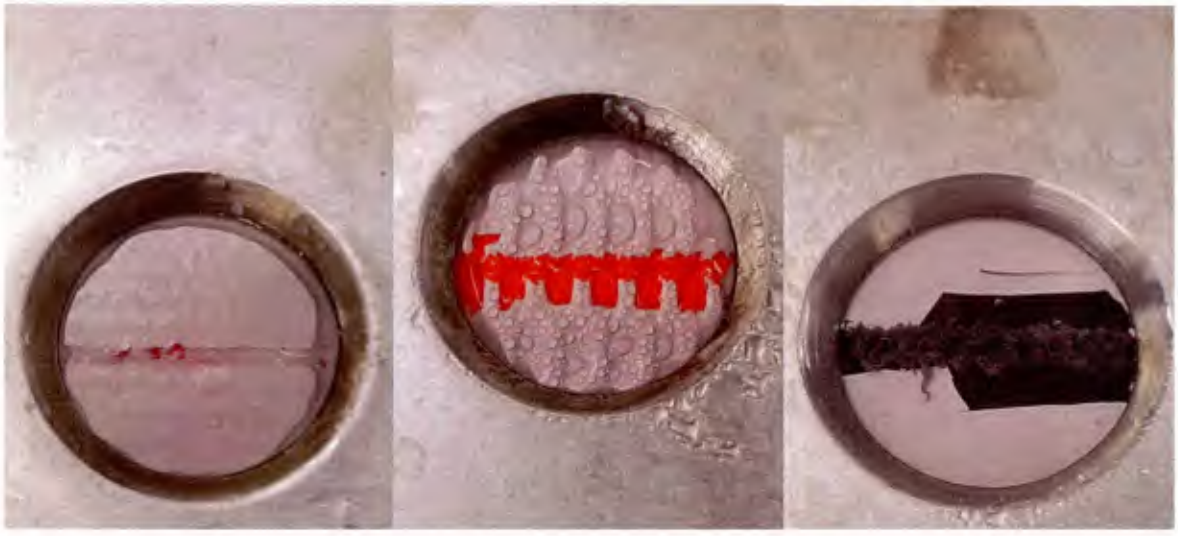

Figure II.13: From left to right: Nexus 1F, Nexus 2F, Nexus 3F failure classifications

Failures for coatings resembled those shown in the above figures and remained the same for essentially all treatment types. Two main factors that did change were the severity of failure at lower pressures, and the overall amount of failure classification at lower pressures for different treatment groups. Substrate type, surface treatment, type of coating, and water pressure were identified as four independent variables that could be used to interpret the results from adhesion testing. Various plots are shown below to illustrate this point. Because of the amount of data that was gathered, only time period 1 (30 days water ageing) will be discussed. There is a slight decrease in performance for several sample combinations, most notably in Intersleek tie-coat samples, with each increase in water ageing time period.

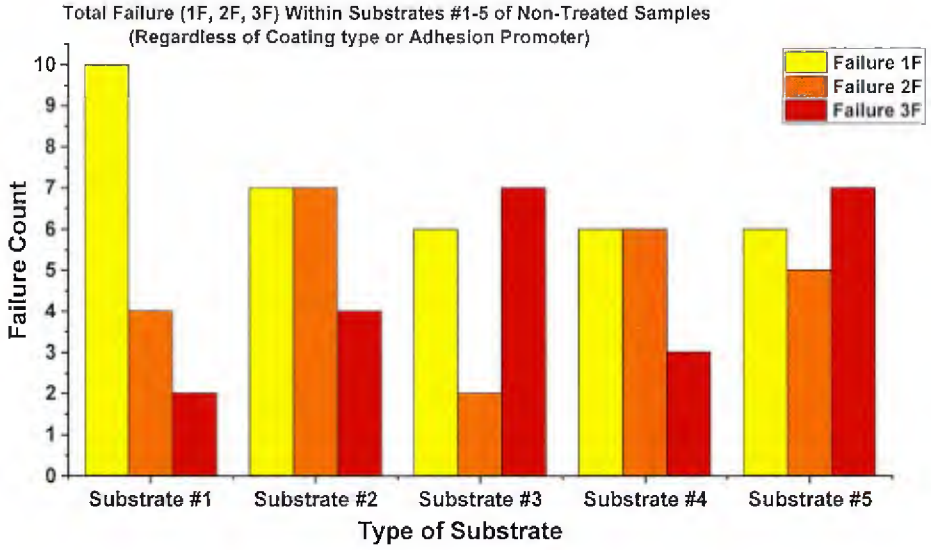

Figure II.14: Total number of failures of non-surface treated samples regardless of coating type or adhesion promoter

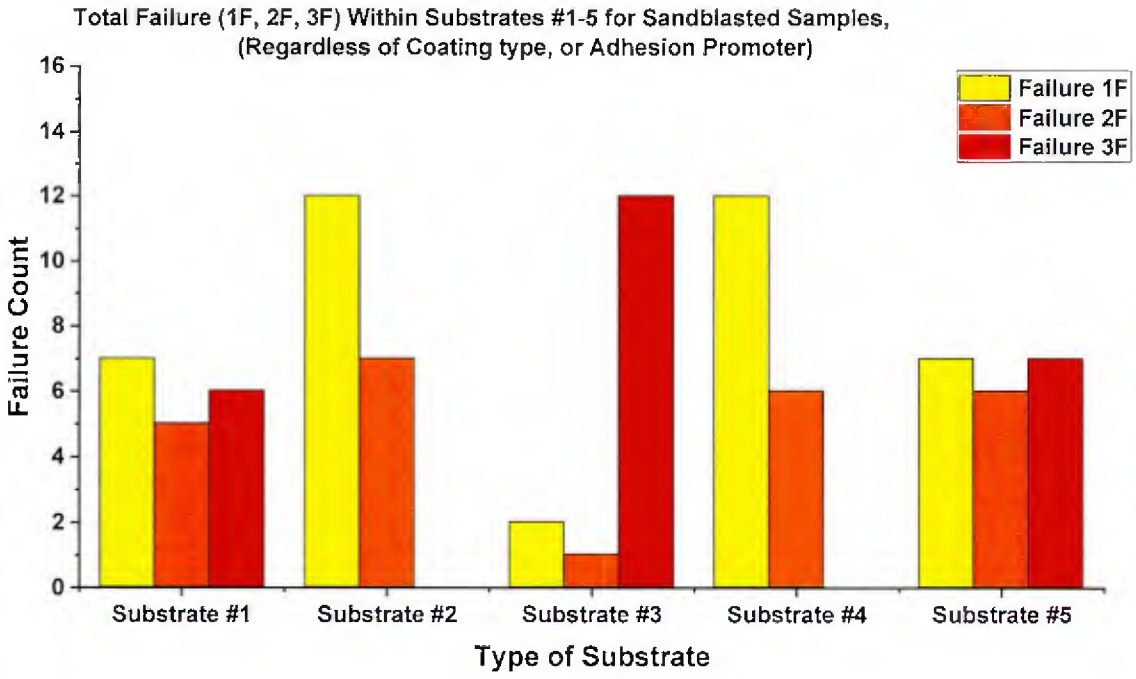

Figure II.15: Total number of failures of sandblast treated samples regardless of coating type or adhesion promoter

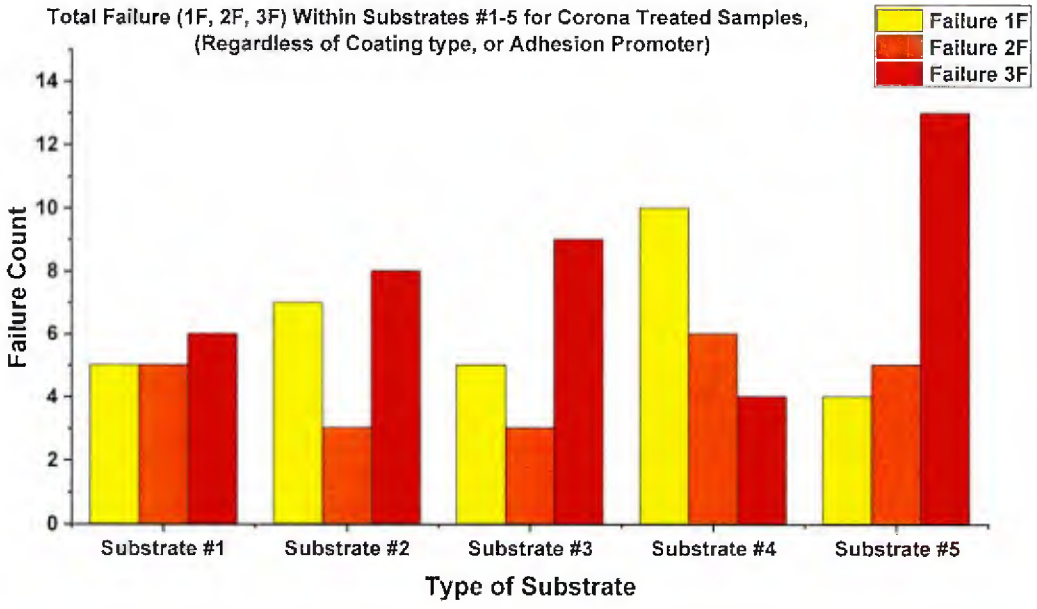

Figure II.16: Total number of failures of corona treated samples regardless of coating type or adhesion promoter.

From **Figures II.14**, **II.15** and **II.16**, a couple observations can be made. First, failure counts for substrate types within the no treatment samples remained consistent. Second, corona treated samples seemed to produce more 3F failures, signaling poorer adhesion than those of non-treated samples. Lastly, sandblasted samples performed the best in terms of overall failure count. Also, the adhesion of coatings to substrates #2 and #4 (EO and DO-T) was improved by sandblasting treatment when comparing the two other treatment groups.

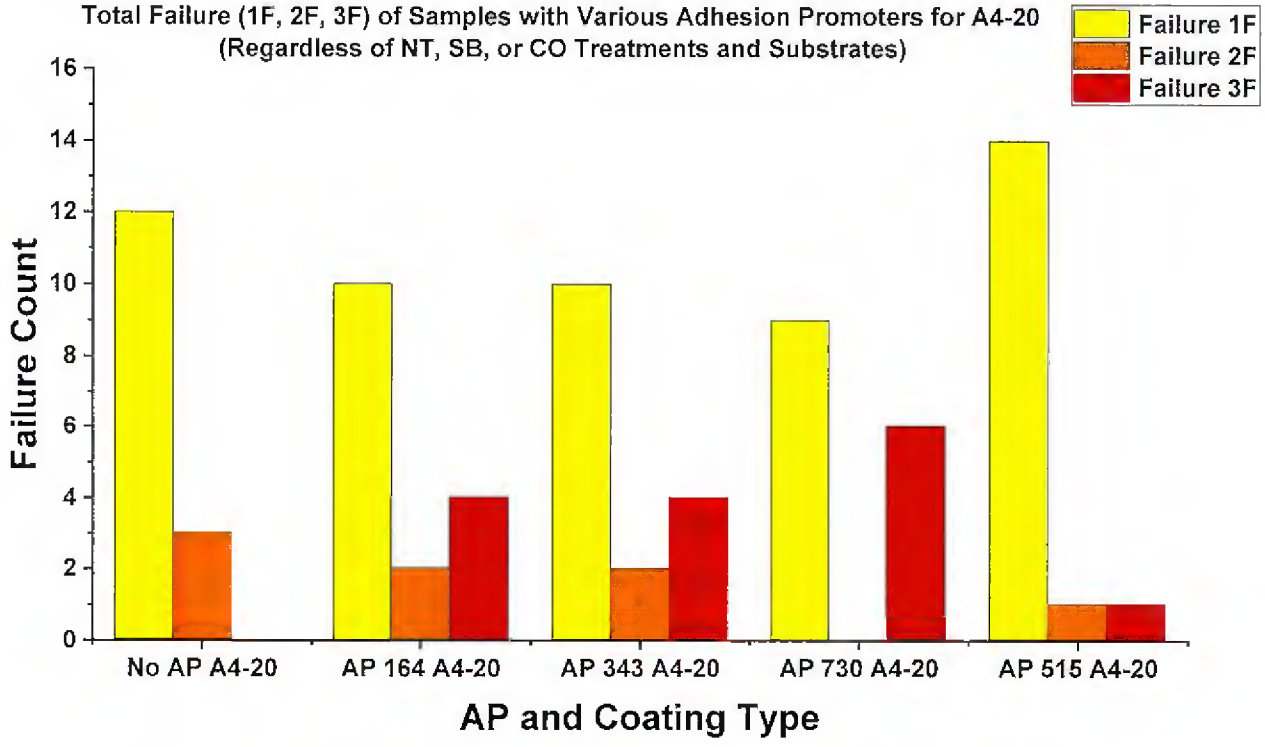

Figure II.17: Total failure count of A4-20 coatings between different adhesion promoters.

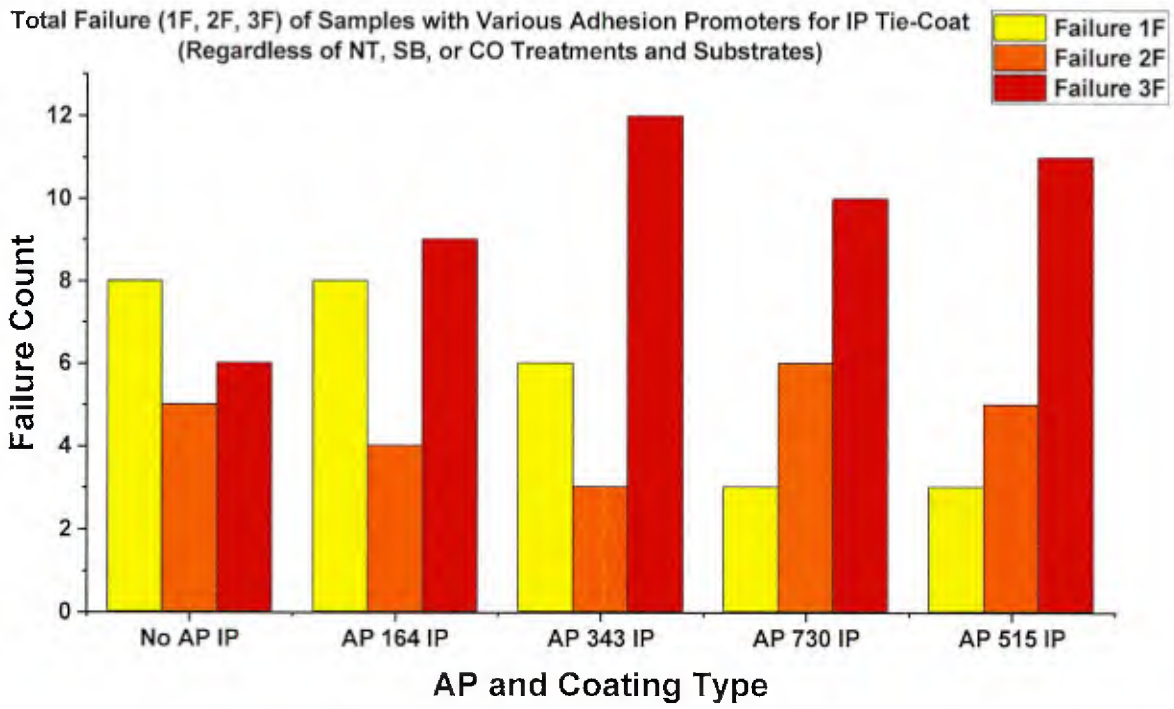

Figure II.18: Total failure count of IP tie-coat coatings between different adhesion promoters.

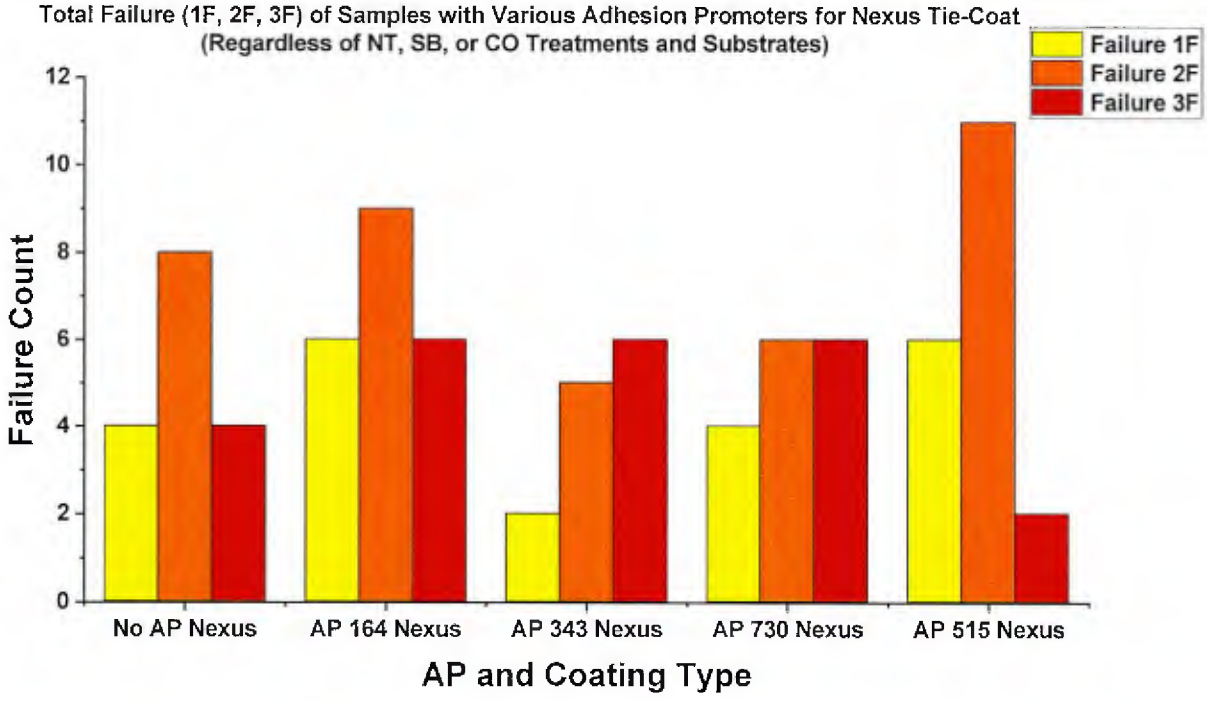

Figure II.19: Total failure count of Nexus tie-coat coatings between different adhesion promoters.

Figures II.17, **II.18** and **II.19** show the total failure counts between different adhesion promoters for the three tested coatings. Overall, it seemed as though the addition of adhesion promoter seemed to cause a decrease in performance. This is seen by increased count of 2F and 3F failures for all coatings, more pronounced for IP and Nexus tie-coats.

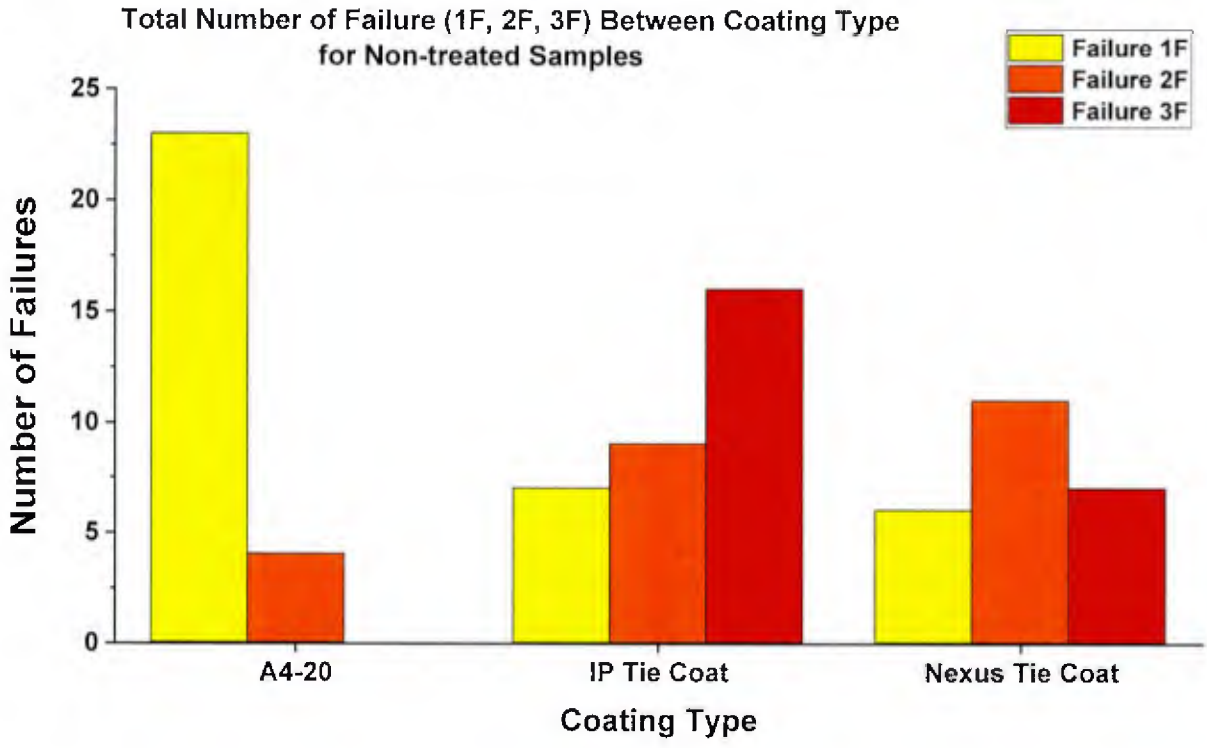

Figure II.20: Total number of failures between different coating types for non-surface treated samples.

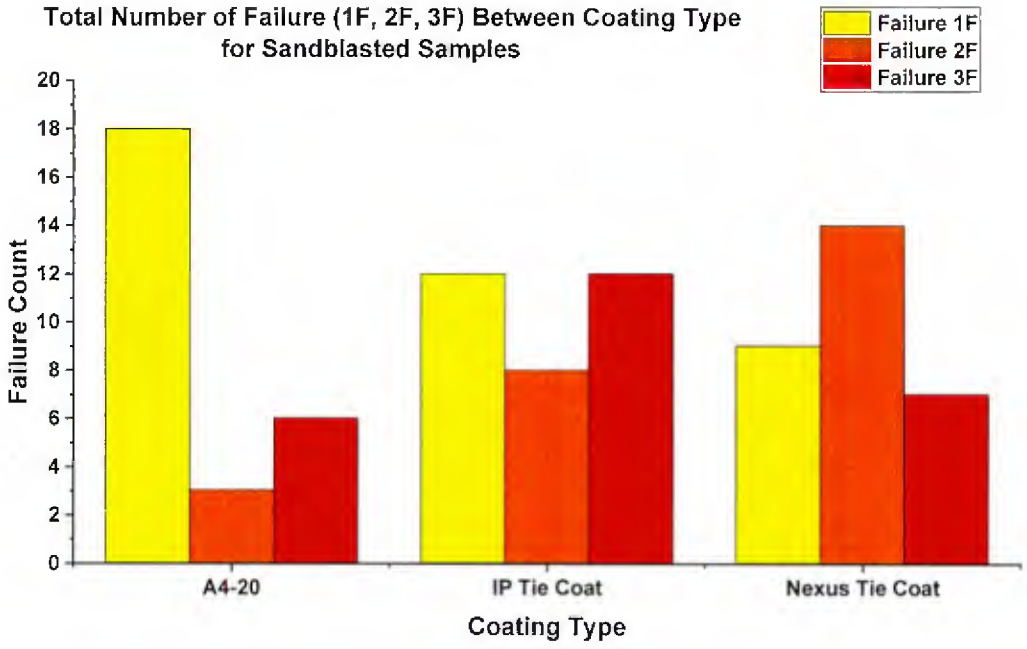

Figure II.21: Total number of failures between different coating types for sandblast treated samples.

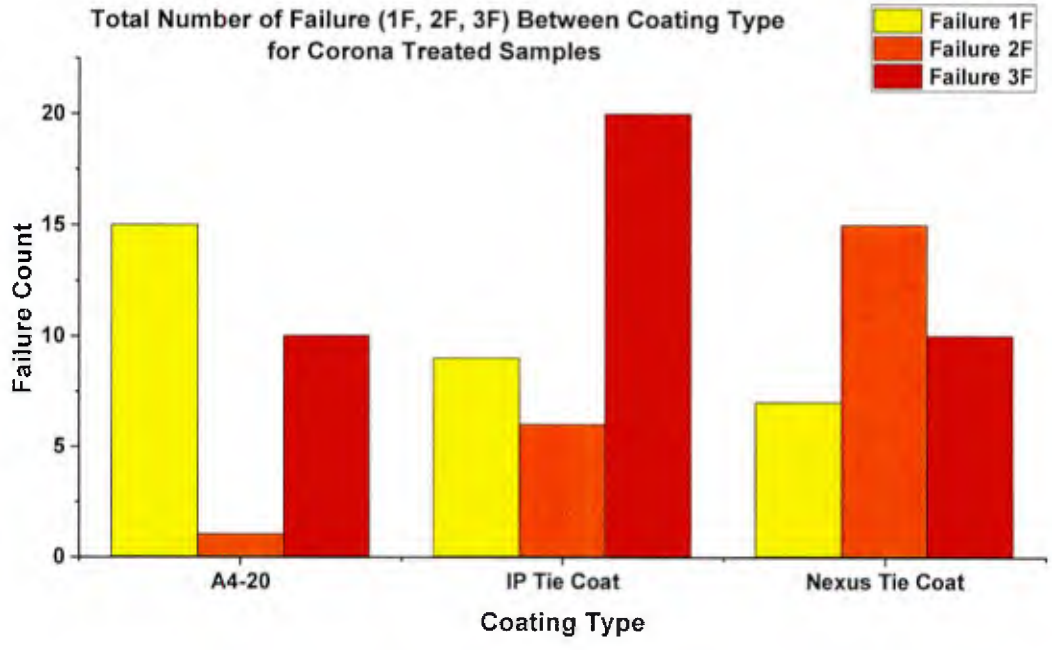

Figure II.22: Total number of failures between different coating types for corona treated samples.

Figures II.20, **II.21** and **II.22** show the differences in failure count and type for the three coatings across the different surface treatment groups. From these figures IP tie-coat performs the worst overall, with higher counts of 3F failures. When samples were subjected to sandblasting, there was slight improvement to adhesion in IP tie-coat by having more 1F and 2F failures and less 3F failures. Corona treatment seemed to negatively affect the adhesion when comparing coating types as significantly more 3F failures were recorded.

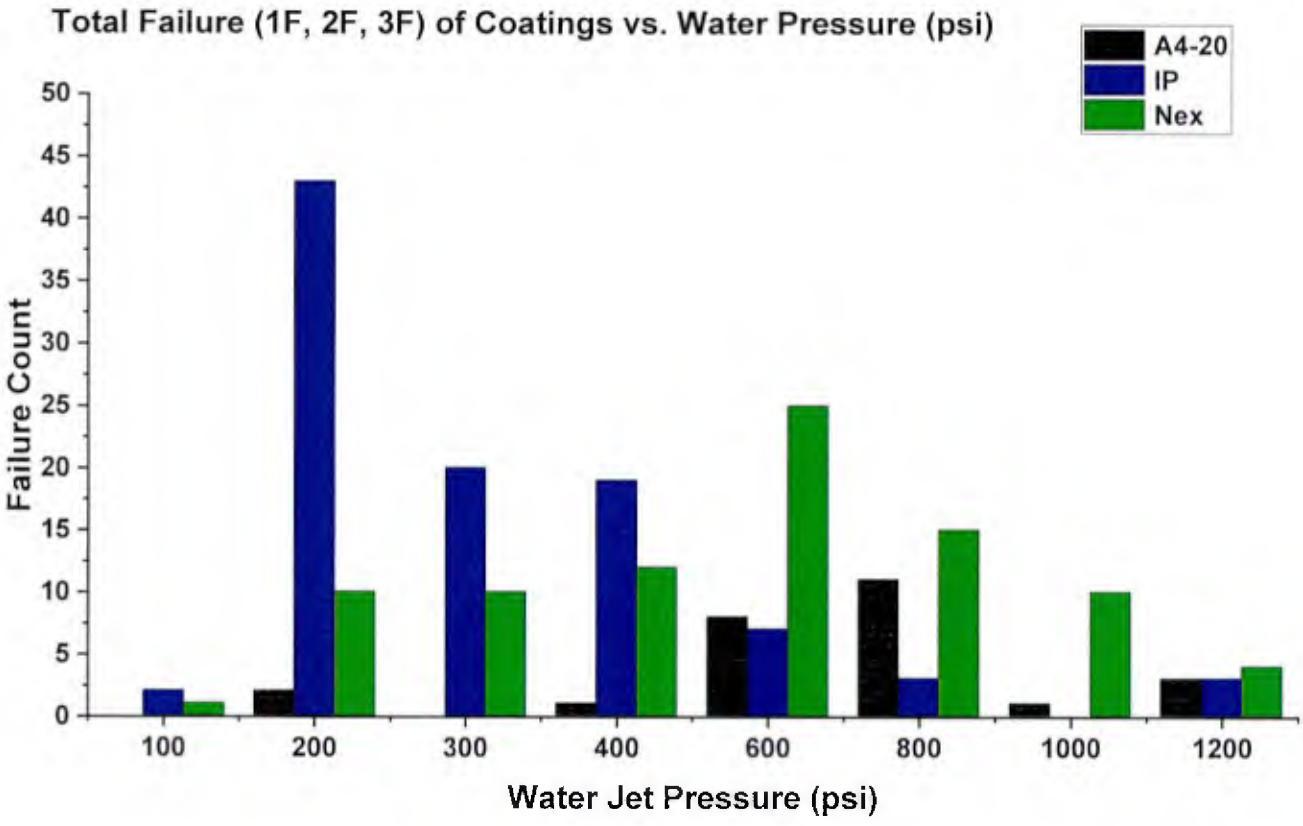

Figure II.23: Total failure counts (1F, 2F, 3F) for the different coating types at increasing pressures regardless of substrate, surface treatment, or adhesion promoter.

From the data shown in **Figure II.23**, IP tie-coat performed significantly worse than the other two coatings with high numbers of failure at the lowest pressures. Nexus tie-coat had steady failure across pressures, with less failure counts than IP tie-coat. Overall, A4-20 coating performed the best at pressures up to 600 psi, pointing to potential superior adhesion to the substrates when considering time period 1.

Further Analysis of Waterjet Adhesion for Determining Field Testing Candidates

After performing waterjet adhesion testing, there was much data to analyze. One of the main objectives of performing these adhesion testing experiments was to identify the best performing

combination of surface treatment, substrate, adhesion promoter, and coating type. Several variations of presenting this data was put forth, such as in **Figures II.14 – II.23**, but identifying a better performing system proved difficult. Therefore, another method was used to group all independent variables into one table. These variables were substrate type, surface treatment, adhesion promoters, coating type, and waterjet pressure. In this table, the other four variables are entered in respective to increasing water pressure (from 100 psi to 1200 psi). A legend for these tables is seen in **Table II.3**.

Table II.3: The four independent variables being entered in with respect to water pressure. The numbers associated with each sample type make visualization easier.

Type of Material	Coating Type	Surface Treatment	Adhesion Promoters
1 - Desmi orange	1 - A4-20	1 - No treatment	1 - No AP
2 - Desmi orange textured	2 - Intersleek tie-coat	2 - Sandblast	2 - AP 164
3 - Desmi black	3 - Nexus tie-coat	3 - Corona	3 - AP 343
4 - Elastec orange			4 - AP 730
5 - Elastec black			5 - AP 515

Each of the recorded 0F, 1F, 2F, and 3F failure ratings recorded from waterjet adhesion testing are color-coded for easier visualization of trends. 0F is colored green, 1F yellow, 2F orange, and 3F is red. **Figure II.24** is an example of how the data was arranged this way for all time periods.

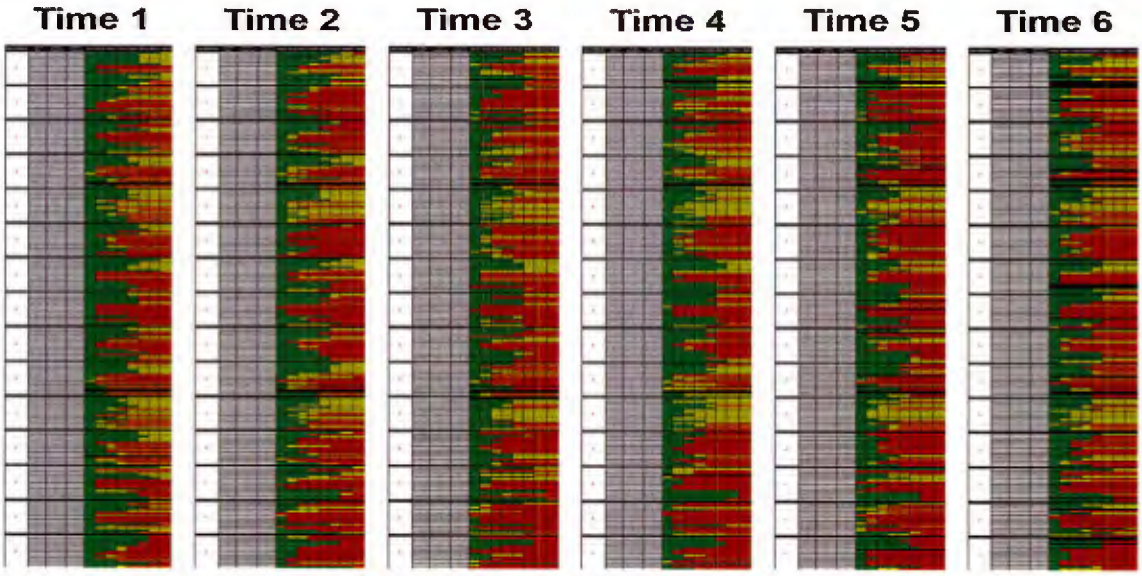

Figure II.24: Data from all independent variables are entered against water pressure. Color-coding of failure categories 0F, 1F, 2F, and 3F are green, yellow, orange, and red. Time periods 1-6 were all entered this way and offer a quicker method for picking out trends in increased adhesion performance

A table showing the data points for substrate type 1, Desmi orange, for time period 1 (30 days water ageing) is shown below in **Table II.4**.

Table II.4: Color-coded failure rating for substrate 1, time period 1. Uncolored numbers correspond to the sample types in **Table II.3**.

Type of Material	Coating Type	Surface Treatment	Adhesion Promoters	100 psi	200 psi	300 psi	400 psi	600 psi	800 psi	1000 psi	1200 psi
1	1	1	1	0	0	0	0	0	0	0	1
	1	1	2	0	0	0	0	0	1	1	1
	1	1	3	0	0	0	0	0	1	1	1
	1	1	4	0	0	0	0	0	1	1	1
	1	1	5	0	0	0	0	0	1	1	1
	2	1	1	0	0	0	0	0	0	0	1
	2	1	2	0	3	3	3	3	3	3	3
	2	1	3	0	3	3	3	3	3	3	3
	2	1	4	0	0	0	2	2	2	2	2
	2	1	5	0	2	2	2	2	2	2	2
	3	1	1	0	0	0	0	0	0	0	1
	3	1	2	0	0	0	0	1	1	1	1
	3	1	3	0	0	0	0	0	0	0	1
	3	1	4	0	0	0	0	0	0	2	2
	3	1	5	0	0	0	0	0	1	2	2
1	1	2	1	0	0	0	1	2	2	2	2
	1	2	2	0	0	0	0	1	1	1	1
	1	2	3	0	0	0	0	2	2	2	2
	1	2	4	0	0	0	0	3	3	3	3
	1	2	5	0	0	0	0	1	1	1	1
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	2	2	3	0	2	2	2	2	2	2	2
	2	2	4	0	3	3	3	3	3	3	3
	2	2	5	0	3	3	3	3	3	3	3
	3	2	1	0	0	0	0	1	1	1	1
	3	2	2	3	3	3	3	3	3	3	3
	3	2	3	0	0	0	0	0	0	2	2
	3	2	4	0	0	0	0	0	2	2	2
	3	2	5	0	0	1	3	3	3	3	3
1	1	3	1	0	0	0	0	0	0	1	1
	1	3	2	0	0	0	0	0	3	3	3
	1	3	3	0	0	0	3	3	3	3	3
	1	3	4	0	0	0	0	0	1	1	1
	1	3	5	0	0	0	0	0	0	1	1
	2	3	1	0	0	1	2	2	2	2	2
	2	3	2	0	3	3	3	3	3	3	3
	2	3	3	0	3	3	3	3	3	3	3
	2	3	4	0	0	3	3	3	3	3	3
	2	3	5	0	0	3	3	3	3	3	3
	3	3	1	0	0	0	0	0	2	2	2
	3	3	2	0	0	0	2	2	2	2	2
	3	3	3	0	0	0	0	0	0	0	2
	3	3	4	0	0	0	0	0	0	2	2
	3	3	5	0	0	1	1	1	1	1	1

By utilizing this method of data visualization, one can determine several parameters for improving adhesion of coatings to oil boom materials. One, the substrate type that had the best overall performance regardless of the other independent variables can be identified. Two, the surface treatment that offers improvement in adhesion performance can be identified. Three, the coating type that offers best overall performance can be identified. Four, the various adhesion promoters that improve adhesion can be identified. This is accomplished by counting the occurrences of greater failure (2F, 3F) during the range of water pressures for each variable being investigated. The greater number, or more orange and red color that is seen, the poorer

the performance. On the other hand, if there is a large concentration of green or yellow, this would constitute better overall performance. Using this method, the better performing combinations of surface treatment, adhesion promoter, and coating type were identified for each substrate type 1-5 as described in **Table II.3**.

Overall, it was seen that the coating A4-20 had the best performance for each substrate type. This is largely due to the flexible, yet tough nature of this self-stratified siloxane-polyurethane. Additionally, the bulk of the coating is primarily comprised of polyurethane, which we assume has more affinity for several of the substrate types due to their compositions containing polyurethane. Because of these reasons, these coatings were more likely to resist damage and adhesion loss through the actions of a forced stream of water. Nexus tie-coat performed slightly worse than A4-20 and undergoes severe coating delamination after initial damage. This is thought to be because this tie-coat is very rigid, and with the introduction of surface cracks, more susceptible to the impact forces of higher water pressure. Intersleek tie-coat performed the worst out of the coating types. This tie-coat is much more flexible and more elastic than the other coating types. Damage to these coatings occurred at very low pressures with complete coating delamination happening well before A4-20 and Nexus coatings.

In terms of surface treatment, there were a couple conclusions. First, for the non-textured, more rigid substrates, it seems as though there was no significant pattern of increased adhesion performance when using sandblasting or corona treated samples. Second, on substrate types 2 and 4 that had both textured polyurethane compositions, sandblasting of samples significantly improved adhesion. Samples had significantly more 1F ratings than 2F or 3F ratings at several water pressures. When evaluating the performance of applying adhesion promoters to the samples before coating application, there is little variation between promoters. However, in all substrate types, Eastman chlorinated polyolefin 515-2 seemed to produce more 1F failure ratings than others.

Lastly, the overall best substrate(s) to use with these combinations of surface treatment, coating type, and adhesion promoter are substrates 2 and 4. These substrates are flexible, less rigid, and have small "pockets" that makeup a textured polyurethane surface. In previous observations it was seen that cracking occurred on these substrates with both A4-20 and Nexus tie-coats. This is typically an indicator of poor adhesion performance, however, throughout waterjet testing these substrates consistently performed better. This is thought to be due to the force of water being directed into the multiple pockets along the fabrics surface. The coating surrounding these pockets on the "flat" portion of the substrate may not be subject to the higher water pressure; thus less delamination is seen. This is illustrated in **Figure II.25**.

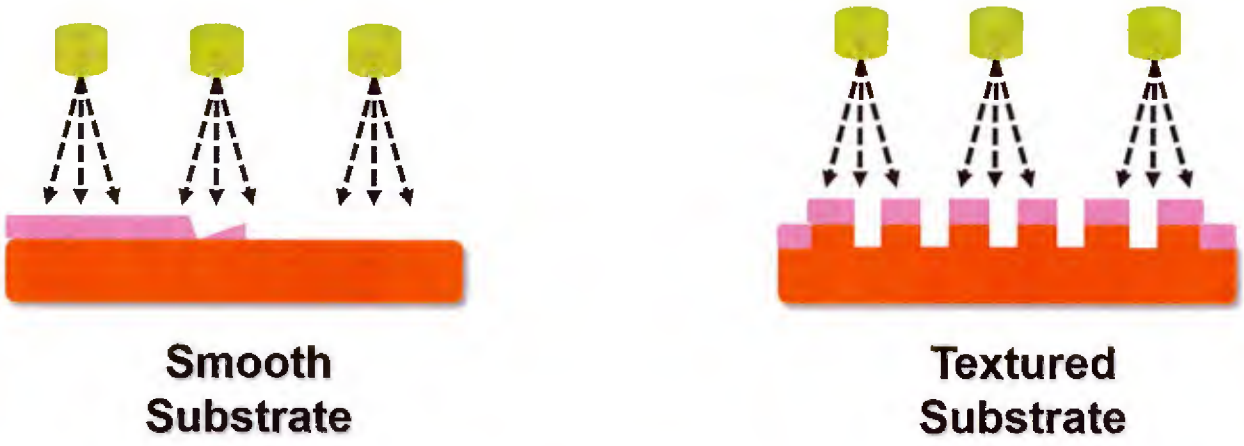

Figure II.25: On smooth substrates, once coating damage occurs the forces of water penetrate under the film more readily, leading to quicker complete delamination. On textured substrates more force is directed into pockets of the substrate and coating on the ridges is less susceptible to adhesion loss and damage.

The combinations of coating type, surface treatment, and adhesion promoter that performed the best overall in adhesion evaluation for each substrate type is shown in **Table II.5**.

Table II.5: Best performing combinations of independent variables for each substrate type

Substrate	Coating Type	Surface Treatment	Adhesion Promoter
1 - Desmi orange	A4-20	No Treatment	AP 515
2 - Desmi orange textured	A4-20	Sand Blasting	AP 515
3 - Desmi black	A4-20	No Treatment	AP 515
4 - Elastec orange	A4-20	Sand Blasting	AP 515
5 - Elastec black	A4-20	No Treatment	AP 515

Field Testing of Larger Samples

After identifying some better performing combinations of the described variables from waterjet adhesion testing, larger samples of these were made and sent to Florida Institute of Technology to be evaluated. Some of the questions that we are interested in are as follows. Do the coatings extend the intervals between necessary cleaning? Do the coatings allow for a gentler cleaning procedure? Do the coatings hold up to repeated cleanings? Can the cleaning be easily done in water? To potentially answer these questions, a total of 63 samples, 6" wide by 24" long, were prepared and their compositions detailed in **Table II.6**. There is a total of four experimental samples, with two control sets used for testing. First is a smooth substrate (substrate #1) that is coated with A4-20. Three different testing periods with three replicates each were chosen. The first period is to let replicates foul continuously for the duration of field testing, second is to clean samples with a fan tip every 4 weeks, and third is to clean every 8 weeks with a fan tip. This

setup for fouling and cleaning periods is repeated for each sample set. The second experimental set is the textured substrate #2, which was sandblasted, and then coated with A4-20. Third and fourth experimental sets are substrate #1 and sandblasted substrate #2 with Nexus tie-coat, followed by the topcoat Hempasil X3+. The last two sets were control samples of both the smooth substrate #1 and the textured #2.

Table II.6: Sample ID's assigned to the various experimental and control sets of samples

Sample ID	Description
1A 1B 1C	Hasibit smooth PU substrate (Substrate #1) coated with A4-20. Replicates are labeled as A, B, and C and will **FOUL CONTINUOUSLY** to determine cleaning interval
2A 2B 2C	Same as in sample 1A, B, C but will be cleaned every **4 WEEKS** with fan tip
3A 3B 3C	Same as in sample 1A, B, C but will be cleaned every **8 WEEKS** with fan tip
4A 4B 4C	Cooley textured PU substrate (Substrate #2), sandblasted and then coated with A4-20. Replicates are labeled as A, B, and C and will **FOUL CONTINUOUSLY** to determine cleaning interval
5A 5B 5C	Same as in sample 4A, B, C but will be cleaned every **4 WEEKS** with fan tip
6A 6B 6C	Same as in sample 4A, B, C but will be cleaned every **8 WEEKS** with fan tip
7A 7B 7C	Hasibit smooth PU substrate (Substrate #1) coated with Hempasil X3+. Replicates are labeled as A, B, and C and will **FOUL CONTINUOUSLY** to determine cleaning interval
8A 8B 8C	Same as in sample 7A, B, C but will be cleaned every **4 WEEKS** with fan tip
9A 9B 8C	Same as in sample 7A, B, C but will be cleaned every **8 WEEKS** with fan tip
10A 10B 10C	Cooley textured PU substrate (Substrate #2), sandblasted and then coated with Hempasil X3+. Replicates are labeled as A, B, and C and will **FOUL CONTINUOUSLY** to determine cleaning interval
11A 11B 11C	Same as in sample 10A, B, C but will be cleaned every **4 WEEKS** with fan tip
12A 12B 12C	Same as in sample 10A, B, C but will be cleaned every **8 WEEKS** with fan tip
13A 13B 13C	Hasibit smooth PU substrate (Substrate #1) control. Replicates are labeled as A, B, and C and will **FOUL CONTINUOUSLY** to determine cleaning interval

14A 14B 14C	Same as in sample 13A, B, C but will be cleaned every **4 WEEKS** with fan tip
15A 15B 15C	Same as in sample 13A, B, C but will be cleaned every **8 WEEKS** with fan tip
16A 16B 16C	Cooley textured PU substrate (Substrate #2) control. Replicates are labeled as A, B, and C and will **FOUL CONTINUOUSLY** to determine cleaning interval
17A 17B 17C	Same as in sample 16A, B, C but will be cleaned every **4 WEEKS** with fan tip
18A 18B 18C	Same as in sample 16A, B, C but will be cleaned every **8 WEEKS** with fan tip

Application of Coatings

Before applying coatings to samples, the substrate types were first cut to size using a band saw and a jigsaw to get near uniform dimensions. After cutting the substrates, a die-punch was used to punch holes into the top of the substrates so that they could be easily bolted to the testing platforms used at Florida Institute of Technology. This can be seen in **Figure II.26**.

Figure II.26: A cut sample with holes punched out for mounting

All samples of substrate #2 were sandblasted prior to coating. Application of coatings was performed in a similar manner for all formulations. First, surfaces of fabrics were brushed clean from any debris. Next, coatings formulations were mixed according to established procedures and applied to the substrates using a 9" roller with ¾" high density polyester nap. Both sides of the substrates were coated and images of the Nexus tie-coat, Hempasil X3+ tie-coat, and A4-20 coating are shown in **Figures II.27-29**.

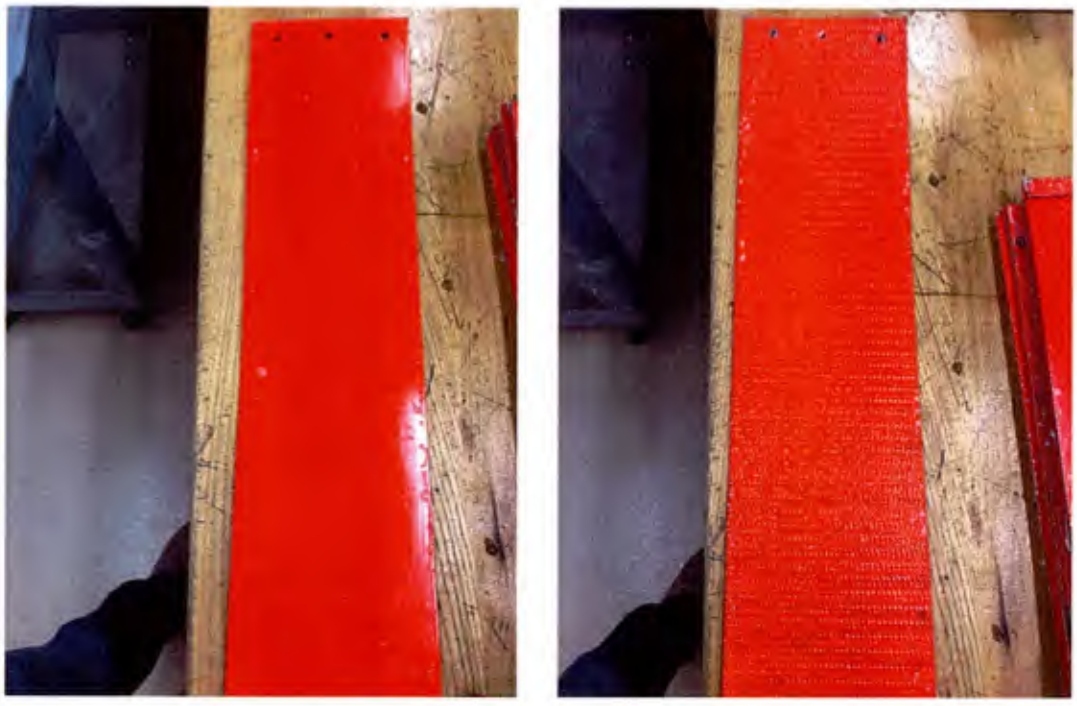

Figure II.27: Left; Smooth substrate #1 coated with transparent A4-20. Right; Textured substrate #2 coated with A4-20

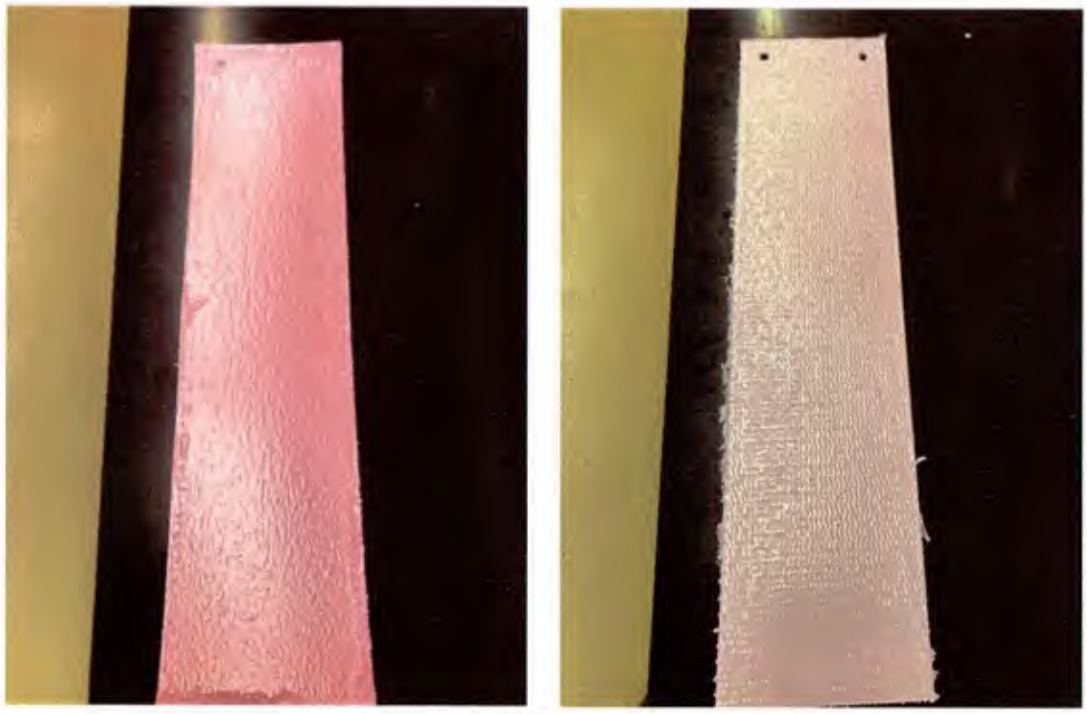

Figure II.28: Left; Smooth substrate #1 coated with Nexus tie-coat. Right; Textured substrate #2 coated with Nexus tie-coat

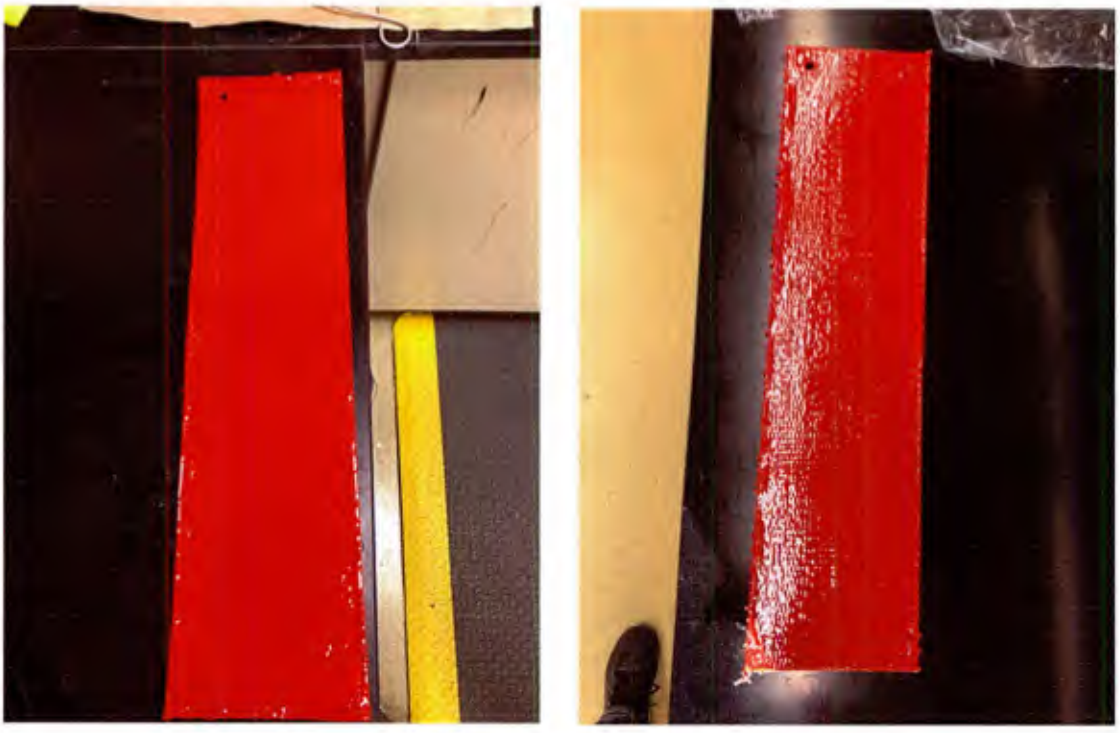

Figure II.29: Left; Smooth substrate #1 coated with topcoat Hempasil X3+. Right; Textured substrate #2 coated with topcoat Hempasil X3+

Coatings were able to be applied to these larger substrates with relative ease. However, when applying the Nexus tie-coat to the smooth substrate #1, which is more rigid and had slight curve to it, cured coatings experienced cracking with slight flexing. This could prove troublesome during deployment of these substrates and is shown in **Figure II.30**.

Figure II.30: Curve seen in substrate #1 samples coated with Nexus tie-coat

After all coatings were applied, additional holes were punched at the top of each sample for placement of ID tags. These aluminum tags were punched with each samples respective ID number/letter combination shown in **Table II.6**. Fastening of these tags to the samples was performed with UV-resistant zip-ties as shown in **Figure II.31**.

Figure II.31: Sample with ID tag attached

These coated samples were then shipped to FIT and were bolted to testing racks and suspended in the marine environment. Analysis of cleaning after four weeks is still pending.

References

1. Bodkhe, R. B.; Thompson, S. E. M.; Yehle, C.; Cilz, N.; Daniels, J.; Stafslien, S. J.; Callow, M. E.; Callow, J. A.; Webster, D. C., The Effect of Formulation Variables on Fouling-Release Performance of Stratified Siloxane-Polyurethane Coatings. *Journal of Coatings Technology Research* **2012,** *9* (3), 235.
2. Tantec LabTEC. http://www.directindustry.com/prod/tantec/product-15078-427306.html (accessed June, 6th).

III. Comprehensive, Biological Laboratory Testing for ONR Principal Investigators

III.1 Technical Objectives

The primary objective of this project task is to provide both NDSU-PIs and ONR-PIs with prompt, robust and practically useful feedback regarding the anti-biofouling properties of environmentally-friendly material/coating technologies that they are developing for the ONR using the systematic approach described below.

III.2 Technical Approach

NDSU employed its state-of-the-art, high-throughput (HT) biological screening workflow to assess the FR performance of new environmentally-benign biofouling control coatings. The rapid biological laboratory assays that comprise this screening workflow have been shown to correlate with static ocean immersion testing conducted at all four ONR affiliated field testing sites, namely, the Florida Institute of Technology (Melbourne, Florida), University of Hawaii (Honolulu, HA), National University of Singapore and California Polytechnic State University (San Louis Obispo, CA). These correlations demonstrate the effectiveness of using the HT biological screening workflow to rapidly assess and down-select coatings for static ocean immersion testing.

Primary screening of the FR properties of coatings was evaluated by assessing their ability to prevent or minimize the attachment and/or growth and adhesion of bacteria and microalgae. ASW suspensions of the microorganisms were dispensed into the wells of the coating array plates and directly onto the coating surfaces. The coating array plates were then incubated at the appropriate conditions to facilitate cell attachment and/or biofilm growth. Bacterial biomass attached to the coating surfaces was quantified using a compatible spectrophotometric assay while the amount of microalgae biomass was determined by measuring the fluorescence of chlorophyll. Bacterial biofilm and microalgae cell adhesion was assessed by treating the coating surfaces with a precisely controlled, pressurized jet of ASW water using a custom-built apparatus. A series of internal and commercial standards (International Paint Intersleek 700, 900 and 1100 SR FR coating systems) were evaluated in parallel with the experimental coatings to aid in the disqualification of poor performing compositions and identification of those that exhibit good AF/FR properties.

In addition to microfouling assessments, an adult barnacle reattachment adhesion was employed as a means to assess the FR properties of coatings towards macrofouling. Adult barnacles were reared on silicone coated glass panels at the Duke University Marine Laboratory (Prof. Daniel Rittschof) and shipped to NDSU for reattachment studies throughout the duration of the project. The adult barnacles were dislodged from the silicone rearing substrate and placed on the surface of experimental coatings prepared in multi-well stamped or fully coated aluminum panels. The panels were then placed in a semi-automated, re-circulating ASW tank for two weeks to facilitate attachment to the coating surfaces. The attached barnacles were measured for adhesion strength using a semi-automated force gauge device, in accordance with ASTM D5618-94. Adult ribbed mussels (3-5 cm) were also received from DUML and were maintained at NDSU for year-round testing. Six mussels were immobilized onto the experimental surfaces and allowed to attach for three days via immersion in ASW tanks. The attached mussels were measured for adhesion strength using an automated tensile force gauge.

III.3 Accomplishments

For the program in its entirety, NDSU completed comprehensive biological laboratory efficacy assessments for 6 ONR PIs and 3 discretionary testing collaborators, comprising 428 unique coating compositions and 12,703 replicates/surfaces analyzed (**Figure III.3-1**).

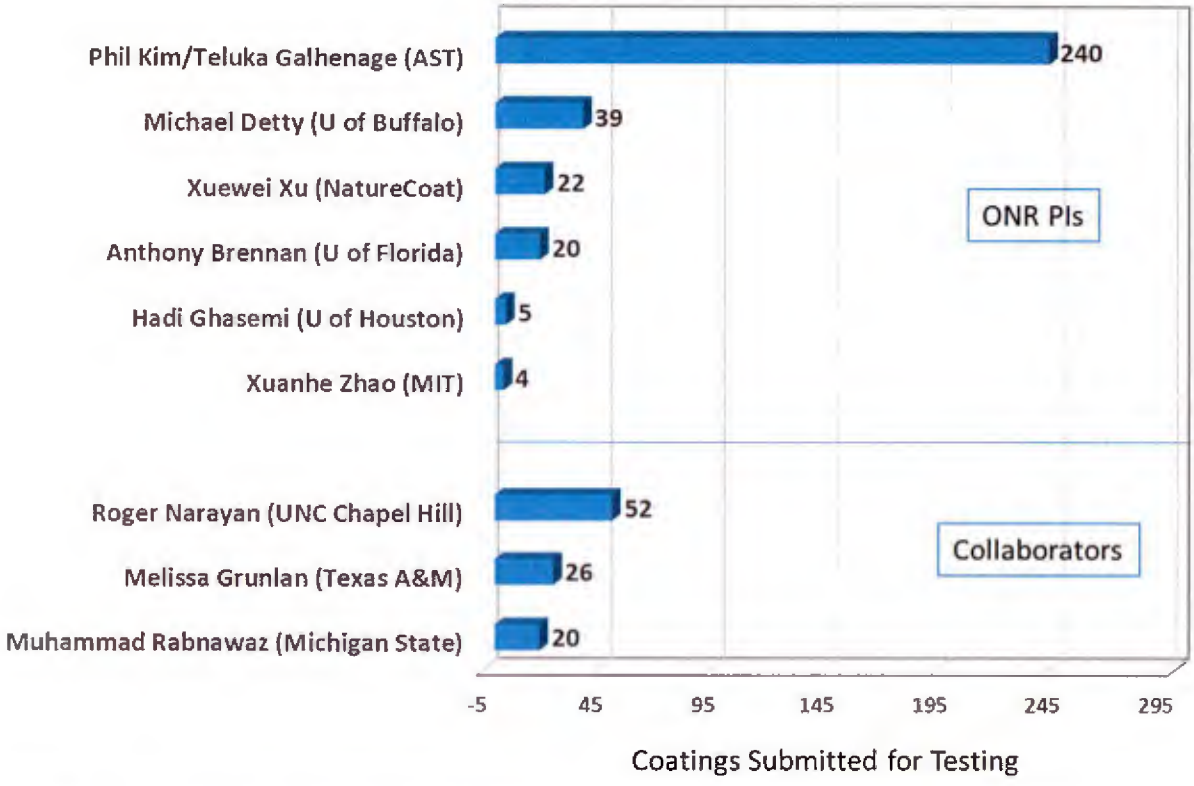

Figure III.3-1. Total number of unique coating compositions submitted by ONR PIs (top) and discretionary testing collaborators (bottom).

The most prolific user of NDSU's testing services among the ONR PIs was Dr. Philseok Kim and Dr. Teluka Galhenage of Adaptive Surface Technologies (AST; formerly SLIPS Inc.), having submitted 240 coatings for assessments toward the full complement of marine fouling organisms; bacteria, microalgae, barnacles and mussels. In this regard, AST leveraged NDSU's rapid testing capability to systematically screen the interactions and impact of multiple formulation variables on the broad-spectrum antifouling properties of SLIPs based marine coatings, including developmental batches of their recently released commercial product SLIPS Foul Protect™, with individual sets comprising up to 37 compositions. Professor Michael Detty at the University of Buffalo submitted a total of 39 coatings over the course of the program to explore optimal ratios of xerogel formulation components for biofouling mitigation while Prof. Anthony Brennan at the University of Florida submitted 20 unique compositions to investigate the antifouling properties of polymer grafted silicone-based coatings. In addition, NatureCoat submitted 22 hydrogel coatings, Prof. Hadi Ghasemi (U of Houston) submitted 5 stress-localized durable coatings, and Prof. Xuanhe Zhao (MIT) submitted 4 actuatable durable hydrogel coatings for general biofouling testing and property assessments.

Discretionary testing for non-ONR funded collaborators included Prof. Roger Narayan at the University of North Carolina-Chapel Hill who submitted 52 antimicrobial treated/coated glass, silicon, surgical gauze and microneedles for transdermal delivery of therapeutics; Prof. Melissa Grunlan at Texas A&M University who submitted 26 unique coatings based on silicones

containing PEO-silane amphiphiles; and Prof. Rabnawaz at Michigan State University who submitted a set of 20 unique coatings based on silicone containing quaternary ammonium compounds.

Since the last interim progress report, AST (Teluka Galhenage) submitted a single set of coatings comprising 9 unique compositions for biofouling assessments toward bacteria, microalgae and mussels. AST also submitted a series of 5 compounds for toxicity testing toward bacteria and microalgae. Two sets of coatings were received Prof. Tonks at the University of Florida comprising 6 unique materials and 1 set of coatings were submitted by NatureCoat comprising 4 unique compositions for testing toward bacteria, microalgae and barnacles. The key highlights and findings from the testing projects are summarized in the following sections.

Adaptive Surface Technologies (AST) – Philseok Kim/Teluka Galhenage

The first set of coatings received from AST consisted of nine unique coating types (designated as 'F1' through 'F9') and were assessed for their antifouling properties toward bacteria, microalgae and mussels. **Figure III.3-2** provides the results of the initial 2 hr cell attachments with the marine microalgae diatom, *Navicula incerta*. Among the experimental coatings, formulations F1, F2, F7 and F9 reduced attachment by approximately 40% when compared to the rest of the formulations and were shown to be comparable to the Intersleek 1100 SR (1100 SR) commercial FR coating from AkzoNobel.

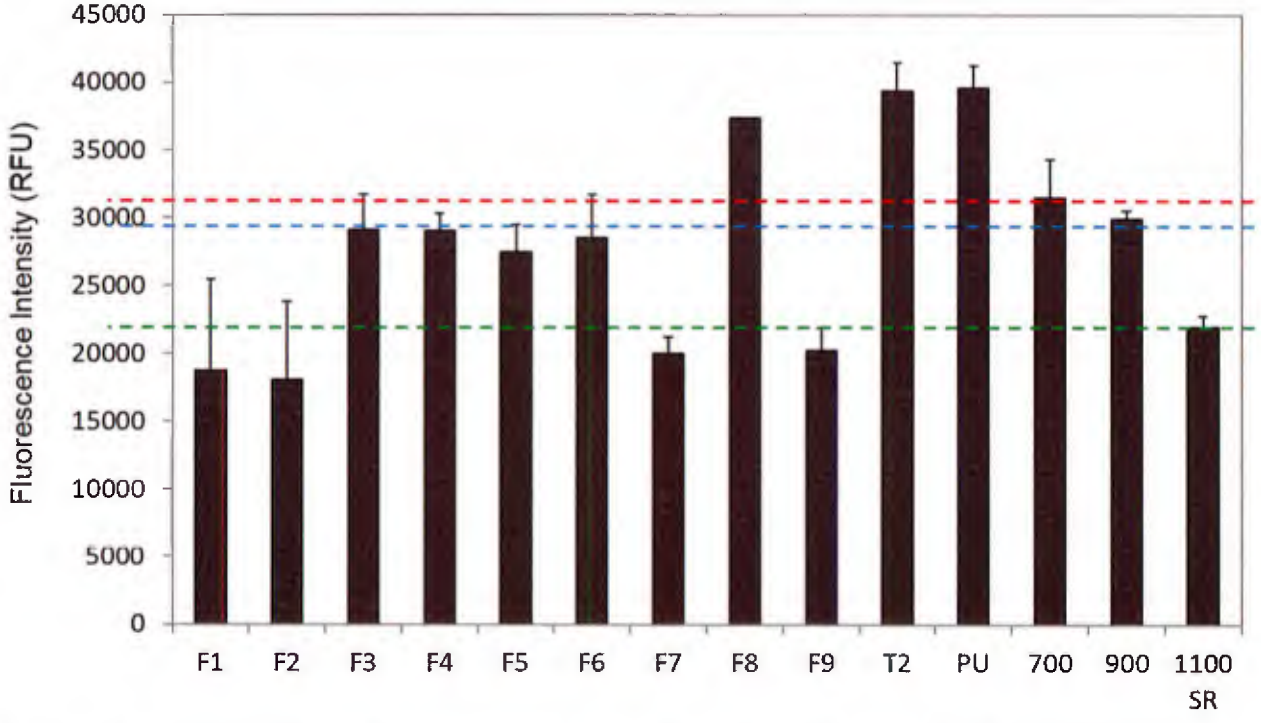

Figure III.3-2. *Navicula incerta* cell attachment as quantified via fluorescence of chlorophyll (excitation: 360nm; emission: 670nm). Each bar indicates the mean relative fluorescence unit (RFU) value of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

The results of the cell adhesion assessments for *N. incerta* after 2 hours of initial attachment are provided in **Figure III.3-3**. All nine of the AST experimental coatings, except F8, facilitated

excellent removal of attached cells with formulations F1, F2, F4, F7 and F9 outperforming the Intersleek 700 (700), Intersleek 900 (900) and 1100 SR commercial FR coating standards at the lower water jetting pressure evaluated (i.e. 10 psi). Formulations F3 and F6 showed comparable performance to 1100 SR while formulation F5 exhibited similar cell removal as 900. At the higher water jetting pressure (i.e. 20 psi), all experimental formulations, except F8, achieved almost complete cell removal and were comparable in performance to the 900 and 1100 SR commercial standards.

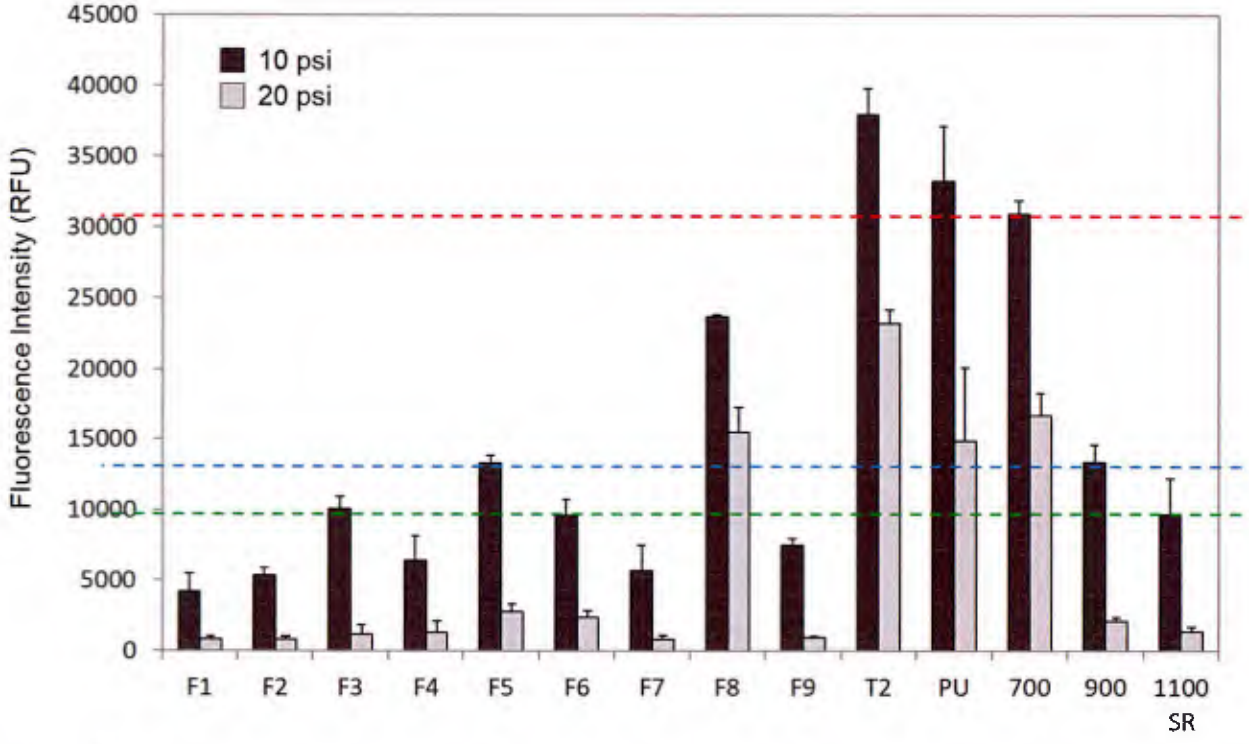

Figure III.3-3. *Navicula incerta* cell adhesion determined with an automated water jetting technique (10 psi and 20 psi, 10 second water jetting duration). Fluorescence measurement of chlorophyll was used to quantify the amount of cells remaining after water jetting treatments (RFU). Each bar indicates the mean cells/biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 10 psi water jetting pressure.

Figure III.3-4 summarizes the results of biofilm growth on the coating surfaces for the marine bacteria, *Cellulophaga lytica*. The accumulation of biofilm growth was similar for all nine formulations, with coating F9 showing the best performance, and exhibited an approximate 40% biofilm growth reduction when compared to the three Intersleek FR standards.

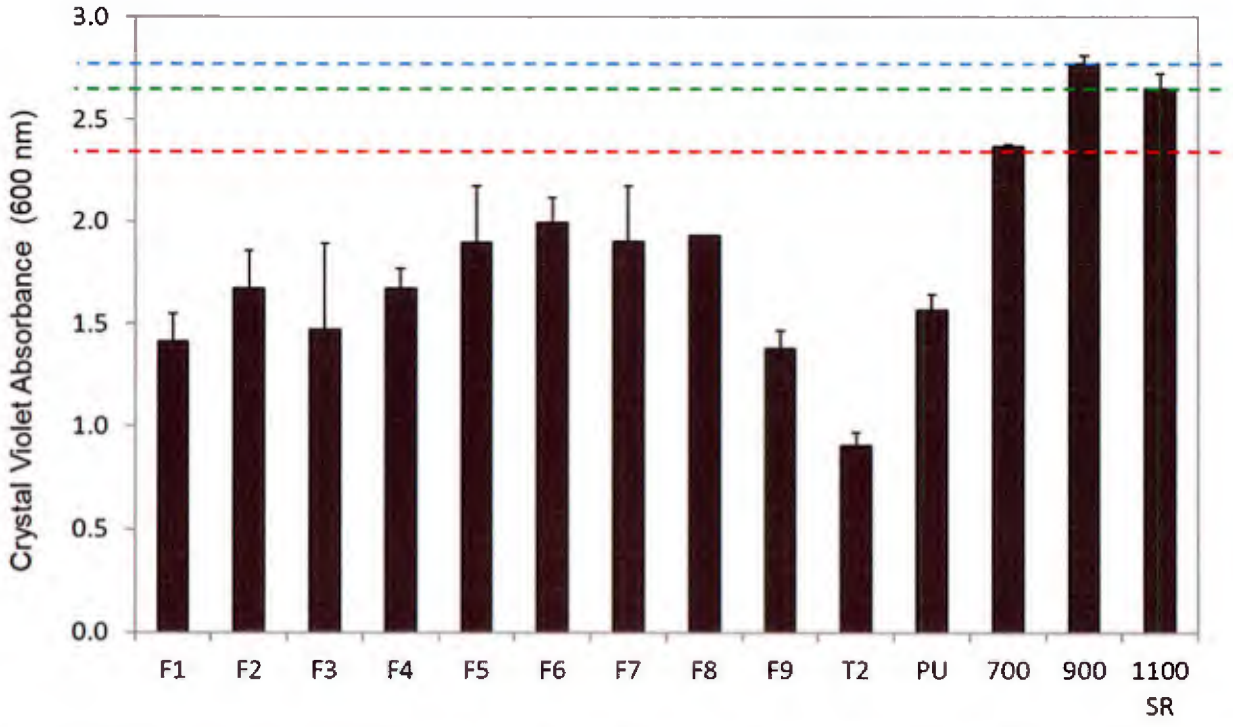

Figure III.3-4. *Cellulophaga lytica* biofilm growth quantified via crystal violet staining. Each bar indicates the mean crystal violet absorbance value (600 nm) of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

The results of the *C. lytica* biofilm retraction assessments are provided in **Figures III.3-5 and III.3-6**. Except for formulation F8, the experimental coatings induced a markedly higher degree of biofilm retraction when compared to the Intersleek FR standards. Retraction was most pronounced for Coating F9 at 10% surface coverage, followed by formulations F1 and F2 at approximately 20% surface coverage. Formulations F3-F7 possessed an approximate 40% surface coverage, while F8 was ineffective at inducing retraction of the attached biofilm (i.e. 100% surface coverage).

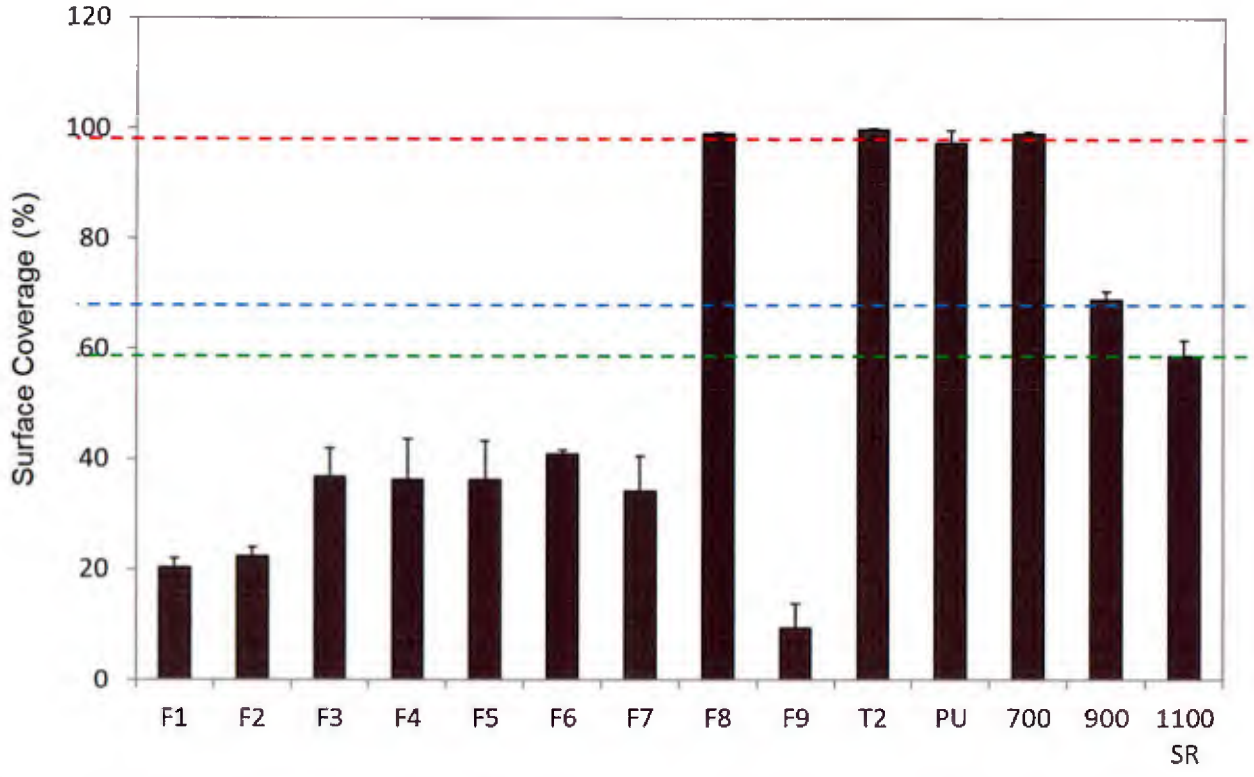

Figure III.3-5. *Cellulophaga lytica* biofilm retraction quantified via crystal violet staining and percent surface coverage measurements. Each bar indicates the mean percent surface coverage value of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

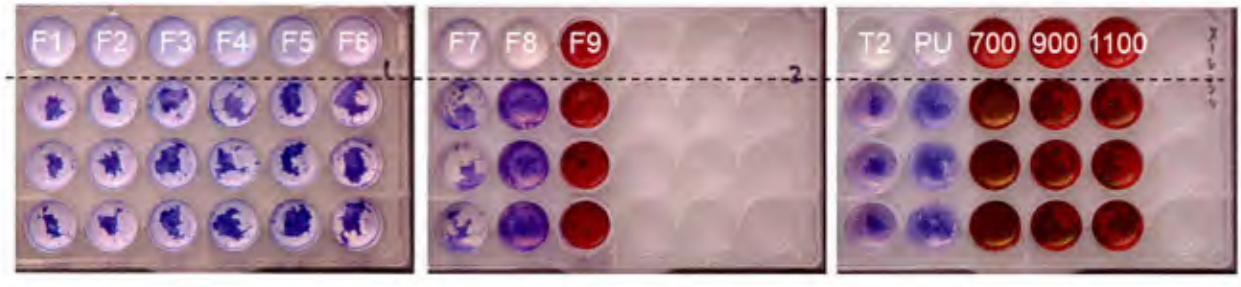

Figure III.3-6. Images of *C. lytica* biofilm retraction after staining with biomass indicator dye crystal violet.

The results of the biofilm adhesion assessments for *C. lytica* are summarized in **Figure III.3-7**. Except for formulation F8, all experimental coating compositions facilitated excellent removal of attached biofilm at the lower 10 psi water jetting pressure and outperformed the Intersleek commercial FR standards. In this regard, formulations F1-F4 achieved almost complete biofilm removal.

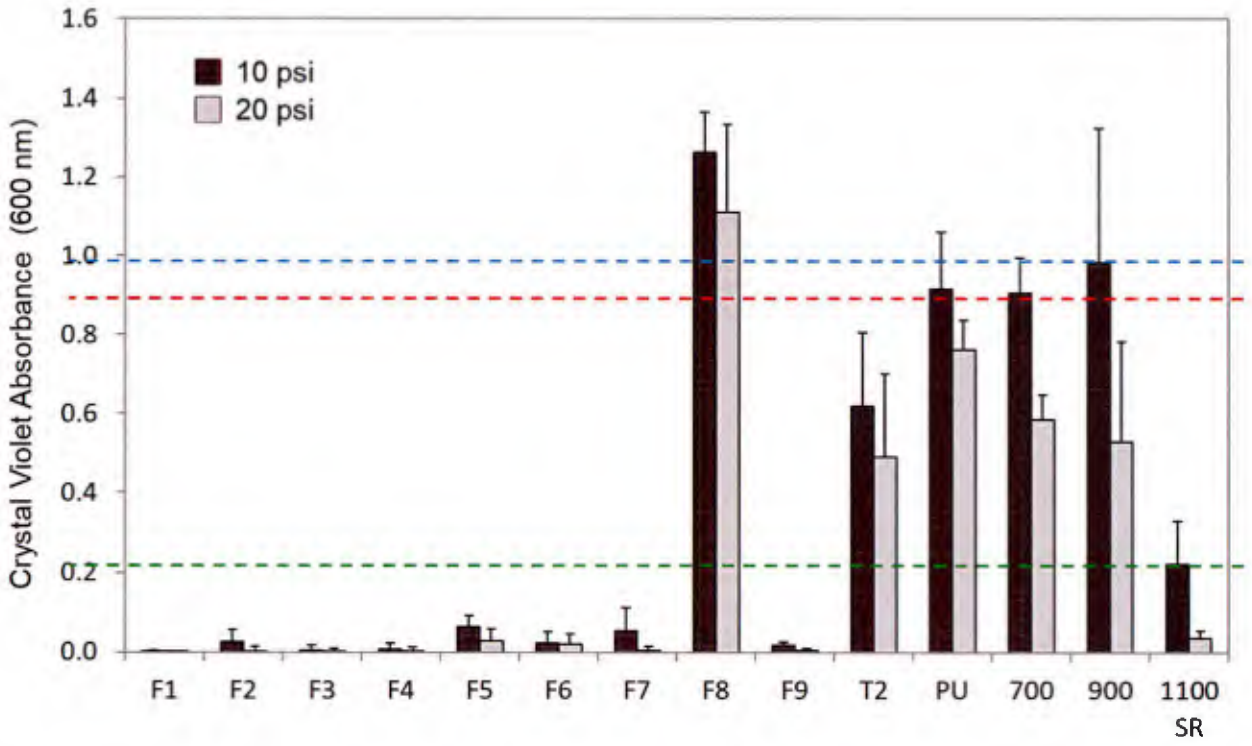

Figure III.3-7. *Cellulophaga lytica* biofilm adhesion determined with an automated water jetting technique (10 and 20 psi, 5 seconds water jetting duration). A crystal violet colorimetric assay was used to quantify the amount of biofilm remaining after water jetting treatments. Each bar indicates the mean biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 10 psi water jetting pressure.

Figure III.3-8 summarizes the results of biofilm adhesion assessments for the marine bacterium, *Halomonas pacifica*. The results of these assessments were nearly identical to those observed with *C. lytica* discussed above (**Figure III.3-7**), with formulations F1-F7 facilitating almost complete removal of the biofilm at the lower 15 psi water jetting pressure and on par with the 1100 SR commercial FR standard.

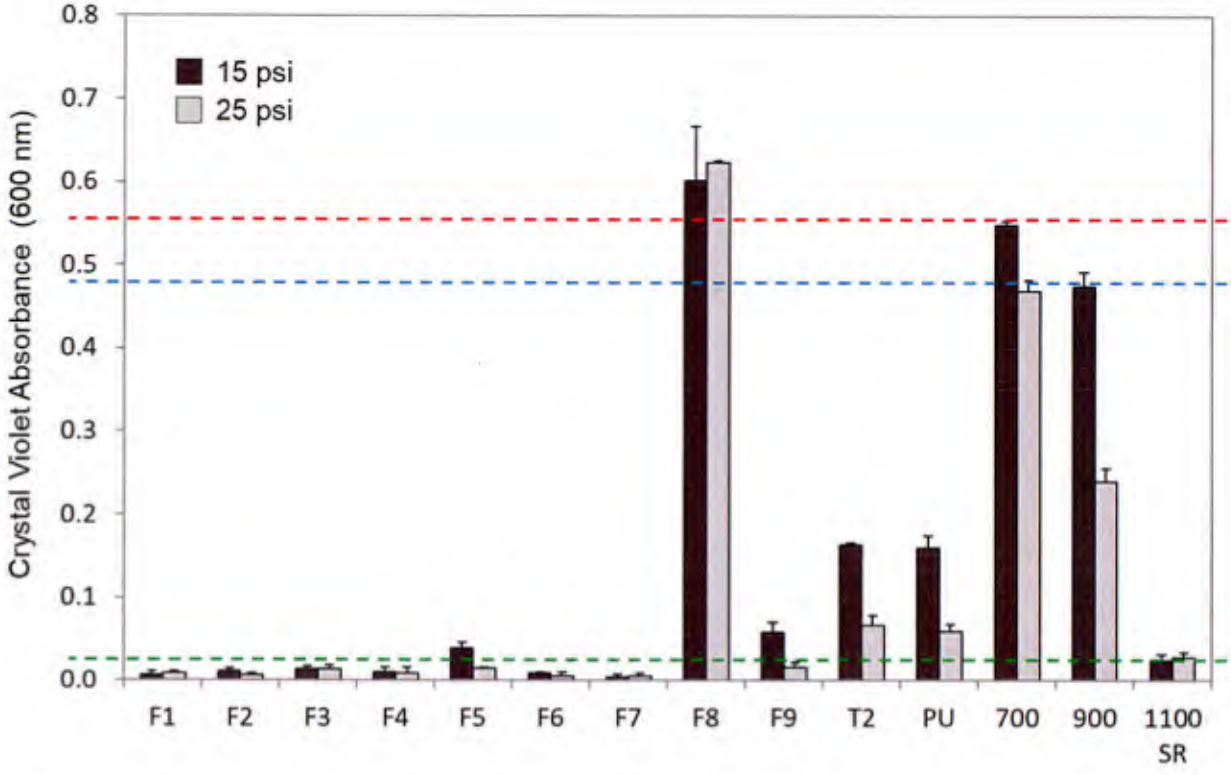

Figure III.3-8. *Halomonas pacifica* biofilm adhesion determined with an automated water jetting technique (15 and 25 psi, 5 seconds water jetting duration). A crystal violet colorimetric assay was used to quantify the amount of biofilm remaining after water jetting treatments. Each bar indicates the mean biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 15 psi water jetting pressure.

The results of the biofilm adhesion assessments for the marine bacterium, *Halomonas marina,* are provided in **Figure III.3-9**. As opposed to the assessments with *C. lytica* and *H. pacifica,* clear differences in biofilm removal were distinguishable among the experimental coatings which separated into general groupings; high/good removal on par with the Intersleek standards (F1, F2, F6, F7 and F9) and low/poor removal that were markedly worse than the Intersleek standards when compared to the entire set of standards (F3, F4, F5 and F8). These disparate results observed with *H. marina* nicely illustrate the species/strain variation and dependency in terms of assessing antifouling properties and comparative performance of new coating technologies.

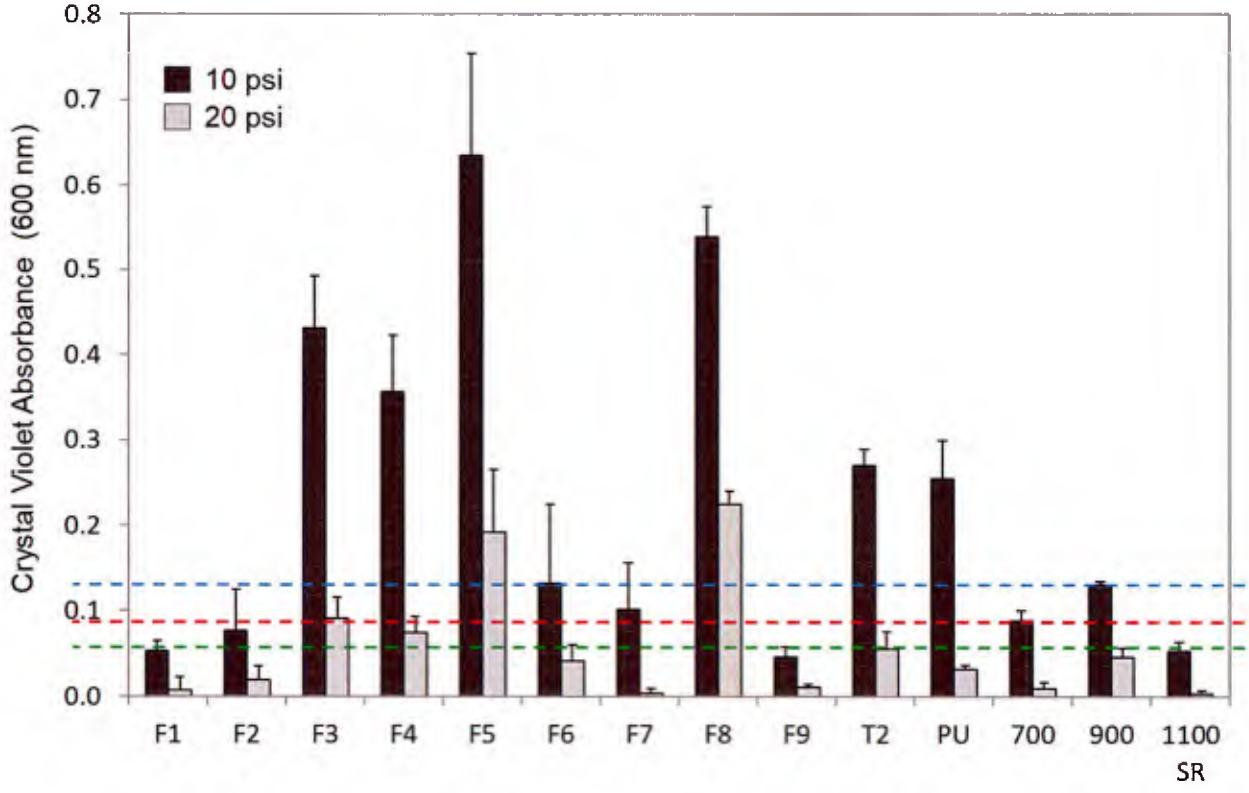

Figure III.3-9. *Halomonas marina* biofilm adhesion determined with an automated water jetting technique (15 and 25 psi, 5 seconds water jetting duration). A crystal violet colorimetric assay was used to quantify the amount of biofilm remaining after water jetting treatments. Each bar indicates the mean biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 10 psi water jetting pressure.

Figure III.3-10 provides the results of the attachment efficiency and average adhesion strength assessments for the marine ribbed mussel, *Geukensia demissa*. With the exception of F8, mussels were unable to attach to the experimental coatings after 3 days of exposure of the animals to the surfaces while immersed in artificial sea water (0%) and were comparable to the 900 (0%) and 1100 SR (33%) Intersleek commercial standards.

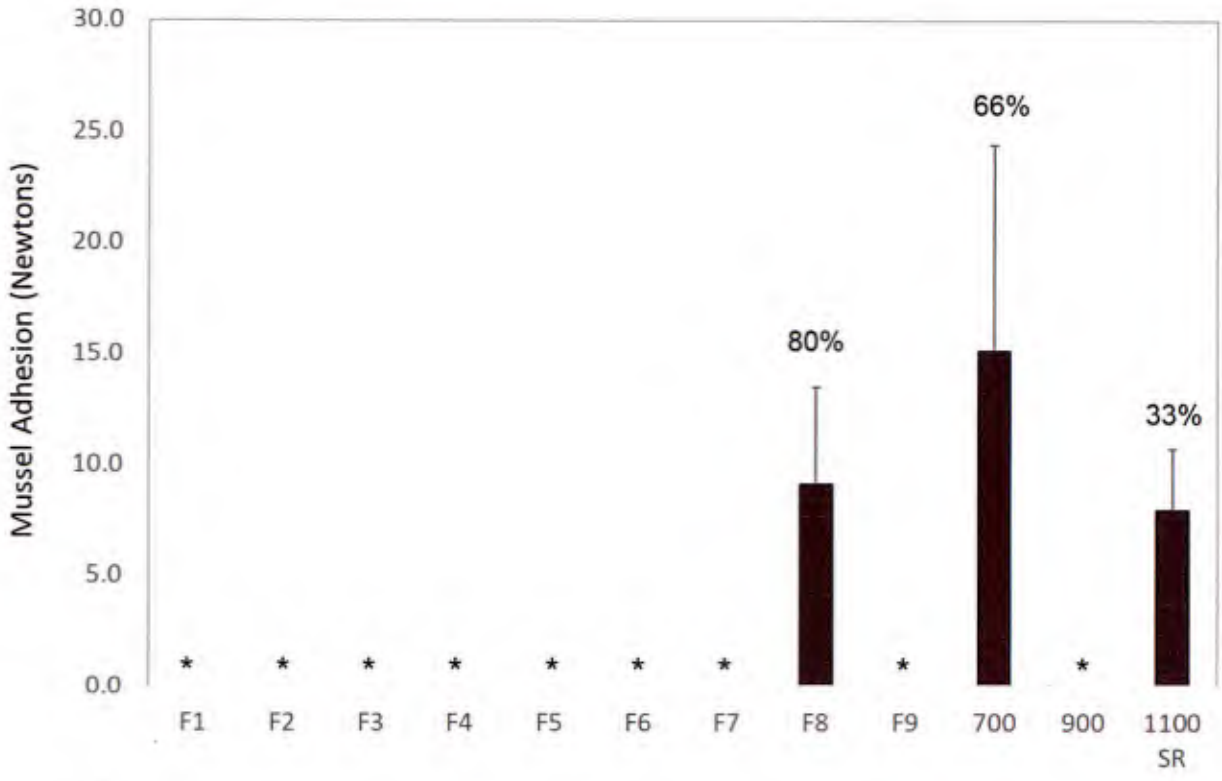

Figure III.3-10. *Geukensia demissa* adhesion after 3 days of attachment. Each bar indicates the mean adhesion value (newtons) of the total number of mussels which yielded a measureable force. Error bars represent one standard deviation of the mean. Asterisk indicates no mussel attachment (n = 6). Percent value above each bar indicates the reattachment efficiency.

In addition to biofouling assessments of coating films, AST submitted 5 coating formulation components/compounds for toxicity assessments toward bacteria and microalgae (designated 'Sample 1' through 'Sample 5'). No measureable or discernable toxicity was observed for the 5 compounds when compared to the Triclosan biocidal control for both *N. incerta* and *C. lytica* (**Figures III.3-11 and III.3-12**). However, a discernable and substantial toxic effect was observed for Sample 1 through Sample 4 across the entire concentration range tested (0.78 ug/mL – 100 ug/mL) and at 100 ug/mL for Sample 5 when tested toward the marine bacterium *Halomonas pacifica* (**Figure III.3-13**). Once again, similar to surface property testing, these disparate results among the three microorganisms demonstrates the strain variation and dependency effects on toxicity assessments of coating formulation components.

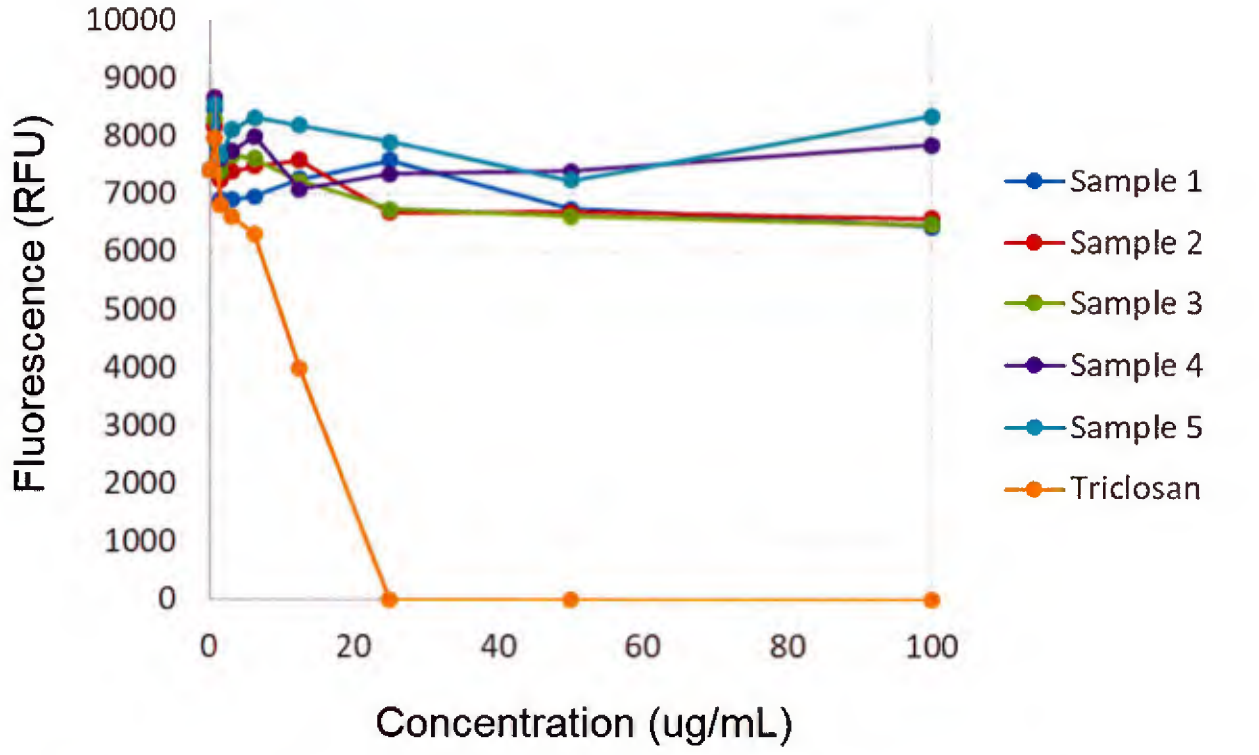

Figure III.3-11. Toxicity testing of coating formulation components/compounds for *Navicula incerta*. Fluorescence measurements of chlorophyll (RFU) was used to quantify the growth (48 hr).

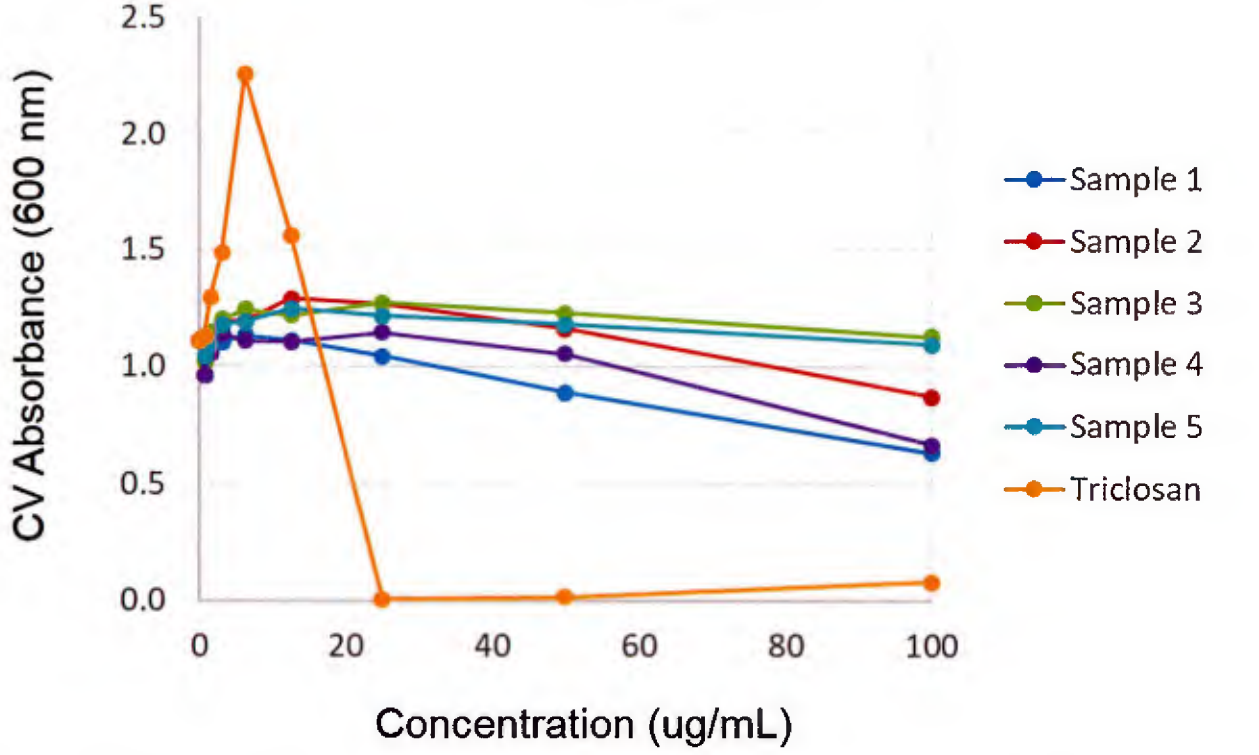

Figure III.3-12. Toxicity testing of coating formulation components/compounds for *Cellulophaga*

lytica. A crystal violet colorimetric assay was used to quantify the amount of biofilm growth (24 hr).

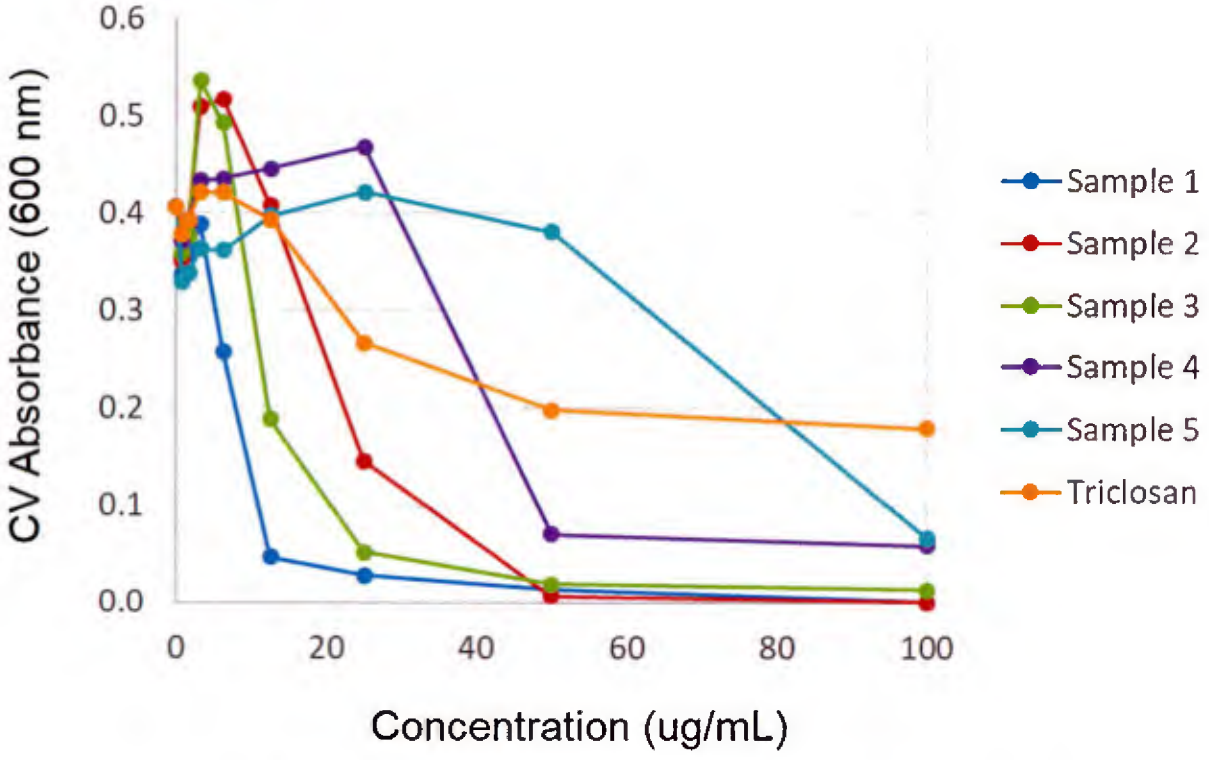

Figure III.3-13. Toxicity testing of coating formulation components/compounds for *Halomonas pacifica*. A crystal violet colorimetric assay was used to quantify the amount of biofilm growth (24 hr).

University of Florida – Prof. Michael Tonks

The first set of coatings received from Prof. Michael Tonk's group consisted of 3 unique coating types that was assessed for antifouling properties toward microalgae, bacteria, and barnacles. **Figure III.3-14** displays the results of the *N. incerta* biofilm growth assessment after 48 hours of culture. The three coatings accumulated substantially less biofilm than the standards (35%-60%), including the Intersleek FR commercial products, with coating HHN (E12) showing the highest reduction in biofilm growth (60%).

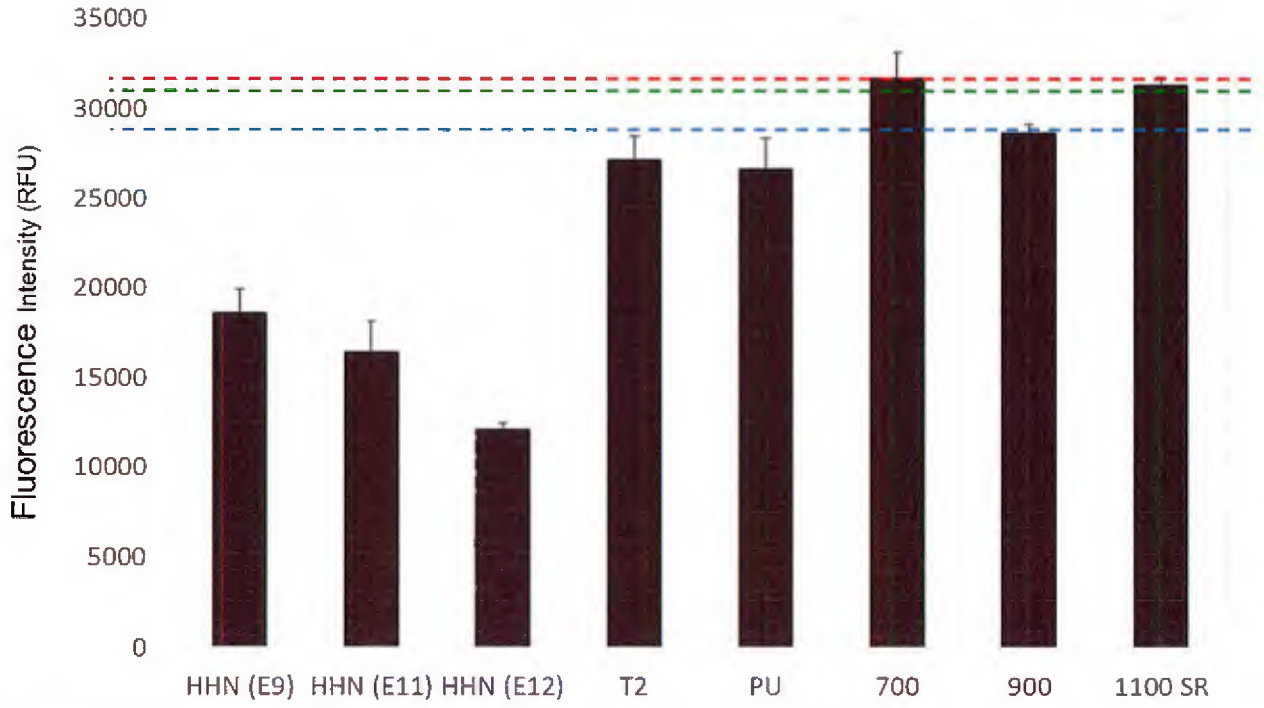

Figure III.3-14. *Navicula incerta* biofilm growth (48 hr) as quantified via fluorescence of chlorophyll (excitation: 360nm; emission: 670nm). Each bar indicates the mean relative fluorescence unit (RFU) value of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

Figure III.3-15 shows the results of the *N. incerta* cell adhesion assessments. None of the experimental coatings performed as well as the 1100 SR Intersleek FR standards at the lower 10 psi water jetting pressure, but were on par with the 900 standard. At 20 psi, coatings HHN (E9) and HHN (E12) facilitated more removal of than formulation HHN (E11), but retained substantially more biomass than the 900 and 1100 SR FR standards.

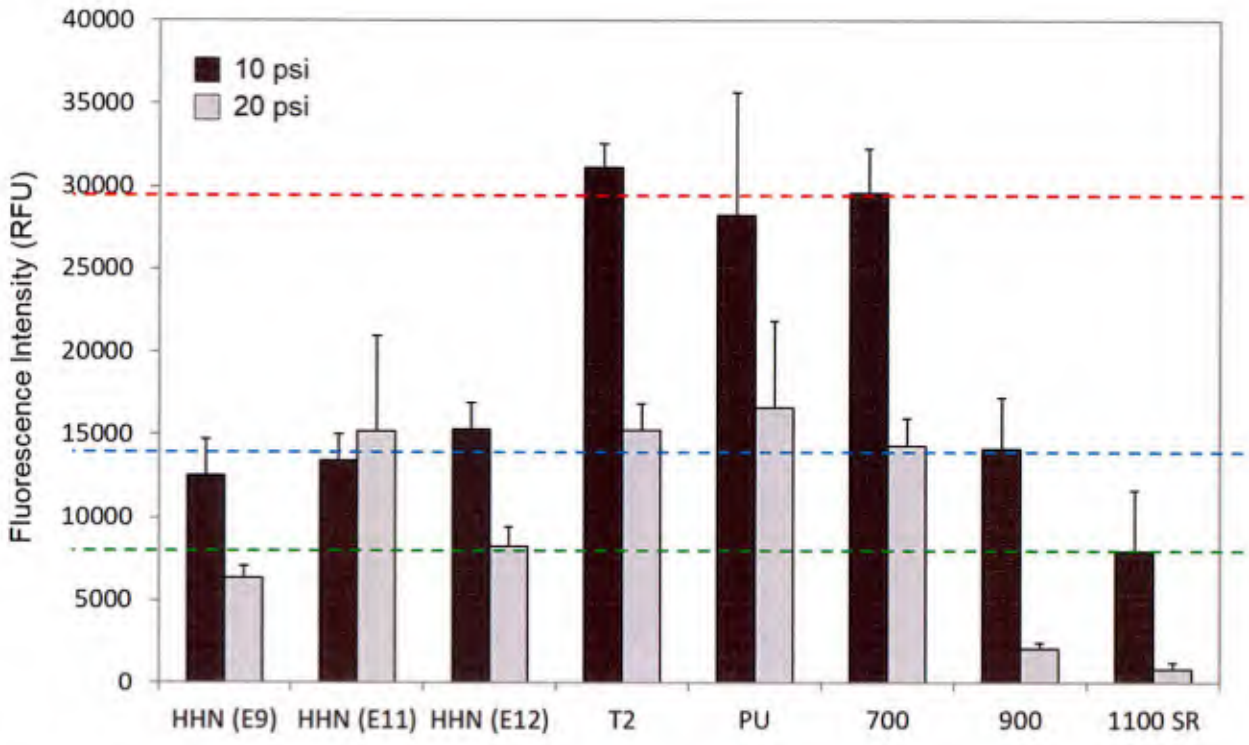

Figure III.3-15. *Navicula incerta* cell adhesion determined with an automated water jetting technique (10 psi and 20 psi, 10 second water jetting duration). Fluorescence measurement of chlorophyll was used to quantify the amount of cells remaining after water jetting treatments (RFU). Each bar indicates the mean cells/biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 10 psi water jetting pressure.

The results of the *C. lytica* biofilm growth assessments are displayed in **Figure III.3-16**. No marked differences in biofilm growth among the experimental coatings was observed and all three coatings exhibited similar biofilm accumulation as the Intersleek FR standards.

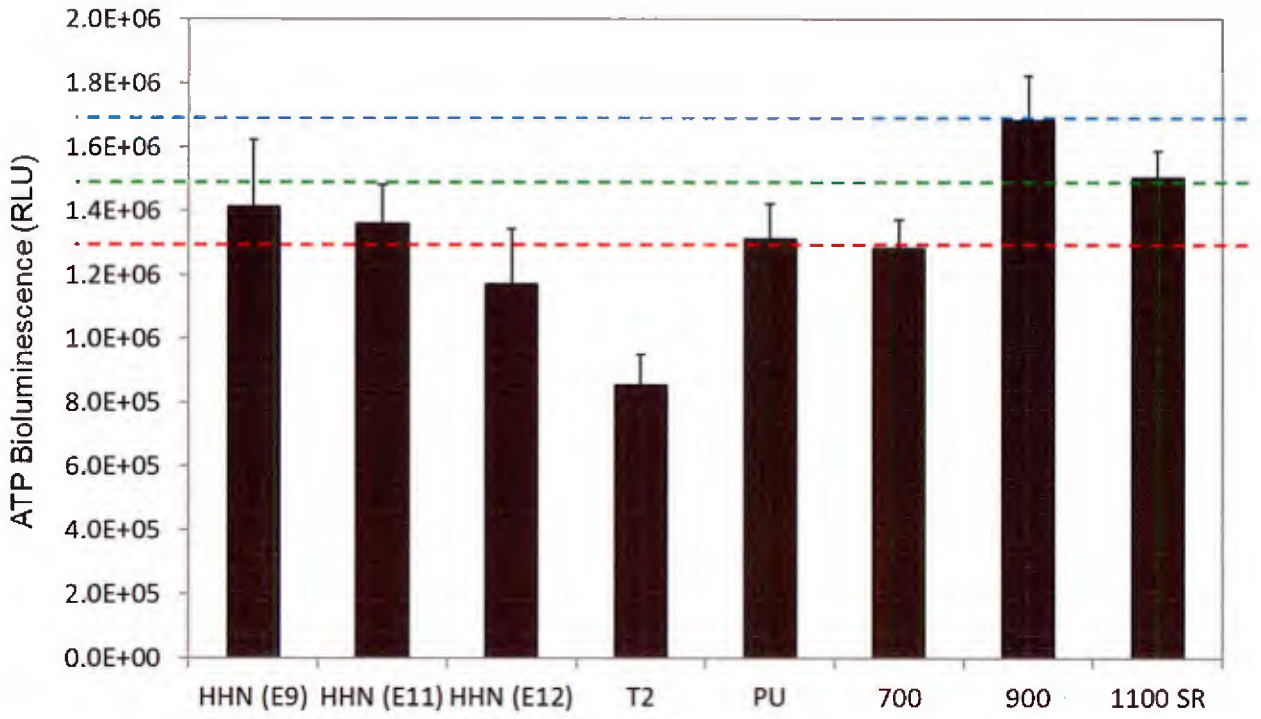

Figure III.3-16. *Cellulophaga lytica* biofilm growth quantified via ATP bioluminescence. Each bar indicates the mean ATP bioluminescence value (RLU) of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

Figure III.3-17 provides a summary of the *C. lytica* biofilm adhesion assessments. A discernable trend in biofilm removal was evident at the lower 10 psi water jetting pressure where biofilm retained after jetting decreased from HHN (E9) to HHN (E12). However, biofilm removal was not as pronounced on the experimental surfaces as it was for the 900 and 1100 SR FR standards; a phenomenon that was more readily apparent at the higher 20 psi water jetting pressure.

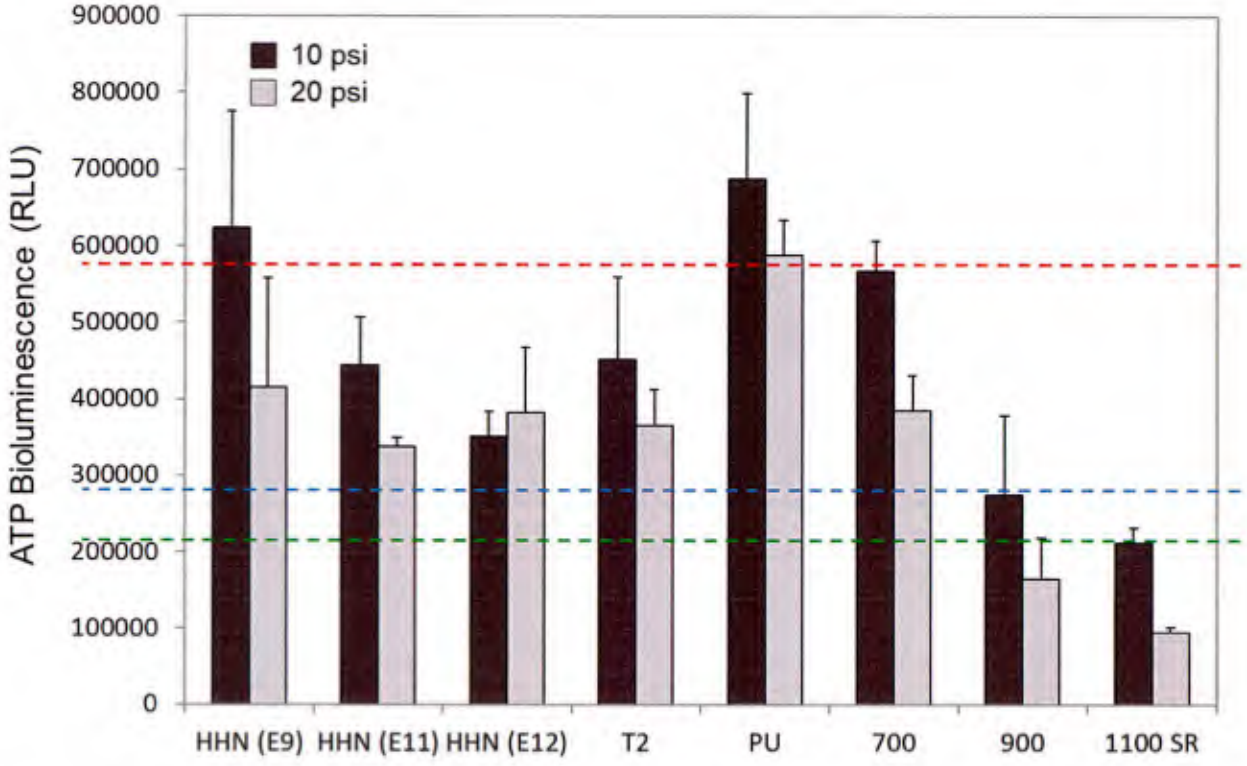

Figure III.3-17. *Cellulophaga lytica* biofilm adhesion determined with an automated water jetting technique (10 and 20 psi at 5 seconds water jetting duration). An ATP bioluminescence assay was used to quantify the amount of biofilm remaining after water jetting treatments. Each bar indicates the mean biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 10 psi water jetting pressure.

The assessment of barnacle reattachment and adhesion after 14 days of artificial sea water immersion/exposure are shown in **Figures III.3-18 and III.3-19**. In terms of reattachment efficiency, all six barnacles attached to HHN (E9) and HHN (E11) while 80% of the barnacles attached to HHN (E12). In comparison, only 66%, 50% and 33% of the barnacles reattached to Intersleek 1100 SR, 700 and 900, respectively. With respect to adhesion mitigation, coating HHN (E12) was dramatically more effective than HHN (E9) and HHN (E11), 0.04 MPa versus 0.16 and 0.17 MPa, respectively. Furthermore, barnacle adhesion was lower on HHN (E12) than the Intersleek FR standards (0.05-0.06 MPa).

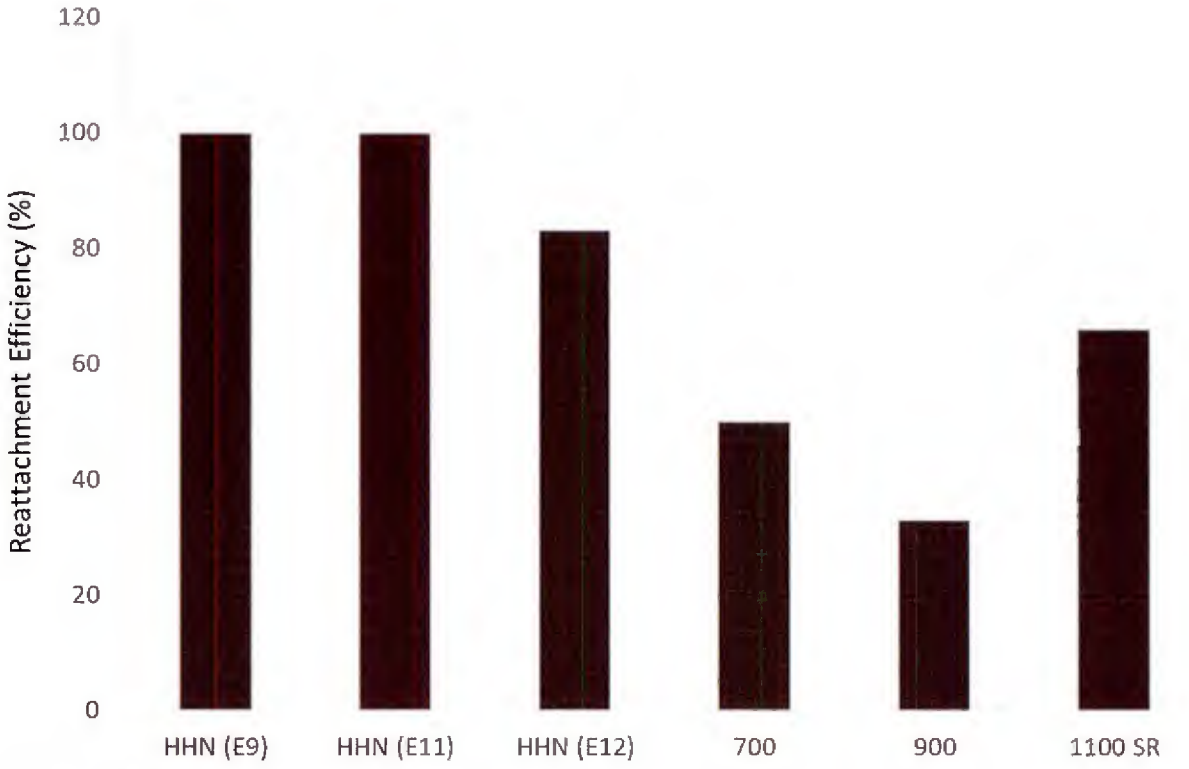

Figure III.3-18. *Amphibalanus amphitrite* reattachment after 14 days of immersion. Each bar indicates the percentage of barnacles that reattached to the coating surface (n = 6).

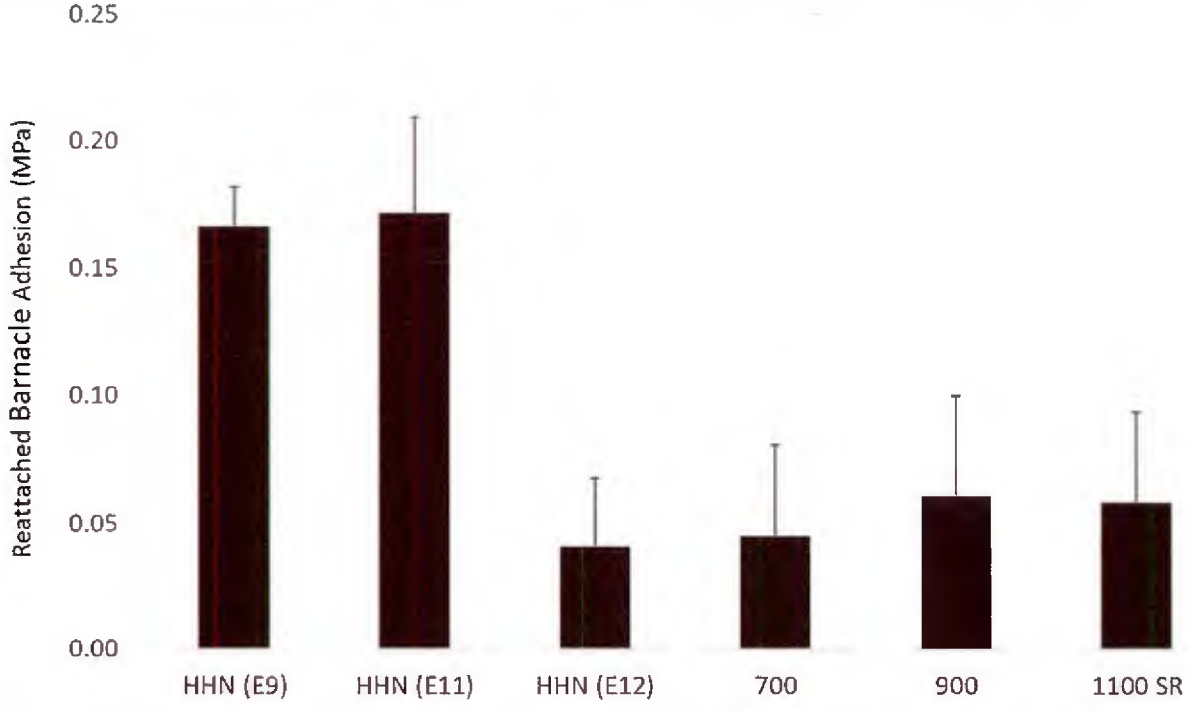

Figure III.3-19. *Amphibalanus amphitrite* adhesion after 14 days of reattachment. Each bar indicates the mean adhesion value (MPa) of the total number of adult barnacles yielding a measureable force. Error bars represent one standard deviation of the mean.

A second set of materials was received from Prof. Tonk's group comprising 3 unique coating types, denoted as 'Coating #1', 'Coating #2' and 'Coating #3'. The results of the *N. incerta* biofilm growth assessments are shown in **Figure III.3-20**. Among three experimental coatings, Coating #3 was most effective at mitigating *N. incerta* biofilm growth, which exhibited a ≥ 70% reduction when compared to the Intersleek FR standards 700, 900 and 1100 SR.

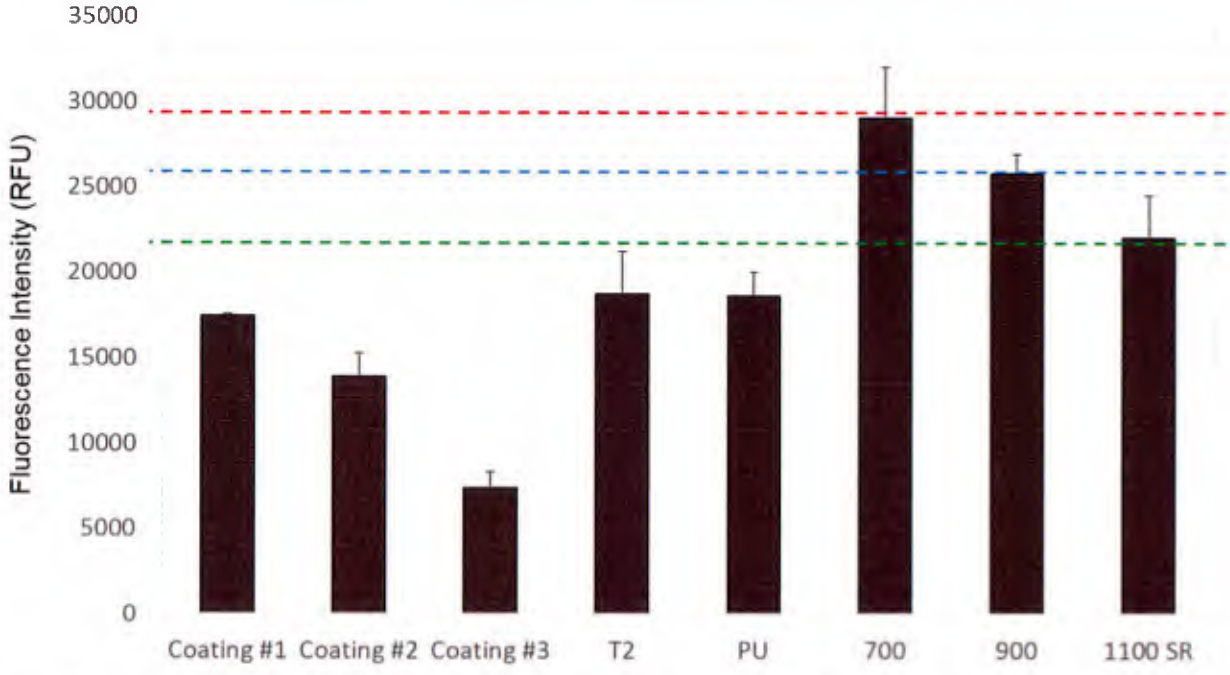

Figure III.3-20. *Navicula incerta* biofilm growth (48 hr) as quantified via fluorescence of chlorophyll (excitation: 360nm; emission: 670nm). Each bar indicates the mean relative fluorescence unit (RFU) value of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

Figure III.3-21 summarizes the results of the *N. incerta* cell adhesion assessments. All three experimental coatings demonstrated comparable cell release properties to the 1100 SR FR standard at the higher 20 psi water jetting pressure, with Coating #1 facilitating the most removal. At the 10 psi water jetting pressure, Coating #1 and Coating #3 enabled markedly more cell removal than Coating #2.

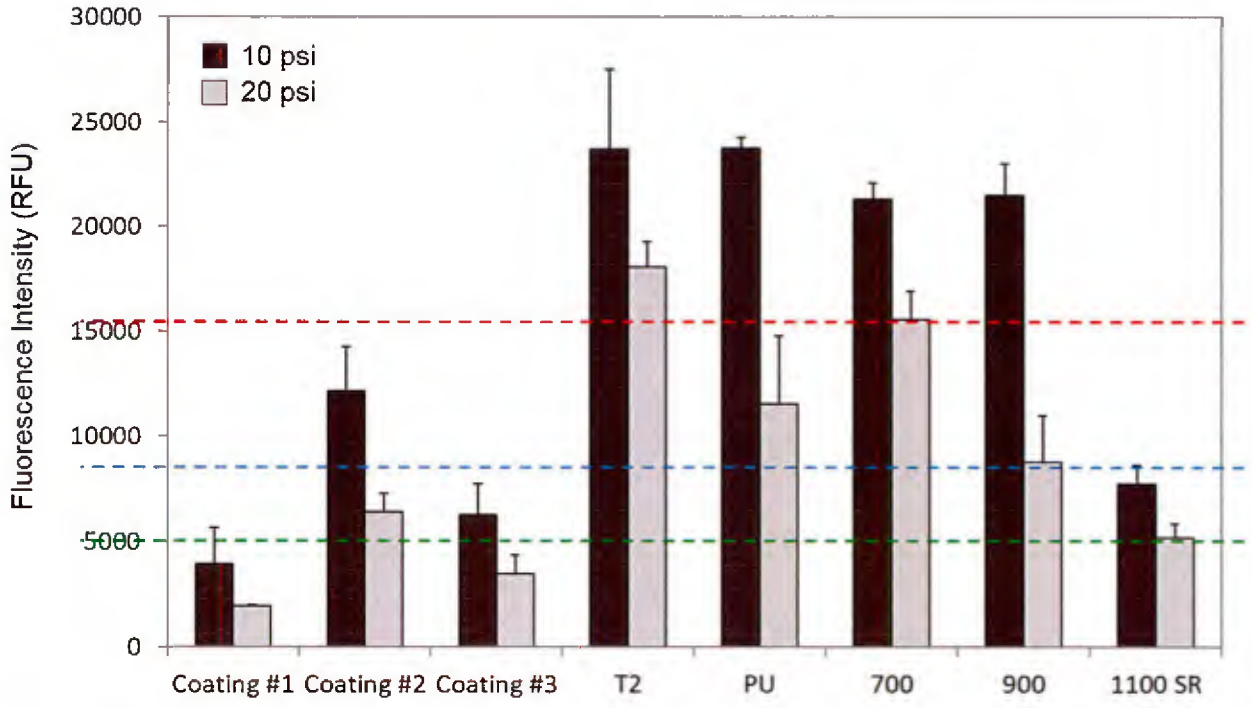

Figure III.3-21. *Navicula incerta* cell adhesion determined with an automated water jetting technique (10 psi and 20 psi, 10 second water jetting duration). Fluorescence measurement of chlorophyll was used to quantify the amount of cells remaining after water jetting treatments (RFU). Each bar indicates the mean cells/biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 20 psi water jetting pressure.

The results of the biofilm growth assessments for *C. lytica* are summarized in **Figure III.3-22**. More biofilm growth was observed on the three experimental coatings when compared to the set of standard coatings. No discernable difference in biofilm growth was observed among the three experimental coatings.

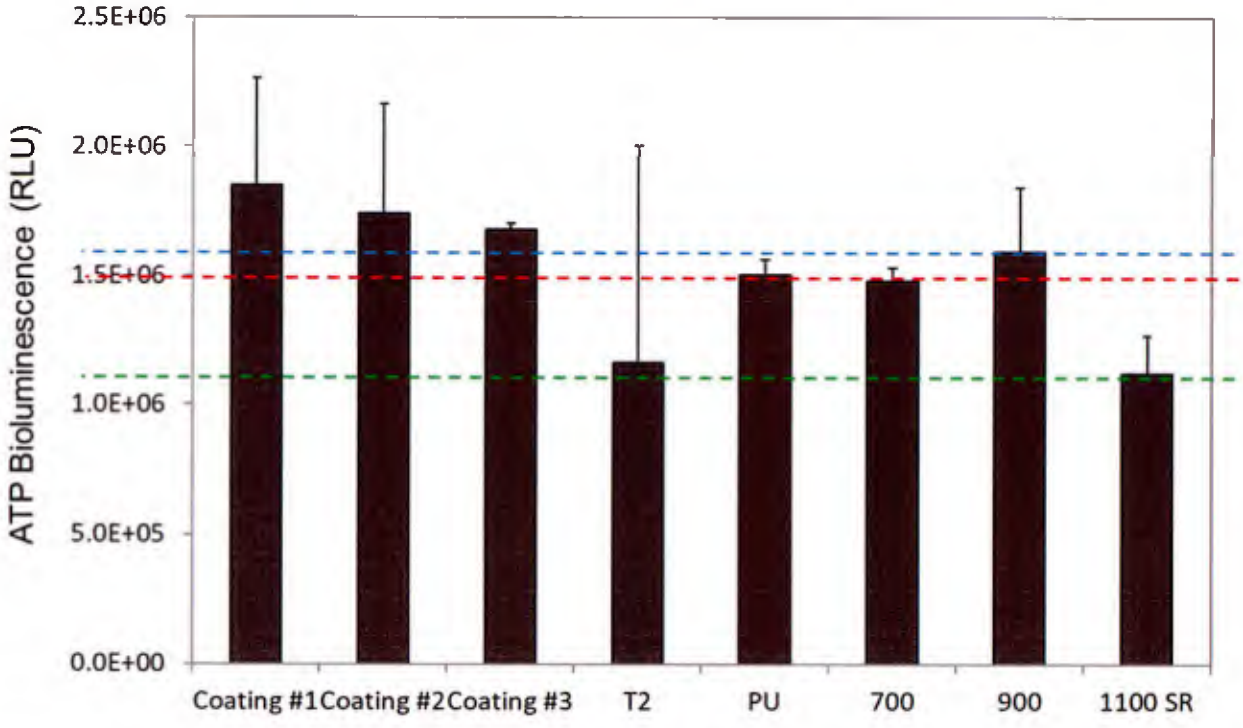

Figure III.3-22. *Cellulophaga lytica* biofilm growth quantified via ATP bioluminescence. Each bar indicates the mean ATP bioluminescence value (RLU) of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

Figure III.3-23 displays the results of the *C. lytica* biofilm adhesion assessments. Among the experimental coatings, Coating #2 and Coating #3 exhibited more biofilm removal than Coating #1 at the higher 20 psi water jetting pressure and showed comparable performance to the 900 FR standard. However, the 1100 SR FR standard facilitated substantially more biofilm removal than these two superior performing compositions.

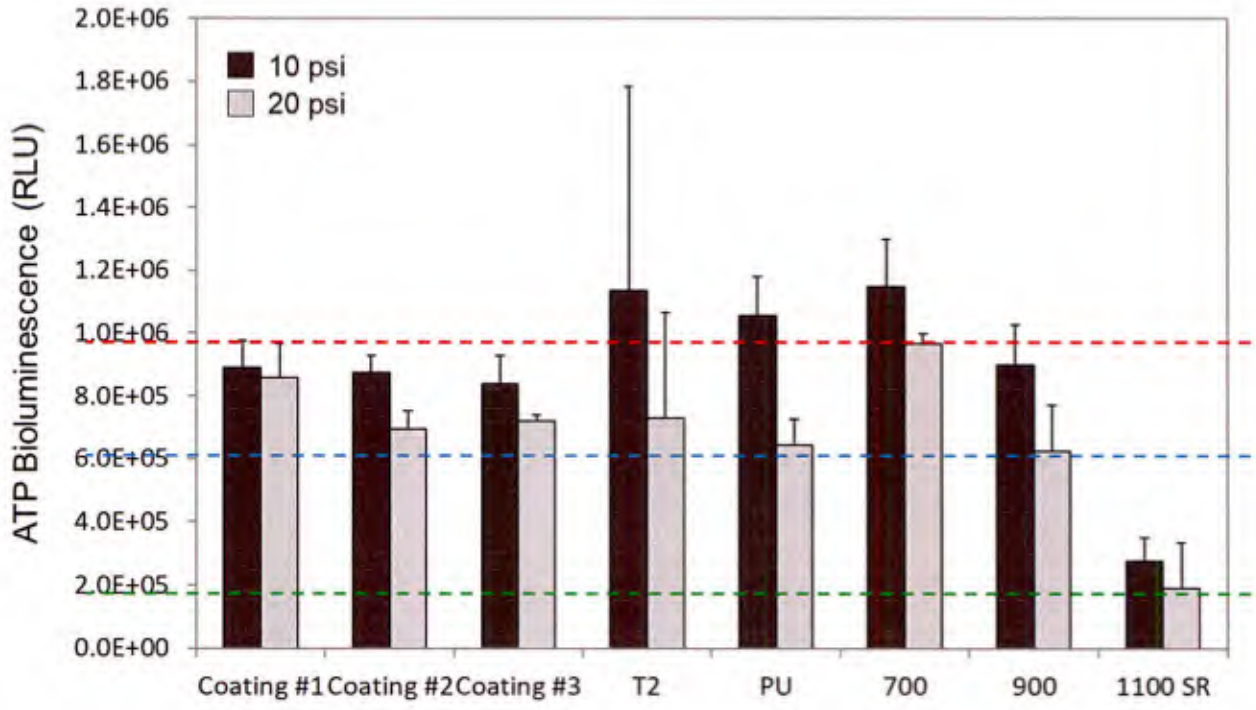

Figure III.3-23. *Cellulophaga lytica* biofilm adhesion determined with an automated water jetting technique (10 and 20 psi at 5 seconds water jetting duration). An ATP bioluminescence assay was used to quantify the amount of biofilm remaining after water jetting treatments. Each bar indicates the mean biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 20 psi water jetting pressure.

The results of the barnacle reattachment and adhesion assessments are provided in **Figures III.3-24 and III.3-25**. Coating #1 (66%) was more effective at mitigating adult barnacle reattachment after 14 days of exposure than Coating #2 (83%) and Coating #3 (83%) and was on par with 1100 SR FR standard. However, a lower degree of attachment was observed for both the 700 (50%) and 900 (33%) standards. With respect to adhesion, the three experimental coatings were not as effective as the Intersleek standards at preventing barnacles from reattaching to their respective surfaces, exhibiting adhesion values >0.095 MPa as opposed to 0.05-0.06 MPa. Among the three experimental formulations, Coating #2 (0.14 MPa) exhibited a markedly higher adhesion value than Coating #1 (0.10 MPa) and Coating #3 (0.096 MPa).

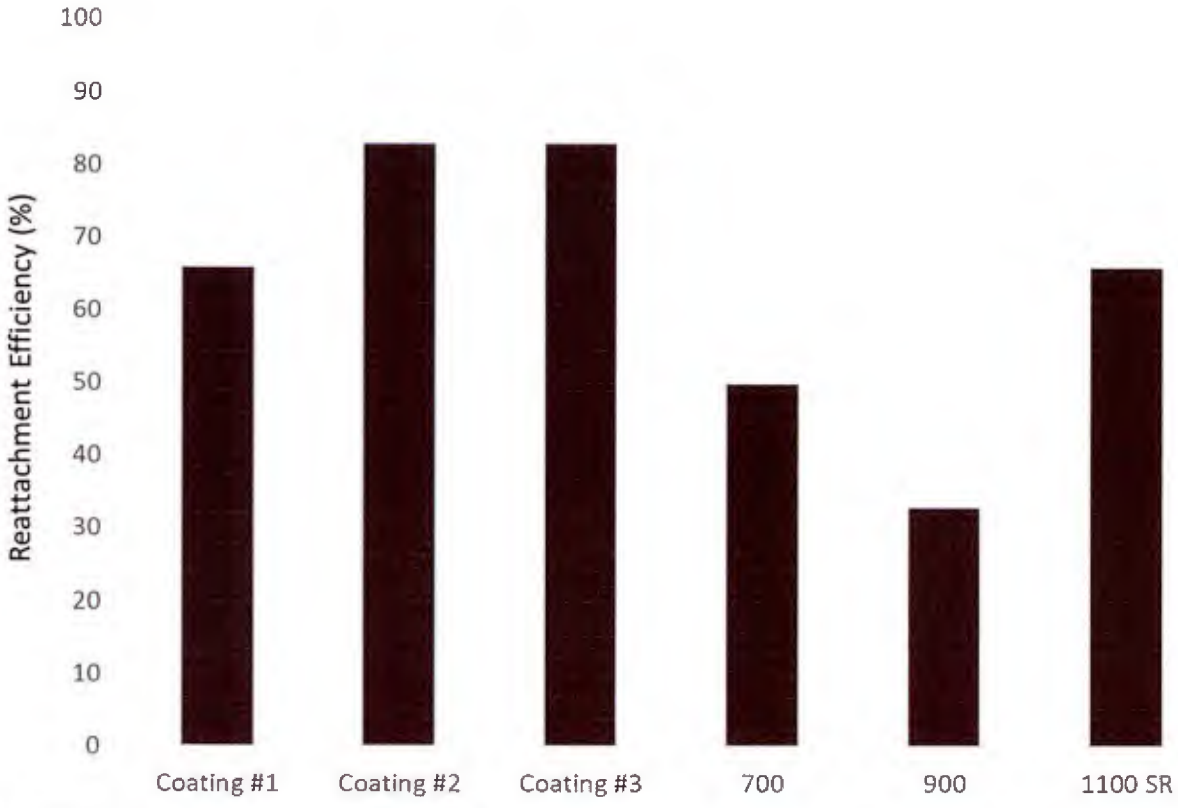

Figure III.3-24. *Amphibalanus amphitrite* reattachment after 14 days of immersion. Each bar indicates the percentage of barnacles that reattached to the coating surface (n = 6).

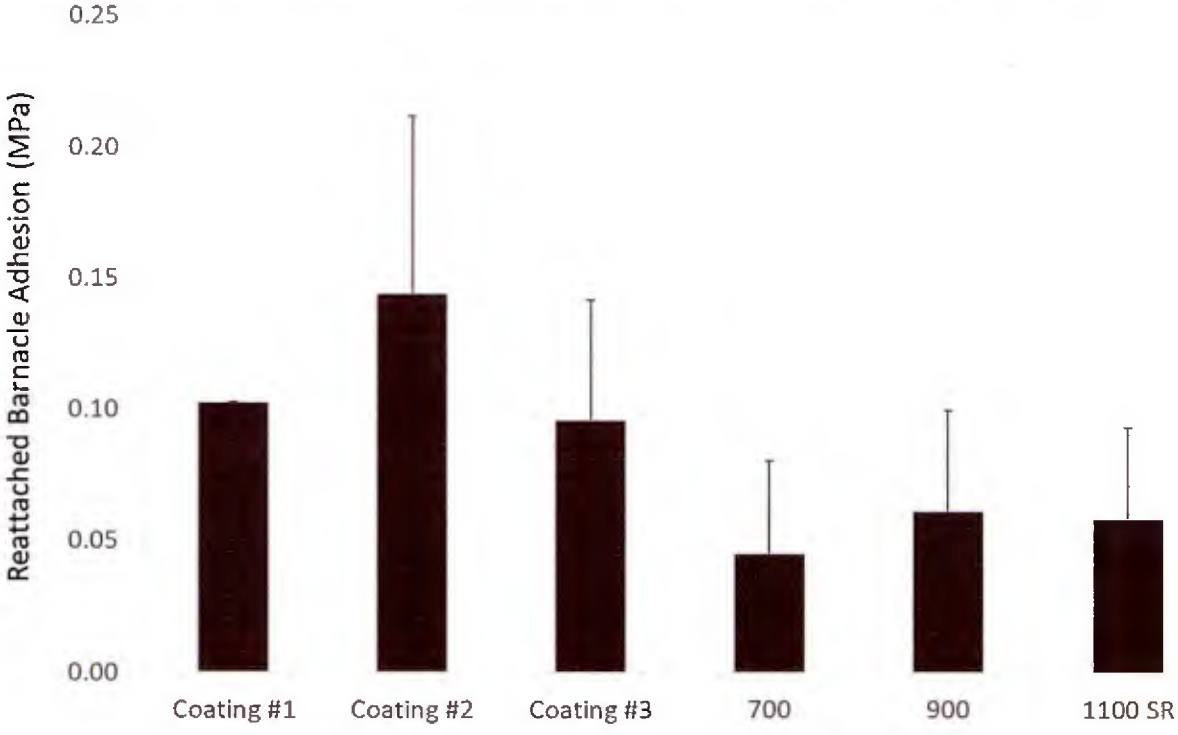

Figure III.3-25. *Amphibalanus amphitrite* adhesion after 14 days of reattachment. Each bar indicates the mean adhesion value (MPa) of the total number of adult barnacles yielding a measureable force. Error bars represent one standard deviation of the mean.

NatureCoat – Xuewei Xu

NatureCoat submitted a set of coatings consisting of 4 unique compositions, NC-1, NC-2, NC-3 and NC-4, that was assessed for antifouling properties toward marine microalgae, bacteria, and barnacles. **Figure III.3-26** shows the results of the *N. incerta* cell attachment assessments which revealed that all three experimental coatings were highly effective at preventing initial cell attachment, achieving >80% reduction as compared to the Intersleek FR commercial standards. There was no discernable difference in performance among the three NatureCoat coatings.

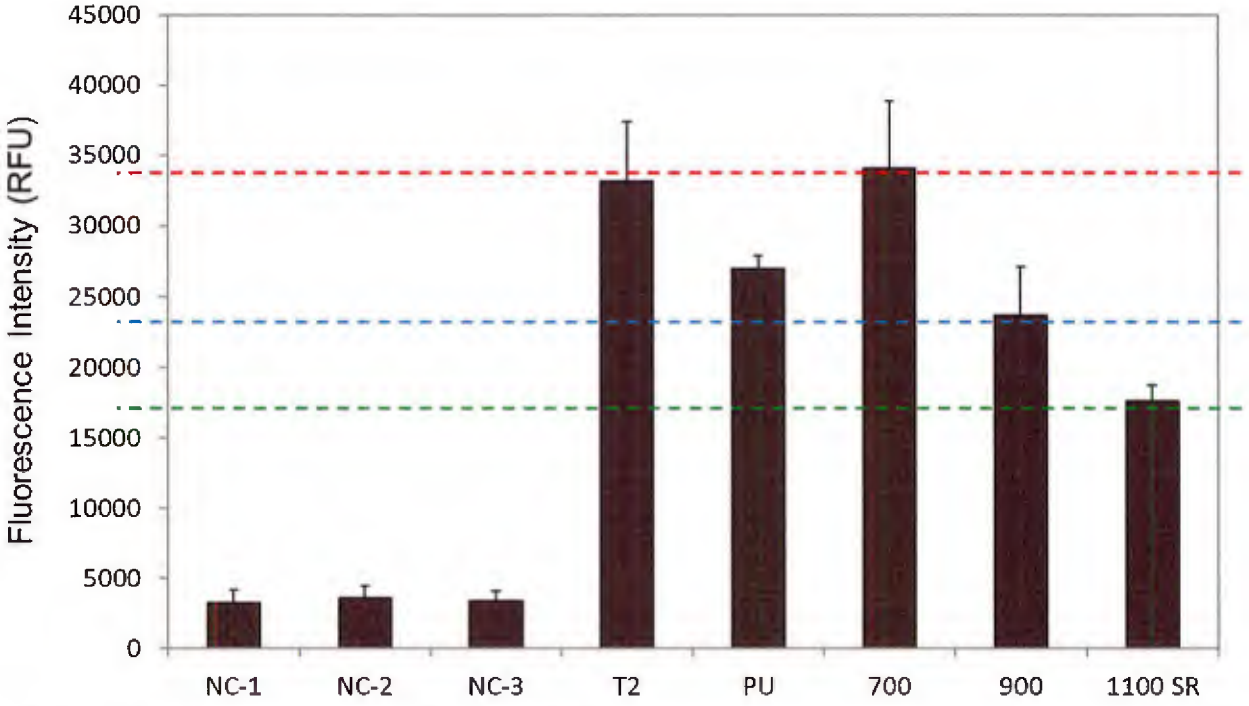

Figure III.3-26. *Navicula incerta* cell attachment (2 hr) as quantified via fluorescence of chlorophyll (excitation: 360nm; emission: 670nm). Each bar indicates the mean relative fluorescence unit (RFU) value of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

Figure III.3-27 summarizes the results of the *N. incerta* cell adhesion assessments. All three experimental coatings exhibited excellent cell adhesion mitigation properties, achieving almost complete removal (< 1000 RFU) of attached *N. incerta* cells at the lower water jetting pressure of 10 psi. In contrast, cell removal from the Instersleek standards was considerably less effective, retaining substantially more biomass after water jetting (i.e. > 5000 RFU). No discernable difference in cell adhesion mitigation was discernable among the three experimental coatings.

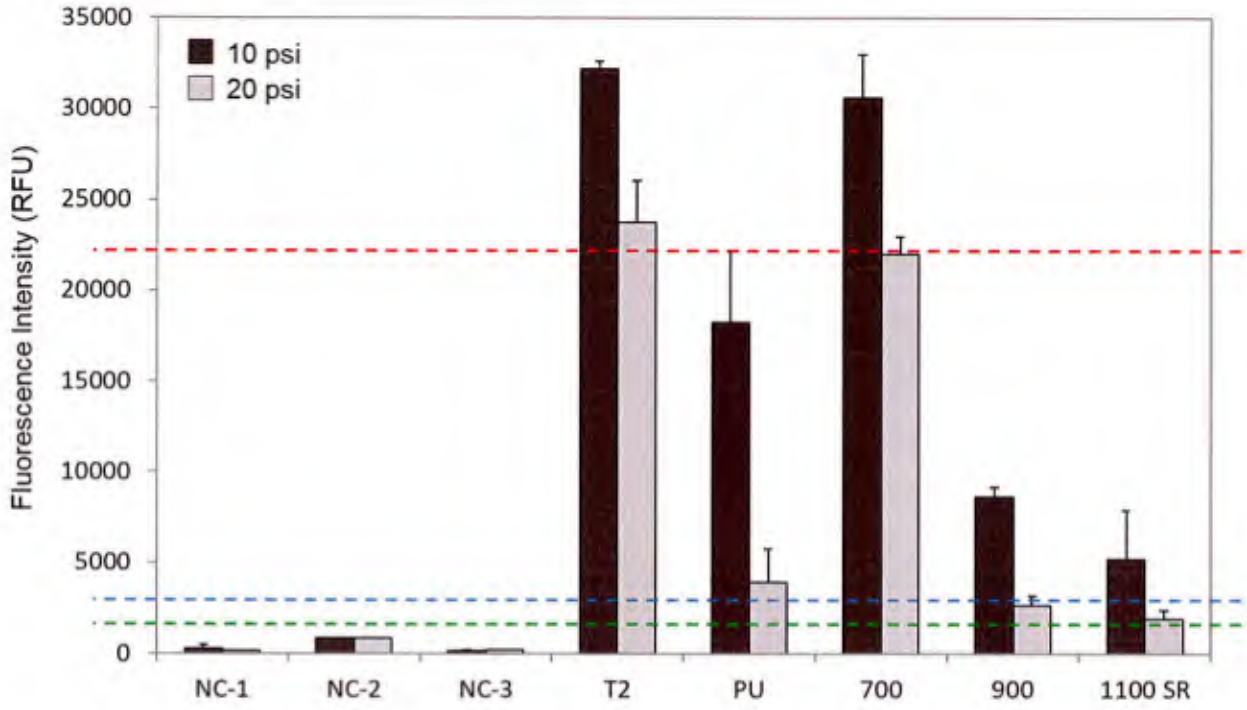

Figure III.3-27. *Navicula incerta* cell adhesion determined with an automated water jetting technique (10 psi and 20 psi, 10 second water jetting duration). Fluorescence measurement of chlorophyll was used to quantify the amount of cells remaining after water jetting treatments (RFU). Each bar indicates the mean cells/biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 20 psi water jetting pressure.

The results of the biofilm growth assessments with *C. lytica* are provided in **Figure III.3-28**. Biofilm growth decreased markedly from NC-1 to NC-3, with NC-3 exhibiting a 15% and 47% reduction when compared to NC-1 and NC-2, respectively. Furthermore, Coatings NC-2 and NC-3 outperformed the Intersleek FR standards, achieving an approximate 50% reduction in biofilm growth as compared to the best performing standard, Intersleek 900.

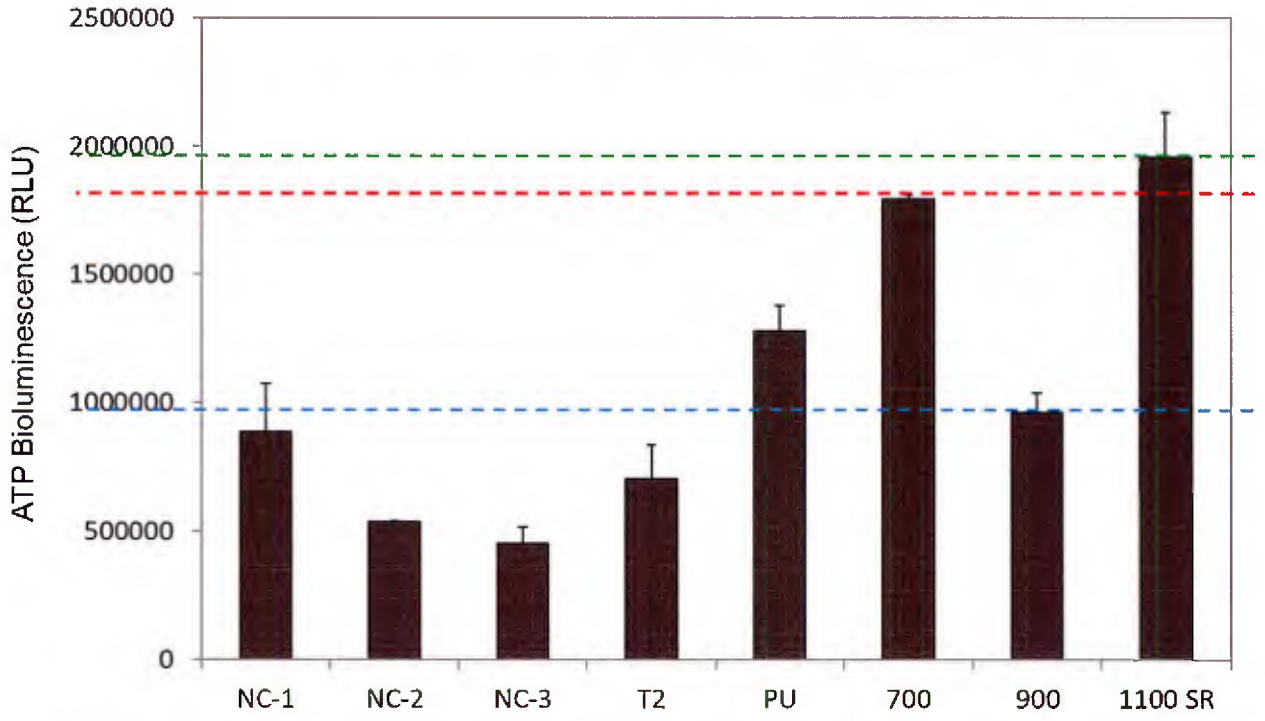

Figure III.3-28. *Cellulophaga lytica* biofilm growth quantified via ATP bioluminescence. Each bar indicates the mean ATP bioluminescence value (RLU) of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards.

Figure III.3-29 summarizes the results of the *C. lytica* biofilm adhesion assessments. Good discrimination of release properties among the experimental coatings was achieved at the lower 10 psi water jetting pressure, with Coating NC-3 retaining 70% and 53% less biofilm than NC-1 and NC-2, respectively. Moreover, coating NC-3 showed similar performance as the 900 and 1100 SR standards, which both exhibited excellent removal of *C. lytica* biofilm.

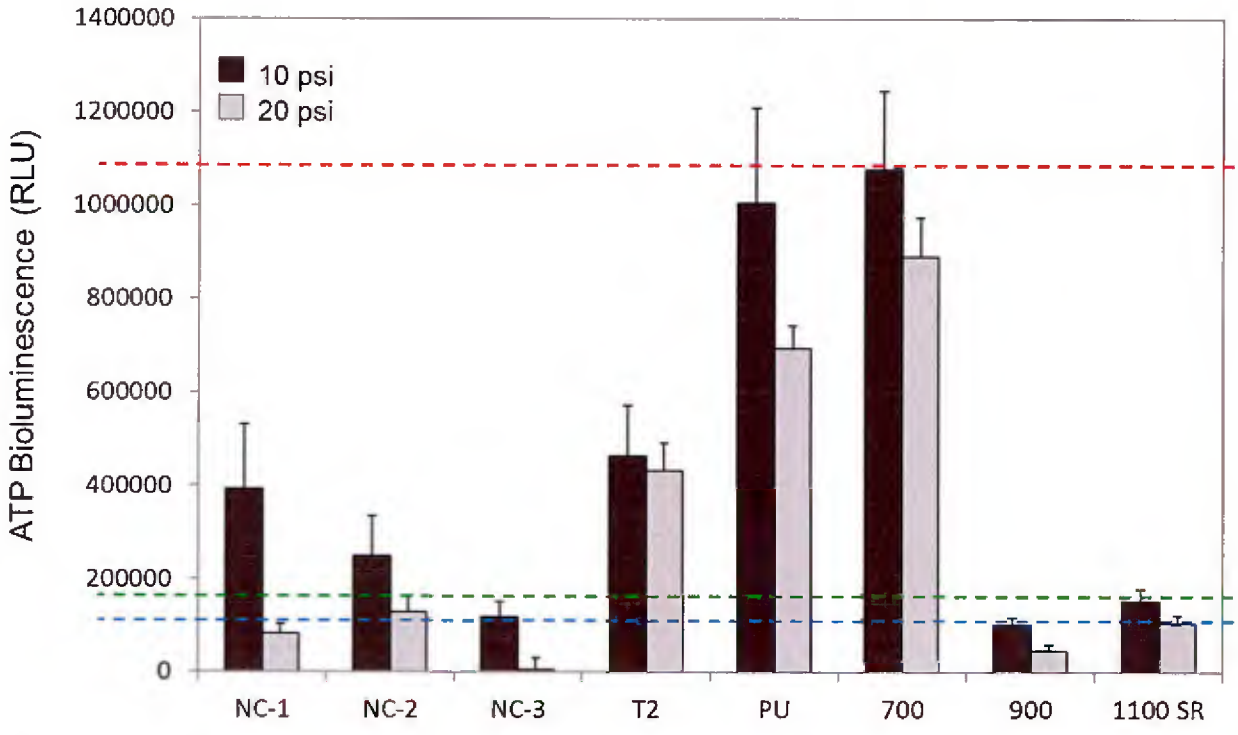

Figure III.3-29. *Cellulophaga lytica* biofilm adhesion determined with an automated water jetting technique (10 and 20 psi at 5 seconds water jetting duration). An ATP bioluminescence assay was used to quantify the amount of biofilm remaining after water jetting treatments. Each bar indicates the mean biomass remaining of three replicate samples. Error bars represent one standard deviation of the mean. Dashed lines indicate performance of 700 (red), 900 (blue) and 1100 SR (green) standards for 10 psi water jetting pressure.

Adult barnacle reattachment and adhesion was assessed for a single formulation, designated as 'NatureCoat' (**Figure III.3-30 and Figure III.3-31**). Reattachment was moderately reduced by NatureCoat exhibiting a 66% efficiency, which was comparable to the 1100 SR commercial standard. However, the 700 and 900 commercial FR standards were more effective than NatureCoat, enabling only 50% and 33% of the barnacles to attach to their respective surfaces. In terms of adhesion, NatureCoat was shown to be quite effective (0.06 MPa) and was comparable to all three commercial FR standards (0.045-0.06 MPa)

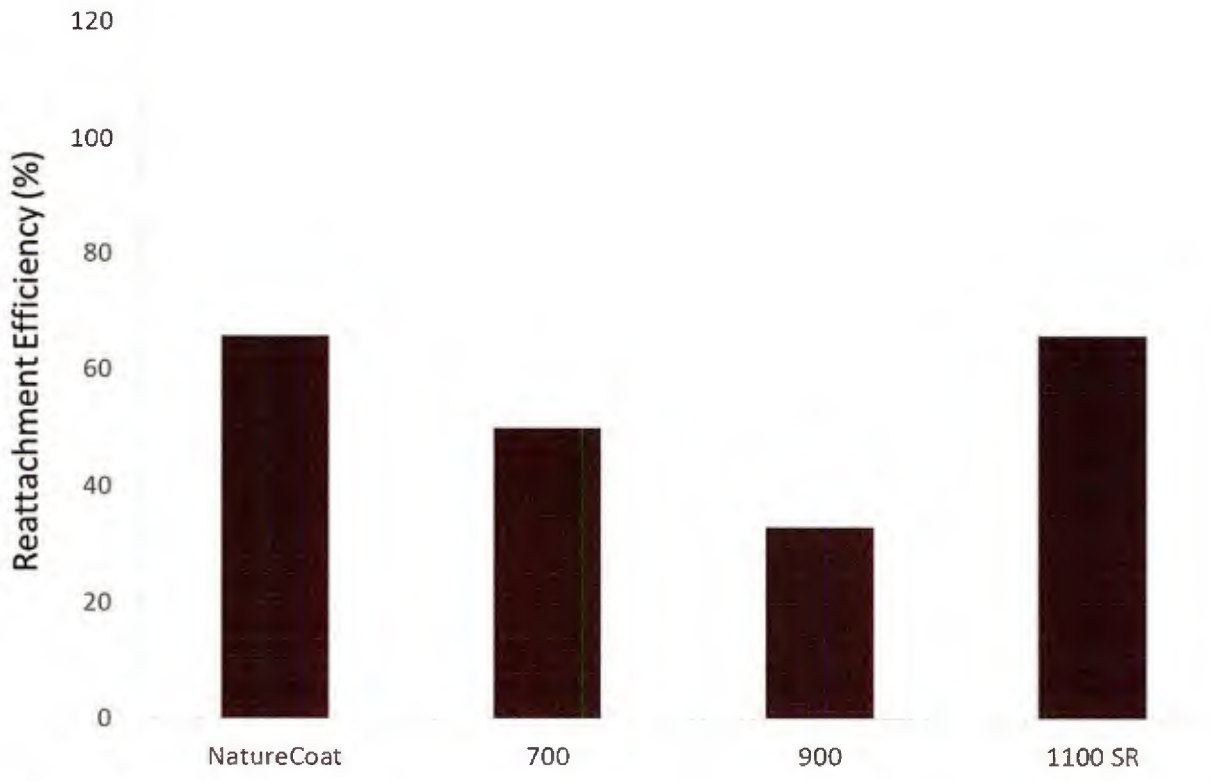

Figure III.3-30. *Amphibalanus amphitrite* reattachment after 14 days of immersion. Each bar indicates the percentage of barnacles that reattached to the coating surface (n = 6).

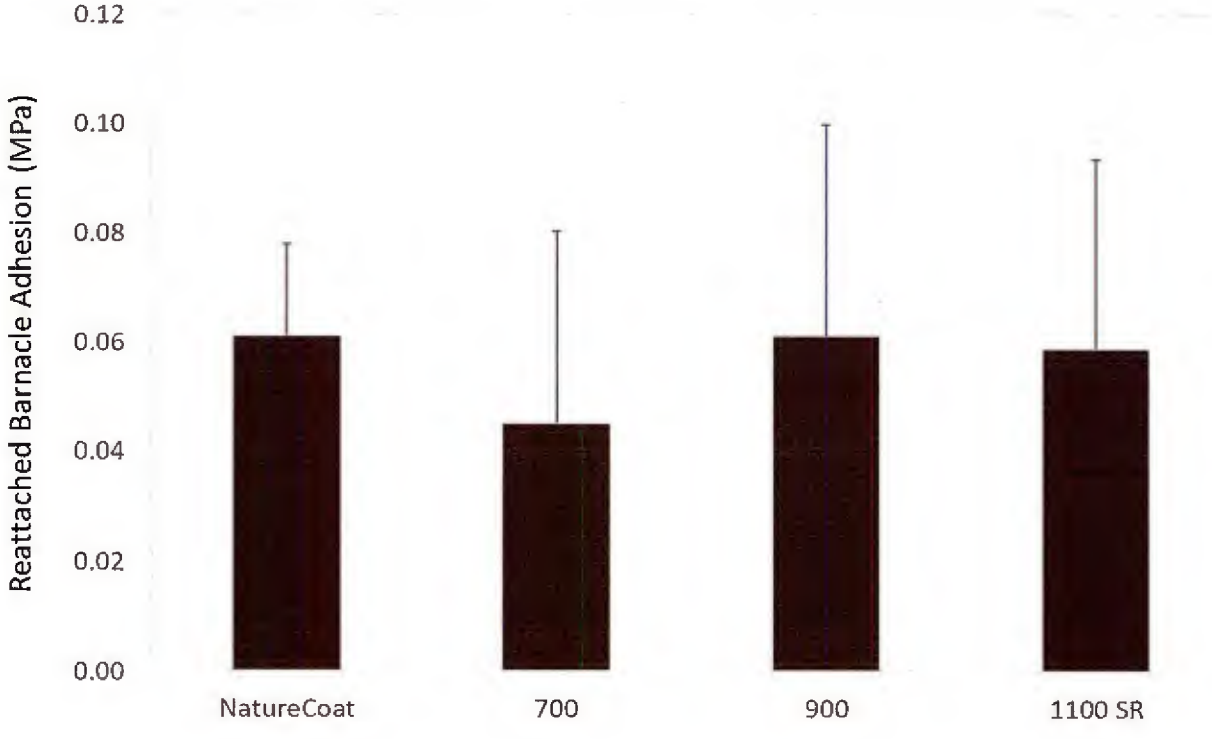

Figure III.3-31. *Amphibalanus amphitrite* adhesion after 14 days of reattachment. Each bar indicates the mean adhesion value (MPa) of the total number of adult barnacles yielding a measureable force. Error bars represent one standard deviation of the mean.

In addition to testing for ONR funded PI's, NDSU engaged in discretionary testing for non-ONR funded academic collaborators (briefly summarized below):

University of North Carolina-Chapel Hill—Prof. Roger Narayan

Four sets of antimicrobial treated aluminum discs and surgical gauze were received from Prof. Roger Narayan, University of North Carolina-Chapel Hill, and were tested for their antibacterial and antifungal properties toward a panel of microorganisms relevant to clinical/healthcare infections and disease, including the Gram-positive bacterium *Staphylococcus aureus,* the Gram-negative bacterium *Pseudomonas aeruginosa*, and the yeast *Candida albicans.*

The first set of materials comprised 6 unique antimicrobial treatments consisting of the antibacterial vancomycin and that antifungal amphotericin B, both individually formulated and in combination. No activity was observed for the antimicrobial treated discs toward *S. aureus* (**Figure III.3-32**) or *P. aeruginosa* (**Figure III.3-33**). However, activity was distinguished for the treatments that contained Amphotericin B toward the opportunistic fungal pathogen *C. albicans* as evidenced by the zones of growth inhibition (**Figure III.3-34**).

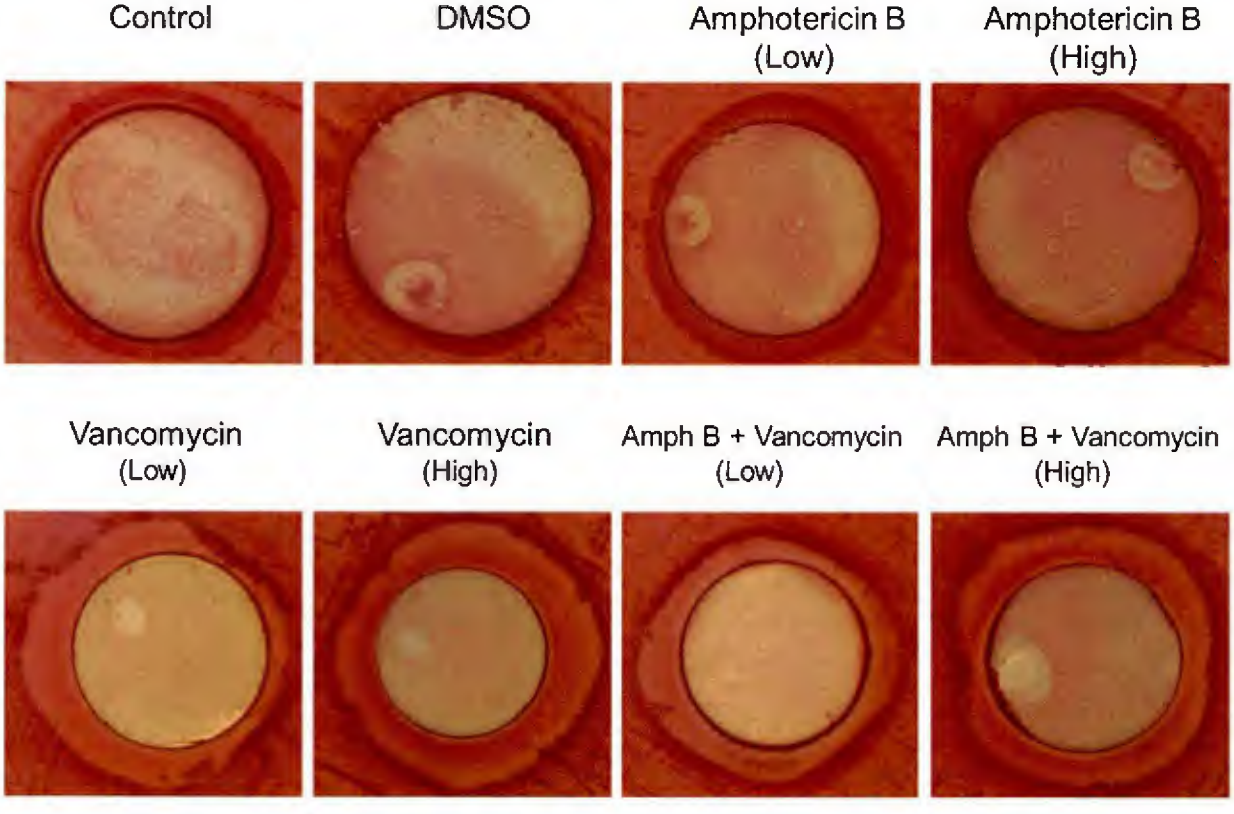

Figure III.3-32. Agar diffusion results toward the Gram-positive bacterium *S. aureus.* Red color indicates viable cell growth. Amph = Amphotericin.

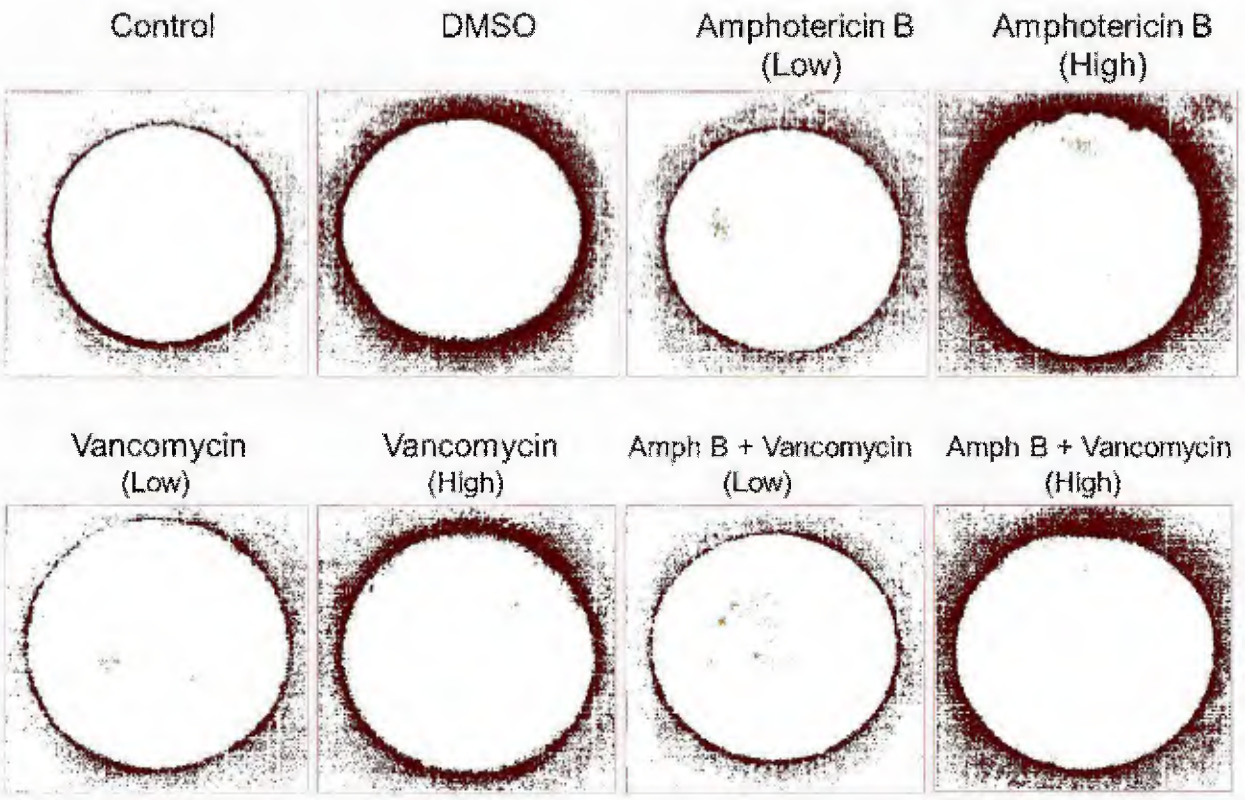

Figure III.3-33. Agar diffusion results toward the Gram-negative bacterium *P. aeruginosa*. Red color indicates viable cell growth. Amph = Amphotericin.

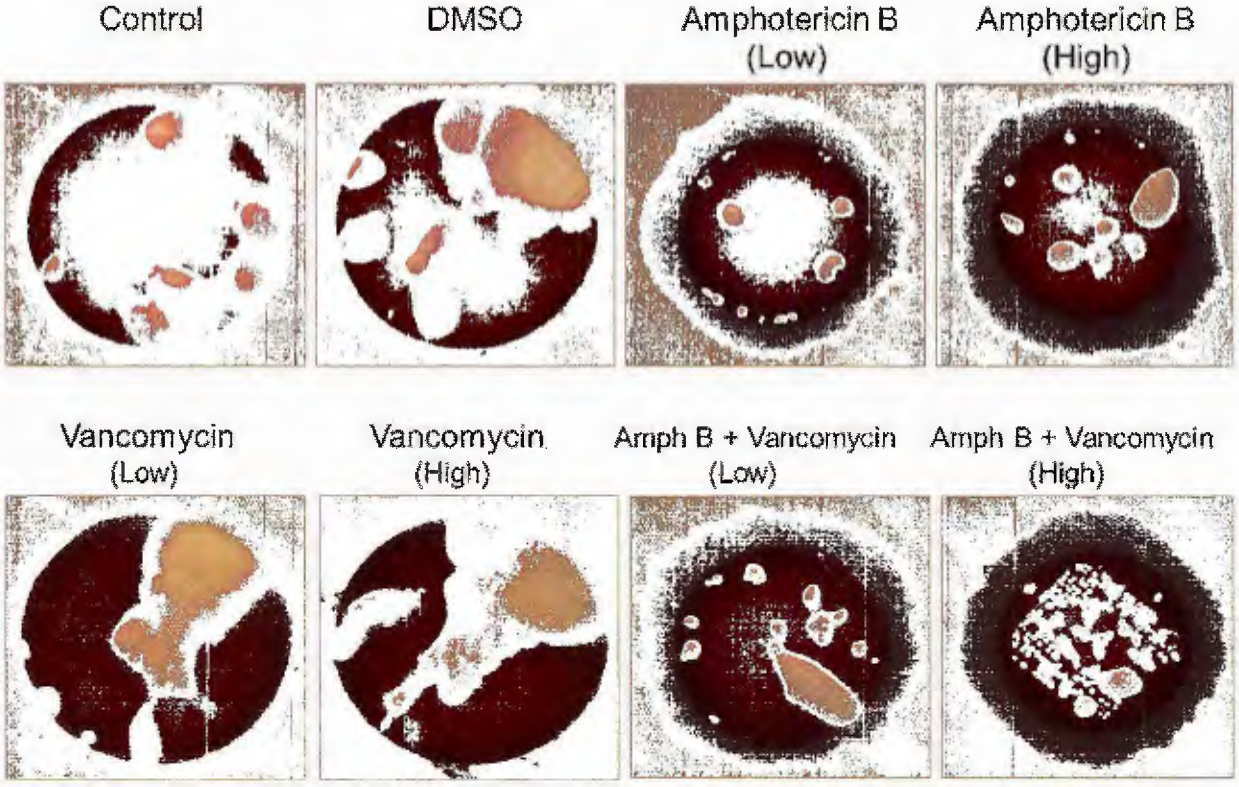

Figure III.3-34. Agar diffusion results toward the yeast *C. albicans*. Red to pinkish color indicates viable cell growth. Amph = Amphotericin.

The second set of materials consisted of a vancomycin treated scaffold and a corresponding untreated control. **Figure III.3-35** shows displays the results of agar diffusion testing toward *S. aureus* and *P. aeruginosa*. The scaffold coated with 15% vancomycin induced a substantial zone of growth inhibition (39 mm) for the Gram-positive bacterium *S. aureus*, but was considerably less effective in preventing the growth of the Gram-negative bacterium *P. aeruginosa* (19 mm). The attenuated activity observed toward *P. aeruginosa* may be expected as vancomycin—a potent peptidoglycan cell wall synthesis inhibitor—is an antibiotic most commonly used to treat multi-drug resistant strains of Gram-positive bacteria, such methicillin tolerant *S. aureus* and *S. epidermidis*. It is interesting to note that the uncoated or untreated scaffold possessed an inherent antibacterial effect, as evidenced by the rather substantial zone of reduced growth.

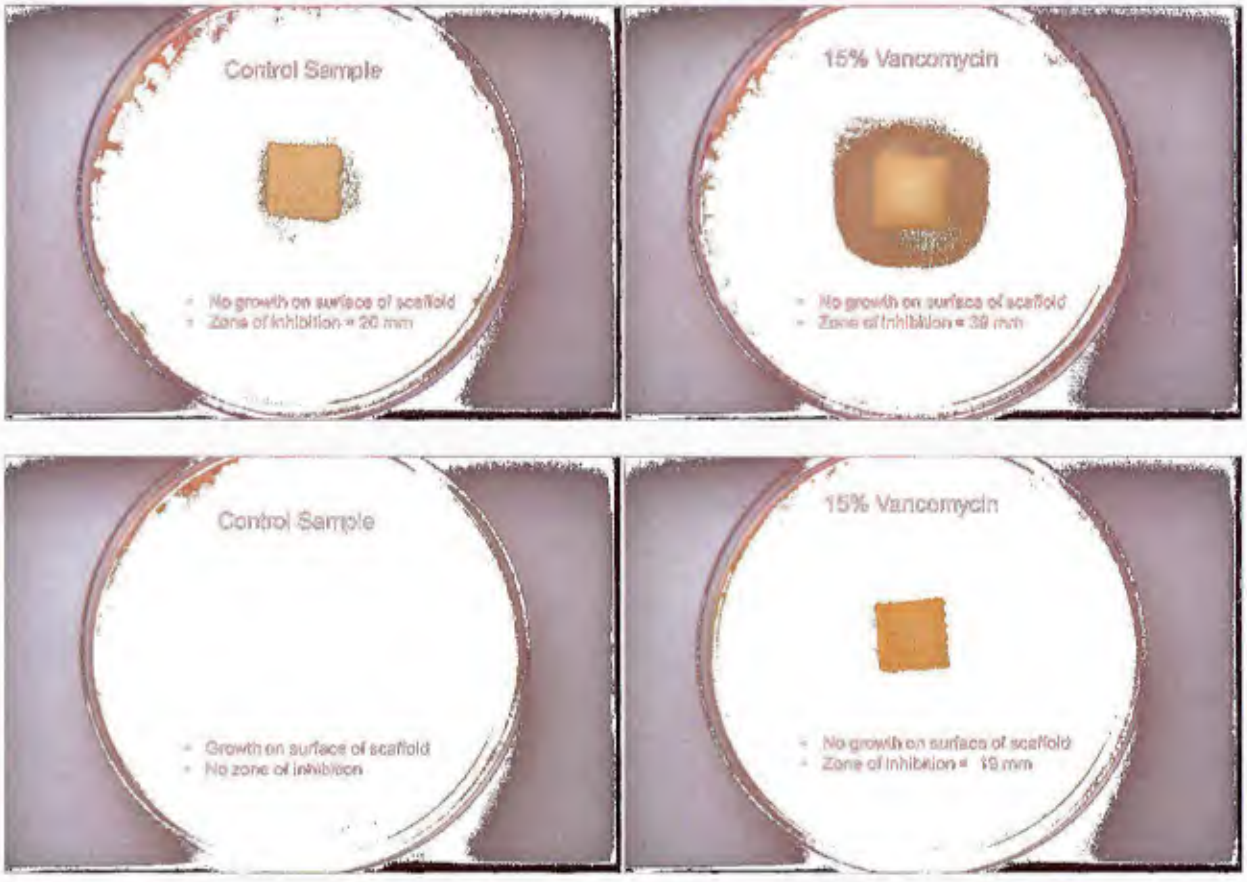

Figure III.3-35. Agar diffusion results toward the Gram-positive bacterium *S. aureus* (top) and the Gram-negative bacterium *P. aeruginosa* (bottom). Red color indicates viable cell growth.

The third set of materials consisted of 5 individual treatments and a non-treated control applied to both aluminum discs and surgical gauze. Figure III.3-36 provides a representative image of the agar plating results for the surgical gauze samples. The combination of 'High' concentrations of azithromycin (Az) + amphotericin B were shown to be most effective at preventing the growth of the three microorganism tested. However, the antimicrobial effectiveness was less pronounced toward the opportunistic fungal pathogen *C. albicans*, with

growth visible beneath the gauze directly in contact with the agar. Thus, these results suggest that the concentration of amphotericin B is too low for practical applications.

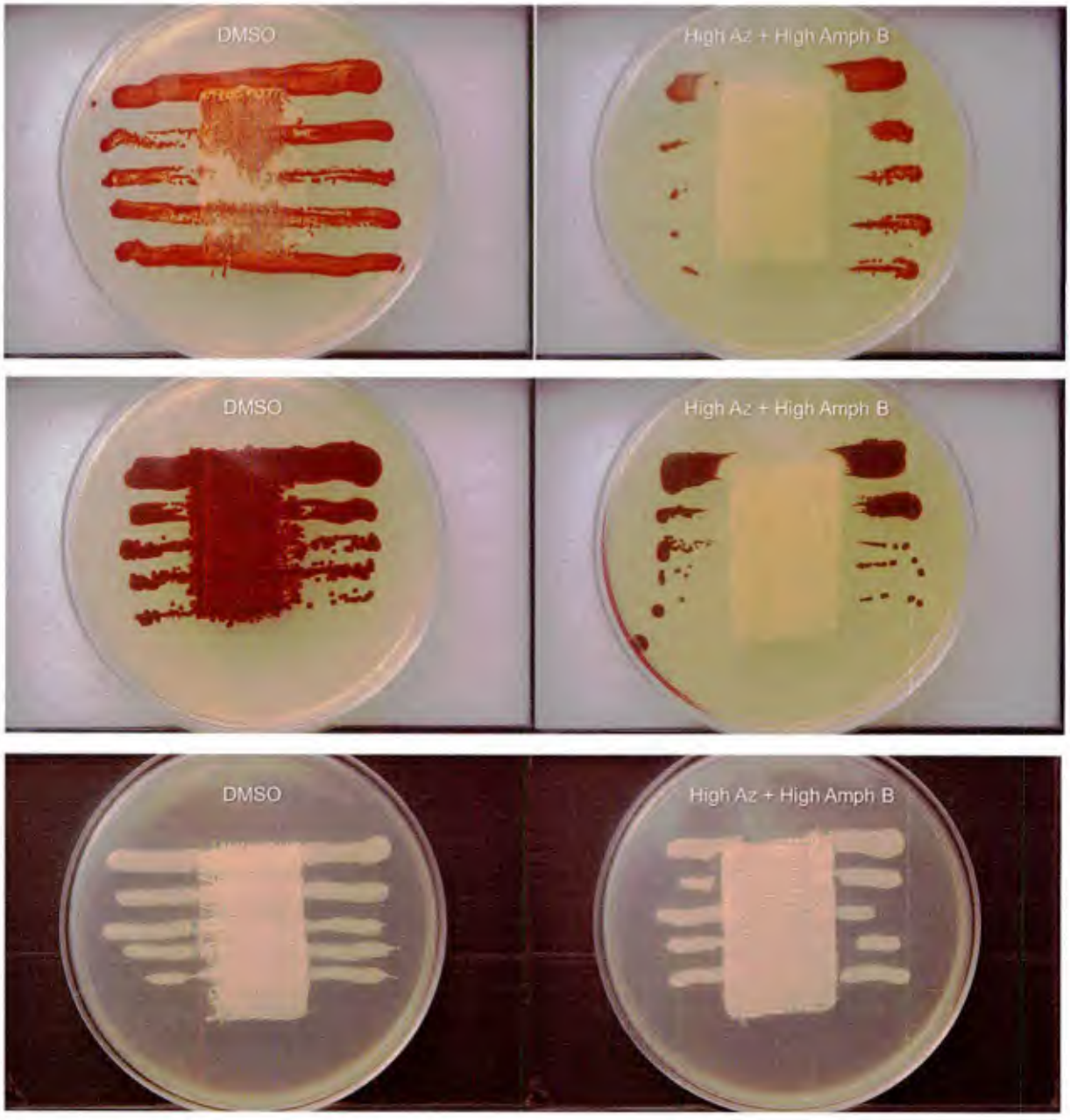

Figure III.3-36. Agar diffusion results toward the Gram-positive bacterium *S. aureus* (top), the Gram-negative bacterium *P. aeruginosa* (middle) and yeast *C. albicans* (bottom). Red color indicates viable cell growth.

The fourth set of materials consisted of 15 antifungal treated substrates that were tested exclusively toward *C. albicans*. **Figure III.3-37** shows the materials that exhibited a discernable degree of growth inhibition, including substrates coated/treated with Isavuconazole, Capsofungin and Ciclopirox Olamine (Ciclopirox). The most effective treatment was Ciclopirox Olamine prepared by a drop casting method, exhibiting a zone of inhibition of 27 mm.

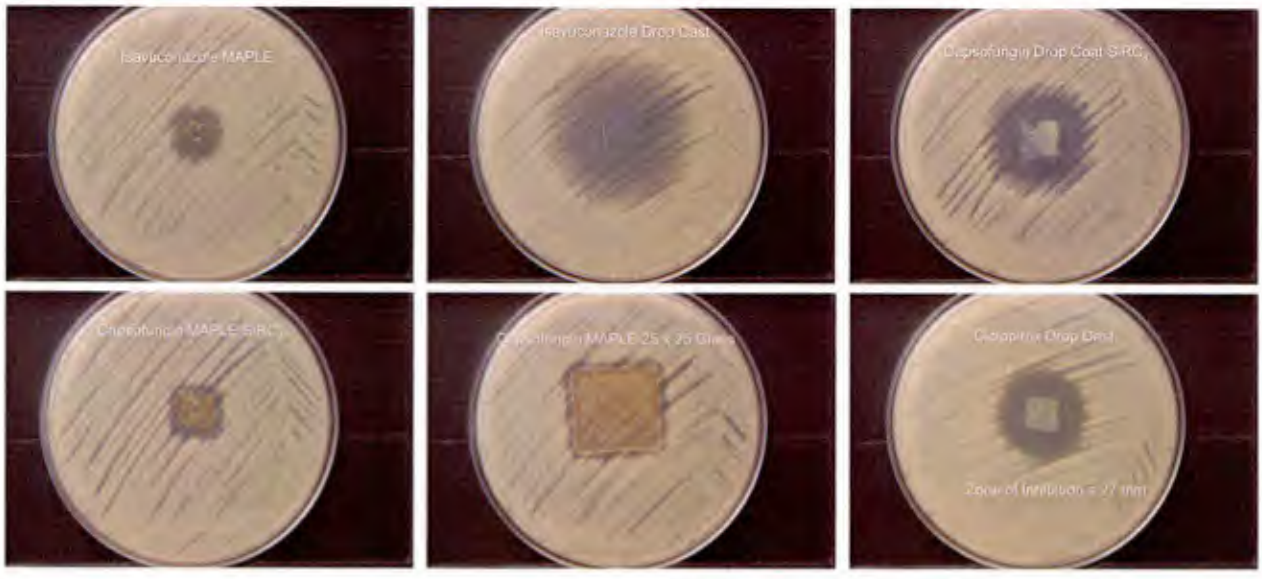

Figure III.3-37. Agar diffusion results toward the yeast *C. albicans.* Red color indicates viable cell growth.