Research Article
Volume 10:3, 2021

Journal of Material Sciences & Engineering

ISSN: 2169-0022 Open Access

Zinc-Based Additives for Biofouling and MIC Protection: Fabrication Method for Long-Term Efficacy

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Abstract

Microbiologically influenced corrosion (MIC) and biofouling both begin with an initial layer of bacteria accumulating on a hard surface exposed to the natural environment. These bacteria quickly form a biofilm which becomes the feeding source for marine life fouling and the root of both of these highly damaging, expensive types of corrosion. Preventative methods for biofilm development is an ongoing field of study due to critical necessity in many industries including healthcare, aerospace, and oil and gas. Today, biofilm inhibitors for the oil and gas industry may include regular cleaning or scraping of the affected surface, electrochemical processes, or biocide injections which have a negative impact on the environment and provide only temporary relief from MIC. This constant need for MIC and fouling remediation creates a great demand and thus market potential for long-term, more environmentally conscious methods to mitigate and control biofilm development. This study investigates the incorporation of well-known biocidal materials as well as one commercial additive into the fabrication process of underwater structures and surfaces. High Density Polyethylene (HDPE) and Fiber Reinforced Plastic (FRP) with antimicrobial additive were processed. Experiments were conducted per ASTM E2149-13a and F895 to evaluate antibacterial efficacy in the laboratory. Field tests were constructed per ASTM D3623 - 78a for material evaluation in offshore fouling conditions. The manufactured materials were tested against gram-positive and gram-negative bacteria and fouling microorganisms to analyze the effectiveness of biofilm prevention. Results showed positive efficacy of biocidal additives incorporated through the fabrication process in all cases including copper, multiple forms of zinc, and titanium dioxide. The commercially available additive produced the largest zone of inhibition and highest reduction of colony forming units in dynamic flow conditions. Fouling tests show that the incorporation of the additive into HDPE and FRP provides a surface protection and thus serves as an agent for material preservation. Results from this study demonstrate innovative and effective methods for surface protection from MIC and biofouling by incorporating antimicrobial additives into the structural matrix during the manufacturing process.

Keywords: High Density Polyethylene • Fiber Reinforced Plastic • MIC

Introduction

Microbes, specifically in the form of biofilms, are a pervasive threat in the oil and gas industry and often result in microbiologically influenced corrosion (MIC) [1-3]. These biofilms form when microorganisms join to form a group and attach themselves to a variety of surfaces including piping, aquatic structures, medical equipment, and even teeth (plaque) [4-7]. The biofilm will begin to react with the chosen substrate of attachment and a deterioration process will take place [8]. MIC clogs up pipelines, increases power consumption and results in equipment corrosion in water injection pipelines [9]. It is well recognized that both chemical and microbiological mechanisms contribute to corrosion and that an estimated 40% of all pipeline corrosion can be attributed to microbiologically influenced corrosion [10,11]. In this type of corrosion, microorganisms cause stress cracking in both metallic and non-metallic materials by forming colonies and eating away at the material surface [11]. MIC has been well documented in substrates exposed to a variety of aqueous environments including seawater, freshwater, soils, and fuels [12-14]. While MIC is commonly caused by sulfate-reducing bacteria (SRB) [15-17], these bacteria can combine with other bacteria and form a more complex, highly aggressive biofilm [1-3,18]

A related phenomenon that occurs when surfaces interact with natural environments such as soil and water is biocorrosion, also known as biofouling [19]. Biofouling was identified more than one hundred years ago, accounts for up to 20% of all corrosion costs, and occurs when a biofilm develops, and marine life begins to attach causing blockages or growths that develop on the hard surface [19-20]. These growths can be made up of microorganisms, algae, plants, and even animals [21]. Biofouling hinders efficiency and increases cost of operation by escalating drag and weight resulting in higher fuel costs and less efficiency as well as the additional cost to remove the buildup on a regular basis [22,23]. Each year, biocorrosion causes billions of dollars of economic losses in the USA [23].

Prevention of chemical corrosion (the most common and well known type) is managed using coatings, sacrificial coatings, linings, environmental controls, corrosion inhibitors, or specific designs and metal choices [24]. These methods are so easily implemented that it is rare to encounter an asset exposed to the environment in oil and gas, aerospace, and the marine industries that is not protected in some way [25]. In 2006, the Trans-Alaska Pipeline suffered from a leak that created significant environmental and economic impact because conventional methods for corrosion prevention were inadequate at prohibiting MIC [26].

Polyethylene is a widely used material in a variety of industries because of its low cost, chemical resistance, and long lifespan and was

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Received date: 01 March, 2021; **Accepted date:** 15 March, 2021; **Published date:** 22 March, 2021

once considered to be a potential solution for MIC [27]. However, it has become clear that the limiting factor of polyethylene is the susceptibility of bacteria growth on the surface [28]. The rates of chemical and physical degradation are higher when compared the damage caused by microbes, but the effects of biodegradation can be more impactful [18,29]. Additionally, combinations of natural factors such as exposure to UV in conjunction with the presence of bacteria and fungi can rapidly accelerate the breakdown of polymers such as polyethylene [30,31]. Studies have shown the path of biodegradation of polymers as 1. attachment of the microorganism to the surface of the polymer, 2. growth of the microorganism using the polymer as the carbon source, 3. initial degradation of the polymer, and 4. Ultimate degradation [30]. Degradation of a polymer material such as HDPE leads to decrease in molecular weight, tensile strength, viscosity, and ultimately, failure. Recent research shows specific degradation rates of HDPE using a variety of offshore and onshore bacteria and fungi exposed to different environmental conditions. Results show that HDPE is susceptible to both MIC and biofouling and that the material failure is greatly enhanced by exposure to high temperatures, humidity, and UV energy [32-34]. Fiberglass has become increasingly accepted as a structural and corrosion solution for offshore and onshore applications because of the inherent qualities of fiberglass, some of which include, slip resistance, electrical and thermal non-conductivity, light weight, non-corrosive, and low maintenance repairs [35,36]. However, resistance to chemical corrosion does not imply resistance to biofouling. When barnacles and other hard shell marine life adhere to fiberglass, they continually grow and exert considerable pressure on the area where they are attached [37]. Fiberglass structures exhibit attractive mechanical properties such as hardness and toughness, but they are not protected against the destructive nature of the barnacle, mollusk, and a wide variety of microorganisms living in the soil and water [38].

For biofouling, the most accepted solution currently is treating the substrate with an antifouling paint or coating [37]. Antifouling paints contain biocides that repel fouling organisms when released at a controlled rate into the water adjacent to the structure [38]. The most common biocides incorporated into the coatings are copper, cuprous oxide, zinc, zinc oxide, and zinc pyrithione [39]. The rate of release of biocides is critical for efficacy; if it is too fast, the antifouling will fail prematurely, especially after a period of intense activity, while if it is too slow, the antifouling will be ineffective, particularly in areas with a high fouling challenge. The fouling organisms must be prevented from attaching and growing on the surface. Once this happens, growth is extremely rapid, and the organisms are beyond the influence of antifouling paints and can only be removed by scrubbing and scraping by underwater divers which is both costly and time consuming [37].

Overall, there is significant literature on studies and products that prevent biofouling through the use of a coating and/or biocide cleaning of the surface [7,10,15,38] The emerging theme, however, is that while the materials are capable of protecting surfaces against biofilm, they only provide protection in the short term and dependent upon the thermal and mechanical performance of the paint and coating and often mixed with complex chemicals that induce environmental risks [40]. This constant need for MIC and fouling remediation creates a great demand and thus market potential for long-term, more environmentally conscious methods to mitigate and control biofilm development [40]. This study investigates the development of such a method incorporating well-known biocidal materials as well as one commercial additive into the fabrication process of underwater structures and surfaces.

Materials and Methods

Biocidal materials

The biocidal materials used in this study were selected based on their documented bactericidal efficacy as well as their accessibility in powder form which was required for the fabrication process [7,10,27,41] These include zinc (44 micron, Belmont Metals, 8024A), zinc oxide (44 micron, ZoChem, ZOX-800), and zinc pyrithione (5 micron, TCI, M0633). The commercial product MIC-GUARD (90 micron, BTG Products, MG) is a MIC and biofouling inhibitor that is advertised as added directly into the coating or lining during the manufacturing process. This product is patent protected, and the technology is proprietary, but the data sheets describe it as a powder additive for biofilm prevention. The MIC-GUARD (MG) additive can be added to coatings and linings according to thickness of material, environment, and cost. This product claims to have enough versatility that it can be added to a variety of material at various percentages to prohibit biofilm development.

Microbiological testing

Microbiological testing was conducted at West Texas A and M University in a certified, third-party laboratory. The test facility used ASTM standards E2149-13a and F895 to evaluate the efficacy of biocides in FRP and HDPE.

ASTM F895 is a test method useful for assessing the cytotoxic potential of new materials and formulations and as part of a quality control program for established medical devices and components. Utilizing this testing method provided qualitative results of the potency of antimicrobial powders. Antimicrobial powders including zinc, zinc oxide, zinc pyrithione, and MIC-GUARD were poured into several wells that had been punched into the inoculated agar. The testing wells were then compared to one another based on the zone of inhibition of cell or spore growth each has created. The petri dishes were place in an incubator at 78 degrees Fahrenheit for 24 hours. The zones of inhibition each well had around itself were then compared. The top two antimicrobial additives from this test were used moving forward.

ASTM E2149 -13a is a test standard for determining antimicrobial activity of biocides under dynamic contact conditions. The 2 × 2 inch samples were placed in sterilized 500 mL flasks with 100 mL of dH2O. For HDPE, gram-positive bacteria, S. aureus was added to each flask at 3 × 106 VC/mL. For fiberglass, gram-negative bacteria, E. coli at approximately 1 × 106 cells/mL was added to each flask. Samples (3 × 0.1 mL) were taken immediately from each flask and spread on TSA plates. The flasks were incubated in a 37°C shaking incubator at 200 rpm. Samples (3 × 0.1 mL) were removed and plated at 1-hr intervals for three hours. Plates were left for growth at 25°C for 60 hr. Colonies were counted using Sphere Flash and the average VC/mL or colony forming units (CFU) were documented.

Sacred Heart Marine Research Center (SHMRC) is a primary test facility for immersion and is located in Karrapad Cove or Tuticorin Bay in south India. This facility is in close proximity to the floating test platforms in the protected bay area and enables SHMRC to expand its research and testing capabilities in marine coatings evaluation and marine research. Samples were sent to SHMRC to be submerged in a static immersion test per ASTM D3623-78a.

ASTM D3623-78a is a test is used to evaluate antifouling panels in shallow submergence. Static immersion remains a necessary step to validate the efficacy of coatings against fouling. The primary fouling organism is the barnacle, Balanus amphitrite amphitrite Darwin. This is also the most common fouling organism found in most parts around the world and likely distributed worldwide by seagoing vessels for many centuries [4]. The seawater temperature remains above 200 degrees Celsius all year and reaches as high as 350 degrees Celsius. The 12 × 6 inch samples are placed two feet below the surface of the water and inspected once a month for growth both qualitatively and quantitatively.

Substrate materials and methods

As the purpose of these experiments was to develop and examine a solution for long-lasting protection, the additive materials were incorporated directly into the substrate during the manufacturing process. This eliminated the dependence of the material performance upon an external coating as well as allowed for a larger matrix which will hold greater amounts of additive and increase the lifespan of the biofilm protection. Two different

fabrication processes were used which include the rotational molding of high-density polyethylene (HDPE) substrates and the laying up of structural fiberglass components.

HDPE powder and manufacturing specifications were provided by a rotational lining company. The HDPE samples were prepared using a lab-scale rotational molder and each sample contained 900 grams of HDPE dry powder. The samples created include a control (no additive), zinc pyrithione at 10%, and MG at 10%. The powder was added to the dry HDPE, poured into a mold, rotationally lined at 350 degrees Fahrenheit for 8 hours, and then slowly cooled over two hours. The samples were cut to microbiological testing facility specifications at 2 × 2 × 0.5 and another set at 12 × 6 × 0.25 inches.

Fiberglass samples were prepared by a company that specializes in fiber reinforced plastic. The additive was added while the plasticizer was in liquid form and before the fiber was added and curing process began. The production of these samples was done externally and unfortunately, only one set of the requested samples was produced. A control sample and MG at 10% was included in this set of samples which were cut to 2 × 2 × 0.5 and 12 × 6 × 0.2 inches.

Results and Discussion

The efficacy of each additive was evaluated for a visual zone of inhibition around the powder well. The results of agar diffusion tests on Escherichia coli are shown in Figure 1. The control sample did not show antimicrobial activity on the bacterial strain. Samples treated with zinc demonstrated insufficient antimicrobial activity and will not be continued in future tests. Samples treated with zinc oxide, MIC-GUARD, and zinc pyrithione resulted effective in inhibiting bacterial growth per the visible zone region created around the sample and will be continued for use in the fabricated substrates.

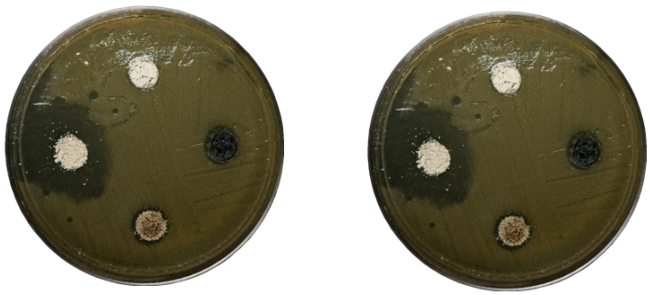


Figure 1. Agar diffusion tests on *E.coli*.

The antibacterial efficacy of samples of rotationally molded HDPE containing the additives was also evaluated through quantitative antibacterial tests on S. aureus (Figures 2 and 3). In Figure 2 representative pictures obtained by the bacteria enumeration tests are reported. The top row of samples is the control at 1 hour, 2 hours, and 3 hours. The plates experience a natural cell death over the course of three hours. The bottom row is the cell growth from the samples containing MG additive. The bacteria are completely neutralized by the second hour. A significant reduction in colony number is clearly visible for the treated samples in comparison with the untreated or control samples.

Figure 3 displays the graphed results of the antimicrobial efficacy of molded HDPE samples including zinc oxide, zinc pyrithione, and MG additives under dynamic flow conditions. The results in Figure 3 indicate that zinc pyrithione and MG are the most efficacious additives in HDPE under these test parameters. The antibacterial efficacy of the zinc pyrithione was 99.9% for S. aureus in dynamic fluid conditions. The MG data sheet indicates that it can be added in quantities ranging from 0.5%-15%. Figure 3 shows data with the MG incorporated at the minimum recommended quantity of 0.5%. The sample resists the bacterial growth for two hours and then the bacteria begin to grow again. This could be because of the low amounts of additive which did not result in a homogenous blend.

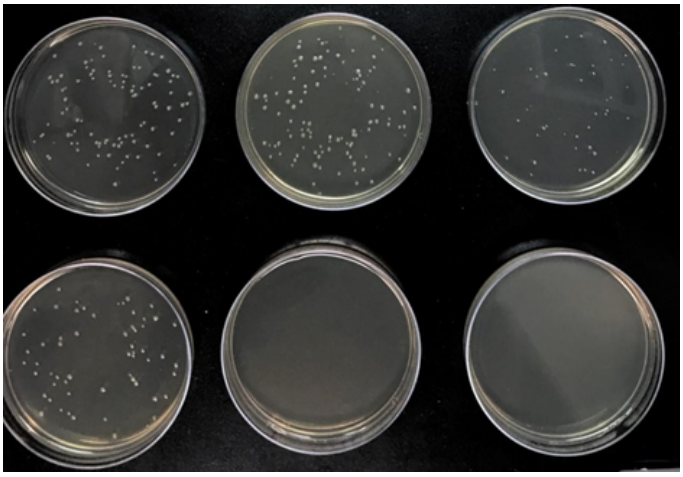


Figure 2. Colony counts for dynamic shaker test on *S. Aureus*.

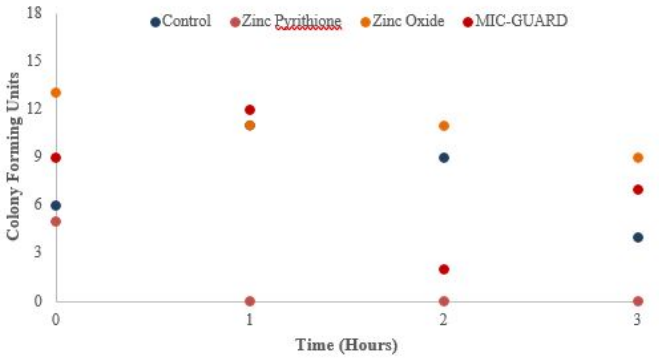


Figure 3. Dynamic shaker tests on *S.aureus*.

Figure 4 shows the results of the antimicrobial efficacy of molded HDPE samples with the MG additives increased to 10% under dynamic flow conditions. After the first hour the antimicrobial HDPE and control HDPE had similar counts. The most dramatic change occurs between two and three hours of exposure when the antimicrobial HDPE begins to significantly lower the S. aureus cell counts. After three hours, the antimicrobial HDPE demonstrated an efficacy of 99.0% with the 10% weight of the additive within HDPE.

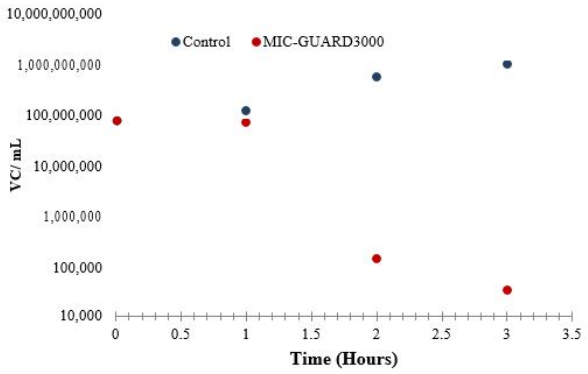


Figure 4. Dynamic shaker tests on S.aureus with 10% MG additive in HDPE samples.

The Table 1 shows the results from the fiberglass samples containing the antimicrobial additive when exposed to dynamic flow conditions. Each sample contains 10% MG by weight.

Sample	Escherichia coli	phical	phical	phical
	VC/mL @1hr	VC/ mL @2hr	VC/ mL @3hr	Antibacterial Efficacy (%)
Fiberglass	1.06 x 10^6	9.6 x 10^5	6.4 x 10^5	-
Fiberglass @ 10% MG	1.71 x 10^5	5.3 x 10^4	0	99.9%
Fiberglass @ 10% MG	7.8 x 10^6	1.25 x 10^5	0	99.9%

Table 1. Antibacterial efficacy of fiberglass samples containing 10% MG on *E.coli*

Figure 5 displays a graphical representation of the quantitative results for efficacy on E. coli of fiberglass samples containing 10% MG. The E. coli experienced a minimal natural death over the three-hour span as seen for the control in Table 1 and Figure 5. The fiberglass samples were duplicated, and both showed the same rates of depletion during the same timeframe indicating high repeatability. Similar to the HDPE, there was hardly any change after one hour of MG exposure to E coli. The rate was most significant between two and three hours taking the counts to zero with an antimicrobial efficacy of 99.9%.

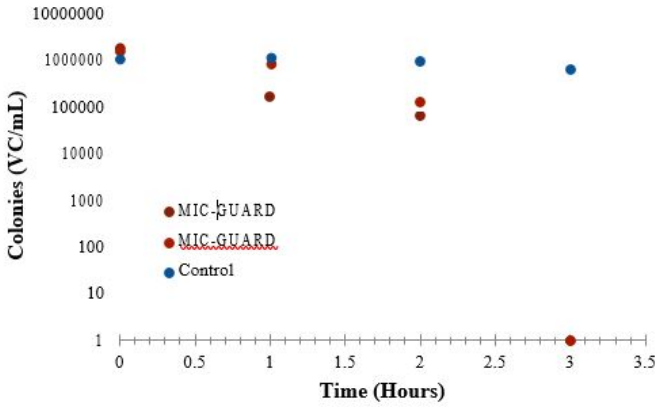
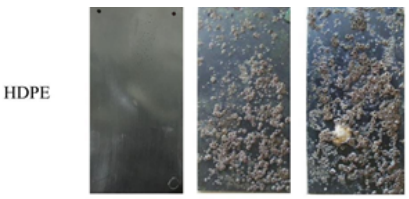


Figure 5. Dynamic shaker tests on *E.coli* with 10% MG additive in fiberglass samples.

E. coli is a gram-negative bacterium that has a more complicated cell wall structure which makes them more difficult to penetrate and mitigate.27 S. aureus is a gram positive bacterium and is naturally more susceptible to biocides therefore easier to control. The surface roughness of fiberglass compared to HDPE could cause also cause a significant change in release rate of antimicrobial between the two materials. Laboratory testing indicated that the zinc pyrithione and MG showed the highest efficacies and performance with both gram-positive and gram-negative bacteria and these two additives will be continued for use in field testing.

Field testing was conducted in Tuticorin Bay off the coast of Southern India and pictures are shown in Figure 6. Samples of HDPE were rotationally molded using zinc pyrithione and MG and placed in shallow submersion tests. On day 120, the HDPE containing no additive (control) shows significant attachment of barnacles, mollusks and oysters. The HDPE sample containing zinc pyrithione additive at 10% has also experienced an abundance of marine growth and is covered with fouling. The HDPE sample containing 10% MG, however, shows minimal attachment of marine life or any type of fouling on the surface.



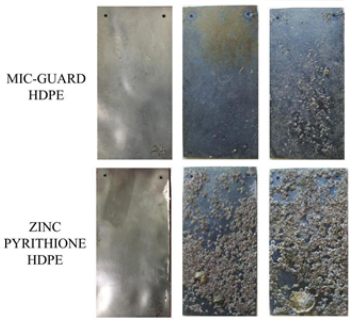


Figure 6. Static submersion tests on marine growth and fouling for HDPE samples.

SHMRC reports the number of barnacle on either side of the duplicated panels, maximum diameter of barnacles on each side of the sample, and the number of oysters attached to the surface of the sample on a monthly basis. The average number of barnacles on each sample is shown in Figure 7. The HDPE substrate with zinc pyrithione collected the largest number of barnacles compared to any other samples at 208 barnacles per side after 30 days. Each sample experienced some marine growth at different times over the 120 days. The control and zinc pyrithione had the largest accumulation during the first month. HDPE samples with MG show a delayed growth of marine life until 90 days where a slight increase was observed. Reports also showed evidence of barnacles detaching from the HDPE with MG samples, so more time in the ocean is needed for a conclusive discussion.

Figures 6 and 7 show that the largest barnacles reside on the control substance indicating that they have had time to attach and grow over the duration of the study. Figure 8 displays the maximum barnacle size per sample over time. Because the results are reported monthly, it is possible to track a single barnacle for attachment and growth. This is important because it gives an indication of duration of attachment. The barnacles on the HDPE samples with additive probably took longer to determine attachment compared to the control so their growth rate is slightly behind in comparison. Results from day 120 show that even though the barnacles that have been attached since day 30, they have not yet grown to the size of 12 mm as seen on the control HDPE. Based on 120 days of ocean data, HDPE molded with zinc pyrithione additive reduces the barnacle growth by 8.7% and HDPE molded with MG additive restricts the barnacle growth by 13%. By reducing the attachment and grow rate of the barnacles, the likelihood of hard-shell fouling is also reduced.

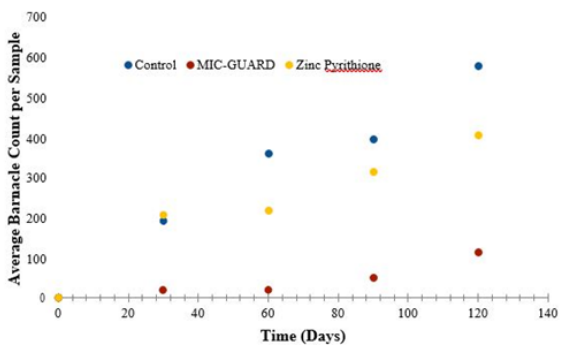


Figure 7. Average barnacle attachment per side of sample over time.

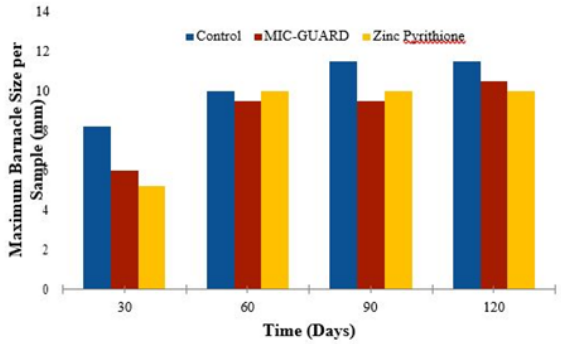


Figure 8. Maximum barnacle size per sample over time.

The MIC-GUARD additive presented the most effective inhibition of marine life attachment and growth per the visible accumulation on its surface and will be continued for use for fiberglass fabrication testing. Fiberglass was fabricated using 10% MG in the resin and samples were sent to SHMRC in Tuticorin Bay. Figure 9 displays the results of fouling efficacy of fiberglass including two samples which contain MG additive and one untreated control after 60 days of static immersion testing.

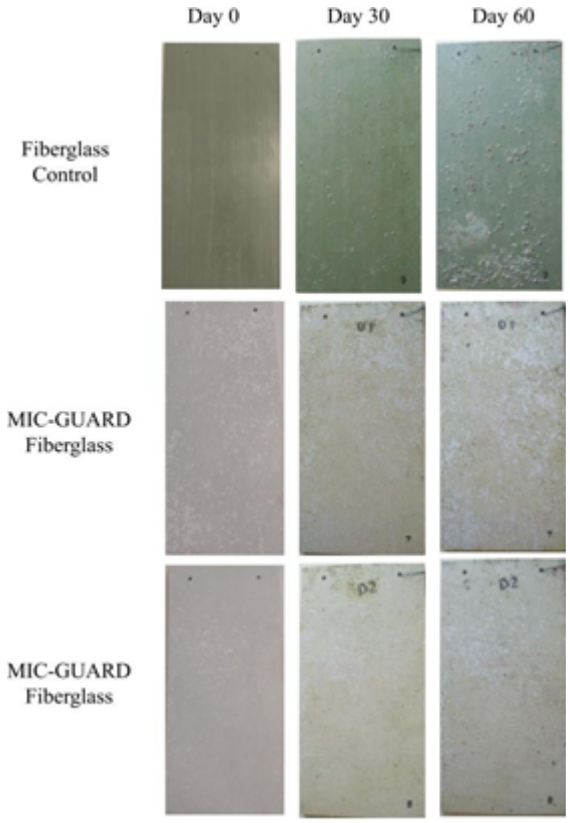


Figure 9. Static submersion tests on marine growth and fouling for fiberglass samples.

Similar to the HDPE, after 60 days of submergence there was hardly any marine life on the samples treated with MG. Figure 10 displays a graphical depiction of the results for barnacle attachment of fiberglass samples containing 10% MG additive. The duplicated fiberglass samples follow the same trend at an average of 7.25 barnacles on MG samples and 135 on untreated fiberglass after an eight-week timeframe.

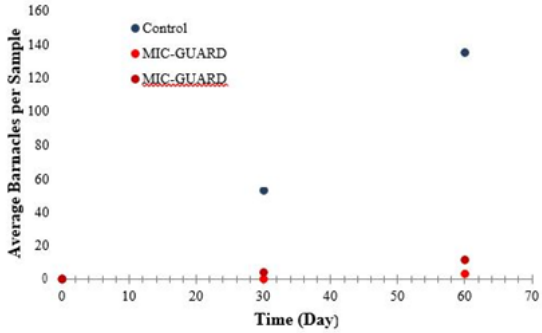


Figure 10. Average barnacle attachment with 10% MG additive in fiberglass samples.

The report from SHMRC also documents the number of oysters that have attached to the samples over time. Oyster larvae navigate waters freely until they determine a suitable location for growth.4 Typically, they attach to a surface that has a healthy biofilm development that serves as food and the platform for attachment.3-5,7-8 Samples of HDPE and fiberglass containing MG additive show no oyster attachment and growth, while all control samples show oyster attachment. These results indicate that MG is preserving the substrate surface by preventing the initial development of a biofilm where marine life can attach, grow, and eventually cause failure.

Conclusion

This study investigates the development of an innovative method for incorporating zinc-based antimicrobial materials into the initial fabrication process of underwater structures and surfaces to protect against biofouling and microbiologically influenced corrosion. Both laboratory and field testing was used to demonstrate the efficacy of the materials against gram positive and gram negative bacteria. Agar diffusion testing highlighted zinc oxide, zinc pyrithione, and a commercially available material called MIC-GUARD as demonstrating the strongest resistance to bacteria based on the size of their inhibiting zone. These three additives were then used in dynamic shaker testing to evaluate the effectiveness of the additives exposed to water. Both zinc pyrithione and MIC-GUARD performed well under dynamic fluid conditions and were used for further field testing. High density polyethylene samples were rotationally molded with zinc pyrithione and MIC-GUARD additives. The samples were submerged in the Indian Ocean and marine fouling was observed over the course of 120 days. The MIC-GUARD additive protected the polyethylene samples from marine attachment and growth indicating that it preserves the substrate by preventing biofilm development. The MIC-GUARD additive was then incorporated into a fiberglass matrix and submerged in the ocean for 60 days. The fiberglass control samples had immediate attachment and growth of barnacles and oysters which increased over the course of testing. The fiberglass samples with MIC-GUARD have minimal barnacle attachment, zero oyster attachment, and show signs of detachment of marine life. This again indicates the lack of biofilm development on the surface of the MIC-GUARD treated samples.

Results from this study fill an increasing void as biofouling and microbiologically influenced corrosion are being recognized as a major problem in a variety of industries including oil and gas, aviation, and healthcare. Expensive coatings and toxic chemicals are being used as a short-term solution, but the ability to fabricate the substrate material with a 'built-in' antimicrobial presents a creative long term solution.

Conflicts of Interest

This work is protected under U.S. Patent Application Serial No. 63/110,880

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How to cite this article: Paige M. Dodge and Emily M. Hunt. Zinc-Based Biofouling and Mic Protection: Fabrication Method for Long-Term Efficacy *J Material Sci Eng 10 (2021)*