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The Effect of Addition of Mangrove *Avicennia Marina* Extract on The Biofouling Activity to Epoxy Coating

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ABSTRACT

Biofouling is one of the problems related to construction maintenance in industrial developments in the maritime construction sector because biofouling adhesion can affect construction productivity. This problem can be overcome by coating using natural-based paints to reduce the ecological impact that will occur. This study aims to determine the effect of the addition of Avicennia marina mangrove extract as an anti-fouling compound mixed with epoxy paint. This research was conducted by mixing Avicennia marina mangrove extract with epoxy paint. Then the alloy is applied as a coating on the ASTM A36 steel specimen. Furthermore, the test was carried out by immersing the specimen in a solution containing Littorina littorea as a biofouling biota. The average results of the biofouling affixing activity test with the addition of mangrove extract concentrations of 0%, 10%, 20%, and 30% were respectively 24.0 gr, 20.5 gr, 13.5 gr, and 6 gr. This test shows that the more the addition of mangrove extracts, the smaller the adhering activity of biofouling to the specimen.

Keywords: *Biofouling*, *Coating,* Epoxy, Mangrove, and *Avicennia marina*.

1. PRELIMINARY

The development of the industry in the maritime construction sector is getting better and better. In practice, metal is one of the primary materials used on a large scale due to its strong physical properties and abundant availability. However, there are still problems related to iron which are often the reason for the increase in production costs to maintain the problems that occur, one of which is biofouling. Biofouling is a collection of living organisms, either macro or micro, which attach to the surface of structures submerged in seawater, such as bacteria, fungi, algae, algae, and shellfish. This continuous adhesion of biofouling can cause further problems such as increasing the corrosion rate of the structure, weakening the carrying capacity of the rig, reducing the readability of the sensor, reducing the maximum speed of the ship, increasing the consumption of materials, and many more. So it takes more maintenance costs which can result in losses, both in cost and time.

An effort that can be made to minimize the adhesion of biofouling to the structure is coating. In general, coatings are used to increase the service life and reliability of structures, such as enhancing the appearance of structures, protecting against corrosion, and slowing down the maintenance life of structures. Since discovering new problems related to biofouling, coatings have been started using anti-fouling paints, namely paints that contain synthetic chemical compounds that are useful for slowing down the adhesion of biofouling.

Initially, anti-fouling paints were made using light chemical compounds, such as arsenic or mercuric oxide derived from linseed oil until it was found that the use of tributyltin (TBT) was considered the most efficient to inhibit the adhesion of biofouling for a long time [2]. Tributyltin contains organotin compounds that are very stable in dark places (for example, the seabed) and do not easily degrade in an environment that impacts the seabed pollution for a long time. TBT is also very toxic to molluscs, crustaceans, and algae species such as shrimp, crab, barnacles, shellfish, and algae. Since 2008, TBT-based anti-fouling paint has been banned by the International Maritime Organization (IMO). Then found another anti-fouling using a mixture of polymer and biocide, which is only toxic to certain biofouling, but this is also still toxic to other marine biota and not yet safe for the environment.

Prioritizing the safety of marine ecology, further research was carried out to address the biofouling problem that noxious anti-fouling paints (such as TBT) are unable to solve. Another thing that can be done to overcome this problem is to use natural ingredients as anti-fouling agents. Natural ingredients contain antibacterial properties that can inhibit the adhesion of biofouling. The use of natural ingredients can also minimize the risk of other environmental pollution because they are environmentally friendly, and their availability is abundant. The use of

natural ingredients as anti-fouling agents is proven by research conducted by Santi et al. (2014) [8], who use seaweed as an anti-fouling agent to show that seaweed extract can inhibit film formation, thereby slowing the process of adhering to biofouling. Research by Bazes et al. (2006) [4] and Purnama et al. (2010) [9] proved that red algae and green algae are capable of being anti-fouling agents because they have active compounds as antibacterial. Research using ketapang leaves conducted by Amin (2017) [2] shows that ketapang leaf extract affects the amount of biofouling attached to the material's surface. Another potential anti-fouling substance is mangroves. Mangroves can neutralize the heavy metal lead (Pb), which pollutes the environment by storing lots of water to dilute the concentration of lead absorbed in the body, thereby reducing the toxicity of the metal spread to the environment. Mangroves contain phenolic compounds in the form of alkaloids, tannins, flavonoids, steroids, and phenolics [6], antibacterial compounds, and can provide toxic effects on biofouling.

In this study, the author has used phenolic compounds in mangrove extract *Avicennia marina* as anti-fouling compounds mixed with epoxy paint to determine the effect of phenolic compounds in mangrove extracts in inhibiting biofouling adhesion. This study used the Mangrove *Avicennia marina* because the availability was mainly in the Wonorejo mangrove area. This research was conducted using ASTM A36 steel as a specimen is often used in structures with variations in the concentration of the extract to mixed in the paint. The results of this final project are expected to determine the effect of phenolic compounds in Avicennia mangrove extracts

2. BASIC THEORY

2.1 ASTM A36 Steel

In low carbon steel or those with low carbon, it is one of the steels most often used in the maritime industry, where its use is in the construction of marine structures and ship construction. A36 steel is included in the low carbon steel classification because it contains carbon with 0.1% - 0.3% [3].

2.2 Coating

The coating is a construction maintenance method to inhibit corrosion rates by creating a coating that prevents the material from coming into direct contact with the environment. In addition, this coating is also helpful for protecting construction materials from biofouling and increasing the construction's aesthetics.

2.3 Biofouling

Biofouling results from the interaction between living mechanisms and solutes on the surface of the material submerged in water, which is characterized by the growth of biofilms. The growth of biofilms is the result of the colonization of bacteria on the surface of the submerged material, which absorbs organic material from the sea. This biofouling process may take up to 3 weeks [10]. The formation process is divided into two stages:

a. Micro fouling formation

This process begins with the attachment of bacteria to the surface of the material; the bacteria attach, multiply, and grows to form colonies, which will later form biofilms followed by adhering to macrofouling. This bacterial growth is caused because the surface area of the material provides molecular films of organic materials such as proteins, photographic, and polysaccharides needed by bacteria. This microfouling takes 1-24 hours to stick to the surface of the submerged material. This microfouling then releases Extracellular Polymeric Substances (EPS) as a result of excretion, which functions to envelop the cells, maintain adhesion to the material, and promote the attachment of macrofouling.

b. Macro fouling Adhesion

This adhesion is caused by the EPS substance secreted by micro fouling, which encourages the macrofouling to stick. This process takes 2-3 weeks to make this biofouling community stick to the surface of the material. According to Abarzua (1995) [1], the attachment of macrofouling is divided into two processes, namely temporary adhesion and permanent adhesion. Temporary adhesion is when the macrofouling is attached to find a suitable substrate for permanent attachment. Suppose in the attachment process, the macrofouling feels incompatible with the substrate. In that case, the macrofouling will automatically release itself, and if in the attachment process, the macrofouling feels it matches the substrate it is attached to. The surface will be attached to the harvester by the macrofouling, and this process is called the permanent attachment process. Macrofouling that sticks to the surface depends on the biota scattered in the environment during the process. Macrofouling that is often found is barnacles, shellfish, and macroalgae.

2.4 Impact of Biofouling Activity

According to the Global Invasive Species Program (2008) [7], based on reports received by IMO, biofouling affects:

- 74% of non-native marine invertebrates in the Hawaiian Islands,
- 42% of marine species are accidentally introduced to Japan,
- 69% arrival of adventitious marine species in New Zealand,
- 78% of marine species introduced in Port Philip Bay, Australia, and
- 70% of the species that have invaded North America are coastal

The impact caused by affixing biofouling to

construction is the need for an increase in maintenance and labor costs. Reduced reliability is faster than design estimates; this also reduces construction productivity.

2.5 Discovery of Antifouling

The best effort that can be done to solve the problem of biofouling is to use an anti-fouling agent. Initially, the anti-fouling agents used were pitch, tar, wax, lead, and arsenic. Since the 1800s, a new coating that is considered the most effective in inhibiting biofouling growth has been discovered, namely the tributyltin compound (TBT). TBT acts as a biocide incorporated into the paint so that it can be removed from the coating, effectively inhibiting the fouling of the hull for up to 5 years. TBT also has a bad impact on other organisms besides biofouling because its toxicity is too high, so since 2008 the use of TBT has been banned by IMO.

Research on the anti-fouling mechanism of marine organisms shows that some secondary metabolites can prevent the adherence of biofouling rather than biocides, so further research is needed in this regard. The discovery of naturally occurring bioactive substances is based on a bioassay-guided fractionation and purification procedure. The choice of test organisms for bioassays is very important and must be ecologically relevant.

2.6 *Avicennia marina Mangrove*

In the past, plants were considered the main ingredient of medicine. Until now, research related to their use is continuing to find innovations. Tropical and sub-tropical areas are practical, such as antimicrobial, antiviral, and anti-fungal. Many medicinal plant extracts have been known to have antimicrobial effects, one of which is mangroves. Mangroves contain toxic compounds such as steroids, triterpenes, saponins, flavonoids, alkaloids, tannins which have been proven by research by Ravikumar et al. (2011) [10], Danata et al. (2014) [6], and Lotlikar (2016) [9] show that mangrove extract does contain antibacterial substances. For this reason, mangroves have the potential to inhibit the growth of micro-fouling films so that they have the potential as an anti-fouling agent. According to research by Mangrio et al. (2016) [10], secondary metabolite compounds contained in mangroves *Avicennia marina* as listed in table 1 below:

Table 1. Phytochemical Screening of Secondary Metabolite Compounds in Mangrove *Avicennia marina*

Metabolite Compounds	Mangrove *Avicennia marina*			
	Flower	Leaf	Branch	Root
Alkaloid	+	++	+	+
Flavonoid	+	++	++	+
Tanin	+	++	+++	-
Terpenoid	-	+	+	-
Saponin	-	+	-	-
Sterol	+	+	+	-

+: have -: do not have

In addition, his research proved that dissolving 2.0 grams of dry *Avicennia marina* mangrove leaves in 10 mL of 70% ethanol solution for 15 minutes produced phenolic compounds 0.5-0.8 mg / mL and produced flavonoids compounds 1.15-1.2 mg / mL. Research conducted by Lincy et al. (2013) [8] also stated that 1 mL of crude extract of *Avicennia marina* mangrove dissolved in 1 mL of Folin Ciocalteu teau and 1 mL of aqueous NaCl for 30 minutes could produce phenolic compounds up to 257 mg / L, and 72 mg / L of compounds. These are flavonoids.

3. MATERIALS AND METHODS

1. Extraction of Mangrove *Avicennia marina*

The extraction process of *Avicennia marina* mangrove is carried out using the maceration method carried out 14x24 hours to get the maximum extraction results. The mangrove branches of the *Avicennia marina* were cleaned (Figure 1 (a)), then oven-dried. Dry twigs are blended into powder (simplicia) and then immersed in 96% ethanol solvent at room temperature for 14 x 24 hours. The ratio between the mass of the extract and the volume of the solution is 1: 4, which means that 1 gram of simplicia is immersed in 4 mL of solvent. In this study, 3 kg of mangrove branches were dissolved using 12 liters of 96% ethanol. Then the solution is filtered to be separated between the filtrate and the immersion pulp. The filtrate obtained was evaporated to obtain *Avicennia marina* mangrove branch extract in the form of liquid which was then prepared into three (3) concentrations to be dissolved in paint, namely 10%, 20%, and 30%.

(a) (b)

Figure 1. (a) *Avicennia marina* mangrove that has been cleaned (b) Extracted from *Avicennia marina* mangrove

Evaporation is carried out on the immersion results after the immersion process is complete to get the extraction results with the appropriate texture. This evaporation is carried out under the sun for 14 x 24 hours to get the extraction result in the form of a liquid with a liquid volume level of 5% (Figure 1 (b)); this is done to make it easier to mix the extraction results with epoxy paint on the coating.

2. Paint Preparation and Mixing

The floating breakwater structure modeled on the paint used in this study is epoxy NIPPON 8048 with two (2) components, namely epoxy resin (Figure 2 (c)) and

Hardener (Figure 2 (b)). The two components are mixed in a ratio of 1: 1, then the paint is added to the extract of the mangrove branches of *Avicennia marina* (Figure 2 (a)) with concentrations of 10%, 20%, and 30% of the total volume of paint, respectively, so that later obtained three (3) paint mixes.

(a) (b) (c)

Figure 2. (a) extracts of Mangrove *Avicennia marina* (b) Hardener (c) Epoxy resin NIPPON 8048

The painting process on steel specimens was carried out using epoxy paint NIPPON 8048 brush, with two applications to get a dry specimen thickness to a dry thickness of 250 µm. The dry thickness is calculated using a Dry Film Thickness Gauge (Figure 3).

Figure 3. Dry Film Thickness Measurement

3. Coating Process

ASTM A36 steel specimens were cut to a size of 90x40x10 mm (Figure 4 (a)), then the surface was cleaned using sandpaper with a 60 grid (Figure 4 (b)) attached to the grinding wheel to speed up and facilitate the sanding process, until the surface is clean (Figure 4 (c)).

(a) (b) (c)

Figure 4. (a) ASTM A36 steel specimen before cleaning (b) the process of cleaning the specimen using sandpaper (c) Cleaned ASTM A36 steel specimens

The coating process is carried out using a brush with two applications. The process takes 2 x 24 hours to get dry specimens. This is because to re-apply the previous coating layer, it must be dry, which according to the technical data sheet NIPPON 8048, repainting can only be done 7 hours after the initial painting. The combination of epoxy paint and mangrove extract carried out in this coating is as follows (Table 2):

Table 2. Alloy Composition for Painting

No	Composition (%)		Specimen Code
	Epoxy	Mangrove Extract	
1	100	0	E100M0-1
2			E100M0-2
3	90	10	E90M10-1
4			E90M10-2
5	80	20	E80M20-1
6			E80M20-2
7	70	30	E70M30-1
8			E70M30-2

4. Biofouling testing

This test was carried out by immersing the specimen for 7 x 24 hours in a 3.5% NaCl solution, given the *Littorina littorea* snail as a biofouling biota. This test is carried out in an aquarium fitted with a filter. The specimens were immersed and given a distance of 4 cm between specimens; then, the *Littorina littorea* snails were evenly distributed to the aquarium (Figure 5). The initial weight of specimens was calculated before soaking and the final weight after soaking to find out how much biofouling was attached to determine the activity of the specimen coating.

Figure 5. Arrangement of specimens and distribution of *Littorina littorea* in an aquarium

4. RESULTS AND DISCUSSION

4.1. Result of Dry Film Thickness

The dry film thickness coating measurement is carried out using a Dry Film Thickness Gauge when the paint is completely dry. In this study, the measurement of Dry Film Thickness at three (3) points, with the following measurement results (Table 3):

Table 3. Result of Dry Film Thickness

No	Spesiment	Result on point (µm)			Range (µm)
		1	2	3	
1	E100M0-1	259	259	260	259.33
2	E100M0-2	260	259	252	257.00
3	E90M10-1	257	256	257	256.67
4	E90M10-2	255	260	254	256.33
5	E80M20-1	254	255	254	254.33
6	E80M20-2	257	255	253	255.00
7	E70M30-1	254	253	256	254.33
8	E70M30-2	254	252	250	252.00

Specimen code is written in the format of epoxy composition (E), mangrove extract composition (M), and test number (1/2).

4.2. Biofouling Testing Analysis

This test was conducted to determine how *Littorina littorea* as biofouling against the epoxy coating layer with the addition of mangrove extracts. This is done by entering *Littorina littorea* in an aquarium with seawater added. Then observed its activity on the coated specimen for 14 days.

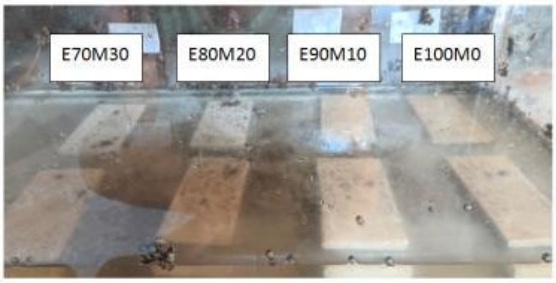

Figure 6. The activity of *Littorina littorea* during 24 hours

Figure 6 shows the activity of *Littorina littorea* at the initial 24 hours. During these 24 hours, the bacteria carried in seawater are adapting to release Extracellular Polymeric Substances (EPS), which functions to attract *Littorina littorea*. During these 24 hours, *Littorina littorea* also adapts to her new environment, actively moving to determine a suitable place. It took seven days for *Littorina littorea* to determine the right area for habitat [16].

Figure 7. Activity of *Littorina littorea* on day 7

The activity of *Littorina littorea* on day 7 (Figure 7), shows that the biofouling has found a suitable place for its habitat. In this picture, the E100M0 specimen shows the most *Littorina littorea* attachment activity, while the E70M30 specimen does not show the presence of *Littorina littorea*.

Figure 8. Measurement of weight gain due to attachment of *Littorina littorea*

After 14 days, there was a very significant difference in the number of *Littorina littorea* attached to the specimen. Figure 8 shows that the E100M0-2 specimen with a 100% epoxy composition without the addition of mangrove extract, almost the entire surface of the specimen was covered by *Littorina littorea*. While the E70M30-2 specimen with an epoxy composition of 70% and the addition of 30% mangrove extracts, very little *Littorina littorea* stuck to its surface. In addition to specimen observations, the added weight of the specimen was also calculated due to the number of *Littorina littorea* attached to the specimen, and the results were obtained (Table 4):

Table 4. Increase in specimen weight due to activity of *Littorina littorea*

No	Specimens	Initial Weight m_1 (gr)	Final Weight m_2 (gr)	Difference Δm (gr)
1	E100M0-1	423	446	23
2	E100M0-2	418	443	25
3	E90M10-1	419	439	20
4	E90M10-2	420	441	21
5	E80M20-1	413	428	15
6	E80M20-2	415	427	12
7	E70M30-1	410	415	5
8	E70M30-2	403	410	7

Furthermore, for each alloy composition, the average weight gain that occurs in the specimen is calculated, shown in the graph (Figure 9):

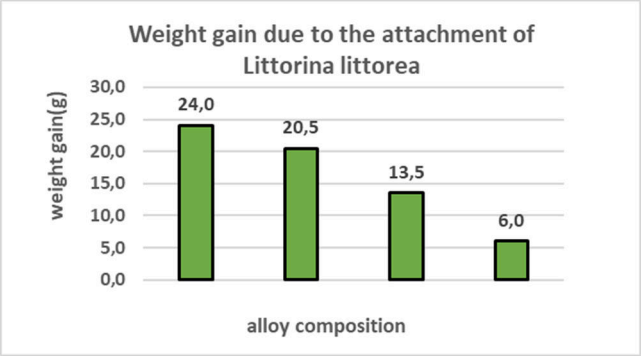

Figure 9. Graph of weight gain due to *Littorina littorea* attachment

The results of measuring the average weight gain in each specimen indicate that the more mangrove extracts were added, the smaller the amount of attachment of *Littorina littorea* to the specimen. This is evidenced by the amount of attachment of *Littorina littorea* to the E100M0 specimen with an additional weight of 24 grams, and decreasing with the addition of mangrove extracts, with an additional weight of 20.5 grams in the E90M10 specimen, 13.5 grams in the E80M20 specimen, and 6 grams in specimen E70M30. This proves that the phenolic compounds in mangrove extracts can inhibit bacterial growth in the specimens. These bacteria will produce proteins which are Extracellular Polymeric Substances (EPS) as a source of nutrients needed by *Littorina littorea* [2].

The attachment of *Littorina littorea* occurs when the Fe2 + ion is oxidized on the surface of the specimen, which O2 then reduces. The oxidation that occurred in this specimen triggered the attachment of *Littorina littorea*. However, the addition of mangrove extracts to the epoxy coating resulted in the inhibition of *Littorina littorea* attachment due to the presence of phenolic compounds contained in it [17].

5. CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

The biofouling affixing test results showed that the E100M0 specimen had the most biofouling bonding activity, with a weight gain of 24 grams, and the specimen with the least amount of biofouling gluing activity was the E70M30 specimen with an additional weight of 6 gr. This proves that the addition of mangrove extracts to the epoxy coating paint has an effect on the binding activity of biofouling on the specimen, with the more mangrove extracts added to the epoxy coating paint, the smaller the biofouling activity occurs and the less mangrove extract is added to the epoxy coating paint, the greater the occurrence. it is affixing biofouling activity.

5.2 Suggestions

This research still has some shortcomings in its implementation procedure, so that some suggestions for further research:

1. It is necessary to add environmental variations to determine how the response occurs in the presence of environmental factors.
2. It is necessary to carry out a coating process with appropriate procedures such as calculating dew point, blasting, calculating roughness, and calculating wet film thickness to determine the proper coating quality.
3. It is necessary to test the number of phenolic compounds contained to determine the effectiveness of using mangrove extracts in this study.

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