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RESEARCH PAPER

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Production and Physicochemical Characterization of Antifouling Biocomposites; The Effect of Chitosan and ZnO to Prevent Marine Biofouling

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Abstract

Adhesion of biofouling organism creates various problems. Efforts to explore the source of the antifouling material from marine organisms have been carried out, but it is non-cultivable mainly; its work efficiency depends on temperature, pH, concentration, and ineffective exposure time. Therefore, it is necessary to explore new sources of antifouling material by developing antifouling biocomposites from chitosan-ZnO. This study aims to determine the effect of differences in concentration of chitosan and ZnO on biocomposite characterization. This study consisted of 3 stages, 1) characterization of chitosan and ZnO; 2) production and characterization of chitosan-ZnO biocomposite; and 3) antifouling activity testing. The results showed that chitosan was completely soluble in 2% acetic acid with a viscosity of 76.4 to 79.6%, a water content of 10.92%, ash of 1.92%, nitrogen of 3.70%, and a deacetylation level of 85%. The ZnO used in this study had a particle size of 396.1-458.7 nm. Biocomposite characteristics indicated that the best treatment was chitosan 1% -ZnO 0.6 g with 0.31% swelling, 0.18% solubility film, 104.50° hydrophobic and 4.50 MPa adhesion. The results of the anti-fouling tests showed that the treatment of chitosan 1% -ZnO 0.6 g had less biofouling than other treatments.

Introduction

Biofouling organisms' adhesion on submerged objects such as boats, fishing nets, and offshore building pillars creates various problems. Antifouling material that is widely used before is tributyltin (TBT). These organotin compounds are very effective as antifouling agents, but are toxic to non-target organisms and the environment (Qian *et al*., 2013). Efforts to explore the source of the antifouling material from the active ingredients of marine organisms have been carried out. Syahputra and Almuqaramah (2019), have investigated the effect of adding mangrove bark extract in oil paint as an antifouling agent. Hasanah *et al*., (2021), succeeded in isolating the active compound from sea cucumber extract (*Phyllophorus* sp.) as an anti-macrofouling agent. But, it is primarily non-cultivable. Its working efficiency is dependent on temperature, pH, concentration, and exposure time which is often ineffective. Efforts to explore sources of antifouling materials that are environmentally friendly and effective needs to do to solve these problems. One of them is by developing an *antifouling* biocomposite.

Composites are a combination of two or more materials that will produce new materials with different mechanical properties and characteristics from the materials they form (He *et al*., 2002). The development of composites is not only from synthetic composites but also leads to natural composites (biocomposites) due to their environmentally friendly nature. The organic biopolymer that is widely researched in biocomposite studies is chitosan.

Chitosan is a biopolymer source that can extract from shrimp shells. The chitosan content of shrimp waste reaches 50% of the shrimp's weight, consisting of the head, shell, and tail. Besides, chitosan is antibacterial, safe, and able to form the film, and the production process is inexpensive (Farouk *et al*., 2012). But, chitosan has structural strength and stability, resulting in the limited application as a coating material. Thus, chitosan needs to be strengthened with zinc oxide (ZnO), which has good mechanical strength.

Zinc oxide (ZnO) register as a safe ingredient according to the FDA (Food and Drug Administration). ZnO material has a wide application, one of which is applied as a coating because it has antibacterial properties, low cost, and safety. ZnO material has toxic activity against organisms such as barnacles and bacteria but does not have environmental toxicity (Bondarenko *et al*., 2013).

Therefore, the development of chitosan-ZnO biocomposite as an active ingredient in environmentally friendly and applicable antifouling paints in the marine industry is an important study to do.

Material and Methods

This study's main ingredients were commercial chitosan, 2% acetic acid (Merck), and ZnO (Merck). This study consisted of 3 stages, namely 1) characterization of chitosan and ZnO; 2) production and characterization of chitosan-ZnO biocomposite; and 3) testing for antifouling activity.

Biocomposite Chitosan-ZnO Preparation

Biocomposite solutions were prepared by dissolving chitosan at different concentrations, 1%, 2%, and 3%. 1 g, 2 g, and 3 g of chitosan powder were added to 40 mL of 2% acetic acid solution and allowed to stand until all of the chitosan swelled. Then distilled water was added to reach a volume of 100 mL and stirred until it was homogeneous (Pranomo *et al*., 2012). ZnO in different treatments, 0.2 g; 0.4 g; and 0.6 g each dissolved in 10 ml distilled water until homogeneous. The biocomposite solution was prepared by adding the previously prepared ZnO solution (0.2 g; 0.4 g; and 0.6 g) to the 1%, 2%, and 3% chitosan solutions. Then the solution is stirred using a hand blender and sonicated for 15 minutes. 0.1 M NaOH is added slowly while stirring using a magnetic stirrer until the mixture's pH reaches 6 (Naamani *et al*., 2017). Biocomposites solutions are analyzed for swelling ratio, solubility film, hydrophobicity, adhesion, and resulting chitosan-ZnO biocomposite treatment was selected. Furthermore, the selected biocomposite treatment was tested for antifouling activity.

Analysis of Swelling Ratio and Solubility Film

Measurement of swelling ratio and solubility film on antifouling coating paint is determined by the method used by Zhong *et al*. (2011). The 25 mm x 75 mm glass slide coated with biocomposite was dried in an oven at 70ºC for 24 hours (until constant weight) to determine the dry weight (M1). Then the dry glass slides were immersed in 50 mL deionized water at room temperature. After 48 hours of immersion, the sample's wet weight (M2) was measured as soon as the excess water was removed by adsorption using paper (Whatman No.1). The glass slides were again dried in an oven at 70ºC for 24 hours (to constant weight) to determine the final dry weight (M3). The percentage of swelling ratio and solubility film is calculated using the following equation:

$$\textit{Swelling ratio } (\%) = \frac{M2 - M1}{M1} \times 100$$

$$\textit{Solubility film } (\%) = \frac{M1 - M3}{M1} \times 100$$

Hydrophobicity Analysis

The hydrophobicity of the biocomposite was determined by measuring the contact angle. The measurement of contact angles was carried out using the static sessile drop method (Carnairo *et al*., 2012). The droplets' results on the substrate's surface coated with antifouling biocomposites were photographed with the camera from the side. Furthermore, from the image obtained, the contact angle was measured using *Image J software* in *the drop analysis menu* by drawing a line between the substrate's surface and the outermost water droplet (right and left corner). Then obtained the value of the contact angle (θ) to the substrate surface.

Analysis of Adhesion

The adhesion test is carried out to determine the strength of a coating. The method used is a pull-off test (Figure 1) based on the American Standard for Testing Materials (ASTM D-4541). The test was carried out by attaching the dolly to the paint layer's surface, drying the glue for two days. After the dolly was firmly attached, peeled off the dolly using a dolly cutter to visible the material's surface. Next, attach the elcometer to the dolly, then rotate the elcometer until the dolly is released from the paint layer's surface so that the paint adhesion value is obtained in MPa units (Maulana, 2015).

Antifouling Activity Testing

Wooden panels measuring 17 cm long, 7 cm wide, and 0.84 cm thick are prepared with jati wood. The wood is then dried and pulverized, with holes on the top and bottom sides. The paint formulations that were tested for antifouling activity included selected chitosan-ZnO biocomposite (15%) and base paint consisting of resin (40%), hardener resin (35%), and thinner (10%). Then stir for 30 minutes. The treatment consisted of 4 parts with different compositions, namely negative control (base paint only with code A), chitosan-based paint (AK), ZnO- based paint (AZ), selected chitosan-ZnO biocomposite and based paint (AZK), and positive control using commercial antifouling paint (Kom). Then the paint formulation was applied with a super fine brush to produce the same thickness of the film and dried at room temperature. The dried wooden panels are submerged at a depth of 50-60 cm from sea level with ropes and tis cables for mounting the panels. Analysis of antifouling activity was carried out according to the method of Bellotti *et al*. (2014). The test panel's surface has been immersed in seawater for one month, then calculated biofouling adhesion such as barnacles and types of shellfish.

Data Analysis

The experiment was designed factorial completely randomized, with different chitosan concentrations (1%, 2%, 3%) and different ZnO concentrations (0.2g; 0.4g; 0.6g) repeated 3 times. The data obtained and analyzed variance (ANOVA) and continued with Duncan test at 5% significance using SPSS 25.

Results and Discussion

Characteristics of Chitosan

Chitosan is biodegradable, biocompatible, antibacterial (Kumirska *et al*., 2011), and is environmentally friendly compared to tributyltin (TBT) which is widely used in antifouling paints (Qian *et al*., 2013). Table 1 shows the results of the measurement of the chitosan quality standard. The solubility of chitosan showed that chitosan was completely soluble in 2% acetic acid. The chitosan used in this study has a fairly high level of solubility, this will facilitate chitosan in its application as an active ingredient in antifouling paint. The acetic acid influences the solubility level of chitosan as a solvent. Acetic acid is included in the weak acid, a carboxylic acid group that contains a carboxyl group (-COOH). The carboxyl group in acetic acid facilitates dissolving chitosan due to the hydrogen interaction between the carboxyl group and the amine group of chitosan. Besides, chitosan's solubility level is also influenced by the short-chain length of chitosan and is closely related to chitosan's deacetylation degree. The deacetylation process will cut the acetyl group in chitin and leave an amine group which is characteristic of chitosan. The presence of a hydrogen element in the amine group facilitates interaction through hydrogen bonds. In contrast, the presence of a carboxyl group in

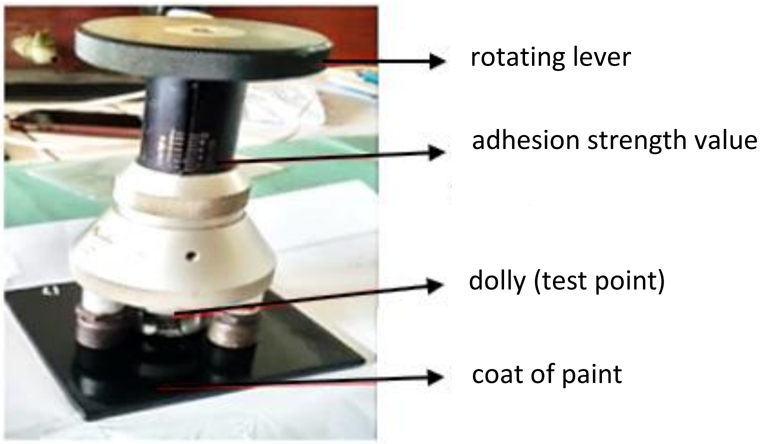

Figure 1. *Pull-off test.*

Table 1. Physicochemical characterization of chitosan

Parameters	Value
Physics	
Solubility in acid (%)	Soluble
Viscosity (cps)	
- Chitosan 1%	76,4
- Chitosan 2%	78,0
- Chitosan 3%	79,6
Chemical	
Water content (%)	10,92
Ash (%)	1,92
Nitrogen (%)	3,70
Deacetylation degree (%)	85,00

acetic acid will facilitate chitosan dissolution due to the hydrogen interaction between the carboxyl group and the amine group (Dunn *et al*., 1997).

Viscosity is an important parameter in chitosan. It is closely related to solubility. The higher the solubility level, the better the viscosity. The results of measuring the chitosan solution's viscosity showed that the higher the chitosan solution's concentration, the higher the viscosity value (Table 1). The concentration of the chitosan solution is directly proportional to its viscosity. The low concentration of chitosan solution has a viscosity level that is more dilute and easy to move, while the high concentration of chitosan solution is thicker and difficult to move. Information on the viscosity of chitosan is related to its application. In the pharmaceutical field, chitosan with low viscosity is needed, while chitosan for thickening or hardening needs chitosan with high viscosity (Dewi & Fawzya, 2006).

The chitosan's water content is expressed as H_2O, bound to the functional groups of the chitosan polymer, especially the amine, N-acetyl, and hydroxyl groups through hydrogen bonds (Dompheipen *et al*., 2016). Chitosan is hygroscopic, so it quickly absorbs water from the surrounding air so that the water content of chitosan depends on the air's relative humidity around the storage area.

Ash content is a parameter to determine the minerals contained in a material. So this can characterize the success of the chitosan demineralization process. The lower value of the ash content, the higher level of chitosan purity (Nugroho *et al*., 2011). Table 1 above shows that the ash content of chitosan used in this study was relatively low (1.92%) when compared to the research of Isa *et al*. (2012) 5.60%; and Mohanasrinivasan *et al*. (2013) 2.28%. It means that the remaining mineral content is very small and indicates that the chitosan used has met the quality standard of chitosan.

The nitrogen content of chitosan used in this study is 3.70%. Nitrogen content is related to the deproteination process. The higher the nitrogen content value indicates that the deproteination process is incomplete. This is due to the amino acid chain that cannot be broken down entirely so that protein denaturation does not properly. According to Abdulkarim et al. (2013), high levels of chitosan protein can be related to the immersion time and the method used during the chitosan-making process.

Deacetylation degree (DD) is a quality parameter of chitosan, which indicates the percentage of acetyl groups released during chitosan deacetylation. The higher the deacetylation degree of chitosan, the lower the acetyl group, so that the interaction between ions and hydrogen bonds are getting stronger (Knoor, 1982). Table 1 above shows that the deacetylation degree of chitosan is 85%. This high deacetylation degree value indicates that many acetyl groups are released from the polymer chain during the deacetylation process. This is very beneficial because the higher the deacetylation degree value, the better the chitosan's solubility will be and will facilitate further application. According to Kusumaningsih (2004), in general, the quality of chitosan used has a DD of 60%, for the technical quality, it is around 85%, for food quality, it is about 90%, and for pharmaceutical, it is 95%. The value of the degree of deacetylation in this study is classified as technical quality.

Characteristics of ZnO

ZnO particle size distribution in Figure 2 in the range of 396.1-458.7 nm. The distribution of ZnO particles was not uniform, with an average particle size of 458.7 nm and an intensity of 94.0%. It can be said that there is agglomeration between the ZnO particles so that their shape is not uniform (Gunalan *et al*., 2011). Foliatini et al. (2015), stated that the size of the ZnO produced is highly dependent on the size of other particles that surround the surface of the nanoparticles. The formation of agglomeration in the ZnO sample shows that the ZnO particle colloid system tends to be less stable so that the average size of the ZnO particles formed is not yet nano-sized, which is below 100 nm. Dumur et al. (2011) stated that needed a strong stabilizer to prevent agglomeration from limiting the growth of larger and fixed nano-sized clusters.

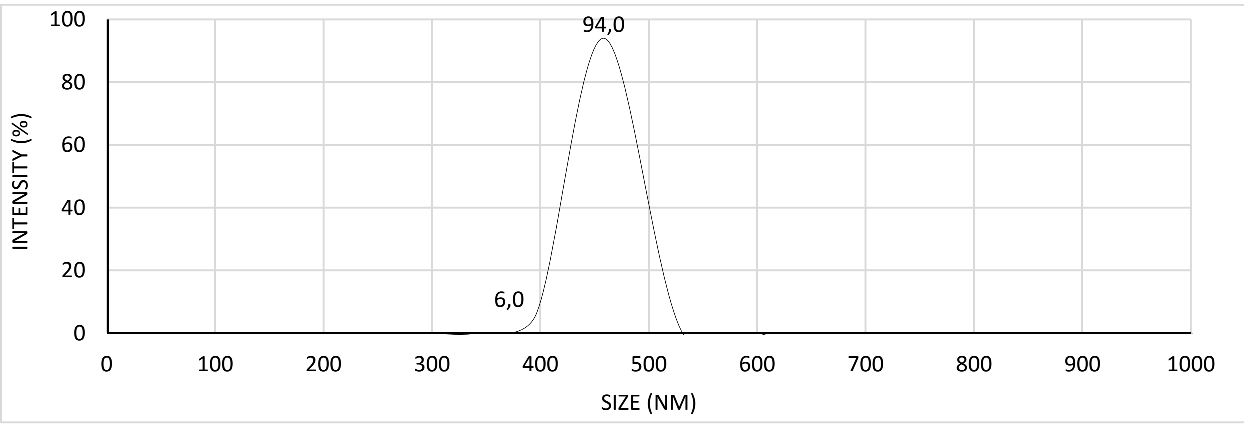

Figure 2. The particle size distribution of ZnO.

The Swelling Ratio of Chitosan-Zno Biocomposite

The swelling ratio is an important characteristic for coating materials because it can affect water resistance. The swelling ratio measurement results in Figure 3 show that the swelling ratio value decreases with the addition of the ZnO concentration. It means that the biocomposite's strength is getting better because of the slight swelling of the biocomposite coating. Meanwhile, along with the addition of chitosan concentration, the value of the biocomposite swelling ratio increased, which means it was terrible because there was swelling in the biocomposite layer which resulted in the coating properties becoming weak and easy to peel off. When ZnO enters the chitosan matrix, the swelling ratio decreases. The decrease in swelling ratio value indicates that ZnO and chitosan combination gives a stability effect to the film matrix. It will reduce the mobility of the chitosan chain and minimize swelling (Reicha et al., 2012). So that the best treatment is the combination of chitosan 1% - ZnO 0.6 g treatment with the smallest swelling ratio value of 0.31%. The swelling ratio is also influenced by water diffusion, ionization of amino or carboxyl groups, dissociation of hydrogen and ionic bonds, and polymer relaxation (Mathew *et al*., 2006).

Solubility Film **of Chitosan-ZnO Biocomposite**

The film's solubility is an important characteristic of the biocomposite coating because it can affect a material's resistance to water. The results of measuring the solubility film in Figure 4 show that ZnO's addition reduces the solubility film significantly. The best treatment was at the concentration of chitosan 1% - ZnO 0.6 g with the lowest film solubility value of 0.18%. The higher the chitosan concentration added, the greater the film's solubility value, while the higher the ZnO concentration added, the smaller the film's solubility value. Chitosan has a hydroxyl group (-OH) which can bind to water molecules through hydrogen interactions.

Meanwhile, ZnO is hydrophobic. In addition to strengthening, it also functions as a filler to cover the cavities or pores on the biocomposite film's surface. It causes the biocomposite film to decrease the solubility of the film. The solubility film is related to its hydrophobicity or water resistance. The higher the hydrophobicity angle, the higher the water resistance, the smaller the solubility film, which means the better. In this study, with the increasing concentration of chitosan, the Zn concentration in the biocomposite decreased. The possibility of the bond between the

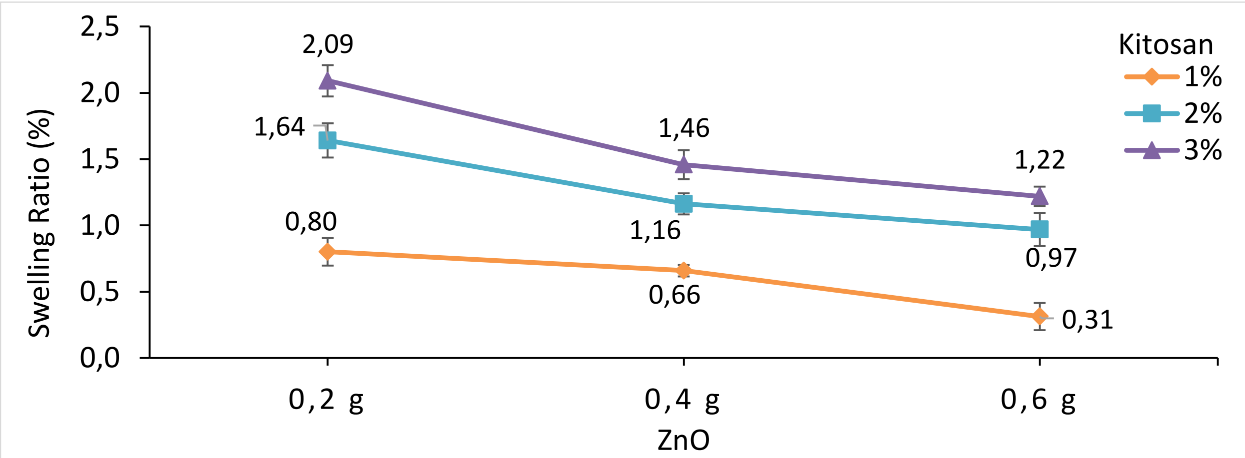

Figure 3. *Swelling ratio* biocomposite.

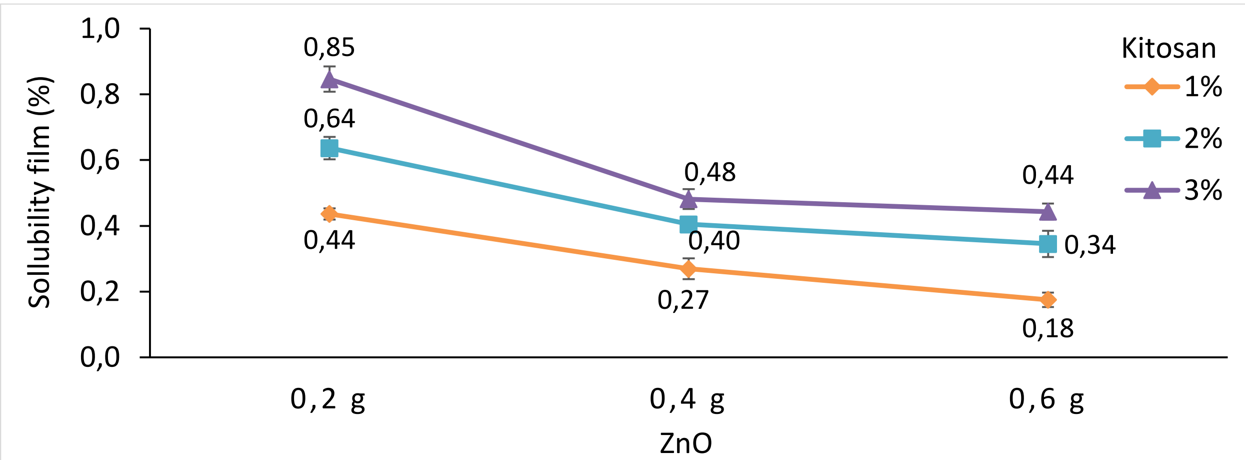

Figure 4. *Sollubility film* biocomposite.

active side of chitosan and Zn is getting smaller, leading to an increase in the solubility value of the film, and the biocomposite becomes brittle.

Hydrophobicity of Chitosan-ZnO Biocomposite

Hydrophobicity is a water repellent property. The biocomposite's hydrophobicity is measured based on the water's contact angle with the coated object's surface. The hydrophobicity of the biocomposite in Figure 5 shows that the higher the ZnO concentration and the lower the chitosan concentration, the higher the degree of hydrophobicity. The higher the degree of hydrophobicity of an object, the greater the water repellency of the object. This hydrophobicity property is important for a coating material. A coating can be hydrophobic or hydrophilic. The treatment with the best hydrophobicity value was the treatment with the concentration of chitosan 1% - ZnO 0.6 g with a contact angle value of 104,50°. According to Yuan & Lee (2013), if a contact angle is more than 90°, it means the surface is hydrophobic. Biocomposites are expected to have hydrophobic properties because these properties will be able to withstand abrasion from seawater. Measurement of a surface's water contact angle is one of the most relevant physicochemical parameters affecting the reduction and adhesion strength of fouling organisms (Lejars *et al*., 2012).

Adhesion of Chitosan-ZnO Biocomposite

The adhesion test results in Figure 6 show that the higher the pressure value that the biocomposite coating can withstand, the higher the level of adhesion of the biocomposite to the substrate's surface. The highest adhesion value was 4.50MPa in the treatment combination, the concentration of chitosan 1% - ZnO 0.2 g. The higher the chitosan concentration, the lower the adhesion of the biocomposite. On the other hand, if the more heightened the ZnO concentration, the higher the biocomposite's adhesive capacity. It is related to the previous test parameters, the swelling ratio, and solubility film. The percentage of the swelling ratio and the small solubility film has a large water repellency. It can maintain the adhesion strength of the biocomposite. The greater the concentration of ZnO, the better the mechanical strength. Conversely, the more the concentration of chitosan, the adhesive power will be weaker or brittle.

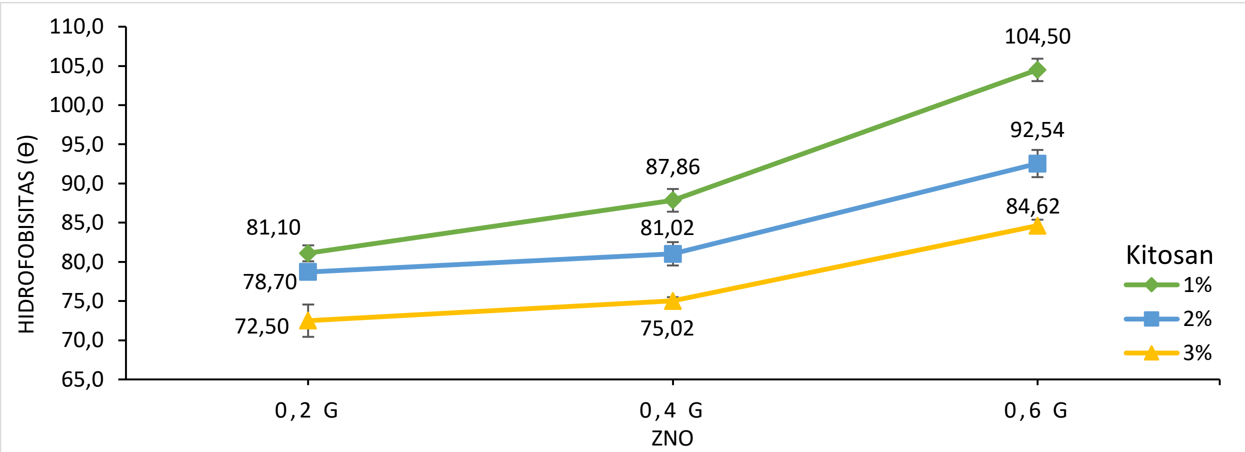

Figure 5. Hydrophobicity of biocomposite.

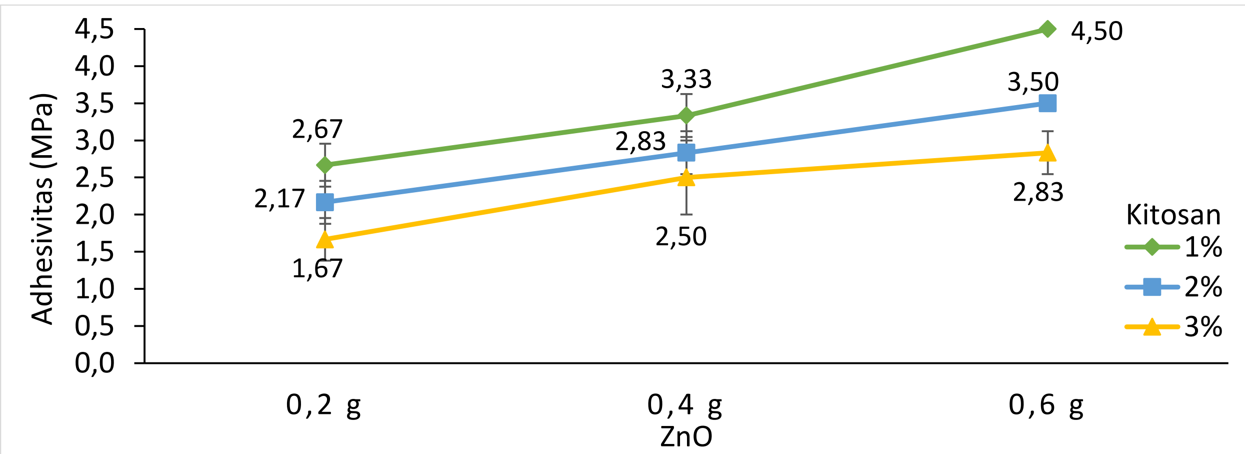

Figure 6. Adhesion of biocomposites.

Antifouling Activity of Chitosan-ZnO Biocomposite

Testing of chitosan-ZnO biocomposite antifouling activity was carried out to measure biocomposites' ability to prevent biofouling growth activities such as barnacles and shellfish. The attachment of biofouling to the surface of the substrate in seawater can occur quickly. Based on the observations of Figure 7, in the fourth week of treatment with the base paint-biocomposite (AZK), about 35 tiny barnacles have started to stick to the wood panel. In contrast, the treatment of base paint-chitosan (AK), base paint-ZnO (AZ), and base paint alone (A) is almost The entire surface of the test panel has been covered with barnacles. For commercial paint (Kom), the surface is still clean from fouling organisms' growth, but the paint has started to become brittle, and even some of the paint has peeled off.

The attachment of bacteria initiates this biofouling growth to the surface of the substrate. The antibacterial effect of biocomposites in this study came from chitosan and ZnO. The ability to inhibit bacterial growth in the chitosan-ZnO biocomposite was due to the interaction between chitosan and Zn metal ions. Chitosan is played by the N atom from the amine group (-NH2) and the O atom from the hydroxy group (-OH). After the complex is formed, the positive charge's density from the chitosan will increase, causing the attraction between the bacterial cell surface to be higher. It is what causes the antibacterial activity of chitosan-ZnO biocomposite applied to the test panel is higher than chitosan or ZnO alone. The chelate reaction formed by chitosan and metal ions will interact with bacteria's outer cell surface, such as proteins, phospholipids, fatty acids, disrupting the cytoplasmic membrane. Disruption of the cytoplasmic membrane causes bacteria's metabolic activity and cell growth to be disrupted (Wang *et al*., 2005).

Attachment of barnacles can result in damage to the coating of the ship's protective paint. According to Sonjaya (2016), the surface of objects affixed with barnacles will be more fragile than those that are not affixed. The affixing activity of biofouling by attaching itself to the object's surface to defend itself from seawater currents will damage the coating's surface. So that the coating is damaged due to the formation of small holes; as a result of the construction of these holes, seawater can enter and weaken the adhesion of

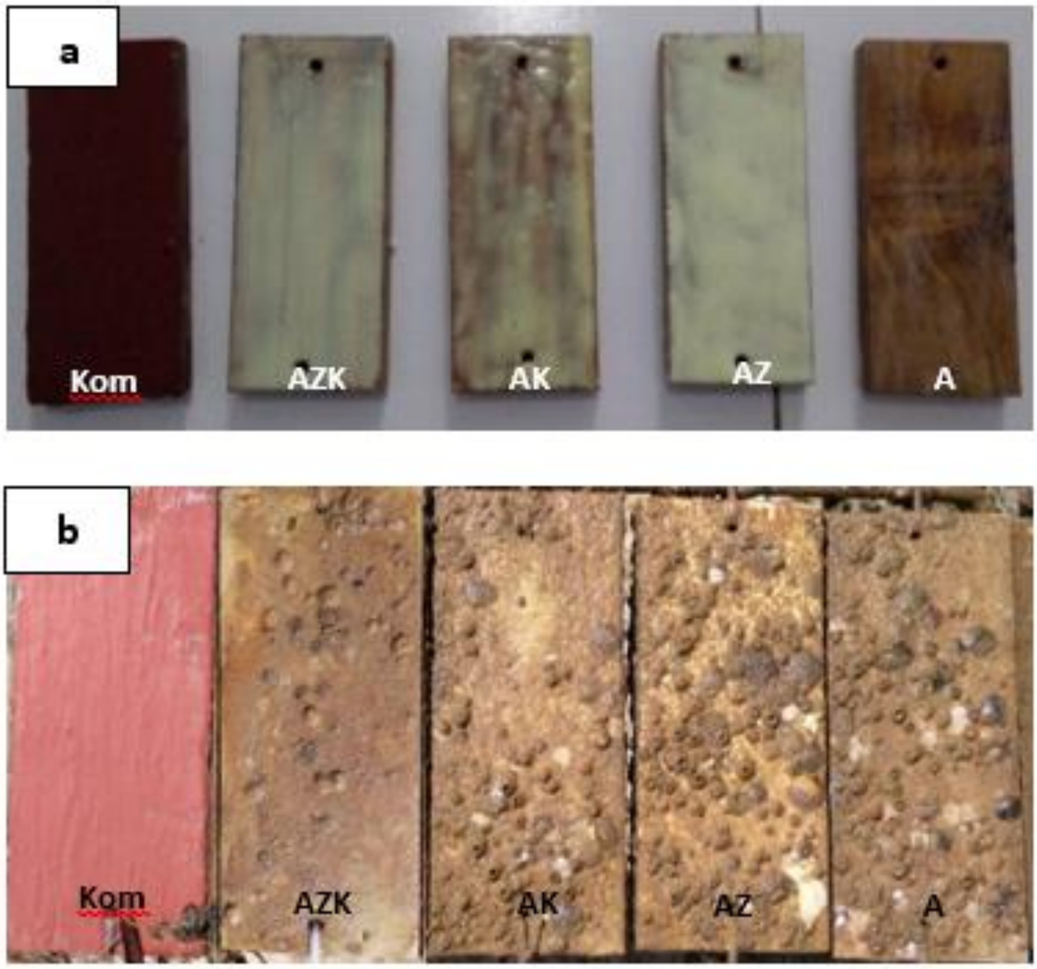

Figure 7. The immersion of antifouling panels testing a) wood panels before immersion, b) wood panels after one-month immersion.

the protective layer from the inside. so that the coating will be damaged and then peeled off. Based on the data from the calculation of the total biofouling that grows (Figure 8), the basic paint-biocomposite (AZK) treatment has a lower total macrofouling value than the other treatments, which is about 35 barnacles, while for commercial paint, the surface is still clean from the growth of fouling organisms, but the paint has started peeling off.

Conclusion

The different treatment of chitosan and ZnO concentrations affected chitosan-ZnO biocomposite characterization as an active ingredient in antifouling paint. The best characteristics were the combination of 1% - ZnO 0.6 g chitosan treatment with a swelling ratio of 0.31%; solubility film 0.18%; hydrophobicity 104.50° and adhesion 4.50 MPa. The results of the observation of the antifouling activity test showed that the combination of 1% - ZnO 0.6 g chitosan treatment was less affixed with biofouling than the other treatments. For further research, it is recommended to experiment using test specimens other than wood materials such as iron or concrete for aquaculture applications, and optimization needs to be done to determine the ratio of the concentration of biocomposite to the optimum base paint so that it can reduce the growth of biofouling more

Ethical Statement

Not applicable

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Author Contribution

First Author: Conceptualization-design, Data Curation, Formal Analysis, Methodology, Writing - original draft, Writing-review and editing; Second Author: Data Curation, Formal Analysis, Investigation, Methodology, Funding Acquisition, Supervision, Writing-review; and Third Author: Formal Analysis, Visualization, Methodology, Supervision, Project Administration, Writing -review and editing.

Conflict of Interest

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, double publication and/or submission have been completely observed by the authors

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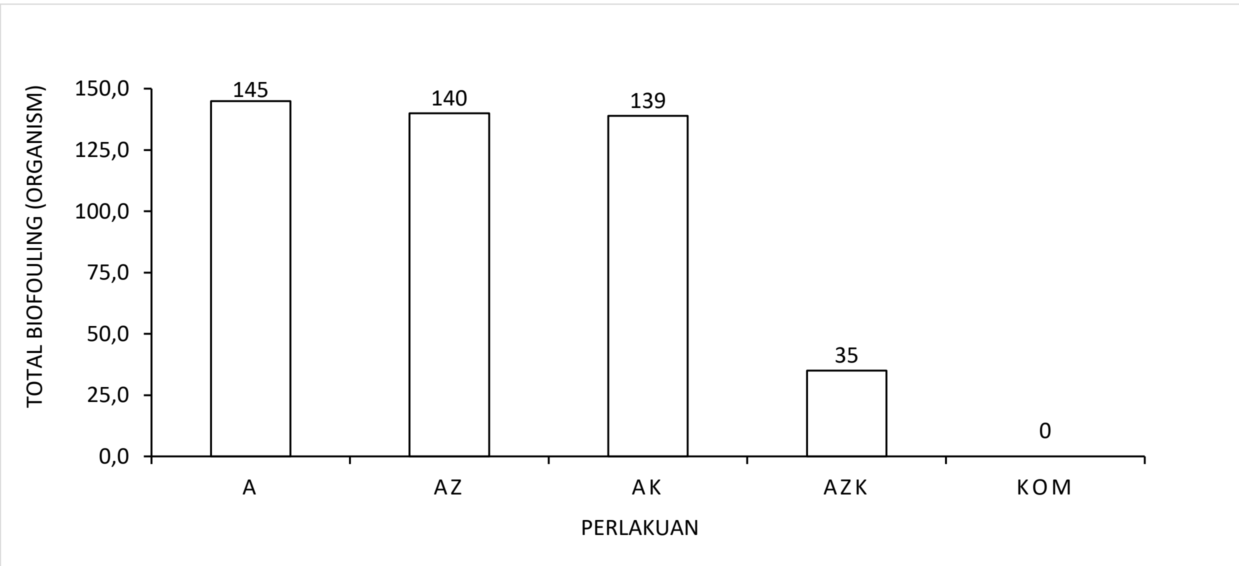

Figure 8. Growth of biofouling.

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