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Carbon Steel Biocorrosion Inhibition by Methanol Extract *Gracilaria Edulis*in Seawater

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ABSTRACT

Biocorossion in terrestrial and marine habitats has been widely studied including the involvement of sulphate reducing bacteria which are of particular concern in the oil and gas industries. One of the sulfate reducing bacteria that triggers biocorrosion is Thiobacillus ferrooxidans. This study examines the effectiveness of Gracilaria edulis macroalgae methanol extract obtained from Sayang Heulang Beach Pamengpeuk District, Garut Regency, West Java as a candidate for carbon steel biocorrosion inhibitors in seawater media. Gracilaria edulis extraction process using Folch extraction method (chloroform: methanol: phosphate buffer, 2:1:0.8 (v/v)). Separation of the crude methanol-water phase extract using silica column chromatography G-60 7733 with n-hexane: acetone (8: 2, 5: 5, 2: 8 (v / v)) and methanol pa from the results of the analysis using a UV spectrophotometer (190-400 nm) there were five combined fractions (Fraction A-Fraction E). Total content of phenolic compounds (TPC) in crude extracts of methanol-water, fraction C and fraction E respectively (410.77; 285.30; 309.51) (mg/gr GAE). The results of the qualitative test of the ability of biocorrosion inhibition of crude extracts of methanol-water phase, fraction C and fraction E by using commercial nails in solid media incubated for four days showed a decrease in the corrosion intensity on nails. Quantitative tests on the ability of biocorrosion inhibition were carried out using the weight-loss method and the results of quantitative tests showed the corrosion rate of fraction C was 12.14 ± 1.09 mpy and the fraction E was 13.94 ± 1.82 mpy for 2. 5% NaCl medium while the corrosion rate of fraction C was 10.73 ± 3.63 mpy and the fraction E was 11.72 ± 0.59 mpy for seawater media. The optimum concentration test results in the inhibition showed the smallest corrosion rate fraction C was found at 0.2 mg / mL TPC levels in both 2.5% NaCl media and seawater media which each had a corrosion rate of 12.91 mpy and 8.09 mpy and the optimum concentration of fraction E was found in TPC levels of 0.4 mg / mL (2.5% NaCl medium) with a corrosion rate of 14.32 mpy and TPC levels of 0.1 mg / mL (sea water medium) with a corrosion rate of 12.14 mpy. By using the optimum fraction C concentration, the results showed that the fraction C had the maximum resistance in inhibiting for 8 days (192 hours). Analysis of carbon steel plates using SEM and EDS showed that T. ferrooxidans bacterial cells formed a biofilm layer on the surface of the carbon steel plate with a localized density but mostly dominated the carbon steel plate surface and by the addition of SEM image inhibitors showed the carbon steel plate forming a thin layer that protects the metal surface and decreases the solubility of iron sulfide in water. The test results using EDS devices also detect the presence of sulfur elements and a decrease in the percentage of iron element mass on carbon steel plates without inhibitors.

Keywords: *Macroalgae, Gracilaria Edulis, Thiobacillus Ferrooxidans, Biocorrosion, Seawater, Nacl 2.5%, Corrosion Rate, Carbon Steel.*

INTRODUCTION

Since the early 17th century steel has become an important and primary material in the construction of infrastructure, ships, railroads and weaponry. But a serious problem that can reduce the usefulness of steel is its susceptibility to corrosion processes. The corrosive environment has a major influence on the mechanical properties of the metal. About 13% of newly processed iron or steel is used annually to replace corroded iron [1]. One of the causes of corrosion comes from the activity of microorganisms. Biocorrosion or corrosion influenced by microbes is a damage caused or accelerated through the presence of microbes and or other microorganisms as well as the result of the metabolic activity of these microbes, especially microorganisms that come in contact with metal

surfaces to form a biofilm layer [2]. The presence of this biofilm modifies the physical and chemical parameters on the metal surface which affect the corrosion process. These parameters include pH, salt concentration, oxygen concentration, organic compound concentration, redox potential and solution conductivity [3].

Several types of bacteria that are related to metals in terrestrial and marine habitats [4] are reducing bacteria (sulfate, iron, and CO2) and oxidizing bacteria (sulfur, iron, manganese) [5]. Among these various types of bacteria, sulfate reducing bacteria are of particular concern in research in the field of biocorrosion because they have an unfavorable impact, there are a number of industries that are affected by the occurrence of biocorrosion including the nuclear power industry, the steel pipe industry, the weapons industry and the oil and gas industry [6]. Of the various industries mentioned, the oil and gas industry are one of the most affected industries because the occurrence of biocorrosion affects the integrity, safety and usability of a number of tools operating in the oil and gas industry.

In 2011, the total cost per year incurred to overcome corrosion in the oil and gas industry was around 13.4 million

US dollars, where biocorrosion contributes US$2 million [7]. This can happen because bacteria in this group have several metabolic activities such as producing hydrogen sulfide which is toxic to the environment and metal surfaces which, if left unchecked, can cause various damage. This damage is caused by the ability of these bacteria to (1) oxidize hydrogen as an electron donor for the continuation of metabolic processes [8]; [9], (2) use Fe3+ and O2 as terminal electron acceptors [10], (3) using aliphatic and aromatic hydrocarbons as carbon sources, (4) only requiring very little water content as a growing medium, (5) accelerating the occurrence of sulfate reduction during magnetite production [11],

Preventive measures against biocorrosion have been carried out, including mechanically through pigging techniques (pipe cleaning) and chemically through biocides, cathode and anode protection. Of all the precautions, for the case of biocorrosion prevention using biocides is the most effective inhibitor but economically it is quite expensive, difficult to degrade and has a negative impact if used continuously in industry which will endanger the industry and the environment [12].

Action that can be used as an appropriate alternative in preventing biocorrosion is to use natural materials by utilizing plant extracts which are rich in bioactive compounds to overcome biocorrosion. One of them is the content of bioactive components of seaweed extract. In the results of research conducted by [13] showed that the crude extract of Sargassum sp. and Gracilaria sp. can act as an inhibitor of biocorrosion. Recent research from [14] stated that extracts of tropical marine macroalgae Gracilaria sp. can be used as a candidate for environmentally friendly biocorrosion inhibitors. The results of this study also support the results of a study conducted by [14] which stated that commercial seaweed crude extracts are capable of inhibiting corrosion in aqueous media with high salinity. However, from various studies that have been conducted, the effectiveness of methanol extract of tropical marine macroalgae as a biocorrosion inhibitor in seawater media has not been studied. Therefore, in this research a study will be carried out on the effectiveness of methanol extract

of macroalgae Gracilaria edulis as an inhibitor of carbon steel biocorrosion in seawater media which is thought to be effective in inhibiting iron-oxidizing microbes so that the wearability of the metal can be maintained.

METHOD

A. Material

The equipment and materials used in this study were obtained from the warehouse of tools and materials for the Biochemistry Laboratory, Chemistry Study Program, FMIPA, ITB. Macroalgae Gracilaria edulis was obtained from Sayang Heulang Beach, Pameungpeuk, Garut, West Java. Thiobacillus ferrooxidans bacteria were obtained from a collection of microbes at the Microbiology Laboratory, SITH, ITB.

B. Gracilaria Edulis Preparation

Gracilaria edulis macroalgae sampling was carried out at Sayang Heulang Beach, Pamengpeuk District, Garut Regency, West Java. The selected Gracilaria edulis had the criteria of being alive, fresh, and brightly colored. After the sample is obtained, the sample is washed and cleaned of impurities such as sand, stones or corals that are still attached to the sample and wrapped in plastic and then stored in the refrigerator at -20oC. Then the samples were air-dried for ± 3 hours and further dried to then be crushed into powder using a blender and the water content of macroalgae was measured and the identification of macroalgae species was carried out morphologically in the Biochemistry Laboratory, ITB Research and Innovation Building.

C. Extraction of Gracilaria Edulis

The extraction process was carried out based on the Folch method [15] with modifications using the main solvents, namely chloroform pa, methanol pa and 0.05 M phosphate buffer pH 7.6. Powdered macroalgae samples were extracted by maceration, namely soaking the samples for 24 hours at 25oC with a mixture of chloroform pa, methanol pa and phosphate buffer with a ratio of 2:1:0.8 (v/v) for each solvent.). The volume ratio of sample and solvent is 1:10 and during the maceration process, shaking is carried out using a shaker at a speed of 150 rpm. Then the extract was centrifuged at 2060 g for 15 minutes. The resulting supernatant and pellets were then filtered using filter paper. The filtered pellets were re-extracted three times with the main solvent mixture at a ratio of 1:1:0.8 (v/v) and the volume ratio of the sample to the solvent was 1:6. The resulting supernatant was centrifuged at 2060 g for 15 minutes. The collected supernatant was then rinsed with mili-Q water at a ratio of 9:2 (v/v) and centrifuged again. The results of centrifugation which formed two phases were then separated to produce crude extracts of the chloroform phase and the methanol phase. The final stage of the extraction process is the evaporation of the methanol solvent with a rotary evaporator and water using a freeze dryer. The collected supernatant was then rinsed with mili-Q water at a ratio of 9:2 (v/v) and centrifuged again. The results of centrifugation which formed two phases were then separated to produce crude extracts of the chloroform phase and the methanol phase. The final stage of the extraction process is the evaporation of the methanol solvent with a rotary evaporator and water using a freeze dryer. The collected supernatant was then rinsed with mili-Q water at a ratio of 9:2 (v/v) and centrifuged again. The results of centrifugation which formed two phases were then separated to produce crude extracts of the chloroform phase and the methanol

phase. The final stage of the extraction process is the evaporation of the methanol solvent with a rotary evaporator and water using a freeze dryer.

D. Fractionation of Methanol Extract of Gracilaria edulis

Separation of the crude extract was carried out using the column chromatography method with the specifications for a column diameter of 1.2 cm and a column height of 11 cm. The column was filled with silica gel G60-7734 as the stationary phase and elution was carried out in stages using hexane pa and acetone pa with a ratio of 8:2, 5:5 and 2:8 (v/v) and methanol pa [16]. Before the elution was carried out, the crude extract was first dissolved with 0.5 mL of n-hexane acetone at a ratio (8:2) before being added to the column. In the separation process, the flow rate is set at 7–8 drops per minute. The fraction was accommodated every 2 mL in a micro tube. These fractions were then observed for their spectra with a UV-Vis spectrometer at a wavelength of 190–400 nm and grouped based on the resulting spectral pattern.

E. Making Gallic Acid Calibration Curves

Gallic acid standard curve was prepared using the modified Folin-Ciocalteu method. First, 50 mL gallic acid mother liquor was prepared with a concentration of 1000 ppm. Then, standard series of gallic acid were made with concentrations (20, 40, 60, 80, 120, 140) ppm using the dilution method of gallic acid mother liquor. Pipette 10 µL from each concentration of the standard series, then add 790 µL of distilled water and 50 µL of Folin-Ciocalteu reagent and shake until homogeneous using a vortex and leave for 8 minutes. After 8 minutes, 150 µL of 20% Na2CO3 was added, then shaken until homogeneous using a vortex and allowed to stand for 30 minutes at room temperature. After 30 minutes,

F. Determination of Total Phenol Content and Fractions

Measurement of the phenol content for the extract was carried out by taking 100 µL of crude methanol extract by pipetting and adding 700 µL of distilled water and 50 µL of Folin-Ciocalteu reagent and shaking until homogeneous using a vortex and allowed to stand for 8 minutes. After 8 minutes, 150 µL of 20% Na2CO3 was added, then shaken until homogeneous using a vortex and allowed to stand for 30 minutes at room temperature. After 30 minutes, the absorption was measured at a wavelength of 765 nm using a UV-Vis spectrophotometer.

Measurement of phenol content for the fractions, namely fraction C and fraction E was carried out by taking 250 µL of the fraction by means of a pipette and adding 650 µL of distilled water and 50 µL of Folin-Ciocalteu reagent and shaking until homogeneous using a vortex and allowed to stand for 8 minutes. After 8 minutes, 150 µL of 20% Na2CO3 was added, then shaken until homogeneous using a vortex and allowed to stand for 30 minutes at room temperature. After 30 minutes, the absorption was measured at a wavelength of 765 nm using a UV-Vis spectrophotometer. Measurements were carried out three times (triplo) so that the phenol content obtained was obtained as milligrams per gram equivalent of gallic acid.

G. Rejuvenation of Thiobacillus Ferrooxidans Bacteria

Bacterial cultivation was carried out in liquid media (modified 9K media) (Harahuc et al., 2000) with the composition of 0.04% K2HPO4, 0.04% MgSO4.7H2O, 3.34% FeSO4.7H2O, 0.4% 2M H2SO4, (NH4)2SO4 0.04% in distilled water. Meanwhile, solid media is prepared with the same composition

as liquid media but added 1% agar. Then the pH of the media was adjusted in the range of 2.5–3.0 with 2 M NaOH. The liquid and solid media were sterilized by autoclaving for 15 minutes at 120oC. This bacterium grows optimally in the dark within 4 days which is indicated by a change in the color of the medium to a cloudy yellow color. After the bacteria have successfully grown, the bacteria are gradually adapted to the media that has been added with NaCl starting from the concentration (0.5%; 1%; 1.5%; 2%; 2.5%) (w/v). In the adaptation process, the OD (Optical Density) of the bacteria was measured until the absorbance reached 0.5 and the bacteria that managed to survive were rejuvenated and the NaCl concentration was increased gradually. The last stage in bacterial rejuvenation is the adaptation of bacteria to media that has been added to seawater using bacterial cultures that have managed to survive in media that has been added to NaCl 2.5% (w/v). The seawater used is seawater taken from Sayang Heulang beach which is first sterilized using 0.2 μm membrane paper and then autoclaved. The last stage in bacterial rejuvenation is the adaptation of bacteria to media that has been added to seawater using bacterial cultures that have managed to survive in media that has been added to NaCl 2.5% (w/v). The seawater used is seawater taken from Sayang Heulang beach which is first sterilized using 0.2 μm membrane paper and then autoclaved. The last stage in bacterial rejuvenation is the adaptation of bacteria to media that has been added to seawater using bacterial cultures that have managed to survive in media that has been added to NaCl 2.5% (w/v). The seawater used is seawater taken from Sayang Heulang beach which is first sterilized using 0.2 μm membrane paper and then autoclaved.

H. Biocorrosion Qualitative Test

Qualitative test of macroalgae Gracilaria edulis extract was carried out on solid media in petri dishes. Thiobacillus ferrooxidans bacteria were spread into 100 μL of solid media. Furthermore, 10 μL of methanol pa, crude extract of Gracilaria edulis methanol, fraction C, and fraction E were put into solid media. Visual observations were made on the intensity of corrosion that occurred on iron nails with an incubation period of 4 days.

I. Biocorrosion Quantitative Test

Quantitative testing of Gracilaria edulis seaweed extract in determining the corrosion rate was carried out using the difference method of reducing sample weight (weight loss) and carried out on two variations of bacterial media, namely media that had been adapted to 2.5% (w/v) NaCl and media that had been adapted to seawater.

J. Sample Weight Reduction Method (Weight Loss)

The grouped fractions were then tested for their inhibition on carbon steel plate weight reduction by immersing the carbon steel plate in a liquid medium for 4 days (96 hours). Quantitative tests were carried out in liquid media with each composition including:

1) containing a liquid medium containing carbon steel plates and no bacteria.
2) containing liquid media with 3% (v/v) bacterial culture and carbon steel plates without added inhibitors.
3) containing liquid media with 3% (v/v) bacterial culture and carbon steel plates with the addition of a biocide inhibitor namely 0.5% (v/v) glutaraldehyde as a positive control.

4) containing liquid media with 3% (v/v) bacterial culture and carbon steel plates with the addition of 0.5% (v/v) Methanol Crude Extract inhibitor.
5) containing liquid media with 3% (v/v) bacterial culture and carbon steel plates with the addition of 0.5% (v/v) fraction C inhibitor.
6) containing liquid media with 3% (v/v) bacterial culture and carbon steel plates with the addition of 0.5% (v/v) fraction E inhibitor.

The carbon steel plate (coupon) used has a total contact surface area of 7.2304 cm2. The method of reducing sample weight (weight loss) was used to determine the corrosion rate and inhibition efficiency of the macroalgae extract fraction against biocorrosion on carbon steel plates.

K. Carbon Steel Morphological Analysis

At this stage, a morphological characterization was carried out on the surface of the carbon steel plate that had been exposed to liquid bacterial media containing inhibitors and without inhibitors using a Scanning Electron Microscopy (SEM) instrument and the chemical composition was determined on the surface of the carbon steel plate by Energy Dispersive Spectroscopy (EDS). The carbon steel plate is carefully removed from the liquid medium for the fixation stage first. The carbon steel plate was immerged for 1 hour in 2% glutaraldehyde solution, dehydrated with 4 ethanol solutions (25%, 50%, 75% and 100%) (v/v) for 15 minutes and then dried at room temperature for 1 night (Bhola et al., 2014).

RESULTS AND DISCUSSION

A. Gracilaria Edulis Preparation

The macroalgae used in this study was Gracilaria edulis which was taken in March 2017. Sampling was carried out during low tide with the coordinate points for sampling at7o40'1.59"-7o40'3.44" South Latitude and 107o41'18.96"-107o41'20.99" East Longitude. During sampling, a number of parameters were also measured and seawater pH was 8.19, salinity was 34.51% and seawater temperature was 27.37oC. Gracilaria edulis was taken in large quantities and observed in detail based on its morphological similarities to literature studies.

To find out if the macroalgae that had been taken were of the Gracilaria edulis type, the samples were analyzed morphologically at the Biochemistry Laboratory, ITB Research and Innovation Building. The results of the morphological analysis that has been carried out show that morphologically the samples taken are indeed Gracilaria edulis (Restika Putri, personal communication) as shown in Figure 3.1.

Figure 3.1 Morphology*Gracilaria edulis*

taxonomymacroalgae Gracilaria edulis. [17] used in this study are as follows.

kingdom : Plantae
Subkingdom : Biliphyta
phylum : Rhodophyta
Class : Florideophyceae
Subclass : Rhdymeniophycidae
Order : Gracilariales
family : Gracilariaceae
genus : Gracilaria
Species : Gracilaria edulis (SG Gmelin) Silva

B. Extraction of Gracilari edulis

Samples of G.edulis in powder form were used in the extraction process by maceration using a solvent mixture of chloroform pa, methanol pa, 50 mM phosphate buffer pH 7.8 with a solvent ratio of 2:1:0.8 (v/v) and a sample ratio : solvent is 1:10 (w/v). The maceration process was carried out for 24 hours at 25oC with shaking at 150 rpm.

After the extraction was carried out, the residue and supernatant were obtained. For the residue, the re-extraction stage was carried out three times to ensure that all polar and nonpolar compounds contained in Gracilaria edulis had been thoroughly extracted. The supernatant was centrifuged at 2060 xg for 15 minutes to separate the compound groups based on their solubility in the methanol-water and chloroform phases. From the results of centrifugation, two phases were formed with the layer boundaries still cloudy in color indicating that the separation had not occurred optimally, so milli-Q water was added with a ratio of 9:2 (v/v) and centrifuged again. The final results of the centrifugation stage show that the crude extract has separated to form two phases with different colors and densities as shown in Figure 3.2.

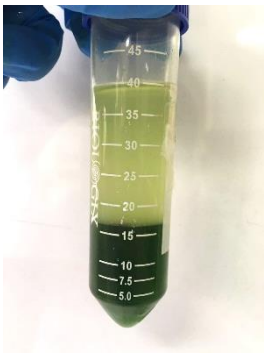


Figure 3.2 Results of centrifugation of Gracilaria edulis

From the centrifugation results it can be seen that the liquid in the upper phase is the methanol-water phase with a lower density than the lower phase. In the methanol-water phase there are a number of polar compounds. The lower phase with a higher density is a dark green extract which dissolves in chloroform. In this phase, compounds with low polarity are dissolved, which are similar

to chloroform which are nonpolar. The methanol-water extract and chloroform extract were carefully separated and the methanol phase was taken to then evaporate the solvent using a rotary evaporator.

The results of the extraction of the macroalgae Gracilaria edulis are shown in Table 3.1. Based on the data in the table it can be seen that Gracilaria edulis has a relatively small yield percent for the crude extract of the methanol-water phase.

Table 3.1 Gracilaria Edulis Extraction Results

Methanol Phase Crude Extract	Dry Mass Before Extraction (grams)	Dry Mass After Extraction (grams)	% Yield (w/w)
	85,62	0.875	1.02

Based on the extraction results, the percent yield of the crude extract of the methanol phase was 1.02%. In the study that has been reported by [17] The percent yield of the crude extract of the methanol phase of Gracilaria edulis from the Tamil Nadu region, India is 3.20%. Meanwhile, from the results from [18] Gracilaria edulis from the Andaman coast, India has a percent yield of the crude extract of the methanol phase of 2.40%. When compared with the percent yield of the results of this study with these studies, it can be reported that the crude extract of the methanol phase of Gracilaria edulis taken from Sayang Heulang beach, Garut, Indonesia has a relatively lower yield percent compared to the percent yield of Gracilaria edulis taken from India. at two different locations. The difference in the yield of this crude extract can be caused by various factors including differences in geographical location which are the main factors affecting the levels of the extract to be obtained. In addition, the selection of the extraction method and the difference in the age of the macroalgae [19] taken when selecting the G.edulis sample will also affect the percent yield obtained.

C. Fractionation of Methanol Extract of Gracilaria edulis

At this stage, the crude extract of the methanol phase was separated by column chromatography method. Column chromatography is a method commonly used when separating and purifying compounds from a mixture. In [20] explained the principle of separation and purification by column chromatography based on the differences in the distribution of components between the mobile phase and the stationary phase. Compounds with weak interactions with the stationary phase will move faster through the column while compounds with strong interactions with the stationary phase will move more slowly and be retained in the column. The collected fractions were then further analyzed using a UV-Vis spectrophotometer (Biochrom) with a wavelength range of 190-400 nm.

Furthermore, the 5 combined fractions were evaporated using a rotary evaporator to evaporate the solvent and the concentrated fraction was obtained. The concentrated fraction was then transferred to a falcon tube and methanol pa was added to a concentration of 10% (v/v) to determine with certainty the volume of the concentrated fraction and then stored at -20oC.Based on a preliminary study of previous research conducted by [14] showed that among the five combined

fractions, fraction C and fraction E were active in inhibiting biocorrosion so that these fractions were further tested for their effectiveness in inhibiting biocorrosion in this study.

D. Making Gallic Acid Calibration Curves

In determining the total phenolic content, gallic acid was used as a standard solution and the Folin Ciocalteu method with slight modifications. In preparing the gallic acid calibration curve, a wavelength of 765 nm was used, which is the maximum absorption of the blue molybdate complex compound [21]. Before measuring total phenol levels,First, a calibration curve for gallic acid standard solution with concentrations (20, 40, 60, 80, 120, 140) ppm was made using the dilution method for gallic acid mother liquor. Figure 3.3 is an acid calibration curve error which will then be used in determining the total phenol content in the crude methanol extract of Gracilaria edulis and several fractions resulting from the fractionation, namely fraction C and fraction E. The total phenol content in the sample can be determined using the regression equation contained in the calibration curve.

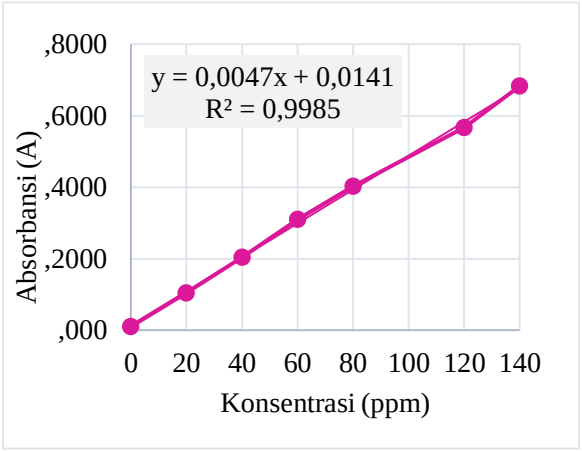


Figure 3.3 Gallic Acid Calibration Curve

Based on the data on the gallic acid calibration curve, the regression equation is obtained where y = 0.0047x + 0.0141 and the coefficient of determination R^2 = 0.9985. The coefficient of determination is 0.9985, which is close to 1, proving that the regression equation is linear and reliable.

E. Determination of Total Phenol Extract and Fraction Levels

The total phenol content of methanol extract, fraction C and fraction E were determined using the Folin Ciocalteu method by measuring the absorbance of the sample at a wavelength of 765 nm. After measuring the absorption, the total phenol content of the sample was calculated using the linear regression equation found in the gallic acid calibration curve. The results of measuring the total phenolic content of methanol extract, fraction C and fraction E are shown in Figure 3.4

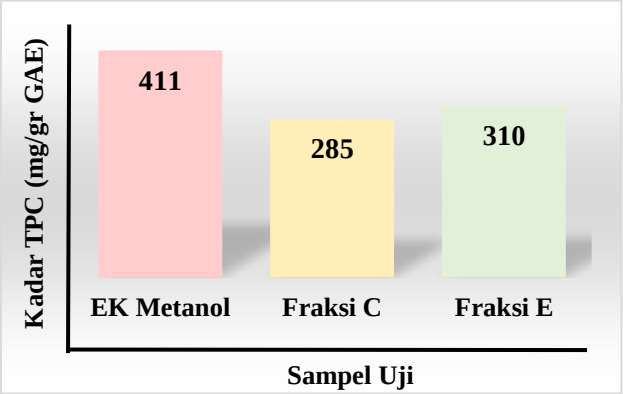


Figure 3.4 Total Phenol Content of Crude Methanol Extract, Fraction C And Fraction E
In Figure 3.4 it can be seen that the total phenol content

There is a difference in the total phenol content between the extract and the fraction caused by the complexity of the structure of the phenolic compounds which will affect the level of solubility of the phenolic compounds in the solvent used during extraction and also the possibility of other groups of non-phenolic compounds being measured when determining the total phenolic content. The presence of water in the crude extract of the methanol phase will likely dissolve nonphenolic compounds containing hydroxyl groups such as carbohydrates [22] which are also detected as metabolites that can be found in Gracilaria edulis [23]. For fraction C, which has relatively lower TPC levels than the TPC content in fraction E, because the probability of phenolic compounds that can dissolve in fraction E is greater than that found in fraction C. It is possible that the phenolic compounds present in fraction C are phenolic compounds with low polarity and a smaller number of phenol groups due to the solvent used to elute fraction C. is a mixture of n-hexane and acetone. Whereas the E fraction was fractionated using methanol as a solvent, making it possible to dissolve various compounds rich in phenol groups with simpler molecular masses.

F. Rejuvenation of Thiobacillus ferrooxidans Bacteria

Rejuvenation of T. ferrooxidans bacteria was carried out in liquid media (modified 9K media) [24] with a specific composition and mixed aseptically with the pH of the media adjusted in the range of 2.5-3.0 [25]. The growth curve for Thiobacillus ferrooxidans bacteria can be seen in Figure 3.5. It can be seen from the growth curve; the adaptation phase of bacteria starts from the first day until the third day. In this phase the bacteria will adapt to the pH and nutrients found in the environment.

The exponential phase starts from the fourth day to the seventh day which is marked by a very rapid increase in the absorbance value which is an indication that there has been bacterial multiplication and metabolic secretion has occurred as a result of bacterial growth which is characterized by a change in the color of the media and the density of the media solution getting thicker. On the seventh to the eighth day the bacteria enter the stationary phase. In this phase, most of the nutrients have been consumed by the bacteria for their growth so that the measured media

density does not show a significant difference because the bacteria have gone through the multiplication phase. On the eighth to the thirteenth day, Bacteria slowly begin to enter the death phase due to the amount of nutrients that have been used up, while more and more metabolites are secreted into the media environment. The metabolic secretion results can be toxic to itself because there are no more nutrients available to defend itself so that gradually the bacteria will die.

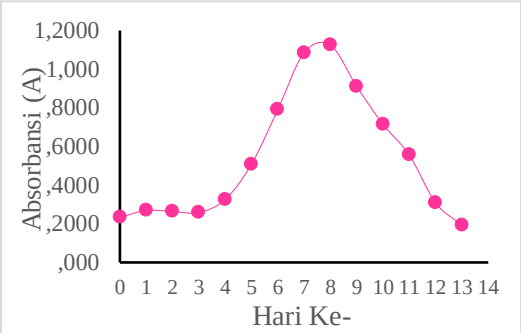


Figure 3.5 Growth Curve of T. ferrooxidans

After making a bacterial growth curve and obtaining a culture that has grown and lived in standard media, the bacteria are then adapted to the media which has been added to the composition of Sodium Chloride in stages, namely (0.5%; 1.0%; 1.5%; 2 .0%, 2.5%) (w/v). The increase in the percentage of NaCl was carried out in stages intended to adapt the bacteria to an environment with a high salt concentration before being adapted to seawater media. Thiobacillus ferrooxidans bacteria are reported to be able to survive in an environment with high salt concentrations if adaptation is carried out gradually [26].

After the bacteria were able to survive in 2.5% NaCl, adaptation was carried out in seawater media. For the adaptation of bacteria to seawater media, there is a slight difference in the composition of the media, namely distilled water and inorganic salts (K2HPO4 0.04%, MgSO4.7H2O 0.04%, and ((NH4)2SO4 0.04%) replaced with seawater because Seawater already contains various minerals which are considered sufficient to meet the nutritional needs of bacteria so that the addition of inorganic salts is not carried out. The results of the adaptation of bacteria to seawater media showed that the bacteria were able to survive in media added to seawater with a very turbid and rusty yellow media color density.

G. Biocorrosion Qualitative Test

Qualitative test of the biocorrosion process was carried out on solid media of T. ferrooxidans bacteria which had been adapted to 2.5% NaCl and had been adapted to seawater by adding each inhibitor, namely the crude extract of the methanol phase, the C fraction and the E fraction evenly into the media. solid filled with bacteria. In the qualitative biocorrosion test, commercial nails were used and the media was incubated for 4 days at room temperature and in the dark. The qualitative test of the biocorrosion process was aimed at visually observing the process of inhibition of the intensity of rusting that occurs on nails with and without the addition of extracts and fractions. In this qualitative test used methanol solvent p. a and media without the addition of bacteria as a comparison because methanol was reported to have antibacterial activity against certain bacteria

[27]. The results of the observation of the qualitative biocorrosion test on 2.5% NaCl media are presented in Table 3.2. Interpretation of visual observations of the intensity of rust that occurs on nails shows that there are differences in the intensity of rusting of nails on media without bacteria, without inhibitors and with the addition of inhibitors.

Table 3.2 Interpretation of Observational Data of Biocorrosion Qualitative Test On 2.5% Nacl Media

Sample	Rusting Intensity
No Bacteria	+ +
Thiobacillus ferrooxidans	+ + + + +
Bacteria + Methanol pa	+ + + + +
Bacteria + EK Methanol	+ + +
Bacteria + C Fraction	+ + +
Bacteria + E Fraction	+ + +

The rustiest nails were found in media in the presence of bacteria and media in the presence of methanol addition. The rusting of nails on the media with the addition of bacteria proves that T. ferrooxidans causes corrosion while the media with the addition of methanol pa solvent is reported to have antibacterial activity, but from the results of this study it is suspected that it has low antibacterial activity against T. ferrooxidans bacteria so rust still occurs. Furthermore, for the media with the addition of inhibitors it was visually observed that inhibition of biocorrosion was indicated by a decrease in the intensity of rust formed on the nails. This indicates that in the crude extract, fraction C and fraction E are thought to have good activity in inhibiting the biocorrosion process.

Table 3.3 Interpretation of Observational Data on Biocorrosion Qualitative Tests in Seawater Media

Sample	Rusting Intensity	Media Observation
No Bacteria	+ +	The media is rust yellow in color with not sharp color intensity.
Thiobacillus ferrooxidans	+ + + + +	3/4 of the surface of the media is rusty yellow and there are rust deposits.
Bacteria + Methanol pa	+ + + +	3/4 of the surface of the media is rusty yellow and there are rust deposits.

Bacteria + EK. methanol	+ + +	2/4 of the surface of the media is yellow rust and the rust deposits are reduced.
Bacteria + C Fraction	+ + +	2/4 of the surface of the media is rust yellow and there are no rust deposits.
Bacteria + E Fraction	+ + +	2/4 of the surface of the media is rust yellow and there are no rust deposits.

For media without bacteria, there was only a change in the intensity of the color of the media and there was no rust precipitate. This indicates that the rust precipitate formed was initiated by the presence of bacteria. The rust precipitate formed is probably the result of iron oxidation by bacteria that has accumulated on the surface of the media. As for the media with the addition of inhibitors, it showed a decrease in the intensity of the color of the media and formed rust precipitates. It is possible that this is caused by the presence of a number of synergistic groups of compounds which have activity in inhibiting biocorrosion which can then reduce the rusting that occurs on nails in corrosive seawater media.

H. Biocorrosion Quantitative Test

The results of the quantitative test for inhibition of biocorrosion by extracts and fractions are shown in Table 3.4. In the testing process with the weight loss method, it is carried out in duplo, namely carrying out two data measurements for each sample to ensure that the results obtained are accurate and reliable. This test also used biocides which are commonly used as biocorrosion inhibitors by the industry and crude extracts of the methanol phase as a comparison to the fractionated fraction.

For testing on media with the addition of 2.5% NaCl it can be seen that fraction C has a corrosion rate of 12.14 ± 1.09 mpy which is the lowest corrosion rate with the greatest inhibition efficiency of 37.21% compared to other inhibitors namely methanol crude extract, fraction E and biocides. The E fraction has a corrosion rate similar to that of biocides, namely 13.94 ± 1.82 mpy. This shows that the E fraction can be used as a better and safer alternative in inhibiting biocorrosion compared to using biocides.

Table 3.4 Quantitative Test Results for Inhibition of Biocorrosion by Extracts and Fractions In 2.5% Nacl Media

Sample	ΔW (grams)	CR (mpy)	%iwt
No Bacteria	0.0294 ± 0.0102	18.88 ± 6.54	-
T. ferrooxidans	0.0337 ± 0.0045	21.61 ± 2.86	-

Biocide	0.0212 ± 0.0034	13.62 ± 2.18	37.00
EK. Methanol	0.0271 ± 0.0053	17.37 ± 3.41	19,61
C faction	0.0167 ± 0.0057	10.73 ± 3.63	50,37
E faction	0.0182 ± 0.0009	11.72 ± 0.59	45,77

The methanol crude extract has a higher corrosion rate with lower inhibition power than fraction C but greater inhibition efficiency when compared to fraction E. This shows that the polarity of the solvent can affect the solubility of compounds that have inhibitory ability. In the methanol crude extract there are still various possible compounds, but the compounds that have better inhibition abilities have different polarities, so that further separation is required through fractionation. Furthermore, the quantitative test results for inhibition of biocorrosion by extracts and fractions in seawater media are shown in Table 3.5.

Sample	ΔW (grams)	CR (mpy)	%iwt
No Bacteria	0.0178 ± 0.0008	11.43 ± 0.55	-
T. ferrooxidans	0.0301 ± 0.0025	19.33 ± 1.64	-
Biocide	0.0217 ± 0.0021	13.94 ± 1.36	27,91
EK. Methanol	0.0207 ± 0.0035	13.30 ± 2.27	31,23
C faction	0.0307 ± 0.0001	12.14 ± 1.09	37,21
E faction	0.0217 ± 0.0028	13.94 ± 1.82	27,91

Table 3.5 Quantitative Test Results for Inhibition of Biocorrosion by Extracts and Fractions in Seawater Media

From table 3.5 it can be seen that a significant decrease in the value of the corrosion rate with the addition of biocide inhibitors, fraction C and fraction E compared to the media without inhibitors and the first position with the lowest corrosion rate is owned by fraction C and then fraction E. A

lower corrosion rate is good in fraction C and fraction E compared to biocides is an indication that there are different mechanisms in inhibiting biocorrosion. Biocides have a greater corrosion rate, possibly caused by the inhibition mechanism by biocides, which kills T bacteria.

Based on the good values of corrosion rate and inhibition efficiency shown by fraction C and fraction E, these two fractions were used in the next test stage to determine the optimum concentration of each fraction in inhibiting biocorrosion. Determination of the optimum concentration is based on the concentration of total phenolic content (TPC) because the group of phenolic compounds is rich in groups that can be donors of free electron pairs so that it is expected to be able to prevent oxidation of metals by stabilizing metals through the formation of complex compounds with metals. In measuring the optimum concentrations of fraction C and fraction E, the same concentrations of TPC were used (0.1; 0.2; 0.3; 0.4 and 0.5) mg/mL so that their effectiveness in inhibition could be compared with one another. Testing the concentration variation to determine the optimum concentration was carried out using the weight loss method with an immersion time of 4 days (96 hours). The results of the concentration optimization test for fraction C and fraction E in 2.5% NaCl media can be seen in the curve in Figure 3.6.

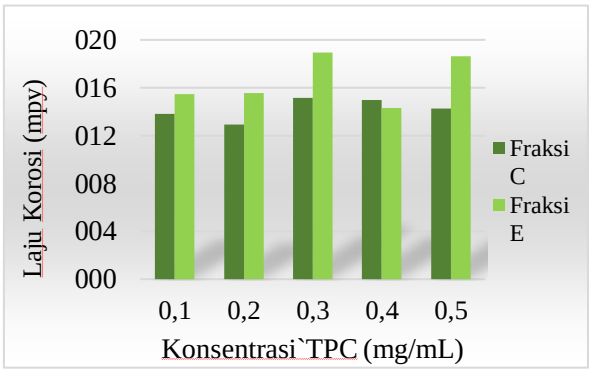


Figure 3.6 Optimization Test Curve for Optimum Concentration of Fraction C And Fraction E In 2.5% Nacl Media

Optimization test results for the concentration of fraction C and fraction E in 2.5% NaCl media showed that fraction data with the smallest corrosion rate was found in fraction C with the optimum concentration at a TPC concentration of 0.2 mg/mL compared to fraction E which had the optimum concentration at a TPC concentration of 0.4 mg/mL. Data from optimizing the concentration of the E fraction shows that the number of fractions needed to optimally inhibit biocorrosion is 0.4 mg/mL. This amount when compared to the C fraction is two times more than the number of fractions needed by the C fraction which will make the E fraction also quite effective as an inhibitor candidate but is less economical to use continuously.

Using the same method and immersion time of 4 days (96 hours), the determination of the optimum concentrations of the C and E fractions was also carried out in seawater. The results of the concentration optimization test for fraction C and fraction E in seawater media can be seen in the curves in Figure 3.7.

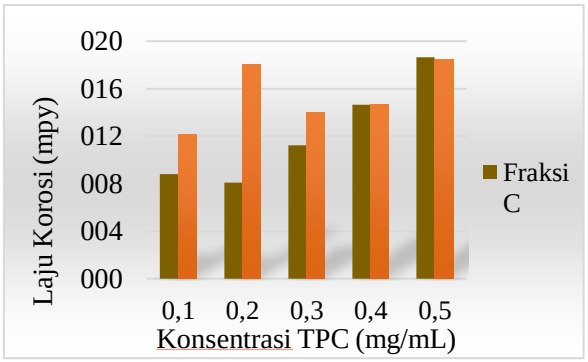


Figure 3.7 Optimization Test Curve for The Optimum Concentration of The Fraction C And E Fraction in Seawater Media.

Optimization test results for the concentration of fraction C and fraction E in seawater media showed a significant difference with the results of tests carried out in 2.5% NaCl media. It is possible that this is due to the seawater media which is more corrosive. The results of optimizing the concentration of the C fraction showed that the optimum concentration of the C fraction was at a TPC concentration of 0.2 mg/mL and this value was the same as that required in 2.5% NaCl media. From these data it can be stated that a TPC concentration of 0.2 mg/mL fraction C can be used both in 2.5% NaCl media and seawater media. The results of the optimization test for the concentration of the E fraction showed that the optimum concentration of the E fraction was at a TPC concentration of 0.1 mg/mL and this concentration was lower than the concentration required in 2.5% NaCl media.

In the variation stage of soaking time, only using bacterial media that had been adapted to seawater and using the C fraction with a TPC concentration of 0.2 mg/mL. The range of variations in immersion time was carried out starting from an interval of 2 days (48 hours) to 16 days (384 hours). Quantitative test results for variations in immersion time can be seen in Figure 3.8.

The corrosion rate of fraction C during immersion time variations from 48 hours to 192 hours showed a relatively low corrosion rate. This shows that fraction C has a long inhibition resistance in suppressing biocorrosion on carbon steel plates. On the 10th day (240 hours) an increase in the corrosion rate was observed, which then periodically experienced a significant increase in the corrosion rate. The occurrence of an increase in the value of the corrosion rate with increasing immersion time is suspected that the group of compounds contained in fraction C experienced a decrease in inhibition ability. Decreased inhibition ability can occur because the amount of fraction C used for testing is only added in a certain volume since the test started and is not added periodically for a certain period of time while there are still some bacteria living in the media which will allow the corrosion process to still occur. This is what causes fraction C to have the maximum wearability in protecting carbon steel plate from biocorrosion for 8 days.

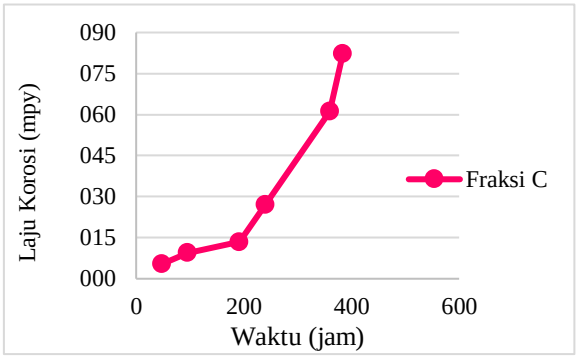


Figure 3.8 Quantitative Test Curve of Fraction C Inhibitor Against Variations in Immersion Time

I. Carbon Steel Morphological Analysis

The carbon steel plate analyzed for its surface morphology and chemical composition after the biocorrosion observation process is a carbon steel plate which has been tested quantitatively for biocorrosion inhibition in seawater using the weight loss method without and with the addition of inhibitor fraction C at a TPC concentration of 0.2 mg/mL with immersion time of 10 days (240 hours). As a comparison, untreated carbon steel plate was also analyzed for its surface morphology and chemical composition. The test results of the SEM tool were carried out with 3 magnifications starting from 500x, 1000x and 5000x magnification for each carbon steel plate analyzed, namely carbon steel plates without treatment, without inhibitors and with the addition of inhibitors.

SEM images of untreated carbon steel plates can be seen in Figure 3.9, those of carbon steel plates without inhibitors in Figure 3.10 and of carbon steel plates with the addition of inhibitors in Figure 3.11.

(a) (b) (c)

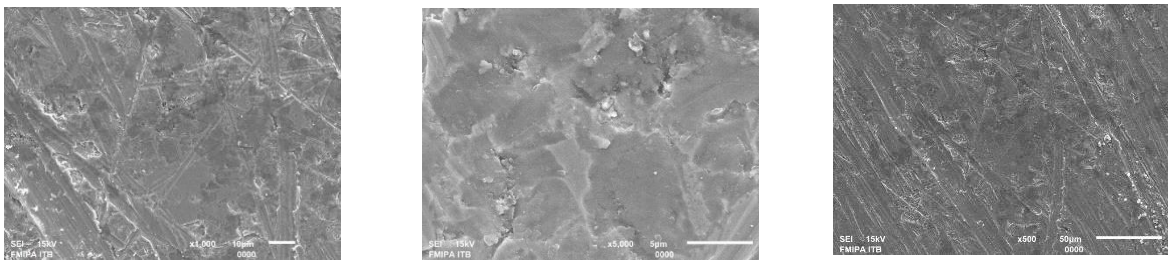


Figure 3.9 SEM image of surface morphology of carbon steel plate without treatment with 500x (a), 1000x (b) and 5000x (c) magnification.

The SEM image results of the surface of the carbon steel plate without any treatment show that the condition of the carbon steel plate tends to be rigid and there is no damage due to the absence of a corrosion process. The lines shown in the image are the results of polishing using sandpaper when cleaning carbon steel plates. The SEM image of carbon steel plate without treatment is then used as a comparison image to the SEM image of carbon steel plate without the addition of inhibitors and with the addition of inhibitors.

The test results in Figure 3.10 are SEM images on carbon steel plates without inhibitors at 500x magnification showing the formation of biofilms on the surface of the carbon steel plate with a very dense density covering the entire surface and there are lines indicating cracks on the surface of the carbon steel plate, presumably this is caused by T. ferrooxidans bacteria which stick to the surface of the carbon steel plate forming a biofilm layer and changing the surface structure of the carbon steel plate.

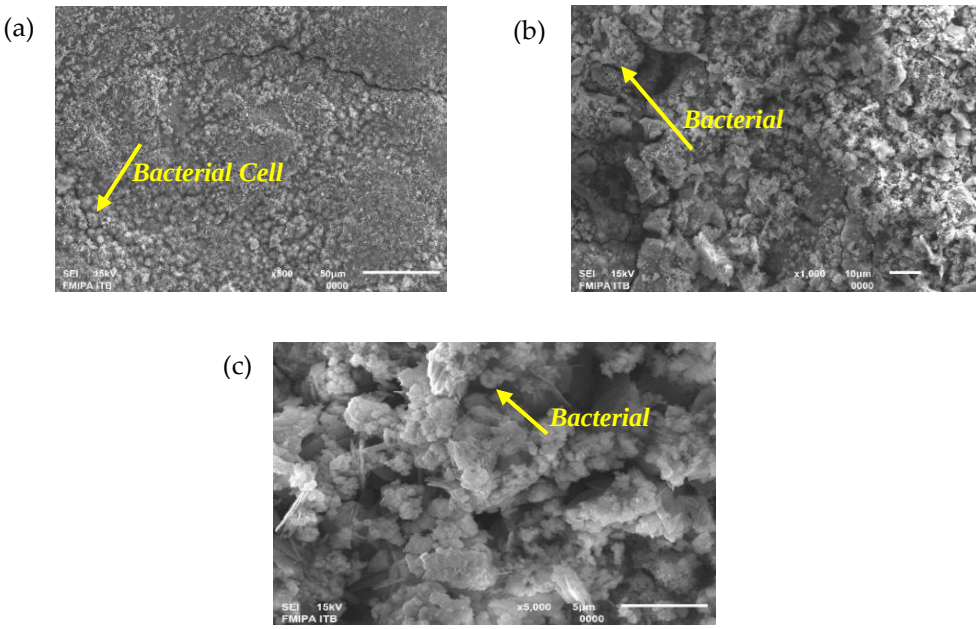


Figure 3.10 SEM image of the surface morphology of carbon steel plates without the addition of inhibitors with magnification 500x (a), 1000x (b), 5000x (c)

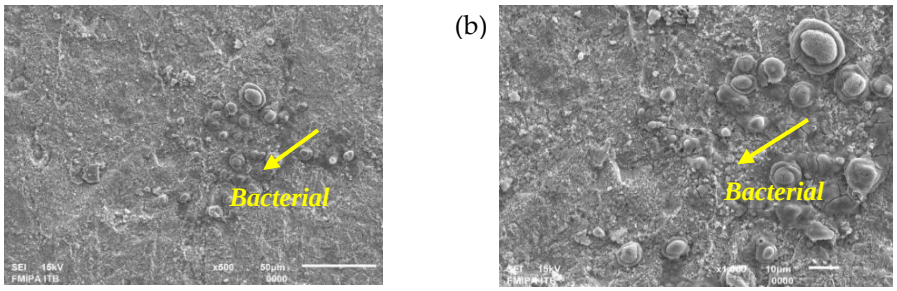
During the process of biofilm formation, the bacteria consume the nutrients available on the surface of the carbon steel plate. When a carbon steel plate is placed in seawater, the process of dissolving iron into iron (II) ions will occur. These iron (II) ions will be dispersed in seawater media which can then be used by bacteria as a source of nutrition and bacterial metabolic activity occurs. The results of the metabolic activity of these bacteria will secrete a number of products which over time will accumulate on the surface and most of the products formed are corrosive which will cause damage to the metal surface. When the SEM image is magnified by 1000 times and 5000 times, T cells can be clearly seen.

For SEM images of carbon steel plates with the addition of C fraction inhibitors (Figure 3.11) the results show a significant difference compared to without the addition of inhibitors. SEM image at 500 times magnification can be seen that most of the surface of the carbon steel plate is covered by a thin layer that protects the metal surface which indicates the role of inhibitors in protecting the metal from corrosion attack by T. ferrooxidans bacteria. In addition, it was also observed that at several points there were a collection of small granules on the surface of the carbon steel plate. It is strongly suspected that this is a side product that accumulates due to the metabolic activity of bacteria that remains on the metal surface. This by-product is thought to be iron sulfide (FeS), a compound that is closely related to the exopolymer compound secreted by bacteria so that it accumulates together

with the exopolymer compound to form a precipitate on the metal surface. Iron sulfide is difficult to dissolve in water so it will be in the form of deposits on the metal surface (Coetser and Cloete, 2005).

With the formation of a thin layer on the surface of the metal which is suspected to be hydrophobic, the solubility of iron sulfide will decrease so that when observed using SEM at 1000x and 5000x magnification, the iron sulfide precipitate formed will be very clear. Iron sulfide is difficult to dissolve in water so it will be in the form of deposits on the metal surface (Coetser and Cloete, 2005). With the formation of a thin layer on the surface of the metal which is suspected to be hydrophobic, the solubility of iron sulfide will decrease so that when observed using SEM at 1000x and 5000x magnification, the iron sulfide precipitate formed will be very clear. Iron sulfide is difficult to dissolve in water so it will be in the form of deposits on the metal surface (Coetser and Cloete, 2005). With the formation of a thin layer on the surface of the metal which is suspected to be hydrophobic, the solubility of iron sulfide will decrease so that when observed using SEM at 1000x and 5000x magnification, the iron sulfide precipitate formed will be very clear.

(a) (b)



(c)

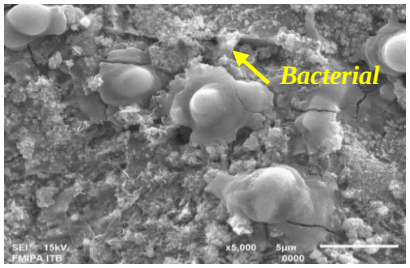


Figure 3.11 Surface Morphology of Carbon Steel Plate with Addition of Inhibitors with Magnification 500x (A), 1000x (B) And 5000x (C)

There is a supporting reference from Beech and Sunner (2004) which shows AFM (Atomic Force Micrograph) image data which observes the occurrence of biocorrosion on metal surfaces caused by the bacterium Desulvovibrio alaskensis, which is a type of sulfate-reducing bacteria group. In the AFM image data studied by Beech and Sunner, it was observed that a collection of FeS particles on the surface of the stainless-steel metal accumulated together with exopolymer compounds secreted by D. alaskensis bacteria.

EDS analysis was also carried out on untreated carbon steel plates, without inhibitors and with the addition of inhibitors to identify the chemical composition of the biofilm layer formed during corrosion. The information that will be known through this EDS analysis is in the form of the atomic mass percent contained in the metal material. The results of the EDS analysis can be seen in Table 3.6.

Table 3.6 Comparison of Data from The Analysis of The Chemical Composition of Carbon Steel Plates Using EDS

Test Treatment	Elemental Composition (%)						Total Mass (%)
	C	O	Si	S	cl	Fe	
No treatment	9.85	10.34	-	-	-	79.80	100
Bacteria + No Inhibitors	6,91	40,44	0.84	1.31	0.24	50,26	100
Bacteria + Inhibitors	10.63	10,13	-	-	0.04	79,20	100

From the data analysis using EDS, it can be seen the differences in chemical composition on the surface of the carbon steel plate without treatment, without inhibitors and with the addition of inhibitors. On the surface of the carbon steel plate without inhibitors, the elemental composition of Fe decreased significantly compared to the elemental composition of Fe on the carbon steel plate without treatment and with the addition of inhibitors.

In addition, on the carbon steel plate without inhibitors, element S was detected which indicated the presence of sulfur-containing compounds on the surface of the carbon steel plate. The detection of elemental sulfur is an indication of metabolic activity by T. ferrooxidans bacteria which reduces sulfate to sulfide and the detection of a decrease in the mass of elemental Fe indicates the oxidation of ferrous metal to form iron (II) ions which are released into solution. The occurrence of iron oxidation is also supported by data on the mass of elemental oxygen which has increased up to four times compared to the mass of elemental oxygen on carbon steel plates without treatment and with the addition of inhibitors. The occurrence of metal oxidation will also affect the percentage composition of other elements which are components of carbon steel, including carbon and silicon elements. It can be seen from the data on the composition of the carbon element contained in the carbon steel plate without inhibitors that there is a decrease in mass and the element Si (silicon) is detected. The element silicon is used as an additional ingredient in the manufacture of carbon steel which will act as a deoxidizer, which will protect the metal layer when corrosion occurs (Holappa, 2014).

The detection of this Si element is an indication that the metal layer is starting to experience damage so that the silicon contained in the metal will carry out its function in protecting the metal surface. It can be seen from the data on the composition of the carbon element contained in the carbon steel plate without inhibitors that there is a decrease in mass and the element Si (silicon) is detected. The element silicon is used as an additional ingredient in the manufacture of carbon steel which will

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From the EDS data it was also obtained that on carbon steel plates with the addition of inhibitors there was an increase in the mass of carbon elements and the mass of Fe elements which was similar to the mass of Fe found in carbon steel plates without any treatment. This is strongly suspected to be due to the role of compounds present in fraction C which can prevent iron oxidation by forming chelating compounds with iron (II) metal so that iron will be stable and not undergo oxidation. From the EDS data it was also detected that the element Cl was found in a smaller amount on the carbon steel plate in the presence of an inhibitor. This shows that the possibility of ions in seawater which are corrosive has a synergistic effect with the possibility of compounds present in the C fraction so that it will help reduce the level of aggressiveness of chloride ions.

J. Identification of Compound Groups in Fraction C and Fraction E

After a series of testing stages, both qualitatively and quantitatively, the ability of fraction C to inhibit, fraction C was analyzed using a UV-Vis spectrophotometer to identify groups of compounds contained in fraction C. In this identification stage, the wavelength range of 190 nm to 400 nm was used. Fraction C is the result of fractionation using n-hexane and acetone at a ratio of 2:8. UV spectrum data for fraction C can be seen in Figure 3.12. From the results of UV spectrum analysis, fraction C has maximum absorption peaks at wavelengths (215, 223, 240, 258, 302) nm. Based on literature, It is possible that compounds with absorption peaks similar to those wavelengths have similarities to the wavelength ranges of various phenolic compounds, including ferulic acid (214 nm and 325 nm), p-coumaric acid (224 nm and 309 nm), apigenin glycosides (235 nm). and 240 nm) and quercetin (256 nm and 354 nm) (Spiridon et al., 2011). Based on the similarity of the research data with the literature, it can be concluded that the group of compounds contained in fraction C is a mixture of several groups of phenolic compounds.

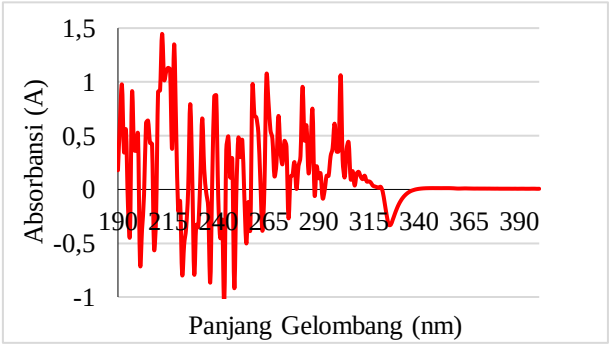

Figure 3.12 UV Spectrum of C Fraction

UV spectrum analysis was also carried out on the E fraction which was also used as an inhibitor in this study. The results of the UV spectrum analysis of the E fraction can be seen in Figure 3.13. Fraction E is the product of fractionation using methanol as eluent pa. Fraction E has maximum absorption at a wavelength of 204 nm, 270 nm and 336 nm.

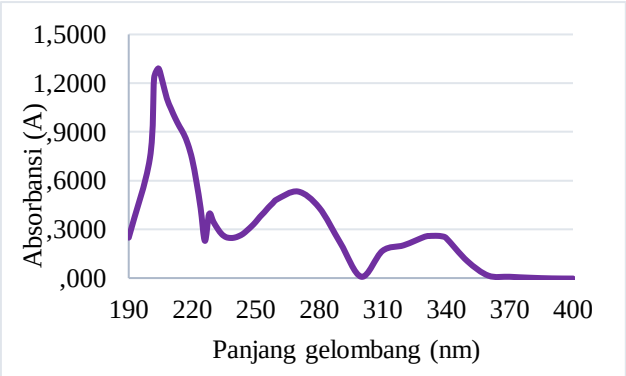

Figure 3.13 UV Spectrum of The E Fraction

In Spiridon et al. (2011) stated that a wavelength of 200 nm to 400 nm is a typical wavelength for detecting groups of phenolic compounds. In relation to the structure of the flavonoids, the maximum absorption peaks obtained can be used as information on possible structures, namely at a wavelength of 200-250 nm for flavonoids with a linear chain, at a wavelength of 250 nm to 295 nm for flavonoids from the aromatic group and a wavelength of 300-400 nm for flavonoids with conjugated double bonds. Based on the results of the UV spectrum analysis, it can be concluded that the E fraction is a group of flavonoid compounds.

K. Biocorrosion Mechanism

After conducting qualitative and quantitative tests on the inhibition process, the possible mechanism of biocorrosion caused by T. ferrooxidants can be identified. Thiobacillus ferrooxidans bacteria is a sulfate reducing bacteria that can reduce sulfate to sulfide. However, to carry out this reduction process, T. ferrooxidans requires free electrons as its energy source, which is obtained from the oxidation of iron (II) to iron (III). The redox reaction mechanism of biocorrosion has been previously described by (Kakooei et al., 2012) which is explained in the following reaction equation.

Anode reaction	$4Fe_{(s)} \rightarrow 4Fe2+(aq) + 8e-$
Water dissociation	$8H2O\ (aq) \rightarrow 8H+(aq) + 8OH-(aq)$
Cathode reaction	$8H+(aq) + 8e- \rightarrow 4H2(g)$
Hydrogen oxidation	$SO42-(aq) + 4H2(g) \rightarrow H2S(aq) + H2O(l) + 2OH-(aq)$
Precipitation reaction (1)	$Fe^{2+(aq)} + H2S(aq) \rightarrow FeS(s) + 2H+(aq)$
Bacterial catalytic reactions	$3Fe2+(aq) \rightarrow 3Fe3+(aq) + 3e-$
Water reduction	$6H+(aq) + 3/2\ O2(g) + 6e- \rightarrow 3H2O(l)$
Precipitation reaction (2)	$2Fe^{3+(aq)} + 6OH-(aq) \rightarrow 2Fe\ (OH)3(s)$

total reaction $4Fe(s) + SO4^{2-}(aq) + 3H2O(l) + 4H^{+}(aq) + 3e^{-} \rightarrow FeS(s) + 2Fe(OH)3(s) + 3OH^{-}(aq)$

From the reaction data contained above, it shows that the free electrons produced by the oxidation of iron on the anode side are used. These free electrons will move towards the cathode and in the presence of water in the environment, a reduction process will occur to form hydrogen gas. Hydrogen gas formed will facilitate the reduction of sulfate ions by bacteria to form sulfide ions. Iron (II) ions that have been released into the environment will react with sulfides to form compounds that have low solubility in water, namely FeS (iron sulfide).

The iron sulfide formed was also observed during the EDS analysis in this study. It can be seen from the EDS data that detected the presence of sulfur elements on the carbon steel plate without the addition of inhibitors, which is thought to be the result of bacterial secretions which have reduced sulfate to sulfide. Iron (II) ions dissolved in water will also be able to react with hydroxide anions (OH-) present in solution to form a compound, namely iron (III) hydroxide (Fe (OH)3). This was observed from the EDS results which showed a significant decrease in the mass percent of Fe elements with the occurrence of biocorrosion.

The results of the study through EDS data showed that with the addition of inhibitors the elemental composition of Fe was almost similar to the elemental composition of Fe carbon steel plates without treatment. This indicates that there is a role of inhibitors in protecting Fe metal from being oxidized to iron (II) ions. The inhibitor mechanism in protecting Fe metal is thought to be through the adsorption of inhibitors on the metal surface to form a chelate compound with Fe metal. Inhibitor fraction C which is rich in phenolic compounds of the phenolic acid group has hydrophobic groups that can form a thin layer with a thickness of several molecules which will be able to inhibit attack by the environment against the metal so that the metal surface becomes protected. This hydrophobic thin layer is thought to interfere with water dissociation on the metal surface so that it can prevent the metal from corrosion attack. In addition, there is a possibility that inhibitors can weaken the presence of an aggressive component, namely chloride anions contained in the media, this can be seen from the corrosion rate data with the addition of the C fraction which experienced a significant decrease in seawater media and also a decrease in the percentage of Cl elemental composition on the plate. carbon steel with added inhibitor. Besides being believed to protect metal surfaces, inhibitors are also thought to have activity in inhibiting the growth of T. ferrooxidans bacteria so that there will be a decrease in the amount of iron (II) ions which are oxidized to iron (III) ions. This is supported by EDS data with the addition of inhibitors,

CONCLUSION

Separation of the crude methanol extract was carried out using column chromatography to produce six groups of combined fractions, namely fraction A-fraction E and fractions C and fraction E were selected as inhibitors used for analysis. From the results of determining the total phenolic content, data was obtained the total phenol content for the crude methanol extract of Gracilaria edulis was 410.77 ± 9.55 mg GAE/g extract, the C fraction was 285.30 ± 2.34 mg GAE/g fraction and the E fraction was 309.51 ± 22.12 mg GAE/g fraction. Qualitative test of the ability of biocorrosion inhibition by the crude extract of the methanol phase, fraction C and fraction E showed that the

inhibition of biocorrosion was observed visually in the form of a decrease in rust intensity that occurred in the commercial nail test medium. The results of the quantitative test for inhibition of biocorrosion by fraction C and fraction E using the weight loss method also showed a decrease in the corrosion rate compared to the corrosion rate in the media without the addition of inhibitors. Optimum concentrations of fraction C and fraction E in inhibiting biocorrosion in seawater media have optimum concentrations of 0.2 mg/mL for fraction C and 0.1 mg/mL for fraction E.T. ferrooxidans bacterial cells form a biofilm layer on the surface of the carbon steel plate with localized density but dominate most of the surface of the carbon steel plate and SEM results when added inhibitors show that the carbon steel plate forms a thin layer that protects the metal surface. The test results using the EDS tool also detected the presence of sulfur and a decrease in the percentage of iron in the carbon steel plate without inhibitors. Identification of the compounds contained in the C and E fractions by UV spectrophotometer showed that the C fraction had a typical maximum absorption peak for the phenolic acid compound group and the E fraction had a specific absorption peak for the phenolic compound group of flavonoids.

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