Evaluation of Bio-Corrosion on Carbon Steel by *Bacillus Megaterium* in Biodiesel and Diesel Oil Mixture

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Highlights:

- Biodiesel affects the growth of microorganisms on carbon steel.
- The growth and metabolism activities of microorganisms on carbon steel induce corrosion.
- Pitting corrosion caused by microbial activity was found on the carbon steel surface.

Abstract: Biodiesel can act as carbon source for bacterial metabolisms, leading to corrosion of carbon steel. In this study, the corrosion of carbon steel by biodiesel blends (B15, B20, B30) was observed in the presence of *Bacillus megaterium*. The effect of biodiesel concentration on microorganism-induced corrosion was investigated by electrochemical impedance spectroscope (EIS), scanning electron microscope (SEM) and digital microscope. The results showed that under various biodiesel concentrations, *Bacillus megaterium* can grow and form biofilm on carbon steel. Based on the impedance analysis, their presence can increase the corrosion rate and cause pitting corrosion because the biofilm can change the electrochemical reactions in the metal or the interface solution and the kinetics of the anodic cathodic reactions. Also, *Bacillus megaterium* produces acid metabolites and can oxidize iron. Besides being influenced by *Bacillus megaterium* activities, the pitting formed on carbon steel depends on the biodiesel concentration. The results showed a great deal of shallow pit formation in B30, exacerbating the severity of metal roughness.

Keywords: *bio-corrosion; biofilm; EIS; hydrocarbons; pitting; SEM.*

1 Introduction

Due to the reduced availability of diesel oil, the Indonesian government has launched a policy to promote the usage of biodiesel through ESDM decree No.

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12 2015. For use as alternative fuel, the biodiesel in a diesel oil mixture is expected to reach 30% v/v. However, problems related to bio-corrosion arise with the use of biodiesel because its hygroscopic properties can easily be contaminated by microorganisms. In addition, carbon steel, a metal that is commonly used for various industrial installations such as storage tanks, easily corrodes, leading to degradation of its strength.

Corrosion can be triggered by microorganisms due to their ability to degrade hydrocarbons as carbon source, form biofilm and produce metabolites that may change the condition of the surrounding environment. Specifically, the biodegradable properties of biodiesel may correspond to the growth and metabolism of microorganisms. Fatty acids contained in biodiesel can be degraded four times faster than diesel oil [1]. The high percentage of unsaturated esters made from palm oil contained in biodiesel, such as 42.4% oleate (18:1), makes it degrade more easily [2]. Among several species of bacteria, *Bacillus sp* is one that may be involved in bio-corrosion, especially of hydrocarbon products [3-6]. In naphtha with 2% of water as medium, inoculated mixed cultures (2.1 x 10^5 CFU/ml) consisting of *Serratia marcescens*, *Bacillus pumilus*, *Bacillus carboniphilus*, *Bacillus megaterium dan Bacillus cereus* affected corrosion of API 5LX steel up to 0.1362 mm/year for 7 days of immersion time. In diesel oil with 2% of water and 1% BH broth as medium and with the presence of *Bacillus cereus* per se (inoculum about 10^6 CFU/ml), the corrosion rate of API 5LX steel reached 0.075 mm/year for 10 days of immersion time. In the presence of microorganisms, the corrosion rate was higher than in the control system (sterile medium) [3,6]. Bio-corrosion prevention measures, such as using inhibitors, are apparently ineffective due to the ability of microorganisms to degrade them [7]. Hence, it is a great challenge to deal with bio-corrosion.

Gravimetric analysis is a method commonly employed for the evaluation of corrosion and the effect of microorganisms on the corrosion rate. This method assumes that the corrosion on the surface is uniform, so the corrosion rate can be calculated based on the metal mass loss. However, in the presence of biofilm, bio-corrosion may occur over heterogeneous surface conditions, frequently initiated by localized pitting corrosion. Electrochemical impedance spectroscopy (EIS) can be used to examine bio-corrosion, providing the correlation between the corrosion rate and the damage of the metal caused by localized corrosion. EIS analysis has been used to examine bio-corrosion [8-10], including the electrochemical processes in metals or biofilm interfaces, corrosion products and biofilm formation related to bio-corrosion.

The investigation of bio-corrosion needs to quantify the pitting corrosion baed on the level of damage to a metal surface that is incompletely coated by biofilms, which can be determined experimentally as reported by Beech, *et al.* [11] and

Steele, *et al.* [12]. Xu, *et al.* [13,14] profiled the changes in the width and depth of pits over time in mild steel corrosion of sulfate-reducing bacteria (SRB). Parthipan, *et al.* [10] and Rajasekar, *et al.* [15] investigated carbon steel API 5L that was exposed to mixed bacterial consortia, especially *Serratia marcescens*, in petroleum. However, these studies conducted pit depth measurement using a light digital microscope instead of an atomic force microscope (AFM), which is widely used to assess the level of corrosion damage.

Although EIS and pit depth measurement have been widely employed in studying bio-corrosion, only a few studies have been conducted to determine the involvement of hydrocarbon-degrading bacteria in the corrosion process of metals, especially related to an increase in biodiesel consumption. Therefore, the purpose of the present research was to understand the impact of the microbial activities of *Bacillus megaterium* on a carbon steel surface with variation of the concentration of biodiesel in a diesel oil mixture using EIS, SEM and a digital microscope. This bacterial species was chosen because it has been identified as the main organism associated with corrosion products of naphtha and diesel oil in piping transport in India [4]. The presence of this bacteria can be predicted to influence carbon steel corrosion, especially in biodiesel.

2 Material and Methods

2.1 Materials

In the experiment, carbon steel ST-37 (Panca Logam Workshop Bandung Indonesia) was used, which was cut to a size of 1 cm^2 and the exposed surfaces were sequentially polished using silicon carbide abrasive paper (grid 240, 400, 600, 800, 1000 and 1200) to obtain smooth surfaces. After polishing, all the specimens were washed with demineralized water and ethanol to remove fat and impurities. Subsequently, they were dried with an electric dryer and stored in a desiccator.

2.2 Preparation of Microorganism

The microorganism used in this research was *Bacillus megaterium* P112, obtained from the National University of Singapore. The bacteria were cultured in a 250-mL Erlenmeyer filled with 100 mL sterilized Bushnell Haas (BH) medium until the beginning of stationary growth and used as inoculum for further experiments. The BH medium composition consisted of (in g L-1) 0.2 $MgSO_4$, 0.02 $CaCl_2$, 1 KH_2PO_4, 1 K_2HPO_4, 1 NH_4NO_3 and 0.05 $FeCl_3$. Two mL inoculum was used in a 500-mL Erlenmeyer containing 300 mL of BH medium and 1 g of sterilized diesel oil as the sole carbon source. The bacteria were then incubated in a rotary shaker at a temperature of 30 °C and 150 rpm for 22 days. Based on total plate count (TPC), the concentration of microorganisms in the solution was 10^6 CFU/ml at the end of the acclimatization process.

The biodiesel and diesel oil used as the immersion medium were supplied by Darmex Agro Jakarta Indonesia and Pertamina Bandung Indonesia respectively. This hydrocarbon is a commercial fuel, which is sold and used in daily life in Indonesia. Thus, these fuels must meet the Indonesian National Standard Quality (SNI) 7182:2015. To remove impurities and microorganisms, the hydrocarbon was filtered with a CA filter membrane with a pore size of 0.45 μm. Then, the diesel oil was blended with 15% (B15), 20% (B20), 30% (B30) v/v biodiesel as different substrates.

2.3 Immersion Process

The immersion experiment was carried out in a 250-mL glass reactor with 200 mL working volume, consisting of a mixture of hydrocarbon, water containing 1% BH broth at a ratio of 2:1 and 2 mL *Bacillus megaterium* in the BH solution from the acclimatization process in view of impedance analysis.

For morphological studies, a 200-mL working volume consisting of a mixture of hydrocarbon and 10% v/v acclimatization solution of BH containing *Bacillus megaterium* was also set up. Soaking was done at a temperature of 30 °C and under anaerobic conditions for 10 days. Anaerobic conditions represented the situation inside the storage tank.

2.4 Characterization of Carbon Steel

EIS studies were performed using Gamry Instruments Reference 3000 Potentiostat/Galvanostat/ZRA (Gamry Instruments, Inc. Warminster, PA, USA) with a three-electrode system for recording. For the EIS measurements, the specimen was connected to a wire and encapsulated on one side. The open surface of the carbon steel acted as the working electrode. The standard calomel electrode (SCE) in saturated KI solution was used as the reference electrode and a platinum wire was used as the counter electrode. The water from the bottom of each reactor was used as the electrolyte solution for EIS measurement. The impedance study was carried out with a scan rate of 10 mV/min and under steady-state conditions in the frequency range 10^2-10^5 Hz. For determining the electrical equivalent circuit (EEC) and fitting Nyquist plots, the Gamry Echem Analyst Software was used.

SEM images of the biofilms and the specimen's surface were captured after 10 days of immersion using Hitachi SU5000 and SU3500 (Hitachi Ltd, Tokyo, Japan). The biofilm morphology was observed by fixing it with formaldehyde in phosphate buffer with a pH of 7.3 for 24 hours and the sample was rinsed with aqua dm followed by ethanol at concentrations of 20%, 50%, 70%, and 98%. The samples were dried with an electric dryer and stored in a desiccator.

Corrosion damage in the form of pitting was captured and measured with VHX Digital Microscope Keyence 5000 (Keyence Corporation Osaka, Japan) after cleaning the metal specimens using a brush and rinsing with 70% ethanol.

2.5 Statistical Analysis

Statistical analysis was performed with one-way ANOVA using Microsoft Excel 2016. Significance level (α) alpha was set at 0.1.

3 Results

Bacillus megaterium activities in the immersion medium of biodiesel can affect the electron transfer and the speed of the running corrosion reaction and hence can be examined through impedance analysis.

The involvement of *Bacillus megaterium* in corrosion can be presented as data on a Nyquist plot that correlates with a proper equivalent circuit simulation. This is based on the concept of EIS, according to which an interface layer can be seen as a combination of passive electrical circuit elements, namely resistance, capacitance and inductance.

One of the difficulties of the impedance technique is determining the specific electrical equivalent circuit (EEC), because the corrosion process involves several processes that run simultaneously. Circuit elements can vary in placement and relationship from one process to another. Here, the EEC was used for describing the effect of *Bacillus megaterium* on corrosion involving biodiesel, as shown in Figure 1.

Based on Figure 1, the biodiesel concentration in the medium did not change the arrangement of the EEC. The EEC consists of two constant phase elements (CPE) to describe the biofilm and the double layer and passive film capacitance. The existing layers also have their respective resistances. The biodiesel concentration influences the corrosion rate, which can be predicted based on the results of the superposition of each variation of the experiment with a Nyquist plot. Since each element of the ECC will produce certain quantities, the effect of *Bacillus megaterium* and biodiesel on the carbon steel corrosion rate can be predicted by determining the value of the charge transfer resistance (Rct) (Table 1).

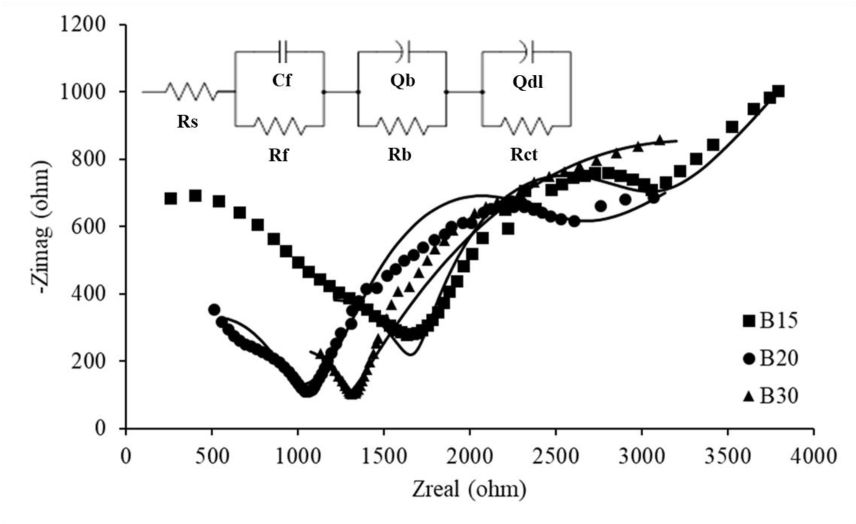

Figure 1 Nyquist Diagram and EEC for carbon steel in the presence of *Bacillus megaterium* at various biodiesel concentrations. Rs (solution resistance), Cf (film capacitance), Qb (constant phase element (CPE) biofilm), Rb (biofilm resistance), Qdl (CPE double-layer), Rct (charge-transfer resistance).

Obviously, the Rct value of carbon steel decreases in proportion to the increase in the biodiesel concentration and the presence of *Bacillus megaterium* in the medium. This means that the decrease in the Rct value can be correlated with an increase in the corrosion rate.

Table 1 Impedance parameters and pit depth of carbon steel immersed in medium containing *Bacillus megaterium.*

Biodiesel concentration	Film resistance R_f (Ω cm^2)	Charge transfer resistance R_{ct} (Ω cm^2)	n_{dl} (x10^{-3})	Average depth of pitting (μm)
B15	920.1	870	867.4	1.61 ± 0.57
B20	729.1	840	787	3.51 ± 1.51
B30	620	739	715	1.10 ± 0.33

The condition of metal surfaces with the increase of biodiesel concentration and activity of *Bacillus megaterium* can be seen in Figure 2 and 3. The formation of biofilm by *Bacillus megaterium* was very visible, as observed using SEM on the metal surfaces. Pitting corrosion is a side-effect damage and there is a significant difference in the level of damage between the surface of metal with variation of the biodiesel concentration and the presence of *Bacillus megaterium*. For concentration B15, small pitting damage as a result of the scattered surface of the specimens was obvious. In contrast, the metal specimen's surface for concentration B20 showed more severe damage. The worst damage was observed for concentration B30, mostly on the surface of the metal specimen, with a great deal of pitting.

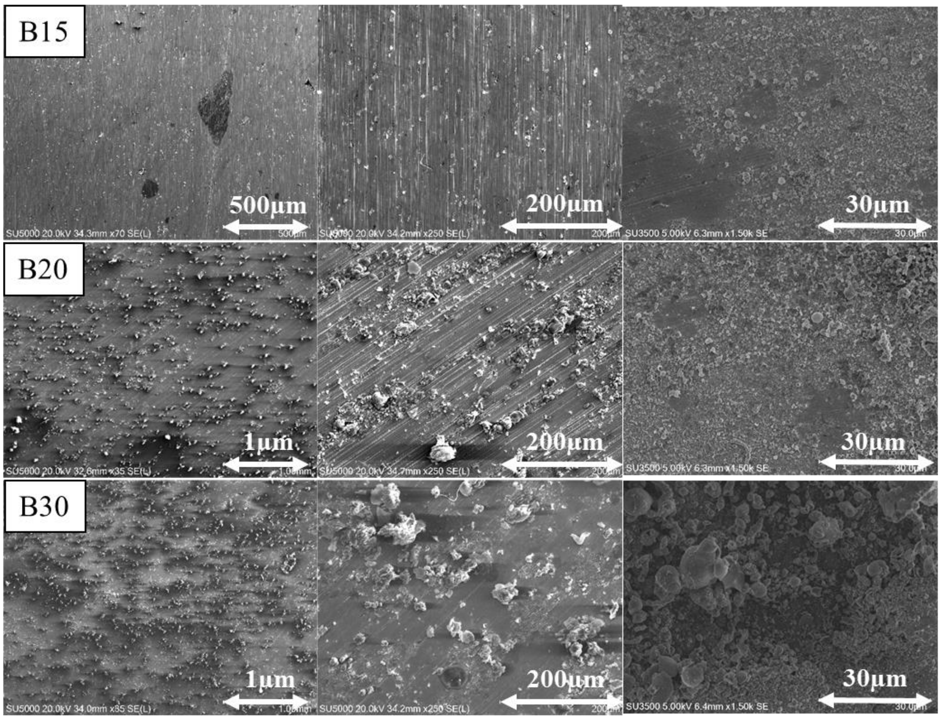

Figure 2 SEM images of carbon steel immersed in diesel oil containing biodiesel as medium with the presence of *Bacillus megaterium.*

Pitting corrosion on metal surfaces under the influence of biodiesel and *Bacillus megaterium* activity was measured by depth at various random positions to determine the level of corrosion damage quantitatively. From the various measured positions, two were selected to describe the size of the pit depth formed. The measurement of the pit depth in the surface scanning graph refers to the deepest point subtracted with the reference point (a clean metal surface). Based on statistical calculation, the *p*-value was 0.089 for the pit depth measurements. Since $p \leq 0.1$ there is statistical evidence that the various biodiesel concentrations affected the pitting corrosion. The average measurement results are shown in Table 1.

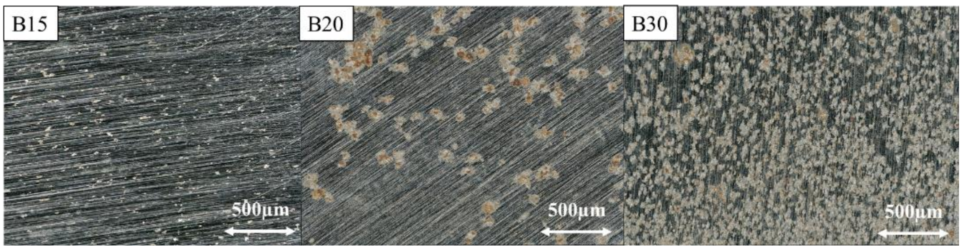

Figure 3 Surface morphology of carbon steel under different biodiesel concentrations with the presence of *Bacillus megaterium.*

The pit depth measured for concentration B15 and the presence of *Bacillus megaterium* in the first location was 1.29 µm, while the depths in the second location were 1.28 µm and 2.27 µm. For concentration B20, the pit depths were 5.13 µm, 2.14 µm, and 3.28 µm, respectively.

In contrast to the pit depth variations for concentration B15, only one pit was shallow while the others for concentration B20 had a greater pit depth. The pit depths measured for concentration B30 were obtained from two different locations, namely 1.33 µm and 0.86 µm. Apparently, the pit depths obtained were lower than the variance in concentrations B15 and B20.

4 Discussion

The hygroscopic nature of biofuel makes it more susceptible to microorganism contamination, including by *Bacillus megaterium*. Free water can trigger the growth of this bacteria, which can increase the risk of corrosion and degradation of hydrocarbons since *Bacillus megaterium* produces enzymes in the form of hydrogen peroxide (H_2O_2) and uses them as carbon source [16]. The addition of biodiesel to diesel oil will increase the bioavailability of the fuel so that it provides a distinct advantage for *Bacillus megaterium*.

The more fatty acid methyl esters (FAME, commonly known as biodiesel) are added, the more unsaturated chain content is obtained in the medium (for instance 53.6% oleic acid in biodiesel produced from palm oil), making it more susceptible to oxidation. Consequently, unsaturated fatty acids degrade into simple acids and other short chain fatty acids. These compounds can naturally be utilized by *Bacillus megaterium* for new cell formation as well as activities that affect the corrosion process.

Importantly, the availability of *Bacillus megaterium* promotes electron transfer in the biodiesel medium and accelerates a variety of corrosion reactions due to the fact that they have the ability to reduce oxygen, sulfate, thiosulfate, Fe (III), oxidize sulfide, ferrous iron, ferment and/or produce acid [17]. Furthermore, on metal surfaces, biofilm can change the surrounding environment such as the local oxygen concentration and pH within biofilm and in bulk [18]. Changes of these environmental conditions lead to different routes for electron movement from the anode to the cathode, thereby changing the electrochemical process on the metal surface, which can be interpreted through impedance analysis with an appropriate EEC using a Nyquist diagram (Figure 1).

The proposed mechanism of bio-corrosion by *Bacillus megaterium* (Figure 4) begins in an aquatic environment, where bacteria prefer to adhere to the carbon steel surface to form biofilm [19]. This is because biofilm is part of the most common survival strategy of microorganisms, allowing bacteria to build their own microenvironment for self-defense, trapping food reserves under it and undergoing metabolic interactions [20]. However, the attachment of microorganisms to the surface can be triggered by the layer in the form of a conditioning film containing proteins and polysaccharide (Stage 1) or a stable oxide film as a passive layer for corrosion resistance [20]. When specimens are immersed in a medium, a passive layer is well-formed and distributed over the surface of the carbon steel, as can be described with the capacitance element (Cf) in the EEC.

With support of the film layer, biofilm formation begins with the interaction of singe-cell bacteria and attachment to the metal surface. Then, the bacteria start to bind and colonize by excreting extracellular polymeric substances (EPS), which are one of the most important components of biofilm (Stage 2). The formation of a biofilm can be depicted with a constant phase element (CPE) (Qb) that affects the structure of the carbon steel surface.

The CPE illustrates that the layers formed on the carbon steel surface are not uniform. When the concentration of biodiesel increases, more carbon sources become available for *Bacillus megaterium*. Consequently, the bacterial growth and thickness of the biofilm increases in variety, as confirmed by the SEM observations in Figure 2.

In all likelihood, the biofilm of *Bacillus megaterium* not only varies in thickness, but also in heterogeneity of the chemical composition over time. Patchiness and heterogeneity of biofilm are forerunners of localized corrosion and accelerate the corrosion rate, because of a non-uniform environment of metabolic products, pH, or dissolved oxygen between the inside and outside of the biofilm [21]. These conditions can generate active electrochemical corrosion cells. Also, corrosion reactions produce products that accumulate on the metal surface (Stages 2 and 3).

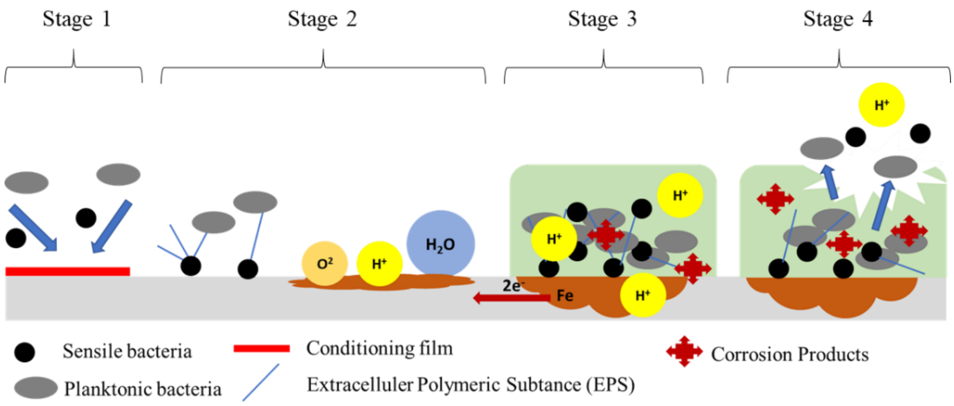

Figure 4 Bio-corrosion mechanism.

Corrosion that can take place in Stages 2 and 3 consists of reactions between the metal and oxygen in free water when biofilm has not yet been formed by *Bacillus megaterium* (Eqs. 1-3) The nature of biodiesel, which is more easily oxidized than diesel oil, can change the medium's pH even in the absence of microorganisms and tends to be acidic (Eq. 4). This affects the amount of carbon steel corrosion.

$$\text{Anode: Fe} \rightarrow \text{Fe}^{2+} + 2e^{-} \tag{1}$$

$$\text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + e^{-} \tag{2}$$

$$\text{Catode: } \frac{1}{2}O_2 + H_2O + 2e^{-} \rightarrow 2OH^{-} \tag{3}$$

$$2\text{H}^{+} + 2e^{-} \rightarrow H_2 \tag{4}$$

Moreover, the oxygen in free water can be used for respiration by *Bacillus megaterium*. As the biofilm thickens over time, the carbon sources and oxygen in the medium makes it difficult to diffuse into biofilm. Nonetheless, oxygen limitations do not have a significant impact on *Bacillus megaterium*'s activities since this bacteria is facultative. Furthermore, under these conditions the bacteria will begin to utilize iron (Fe), which is oxidized to Fe^{2+} and Fe^{3+} (Eq. 1 and 2) as electron donors to obtain the energy used in the metabolic process (Stage 3). The process of electron transfer through the cell wall to the cytoplasma can occur through direct transfer electrons using protein cell membranes, such as c-cytochrome, conductive nanowires (pili), or through transfer electron mediators, namely electron carriers. Oxidized iron on the anode side can react with acid metabolites produced by *Bacillus megaterium* to form pitting and corrosion products underneath the biofilm.

Iron oxide corrosion products such as FeOOH, Fe_2O_3 and Fe_3O_4 can be formed from the activity of *Bacillus megaterium* (Eqs. 5-9). Hermatite has a red color, while magnetite tends to be blackish [24]. Meanwhile, FeOOH tends to be yellow. The combination of these corrosion products was observed on the carbon steel surface using a digital microscope (Figure 3).

Corrosion product formation:

$$Fe + \frac{3}{4} O_2 + \frac{1}{2} H_2O \rightleftarrows FeOOH \quad (5)$$

$$FeOOH + e^- \rightarrow Fe_3O_4 + H_2O + OH^- \quad (6)$$

$$Fe^{2+} + 2OH^- \rightleftarrows Fe(OH)_2 \quad (7)$$

$$Fe(OH)_2 + O_2 + xH_2O \rightleftarrows Fe_2O_3.xH_2O \quad (8)$$

$$Fe^{2+} + 8FeOOH + 2e^- \rightleftarrows 3Fe_3O_4 + 4H_2O \quad (9)$$

The value of Rct for various concentrations of biodiesel and the presence of *Bacillus megaterium* can be used to predict the corrosion rate. In this case, the resistance of the biodiesel and diesel oil mixture as the test solution is still too large. Consequently, the EIS analysis cannot be accurate. Large solution resistance means that the absorbed water is not completely dissolved due to the hygroscopic nature of biodiesel.

Therefore, analysis of the carbon steel's impedance was done in the water phase at the bottom of an immersion reactor. For this reason, the solution's resistance value in this experiment was ignored. Consequently, the increasing corrosion rate could be described by the slope in the Rct value up to 739 Ω cm^2, while speeding up the corrosion rate in the presence of *Bacillus megaterium*. Additionally, pitting corrosion can be examined from the NDL parameter. A decrease in n_{dl} value indicates that the surface of the carbon steel is increasingly rougher or non-uniform, which can be caused by pitting corrosion.

The deeper size of the pits for concentration B20 can be related to the biodiesel content in the medium. Increased biodiesel concentrations are also in line with an increase in oxidized unsaturated chains that produce simple acids. These simple fatty acids are favorable for the growth and activity of *Bacillus megaterium*, which affects corrosion. Furthermore, the deeper size of the pits can be caused by the reaction of water, biodiesel oxidation products and the metal surface when the biofilm has not yet been fully formed by *Bacillus megaterium*. The increase in productivity of *Bacillus megaterium* produces EPS as the increasing concentration of biodiesel forms a biofilm on the surface of the specimens that can be protected due to the corrosive environment.

The formation of a biofilm that covers almost the entire surface of the specimen can minimize the contact between the metal surface and the acidic medium as a result of the oxidation process and water that is not completely dissolved in the medium. Thus, the pitting depths formed at various concentrations of biodiesel tend to be shallow, because they are only caused by the reaction between the *Bacillus megaterium* metabolites trapped under the biofilm on the metal surface.

Moreover, the pitting formation spreads more over the surface of the specimen corresponding to the variation of biodiesel concentration. In this case, the biofilm was formed more abundantly at high concentrations of biodiesel, as observed by SEM. Still, the protective properties of these biofilms are not sustained very long. The biofilm will undergo lysis and microorganisms released into the immersion medium will initiate biofilm formation on other metal surfaces (Stage 4). When lysis occurs, the metal surface is no longer protected and easily reacts with a corrosive environment. Especially when pitting has been formed under the biofilm, the depth of pitting can increase due to reactions with the medium.

5 Conclusion

Biodiesel and biofilm of *Bacillus megaterium* can influence the corrosion of carbon steel. The development of the biofilm is a dynamic and continuous process with an increase in heterogeneity on the surface of the carbon steel. Biofilm can induce the loss of corrosion resistance, affect the distribution, the depth of the formed pits and the roughness of the metal surface.

Acknowledgements

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