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Research Article

Biofilm Formation and Bio Corrosion of Carbon Steel in Diesel-Biodiesel Storage Tank

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Abstract

Biodiesel is potential to blend with petroleum diesel as an alternative blended fuel. Biodiesel is usually stored in carbon steel storage tank which easily corroded by microorganisms. Microorganisms can use blended fuels as carbon source and water from biodiesel which is hygroscopic for growth and metabolism. Thus, degradation of fuel may occur and lead to biocorrosion by microorganisms such as *Bacillus megaterium*. This research was conducted to determine the effect of biodiesel concentration of blended fuel on biofilm formation and biocorrosion by *Bacillus megaterium*. The experiments were carried out by immersing carbon steel specimens in immersion medium for 21 days with variation of biodiesel concentration (B0, B20, B30, and B100). Biofilms that form on the metal surface cause areas with non-uniform oxygen concentrations and form anodic/cathodic conditions, raised to potential differences and biocorrosion occurred. The rate of corrosion increased along with biodiesel concentration which it enhanced the bioavailability of blended fuel. The average corrosion rates were 0,035 ± 0,03; 0,533 ± 0,33; 0,642 ± 0,28; 0,109 ± 0,04 mm/year achieved by B0, B20, B30 and B100 respectively. Microorganism activity also caused damage to the metal surface by forming pitting corrosion on B30 and B100.

Keywords: bacillus megaterium, biocorrosion, biodiesel, biofilm, blended fuel

1. Introduction

Biodiesel is potential renewable fuel to be applied as alternative energy in Indonesia since palm oil is Indonesia's biggest commodity. Palm oil is one of the best raw material to produce biodiesel beside rapeseed, canola, and castor oil (Hoekman *et al*., 2012; Kurnia *et al*., 2016; Kuss *et al*., 2015). Fuel's high demand from industrial and transportation sectors triggers the utilization of biodiesel instead of petroleum diesel. Utilization of biodiesel starts from biodiesel-diesel blends by mixing it with petroleum diesel in some degree of comparison. B-level is the biodiesel level in the fuel blend, for example B20 means that the fuel blend contains 20% volume of biodiesel. In practice, the utilization of blended fuel meets various obstacles, such as fouling or blockage in pipe or engine.

The difference characteristic between two fuel depends on the compounds; methyl ester hydrocarbon compound (FAME) in biodiesel and aromatic & aliphatic hydrocarbon compound in petroleum diesel; causing significant differences in fuel's physical and chemical properties such as density, cetane number, oxidative stability, and saturated acid content (Abbaszaadeh *et al*., 2012; Arbab *et al*., 2013; Hoekman *et al*., 2012). Biodiesel, known less stable than petroleum diesel, makes fuels easier to initiate an auto-oxidation process. It causes fuel degradation by forming oxidation products such as aldehydes and alcohol, etc. This phenomenon is due to the unsaturated fatty acid content of biodiesel which leads the fuel acidic and forms insoluble gum and sediment which cause blockage at advanced

(Monyem & Gerpen, 2001). Hygroscopic properties of biodiesel also enhance this condition because it increase dissolved water level in biodiesel (Fregolente *et al*., 2012; He *et al*., 2007; Oliveira *et al.*, 2008). Corrosion may occur as the water precipitate as free water layer at the bottom of the tank and trigger of sludge formation. Moreover, the presence of water increases the growth of microorganisms that can cause biocorrosion (Groysman, 2014).

Biologically, corrosion is caused by the activity of microorganisms. Biofilm as microorganisms' acts in corrosion mechanism on metal surface induced the tendency of the metal to return to its original condition as a result of their survival strategies (Alasvand Zarasvand & Rai, 2014; Beech & Sunner, 2004; Liengen *et al*., 2014). The blended fuels as mention before are potential to be used as carbon source for microorganisms to grow and metabolize. In the other hand, the percentage of biodiesel on blended fuel may cause bio-corrosion.

Common metals used as storage tank material are carbon steel. Carbon steel has tendency to form an oxide layer which is unfortunately easily corroded as it's the ideal place for microorganisms to attach at metal surface (Heyer *et al*., 2013). The presence of biofilm may cause a non-uniform area thus induced vary of oxygen concentration on the surface (Lewandowski & Beyenal, 2008). The lack of oxygen area forms an anaerobic anodic condition and the rich oxygen area forms cathodic condition cause a potential difference thus corrosion occurs.

Many studies have developed to observe microorganisms which survive on fuel or hydrocarbon medium. *Bacillus megaterium* is one of them which have found in pipelines and hydrocarbon storage systems and have caused corrosion and degradation of fuels (Das & Chandran, 2011; Maruthamuthu *et al*., 2011; Rajasekar *et al*., 2005). This research was conducted to determine the activity of *B. megaterium* in blended fuel medium with various biodiesel concentrations.

2. Materials and Methods

Blended fuel used were FAME from palm oil produced by Chemical Engineering ITB Laboratory and petroleum diesel from Pertamina. Both fuels used as immersing medium which were sterilized by 0.45 μm pore size membrane first. Carbon steel ST-37 as metal specimens were polished by 240 to 1200 grids abrasive papers. The microorganism, *Bacillus megaterium PI12,* was from Chemical Engineering ITB Laboratory. *B. megaterium* was cultured at sterilized Bushnell Haas (BH) medium 100 ml consisted of $MgSO_4$ 0,2 g/L, $CaCl_2$ 0,02 g/L, KH_2PO_4 1 g/L, K_2HPO_4 1 g/L, NH_4NO_3 1 g/L, and $FeCl_3$ 0,05 g/L. The acclimatization process was done by inoculating microbial culture 2 ml at 300 ml BH medium and 1 gram diesel as carbon source. Incubation was done in the rotary shaker at 27 ° C 200 rpm for 30 days.

Immersion process was conducted in closed acrylic reactor. To imitate the storage condition, metal specimens were in direct contact with blended fuel which first was mixed with inoculum. Metal specimens were located on the middle of fuel and hung by glass hooks. The number of specimens was calculated according to ASTM G 31-72 standard in 1×1 cm size.

In order to study the effect of blended fuel to biofilm formation and bio-corrosion process, the experiments were done by four blended fuel; 0% biodiesel (B0), 20% biodiesel (B20), 30% biodiesel (B30), and 100% biodiesel (B100). Immersion reactor had 800 ml working volume and was stored at room temperature for 21 days. We used Total Plate Count (TPC) method to analyze the number of bacterial colonies on biofilms, Scanning Electron Microscopy (SEM) SU3500 to analyze biofilm and corrosion morphology, Fourier Transform Infrared (FTIR) IR Prestige-21 to analyze biofilm composition, and gravimetric method to determine corrosion rates. To analyze biofilm morphology, specimens were first washed by sterilized water then preserved by formaldehyde solution.

The rate of corrosion is calculated by gravimetric method. The data obtained is quantitative and determined by equation:

$$r = 8.76 \times \Delta m / (\rho \times t \times A)$$

r = corrosion rate ($mg/cm^2.day$)
Δm = mass loss (mg)
A = specimen surface area (cm^2)
T = immersion time (days)

3. Result and Discussion

3.1 The Growth Profile of *Bacillus Megaterium* in The Medium

Biofilms are thin layers of microorganisms that were formed and attached to metal surfaces of each blended fuel. The growth profile of *Bacillus megaterium* in the medium was determined by doing calculation of live microorganisms from biofilm samples. Figure 1 presents the number of *Bacillus megaterium* colonies on biofilms formed on metal surfaces by TPC method.

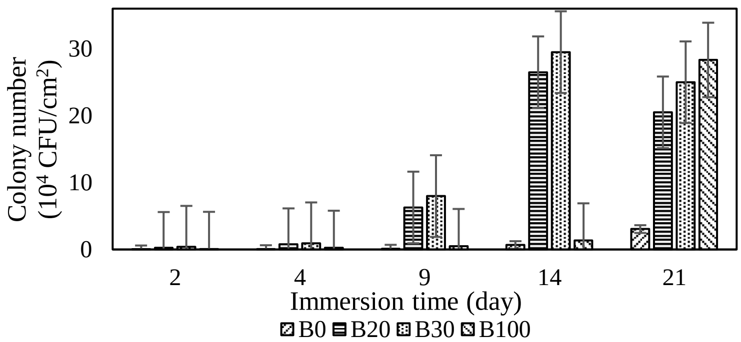

Figure 1. The number of *Bacillus megaterium* colonies on biofilms formed on metal surfaces for each blended fuel at the same interval days

Significant difference in the number of *Bacillus megaterium* colonies can be seen in each blended fuel. At the beginning, the adaptation phase occurred in all biodiesel concentration. In the following days, the exponential phase began and gave differences in each concentration. *Bacillus megaterium* experienced a very slow growth phase in the B0 medium. Diesel was a complex structure consisting of saturated chain hydrocarbons of C_9 – $>C_{30}$ and aromatic hydrocarbons such as benzene, xylene and naphthalene. The complex structure of hydrocarbons was stable so that microorganisms needed extra energy to penetrate into the structure. Therefore, diesel is more complicated to convert into carbon source for microorganisms when compared to biodiesel (Fazal *et al*., 2010).

In B20 and B30, microorganisms had rapid adaptation phase and then grow fast in the exponential phase. Addition of biodiesel in the medium increased bioavailability of fuel (Owsianiak *et al*., 2009; Wu *et al*., 2016). In addition, the more unsaturated chain content in biodiesel FAME, the more susceptible to oxidation (Lapuerta *et al*., 2012; Passman, 2013; Wu *et al*., 2016). FAME was degraded into simple carbohydrate, amino acids, acetic acid and fatty acids which were able to be utilized by microorganisms naturally for respiration reactions and formation of new cells (Aktas *et al*., 2017; Lutz *et al*., 2006).

The presence of oxygen function groups in biodiesel causes oxidation of metals (Fazal *et al*., 2010). Consequently, layer of iron oxide was formed, which act as the attachment site of microorganisms to initiate biofilm. The decline in the number colonies of microorganism at the end of the immersion time indicates a lysis. Lysis is the last stage of the biofilm formation, which mature biofilm had modification by breaking of the biofilm layer on the metal surface, so microorganisms spread into the immersion medium (Heyer *et al*., 2013).

The growth of *Bacillus megaterium* in B100 had a different profile when compared to mixed fuel and pure diesel. The adaptation phase proceeded slowly until the 14th day, then increased rapidly. We investigated that *Bacillus megaterium* could live well in biodiesel medium but required a long adaptation phase as the acclimatization process was only done by diesel as carbon sources. *Bacillus megaterium* is a microorganism that is originally found in hydrocarbons. Therefore, it will undergo an adaptation phase if the medium only consists of biodiesel. This because microorganisms need more specific carbon source, so that the difference structure can cause long adaptation time (Owsianiak *et al.*, 2009).

3.2 Biofilm Morphology

The morphology of biofilms attached to the metal surface was observed by SEM (*Scanning Electron Microscopy*) analysis. SEM analysis was investigated 3 times of each blended fuel at day 1, 11, and 21. Morphology of the biofilm at the first day of immersion is presented in Figure 2.

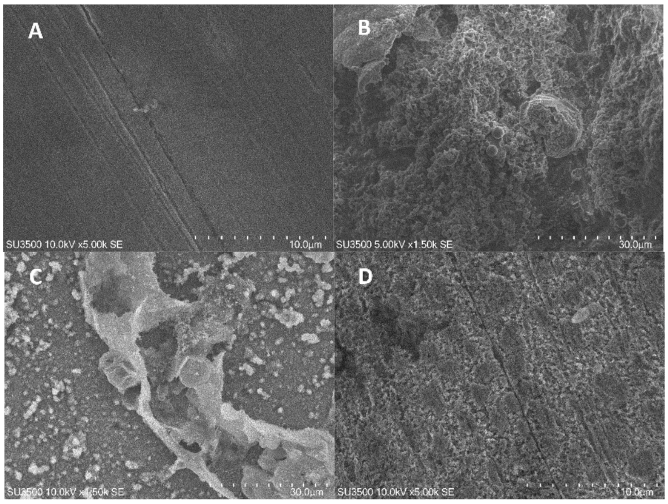

Figure 2. Morphology of biofilms on metal surfaces at the first day of immersion (a) B0, (b) B20, and (c) B30, and (d) B100 were taken at 1,500 times magnification

Figure 2 shows there is no visible biofilm layer on the metal surface at B0, only visible dots or spots that indicate the presence of a single cell of microorganisms which begin to form colonies. Biofilm layers began to appear at B100, although they were still thin and had not completely covered the metal surface. Whereas at B20 and B30, biofilm layer has formed on the metal surface.

The morphology of biofilms on the second observation at 11th day of immersion are presented in Figure 3. In B0, there were no significant changes from day 1. Single cell microorganisms were still visible at some point and still did not form a biofilm layer. In other blended fuel, biofilm layer was already seen covering the metal. A neat biofilm coating showed that biofilm had entered a maturation phase. In B20 and B30, also clearly seen that there was lysis process, which biofilms broke and microorganisms released into the immersion medium initiating the formation of new biofilms on the other surfaces. *Bacillus megaterium* was observed to undergo lysis on the 10th day of immersion with diesel-biodiesel medium (Pusparizkita *et al.*, 2018). Despite the lysis, the biofilm layer was still seen covering the metal tightly, and the number of colonies increased on the 14th day.

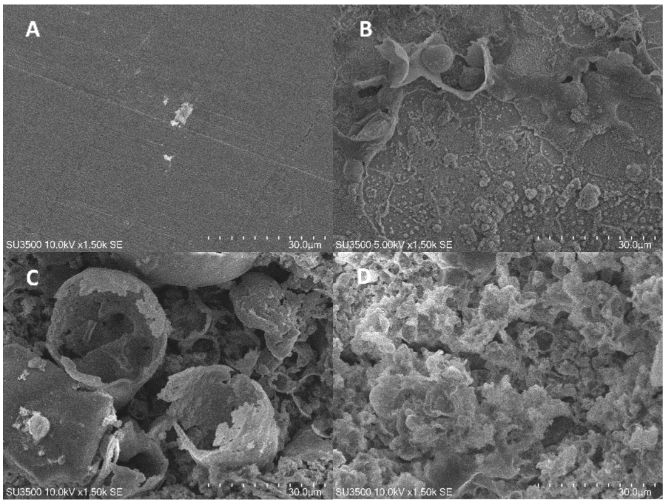

Figure 3. Morphology of biofilms on metal surfaces at the 11th day of immersion (a) B0, (b) B20, and (c) B30, and (d) B100 were taken at 1,500 times magnification

On the 21st day, the last day of the immersion, the morphology of the biofilm layer are presented in Figure 4. There were still no significant biofilm layers at B0 that had not indicated the formation of biofilm. Whereas at B20, B30, and B100, the biofilm layers were seen covering the surface of the metal as full and thick layers. The biofilm layer at B100 were the thickest and densest among all which showed the accumulation of EPS (exopoly-saccharides) in line with the number of *Bacillus megaterium* colonies that grew very rapid by TPC analysis.

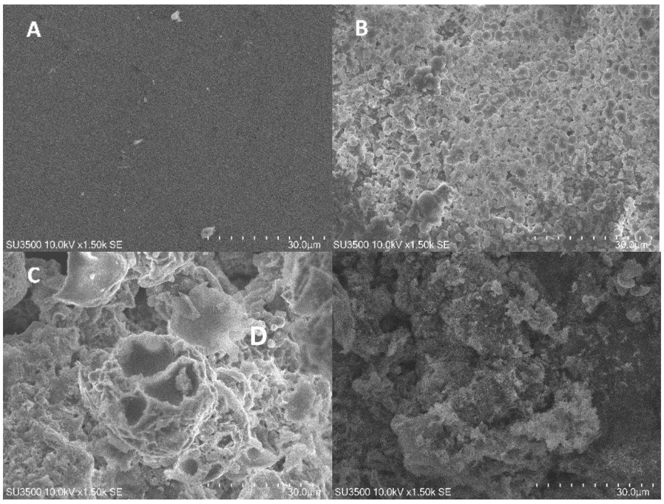

Figure 4. Morphology of biofilms on metal surfaces at the 21th day of immersion (a) B0, (b) B20, and (c) B30, and (d) B100 were taken at 1,500 times magnification

3.3 Biofilm Composition

Biofilm composition analysis was performed by SEM/EDX (*Scanning Electron Microscopy / Energy Dispersive X-ray Spectroscopy*) and FTIR (*Fourier Transform Infrared Spectroscopy*) at 21st day. Table 1 presents the weight percent of chemical elements detected in the biofilm layer at each blended fuel.

Table 1. Composition of chemical elements detected on biofilms

No	Element	% weight			
		B0	**B20**	**B30**	**B100**
1	C	<1	44.57	47.47	41.54
2	O	<1	49.95	46.54	41.54
3	Fe	±100	5.48	5.99	15.09

In B0, due to the small number of microorganism colonies formed as observed in Figure 4.a, the SEM/EDX showed that the chemical element detected on the metal specimen was only the carbon steel itself, Fe. The other blended fuels indicated that there were elements Fe, O and C. Figure 5 shows the distribution of elements detected in SEM/EDX analysis. Biofilm was observed as combination of elements C and O in purple and green color index and corrosion products was observed as combination of Fe and O in orange and green color index. Weight % of C/Fe in B20 was slightly lower than B30. Due to the rapid adaptation phase, B20 and B30 had pile of biofilm and had massive production of EPS. This phenomenon and also the corrosion products made the metal surface got more covered.

On B100, weight % of C and Fe have the same value. But in EDX, element O, as green color, spreads in almost area along with purple (C) and orange (Fe) dots, indicated the metal surface was more covered by both biofilm and corrosion products. This happened because the number of colonies increased dramatically on the 21st day. Thus, the EPS covered over the surface and mixed with corrosion products that were also on the metal surface.

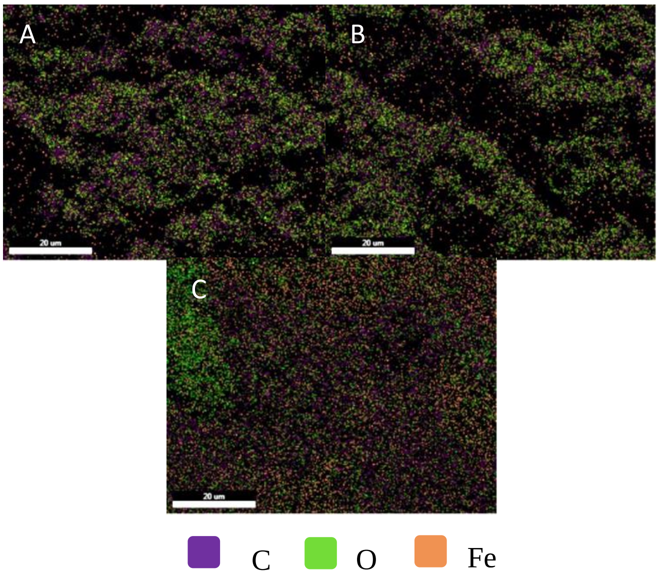

Figure 5. EDX biofilm on metal surfaces at 21st day immersion (a) B20, (b) B30, and (c) B100

The second analysis to determine biofilm composition was carried out by FTIR (*Fourier Transform Infrared Spectroscopy*). Figure 6 shows graphs which contain peaks aligned with the frequency of the bonding of atoms of biofilm material on 21st day immersion. We can see that each blended fuel shows same peak area indicated that the composition of biofilms formed did not provide a significant difference. The peak area shows groups of compounds forming from biofilms adhered to the metal surface.

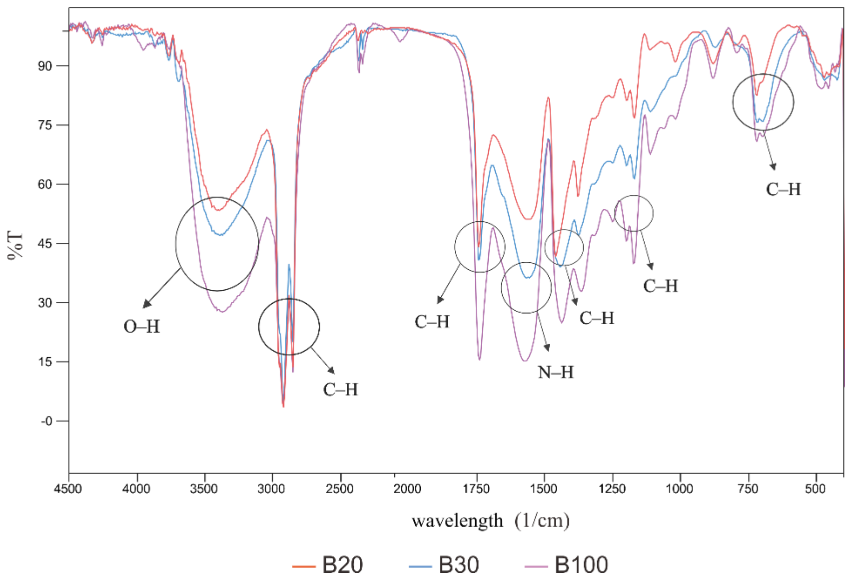

Figure 6. FTIR of biofilm on metal surfaces at 21 days immersion at B20, B30, and B100

It can be seen that many function groups were detected in biofilms attached to metal surfaces include OH hydroxyl groups at wavelengths of 3,390.86 - 3,412.08 cm^{-1}, CH groups at wavelengths of 2,852.72 and 2,924.09 - 2,926.01 cm^{-1} and fatty acid ester group at a wavelength of 1,739.79 - 1,743.65 cm^{-1} (Schmitt & Flemming, 1994). Amide II groups were detected at wavelengths of 1,564.27 - 1,571.99 cm^{-1}, CH groups at 1,454 - 1,460.11 cm^{-1} and carboxylic groups at wavelengths of 1,365.6 - 1,377.17 cm^{-1} (Lu *et al.*, 2011). Polysaccharide and $(CH_2)n$ groups were also detected at wavelengths of 1170.79 and 717.52 - 721.38 cm^{-1} respectively.

The existence of those groups in the biofilm shows an interaction between *Bacillus megaterium* with diesel and biodiesel medium. Alcohol, fatty acid esters, and carboxylates groups are products as the oxidation of biodiesel which were used as a carbon source by microorganisms. Utilizing the oxidation medium as a carbon source, microorganisms produced EPS which composed of carbohydrate or polysaccharide groups and amide group II as protein compound and detected in FTIR graphs (Lu *et al.*, 2011; Rajasekar *et al.*, 2007).

3.4 Bio-corrosion of Carbon Steel

The bio-corrosion process was observed by gravimetric analysis by comparing mass of metal specimens before and after immersion. The addition of microorganisms increases metal specimens' mass loss, which indicated the role of microorganisms in the corrosion process in each variation of biodiesel concentration. The longer the immersion times, the higher the mass loss. This mass loss was used as a parameter to calculate the rate of corrosion at B0, B20, B30, and B100 by equation 1 and was presented in Figure 7.

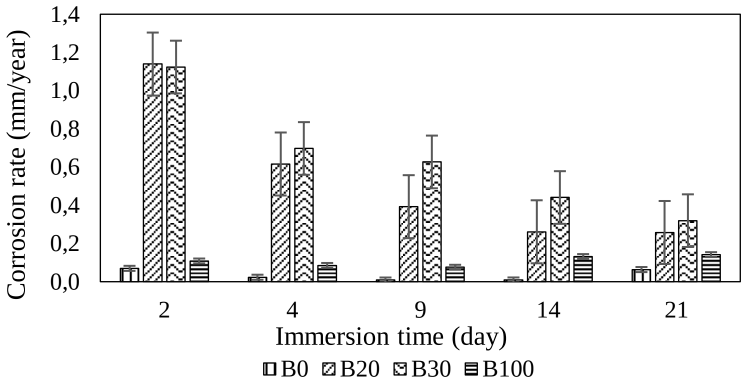

Figure 7. The rate of corrosion of B0, B20, B30, and B100 at 21 days immersion

High corrosion rate at the beginning of immersion occurred due to direct contact between metal specimens and the blended fuel medium. Both B20 and B30 have the highest corrosion rate values compared to pure diesel and biodiesel medium. Biodiesel is hygroscopic and has high oxide compound and polarity which increasing the corrosivity of metal (Fazal *et al.*, 2010). In addition, auto oxidation on biodiesel hydrolyzes acidic compounds thus increase corrosion rates.

Corrosion involves an electrochemical reaction that is a chemical reaction that occurs because of the transfer of electrons. The electron transfer process takes place through a series of oxidation reactions at the anode and reduction at the cathode. The oxidation reaction of metals into ions in the anodic process, and the reduction reaction of solutions (biodiesel and biofilm) in contact with the metal in the cathodic process (Beech & Sunner, 2004). Metal ions concentrate from the aqueous phase or the substratum into the biofilm increase corrosion rates by providing an additional cathodic reaction (Heyer et al., 2013).

Anodic reaction : $M \rightarrow M^{2+} + 2e^-$

Cathodic reaction : $2H^+ + 2e^- \rightarrow H_2$

The decline in corrosion rate is proportional to the increase in the number of microorganism colonies in the biofilm. In B20 and B30, the most obvious decrease in corrosion rate was observed among the others. Growth profile of microorganisms on both of these mediums increased over the immersion time, except the decreased on the 21st day due to lysis. The biofilm was evenly distributed covered the entire surface of metal specimens, according to morphological analysis of biofilms by SEM. It could minimize contact between the metal and the medium that was acidic due to the oxidation process. In addition, the minimum contact of biodiesel with metals, minimizes oxidation since the metals worked as catalysts in oxidation reactions (Jakeria *et al.*, 2014).

The average corrosion rate for each concentration is presented in Table 2. The corrosion rate in the inoculated medium increased if compared to the control which proven the role of microorganisms in the bio-corrosion process. Adding biodiesel to medium caused an increase in the rate of corrosion, but this did not apply to the medium of 100% biodiesel.

Table 2. The average corrosion rate on the variation of biodiesel concentrations in the control and inoculated medium

No	Blended Fuel	Corrosion Rate (mm/year)	
		Control	Inoculated
1	B0	0,0004±0,001	0,035±0,03
2	B20	0,0574±0,020	0,533±0,33
3	B30	0,0596±0,015	0,642±0,28
4	B100	0,0009±0,002	0,109±0,04

In B0, corrosion rate increases by the addition of microorganisms although no biofilm was detected in SEM analysis. Based on the TPC analysis, the highest number of colonies on B0 was 3.05×10^4 CFU/cm^2 on the 21st day. *Bacillus megaterium* can degrade diesel with % BE of 68.78%, and the number of colonies detected in the medium is 1.0×10^6 CFU/ml with 18 days incubation period (Rajasekar, 2007). This shows that most microorganisms live and grow on the medium, degrade the diesel fuel and cause corrosion.

The highest corrosion rate is achieved by B30, then followed by B20. The presence of an iron oxide layer which covering metal surface as deposits of corrosion products as the implication of the easily corroded carbon steel. It caused the tendency of microorganisms to attach to the metal as the initiation of biofilms formation (Heyer et al., 2013). The rate of corrosion increases due to the activity of microorganisms which produce acidic metabolite products then accumulate on the surface such as carboxylic acids, aldehydes, alcohols and saturated fatty acids (Jakeria et al., 2014; Sousa et al., 2009). Biofilms over the metal made microorganisms difficult to access the medium as a carbon source and thus utilize the electrons from iron oxide as electron donors (Xu & Gu, 2014). The microorganisms began to use Fe, which is oxidized to Fe^{2+} and Fe^{3+} as electron donors to obtain the energy applied in the metabolic process. This causes an increase in mass loss during the immersion time.

The decline in the rate of corrosion occurred on B100 and this was in line with the results of the analysis of the number of colonies, which the adaptation phase went very slowly. The slow adaptation phase was caused by the acclimatization phase of the *Bacillus megaterium* due to carbon sources. The difference structure of diesel and biodiesel causes the need for more specific microorganisms in each biodiesel carbon source in the medium (Owsianiak *et al*., 2009).

The surface damage of metal specimens is the impact of the biocorrosion process. The morphology of the metal surface after immersion are used to observe the corrosion form caused by the activity of *Bacillus megaterium* and the concentration of biodiesel. Corrosion form was analyzed by SEM and it was carried out on each variation after 21 days. Metal specimens first went through the pickling process to remove adhered corrosion products. Corrosion form then analyzed by comparing SEM of metal specimens before and after immersion in B0, B20, B30, and B100. The results of SEM analysis on the surface of metal specimens are presented in Figure 8.

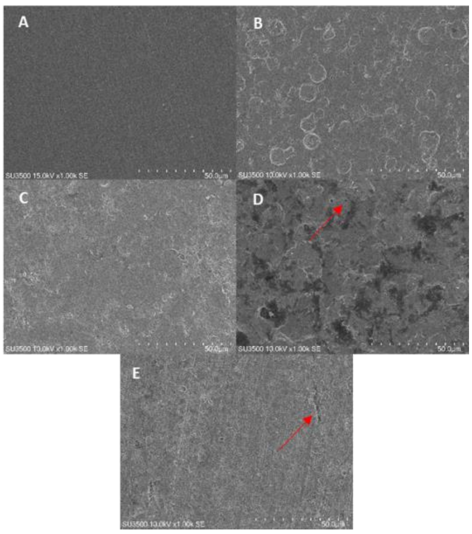

Figure 8. Morphology of corrosion on metal surfaces at the 1st day of immersion (a), at the 21th day of immersion (b) of B0, (c) of B20, and (d) of B30, and (e) of B100 were taken at 1,000 times magnification

In B0 and B20, the damage was almost evenly distributed on the surface. Meanwhile, at B30 and B100, metal damage was seen with a certain depth as marked by red arrows at Figure 8. The damage were caused by the activity of *Bacillus megaterium* is classified as a *pitting corrosion*. Pitting corrosion occurs due to the activity of microbes that produce metabolite products as shown in the FTIR data and the thick layer of biofilm on the SEM image of the biofilm. The growth of the pit is under the thickness of biofilm and corrosion products or in anodic area, therefore depth and size of the pit grows rapidly. Pitting corrosion had been reported causing losses in because a leakage may occur when undetected pits grow deeper and transform into holes, breaking through the materials (Aktas et al., 2017; Beech & Sunner, 2004; Groysman, 2014).

4. Conclusions

Biodiesel addition enhance the bioavailability of blended fuel. The number of colonies on the biofilm increased and the corrosion took place on carbon steel which depend on the biodiesel concentration. The corrosion rate escalated as function of exposure time in blended fuel.

The biofilm composed of biodiesel degradation compounds and the building blocks of EPS in carbohydrates and proteins forms. Biofilm formation provided corrosion inhibition properties, whereby the corrosion rate decreased by time by blocking contact among metal surface and blended fuel. Nevertheless, microorganism activity caused another damage to the metal surface by forming pitting corrosion. Thus, further studies regarding to the pitting corrosion on its depth and size and study about bioavailability to other biodiesel concentration on blended fuel are required.

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