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EFFECT OF DIETANOLAMIDE (DEA) SURFACTANT ADDITION AND DEEP-SEA BACTERIA ACTIVITIES ON THE BIODEGRADABILITY OF ARTIFICIAL OILY WASTEWATER IN SEAWATER MEDIA

PENGARUH PENAMBAHAN SURFAKTAN DIETANOLAMIDA (DEA) DAN AKTIFITAS BAKTERI LAUT-DALAM TERHADAP BIODEGRADASI LIMBAH CAIR BERMINYAK ARTIFISIAL DI DALAM MEDIA AIR LAUT

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ABSTRAK

Tumpahan minyak bumi di lingkungan laut akan berdampak buruk bagi biota yang ada di dalamnya. Mitigasi tumpahan minyak yang aman, efisien, relatif murah dan mudah penerapannya adalah degradasi tumpahan minyak secara biologi dengan menggunakan mikroorganisme atau dikenal bioremediasi. Minyak bumi akan lebih mudah terdispersi dalam air bila ditambahkan surfaktan. Surfaktan memiliki kemampuan untuk meningkatkan bioavalibilitas minyak bumi sehingga memudahkan bakteri kontak dengan sumber karbon sebagai makanannya. Tujuan dari penelitian ini adalah menguji pengaruh penambahan surfaktan dietanolamida (DEA) guna meningkatkan kemampuan bakteri laut dalam untuk mendegradasi senyawa hidrokarbon minyak bumi pada media air laut. Uji biodegradasi senyawa hidrokarbon dilakukan pada media air laut sebanyak 8 liter, kemudian dilakukan pengamatan kemampuan surfaktan DEA dalam menurunkan tegangan permukaan, kandungan minyak, pH dan nutrien pada hari 0, 1, 3, 6 dan 10. Analisis GC-MS dilakukan untuk mendeteksi perubahan komponen kimia pada minyak bumi. Bakteri yang digunakan adalah konsorsium bakteri yang terdiri atas Enterobacter sp.,Pseudomonas sp., dan Raoultella sp. Senyawa hidrokarbon terdegradasi hingga 65.52% dengan laju biodegradasi k= -0.1054t pada media dengan penambahan surfaktan DEA. Fraksi alifatik yang terdeteksi adalah senyawa n-alkana $C_{17} - C_{31}$ dan setelah biodegradasi menjadi $C_{20} - C_{31.}$ Hasil penelitian menunjukkan bahwa surfaktan DEA mampu meningkatkan kemampuan konsorsium bakteri dalam mendegradasi senyawa hidrokarbon minyak bumi.

Kata Kunci: *bakteri laut dalam, biodegdarasi, surfaktan DEA, senyawa hidrokarbon*

ABSTRACT

Marine oil spills have bad impacts on the marine biota. Oil spill mitigation that is currently safe, efficient, relatively cheap and easy to implement is bioremediation, that is degradation of oil spills biologically using microorganisms. Petroleum will be more easily dispersed in water when surfactants are added. The surfactants have the ability to increase the bioavailability of petroleum to facilitate

bacteria contact with carbon sources as their feed. This study was intended to test the effect of addition of diethanolamide (DEA) surfactants to improve the ability of bacteria to degrade hydrocarbon compound in the seawater media. The biodegradation experiment was conducted in 8-liter seawater media and the ability of DEA surfactants to reduce surface tension, oil content, pH and nutrients on days 0, 1, 3, 6 and 10 were observed. GC-MS analysis was conducted to detect chemical component changes in petroleum. A bacterial consortium of *Enterobacter* sp., *Pseudomonas* sp., and *Raoultella* sp. was utilized. The oil was degraded up to 65.52% with biodegradation rate k = -0.1054 t in the media added with DEA surfactants. The aliphatic fraction detected was C_{17}-C_{31} n-alkane compound and after biodegradation it became C_{20}-C_{31}. The results showed that DEA surfactants were able to improve the ability of bacterial consortium to degrade petroleum.

Keywords: deep sea bacteria, biodegradation, surfactant DEA, hydrocarbon compounds

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I. INTRODUCTION

Indonesia's marine waters are one of the vulnerable areas in the world to the oil spill disaster, especially Malacca Strait, Lombok Strait, Sunda Strait, Makassar Strait, and South Coast of Java (Indian Ocean). Those waters are the main routes of oil transportation and have more than 82 onshore and offshore exploration activities (Darmayati et al. 2015). A marine oil spill can severely impact the living aquatic organisms. It is due to the crude oil contains 50 – 95% hydrocarbon compounds which have toxic, and in some cases, it is carcinogenic for biota and human. In general, crude oil is a mixture of complex hydrocarbons consist of aliphatic, aromatic, asphaltene, and resins compounds (Sakthipriya et al. 2015).

Oil spill mitigation can be done in three ways: physically, chemically and biologically. Physical mitigation of oil spill cannot eliminate oil from the environment completely. On the other hand, chemical mitigation of oil spill is also ineffective because it adds a chemical compound and can give a severe impact on the environment, especially compounds that are not environmentally friendly. The alternative of oil spill mitigation which is safe, efficient, relatively cheap, and easily applicable through biological degradation using microorganisms, also known as bioremediation. Microorganisms such as bacteria have the ability to degrade hydrocarbon compounds and use them as energy sources (Doerffer 2013).

According to Dwinovantyo et al (2015), bacterial isolates from sediments with complex habitats and many organic materials such as Enterobacter sp., Pseudomonas sp., and Raoultella sp. has a potency to degrade the crude oil. The results of the previous research indicate that the consortium of these three bacteria can degrade the oil up to 88.64% on the test media with 150 ml volume of seawater for seven days of observation (Dwinovantyo 2015). However, the biodegradation rate decreased to 49.38% when applied on a larger scale with 8 liters of test media volume (Rahmaniar 2016).

Information on the utilization of bacteria from deep-sea sediments for the processing of petroleum wastewater and other activities is still very limited. Deep-sea sediments contain the largest microbial habitat on earth where more than 1/3 of all microbial biomass is on planet Earth. It was recently discovered for the first time that these cells actually divide and are not inactive. This means that the microbial biosphere has activity and is likely to play an important role in the elemental cycle globally over the geological timeframe. In addition, these bacteria have certain mechanisms so that they are able to adapt and survive in extreme environments and are not environmentally friendly; live under high pressure, with limited nutrient content and oxygen (Poly. A 2017).

Beside that crude oil which has water-soluble characteristic (hydrophobic) can reduce bioavailability or ability of the oil to be utilized by

bacteria which can lead to limiting the biodegradation process. The oil spill can be more easily dispersed in water when the surfactant is added. Surfactant (surface active agent) is a complex compound consisting of hydrophilic and hydrophobic groups which are soluble in water and oil. The surfactant will bind oils to the non-polar compounds and water to the polar compounds thus increasing solubility of oil in water to facilitated contact between microorganism dan carbon sources of petroleum as its nutrition. (Syafrizal et al. 2015).

In addition, surfactants also have the ability to increase the bioavailability of petroleum so that it makes it easier for bacteria to contact carbon sources as food. Dietanolamide surfactant (DEA) used in the study is one type of surfactant that is natural and renewable because it is made from palm oil. According to Elvina (2015), DEA surfactants have biodegradable properties, are environmentally friendly and are good dispersing agents because they contain hydrophilic and hydrophobic groups on their molecules.

This study was conducted to determine the effect of adding diethanolamide (DEA) surfactant to increase the ability of deep-sea bacteria to degrade hydrocarbon compounds in seawater media. In addition, this study also aims to determine changes in the characteristic of hydrocarbon compounds after the biodegradation process, which was analyzed using GCMS.

II. METHODOLOGY

A. Critical Micelle Concentration (CMC) Measurement Based On Surface Tension Test

The surfactant diethanolamide (DEA) were prepared by solution at concentrations of 2.0, 1.6, 1.2, 0.8, 0.4, 0.1, 0.05, 0.01, 0.005, and 0.001% (v/v) was tested for its ability on decreasing surface tension using a tensiometer. Measurements were made ten replications at each concentration, starting from water to the highest concentration of surfactant solution.

B. Bacterial Culture Preparation

The three selected strains bacteria i.e. *Enterobacter sp.*, *Pseudomonas sp.*, and *Raoultella sp.* were obtained from Research Development Center for Oil and Gas Technology "LEMIGAS" isolated by Dwinovantyo *et al.* (2015) from the deep-sea sediments were activated on agar nutrient media. Then, bacterial culture was incubated using an incubator for 48 hours at 37°C. The mixed three strains consortium of bacteria was cultivated in 100 ml of nutrient broth (NB) media. The composition of NB media consists of 5 g/l peptone and 3 g/l of meat extract.

The bacterial consortium adaptation was carried out on the seawater media. The seawater media for adaptation containing 100 ml of sterile seawater, 0.5% (v/v) crude oil with the value of American Petroleum Institute (API°) 33.13 and specific gravity of 0.8595 g/cm^3, 0.1 ml of 1000 ppm NPK solution, 0.2 ml of 1300 ppm urea solution, 0.1% (v/v) yeast extract, and 0.05% (v/v) surfactant diethanolamide (DEA) solution. The media was stirred using an automatic shaker for 72 hours at a rate of 120 rpm and room temperature (±30°C). The bacterial consortium can survive and grow well and can be used for the biodegradation process if the bacterial population is more than 10^6 CFU/ml (Okoro 2010).

C. Experimental Design

The microcosm experiment was conducted on 8 liters of seawater media (the total amount of experimental media) using a glass vessel with dimensions 19 cm (L) x 19 cm (W) x 36 cm. This study was divided into two groups i.e. with the addition and without the addition of DEA surfactant, each group has its own control media without bacteria inoculated.

Sterile seawater with a salinity of 31 psu was put into a glass vessel. Then, added with crude oil 5000 mg/l, solution of NPK and urea with a ratio of 5:1, 0.1% (v/v) yeast extract, 1% (v/v) the bacterial consortium that has been adapted, and 0.05 % (v/v) surfactant diethanolamide (DEA) solution. The medium was stirred using an automatic stirrer at 200 rpm and aerated using an underwater aerator.

D. Oil & Grease Concentration Analysis

Oil & grease was determined gravimetrically using a separatory funnel. Extraction was carried out by inserting 100 ml of sample into separating funnel, then added with pro analysis grade n-hexane as a solvent. Anhydrous sodium sulfate (Na_2SO_4) was used to absorb remained water in samples. The extracted samples were evaporated using an automatic rotary evaporator with a temperature ranging from 50 - 60°C. The remained n-hexane in the boiling flask was evaporated using an oven at temperature 55 - 60 ° C for 24 hours.

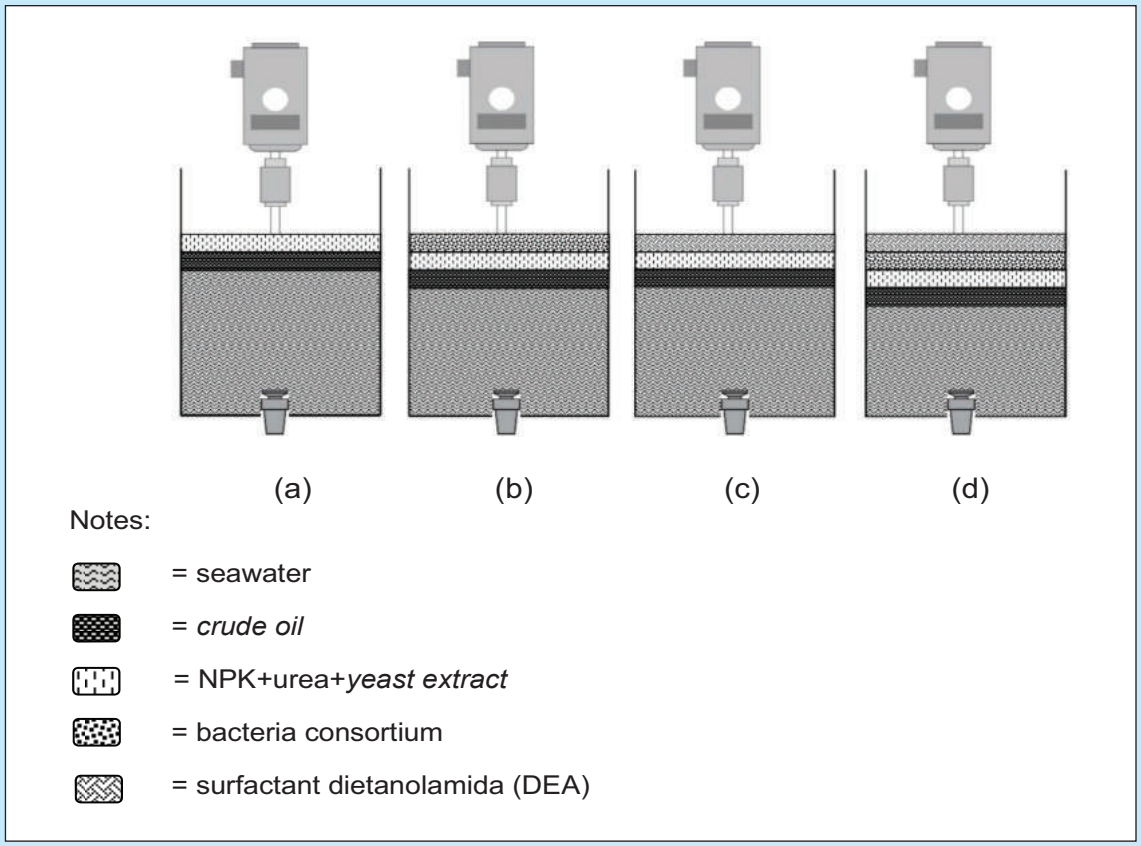

Figure 1
Illustration of oil biodegradation test without adding DEA surfactant in media (a) control, (b) inoculation of bacteria and with adding surfactant DEA in media, (c) control dan (d) inokulasi bakteri.

$$Oil\ and\ grease\ (\text{mg/l}) = \frac{(W1\text{-}W0)\ x\ 1000}{V} \quad (1)$$

Noted:

W0 = The boiling flask is empty (g)
W1 = The bolilng flask with oil (g)
V = Sample vol (L)

$$\text{Biodegradation rate ;} \quad \frac{Nt}{N_0} = exp(-kt) \quad (2)$$

Noted:

N_0 = Oil dan grease before biodegradation process proses (mg/l)
N_t = Oil dan grease after biodegradation process proses (mg/l)
k = Contstan of biodegradation rate
t = Priode of time from N_0 ke N_t (day)

E. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The gas chromatography-mass spectrometry (GC-MS) analysis was carried out using Agilent GC System 6890N with Agilent Mass Selective Detector 5973 inert and HP-5MS column (30 m long, 250 µm diameter and 0.25 µm film thickness). A sample of 1 µl was injected in a splitless mode with a temperature of 300° C and an active purge valve 45 seconds after the sample was injected. The GC-MS column is set at a temperature starting at 100 °C, then the temperature was increased to 280°C at a rate of 6°C for 20 minutes, then left constant for 50 minutes. Helium gas (He) was used as a carrier gas with a column flow of 1 ml/min, at a pressure of 10,523 psi. The solvent delay was set for 2 minutes.

F. Parameter and Statistical Analysis

Data analysis was determined by comparing the ability of DEA surfactant in

reducing surface tension, oil & grase content, pH and GC-MS chromatogram. Statistical analysis was determined by using one-way analysis of variance (ANOVA) followed with the further tests, i.e. T-test and Duncan test.

III. RESULTS AND DISCUSSION

A. Critical Micelle Concentration (CMC)

Critical Micelle Concentration (CMC) is a condition when the surface tension value is almost saturated and shows a surfactant critical concentration limit in a solution. As stated by Adlina (2016) the value of CMC represents a surfactant saturation point that can work to bind water and oil. The effectiveness of a surfactant is measured based on its ability to reduce surface tension. The surface tension is the energy needed to increase the surface area of liquid in area unit. The effect of DEA surfactant addition in various concentrations (%) on the surface tension value (mN/m) can be seen in Figure 2.

The surface tension value of DEA surfactants was measured at a concentration of 0.001 - 2%. The surface tension value of pure water without DEA surfactant is 73.55 mN/m. As maintained by Beattie *et al*. (2014) the surface tension value of pure water is 72.70 mN/m and is used as a standard for surface tension measurement. Water has a higher surface tension value compared to most other liquids, because of its greater cohesive force based on its hydrogen bond. The results of measurement of surface tension value of DEA surfactants at concentrations of 0.001, 0.005, 0.01, 0.05, 0.1, 0.4, 0.8, 1.2, 1.6, and 2% are 30.63, 30.03, 29.14, 28.10, 28.23, 28.90, 28.77, 29.15, 29.13 and 29.26 mN/m respectively (Figure 2). Based on the study of characteristics of DEA surfactants conducted by Elvina (2015), DEA surfactant at 1% concentration has a surface tension value of 27.52 mN/m.

Addition of a surfactant to a solution will decrease its surface tension. As seen in Figure 2, with DEA surfactant at initial concentration of 0.001%, the surface tension value decreased to 30.63 mN/m. The surface tension value of DEA surfactant continued to go down to 0.05% concentration which was the concentration with the lowest surface tension value of 28.10 mN/m, while the surface tension value increased again to the highest concentration of surfactant used. This surface tension decrease is because of the cohesion and adhesion forces on the water surface, namely the adhesion forces cause the molecules on the surface and the molecules under the surface attract one another (Elvina 2015). The surfactant concentration with this lowest surface tension value is thought as the CMC value. As argued by Elvina (2015) the lowest surface tension

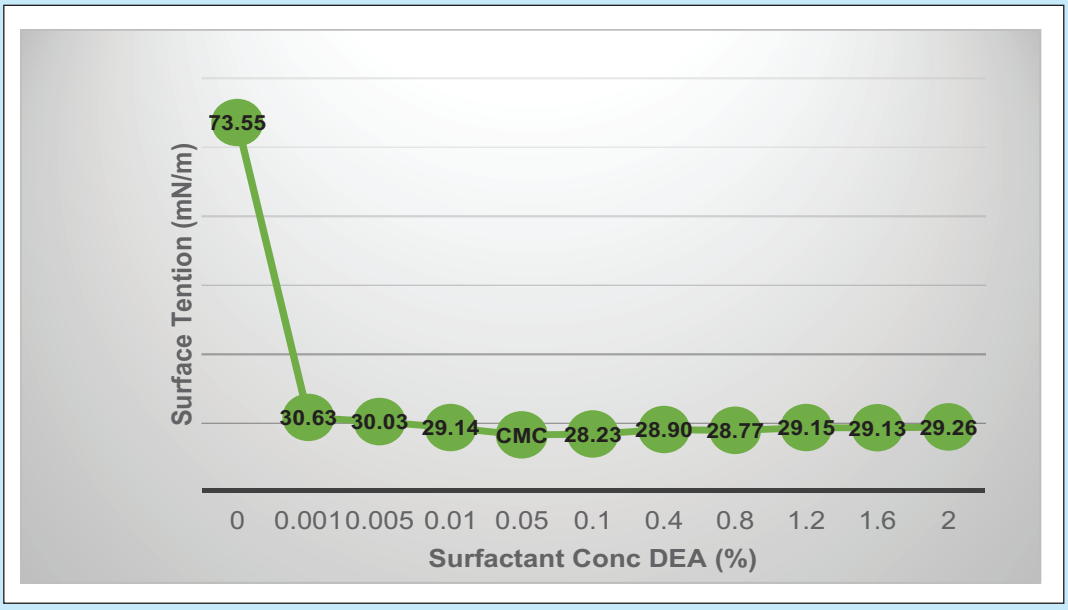

Figure 2
Effect of DEA surfactant concentration (%) on surface tension value (mN/m).

value shows the surfactant effectiveness in reducing the surface tension, meaning that the concentration is close to the CMC value. After reaching a certain concentration, the surface tension will be constant and tends to increase again even though the surfactant concentration is increased. If the surfactant is added far exceeding the CMC value, the surfactant will form an aggregate or micelle. Tian et al. (2016) reported that the formation of micelles can block bacteria from contacting with oil so that oil biodegradation is inhibited. According to Sajna *et al*. (2015) at a higher concentration than the CMC value, most surfactants show toxicity and may affect bacterial metabolism. The use of surfactants that exceed the CMC value is economically inefficient, but it may also lead to reemulsification (Adlina 2016). This CMC value is

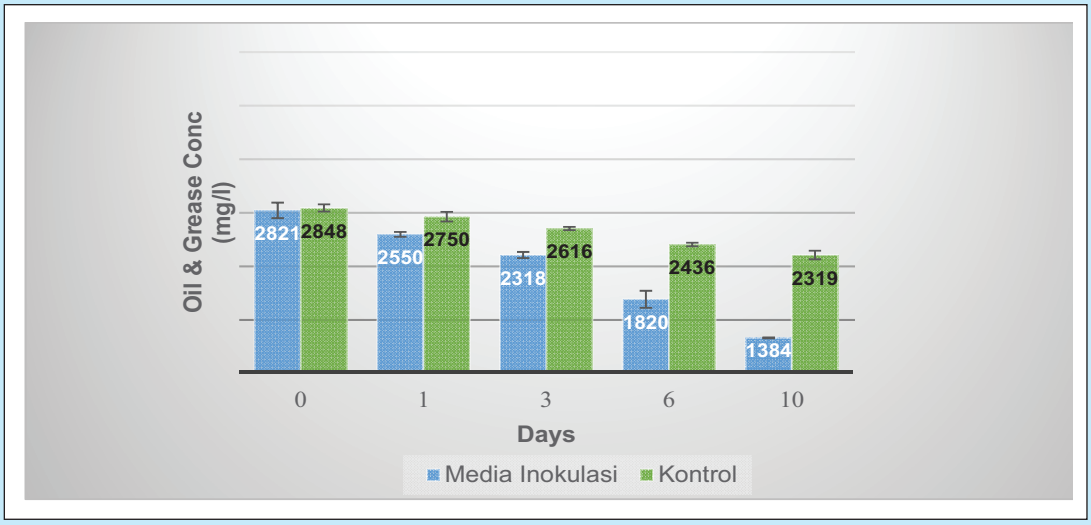

Figure 3
Mean and standard deviation of oil concentration (mg/l) not added with diethanolamide (DEA) surfactant on bacterial inoculation media and control during 10 (ten)-day observation.

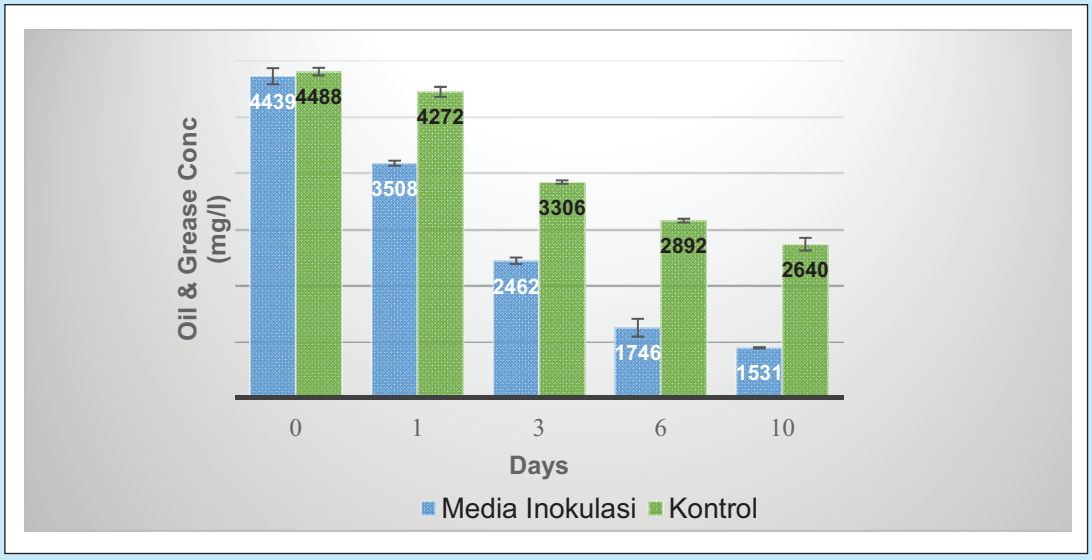

Figure 4
Mean and standard deviation of Oil concentration (mg/l) added with diethanolamide (DEA) surfactant on bacterial inoculation media and control during 10 (ten) days of observation.

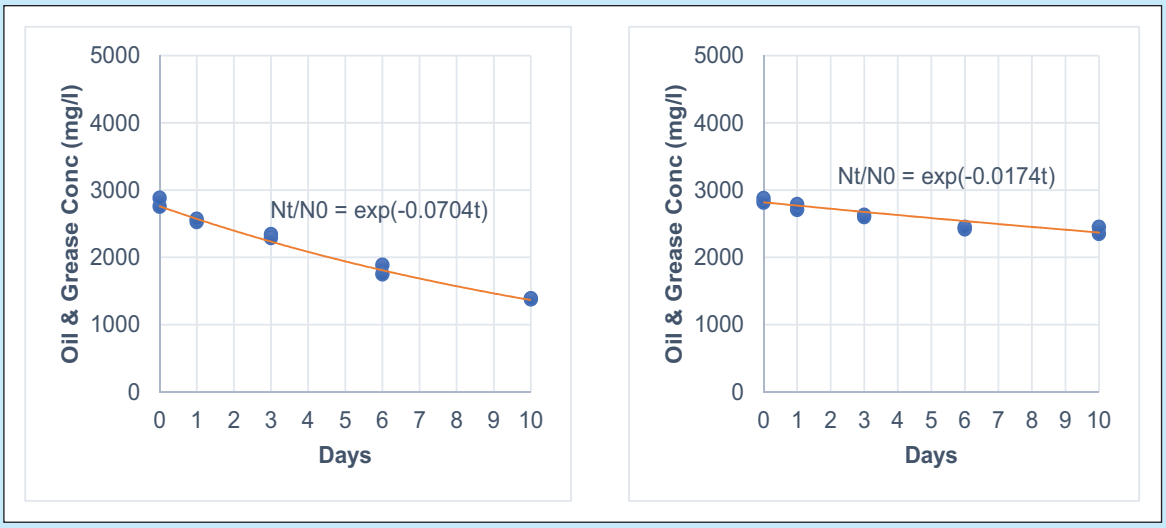

Figure 5
Oil biodegradation rate without DEA surfactant in (a) bacterial inoculation media and (b) control media during the 10 (ten)-day observation.

chosen as the DEA surfactant concentration utilized in the study.

B. Oil Biodegradation Experiment

Oil biodegradation experiment was conducted by comparing oil concentration (mg/l) on day 0 to day 10 on each treatment and control media. The average value and standard deviation of oil concentrations (mg/l) without addition and with DEA surfactant on bacterial inoculation media along with the control during the 10-day observation can be seen in Figure 3 and Figure 4.

As indicated in Figure 3 and Figure 4, it can be seen that there are differences in oil concentrations at the beginning of observation (day 0) in each treatment. Oil concentration on day 0 in treatment without DEA surfactant ranges from 2821 - 2848 mg/l, while treatment with DEA surfactant ranges from 4439 - 4488 mg/l. The oil concentration measured in the media not added with DEA surfactant is lower than that in the media added with DEA surfactant, due to poor oil dispersion and oil attached to the glass vessel wall. The addition of DEA surfactant caused the oil turn into smaller, finer and better dispersed granules in the water, and there was only a little oil attached to the glass vessel wall, so that the measured oil concentration was much larger.

The results showed that the percentage of oil dispersion on day 0 ranged between 56.42 and 56.96% in the treatment without DEA surfactant, while in the treatment with DEA surfactant ranged between 88.78 and 89.76%. Elvina *et al*. (2016) reported that the use of Oil Spill Dispersant (OSD) which was a mixture of DEA surfactant and MES surfactant had percentage of oil dispersion of 87.92 - 92.44%. As argued by Elvina (2015), DEA surfactant is good dispersing agent because it contains hydrophilic (polar) and hydrophobic (non-polar) groups in their molecules. The surfactant will bind water to polar groups and oils in non-polar groups.

The percentage of oil concentration reduction without DEA surfactant in bacterial inoculation media (Figure 3) during the 10 (ten)-day observation with oil concentration at the beginning and at the end of the observations of 2821 mg/l and 1384 mg/l was 50.93% with biodegradation rate k = -0.0704 t (Figure 5a), while the control media with oil concentration at the beginning and at the end of observation of 2848 mg/l and 2319 mg/l was 18.59% with biodegradation rate k = -0.0174 t (Figure 5b). The percentage of oil concentration reduction with DEA surfactant in bacterial inoculation media (Figure 4) during the 10 (ten)-day observation with oil concentration at the beginning and at the end of observation of 4439 mg/l and 1531 mg/l respectively was 65.52% with biodegradation rate k = -0.1054 t (Figure 6a), while the control media with oil concentration at the beginning and at the end of observation of 4488 mg/l and 2640 was 41.19% with biodegradation rate k = -0.0547 t (Figure 6b).

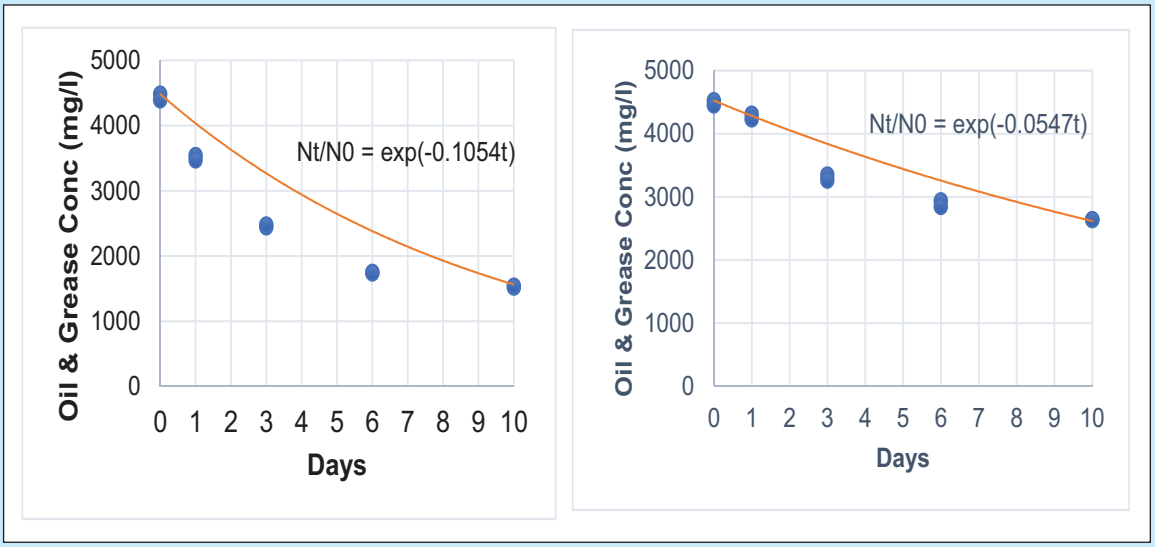

Figure 6
Oil biodegradation rate with the addition of DEA surfactant in (a) bacterial inoculation media and (b) control media during the 10 (ten)-day observation.

The results showed that the percentage of oil concentration reduction and biodegradation rate in the media with bacterial inoculation was higher than that in the control media (without bacterial inoculation). This happened because bacteria had the ability to degrade hydrocarbon compounds and utilized them as energy sources (Bao et al. 2014). As argued by Marsandi and Estuningsih (2016), bacteria had the ability to convert complex hydrocarbon compounds contained in petroleum, into simpler compounds with the final product in the form of CO_2, water and energy. The bacteria used in this study were consortium bacteria (mixed culture) consisting of *Enterobacter* sp., *Pseudomonas* sp., and *Raoultella* sp. The bacterial consortium was isolates selected from deep sea sediments and had the ability to degrade petroleum by 88.64% during 7-day observation (Dwinovantyo 2015).

The pure culture of bacteria isolated from deep sea sediments has the ability to degrade oil, however the use of bacterial consortium is far more efficient than the pure culture (Kumar et al. 2014). Combination of different bacterial cultures (consortium) has more genetic information needed for metabolism, so it has greater potential to degrade petroleum contaminants. The use of bacterial consortium on petroleum biodegradation will occur in two possibilities that can affect the biodegradation process, namely synergism or antagonism (Bao et al. 2014). If there is synergism in the bacterial consortium, the oil biodegradation process will increase, on the contrary if there is antagonism, the biodegradation process will decrease (Marsandi and Estuningsih 2016). Patowary. 2016 also reported that bacterial consortium was able to produce synergistic effects to improve the oil biodegradation process. The research conducted by Dwinovantyo (2015) showed that the consortium of the three bacteria was able to synergize in degrading petroleum. Statistically, the study performed with bacterial inoculation t-test on biodegradation rate indicated that the media with bacterial inoculation was significantly different from the media without bacterial inoculation. This was stated with Sig value of 0.024 ($P < 0.05$) meaning that the media with bacterial inoculation had a significant effect on the oil biodegradation rate.

The bacterial consortium in the media added with DEA surfactant has a higher biodegradability compared to the media not added with DEA surfactant. This can be seen from the percentage of reduction in oil concentration of 65.52% in bacterial inoculation media added with DEA surfactant, whereas in bacterial inoculation media not added with DEA surfactant the percentage of oil concentration decreased by 50.93%. As argued by Syafrizal et al. (2015), surfactants were able to increase oil solubility in water so that the bioavailability of petroleum to be utilized by bacteria increased and made the bacteria more easily degrade hydrocarbon compounds. Tian et al. (2016) also reported that surfactants had the

ability to increase bioavailability, solubility and biodegradation of petroleum pollutants.

The controls of each media also decreased oil concentration with percentage of 18.59% in the media not added with DEA surfactant and 41.19% in the media added with DEA surfactant. The decrease in the oil concentration in the respective control media was because of physical factor, namely weathering due to effect of automatic stirring with overhead stirrer. In addition, the decrease in oil concentration in the control media was thought due to the growth of bacteria contained in crude oil, so that biodegradation activities also occurred. On bacterial gradual isolation from petroleum samples indicated that the isolates of bacteria contained in petroleum had the ability to degrade oil. Statistically, the study conducted with surfactant t-test on biodegradation rate showed that the media added with DEA surfactant were significantly different from one not added with DEA surfactant. This was stated with a Sig value of.118 ($P < 0.15$) meaning that the media added with DEA surfactant had a significant effect on the oil biodegradation rate.

Another factor that also plays an important role in the petroleum biodegradation process is the acidity (pH) level in the environment (Shahian *et al*. 2013). The pH value affects the ability of bacteria to maintain cellular activity continuity, transport of cell membranes and equilibrium of reactions catalyzed by enzymes. The average pH value and standard deviation with and without DEA surfactant on the bacterial inoculation media along with the control during the 10 (ten)-day observation can be seen in Figure 7 and Figure 8.

The results showed that the pH value in the media added with DEA surfactant (Figure 8) ranging from 7.24 to 7.82 which was higher than that in the media not added with DEA surfactant (Figure 7) which ranged between 6.53 and 7.10. The difference in the range of pH values was due to addition of DEA surfactant that had alkaline properties, so that the media added with DEA surfactant had a higher pH value. The pH value of DEA surfactant at a concentration of 2% (b/v) measured with a pH meter was 10.67. Based on the study on characteristics of DEA surfactant conducted by Elvina (2015), DEA surfactant has a pH value of 10.87.

The pH value of bacterial inoculation media in each treatment (Figure 7 and Figure 8) tended to fluctuate. The bacterial inoculation media not added with DEA surfactant on day 0 had a pH value of 6.73, and it then increased on day 1 reaching 7.10, while on day 3 to 10 it continued to decline reaching 6.53 (Figure 7). The bacterial inoculation media added with DEA surfactant on day 0 had a pH value of 7.67, and it then decreased on day 1 reaching 7.44, and on day 3 it increased again up to 7.62, and on day 6 to 10 it declined reaching 7.24 (Figure 8). The

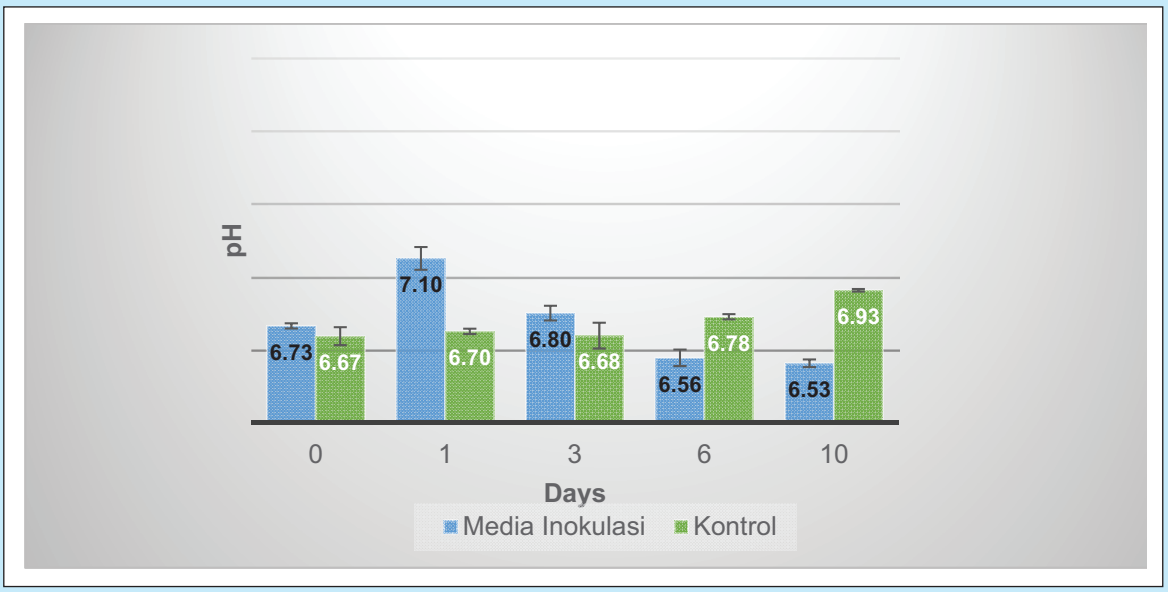

Figure 7
Average pH value and standard deviation without DEA surfactant to bacterial inoculation media and control during the 10 (ten)-day observation.

pH value of the control media not added with DEA surfactant on day 0 was 6.67, and I tended to increase until day 10 reaching 6.93 (Figure 7). In contrast to the control media not added with DEA surfactant, the pH value of the control media added with DEA surfactant tended to decrease on day 0 reaching 7.82, and it then continued to decline until day 10 reaching 7.49 (Figure 8).

Decreasing in pH value is due to activity of bacterial consortium that form acid metabolites. Biodegradation of n- alkali contained in petroleum will form alcohol and then become fatty acids. The fatty acids resulting from degradation of n- alkali will further form acetic acid and propionic acid, thus reducing the pH value of the media (Rosenberg et al. 1992). An increase in pH value is due to decomposition of nitrogen-containing compounds to form ammonium carbonate. The ammonium carbonate will break down into ammonia, CO_2 and water. Ammonia reacts with water to becomes NH_4OH so that the pH in the media increases As argued by Darsa *et al*. (2014) the optimum pH value in the petroleum bioremediation process ranges from 6 to 8, so changes in pH values during oil biodegradation process experiment are still within the optimum range for petroleum bioremediation process.

The results of previous study conducted by Dwinovantyo (2015) showed that percentage of oil concentration decreases by 88.64% with biodegradation rate k = -0.3134 t during seven-day observation with 150 ml experiment media. The percentage of oil concentration decreased to 49.38% with biodegradation rate k = -0.0972 t in the 8 liter seawater nutrient broth media (total experiment media) during seven-day observation, while in the media of water the percentage of oil concentration decreased by 38.78 % with biodegradation rate k = -0.0701 t (Rahmaniar 2016). When compared to Rahmaniar's (2016) research, there is a significant difference. The percentage of decrease in oil concentration is 65.52% with biodegradation rate k = -0.1054 t in the formatted water media with addition of diethanolamide (DEA) surfactant. The results showed that the addition of diethanolamide (DEA) surfactant was quite effective in increasing the percentage of concentration reduction and oil biodegradation rate. Based on the Duncan test of treatment media on biodegradation rate, it is known that all treatment media have a significant effect on biodegradation rate. This is indicated by the different subset locations in each treatment media. The bacterial inoculation media added with DEA surfactant is the best one because it is located in

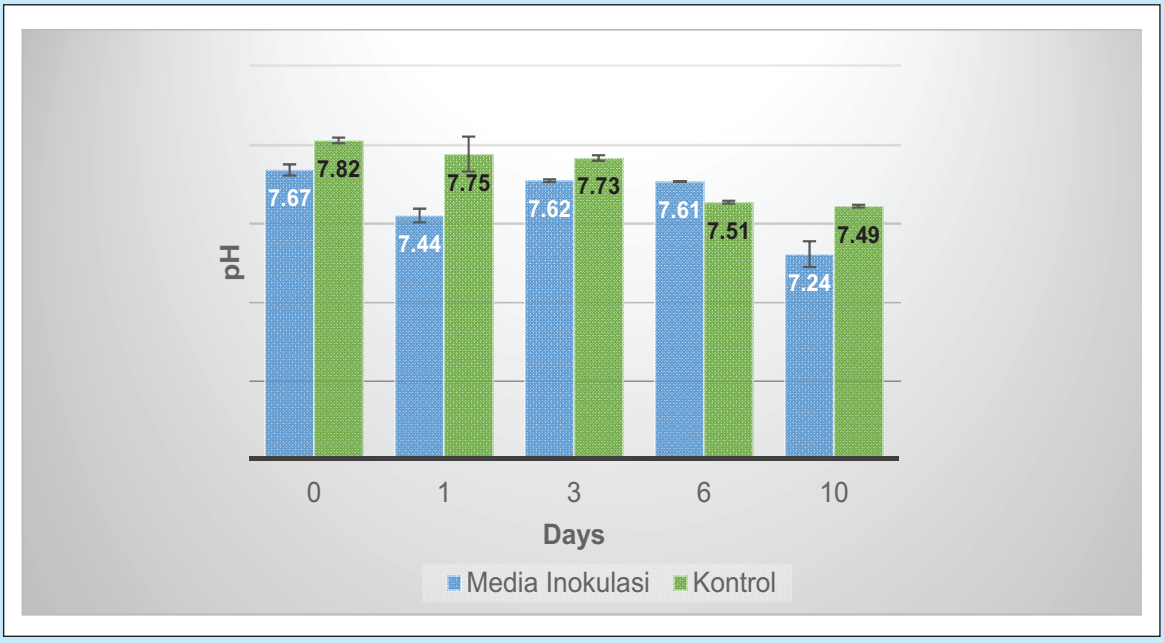

Figure 8
Average pH value and standard deviation with DEA surfactant to bacterial inoculation media and control during the 10 (ten)-day observation.

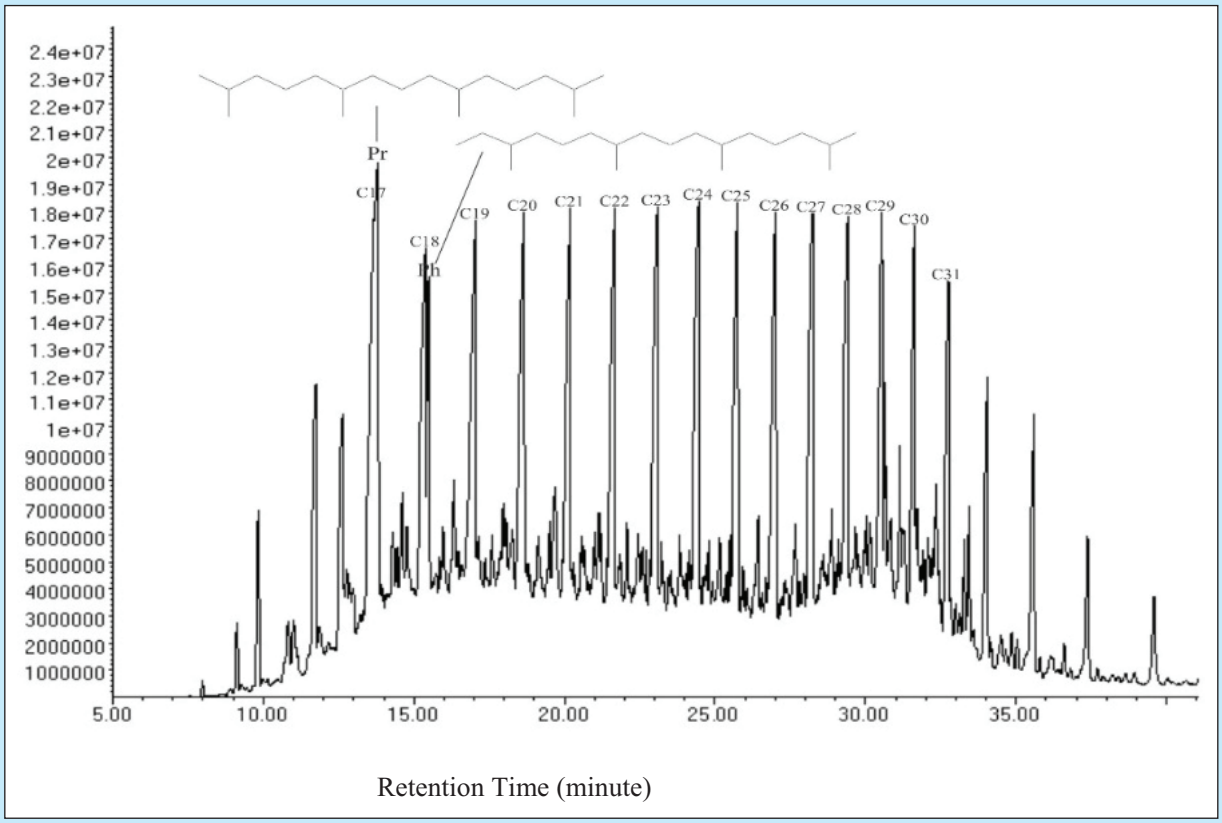

Figure 9
Aliphatic fraction chromatogram before biodegradation process.

the first subset with average biodegradation rate of k = -1.0545 on day 10, while bacterial inoculation media not added with DEA surfactant is located in the second subset with average biodegradation rate of k = -0.7045 on day 10. The control media with and without DEA surfactant are located in the third and fourth subset with average biodegradation rate of k = -0.5470 and k = -0.1735 respectively on day 10.

C. Gas Chromatography-Mass Spectrometer (GC-MS) Analysis

Gas Chromatography-Mass Spectrometer (GC-MS) Analysis is conducted to detect changes in chemical components that occur in petroleum after biodegradation process. Petroleum is a mixture of complex hydrocarbons composed of aliphatic, aromatic, asphaltene and resins fractions (Sakthipriya et al. 2015). The aliphatic fraction is a saturated hydrocarbon with a single bond which is divided into alkanes and cycloalkanes. Alkanes have a general formula C_nH_{2n+2} and are arranged in a straight (n- alkane) and branched chain. In the oil industry, alkane is better known as paraffin. Cycloalkanes or often called naphthenes have one or more rings of carbon atoms with general formula of C_nH_{2n}.

This study only focuses on the aliphatic fraction only because this fraction is most easily degraded and has the largest content. The aliphatic fraction is the most dominant petroleum component, and gives the largest contribution to the total oil weight. The aliphatic fraction is a fraction that is easily degraded, long branched and long chain aliphatic fractions are more difficult to degrade by microorganisms (Zam 2011). The ability of biodegradation of petroleum by microorganisms will decrease if the chain and number of branches are getting longer. The GC-MS chromatogram of aliphatic fraction before and after biodegradation process can be seen in Figure 9 and Figure 10.

The aliphatic fraction detected by GC-MS before biodegradation process was dominated by n-alkanes with number of C_{17}-C_{31} carbon atoms (Figure 9) in the retention time range of 12.63-32.76 minutes. After the biodegradation process observed for ten days, the aliphatic fraction detected to be C_{20}-C_{31} (Figure 10) in the retention period of 18.65

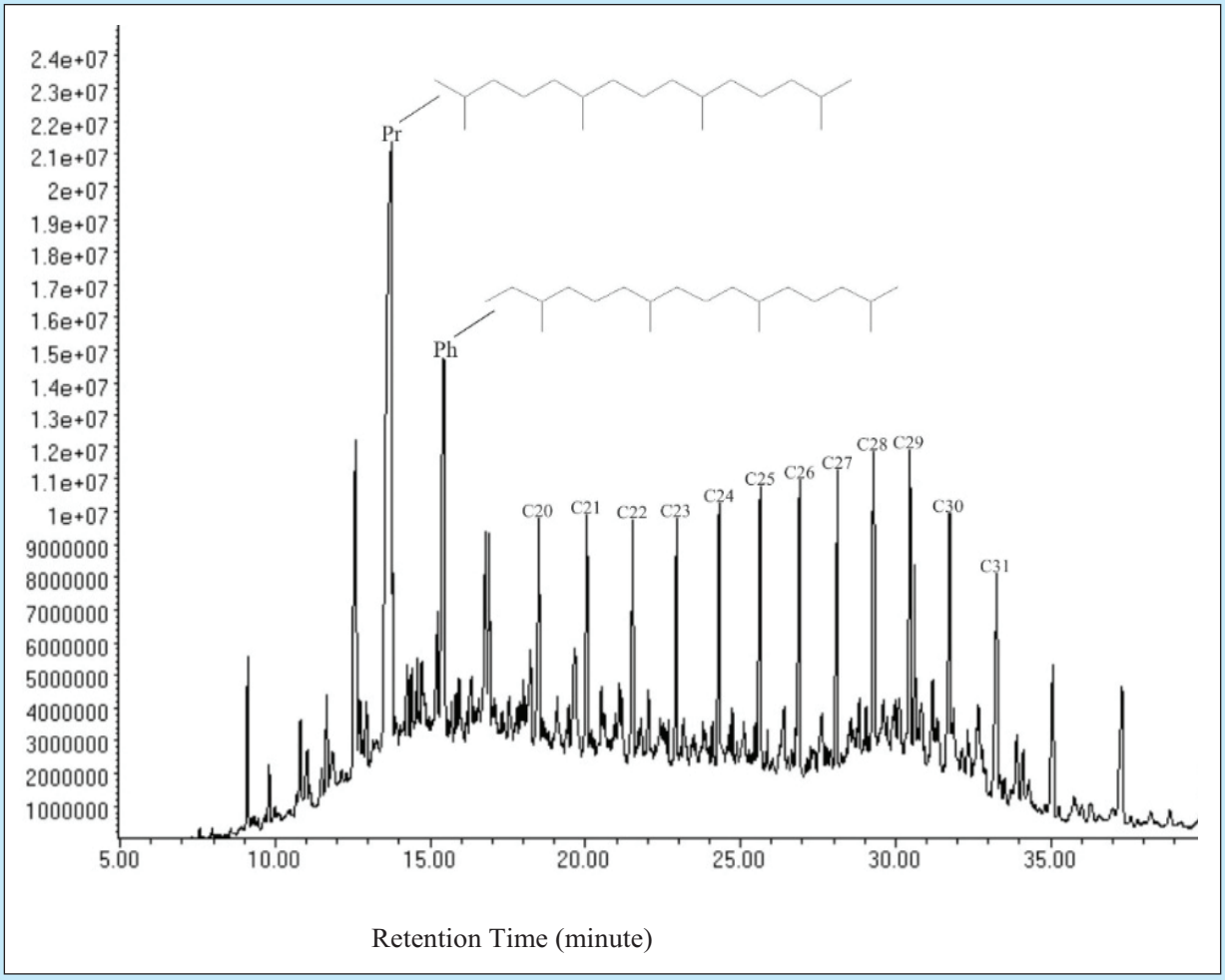

Figure 10
Aliphatic fraction chromatogram after biodegradation proces.

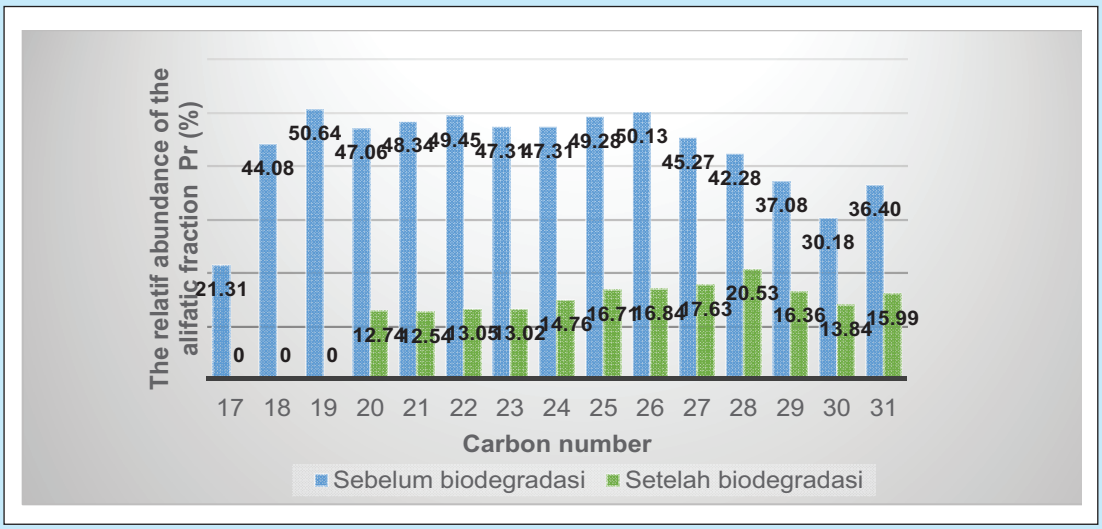

Figure 11
Percentage of relative abundance of aliphatic fraction of pristane (Pr) before and after biodegradation process.

- 32.76 minutes. The compounds with number of carbon atoms C_{17}-C_{19} disappeared because they had degraded completely.

The percentage of biodegradation of aliphatic fraction was calculated based on the relative abundance of pristane (Pr), by dividing the peak area of aliphatic compound with that of pristane compound before and after this biodegradation. This technique is commonly called as the normalization ratio. Normalization method for one of the hydrocarbon components (in this study, pristane compound) is needed to eliminate the influence of chromatographic process instability (Desrina 2010). As argued by Diaz et al. (2000), pristane (Pr) and phytana (Ph) compounds are more resistant to biodegradation than n-alkanes. Pristane and phytana compounds can be used as biomarkers or biodegradation indices, because they have stable properties and tend not to be biodegradable (Bao et al. 2014). The percentage of relative abundance of aliphatic fraction against pristane before and after biodegradation process can be seen in Figure 11.

As seen on Figure 11, n-alkane compounds with number of carbon atoms C17-C19 are degraded entirely. The percentage of biodegradation of n-alkane compounds with number of carbon atoms C20 - C31 ranges from 51.44 to 74.06%. The n-alkane compound with the highest percentage of biodegradation is heneicosane (C_{21} H_{44}) with percentage of biodegradation of 74.06%, while the compound with the smallest percentage of biodegradation is octacosane ($C_{28}H_{58}$) with percentage of biodegradation of 51.44%.

The decrease in percentage of abundance of aliphatic fraction after biodegradation process indicates that the compound has been degraded by bacteria. N-alkaline compound is saturated hydrocarbon with single bond that is most easily degraded by microorganisms (Syafrizal *et al*. 2015). As argued by Celovsky. 2013, in the aliphatic fraction the order of the most easily degraded compound to the most difficult degraded compound is n-alkane > branched -chain alkanes > cycloalkanes.

IV. CONCLUSIONS

Diethanolamide (DEA) surfactant is able to enhance the ability of bacterial consortium of *Enterobacter* sp., *Raoultella* sp., And *Pseudomonas* sp. in degrading petroleum in the seawater media. The bacterial consortium is able to degrade oil up to 50.93% in the media without DEA surfactant with biodegradation rate k= -0.0704t and 65.52% in the media added with DEA surfactant with biodegradation rate k = -0.1054 t during ten-day observation.

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