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BIOREMEDIATION OF CRUDE OIL CONTAMINATED SEAWATER WITH THE APPLICATION OF BIOSURFACTANT AND BIOSTIMULATION

BIOREMEDIASI AIR LAUT TERKONTAMINASI MINYAK BUMI DENGAN APLIKASI BIOSURFAKTAN DAN BIOSTIMULASI

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ABSTRAK

Minyak bumi merupakan salah satu sumber pencemar yang dapat ditemukan di lautan dan berdampak negatif terhadap ekosistem laut. Diperlukan kondisi khusus untuk mereduksi polutant tersebut, salahsatu proses yang terjadi adalah biodegradasi, yaitu proses degradasi komponen-komponen hidrokarbon minyak bumi oleh akitivitas mikroorganisme sehingga ekosistem laut kembali normal. Remediasi air laut yang terkontaminasi minyak bumi melalui aplikasi biosurfaktan dan biostimulasi merupakan salah satu cara untuk meningkatkan efektivitas biodegradasi minyak bumi. Untuk meneliti aspek tersebut dilakukan percobaan skala laboratorium menggunakan media dasar air laut yang ditambahkan bahan pencemar minyak. Terdapat empat perlakuan: P0 (media dasar tanpa penambahan biosurfaktan dan nutrien), P1 (penambahan biosurfaktan), P2 (penambahan biosurfaktan dan nutrien), dan P3 (penambahan nutrien). Hasil pengamatan selama 21 hari inkubasi menunjukkan bahwa perlakuan penambahan biosurfaktan tidak menunjukkan peningkatan biodegradasi yang signifikan. Peningkatan biodegradasi yang signifikan terjadi pada pada perlakuan dengan penambahan biostimulan dan lebih meningkat lagi degradasinya bila biostimulasi dikombinasikan dengan biosurfaktan.

Kata Kunci: *bioremediasi, biodegradasi, hidrokarbon minyak bumi, biosurfaktan, biostimulasi*

ABSTRACT

Petroleum is one of the pollutant sources that can be found in the oceans and has a negative impact on the marine ecosystem. Special conditions are needed to reduce the pollutant, one of the processes that occurs is biodegradation, which is the process of degradation of petroleum hydrocarbon components by the activity of microorganisms so that the marine ecosystem returns to normal. Remediation of seawater contaminated with petroleum through the application of biosurfactants and biostimulation is one way to increase the effectiveness of petroleum biodegradation. To examine these aspects laboratory-scale experiments were carried out using basic seawater media which added oil pollutants. There are four treatments: P0 (basic media without the addition of biosurfactant and nutrients), P1 (addition of biosurfactant), P2 (addition of biosurfactant and nutrients), and P3 (addition of nutrients). The results of observations for 21 days of incubation showed that the addition of biosurfactant treatment did not show a significant increase

in biodegradation. Significant increase in biodegradation occurs in the treatment with the addition of biostimulants and more degradation if biostimulation is combined with biosurfactant.

Keywords: bioremediation, biodegradation, petroleum hydrocarbons, biosurfactant, biostimulation

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I. INTRODUCTION

Petroleum hydrocarbons are among the major contaminant source in marine ecosystems that negatively affecting the environment. Spilled oil could damage marine and land ecosystems, causing serious impact to the organisms because of its toxic features and could also possibly affecting humans whose life depend on the exploitation of marine resources. Approximately 3,2 million ton of petroleum hydrocarbon enters the ocean every year from variety of sources, including transport activity, oil drilling, oil seepage and accidental explosion of oil wells (Saadoun 2015).

Once oil enter the sea, it will undergo several physical, chemical and biological processes that are instrumental on the disappearence of oil from the environment. Naturally occuring biodegradation is the most important factor in oil removal from a polluted marine environment (Hassanshahian and Capello 2013). Biodegradation role in oil removal process encourage the use of bioremediation as a method for environmental recovery.

One common approach in oil biodegradation is biostimulation, which is through the addition of appropriate nutrients so that it influences the increase in bacterial growth and accelerates biodegradation. On the other hand the availability of oil to bacteria can be an obstacle to biodegradation being slow. A method is needed to increase availability so that biodegradation of oil is faster, namely through the addition of biosurfactants which can increase the surface area between oil and bacteria. This study aims to determine the effect of biosurfactant and biostimulation applications on petroleum biodegradation. This research is expected to provide information on the role of biosurfactants and the addition of nutrients in increasing the biodegradation of oil spill.

II. METHODOLOGY

A. Preparations

Materials used in this research are seawater taken from Muara Angke added by light crude oil API 34^{o} from "x" oil field, biosurfactant, and nutrients. Crude oil was used as the source of hydrocarbons contaminant. The addition of biosurfactant and nutrients served as treatments. The sources of nutrients consist of urea (46% nitrogen) and NPK (16:16:1).

All materials and instruments used in this experiment were sterilized to prevent cross contamination and sterilize unwanted bacteria. Instruments were sterilized using autoclave at 121^{o}C in 15 psi for 20 minutes. The same sterilization procedure was also applied to microbial growth media (Brown 2010).

Erlenmeyer flasks were used as seawater container. Sterile erlenmeyers were labeled according to the treatments applied. 5 ml of crude oil were added to 100 ml of filtered seawater as a source of pollution. Rotary shaker was used to simulate the dynamics of marine environments.

B. Treatments

This research carried out experimentally in a laboratorium using completely randomized design. Four treatments was applied:

P0: seawater without the addition of biosurfactant and nutrients

P1: seawater with biosurfactant

P2: seawater with biosurfactant and nutrients

P3: seawater with nutrients

Biosurfactant was added to reduce the surface tension between oil and seawater. Nutrients was added as a source of nitrogen and phospor. Thirty-six erlenmeyer flasks were grouped according to

the four treatments. 500 ppm of biosurfactants were added to P1 and P2 (Zulkifliani et al. 2013). NPK and urea fertilizers were used as the source of nutrients for biostimulation using C:N:P ratio of 100:10:1. All treatments were replicated three times and sampled for analysis during the 7th, 14th and 21st days of incubation.

C. Total Petroleum Hydrocarbons (TPH) Measurements

Hydrocarbons analysis were measured quantitatively using gravimetric method (SNI, 2004). Boiling flasks were weighted before used as extraction container. The analyzed sample was poured inside separatory funnel. N-hexane solvents were added to erlenmeyer, the sample container, and shaked evenly before being poured into separatory funnel. The extraction of water was done by shaking the separatory funnel vigorously for approximately two minutes. The solution would then separated into two layers, the oil-solvent and water. Water layer was put back into the erlenmeyer. The oil-solvent layer was filtered using filter paper that had been added by Na_2SO_4 into the boiling flask. Oil and solvent were separated using rotary evaporator at 70°C. The extraction process ended after there was no more solvent dripping from the condenser. The boiling flask would then heated in the oven remove the last possible adhering water or excess solvent. The boiling flask was then cooled in the desiccator before being weighted to the nearest miligram. Residual oil (mg/L) were calculated using this formula:

$$\frac{(B - A)}{C} \times 1000$$

where:

A = weight of boiling flask (mg)

B = weight of boiling flask after solvent extraction (mg)

C = volume of sample (L).

D. Total Bacterial Count

The amount of bacteria colonies were calculated using spread plate method (Seil and Webster 2012). In this method, the sample containing bacteria solution was diluted several times. 0,1 ml of the sample that had been diluted was pipetted into agar medium in the plate dan spread evenly using drigalski rod. Plates were incubated at 30°C for 48 hours. Bacteria colonies were calculated after incubation. Only the plates with no fewer than 30 and no more than 300 visible colonies would be counted. Colonies per ml (CFU/ml) were calculated by multiplying the colony count with the dilution factor:

$$\frac{\text{CFU}}{\text{ml}} = \frac{\text{bacterial colonies x dilution factor}}{\text{innoculation volume}}$$

E. Data Analysis

The percentage of biodegradation was defined as ratio between the initial oil concentration minus residual oil concentration with the initial oil concentration:

$$\text{Biodegradation (\%)} = \frac{Co - C}{Co} \times 100$$

where C_0 was the initial concentration (mg/L) and C was the residual concentration (mg/L).

Biodegradation rate was assumed as first order reaction calculated with this formula:

$$C = C_0 e^{-kt}$$

where C_0 was the initial oil concentration (mg/L).The following formula was used to calculate the reaction rate constant k:

$$\ln C = -kt + \ln C_0$$

(Yudono et al. 2009).

The treatment response was the comparison between biodegradation percentage in each treatments against P0 (without biosurfactants and nutrients):

$$\frac{\text{Biodegradation P} - \text{Biodegradation P0}}{\text{Biodegradation P0}} \times 100$$

III. RESULTS AND DISCUSSIONS

A. Hydrocarbons Biodegradation

The percentage of biodegradation and biodegradation rate constant was calculated based on the change of hydrocarbons concentration.

umbers are mean ± standard deviation with P0 = treatment without an addition of biosurfactant and nutrients; P1 = with an addition of biosurfactant; P2 = with an addition of biosurfactant and nutrients; P3 = with an addition of nutrients. Same letter at the end of numbers indicate that the treatments are not significantly different at $p < 0,05$.

Day	Treatments			
	P0	P1	P2	P3
T_7	17,594 ± 6,804[a]	19,023 ± 0,071[a]	26,734 ± 4,519[ab]	48,122 ± 12,143[b]
T_{14}	21,030 ± 2,667[a]	19,662 ± 0,681[a]	33,390 ± 2,852[ab]	49,671 ± 12,920[b]
T_{21}	23,471 ± 2,003[ab]	22,520 ± 0,781[b]	65,593 ± 11,843[a]	63,161 ± 13,268[a]
Rate	0,012	0,011	0,047	0,043

Numbers are mean ± standard deviation with P0 = treatment without an addition of biosurfactant and nutrients; P1 = with an addition of biosurfactant; P2 = with an addition of biosurfactant and nutrients; P3 = with an addition of nutrients. Same letter at the end of numbers indicate that the treatments are not significantly different at p < 0,05.

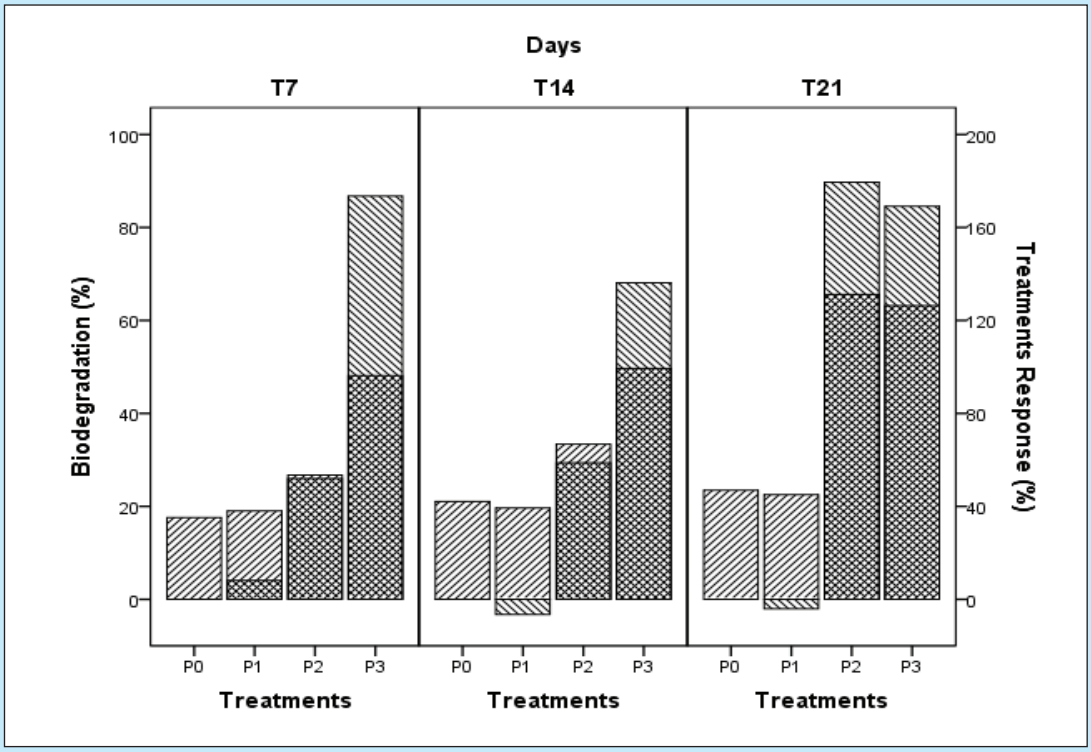

Fig.ure 1
.Average Treatments Response (▨) and Biodegradation Percentage (▨).

The results show that for 21 days the incubation of the amount of hydrocarbons in P2 treatment experienced the greatest biodegradation of 65.59%, followed by P3 of 63.16% while P0 had biodegradation of 23.45% and P1 has the lowest biodegradation of 22.52%. The 14th day of incubation showed that biodegradation of P1, P2, and P3 had the lowest increase compared to other days. P2 and P3 continued to show an increase in biodegradation until the 2st day incubation, whereas at P0 and P1 significant changes only occurred on the first seven days of the study. The largest rate of biodegradation constant occurred in P2, followed by P3, P0, and P1. Then on the 7 day of incubation, there was an increase in the percentage of hydrocarbon biodegradation in each treatment. P0 and P1 get a biodegradation percentage of less than 20% while P2 exceeds 25% and P3 almost 50%. Hydrocarbons lost at the start of the study are thought to be saturated hydrocarbons which are more easily degraded. According to Head et al. (2006), biodegradation of petroleum in surface area resulted in changes in petroleum composition. The characteristics of biodegradation are characterized by the disappearance of saturated hydrocarbons accompanied by an increase in polar fractions which are more difficult to degrade. Olajire

and Essien (2014) reported that biodegradation of oil by microbes generally occurs in light oil fractions, whereas in heavier oil fractions biodegradation will occur more slowly. In addition, in the first phase of testing (exponential phase) the occurrence of good hydrocarbon biodegradation involves bacteria that can grow rapidly and slow down at a later stage (stationary phase and mature phase), this is due to reduced hydrocarbon and nutrient components in the media during incubation.

B. Treatments Response

Treatments response by the addition of biosurfactant and nutrients to hydrocarbons biodegradation were obtaines by comparing the biodegradation percentage of each media that were given treatment to P0. During the 14 days of incubation, P3 showed the biggest response as opposite to P1, while at the 21 days of incubation P2 showed the biggest response. Meanwhile, P1 showed negative responses in both the 14 and 21 days of incubation and P0 did not change the percentage of biodegradation in a significant amount. This happens because of the limiting factor in the biodegradation process in the treatment. P0 simulates the natural conditions of the aquatic environment impact by oil spills. In these conditions the biodegradation generally occurs very slowly. This can be caused by reduced nutrition which can support the growth of oil degrader bacteria. Therefore, the biodegradation can still occur but under conditions that are slow and require a long time (Szulc et al. 2014). The treatment response that was not significant was indicated by P1 with the addition of biosurfactant. The addition of biosurfactant aims to emulsify oil to penetrate bacteria access to oil, which according to Chekroud et al. (2011), biodegradation will take place faster when the oil is emulsified.

Biosurfactants are known to damage microbial cell membranes until lysis occurs by increasing membrane permeability resulting in cell metabolic disorders. The inability of bacteria to adapt to the presence of biosurfactants can be one of the causes of failure to use biosurfactants to increase the biodegradation of oil spills in the sea. The results of the study conducted by McKew et al. (2007) show that the use of rhamnolipid biosurfactant obtained the lowest biodegradation percentage. This is due to the limited number of nutrients so that the increase in oil availability caused by emulsification only has a minimal impact on biodegradation. Chen et

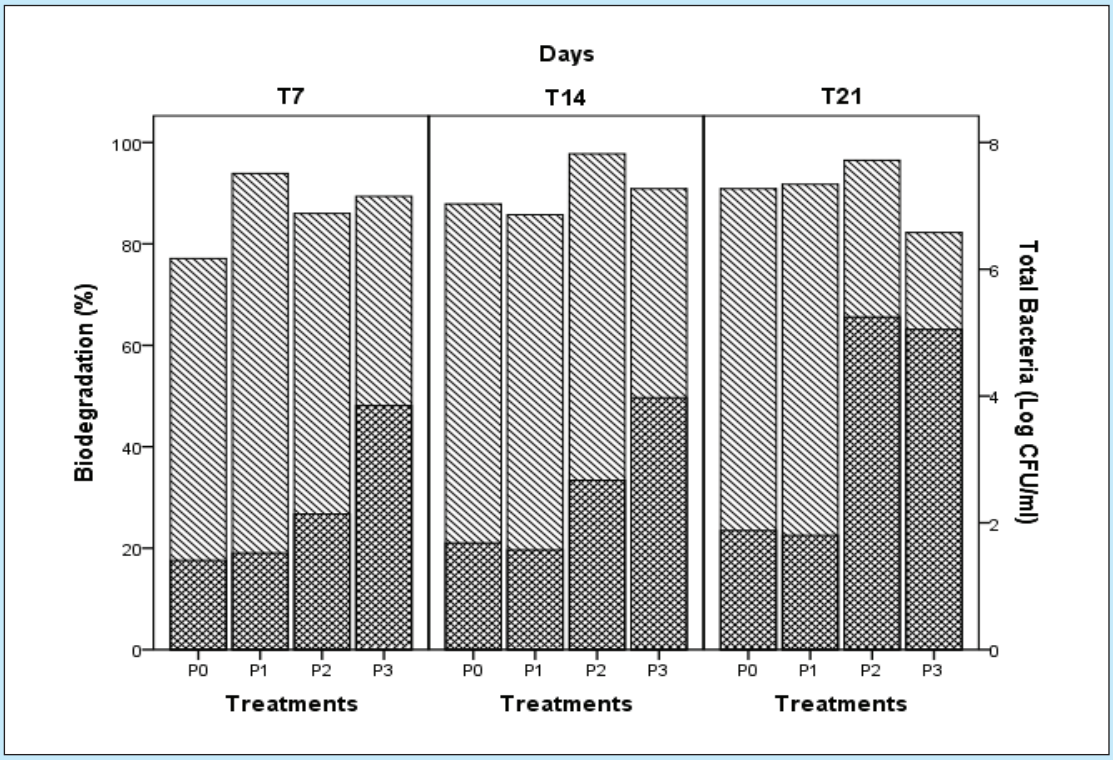

Figure 2
Average Total Bacteria (▨) and Biodegradation Percentage (▨).

al. (2013) reported that the biosurfactant ability of rhamnolipid species in increasing oil biodegradation efficiency was 5.63%.

P2 and P3 showed a positive response until the 21 days of incubation. The success of P2 and P3 in degrading hydrocarbons is estimated to occur due to adequate nutrient intake for the growth of oil degrader bacteria and combined with biosurfactant. The success of adding nutrients in increasing the percentage of biodegradation is supported by several previous studies. The research conducted by Hassashahian et al. (2013) show that the addition of inorganic nutrients resulted in an increase of biodegradation up to 80% within 20 days of incubation. Meanwhile, research conducted by Nikolopoulou et al. (2013) compared the use of two different nutrient sources, namely NPK and uric acid and lecithin. The results of the study indicate that the potential use of NPK and uric acid and lecithin as a source of nutrition can stimulate indigenous bacteria to degrade 80% of the saturated hydrocarbon fraction within 30 days of incubation. The use of different nutrients will have different effects. In this study, urea and NPK were used as sources of water-soluble nutrients. The nutrients type is easily availability, their concentrations are easily manipulated and inorganic compound, but are more susceptible to waves and tides. Appropriate nitrogen and phosphorus concentrations will select bacteria communities that will be dominated by organisms that have the ability to utilize nutrients more in accordance with polluted environmental conditions.

C. Dynamics of Bacteria

The results of the observation show that the average number of the largest bacteria was obtained by P2, followed by P0, P1, and P3. While P2 and P3 achieve the highest average number of bacteria on the 14 days of incubation. P0 shows the highest average at the initial stage of testing while P1 achieve the highest average number of colonies on the 7 day of incubation. Based on Figure 2, the increase in the total number of bacteria is not linear with an increase in the percentage of biodegradation.

IV. CONCLUSIONS

Biostimulation through the addition of nutrients can increase biodegradation of petroleum hydrocarbons in seawater. The combination of adding nutrients with the addition of biosurfactants has a positive effect on biodegradation. The percentage of biodegradation of petroleum hydrocarbons for 21 days of incubation reached 65.59% with the addition of biosurfactants and nutrients and the addition of nutrients reached 63.16%. Meanwhile, the percentage of biodegradation with the addition of biosurfactant was only able to reached 22.52%.

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