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Effectiveness of *Chetoceros calcitrans* and *Skeletonema costatum* in degrading diesel fuel on laboratory-scale Test

Bambang Budi Sasmito[1], Bambang Budi Sasmito Dwi Candra Pratiwi*[2,3], Rarasrum Dyah Kasitowati [2,3], Titik Dwi Sulistyati[1] and Dian Fitri Nuryani[2]

[1]Fisheries and Product Technology, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Malang 65145, Indonesia
[2] Marine Science, Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Malang 65145, Indonesia
[3]Coastal Resilience and Climate Change Adaptation (CORECT) Research group, Universitas Brawijaya, Malang 65145, Indonesia

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ABSTRACT

Our current marine waters are very vulnerable to environmental pollution mainly due to diesel fuel waste from fishing boats or other domestic activities. Degradation of pollutants in aquatic waters can be done using physical, chemical, or biological methods. Scientists often use chemical methods because of their relatively faster use, but this method will produce new waste not environmentally friendly. The purpose of this study was to determine the effectiveness of microalgae as an environmentally friendly biological method in degrading diesel fuel; species of microalgae used was *Skeletonema costatum* and *Chetoceros calcitrans* species. The t-test results show no significant difference in environmental parameters for both species to grow. The growth of *Skeletonema costatum* and *Chaetoceros calcitrans* overall increased for 7 days. The R2 (R-Square) value of the *Chaetoceros calcitrans* species (0.9495) was very close to 1 when compared to the value of R2 of *Skeletonema costatum* (0.8115). The R-Square values shows that the four treatments (control, the addition of 5 mL, 10 mL, and 20 mL diesel fuel) are more applicable to the *Chaetoceros Calcitrans* species. Treatment differences (oil concentrations) and species differences gave a significantly different effect on percentage of degradation at 95% and 90% confidence intervals respectively.

Key words : *Phytoplankton, Degradation, and Diesel Fuel*

Introduction

Our marine waters are currently very susceptible to pollution from various types of contaminants. According to Pratiwi *et al.* (2017), with the addition of hydrocarbons, one of which is diesel fuel waste from ships, it will cause very chronic effects on organisms in the oceans. According to Raouf *et al*., (2017), the inclusion of organic and inorganic materials in waters is caused by various human activities. The waste material is caused by domestic, agricultural, and industrial activities. The use of microalgae as biodegradator is very effective in some countries, as it uses inorganic nitrogen and phosphorus as a growth medium. In addition, microalgae are also able to transmit heavy metals and toxic compounds in waters. Commercially microalgae have been widely used for handling of liquid waste for example using *Chlorella* and *Dunaliella* species.

***Corresponding author's email:** dwicandra@ub.ac.id

Degradation of pollutants in the marine environment can use physical, chemical, and biological techniques. Scientists generally use chemical techniques because their use is relatively easier and faster. However, this chemical method can threaten the ecosystem in the oceans. Biological method is one of the alternatives capable of degrading pollutants in the waters without endangering the ecosystems (Pratiwi *et al.*, 2017b). Microalgae can be used as one of the pollutant biodegradation agents. Microalgae can collect heavy metals, herbicides, insecticides, and phenols (Jin *et al*., 2011). Diesel fuel has been widely used in various parts of the world primarily for transportation activities. Most of these diesel machines are used for agricultural, industrial, or marine engine, in which 90% of the world's use of diesel fuel is dominated by marine activity. America has used diesel fuel as much as 600 million liters per day by 2016 and is predicted to increase by 110 million liters per day by 2040. However, the combustion process will produce waste that endangered marine life (Aktas *et al.*, 2017)

Many cases of oil spills have prompted scientists to invent physical and chemical methods, as well as biotechnology applications that could remove the former oil spill. One way to reduce the presence of oil spills is by bioremediation method. According to Xiong *et al*. (2016), several studies have shown that microalgae can effectively be used for bioremediation of organic and inorganic pollutants.

Biodegradation of pollutants using bacteria and fungi has been done since several years ago. However, when compared to using microalgae, these algae are much more adaptive because they can grow in autotroph and heterotroph condition. In addition, microalgae can also accelerate the micro biota growth; therefore, it can reduce pollutants so quickly. Microalgae are also able to live in extreme environmental conditions and environmental conditions with low nutrients (Hamouda *et al*., 2016). The purpose of this study was to determine the effectiveness of microalgae, especially *Skeletonema costatum* and *Chetoceros calcitrans* species, in degrading diesel fuel waste.

Research Method

Preparation of Sample

Microalgae obtained from selective culture

East Java Sea water sample. Pollutants were tested by hydrocarbons in the form of diesel fuel. Biodegradation was done for 7 days at Laboratory. The microalgae obtained were put into liquid culture using a bottle container. The culture was added with sterile sea water which previously had been in autoclave (added with nutrient in the form of diatom fertilizer into the reaction tube as much as ¾ part). Fertilizer used was much as 1 ml/L of the water culture medium; it was added with aeration and then placed on the shaker under lights. The culture aims to multiply phytoplankton before it was tested.

Experimental Design

Microalga cells taken from the study sites were included in each of the 12 glass bottles with the following composition: 10 mL microalgae, 1 mL diatom fertilizer, 1 mL of vitamins, 1 mL of silicate, and the remaining sterile seawater already boiled. Each bottle containing phytoplankton was treated with diesel fuel with concentration 0 mL, 5 mL, 10 mL, and 20 mL of 3 repetitions. Before the diesel fuel was put into the bottle, the levels were calculated. Biodegradation process was done at incubator shaker by using aeration and lighting as photosynthetic process of phytoplankton. Lighting on the incubator shaker used white fluorescent lights and the process was performed in a sterile room with a temperature of 18-25°C. During the degradation process, water quality parameters were measured, such as measurement of temperature, salinity, dissolved oxygen content (DO), pH, and phytoplankton density of 1 x 24 hour.

Exposure Test

Biodegradation test was done using sterile seawater that previously had been added diatom fertilizer, silicate, and vitamin as nutrients needed by phytoplankton in metabolism process and growth. Seawater added diatom fertilizer, silicate, and vitamins were added with diesel fuel with different concentrations of 5 mL, 10 mL, 20 mL then added phytoplankton as biodegradation agent from culture results as much as 10 mL. The sterile seawater was put into the shaker in order to condition the biodegradation medium to be more dynamic and to homogenize the phytoplankton to get the nutrients equally. The shaker speed was 80 rpm and aerated as a source of oxygen for 24 hours. Calculation of the phytoplankton growth phase was done by observing the Optical Density on the first day until the seventh day. Observation of the density of this phytoplankton was performed using Spectrophotometer. The Optical was added with 10 grams of anhydrous Na_2SO_4. If clear layer of solvent is not obtained and emulsion resulted is not more than 5 mL, centrifuge it for 5 minutes at 2400 rpm. Then, we refined and rinsed the filter paper with n-Hexane and made one with filtrate extract into the distillation flask. The next step was distillation with hotplate at 85 °C up to evaporate. Then, it was lifted and refrigerated on the desiccator for 30 minutes, before weighed and recorded for the remaining diesel weight. Residual diesel fuel content was measured using the following formula:

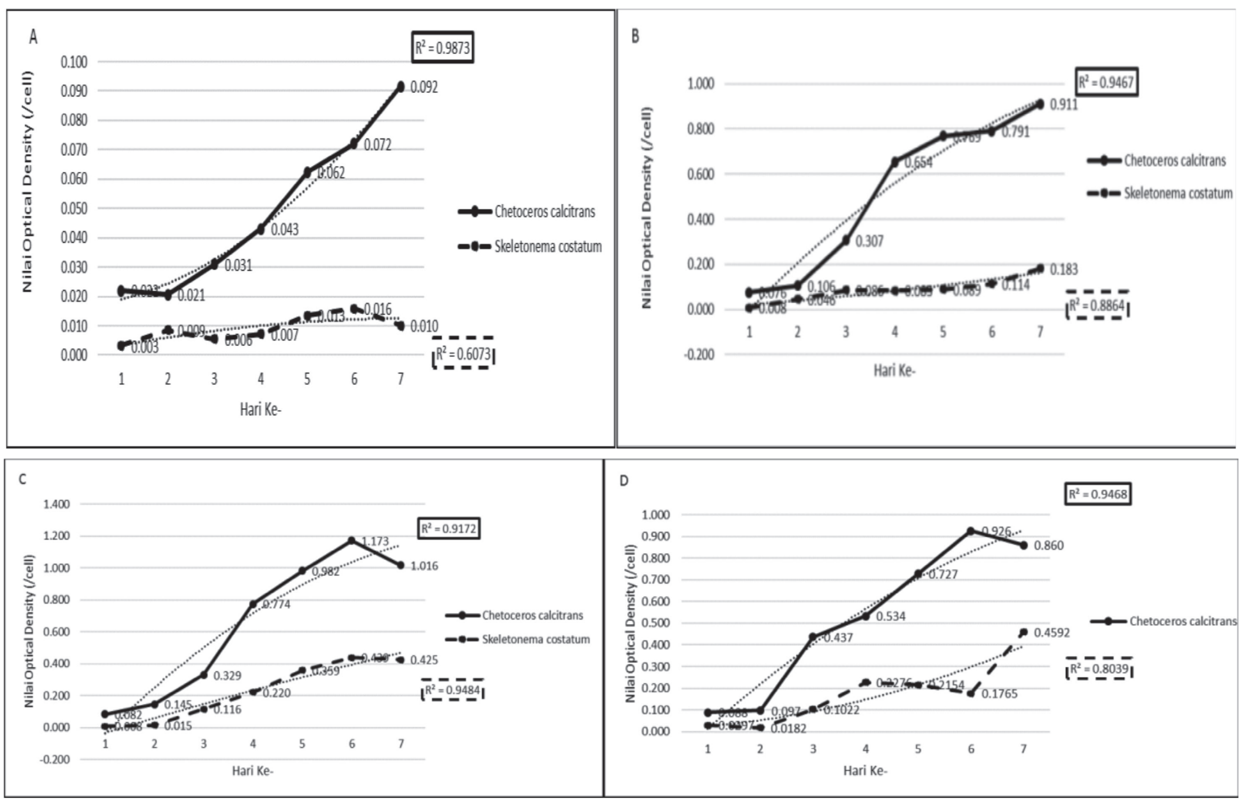
Density was read through the resulting absorbance value. The wavelength used was 650 nm.

Gravimetric Test

Gravimetric method is used to determine the oil content in a solution. Gravimetric analysis or quantitative analysis is the process of isolation and weighing of an element or a particular compound of an element in the purest possible form. The element or compound is separated from a portion of the investigated substance that has been weighed. The tools in gravimetric method are distillation pumpkin, pipettes, funnels, and separating flasks; they were dried into the oven for 15-20 minutes before cooled into the desiccator for 1 hour and weighed using the analytical balance as the initial weight. The next step was to take water sample and put into separator funnel and added with 30 mL n-Hexane solvent into test water and into separator funnel. The mixed solution was shaken for 2 minutes until the layer separated. Then, we removed the water layer. The result of the shaken solution was filtered by organic fraction (top) with filter paper.

Statistical Analysis

The t-test was used to test the difference in the sig-



In which:
KM: residual fuel (%)
A: initial diesel fuel content (mg/L)
B: diesel fuel content after degradation (mg/L)
Decrease for diesel fuel is expressed in percentage (%). The amount of degradation of diesel oil can be calculated using the following formula:
*C0 : initial concentration; C1 : final concentration

Table 1. Water Quality Parameters

Environmental Parameters	*Chetoceros calcitrans*	*Skeletonema costatum*
Temperature	25.2±0.06	26.9±0.15
Salinity	38.0±0.19	40.0±0.61
pH	7.47±0.01	6.55±0.11

*standard deviation : ±

nificance of environmental parameters between the two species. The RAK (Randomized Block Design) Test was used to determine the difference of percentage in degradation of phytoplankton in different treatments of diesel fuel concentration.

Discussion

Measurement of Water Quality Parameters

Measurement of water quality parameters is needed to determine the effect in the growth of microalgae. The results of the temperature, salinity, and pH measurements on the medium are shown in Table 1.

Marine waters are currently very susceptible to pollution from various types of contaminants. One of the pollutants most potential to damage the balance of ecosystems and biota life in marine waters is oil. Microalgae can be used as one of the pollutant biodegradation agents. Microalgae can absorb heavy metals, herbicides, insecticides and phenols (Ji *et al.*, 2014). The ability of microalgae in biodegradation (*Skeletonema costatum* and *Chetoceros calcitrans*) is influenced by environmental factors. One of the most significant environmental factors affecting microalgae biodegradation is salinity, pH, and temperature.

The results of measurements of environmental parameters on the culture medium of the four treatments on microalgae show no significant difference. The standard deviation of all environmental parameters shows that *Skeletonema costatum* species has greater standard deviation value than that of *Chetoceros calcitrans* species. This suggests the increasingly diverse data in *Skeletonema costatum* species over *Chetoceros calcitrans* species. The same thing also happened on the average data of environmental parameters of both species. The smallest standard deviation is ± 0.01 on pH for *Chetoceros calcitrans* species, whereas the largest standard deviation value is ± 0.61 on salinity for *Skeletonema costatum* species. The mean temperature of culture media in both species ranged from 25.2 to 26.9 °C, salinity ranged from 38 to 40 ppm and for pH values of 6.55 to 7.47. The result of t-test of environmental parameters of both species using 95% confidence interval showed that the average value of the three environmental parameters of both species show significant differences.

According to Jankowska *et al.* (2017), there are several factors that may affect the growth of microalgae, i.e. concentration and nutrient quality, CO_2, water flow, temperature (16-27 °C), water brightness, pH level (4 -11), density, salinity (12-40 g/L), turbidity, as well as biological factors. It is regardless of toxic factors, or heavy metals in the waters. As explained in Aslam *et al.* (2017), microalgae during the exponential growth phase can increase body biomass 2-fold under the ideal

Table 2. Results of Degradation of Diesel Fuel in Four Different Treatments

Concentration of Diesel Fuel	Initial Concentration of Diesel Fuel (mL)		Initial Concentration of Diesel Fuel		Concentration of Residue (%)		% Degradation	
	Species1	Species2	Species1	Species2	Species 1	Species 2	Species 1	Species 2
A	0	0	0	0	0	0	0	0
B	5	5	2.1	1.6	42	32	58	68
C	10	10	7.1	6.21	71	62	29	37.9
D	20	20	14.3	13.02	71.5	65	28.5	34.9

Note A: Diesel fuel concentration 0 ml B: Diesel fuel concentration 5 ml C: Diesel fuel concentration 10 ml D: Diesel fuel concentration 20 ml Species 1: *Skeletonema costatum*
Species 2: *Chetoceros calcitrans*

temperature, light, and nutrients, whereas in micro lagging activities, low pH or low salinity may lead to a condition an extreme adaptation due to the carbon content.

Microalgae Growth

Growth patterns on *Skeletonema costatum* and *Chaetoceros calcitrans* overall increased over the 7 days. The mean value of R2 (R-Square) of the *Chaetoceros calcitrans* species (0.9495) is very close to 1 when compared to the value of R2 of *Skeletonema costatum* (0.8115). This shows that the four treatments (control, the addition of 5 mL, 10 mL, and 20 mL diesel fuel) are more suitable to be applied to *Chaetoceros calcitrans* species.

Note: A: Diesel fuel concentration 0 mL B: Diesel fuel concentration 5 mL C: Diesel fuel concentration 10 mL D: Diesel fuel concentration 20 mL

According to Wang *et al*. (2016) phenol is one of the existing pollutant compounds in the aquatic environment. In normal conditions, the microalgae will have a low tolerance and relatively low growth under conditions of phenol concentration in sufficiently high waters. Microalgae can grow well without pollutant concentrations i.e. at phenol levels below 500 mg/L or at most 700 mg/L. The concentration of biomass produced at phenol concentration 500 mg/L was 3.4 g/L, while at the concentration of phenol 700 mg/L, the resulting biomass was 2.7 g/L.

Gravimetric Analysis

Gravimetric analysis is one of the methods used to determine the level of oil in waters. This study uses gravimetric analysis to determine the concentration of oil on bioremediation test media. The gravimetric method is the method used to determine the total fat present in the microalgae. Based on gravimetric analysis, we can determine microalgae potentially good in producing biodiesel, which determined based on result of optimum fat of microalgae.

The study shows that phytoplankton of *Chetoceros calcitrans* species could degrade diesel fuel with an average value of 46.9% when compared with *Skeletonema costatum* species with an average value of 38.5%. Of both species, the effectiveness of degradation began to occur in treatment C and D with the concentration of 10 mL and 20 mL diesel fuel. This suggests that diesel oil hydrocarbons with low concentrations are slightly easier to degrade than the high concentrations of 10 and 20 mL. The results of Randomized Block Design (RAK) showed that the treatment difference (oil concentration) gave a significantly different effect on percentage of degradation at 95% confidence interval. Different species also gave significant different effect to the percentage of degradation when tested using RAK at 90% confidence interval.

Biodegradation test results reveal that 1% oil-treated organisms have a decrease in oil content higher than 3% of oil concentration. This is because at 1% oil concentration the hydrocarbon amount is lower than at the 3% oil concentration, so the oil at 1% concentration is more easily broken down and utilized by the organism as an energy source. This means that hydrocarbon composition in the oil has an effect on the biodegradation process so that at higher concentrations it will take longer to degrade. According to Graham *et al*. (2017), some cases show the growth and production of microalgae in wastewater media is the same, even better, than that of laboratory media.

Conclusion

Chetoceros calcitrans effectively degrade oil diesel contamination compared to *Skeletenoma costatum* on controlled environment. Further more, enrichment the present of it can be benefit to the environment.

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