SCREENING OF INDIGENUS BACTERIA'S DEGRADING OF HYDROCARBON PETROLEUM FROM POLLUTED SEA AT BANDAR LAMPUNG'S DOCK

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ABSTRACT

Indonesia is committed to achieving the SDGs in 2030. One of SDGs is protect the marine ecosystem. Bandar Lampung which has a coastal area in a form of a dock for sea transportation and the anchoring of fishing boats. These area are starting to be polluted due to waste, one of which is the fuel waste of ships or fishing boats that are in the area. When the quality of sea water decreases due to pollution it will have an impact on population and diversity of marine life. If this happens the sea will be damaged and the income of fishermen will decrease. Various studies have been conducted to overcome problems caused by oil (petroleum hydrocarbons), especially research on the use of microbiology in remediating polluted environments. Therefore, this study was conducted to test P1 isolates isolated from sea water in docks around Bandar Lampung. The Measurement was carried out at The Integrated Laboratory of ITERA by observing the growth of the bacteria (population of bacteria) on selective media that had been added with diesel oil as much as 2% (v/v). This measurement was carried out on day 1, day 4 and day 7 after incunbation for population and for % TPH was measurement in the first day and the last day of incubation. The results obtained that the GT1 isolate have a good result in population and decreasing of TPH than the other isolate.

Keywords: *Sea water Pollution, Fuel waste, Coastal area, Bioremediation, Petroleum hyrocarbon*

1. INTRODUCTION

Indonesia has a wide sea area with various biota that live in it. Bandar Lampung has a coastal area and it supplies most of the demand for seafood in the city of Bandar Lampung. The activities of fishermen who are quite active in the area have a negative impact on the quality of sea water.

The pollution index value (IP) at Lampung bay in 2015 ranged from 2.96 - 7.25 which is categorized as low to moderate, TPI Lempasing has the highest IP value compared to other regions (Tugiyono, *et al.,* 2015). The quality of sea water has an impact on the biota that lives in it. When the quality of sea water decreases due to pollution, it will have an impact on the amount and diversity of marine biota. If this happens the fishermen's income will decrease.

Fisherman activities such as fishing or other activities can produce waste. Waste is one of the environmental problems and become the main concern now. The increasing in human population is also followed by increasing in amount of waste which produced due to human activities. Waste becomes a source of pollutants and even a source of disease that can endanger the health of humans and other living organisms and disturb the stability of environment.

Marine pollution due to waste containing hydrocarbon compounds such as petroleum or its derivative products is very common, especially in the port and dock areas. This pollution occurs because of several natural events or due to human activities such as ships/boats, oil terminals, piping failures and others (Santisi, *et al.*, 2015). Pollution due to hydrocarbon fuels is categorized as a major pollutant and its remediation in the environment is a global problem.

Now, Indonesia is one of the countries that supports the achievement of

Sustainable Development Goals (SDGs) in 2030. Marine pollution is contrary to the purpose of the 14th SDGs, which is to conserve and sustainably utilize marine, oceanic and maritime resources for sustainable development. Pollution due to fuel at sea can have a negative impact on marine life both directly and indirectly. The direct impact can be in the form of poisoning while the indirect effect can inhibit the growth of the biota itself (Macaulay and Rees, 2014). Therefore, pollution caused by fuel needs to be a concern.

Bioremediation is one method to restore the environment due to pollution of hydrocarbon compounds. This method utilizes microbes (bacteria and fungi) to degrade pollutants. Bioremediation is used as a way to overcome fuel pollution due to several reasons such as being environmentally friendly and more economical than other methods (Macaulay and Rees, 2014). Microbial degradation is one method that is suitable for dealing with pollution due to diesel fuel (Kauppi, 2011).

Utilization of microbial from the location of polluted sea (Indigenous microbial) is the main focus of this study because bioremediation can naturally occured. So, in sea water contaminated with fuel will be found microbes that have the ability to degrade hydrocarbon compounds. Research Santisi, *et al.* (2015) isolates bacteria from sea water polluted by crude oil and the isolates obtained proved to be able to degrade crude oil.

The coastal area of Bandar Lampung city has been a center for fish and other marine products since a long time a go. In addition, it also has a port and a dock which is the center of sea transportation. The high activities in that area cause pollution due to fuel and it has been going on for a long time. Therefor, this study was conducted to obtain indigenous isolates that have the ability to degrade hydrocarbon compounds (fuels) which have naturally adapted to environmental conditions in the region. In addition, through this research, it will be known how much the ability of isolates resulting from isolation in degrading hydrocarbon compounds.

Indonesia has a wide sea area with various biota that live in it. Bandar Lampung has a coastal area, most of its inhabitants use the sea as a source of livelihood and supply most of the demand for seafood in the city of Bandar Lampung. The activities of fishermen who are quite active in the area have a negative impact on the quality of sea water.

The pollution index value in Lampung Bay ranges from 2.96 - 7.25 which belongs to the category of mild to moderate polluted (Tugiyono, *et al.,* 2015). When the quality of sea water is good, various types of marine life will live there. However, when the quality of sea water decreases due to pollution it will have an impact on the decrease in the amount and diversity of marine biora. If this happens the fishermen's income will decrease.

Human activities such as fishing, fish processing or other activities can produce waste. Waste is one of the environmental problems and is the main concern at this time. The increase in human population is also followed by an increase in the amount of waste produced due to human activities. Waste becomes a source of pollutants and even a source of disease that can endanger the health of humans and other living things and disturb the stability of the environment.

Marine pollution due to waste containing hydrocarbon compounds such as petroleum or its derivative products is very common, especially in the harbor and dock areas. This pollution occurs because of several natural events or due to human activities such as ships / boats, oil terminals, piping failures and others (Santisi, *et al.*, 2015). In 2008, global demand for fuel was 85.62 million barrels / day so that pollution caused by hydrocarbon compounds was categorized as a major pollutant and

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Bioremediation is one method to restore the environment due to pollution of hydrocarbon compounds. This method utilizes microbes (bacteria and fungi) to degrade pollutants. Bioremediation is used as a way to overcome fuel pollution due to several reasons such as being environmentally friendly and more economical than other methods (Macaulay and Rees, 2014). Microbial degradation is one method that is suitable for dealing with pollution due to diesel fuel (Kauppi, 2011).

Utilization of microbial originating from the location of pollution (microbial indigenus) is the main focus of this study because bioremediation can naturally occur. So that in seawater contaminated with fuel will be found microbes that have the ability to degrade hydrocarbon compounds. Research Santisi, *et al.* (2015) isolates bacteria from seawater polluted by crude oil and the isolates obtained proved to be able to degrade crude oil.

The coastal city of Bandar Lampung, which has long been a center for fish and other seafood production. In addition, it also has a port and a pier which is the center of sea transportation. The high activity in the region so that pollution due to fuel has always occurred and has been going on for a long time. For this reason, this study was conducted to obtain indigenic isolates that have the ability to degrade hydrocarbon compounds (fuels) which have naturally adapted to environmental conditions in the region. In addition, through this research, it will be known the ability of isolates from isolation in degrading hydrocarbon compounds.

2. METHOD

This research was conducted in This research will be carried out in March - July 2019. Samples of sea water contaminated are taken from coastal areas in Bandar Lampung (Pulau Pasaran, Gudang Lelang and TPI Lempasing). Sea water sampling is carried out in the morning. Bacterial isolation indigenous and bacterial selection for the degradation of hydrocarbon compounds will be carried out in an integrated laboratory of Institut Teknologi Sumatera. Screening of potential bacteria is carried out by measurement of bacteria growth, biochemical, gram staining, hemolytic activity, oil spreading assay and biodegradation potential of diesel oil (Lee, *et al*., 2018).

2.1. Isolation and Growth of Bacteria

Isolation preparation is done by sterilizing tools and selective medium using an autoclave at 121^{o}C at pressure of 1 atm for 20 minutes. Isolation of bacteria degrading hydrocarbon compounds was carried out using Mineral Salt Medium pH 6.8 - 7.0. Diesel oil of 2% (v/v) was added in the media as a carbon source. Isolates from isolation inoculated in selective medium with diesel oil for 7 days and measurement of bacteria popullation was done in fourth and seventh days.

2.2. Degradation of Diesel Oil by Bacteria

Measurement of oil degradation is done using the gravimetric method.

Measurement of oil content is according to SNI 06 6989.10-2004. Oil content can be calculated as follows:

$$Oil\ Content\ \left(\frac{mg}{L}\right) = \frac{A - B}{ml\ sample}\ x\ 1000$$

Note :

A = vial bottle weight + extract (mg)

B = Empty vial bottle weight (mg)

to determine the percentage of biodegradation of oil that occurs can be determined by using :

$$\%\ TPH = \frac{Bmo - Bmn}{Bmo}\ x\ 100$$

Note:

% TPH = Percentage of Biodegradation

Bmo = Weight of initial petroleum (g)

Bmn = Weight of final petroleum (g)

2.3. Characterization of Bacteria

Characterization of bacteria was done by measurement of biochemical bacteria, gram staining and hemolytic activity.

3. RESULT AND DISCUSSION

3.1. Isolation and Growth of Bacteria

There are three isolates from isolation. GT1 Isolate was isolated from Gudang Lelang and TPI Lempasing, T1 isolate was isolated from TPI Lempasing and P2 was isolated from Pasaran Island.

The growth of bacteria was carried out by measurement of the isolates population on liquid minimum medium that had been added with diesel oil 2% (v/v). The results can be seen in Figure 1.

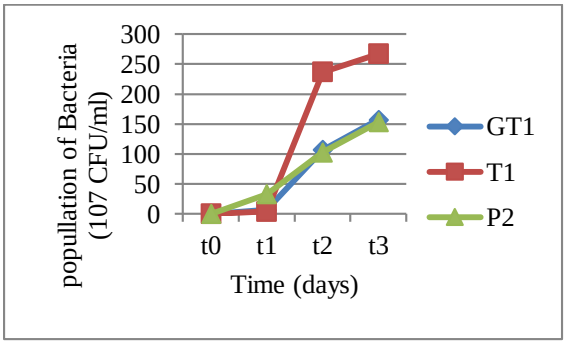

Figure 1. The Growth of bacterial on selective medium with diesel oil 2% (v/v) for 7 days (t_0 = day 0 of incubation, t_1 = day 1 of incubation, t_2 = day 4 of incubation, t_3 = day 7 of incubation)

Figure 1 shows that the growth of each isolate grown on selective medium added diesel oil increase in population over time. Although not all isolates show a significant increase. Based on this result, it can be seen that T1 isolates showed the best growth with the highest population (at t1 4 x 107 CFU / ml, t2 237 x 107 CFU / ml and t3 267 x 107 CFU / ml) compared to other isolates followed by isolates followed by GT1 (at t1 7 x 107 CFU / ml, t2 107 x 107 CFU / ml and t3 157 x 107 CFU / ml) and P2 (at t1 33 x 107 CFU / ml, t2 103 x 107 CFU / ml and t3 153 x 107 CFU / ml). 3 other isolates have good growth ability in selective medium with pollutants. There are several factors that influence bacterial growth and also the effectiveness of bioremediation including the diversity of existing microorganisms, the availability of contaminants to be degraded by microbes, environmental conditions, concentration and toxicity of pollutants (Ganjar and Kardena, 2014).

3.2. Degradation of Diesel Oil by Bacteria

Measurement of isolates's ability to degrade hydrocarbon compounds was carried out by measuring Total Petroleum Hydrocarbons (TPH) with gravimetric methods at the beginning (t_1) and end day of growth (t_7).

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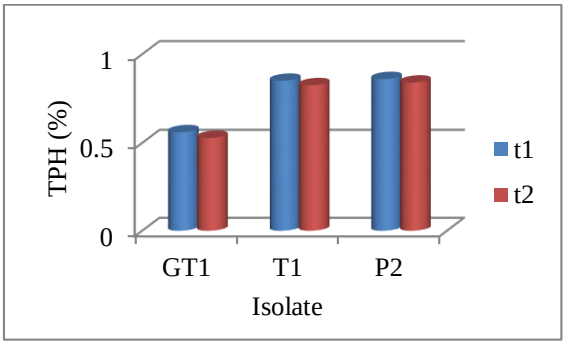


Figure 2. The bioegradation of 2% (v/v) diesel oil by Isolates (t_1 = the first day of incubation, t_2 = day 7 of incubation)

Based on Figure 2, it can be seen that GT1 isolate show the highest percentage of TPH reduction (3,2%) compared to the other isolates. These results are consistent with the results of the growth of the previous which show that GT1 isolate is one of isolates that have higher growth ability compared to other isolates.

Decreasing of Total Petroleum Hydrocarbon shows the ability of bacteria to degrade hydrocarbon compounds. Many bacteria are known to have the ability to degrade hydrocarbon compounds. *Bacillus cereus, Pseudomonas aeruginosa, Acinetobacter calcoaceticus,* and *Basillus subtilis* are examples of bacteria that can degrade hydrocarbon compounds (Manalu, *et al.*, 2016; Tian, et al., 2016). In addition, *Bacillus simplex* and *Bacillus firmus* are known to have a good ability to degrade various petroleum hydrocarbon products (Ganjar and Kardena, 2014).

Hydrocarbon compounds in petroleum are a source of carbon for the growth of microorganisms, so they can be degraded well (Handrianto, 2018).

3.3. Characterization of Bacteria

One isolate from three isolate was selected based on bacterial growth data on media with diesel oil and the decreasing of TPH that was successfully measured. one isolate showed the highest growth and decreasing of %TPH compared to other isolates.

Selected bacterial isolates were performed microscopic characterization and biochemical tests. Microscopic characterization of bacteria is done by gram staining so that it can be seen that the isolate is classified as gram positive or negative. The results of the test are shown in the following table.

Table 1. The results of characterization of GT1 Isolate

No.	Parameters	GT1 Isolate
1	Gram staining	-
2	Cell Form	Basil
4	Lysine	+
5	H2S	-
6	Glucose	+
7	Mannitol	+
8	Xylose	+
9	Indole	-
10	Urease	+
11	Citrate	+
12	Gelatin	-
13	Lactose	+
14	Sucrose	+
15	Hemolysis	ɣ

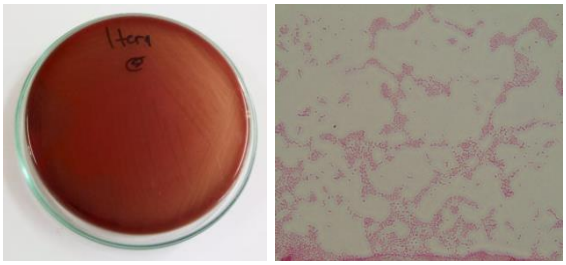
In the biochemical test of Triple Sugar Iron Agar (TSIA) against bacterial isolates, the isolate was able to ferment all carbohydrates (glucose, lactose and sucrose). In this research observation glucose test was found at the bottom (butt) of the media which was yellow (acid) while for the lactose and sucrose test was on the slope of the media (slant) which had a yellow color. According to Ismail (2015) if the TSIA media does not experience a yellow discoloration, then the bacteria cannot ferment carbohydrates. Conversely, if the media changes yellow color, the bacteria are able to ferment carbohydrates.

The observation of isolates by carrying out the Simon Citrate Agar (SCA) test resulted in a positive reaction, because the bacteria used citrate as a carbon source in its metabolism, which was shown by the change in the initial green media to turn blue. Positive reaction, bacteria have the ability to use citric acid as their carbon source.

Based on the results of hemolysis tests, GT1 isolate did not show ability to hemolysis in blood agar media because they did not show changes in color in the blood agar media (Gamma/ɣ Hemolysis). Gram

staining of GT1 shows that the isolate is gram negative bacteria.

Figure 3. ɣ Hemolysis (left) and gram staining (Right) of GT1 isolate



4. CONCLUSION

The results obtained that the T1 (at t1 4 x 10^7 CFU / ml, t2 237 x 10^7 CFU / ml dan t3 267 x 10^7 CFU / ml) dan GT1 isolates was the highest population GT1 (at t1 7 x 10^7 CFU / ml, t2 107 x 10^7 CFU / ml and t3 157 x 10^7 CFU / ml) and decreasing of TPH (3,2%) GT1 isolate was also higher than the other isolate.

5. ACKNOWLEDGE

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