Hydrocarbon-Degrading Bacteria’s Selection from Soil Contaminated Engine Oil Waste of South Lampung

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Abstract. Waste containing hydrocarbon compounds is classified as hazardous and toxic waste. Waste management must be done properly to prevent environmental pollution that ultimately has an impact on environmental damage. Engine oil is one of the hydrocarbon compound products that are widely used by motor vehicles. Waste engine oil from automotive repair shops has not been properly managed because waste engine oil is often thrown away on the ground around the workshop. This condition has been going on for a long time due to soil contamination by waste engine oil. Thus, most microbes cannot live in polluted soils. Isolation of microorganisms in polluted soils is carried out to obtain bacteria that can grow in hydrocarbon-contaminated soils and have the potential to degrade hydrocarbon compounds. After isolation bacteria from two selected locations (Way Hui and Way Kandis), this research got four bacteria. These four isolates were selected by growing on selective media which added with engine oil with a concentration of 2% for 7 days. Selection is done by counting the population of each isolate that lives in the media. Bacteria population was calculated on t1 (day 1), t4 (day 4) and on t7 (day 7). In addition, the selection is also done by calculating TPH (Total Petroleum Hydrocarbon) at t1 and t7. From these stages, it got one isolate (B7) which showed the highest results in population and three isolates showed the highest TPH decreasing (B1, B6 and B7).

Keywords: environmental, isolation, microorganisms, TPH, petroleum hydrocarbon

1. Introduction

Petroleum, gasoline, diesel, engine oil and other hydrocarbons are hazardous and toxic substances. Used engine oil is one of the many wastes generated both in industry and other business activities. Motorcycle repair shop is one of the producers of used lubricating oil waste because in a certain period the motor must replace the engine oil so that the waste of used engine oil is generated quite a lot.

Waste oil used as a hazardous and toxic waste should be managed in accordance with government regulation no. 101 of 2014 [1]. Waste of used engine oil must be managed by those who have management permits such as storage, collector, beneficiary, processor or landfill. However, used engine oil waste generated by the motorbike repair shop is still not well managed [2][3]. Most of the workshops in South Sumatera still dump their used engine oil waste to the ground around the workshop.

Poor management of hazardous and toxic waste can adversely affect the environment, one of which is soil pollution. The characteristics of hazardous and toxic waste cause a significant impact on the environment. Land that has a function as a place to live for various living things can be disrupted. Prolonged soil pollution can also affect groundwater so that it can affect the health of humans and other living things [3].

Pollution of land around the motor and car repair shops by waste of used engine oil for a long time resulted in the soil no longer have the same characteristics as the uncontaminated land. Microbes in contaminated soil are not as much as microbes in uncontaminated soil. Not only are the numbers different, but the types of microbes that live are also different. This is because not all microbes can live in these critical conditions. Several studies have found that microbes can live in soils contaminated with hydrocarbon compounds and are able to utilize hydrocarbon compounds as a source of food and energy [4] [5]. Microbes isolated from contaminated soil hydrocarbon compounds can be used as biological agents in the remediation of polluted soils through bioremediation techniques. Therefore, the selection of microbes that have the ability to degrade hydrocarbon compounds in polluted soils originating from this workshop needs to be done in order to obtain superior isolates that have a high degradation ability and reproductive ability.

2. Method

2.1. Sampling
This study was conducted from April 2019 in the Integrated Laboratory of the Sumatra Institute of Technology. Sampling was done by purposive sampling of soil that was contaminated with used engine oil from an automotive repair shop around ITERA in two locations (Way Kandis and Way Hui). Sampling locations selected that have been polluted for more than 5 years. Soil samples were taken with criteria, such as oily, black and thick oily odor. Tools and materials needed such as glass tools, ose, bacterial selective medium, cotton, aluminum foil, autoclave, incubator, etc.

2.2. Isolation of Bacteria
The bacteria were isolated from soil samples by the enrichment culture technique using engine oil waste as a carbon source. Counts of heterotrophic bacterial were determined by plating serially diluted samples on Bushnell-Hass agar medium. Oil-degrading bacteria were enumerated on the Bushnell-Hass medium using engine oil as carbon source as previously reported. 10 g of oil-contaminated soil sample was taken, gradual dilution was carried out to 10^-7. After that, 1 ml of dilution was taken using a micropipette to be inoculated by pour plate on and Bushnell-Hass agar modified with the addition of oil, tween, and then incubated at 37°C for 72 hours. Observed the presence of bacteria on medium.

2.3. Selection of Bacteria
Four isolates, B1, B3, B6 dan B7 were inoculated in 100 ml Bushnell-Hass medium contained 2% of engine oil. Then, incubated and shaker for 7 days. The most number of bacteria population were selected as the candidate of oil degrading bacteria. Measurement was done at 1 day, day 4 and day 7 after inoculation.

3. Results and Discussion

3.1. Bacterial Selection
The selection of bacteria was done by using the data of the bacteria population and the ability to degrade the hydrocarbon compound. The isolate which had the highest population and decreasing of Total Petroleum Hydrocarbon was selected as the superior isolate.

3.2. The Growth Rate of Bacteria in Medium Contaminated Engine Oil Waste
There are four bacteria which isolated from soil contaminated engine oil in Way Kandis and Way Hui. The four bacteria were culture in liquid medium added 2% (v/v) engine oil for one week. The growth of bacteria measured by measuring bacteria population using the pour plate method. The result of this measurement is shown in figure 1.
Figure 1 shows that B7 isolate has the ability to grow higher than other isolates. It also showed that B7 isolate had the ability to grow in extreme condition. But, the population of B7 isolate decreasing from \( t_1 \) (324 x 10\(^7\) CFU/ml) to \( t_7 \) (279 x 10\(^7\) CFU/ml). This number was still higher than the others isolates. The reason for the bacteria population decreasing maybe because the isolate has passed the log phase or the isolate need to adapted in this extreme medium.

Microbes that have the ability to degrade hydrocarbon compounds are widely distributed both in soil and in water, but degrading microorganisms may not necessarily be present in sufficient quantities in these polluted areas [4]. Hydrocarbon degrading microbes can be used as bioremediation agents for the protection of soil and water against hydrocarbon waste pollution [5] [6].

### 3.3. The Decreasing of Total Petroleum Hydrocarbon

Isolate culture in Bushnell Haas liquid medium added 2% (v/v) engine oil waste for seven days. The total petroleum hydrocarbon had been measured on the first day of cultured (\( t_1 \)) and the last day of cultured (\( t_7 \)). The result of a measurement can be seen in Table 1.

**Table 1.** The result of Total Petroleum Hydrocarbon (TPH) measurement from the medium containing engine oil

| Isolate | \( t_1 \) | \( t_7 \) | % TPH decreasing |
|---------|---------|---------|-----------------|
| B1      | 0.740   | 0.730   | 1.0%            |
| B3      | 0.290   | 0.285   | 0.5%            |
| B6      | 0.740   | 0.735   | 0.5%            |
| B7      | 0.870   | 0.855   | 1.5%            |

\*\( t_1 \) = population of bacteria at day 1 after inoculation
\( t_7 \) = population of bacteria at day 7 after inoculation

Table 1 shows that the ability of isolates in degrading hydrocarbon compounds was low and relatively same for all isolates. The decreasing of TPH showed the ability of bacteria in degrading offhydrocarbon compound because bacteria can use hydrocarbon compound from oil as carbon source and also the source of their energy. But, it is not every bacteria have that ability. This result showed the low ability of bacteria in decreasing oil (hydrocarbon compound). The low ability to degrade hydrocarbon compounds is influenced by several things. In addition to the nutrient aspect, several other environmental factors also play a role in optimizing the degradation of contaminants [7].

The growth of bacteria shows that B7 isolate had the highest population than the others and the decreasing of total petroleum hydrocarbon also had a good result than some others isolate. So, from these results B7 isolate was selected because it is can be adapted in extreme conditions and had the ability to degraded hydrocarbon compounds.
4. Conclusion

Bacteria population was calculated on t1 (day 1), t4 (day 4) and on t7 (day 7). In addition, the selection is also done by calculating TPH (Total Petroleum Hydrocarbon) at t1 and t7. From these stages, it got one isolate (B7) which showed the highest results in population and three isolates showed the highest TPH decreasing (B1, B6, and B7).

5. Acknowledgements

The authors would like to appreciate to Institut Teknologi Sumatera’s grant “ITERA Smart” for financial support to present this study.

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