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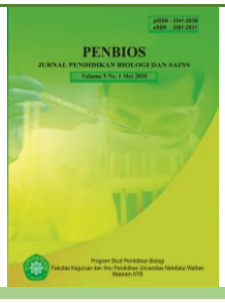

Research Article

Isolation and Identification of Hydrocarbon Degrading Bacteria From Used Lubricant Oil

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ABSTRACT

Hydrocarbons are organic compounds consisting of carbon and hydrogen. Petroleum in particularly lubricant oil is a group of hydrocarbon compounds which can be grouped into aliphatic, alicyclic, and aromatic hydrocarbons. The use of oil as a lubricant will leaving used lumbricant oil that if not managed properly will cause environmental pollution. This research aims to isolate and identifying of hydrocarbon degrading bacteria from used lubricant oil. Type of research is descriptve explorative with qualitative approach. Isolation is done by adding the sample of used lubricant oil into the NA medium (Natrient Agar) by observing the clear zone formed. Bacterial identification is done by observing morphology and biochemical activity. Four isolates were obtain and two of them were selected to identification are isolate I and III. The result from bacterial identification was performed based on morphological and biochemical characterization show isolate I is *Bacillus brevis* and isolate III is *Bacillus macerans*. These two isolates were from the genus Bacillus which is known to be quite good at degrading petroleum.

INTRODUCTION

Oil is a product of petroleum processing that is rich in aliphatic and aromatic hydrocarbons (Ahda & Fitri, 2017). This oil is usually used by the community in terms of transportation to support daily activities. In Indonesia, the use of motorized vehicles has increased every year. Based on the Central Bureau of Statistics, the number of motorized vehicles in Indonesia from 2011 to 2013 increased by about 23% from 68,839,341 to 84,732,652 vehicles (BPS, 2013). Meanwhile, in West Nusa Tenggara Province the number of motorized vehicles also increased from 2013 to 2014 by 5.3% from 1,278,620 to

1,347,545 vehicles (NTB, 2015). This shows that the need for lubricating oil or oil also increases every year.

The increase in the need for lubricating oil or oil will increase the amount of residue or waste from the lubricating oil or oil. Lubricating oil or oil that has been used and not reused will usually be placed in storage drums which will then be taken by used oil collectors to be reprocessed into asphalt mixture for road repair projects. The storage and management of used oil must be carefully considered so as not to harm and harm living things and the surrounding environment because the hydrocarbon compounds contained in used oil are difficult to decompose. When hydrocarbon compounds are on the soil surface and for a long time these compounds will settle as toxic substances. This will cause disruption of the ecosystem and the water cycle in the soil (Karwati, 2009). However, the management and utilization of used oil require a fairly expensive cost so that only a few industries can carry it out (Ratman, 2010). Therefore, it is necessary to use an environmentally friendly and inexpensive method of managing used oil, namely biologically one of them by utilizing microbes such as bacteria.

Bacteria are living things that are abundant in nature and can live in almost all habitats. Each bacterium has its own characteristics. The characteristics of bacteria that can degrade hydrocarbons contained in used oil are bacteria that have the ability to utilize hydrocarbon compounds as a source of energy needed for their growth (Aliyanta et al., 2012). The process of degradation using the help of microbes is relatively easy with low cost and environmentally friendly (Hafiluddin, 2011).

Previous research conducted by Nusyirwani and Amolle succeeded in isolating three types of bacteria from Dumai waters that have potential as petroleum bioremediation agents, namely Providencia vermicola, Burkholderia cepacia and Myroides odoratimimus (Copper, 2010). There is also a study conducted by Elyza who succeeded in characterizing two bacterial isolates that have the potential as petroleum bioremediation agents, namely Pseudomonas alcaligens and Alcaligens facealis (Mukharomah et al., 2016). Feliatra in Nusyirwani and Amolle succeeded in isolating and identifying bacteria that have the potential to degrade petroleum from the waters of the Malacca strait, namely Acinetobacter sp., Arthrobacter sp., Micrococcus sp., Pseudomonas sp., Bacillus sp. Meanwhile, Arsanti in Nusyirwani and Amolle also found potential bacteria for petroleum bioremediation such as Azotobacter sp., Alcaligens sp., Chromobacterium sp., Planococcus sp., and Micrococcus sp (Copper, 2010).

This study aims to isolate and identify hydrocarbon-degrading bacteria from used oil. The result of this research is the availability of pure isolates of hydrocarbonoclastic bacteria on a laboratory scale.

METHOD

1. Sampling Location

Used oil samples were taken from CV Wahyu Nusantara Indah, Pagutan Village, Ampenan District, Mataram City. Sampling was carried out on September 6, 2017.

2. Used Oil Sampling

Used oil samples were taken from CV Wahyu Nusantara Indah, Pagutan Village, Ampenan District, Mataram City. A sampling of used oil uses a purposive sampling tech-

nique with the criterion of the longest remaining used oil.

3. Used Oil Bacteria Isolation

The isolation technique used is the pour plate technique (Yolantika et al., 2015). A total of 1 ml of used oil sample was inoculated into NA selective medium. Incubation was carried out for 120 hours at 37^0C. Then observe the clear zone formed.

4. Used Oil Bacteria Purification

After incubation, the representative bacterial colonies isolated were streaked on NA medium in a petri dish using the quadrant streak method. The clear zone formed around the isolate is an indicator of the ability of bacteria to degrade hydrocarbons (Permana et al., 2016). The isolates to be used for biochemical characterization and hydrocarbon analysis were selected based on the similarity of the morphology of the colony.

5. Bacteria Identification

Bacterial colonies were inoculated by means of a quadrant streak plate on NA in a petri dish, then incubated at 37°C for 24 hours. Macroscopic observations on NA medium in petridishes included colony shape, colony color, colony edge, texture, and surface. Observation of microscopic morphology by observing cell shape, gram, cell length, distribution, endospore, and spore. While observing the biochemical characteristics by conducting several tests, namely motility test, aerobic growth, H2S production, citrate use test, urea test, glucose test, sucrose test, lactose test, mannitol test, indole production test, methyl red test, Voges Proskauer test, test starch, 6.5% NaCl test, and catalase test. The results of macroscopic, microscopic, and biochemical observations were then compared with the flowchart of Bergey's Manual of Determinative Bacteriology.

6. Data analysis

The data obtained were presented descriptively based on macroscopic, microscopic, and biochemical characteristics. The data obtained are presented in the form of figures and tables.

RESULTS AND DISCUSSION

1. Isolation of Hydrocarbon Degrading Bacteria

A sampling of used oil was carried out at the used oil stockpile of CV Wahyu Nusantara Indah, Mataram city by using a purposive sampling technique with criteria for used oil that had been stockpiled for a long time. The medium used in the isolation stage was NA medium as a source of bacterial nutrition.

Based on the isolation results obtained 4 isolates that can be isolated from used oil and named isolates I, II, III, and IV. The indicator for taking bacterial isolates is in the presence of a degradation zone in the form of a clear zone (**Figure 3.1**). The formation of a clear zone means that bacteria are able to degrade used oil (hydrocarbons). Bacteria capable of degrading hydrocarbons have the ability to produce biosurfactants. Biosurfactants are a group of molecules that have surface-active properties. This property is due to biosurfactants are complex molecules that have hydrophilic and hydrophobic groups, so that they can reduce the surface tension in the space between water and oil. Biosurfactants can play a role in dissolving hydrophobic compounds such as petroleum by forming micelle structures. This causes the level of dispersion and emulsification of petro-

leum to increase in water (Permana et al., 2016).

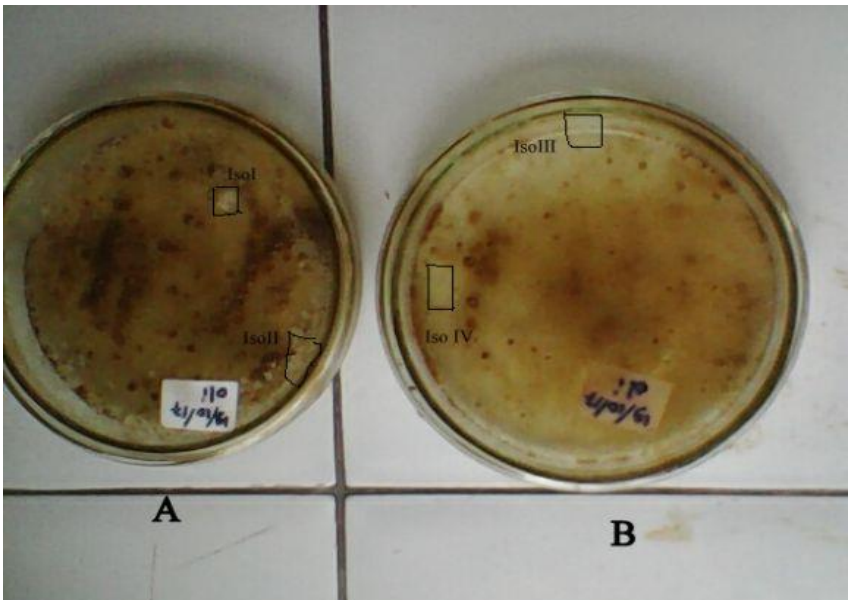

Figure 3.1 Formation of a degradation zone (A: isolates I & II, B: isolates III & IV)

2. Macroscopic Morphological Characteristics

The four isolates were then transferred to NA (Nutrient Agar) medium. The selection was carried out on 4 bacterial isolates obtained to select isolates that have the potential to degrade hydrocarbons. The selection of these isolates was carried out based on the morphological character of the bacterial isolates, namely the shape of the colony. The colony formation of the four isolates can be seen in the following **Table 3.1.**

Table 3.1 Macroscopic Observation Of Isolates I-IV

Bacteria Identification	Isolate I	Isolate II	Isolate III	Isolate IV
Form	Medium-small round	Medium-small round	Medium round	Medium round

Based on the observation of the colony morphology of the isolates, it was found that the colony forms of isolates I and II had small-medium round colonies, and isolates III and IV had medium-spherical colonies. As many as two of the four isolates were obtained, each colony shape was medium-small (Isolate I) and medium spherical (Isolate III). Morphological observations of the two isolates were as follows (**Table 3.2**).

Table 3.2 Observation Of Macroscopic Morphology Of Isolates I and III

Bacteria Identification	Isolate I	Isolate III
Form	Medium-small round	Medium round
Colour	Cream-White	Cream-White
Texture	Strongly attached to the media	Strongly attached to the media
Edge	Uneven	Uneven
Surface	Flat (dry at the center of gathering)	Uneven (dry)

Morphological observations were carried out on isolates I and III aged 24 hours, on NA (Nutrient Agar) medium at room temperature (**Figure 3.2**). The results of macroscopic observations showed that isolate I had a medium-small round shape, a cream-white color, a texture that adhered strongly to the media, uneven edges, and a flat surface with dry in the middle and clumped together. In isolate III, the results of macroscopic observations were a medium round shape, cream-white color, texture firmly attached to the media, uneven edges, uneven and dry surface.

Figure 3.2 Macroscopic observations (A: isolate I, B: isolate III) on NA medium in petri dishes

3. Microscopic Morphological Characteristics

Table 3.3 shows the microscopic characteristics of isolates I and III. Isolate I had a medium fat rod shape, gram-positive with a cell length of 2.62 m, the cells were spread, had spores and endospores. Isolate III had microscopic characteristics, namely the shape of the stem cells were slender, gram-positive with a cell length of 2.8 m, the cells were spread, had spores and endospores.

Table 3.3 Microscopic Observation Of Isolates I and III

No.	Bacteria Identification	Isolate I	Isolate III
	Morphological Characteristics Microscopic		
1.	Cell shape	Stems are fat	Stem is slender
2.	Gram	+	+
3.	cell length	2,62 µm	2,82µm
4.	Deployment	Spread	Spread
5.	Endospore	Available	Available
6.	Spore	Available	Available

4. Biochemical Characteristics of Hydrocarbon Degrading Bacteria

Table 3.4 shows the biochemical activity of isolates I and III. In isolate I grew under aerobic, motile conditions, the H2S test, glucose, sucrose, starch, 6.5% NaCl and catalase were positive. Negative results were seen in the use of citrate, urea, lactose,

mannitol, indole, methyl red and Voges Proskauer tests. Meanwhile, isolate III showed that the biochemical activity of isolate III grew under aerobic, motile conditions, H2S test, glucose, sucrose, starch, mannitol test, 6.5% NaCl, and catalase test were positive. Negative results were seen in the use of citrate, urea, indole test, methyl red test, and Voges Proskauer test.

Table 3.4 Observation Of Biochemical Activity

No.	Bacteria Identification	Isolate I	Isolate III
Biochemical Characteristics			
1.	Motility Test	+	+
2.	Aerobic Growth	+	+
3.	H2S Produksi Production	-	-
4.	Citrate use test	-	-
5.	Urea	-	-
6.	Glucose	+	+
7.	Sucrose	+	+
8.	Lactose	-	-
9.	Mannitol	-	+
10.	Indo Production	-	-
11.	Methyl Red Uji Test	-	-
12.	Voges Proskauer Test	-	-
13.	Starch	+	+
14.	6.5% NaCl	+	+
15.	Catalase Test	+	+

Description: +/- = positive/negative reaction

Based on macroscopic, microscopic, and biochemical morphological characteristics, the two isolates were *Bacillus brevis* and *Bacillus macerans* bacteria. Various species of Bacillus have been known to grow well at thermophilic temperatures. *Bacillus brevis* and *Bacillus macerans* are two species of the genus Bacillus. *Bacillus brevis* is one type of the genus Bacillus which is known to be quite good at degrading petroleum (Pikoli et al., 2000). In addition, *Bacillus brevis* is also a biosurfactant so that it is able to grow on oil and is able to emulsify it. At optimum conditions, *Bacillus brevis* is able to produce biosurfactants up to 79.96% with an emulsification level of 71.89 ± 0.56% (Mouafi et al., 2016).

Bacillus macerans in Bergey's Manual of Systematic Bacteriology 2nd 2004 changed its name to *Paenibacillus macerans*, but is still in Class Bacilli. Several studies on *Paenibacillus macerans*, among others, state that *Paenibacillus macerans* has biosurfactant ability. This biosurfactant was able to reduce the surface tension of water from 72.30 to 35.34 mN/m at a concentration of 2.76 g/L and achieved an emulsification index of 56% after a 24-hour reaction with engine oil (Liang et al., 2014).

CONCLUSION

Based on the results of the isolation and identification of hydrocarbon-degrading bacteria from used oil, it can be concluded that the two isolates were *Bacillus brevis* and *Bacillus macerans.*

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