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Original research

Isolation and Morphological Characterization of Oil Degrading Bacteria from Waste Cooking Oil Contaminated Soil

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Abstract:
Oil is used as an important component in human life as an energy source. The fried oil gives a delicious taste for consumption. However, the remaining cooking oil that has been cooked is discarded in the surrounding environment which is the cause of soil contamination by the oil. Thus, bioremediation is carried out to degrade contaminants with the ability of microorganisms or macroorganisms into non-toxic compounds. The purpose of this research was to isolate and characterize the morphology of hydrocarbon-degrading bacteria from the environment or soil that had used cooking oil spills. The research method used was purposive sampling and descriptive qualitative. The results of the research obtained that two bacterial isolates had a round shape, optically opaque, and flat edges. BCO-1 isolate has a brown color with a flat elevation and BCO-2 is white with a convex elevation. Bacterial isolate BCO-1 had a change in oil height of 0.81 cm, while isolate BCO-2 had 0.88 cm. This indicates that bacteria in BCO-1 isolates had a significant ability to degrade used cooking oil that was contaminated in the soil compared to BCO-2 isolates based on changes in the level of oil fluid.

Keywords: Bacteria Degrading, Bioremediation, Cooking Oil

Introduction

Cooking oil is an important part of the human diet and has various nutritional functions. Cooking oil is a high-density energy source (9 calories/g) that helps increase the calorie density of foods. Cooking oil is an oil that has undergone a purification process such as degumming, neutralization, bleaching, and deodorization (Gargouri *et al.,* 2014; Yolantika *et al.*, 2015).

In food processing, cooking oils can be used as heat conductors, flavor enhancers, improve food texture, and increase nutritional value. The use of cooking oil for daily cooking is inherent in the community because fried foods have a better taste compared to foods that are boiled, steamed, or burned (Amalia *et al.*, 2010; Yolantika *et al.*, 2015; Galanakis, 2020). This makes most people, especially housewives and food sellers rely on cooking oil.

Leftover cooking oil, also known as wasted cooking oil has not received special attention from the public. The behavior of people who tend to repeatedly use cooking oil or throw inappropriate oil into waterways or yards (Al-Hawash *et al.*, 2018). This harms health and the environment because repeated use of cooking oil can cause cancer and narrowing of blood vessels, which can lead to coronary heart disease, stroke, and high blood pressure. At the same time, dumping waste cooking oil into waterways or yards can cause water pollution and soil fertility (Afianti *et al.*, 2019; Al-Hawash *et al.*, 2018; Amalia *et al.*, 2010). Soil contamination by hydrocarbons can cause damage to local ecosystems because these compounds accumulate in plants and animals around contaminated sites (Luo *et al.*, 2013; Palanisamy *et al.*, 2014; Iravanian and Ravari, 2020). Therefore, it is necessary to work hard to overcome the problem of wasted cooking oil that pollutes the soil. One of the efforts that can be done is to use bioremediation.

Bioremediation is a method that uses the ability of microorganisms or large organisms to transform into other forms of non-toxic and harmful compounds to degrade pollutants in certain media (Rahayu *et al.*, 2019; Novianty *et al.*, 2020; Hossain *et al.*, 2022). In nature, microbes live or congregate in media such as soil, water, air, animal waste, garbage, plants, animals, and humans (Pi *et al.*, 2018; Yan *et al.*, 2013). Examples of bacteria that can degrade hydrocarbons include *Pseudomonas, Cammonas, Enterococcus, Entrobacter, Acinetobacter, Sphingomyces, Bacillus, Rhodococcus, Providencia,* and *Citrobacter* (Hossain *et al.*, 2022; Rahayu *et al.*, 2019; Ghoreishi *et al.,* 2017; Moorthy, 2010; Cai *et al.*, 2021). These hydrocarbon bacteria can live on contaminated soil because these bacteria have lipase enzymes that can use or consume substrates (contamination) as materials to live in polluted soil. Unfortunately, there is no monitoring of soil pollution due to wasted cooking oil. Thus, it is hoped that microbial isolates will emerge that can be the key to bioremediation in polluted soil. Therefore, the purpose of the research was to isolate and determine the morphological characteristics of hydrocarbon-degrading bacteria from the environment or soil containing wasted cooking oil spills.

Material and Methods

The method used in this research is purposive sampling and descriptive qualitative. Purposive sampling was done by determining where the sample will be taken on soil contaminated with wasted cooking oil. Qualitative description by describing the morphology of bacteria degrading through the characteristics of the samples obtained. In this study, tools and materials were prepared in the form of Erlenmeyer, shaker, test tube, test tube rack, ruler, micropipette, LAF (Laminar Air Flow), Ose needle, Petri dish, micropipette tip, mineral salt media (MSM), NA (Nutrient Agar) media, and soil samples from the environment contaminated with wasted cooking oil in the Street Market Area Cipayung District, East Jakarta, Indonesia.

Soil microbes were isolated by taking soil samples aseptically from an environment contaminated with wasted cooking oil at depth of 5-10 cm. Mineral salt media (MSM) was prepared with the composition (g/l): K_2HPO_4, 0.8 g; KH_2PO_4, 0.2 g; $CaCl_2$, 0.05 g; $MgCl_2$, 0.5 g; $FeCl_2$, 0.01 g; $(NH_4)_2SO_4$, 1.0 g; NaCl, 5.0 g (Saimmai *et al.*, 2012). Then, an enrichment culture was made by inserting 10 grams of soil sample into MSM media and added 1% used cooking oil aseptically. The mixture was incubated for five days in a shaker at room temperature, 30^{o}C. Microbial isolation was performed from enrichment culture on mineral salt media (MSM) with 1% used cooking oil addition. Bacterial culture then incubated at 30 ^{o}C for 48 hours. Bacteria was purified and made a pure culture of bacterial isolates on NA media. The morphological characteristics of the oil degrading bacteria were observed and the ability to degrade hydrocarbon was tested by adding 1 ml of used cooking oil into 9 ml of liquid MSM culture (7 days) in a test tube. The height of the wasted cooking oil in the test tube was measured using a ruler. One Ose of each bacterial isolate was taken and incubated for seven days at room temperature, 28^{o}C. Observations were made for a week by measuring the height of the wasted oil.

Results and Discussion

Isolation of samples contaminated with wasted cooking oil obtained two pure isolates with codes BCO-1 and BCO-2. The naming BCO isolate stands for Bioremediation of Cooking Oil. The two pure isolates were observed for their morphological characteristics. Morphological characterization aims to observe both colony morphology and bacterial cell morphology in bacterial isolates that have passed the selection. Microorganisms grown on various media will show different macroscopic appearances in their growth (Cappucino and Sherman, 2020). These differences are called culture characteristics, which are used as the basis for separating microorganisms into taxonomic groups (Manalu *et al.*, 2016; Saibdaingsih *et al.*, 2013). The bacterial isolates were observed for colony morphology by observing colony shape, color, optics, edges, and elevation. Observation of colony morphology is shown in Table 1.

Table 1. Morphological characteristics of bacteria in Petri dish from soil contaminated with wasted cooking oil

Isolate	Shape	Elevation	Optics	Pigmentation	Edge
BCO-1	Round	Flat	Opaque	Brown	Entire
BCO-2	Round	Convex	Opaque	White	Entire

Based on the results of morphological observations from Petri dish, it showed a round shape, flat and convex elevation, optically opaque, and entire colony edges. Pigmentation on isolates BCO-1 is brown and BCO-2 is white which can be seen in Figure 1. These character bacteria isolates based on Cappucino and Sherman (2020) state that the general shape of bacterial colonies is circular, irregular, filamentous, and rhizoid. The elevations are raised, convex, flat, umbonate, and crateriform. The edges are entire, undulate, filiform, curled, and lobate. The ability of bacteria to degrade wasted cooking oil from polluted soil in BCO-1 isolates showed a significant change in oil height degrading used cooking oil compared to BCO-2 isolates which can be seen in Table 2. Turbidity in BCO-1 isolates showed more turbidity than in BCO-2 isolates. BCO-2 isolate after shaking which can be seen in Figure 2. This is due to the activity of enzymes in bacteria that can degrade the oil.

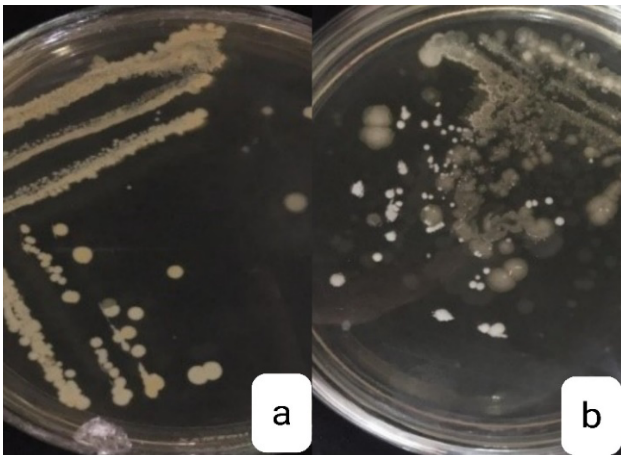

Figure 1. Bacterial isolate a) BCO-1 and b) BCO-2

Enzymes that can degrade the oil are found in the research results of (Elyza *et al.*, 2015) which stated that the decrease in oil content was due to the activity of lipolytic bacteria in utilizing CPO contained in the media as an energy source. Lipase enzyme is important for bacteria in the biodegradation of oil-contaminated land. The lipase enzyme is used by bacteria to be in direct contact with a substrate containing lipids, this oil will be used by bacteria as a carbon source for their growth. The presence of this lipase enzyme depends on various factors, which is this factor that determines the presence of the lipase enzyme work (Davoodi *et al.*, 2020; McGenity, 2014; Pi *et al.*, 2016).

Table 2. The ability of bacteria to degrade wasted cooking oil

Isolate	Change of Oil Height (cm) (Mean±SE)
BCO-1	0,81±0,10
BCO-2	0,88±0,10

Both of these bacteria can degrade wasted cooking oil. However, based on Table 2, BCO-1 isolate is better in wasted cooking oil degradation activity than BCO-2 isolate. Because of the differences in oil degradation observed at the level of oil fluid. Besides that, various factors can affect the work of enzymes in bacteria. Enzyme activity is influenced by substrate concentration, enzyme concentration, isolate, and pH. While the enzyme reaction speed is influenced by enzyme work, the more enzyme concentration, the faster the reaction, so more substrates are used (Vitolo, 2020; Robinson, 2015).

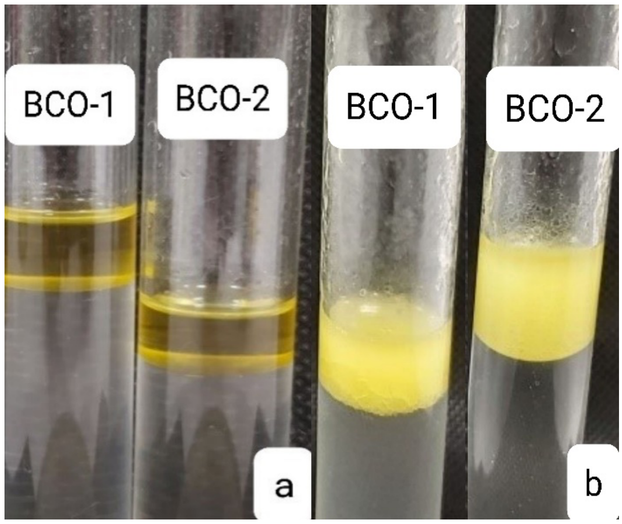

Figure 2. The ability to degrade oil by bacteria under condition of a) before degradation and b) after degradation

Changes in oil height indicate the decomposition of the substrate which in this case is used as wasted cooking oil found in soil contaminated by bacterial isolates with lipase enzymes through their metabolic pathways (Manalu *et al.*, 2013; Biswas *et al.*, 2016). Thus, wasted cooking oil can decrease along with the decomposition of the substrate. The first step in the biodegradation process by bacterial metabolism is that the substrate enters the cell membrane through a diffusion mechanism (Yan *et al.*, 2013; Okino-Delgado *et al.*, 2017; Somboon *et al.*, 2020). Macromolecules will be retained in the cell wall and some are degraded by exoenzymes released through the cell wall. Furthermore, reactions occur in the cell cytoplasm to decompose organic compounds (Handrianto, 2018; Novianty *et al.*, 2020).

Conclusion

Isolation of bacteria degrading from soil contaminated with wasted cooking oil obtained two pure isolates, which are BCO-1 and BCO-2. The morphological characterization of these bacterial isolate has a round shape, optically opaque, and flat edges with brown color on BCO-1 and white on BCO-2. Both isolates can degrade wasted cooking oil based on changes in the liquid height of the oil.

Author Contribution

Imam Safir Alwan Nurza: Conceptualization, Formal analysis, Methodology, Validation, Visualization.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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