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Diesel Fuel Degradatiom Activity Of Hydrocarbon-Degrading Bacteria From Nusantara Fisheries Port Brondong Lamongan

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ABSTRAK

Pelabuhan Perikanan Nusantara (PPN) Brondong Kabupaten Lamongan merupakan pelabuhan dengan aktivitas kapal yang tinggi. Hal ini menyebabkan pencemaran hidrokarbon berupa bahan bakar disel maupun oli yang terjadi di perairan PPN Brondong. Usaha awal yang dapat dilakukan dalam penanganan pencemaran adalah dengan melakukan isolasi dan karakterisasi bakteri pengurai hidrokarbon yang hidup di perairan tersebut. Penelitian ini merupakan langkah strategis dalam penanggulangan tumpahan hidrokarbon petroleum di perairan tersebut melalui proses bioremediasi, baik secara bioaugmentasi maupun biostimulasi untuk penelitian lebih lanjut. Metode penelitian ini adalah dengan mengisolasi bakteri pendegradasi hidrokarbon dari perairan PPN Brondong dengan menggunakan metode isolasi *spread plate*. Karakter koloni yang berbeda dipilih untuk dilakukan pemurnian isolat bakteri. Isolat bakteri yang didapatkan selanjutnya dilakukan aktivitas pendegradasi hidrokarbon dengan mengkulturkan di dalam medium minimal yang mengandung bahan bakar disel. Aktivitas degradasi hidrokarbon dilakukan dengan pengujian *total petroleum hydrocarbon* (TPH). Hasil dari penelitian ini didapatkan 7 isolat berbeda dari situs yang tercemar bahan bakar minyak di PPN Brondong. Kultur dengan medium minimal yang mengandung bahan bakar disel mendapatkan hasil bahwa seluruh isolat memiliki kemampuan dalam pendegradasian hidrokarbon dengan adanya penurunan TPH.

Kata kunci : Bioremediasi, Degradasi hidrokarbon, Isolasi bakteri, Pencemaran petroleum, Pelabuhan Perikanan Nusantara

ABSTRACT

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I. INTRODUCTION

1. Research Background

The Nusantara Fisheries Port (Pelabuhan Perikanan Nusantara - PPN) Brondong in Lamongan Regency is a strategic port serving fishing vessels and playing a vital role in the development of capture fisheries in the region. As the center of fish trade in Lamongan Regency, the port handles significant fishery production, reaching 56,000 tons in 2018, including various fish species such as *swanggi*, *kurisi*, *kuniran*, squid, grouper, and red snapper. The high activity of fishing vessels, averaging 11,000 trips annually between 2014 and 2018, contributes to the port's economic importance but also leads to environmental challenges (KKP, 2018).The intensive operation of fishing vessels at PPN Brondong has resulted in substantial pollution, primarily from diesel fuel and residual engine oil discharged directly into the waters. Research indicates that the Water Quality Index in the area is classified as poor, negatively impacting macrozoobenthos and aquatic ecosystems (Muktini, 2003). Additionally, nutrient concentrations, such as nitrate and phosphate, in Brondong's coastal waters exceed government standards, further exacerbating the environmental degradation (Choirun et al., 2015).Petroleum hydrocarbon pollution poses severe risks to aquatic life and human health. Contaminated waters can lead to the mortality of marine organisms, damage fish eggs and larvae, and harm humans who consume contaminated fishery products (Adams et al., 2020; Blackburn et al., 2014; Fodrie et al., 2014). Addressing this pollution is critical to safeguarding the aquatic environment and ensuring the sustainability of fishery resources.Bioremediation, particularly using hydrocarbon-degrading bacteria, offers a promising solution to mitigate pollution. Bacteria such as *Pseudomonas*, *Marinobacter*, *Rhodococcus*, and others have demonstrated the ability to degrade hydrocarbon compounds effectively (Chaerun et al., 2004; Varjani & Upasani, 2017). Indigenous bacteria from polluted sites, such as PPN Brondong, are particularly advantageous due to their adaptive capabilities and high degradation potential (Leahy & Colwell, 1990; Wahyuni, 2019).To address pollution in PPN Brondong, initial efforts should focus on isolating and characterizing indigenous hydrocarbon-degrading bacteria from the waters. This research will provide insights into the bacteria's degradation activity and serve as a foundation for implementing bioremediation strategies, such as bioaugmentation or biostimulation, to restore the aquatic environment and ensure the sustainability of fishery resources.

2. Problem Formulation

1. Are hydrocarbon-degrading bacteria present in PPN Brondong?
2. What is the capability of the isolated hydrocarbon-degrading bacteria from PPN Brondong in degrading diesel fuel?

3. Research Objectives

1. To identify hydrocarbon-degrading bacteria in PPN Brondong.
2. To determine the degradation capability of the isolated hydrocarbon-degrading bacteria from PPN Brondong against diesel fuel.

4. Research Benefits

This research provides valuable information on the potential of hydrocarbon-degrading bacteria from PPN Brondong and offers an alternative solution to address pollution issues in PPN Brondong and other similar ports.

II. RESEARCH METHOD

Place and Time

This research was conducted from June to October 2023. Seawater samples contaminated with hydrocarbon pollutants were collected from PPN Brondong in Lamongan Regency on GPS coordinate -6.868788, 112.288821. The isolation and characterization of hydrocarbon-degrading bacteria were carried out in the Microbiology Laboratory at the University of Muhammadiyah Lamongan.

Material and Equipment

The materials used included saline water, Nutrient Agar (NA) medium, peptone, yeast extract, diesel fuel, n-hexane, Gram stain, ice, labeling paper, and aluminum foil. The equipment used in this research consisted of sterile bottles, rope, an ice box, test tubes, micropipettes, Petri dishes, spreaders, an incubator, a rotary shaker, a microscope, beakers, a separatory funnel, an oven, an analytical balance, Gram staining tools, and an autoclave.

Research Stages

Water Sampling

Samples were collected using tightly sealed sterile bottles with a string attached to the neck of the bottle. Water sampling was conducted from aboard a ship/boat by opening the sterile bottle and slowly lowering it into the water to collect 500 ml of water. The bottle was then tightly sealed and placed in an ice box. Additionally, 1 liter of non-sterile water was collected for Total Petroleum Hydrocarbon (TPH) testing.

Figure 1 water sampling point at sea water of PPN Brondong

Isolation of Bacteria

A 1 ml sample was added to a tube containing 9 ml of saline water. The sample was then serially diluted up to a dilution of 10^{-6}. The last three dilutions were plated using the spread plate method onto the surface of solid Nutrient Agar (NA) medium, each in duplicate. The cultures were incubated for 48 hours at room temperature.

Macroscopic and Microscopic Observation

Colonies growing on the NA medium were observed for their morphological characteristics, including color, shape, size, and elevation. Each distinct colony was labeled as a strain and purified using the streak plate method on solid NA medium, followed by incubation for 24-48 hours. Each purified colony was then subjected to microscopic observation using Gram staining.

Hydrocarbon Degradation Capability of Microbial Strains

Each obtained strain was cultured in a minimal medium containing 5 g peptone, 5 g yeast extract, and 2 g diesel fuel at room temperature and agitated at 160 rpm using a rotary

shaker. Petroleum hydrocarbon degradation was measured using the Total Petroleum Hydrocarbon (TPH) method at the end of the incubation period. A liquid medium not inoculated with bacterial strains was used as a control.

Measurement of Total Petroleum Hydrocarbon (TPH)

A 10 g sample was placed into a 250 ml beaker, and 50 ml of n-hexane was added. The mixture was poured into a separatory funnel and shaken until the oil separated. The sample was then transferred back into the beaker and evaporated in an oven at 70 °C until the n-hexane completely evaporated. The remaining oil residue was weighed using an analytical balance. This process was used to determine the amount of oil remaining in the sample after evaporation. The total hydrocarbon degradation was calculated using the following formula, with TPH_0 = Initial TPH and TPH_n = Final TPH.

$$\text{petroleum degradation (\%)} = \frac{TPH_0\text{-}TPH_n}{TPH_0} \times 100\%$$

Research Design

This study is exploratory research aimed at identifying the characteristics of bacteria inhabiting hydrocarbon-contaminated waters at PPN Brondong. The hydrocarbon degradation capability was tested using one-way ANOVA with an acceptance interval of $P < 0.05$.

III. RESULTS AND DISCUSSION

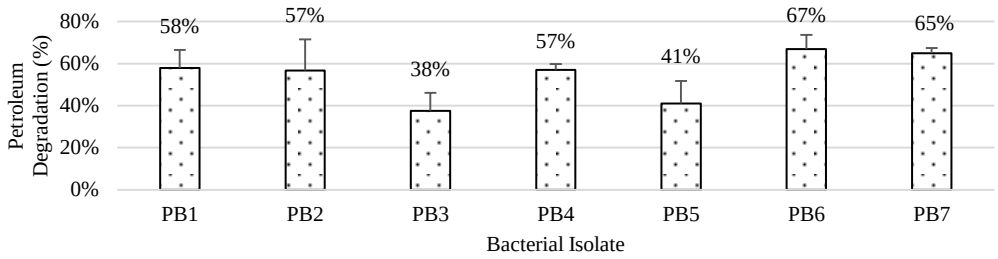
The isolation of bacteria from contaminated water samples in the waters of PPN Brondong successfully identified 7 distinct bacterial colonies (Table 1), with diverse microscopic characteristics. Among these seven colonies, 2 Gram-positive bacterial isolates were found, exhibiting two cell shapes: cocci (spherical) and bacilli (rod-shaped). This finding highlights the diversity of bacteria present in the contaminated waters.

Table 1 macroscopic and microscopic characteristic of bacterial isolate

Bacterial Isolate	Macroscopic character	Microscopic Character		
	Colony form	Cell shape	Gram stain	Catalase activity
PB1	Circular	Cocci	+	+
PB2	Punctiform	Bacilli	+	+
PB3	Irregular	Bacilli	+	+
PB4	Spidel	Bacilli	+	+
PB5	Rhizoid	Bacilli	+	+
PB6	Umbunate	Bacilli	+	+
PB7	Entire	Cocci	+	+

The bacterial isolates obtained from hydrocarbon-contaminated waters at PPN Brondong demonstrate potential in degrading hydrocarbon compounds. A Gram-positive, rod-shaped bacterium isolated from the site was found to convert hydrocarbon chains into fatty acids through the beta-oxidation (β-oxidation) pathway. This process is advantageous for the bacteria, as the fatty acids produced can be utilized as a carbon source for energy and as precursors in cellular metabolism. This mechanism has been widely reported in various studies as a primary pathway for hydrocarbon degradation by bacteria (Rojo, 2009; Varjani, 2017). The degradation activity of indigenous bacteria against diesel fuel varied between 38% and 67% (Figure 2). Isolate PB6 exhibited the highest activity (67%), while PB3 showed the lowest (38%). Despite this variation, statistical analysis revealed no significant difference ($P < 0.05$) among the isolates in their degradation capabilities. This

indicates that all isolates possess considerable potential for hydrocarbon degradation. Recent studies have also reported that indigenous bacteria often exhibit high degradation efficiency due to their adaptation to polluted environments (Ghosal et al., 2016; Varjani & Upasani, 2017).



Gambar 2 **Percentage degradation of diesel fuel from bacterial isolate. No significant different among them (P<0,05).**

The degradation process of diesel fuel can also be observed through changes in the pH of the medium. Although pH fluctuations occurred during the degradation process, the differences in pH among the isolates were not significant. This aligns with findings that the pH of the medium tends to remain stable during hydrocarbon biodegradation, due to the balanced metabolic activity of the bacteria (Das & Chandran, 2011).

Table 2. culture pH at end of incubation

Bacterial Isolate	pH		
PB1	6,58	±	0,85
PB2	7,23	±	0,63
PB3	6,47	±	0,61
PB4	7,23	±	0,08
PB5	6,62	±	0,86
PB6	7,48	±	0,31
PB7	7,97	±	0,11

The use of indigenous bacteria in bioremediation has proven effective in addressing oil pollution across various locations. For instance, in the Gulf of Mexico, indigenous bacteria were successfully employed to clean up the Deepwater Horizon oil spill in 2010. Within 90 days, hydrocarbon concentrations in the waters were significantly reduced (Hazen et al., 2010). Additionally, bioaugmentation using bacteria from other contaminated sites has been successfully applied in locations such as the coast of Alaska and the Mediterranean Sea (Atlas & Hazen, 2011).Several factors influence the efficiency of hydrocarbon bioremediation, including temperature, nutrients, oxygen availability, salinity, pH, moisture, and the presence of indigenous bacteria. Recent studies indicate that the addition of nutrients (such as nitrogen and phosphorus) and aeration can enhance hydrocarbon degradation rates by up to 70% (Ghosal et al., 2016; Varjani, 2017). Various Gram-positive and Gram-negative bacteria have been reported to degrade hydrocarbon compounds in diesel fuel. Gram-positive bacteria such as *Rhodococcus* spp., *Bacillus* spp., and *Mycobacterium* spp. have demonstrated effectiveness in degrading alkanes and polycyclic aromatic hydrocarbons (PAHs). For example, *Rhodococcus erythropolis* can degrade 80% of alkanes in diesel fuel within 14 days through the β-oxidation pathway (Varjani & Upasani, 2017). Meanwhile, *Bacillus subtilis* produces biosurfactants that enhance hydrocarbon solubility, accelerating the degradation process with an efficiency of

65-70% (Ghosal et al., 2016). *Mycobacterium vanbaalenii*, on the other hand, exhibits specialized capabilities in degrading PAHs with an efficiency of up to 75% (Rojo, 2009). Among Gram-negative bacteria, *Pseudomonas* spp., *Acinetobacter* spp., and *Alcanivorax* spp. are the most extensively studied. For instance, *Pseudomonas putida* can degrade 90% of alkanes in diesel fuel within 21 days through the production of alkane hydroxylase enzymes (Varjani, 2017). *Acinetobacter calcoaceticus* also shows significant degradation efficiency (60-70%) against diesel fuel, primarily due to its ability to produce biosurfactants (Das & Chandran, 2011). *Alcanivorax borkumensis*, a marine bacterium specialized in alkane degradation, has been successfully used in the bioremediation of oil spills in marine environments, such as the Deepwater Horizon spill in the Gulf of Mexico, with a degradation efficiency of 85% (Hazen et al., 2010). The potential applications of these bacteria are vast, particularly in the context of bioaugmentation and the use of bacterial consortia. Combinations of bacterial species, such as *Pseudomonas putida* and *Rhodococcus erythropolis*, have demonstrated degradation efficiencies of up to 95% against diesel fuel (Varjani, 2017). Additionally, the production of biosurfactants by bacteria like *Bacillus subtilis* and *Pseudomonas aeruginosa* can be utilized to enhance hydrocarbon solubility, thereby accelerating bioremediation processes (Ghosal et al., 2016). Genetic engineering also presents a promising approach, where bacteria such as *Pseudomonas putida* can be modified to increase the production of degradative enzymes, further improving bioremediation efficiency (Rojo, 2009).

IV. CONCLUSION

Isolation of bacteria from contaminated water samples in PPN Brondong identified 7 distinct bacterial colonies, with 2 Gram-positive isolates exhibiting cocci and bacilli morphologies. These isolates demonstrated hydrocarbon degradation capabilities, particularly for diesel fuel, with degradation rates ranging from 38% to 67%. Isolate PB6 showed the highest activity (67%), while PB3 had the lowest (38%). Despite variations, no significant differences ($P < 0.05$) were observed among the isolates, indicating their high degradation potential. Additionally, pH changes during degradation were stable across isolates, reflecting balanced metabolic activity during biodegradation.

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