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Research Article

Petroleum Degradation by Bacteria Explored from Logending Mangrove Sediments

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ABSTRACT

Oil spills resulting from shipping activities, tanker-based oil transportation, and fuel oil usage can cause coastal pollution, particularly in sensitive ecosystems such as mangroves. More than 90% of petroleum consists of hydrocarbons with complex carbon chain structures, making them difficult to decompose. Biological remediation using microorganisms offers a promising alternative for pollution mitigation, as microbes can degrade petroleum components and oxidizing hydrocarbons. This study aimed to evaluate the petroleum-degrading ability of selected bacterial isolates obtained from mangrove sediments at Logending Beach. The research employed experimental and survey methods. The primary parameter measured was Total Petroleum Hydrocarbons (TPH), while supporting parameters included pH and bacterial population density. The study consisted of several stages, including bacterial isolation, screening, and evaluation of the petroleum degradation capacity of selected isolates. The results identified two potential bacterial isolates capable of degrading crude oil. Isolate LG62 exhibited a degradation efficiency of 71.40%, while isolate LG105 showed a degradation efficiency of 57.10%. Petroleum concentrations of 2% (v/v) and 5% (v/v) were degraded more effectively than higher concentrations. Overall, the two bacterial isolates (LG62 and LG105) from Logending mangrove sediments demonstrated significant potential as bioremediation agents for petroleum hydrocarbon contamination.

Keywords: *Colocasia esculenta; diabetes mellitus; motoric balance; oxidative stress; sensory function.*

Introduction

Petroleum is one of Indonesia's primary and most significant energy sources [1–3]. It contains various elements, including carbon (C), sulfur (S), zinc (Zn), copper (Cu), nickel (Ni), mercury (Hg), aluminum (Al), cadmium (Cd), lead (Pb), chromium (Cr), and others [4], [5]. Petroleum also contains numerous organic compounds with carbon chains, such as cycloalkanes, aliphatic hydrocarbons, aromatic hydrocarbons, and polyaromatic compounds [6]. Crude oil has a complex carbon chain structure that is difficult to

decompose, leading to soil and water pollution [7], [8].

Oil spills from shipping activities, oil transportation by tankers, and fuel oil usage can cause pollution along coastlines, including sensitive ecosystems such as mangroves [9], [10]. Petroleum waste pollution can be addressed through physical, chemical, and biological approaches [11], [12]. Among these, hydrocarbonoclastic bacteria represent an effective biological solution [13], [14], as they function as bioremediation agents capable of mitigating petroleum contamination in coastal environments. These bacteria degrade petroleum components by oxidizing hydrocarbons [15], [16]. This process involves the breakdown of long-chain hydrocarbons into shorter-chain compounds, transforming petroleum into lighter fractions and consequently reducing its density and viscosity [17], [18].

Despite numerous studies reporting the use of hydrocarbon-degrading bacteria for petroleum bioremediation, most existing research focuses on general bacterial isolates or single-species approaches under controlled laboratory conditions. In contrast, information on indigenous hydrocarbonoclastic bacterial communities that are naturally adapted to coastal environments subjected to chronic oil contamination remains limited, particularly within Indonesia's mangrove ecosystems. Understanding the diversity and specific degradation potential of these native bacterial strains is crucial for the development of site-specific and sustainable bioremediation strategies. Therefore, this study aims to isolate and characterize hydrocarbonoclastic bacteria from oil-contaminated mangrove sediments and to evaluate their biodegradation capacity toward complex petroleum hydrocarbons, thereby providing new insights into the ecological adaptation and functional potential of native bacterial consortia in tropical coastal systems.

Materials and Methods

The equipment used in this study included inoculating loops, Petri dishes, test tubes and racks, serological pipettes, an incubator, an incubator shaker, a universal pH meter, Erlenmeyer flasks, a microwave, measuring cylinders, separating funnels, filter paper, beakers, an analytical balance, and a hot plate. The materials used comprised fifteen bacterial isolates obtained from mangrove sediments at Logending Beach, Nutrient Broth (NB), Nutrient Agar (NA), solid and liquid Stone Mineral Salt Solution supplemented with yeast extract (SMSSe), petroleum, n-hexane, and distilled water.

Screening of bacterial ability to degrade petroleum

A total of fifteen pure bacterial cultures isolated from mangrove sediments were inoculated using one ose and the streaking technique onto Stone Mineral Salt Solution supplemented with yeast extract (SMSSe) agar containing 2% (v/v) petroleum. Each treatment was performed in triplicate. The cultures were incubated at room temperature for three consecutive incubation periods of 24 hours each. Bacterial isolates capable of growing on and degrading petroleum were identified by the presence of a clear zone surrounding the colonies [19].

Ability test of potential bacteria in degrading petroleum

A 1 mL aliquot of the bacterial suspension (10^8 cells/mL) was inoculated into an Erlenmeyer flask containing 150 mL of liquid Stone Mineral Salt Solution supplemented with yeast extract (SMSSe) medium with three petroleum concentrations, namely 2% (v/v), 5% (v/v), and 10% (v/v). Each treatment was conducted in triplicate. The cultures were incubated in a shaker incubator at 120 rpm for 14 days. Measurements of pH, total bacterial counts using the Total Plate Count (TPC) method, and crude oil content

analysis using the gravimetric method were performed on days 7 and 14.

Data analysis

Data representing the petroleum degradation capacity of the bacterial isolates were subjected to statistical analysis using analysis of variance (ANOVA) to assess significant differences among treatments. When the ANOVA indicated significant effects ($p < 0.05$), mean values were further compared using Duncan's Multiple Range Test (DMRT) at a 95% confidence level to identify specific differences among bacterial isolates and petroleum concentrations. This combined statistical approach ensured a robust evaluation of degradation performance and provided a quantitative basis for distinguishing the most effective bacterial strains for petroleum bioremediation.

Results and Discussion

The screening of fifteen bacterial isolates revealed that isolates LG62 and LG105 exhibited robust growth on SMSSe medium (Table 1). These isolates also demonstrated petroleum-degrading activity, as indicated by the formation of clear zones surrounding the colonies. In contrast, isolates LG7, LG19, LG42, LG64, LG89, and LG113 were able to grow along the streaked inoculation lines but did not produce clear zones, suggesting that they were unable to utilize petroleum hydrocarbons as a carbon source and instead relied on nutrients provided by the growth medium. Several other isolates, namely LG2, LG4, LG6, LG35, LG75, LG78, and LG116, showed no visible growth along the inoculation lines, likely because the presence of petroleum in the medium was unsuitable for their growth and inhibited bacterial activity [20]. Based on these screening results, isolates LG62 and LG105 were selected for further evaluation of their petroleum degradation capability.

Gravimetric analysis of crude oil content demonstrated that isolates LG62 and LG105 were capable of degrading crude oil, as indicated by a significant increase in the percentage of petroleum degradation on day 14 (Figure 1). Isolate LG62 achieved degradation rates of up to 71.4% at a petroleum concentration of 2% (v/v) and 65.1% at 5% (v/v). However, at a higher concentration of 10% (v/v), the degradation efficiency markedly decreased to 14.4%. Similarly, isolate LG105 degraded 57.1% of crude oil at 2% (v/v) and 50.6% at 5% (v/v), while the degradation rate declined to 15.3% at a concentration of 10% (v/v).

Table 1. The result of the screening of potential petroleum-degrading bacterial isolates

Isolate	Growth	Clear Zone
LG2	-	-
LG4	-	-
LG6	-	-
LG7	+	-
LG19	+	-
LG35	-	-
LG42	+	-
LG62	+	+
LG64	+	-
LG75	-	-
LG78	-	-
LG89	+	-
LG105	+	+
LG113	+	-
LG116	-	-

The ANOVA results indicated that isolate variation, petroleum concentration, and the interaction between isolates and petroleum concentrations significantly affected the reduction in petroleum content. Further analysis using Duncan's Multiple Range Test (DMRT) showed that isolates LG62 and LG105 exhibited relatively high petroleum degradation efficiency at petroleum concentrations of 2% (v/v) and 5% (v/v). However, their degradation performance significantly decreased at a petroleum concentration of 10% (v/v) (Table 2). Higher petroleum levels in the medium resulted in lower degradation

percentages, likely because longer incubation times are required for complete degradation. In addition, the reduced degradation rate may be attributed to limited nutrient availability and decreased oxygen diffusion in the medium [17], [21].

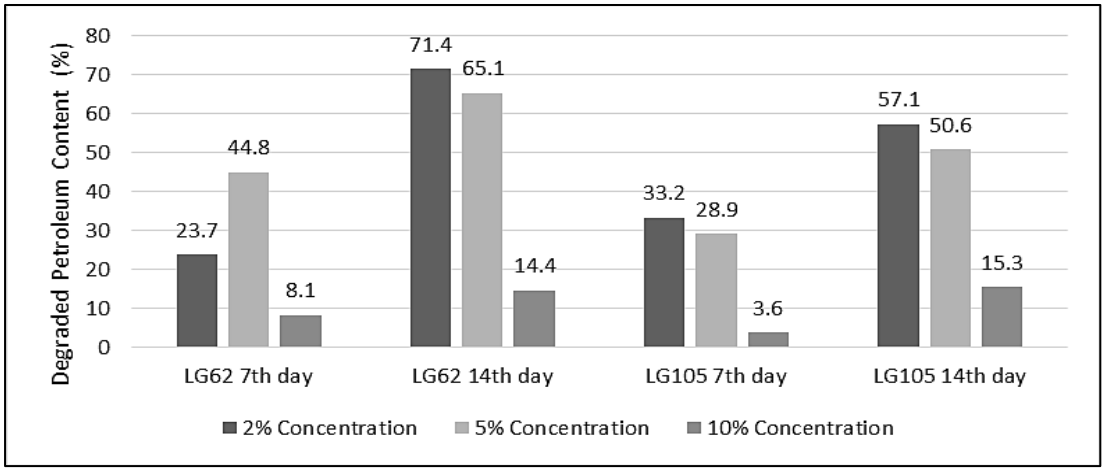

Figure 1. Percentage of crude oil levels degraded by bacterial isolates LG62 and LG105.

Bacterial population counts increased across most treatments, except for isolate LG105 at a petroleum concentration of 2% (v/v), where the population declined from 23.29 × 10^5 CFU/mL to 8.83 × 10^5 CFU/mL (Figure 2). Overall, isolate LG62 exhibited a faster growth rate than LG105, which corresponded with the higher percentage of petroleum degradation observed in the samples. However, in the treatment involving isolate LG62 at a petroleum concentration of 10% (v/v), the bacterial population (53.5 × 10^5 CFU/mL) did not correspond proportionally to the relatively low petroleum degradation rate of 14.4%.

Table 2. The ability of potential bacterial isolates to degrade petroleum

Isolate	Oil Concentration (%)	Graded Crude Oil Content (%) (Day 14)
Control	2	0.00 b
	5	0.00 b
	10	0.00 b
LG62	2	71.40 a
	5	65.17 a
	10	14.40 b
LG105	2	57.10 a
	5	50.67 a
	10	15.30 b

The results indicated that higher levels of petroleum degradation were generally associated with increased bacterial populations, suggesting that the bacteria were able to utilize petroleum-derived carbon as a nutrient source. These bacteria produce enzymes capable of breaking down complex organic compounds into simpler molecules [22]. The decline in the LG105 bacterial population at a petroleum concentration of 2% (v/v) was likely attributable to a decrease in the medium pH to an acidic level of 5.8, which can inhibit the growth of bacteria that are intolerant to acidic conditions (Figure 3) [23].

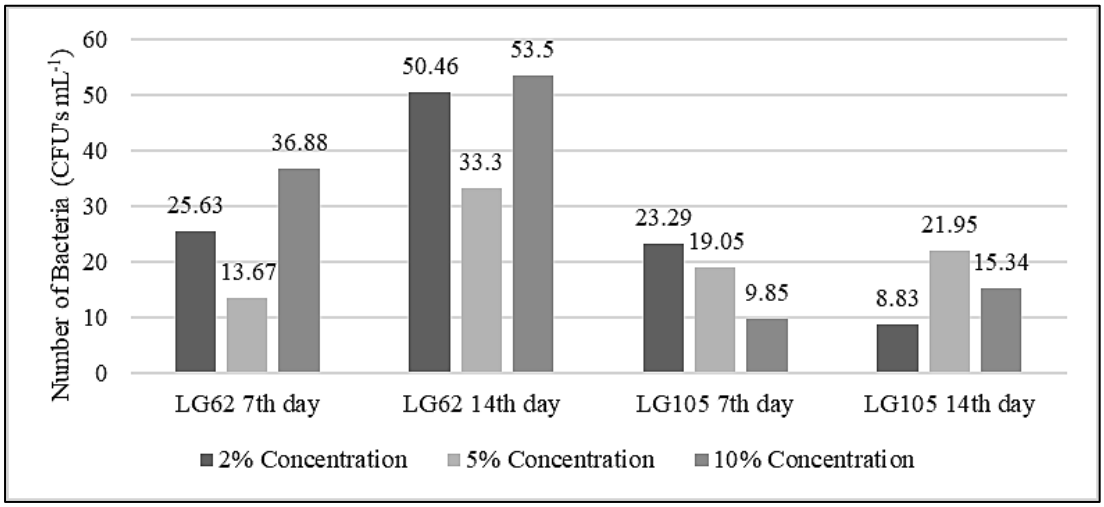

Figure 2. Number of bacterial isolates LG62 and LG105 at various oil concentrations.

The relatively high bacterial population observed for isolate LG62 under the 10% (v/v) petroleum treatment may be attributed to uneven homogenization of the culture medium, which could have resulted in localized zones with elevated bacterial densities. Such heterogeneity may have promoted the formation of micro-niches that favored bacterial aggregation and growth, particularly for strains capable of effectively colonizing oil water interfaces. Under these conditions, petroleum-degrading bacteria can form dense microcolonies that enhance substrate accessibility and nutrient exchange, leading to higher apparent cell densities in samples collected from oil-rich regions of the medium. Moreover, petroleum-degrading bacteria, including members of the genera Pseudomonas, Bacillus, and Acinetobacter, are known to secrete biosurfactants composed of both hydrophilic and hydrophobic moieties, which facilitate the emulsification and solubilization of hydrophobic hydrocarbons [24].

The hydrophobic moieties of these biosurfactants facilitate bacterial adhesion to oil droplets, enabling cells to anchor directly onto petroleum surfaces and utilize hydrocarbons as carbon and energy sources. This adhesion process increases bacterial population density in regions with higher oil concentrations, as biosurfactant-mediated colonization enhances both hydrocarbon uptake and degradation efficiency [24], [25]. Consequently, the elevated cell counts observed under the 10% petroleum treatment likely reflect an adaptive microbial response to hydrocarbon abundance, driven by the production of surface-active compounds that promote stable oil–bacteria interactions.

After 14 days of incubation, distinct pH variations were observed between media inoculated with isolates LG62 and LG105, indicating differences in their metabolic responses during petroleum degradation. The medium containing isolate LG62 exhibited a gradual increase in pH from 7.0 to 8.0 across all petroleum concentrations, suggesting active hydrocarbon oxidation processes that generated alkaline by-products or consumed acidic intermediates. Such alkalinization is commonly associated with the production of basic metabolites, including ammonia and hydroxide ions, as well as the release of CO_2 and other gaseous products during aerobic degradation.

In contrast, the medium inoculated with isolate LG105 showed a decrease in pH from 7.0 to 5.8, indicating the accumulation of organic acids and partially oxidized hydrocarbon intermediates. This acidification is typically linked to incomplete hydrocarbon mineralization and the predominance of metabolic pathways favoring acidogenic processes. The contrasting pH shifts observed between isolates LG62 and LG105 therefore reflect

fundamental differences in their catabolic pathways and enzymatic mechanisms for petroleum degradation, with LG62 likely promoting a more complete oxidative metabolism characterized by alkalinization of the medium. and LG105 demonstrating a more acidogenic degradation route.

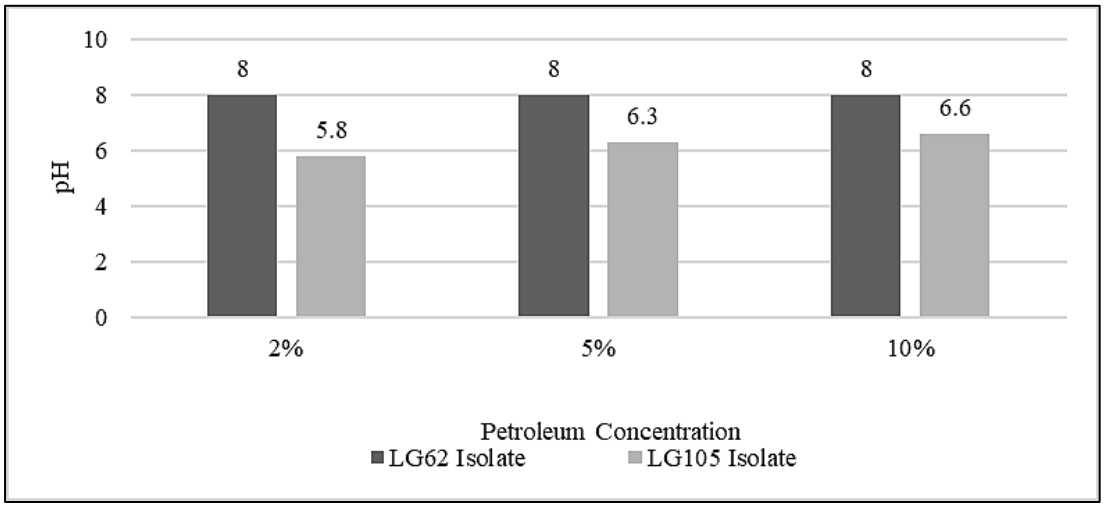

Figure 3. Correlation between the percentage of degraded petroleum and pH.

The variation in pH values observed in the culture medium (Figure 3) treated with different bacterial isolates reflects distinct metabolic pathways and biochemical mechanisms involved in petroleum degradation. Each bacterial strain possesses specific enzymatic systems that govern the nature of intermediate metabolites produced during hydrocarbon breakdown. Some isolates may predominantly employ oxidative pathways that lead to the accumulation of alkaline by-products, whereas others rely on fermentative or acidogenic routes, resulting in the production of organic acids. These differences in metabolic strategies ultimately determine the direction and magnitude of pH changes in the culture medium during the biodegradation process.

An increase in pH during incubation may be attributed to the formation of basic intermediates or gaseous by-products, such as carbon dioxide (CO_2), methane (CH_4), and ammonia (NH_3), generated during hydrocarbon oxidation. In addition, the utilization of nutrient sources such as NH_4NO_3 and KH_2PO_4 in the SMSSe medium may contribute to the release of hydroxide ions (OH^-), thereby further promoting an alkaline shift in the medium. An increase in alkalinity generally indicates effective hydrocarbon metabolism under aerobic conditions, in which the oxidative cleavage of long-chain hydrocarbons produces less acidic intermediates and is accompanied by enhanced microbial respiration rates [21].

Conversely, a decrease in pH may indicate the accumulation of acidic intermediates, such as carboxylic acids, aldehydes, and ketones, generated during the β-oxidation of hydrocarbon chains. These organic acids are typical by-products of partial hydrocarbon degradation, particularly when catabolic processes occur under conditions of limited oxygen or nutrient availability. The observed acidification therefore suggests active microbial utilization of hydrocarbons through metabolic pathways that favor acidogenesis rather than complete mineralization. This phenomenon is consistent with previous studies [21], [26], which reported that hydrocarbon-degrading bacteria, especially species of *Pseudomonas* and *Acinetobacter*, can lower medium pH as a result of organic acid production during petroleum biotransformation.

Conclusion

Bacterial isolates LG62 and LG105 obtained from Logending mangrove sediments demonstrated the ability to degrade petroleum hydrocarbons by 71.4% and 57.1%, respectively, at a petroleum concentration of 2% (v/v). The degradation efficiency of both isolates was strongly influenced by petroleum concentration, with effectiveness decreasing as oil concentration increased, likely due to nutrient limitation and increased environmental stress. These findings confirm the potential of indigenous hydrocarbonoclastic bacteria as effective bioremediation agents for oil pollution in coastal environments. Future studies should focus on optimization strategies, including co-culture systems, nutrient supplementation, and genetic or metabolic enhancement, to improve bacterial tolerance and degradation efficiency under high petroleum concentrations.

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Conflict of interest

The authors declare that no conflict of interest in this study.

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