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Bioremediation of Oil-Contaminated Soil with Variations of Bacterial Cultures and Nutrient Ratio-Field-Scale Experimental Research

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Abstract:

The spread of hydrocarbon contamination in the form of oil-contaminated soil originates from waste from drilling and oil production. Hydrocarbon contaminants are categorized as hazardous and toxic waste that has long been deposited in the soil, disrupting the soil ecosystem and the groundwater cycle. Remediation of oil-contaminated soil is carried out using ex-situ bioremediation technology. Oil-contaminated soil is excavated using heavy equipment, collected at the contaminated site, and then transported to a licensed waste treatment facility. The study aims to evaluate the degradation of Total Petroleum Hydrocarbon (TPH) over time using biostimulation and bioaugmentation methods to meet the success parameters of the quality standards for the Total Concentration (TC) of long-chain Petroleum Hydrocarbon (PH) (with a PH value ≤ TC-B 0.5%. Biostimulation uses urea fertilizer as a source of N and SP-36 fertilizer as a source of phosphorus with treatment variations of C:N:P ratios of 100:10:1 and 100:5:1. Bioaugmentation uses variations in the treatment of indigenous bacteria that are naturally available in contaminated soil and variations with the addition of non-indigenous bacteria from bacterial cultures. The results of the study showed that the fastest bioremediation time to achieve the success parameters of the quality standards was 28 days with an average degradation rate of 3%/day. The 2-factor Analysis of Variance (ANOVA) statistical test with 4 replications produced a P-value = 0.257 (> 0.05) so that the C:N:P ratio did not have a significant effect on the decrease in concentration of TPH. The C:N:P ratio of 100:5:1 showed a higher percentage of TPH degradation compared to the C:N:P ratio of 100:10:1. There was no significant difference in the rate of TPH degradation between the treatment variations of indigenous bacteria and mixing with non-indigenous bacteria. The approach to calculating the population spike of non-indigenous bacteria mixing using logarithmic units showed a population spike of $0.06\ log_{10}$ ($1.14\ times$)$<0.5\ log_{10}$ ($3.16\ times$). The non-indigenous bacterial inoculum survived but did not dominate the degradation pathway due to bacterial competition.

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Introduction

The contaminated soil with petroleum hydrocarbons is one of the most significant pollution problems, which can be caused by production, storage, and utilization activities of crude oil(Lirong et al., 2023). Hydrocarbon contaminants found in the soil and water can lead to significant environmental impacts and pose great risks to humans and other living organisms in the affected environment, resulting in various environmental issues, ecological disasters, and even global disasters(Hasan et al., 2025) . Approximately 2 kg of petroleum hydrocarbons is released into the environment for every 1 ton of crude oil produced and transported (Dey et al., 2024). In Indonesia, the crude oil explored contains many hydrocarbons with very low sulfur levels. The main composition of crude oil consists of 80–89% carbon, 12–14% hydrogen, 2–3% oxygen, 0–3% sulfur, and 0.3–1% nitrogen. In addition, crude oil also contains pollutants such as Cl, Ni, Mo, Fe, Na, and other substances with varying physical and chemical properties depending on the origin of the crude oil (Chen et al., 2023). According to the Indonesian Government Regulation (PP) No. 22 of 2021, attachment IX, petroleum waste falls under the category of hazardous and toxic waste (B3).

Naturally, the environment has the ability to degrade contaminants through biological and chemical processes; however, often the contamination burden exceeds the degradation rate, leading to accumulation of contaminants, which then requires human intervention with available technologies to address these environmental issues(Mohanta et al., 2024) . Total Petroleum Hydrocarbon (TPH) represents the identification of hydrocarbons found in crude oil (US EPA, 2023). According to the Ministry of Environment and Forestry of the Republic of Indonesia Regulation No. P.101/MENLHK/SETJE/KN/1/11/2018, TPH values are one of the parameters that need to be tested to determine the success of crude oil cleanup based on the total concentration of Petroleum Hydrocarbons (PH) for the long-chain $C_{10} - C_{36}$.

Remediation is a process designed to improve or stop environmental damage, reduce risks to humans and the environment to an acceptable level (Negi & Singh, 2023). Bioremediation technology accelerates the natural degradation of chemical contaminants and provides a better cleanup strategy for various types of pollutants. This environmentally friendly and cost-effective process is used to remove hydrocarbons in contaminated soils (Wei et al., 2023).

Bioremediation techniques involve microorganisms that use the contaminants as a food and energy source for growth and development. This process includes modifying the soil's conditions (mainly oxygen, moisture, nutrients, sulfur, and pH) to stimulate microbial activities responsible for degrading contaminants(Liu et al., 2023) . There are two types of bioremediation techniques: in-situ bioremediation, which is conducted without excavation or transportation of contaminated soil, effective up to a depth of 30–60 cm, as deeper layers experience limited oxygen availability; and ex-situ bioremediation, which involves excavation, transport, treatment, and re-disposal of the soil (Limbong & Tangahu, 2025).

Two approaches used in bioremediation for oil spills are: biostimulation, which stimulates the growth of indigenous hydrocarbon-degrading microorganisms by adding nutrients or improving habitat conditions; and bioaugmentation, which introduces additional microorganisms to complement the existing microbial population(Helmy & Kardena, n.d.).Biostimulation enhances biodegradation processes by providing necessary nutrients and modifying environmental conditions (Rua & Nahak, 2024). Research by (Puspitasari, 2024) showed that laboratory-scale bioremediation of oil with nutrient variations C:N:P 100:10:1 and 100:5:1 resulted in the highest TPH degradation. According to (Limbong & Tangahu, 2025), the most efficient biodegradation with an optimal C:N:P ratio of 100:10:1 was observed.

Bioaugmentation involves introducing microorganisms that can break down hydrocarbons as an energy source (Osadebe et al., 2023). Effective biodegradation occurs between the 15th and 30th day of bioaugmentation, achieving 96.5% biodegradation after 60 days in 1% oil-contaminated soil (Rahmati et al., 2022). Biologically active species used for bioremediation include bacteria, archaea, and fungi. Most studies focus on bacterial-based bioremediation processes. (Laczi et al., 2020)states that bacteria can utilize hydrocarbon contaminants in the environment and begin self-propagating when conditions are suitable.

The main objective of this research is to evaluate the degradation of Total Petroleum Hydrocarbon (TPH) over time using biostimulation and bioaugmentation methods to meet the standard quality parameter for Total Petroleum Hydrocarbon (PH) concentration for long chains $C_{10} - C_{36}$ with PH values ≤ TC-B 0.5%. Biostimulation uses urea fertilizer as a nitrogen source and SP-36 fertilizer as a phosphorus source with C:N:P ratio variations of 100:10:1 and 100:5:1. Bioaugmentation involves varying indigenous bacteria naturally found in contaminated soils and the addition of non-indigenous cultured bacteria. This field-scale experimental research was conducted over 28 days with initial TPH concentrations of 3% and 5%.

Methods

Research location

The research was conducted in an oil mining area, a tropical region with an average ambient temperature of 25-35°C, humidity of 75-80%, and a pH of 6-8. The contaminated soil had been contaminated with oil for over 50 years. The oil-contaminated soil was excavated using heavy equipment, collected at the contaminated site, and then transported to a licensed hazardous waste treatment facility.

Research design

The research uses three types of variables, namely:

a. Independent variables, consisting of biological agents (indigenous and non-indigenous bacteria), C:N:P ratio (100:10:1 and 100:5:1) and TPH concentration (3% and 5%)
b. Dependent variables, consisting of pH, temperature, humidity, and TPC
c. Control variable, namely the time required to reach the quality standard

The study used 12 treatment variations, each measuring 4m x 4m x 0.3m. The research matrix is as follows:

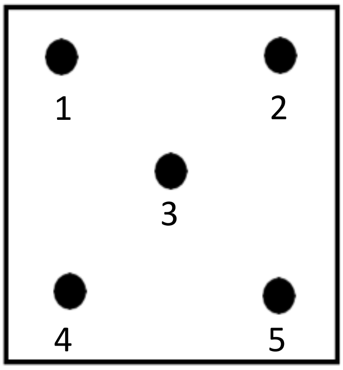
Table1.Research matrix

C:N:P ratio	Z0		B0	
	3%	5%	3%	5%
X0	Z0X0(3)	Z0X0(5)	B0X0(3)	B0X0(5)
Y1	Z0Y1(3)	Z0Y1(5)	B0Y1(3)	B0Y1(5)
Y2	Z0Y2(3)	Z0Y2(5)	B0Y2(3)	B0Y2(5)

X0: no nutrients; Y1: nutrients (100:10:1);
Y2: nutrient (100:5:1); Z0: indigenous bacteria;
B: non-indigenous bacteria

Figure 1.Research location

TPH sampling analysis



Sampling was conducted at five points using a hand auger, then the five samples were composited. The soil test samples were sent to the laboratory for TPH (%) testing using the US EPA 8015C:2007 method.

Sampling was conducted over a 7-day period for each treatment variation. Mathematical calculations were performed using the formula, where TPH_{final} and $TPH_{initial}$ represent the initial and final TPH concentrations for each treatment variation:

TPH degradation (%): $\frac{TPH_{final} - TPH_{initial}}{TPH_{initial}}\ x\ 100\%$

Degradation rate over time: $\frac{TPH\ degradation\ (\%)}{total\ days}$

Calculation of bacterial population

The measurement of the bacterial population is calculated by Total Plate Count (TPC) analysis. The TPC value indicates the population of microorganisms that contribute to the hydrocarbon decomposition process. (Lara-Moreno et al., 2022) showed an increase in bacterial population 0,5 log_{10} ≥ or 3.16 times as the minimum requirement to declare bioaugmentation settled, active and significant increase during bioaugmentation. (Lohri et al., 2015) stated that it is used to determine whether there is a synergistic effect or competition between mixed strains.

Bacterial population growth (Δlog_{10}): $log_{10}(TPC\ final) - \ log_{10}(TPC\ initial)$.

Results

TPH Degradation

Figure 2 shows the results of the observation of TPH degradation from day 0 to day 28 of the study. The research findings indicate that over time, the TPH values showed variations and changes based on the treatments applied.

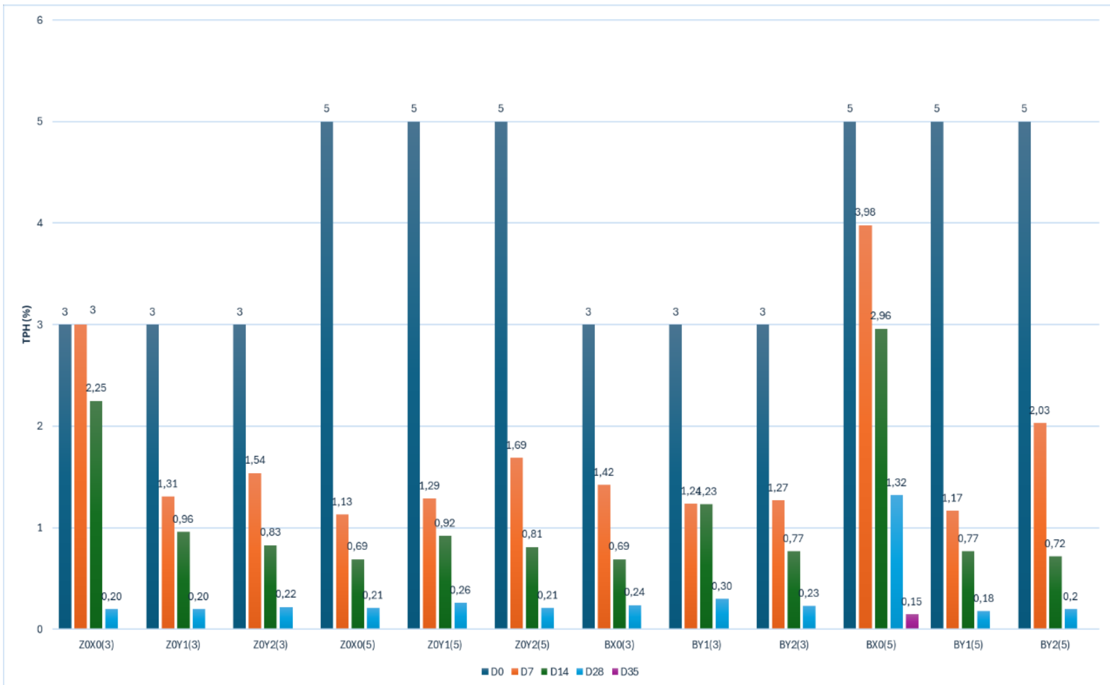


Figure 2.TPH Degradation Over Time

In the initial phase of bioremediation testing, the average TPH degradation for all treatment variations was 54%. (Platt et al., 2021) stated that the use of Bacillus sp. bacteria can reduce petroleum levels by up to 55% after a 7-day incubation period. Variations in indigenous bacterial treatments identify local bacterial populations found in active soil, and recovery activities occur through natural attenuation with sufficient nutrient availability and effective bacterial growth without external bacterial intervention.

Naturally occurring indigenous bacteria are already adapted to metabolize hydrocarbons. Research by (Maqbool et al., 2012) showed that soil with an adaptive indigenous microbial community can degrade TPH without additional treatment. (Ite & Ibok, 2019) stated that "natural attenuation" is often quite effective in the initial stages, especially in long-contaminated environments. (Limbong & Tangahu, 2025) explained that in conditions without bacterial addition, indigenous bacteria are more efficient in initiating degradation because they do not have to re-adapt. The addition of bacteria can disrupt the indigenous bacterial ecosystem and slow the initial degradation process. According to , hydrocarbon degradation occurs most rapidly in the initial phase due to high bioavailability and the continued availability of light hydrocarbon fractions that are easily dissolved and absorbed.

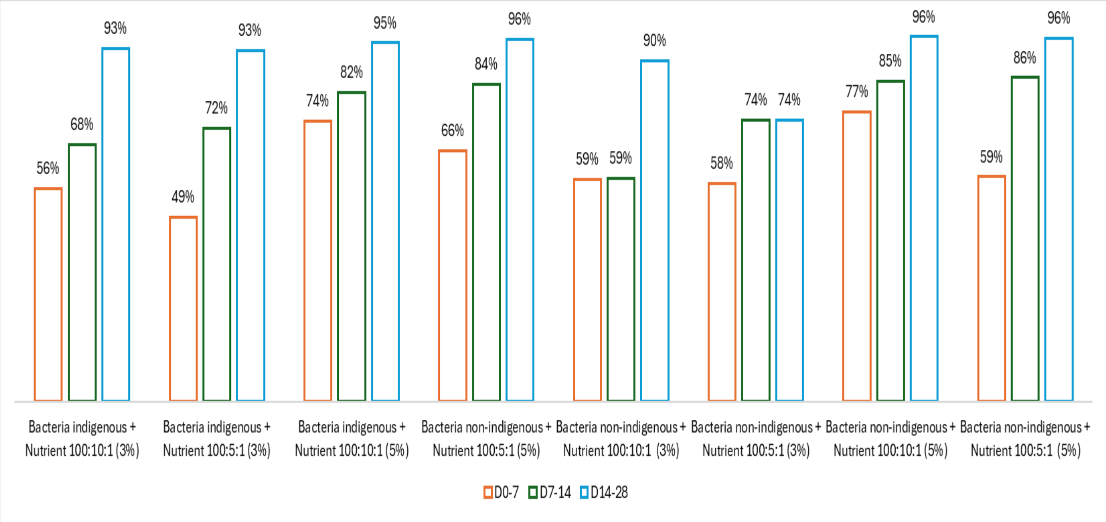


Figure 3.Cumulative TPH Degradation

Figure 3 shows cumulative variations in the treatment of indigenous and non-indigenous bacteria with nutrient ratios of 100:10:1 and 100:5:1, indicating an average increase in the percentage of degradation of 20% for each day of the study. These results indicate a short lag phase (adaptation), and microorganisms remain in the log phase (growth) longer under environmental conditions that support continuous enzymatic activity (pH, humidity, oxygen). Biodegradation was consistent and progressive throughout the 28 days of the study.

C:N:P ratio to TPH degradation

The addition of bacteria does not always increase TPH degradation consistently. Figure 4 shows that a C:N:P ratio of 100:5:1 has a higher percentage of TPH degradation than a C:N:P ratio of 100:10:1. Research by (Wang & Bartha, 2018) states that indigenous bacterial populations in soils that have been exposed to hydrocarbons for a long time can adapt and carry out nutrient scavenging efficiently, so that the addition of large amounts of nutrients (high ratio) does not always increase degradation activity significantly. stated that native microbes that have lived for a long time in polluted locations tend to have reached a functional balance with the contaminant and have the capacity to survive and metabolize without being highly dependent on nutrient supplementation.

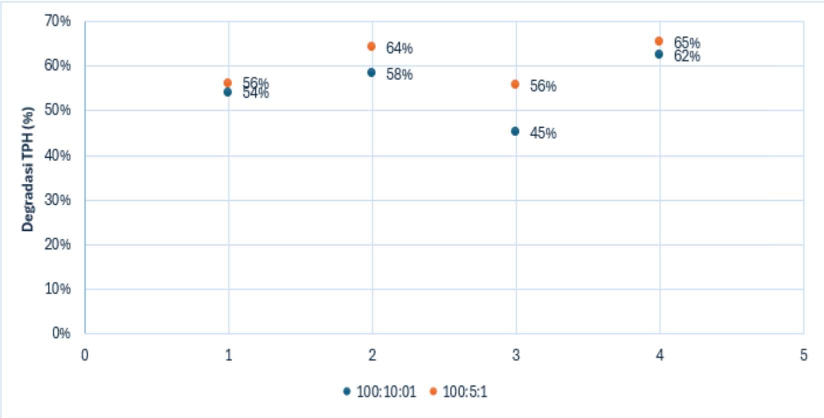


Figure 4.C:N:P ratio to TPH degradation

Analysis of variance (ANOVA) statistical test was conducted using Minitab18 software to determine whether the C:N:P ratio had a significant effect on TPH degradation. The C:N:P ratios of 100:5:1 and 100:10:1 were used as factors, and the % TPH degradation was used as the response.

Table2.Factors and responses

Factors	Responses (% TPH degradation)			
100:5:1	56	64	56	65
100:10:1	54	58	45	62

$H_0 = \mu_{100:5:1} = \mu_{100:10:1}$

$H_1 = at\ least\ one\ means\ different$

Minitab simulation produced a P-value = 0.257 (> 0.05) so that the C:N:P ratio did not have a significant effect on reducing TPH concentration.

Mixed bacterial population

Table 3 shows a comparison of TPC values before and after bioremediation, as well as the resulting bacterial population spike. The post-bioremediation increase was only 14%. The bacterial mix resulted in an inoculum of non-indigenous bacteria that survived but did not yet dominate the degradation pathway due to bacterial competition. Six out of seven colonies counted on day 28 still belonged to the indigenous bacterial community, while one colony was an introduced strain of non-indigenous bacteria.

Table 3. Bacterial population growth

Parameter	TPH 3%	TPH 5%
Initial TPC (cfu/g)	1.13×10^6	1.24×10^6
Final TPC (cfu/g)	1.29×10^6	1.41×10^6
Population growth	1.60×10^5	1.70×10^5
Δlog_{10}	0.06	0.03

Increase factor (× fold)	1.14 times	1.07 times

Conclusion

Bioremediation of long-standing oil-contaminated soil can occur through natural attenuation by utilizing indigenous bacteria and sufficient nutrient availability. The addition of non-indigenous bacteria can trigger competition with indigenous bacteria that have naturally adapted to the contaminated environment. Optimal nutrient balance is more crucial than simply adding bacteria.

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