

Optimization of Bioremediation of Used Engine Oil-Contaminated Soil Using *Pseudomonas Putida*

Eman Falih Nimmah^{1*}, Melad Khalaf Mohammed²

¹ Ministry of education, general directorial of education in wasit, yakoot school, wasit, Iraq.

² Ministry of Higher Education, Department of Biology, College of Science, Wasit University, Iraq.

ABSTRACT

Background: Used engine oil is highly resistant to natural degradation processes due to the presence of metals generated from engine component corrosion and friction. While various techniques exist for treating oil-contaminated soil, bioremediation remains the most cost-effective and environmentally friendly approach for soil remediation. **Objective:** This study aimed to evaluate the bioremediation potential of *Pseudomonas putida* under optimal conditions for degrading used engine oil in contaminated soil. **Methods:** *Pseudomonas putida* was employed to bioremediate soil contaminated with used engine oil. Optimal conditions for bacterial growth and degradation efficiency were determined by varying parameters, including carbon source, pH, temperature, and incubation time. Glucose served as the carbon source, and experiments were conducted at pH 7, 35°C, with a 2% (v/v) oil concentration, and an incubation period of 144 hours. **Results:** The data indicated that *Pseudomonas putida* effectively degraded used engine oil under the specified conditions. A 2% (v/v) oil concentration, with glucose as the carbon source, supported optimal bacterial growth and bioremediation activity, highlighting the strain's potential for environmental cleanup of oil-contaminated soils. **Conclusion:** This study demonstrates the effectiveness of *Pseudomonas putida* in bioremediating used engine oil-contaminated soil under optimal conditions. These findings underscore the potential of bioremediation as a sustainable and efficient method for mitigating environmental pollution caused by hydrocarbons.

Keywords: Petroleum Hydrocarbons, Bioremediation, *Pseudomonas Putida*, Soil, Engine Oil

INTRODUCTION

Bioremediation is an effective, secure, and cost-effective alternative approach for cleaning up contaminated sites and restoring the naturally occurring ecosystem in the environment; and it is highly efficient, but it still takes several weeks or months to complete; the most common microorganism used in bioremediation is bacteria. The chemical structure of engine oil is composed of about 80% lubricating oil. Large amounts of lubricating oils, make up long-chain saturated hydrocarbons, and additives are used in car engines, the chief components of the base oil are cyclic alkanes (c-alkanes), long-chain hydrocarbons, and alkanes are known as recalcitrant to microbial. Hydrocarbons are potentially toxic organic compounds, and their presence in the soil has negative effects on both ecological quality and human health. For optimum biodegradation conditions; it is important to know basic information such as residual oil concentration in the soil, population density of the bacterial - and the biodegradation potential. The main factors influencing bioremediation process are adaptation, the makeup of pollutants, and the availability of nutrients. As a result, the biological breakdown of petroleum hydrocarbons may be significantly impacted by a variety of parameters, including temperature, viscosity, oxygen supply, salinity, and the viscosity of pollutants [1]. In bioremediation technology where bacteria are cultured in the existence of hazardous pollutants to enhance the breakdown or removal of inorganic and organic pollutants. been done on the *Pseudomonas putida*, its capable to decompose PAHs. Also, the Bacillus group bacteria living in the contaminated environments capable of hydrocarbons biodegradation. These bacteria can certainly adapt to the hydrocarbon-polluted sites and use the hydrocarbons as a source of carbon and energy for their

metabolism and growth [2]. the biodegradation in bacteria depended on degradation genes to degrade PAHs.

In microbial bioremediation uses genes like *gntR*, *alkS*, and *lipS*, which are linked to the breakdown of different pollutants and the bacterial resistance to harmful substances. Remediation of engine oil-contaminated soil can be done in several ways including using physicals, chemicals, in general; these methods can eliminate petroleum waste quickly but is not environmentally friendly and expensive. Remediation technologies of soil hydrocarbons: natural attenuation, bio stimulation, and bioaugmentation, and natural attenuation protocol. The goal of this study was to create an effective bioremediation system by *pseudomonas putida*. *Pseudomonas putida* is classified as a chemo-organotroph Because of its capacity to eliminate a broad variety of carbon molecules, including petrol hydrocarbons.

MATERIALS AND METHODS

Eleven soil samples contaminated with used engine oil were investigated. In which hydrocarbon-degrading bacteria were isolated on Enhancement media Mineral Salt Medium (MSM) and nutrient agar was done as described by Udgire et al. [3]. After that isolate identification was carried out; phenotypic characters [4] and biochemical tests were performed by the standard procedures outlined in Bergey's Manual of Systematics and PCR technique to determine *P.putida* using specified primers the 16S rRNA gene. sample O9 was chosen as a representative sample. The bacteria (*Pseudomonas putida*) were obtained from O9isolate. That sample was subjected to

determined microbiological analyses and Optimal parameters such as the Effect of Incubation Periods, temperature, carbon sources, and pH.

Optimal Condition for Bioremediation of Hydrocarbon Pollutants

Effect of Incubation Periods

Fifty ml of Bushnell-Hass medium broth was placed in 250 ml Erlenmeyer flasks, supplemented with 1% engine oil as a hydrocarbon source, and each flask was inoculated with 5 ml of bacterial inoculum of HD2 (three replicates were made). All flasks were incubated in a shaker incubator at 30 °C for different periods of incubation (0,24,72,120,144) hours, [5]. All experiments of optimal conditions for hydrocarbon remediation were performed in the same procure with three replicates of bacterial isolate (O9). Such as the Effect of Temperature (20,25, 30, 35, 37and 40°C) for (9 days) and the effect of Different pH Values (The BH medium was distributed in the amount of 50 ml, and the pH values of this medium were adjusted to become (5, 6, 7, 8, 9). using 0.1 N of HCl or 0.1 N of NaOH solutions) [6]. Effect of Different Carbon Sources (The medium components were modified by adding (2%) from different carbon sources (maltose, glucose, sucrose, xylose, fructose, and soluble starch) and using separate flasks for each carbon source [7]. After the end of each period bacterial growth (O.D at 600nm), gravimetric analysis for engine oil degradation, biomass, and emulsification index were measured for bacterial culture (O9)and control [5]. The control flask contains (50 ml BHM and 1% engine oil without adding bacterial inoculum (O9).

Measurement of Bacterial Growth

The absorbance (optical density) data were taken at 600 nm using a UV- VIS spectrophotometer to measure the cell turbidity of samples from the bacterial culture (O9) and the control [7].

Measurement of Engine Oil Remediation by Gravimetric Method

It was measured depending on the method [8].

1-engine Oil Remediation Bushnell Hass medium 50 ml was poured into 250 ml conical flasks and added with 1% motor oil to examine the oil cleanup. The medium was shook at 150 rpm for nine days at 30°C after being infected with bacteria (O9).

2-Engine oil Extraction from Test Flask: After the end of the incubation period, the cells were harvested, the supernatant with

engine oil was extracted, and 5ml of n-hexane was added for estimation of oil remediation rates. The extract was treated with 0.5g of sodium sulfate, decanted, and evaporated to dryness.

3-Gravimetric Analysis, the amount of residual engine oil was measured after extraction of oil from the medium and evaporating it to dryness. The volume of extracted oil was deducted from the previously weighed beaker.

Weight of residual engine oil = Weight of the beaker containing extracted engine oil - Weight of empty beaker

Amount of engine oil remedied = Weight of engine oil in the media - Weight of residual engine oil

$$\text{The percentage of remediation (\%)} = \frac{\text{Amount of engine oil remedied}}{\text{Amount of engine oil in the media}}$$

Results and Discussion

Optimal Conditions of Hydrocarbon Pollutants Bioremediation by *Pseudomonas Putida*

Effect of Incubation Period

BHM supplemented with 1% engine oil as a carbon source and culturing the isolate for multiple periods of incubation (0,24,72,120, and 144 hours) at 30 °C and 150 rpm/min. The best hydrocarbon biodegradation of *P. Putida* in 144 hours of incubation, was cell count (6.8×10^5 and 0.6 g/l) in 120 hours respectively. The uppermost values of the bacterial growth biomass, Cell count, and Protin contents were (12, 8.7×10^5 and 0.9 mg/l) respectively in (144 hours) of the incubation period compared to other periods as shown in Table (1).

Muhammad et al. [9] found that all seven isolates reached their maximum levels after 7 days of incubation, indicating high potential hydrocarbon degradations. It is found that increased incubation periods reduced the total hydrocarbon content of the medium, which was used to monitor the biodegradation potentials of *Pseudomonas* sp. and *Bacillus* species indicate bacterial biodegradation. Showing Kebede et al., [2]. The number of bacteria lower than 103 CFU per gram of soil showed less biodegradation potential.

Table 1: Total Bacterial Count of Soil Samples on Nutrient Agar

Grow Time (Hrs)	Dry Biomass (g/L)	Cell Count	Protein Content (mg/L)
0	0.1	1×10^5	ND
24	0.2	2×10^5	0.1
72	0.6	5×10^5	0.2
120	0.7	6.8×10^5	0.6
144	1.2	8.7×10^5	0.9

Effect of pH

This study declared differences in engine oil bio-degradation and bacterial growth observed at different pH values (5, 6, 7, 8, 9). The best pH for petroleum hydrocarbon remediation by *Pseudomonas putida* at PH (7) biomass (1.3g/l). In an earlier study by Li *et al.*, [10] alternatives in pH can affect enzymatic activity and bacterial community structure. Additionally, pH affects the soil's component solubility and accessibility, which indirectly affects biological activity [11]. Figures (1). The results presented in Figures (3-8) Sattar *et al.*, [1] documented that most microbes could be PAH (polyaromatic

hydrocarbons) degraded at neutral7 pH. In contrast, our results come in agreement with Sharma & Pandey [12] reported high degradation rates of HCs occur at a pH range of (6–9).

Effect of Carbon Source

Different carbon sources used for bacterial growth as nutrients. Show figure (3) The maximum percentage of engine oil remediation and optical density of bacterial growth by *Pseudomonas putida* was detected in flasks containing BH broth modified by carbon sources glucose and xylose. Abdel-Shafy & Mansour [13] highlighted

Pseudomonas sp. bacterial's diverse physiological and metabolic properties, along with their complex enzymatic system, which allows

them to depend on various hydrocarbon compounds as their sole carbon source.

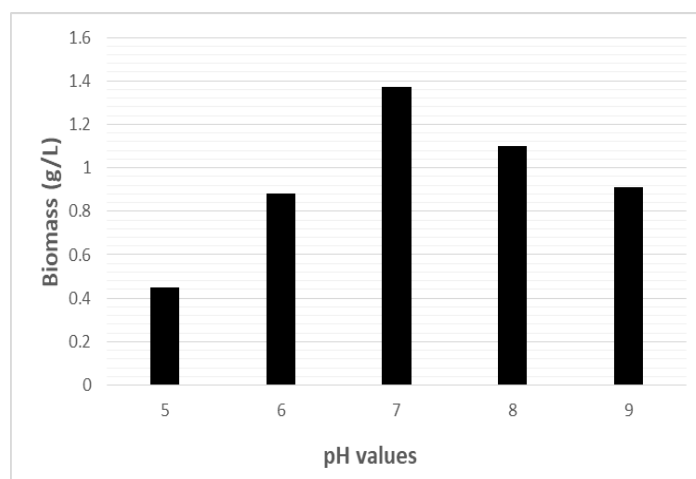


FIGURE 1: Role of Different pH Values on *Pseudomonas Putida*'s Decomposition Hydrocarbon Pollutants. Relationship between pH and Biomass.

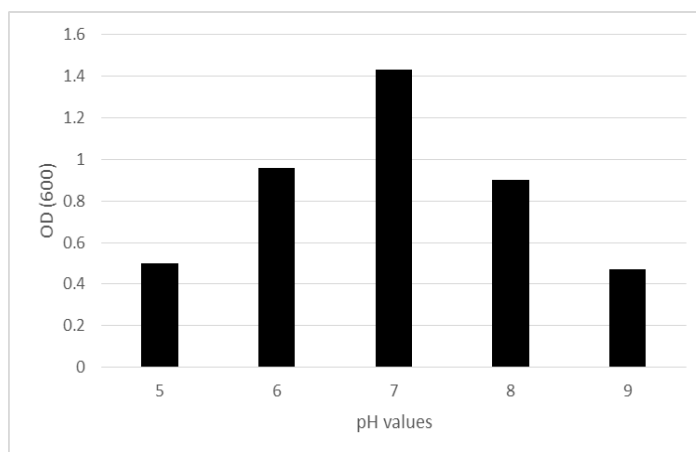


FIGURE 2: A-The Connection between Optical Density and PH Values

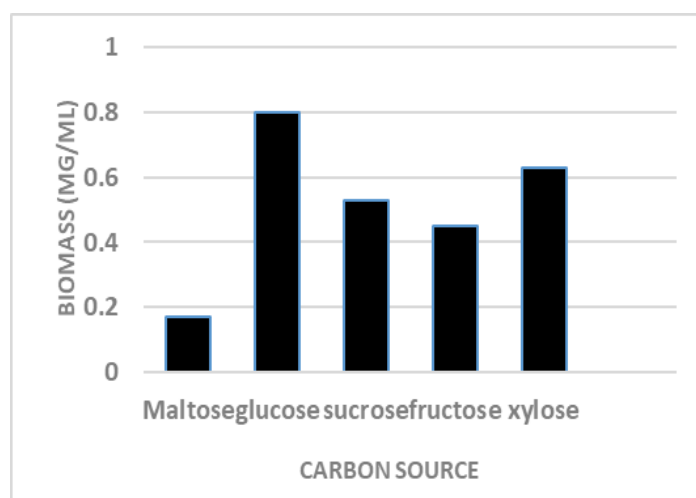


FIGURE 3: B- The Connection between Biomass and Carbons Course

Effect of Temperature

Temperature affects the viscosity of petroleum wastes, increasing solubility and volatility, leading to increased biodegradation rates. The optimal temperature for bioremediation of engine oil is 35°C, where maximum growth and hydrocarbon biodegradation occur.

The results are presented in Figure (4). Kebede et al., [2] showed higher temperatures reduce oxygen solubility and weaken aerobic bacteria metabolic activity, decreasing hydrocarbon biodegradation rates. Higher temperatures also accelerate hydrocarbon metabolism [14]. Extreme degradation occurs at a temperature array of 30–40 [7].

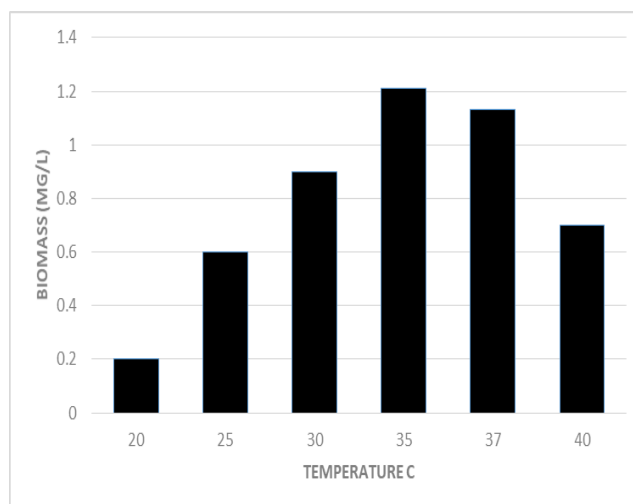


FIGURE 4: The Connection between Biomass and Temperature

GC-MS Chromatogram of Bioremediation of the Spent Engine Oil

In this study, alternative carbon sources used glucose, sucrose, xylose, and fructose. intensive biodegrading was observed. The bioremediation for SEO samples by *pseudomonas putida* shows a high percentage 100 percentage for Easter2, and Easter3 when using alternative carbon sources. This suggests these chemicals may have broken down into more ones that are volatile and fled. Ester components are degraded rapidly due to their fast solubility and short-carbon chain. Also, polymerase degradation Percentage was 89.52% in glucose carbon sources, in Xylose 88.95%, fructose 74.51%, 91and scruse73%. Alkanes and PAH had slowing degradation Percentages because these have a high percentage of long-chain carbons and were insolubility so biodegradation was late. The results are presented in Figure (5). It has used the bioremediation technique, using *Pseudomonas* sp. as an active bioremediation agent to remove hydrocarbon contaminants from contaminated sites. Umoren *et al.* [15] reported that bacteria have some genes encoded for degradation enzymes that aid them to break down and use hydrocarbons as a carbon and energy source in bioremediation [16]. During remediation methods, as much as 40% of hydrocarbons may evaporate quickly due

to light oil components. The petroleum's most readily broken down constituents are n-alkanes and cycloalkanes, whereas the aromatic portion was weakly degradable [17]. In another study early biodegradation processes, for alkanes and alkenes; because of readily degradable short-chain alkanes and alkenes; while weakly degradable long-chain alkanes, branched alkanes, and cyclic alkanes have a lengthy, lag time for microbiological adaptation for the oxidation, these are the most impervious to microbial degradation [16].

Conclusion

Microorganisms, mainly bacteria have the capacity of bioremediating. Data from this work provide strong proof that *Pseudomonas putida* can break down the chemical components in engine oil and use them as a source of carbon, demonstrating the bacteria's potential for engine oil bioremediation in contaminated soil. However, their degradation ability depends on the Optimal conditions such as pH, temperature, carbon sources, and incubation time. In this study, the optimized growth parameters are the temperature of 35 °C, pH 7, and incubation time of 144 hours.

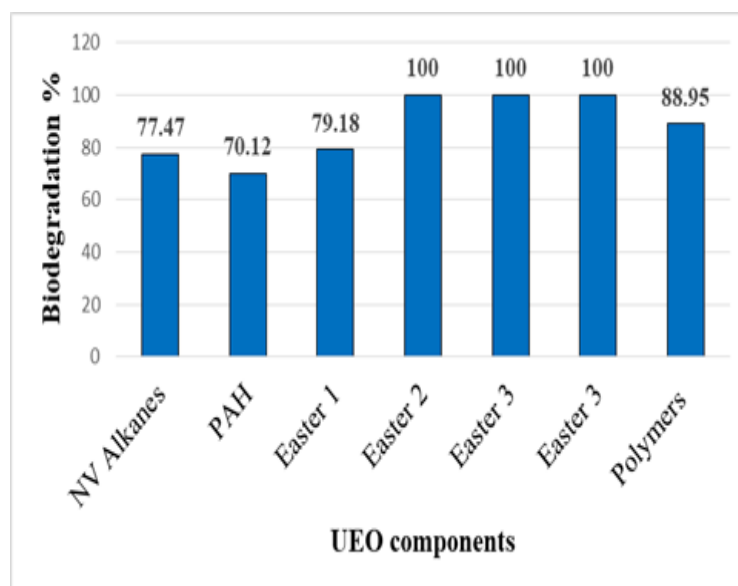


FIGURE 5: Percentage of Hydrocarbon-Degrading Bacteria Strain Alternative Carbon Sources A- gl

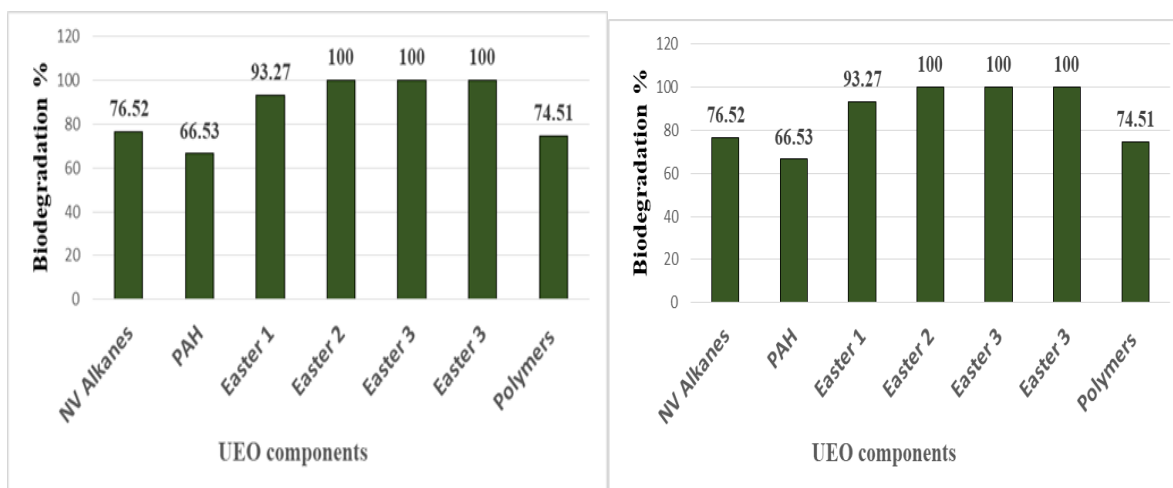


Figure 6: B-Xylose

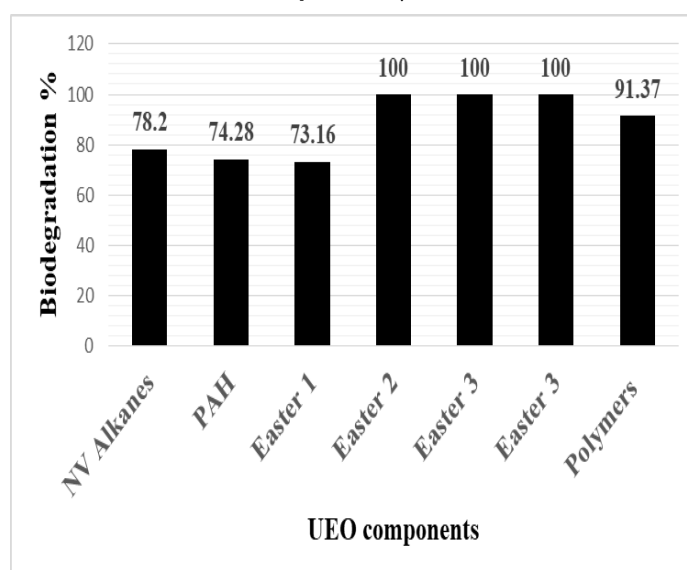


Figure 7: D-Fructose

REFERENCES

- [1] S. Sattar *et al.*, "Composition, impacts, and removal of liquid petroleum waste through bioremediation as an alternative clean-up technology: A review," *Heliyon*, vol. 8, no. 10, 2022.
- [2] G. Kebede, T. Tafese, E. M. Abda, M. Kamaraj, and F. Assefa, "Factors influencing the bacterial bioremediation of hydrocarbon contaminants in the soil: mechanisms and impacts," *Journal of Chemistry*, vol. 2021, no. 1, p. 9823362, 2021.
- [3] M. Udgire, N. Shah, and M. Jadhav, "Enrichment, isolation and identification of hydrocarbon degrading bacteria," *Int. J. Curr. Microbiol. App. Sci*, vol. 4, no. 6, pp. 708-713, 2015.
- [4] D. H. Bergey, *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins, 1994.
- [5] S. Naveenkumar, N. Manoharan, S. Ganesan, S. Manivannan, and G. Velsamy, "Isolation, screening and in vitro mutational assessment of indigenous soil bacteria for enhanced capability in petroleum degradation," *International journal of environmental sciences*, vol. 1, no. 4, pp. 498-513, 2010.
- [6] M. S. H. Zavareh, G. Ebrahimipour, M. S. Moghadam, J. Fakhari, and T. Abdoli, "Bioremediation of crude oil using bacterium from the coastal sediments of Kish Island, Iran," *Iranian journal of public health*, vol. 45, no. 5, p. 670, 2016.
- [7] P. Datta, P. Tiwari, and L. M. Pandey, "Isolation and characterization of biosurfactant producing and oil degrading *Bacillus subtilis* MG495086 from formation water of Assam oil reservoir and its suitability for enhanced oil recovery," *Bioresource technology*, vol. 270, pp. 439-448, 2018.
- [8] R. Latha and R. Kalaivani, "Bacterial degradation of crude oil by gravimetric analysis," *Advances in Applied Science Research*, vol. 3, no. 5, pp. 2789-2795, 2012.
- [9] R. Muhammad, J. Mohammed, I. Muhammad, and A. Hamzat, "Stimulated bioremediation of soil contaminated with spent engine oil using organic wastes," *Science World Journal*, vol. 17, no. 2, pp. 308-314, 2022.
- [10] Q. Li, J. Liu, and G. M. Gadd, "Fungal bioremediation of soil co-contaminated with petroleum hydrocarbons and toxic metals," *Applied Microbiology and Biotechnology*, vol. 104, no. 21, pp. 8999-9008, 2020.
- [11] A. E. AJIBOYE, H. O. SULAYMAN, and A. T. AJAO, "Bioremediation of spent engine oil on selected contaminated soils within ilorin metropolis," *Advanced Journal of Graduate Research*, vol. 8, no. 1, pp. 91-104, 2020.
- [12] S. Sharma and L. M. Pandey, "Biodegradation kinetics of binary mixture of hexadecane and phenanthrene by the bacterial microconsortium," *Bioresource Technology*, vol. 358, p. 127408, 2022.

- [13] H. I. Abdel-Shafy and M. S. Mansour, "Microbial degradation of hydrocarbons in the environment: an overview," *Microbial action on hydrocarbons*, pp. 353-386, 2018.
- [14] C. Muangchinda *et al.*, "Abundance and diversity of functional genes involved in the degradation of aromatic hydrocarbons in Antarctic soils and sediments around Syowa Station," *Environmental Science and Pollution Research*, vol. 22, pp. 4725-4735, 2015.
- [15] A. S. Umoren *et al.*, "Long-term effects of crude oil spillage on selected physicochemical properties including heavy metal contents of sandy tropical soil," *Bulletin of environmental contamination and toxicology*, vol. 102, pp. 468-476, 2019.
- [16] S. Abha and C. S. Singh, "Hydrocarbon pollution: effects on living organisms, remediation of contaminated environments, and effects of heavy metals co-contamination on bioremediation," *Introduction to enhanced oil recovery (EOR) processes and bioremediation of oil-contaminated sites*, vol. 318, 2012.
- [17] S. Maletić *et al.*, "Characterization of weathered petroleum hydrocarbons during a landfarming bioremediation study," *Journal of the Serbian Chemical Society*, vol. 77, no. 11, pp. 1671-1685, 2012.



This is an open-access article distributed under the terms of the Creative Commons Attribution License.