Isolation, Biochemical And Biophysical Characterisation of Hydrocarbonoclastic Bacteria From Diesel Contaminated Environment

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Abstract. Environmental contamination by diesel fuel poses substantial ecological challenges, compromising both terrestrial and aquatic ecosystems due to its persistent toxic properties. Biological remediation offers a sustainable and environmentally conscious solution to address these environmental concerns by utilising hydrocarbon-metabolising microorganisms. This research examines the bioremediation capabilities of *Paenibacillus polymxa*, obtained from a diesel-polluted location, for addressing diesel contamination issues. The isolated bacterial strain developed prominent, cream-coloured, textured colonies when grown on Bushnell-Haas medium and exhibited elongated, Gram-positive, motile characteristics with spore-forming capabilities. Biochemical analysis demonstrated broad metabolic capabilities, showing positive responses for catalase, oxidase, urease, casein degradation, and starch breakdown activities. Maximum diesel breakdown efficiency was observed at 5% diesel concentration, pH 9, and 37°C temperature, achieving a peak degradation rate of 70%. These results demonstrate the organism's durability and effectiveness in hydrocarbon metabolism, establishing it as a viable option for environmentally sustainable remediation of diesel-contaminated areas. Subsequent research should emphasise genetic optimisation, nutrient enhancement, consortium development, and field validation to confirm practical applications and commercial viability.

Keywords: Biological remediation; hydrocarbon metabolism; diesel pollution; *Paenibacillus polymxa;* environmental restoration; microbial degradation.

INTRODUCTION

Diesel fuel represents one of the most extensively utilised petroleum derivatives worldwide [1]. Persistent low-level diesel releases from transportation systems, storage vessels, or containment facilities during fuel transfer operations can lead to severe environmental contamination issues. These incidents often remain undetected, creating significant ecological risks due to the accumulation of hydrocarbons [2]. Biological remediation serves as an efficient, cost-effective, and eco-friendly treatment approach utilising microorganisms for hydrocarbon breakdown [2].

Multiple variables affect microbial proliferation rates, including soil hydration, temperature conditions, community composition, pH levels, oxygen availability, and nutritional content. Carbon, nitrogen, and phosphorus concentrations represent critical elements in successful hydrocarbon remediation within soil systems. Microorganisms capable of biodegrading petroleum hydrocarbon components such as polycyclic aromatic hydrocarbons (PAHs), naphthalene, single-ring aromatic compounds like toluene, or straight-chain hydrocarbons including n-alkanes, can be easily obtained from environmental sources, particularly from petroleum-impacted locations.

Hydrocarbon compounds, including polycyclic aromatic hydrocarbons (PAHs), benzene, kerosene, and diesel fuel, constitute significant organic contaminants and serve as energy sources for various industrial processes, transportation systems, and domestic applications [3]. Diesel fuel is widely used in engines, fuel systems, and industrial operations. It constitutes one of the petroleum products generated through crude oil fractional distillation, comprising carbon chain mixtures ranging from 9 to 25 carbon atoms, which encompass both aromatic and aliphatic hydrocarbon components [4].

Research Problem Statement. Environmental diesel contamination poses a significant threat to ecological systems and human health due to the persistent and toxic characteristics of hydrocarbon pollutants [5]. Traditional remediation methods often prove costly, inefficient, and potentially environmentally harmful. Therefore, growing attention focuses on bioremediation as an environmentally friendly alternative. Hydrocarbonoclastic bacteria, which are capable of degrading hydrocarbons, present a promising approach. However, enhanced understanding of these bacteria, particularly those obtained from diesel-contaminated locations, remains crucial for improving their bioremediation applications [6].

Research Justification. Numerous bioremediation studies have been conducted globally; however, limited research exists on the bioremediation of diesel-contaminated environments in Katsina State, Nigeria. Effective isolation and characterisation of hydrocarbonoclastic bacteria will enhance our understanding of their biodegradation roles. This knowledge can facilitate the development of more effective and environmentally sustainable bioremediation approaches for areas contaminated with diesel.

Research Aims and Objectives. This research aims to isolate, screen, and characterise hydrocarbon-degrading bacteria from diesel-contaminated environments.

Specific Objectives:

- To quantify diesel-degrading bacteria from contaminated soil samples;

- To isolate and characterise bacteria demonstrating hydrocarbon-degrading capabilities from diesel-contaminated environments;

- To establish optimal pH, temperature, and substrate concentration conditions for hydrocarbon-degrading bacterial isolates.

Environmental and Health Consequences of Hydrocarbon Contamination. Hydrocarbon pollution is a global issue that affects the health of animals, the environment, and humans. Recently, the potential for hydrocarbon contamination has gained increased attention, particularly in aquatic, marine, and terrestrial environments. Research indicates that hydrocarbons can have a significantly adverse impact on ecosystems [7]. Hydrocarbon contamination causes deterioration in ecosystem function and affects both living (biotic) and non-living components.

When hydrocarbons enter soil systems, they can impede the availability of water, nutrients, oxygen, light, and other parameters for biological processes. This affects soil productivity (plant development and seed viability) and consequently agricultural output. Hydrocarbon contaminants can cause immediate or delayed effects, including genetic alterations, immune system toxicity, developmental abnormalities, cancer development, high bioaccumulation potential, and deterioration of ecosystem function.

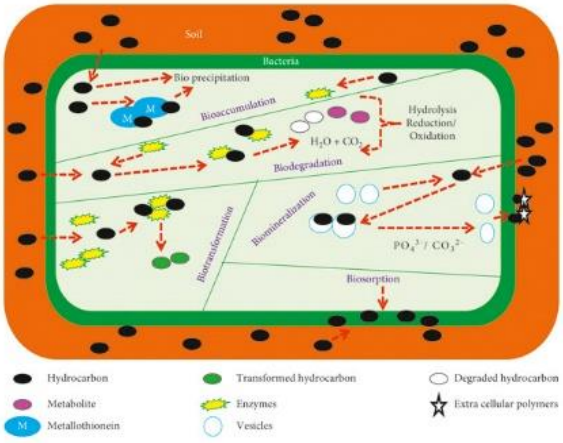

Figure 1 –The mechanisms of microbial remediation used for reducing heavy metals

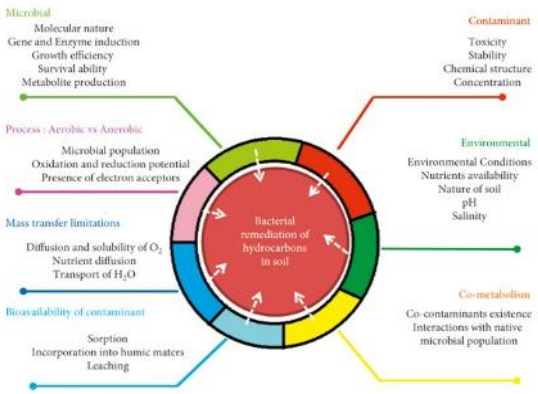

Figure 2 – Major classes of factors influencing the bacterial remediation of hydrocarbons in soil

Hydrocarbon Bioremediation Processes. Bioremediation involves contaminant removal, destruction, immobilisation, mineralisation, or transformation from soil using plants, protozoa, fungi, microalgae, and particularly bacteria [8] and their metabolic products under optimal conditions. Various bioremediation methods are broadly classified into natural attenuation, bioaugmentation, and biostimulation.

Bioaugmentation involves introducing selected hydrocarbon-degrading microbial strains or communities into contaminated environments to enhance the biodegradation potential of the existing microbial community [9]. Biostimulation involves the amendment of macro- and micronutrients, the maintenance of physical parameters (pH, temperature, and aeration), and the provision of surface-active substances (surfactants) in contaminated sites to optimise soil conditions and enhance biodegradation.

Multiple studies have demonstrated that diverse microorganisms, including bacteria, fungi, yeasts, protozoa, and algae, significantly contribute to the biodegradation of hydrocarbon pollutants, with bacteria being the most dominant and active degraders [10]. Hydrocarbon-degrading bacteria are widespread, with well-known genera including Achromobacter, Marinobacter, Actinobacter, Alcaligenes, Mycobacterium, Arthrobacter, Bacillus, Rhodococcus, Corynebacterium, Micrococcus, Flavobacterium, Nocardia, Bravibacterium, Streptococcus, Stenotrophomonas, Methylobacterium, Enterobacter, *and* Pseudomonas.

Bacterial Bioremediation Influencing Factors. Multiple factors influence the efficiency of bacterial bioremediation and biodegradation rates. Primary factors include microbial characteristics (community composition, metabolic capacity, population density, biosurfactant production ability, and competition), contaminant physicochemical properties (chemical structure, concentration, toxicity, and bioavailability), and environmental conditions (soil type, temperature, pH, oxygen availability, salinity, nutrients, and water availability).

Hydrocarbon Properties: The physical and chemical characteristics of hydrocarbon pollutants significantly influence biodegradation processes. High molecular weight polyaromatic hydrocarbons (four or more rings) and highly condensed cycloalkane compounds show greater resistance than unbranched alkanes and lighter PAHs to microbial degradation. Hydrocarbon biodegradation rates follow the following order: n-alkanes > branched alkanes > low-molecular-weight aromatics > high-molecular-weight aromatic hydrocarbons > asphaltenes.

Physical Parameters:

1) Soil Properties: Soil physicochemical characteristics determine the nature of the indigenous microbes, bacterial community composition, and functional gene makeup.

2) Temperature: Significantly affects bacterial growth rates, enzyme activity, pollutant chemistry, and bacterial community diversity.

3) pH: Most hydrocarbon-degrading bacteria prefer neutral to slightly alkaline pH (6-8) for optimal bioremediation.

4) Nutrient Availability: Bacteria require nutrients for metabolism and growth, with recommended C:N:P ratios of 100:10:1 to 100:20:1.

5) Oxygen Availability: Aerobic catabolism produces higher biodegradation rates than anaerobic metabolism.

METHODOLOGY

Study Location. This research was conducted in Katsina metropolis at longitude 007°26'E and latitude 12°17.00'N to 12°17.84'N, Katsina State, Northwestern Nigeria.

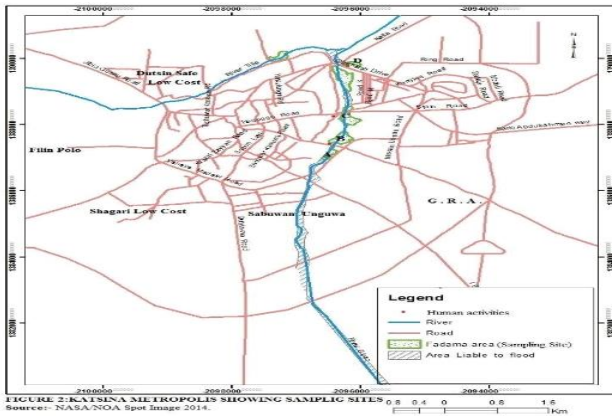

Figure 3 – Study area map of Katsina metropolis, Northwestern Nigeria

Sample Collection and Processing. Soil samples were obtained from nearby automotive workshops, fuel storage facilities, and refinery locations within Katsina metropolis using random sampling methodology. A sterilised hand trowel collected 2 kg of soil from a 0-30 cm depth, and placed it aseptically into polythene containers. Collected samples were transported to Umaru Musa Yar'adua University, Katsina microbiology laboratory, where enrichment cultures were immediately established.

Sample Preparation and Serial Dilution. From each collected sample (oil-contaminated soil), 1 g was measured and added to sterilised test tubes containing 9 millilitres of distilled water, creating the 10^{-1} dilution (stock solution). This was allowed to soak for 10 minutes with gentle agitation using a rotary shaker. Subsequently, 1 millilitre was transferred to clean, sterilised test tubes containing 9 millilitres of distilled water, obtaining the 10^{-2} dilution. This process continued consistently in subsequent tubes until 10^{-3} to 10^{-6} dilutions were obtained.

Media Preparation and Sample Inoculation. Nutrient agar was prepared according to the manufacturer's instructions. Plating utilised the pour plate method: 1ml from each 10^{-4}, 10^{-5}, and 10^{-6} dilutions was dispensed into clean Petri dishes. Medium was added, the plates were swirled, and the medium was allowed to solidify. Incubation proceeded at 37°C for 24 hours in an autoclave.

Bacterial Enumeration. After 24 hours, colonies from incubated plates were counted using the Electronic Colony Counter SC6PLUS Model (Stuart Equipment, UK). Colony Forming Units = Number of colonies counted × 1/dilution factor.

Bacterial Sub-culture. The pure bacterial colonies obtained were inoculated onto fresh nutrient agar plates to establish pure cultures for each colony. Plates were incubated at 37°C in an autoclave for 24 hours.

Hydrocarbon-Degrading Bacteria Isolation

Bushnell-Haas Agar Preparation. The prepared mineral salt medium was Bushnell-Haas Agar (BHA), containing Plain Agar Powder (15 g); KH_2PO_4 (1 g); K_2HPO_4 (1 g); NH_4NO_3 (1 g); $MgSO_4$ (0.2 g); $FeCl_3$ (0.05 g) and $CaCl_2$ (0.02 g), dissolved in one liter of distilled water and sterilised by autoclaving at 121°C for 15 minutes. The medium was supplemented with 2.5% petroleum as a carbon/energy source and 2.5% diesel as a carbon source.

Bacterial Screening and Isolation. Pure bacterial isolate cultures were streaked onto prepared Bushnell-Haas Agar plates and incubated at 37°C for 96 hours. Colonies from each plate were observed, enumerated, and subcultured onto fresh nutrient agar slants, which were then maintained at 4°C for further analysis.

Morphological Analysis. Gram staining followed standard protocols. Briefly, bacterial smears were prepared on glass slides, air-dried, and heat-fixed over a flame. Crystal violet drops were added and allowed to stand for 1 minute. Slides were rinsed in water for five seconds, then Gram's iodine was applied to the smears and allowed to stand for one minute, enabling the formation of a dye-iodine complex in the bacterial cell cytoplasm. Slides were tilted and decolourised with solvent (acetone solution) for five seconds, then rinsed and shaken to remove excess. Slides were finally treated with safranin (counter-stain) and allowed to stand for 1 minute, then washed briefly with water and shaken to remove excess. They were dried before microscopic examination.

Biochemical Tests

Catalase Test. Three drops of hydrogen peroxide (3%) were added to overnight-grown cultures in test tubes, with vigorous bubble formation indicating catalase activity.

Casein Hydrolysis. Isolates were grown overnight in nutrient broth and then inoculated onto skim milk agar, which was incubated at 30°C for 48 hours. Clear zone formation around isolates against white backgrounds indicated isolate casein hydrolysis activity.

Urease Test. Pure bacterial isolates were inoculated into urea broth and incubated at 30°C for 24 to 48 hours – a colour change from yellow to pink indicated urease production.

Starch Test. Isolates were grown overnight in nutrient broth and then inoculated onto starch agar medium, which was incubated at 30°C for 48 hours. Plates were flooded with Gram stain. Clear area formation around isolates against blue-black backgrounds indicated starch hydrolysis.

RESULTS AND DISCUSSION

Bacterial Isolation and Identification. The bacterial isolate obtained from diesel-contaminated sites grew successfully on Bushnell-Haas agar, producing prominent, cream-colored, textured colonies. Microscopic examination revealed rod-shaped, Gram-positive, motile cells with the capability to form endospores, a characteristic of bacteria in the *Paenibacillus* genus. Based on morphological characteristics and biochemical tests, the isolate was identified as *Paenibacillus polymxa*. This identification was further confirmed by its efficient growth in the presence of diesel, indicating its potential as a hydrocarbon-degrading bacterium.

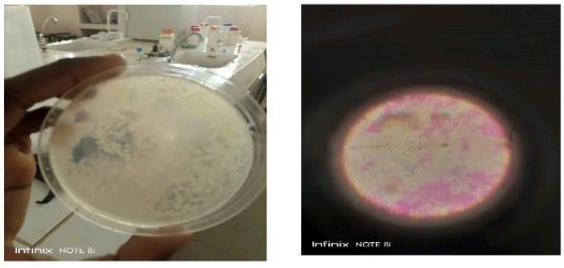

Figure 4 –Morphological characteristics of isolated Paenibacillus polymyxa on selective medium

Table 1 – Biochemical Test Results

No	Enzyme	Result
1	Catalase	Positive (+)
2	Oxidase	Positive (+)
3	Urease	Positive (+)
4	Casein hydrolysis	Positive (+)
5	Starch hydrolysis	Positive (+)

Table 2 – Optimum Conditions for Hydrocarbon-Degrading Bacterial Growth

Parameters	Optimal Conditions	Colony Count	Degradation, %
Substrate concentration	1%	1.04×10^2	15%
	2.5%	3.04×10^2	40%
	5%	5.72×10^2	60%
pH	5	5.0×10^1	10%
	7	4.16×10^2	40%
	9	6.88×10^2	65%
Temperature	0°C	No growth	0%
	20°C	5.2×10^1	15%
	30°C	3.06×10^2	50%
	37°C	6.02×10^2	70%

Bacterial Identification and Characteristics. The successful isolation and identification of *Paenibacillus polymxa* from diesel-contaminated sites demonstrates its considerable bioremediation potential. The bacterium's ability to produce prominent, cream-coloured, textured colonies on Bushnell-Haas agar, combined with its rod-shaped, Gram-positive, motile, and endospore-forming characteristics, aligns with established features of the Paenibacillus genus [11]. These characteristics indicate that *P. polymxa* is well-adapted for survival and activity in harsh hydrocarbon-contaminated environments.

Metabolic Diversity and Enzymatic Functions. Biochemical characterisation demonstrated the isolate's metabolic diversity, with positive results for catalase, oxidase, urease, casein hydrolysis, and starch hydrolysis tests. These enzymatic functions are crucial for the degradation of hydrocarbons. For example, catalase and oxidase reduce oxidative stress, while hydrolytic enzymes facilitate the breakdown of complex organic compounds into simpler forms [12]. Such metabolic flexibility enhances the organism's ability to thrive in environments rich in hydrocarbons and efficiently degrade diesel.

Environmental Condition Optimisation. Evaluation of *P. polymyxa* under varying environmental conditions revealed optimal performance at *a* 5% diesel concentration, pH 9, and 37°C, achieving maximum degradation rates of 60%, 65%, and 70%, respectively. These findings align with recent studies that highlight the importance of neutral to alkaline pH and mesophilic temperatures in enhancing hydrocarbon degradation by supporting enzyme activity and bacterial metabolism [13, 14].

P. polymxa's ability to grow and degrade diesel at varying concentrations further emphasises its

adaptability and potential for application in diverse contaminated environments. The observed increase in diesel degradation with rising pH values and temperatures highlights the importance of optimising environmental factors for bioremediation.

Significance and Practical Applications. The maximum degradation at pH 9 and 37°C reflects the bacterium's preference for slightly alkaline and moderate-temperature conditions, which are common characteristics of effective hydrocarbon-degrading bacteria [15]. These properties make *P. polymxa* a promising candidate for the remediation of diesel-contaminated sites, especially in tropical and temperate regions.

The significance of these findings lies in their practical applications. Diesel contamination poses serious environmental hazards, including soil and water pollution. Bioremediation using *P. polymxa* offers an eco-friendly and cost-effective approach to mitigate these effects. Furthermore, the bacterium's robust metabolic and enzymatic profile provides opportunities for its application in industrial-scale remediation efforts [16].

Comparison with Previous Studies. The identified strain *Paenibacillus polymxa* showed remarkable hydrocarbon degradation capabilities compared to other studies. The maximum degradation rate of 70% at optimal conditions is competitive with those reported for other hydrocarbon-degrading bacteria. The bacterium's ability to maintain activity across a range of environmental conditions makes it particularly suitable for field applications where conditions may vary significantly.

CONCLUSIONS

This study's findings demonstrate that *Paenibacillus polymyxa,* isolated from diesel-contaminated sites, possesses significant potential for bioremediation of diesel-contaminated environments. The bacterium's ability to grow efficiently on Bushnell-Haas agar and its positive biochemical test results confirm its metabolic adaptability and hydrocarbon-degrading capacity.

The study further revealed that *P. polymxa* achieves optimal diesel degradation under specific environmental conditions, including 5% diesel concentration, pH 9, and 37°C temperature, with a maximum degradation rate of 70%. These results emphasise the bacterium's resilience and efficiency, making it a promising candidate for mitigating environmental hazards posed by diesel contamination.

To maximise *Paenibacillus polymxa* potential as a bioremediation agent, several key steps are recommended:

Further Optimisation: Research should focus on optimising the bacterium's degradation efficiency through genetic engineering, adaptive evolution, or nutrient supplementation to enhance its hydrocarbon-degrading capabilities.

Field Studies: Field studies in diesel-contaminated environments are crucial for validating laboratory findings and evaluating the bacterium's performance under real-world conditions.

Microbial Consortium Development: Developing microbial consortia that include P. polymxa and other hydrocarbon-degrading bacteria could yield synergistic effects, resulting in faster and more efficient diesel degradation.

Environmental Assessment: A Comprehensive environmental assessment is crucial to ensure that the P. polymxa application does not disrupt native microbial communities or other ecological processes.

Industrial Scale-up: Scaling up these findings to industrial and environmental settings should be prioritised, focusing on creating cost-effective and sustainable bioremediation strategies.

These steps will advance the *P. polymxa* application and contribute significantly to addressing environmental challenges posed by diesel contamination.

REFERENCES

1. Abram, O. H., Tallei, T. E., De Queljoe, E., & Kolondam, B. J. (2014). Identification Of Potential Diesel Oil-Degrading Bacteria Isolated From Manado Sea Port Based On 16s Rrna Gene. *Jurnal Ilmiah Sains, 14*(2), 73. doi: 10.35799/jis.14.2.2014.5932

2. Geetha, S. J., Joshi, S. J., & Kathrotiya, S. (2013). Isolation and Characterisation of Hydrocarbon Degrading Bacterial Isolate from Oil Contaminated Sites. *APCBEE Procedia, 5*, 237–241. doi: 10.1016/j.apcbee.2013.05.041

3. Ashikodi, A. O., & Abu, G. O. (2019). Hydrocarbon degradation potential of some hydrocarbon-utilising bacterial species associated with Kenaf (Hibiscus cannabinus L.) plant. *International Research Journal of Biological Sciences, 8*(1), 10–19.

4. Bekele, G. K., Gebrie, S. A., Mekonen, E., Fida, T. T., Woldesemayat, A. A., Abda, E. M., Tafesse, M., & Assefa, F. (2022). Isolation and Characterisation of Diesel-Degrading Bacteria from Hydrocarbon-Contaminated Sites, Flower Farms, and Soda Lakes. *International Journal of Microbiology, 2022*, 1–12. doi: 10.1155/2022/5655767

5. Mnif, I., Sahnoun, R., Ellouz-Chaabouni, S., & Ghribi, D. (2017). Application of bacterial biosurfactants for enhanced removal and biodegradation of diesel oil in soil using a newly isolated consortium. *Process Safety and Environmental Protection, 109*, 72–81. doi: 10.1016/j.psep.2017.02.002

6. Peter, O. (2011). Biological Remediation of Hydrocarbon and Heavy Metals Contaminated Soil. Soil Contamination. doi: 10.5772/24938

7. Abbasian, F., Lockington, R., Mallavarapu, M., & Naidu, R. (2015). A Comprehensive Review of Aliphatic Hydrocarbon Biodegradation by Bacteria. Applied Biochemistry and Biotechnology, 176(3), 670–699. doi: 10.1007/s12010-015-1603-5

8. Mekonnen, B. A., Aragaw, T. A., & Genet, M. B. (2024). Bioremediation of petroleum hydrocarbon contaminated soil: a review on principles, degradation mechanisms, and advancements. *Frontiers in Environmental Science, 12*. doi: 10.3389/fenvs.2024.1354422

9. Sihag, S., Pathak, H., Jaroli, D. P. (2014). Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. *International Journal of Pure & Applied Bioscience, 2*(3), 185–202.

10. Ghazali, F. M., Rahman, R. N. Z. A., Salleh, A. B., & Basri, M. (2004). Biodegradation of hydrocarbons in soil by microbial consortium. *International Biodeterioration & Biodegradation, 54*(1), 61–67. doi: 10.1016/j.ibiod.2004.02.002

11. Chen, S.-C., Musat, F., Richnow, H.-H., & Krüger, M. (2024). Microbial diversity and oil biodegradation potential of northern Barents Sea sediments. *Journal of Environmental Sciences, 146*, 283–297. doi: 10.1016/j.jes.2023.12.010

12. Ajona, M., & Vasanthi, P. (2021). Bioremediation of petroleum contaminated soils – A review. *Materials Today: Proceedings, 45*, 7117–7122. doi: 10.1016/j.matpr.2021.01.949

13. Mangla, H., Sharma, H., Dave, S., Sudan, J., & Pathak, H. (2021). Microbial mechanism of petroleum hydrocarbons degradation: "An Environmental perspective." *Applied Environmental Biotechnology, 6*(2), 32–42. doi: 10.26789/aeb.2021.02.005

14. Bamforth, S. M., & Singleton, I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology & Biotechnology, 80*(7), 723–736. doi: 10.1002/jctb.1276

15. Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N., & Naidu, R. (2011). Bioremediation approaches for organic pollutants: A critical perspective. *Environment International, 37*(8), 1362–1375. doi: 10.1016/j.envint.2011.06.003

16. Das, N., & Chandran, P. (2011). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International, 2011*, 1–13. doi: 10.4061/2011/941810