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Research Article

Biodegradation of PAHs in Used Engine Oil Using *Pseudomonas aeruginosa* and *Bacillus licheniformis* Isolated from Contaminated Soil of a Mechanic Workshop in Birnin Kebbi Metropolis

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Abstract

Background: Polycyclic Aromatic Hydrocarbons (PAHs) are toxic, carcinogenic, and persistent organic pollutants commonly found in Used Engine Oil (UEO), posing significant environmental and health threats. Bioremediation using hydrocarbon-degrading bacteria provides an eco-friendly alternative for mitigating petroleum pollution. This study investigated the biodegradation potential of Pseudomonas aeruginosa and Bacillus licheniformis isolated from contaminated soil in mechanic workshops within Birnin Kebbi metropolis.

Materials and Methods: Surface soil samples (0-5cm) were collected from five mechanic workshops in Birnin Kebbi, Nigeria, using grab sampling techniques. The samples were homogenized, sieved (2-4 mm), and stored at 4°C. Bacterial isolates were obtained through serial dilution and spread plate methods on nutrient agar, followed by identification via morphological, Gram, and biochemical characterization. The isolates were tested for their ability to degrade UEO in both minimal salt media and soil systems. The degradation process was monitored for 14 days in liquid media and for six weeks in soil pots. Residual hydrocarbons were extracted via Soxhlet extraction using dichloromethane/methanol (93:7 v/v), quantified gravimetrically, and analyzed for PAHs composition using Gas Chromatography-Mass Spectrometry (GC-MS).

Results: Two bacterial species, *Pseudomonas aeruginosa* (Gram-negative) and *Bacillus licheniformis* (Gram-positive), were identified as the predominant hydrocarbon-degrading bacteria. Both species demonstrated the ability to utilize UEO as the sole carbon source. In minimal salt media, *P. aeruginosa* exhibited sustained growth and higher degradation efficiency than *B. licheniformis*. Gravimetric analysis showed that P. aeruginosa degraded up to 55.74% of the used engine oil, while B. licheniformis achieved a maximum degradation rate of 48.8%. GC-MS analysis revealed a significant reduction in both high- and low-molecular-weight PAHs, with degradation products indicating transformation into oxygenated and chlorinated PAH metabolites. The biodegradation efficiency was positively correlated with bacterial cell concentration (CFU/mL). Conclusion: This study demonstrated the effective biodegradation of used engine oil and associated PAHs by *Pseudomonas aeruginosa* and *Bacillus licheniformis* isolated from hydrocarbon-contaminated soil. *P. aeruginosa* exhibited superior degradation potential, suggesting its suitability for bioremediation applications in hydrocarbon-polluted environments. The findings support the use of indigenous microbial strains as sustainable agents for mitigating petroleum hydrocarbon pollution in Nigerian ecosystems.

Keywords: Biodegradation, *Pseudomonas aeruginosa*, *Bacillus licheniformis*, Used engine oil, Polycyclic Aromatic Hydrocarbons (PAHs)

Introduction

In developing nations, the establishment of designated automotive service zones such as mechanic and repair workshops has become increasingly common [1]. Within these environments, accidental spills, illegal dumping, and improper handling of Used Engine Oil (UEO) are major sources of environmental pollution, particularly with Polycyclic Aromatic Hydrocarbons (PAHs), which are persistent organic pollutants of global concern [2]. Fresh motor oil contains a high concentration of volatile, water-soluble, and lighter hydrocarbons, posing short-term (acute) toxicity risks to exposed organisms. However, used motor oil accumulates heavier PAHs at concentrations several orders of magnitude higher than those in fresh oil. For instance, concentrations of dibenzo(a,c) anthracene, fluoranthene, benzo(a)anthracene, benzo(a)pyrene, benzo(e)pyrene, benzo(g,h,i) perylene, and 4-methylpyrene have been reported to be 36, 253, 720, 1,112, 4,770, and 7,226 times higher, respectively, in used oil than in fresh oil [3].

Used Engine Oil also contains gasoline residues, diverse aliphatic and aromatic hydrocarbons, additives (detergents, oxidation inhibitors, and rust preventives), sulfur and nitrogen compounds, and heavy metals such as Pb, Zn, Cu, Cr, Ca, Ba, and Mg. These arise from engine wear, oxidation, and thermal degradation during engine operation [4]. UEO, defined as lubricating oil removed from the crankcase of internal combustion engines, comprises approximately 80-90% hydrocarbons and 10-20% performance-enhancing additives [5]. During use, these additives degrade and the oil becomes contaminated with combustion products and metallic wear debris [6]. Globally, used motor oil is one of the most frequently spilled petroleum products, posing an even greater challenge in developing nations where recycling practices are inadequate. In Nigeria, approximately 20 million gallons of UEO are generated annually, contributing significantly to environmental pollution [7]. Although fresh motor oil exhibits acute toxicity due to volatile hydrocarbons, UEO contains more persistent and toxic compounds, including PAHs and heavy metals, which cause chronic toxicity to ecosystems [8].

Several studies have characterized the composition of UEO, reporting densities around 0.828 g/mL and compositions of 14% aromatic and 65.4% aliphatic hydrocarbons by weight. The total PAH content represents approximately 1.2% of the aromatic fraction [8]. PAHs are well known for their carcinogenic, mutagenic, and teratogenic properties, as well as for impairing the reproductive capacity of exposed organisms [9]. In developed nations, recycling and "do-it-yourself" automotive service awareness have reduced environmental oil discharge. Conversely, in many developing regions, used motor oil is irresponsibly discarded into gutters, drains, vacant lots, and waterways [10]. PAHs are broadly categorized by molecular weight. Low-Molecular-Weight (LMW) PAHs consist of two to three benzene rings, whereas High-Molecular-Weight (HMW) PAHs contain four or more rings [11]. Humans and wildlife are rarely exposed to single PAHs; instead, they encounter mixtures that can be transformed within the body into metabolites-some of which are more toxic than their parent compounds [12]. Given their persistence, toxicity, and environmental ubiquity, there is a growing interest in the use of microorganisms-particularly *Pseudomonas* and *Bacillus* species-for biodegradation of UEO and associated PAHs.

Materials and Methods

Soil Sample Collection

Surface soil samples (0-5 cm depth) were collected using a grab sampler from five different locations within Birnin Kebbi Metropolis. Samples were collected in duplicates, with each sampling point at least 5 m apart. The collected soils were wrapped in aluminum foil to prevent contamination and immediately transported to the laboratory. In the laboratory, the samples were homogenized, sieved through a 2-4 mm mesh to remove stones and debris, and stored at 4°C until further analysis [2].

Isolation and Identification of Microorganisms

Microorganisms were isolated following the method described by *Ogumbayo, et al.,* [1]. Briefly, a serial dilution (10^1-10^5) was prepared from 1g of each soil sample. The total heterotrophic bacterial population was determined using the spread plate technique, by plating 0.1mL of each dilution onto nutrient agar and incubating at 37 °C for 24 hours. Distinct colonies were sub-cultured repeatedly to obtain pure isolates. The bacterial isolates were identified based on their colonial morphology, Gram staining, and standard biochemical tests [13].

Biodegradation of Used Engine Oil in Minimal Salt Medium

The ability of the isolated bacteria to utilize hydrocarbons present in Used Engine Oil (UEO) was assessed following the procedure of *Ogumbayo, et al.,* [1]. A basal Minimal Salt Medium (MSM) was prepared using 0.5g yeast extract, 0.5g $(NH_4)_2$ SO_4, 0.1g KH_2PO_4, 0.1g K_2HPO_4, 0.1g $MgSO_4$, 0.1g NaCl, and 0.1g $CaCl_2$, dissolved in 150mL of distilled water. The medium was dispensed into 250 mL conical flasks and sterilized. After cooling, 1mL of UEO was aseptically introduced into each flask as the sole carbon source. Overnight broth cultures of the bacterial isolates were inoculated into the flasks and incubated in a gyratory shaker at 150rpm and 30 °C. Utilization of the UEO was monitored every 24 hours for 14 days by withdrawing aliquots of the culture and plating on nutrient agar, followed by incubation at 37°C for 24 hours to determine viable counts.

Biodegradation of Used Engine Oil in Soil Medium

Uncontaminated soil was collected and sieved through a 2mm mesh to ensure uniformity. Artificial contamination was achieved

by mixing 50mL of UEO with 1 kg of soil, followed by intermittent watering and weathering for one week. A total of 24 experimental pots were prepared, each containing 1kg of unpolluted soil. Twenty pots were spiked with 50 mL of UEO, while four unpolluted pots served as controls. Bacterial inocula of *Pseudomonas aeruginosa* and *Bacillus licheniformis* were separately cultured in 50 mL of nutrient broth within 250 mL conical flasks. The cells were harvested, washed, and standardized using McFarland standards to obtain inocula ranging from 0.5×10^8 to 4.0×10^8 CFU/mL. The standardized bacterial suspensions (100 mL) were introduced onto the surface of the UEO-contaminated soils. Control pots received 100 mL of sterile distilled water. The pots were maintained under optimum environmental conditions for six weeks with periodic watering, after which residual UEO was extracted for analysis [14].

Soxhlet Extraction of Residual Used Engine Oil

Residual UEO was extracted using the method described by Daniel and Nwaichi [15]. Approximately 625 mL of squalane solution was added to each soil sample as an extraction standard, mixed thoroughly with the soil slurry, and transferred into a thimble. Extraction was performed using a 200 mL Dichloromethane (DCM) and methanol solvent mixture (93:7, v/v) in a Soxhlet apparatus.

Gravimetric Analysis of Extractable Organic Matter

The amount of UEO degraded was determined following the gravimetric method of Latha and Alavaina [16]. The extract was concentrated to 10 mL with DCM, and 2 mL aliquots were evaporated to dryness in pre-weighed vials. The weight of the residual oil was determined, and the percentage degradation was calculated using the following equations:

Weight of residual oil = Weight of vial with extract-Weight of empty vial

Amount degraded = Weight of UEO added-Weight of residual oil

$$\%\text{Degradation} = \frac{\text{Amount degraded}}{\text{Amount Added}} \times 100$$

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Quantitative analysis of Polycyclic Aromatic Hydrocarbons (PAHs) was performed using the method of *Terry, et al.,* [17]. A Hewlett-Packard 5890 Gas Chromatograph (GC) coupled with a 5971-mass spectrometer (MS) was used, operating at 70 eV and 280°C. Separation was achieved using an HP-MS 5 capillary column. The injector temperature was set at 280°C. The oven temperature was initially held at 70°C for 4 minutes, then increased at 7°C/min to 280°C, and maintained for 20 minutes. PAHs were detected in selective ion monitoring mode, and identification was based on molecular ion peaks corresponding to specific PAH compounds.

Results

The microbiological and biodegradation analyses of soil samples collected from a mechanic workshop revealed the presence of both *Pseudomonas aeruginosa* and *Bacillus licheniformis* as the predominant bacterial isolates responsible for the degradation of Used Engine Oil (UEO). The biochemical and morphological characterization (Table 1) showed that *P. aeruginosa* appeared as Gram-negative, rod-shaped, and scattered cells, while *B. licheniformis* was identified as Gram-positive, rod-shaped, and scattered. During the biodegradation assay, both isolates demonstrated progressive bacterial growth with increasing incubation time (Table 2). *P. aeruginosa* exhibited a higher plate count, which peaked at 123,606 CFU after 336 hours, compared to *B. licheniformis*, which reached a maximum count of 10,120 CFU within the same period. This suggests that *P. aeruginosa* possessed a greater capacity to utilize hydrocarbons in the UEO as a carbon source compared to *B. licheniformis*.

The extent of oil degradation (Table 3) further confirmed the superior biodegradation potential of *P. aeruginosa*. At an inoculum concentration of 9.0×10^8 CFU/mL, *P. aeruginosa* degraded up to 55.74% of UEO, while *B. licheniformis* achieved a maximum of 48.8% degradation at 6.0×10^8 CFU/mL. The control samples showed minimal degradation (1.5% and 1.07%, respectively), indicating that biodegradation was primarily driven by microbial activity. Gas Chromatography-Mass Spectrometry (GC-MS) chromatograms (Figures 1 and 2) illustrated the breakdown of Polycyclic Aromatic Hydrocarbons (PAHs) metabolites in UEO following biodegradation by both bacteria. The chromatographic profiles revealed a significant reduction in the concentration of both low- and high-molecular-weight PAHs, with residual metabolite concentrations ranging between 0.5 and 2.5 ppm. The degradation efficiency increased proportionally with inoculum concentration, indicating enhanced metabolic activity at higher bacterial densities. Figure 3 summarizes the comparative degradation efficiency of both isolates, showing that *P. aeruginosa* consistently outperformed *B. licheniformis* across all tested concentrations.

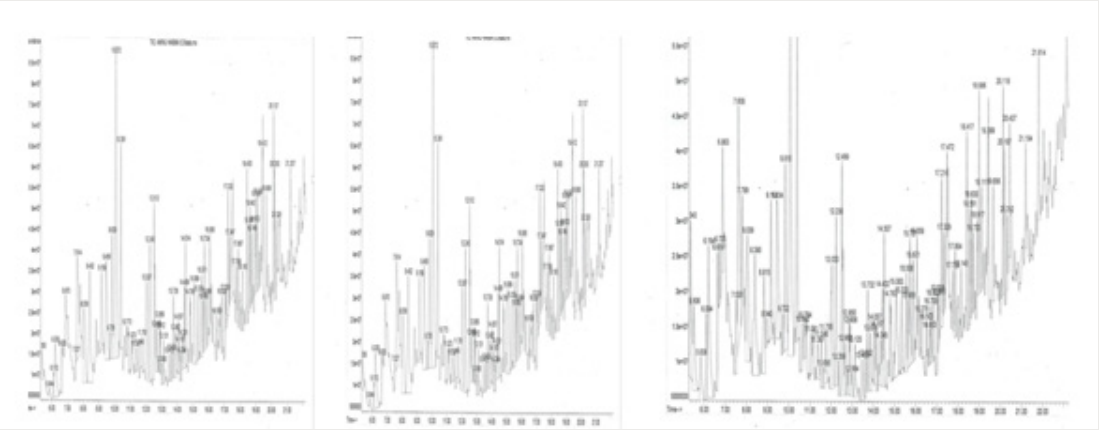

Figure 1: GC-MS Chromatogram of PAHs metabolites in Used Engine Oil degraded by Psuedomonas aeruginosa at (ai) 1.5, (aii) 3.0, and (aiii) 12.0 CFU/mL.

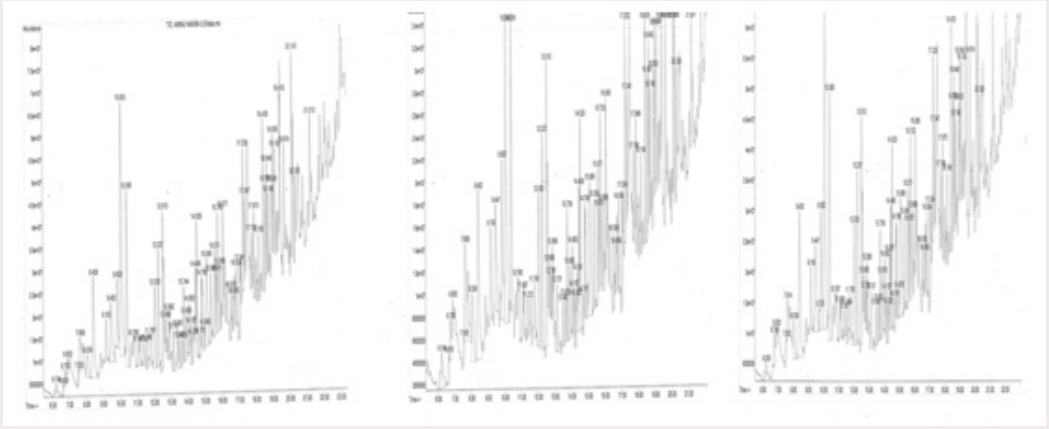

Figure 2: GC-MS Chromatogram of PAHs metabolites in Used Engine Oil degraded by Bacillus licheniformis at (bi) 1.5, (bii) 3.0, and (biii) 12.0 CFU/mL.

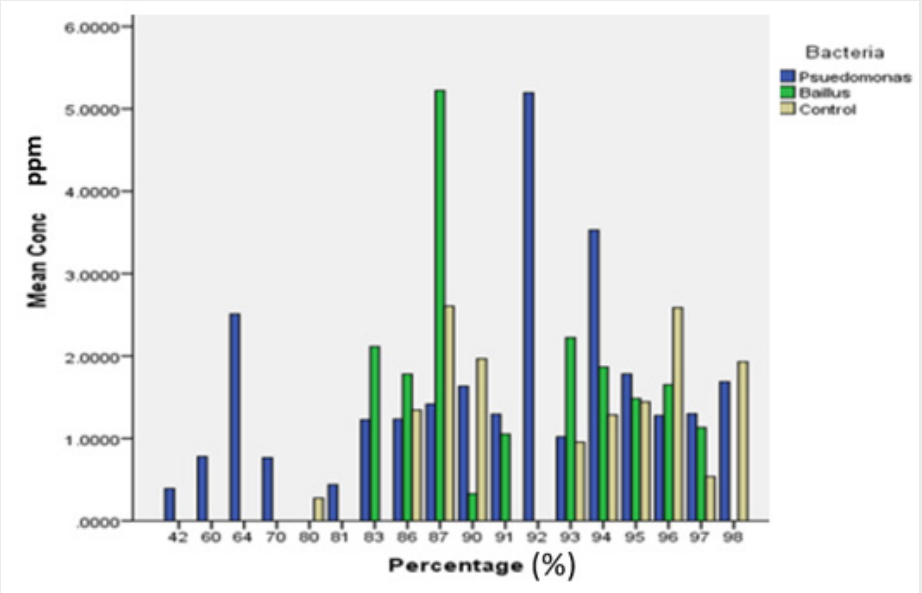

Figure 3: Percentage Degradation of PAHs in Used Engine Oil by Pseudomonas aeruginosa and Bacillus licheniformis.

Table 1: Gram reaction, morphology, and Biochemical Characterization of the isolated bacteria from the Soil of the mechanic workshop.

S/N	Sample	Gram staining	Shape	Cell arrangement	Inference
1	S1	-	Rod	Scattered	*Pseudomonas aeruginosa*
2	S2	+	Rod	Scattered	*Bacillus licheniformis*
3	S3	+	Rod	Scattered	*Bacillus licheniformis*
4	S4	-	Rod	Scattered	*Pseudomonas aeruginosa*
5	S5	-	Rod	Scattered	*Pseudomonas aeruginosa*

Table 2: Plate Count during Biodegradation of Used Engine Oil in Minimal Salt Media (48-336 CFU/hrs).

Organism	Time (h)						
	48	96	144	192	240	288	336
P. aeruginosa	490	528	464	2706	5000	10824	12564
P. aeruginosa	942	1044	1088	2710	6421	10694	123606
P. aeruginosa	592	9574	1045	2605	6155	11184	121455
B. licheniformis	1294	1064	1576	2018	3486	8072	9158
B. licheniformis	1314	1330	2816	2120	4276	8480	10120

Table 3: Percentage of Used Engine Oil degraded by *P. aeruginosa* and *B. licheniformis* at varying CFU/ml.

NO. of Cells (CFU/ml)	% oil degraded	% oil degraded
	P. aeruginosa	***B. licheniformis***
1.5×10∧8	51.87	40.45
3.0×10∧8	54.45	42.75
6.0×10∧8	53.15	48.8
9.0×10∧8	55.74	43.94
12×10∧8	55.51	44.86
0.00 (Control)	1.5	1.07

Discussion

This study focused on isolating hydrocarbon-degrading bacteria from soils contaminated with Used Engine Oil (UEO) and assessing their potential for Polycyclic Aromatic Hydrocarbon (PAH) degradation. The morphological and biochemical characterization revealed that all isolates were rod-shaped with scattered cell arrangements. *Pseudomonas aeruginosa* was Gram-negative, while *Bacillus licheniformis* was Gram-positive, consistent with classical taxonomic descriptions of both genera. These findings are supported by previous studies, which also identified *Pseudomonas* and *Bacillus* species as predominant hydrocarbon-degrading bacteria in soils contaminated with engine oil or automotive waste in Nigeria [14,18]. The agreement between our characterization and earlier reports validates the selection of these isolates for biodegradation studies. Both *P. aeruginosa* and *B. licheniformis* effectively degraded used engine oil in Minimal Salt Medium (MSM) and soil media using UEO as the sole carbon source. However, *P. aeruginosa* demonstrated superior degradation efficiency, showing continuous growth from 48 to 336 hours and achieving approximately 60% oil reduction in soil media, while *B. licheniformis* achieved 40-48%. Similar high degradation capacity has been reported for *P. aeruginosa* in Nigeria and India, where the bacterium achieved between 68% and 90% degradation in optimized liquid culture systems [19,20]. Although our observed degradation (≈60%) is slightly lower, it remains consistent with literature considering the experiment was conducted in a soil matrix-a more complex environment than liquid media-and over a shorter period (14 days).

Fewer studies have focused specifically on *B. licheniformis* in UEO degradation. Nonetheless, members of the *Bacillus* genus have been implicated in hydrocarbon and PAH degradation. The 40-48% degradation observed in this study aligns with the findings of Kong et al., who reported 12-20% PAH degradation by *Bacillus* sp. PAH-2 [21]. Additionally, *Ihejirika, et al.,* [22] found that *P. aeruginosa*

and *B. licheniformis* achieved nearly 99.9% degradation of low-pour fuel oil under nutrient-stimulated conditions, suggesting that the lower degradation rate in our study likely reflects differences in medium type, contamination complexity, and nutrient availability. The growth and degradation dynamics observed-steady growth and higher degradation by *P. aeruginosa* versus rapid initial growth followed by decline for *B. licheniformis*-are noteworthy. The initial rapid growth of *B. licheniformis* may reflect its utilization of simpler hydrocarbons, while the subsequent decline could result from inhibitory metabolite buildup or depletion of accessible carbon fractions. Conversely, *P. aeruginosa* sustained growth and degradation, likely due to its broader enzymatic versatility, biosurfactant production, and tolerance to toxic intermediates. These findings agree with earlier reports identifying *Pseudomonas* species as dominant hydrocarbon degraders owing to their metabolic diversity and biosurfactant production that enhance hydrocarbon bioavailability [23].

GC-MS analysis revealed notable concentrations of both high and low molecular weight PAHs in UEO, ranging from approximately 0.5 to 2.5 ppm. Treatments inoculated with *P. aeruginosa* produced oxygenated and chlorinated PAH intermediates, indicating partial transformation rather than complete mineralization. This pattern aligns with established PAH biodegradation pathways involving oxidation and ring cleavage before mineralization [24]. Higher inoculum densities produced greater metabolite formation, corroborating findings that microbial abundance and bioavailability enhancement promote hydrocarbon breakdown [25]. Although detailed mineralization tracking was not performed, the detection of transformation products confirms active PAH degradation. The superior PAH degradation performance of *P. aeruginosa* over *B. licheniformis* in this study supports previous comparative findings where *Pseudomonas* species outperformed *Bacillus* in petroleum hydrocarbon degradation [26,27]. Thus, *P. aeruginosa* remains a promising candidate for UEO and PAH bioremediation, while *B. licheniformis* may function effectively as part of a microbial consortium.

Despite the promising results, several limitations must be noted. Gravimetric analysis, while useful for total oil degradation estimation, does not distinguish between different hydrocarbon fractions. Future studies should incorporate detailed PAH profiling and mineralization quantification to better assess complete biodegradation. Furthermore, environmental factors in the soil medium, such as oxygen limitation, nutrient depletion, and metal interference, likely constrained microbial activity compared to optimized laboratory conditions. Studies have shown that nutrient supplementation and biosurfactant enhancement can significantly increase degradation efficiency [22]. This study demonstrates that *P. aeruginosa* and *B. licheniformis* isolated from contaminated soils in Birnin Kebbi possess intrinsic hydrocarbon-degrading capabilities. The efficiency of *P. aeruginosa*, in particular, indicates strong potential for application in bioremediation of oil-contaminated workshop soils in Nigeria. Nevertheless, further optimization, including nutrient amendment, inoculum size adjustment, biosurfactant addition, and mixed-culture use, could enhance degradation efficiency. A sequential biodegradation strategy utilizing both bacteria could also be effective, with *B. licheniformis* targeting easily degradable fractions and *P. aeruginosa* handling more recalcitrant PAHs.

Conclusion

This study reaffirms *Pseudomonas aeruginosa* as a potent hydrocarbon-degrading bacterium and extends the understanding of its capacity to degrade used engine oil and associated PAHs in contaminated soils. The moderate performance of *Bacillus licheniformis* suggests its potential as a co-degrader in mixed consortia. Morphological and biochemical characterization confirmed reliable isolate identification, while gravimetric and GC-MS analyses demonstrated active degradation and transformation of PAHs. Future research should aim to quantify mineralization rates, evaluate metabolite toxicity, and optimize treatment conditions for field-scale application. Such advancements could bridge the gap between laboratory success and effective environmental bioremediation of hydrocarbon-contaminated soils.

Acknowledgements

None.

Conflict of Interest

None.

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