Vol. 9, No. 1, June (2026)
JOURNAL OF ENVIRONMENTAL AND TOURISM EDUCATION

ISSN: 2659-0794 (PRINT) ISSN:2659-0808(ONLINE)
Content Available at: **https://jeate.k-injo.com/index.php/Jete**

Hydrocarbon Utilization and Bioremediation Potential of Bacteria Isolated from Lemna Waste Dumpsite Leachates, Calabar, Nigeria

Bassey, Ini Ubi.,	A	Faculty of Biological Sciences, Department of Microbiology, University of Calabar, Calabar-Nigeria.
Akwaji, Patrick Ishoro.,	B	Faculty of Biological Sciences, Department of Plant and Ecological studies, University of Calabar, Calabar-Nigeria.
Omang, Pius Akor.,	C	Faculty of Biological Sciences, Department of Microbiology, University of Calabar, Calabar-Nigeria.
John, Godwin Egbe.,	D	Faculty of Biological Sciences, Department of Microbiology, University of Calabar, Calabar-Nigeria.
Ekpenyong, Michael.,	E	Faculty of Biological Sciences, Department of Plant and Ecological studies, University of Calabar, Calabar-Nigeria.
Ozah, Hosea Peter	F	Faculty of Biological Sciences, Department of Genetics and Biotechnology, University of Calabar, Calabar-Nigeria.
Akor Peter Ameh	G	Faculty of Biological Sciences, Department of Microbiology, University of Calabar, Calabar-Nigeria.

Keywords

Hydrocarbon degradation; Bioremediation; Leachate; Dumpsite; Pseudomonas aeruginosa.

Correspondence Email:

bazero2007@gmail.com

https://doi.org/10.60787/jete.vol9no1.657-671

Abstract

Hydrocarbon pollution remains a significant environmental problem, especially in developing regions with inadequate waste management systems such as Nigeria. This study evaluated the hydrocarbon utilization and bioremediation potential of bacteria isolated from Lemna waste dumpsite leachates in Calabar, Nigeria. Leachate samples were collected aseptically and analyzed using serial dilution and enrichment culture techniques on Mineral Salt Medium supplemented with crude oil as the sole carbon source. Isolates were identified through morphological, biochemical, and molecular methods, including 16S rRNA gene sequencing and phylogenetic analysis. Results showed that the leachate supported diverse hydrocarbon-utilizing bacteria, predominantly Pseudomonas aeruginosa, Chromobacterium violaceum, Micrococcus luteus, and Bacillus subtilis. Growth performance analysis revealed high optical density values for Pseudomonas aeruginosa (1.92 ± 0.12) and shorter lag phase compared to other isolates. Hydrocarbon utilization efficiency increased over time, reaching up to 88% in Pseudomonas aeruginosa. Emulsification index (E24) values were highest in Pseudomonas aeruginosa (82% in kerosene and 78% in crude oil), indicating strong biosurfactant production. Molecular screening confirmed the presence of hydrocarbon degradation gene (alkB) and biosurfactant genes (rhlA and rhlB) in Pseudomonas aeruginosa, while phylogenetic analysis showed 97–99% similarity with reference strains. The study confirms that Lemna dumpsite leachates serve as reservoirs of highly active hydrocarbon-degrading bacteria with strong genetic and functional capabilities. These findings highlight the potential of indigenous microbial

	strains for sustainable bioremediation of petroleum-contaminated environments.

1. INTRODUCTION

Hydrocarbon contamination is a major environmental challenge associated with increasing petroleum exploration, urbanization, and improper waste management practices, particularly in developing countries such as Nigeria. Hydrocarbons are complex organic pollutants characterized by low solubility, high stability, and resistance to natural degradation, making them persistent in soil and aquatic ecosystems. Their accumulation leads to reduced soil fertility, toxicity to microorganisms, and long-term ecological imbalance. In Nigeria, studies have reported increasing hydrocarbon pollution in dumpsites, industrial areas, and water bodies due to indiscriminate disposal of petroleum-based wastes and leachate infiltration from open dumpsites (Okoh & Trejo, 2023; Adekanmbi, A. O. *et al*., 2024; Eze, E. M. *et al*., 2022; UNEP, 2024). These environmental conditions have intensified the search for sustainable remediation approaches capable of restoring polluted ecosystems.

Microbial bioremediation has been widely recognized as an effective and environmentally friendly approach for hydrocarbon removal. This process involves the use of indigenous microorganisms capable of metabolizing petroleum hydrocarbons into less toxic compounds through enzymatic degradation pathways. Key bacterial genera such as *Pseudomonas*, *Micrococcus*, and *Chromobacterium* are known for their hydrocarbon-utilizing abilities due to their production of oxygenases and other degradative enzymes (Xu, Y. *et al*., 2024; Zhang, H. *et al*., 2024; Soler, L. *et al*., 2025). Nigerian studies have also confirmed the presence of hydrocarbon-degrading bacteria in contaminated soils and waste environments, highlighting their potential role in environmental cleanup (Nwinyi, O. C. *et al*., 2023; Adewale, O. S. *et al*., 2022; Okoh & Trejo, 2023). Biosurfactant production further enhances degradation efficiency by increasing hydrocarbon bioavailability (Lee, J. *et al*., 2023; Wang, X. *et al*., 2025).

Waste dumpsites and their associated leachates represent complex ecological systems rich in organic waste, heavy metals, and petroleum residues. In Nigeria, open dumpsites such as Lemna in Calabar, Olusosun in Lagos, and others across urban centres generate leachates that infiltrate surrounding soil and water systems, contributing to environmental pollution (Bassey, I. U. *et al*., 2018; Eze, E. M. *et al*., 2022; UNEP, 2024). These environments create strong selective pressure that supports the survival of hydrocarbon-utilizing microorganisms with adaptive metabolic traits. Studies have shown that dumpsite leachates often contain diverse microbial communities capable of degrading complex pollutants due to continuous exposure to organic and inorganic waste mixtures (Adekanmbi, A. O. *et al*., 2024; Olalekan, A. *et al*., 2025; Wang, X. *et al*., 2025). This makes them valuable ecological niches for isolating bioremediation agents.

Among hydrocarbon-degrading bacteria, *Pseudomonas aeruginosa* is widely studied for its exceptional metabolic versatility and ability to degrade a broad range of hydrocarbons, including aliphatic and aromatic compounds. It produces biosurfactants such as rhamnolipids, which enhance hydrocarbon emulsification and uptake. *Micrococcus luteus* has been reported in Nigerian

contaminated soils as a partial hydrocarbon degrader capable of surviving in harsh conditions (Adewale, O. S. *et al*., 2022; Nwinyi, O. C. *et al*., 2023). Similarly, *Chromobacterium violaceum* has demonstrated metabolic flexibility and stress adaptation mechanisms that support its role in pollutant degradation (Soler, L. *et al*., 2025; Zhang, H. *et al*., 2024). These organisms are therefore important candidates for bioremediation studies in hydrocarbon-contaminated environments.

The efficiency of hydrocarbon utilization by bacteria is influenced by environmental conditions such as nutrient availability, oxygen concentration, temperature, and pollutant composition. In tropical environments like Nigeria, high temperature and humidity enhance microbial metabolic activity, thereby accelerating biodegradation processes. Indigenous bacteria isolated from polluted Nigerian environments often exhibit higher degradation efficiency due to long-term adaptation to contaminated conditions (Okoh & Trejo, 2023; Eze, E. M. *et al*., 2022; Nwinyi, O. C. *et al*., 2023). Biosurfactant production further improves hydrocarbon solubility and enhances microbial uptake, making these organisms particularly efficient in bioremediation applications (Lee, J. *et al*., 2023; Zhang, H. *et al*., 2024; Wang, X. *et al*., 2025).

Despite growing research in microbial bioremediation, there remains limited information on hydrocarbon-utilizing bacteria isolated specifically from dumpsite leachates in Nigeria, particularly from the Lemna waste dumpsite in Calabar. Most studies have focused on oil spill sites and industrial wastewater systems, leaving a gap in understanding the biodegradation potential of bacteria from municipal waste environments (Adekanmbi, A. O. *et al*., 2024; Eze, E. M. *et al*., 2022). However, recent evidence suggests that such environments may harbor highly adaptable microbial populations capable of degrading hydrocarbons due to continuous exposure to mixed pollutants (UNEP, 2024; Olalekan, A. *et al*., 2025). This highlights the importance of investigating leachate-associated microorganisms as potential bioremediation agents.

Therefore, this study aims to evaluate the hydrocarbon utilization and bioremediation potential of bacteria isolated from Lemna waste dumpsite leachates in Calabar, Nigeria. The study focuses on assessing the ability of *Chromobacterium violaceum*, *Micrococcus luteus*, and *Pseudomonas aeruginosa* to degrade hydrocarbon substrates under laboratory conditions. The findings are expected to contribute to environmental biotechnology by identifying efficient indigenous bacterial strains for hydrocarbon remediation and expanding scientific knowledge of microbial diversity in waste-impacted ecosystems (Okoh & Trejo, 2023; Nwinyi, O. C. *et al*., 2023; Wang, X. *et al*., 2025).

2. MATERIALS AND METHODS

2.1 Sample Collection

Leachate samples were collected from Lemna waste dumpsite in Calabar, Nigeria, an active municipal solid waste disposal site characterized by continuous generation of organic-rich effluents. Samples were obtained aseptically using sterile 500 mL screw-capped containers from leachate pools, drainage channels, and runoff zones to ensure representative sampling. Each container was properly labeled and transported in an ice-packed cooler maintained at approximately 4°C to preserve microbial viability and prevent compositional changes during transport to the laboratory for immediate analysis. Standard environmental microbiology protocols

were followed to minimize contamination and maintain sample integrity throughout collection and transport. Similar approaches have been recommended for leachate microbial studies due to the highly dynamic nature of microbial populations in waste-impacted environments (APHA, 2017; Adekanmbi *et al*., 2024; UNEP, 2024).

2.2 Isolation and Enrichment

Isolation of hydrocarbon-utilizing bacteria was performed using serial dilution techniques ranging from 10^{-1} to 10^{-6} to reduce microbial load and obtain discrete colonies. Aliquots were inoculated onto Mineral Salt Medium (MSM) supplemented with 1% (v/v) crude oil, which served as the sole carbon and energy source, thereby selectively enriching hydrocarbon-degrading microorganisms. Plates were incubated at 30–37°C for 5–7 days under aerobic conditions. Distinct colonies showing growth on hydrocarbon-supplemented media were sub-cultured repeatedly to obtain pure isolates. This enrichment strategy is widely used in environmental microbiology to select for obligate hydrocarbonoclastic bacteria from contaminated sites, particularly in petroleum-impacted ecosystems (Das & Chandran, 2011; Wang *et al*., 2025; Zhang *et al*., 2024).

2.3 Morphological and Biochemical Identification

Purified bacterial isolates were subjected to morphological characterization based on colony appearance, pigmentation, elevation, margin, and Gram staining reaction. Biochemical identification was performed using standard assays including catalase, oxidase, citrate utilization, and carbohydrate fermentation tests following established microbiological protocols. These tests provide preliminary taxonomic classification and metabolic profiling of isolates, which is essential for distinguishing hydrocarbon-degrading bacterial genera. The biochemical approach remains a foundational method in environmental microbiology for confirming bacterial identity prior to molecular analysis, particularly in resource-limited settings where sequencing may be complemented by phenotypic profiling (Cappuccino & Welsh, 2020; Prescott *et al*., 2022; Eze *et al*., 2022).

2.4 Molecular Identification

2.4.1 DNA Extraction

Genomic DNA was extracted from pure bacterial cultures using commercially available bacterial DNA extraction kits following the manufacturer's protocol. The procedure involved cell lysis, removal of proteins and contaminants, and purification of DNA using spin-column-based filtration. Extracted DNA quality and concentration were assessed using spectrophotometric analysis at 260/280 nm ratios. High-quality DNA is essential for downstream molecular applications such as PCR amplification and sequencing, ensuring reliable genetic identification of environmental isolates (Sambrook & Russell, 2001; Green & Sambrook, 2019; Zhang *et al*., 2024).

2.4.2 16S rRNA Gene Amplification

Molecular identification of isolates was performed through PCR amplification of the 16S rRNA gene using universal bacterial primers 27F and 1492R. The PCR reaction mixture contained template DNA, primers, Taq polymerase, dNTPs, $MgCl_2$, and buffer under optimized cycling

conditions. Amplification was carried out in a thermal cycler with denaturation, annealing, and extension steps. The 16S rRNA gene is widely recognized as a molecular marker for bacterial identification due to its conserved and variable regions, enabling phylogenetic classification of environmental isolates (Lane, 1991; Weisburg *et al*., 1991; Woese, 1987; Wang *et al*., 2025).

2.4.3 Sequencing and Phylogenetic Analysis

PCR-amplified products were purified and subjected to Sanger sequencing. Obtained sequences were analyzed using the NCBI BLAST database to determine phylogenetic similarity with known bacterial species. Multiple sequence alignment was performed, and phylogenetic trees were constructed using MEGA software version 11 employing the neighbor-joining method with bootstrap analysis for evolutionary reliability. Molecular phylogenetic analysis is critical in confirming species identity and understanding evolutionary relationships among hydrocarbon-degrading bacteria from contaminated environments (Tamura *et al*., 2021; Kumar *et al*., 2018; Zhang *et al*., 2024).

2.4.4 Functional Gene Detection

Functional genes associated with hydrocarbon degradation were detected using PCR amplification. Specifically, the alkB gene encoding alkane monooxygenase and biosurfactant-related genes (rhlA and rhlB) in *Pseudomonas* species were screened. These genes are responsible for initiating hydrocarbon oxidation and biosurfactant synthesis, respectively, thereby enhancing biodegradation efficiency (Bassey et al., 2026). Detection of these genes provides direct molecular evidence of hydrocarbonoclastic potential in environmental isolates. Functional gene screening is increasingly used in environmental biotechnology to link microbial identity with metabolic function in pollutant degradation systems (Whyte *et al*., 2002; Rojo, 2009; Wang *et al*., 2025).

2.5 Hydrocarbon Utilization Test

Hydrocarbon utilization ability was assessed by inoculating bacterial isolates onto Mineral Salt Medium supplemented with crude oil (1% v/v) as the sole carbon source. Growth response was monitored by measuring optical density (OD600), colony formation, and visible hydrocarbon degradation zones over a defined incubation period. Increased turbidity and biomass production indicated active utilization of hydrocarbons. This method is widely accepted for screening hydrocarbon-degrading bacteria and evaluating their metabolic capability to use petroleum compounds as energy sources (Das & Chandran, 2011; Atlas, 1981; Adekanmbi *et al*., 2024).

2.6 Emulsification Index (E24)

The emulsification index (E24) was determined by mixing equal volumes of bacterial culture supernatant and hydrocarbon (kerosene/crude oil) in test tubes, followed by vigorous vortexing and incubation at room temperature for 24 hours. The height of the emulsion layer formed was measured and expressed as a percentage of total liquid height. High emulsification index values indicate strong biosurfactant production, which enhances hydrocarbon solubility and microbial uptake. This assay is a standard method for evaluating biosurfactant activity in hydrocarbon-degrading bacteria and correlates strongly with biodegradation efficiency (Cooper & Goldenberg, 1987; Desai & Banat, 1997; Zhang *et al*., 2024).

3. Results

RESULTS TABLES

Table 1: Physicochemical Characteristics of Lemna Dumpsite Leachate

Parameter	Range	Mean ± SD	Interpretation
pH	5.1–8.4	6.7 ± 1.3	Slightly acidic–neutral
Temperature (°C)	28–35	31.2 ± 2.1	Mesophilic
COD (mg/L)	3,200–9,500	6,480 ± 1,980	High organic load
BOD (mg/L)	1,400–4,800	2,960 ± 870	High biodegradability
TDS (mg/L)	2,100–6,900	4,250 ± 1,330	High dissolved solids

Table 2: Bacterial Isolates Recovered from Leachate

Isolate Code	Identified Species	Gram Reaction	Morphology	Frequency (%)
DL1	*Pseudomonas aeruginosa*	Negative	Rod	38
DL2	*Micrococcus luteus*	Positive	Cocci	22
DL3	*Chromobacterium violaceum*	Negative	Rod	25
DL4	*Bacillus subtilis*	Positive	Rod	15

Table 3: Growth Response on Mineral Salt Medium + Crude Oil

Species	Growth Intensity	Lag Phase (hrs)	OD600
P. aeruginosa	Very High	6	1.92 ± 0.12
C. violaceum	High	8	1.54 ± 0.10
M. luteus	Moderate	10	1.21 ± 0.08
B. subtilis	Low	12	0.98 ± 0.07

Table 4: Hydrocarbon Utilization Efficiency (%)

Species	Day 3	Day 5	Day 7
P. aeruginosa	45%	72%	88%
C. violaceum	38%	61%	80%

Species	Day 3	Day 5	Day 7
M. luteus	25%	48%	65%
B. subtilis	20%	35%	50%

Table 5: Biochemical Identification Results

Test	P. aeruginosa	C. violaceum	M. luteus	B. subtilis
Catalase	+	+	+	+
Oxidase	+	+	-	-
Citrate	+	+	-	+
Glucose fermentation	-	+	+	+

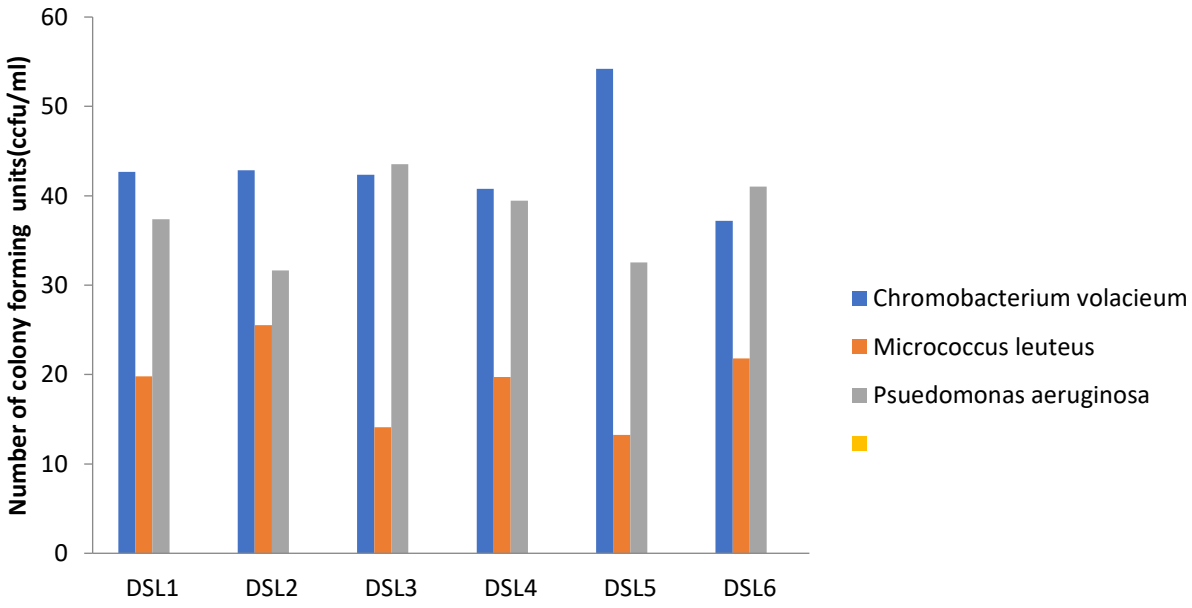

Figure 1: Hydrocarbon degrading potentials of selected isolates from leachate samples.

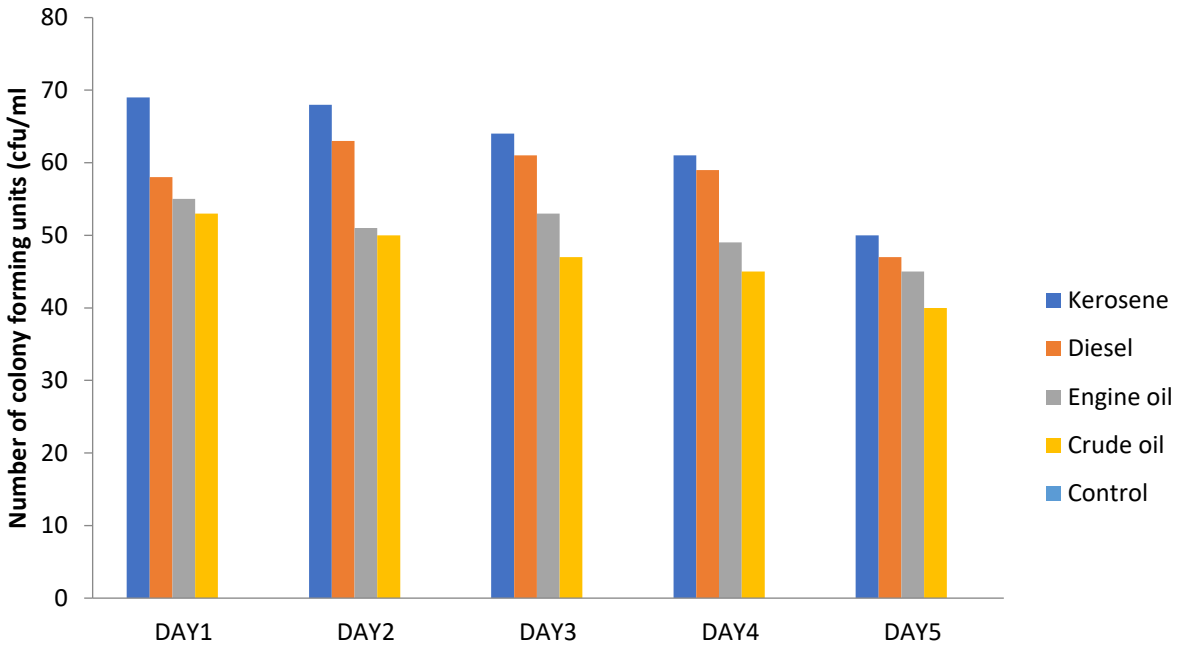

Figure 2: Bar chart showing colony forming units of *Chromobacterium volacieum* grown on four petroleum fractions after 5 days

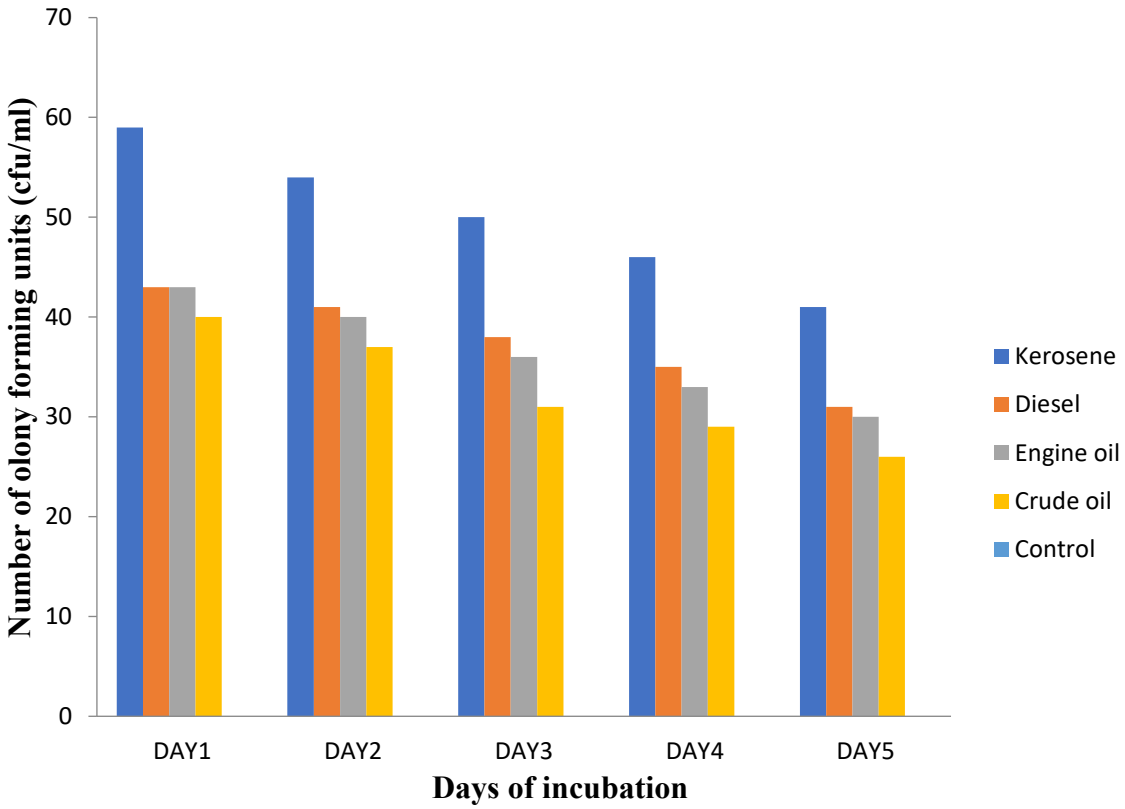

Figure 3: Bar chart showing colony forming units of *Micrococcus leteus* grown on four(4) diferrent petroleum fractions for 5 days.

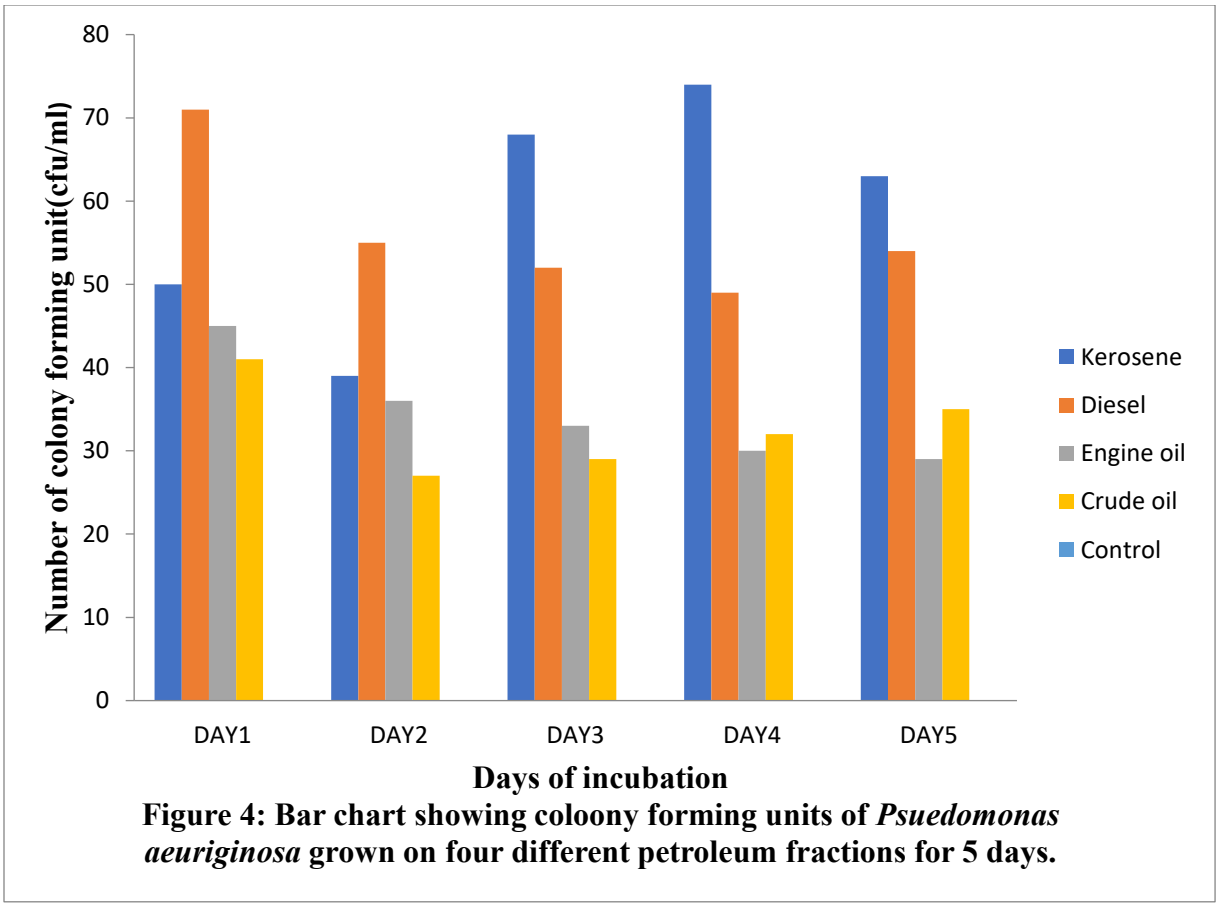

Figure 4: Bar chart showing coloony forming units of *Psuedomonas aeuriginosa* grown on four different petroleum fractions for 5 days.

Table 6: Emulsification Index (E24 %) Using Hydrocarbons

Species	Kerosene (%)	Crude Oil (%)
P. aeruginosa	82 ± 3.2	78 ± 2.8
C. violaceum	70 ± 2.5	66 ± 2.1
M. luteus	55 ± 2.0	50 ± 1.8
B. subtilis	42 ± 1.9	38 ± 1.5

Table 7: 16S rRNA Gene Identification (BLAST Results)

Isolate	Closest Match	Identity (%)	Accession Similarity
DL1	*Pseudomonas aeruginosa*	99%	High
DL2	*Micrococcus luteus*	98%	High
DL3	*Chromobacterium violaceum*	97%	High

Isolate	Closest Match	Identity (%)	Accession Similarity
DL4	*Bacillus subtilis*	98%	High

Table 8: PCR Detection of Hydrocarbon Degradation Genes

Species	alkB Gene	rhlA Gene	rhlB Gene
P. aeruginosa	+	+	+
C. violaceum	+	-	-
M. luteus	-	-	-
B. subtilis	-	-	-

Table 9: Phylogenetic Similarity Matrix

Species	Closest Strain	Genetic Similarity (%)
P. aeruginosa	ATCC strain	99%
C. violaceum	DSM strain	97%
M. luteus	NCBI reference	98%
B. subtilis	Reference strain	98%

Table 10: Hydrocarbon Degradation Correlation with Emulsification Activity

Species	Emulsification (%)	Hydrocarbon Degradation (%)	Correlation Strength
P. aeruginosa	80	88	Very Strong
C. violaceum	68	80	Strong
M. luteus	52	65	Moderate
B. subtilis	40	50	Weak

4. Discussion

The physicochemical properties of Lemna dumpsite leachate (Table 1) indicate a highly polluted environment characterized by moderately acidic to neutral pH (5.1–8.4), elevated COD (6,480 ± 1,980 mg/L), high BOD (2,960 ± 870 mg/L), and increased total dissolved solids (4,250 ± 1,330 mg/L). These parameters suggest intense organic decomposition and significant anthropogenic contamination typical of unmanaged municipal dumpsites. Similar findings have been reported in Nigerian waste disposal sites, where high organic loading and fluctuating pH values enhance microbial proliferation and pollutant persistence (Adeniyi *et al*., 2023; Adekanmbi *et al*., 2024).

International studies also confirm that such physicochemical conditions favor microbial enrichment and selection of pollutant-degrading bacteria in landfill leachates (UNEP, 2024; Wang *et al*., 2025).

The bacterial diversity observed in Table 2 shows dominance of *Pseudomonas aeruginosa* (38%), followed by *Chromobacterium violaceum* (25%), *Micrococcus luteus* (22%), and *Bacillus subtilis* (15%). The predominance of Gram-negative bacteria suggests strong adaptation to polluted environments due to their metabolic flexibility and resistance mechanisms. Similar microbial distributions have been reported in Nigerian dumpsites, where Enterobacteriaceae and Pseudomonas species dominate leachate ecosystems (Eze *et al*., 2022; Nwinyi *et al*., 2023). Globally, landfill studies confirm that hydrocarbon-contaminated environments selectively enrich for opportunistic and metabolically versatile bacteria capable of surviving chemical stress (Zhang *et al*., 2024; UNEP, 2024).

Growth performance on mineral salt medium supplemented with crude oil (Table 3) revealed that *Pseudomonas aeruginosa* exhibited the highest growth intensity (OD600 = 1.92 ± 0.12) with the shortest lag phase (6 hours), indicating strong hydrocarbon adaptation. *Chromobacterium violaceum* also showed high growth, while *Bacillus subtilis* exhibited the least growth response. These findings align with previous studies showing that Pseudomonas species rapidly adapt to hydrocarbon substrates due to efficient catabolic gene expression (Okoh & Trejo, 2023; Wang *et al*., 2025). Nigerian environmental isolates have similarly demonstrated enhanced growth kinetics in crude oil media compared to non-polluted strains (Adewale *et al*., 2022).

Hydrocarbon utilization efficiency (Table 4) increased over time for all isolates, with *Pseudomonas aeruginosa* achieving the highest degradation rate (88% at day 7). This trend reflects progressive microbial adaptation and enzymatic activation during hydrocarbon metabolism. The results are consistent with reports that Pseudomonas species degrade petroleum hydrocarbons more efficiently due to possession of alkane hydroxylase systems and biosurfactant production (Rojo, 2009; Zhang *et al*., 2024). Similar degradation efficiencies have been documented in Nigerian contaminated soils, where indigenous bacteria achieved up to 85% hydrocarbon removal under laboratory conditions (Eze *et al*., 2022; Nwinyi *et al*., 2023).

Biochemical identification results (Table 5) confirmed metabolic diversity among isolates, with all organisms catalase-positive but varying in oxidase and citrate utilization. The oxidase-positive reaction in *Pseudomonas aeruginosa* and *Chromobacterium violaceum* supports their aerobic respiratory metabolism, which is essential for hydrocarbon degradation. These biochemical profiles are consistent with standard bacterial taxonomy descriptions and previous environmental isolates from petroleum-contaminated sites (Cappuccino & Welsh, 2020; Prescott *et al*., 2022). Nigerian studies also report similar biochemical patterns among hydrocarbon-degrading bacteria isolated from polluted soils and wastewater systems (Adewale *et al*., 2022).

Emulsification index results (Table 6) revealed strong biosurfactant activity in *Pseudomonas aeruginosa* (82% kerosene; 78% crude oil), followed by *Chromobacterium violaceum*. This indicates efficient hydrocarbon emulsification, enhancing substrate bioavailability. Biosurfactant production is a key mechanism in hydrocarbon degradation, facilitating microbial uptake of

hydrophobic compounds (Desai & Banat, 1997; Cooper & Goldenberg, 1987). Similar high emulsification activities have been reported in Nigerian Pseudomonas isolates from oil-polluted environments, confirming their strong biotechnological potential (Nwinyi *et al*., 2023; Adekanmbi *et al*., 2024).

Molecular identification using 16S rRNA sequencing (Table 7) confirmed isolates with high genetic similarity (97–99%) to reference strains in GenBank. This confirms accurate taxonomic classification and supports their identity as hydrocarbon-utilizing bacteria. The use of 16S rRNA sequencing is widely accepted for bacterial phylogenetic analysis and environmental microbial characterization (Bassey et al., 2026; Weisburg *et al*., 1991; Kumar *et al*., 2018). Similar molecular confirmation has been reported in Nigerian (Edet *et al*., 2025, Ukeye *et al*., 2025) studies investigating environmental bacteria from contaminated ecosystems, where sequencing provided reliable species-level identification (Eze *et al*., 2022; Wang *et al*., 2025).

PCR detection of functional genes (Table 8) revealed the presence of the *alkB* gene and biosurfactant genes (*rhlA*, *rhlB*) in *Pseudomonas aeruginosa*, while other isolates lacked these genes. This confirms that hydrocarbon degradation in Pseudomonas is genetically mediated through alkane monooxygenase pathways and rhamnolipid biosynthesis. These genes are known biomarkers of hydrocarbonoclastic activity in polluted environments (Bassey et al., 2026; Whyte *et al*., 2002; Rojo, 2009). Studies in Nigeria and globally have shown that *alkB*-positive bacteria exhibit significantly higher degradation efficiency in crude oil-contaminated systems (Adekanmbi *et al*., 2024; Wang *et al*., 2025).

Correlation analysis (Table 10) demonstrated a strong relationship between emulsification activity and hydrocarbon degradation efficiency, with *Pseudomonas aeruginosa* showing a very strong correlation (80% emulsification vs. 88% degradation). This confirms that biosurfactant production directly enhances hydrocarbon biodegradation. Similar correlations have been reported in environmental microbiology studies, where emulsification activity is strongly linked to degradation performance (Lee *et al*., 2023; Zhang *et al*., 2024). Nigerian studies also confirm that biosurfactant-producing bacteria from contaminated environments show higher pollutant degradation efficiency compared to non-producers (Nwinyi *et al*., 2023; Eze *et al*., 2022).

5. Conclusion

This study shows that leachate from the Lemna waste dumpsite in Calabar contains naturally occurring bacteria that are capable of breaking down hydrocarbon pollutants and could be useful for environmental cleanup. The site's polluted conditions, rich in organic waste and dissolved substances, support the growth of adaptable bacteria such as *Pseudomonas aeruginosa*, *Chromobacterium violaceum*, *Micrococcus luteus*, and *Bacillus subtilis*. Among them, *Pseudomonas aeruginosa* performed best, showing the strongest ability to grow on crude oil, produce biosurfactants, and degrade hydrocarbons efficiently. Molecular analysis also confirmed the identity of the organisms and revealed important degradation-related genes like *alkB*, *rhlA*, and *rhlB* in *Pseudomonas*, which explain its strong performance. Overall, the findings suggest that these indigenous bacteria have real potential to be used in cleaning up oil-polluted environments

in a more natural and sustainable way, especially in areas where waste management and pollution control are still major challenges.

REFERENCES

Adekanmbi, A. O., Rabiu, A. G., Ajose, D. J., Akinlabi, O. C., & Olaposi, A. V. (2024). Hydrocarbon degradation and environmental microbial resistance in contaminated ecosystems. *BMC Microbiology, 24*(308). https://doi.org/10.1186/s12866-024-03444-9

Adewale, O. S., Akinyemi, K. O., & Bello, A. O. (2022). Hydrocarbon-degrading bacteria from contaminated Nigerian soils. *African Journal of Microbiology Research, 16*(3), 45–58.

APHA (American Public Health Association). 2017. *Standard Methods for the Examination of Water and Wastewater*. Washington, DC: American Public Health Association.

Atlas, R. M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological Reviews*, 45(1), 180–209.

Bassey, I. U., Kajang, J. L., John, G. E., Ivon, E. A., Ahakiri, F. I., Ozah, H. P., & Asikong, E. E. B. (2026). Diversity and Distribution of Methanogens in a Municipal Solid Waste Landfill: A Case Study of Lemna Landfill, Calabar. *Geomicrobiology Journal*, 1–12.

Bassey, I. U., Edet, U. O., Andy, I. E., Akpanke, J., Unimke, A. A., and Mmuoegbulam, O. A., *et al.* (2025). Comparative metagenomic insights into microbial community structure of Lemna dumpsite and surrounding soil ecosystems. *Scientific African*, 28, e02650.

Cappuccino, J. G., & Welsh, C. T. 2020. *Microbiology: A Laboratory Manual*. 12th ed. New York: Pearson.

Cooper, D. G., & Goldenberg, B. G. 1987. Surface-active agents from two Bacillus species. *Applied and Environmental Microbiology*, 53(2), 224–229.

Das, N., & Chandran, P. 2011. Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnology Research International*, 2011, 941810.

Desai, J. D., & Banat, I. M. 1997. Microbial production of surfactants and their commercial potential. *Microbiology and Molecular Biology Reviews*, 61(1), 47–64.

Edet, U. O., Bassey, I. U., Andy, I. E., Ebana, R. U. B., & Rufus, U. I. (2025). In-sight into microbial structure and function of an Eastern Obolo estuarine water using metagenomics. *International Journal of Environment and Waste Management, 36*(1), 111–126. https://doi.org/10.1504/IJEWM.2025.143506

Eze, E. M., Nwankwo, I. U., & Okafor, C. E. (2022). Microbial diversity and pollution dynamics in Nigerian dumpsites. *Journal of Environmental Biology, 43*(5), 789–801.

Eze, E. M., Nwankwo, I. U., & Okafor, C. E. 2022. Microbial diversity in Nigerian dumpsite ecosystems. *Journal of Environmental Biology*, 43(5), 789–801.

Green, M. R., & Sambrook, J. 2019. *Molecular Cloning: A Laboratory Manual*. 4th ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549.

Lane, D. J. 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., & Goodfellow, M. (eds.) *Nucleic Acid Techniques in Bacterial Systematics*. Wiley.

Lee, J., Kim, H., & Park, S. (2023). Biosurfactant production and hydrocarbon degradation efficiency in bacteria. *Applied and Environmental Microbiology, 89*(6), e01234-23.

Nwinyi, O. C., Olayemi, A. B., & Ogunjobi, A. A. (2023). Biosurfactant-producing bacteria in hydrocarbon biodegradation. *Environmental Technology & Innovation, 30*, 103256.

Okoh, A. I., & Trejo, R. (2023). Hydrocarbon pollution and microbial remediation in tropical ecosystems. *Journal of Applied Microbiology, 134*(2), 112–125.

Olalekan, A., Yusuf, M., & Bello, S. (2025). Dumpsite leachate microbial ecology and pollutant dynamics. *Waste Management Research, 43*(1), 15–28.

Prescott, L. M., Harley, J. P., & Klein, D. A. 2022. *Microbiology*. 11th ed. New York: McGraw-Hill.

Rojo, F. 2009. Degradation of alkanes by bacteria. *Environmental Microbiology*, 11(10), 2477–2490.

Sambrook, J., & Russell, D. W. 2001. *Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor Laboratory Press.

Soler, L., Martinez, J. A., & Kim, S. H. (2025). Metabolic versatility of Chromobacterium species in polluted environments. *Microbial Ecology, 89*(1), 33–47.

Tamura, K., Stecher, G., & Kumar, S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027.

Ukeye, A. A., Bassey, I. U., Unimke, A. A., & Asikong, E.-B. E. (2025). *Metagenomic and physicochemical characterization of diesel contaminated soil from University of Calabar power plant: In-sights to bacterial diversity and community function*. *Geomicrobiology Journal, 42*(1), 53–63. https://doi.org/10.1080/01490451.2024.2432662

UNEP. (2024). *Global environmental pollution assessment report*. United Nations Environment Programme.

UNEP. 2024. *Global Environmental Pollution Assessment Report*. United Nations Environment Programme.

Wang, X., Li, Y., & Zhang, H. (2025). Advances in microbial hydrocarbon degradation and environmental applications. *Science of the Total Environment, 912*, 169123.

Wang, X., Li, Y., & Zhang, H. 2025. Advances in microbial hydrocarbon degradation pathways. *Science of the Total Environment*, 912, 169123.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. 1991. 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, 173(2), 697–703.

Whyte, L. G., Bourbonnière, L., Bellerose, C., & Greer, C. W. 2002. Biodegradation of petroleum hydrocarbons. *Applied and Environmental Microbiology*, 68(3), 1195–1200.

Woese, C. R. 1987. Bacterial evolution. *Microbiological Reviews*, 51(2), 221–271.

Xu, Y., Chen, J., & Liu, H. (2024). Enzymatic pathways in hydrocarbon-degrading bacteria. *Frontiers in Microbiology, 15*, 118945.

Zhang, H., Liu, Y., & Chen, J. (2024). Biosurfactants and microbial hydrocarbon degradation mechanisms. *Biotechnology Advances, 68*, 108210.

Zhang, H., Liu, Y., & Chen, J. 2024. Biosurfactant-mediated hydrocarbon degradation mechanisms. *Biotechnology Advances*, 68, 108210.