Journal of Basics and Applied Sciences Research (JOBASR)
ISSN (print): 3026-9091, ISSN (online): 1597-9962
Volume 4(4) July 2026
DOI: https://dx.doi.org/10.4314/jobasr.v4i4.17

# Crude Oil Biodegradation Potential by Mixed Consortia of *Pseudomonas Lurida* and *Bacillus Endophyticus*

**Mustapha Gani[1*], Zubaidat A. Binji[2], Mudassiru Salihu[3] & Abdulkadir Nafi'u[4]**
**[1,2,3,4]Department of Microbiology Sokoto State University, Sokoto, Nigeria.**
[*]Corresponding Author Email: **Mustapha.gani@ssu.edu.ng**

**ABSTRACT**
Crude oil contamination poses significant environmental challenges due to the persistence and toxicity of its hydrocarbon components. This study evaluated the biodegradation potential of a mixed bacterial consortium comprising *Pseudomonas lurida* (DSM 15835) and *Bacillus endophyticus* (DSM 13796) using gas chromatography–mass spectrometry (GC–MS). Sterile sediment microcosms amended with 10% Bonny light crude oil were inoculated with approximately $5 \times 10^7$ CFU of the consortium and monitored over five weeks. GC–MS analysis revealed substantial degradation of hydrocarbons ranging from C7 to C44, with marked reductions in peak abundance and the emergence of intermediate metabolites such as octane derivatives and alcohols. The consortium demonstrated enhanced biodegradation efficiency compared to single strains, indicating synergistic interactions. Growth curve analysis showed rapid exponential growth followed by an early stationary phase, suggesting efficient utilization of readily degradable hydrocarbons. The findings highlight the effectiveness of mixed microbial consortia in the bioremediation of petroleum-contaminated environments.

**Keywords:**
Biodegradation,
GC–MS, Crude oil,
Microbial consortium,
Pseudomonas lurida,
Bacillus endophyticus.

## INTRODUCTION

Petroleum hydrocarbons are among the most prevalent environmental pollutants due to increasing industrialization, oil exploration, and accidental spills (Ahmed & Fakhruddin, 2018). Crude oil contamination adversely affects soil structure, reduces agricultural productivity, and poses serious ecological and public health risks due to the toxicity and persistence of its components (Varjani, 2017; Bacosa *et al*., 2020). These hydrocarbons are complex mixtures consisting of aliphatic, aromatic, and polycyclic aromatic hydrocarbons (PAHs), many of which are recalcitrant and resistant to natural degradation processes (Das & Chandran, 2011).

Conventional physicochemical methods for remediation, such as excavation, incineration, and chemical treatments, are often expensive and may generate secondary pollution (Kuppusamy *et al*., 2016). In contrast, bioremediation has emerged as an environmentally sustainable and cost-effective alternative that utilizes microorganisms to transform or mineralize toxic pollutants into less harmful products (Azubuike *et al*., 2016). Microbial degradation of hydrocarbons occurs through enzymatic processes that convert complex organic compounds into simpler molecules, ultimately leading to carbon dioxide and water under aerobic conditions (Das & Chandran, 2011).

Among hydrocarbon-degrading microorganisms, species of the genus *Pseudomonas* are widely recognized for their metabolic versatility and efficiency in degrading a broad spectrum of hydrocarbons, including alkanes and aromatic compounds ( Ivanova *et al*., 2022). *Pseudomonas lurida*, a Gram-negative bacterium, has been reported to possess catabolic genes involved in hydrocarbon degradation and exhibits adaptability to contaminated environments (Garrido-Sanz *et al*., 2016). These organisms utilize hydrocarbons as carbon and energy sources through pathways such as terminal oxidation and β-oxidation, enabling the breakdown of complex petroleum fractions (Victor *et al*., 2020).

Similarly, *Bacillus endophyticus*, a Gram-positive, spore-forming bacterium, plays a crucial role in hydrocarbon biodegradation, particularly through the production of biosurfactants. Biosurfactants enhance the bioavailability of hydrophobic compounds by reducing surface tension and increasing solubility, thereby facilitating microbial access to hydrocarbons (Mnif & Ghribi, 2015). Additionally, *Bacillus* species are known for their resilience under harsh environmental conditions and their ability to degrade a wide range of organic pollutants (Das *et al*., 2024).



**How to cite this article:** Mustapha G., Zubaidat A. B., Mudassiru S. & Abdulkadir N. (2026). Crude Oil Biodegradation Potential by Mixed Consortia of *Pseudomonas Lurida* and *Bacillus Endophyticus*. *Journal of Basics and Applied Sciences Research*, *4*(4), 150-157. https://dx.doi.org/10.4314/jobasr.v4i4.17

Recent studies have emphasized that mixed microbial consortia exhibit superior biodegradation performance compared to individual strains due to synergistic interactions, metabolic cooperation, and the presence of complementary enzymatic systems (Chaudhary *et al*., 2021; Varjani & Upasani, 2017). In such consortia, one microorganism may initiate the breakdown of complex hydrocarbons into intermediate compounds, which are subsequently utilized by other members of the consortium, thereby enhancing overall degradation efficiency. Gas chromatography–mass spectrometry (GC–MS) is a widely used analytical technique for monitoring hydrocarbon degradation, as it enables the identification and quantification of individual components within complex mixtures (Hubschmann, 2026). It also provides insights into the formation of intermediate metabolites during biodegradation processes (Wang *et al*., 2018).

In this study, the biodegradation potential of a mixed consortium of *Pseudomonas lurida* and *Bacillus endophyticus* was evaluated using GC–MS analysis. The study aims to assess the efficiency of this consortium in degrading crude oil hydrocarbons in contaminated sediment and to elucidate the changes in hydrocarbon composition over time. The findings are expected to contribute to the development of effective microbial strategies for the remediation of petroleum-contaminated environments.

## MATERIALS AND METHODS

### Sample collection and processing for the biodegradation of crude oil

The bonny light crude oil used in this study was supplied by the Microbiology Bayero University Kano, Nigeria. Also, the soil samples used in this study were autoclaved repeatedly until there were no viable bacterial cells on agar plates (Rahman *et al*., 2002; Das & Mukherjee, 2007). Similarly, a test run experiment was conducted on these bacterial species using the collected sediment sample to determine the suitability of the sediment for the present study.

### Preparation of Bacterial Inoculum

For this study, viable pure cultures of *Pseudomonas lurida* (DSM15835) and *Bacillus endophyticus* (DSM13796) were purchased from DMSZ Leibniz Institute, Germany these species were inoculated individually in a sterile 250 ml flask containing fresh LB broth (Thermo Fisher Scientific) prepared according to manufacturer's instructions. The flasks were incubated in a shaker at 170 rpm at 37°C for 24 hours. A solution of Phosphate Buffer Saline (PBS) was prepared by dissolving a tablet in a 200 ml of deionized water which as placed on magnetic stirrer and were immediately autoclaved for 15 minutes at 121°C. Bacterial cells were harvested by pipetting 1 ml of culture in sterile Eppendorf tubes and were centrifuged at 1000 rpm at 4°C for 5 minutes and supernatant was discarded, autoclaved ice-cold PBS solution as used to wash the pallets. Thus, 1 ml of autoclaved ice-cold PBS was added in the Eppendorf tubes containing the bacterial pallets and the tubes were centrifuged at 1000 rpm at 4°C for 5 minutes. This procedure was repeated three times in order to get rid of all nutrients from the medium were left behind (Atlas & Hazen, 2011; Varjani & Upasani, 2017).

Following that, all pallets were suspended in 1 ml of minimal salt medium (MSM). Thereafter, serial dilution was carried out to determine the bacterial colony-forming unit (CFU) per ml for the study of the microcosm. This was done by adding 1 ml of bacterial cells suspended in MSM into autoclaved tubes containing 9 ml of deionized water. Standard plate counting was performed by pipetting 100 µl from each serial dilution tubes into LB agar (ThermoFisher Scientific) plates prepared according to manufacturer's instruction. All plates were incubated at 37°C for 24 hours (Cappuccino & Sherman, 2014).

### Microcosms set up

For this study, 30g of autoclaved sediment were added in sterile Petri dishes with 10% crude oil sterilized by passing through a 0.2µm filter. Thereafter, $5x10^7$ Colony Forming Unit of a mixed culture of *P. lurida* and *B. endophyticus* were inoculated in the experimental microcosm and runs in three biological replicates (Head et al., 2006; Varjani, 2017). The soil sample was used as a negative control was not inoculated with any bacterial cells. Day one (T0) samples were taken for GC-MS analysis after which the plates were incubated at 37°C. Samples for the GC-MS were taken after every seven days through the period of five weeks (Chaudhary *et al*., 2021).

### Sample Preparation and Gas Chromatography-Mass Spectrometry Analysis

For this study, 100 µl of each of the hydrocarbon standards (n-Cyclopantane, n-Hexane, n-Octane, n-Nonane, n-Decane, n-Undecane, n-Dodecane and then Tetradecane) and 200 µl of dichloromethane (DMC) were added in a tube to make 1mL in total. After that, 10 µl of the hydrocarbon standard mixture was added to a GC-MS vial containing 990 µl of dichloromethane to give a concentration of 10 µl per mL. This was repeated to give to 20 µl per mL, up to 50 µl per mL. After that, the GC-MS vials were sonicated for about 10 minutes (Wang *et al*., 2018; Prince, 2015). After which that, 1.0 µl was injected into the GC-MS for calibration using the same program/settings as the experimental samples described below.

Crude oil-polluted sediment (2g) from each of the microcosms was combined with 12 mL of dichloromethane and the mixture was sonicated for 3 minutes. The extract was centrifuged and filtered through

a cotton cool plug contained within a sterile glass pipette. Sodium sulphate was used to remove moisture from the extracted mixture. This is done by adding the sodium sulphate in the Falcon tube containing the mixture and was shaken vigorously. After that, 2 µl of each microcosm sample was injected into a GC containing an HP-5MS column (Agilent) for the analysis of crude oil degradation by these bacteria. A temperature of 280°C was used for the injector while a temperature of 300°C was also used for the detector. Similarly, a holding temperature for 2 minutes at 4°C and 45°C to 310°C per minute as well as at 310° C for 25 minutes were maintained throughout the GC-MS runs. This analysis and the identification of the hydrocarbon compounds was done using CHEMSTATION software as well as NIST 2012 WILEY 2009 using default settings (Wang *et al*., 2018; Bacosa *et al*., 2020).

**RESULTS AND DISCUSSION**

The chromatograms and bacterial growth curve below were obtained from the study of the microcosm with a mixed consortium of *P. lurida* and *B. endophyticus* incubated at 30°C for five weeks. The T0 chromatogram represents the crude oil profile on day one before being incubated while T5 showed the biodegradation profile of the components of crude in the fifth week when the experiment was terminated.

The GC–MS chromatogram at $T_0$ (Figure 1) illustrates the baseline hydrocarbon profile of the crude oil before incubation. A complex distribution of peaks was observed, representing aliphatic hydrocarbons across a broad carbon range (C7–C44). The dominance of sharp and intense peaks indicates a high concentration of undegraded hydrocarbons, including both light and heavy fractions. This initial profile serves as a reference for assessing the extent of biodegradation in subsequent sampling periods.

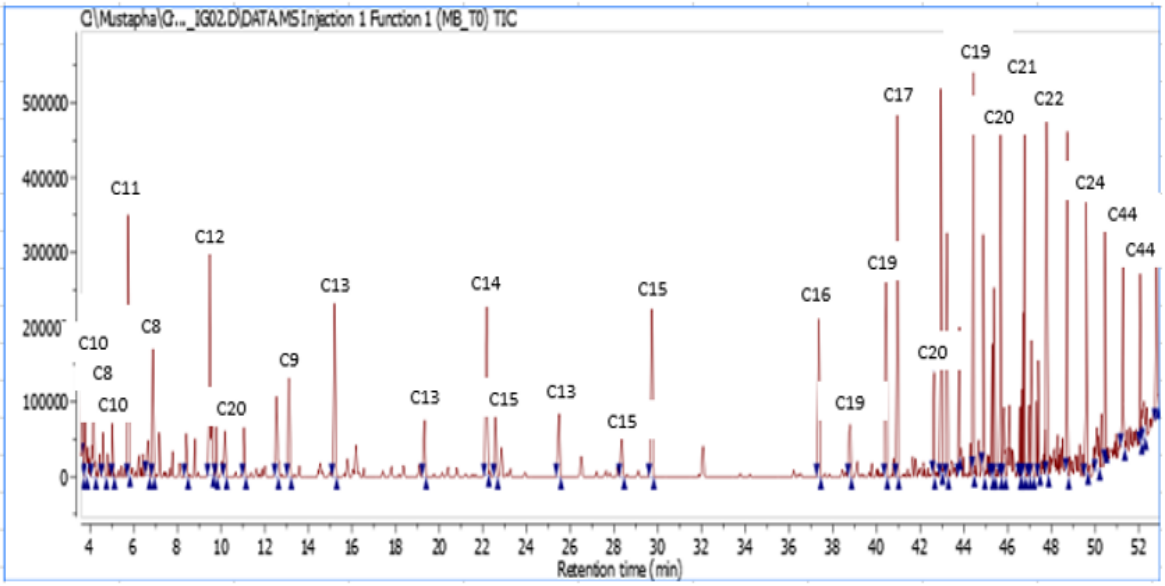


**Figure 1:** GC–MS chromatogram of crude oil at day 0 ($T_0$)

The GC–MS chromatograms revealed a substantial reduction in hydrocarbon components following incubation with the mixed microbial consortium (Figure 2). The initial chromatogram ($T_0$) showed a complex mixture of hydrocarbons ranging from C7 to C44, characteristic of crude oil composition. After five weeks ($T_5$), a significant decrease in peak intensity and number was observed, particularly within the C7–C22 range, indicating effective degradation of low molecular weight hydrocarbons. Additionally, notable reductions in peak areas of higher molecular weight hydrocarbons (C11–C44) were observed, suggesting partial degradation of more recalcitrant fractions. Specific transformations included the breakdown of C13 to C8, C16 to C11, C20 to C18, and C22 to C17, indicating active metabolic conversion of hydrocarbons into simpler compounds. The post-treatment chromatogram (Figure 2) also showed the emergence of new peaks corresponding to intermediate metabolites such as Octane-3,6-dimethyl-, 2-Octanol, Octadecane, and sulphur-containing esters. The presence of these compounds indicates ongoing biodegradation processes involving oxidation and fragmentation pathways.

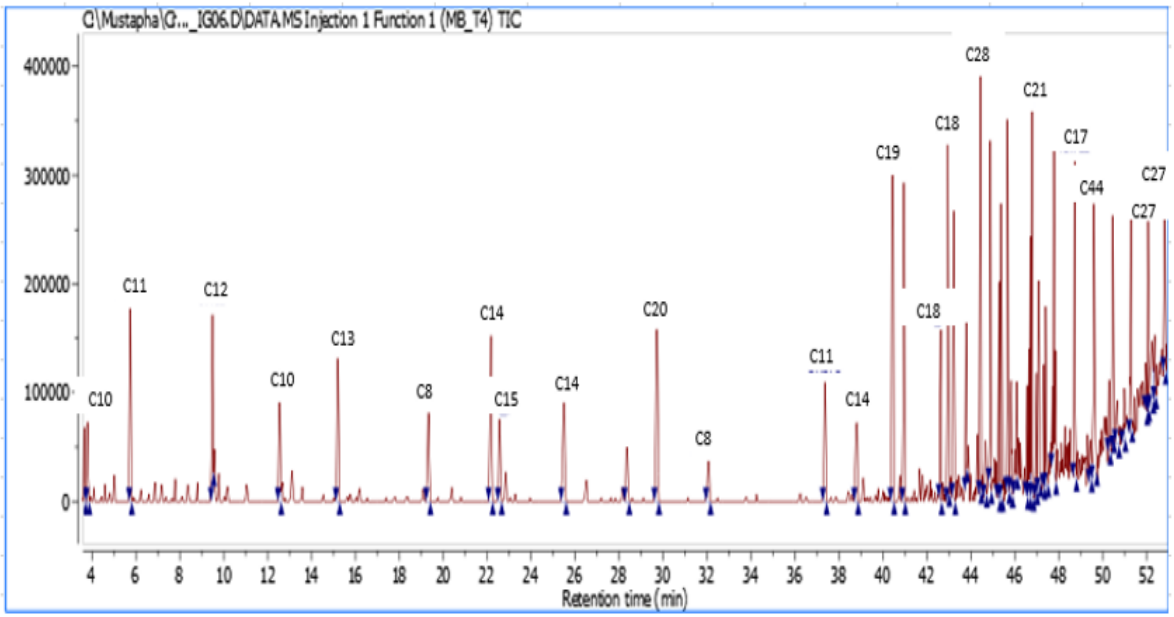


**Figure 2:** GC-MS chromatograms of crude oil extracted at week five (B) from the experimental microcosms in biodegradation study with a mixed consortium of *P. lurida* and *B. endophyticus*

The bacterial growth curve (Figure 3) showed a distinct exponential phase between T1 and T2, indicating rapid utilization of readily degradable hydrocarbons. This was followed by a stationary phase (T2–T3) and a decline phase, suggesting depletion of accessible substrates and accumulation of more recalcitrant compounds.

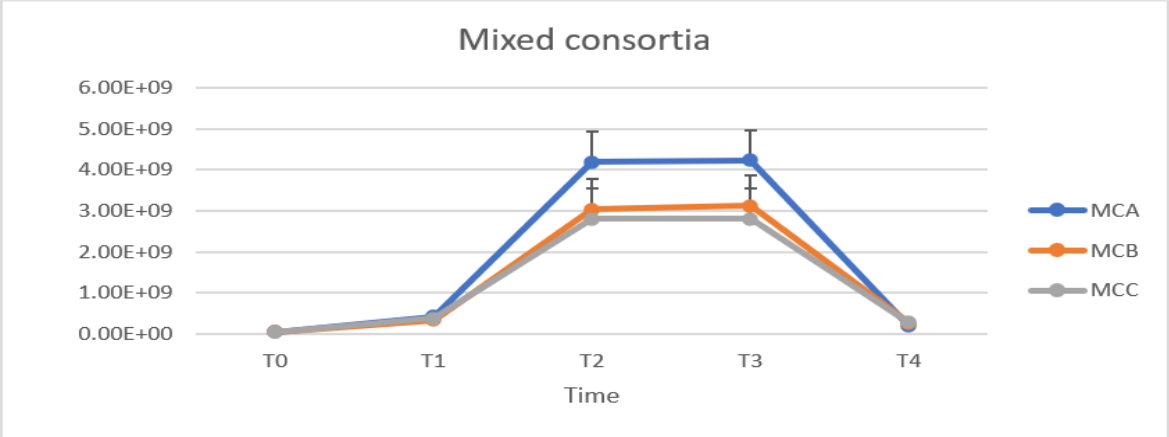


Figure 3: Bacterial growth curve during the experimental period

Table 1 presents the classification of hydrocarbons identified in the crude oil samples into degraded, partially degraded, and non-degraded compounds following treatment with the microbial consortium. The results show that several hydrocarbons, including compounds such as 1,2-cyclohexanediol derivatives, phenethyl alcohol, and o-xylene, were completely degraded during the experimental period. A number of hydrocarbons, particularly medium- to high-molecular-weight alkanes such as nonadecane, hexadecane, and pentadecane, were only partially degraded, indicating incomplete breakdown of more complex compounds. In contrast, some hydrocarbons, including undecane and certain branched alkanes, remained largely unaffected, suggesting resistance to microbial degradation. Overall, the distribution of degraded and partially degraded compounds demonstrates the capability of the microbial consortium to act on a broad spectrum of hydrocarbons, although the persistence of some compounds highlights the recalcitrant nature of specific crude oil components.

Table 1: List of degraded, partially degraded and non-degraded compounds by *P. lurida* and *B. endophyticus*

| S/No | Degraded Compounds | Partially Degraded Compounds | Not Degraded Compounds |
|---|---|---|---|
| 1 | 1,2, cyclohexanedoil,1-methyl-, trans | Nonadecane | Undecane |
| 2 | Phenethyl alcohol | Undecane, 5,5-dimethyl- | Dodecane |
| 3 | Decane,2-cyclohexyl | Hexadecane | Tridecane |
| 4 | o-xylene | Pentadecane | Pentadecane,2,6,10,14-tetramethyl- |
| 5 | Eicosyne | Undecane, 4,8-dimethyl- | Hexadecane,2,6,10,14-tetramethyl- |
| 6 | Benzene,1,3,5-trimethyl- | Tetratetracontane | Heneicosane |
| 7 | 2-pentanone,4-cyclohexylidene-3,3-diethyl- | Nonadecane | |
| 8 | Heptadecane | | |
| 9 | Eicosane | | |
| 10 | Docosane | | |

The findings of this study demonstrate the effectiveness of the mixed microbial consortium in the biodegradation of crude oil hydrocarbons, as evidenced by both GC–MS chromatographic analysis and compound classification. The substantial reduction in hydrocarbon peaks across a wide carbon range (C7–C44) confirms the ability of the consortium to degrade both low and high molecular weight hydrocarbons. Similar broad-spectrum degradation has been reported in studies involving microbial consortia, where metabolic diversity enhances the breakdown of complex petroleum mixtures (Chaudhary *et al*., 2021; Varjani, 2017; Ghosal *et al*., 2016).

The GC–MS analysis of the mixed consortium of *Pseudomonas lurida* and *Bacillus endophyticus* revealed substantial elimination of hydrocarbon peaks ranging from C7 to C22, alongside significant reductions in peak areas of higher molecular weight hydrocarbons between C11 and C44 (Figure 2). This observation is consistent with previous studies which reported that microbial consortia are capable of degrading both low- and high-molecular-weight hydrocarbons due to their diverse enzymatic systems (Chaudhary *et al*., 2021; Varjani, 2017). The reduction and transformation of hydrocarbons, such as C13 to C8 and C20 to C18, indicate active metabolic degradation pathways, particularly β-oxidation and enzymatic cleavage processes, as similarly reported by Das and Chandran (2011).

The degradation pattern observed, including the transformation of hydrocarbons such as C13 to C8 and C20 to C18, suggests active enzymatic processes such as terminal oxidation and β-oxidation. These pathways are well documented in hydrocarbon-degrading microorganisms and are responsible for converting long-

chain hydrocarbons into shorter, more metabolizable compounds (Das & Chandran, 2011; Xu *et al*., 2018). The detection of intermediate metabolites such as alcohols and branched alkanes further supports the occurrence of stepwise biodegradation, which is consistent with earlier findings by Bacosa et al. (2020) and Prince (2015), who reported the formation of oxygenated intermediates during microbial degradation of crude oil.

The emergence of new compounds, including Octane-3,6-dimethyl-, 2-Octanol, and Octadecane, further confirms the biodegradation process through the formation of intermediate metabolites. Comparable findings have been reported by Bacosa et al. (2020), who observed the generation of oxygenated intermediates during microbial degradation of crude oil, indicating progressive breakdown of complex hydrocarbons into simpler and less toxic compounds.

The marked reduction in hydrocarbon peak abundance up to C44 suggests a strong synergistic interaction between *P. lurida* and *B. endophyticus*, enabling the consortium to degrade a wider spectrum of hydrocarbons than individual strains. This agrees with findings by Varjani and Upasani (2017), who emphasized that microbial consortia enhance biodegradation efficiency through metabolic cooperation and substrate sharing. The ability of the consortium in this study to reduce pentadecane, which is often resistant to degradation by single strains, further highlights this synergistic effect.

The bacterial growth pattern observed, with exponential growth between T1 and T2 followed by a plateau and decline, suggests rapid utilization of readily degradable hydrocarbons. Similar growth trends have been reported in hydrocarbon biodegradation studies, where microbial populations initially increase due to substrate availability and subsequently decline as nutrients become limiting (Nwinyi *et al*., 2016). The early decline phase in this study may indicate exhaustion of easily metabolizable hydrocarbons and the persistence of more recalcitrant compounds, which are more resistant to microbial attack.

In comparison to other studies, the relatively rapid degradation observed within five weeks suggests that the mixed consortium is highly efficient. However, the inability of the consortium to completely degrade all hydrocarbon fractions aligns with reports that high molecular weight and branched hydrocarbons often require longer treatment periods or additional microbial species for complete mineralization (Varjani, 2017; Das & Chandran, 2011). This indicates that while the consortium is effective, further enhancement strategies such as nutrient supplementation or the inclusion of additional hydrocarbon-degrading microorganisms may improve overall biodegradation efficiency.

In contrast, the partial degradation of medium to high molecular weight hydrocarbons such as nonadecane, hexadecane, and pentadecane suggests that these compounds require longer degradation periods or more specialized enzymatic systems. Similar findings have been reported by Varjani and Upasani (2017) and Hassanshahian et al. (2012), who noted that long-chain and branched hydrocarbons exhibit greater resistance to microbial degradation due to their structural stability and low solubility.

The observed reduction in pentadecane, which is often resistant in single-organism systems, suggests enhanced degradation capability of the consortium. This supports the concept of synergistic interactions in microbial consortia, where metabolic cooperation enables the utilization of a wider range of substrates (Chaudhary *et al*., 2021; Varjani & Upasani, 2017). Such interactions may involve cross-feeding mechanisms, where intermediate metabolites produced by one group of microorganisms are further degraded by others, leading to improved overall efficiency.

The bacterial growth dynamics observed in this study further support the biodegradation process. The exponential growth phase between T1 and T2 indicates rapid utilization of readily available hydrocarbons, which serve as carbon and energy sources. This is consistent with reports by Nwinyi et al. (2016) and Rahman et al. (2002), who observed similar growth patterns during hydrocarbon biodegradation. The subsequent plateau and decline phases suggest depletion of easily degradable substrates and accumulation of more recalcitrant compounds. The exponential growth observed between T1 and T2 (Figure 3) suggests efficient utilization of readily biodegradable (preferential) crude oil components by the two bacterial strains. This rapid increase in biomass indicates that the consortium was able to effectively access and metabolize low molecular weight hydrocarbons during the early phase of the experiment. The bacterial growth curve subsequently exhibited a plateau between T2 and T3, followed by a decline phase. This pattern is typical of hydrocarbon biodegradation systems, where microbial growth initially increases due to substrate availability and later stabilizes as nutrients become limiting (Das & Chandran, 2011; Nwinyi *et al*., 2016).

The early decline in bacterial growth observed in this microcosm suggests that the consortium rapidly exhausted the easily degradable hydrocarbon fractions and encountered difficulty in metabolizing the more complex and recalcitrant components. Similar observations have been reported by Varjani (2017), who noted that high molecular weight and branched hydrocarbons are more resistant to microbial degradation and often require longer adaptation periods or specialized enzymes.

Additionally, nutrient limitation may have contributed to the decline in microbial growth. The availability of nitrogen and phosphorus is critical for sustaining microbial metabolism during biodegradation, and their depletion can significantly reduce degradation efficiency

(Bacosa *et al*., 2020; Tyagi *et al*., 2011). This implies that nutrient supplementation (biostimulation) could further enhance the biodegradation performance of the consortium.

Furthermore, the inability of the bacterial population to sustain prolonged growth may also be attributed to nutrient limitation, particularly nitrogen and phosphorus, which are essential for microbial metabolism in hydrocarbon-contaminated environments (Bacosa *et al*., 2020). In comparable studies, supplementation with inorganic nutrients has been shown to extend the exponential phase and enhance overall biodegradation efficiency.

The observed growth trend in this study aligns with findings by Chaudhary et al. (2021), who reported that mixed microbial consortia often exhibit rapid initial degradation followed by a decline due to substrate depletion and accumulation of intermediate metabolites. This suggests that, although the consortium demonstrated high efficiency in degrading accessible hydrocarbons, extending the experimental period beyond five weeks may not significantly improve degradation without additional interventions such as nutrient amendment or inclusion of more specialized hydrocarbon-degrading microorganisms.

The results presented in Table 1 provide additional insight into the degradation efficiency of the consortium. The complete degradation of certain compounds, including aromatic and low molecular weight hydrocarbons, indicates that these compounds are more susceptible to microbial attack. This observation aligns with previous studies showing that simpler hydrocarbons are more readily degraded due to their higher bioavailability and lower structural complexity (Atlas & Hazen, 2011; Das & Mukherjee, 2007).

The presence of non-degraded compounds, as indicated in Table 1, further highlights the recalcitrant nature of certain hydrocarbon fractions. These compounds may persist due to limited bioavailability, toxicity, or lack of specific degradative enzymes within the microbial consortium. Previous studies have emphasized that complete mineralization of such compounds often requires extended incubation periods, co-metabolism, or the inclusion of additional microbial species with specialized degradation capabilities (Head *et al*., 2006; Yakimov *et al*., 2007).

## CONCLUSION

The study demonstrated that a mixed microbial consortium effectively degrades crude oil hydrocarbons, as confirmed by GC–MS analysis showing significant reduction in compounds from C44 to C7. The results indicate strong synergistic interactions and metabolic cooperation within the consortium. Although rapid degradation of easily available hydrocarbons was observed, the persistence of more complex compounds and the decline in microbial growth suggest the need for additional strategies, such as nutrient supplementation, for complete remediation. Overall, the consortium shows strong potential for application in the bioremediation of oil-contaminated environments.

## REFERENCE

Ahmed, F., & Fakhruddin, A. N. M. (2018). A review on environmental contamination of petroleum hydrocarbons and its biodegradation. *International Journal of Environmental Sciences & Natural Resources, 11*(3), 1–7.

Atlas, R. M., & Hazen, T. C. (2011). Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. *Environmental Science & Technology, 45*(16), 6709–6715. https://doi.org/10.1021/es2013227

Azubuike, C. C., Chikere, C. B., & Okpokwasili, G. C. (2016). Bioremediation techniques—classification based on site of application. *World Journal of Microbiology and Biotechnology*, *32*(11), 180. https://doi.org/10.1007/s11274-016-2137-x

Bacosa, H. P., Suto, K., Inoue, C., & Matsumoto, K. (2020). efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa*. *Bioresource Technology, 98*(7), 1339–1345.

Cappuccino, J. G., & Sherman, N. (2014). *Microbiology: A laboratory manual* (10th ed.). Pearson Benjamin cummings.

Chaudhary, D. K., Bajagain, R., Jeong, S. W., & Kim, J. (2021). Biodegradation of petroleum hydrocarbons by bacteria and their consortia. *Chemosphere, 285*, 131416. https://doi.org/10.1016/j.chemosphere.2021.131416

Das, A., Das, N., Rajkumari, J., Pandey, P., & Pandey, P. (2024). Exploring the bioremediation potential of *Bacillus* spp. for sustainable mitigation of hydrocarbon contaminants. *Environmental Sustainability, 7*(2), 135–156.

Das, K., & Mukherjee, A. K. (2007). Crude petroleum-oil biodegradation of petroleum hydrocarbons by bacterial consortia. *Environmental Science and Pollution Research, 27*(28), 34987–34999.

Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants. *Biotechnology Research International*, 2011, 941810. https://doi.org/10.4061/2011/941810

Garrido-Sanz, D., Meier-Kolthoff J. P., Goker M. & Martin M. (2016). Genomic insights into the

environmental adaptability of *Pseudomonas* species. *Environmental Microbiology Reports, 8*(5), 708–718.

Ghosal, D., Ghosh, S., Dutta, T. K., & Ahn, Y. (2016). Current state of knowledge in microbial degradation of hydrocarbons. *Bioresource Technology, 210*, 1–15.

Hassanshahian, M., Emtiazi, G., & Cappello, S. (2012). Isolation and characterization of crude-oil-degrading bacteria. *Marine Pollution Bulletin, 64*(1), 7–12.

Head, I. M., Jones, D. M., & Röling, W. F. (2006). Marine microorganisms make a meal of oil. *Nature Reviews Microbiology, 4*(3), 173–182.

Ivanova, A. A., Mullaeva, S. A., Sazonova, O. I., Petrikov, K. V., & Vetrova, A. A. (2022). Current research on simultaneous oxidation of aliphatic and aromatic hydrocarbons by bacteria of the genus *Pseudomonas*. *Folia Microbiologica, 67*(4), 591–604.

Kuppusamy, S., Palanisami, T., Megharaj, M., Venkateswarlu, K., & Naidu, R. (2016). Ex-situ remediation technologies for environmental pollutants: A critical perspective. *Reviews of Environmental Contamination and Toxicology, 236*, 117–192.

Mnif, I., & Ghribi, D. (2015). Microbial derived surfactants: Biosynthesis and applications. *Biotechnology Research International*, 2015, 1–17.

Nwinyi, O. C., Ajayi, O. O., & Amund, O. O. (2016). Petroleum hydrocarbon degradation by microbial communities. *Brazilian Journal of Microbiology, 47*(3), 551–562.

Prince, R. C. (2015). Oil spill dispersants: Boon or bane? *Environmental Science & Technology, 49*(11), 6376–6384.

Rahman, K. S. M., Rahman, T. J., Kourkoutas, Y., Petsas, I., Marchant, R., & Banat, I. M. (2002). Enhanced bioremediation of petroleum sludge. *Biotechnology Letters, 24*, 839–844.

Röling, W. F. M., & van Bodegom, P. M. (2014). Toward quantitative understanding of microbial communities. *ISME Journal, 8*, 221–232.

Singh, A., & Ward, O. P. (2004). Biodegradation and bioremediation. *Springer*.

Tyagi, M., da Fonseca, M. M. R., & de Carvalho, C. C. C. R. (2011). Bioaugmentation and biostimulation strategies. *Chemosphere, 74*, 184–190.

Varjani, S. J. (2017). Microbial degradation of petroleum hydrocarbons. *Bioresource Technsology, 223*, 277–286.

Varjani, S. J., & Upasani, V. N. (2017). A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. *International Biodeterioration & Biodegradation, 120*, 71–83.

Victor, I. A., Iwok, E. O., Archibong, I. A., Effiom, O. E., Okon, E., & Andem, A. B. (2020). The biochemical mechanisms of petroleum degradation by bacteria. *International Journal of Engineering Research, 11*, 1258–1275.

Wang, Z., Fingas, M., & Page, D. S. (2018). Oil spill identification. *Journal of Chromatography A, 843*, 369–411.

Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H., & Yu, H. (2018). Petroleum hydrocarbon degradation by bacteria. *Environmental Pollution, 242*, 531–540.

Yakimov, M. M., Timmis, K. N., & Golyshin, P. N. (2007). Obligate oil-degrading marine bacteria. *Current Opinion in Biotechnology, 18*(3), 257–266.