



HYDROCRBON DEGRADATION POTENTIALS OF FUNGI ISOLATED FROM OIL-CONTAMINATED SOIL OF MECHANIC WORKSHOP

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Abstract

*Petroleum hydrocarbon pollution poses a significant threat to ecosystems due to its persistence and toxicity. This study explores the potential of fungi to catabolize and metabolize diesel, with a view to offering a promising biotechnological solution for environmental clean-up. Soil samples were collected from mechanic workshops within Kaduna metropolis. Fungi were isolated and screened for their ability to degrade diesel. Sixteen (16) fungal isolates were recovered and screened for diesel utilization which include *Rhizopus stolonifer*, *Candida albicans*, *Aspergillus fumigatus*, *Penicillium sp.*, *Exophiala sp.*, *Fusarium sp.* and *Saccharomyces cerevisiae*. Among these, *Aspergillus flavus* and *Exophiala sp.* demonstrated the highest degradation potential with residual oil of 0.39 and 0.41 respectively. The best performing isolates were further subjected to biodegradation study for 15 days using gravimetric method. From the results obtained, 68% and 63% degradation was recorded for *Aspergillus flavus* and *Exophiala sp.* respectively. The findings underscore the enzymatic versatility of fungi and their capacity to thrive in polluted environments, making them ideal agents for sustainable waste management. This study contributes to the growing body of research advocating for eco-friendly, low-cost alternatives remediation approach.*

Keywords: *Biodegradation, Fungi, Hydrocarbon, Mechanic workshop*

Introduction

Polycyclic aromatic hydrocarbons (PAHs) have increasingly contaminated the environment due to rising industrialisation and improper disposal of petroleum products. These compounds are known for their persistence, carcinogenicity, and toxicity which pose serious risk to both humans, animals and the ecosystem. Because to their chemical stability and hydrophobicity, PAHs tends to accumulate in air, soil, and water. They primarily originate from crude oil, petroleum derivatives, and incomplete combustion of organic materials (Al-Hawash *et al.*, 2018). Soil contaminated with petroleum associated organic pollutants, have high potential health due to their capability to infiltrate the food chain and bioaccumulation in living organisms (Daasii & Almaghribi, 2023). In Nigeria, oil spills and leakage from auto-mechanic workshops have been poorly managed for decades, resulting to gradual build-up of petroleum pollutants. Waste motor oil, which often contains carcinogenic compounds such as benzene and toxic metals such as lead, arsenic, and cadmium, can seep into groundwater and pose serious environmental and public health hazards (Onohoa *et al.*, 2011). Although, these workshops play an important role in vehicle maintenance, they have become major contributors to hydrocarbon pollution as a result of improper disposal of petroleum products (Okoye & Abba, 2024). Such practice adversely affects the microbiological balance and physicochemical properties of the soil. Consequently, with the increasing number of mechanic workshops and their uncontrolled discharge of used into the environment, there is need to consider options for their removal from the environment due to the environmental hazards associated with petroleum products (Veerapagu *et al.*, 2019).

Conventional pollution control strategies employing physicochemical methods have often exacerbated environmental problems rather than resolving them. Similarly, mechanical techniques for mitigating hydrocarbon pollution are typically expensive, labor-intensive, and time-consuming. In contrast, environmental restoration through bioremediation offers a more effective, efficient, and eco-friendly alternative that requires less energy and time (Ekanem & Ogunjobi 2017). In contrast, bioremediation, which involves the use of living organisms and their metabolic products to detoxify or completely remove pollutants from contaminated environments, provides an efficient and eco-friendly alternative (Bello *et al.*, 2020). Microorganisms such as bacteria and fungi are particularly well recognized for their ability to utilize diverse organic compounds ranging from simple substrates to complex hydrocarbons as sources of carbon and energy. This metabolic

versatility makes them excellent candidates for the degradation of various organic pollutants, including pesticides, hydrocarbons, and other xenobiotics (Espinosa-Ortiz *et al.*, 2021). Among these, fungi demonstrate exceptional potential for hydrocarbon degradation due to their ability to secrete extracellular oxidative enzymes such as lignin peroxidase (*LiP*), manganese peroxidase (*MnP*), and laccase that enable them to breakdown recalcitrant hydrocarbon pollutants resistant to bacterial degradation (Ramdass & Rampersad 2021; Al-Zaban *et al.*, 2021). These enzymes, produced by fungal genera such as *Aspergillus*, *Penicillium*, *Fusarium*, and *Trichoderma*, enable them to decompose highly stable hydrocarbon pollutants that are otherwise difficult to remove (Rezaei & Moghimi, 2024). The efficiency of biodegradation depends on several environmental factors including temperature, nutrient availability, substrate concentration, and aeration all of which must be maintained under optimal condition (Nanekar *et al.*, 2023). Microorganisms capable of degrading petroleum hydrocarbons possess remarkable adaptive abilities that allow them to survive and function effectively in contaminated environments (Novianty *et al.*, 2021).

Investigating microbial interactions with hydrocarbons enhances our understanding of microbial ecology and biotechnological processes, particularly in the area of hydrocarbon metabolism. Insights gained from such studies can inform the development of bioaugmentation or biostimulation strategies tailored to local mechanic workshop environments, thereby supporting community-level clean-up programs and evidence-based environmental policies. Therefore, this study aims to evaluate the hydrocarbon degradation potential of fungi isolated from mechanic workshop soils, contributing to the development of sustainable and locally adaptable bioremediation strategies.

Materials and Methods

Collection and Preparation of Sample

The soil samples were collected in sterile zip lock bags from 5 different mechanic workshops within Kaduna metropolis. Serial dilution of each sample was carried out. One gram (1g) of soil samples was aseptically weighed into 9 ml of sterile distilled water in a test tube. The samples were vortexed to homogenize and allowed to stand for 10 minutes. From the initial dilution, 10-fold serial dilutions were carried out in clean sterile test tubes containing 9 ml of sterile distilled water (Makut *et al.*, 2022)

Isolation of Fungi from Oil-contaminated Soil

Aliquot (0.1ml) from 10^{-5} 10^{-6} and 10^{-7} dilutions were inoculated on potato dextrose agar plates by spread plate method and incubated at room temperature for 5 days. Distinctive colonies were sub-cultured onto fresh plates for purification. The pure isolates were maintained in agar slants and stored in a refrigerator until further characterization and identification (Saidu *et al.*, 2019). Fungi were characterized and identified by observing their cultural and morphological characteristics. The characteristics observed were compared with description from Atlas of Clinical Fungi (Bello *et al.*, 2020).

Screening for Hydrocarbon Utilization on Solid Media

Fungal isolate was inoculated on the prepared mineral salt oil agar medium using a sterile inoculating needle. The plates were incubated at ambient temperature for 10 days. The fungal growth was monitored and determined by visual observation (Odili *et al.*, 2019).

Screening for Hydrocarbon Utilization on Liquid Media

The isolates were screened for the ability to utilize diesel as sole carbon source using mineral salt medium. The medium (10ml) was supplemented with 10% diesel (AIDisi *et al.*, 2016). The media were inoculated with 0.1ml of fungal inoculum and incubated in Shaker Incubator set at 150rpm and 25°C. The amount of oil left was determined by weighing the residual oil after 8 days' incubation (Essien *et al.*, 2023).

Biodegradation Study

The rate and extent of biodegradation of the hydrocarbon by potential isolates was assessed using the gravimetric method. A standardized inoculum (1% v/v) was inoculated into Erlenmeyer flasks containing 100mL of mineral salt medium enriched with 1% v/v diesel and 1% v/v Tween 80 as well as control flask without inoculum were incubated at 25°C with shaking at 150rpm for 15 days (Mbachu *et al.*, 2016). The residual petroleum oils in all flasks were recovered through liquid-liquid extraction. The oil was extracted with 40 ml n-hexane in a separating funnel and shaken vigorously to separate hydrocarbon. After that, the contents were allowed to settle, the top layer containing solvent mixed with diesel was taken out and the solvent was allowed to evaporate under

reduced pressure (Vinothini *et al.*, 2015). Monitoring of degradation was carried out in intervals of 5 days (day 0, 5, 10, 15) and the amount of oil left was weighed (Essien *et al.*, 2023).

Percentage weight loss of the oil was calculated as;

$$\% \text{ degradation} = \frac{\text{initial weight (W1)} - \text{final weight (W2)}}{\text{initial weight (W1)}} \times 100$$

Results

Table 1 represents the colonial and microscopic characteristics of fungal isolates obtained. A total of and identified based on colonial and microscopic features. The identified fungi *Rhizopus stolonifer*, *Candida albicans*, *Aspergillus fumigatus*, *Penicillium* sp., *Exophiala* sp., *Fusarium* sp. and *Saccharomyces cerevisiae*. Identification was achieved by comparing the observed characteristics with standard description in the atlas of clinical fungi.

Table 1: Colonial Morphology and Microscopic Characteristics of the Fungal Isolates

Isolate's code	Morphology	Microscopic appearance	Probable organism identified
AF1	White, cottony mycelium to black	Non septate hyphae, spherical sporangia containing sporangiospores	<i>Rhizopus</i> sp.
AF2	Creamy, large, round, smooth	Oval yeast cells with buds	<i>Candida albicans</i>
AF3	Green with white edges, large, round, cottony	Septate hyphae with conidiophore	<i>Aspergillus fumigatus</i>
AF4	Green, round, cottony, large	Branched, septate hyalinated hyphae with round conidia	<i>Penicillium</i> sp.
AF5	Black, large, round, mucoid	Small oval yeast cells	<i>Exophiala</i> sp.

BF1	Creamy, large, round, mucoid	Small, oval yeast cells with buds	<i>Candida albicans</i> .
BF2	Fast growing colonies with cottony aerial mycelium, pink with white edges	Septate hyphae, oval conidia	<i>Fusarium</i> sp.
CF1	Creamy, large, irregular, mucoid	Small oval yeast cells	<i>Candida</i> sp.
CF2	Dark green, large, round, cottony, powdery, raised	Septate hyphae with conidiophore	<i>Aspergillus</i> sp.
CF3	Yellow- green colonies	Septate hyphae with conidia in chains	<i>Aspergillus flavus</i>
DF1	Creamy, large, round, mucoid	Oval yeast cells	<i>Candida</i> sp.
DF2	Grey, large, cottony, round	Branched septate hyphae	<i>Aspergillus fumigatus</i>
DF3	Green, large, cottony, round	Elongated septate hyphae with conidiophore	<i>Aspergillus</i> sp.
EF1	Off white, large, smooth, round	Oval yeast cells with buds	<i>Saccharomyces cerevisiae</i>
EF2	Yellow, large, round, mucoid	Oval yeast cells with buds	<i>Candida lipolitica</i>
EF3	Fast growing, brown mycelia, dry surface with sclerotia	Elongated, branched, septate hyphae with conidiophore	<i>Aspergillus terreus</i> .

KEYS: AF- Sample A fungi, BF: Sample B fungi, CF- Sample C fungi. DF- Sample D fungi, EF- Sample E fungi

The fungal isolates were assessed for their ability to utilize hydrocarbon using mineral salt medium supplemented with diesel oil as sole carbon source. The diesel utilization potential of each isolate was evaluated by growth on oil agar which was recorded qualitatively based on degree of visible growth as -: no growth, +: low growth, ++: medium growth and +++: high growth, followed by

gravimetric analysis to determine residual oil after 8 days' incubation. All fungal species showed varying levels of growth and degradation on mineral salt medium amended with diesel. *Aspergillus flavus* and *Exophiala* sp. showed the highest degradation efficiencies with residual oil of 0.39 g and 0.41 g, respectively as shown in table 2.

Table 2: Hydrocarbon Utilization of the Different Fungal Isolates

Isolate code	Potential organism	Diesel utilization on oil agar	Residual oil(g)
AF1	<i>Rhizopus</i> sp.	+++	0.46
AF2	<i>Candida albicans</i>	+	0.66
AF3	<i>Aspergillus fumigatus</i>	+++	0.52
AF4	<i>Penicillium</i> sp.	+	0.58
AF5	<i>Exophiala</i> sp.	+++	0.41
BF1	<i>Candida albicans</i>	+	0.59
BF2	<i>Fusarium</i> sp.	+++	0.48
CF1	<i>Candida</i> sp.	-	0.67
CF2	<i>Aspergillus</i> sp.	-	0.60
CF3	<i>Aspergillus flavus</i>	+++	0.39
DF1	<i>Candida</i> sp.	+	0.63
DF2	<i>Aspergillus fumigatus</i>	+++	0.58
DF3	<i>Aspergillus</i> sp.	+	0.59
EF1	<i>Saccharomyces cerevisiae</i>	++	0.61
EF2	<i>Candida lipolitica</i>	++	0.57
EF3	<i>Aspergillus</i> sp.	+++	0.56
control		-	0.78

Initial concentration oil: 0.821g

Key: Low growth +, Moderate growth: ++, High growth: +++

Biodegradation study was carried out using gravimetric analysis for 15 days and readings were taken at 5 days' intervals. Both *Aspergillus flavus* and *Exophiala* sp. showed significant diesel

degradation. *A. flavus* achieved the highest early degradation, increasing from 23.1% at day 5 reaching 68.6% by day 15 followed by *Exophiala* sp. showed degradation from 19.8% at day 5 to 63.8% by day 15. The control exhibited only minimal natural attenuation (4.4 – 5.4% over 15 days), confirming that most degradation was biologically mediated by the isolates as shown in table 3.

Table 3: Percentage degradation potential of the best performing isolates

Isolates	Percentage degradation (%)		
	Day 5	Day 10	Day 15
<i>Aspergillus flavus</i>	23.1	57.0	68.6
<i>Exophiala</i> sp.	20.7	40.5	63.8
Control	4.4	4.6	5.4

Discussion

A total of sixteen fungal isolates were recovered from oil-contaminated soil samples. The isolates demonstrated diversity in form and taxonomy, reflecting fungal adaptation to hydrocarbon polluted environments. The identified genera include *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium*, *Fusarium* and *Rhizopus* species all of which have been documented for their hydrocarbon degrading abilities. Several yeast species including *Saccharomyces cerevisiae*, *Candida albicans*, *Candida lipolytica* and *Exophiala* sp. were isolated. Yeasts are increasingly recognized in hydrocarbon degradation, especially in diesel and engine oil-polluted soils. This result is in agreement with Okigbo and Okafor (2022) & Ameen *et al.*, (2020) who isolated similar genera of hydrocarbon utilizing fungi.

Screening results revealed varying levels of hydrocarbon degradation by different fungi isolates over an eight-day period. Microbial growth on oil agar was categorized qualitatively (+ to +++) based on growth, and quantitatively by the weight of residual oil. Most isolates showed ability to degrade hydrocarbon on both solid and liquid media amended with diesel. *Aspergillus flavus* and *Exophiala* sp. showed the highest degradation efficiencies with residual oil of 0.39 g and 0.41 g, respectively. These isolates likely express diverse enzymes like oxygenases and peroxidases that

facilitate hydrocarbon breakdown (Prenafeta-Boldú *et al.*, 2019). Similar findings were reported by Elewu *et al.*, (2025). Among these, *Aspergillus* spp. are among the most frequently reported filamentous fungi that are able to utilize hydrocarbon as carbon source (Jasme *et al.*, 2023; Elewu *et al.*, 2025). The dominance of *Candida* and *Aspergillus* species indicates strong fungal adaptation to hydrocarbons via bio surfactant production, enzymatic degradation, and resistance traits. Their presence supports the idea that fungal bioaugmentation could enhance hydrocarbon remediation in workshop soils. The fact that these organisms could degrade diesel efficiently points to their potential usefulness in cleaning oil spills in tropical soil.

The biodegradation study further confirmed the ability of selected isolates to degrade diesel over a 15-day incubation period. A progressive increase in diesel degradation across isolates from day 0 to day 15, indicating active microbial adaptation, while sharp increase after day 10 reflects active enzymatic breakdown of diesel components. This trend agrees with findings of Al-Hawash *et al.*, (2018), Mohesien *et al.*, (2018), and Odili *et al.*, (2020) who noted that the hydrocarbon degradation rate could be enhanced with a longer incubation period after treatment. After 15 days, *Aspergillus flavus* showed 68.6% degradation and *Exophiala* sp. 63.5 %. The control recorded only 5.4%, confirming that natural attenuation alone is insufficient within this time frame. This supports findings by Essien *et al.*, (2023) that fungi degrade hydrophobic hydrocarbons through extracellular ligninolytic and peroxidase enzymes. The performance of *Aspergillus flavus* in this study (64.6% after 15 days) is comparable to degradation rates reported for other *Aspergillus* species. This agrees with findings of Al-Dossary *et al.*, (2019) who reported 60% degradation of crude oil by *Aspergillus flavus* in 14 days and Aljawhari *et al.*, (2014) who reported fungal degradation percentage of 65% after 14 days of incubation. Biodegradation of hydrocarbon by *Exophiala* spp. has been reported by other studies. Ide-Perez *et al.*, (2020) reported that *Exophiala* strain could remove up to 80% polyaromatic hydrocarbons. Prenafeta-Boldú *et al.* (2019) reviewed fungal communities like *Exophiala* spp. noting their ability to degrade complex PAHs via extracellular enzymes like laccases and peroxidases.

The high degradation rates observed in this study indicate that fungi possess strong potential for application in hydrocarbon contaminated environment. Their resilience and enzymatic versatility make them promising agents for eco-friendly restoration of polluted environment.

Conclusion

A total of sixteen fungal species were isolated including *Rhizopus stolonifer*, *Candida albicans*, *Aspergillus fumigatus*, *Penicillium* sp., *Exophiala* sp., *Fusarium* sp. and *Saccharomyces cerevisiae*. Among these, *Aspergillus flavus* and *Exophiala* sp. demonstrated the highest degradation capabilities, achieving 68% and 63% diesel degradation respectively after 15 days of incubation. The results affirm that fungal species from oil polluted soils can serve as efficient biocatalysts for eco-friendly remediation of petroleum contaminated environments.

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