Biosurfactant Production in Soil Amended With Diesel Using Fungi Isolated from a Mechanic Workshop Soil in Dan-Magaji Zaria, Kaduna State

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ABSTRACT

The increasing contamination of soils by petroleum hydrocarbons has intensified the search for environmentally sustainable remediation technologies. Biosurfactants, which are surface-active compounds produced by microorganisms, have gained significant attention due to their biodegradability, low toxicity, and effectiveness under extreme environmental conditions. This study investigated fungal biosurfactant production from hydrocarbon-contaminated soils collected from mechanic workshops in Danmagaji, Zaria, Kaduna State, Nigeria. Physicochemical analysis of the soil revealed slightly acidic conditions, moderate electrical conductivity, and low moisture content, and elevated temperatures typical of hydrocarbon-polluted environments. Fungal isolation yielded four species: *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, and *Fusarium oxysporum*. Screening for biosurfactant production using oil spreading, drop collapse, and emulsification index (E24) assays identified *Aspergillus flavus* and *Fusarium oxysporum* as positive producers. Biosurfactant production in Mineral Salt Medium (MSM) amended with diesel demonstrated higher yields in *A. flavus* (0.00152 g/mL) compared to *F. oxysporum* (0.00136 g/mL). The findings confirm the potential of indigenous fungal isolates to utilize diesel as a carbon source for biosurfactant synthesis and highlight their applicability in the bioremediation of hydrocarbon-contaminated soils.

Keywords: Biosurfactants, Hydrocarbon contamination, Bioremediation, *Aspergillus flavus*, *Fusarium oxysporum*

INTRODUCTION

Hydrocarbon contamination resulting from petroleum exploration, automobile servicing, and industrial discharge remains one of the most significant environmental challenges worldwide. Mechanic workshops, in particular, contribute substantially to soil pollution through the indiscriminate disposal of diesel, engine oils, lubricants, and related petroleum products. Such contamination alters soil physicochemical properties, suppresses native biodiversity, and poses severe ecological and public health risks. Conventional remediation techniques, including chemical oxidation, incineration, and physical excavation, are often expensive and environmentally disruptive. Consequently, attention has shifted toward biological alternatives that are eco-friendly and cost-effective. Among these alternatives, biosurfactants have emerged as promising agents for hydrocarbon remediation due to their ability to reduce surface and interfacial tension, thereby enhancing the bioavailability of hydrophobic pollutants Uyar & Saglam (2021).

Biosurfactants are amphiphilic compounds produced by microorganisms and consist of hydrophilic and hydrophobic moieties that facilitate emulsification of immiscible substances such as hydrocarbons and water. Unlike synthetic surfactants, biosurfactants are biodegradable, less toxic, and stable under varying environmental conditions including extreme pH, salinity, and temperature. Their production is frequently induced in hydrocarbon-rich environments, making contaminated soils important reservoirs of biosurfactant-producing

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microorganisms. Although bacterial biosurfactants have been extensively investigated, fungal biosurfactants remain comparatively underexplored despite the remarkable adaptability of fungi in polluted environments. Filamentous fungi possess extensive enzymatic systems and hyphal networks that enable survival and metabolic activity in harsh ecological niches. Species of *Aspergillus*, *Penicillium*, and *Fusarium* have repeatedly been isolated from hydrocarbon-polluted soils and have demonstrated significant biodegradation capabilities Uyar & Saglam (2021).

This study therefore aimed to isolate and identify fungi from hydrocarbon-contaminated soils collected from mechanic workshops in Danmagaji, Zaria, Kaduna State, Nigeria, evaluate their biosurfactant-producing potentials, and assess their suitability for diesel bioremediation applications.

MATERIALS AND METHODS

Study Area and Sample Collection

Soil samples were collected from mechanic workshops located in Danmagaji, Zaria, Kaduna State, Nigeria (11°04′0″N, 7°41′17.5″E). The area is characterized by extensive hydrocarbon contamination resulting from automobile repair activities involving diesel and engine oils. Surface soil samples (0–15 cm depth) were collected aseptically using clean hand trowels. Approximately 500 g of soil was obtained from each sampling point, placed in a clean polyethylene bags, and transported to the Microbiology laboratory at 4°C for further analysis.

Physicochemical Analysis

Soil physicochemical parameters including temperature, pH, electrical conductivity, and moisture content were determined using standard analytical procedures to assess the environmental conditions influencing fungal isolation and biosurfactant production (Aleruchi *et al.,* 2022; Izomor *et al.,* 2024).

Temperature

Soil temperature was measured in situ at the time of sampling using a digital soil thermometer (Hanna Instruments HI98501) inserted to a depth of 5 cm. Readings were taken immediately after exposure to avoid atmospheric influence, and the average of three measurements per site was recorded. This method aligns with standard on-site determinations for soil microbial studies, where temperature affects hydrocarbon degradation rates (Aleruchi *et al.,* 2022; Izomor *et al.,* 2024).

pH

Soil pH was determined using the electrometric method in a 1:2.5 soil-water suspension. Ten grams of air-dried soil (<2 mm sieve) were mixed with 25 mL of distilled water, stirred for 30 minutes, and allowed to settle for 1 hour. The pH was measured using a calibrated glass electrode pH meter standardized with buffer solutions of pH 4.0 and 7.0. This protocol follows EPA Method 9045D for soil pH analysis (U.S. Environmental Protection Agency, 2000) and is consistent with measurements in contaminated soils (Aleruchi *et al.,* 2022).

Moisture

Soil moisture content was assessed gravimetrically. Five grams of fresh soil were weighed into a pre-weighed aluminum dish and oven-dried at 105°C for 24 hours until constant weight was achieved. The moisture content was calculated as:

Moisture (%) = [(Wet weight - Dry weight) / Wet weight] × 100

This standard oven-drying method is widely used for soil moisture determination in microbial ecology studies (Aleruchi *et al.,* 2022).

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Electrical Conductivity

Electrical conductivity (EC) was measured in a 1:5 soil-water suspension. Ten grams of air-dried soil were mixed with 50 mL of distilled water, shaken for 1 hour, and allowed to settle. The supernatant was analyzed using a calibrated conductivity meter (Hanna Instruments HI8733) at 25°C. Results were expressed in dS/m, with corrections for temperature if needed (EC decreases by approximately 2.2% per °C below 25°C). This procedure follows the FAO GLOSOLAN standard operating procedure for soil EC (Food and Agriculture Organization, 2021) and is applied in hydrocarbon-polluted soil assessments (Aleruchi *et al.,* 2022).

Isolation and Identification of Fungi

Sample Collection and Preparation

Soil (25g) was suspended into a conical flask containing 225ml of sterile distilled. The mixture was vortexed to homogenize the suspension. Further serial dilutions up to 10^{-4} were prepared as described by Christopher *et al.,* (2021).

Isolation of Total Heterotrophic Fungi

Aliquots (0.1 ml) from the 10^{-2} and 10^{-3} dilutions were aseptically inoculated onto PDA plates supplemented with chloramphenicol (250 mg L^{-1}) to suppress bacterial growth. The inoculum was spread evenly using a sterile glass rod. Plates were incubated at 25–28°C for 5–7 days. After incubation, distinct colonies were counted and recorded as colony-forming units (CFU/g soil) (Christopher *et al.,* 2021). Pure isolates were obtained by sub-culturing onto fresh PDA plates.

Identification of Fungal Isolates

Fungal isolates were identified based on macroscopic and microscopic characteristics. Colony morphology, colour, texture, and sporulation were examined on PDA. Microscopic identification was done using Lactophenol Cotton Blue (LPCB) staining as described by Sarah *et al.,* (2016). A small portion of aerial mycelium was mounted on LPCB, covered with a cover slip, and examined under ×10 and ×40 objectives. Identification of fungal isolates was performed based on macroscopic and microscopic characteristics. Macroscopic observations included colony colour, texture, and growth rate, while microscopic analysis involved examining hyphal structures, spore morphology, and conidial arrangements under a compound microscope using standard taxonomic keys (Sarah *et al.,* 2016; George-Okafor *et al.,* 2009) and and compared with identification manuals such as Descriptions of Medical Fungi by Sarah *et al.*, (2016).

Screening of Biosurfactant-Producing Fungi

The screening of biosurfactant-producing fungi was conducted using Potato Dextrose Broth (PDB) as the basal medium, amended with diesel (20 g L^{-1}) as the carbon source and yeast extract (10 g L^{-1}) as the nitrogen source. Erlenmeyer flasks (125 mL) containing 25 mL of this medium were inoculated with fungal spores at a final concentration of 1×10^4 spores mL^{-1}. The flasks were incubated without shaking at room temperature (25 ± 2°C) for 7 days, after which the cultures were filtered using quantitative filter paper (pore size 28 µm) to remove fungal biomass, yielding a cell-free filtrate. Biosurfactant production was assessed using three screening methods; the oil spreading assay, the drop collapse test, and the emulsification index (E24). These methods were adapted from Cameron *et al.,* (1988); Bodour and Miller-Maier (1998) respectively, to evaluate the surface-active properties of the fungal filtrates.

Oil Spreading Assay: This assay was performed by adding 40 mL of distilled water to a Petri dish, followed by the gentle addition of 20 µL of diesel oil to form a thin layer on the surface. Subsequently, 10 µL of the cell-free filtrate was placed onto the oil surface. The diameter of the clear zone formed due to the displacement of oil by the biosurfactant was measured in millimeters. A larger clear zone indicated higher biosurfactant activity, with distilled water serving as a negative control and Sodium Dodecyl Sulfate (SDS) at 25% as a positive control.

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Drop Collapse Test: A polystyrene 96-well microplate was prepared by washing with hot water, ethanol, and distilled water. Each well was prefilled with 2 µL of diesel oil and left at room temperature for 24 hours. Then, 5 µL of the cell-free filtrate was added to each well. After 1 minute, the shape of the drop was observed visually. A positive result was recorded if the drop collapsed, indicating biosurfactant presence, while a beaded drop indicated no activity. SDS (25%) served as a positive control, and distilled water as a negative control.

Emulsification Index (E24): The emulsification capacity was determined by mixing 4 mL of the cell-free filtrate with 6 mL of diesel in a screw-cap test tube. The mixture was vigorously shaken for 2 minutes using a vortex mixer. After 24 hours of incubation at room temperature, the height of the emulsion layer was measured and compared to the total height of the liquid. The emulsification index was calculated using the formula: E24=height of emulsion/total height X 100

A higher E24 value indicated greater emulsification capacity, with Tween 80 (0.2% w/v) used as a positive control.

Fungal isolates exhibiting positive results in at least two of the three assays were selected for further analysis. The isolate demonstrating the highest E24 value was chosen for subsequent experiments.

Biosurfactant Production in Mineral Salt Medium (MSM)

Biosurfactant production was optimized using Mineral Salt Medium (MSM), which was prepared with the following composition per litter of distilled water: 1.0 g NH_4NO_3, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.5 g K_2HPO_4, and 0.5 g KH_2PO_4, adjusted to pH 6.0. The medium was amended with diesel (20 g L^{-1}) as the carbon source and yeast extract (10 g L^{-1}) as the nitrogen source. Erlenmeyer flasks (250 mL) containing 50 mL of MSM were sterilized at 121°C for 15 minutes and inoculated with 1×10^6 spores mL^{-1} of the selected fungal isolate. The cultures were incubated at room temperature on a rotary shaker at 150 rpm for 7 days. After incubation, the broth was centrifuged at 5000 rpm for 20 minutes to remove fungal biomass, and the supernatant was collected for biosurfactant extraction.

Biosurfactant extraction involved adjusting the pH of the supernatant to 2.0 with 6N HCl, followed by the addition of an equal volume of ethyl acetate. The mixture was shaken vigorously and allowed to separate into two phases. The organic phase containing the biosurfactant was collected, and the solvent was evaporated under reduced pressure at 40°C using a rotary evaporator. The crude biosurfactant was resuspended in a minimal volume of distilled water and stored at 4°C for further analysis. Semi-purification of the biosurfactant was conducted by pooling the crude extracts from the replicate cultures grown under optimized MSM conditions. The pooled extract was filtered through Whatman No. 1 filter paper and precipitated with ethanol (1:4 v/v) at 4°C for 48 hours. The mixture was centrifuged at 5000 rpm for 20 minutes, and the resulting precipitate was collected and air-dried to obtain a semi-purified biosurfactant. The emulsification index (E24) was then measured using the method described earlier.

RESULTS

The physicochemical properties of the sampling site was presented in table 1 with a recorded temperature of a 32°C, pH 6.21, an electrical conductivity 1.10 dS/m, and a moisture content of 4.68%. Four fungal species were identified; *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum,* and *Fusarium oxysporum* according to their unique morphological features as shown in table 2. The biosurfactant screening identified *Aspergillus flavus* and *Fusarium oxysporum* as the only positive producers, demonstrating clear zones of 20 mm and 15 mm, respectively, along with positive drop-collapse and emulsification results. *Aspergillus niger* and *Penicillium notatum* showed no activity and were deemed non-producers table 3. Table 4 shows biosurfactant production in liquid medium amended with diesel; *A. flavus* demonstrated a higher production yield (0.00152 g/mL) compared to *F. oxysporum* (0.00136 g/mL).

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Table 1. Physicochemical Properties of Soil Sample

Properties	**Value**
Temperature (°C)	32
pH	6.21
Electrical Conductivity (ds/m)	1.10
Moisture (%)	4.68

Table 2 Cultural and Microscopic characteristics of Fungi isolated from hydrocarbon-contaminated soil

Isolate code	**Macroscopic characteristics**	**Microscopic characteristics**	**Inference**
F1	Yellow-green, velvety colony; Pale yellow	Biseriate phialides on vesicles; rough globose conidia	*Aspergillus flavus*
F2	Jet-black, powdery colony; pale yellow	Radiate black conidial heads; biseriate phialides	*Aspergillus niger*
F3	Green-blue velvety colony; smooth margin	Brush-like penicillus; ovoid conidia	*Penicillium notatum*
F4	Floccose white colony turning pink/purple	Canoe-shaped macroconidia; monophialides; chlamydospores	*Fusarium oxysporum*

Table 3 Screening for Biosurfactant production Using Three Different Assay Techniques

Isolate Code	Oil spreading assay (mm)	Drop collapse	Emulsification index
Aspergillus flavus	20	+	+
Aspergillus niger	0	-	-
Penicillium notatum	0	-	-
Fusarium oxysporum	15	+	+

Key: + = Positive, - = negative.

Table 4. Biosurfactant produced from isolated fungi

Fungi	Inoculum size (cfu/mL)	Biosurfactant produced (g/mL)
Aspergillus flavus	1.0×10^6	0.00152
Fusarium oxysporum	1.0×10^6	0.00136

DISCUSSION

This study provides valuable insights into the biosurfactant-producing potential of indigenous fungal isolates obtained from hydrocarbon-contaminated mechanic workshop soils in Danmagaji, Zaria, Nigeria. The use of

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multiple screening assays and diesel-amended mineral salt medium strengthens the reliability and environmental relevance of the findings. However, limitations such as the restricted sampling scope, absence of molecular identification and advanced biosurfactant characterization, as well as the lack of field-scale validation, may constrain the generalizability of the results. Future studies should integrate molecular, biochemical, and pilot-scale approaches to enhance understanding and practical application of fungal biosurfactants in environmental remediation.

The physicochemical parameters of the soil (slightly acidic pH, moderate electrical conductivity, low moisture content, and elevated temperature) are characteristic of hydrocarbon-contaminated sites and align with findings from similar ecological niches. The slightly acidic pH observed is consistent with reports by Aleruchi *et al.,* (2022) in their assessment of mechanic workshop soils in Port Harcourt. This acidity often arises from the accumulation of acidic intermediates during the incomplete microbial degradation of petroleum hydrocarbons, a process that can alter soil chemistry over time. The elevated temperatures recorded are a significant ecological factor, as noted in tropical contamination studies. Higher ambient temperatures can reduce hydrocarbon viscosity, thereby increasing their bioavailability to microorganisms, a factor implied in the selective enrichment of thermotolerant species like *Aspergillus* and *Fusarium*.

The low moisture content is a typical feature of surface soils in tropical savanna climates and creates a selective pressure that favors filamentous fungi. This aligns with the observations of Aleruchi *et al.,* (2022), who documented similar conditions and the consequent dominance of fungal populations in mechanic workshop environments. The hyphal network of fungi is a key adaptation for traversing air-filled pores in such semi-arid soils, providing a competitive advantage for nutrient and water access, which explains their pronounced role in these contaminated ecosystems. The moderate electrical conductivity values suggest that soluble salt concentrations are not inhibitory and may in fact support the osmotic balance and metabolic processes of adapted microorganisms, a factor often overlooked but crucial for microbial activity in stressed environments.

The isolation of *Aspergillus flavus, Aspergillus niger, Penicillium notatum,* and *Fusarium oxysporum* from the diesel-contaminated soil is strongly supported by the literature on hydrocarbon-impacted environments. This mycological profile is highly consistent with the findings of April *et al.,* (2000), who isolated similar genera, including Aspergillus and Penicillium, from flare pit soils in Canada, and Obire *et al.,* (2020), who reported their presence in oilfield environments. More recently, Aleruchi *et al.,* (2022) confirmed the prevalence of these genera in automobile workshop soils in Port Harcourt, indicating a global distribution of these resilient taxa in petroleum-rich niches. The repeated isolation of these specific genera from disparate geographic locations underscores a strong evolutionary selection for traits such as robust cell walls, versatile enzymatic profiles, and efficient stress response systems in the face of hydrocarbon toxicity.

The prevalence of *Aspergillus* species, corroborates their well-documented metabolic versatility and tolerance to environmental stressors, as highlighted by Tomilayo *et al.,* (2025). The coexistence of multiple species within the same genus suggests potential niche differentiation, a phenomenon observed in complex microbial communities where related taxa utilize different hydrocarbon fractions or employ complementary degradation strategies. The isolation of *Fusarium oxysporum* further supports its documented hydrocarbon-degrading capabilities in various polluted environments. The successful cultivation of these fungi on standard media underscores their viability and robust physiological adaptation to contamination, a testament to the selective pressure exerted by diesel, as described by Izomor *et al.,* (2024). This adaptation is not merely survivalist; it is indicative of a metabolic reprogramming where hydrocarbons are recognized as a primary carbon source, a prerequisite for their application in bioremediation.

The screening results, which identified *Aspergillus flavus* and *Fusarium oxysporum* as positive biosurfactant producers, are in line with the capabilities of fungi from contaminated sites. However, the fact that *Aspergillus niger* and *Penicillium notatum* tested negative in all assays presents a point of divergence from some literature. For instance, Mahmoud *et al.,* (2024) reported biosurfactant production by *Aspergillus niger* isolated from petrochemical waste. This discrepancy underscores a critical point in microbial ecology and biotechnology that biosurfactant production is a strain-specific trait rather than a universal characteristic of a genus. It is not guaranteed that all isolates of a species will possess or express this capability under standard screening conditions, a variability noted in broader microbial studies. This highlights the importance of extensive screening

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within a species to find the most potent strains, rather than assuming functionality from taxonomic identification alone.

The use of multiple screening assays (oil spreading, drop collapse, emulsification index) follows established protocols and strengthens the validity of the results. The methods are consistent with those used by Pieprzyca & Ziembińska-Buczyńska (2018) and Izomor *et al.,* (2024) for screening environmental isolates. The positive emulsification index (E24) for the potent isolates is particularly significant, as it demonstrates a functional property critical for bioremediation (the ability to form stable emulsions and increase hydrocarbon bioavailability). This principle is central to the role of biosurfactants as described by Uyar & Saglam (2021), who emphasized their function in pseudo solubilization, effectively making the diesel more accessible to the enzymatic machinery of the degrading microorganisms. The oil-spreading assay, which directly demonstrates tension-reducing activity, aligns with the foundational work of Mulligan *et al.,* (1984), confirming that the produced metabolites are true surface-active agents.

The successful production of biosurfactants in a Mineral Salt Medium (MSM) with diesel as the sole carbon source is a key finding that aligns with the fundamental work of Desai & Banat (1997), who established that hydrophobic substrates could induce biosurfactant synthesis. This confirms the adaptive response of these indigenous fungi to utilize diesel not only for growth but also to produce value-added metabolites, a concept further elaborated by Chandran & Das (2010) in their work with the yeast *Trichosporon asahii*. The production process itself, involving acid precipitation and solvent extraction, is a standard downstream processing technique widely reported in the literature for recovering diverse biosurfactants.

The extraction of a crude biosurfactant with a milky, oil-like appearance is a common physical description for many microbial glycolipids and lipopeptides. While this study provided a physical characterization, the literature suggests a path for further analysis. For example, Rikalović *et al.,* (2012) and Faisal *et al.,* (2023) utilized advanced techniques like FTIR and GC-MS to precisely characterize the chemical structure of biosurfactants from *Pseudomonas* species, revealing them to be rhamnolipids. Similarly, Chandran & Das (2010) confirmed the glycolipid nature of their biosurfactant from *T. asahii* through similar analyses. Applying these characterization methods to the biosurfactants from *Aspergillus flavus* and *Fusarium oxysporum* in future work would allow for a direct structural comparison with these well-defined molecules, potentially classifying them into known groups like sophorolipids or mannosylerythritol lipids, which are known to be produced by fungi.

The differential yield between the two species highlights the importance of strain selection, a factor critical for process optimization as seen in bacterial systems (Rikalović *et al.,* 2012). The yields reported here are from a non-optimized system. The literature strongly indicates that yields can be significantly enhanced through systematic optimization of cultural parameters. Mahmoud *et al.,* (2024), for instance, optimized carbon and nitrogen sources for *Aspergillus* species, while Rikalović *et al.,* (2012) used response surface methodology to maximize rhamnolipid production. This presents a clear opportunity for future research to build upon these foundational findings by exploring the impact of variables such as the C/N ratio, trace elements, pH, and aeration on the biosurfactant yield from these fungal isolates. Furthermore, exploring the stability of these biosurfactants under various pH, temperature, and salinity conditions, as was done by Rikalović *et al.,* (2012), would be a crucial next step to assess their robustness for real-world environmental applications.

Based on the findings of this study, the following conclusions were drawn in direct relation to the stated objectives: The soil from the mechanical workshop in Dan-Magaji, Zaria, is characterized by a slightly acidic pH, elevated temperature, moderate electrical conductivity, and low moisture content, which are conditions influencing microbial activity in contaminated environments.

The hydrocarbon-contaminated soil harbors a distinct fungal community, evidenced by the successful isolation and identification of *Aspergillus flavus, Aspergillus niger, Penicillium notatum,* and *Fusarium oxysporum.*

Among the isolated fungi, *Aspergillus flavus* and *Fusarium oxysporum* were identified as promising biosurfactant producers, demonstrating significant surface and emulsifying activities across multiple screening assays.

Both *Aspergillus flavus* and *Fusarium oxysporum* can utilize diesel as a sole carbon source to produce biosurfactants in a mineral salt medium, confirming their potential role in the bioremediation of diesel-contaminated soils.

Based on the findings of this study, the following recommendations were made:

To significantly enhance biosurfactant yield from *Aspergillus flavus* and *Fusarium oxysporum*, a systematic optimization of key cultivation parameters is recommended. This should focus on determining the optimal concentrations of the diesel amendment and nitrogen source to establish the ideal carbon-to-nitrogen (C/N) ratio, alongside the effects of initial pH, temperature, and fermentation duration.

Furthermore, for a long-term strategy, the genetic engineering potential of these potent isolates should be explored. Molecular techniques, such as targeted gene editing (e.g., CRISPR-Cas9), could be employed to overexpress key genes in the biosurfactant synthesis pathway or to disrupt regulatory genes that limit production, potentially leading to the development of superior, high-yielding fungal strains.

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