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BIODEGRADATION OF DIESEL OIL BY INDIGENOUS BACTERIAL SPECIES ISOLATED FROM CONTAMINATED SOIL

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Abstract
This study investigated the biodegradation potential of indigenous bacterial species isolated from diesel-contaminated soils. Soil samples were collected from hydrocarbon-polluted sites in Sokoto State, Nigeria, and analysed for hydrocarbon-utilizing bacteria using enrichment culture techniques. Five bacterial species *Rhodococcus*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, and *Staphylococcus aureus* were isolated and identified through morphological and biochemical characterization. These isolates were evaluated in a soil microcosm experiment. Fourier Transform Infrared (FTIR) analysis showed significant reduction and transformation of hydrocarbon functional groups including alkenyl (C=C) stretching and cycloalkane (C–C) vibrations, alongside the emergence of hydroxyl (O–H) and methylene (C–H) groups after 14 days of incubation, indicating active biodegradation. The mixed consortium showed the most pronounced spectral changes, followed by *Rhodococcus* sp. and *Bacillus subtilis*, while the control showed minimal changes. These findings indicate that biodegradation efficiency was highest in the consortium based on FTIR spectral transformations, highlighting the importance of synergistic microbial interactions in the bioremediation of diesel-contaminated soils. The findings demonstrate the effectiveness of indigenous bacteria in bioremediation and highlight the enhanced performance of microbial consortia.
Keywords: Biodegradation, Diesel oil, Indigenous bacteria, Bioremediation, FTIR, Hydrocarbon degradation

INTRODUCTION

Diesel oil contamination is a major environmental concern due to its complex composition of aliphatic and aromatic hydrocarbons, many of which are toxic, mutagenic, and persistent in the environment (Kaur *et al*., 2018; Hewelke *et al*., 2018). Increased industrialization and transportation activities have led to frequent oil spills, resulting in severe soil and water pollution (Chaudhary and Kim, 2019).

Bioremediation has emerged as an environmentally friendly and cost-effective approach for the remediation of hydrocarbon-contaminated environments (Das and Chandran, 2011). Indigenous microorganisms play a crucial role in this process due to their ability to utilize hydrocarbons as a carbon and energy source (Mnif *et al*., 2015). Bacterial genera such as *Pseudomonas*, *Bacillus*, and *Rhodococcus* have been widely reported for their hydrocarbon-degrading capabilities (Yergeau *et al*., 2012; López-Echartea *et al*., 2021).

The efficiency of biodegradation depends on environmental factors such as nutrient availability, pH, temperature, and microbial population dynamics (Sutton *et al*., 2013). Furthermore, microbial consortia have been shown to outperform single isolates due to synergistic metabolic interactions (Bacosa *et al*., 2018). This study aims to isolate, identify, and evaluate the biodegradation potential of indigenous bacterial species from diesel-contaminated soils using microcosm experiments and FTIR analysis.

MATERIALS AND METHODS

Study Area and Sample Collection

Soil samples were collected from diesel-contaminated site at Road, Sokoto State, Nigeria (Latitude 13.0277921N, Longitude 5.2145076E). Control samples were obtained from an uncontaminated site. Samples were collected at a depth of 5cm.

Isolation of Hydrocarbon-Utilizing Bacteria

Serial dilution and spread plate techniques were used to isolate bacteria on Luria-Bertani agar following standard microbiological procedures (Cheesbrough, 2000). Hydrocarbon-utilizing bacteria were selected using mineral salt medium (MSM) supplemented with diesel as the sole carbon source (Atlas and Bartha, 1992).

Identification of Isolates

Bacterial isolates were identified based on morphological characteristics, Gram staining, and biochemical tests including catalase, citrate, indole, methyl red (MR), Voges–Proskauer (VP), urease, and triple sugar iron (TSI) tests, following standard methods (Cheesbrough, 2000; Oyeleke and Manga, 2008).

Biodegradation Screening

The biodegradation potential of isolates was assessed based on their growth in MSM containing diesel oil as the sole carbon source, as described by (Nwinyi *et al*., 2016).

Soil Microcosm Experiment

Sterile soil microcosms (20 g) were prepared and inoculated with selected isolates (*Rhodococcus* sp. and *Bacillus subtilis*). Diesel oil (2 ml) was added, and samples were incubated at 35°C for 14 days following standard microcosm procedures (Agarry and Latinwo, 2015).

FTIR Analysis

FTIR spectroscopy was used to determine changes in hydrocarbon functional groups before and after

biodegradation, following methods described by (Wang *et al.*, 2020).

RESULTS

Isolation and Enumeration of Hydrocarbon-Utilizing Bacteria (HUB)

Table 1 shows the total viable count of hydrocarbon-utilizing bacteria (HUB) isolated from the sampled soils. The results indicate that diesel-contaminated samples recorded higher bacterial counts, ranging from 10^3 to 10^4 CFU/g, compared to the control samples which ranged from 10^2 to 10^3 CFU/g. The highest count was observed in sample B3, while the lowest was recorded in control sample D3. This suggests that hydrocarbon contamination enhances the proliferation of specialized degrading bacteria.

Identification of Bacterial Isolates

Table 2 shows the morphological characteristics of the bacterial isolates obtained from the study area. The isolates exhibited diverse colony morphologies, including circular and irregular shapes with creamy to whitish pigmentation. Most colonies were smooth or rough in texture with entire or undulate margins. Microscopic examination revealed that the majority of isolates were rod-shaped, while *Staphylococcus aureus* appeared as cocci in clusters. All isolates were Gram-positive.

Table 3 shows the biochemical characteristics of the bacterial isolates.

All isolates tested positive for catalase, indicating aerobic metabolism. Variations were observed in citrate utilization, urease activity, and methyl red reactions, which aided in differentiating the isolates. Indole production was negative across all isolates. These biochemical profiles were used to identify the organisms as *Rhodococcus* sp., *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, and *Staphylococcus aureus*.

Screening for Diesel Degradation Potential

Figure 1 shows the screening of bacterial isolates for diesel degradation potential.

All isolates demonstrated growth in mineral salt medium supplemented with diesel oil, confirming their ability to utilize hydrocarbons as a carbon source. However, *Rhodococcus* sp. and *Bacillus subtilis* showed significantly higher growth compared to other isolates, indicating superior degradation potential.

FTIR Analysis

Figure 2A shows the FTIR spectrum of diesel-contaminated soil inoculated with *Bacillus subtilis* before incubation. The spectrum revealed characteristic hydrocarbon peaks, indicating the presence of complex compounds.

Figure 2B shows the FTIR spectrum of diesel-contaminated soil inoculated with *Bacillus subtilis* after 14days of incubation. Changes in peak intensity and the appearance of new peaks were observed.

Figure 3A shows the FTIR spectrum of diesel-contaminated soil inoculated with *Rhodococcus* sp. before incubation. The spectrum showed peaks associated with inorganic and organic functional groups.

Figure 3B shows the FTIR spectrum of diesel-contaminated soil inoculated with *Rhodococcus* sp. after 14days of incubation. New peaks and shifts in functional groups were observed.

Figure 4A shows the FTIR spectrum of diesel-contaminated soil inoculated with mix consortium before incubation. The spectrum revealed peaks corresponding to hydrocarbon structures.

Figure 4B shows the FTIR spectrum of diesel-contaminated soil inoculated with mix consortium after 14days of incubation. Noticeable spectral changes were observed.

Figure 5A shows the FTIR spectrum of diesel-contaminated soil without inoculum before incubation. The spectrum showed peaks corresponding to silicate and hydrocarbon groups.

Figure 5B shows the FTIR spectrum of diesel-contaminated soil without inoculum after 14days of incubation. Only slight variations in peak positions were observed.

Table 4A shows the peak assignments before incubation, where functional groups such as: Cyclohexane ring vibrations (1032.5 cm^{-1}), Primary amine N–H bending (1638.2 cm^{-1}) and Aliphatic C–F stretching were identified, confirming the presence of diverse hydrocarbon components.

Table 4B shows the peak assignments after incubation, which include: Vinyl C–H bending (911.3 cm^{-1}), Methylene C–H bending (1455.5 cm^{-1}), Alkenyl C=C stretching (1634.4 cm^{-1}), Methylene C–H stretching (2847.7 cm^{-1}) and Hydroxyl (O–H) stretching (3319.2 cm^{-1}). These changes indicate transformation of hydrocarbon compounds during incubation.

Table 5A shows the peak assignments before incubation, including: Silicate ion (911.3 cm^{-1}), Phosphate ion (1004.3 cm^{-1}) and Aliphatic phosphate (1026.9 cm^{-1}).

Table 5B shows the peak assignments after incubation, including: Alkenyl C–H bending (678.4 cm^{-1}), Vinyl C–H bending (911.3 cm^{-1}), Primary amine C–N stretching (1023.2 cm^{-1}), Methoxy C–H stretching (2847.7 cm^{-1}) and Hydroxyl (O–H) stretching (3345.3–3626.7 cm^{-1}).

Table 6A shows the peak assignments before incubation, including: Cyclohexane ring vibrations (1000.8 cm^{-1}) and Alkenyl C=C stretching (1638.2 cm^{-1})

Table 6B shows the peak assignments after incubation, including: Vinyl C–H bending (911.3 cm^{-1}), Cyclohexane ring vibrations (997.1 cm^{-1}), Amide group formation (1634.4 cm^{-1}) and Hydroxyl (O–H) stretching (3298.7 cm^{-1}).

Table 7A shows the peak assignments before incubation, including: Silicate ion (907.6 cm^{-1}) and Alkenyl C=C stretching (1634.4 cm^{-1}).

Table 7B shows the peak assignments after incubation, including: Methylene rocking (747.3 cm^{-1}), Skeletal C–C vibrations (907.6 cm^{-1}), Cyclohexane ring vibrations (1000.8 cm^{-1}) and Aliphatic phosphate (1032.5 cm^{-1}).

Table1: Total Viable Count of Hydrocarbon-Utilizing Bacteria (HUB)

Sample Code	Colony Count (CFU/g)
A1	4.5×10^3
A2	5.2×10^3
A3	4.8×10^3
B1	3.0×10^4
B2	2.8×10^4
B3	3.5×10^4
C1	4.0×10^3
C2	3.8×10^3
C3	4.2×10^3
D1 (Control)	1.2×10^3
D2 (Control)	1.0×10^3
D3 (Control)	9.0×10^2

Table 2 Morphological Characteristics of Hydrocarbon-Utilizing Bacterial Isolates

Isolate Code	Presumed Organism	Colony Shape	Colony Colour	Elevation	Margin	Surface Texture	Cell Shape	Gram Reaction
A1	*Rhodococcus* sp.	Circular	Creamy-white	Convex	Entire	Smooth	Rod	Gram-positive
A2	*Bacillus megetarium*	Circular	Off-white	Flat	Irregular	Rough	Rod	Gram-positive
B1	*Bacillus subtilis*	Irregular	Creamy	Raised	Undulate	Rough	Rod	Gram-positive
B2	*Bacillus subtilis*	Irregular	Creamy	Raised	Undulate	Rough	Rod	Gram-positive
C1	*Staphylococcus aureus*	Circular	Golden-yellow	Convex	Entire	Smooth	Cocci	Gram-positive
C2	*Bacillus cereus*	Irregular	Whitish	Flat	Lobate	Rough	Rod	Gram-positive
D1	*Rhodococcus* sp.	Circular	Creamy-white	Convex	Entire	Smooth	Rod	Gram-positive
D2	*Staphylococcus aureus*	Circular	Yellow	Convex	Entire	Smooth	Cocci	Gram-posit

Table 3 Biochemical Characteristics of Hydrocarbon-Utilizing Bacterial Isolates

Isolale Code	A1	A2	B1	B2	C1	C2	D1	D2
Catalase	+	+	+	+	+	+	+	+
Cougulase	+	+	+	+	+	+	+	+
Indole	–	–	–	–	–	–	–	–
Citrate	–	–	+	+	–	+	–	–
Motility	–	+	+	+	–	+	–	–
MR	+	–	–	–	+	–	+	+
VP	–	+	+	+	–	+	–	–
Urease	+	+	–	–	–	–	+	–
TSI gas	+	+	+	+	+	+	+	+
H2S	–	–	–	–	–	–	–	–
Glucose	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+
Isolate	*Rhodococcus*	*Bacillus megterium*	*Bacillus Subtillis*	*Bacillus Subtillis*	*S. Aureus*	*Bacillus cereus*	*Rhodococcus*	*S.Aureus*

Figure 1: Screened bacterial isolates on mineral salt medium supplemented with diesel oil.

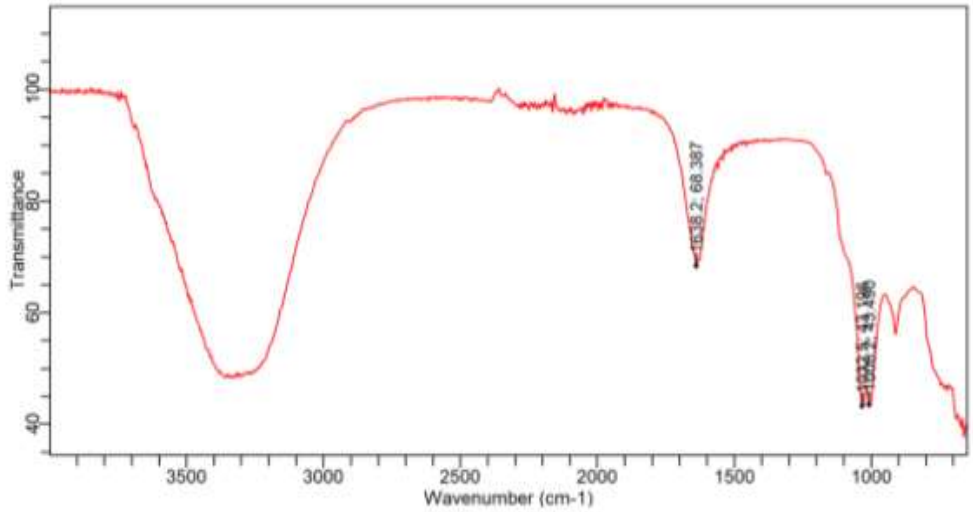

Figure 2A: FTIR spectral profile of microcosm study excited with *Bacillus Subtillis* before incubation.

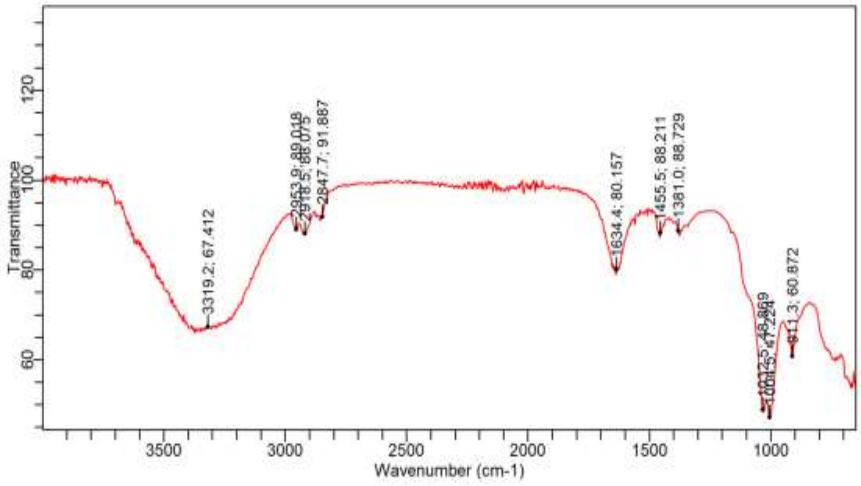

Figure 2B: FTIR spectral profile of microcosm study excited with *Bacillus Subtillis* after 14 days of incubation.

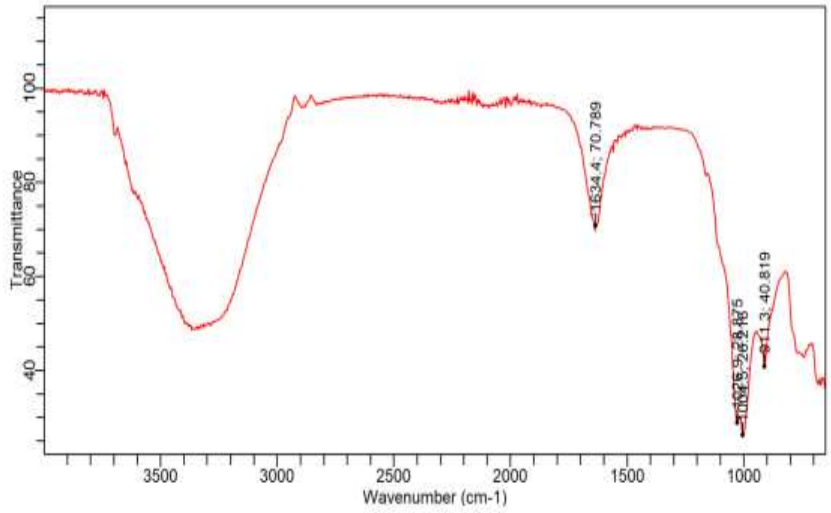

Figure 3A: FTIR spectral profile of microcosm study excited with *Rhodococcus* sp. before incubation.

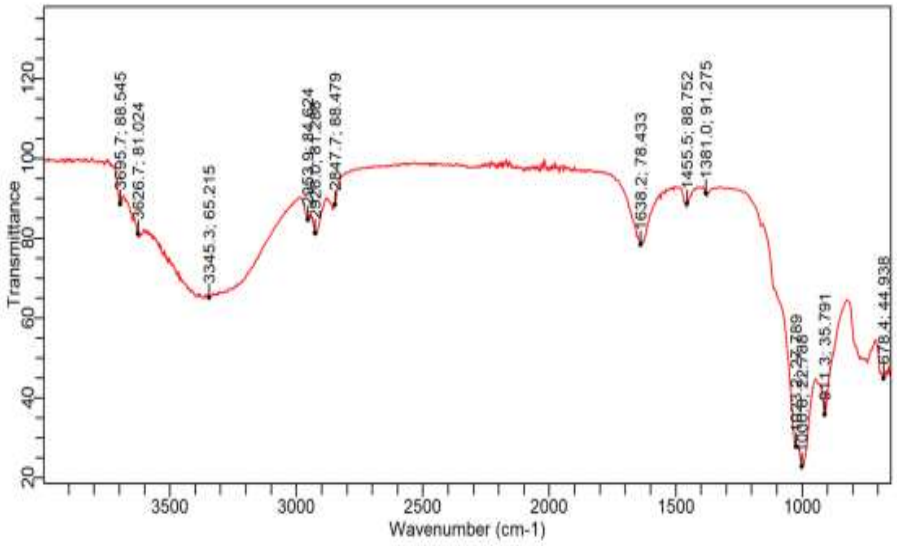

Figure 3B: FTIR spectral profile of microcosm study excited with *Rhodococcus* sp. after 14 days of incubation.

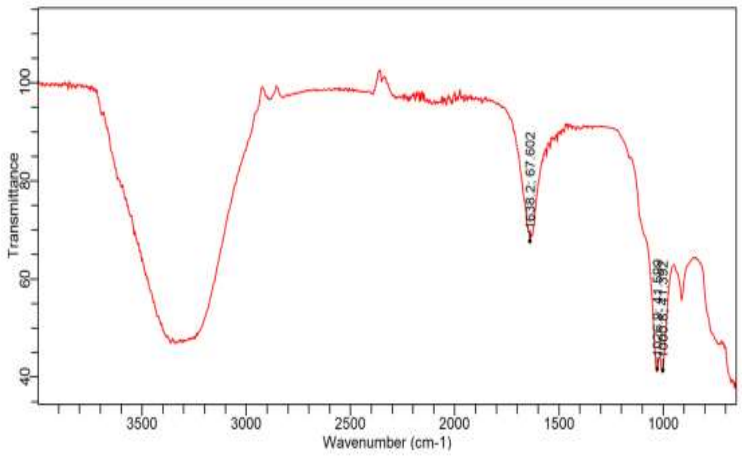

Figure 4A: FTIR spectral profile of microcosm study excited with mix consortium before incubation.

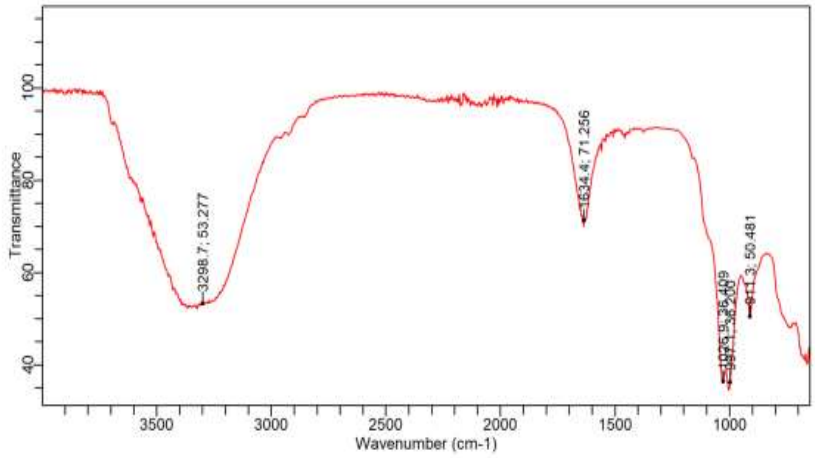

Figure 4B: FTIR spectral profile of microcosm study excited with mix consortium after 14 days of incubation.

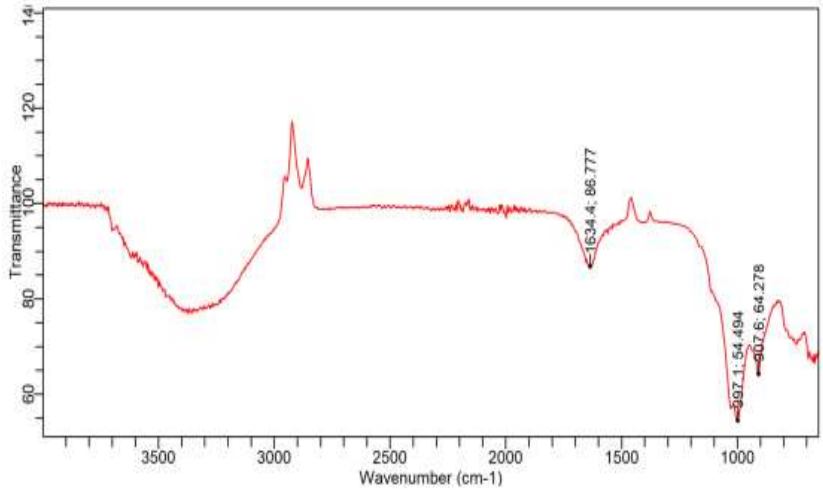

Figure 5A: FTIR spectral profile of microcosm study without inoculum before incubation.

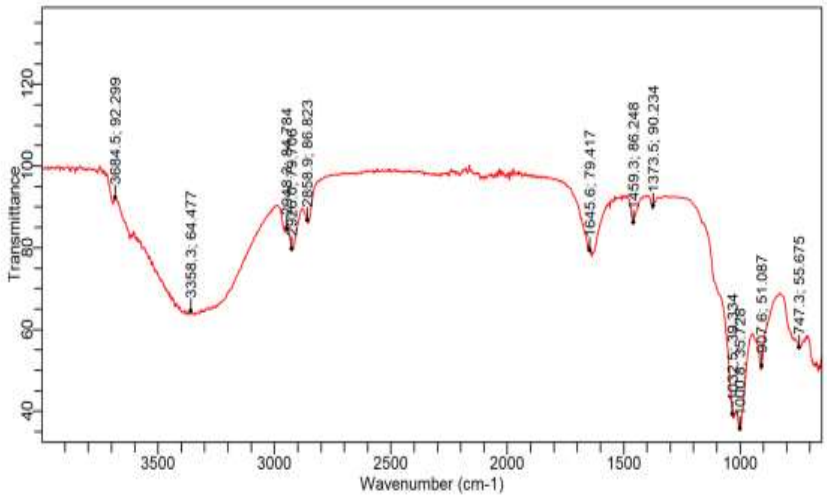

Figure 5B: Figure 5A: FTIR spectral profile of microcosm study without inoculum after 14 days of incubation.

Table 4A: Peak assessment in respect to their functional group excited with *Bacillus Subtillis* before incubation

WAVE NUMBER	PEAK	FUNCTIONAL GROUP
1055 _ 1000	1032.5	Cyclohexane ring vibrations
1680 _ 1620	1638.2	Primary amine N-H bend
1150 _ 1000	1008.2	Aliphatic fluoro compounds, C - F stretch

Table 4B: Peak assessment in respect to their functional group excited with *Bacillus Subtillis* after 14 days of incubation

WAVE NUMBER	PEAK	FUNCTIONAL GROUP
915 _ 890	911.3	Vinyl C-H out of plane bend
1055 _ 1000	1032.5	Cyclohexane ring vibrations
1385 _ 1380	1381.0	Gem Dimethyl or "iso"_ (doublet)
1485 _ 1445	1455.5	Methylene C-H bend
1680 _ 1620	1634.4	Alkenyl C=C stretch
2865 _ 2845	2847.7	Methylene C-H asym./sym. Stretch
3400 _ 3200	3319.2	Normal "polymeric" OH stretch

Table 5A: Peak assessment in respect to their functional group excited with *Rhodococcus* sp. before incubation

WAVE NUMBER	PEAK	FUNCTIONAL GROUP
1100 _ 900	911.3	Silicate ion
1100 _ 1000	1004.3	Phosphate ion
1050 _ 990	1026.9	Aliphatic phosphates (P-O-C stretch)

Table 5B: Peak assessment in respect to their functional group excited with *Rhodococcus* sp. after 14 days of incubation

WAVE NUMBER	PEAK	FUNCTIONAL GROUP
680 _ 610	678.4	Alkenyl C-H bend
915 _ 890	911.3	Vinyl C-H out of plane bend
1090 _ 1020	1023.2	Primary amine CN stretch
1690 _ 1590	1638.2	Open chain imino C-C=N-
2820 _ 2810	2847.7	Methoxy, C-H stretch (CH3-O-
3400 _ 3200	3345.3	Normal polymeric OH stretch
3620 _ 3540	3626.7	Secondary alcohol, OH stretch

Table 6A: peak assessment in respect to their functional group excited with mixed consortium before incubation

WAVE NUMBER	PEAK	FUNCTIONAL GROUP
1050 _ 1000	1000.8	Cyclohexane ring vibrations
1680 _ 1620	1638.2	Alkenyl C=C stretch

Table 6B: Peak assessment in respect to their functional group excited with mix consortium after 14 days of incubation

WAVE NUMBER	PEAK	FUNCTIONAL GROUP
915 _ 890	911.3	Vinyl C-H out of plane bend
1055 _ 1000	997.1	Cyclohexane ring vibrations
1680 _ 1630	1634.4	Amide
3400 _ 3200	3298.7	Normal polymeric OH stretch

Table 7A: Peak assessment in respect to their functional group without inoculum before incubation

WAVE NUMBER	PEAK	FUNCTIONAL GROUP
1100 _ 900	907.6	Silicate ion
1680 _ 1620	1634.4	Alkenyl C=C stretch

Table 7B: Peak assessment in respect to their functional group without inoculum after 14 days of incubation

WAVE NUMBER	PEAK	FCTIONAL GROUP
750 _ 720	747.3	Methylene_(CH2)n rocking (n>3)
1300 _ 700	907.6	Skeletal C-C vibrations
1055 _ 1000	1000.8	Cyclohexane ring vibrations
1050 _ 990	1032.5	Aliphatic phosphates (P-O-C stretch)

DISCUSSION

The results of this study demonstrated that diesel-contaminated soils harbored a higher population of hydrocarbon-utilizing bacteria compared to the control samples. This indicates that hydrocarbon pollution enhances the growth and adaptation of specialized microbial communities capable of utilizing petroleum hydrocarbons as a carbon source. Similar findings were reported by Das and Chandran (2011), who observed that contaminated environments selectively enrich hydrocarbon-degrading microorganisms. This implies that polluted soils naturally develop microbial populations suitable for bioremediation.

The identification of bacterial isolates such as *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Rhodococcus sp.*, and *Staphylococcus aureus* indicates the presence of diverse microbial species with varying metabolic capabilities. The predominance of *Bacillus* and *Rhodococcus* species suggests their adaptability to hydrocarbon-contaminated environments. This observation agrees with López-Echartea et al. (2021) and Mnif et al. (2015), who reported that these genera possess enzymatic systems capable of degrading complex hydrocarbons. This implies that these organisms play a significant role in the natural attenuation of petroleum pollutants. The screening results showed that *Rhodococcus* sp. and *Bacillus subtilis* exhibited higher growth in diesel-supplemented media compared to other isolates. This indicates their superior ability to utilize hydrocarbons as an energy source. Similar observations were made by Mnif et al. (2015), who reported enhanced hydrocarbon degradation by these species due to their production of biosurfactants and degradative enzymes. This implies that these isolates are promising candidates for bioremediation applications.

The FTIR results for *Bacillus subtilis* showed that before incubation, the spectrum was characterized by peaks corresponding to cyclohexane ring vibrations, primary amine groups, and aliphatic compounds, indicating the presence of complex hydrocarbons. After 14 days of incubation, new peaks such as vinyl C–H bending, methylene C–H stretching, alkenyl C=C stretching, and hydroxyl (O–H) groups were observed, along with changes in peak intensity. This indicates that *Bacillus subtilis* transformed complex hydrocarbons into simpler compounds. This finding is consistent with Mnif et al. (2015) and Das and Chandran (2011), who reported that *Bacillus* species degrade hydrocarbons through enzymatic activity and biosurfactant production, enhancing hydrocarbon breakdown. This implies that *Bacillus subtilis* is an effective hydrocarbon degrader and can be utilized in bioremediation of diesel-contaminated soils.

The FTIR spectral profile for *Rhodococcus* before incubation showed peaks associated with silicate and phosphate groups. After incubation, new peaks such as alkenyl C–H bending, primary amine C–N stretching, methoxy groups, and hydroxyl groups were observed. These changes indicate degradation and transformation of hydrocarbon compounds. This agrees with López-Echartea et al. (2021), who reported that *Rhodococcus* species possess diverse metabolic pathways enabling degradation of complex hydrocarbons. Wang et al. (2018) also reported similar FTIR changes during microbial degradation. This

implies that *Rhodococcus* sp. has strong capability to degrade recalcitrant hydrocarbons and is highly suitable for bioremediation.

The FTIR spectra of the mixed consortium before incubation showed hydrocarbon-related peaks, while after incubation, new functional groups such as vinyl C–H, amide, and hydroxyl groups appeared, along with more pronounced spectral changes compared to individual isolates. This indicates enhanced degradation of hydrocarbons. This observation is supported by Bacosa et al. (2018) and Sutton et al. (2013), who reported that microbial consortia improve biodegradation efficiency through synergistic interactions and complementary metabolic activities. This implies that microbial consortia are more effective than single isolates and should be preferred for large-scale bioremediation applications.

The FTIR spectra of the control sample showed minimal changes before and after incubation, with only slight variations in functional groups such as methylene rocking and skeletal vibrations. This indicates negligible degradation in the absence of microbial inoculation. This finding is consistent with Agarry and Latinwo (2015), who reported that natural attenuation without microbial enhancement is slow and limited. This implies that microbial activity is essential for effective biodegradation of hydrocarbons.

Overall, the results indicate that biodegradation was most effective in the mixed consortium, followed by *Rhodococcus* sp. and *Bacillus subtilis*, while the control showed minimal changes. This demonstrates that microbial interaction enhances degradation efficiency and supports the application of combined bacterial systems in environmental bioremediation.

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