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Effect of Cornstover Char and Indigeneous Bacteria on the Physiochemical Properties and Hydrocarbon Degradation of Crude Oil - Contaminated soil

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ABSTRACT: Bioremediation offers a sustainable approach to addressing crude oil contamination in soil, leveraging microbial activity and nutrient amendments to enhance hydrocarbon degradation. Hence, the objective of this paper is to evaluate the effects of Cornstover char and indigenous Bacteria on the physiochemical properties and hydrocarbon degradation in crude oil-contaminated soil using appropriate standard procedures. Results revealed significant increases in pH, notably from 9.3 to 11.85 in the SCCC setup, while setups like SCCS showed a transient spike to 12.15 by Week 4 before stabilizing at 11.3. Electrical conductivity decreased significantly across most setups, such as SC, which dropped from 470 to 109.5 µS/cm, indicative of hydrocarbon breakdown and nutrient utilization. The SCCC and SCCN setups achieved the highest TPH degradation rates, 93.99% and 94.60%, respectively, while natural attenuation in SC showed a limited reduction of 49.28%. PAH degradation followed a similar trend, with SCCC and SCCN setups achieving 74.71% and 91.47% degradation, respectively. This study underscores the synergistic effects of microbial augmentation and nutrient supplementation in optimizing hydrocarbon degradation. The findings provide valuable insights for developing scalable, site-specific bioremediation strategies. Further exploration of long-term soil health and microbial dynamics is recommended to enhance bioremediation practices.

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Crude oil contamination represents a persistent environmental challenge, particularly in regions heavily dependent on petroleum exploration and production activities. The release of crude oil into terrestrial ecosystems results in severe soil degradation, leading to the loss of soil fertility and the disruption of microbial communities essential for ecological balance. This contamination also poses serious threats to human health and biodiversity by infiltrating water sources and bioaccumulating in food chains (Ali *et al*., 2020). While traditional remediation methods, such as soil excavation, chemical treatments, and incineration, have been employed to address oil contamination, these techniques are often prohibitively expensive and environmentally invasive, prompting a shift towards sustainable alternatives. Bioremediation has emerged as a promising eco-friendly solution for the restoration of crude oil-contaminated soils. This process exploits the metabolic activities of

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microorganisms to transform harmful hydrocarbons into non-toxic compounds such as carbon dioxide, water, and biomass. Indigenous bacteria, naturally present in contaminated environments, are particularly advantageous in bioremediation because they are already acclimated to site-specific conditions and possess enzymatic pathways for hydrocarbon degradation. Studies have shown that these native microbial populations can significantly reduce hydrocarbon concentrations while improving soil health, making them a critical component in sustainable remediation strategies (Ali *et al*., 2020).

In addition to microbial approaches, biochar has gained recognition as a complementary agent in bioremediation. Biochar, a carbon-rich material produced through the thermal decomposition of organic biomass under low oxygen conditions, has unique physicochemical properties that make it an effective remediation tool. Its high porosity and large surface area enable the adsorption of petroleum hydrocarbons, thereby reducing their mobility and bioavailability. Furthermore, biochar can improve soil properties by enhancing water retention, nutrient availability, and pH balance, which are essential for optimal microbial activity. Research highlights the synergistic effects of combining biochar with microbial agents, noting that such integrations not only enhance hydrocarbon degradation but also promote long-term soil recovery (Song *et al*., 2024). Cornstover char, a type of biochar derived from the pyrolysis of corn residues, is particularly promising due to its availability and sustainability. Utilizing agricultural waste such as cornstover for biochar production addresses waste management challenges while offering a renewable resource for environmental remediation. Cornstover char has demonstrated strong adsorption capacities for hydrocarbons and can create conducive environments for microbial proliferation, further supporting the degradation process. Its application aligns with global sustainability goals, contributing to carbon sequestration and the mitigation of greenhouse gas emissions (Song *et al*., 2024). Hence, the objective of this paper is to evaluate the effects of Cornstover char and indigenous on the physiochemical properties and hydrocarbon degradation in crude oil-contaminated soil

MATERIALS AND METHODS

Collection of uncontaniated Soil, and Corn stover char: Pristine soil samples (uncontaminated soil sample) was collected from a virgin farm land in Egini (attitudes 5º 28’ 00” N; longitudes 5º50’ 00”), located in Udu Local Government Area, Delta State, southern Nigeria. While hydrocarbon Contanminated Soil Sample was collected at 1m depth using a soil auger from hydrocarbon consternated soils obtained from a mechanic workshop located along refinery Road, Effurum, Uvwie L.G.A, Delta State, N 5^{o} ‘0347.3722’ E 5^{0} 46’28.61544’. The collected soil samples were carefully packaged in a sterilized bag to avoid contamination; and were immediately transported to the laboratory for analyses.

Corn Stover Char was collected from a local corn seller who sells corn in Ugborikoko market (Latitude N5^{o} 32’ 28. 11552, Longitude E 5^{o} 45’49.33 764”), Upon collection of the cornstover from the locals, it was crushed and air dried. Once dried, the cornstover were subjected to charring to convert them into charcoal form. A muffle burner operating at temperature ranging from 700℃ to 1000℃ was utilized for this purpose. The grinded cornstover char was kept in an air-tight container until when required for use. NPK (15:15:15) fetilizer was purchased from a trader who sells agriculture products in Ogborikoko market.

Standard methods (APHA, 1998; USEPA, 1996) were employed to assess the physical and chemical properties of the samples, including pH, electric conductivity, total nitrogen, phosphorous, total petroluem hydrocarbon (TPH), Polycyclic Aromatic hydrocarbon (PAH), Organic carbon content, moisture content, total heterotrophic bacteria (THB), and hydrocarbon-utilizing bacteria (HUB).

Isolation and Purification of Microorganisms: Bacteria capable of degrading hydrocarbon were isolated from hydrocabon contimnated soil using the enrichment and streak methods (Siddique *et al*., 2003; Avishai and Charles, 2014; Ibezute *et al*., 2024). One gram of soil from hydrocarbon contaminated sample was aseptically suspended in 10 ml of distilled water and mixed well for 15 minutes and vortexed. The suspension was aseptically inoculated into mineral salt medium (MSM) broth, which was supplemented with the combination of petrol, disel and engine oil in equal ratio as the sole carbon source, and incubated at 37°C for 7 days in a rotary shaker at 120 rpm (Atlas, 1981; Fulekar *et al.*, 2017; Ibezute *et al*., 2024).

This process was repeated through successive subcultures in fresh MSM to progressively enrich hydrocarbon-degrading bacteria (Cerniglia, 1992). After enrichment, the culture was inoculated onto MSM agar plates containing a thin layer of spent engine oil, and the appearance of clear zones indicated bacterial hydrocarbon degradation. The isolates were purified using the streak plate method on nutrient agar, incubated at 37°C for 48 hours, and

well-isolated colonies were selected and transferred to fresh plates (Baron and Finegold, 1990). The pure isolates were preserved on nutrient agar slants for long-term storage (Cappuccino and Sherman, 2008; Ibezute *et al*., 2024).

Morphological, Biochemical Characterization of Microorganisms and Inoculum Development: Bacterial isolates obtained from nutrient agar were initially identified through standard morphological, microscopic (Gram staining), and biochemical tests, following the methods outlined in Bergey's Manual of Determinative Bacteriology (Zhou *et al*., 2008; Ibezute *et al*., 2024). For inoculum development, a loopful of each selected colony was inoculated into 5 mL of sterile 0.1% NaCl solution and centrifuged at 4,000 rpm to pellet the bacterial cells. The pellets were washed twice with sterile distilled water to remove impurities, and the final suspension was adjusted to an absorbance of 0.1 at 600 nm using a UV-Vis spectrophotometer to standardize the bacterial concentration before inoculation (Ekundayo *et al*., 2012; Al-Wasify; El_Naas, 2015; Ibezute *et al*., 2024).

Experimental setup: The contaminated soil was divided into groups and setup as described in table 1. In each setup, the mixture was left undisturbed for two days to allow the volatilization of the oil's toxic components, upon which the moisture content of the mixture was adjusted by adding 200mL of distilled water. The adjusted mixture was incubated at room temperature, (28 ± 2 °C).

Table 1: Experimental Design

S/N	Code	Interpretation
1	SC	1kg Soil + 50ml Crude Oil – Control (Natural attenuation)
2	SCCT	1kg Soil + 50ml Crude Oil + 100ml bacteria consortium – (Bioaugumentation)
3	SCCS	1kg Soil + 50ml Crude Oil + 25g corn stover char – (Biostimulation)
4	SCN	1kg Soil + 50ml Crude Oil + 25g NPK – (Biostimulation)
5	SCCC	1kg Soil + 50ml Crude Oil + 100ml bacteria consortium + 25g corn stover char – (Biostimulation and Bioaugumentation)
6	SCCN	1kg Soil + 50ml Crude Oil + 100ml bacteria consortium + 25g NPK - (Biostimulation and Bioaugumentation)

The content of each vessel was tilled twice a week for aeration and the moisture maintained by the addition of sterile distilled water. The experiment was set up in triplicate. Biodegradation of TPH and remediation of spent engine oil contaminated soil was monitored at four-week interval (0, 4 and 8 weeks) by analysing the following parameters; pH, electric conductivity, total nitrogen, phosphorous, total petroluem hydrocarbon (TPH), Polycyclic Aromatic hydrocarbon (PAH), Organic carbon content, moisture content, total heterotrophic bacteria (THB), and hydrocarbon-utilizing bacteria (HUB). Composite samples were obtained by mixing 5g of soil collected from four different areas of the glass container for isolation and enumeration of hydrocarbon utilizing bacteria and determination of total petroleum hydrocarbon.

Data Analysis: All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data are presented as mean ± SE. One-way ANOVA was used to determine the differences among various groups. Percentage total petroleum hydrocarbon (TPH) loss was calculated using the formula

$$\%\, TPH\ Loss = \frac{TPH\ Initial - TPH\ Final}{TPH\ Initial} X \frac{100}{1} \quad (1))$$

RESULT AND DISCUSSION

Physicochemical characteristics: The physical and chemical characteristics of dumpsite leachate, soil and water hyacinth are shown in Table 2. The leachate is characterized by its alkalinity (pH of 8.01), high nitrate (55.82 ± 12.31 mg/kg) and moisture content. The soil was more acidic (pH of 6.12), with moderate phosphate (21.8 ± 1.5 mg/kg), low moisture, and a diverse bacterial community. The water hyacinth is slightly acidic (pH of 6.54), with high nitrate (1550.5 ± 0.03 mg/kg), high phosphate (2783.36 ± 1.80 mg/kg), high moisture, and the lowest bacterial count. These findings provide insights into their ecological roles and potential impacts on surrounding ecosystems, underscoring the importance of understanding their chemical and biological dynamics in environmental studies and management practices.

Table 2. Baseline line results of the physiochemical and microbial parameters of the non-polluted soil and cornstover ash samples

S/N	Parameters	Non-contaminated soil	Corn Stover Ash
1	pH	6.70	10.90
2	Moisture (%)	17.50	11.50
3	Sulphur(mg/kg)	59.00	78.00
4	Phosphorus(mg/kg)	20.90	30.30
5	Nitrogen(ma/kg)	12.32	1.17
6	Organic Content(%)	2.61	3.27
7	Total petroleum hydrocarbon(ppm)	20.05	1.30
8	Total heterotrophic bacteria (cfu/g)	5.3×10^7	8.8×10^6
9	Hydrocarbon utilizing bacteria(cfu/g)	6.1×10^4	NIL

Isolation, Morphological and Biochemical characterization of microorganisms from hydrocarbon contaminated soil: After Isolation by the direct culture and enrichment technique(s), several distinct pure cultures each were obtained and identified by their morphological and biochemical test. However, some responded similarly displaying overwhelming similarity based on which 4 isolates were selected and reported in Table 3, A total of three identified isolates were gram negative with one gram positive. The gram positive isolates were *Myroides* sp, *Providencia* sp *and Proteus* sp; while the gram positive isolate was *Bacillius* Sp The isolates were predominantly rod shaped.

Table 3. Cultural, Morphological, Biochemical and Physiological Characteristics of Bacterial Isolates

Characteristics	**MWS1**	**MWS2**	**MWS3**	**MWS4**
Cell morphology	Rod	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Single
Gram reaction	Negative	Negative	Positive	Negative
Motility	+	-	-	+
Test for enzymes				
Catalase production	+	+	+	+
Spore formation	-	-	+	-
Oxidase	+	-	-	-
Coagulase	-	-	-	-
Citrate utilization	-	+	+	+
Indole	+	-	+	-
Nitrate reduction	-	-	+	+
Urease	-	+	-	+
Acid test	-	+	+	+
Sugar fermentation				
Lactose	-	+	-	-
Glucose	-	+	+	+
Galatose	-	+	+	-
Maltose	+	+	+	-
Mannitol	-	+	+	-
Probable Identity	*Myroides* sp	*Providencia* sp	*Bacillius* Sp	*Proteus* sp

The result of the physiochemical changes observed during bioremediation of crude oil polluted soil. The changes associated with pH, electric conductivity, nitrogen, potassium, phosphorous, soil organic matter, total TPH, total PAH, total hydrocarbon bacterial and total hydrocarbon utilizing bacterial is shown from Fig 1 to fig 12. pH increased during bioremediation in all setups. In SC setup, it increased from 8.6 to 9.6 over the 8-week period; SCCT the pH increased from 9.1 to 9.8 over 8 weeks; in SCN, it increased from 8.0 to 8.75 over 8 weeks; SCCS, there was a significant increase in pH from 8.1 to 12.15 at Week 4, followed by a decrease to 11.3 by Week 8; SCCN, pH fluctuated from 8.9 to 9.05 over 8 weeks; and in SCCC, there significant increase in pH from 9.3 to 11.85 over 8 weeks. In SC, Electrical Conductivity (EC) decreased from 470 to 109.5 over the 8-week period; SCCT, EC decreased from 312.5 to 66.83 over 8 weeks; in SCN, EC decreased from 400 to 111 over 8 weeks; in SCCS, EC decreased from 372.5 to 146.5 over 8 weeks; SCCN, EC increased from 376 to 320.5 and then decreased to 139.5 over 8 weeks; in SCCC, EC increased from 389.5 to 292.5 and then increased further to 282 over 8 weeks.

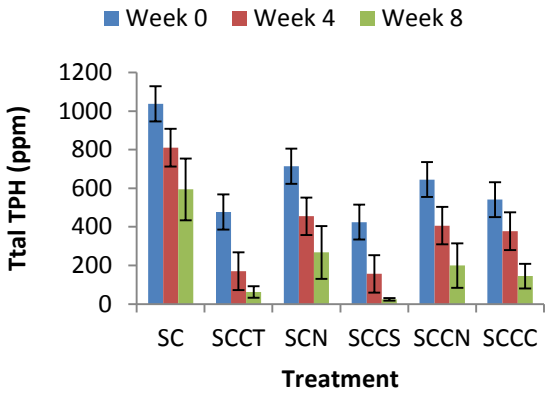

Fig 1: Effects of cornstover char and indigeneous bacteria in the total petroleum hydrocarbon (TPH) concentration during bioremediation of hydrocarbon contaminated soil

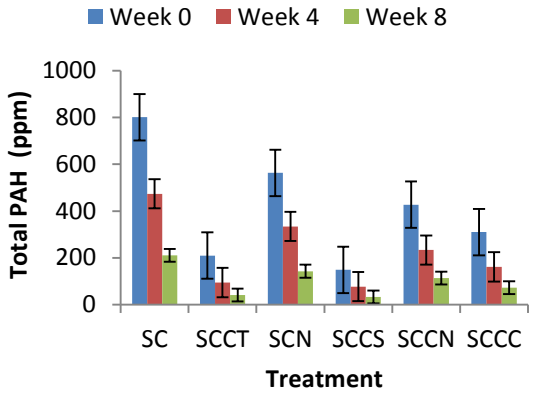

Fig 2: Effects of cornstover char and indigeneous bacteria in the total polyaromatic hydrocarbon (PAH) concentration during bioremediation of hydrocarbon contaminated soil

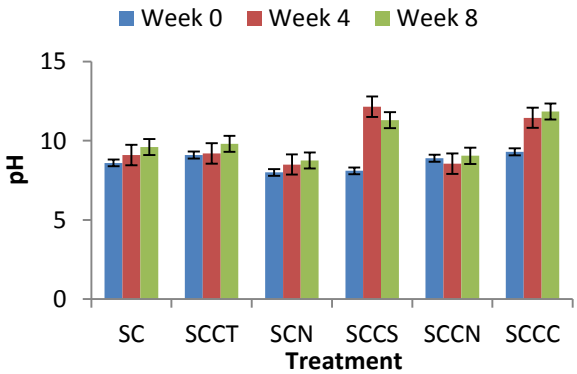

Fig 3: Effects of cornstover char and indigeneous bacteria in the pH during bioremediation of hydrocarbon contaminated soil

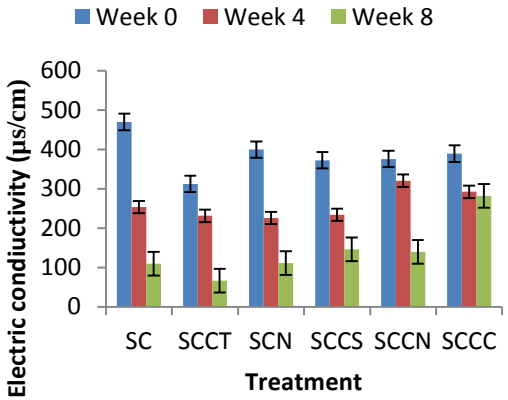

Fig 4: Effects of cornstover char and indigeneous bacteria in the electric conductivity during bioremediation of hydrocarbon contaminated soil

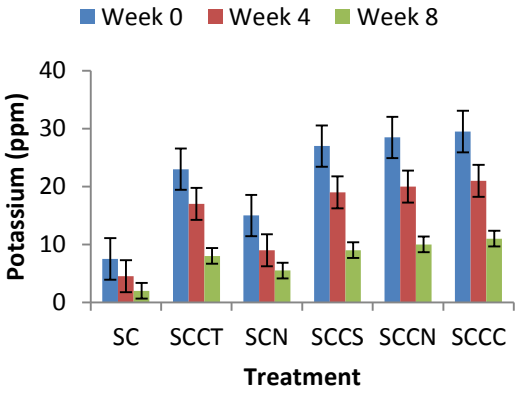

Fig 7: Effects of cornstover char and indigeneous bacteria in the potaqssium concentration during bioremediation of hydrocarbon contaminated soil

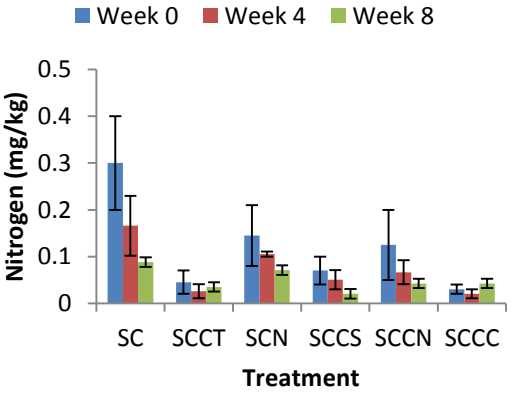

Fig 5: Effects of cornstover char and indigeneous bacteria in the nitrogen concentration during bioremediation of hydrocarbon contaminated soil

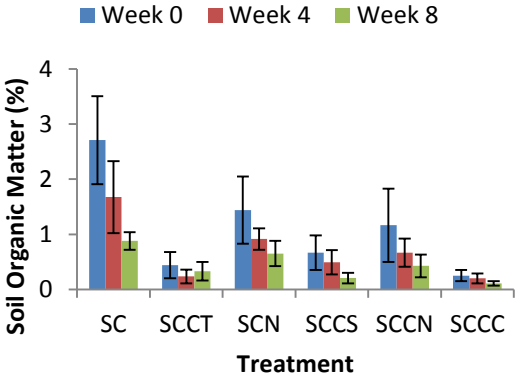

Fig 8: Effects of cornstover char and indigeneous bacteria in the soil organic matter during bioremediation of hydrocarbon contaminated soil

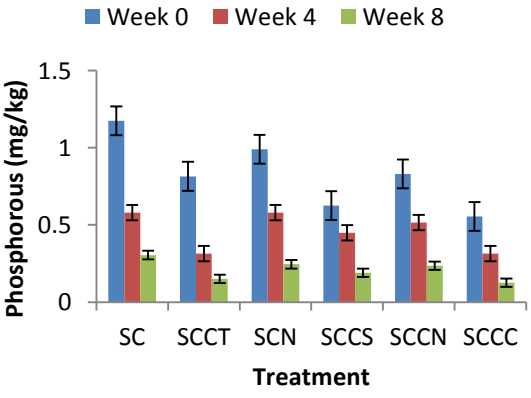

Fig 6: Effects of cornstover char and indigeneous bacteria in the phosphorous concentration during bioremediation of hydrocarbon contaminated soil

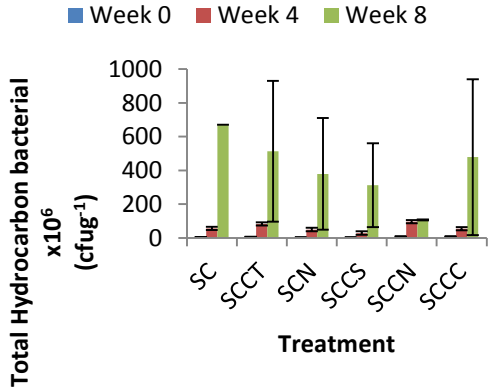

Fig 9: Effects of cornstover char and indigeneous bacteria in the total heterotrophic bacteria count during bioremediation of hydrocarbon contaminated soil

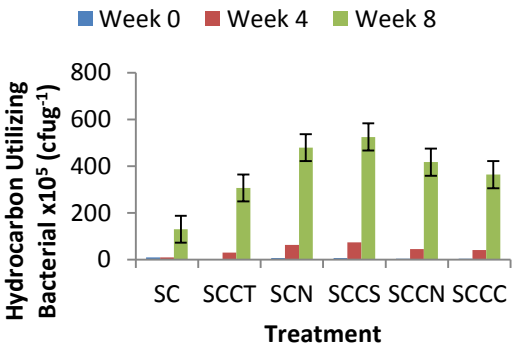

Fig 10: Effects of cornstover char and indigeneous bacteria in the hydrocarbon utilizing bacteria during bioremediation of hydrocarbon contaminated soil

The findings of this study revealed a predominance of Gram-negative bacterial isolates, including *Myroides* sp., *Providencia* sp., and *Proteus* sp., alongside one Gram-positive bacterium, *Bacillus* sp., obtained through both direct culture and enrichment techniques. These results enhance the understanding of microbial diversity in hydrocarbon-contaminated environments and their ecological implications. The dominance of Gram-negative bacteria aligns with existing literature, which consistently highlights their metabolic versatility and structural adaptations, such as an outer membrane that provides resilience against hydrophobic organic compounds (Das and Chandran, 2011). Their extensive genetic adaptability further enables the degradation of diverse hydrocarbons (Varjani, 2017). The identification of *Bacillus* sp., a Gram-positive bacterium, underscores its critical role in biodegradation. Known for its ability to form endospores, Bacillus sp. demonstrates remarkable survival under extreme environmental conditions, which aligns with previous findings on its versatility in hydrocarbon degradation (Awasthi *et al*., 2014). Furthermore, its production of biosurfactants enhances hydrocarbon solubilization, potentially complementing the metabolic activities of Gram-negative bacteria (Das and Chandran, 2011).

Morphological and biochemical analyses revealed that the isolates were predominantly rod-shaped, a feature advantageous for nutrient uptake due to their high surface-area-to-volume ratio (Madigan *et al*., 2018). This morphology is particularly beneficial in hydrocarbon-contaminated environments where resources are often limited. The identification of *Myroides* sp., *Proteus* sp., and *Providencia* sp. corroborates their documented roles in hydrocarbon degradation, particularly for metabolizing recalcitrant hydrocarbons, such as polycyclic aromatic hydrocarbons (PAHs) (Kim *et al*., 2012; Varjani, 2017). These results contribute to the understanding of microbial dynamics in hydrocarbon-contaminated environments, supporting findings by Margesin and Schinner (2001) on the dominance of Gram-negative bacteria. However, the inclusion of Bacillus sp. adds to the evidence that Gram-positive bacteria, though less prevalent, play significant roles in biodegradation (Awasthi *et al*., 2014). Contrasting studies reporting a higher prevalence of Gram-positive bacteria in specific conditions (Banerjee *et al*., 2021) suggest that microbial community structures are influenced by environmental factors such as hydrocarbon type and soil properties.

The observed physicochemical changes during bioremediation provide valuable insights into the dynamics of crude oil degradation in soil environments. Trends in pH, electrical conductivity (EC), and nutrient availability, highlight the effectiveness of various bioremediation strategies and their influence on microbial activity and hydrocarbon breakdown. The increase in pH across all setups indicates active microbial metabolism and its influence on soil chemistry. Notably, setups like SCCS and SCCC demonstrated significant shifts, reflecting robust microbial activity facilitated by bioaugmentation and biostimulation treatments. In contrast, the moderate pH fluctuations in SCN and SCCN suggest slower metabolic processes or limited nutrient availability. The decline in EC observed in setups such as SC and SCN indicates the consumption of soluble ions during hydrocarbon degradation by microbial consortia. Conversely, setups like SCCC demonstrated nutrient mobilization and solubilization, attributed to the synergistic effects of bioaugmentation agents and organic amendments.

These results align with existing literature, where increases in pH are associated with the production of alkaline byproducts, such as ammonia, during hydrocarbon degradation (Das and Chandran, 2011). Similarly, the observed decrease in EC corresponds to nutrient uptake by hydrocarbon-degrading bacteria (Chikere *et al*., 2012). Enhanced pH changes in SCCS and SCCC corroborate findings that organic amendments and bioaugmentation agents stimulate microbial communities, thereby accelerating hydrocarbon degradation (Varjani, 2017). While the stability of pH in SCN and SCCN suggests limited degradation processes, it highlights the need for complementary strategies. Nutrient amendments alone, as seen in SCN and SCCN, may not sufficiently enhance bioremediation without concurrent bioaugmentation. This challenges earlier assertions that nutrient amendments are the primary

drivers of hydrocarbon degradation (Atlas and Bartha, 1992).

Conclusion: This study has demonstrated the effectiveness of various bioremediation strategies in enhancing the degradation of crude oil in contaminated soil through the dynamic interplay of physiochemical parameters and microbial activity. Significant changes in soil pH, electrical conductivity, nutrient content, and hydrocarbon degradation rates were observed across the experimental setups. The results highlighted that bioaugmentation and biostimulation, particularly in combination, significantly enhanced microbial activity and hydrocarbon degradation compared to natural attenuation. These insights contribute to the development of effective, scalable bioremediation strategies for managing crude oil-contaminated soils. Future studies could further explore the long-term impacts of these treatments on soil health, microbial community structure, and ecosystem recovery, as well as their application to diverse contamination scenarios. This study underscores the potential of integrating bioaugmentation and biostimulation to enhance bioremediation efficiency and provides a robust framework for improving environmental management practices.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the first author or corresponding author or any of the other authors

REFERENCES

Ali, N; Dashti, N; Khanafer, M; Al-Awadhi, H; Radwan, S (2020). Bioremediation of soils saturated with spilled crude oil. *Sci. Rep.*, *10*(1), 1116. https://doi.org/10.1186/s13213-020-01580-x

Al-Wasify, RS; El-Naas, MH (2015). Bioremediation of crude oil contamination in soil: A review. *Int. J. Chem. Eng.*, 1–18. https://doi.org/10.1155/2015/715464

Atlas, RM; Bartha, R (1992). Hydrocarbon biodegradation and oil spill bioremediation. *Adv. Microb. Ecol.*, *12*(1), 287–338.

Atlas, RM (1981). Microbial degradation of hydrocarbons in the environment. *Adv. Appl. Microbiol.*, *27*, 77–100. https://doi.org/10.1016/S0065-2164(08)70261-4

Awasthi, M; Singh, M; Soni, SK; Singh, R; Kalra, A (2014). Biodegradation of polycyclic aromatic hydrocarbons by *Bacillus subtilis* isolated from hydrocarbon-contaminated soil. *J. Appl. Microbiol.*, *116*(3), 593–604.

Awasthi, M; Singh, S; Jain, A. (2014). Biosurfactant-enhanced degradation of petroleum hydrocarbons by bacterial consortium. *Int. Biodeterior. Biodegrad.*, *89*, 1–9. https://doi.org/10.1016/j.ibiod.2014.01.003

Avishai, B; Charles, ED (2014). Estimation method for serial dilution experiments. *J. Microbiol. Methods.* https://doi.org/10.1016/j.mimet.2014.08.023

Banerjee, A; Ghoshal, AK; Pal, S (2021). Hydrocarbon bioremediation by Gram-positive bacteria in petroleum-polluted soil. *Environ. Technol. Innov.*, *21*, 101225.

Banerjee, S; Joshi, SR; Mandal, S (2021). Gram-positive bacteria as potent hydrocarbon degraders: A review. *Environ. Sci. Pollut. Res.*, *28*(2), 1236–1247. https://doi.org/10.1007/s11356-020-10619-1

Baron, EJ; Finegold, SM (1990). *Bailey & Scott's Diagnostic Microbiology* (8th ed.). Mosby.

Cappuccino, JG; Sherman, N (2008). *Microbiology: A Laboratory Manual* (9th ed.). Pearson/Benjamin Cummings. Retrieved from https://www.pearson.com

Cerniglia, CE (1992). Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation*, *3*(1), 35–48. https://doi.org/10.1007/BF00129093

Chikere, CB; Okpokwasili, GC; Chikere, BO (2012). Monitoring of microbial hydrocarbon remediation in soil. *3 Biotech.*, *2*(3), 127–138. https://doi.org/10.1007/s13205-011-0033-0

Das, N; Chandran, P (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnol. Res. Int.*, *2011*, 1–13. https://doi.org/10.4061/2011/941810

Ekundayo, EO; Obuekwe, CO; Okoh, F (2012). Impact of crude oil pollution on soil physicochemical and microbiological properties. *Environ. Manage.*, *14*(5), 597–602. Retrieved from

https://www.environmentalmanagementjournal.com

Fulekar, MH; Singh, A; Bhaduri, AM; Kalyani, DC (2017). Bioremediation of hydrocarbon-contaminated sites. In: *Bioremediation of Industrial Waste for Environmental Safety* (pp. 155–182). Springer. https://doi.org/10.1007/978-981-10-4245-4_7

Ibezute, SU; Tudararo-Aherobo, LE; Okorhi-Damisa, FB (2024). Bioremediation of spent engine oil-contaminated soil with bacteria isolated from municipal waste dumpsite amended with water hyacinth (*Eichhornia crassipes*) leaves as biostimulant. *J. Appl. Sci. Environ. Manage.*, *28*(10B Suppl.), 3429–3437.

Kim, SJ; Kweon, O; Jones, RC; Freeman, JP; Edmondson, RD; Cerniglia, CE (2012). Complete genome sequence of *Myroides odoratimimus* strain OD-1. *J. Bacteriol.*, *194*(3), 764–765. https://doi.org/10.1128/JB.06156-11

Madigan, MT; Martinko, JM; Bender, K; Buckley, D; Stahl, D (2018). *Brock Biology of Microorganisms* (15th ed.). Pearson.

Margesin, R; Schinner, F (2001). Biodegradation and bioremediation of hydrocarbons in extreme environments. *Appl. Microbiol. Biotechnol.*, *56*(5–6), 650–663. https://doi.org/10.1007/s002530100701

Siddique, T; Okeke, BC; Arshad, M; Frankenberger, WT (2003). Enhanced degradation of hydrocarbons in soil by microbial augmentation. *Bioremediat. J.*, *7*(3–4), 297–308. https://doi.org/10.1080/713607990

Song, H; Chen, SF; Si, G; Bhatt, K; Chen, SH; Chen, WJ (2024). Removal of environmental pollutants using biochar: Current status and emerging opportunities. *Environ. Geochem. Health.*, *46*, 384. https://doi.org/10.1007/s10653-024-02142-9

Varjani, SJ (2017). Microbial degradation of petroleum hydrocarbons. *Bioresour. Technol.*, *223*, 277–286. https://doi.org/10.1016/j.biortech.2016.10.037

Zhou, M; Li, Y; Xie, X (2008). *Bergey's Manual of Determinative Bacteriology* (9th ed.). Williams & Wilkins. Retrieved from https://www.wolterskluwer.com