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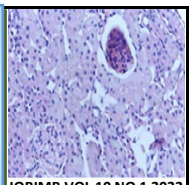

Studies on the Bioremediation Potential of Bacteria Isolated from Diesel-contaminated Soils in Kano

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ABSTRACT

Bioremediation is pollution control technology that uses microorganism to clean up contaminated environment. This study was aimed at assessing the bioremediation potential of bacteria isolated from diesel-contaminated soils and screen them for the ability to remediate diesel contaminated environment using their potential to degrade diesel as carbon and energy source. Diesel-contaminated soil samples were collected using standard method. Bacteria were isolated, characterized and identified using standard microbiological procedures. The identified bacteria species were subsequently screened for diesel biodegradation potential in Bushnell Haas Media (BHM). Optimum conditions (pH, temperature, and diesel concentrations) for biodegradation were determined. The results showed that *Bacillus subtilis* and *Bacillus megaterium* were the most potent species identified. *Bacillus subtilis* and *Bacillus megaterium* were observed to utilize diesel as the sole carbon source in which they degrade 79% and 80% diesel during the 25 days incubation study respectively. Optimum diesel biodegradation for *Bacillus subtilis* and *Bacillus megaterium* were temperatures of between 30 and 35 °C, pH between 6 and 7, diesel concentrations between 3% and 2%. The findings of this study demonstrated the species' ability to digest fuel, suggesting their potential utility in the broad-scale bioremediation of diesel-contaminated soils.

INTRODUCTION

Over the past two decades, there have been significant technological developments in the field of bioremediation. These developments are aimed at restoring contaminated ecosystems as a result of growing human activity on energy reservoirs, dangerous farming methods, and rapid industrialization. In terms of bioremediation of polluted places, utilizing has been shown to be effective and reliable due to its eco-friendly characteristics and low cost [1–3]. Although many different kinds of microorganisms can use crude oil as a growth and metabolic substrate, previous studies have shown that microbes acclimated to the same conditions are more effective at doing so [4,5].
The accumulation of pollutants in animal and plant tissue can cause the death or mutation of progeny, therefore soils poisoned by petroleum chemicals have severe consequences for local ecosystems [6–9]. Toxic components of the petroleum turn fertile soil into wastelands and ruin the natural beauty of the area. Oil pollution has other negative environmental repercussions, such as harm to soil microflora and contamination of ground water. These leaks cause ecological changes, such as the extinction of some species and the disruption of microbial growth and reproduction cycles [10–13].

To foster a sustainable development with minimal to no environmental impact, it is crucial that all petroleum-related contaminants and wastes be removed from the environment. The metabolic diversity of microorganisms plays a crucial role in the elimination of pollutants through biological processes. In the present study, the potency of *Bacillus* species to be used in bioremediation was determined.

MATERIALS AND METHODS

Sample Collection

Diesel-contaminated soil samples were collected randomly using an auger at a depth of 0-15 cm from generator houses of Bayero University Kano New Campus (N11°57.886' E008°25.884', 1555ft), ecological garden of Bayero University (N11°58.841' E008°28.496', 1505ft), Bayero University Old Campus (N11°58.895'E008°28.917', 1660ft), and Hotoro Trailer Garage (N11°58.568'E008°34.039', 1645ft), Kano, Nigeria. Samples were homogenized, sieved through a 2mm sieve iron mesh to remove debris and stones and stored in black polythene bags at room temperature.

Isolation, characterization and identification of bacterial isolates

The bacteria were isolated using standard microbiological procedures and the isolates obtained were identified using Gram reaction, morphological and biochemical characteristics. The biochemical tests: indole production, urease production, Voges–Proskauer, methyl red, nitrate reduction, starch hydrolysis, oxidase, citrate utilization, catalase and motility tests of the bacterial isolates were carried out according to the method described by Cheesbrough [14]. Gram staining of each bacterial isolate was performed as described by Abubakar *et al.* [15].

Screening of bacterial isolates for diesel degradation

The method developed by Afuwale and Modi was used to test pure isolates for diesel metabolism[10]. Bushnell Haas Medium (BHM) supplemented with diesel as the sole carbon source was used. An aliquot (100 µl) of bacterial culture was then transferred to 250 mL conical flasks containing 100mL BHM with (0.5 mL) diesel added and incubated at 37°C in an incubator. BH media composition {g l-1:$MgSO_4$ (0.2), $CaCl_2$ (0.02), KH_2PO_4 (1.0), K_2HPO_4 (1.0), NH_4NO_3 (1.0), $FeCl_3$ (0.05) final pH 7}. Un-inoculated medium in the conical flask were kept as control. Bacterial isolates were screened for their ability to degrade diesel in 25 days incubation period. The turbidity and optical densities of the isolates were also observed using spectrophotometer at 600nm.

Determination of total petroleum hydrocarbon

Following Agarry *et al.* instructions [11], diesel was extracted from experimental samples using n-hexane to examine its deterioration. The remaining diesel oil in the sample broth solutions was extracted by adding 5 mL of hexane and shaking the mixture violently for 5 minutes. The extraction was centrifuged at 2500 rpm, and the resulting filtrate was filtered. An upper organic phase layer and a bottom medium layer separated. The biodegraded diesel oil was found in the top organic phase layer. After employing a separating funnel, the organic phase layer was collected in a beaker. One milliliter of organic phase extract (filtrate) was mixed with fifty milliliters of hexane to make a dilution. Absorbance spectrophotometry was used to determine the concentration of organic compounds in a solution at a wavelength of 400 nm. with HACH DR/2010 Spectrophotometer using n-hexane as blank.

Percent degradation (D) was calculated using the following formula:

$$D = \frac{TPH_i - TPH_r}{TPH_i} \times 100$$

Where,

TPHi and TPHr are the initial and residual TPH concentrations respectively.

Optimization Study

Effect of pH on bacterial growth during biodegradation of diesel oil

Two different bacterial isolates were tested in a 50 mL BHM with 1% diesel (v/v) oil as the sole carbon source to investigate the influence of pH on growth. Autoclaved BHM with pH values between 6.0 and 8.0 was injected with bacterial inocula (100 uL) that had been grown overnight in nutritional broth (NB).equating to 6, 6.5, 7, 7.5, and 8. The pH values were adjusted appropriately using 1 M NaOH and 1 M HCl. The test was conducted at 37°C for a period of 5 days in an incubator. Optical density was recorded using spectrophotometer (SpectrumLab 752s) readings at 600 nm [16].

Effect of temperature on bacterial growth during biodegradation of diesel oil

The substrate concentration was kept constant at 1% (diesel) while the temperatures were changed (25^0C, 30°C, 35°C 40°C and 45°C) alongside control tests. Five days of bacterial growth were observed by spectrophotometer (SpectrumLab 752s) readings at 600 nm [16].

Effect of various concentrations on bacterial growth during biodegradation

Sterile BHM was supplemented with 0.5, 1, 3, and 5 percent diesel oil at 30 degrees Celsius to examine the effect of substrate concentration on the growth of the two bacterial isolates. For 5 days, the rate of bacterial growth in an incubator was monitored. The development was measured by spectrophotometric (SpectrumLab 752s) readings at 600 nm .

Statistical Analysis

The experimental data were analyzed using a single-factor ANOVA in SPSS version 19.0 [16], and all tests were performed in triplicate. Post-Hoc test (Turkey's test) was used to examine statistical significance between treatment group means at $P<0.05$ [17].

RESULTS

Morphological and biochemical characteristics of the potent bacterial isolates

Morphological and biochemical characteristics of the potent bacterial isolates was presented in **Table 1**. The isolates giving the potential to degrade diesel were identified as *Bacillus megaterium* and *Bacillus subtilis* (**Table 1**).

Percentage degradation of diesel by bacteria isolates

The results of the percentage degradation showed decrease in diesel content in the soil samples during the 25-days study period. At the initial phase of the study, there was approximate equal volume of diesel in the soil samples acted by bacterial isolates *Bacillus subtilis* and *Bacillus megaterium*. The percentage degradation in the diesel contaminated soil sample acted by *Bacillus subtilis* increased from the initial concentration of 0% to 79% after the 25-days incubation period. The result also showed the percentage degradation of the diesel contaminated soil acted upon by the species to be (0%, 33%, 38%, 73%, 75% and 80%) for the 0, 5, 10, 15, 20 and 25th day incubation period respectively (**Table 2**). *Bacillus megaterium* bacterial isolate was observed to have higher diesel degrading potentials compared to *Bacillus subtilis* as shown in **Table 2**.

Optimization of growth conditions for diesel degrading bacteria

In order to enhance the bacterial growth, optimization of environmental conditions was very significant. The bacterial isolates in the culture media were subjected to five different pH levels to monitor the effect of pH variation on bacterial growth. In addition, the bacterial isolates were subjected to varying temperature levels and also varying substrate (diesel) concentration.

Effect of pH on diesel biodegradation by *B. subtillis* and *B. megaterium*

The effect of pH treatments on the growth of bacterial isolates revealed that there was steady increase in *Bacillus subtilis* and *Bacillus megaterium* bacterial growth between the pH levels of 6–7. However, beyond the pH of 7, there was decline in the growth of the *Bacillus megaterium*. The species were observed to have optimum pH of 7 as *Bacillus subtilis* and *Bacillus megaterium* and had the optimum pH of 0.249±0.00 and 0.268±0.00 respectively as shown in **Table 3**.

Effect of temperature on diesel biodegradation by *B. subtillis* and *B. megaterium*

Temperature optimization affects the growth of bacterial isolates as shown in **Table 4**. The result observed indicated that *Bacillus subtilis* and *Bacillus megaterium* experienced optimal growth at the temperature of 30 °C and 35 °C respectively with optical density of 0.244±0.00 nm for *Bacillus subtilis* and 0.268±0.00nm for *Bacillus megaterium*. However, beyond the optimal temperature of the two isolates, the growth of *Bacillus subtilis* declined from 0.200±0.00 nm to 0.190±0.00nm at the temperature of 45 °C. Similarly, the growth of *Bacillus megaterium* declined from 0.268±0.00 nm at the temperature of 30 °C to 0.210±0.01nm at the temperature of 45 °C.

Effect of concentrations on diesel oil biodegradation by *B. subtillis* and *B. megaterium*

The effect of substrate concentration (diesel oil) on the growth of two bacterial isolates and their ability to degrade diesel oil was determined at optimum pH 7 and supplemented with various concentration of diesel oil i.e. 0.5, 1, 2, 3 and 4% at 30°C. The results as presented in **Table 5** indicated that isolate BNC2 had a continual growth with increase in substrate concentration from 0.261±0.01 OD at 0.5% to 0.321±0.06 OD at 2% diesel concentration. However, there was decline in growth at 3% (0.302±0.02 OD) and 4% diesel concentration (0.288±0.01 OD). Similarly, BOC4 had optimal growth of 0.305±0.01 (OD) at 3% increase in diesel concentration. The results also showed that there was decline in optical density after the optimum concentration of 3% diesel concentration to 0.278±0.01 OD at 4%.

Table 1. Morphological and biochemical characteristics of the potent isolates.

Morphological and Biochemical Characteristics	Isolates	
	BOC_4	BNC_2
Gram Reaction	+	+
Cell type	Rod	Rod
Motility Test	+	+
Voques-Proskauer Test	-	+
Indole Production Test	-	-
Citrate Utilization Test	-	+
Starch Hydrolysis Test	+	+
Nitrate Reduction Test	-	+
Catalase Test	+	+
Oxidase Test	+	+
Methyl Red Test	+	-
Urease Production Test	+	-
Coagulase Test	-	+
Identified Organisms	*B. megaterium*	*B. subtilis*

Table 2. Percentage degradation of diesel by bacteria isolates.

Incubation period (Day)	Diesel Degradation (%)	
	Bacillus subtilis	*Bacillus megaterium*
0	0	0
5	28	33
10	29	38
15	73	73
20	77	75
25	79	80

Table 3. Effect of pH treatment on diesel oil biodegradation by *B. subtillis* and *B. megaterium.*

Organisms	pH Levels				
	6	6.5	7	7.5	8
B. subtilis	0.219±0.00^a	0.233±0.00^a	0.249±0.00^a	0.228±0.00^a	0.200 ±0.00^a
B. megaterium	0.230±0.00^a	0.251±0.00^a	0.268±0.00^a	0.238±0.00^a	0.214±0.00^a

Note: Values are Mean ± SD of replicate estimations. Values followed by the different letters along the same column are significantly different at P < 0.05 (Turkey's test).

Table 4. Effect of temperature on diesel oil biodegradation by *B. subtillis* and *B. megaterium.*

Bacteria spp.	Temperature (°C)				
	25	30	35	40	45
Bacillus subtilis	0.230±0.001^a	0.244±0.00^a	0.220±0.001^a	0.200±0.004^a	0.190±0.00^a
Bacillus megaterium	0.243±0.00^a	0.240±0.001^a	0.268±0.00^b	0.228±0.005^b	0.210±0.001^b

Note: Values are Mean ± SD of replicate estimations. Values followed by the different letters along the same column are significantly different at P < 0.05 (Turkey's test).

Table 5. Effect of concentration on diesel oil biodegradation by *B. subtillis* and *B. megaterium.*

Bacteria spp.	Substrate Concentration (%)				
	0.50	1	2	3	4
Bacillus subtilis	0.247±0.10^a	0.268±0.04^a	0.284±0.01^a	0.305±0.03^a	0.278±0.01^a
Bacillus megaterium	0.261±0.01^a	0.285±0.02^a	0.321±0.06^b	0.302±0.02^a	0.288±0.01^a

Note: Values are Mean ± SD of replicate estimations. Values followed by the different letters along the same column are significantly different at P < 0.05 (Turkey's test).

DISCUSSION

There are so many bacteria possessing the ability to utilize hydrocarbon as their sole source of carbon, thus transforming hazardous component into non-hazardous, biodegradable and ecofriendly compounds [18]. The result of the percentage biodegradation potential of *Bacillus megaterium* and *Bacillus subtilis* indicated that *Bacillus megaterium* and *Bacillus subtilis* can degrade diesel contaminated soil up to 80% and 79%

respectively. The bacteria were identified as *Bacillus subtilis* and *Bacillus megaterium* by morphological and biochemical characteristics (**Table 1**). The result of the current research corresponded to the findings of Chithra and Shenpagam [19] who observed that *Bacillus* species were found to degrade diesel oil better than other bacterial isolates found in the soil. Similarly, according to Jyothi *et al.* [8] *Bacillus* species are potent degraders of hydrocarbons (gasoline and diesel) as they are able to use diesel as carbon and energy source for their metabolism.

In order to enhance the bacterial growth, optimization of environmental conditions is very significant. The pH variation in culture medium is due to the accumulation of metabolic waste products by the bacterial cells which strongly affects its growth. Hence it is very important to maintain optimum pH condition for bacterial growth medium. Different buffers with pH such as 6, 6.5, 7, 7.5 and 8 were used to study the optimal growth of the two bacterial isolates. At pH 7, *Bacillus subtilis* showed a higher optical density compared to *Bacillus megaterium* ($P < 0.05$). This agreed with the result of Ueno *et al.* [20]; Kwapisz*et al.* [21] where they reported the optimal growth of many diesel degrading bacteria at neutral or near neutral pH. Nwayi *et al.* [22] also reported that optimal pH for bacterial growth on diesel contaminated soil to be neutral.

Bacterial strains from hydrocarbon contaminated soil able to grow in a wide range of temperature [23]. The selected two bacterial isolates grew at different temperature conditions, 25 °C, 30 °C, 35 °C and 40 °C. Higher growth was observed at 30 °C and 35 °C for *Bacillus subtilis* and *Bacillus megaterium* respectively and showed a positive correlation between the temperature and the bacterial growth ($P<0.005$). Similarly, the optimal temperature for bacterial growth on diesel degradation was reported at 30 °C [23,24].

While diesel oil is an essential carbon source, it can become poisonous to microorganisms if it has been contaminated to a certain extent, due to the solvent action that can break bacterial cell membranes. As a result, researchers have conducted numerous diesel biodegradation investigations with diesel contamination levels between 0.5% and 1.5%. Degradation is typically slowed by a factor of 1.5 for every percentage point increase in concentration [24]. However, in this study, optimization of different substrate concentration revealed that, 2% and 3% diesel concentrations supported the excellent growth of *Bacillus megaterium* and *Bacillus subtilis* with no significant difference ($P < 0.05$). Findings were consistent with those reported by Nwinyi *et al.* [22], whereby fuel-degrading bacteria of the same species were shown to exist.

Hydrocarbon stresses on cell membranes, influencing permeability and, subsequently, energy generation, may explain why bacterial growth slowed after the optimal concentration of 2% and 3% for *Bacillus subtilis* and *Bacillus megaterium*, respectively. Diesel oil breakdown by bacteria species was also shown to be concentration dependent, with lower percentages recorded at higher concentrations of diesel. This trend may be attributable to the presence of extremely persistent aromatic alkanes as reported by Molnar *et al.* [25]. But during the course of the experiment, every bacterium showed idiophatic growth patterns, which pointed to a metabolic shift on the part of the microbes in relation to the utilization of metabolites from diesel decomposition [26].

CONCLUSION

Bacillus subtilis and *Bacillus megaterium* were shown to be the two most effective bacterial isolates for breaking down diesel in this study. It was found that the species relied on diesel oil as its sole carbon source. The two effective degraders, *Bacillus megaterium* and *Bacillus subtilis*, were used to optimize culture conditions, and it was found that 2 percent diesel concentrations were optimal for *Bacillus megaterium*, and 3 percent diesel concentrations were optimal for *Bacillus subtilis*, in the pH 7 and temperature range of between 30 and 35°C. During the 25-day incubation period, the bacterial species were also able to break down up to 79% and 80% of diesel, respectively. These results provided strong evidence for the potential of the discovered bacterial species to be used in the development of an environmentally benign mitigation method for petroleum hydrocarbon pollution.

REFERENCES

1. Nawawi NM, Ahmad SA, Shukor MY, Syed MA, Ibrahim ML. Isolation and characterization of phenol-degrading microorganism: recent advances. J Environ Bioremed Toxicol. 2014;2(1):11–22.
2. Othman AR, Yusof MT, Shukor MY. Biodegradation of Sodium Dodecyl Sulphate (SDS) by Serratia marcescens strain DRY6. Bioremed Sci Technol Res. 2019 Dec 28;7(2):9–14.
3. Habib S, Iruthayam A, Abd Shukor MY, Alias SA, Smykla J, Yasid NA. Biodeterioration of Untreated Polypropylene Microplastic Particles by Antarctic Bacteria. Polymers. 2020 Nov;12(11):2616.
4. Alhassan AY, Babandi A, Uba G, Yakasai HM. Isolation and Characterization of Molybdenum-reducing *Pseudomonas* sp. from Agricultural Land in Northwest-Nigeria. J Biochem Microbiol Biotechnol. 2020 Jul 31;8(1):23–8.
5. Yakasai HM, Babandi A, Uba G. Inhibition Kinetics Study of Molybdenum Reduction by *Pantoea* sp. strain HMY-P4. J Environ Microbiol Toxicol. 2020 Dec 31;8(2):24–9.
6. April TM, Foght JM, Currah RS. Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada. Can J Microbiol. 2000;46(1):38–49.
7. Chaillan F, Le Flèche A, Bury E, Phantavong YH, Grimont P, Saliot A, *et al.* Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Res Microbiol. 2004;155(7):587–95.
8. Jyothi K, Babu SK, Clara N, Kashyap A. Identification and isolation of hydrocarbon degrading bacteria by molecular characterization. Helix. 2012 Jan 1;2:105–11.
9. Liu B, Ju M, Liu J, Wu W, Li X. Isolation, identification, and crude oil degradation characteristics of a high-temperature, hydrocarbon-degrading strain. Mar Pollut Bull. 2016;106(1–2):301–7.
10. Jones DM, Douglas AG, Parkes RJ, Taylor J, Giger W, Schaffner C. The recognition of biodegraded petroleum-derived aromatic hydrocarbons in recent marine sediments. Mar Pollut Bull. 1983;14(3):103–8.
11. Wattayakorn G. Petroleum pollution in the Gulf of Thailand: A historical review. Coast Mar Sci. 2012 Jan 1;35:234–45.
12. Boz DT, Yalçın HT, Çorbacı C, Uçar FB. Screening and molecular characterization of polycyclic aromatic hydrocarbons degrading yeasts. Turk J Biochem. 2015;40(2):105–10.
13. Shetaia YMH, El khalik WAA, Mohamed TM, Farahat LA, ElMekawy A. Potential biodegradation of crude petroleum oil by newly isolated halotolerant microbial strains from polluted Red Sea area. Mar Pollut Bull. 2016;111(1–2):435–42.
14. Cheesbrough M. District Laboratory Practice in Tropical Countries [Internet]. 2nd ed. Cambridge: Cambridge University Press; 2006 [cited 2022 Jul 21]. Available from: https://www.cambridge.org/core/books/district-laboratory-practice-in-tropical-countries/6F6FE267756B004A4826209C692BD5B3
15. Abubakar YU, Taura DW, Muhammad BA, Ibrahim TS, Njobdi AB, Umar M, *et al.* In situ biodegradation of diesel by bacteria isolated from Broilers Chicken droppings. Adv Pharm J, 2020; 5(5):164-171. Adv Pharm J. 2020;5(5):164–71.

16. Mwaura AN, Mbatia BN, Muge EK, Okanya PW. Screening and Characterization of Hydrocarbonoclastic Bacteria Isolated from Oil-contaminated Soils from Auto Garages. Int J Microbiol Biotechnol. 2018 Mar 23;3(1):11.
17. Agarry S, Latinwo GK. Biodegradation of diesel oil in soil and its enhancement by application of bioventing and amendment with brewery waste effluents as biostimulation-bioaugmentation agents. J Ecol Eng. 2015 Feb 8;16(2):82–91.
18. Vignesh R, Arularasan A, V G, Deepika C. Isolation identification and characterization of potential oil degrading bacteria from oil contaminated sites. 2016 Jan 1;
19. Chithra S, Hema N. Isolation and identification of oil degrading bacteria from oil contaminated soil and comparison of their bioremediation potential. Glob J Res Anal. 2012 Jun 15;3:181–4.
20. Ueno A, Ito Y, Yumoto I, Okuyama H. Isolation and characterization of bacteria from soil contaminated with diesel oil and the possible use of these in autochthonous bioaugmentation. World J Microbiol Biotechnol. 2007 Oct 1;23.
21. Kwapisz E, Wszelaka J, Marchut-Mikolajczyk O, Bielecki S. The effect of nitrate and ammonium ions on kinetics of diesel oil degradation by Gordonia alkanivorans S7. Int Biodeterior Biodegrad. 2008 Apr 1;61:214–22.
22. Nwinyi OC, Kanu IA, Tunde A, Ajanaku KO. Characterization of diesel degrading bacterial species from contaminated tropical ecosystem. Braz Arch Biol Technol. 2014 Oct;57:789–96.
23. Shukor MY, Dahalan FA, Jusoh AZ, Muse R, Shamaan NA, Syed MA. Characterization of a diesel-degrading strain isolated from a hydrocarbon-contaminated site. J Environ Biol. 2009;30(1):145–50.
24. Lee M, Kim MK, Singleton I, Goodfellow M, Lee ST. Enhanced biodegradation of diesel oil by a newly identified Rhodococcus baikonurensis EN3 in the presence of mycolic acid. J Appl Microbiol. 2006 Feb;100(2):325–33.
25. Molnár M, Leitgib L, Gruiz K, Fenyvesi E, Szaniszló N, Szejtli J, *et al.* Enhanced biodegradation of transformer oil in soils with cyclodextrin--from the laboratory to the field. Biodegradation. 2005 Mar;16(2):159–68.
26. Palanisamy N, Ramya J, Kumar S, Vasanthi N, Chandran P, Khan S. Diesel biodegradation capacities of indigenous bacterial species isolated from diesel contaminated soil. J Environ Health Sci Eng. 2014 Dec 12;12(1):142.