

Original Research Article

HYDROCARBON DEGRADATION AND BIODEGRADERS IN OIL SPILLAGE SITES IN PORT HARCOURT

Abstract

Information on remediation of soils polluted from the activities of artisanal crude oil refining is lacking. The functional capability of the indigenous microbial community and prospects for recovery of important bio-resources has also not been explored. The aim of this study was to identify the hydrocarbon biodegrader present in oil spillage sites in Port Harcourt. Samples were taken from various sites (labelled ss1, ss2, ss3 and controls) in Port Harcourt and the samples were transported to the lab for bacteriological assessment. The samples were cultured in Nutrient agar and Basal Salt Medium (BSM). The total heterotrophic bacteria counts were enumerated by serially diluting the fluid sample. Microbial characterization was done based on morphological, physiological and biochemical tests. From the results, *Pseudomonas* species and *Bacillus* species were the probable isolated from the culture of hydrocarbon contaminated environment. The results also revealed increased compared to the control organism that maintained the same growth level throughout the 5 days of incubation, the isolated bacteria (*Pseudomonas* species and *Bacillus* species) had steady bacterial growth. This study has shown that *Bacillus* species and *Pseudomonas aeruginosa* are potential agents of bioremediation of environmental hydrocarbon pollution.

Keywords: *bacteria, count, environment, isolate, pollution.*

INTRODUCTION

Hydrocarbon pollution resulting from decades of crude oil exploration, production, transportation and processing in the Niger Delta has led to devastating degradation of agricultural lands, aquaculture and vegetation [1-7]. The increasing resort to artisanal refining, usually undertaken under very primitive conditions has also been reported to be substantially contributing to the already existing oil-pollution challenge [1-7]. Information on remediation of soils polluted from the activities of artisanal crude oil refining is lacking. The functional capability of the indigenous microbial community and prospects for recovery of important bio-resources has also not been explored. Bioremediation has been established to be a reliable cost-effective method for oil spill remediation in the Niger Delta [8]. This is owing to the favourable tropical conditions which include presence of abundant hydrocarbon degrading microbes, adequate rainfall and sunlight. Bioremediation involves the improvement of the natural ability of microorganisms to degrade environmental contaminants, thereby reducing the degradation half-life and associated risk of the pollutant. This is achieved through bioaugmentation (addition of known degraders of the contaminant) and or biostimulation which involves the addition of nutrients in the form of fertilizers. Several studies involving the assessment of the capacity of indigenous microbial communities to detoxify hydrocarbon contaminants both in ex-situ and field-scale remediation studies have been undertaken in the Niger Delta with success [8]. The hydrocarbon fractions with established microbial pathways for activation and degradation cut across both saturates (short-chain, long-chains, isoalkanes, cycloalkanes) and aromatic hydrocarbons (volatile monoaromatics, polycyclic aromatics and aromatic sulphur-containing compounds). Also, microbial domains known to contribute to hydrocarbon degradation in both aerobic and

anaerobic environments include bacteria, fungi and archaea [9,10]. The close proximity of oil activities, including artisanal refining, to water bodies particularly wetlands, mangroves and estuaries require the use of special fertilizer formulations for enhancing biodegradation. This is important for improved nutrient utilization efficiency and reduced risk of secondary contamination. The use of inorganic fertilizers for hydrocarbon remediation in the Niger Delta is highly discouraged because it can lead to further acidification of the oil-polluted soil [8]. Secondly, its poor nutrient efficiency and a possible secondary contamination of surrounding waters can be aggravated by the frequent rainfall usually experienced in the Niger Delta. Several studies have employed the use of organic alternatives as nutrient source for improved microbial-dependant hydrocarbon degradation. Over the years, numerous studies have described the application of microbial consortia for crude oil degradation throughout the world but studies on degradation of crude oil by employing indigenous bacterial consortia from this petro-chemically important geographical region are very limited, hence this study.

MATERIALS AND METHODS

In this study, samples of oil spillage contaminated soil were taken from various locations in Port Harcourt and labelled as ss1, ss2, ss3 and controls where "ss" represents sampling station. Port Harcourt is the capital city of Rivers State, the hub of oil activity in the Niger Delta and the country at large. The collected samples were transported to the laboratory where the bacteriological studies were carried out.

All glassware's (Conical flasks, Measuring cylinders, Beakers, Petri dishes and Test tubes etc) were washed and sterilized in an oven. All culture media were sterilized using the autoclave at 121°C, 15psi for 15 minutes. The media used were Nutrient agar and Basal Salt Medium (BSM). They were prepared according to the manufacturer's instructions.

Total heterotrophic bacteria counts

The total heterotrophic bacteria counts were enumerated by serially diluting the fluid sample from the settled mixture in normal saline at dilution 10^{-2} – 10^{-6} and 0.1 mL of aliquots and each dilution was inoculated into nutrient agar plates. The plates were then incubated at room temperature, ($28 \pm 2^\circ\text{C}$) for 24 - 48 hours after which colony counts were taken.

Total hydrocarbon utilizing bacteria counts

Similarly, the population of hydrocarbon utilizing bacteria was estimated on mineral salt medium (BSM). The medium contained (in g/L) K_2HPO_4 , 0.38 g; KH_2PO_4 , 0.6 g; NH_4Cl 1 g, FeCl_3 , 0.05 g, Agar 20 g and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.20 g. The pH of the medium was adjusted to 7.2 for bacterial estimation. The MS medium was fortified with nystatin ($50\mu\text{g}/\text{mL}$) to suppress fungal growth and 0.1 mL of aliquots of serially diluted sample was inoculated on MS medium.

Characterization and identification of isolates

Isolated microbial strains were characterized to identify the family they belong. Microbial characterization was done based on morphological, physiological and biochemical tests.

Data analysis

Data obtained from the study were simply described with no inferential statistics by using relevant formulas to determine the colony forming unit per gram (Cfu/g) and Log cfu/g.

RESULTS

Total Heterotrophic Bacterial Nutrient Agar

Table 1 shows the enumeration of bacterial count from the six samples in Nutrient agar

Table 1: Heterotrophic Bacteria Count in Nutrient Agar

Samples	Dilution factor	Colony count	Cfu/g	Log cfu/g
(control) 0-15	10 ⁴	82	8.2 x10 ⁶	6.91
(ss1) 05-30	10 ⁴	36	3.6x10 ⁶	6.55
(ss1) 0-15	10 ⁴	45	4.5 x10 ⁶	6.65
(ss2) 0-15	10 ⁴	24	2.4 x10 ⁶	6.38
(ss3) 15-30	10 ⁴	14	1.4 x10 ⁶	6.14
(control) 15-30	10 ⁴	32	3.2 x10 ⁶	6.50

ss: sampling station/site

Macroscopic And Biochemical Test Identification Of The Isolates

Table 2 shows the cultural morphological features of the different organisms isolated differently by their varying cultural features like shape, colour, opacity, elevation, size, margin and surface subject to biochemical test identification.

TABLE 2: Colonial Morphology Of Total Heterotrophic Bacteria Isolates

Isolate code	Surface	Shape	Elevation	Size	Color	Opacity
A	Mucoid	Circular	Convex	3mm	Creamy white	Translucent
B	Dull/smooth	Circular	Flat	Large	White	Opaque
C	Dry/mucoid	Circular	Flat	3mm	Creamy white	Opaque
D	Shiny	Irregular	Flat	Large	White	Translucent
E	Dry/mucoid	Circular	Flat	3mm	Creamy white	Opaque
F	Shiny	Circular	Raised	2mm	Cream	Translucent

TABLE 3: Biochemical Test Result

Isolate code	Gram rxn	catalase	oxidase	MR	VP	Indole	Citrate	Glucose	lactose	Sucrose	H2S	Motility	Probable organisms
A	-	+	-	-	+	-	+	+	+	+	-	-	<i>Klebsiella sp</i>
B	-	+	+	-	-	-	+	-	-	-	+	-	<i>Pseudomonas aeruginosa</i>
C	+	+	-	-	+	-	+	+	-	+	-	+	<i>Bacillus sp</i>
D	+	+	-	-	+	-	+	+	-	+	-	+	<i>Bacillus sp</i>
E	+	+	-	-	-	-	+	-	-	-	+	-	<i>Pseudomonas sp</i>
F	-	+	-	-	+	-	+	+	+	+	-	-	<i>Klebsiella sp</i>

Table 4: Hydrocarbon degradation by consortium from day 1 – day 5

Isolate code	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
A	1.522	1.527	1.530	1.531	1.531
B	0.459	0.737	0.981	0.121	1.290
C	0.386	0.592	0.781	0.914	1.158
D	0.328	0.526	0.712	0.901	1.140
E	0.304	0.611	0.807	0.956	1.268

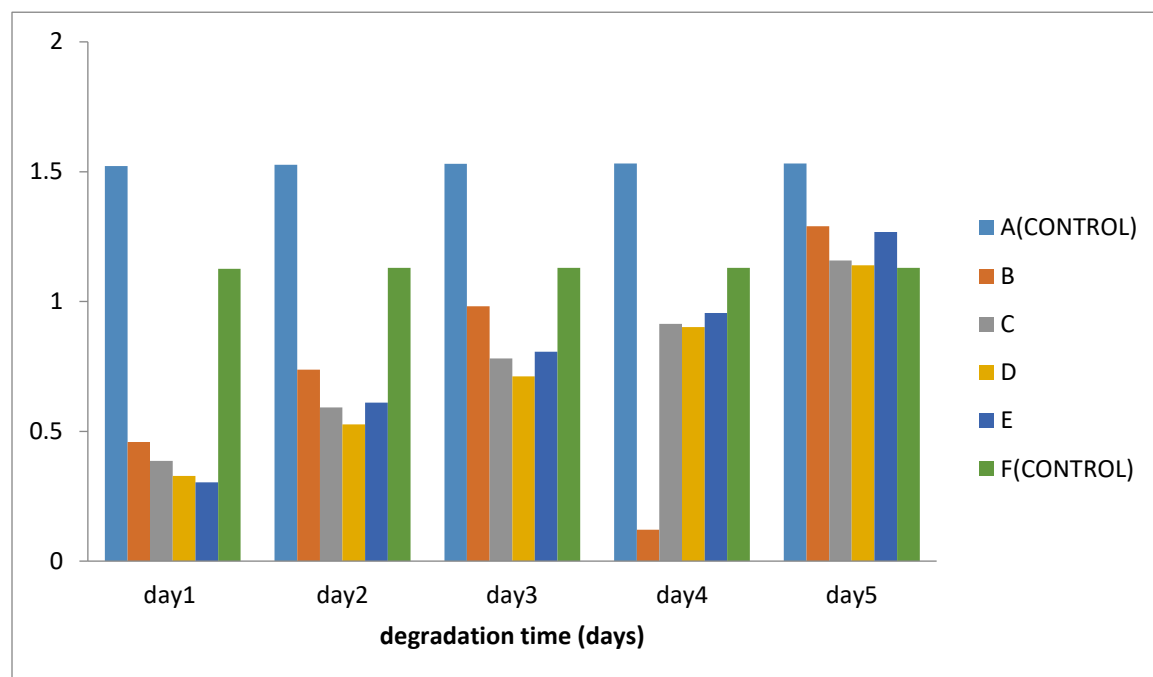


Fig1: hydrocarbon degradation by consortium

DISCUSSION

This project focused consortium with potential for hydrocarbon degradation by indigenous microbes from an arsenical oil polluted soil. The problem caused by oil spills is of major global concern because this does not affect the environment alone but has adverse health effects on humans [1-7). Petroleum hydrocarbons persist in the environment due to the low numbers of hydrocarbon utilizing organisms, environmental conditions and also poor knowledge about the abilities of naturally occurring microorganisms to degrade the oil in polluted environments [11]. The microorganisms capable of surviving in such a polluted environment are those that develop specific enzymatic and physiological responses that allow them to use the hydrocarbon compounds as substrates [12].

After the incubation process on the media, different bacteria colonies were seen on the media as shown in Table 1 and 2 where colony counts were presented, indicating evidence of bacteria presence and the morphological features of the isolated bacteria. Bacteria colony count was carried out and for each sample and recorded. After this incubation and colony counting process, biochemical test was carried out in order to determine the probable isolate from the samples as revealed from Table 3. The study revealed that different organism were isolated in hydrocarbon environment and as such may be implicated in biodegradation of hydrocarbon since the survival of an organism in that environment is suggestive that it possess the enzymes to enable it degrade the hydrocarbon or perish [12]. The isolated bacteria were identified to be *Pseudomonas aeruginosa*, *Pseudomonas sp* and *Bacillus sp*.

Except for the controls that maintained relatively constant level of bacterial growth throughout the five days as seen in Figure 1, other isolates (had a steadily increasing growth from day 1 to day 5, implying that the isolates thrived favourably in hydrocarbon environment unlike the control organism, *Klebsiella species*. This means that *Pseudomonas sp* and *Bacillus*

sp are hydrocarbon environment-friendly and as such may be involved in the degradation of hydrocarbon from oil spillage. This finding aligns with the report by other authors who said *Bacillus sp* and *Pseudomonas sp* are used to degrade hydrocarbon and lubricant oil [13]. The find was also supported by the study conducted by Gopinath on bioremediation of lubricant oil pollution in water by *Bacillus megaterium*, where he also reported the efficacy of *Bacillus species* in degrading hydrocarbon [14].

CONCLUSION

In understanding the role of bacteria isolates in bioremediation of oil spillage, this study has revealed that *Bacillus species*, *Pseudomonas species* and *Pseudomonas aeruginosa* may be suitable in biodegradation of hydrocarbon following their ability to thrive in hydrocarbon polluted environment.

REFERENCES

Kurnia DR, Mangunwardoyo W, Ambarsari H. Biodegradation of used lubricant oil hydrocarbons using *Bacillus subtilis* InaCC B289 and *Pseudomonas aeruginosa* InaCC B290 in single or mixed cultures. In AIP Conference Proceedings 2018 Oct 17 (Vol. 2021, No. 1). AIP Publishing.

Gopinath SM, Shareef MIA, Ganessin A. Bioremediation of lubricant oil pollution in water by *Bacillus megaterium*. IJIRSET. 2007; 8:6773–6780

References

1. Onwuli D, Ajuru G, Holy B, Fyनेface CA. The concentration of lead in periwinkle (*Tympanotonos fuscatus*) and river sediment in Eagle Island River, Port Harcourt, Rivers state, Nigeria. American Journal of Environmental Protection, 2014; 2(2): 37-40
2. Onengiyeofori I, Fyनेface CA. Assessment of Serum Levels of some Heavy Metals in Carpenters Residing in Port Harcourt in Relation to their Lifestyle. Asian Journal of Research in Medical and Pharmaceutical Sciences. 2018; 4(4): 1-7
3. Fyनेface CA, Emeji R, Osere H, Nwisah L. Concentrations of Nickel in Sediment and Periwinkle of Eagle Island River, Port Harcourt. Asian Journal of Fisheries and Aquatic Research, 2018; 1(4): 1-5
4. Biambo KG, Nyebuchi J, Roseline E, Fyनेface AC, Lauretta N. Zinc Composition in Breast Milk of Lactating Mothers in Urban and Sub-urban Areas in Rivers State. Asian Journal of Research in Nursing and Health, 2021; 4(4): 165-169
5. Catherine I, Biambo KG, Nyebuchi J, Fyनेface AC, Goodnews N. Evaluation of Nutrient Composition in Breast Milk of Breast Feeding Mothers in Urban and Sub-urban Subjects in Rivers State. Asian Journal of Pediatric Research, 2021; 7(2): 39-44
6. Faith D, Biambo GK, Nyebuchi J, Amadi FC, Konne FE. Comparative Study of Heavy Metals in Breast Milk of Breast Feeding Mothers in Urban and Sub-Urban Subjects in Rivers State. Journal of Applied Life Sciences International. 2021;24(8): 31-36
7. Amadi CF, Aleruchi-Didia TN, Konne FE, Nyenke CU. Environmental Effect of Kpo-fire in Niger Delta and Future Health Implications. Biotechnology Journal International, 2022; 26(2): 18-24

8. Panel IN. Sustainable remediation and rehabilitation of biodiversity and habitats of oil spill sites in the Niger Delta: Main report including recommendations for the future. A Report by the Independent IUCN–Niger Delta Panel (IUCN–NDP) to the Shell Petroleum Development Company of Nigeria (SPDC). 2013 Jul.
9. Hamza UD, Mohammed IA, Sale A. Potentials of bacterial isolates in bioremediation of petroleum refinery wastewater. *Journal of Applied Phytotechnology in Environmental Sanitation*. 2012 Apr 1;1(3).
10. Sonune NA, Garode AM. Bioremediation potential of bacterial isolates for municipal wastewater treatment. *Current world environment*. 2015 Aug 1;10(2):619-25.
11. Adebusoye SA, Ilori MO, Amund OO, Teniola OD, Olatope SO. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World Journal of Microbiology and Biotechnology*, 2007; 23(8): 1149–1159.
12. Chrzanowski Ł, Kaczorek E, Olszanowski A. The ability of *Candida maltosa* for concentrations of hexadecane by *Aspergillus niger* in a solid-state system: Kinetic analysis. *BioresTechnol*. 2006; 94(14): 1583–1591
13. Kurnia DR, Mangunwardoyo W, Ambarsari H. Biodegradation of used lubricant oil hydrocarbons using *Bacillus subtilis* InaCC B289 and *Pseudomonas aeruginosa* InaCC B290 in single or mixed cultures. In AIP Conference Proceedings 2018 Oct 17 (Vol. 2021, No. 1). AIP Publishing.
14. Gopinath SM, Shareef MIA, Ganessin A. Bioremediation of lubricant oil pollution in water by *Bacillus megaterium*. *IJIRSET*. 2007; 8:6773–6780

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