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. The results showed that based on bacteria growth, *Bacillus species* and *Pseudomonas aeroginosa* were identified as the biodegraders from their study growth trends in hydrocarbon polluted samples. This study as shown that *Bacillus species* and *Pseudomonas aeroginosa* 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. 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 lab 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 121^0C, 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 of each dilution was inoculated into nutrient agar plates. The plates were then incubated at room temperature, (28 ± 2°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_2HPO4, 0.38 g; $KH2PO_4$, 0.6 g; NH_4Cl 1 g, $FeCl_3$, 0.05 g, Agar 20 g and $MgSO_4 .7H_2O$, 0.20 g. the pH of the medium was adjusted to 7.2 for bacterial estimation. The MS medium was fortified with nystatin (50µg/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	8.2×10^6	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	614
(control) 15-30	10^4	32	3.2 x10^6	6.50

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	Motiity	Probable organisms
A	-	+	-	-	+	-	+	+	+	+	-	-	*Klebsiella sp*
B	-	+	+	-	-	-	+	-	-	-	+	-	*Pseudomonas aeroginosa*
C	+	+	-	-	+	-	+	+	-	+	-	+	*Bacillus sp*
D	+	+	-	-	+	-	+	+	-	+	-	+	*Bacillus sp*
E	+	+	-	-	-	-	+	-	-	-	+	-	*Pseudomonas sp*
F	-	+	-	-	+	-	+	+	+	+	-	-	*Klebsiella sp*

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
F	1.126	1.129	1.130	1.130	1.130

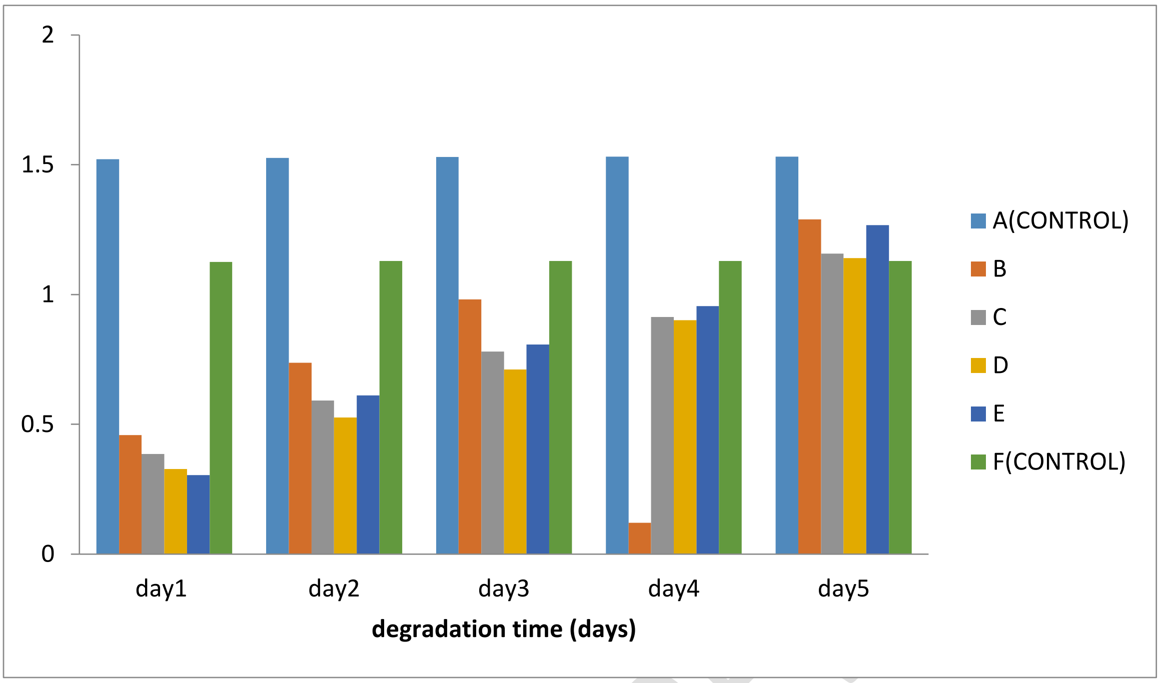

Fig1: hydrocarbon degradation by consortium

DISCUSSION

This project focused consortium with potential for hydrocarbon degradation by indigenous microbes from an artesenal 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. 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 other to determine the probable isolate from the samples.

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 hydocarbon [14].

CONCLUSION

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

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