Original Research Article

ISOLATION AND IDENTIFICATION OF SOME HYDROCARBON UTILIZING BACTERIA ISOLATED FROM CONTAMINATED SOIL IN ZUMA, BWARI AREA COUNCIL, FCT, ABUJA, Nigeria.

Abstract

The wide spread use of petroleum products leads to contamination of soil and aquatic environments, thereby posing a serious threat to all life forms including humans. Therefore, isolation of oil-degrading microorganisms and optimizing conditions for biodegradation process are important. Thus, this study was aimed at isolating and characterizing hydrocarbon utilizing bacteria from hydrocarbon contaminated soil in Bwari area council, Federal Capital Territory, Abuja, Nigeria, using three different petroleum products (petrol, kerosene and diesel) thereby checking their catabolic capacities on the products. Enrichment culture technique (using mineral salt medium; MSA) was employed to obtain fourteen (14) bacterial isolates capable of utilizing the petroleum products. The ability to degrade petroleum hydrocarbons is not restricted to a few microbial genera; a diverse group of bacteria and fungi have been shown to have this ability. The most important (based on frequency of isolation) genera of hydrocarbon utilizers in environments were Pseudomons spp. 21.43%, Bacillus spp. 21.43%, Acinetobacter spp. 7.14%, Corynebacterium spp. 7.14%, Staphylococci spp. 7.14%, Citobacter spp. 14.29%, Aeromonas spp. 14.29%, Flavobacterium spp. 7.14%. The hydrocarbon utilizing bacteria count ranged from 1.8×10^4 to 1.5×10^8 cfu/ml showing a high range of different bacteria found in polluted soil due to formation of consortia to degrade the substrates. The highest growth was found in petrol sample (PS) followed by kerosene sample (KS) and then diesel sample (DS), implying the ability of these organisms to rapidly utilize lower molecular and low density hydrocarbon than higher ones. Applications of this adaptation of microorganisms have been seen in bioremediation, oil recovery, indicators of environmental pollution of petroleum hydrocarbons, etc which are all environmental friendly and cost effective.

Key words: Bacteria, Soil, Hydrocarbon, Nigeria.

1.0 Introduction

Oil spillage and oil pollution in water environment have been a major threat to the ecosystem and human being through the transfer of toxic materials including polycyclic aromatic hydrocarbons (PAHs) into the food chain (Sei and Fathepure, 2009). Presence of polycyclic aromatic hydrocarbons in the soil and water is a major problem as environmental contaminants and most of these PAHs are recalcitrant in nature.In "Polycyclic Aromatic Hydrocarbons Degradation Techniques:A Review" by Ukiwe, *etal*(2013)"A critical over-view of several degradation techniques has been presented in this review. These techniques include; chemical degradation, biodegradation, phytodegradation as well as combined degradation methods. Other developing degradation methods that has gained wide public acceptance include; solar ultraviolet radiation,

direct photolysis and ultrasound frequency degradation. However, some non-chemical degradation, photocatalytic degradation as well as current density enhanced degradation is novel approaches that have been tested successfully for the compound's removal". These methods are rarely successful in rapid removal and cleaning up PAHs (Dasgupta *et al.,* 2013), and also are not safe and cost effective when compared to microbial biodegradation. In many ecosystems there is already an adequate indigenous microbial community capable of extensive oil biodegradation, provided that environmental conditions are favourable for oil-degrading metabolic capacity (Capelli *et al.,* 2004, Kim *et al.,* 2004). There are several advantages relying on indigenous microorganisms rather than adding microorganisms to degrade hydrocarbons. First, natural populations have developed through many years. These microorganisms are adapted for survival and proliferation in that environment. Secondly, attention has been focused on marine environmental factors which influence since the world's oceans are the largest and ultimate receptors of hydrocarbon pollutants.Most previous reviews concerning the microbiology of petroleum pollutants have been concerned with the marine environment. This field expands the scope to include consideration of the fate of petroleum hydrocarbons in freshwater and soil ecosystems （Ronald M. Atlas, 1982）. A number of hydrocarbon-degrading microorganisms produce emulsifying agents (Rahman *et al.,* 2002, Sood and Banwari, 2009, Venvolden and Copper, 2003 and Jain, 2011). Some of these bioemulsifiers have been considered for use in cleaning oil storage tanks, such as supertankers (Zhang *et al.,* 2012). In some cases, the emulsifying agents appear to be fatty acids or derivatives of fatty acids; in other cases, more complex polymers are active emulsifying agents. Although the production of emulsifying should increase the susceptibility of hydrocarbons in oil to microbial degradation, microbial strains which effectively emulsify oil often do not extensively degrade the hydrocarbons in the oil.

2.0 Materials and Methods

Sampling area

The sampling area was Zuma in Bwari area council of Federal Capital Territory, Abuja, lying between latitude 8.25 and 9.20 north of the equator and longitude 6.45 and 7.39 east of the Greenwich meridian, Abuja is geographically located in the center of the country.

Sample collection

Soil sample was obtained from the generator house in Liberty Hotel from Zuma in Bwari area council of Federal Capital Territory, Abuja, Nigeria. The samples were aseptically collected

using sterile spatula in different portions and depth of 15-20cm. The samples were stored in sterile aluminum foils and transported to the laboratory within 24 hours of collection.

Isolation procedure

The modified method of Francy et al., (2000) was employed. About 20.0 grams of the contaminated soil sample was inoculated into 180ml of mineral salt broth (MSB) in a 250 ml conical flask. The mixture was then shaken thoroughly to produce a well dispersed suspension and this was incubated at 30˚C for 72 hours. This aid at resuscitating the stressed microorganisms present in the sample. The mixture was serially diluted (10^{-1} to 10^{-6}) from the stock sample using sterile distilled water and plated in triplicates on mineral salt agar (MSA) incorporated with nystatin to inhibit the growth of fungi. A filter paper (Whatman No. 1) saturated with the different hydrocarbon/petroleum products (kerosene, diesel, and petrol) was aseptically placed on the plates over the lid of the same Petri dish and these were incubated while inverted (vapour phase transfer method) for 14 days at 30˚C.Pure cultures of each isolate were prepared on agar slants for further analysis.

3.0 Results

Table 2.1 below shows total heterotrophic count which ranges from $1.8x10^4$to $1.5\ x10^8$cfuml^{-1}, isolate code number PS had the highest number of hydrocarbon utilizing bacteria count followed by KS and DS, these varies from the average colony count.

Table 2.1: Total Heterotrophic Count（Colony forming unit per milliliter）

Isolates	Colony count			Average colony count	Dilution Factor	Total Count (CFU/ml)
DS 1	18	25	11	10	10^{-2}	1.8×10^{-4}
PS 1	55	51	62	56	10^{-2}	5.6×10^{-4}
KS1	20	23	23	23	10^{-2}	2.3×10^{-4}
DS 2	18	17	10	15	10^{-3}	1.5×10^{-5}
PS 2	38	54	41	44	10^{-3}	4.4×10^{-5}
KS 2	21	19	23	21	10^{-3}	2.1×10^{-5}
DS 3	12	8	10	10	10^{-6}	1.0×10^{-8}
PS 2	13	21	11	15	10^{-6}	1.5×10^{-8}
KS 3	6	11	10	9	10^{-6}	9.0×10^{-7}

Fig 1 below shows the comparison of the number of hydrocarbon utilizing bacteria on mineral salt medium with the different carbon sources. It can be deduced that for all the dilutions, the highest number of hydrocarbon utilizing bacteria was observed in the plate having petrol as the carbon source(PS) followed by that with kerosene (KS) and then lowest in that with diesel (DS). This consistency shows that for all the petroleum products used, petrol was the most preferred followed by kerosene and then diesel.

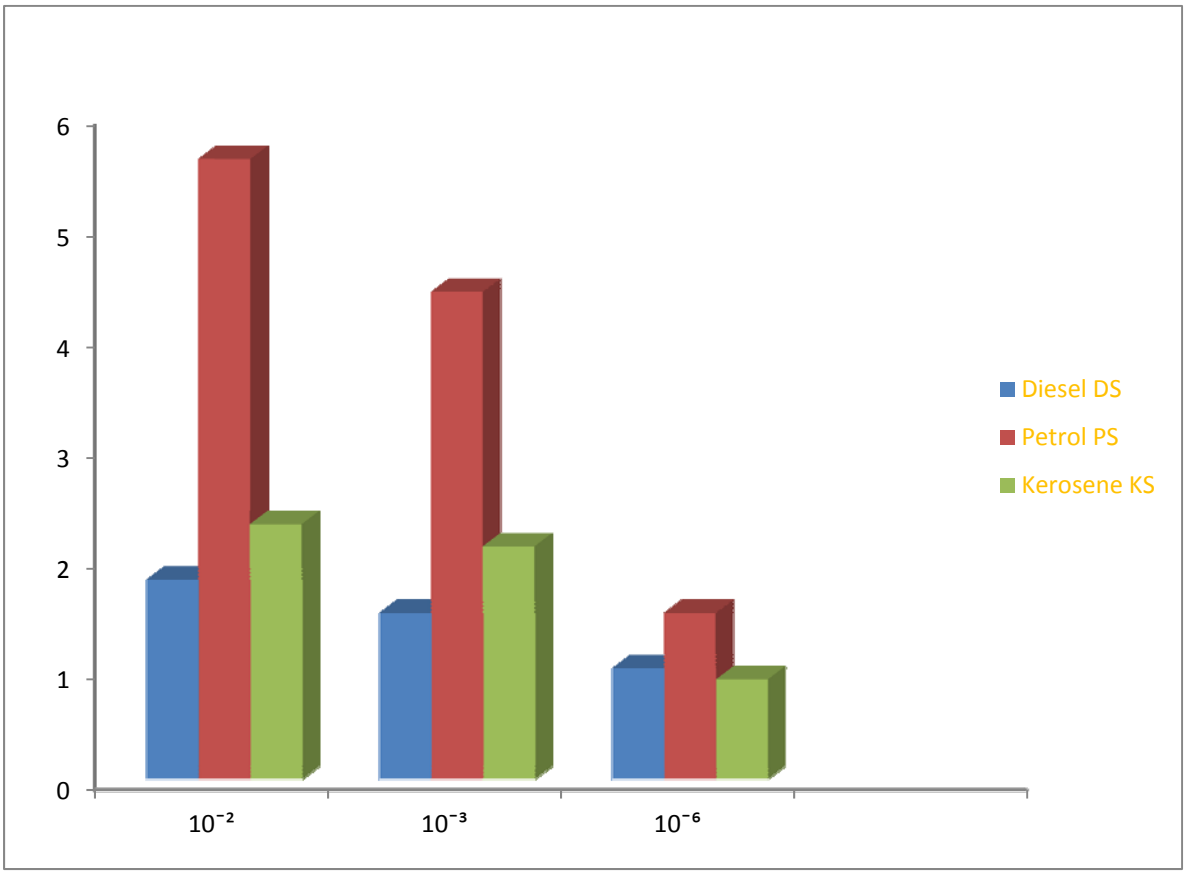

FIG I: Graph showing the cfu/ml of the hydrocarbon utilizing bacteria on mineral salt medium having the different carbon sources.

Table 2.2 below shows the colonial morphology of the test isolates. Out of the 14 isolates, 8 were convex, and 6 were flat in terms of elevation. All the isolates displayed circular shapes, 8 isolates were smooth with regular shaped edges like ring and the remaining 6 isolates showed irregular shaped edges. In terms of their characteristic colouration, 6 isolates showed cream colouration, 1 was milky white in colours while 3 showed brownish white colouration, 2 had a

greenish white colouration, 1 was creamy yellow and 1 was brownish yellow and the diameter of the isolates ranges from 0.1 to 3.7 cm in sizes. 11 isolates were opaque and 3 transparent.

S/N	Isolate code number	Shape	Elevation	Transparency	Colour	Edge	Size (cm)
1	DS a	Circular	Flat	Opaque	Cream	Rough	3.7
2	DS b	Circular	Convex	Transparent	Cream	Smooth	2.3
3	KS c	Circular	Convex	Opaque	Cream	Smooth	2.7
4	DS d	Circular	Flat	Opaque	Greenish white	Rough	0.6
5	KS e	Circular	Flat	Opaque	Cream	Rough	3.1
6	PS f	Circular	Convex	Opaque	Brownish white	Smooth	1.3
7	PS g	Circular	Convex	Transparent	Cream	Smooth	0.1
8	KS h	Circular	Convex	Opaque	Brownish white	Smooth	0.4
9	PS i	Circular	Flat	Opaque	Milky white	Rough	2.5
10	PS j	Circular	Flat	Opaque	Cream	Rough	0.8
11	KS k	Circular	Flat	Opaque	Greenish white	Rough	0.5
12	PS l	Circular	Convex	Transparent	Creamish yellow	Smooth	1.2
13	KS m	Circular	Convex	Opaque	Brownish white	Smooth	0.3
14	PS n	Circular	Convex	Opaque	Brownish yellow	Smooth	0.3

Key:

PS1 – Petrol Sample plate 1 PS2 –Petrol Sample plate 2 PS3 – Petrol sample plate 3

KS1 –Kerosene Sample plate 1 KS2 –Kerosene Sample plate 3 KS3 –Kerosene Sample plate 3

DS1 –Diesel Sample plate 1 DS2 –Diesel Sample plate 2 DS3 –Diesel Sample plate 3

Table 2.3 below shows the cellular morphology, biochemical characterization and Gram reaction of the isolates from this study with reference to Bergey's manual of determinative bacteriology 9th edition (1995). It was observed that *Bacillus* and *Pseudomonas* species were the predominant isolates in the whole of the hydrocarbon contaminated soil samples screened followed by *Citrobacter* and *Aeromonas* species, then *Acinetobater, Corynebacterium, Staphylococcus* and *Flavobacterium* species.

114

115

Table 2.3: Gram stain, Morphology and Biochemical characteristics of isolates

S/N	Isolates	Cell morphology	Grams Reaction	Catalase	Indole	MR	VP	H_2S	Spore test	Motility	Citrate	Glucose	Sucrose	Lactose	Mannitol	Urea	Coagulase	Heamolysis	Probable genera
1	DSa	Rods	+	+	-	+	+	-	+	-	+	A/G	-	A	+	-		β	*Bacillus* s
2	DSb	Rods	-	+	+	+	+	+	-	-	-	A/G	-	-	+	-		α	*Aeromonas*
3	KSc	Rods	-	+	+	+	+	+	-	-	-	A	-		+	-		α	*Aeromonas*
4	DSd	Rods	-	+	-	-	-	-	-	+	+	A	-	-	-	-		α	*Pseudomonas*
5	KSe	Rods	-	+	-	+	+	-	+	+	+	A/G	-	-	+	-		β	*Bacillus* s
6	PSf	Coccobacilli	-	+	-	-	-	-	-	-	+	A/G	-	A	+	-		γ	*Acinetobacter* spp.
7	PSg	Rods	+	+	-	+	-	-	-	-	+	A/G	-	A	-	-		γ	*Corynebacterium* spp.
8	KSh	Rods	-	+	-	+	-	+	-	+	+	A/G	A	A	+	-		γ	*Citrobacter* spp.
9	Psi	Rods	+	+	-	+	+	-	+	+	+	A/G	-	A	+	-		β	*Bacillus* spp.
10	PSj	Rods	-	+	-	-	-	-	-	+	+	A	-	-	+	-		α	*Pseudomonas* spp.
11	KSk	Rods	-	+	-	-	-	-	-	+	+	A	-	-	+	-		α	*Pseudomonas* spp.
12	PSl	Cocci	+	+	-	+	+	-			+	A	A	A	+	-	+	β	*Staplylococcus aureus*
13	KSm	Rods	-	+	-	+	-	+	-	-	+	A/G	A	A	+	-		γ	*Citobacter* spp
14	PSn	Rods	-	+	-	-	+	-	-	-	-	A	-	A	+	-		γ	*Flavobacterium* spp.

Statistical Analyses: Simple percentage was used to calculate the frequency of occurrence of each isolate.

Discussion

Soil had been known to be a favourable habitat for the proliferation of microorganisms, with micro colonies developing around soil particles, but the addition of refractory humic substances slow down the activities of these organisms, thus giving room to organisms that have the ability of metabolizing such products and limiting the growth of non-metabolizers of the products (Obire and Nwaaubeta, 2002).Biodegradation has been considered as efficient, economic,

versatile and environmentally sustainable treatment. Degradation of petroleum hydrocarbons by environmental micro flora involves microorganisms having specialized metabolic capacities.

Contaminated soil was analyzed for hydrocarbon utilizing bacteria. The hydrocarbon utilizing bacteria ranged from 1.5×10^3 to 2.8×10^7 cfu/ml.Thus the counts of hydrocarbon- utilizing bacteria obtained from the contaminated soil were very high. This may be attributed to the fact that contaminated soils often harbour vast array of microbial flora that is capable of utilizing the hydrocarbon as energy and carbon source and also due to the presence of residual hydrocarbon in the polluted soil which boosts the carbon supply in the soil, hence favours the growth of the hydrocarbon utilizing bacteria (HUB) which is in line with the report of Ijah and Abioye (2003). It has been observed that hydrocarbon discharge to the ecosystem may result in the high microbial population in the soil (Okpokwasili, 2000). Fourteen bacteria isolates which include organisms from the genera *Corynebacterium, Acinetobacter, Pseudomonas, Citobacter, Staphylococci, Bacillus, Flavobacterium* and *Aeromonas* isolated from the contaminated soil were similar to the reports of Obire and Nwaauebeta. The genera *Pseudomonas* and *Bacillus* were the most predominant as expected because they are commonly ubiquitous especially the ones in oil polluted areas (Makkar and Cameotra, 2002). Previous reports have proved extensive microbial diversity with population estimated between approximately 4×10^3 to 10^4 species per g of uncontaminated soil (Adesemoye *et al.,* 2006). Some microorganisms are more abundant in areas of high concentration of hydrocarbons as they are very resistant such as *Pseudomonas* spp. able to form adaptive structures such as spores as seen in *Bacillus* spp. and their ability to use nitrate as an electron acceptor and in the process producing or giving off hydrogen sulphide gas as seen in *Citobacter* spp. and *Aeromonass* spp. The microorganisms isolated have been implicated in hydrocarbon degradation and are among such organisms. The hydrocarbon-utilizing bacteria isolated were dominated by Gram negative bacteria belonging to a wide range of taxa which is in accordance with the report of Adesemoye *et al.,* (2006). On the basis of the growth data, we could infer that petrol was highly preferred by the isolates in relation to the reports of Amanchukwu *et al.,* (2000) and Okpokwasili (2000). This accounts for the ability to rapidly degrade low density and low molecular weight hydrocarbon as compared to those of kerosene and diesel which were utilized but at a much slower rate.

Conclusion

Cleaning up of petroleum hydrocarbons in the subsurface environment is a real world problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants. Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. This paper describes the study on the isolation and identification of petroleum (crude) oil-degrading bacterium from contaminated oil region. Out of the total fourteen bacterial isolates, the eight genera isolated are promising organisms with degradation capability and this selected bacterial isolates could be effective in cleaning oil spills or oil contaminated soils accordingly with their prevalence; *Pseudomonas* spp. 21.43%, *Bacillus* spp. 21.43%, *Aeromonas* spp. 14.29%, *Citrobacter* spp. 14.29%, *Acinetobacter* spp. 7.14%, *Corynebacterium* spp. 7.14%, *Staphylococci* spp. 7.14%, and *Flavobacterium* spp. 7.14%. The result of the present study revealed that the soil in Zuma, Bwari area council, Federal Capital Territory, Abuja, in Nigeria harbours hydrocarbon degraders which are useful in bioremediation of oil polluted soils in the region. The biases associated with culture dependent microbial enumeration techniques limited the full description of the bacterial diversity in these soil samples (Chikere *et al*., 2009a, 2009b; Lawson *et al*., 2013 and Ekhaise and Nkwelle, 2011). It is an established fact that more than 90% of micro-organisms in the environment may not be culturable and as such, can only be detected with molecular methods used in the field of metagenomics (Edlund and Jansson, 2006).

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