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Full Length Research Paper

Degradative activity of bacteria isolated from hydrocarbon-polluted site in Ilaje, Ondo State, Nigeria

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A study was carried out to isolate hydrocarbon-degrading bacteria associated with environmental samples collected from Ilaje coastal area, Nigeria. The samples were analyzed microbiologically using standard microbiological techniques. These organisms were further studied to determine their biodegrading activities on hydrocarbons (diesel, kerosene, petrol) using enrichment medium. The microbial growths were determined using spectrophotometer blanked at 600 nm. The nine bacteria isolated from environmental samples were *Enterobacter aerogenes, Pseudomonas aeroginosa, Aerococcus viridian, Clostridium sporogenes, Staphylococcus aureus, Lactobacillus acidophilus, Micrococcus luteus, Streptococcus faecalis* and *Bacillus* sp. It was observed from the result that the length of incubation had significant effect on degradation as well as the cell load. For all the bacteria, there was general increase in length of incubation with the various hydrocarbons. The results showed that there was degradation of oil, mostly between days 1 and 3. It was also observed that there was a gradual decline in the concentration of the broth, between days 4 and 7, which suggests decrease in the bacterial population and that the oil was being degraded. The test on the degrading activity of isolates on hydrocarbon revealed that *S. aureus, C. sporogenes*, *S. faecalis* and *Bacillus* sp. were the best degraders of kerosene, petrol and diesel, respectively. The ability of these isolates to degrade hydrocarbons is clear evidence that their genome harbors the relevant degrading gene. However, an important limiting factor is the slow rate of degradation which often limits the practicality of using microorganisms in remediating hydrocarbon impacted environment. Further research in this area can make a marked improvement.

Key words: Biodegrading activity, bioremediation, microorganisms, incubation period, oil spill.

INTRODUCTION

Petroleum (crude oil) is a complex mixture of hydrocarbons, mostly saturated or aromatic. The molecular sizes are separated into fractions based on boiling points. The components are dissolved natural gas, gasoline, benzene, xylenes, naphthalenes, octanes, camphor, kerosene, diesel, fuel, heating oil and tars (Wilhelm and Bloom, 2000; Dubey, 2009). Since the discovery of petroleum in 1956 in Nigeria, it is known to have much economic importance, producing at a rate of 818 million barrels in 2004 from more than 150 oil fields, mostly in the Niger Delta (Microsoft Encarta, 2009). It is a major source of Nigeria's export earnings. Virtually, 100% of export earnings and about four-fifths of government revenues are derived from petroleum (Microsoft Encarta, 2009). In fact, the benefits of crude oil cannot be over-emphasized. It has been the major sources of revenue, energy and employment generation in Nigeria. Oil exploration and exploitation are increasing across the globe because of the ever-increasing demand for energy. These are known to have a lot of good impact on the nation's economy. However, exploration, exploitation, production, transportation and storage of crude oil often results in oil spill incidents, which could be accidental. There are often deliberate or indiscriminate discharges of

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crude oil into the ecosystem such as; sabotage, oil theft/ bunkering, operational error, and vandalism. These are known to cause ecological problems of great dimension in the ecosystem. It is obvious therefore that the growth and activities of petroleum associated industries in Nigeria and in other parts of the world has led to increased oil pollution in the environment.

Ilaje community, located in the Southern part of Ondo State, closer to the Atlantic Ocean, is one of the oil producing communities in the Niger Delta region of Nigeria. The major occupation of the indigenes is fishing; hence, a large fish market is located in the area that attracts buyers from all parts of the country. Oil explorations in these communities sometimes lead to pollution of lands and water bodies due to spillage. The continuous exploration, production and processing of crude oil and their transportation exposes the environment to constant threat of oil pollution. Crude oil is among the most ubiquitous and persistent environmental contaminants (Afolabi et al., 1985; Ekweozor, 1989; Onwuka, 2005) because it is capable of causing serious damages to human and the ecosystem. It has been reported that the greatest single environmental problem connected with crude oil exploration in Nigeria is oil spillage both on shore and off shore (Okpokwasili, 1996).

Oil spillage has detrimental effects on both plants and animals. It is reported that oil spillage has caused constant threat to farmlands, crop plants, forest tree species and other vegetations in oil producing areas in Nigeria (Ogri, 2001; Agbogidi, 2003). There have been over 4,000 oil spills in the Niger-Delta area of Nigeria since 1960 (N. C. A. B, 2003). Toxicity of crude oil depends on its physical and chemical composition, the amount of the oil, the plant species and time of application as well as other environmental conditions (Baker, 1970; Nagaele, 1987; Agbogidi et al., 2005b). The direct effect of oil spillage on the ecosystem includes damage of fur and feathers of birds making them prone to death by freezing, accidental poisoning, blindness, liver damages, disabilities and infertility. It affects soil fertility adversely, causes alterations in soil physiochemical and microbiological properties, thereby having detrimental effects on the terrestrial and aquatic ecosystems.

In order to reduce or eliminate the effect of oil spillage on the environment and living organisms, efforts such as application of chemical dispersants, skimming of the surface oils, application of biological oil agents and inoculating the spilled areas with relevant bacteria are the outcomes of intensive research. The most promising of many researches carried out to deal with large scale oil spillage is the use of microorganisms to provide an effective alternative (Singh et al., 2001). This is bioremediation, a process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition (Okon and Trejo-Hernandez, 2006). Dua et al. (2002) reported that microorganisms are capable of using organic substances, natural or synthetic, as sources of nutrients and energy hence, exhibiting remarkable range of degrading capabilities. For bioremediation to be considered as an applicable technology to the clean-up of a specific pollutant, it is necessary to show that a specific chemical or chemical mixture is biodegradable and that the process of bioremediation will not result in outward ecological side effect (Atlas and Bartha, 1998). The qualitative and quantitative aspects of bioremediation of pollutants are dependent on the composition of the indigenous microbial community, the ambient and seasonal environmental conditions. The temperature, pH, adequate inorganic nutrients and relative humidity of the environment are factors that affect the growth of these microorganisms (Dubey, 2009). These microbes derive nutrients and energy for optimal growth and reproduction from the hydrocarbons so as to degrade them. Biodegradation, which is the destruction of organic compounds by microorganisms is carried out largely by diverse bacterial populations, mostly *Pseudomonas* species. The hydrocarbon-degrading populations are widely distributed in the lands and water bodies. In a research carried out by Ojo (2006), hydrocarbons-utilizers detected included *Bacillus megaterium, Pseudomonas putida, Micrococcus luteus, Bacillus brevis, Bacillus pumilis and Enterobacter aerogenes.* When an environment is contaminated with petroleum, the proportion of hydrocarbon-degrading microorganisms increases rapidly. In particular, there is an increase in the proportion of bacterial populations with plasmids containing genes for utilization. It is reported that the proportion of hydrocarbon-degrading bacterial population in hydrocarbon-contaminated environments often exceeds 10% of the total bacterial population (Atlas, 1995).

There is the need to test for the biodegrading capabilities of microorganisms associated with oil spill because not all microbes isolated from hydrocarbons are able to degrade/utilize them. The ability of an organism to degrade a specific substrate is clear evidence that its genome harbors the relevant degrading gene (Cowan and Strafford, 2007). The aim of this study therefore, is to isolate and identify hydrocarbon-degrading bacteria associated with oil polluted site in Awoye village, Ilaje, Nigeria and test their ability to degrade different hydro-carbons. The expected outcome of this study will provide information on the bacterial population, hydrocarbon-degrading microorganisms and their degrading ability on kerosene, diesel and petrol.

MATERIALS AND METHODS

Source and collection of samples

Environmental samples (soil and water) were collected from Awoye village in Ilaje, Ondo State, Nigeria after 24 months of oil spillage. Awoye village is situated between 6° latitude and 5° longitude. It is located in the Southern part of Ondo State very close to the Atlantic Ocean. The area was reported to have been polluted with hydrocarbon in 2004 (Nigerian Tribune of Tuesday, 12th August,

2004). The soil samples (AWS1 – AWS10) were collected by means of soil auger into sterile cellophane bags, while the water samples were collected aseptically into screw-capped containers from twenty different locations and depths. The depths considered were 0 to 10 cm (AW1D10 – AW9D10) and 11 to 20 cm (AW10D20- AW20D20) from the water surface. Unpolluted samples AWS5C and AW21DC were also collected from the area for soil and water, respectively. These samples were taken to the laboratory for microbiological analyses.

Bacterial counts

The samples were microbiologically analyzed using the pour plate method (Song and Bartha, 1990). 1 g of the moist soil and 1 ml of water samples were used to make ten fold dilution series in triplicates. 1 ml each from dilution 10^{-4} was seeded into nutrient agar plates for soil and water and incubated at 37°C for 24 h. The bacterial counts were thereafter, enumerated on nutrient agar.

Isolation and characterization of isolates

Pure isolates were obtained using streak techniques and stored at 4°C in agar slant. Nutrient agar, composed of meat extract (1 g/L); peptone (5 g/L); yeast extract (2 g/L); sodium chloride (8 g/L); agar (15 g/L); and distilled water (1 L) was used to prepare the agar slant. Individual colonies were identified by morphological and biochemical techniques using the taxanomic scheme of Beygey's Manual of Determinative Bacteriology (Holt et al., 1994).

Enumeration of oil-degrading bacteria

The mineral salt medium (MSM) as described by Ijah and Abioye, (2003) was used. The composition of the MSM is as follows: K_2HPO_4 (1.8 g/L); NH_4Cl (4 g/L); $MgSO_4.7H_2O$ (0.2 g/L); NaCl (0.1 g/L); $Na_2SO_4.7H_2O$ (0.01 g/L); agar (20 g/L); carbon source (1%); and distilled water (1L) with pH 7.2. The medium was sterilized by autoclaving at 121°C for 15 min. The samples were serially diluted and 1 ml from 10^{-4} dilutions was seeded in the MSM agar. The medium was supplemented with 1% (v/v) filter sterilized hydrocarbons (kerosene, petrol and diesel) to serve as the only source of carbon and energy (Ijah and Abioye, 2003). The medium was incubated at room temperature for 3 days.

Biodegrading activities of bacteria on oil

The degrading activities of each isolates were obtained by using Mineral salt broth (MSB) in which 1% of each hydrocarbon (petrol, kerosene and diesel) was added and incubated at room temperature for seven days. The optical density (OD_{600nm}) was read using spectrophotometer after which the degrading activities (Unit/mL/hr) for each isolates were calculated.

RESULTS

Bacterial counts

The bacterial counts ($x10^4$) ranged between 20.14 ± 18 and 41.11 ± 2 Cfu/ml for soil samples, while the bacterial counts ($x10^4$) for the unpolluted soil was 18 ± 2.3 (Table 1). The bacterial counts ($x10^4$) for the water samples collected at depths 0 to 10 cm varied between 13.33 ± 2.8 and 21.66 ± 2.8 Cfu/ml. The samples collected from the depths (11 to 20 cm) had bacterial counts ($x10^4$) between 1.33 ± 1.2 and 11.66 ± 1.7 Cfu/ml, while the samples collected from the unpolluted sample had bacterial counts of 9.88 ± 1.8 Cfu/ml (Table 2). It could be observed from the results that the bacterial counts obtained decreased as the depth increased. The study reveals that bacteria were present in the polluted as well as unpolluted samples.

Table 1. Enumeration of microorganisms from oil-polluted soil.

Samples	Mean of bacterial counts ($x10^6$ Cfu/mL)
AWS1	23.24 ± 1.6
AWS2	34.45 ± 1.2
AWS3	24 ± 2.2
AWS4	38.33 ± 3.4
AWS5C	18 ± 2.3
AWS6	34.22 ± 1.7
AWS7	32.45 ± 2.4
AWS8	20.14 ± 18
AWS9	30.12 ± 2.3
AWS10	41.11 ± 2

AW -Awoye, S- Soil, C -Positive control.

Identification of bacteria

Table 3 shows the morphological and biochemical characteristics of the nine bacterial isolates found in the soil and water samples. The seven bacteria isolated from the soil samples were *Enterobacter aerogenes, Pseudomonas aeroginosa, Aerococcus viridian, Clostridium sporogenes, Staphylococcus aureus, Lactobacillus acidophilus* and *Bacillus* sp., while the seven isolated from water samples were *M. luteus, Aerococcus viridans, C. sporogenes, S. aureus, Streptococcus faecalis, L. acidophilus* and *Bacillus* sp. All the isolates were gram positive with the exception of *E. aerogenes* and *P. aeroginosa.* Only *C. sporogenes, S. aureus,* and *Bacillus* sp. were positive to spore stain, while only *S. aureus* was coagulase positive.

Hydrocarbon-degrading bacteria

Table 4 shows the population of hydrocarbon-degrading bacteria in soil, while Table 5 shows the population of hydrocarbon-degrading bacteria in water. The mean of kerosene-degrading bacteria ($x10^4$) Cfu/ml ranged from 11.00 ± 2.1 to 37.33 ± 5.7 and 8.00 ± 2.3 to 21.33 ± 3.5 in soil and water, respectively. In diesel, the mean of degrading bacteria ($x10^4$) Cfu/ml ranged from 17.00 ± 2.4 to 32.66 ± 1.8 and 5.00 ± 1.3 to 14.66 ± 3.6; while the mean of petrol-degrading bacteria ($x10^4$) Cfu/ml ranged from 17.66 ± 4.3 to 40.00± 3.4 and 10.33 ± 2.1 to 24.00± 2.3 in

Table 2. Enumeration of microorganisms from oil-polluted water.

Oil-polluted water samples	Mean of bacterial counts (x10^{4}Cfu/ml)	Oil-polluted water samples (contd.)	Mean of bacterial counts (x10^{4}Cfu/ml)
AWID10	21.66 ± 2.8	AW12D20	10.33 ± 2.4
AW2D10	19.33 ± 1.2	AW13 D20	9.66 ± 1.8
AW3D10	21.33 ± 3.4	AW14 D20	4.33 ± 1.2
AW4D10	19.66 ± 2.2	AW15 D20	3 ± 2.2
AW5D10	17 ± 1.8	AW16 D20	3.66 ± 2.4
AW6D10	18.66 ± 1.4	AW17 D20	5.66 ± 1.8
AW7D10	15.66 ± 1.7	AW18 D20	2.33 ± 1.4
AW8D10	17 ± 3.5	AW19 D20	5.33 ± 3.4
AW9D10	13.33 ± 2.8	AW20 D20	1.33 ± 1.2
AW10D20	11.66± 1.7	AW21DC	9.88 ± 1.8
AW11D20	10.66 ± 2.4		

AW -Awoye, D10 -Depth (0 – 10) cm, D20 -Depth (11 – 20) cm, DC -Positive control.

Table 3. Morphological and biochemical characteristics of bacteria isolated from oil polluted soil and water.

Probable organism	*Micrococcus luteus*	*Enterobacter aerogenes*	*Pseudomona saeroginosa*	*Aerococcus viridans*	*Clostridium sporogenes*	*Staphylococcus aureus*	*Streptococcus feacalis*	*Lactobacillus acidophilus*	*Bacillus* sp.
Gram stain	+	-	-	+	+	+	+	+	+
Spore stain	-	-	-	-	+	+	-	-	+
Catalase	+	+	-	-	+	-	-	-	+
Motility	-	+	+	-	-	-	-	-	+
Coagulase	-	-		-	-	+	-	-	
Indole		-	-			-		-	-
MR-VP		-/+	-/+			-/+			-
Starch hydrolysis	+	-	-	+	+	-	+		-
Glucose	-	AG	AG	A	A	A	A	AG	AG
Lactose	+	AG	AG	+	+	-	=	+	A
Sucrose	+	-	AG	+	+	A	+	-	AG
Maltose	+	A	-	+	+	AG	+	-	A
Arabinose	+	AG	A	+	+	A	+	-	AG
Fructose		A	A			A			A
Oxidase		-	-			-	-		-
Shape	Round in clusters	Rods	Short rod	Spherical	Rod	Coccus	Round	Rod	Rod
Edge	Dented	Serrated	Dented	Dented	Dented	Dented	Dented	Dented	Entire
Elevation	Raised	Flat	Raised	Raised	Raised	Raised	Raised	Raised	Raised

Table 3. Contd.

Surface Pigmentation	Smooth Creamy	Smooth White	Smooth White	Smooth Creamy	Smooth Creamy	Smooth Creamy	Rough Pinkish	Smooth Creamy white	Entire margin Creamy

+ -positive, - negative, A -Acid production, G -Gas production.

Table 4. Enumeration of oil-degrading bacteria isolated from oil-polluted soil samples.

Samples	Mean of kerosene-degrading bacteria ($x10^4$ Cfu/mL)	Mean of diesel-degrading bacteria ($x10^4$ Cfu/mL)	Mean of petrol-degrading bacteria ($x10^4$ Cfu/mL)
AWSI	31.33 ± 3.6	22.00 ± 3.6	39.33 ± 2.5
AWS2	37.33 ± 5.7	25.33 ± 2.5	40.00 ± 3.4
AWS3	17.33 ± 4.4	20.66 ± 5.3	25.66 ± 1.5
AWS4	31.33 ± 2.3	17.00 ± 2.4	33.33 ± 1.0
AWS5C	11.00 ± 2.1	22.00 ± 1.5	23.33 ± 5.3
AWS6	33.00 ± 3.2	20.33 ± 3.5	37.33 ± 5.0
AWS7	17.66 ± 4.0	22.66 ± 2.2	27.33 ± 2.6
AWS8	12.66 ± 2.5	21.33 ± 2.6	17.66 ± 4.3
AWS9	21.00 ± 1.4	23.00 ± 1.3	21.00 ± 3.4
AWS10	31.00 ±± 3.2	32.66 ± 1.8	40.00 ± 2.3

Table 5. Enumeration of oil-degrading bacteria isolated from oil-polluted water samples.

Samples	Mean of kerosene-degrading bacteria ($x10^4$ Cfu/mL)	Mean of diesel-degrading bacteria ($x10^4$ Cfu/mL)	Mean of petrol-degrading bacteria ($x10^4$ Cfu/mL)
AWID10	21.33 ± 3.5	14.66 ± 3.6	24.00 ± 2.3
AW2D10	10.33 ± 1.1	10.66 ± 2.4	12.66 ± 1.1
AW3D10	17.33 ± 3.1	11.00 ± 2.1	13.33 ± 1.7
AW4D10	13.00 ± 1.4	10.00 ± 1.7	11.00 ± 1.8
AW5D10	14.66 ± 1.3	12.22 ± 1.2	14.33 ± 2.6
AW6D10	18.00 ± 1.5	8.33 ± 1.8	15.00 ± 1.1
AW7D10	9.66 ± 4.5	8.66 ± 2.4	12.33 ± 2.7
AW8D10	9.33 ± 1.1	7.00 ± 3.2	12.66 ± 2.3
AW9D10	12.00 ± 2.2	9.0 ± 2.5	17.00 ± 2.5
AW10D20	8.00 ± 2.3	10.66 ± 2.3	14.33 ± 1.2
AW11D20	15.33 ± 1.4	7.33 ± 2.4	13.66 ± 1.8
AW12D20	8.66 ± 3.4	6.66 ± 3.3	14.33 ± 1.2

Table 5. Contd.

AW13 D20	12.33 ± 2.3	5.0 ± 1.3	13.66 ± 2.5
AW14 D20	11.33 ± 1.4	9.66 ± 2.5	12.33 ± 2.8
AW15 D20	11.66 ± 1.1	6.33 ± 2.4	12.00 ± 2.7
AW16 D20	13.00 ± 2.3	6.33 ± 2.2	10.00 ± 1.7
AW17 D20	11.00 ± 2.3	7.66 ± 1.5	10.66 ± 1.8
AW18 D20	16.00 ± 2.4	7.33 ± 1.7	11.00 ± 1.8
AW19 D20	10.33 ± 2.2	7.60 ± 2.6	10.33 ± 2.1
AW20 D20	11.33 ± 2.3	7.33 ± 2.4	11.33 ± 2.5
AW21 DC	4.5 ± 2	4.2 ± 2.2	5.5 ± 1.2

soil and water, respectively.

Biodegrading activity and incubation period of each isolates on hydrocarbons

Figures 1 to 3 show the biodegrading activity of each isolates on hydrocarbons (kerosene, diesel and petrol). *S. aureus* demonstrated the greatest ability to degrade kerosene with degrading activity of 12.6 Unit/ml/h on day 1, while *C. sporogenes* demonstrated the greatest ability to degrade both diesel and petrol with degrading activities of 14.7 and 14.3 Unit/ml/h, respectively on day 1. The effects of incubation period on the degrading activity of the oil-degrading bacteria are also illustrated in the figures.

DISCUSSIONS

It is obvious from this study that when the environment was contaminated with petroleum, the proportion of hydrocarbon-degrading micro-organisms increased rapidly. High numbers of certain hydrocarbon-degrading microorganisms from an environment implies that those organisms are the active degraders of that environment (Okerentugba and Ezeronye, 2003). In particular, the proportion of bacterial populations with plas-mids containing genes for utilization are increased (Atlas, 1995).

The fact that these organisms were isolated from the hydrocarbon-polluted environ-mental samples showed that there were able to exist in the oil spilled environment, while microorganisms that could not survive in this environment were eliminated by the unfavourable condition caused by the oil spill. Most of the isolates are predominantly indigenous micro-organisms of coastal region, which are constantly exposed to different petroleum contaminants. The presence of oil-degrading organisms in the polluted soil and water is clear indication that the indigenous microbes were carrying out their metabolic activity. The activities of these microorganisms could be responsible for the bioremediation of the environment (Ojo, 2006).

Effects of incubation period on the degrading activity of the oil-degrading bacteria are illustrated in Figures 1 to 3. Our results showed that all the organisms maximally utilized all the hydrocarbon substrates (petrol, kerosene and diesel) when supplied as the sole source of carbon and energy. Although, the level of utilization differs from one microbe to another (due to differences in their growth) and from one hydrocarbon substrate to the other; due to the obvious differences in their molecular sizes. The bacterium with the highest degrading activities on kerosene was *S. aureus*, while the least was *L*, *acidophilus. C. sporogenes* exhibited the highest degrading activity on diesel and petrol, while the least hydrocarbon-degrading bacteria were *E. aeroginosa* and *L. acidophilus*, respectively. These degrading capabilities on different hydrocarbons revealed that the micro-organisms isolated from the soil and water samples were able to degrade hydrocarbons. The cells were able to multiply within the days of study, indicating that they were able to degrade and utilize the oil for their growth and develop-ment, hence the concomitant increase in the concentration of the broth (turbidity). This gradual increase in the concentration of the broth indicates bacterial growth (Aneja, 2007), hence degradation of hydrocarbons, mostly between days 1 and 3 and gradual decline in the concen-tration of the broth suggests decrease in the bacterial population and that the hydrocarbon has been degraded, mostly between days 4 and 7. It was observed that in kerosene, *S. aureus, S. faecalis* and *Bacillus* sp. had highest degrading activities on day 1; *M. luteus* and *A. viridans* on day 3, while *P. aeroginosa, S. faecalis*, *C. sporogenes* and *L. acidophilus* had the highest

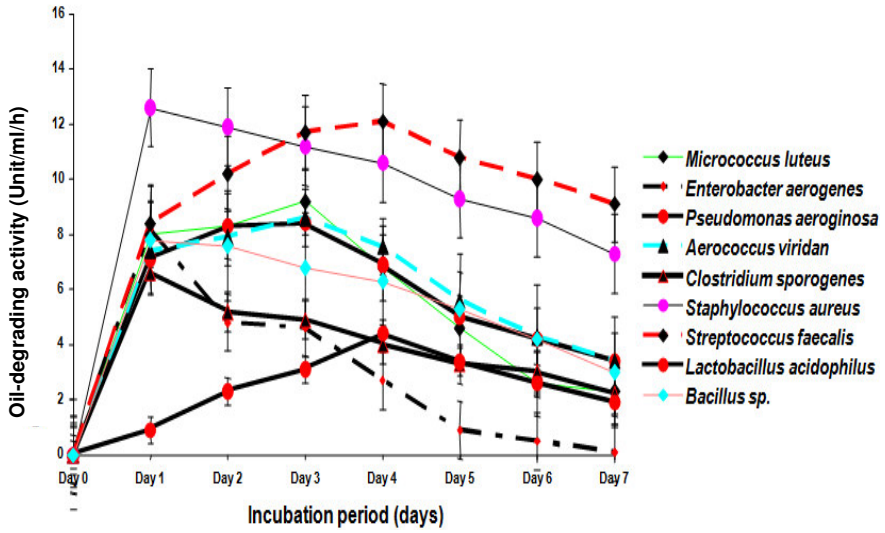

Figure 1. Effect of incubation period on degrading ability of isolates on kerosene.

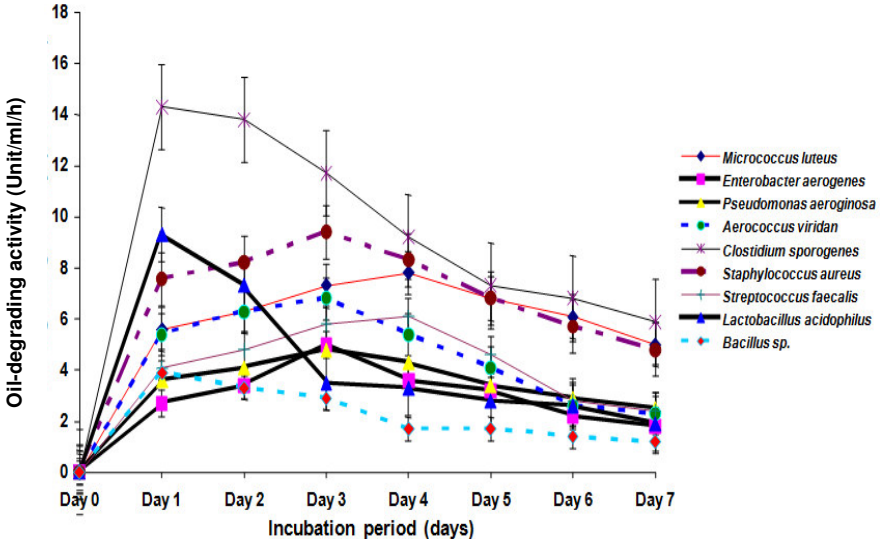

Figure 2. Effect of incubation period on degrading ability of isolates on diesel.

degrading activities on day 4. The degrading activity on diesel shows that *C. sporogenes* and *L. acidophilus* had the highest degrading activities on day 1; *S. aureus, A. viridans, Bacillus* sp, *E. earogenes* and *P. aeroginosa* had the highest on day 3, while *M. luteus* and *S. faecalis* had the highest on day 4. On petrol, *C. sporogenes,* and *Bacillus* sp. exhibited the highest degrading activity on day 1; *S. aureus, A. viridans, E. earogenes* and *P. aeroginosa* on day 3; and *S. faecalis* and *M. luteus* on day 4. The test on the degrading activity of isolates on hydrocarbon revealed that *S. aureus, C. sporogenes*, *S. faecalis* and *Bacillus* sp. were the best degraders of kerosene, petrol and diesel, respectively. The statistical analysis showed that, given similar conditions, there was significant difference in the degrading activity of the isolates on the three hydrocarbons at 95% confidence interval and that the incubation period had significant difference on the degrading activity of the isolates at 95%confidence interval. All the isolates degraded the hydrocarbons, suggesting similarities in the gene that encodes degradation in their genetic make up.

It is evident from this study that, hydrocarbon-degrading organisms can be isolated from hydrocarbon-polluted sites and that the degrading ability demonstrated by the microorganisms is a clear indication that they can be applied in the bioremediation process. Bioremediation is one of the most rapidly growing areas of environmental biotechnology, which has been used for the cleaning up

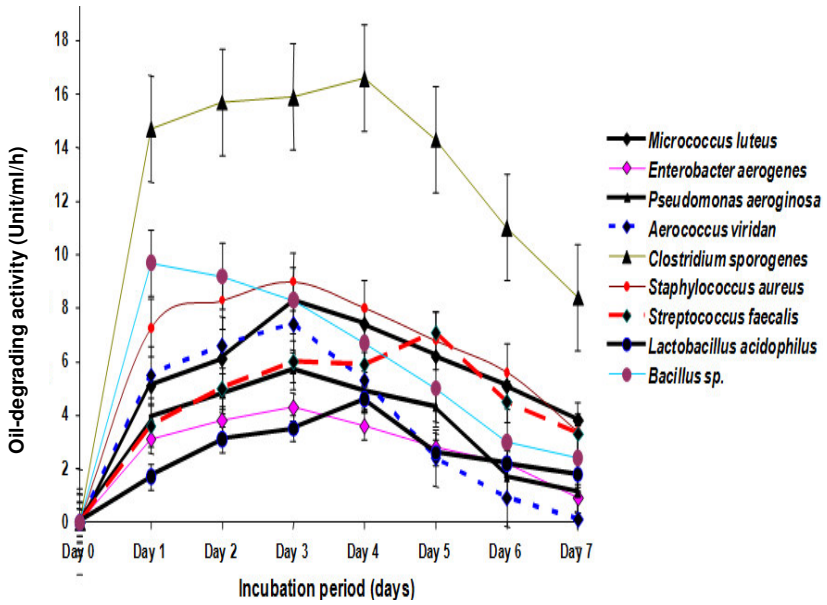

Figure 3. Effect of incubation period on degrading ability of isolates on petrol.

of pollutants. This is because of its low costs and its public acceptability (Grazyna et al., 2001). However, an important limiting factor is the slow rate of degradation, which often limits the practicality of using microorganisms in remediating hydrocarbon impacted environment. Further research in this area can make a marked improvement.

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