International Journal of Academic Research and Development
ISSN: 2455-4197, Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 2; Issue 4; July 2017; Page No. 308-313

Bacterial biodegradation of petroleum hydrocarbon using local isolates from tropical polluted environmental media

[1] George Ubong, [2] Inyang-Etoh Aniema, [3] Ekpo Antai
[1] Department of Zoology & Environmental Biology, University of Calabar, Calabar, Cross River State, Nigeria
[2, 3] Faculty of Oceanography, University of Calabar, Calabar, Cross River State, Nigeria

Abstract

Studies on bacterial biodegradation of petroleum hydrocarbon using local isolates from tropical polluted environmental media (soil and water) in Ibeno Local Government Area, Akwa Ibom State was conducted between October to December, 2016 with the aim of isolating bacteria strain that has the ability to biodegrade petroleum hydrocarbon in the event of pollution. In the laboratory standard procedures for isolating and identification of bacteria strains were used. The bacteria were identified as species of Baciillus (60%), Micrcoccus (30%) and Alcaligenes (10%). The aliphatic hydrocarbon was more readily utilized than the aromatics. Further studies revealed that *Bacillus megaterium* CDB5 and *Micrococcus luteus* CDB2 degraded the both type of diesel oil effectively. However, *B. megaterium* showed considerably higher ability in degrading both light and thick diesel oil than *M. luteus*. *B. megaterium* was able to cause weight losses of 49.5% and 41.9% of transniger pipeline and bonny light diesel oil respectively after 10 days of incubation while *M. luteus* cause weight losses of 35.5% and 29.1% of transniger pipeline and bonny light diesel oil respectively over the same incubation period and environmental conditions. *B. megaterium* also emulsify the both types of diesel oil while *M. luteus* could not emulsify any of the diesel oil used for the study. The result shows that *B. megaterium* CDB5 could serve as a potential candidate in seedling of oil spill environment in the event of pollution.

Keywords: bacteria, biodegradation, petroleum hydrocarbon, local isolates, tropical, environmental media

1. Introduction

In the marine environment, bacteria are considered to be the most efficient microorganism in degrading petroleum hydrocarbons (Atlas & Bartha, 1973) [2]. The most prevalent degrading bacteria in the marine ecosystem are Pseudomonas, Achromobacter, micrococcus, Nocardia, Vibro, Acienetobacter, Brovibacterium, Coryen -bacterium and Flavobacterium while Candida, Rhodotorula, Sporobolomyces and Clasdosparium are most prevalent hydrocarbon degrading fungi in the marine environment (Atlas & Bartha, 1973) [2].

Numerous bacteria strains, yeast, filamentous fungi, actinomycetes have been isolated from oil polluted environments, with bacteria yeast being the organisms isolated with great regularity (Zobell, 1969) [16]. These organisms have been reported to utilize various hydrocarbons. It has been shown that given favourable environmental conditions, microorganism will degrade a substantial portion (40-50%) of petroleum hydrocarbon.

When diesel oil spill in water due to nefarious activities such as, tanker operation and accident, offshore exploration and production, deliberate disposal of petroleum waste, corrosion of petroleum pipeline and sabotage, the oil spread on the water surface, volatile substances escape rapidly, whereas water soluble materials disperse. The in-dispersed and volatile crude oil components are subjected to microbial degradation (Odu, 1981) [11]. For bioremediation to take place in the event of spillage, microorganism capable of degrading and utilizing the hydrocarbon as a source of carbon must be present besides favourable environmental factors such as oxygen, temperature, salinity and nutrients levels (Odu, 1981) [11].

The objectives of this study was to evaluate the potential for use of indigenous petroleum oxidizers / utilizers in bioremediation of petroleum hydrocarbon polluted soils and water, also the potential enhancement of petroleum hydrocarbon removal from soil and water by controlled addition of lead chloride ($PbCl_2$) and zinc sulphate ($ZnSO_4$).

2. Materials and Methods

2.1 Collection of Samples

Oil polluted soil and water samples used were collected from oil spilled site at Ibeno, Akwa Ibom State, Nigeria and bulked. Equally, non-oil polluted soil samples were collected. The soil and water samples were collected in Ziploc bags and plastic bottles respectively and then transported to the laboratory for the isolation of bacteria in an ice chest. The petroleum hydrocarbons used were; Bonny light diesel oil, Transniger pipeline diesel oil (TNP), other petroleum products like kerosene, toluene, benzene and hexane were used. The petroleum hydrocarbons were collected from Port Harcourt refinery, Alesa-Eleme, Rivers State in sterile sampling bottles and stored at room temperature of (28±2ºC). The metal salts lead chloride ($PbCl_2$) and zinc sulphate ($ZnSO_4$) used were obtained from the Department of Pure and Applied Chemistry, University of Calabar, Calabar.

2.2 Sterilization of Media

All the media and diesel oil used in this study were sterilized by autoclaving at 121ºC for 15 minutes. Petroleum products were sterilized by filtration through membrane filter with 0.45m pore size (SM 16510 Satorius, D-34000 Germany).

2.3 Isolation and Identification of Bacteria
Bacteria were isolated by soil enrichment method of Frederick and Westlake (1981) [11]. In this method, 2ml of Bonny light diesel oil was introduced into a 500ml capacity conical flask containing 200ml of mineral salt medium. The content of the flask was then sterilized by autoclaving at 121ºC for 15 minutes. After sterilization, it was allowed to cool to about 45ºC then 2g of the soil sample was inoculated into the medium. The flask was then incubated at 28 ºC for 4 days in a Shaker incubator (Model Bks-300-010F, Gallenkamp, England), operated at 200rpm.

At the end of the incubation period, 0.1ml of the enrichment medium was plated in nutrient agar plate using spread plate method. The plates were then incubated at room temperature (28±2ºC) for 24 to 48 hours. Discrete colonies were picked and cultured on fresh nutrient agar plates (Zobell, 1973) [17].

2.4 Purification of Isolates
The isolates obtained were purified by repeated sub-culturing onto nutrients agar plates. The pure cultures obtained were maintained on nutrients agar slants in the refrigerator at 8^0C for further studies.

2.5 Characterization and Identification of Bacterial Isolates
Characterization of the bacterial isolates was based on microscopic technique and biochemical test (Gram's stain, motility test, catalase test, oxidase test, coagulase test, spore stain and nitrate reduction test) as described by Cowan (1974) [4] and Cruickshank *et al.* (1975) [5].

2.6 Screening Test for the Utilization of various Petroleum Hydrocarbons by Bacteria
The ability of the bacterial isolates to utilize the various petroleum hydrocarbons was determined by the method of Okpokwasili and Okorie (1988) [12] using sterile mineral salts medium of Zajic and Suplison (1972) [15]. Five millimeters of the mineral salts medium was pipette into each test tubes and 0.1ml (3.2 x 10^6 cells) of 24 hours old nutrient broth grown bacteria culture added. Duplicate test tubes were left un-inoculated to serve as control. All the test tubes were incubated without shaking at room temperature (28±2ºC) for 10 days. After 24 hours of incubation the cultures were observe usually for turbidity (as evidence of bacterial growth) as compared to the un-inoculated control. The extent of bacterial growth to grow in the hydrocarbon medium was represented as maximum growth (+++), moderate growth (++), minimal growth (+) and no growth (-). To confirm the results, each bacteria culture was sub-culture into nutrients agar plates and incubated at room temperature (28±2ºC) for 24 – 48 hours.

2.7 Enhanced Biodegradation and Emulsification Studies
2.7.1 Biodegradation of Diesel Oil
Based on rapid growth on diesel oil medium, two bacterial isolates *Bacillus megaterium* and *Micrococcus luteus* were selected for further studies. Degradation of the diesel oil was determined by the gravimetric analysis method of Mulkins-Phillips and Stewart (1974) [10]. In this procedure, 0.1ml (3.2 x 10^6 cells) of 24 hours old nutrient broth bacterial cultures was inoculated into 150ml mineral salt medium in a 250ml conical flask containing 1.5ml of either bonny light or transniger pipeline diesel oil as a sole source of carbon and energy. Mineral salts medium with diesel oil added but without bacterial culture served as control. All the experiment were set up in duplicates and incubated at 28^0C in a shaker incubator (Model Bks-300-010F, Gallenkamp, England), operated at 200rpm. Every 2 days the amount of diesel oil left was determined by extracting the residual diesel oil with 15ml di-ethly either into a reweighed bottle and left to evaporate at room temperature (28±2^0C) overnight.

The weighed of the bottle and content (residual diesel oil) was recorded using analytical balance (Model P165 Mettler instrument AG. Switzerland). The experiment was terminated after 10 days. The weighed of the residual diesel oil was calculated by subtracting the weighed of the empty bottle from the weighed of the empty bottle containing residual diesel oil.

Percentage degradation of the incorporated diesel oil was calculated thus; weight of diesel oil (control) minus weight of diesel oil (degraded) divided by weight of diesel oil (control) multiplied by 100.

2.7.2 Emulsification of Diesel Oil
Like other hydrocarbons the emulsification of bonny light and transniger pipeline diesel oil by *Bacillus megaterium* (CDB5) and *Micrococcus luteus* (CDB2) was determined by the method of Broderick and Cooney (1982) [3]. This method involved visual inspection of the opaque layer of the medium caused by emulsified oil droplets. The visual inspection was done at 2 days interval for 10 days.

2.8 Data Analysis
Data obtained was subjected to Analysis of variance (version 20.0) and Least Significant difference (LSD) test were employed to separate significant differences in mean values computed for the two metal salts. The probability level was set at $p = 0.05$.

3. Results
3.1 Characteristic and Identification of Bacterial Isolates
The bacteria isolates from water and soil were characterized based on some important biochemical test. The characteristics of the bacterial isolates are presented in Table 1. The bacteria isolates were identified as

species of Bacillus, Micrococcus and Alcaligenes (Table 1). The results revealed that 60% of the isolates were Bacillus spp., followed by Micrococcus spp with 30% while Alcaligenes spp. Formed 10% of the total isolates.

3.2 Bacterial Utilization of Petroleum Hydrocarbon

The results of the ability of the bacteria isolates to utilize various petroleum hydrocarbons as a sole source of carbon and energy are presented in Table 2.Kerosene oil and Hexane supported the growth of all the ten bacterial isolates tested. The ten bacteria isolates showed maximum growth to minimal growth in the different petroleum hydrocarbon exception of CDB3 that was able to utilize only kerosene oil and hexane (Table 2). The results revealed that hexane was highly preferred by the organism while toluene was the least preferred petroleum products (Table 2).

3.3 Degradation of Transniger Pipeline and Bonny Light Diesel Oil by *Bacillus megaterium* CDB5 and *Micrococcus luteus* CDB2

The results obtained from the degradation of transniger pipeline diesel oil revealed that the two organisms degraded the diesel oil effectively. However, *B. megaterium* exhibited considerably higher ability in degrading the oil than *M. luteus* (Table 3). *B. megaterium* caused a 49.5% decrease in weight of the oil after 10 days of incubation, while *M. luteus* caused a 35.5% decrease in weight of the same diesel oil over the same incubation period.

The results of the degradation of bonny light diesel oil by *B. megaterium* and *M. luteus* is presented in Table 4. The results revealed that *B. megaterium* degraded bonny light diesel oil more efficiently than *M. luteus*. *B. megaterium* caused a 41.9% decrease in weight of the bonny light diesel oil after 10 days of incubation, while *M. luteus* caused a 29.1% decrease in weight of the same diesel oil over the same incubation period. The results (Table 3 and Table 4) revealed that the two organisms degraded transniger pipeline diesel oil more efficiently than bonny light diesel oil under the same environmental conditions and incubation time.

3.4 Emulsification of Transniger Pipeline and Bonny Light Diesel Oil by *Bacillus megaterium* CDB5 and *Micrococcus luteus* CDB2

The results of the emulsification of transniger pipeline and bonny light diesel oil by *B. megaterium* and *M. luteus* is presented in Table 5. The results revealed that only *B. megaterium* was able to emulsify the two types of the diesel oil. However, transniger pipeline diesel oil was greatly emulsified by the organism than bonny light diesel oil. Transniger pipeline diesel oil was emulsified by the organism within 2 days of incubation while bonny light diesel oil was emulsified by the same organism after 4 days of incubation.

3.5 Effects of Heavy Metal on the Degradation of Bonny Light Diesel Oil

The results of the effects of heavy metals ($PbCl_2$ and $ZnSO_4$) on the degradation of bonny light diesel oil *B. megaterium* showed that 0.05% and 0.1% of $PbCl_2$ enhanced diesel oil degradation by the organisms while 0.5% of the metal salt decreases oil biodegradation. Similar results were obtained when the diesel oil medium was supplemented with different concentration of $ZnSO_4$. However, 0.1% $ZnSO_4$ supported more diesel oil degradation by the organism than 0.1% $PbCl_2$. The organism degraded 54.6% of the diesel oil after 10 days when the medium was supplemented with 0.1% of $PbCl_2$ while 61.7% degradation was observed over the same incubation period when 0.1% of $ZnSO_4$ was added to the medium (Table 6 and Table 7). Statistical analysis using analysis of variance (Version 20.0) shows that the different concentrations of the two metal salts used significantly ($p<0.05$) influenced the degradation of the oil by the organism. Least significant test (LSD) revealed that the rates of degradation of the oil as influenced by the various concentrations of $PbCl_2$ and $ZnSO_4$ were significantly ($p<0.05$) different from each other. Analysis however, indicated that the rate of oil degradation by the organism when the medium was supplemented either with 0.05% $PbCl_2$ or 0.05% $ZnSO_4$ were not significantly ($p<0.05$) different from the rates of diesel oil degradation by the same organism when the oil medium was not supplemented with the metal salt.

Table 1: Characterization and Identification of Bacterial Isolates

															Carbohydrate Utilization					
Isolates tested	**Cell shape**	**Gram's reaction**	**Mobility**	**Catalase**	**Oxidase**	**Coagulase**	**Spore stain**	**Starch hydrolysis**	**Nitrate reduction**	**Indole test**	**Urease**	**Citrate**	**M.R**	**V.P**	**Glucose**	**Lactose**	**Sucrose**	**Xylose**	**Maltose**	**Probable Organism**
CDB1	Rod	-	+	+	+	-	+	+	+	-	-	-	-	+	-	-	-	-	-	*Alcaligenes SP*
CDB2	Cocci	+	-	+	-	-	-	-	+	-	+	-	+	-	A	-	A	-	A	*Micrococcus luteus*
CDB3	Cocci	+	-	+	-	-	-	-	-	-	+	-	+	-	+	+	+	+	+	*Micrococcus varians*
CDB4	Rod	+	+	+	-	-	+	+	+	-	+	+	-	+	+	-	A	+	A	*Bacillus licheniformis*
CDB5	Rod	+	+	+	-	-	+	+	+	-	+	+	-	+	+	-	A	+	+	*Bacillus megaterium*
CDB6	Rod	+	+	+	+	-	+	+	+	-	-	+	+	+	A	A	A	A	A	*Bacillus*

																				subtilis
CDB7	Rod	+	+	+	+	-	+	+	+	-	-	+	-	+	+	-	-	+	A	*Bacillus coagulans*
CDB8	Cocci	+	-	+	-	-	-	-	+	-	+	-	+	-	-	-	+	-	+	*Micrococcus roseus*
CDB9	Rod	+	+	+	+	-	+	-	-	-	-	+	+	+	A	A	A	A	A	*Bacillus Sp*
CDB10	Rod in chains	+	+	+	+	-	+	+	-	+	-	-	+	+	A	A	A	A	A	*Bacillus Sp*

+: Positive result
-: Negative result
A: Acid production

Table 2: Utilization of Petroleum Hydrocarbons by Bacteria

Bacteria Isolates	Kerosene	Diesel	Benzene	Hexane	Crude Oil	Toulene
Alcaligene Sp. (CDB1)	++	++	++	++	++	++
Micrococcus luteus (CDB2)	++	++	++	++	++	++
Micrococcus varians (CDB3)	+	-	-	+	-	-
Bacillus licheniform (CDB4)	+++	+++	++	+++	++	++
Bacillus megaterium (CDB5)	+++	+++	+++	+++	++	+
Bacillus subtilis (CDB6)	++	++	+++	+++	++	+
Bacillus coagulans (CDB7)	+++	+++	+++	+++	++	+
Micrococcus rseus (CDB8)	++	++	++	++	++	+
Bacillus Sp. (CDB9)	+++	+++	+++	+++	++	+
Bacillus Sp. (CDB10)	+++	+++	+++	+++	++	+

+++: Maximum growth
++: Moderate growth
+: Minimal growth
-: No growth

Table 3: Weight Loss of Transniger Pipeline Diesel Oil resulting from the Growth of *Bacillus megaterium* (CDB5) and *Micrococcus luteus* (CDB5)

Incubation Time (Days)	Weight Loss of Transniger Pipieline diesel Oil (%)	
	B. megaterium	***M. luteus***
2	9.8±1.1	5.6±0.8
4	19.1±1.3	9.3±0.3
6	27.1±0.8	18.8±0.8
8	39.5±0.8	22.5±0.8
10	49.5±0.7	35.5±0.7

Table 4: Weight Loss of Bonny Light Diesel Oil resulting from the Growth of *Bacillus megaterium* (CDB5) and *Micrococcus luteus* (CDB5)

Incubation Time (Days)	Weight Loss of Bonny Light diesel Oil (%)	
	B. megaterium	***M. luteus***
2	7.0±0.7	3.5±0.6
4	15.6±0.8	9.1±0.6
6	20.9±1.0	12.6±0.8
8	32.7±0.6	19.3±1.0
10	41.9±0.8	29.1±0.6

Table 5: Emulsification of Transniger Pipeline and Bonny Light Diesel Oil by *Bacillus megaterium* (CDB5) and *Micrococcus luteus* (CDB5)

Bacteria Isolates	Rate of Emulsification									
	Transniger Pipeline Diesel Oil						Bonny Light Diesel Oil			
	2	4	6	8	10	2	4	6	8	10
Bacillus megaterium (CDB5)	+	+	++	+++	+++	-	+	+	++	++
Micrococcus luteus (CDB5)	-	-	-	-	-	-	-	-	-	-

+++: Maximum emulsification
++: Moderate emulsification
+: Minimal emulsification
-: No emulsification

Table 6: Effects of Lead Chloride ($PbCl_2$) on the Degradation of Bonny Light Diesel Oil by *Bacillus megaterium* (CDB5)

Incubation Period (Days)	Concentration of Lead Chloride (%)			
	0.00	0.05	0.10	0.50
2	7.1±0.7	7.1±0.2	9.0±0.1	3.4±0.4
4	15.6±0.8	18.3±0.4	29.3±0.2	12.2±0.2
6	20.9±1.0	25.5±0.6	31.5±0.3	15.3±0.3
8	32.7±0.6	36.4±0.5	37.4±0.3	25.7±0.4
10	41.9±0.8	47.8±0.2	54.6±0.4	30.6±0.2

Table 7: Effects of Zinc Sulphate ($ZnSO_4$) on the Degradation of Bonny Light Diesel Oil by *Bacillus megaterium* (CDB5)

Incubation Period (Days)	Concentration of Zinc Sulphate (%)			
	0.00	0.05	0.10	0.50
2	7.0±0.7	7.7±0.1	8.5±0.3	5.1±0.1
4	15.6±0.8	17.0±0.1	21.4±0.5	11.1±0.3
6	20.9±1.0	25.5±0.5	39.3±0.4	11.1±0.3
8	32.7±0.6	37.0±0.3	53.4±0.6	25.7±0.2
10	41.9±0.8	43.8±0.1	61.7±0.3	36.9±0.2

4. Discussion

The petroleum industry in Nigeria came to limelight not too long after independence and has ever remained paramount in its influence in the economic and physical landscape of Nigeria. The Niger Delta has been the region most prone to oil contamination because of the high rate of petroleum related activities in that region. Microbial degradation of hydrocarbon has contributed greatly to the disappearance of oil spills from our environment.

The organisms used in this study were isolated from oil polluted water and soils. The microorganisms identified in this study are naturally occurring populations that participate in soil processes and having inherent ability to degrade petroleum hydrocarbons (Arvanitis, *et. al*., 2008) [1]. Similar organisms were also isolated by other investigators (Satishkumar, *et al*. 2008; Malik, and Ahmed, 2012; Jasmine and Mukherji, 2014) [14, 9, 8]. The varying capabilities of the isolates in metabolizing the oil may be due to their hydrocarbon degradation enzyme systems. While some isolates may have strong affinity for the hydrocarbon substances, some may have to undergo a long period of lag due to inhibitory components of the oil before onset of biodegradation.

The results for the utilization of various petroleum hydrocarbon revealed that hexane (Aliphatic hydrocarbon), kerosene and diesel oil (mixture of aliphatic hydrocarbon) supported more bacteria growth than benzene and toluene (Aromatic hydrocarbon). The result of this study is consistent with earlier assertion made by Atlas and Bartha (1973) [2] that n-alkanes are the most readily degraded components in hydrocarbon mixture, while the aromatics especially the condensed polyaromatic hydrocarbon are known to be recalcitrant to microbial attack. The aromatics therefore, proved to be poor sources of carbon and energy. However, benzene was utilized by the bacteria better than toluene. This is attributed to the methyl group present in toluene. Toluene is made up of a benzene ring with a methyl substitution of the benzene ring. This attribute of toluene increases resistance to degradation and hence, recalcitrance of the compound.

Studies revealed that however, *B. megaterium* showed a considerably higher ability in degrading transniger pipeline and bonny light diesel oil than *M. luteus*. The result of these findings is similar to earlier report made by Riskuwa-Shehu and Ijah (2016) [13] during their studies on enhanced removal of crude oil in soil by mixed culture of *Bacillus megaterium* UL05 and *Pseudomonas aeruginosa* UL07. The efficient ability of *B. megaterium* CDB5 in degrading the two types of oil is probably due to the fact that the organism produced surface active agents which emulsified the oil thereby creating a large surface area for microbial colonization. *M.luteus* CDB2 was unable to emulsify the two type of diesel oil used. This means that it could not produce surface active agents. Gutrick and Rosenberg (1977) [7] reported that emulsification enables hydrocarbon utilizing microorganisms to efficiently degrade hydrocarbons.

The present study has pointed to *B. megaterium* as a better candidate in seedling oil polluted water and soil. It was also observe that *B. megaterium* CDB5 and *M. luteus* CDB2 degraded transniger pipeline diesel oil better than bonny light diesel oil probably due to the varying chemical constituents of the oil.

5. Conclusion

The environmental effects of oil spillage have drawn considerable attention to microbial degradation as the most important and environmental friendly approach to oil spill containment. It is on this background that this research was conceived to identify various microorganisms that can be used in the abatement of oil spill.

However, *B. megaterium* CDB5 showed considerably higher ability in degrading transniger pipeline and bonny light diesel oil than *M. luteus* CDB2. *B. megaterium* CDB5 caused a 49.5% decrease in weight of transniger pipeline diesel oil and 41.9% decrease in weight of Bonny light diesel oil after 10 days of incubation while *M. luteus* CDB2 caused weight losses of 35.5% and 29.1% of transniger pipeline and bonny light diesel oil respectively, over the same environmental conditions and incubation period.

The efficient ability of *B. megaterium* CDB5 in degrading the two types of oil is probably due to the fact that the organism produced surface active agents which emulsified the oil thereby creating a large surface area for microbial colonization. *M.luteus* CDB2 was unable to emulsify the two type of diesel oil used.

Thus, *B. megaterium* CDB5 could be recommended as the better candidate for the bioremediation of oil contaminated soil and water within this active oil producing region.

6. References

1. Arvanitis N, Katsifas EA, Chalkou KI, Meintanis C, Karagouni AD. A Refinery Sludge Deposition site: presence of nahH and alkJ genes and crude oil biodegradation ability of bacterial isolates. Biotechnology Lett. 2008; 30:2105-2110.
2. Atlas RM, Bartha R. Fate and Effects of Polluting Petroleum in the Marine Environment. Microbiological Revieews. 1973; 49:49-85.
3. Broderick LS, Cooney JJ. Emulsification of Hydrocarbons by Bacteria from Freshwater Ecosystem. Developments in Industrial Microbiology. 1982; 23:225-427.
4. Cowan ST. Manual for the Identification of Medical Bacteria (2nd ed.). Cambridge University Press, New York, 1974.
5. Cruickshank R, Duguid JP, Mavinion BP. Medical Micrbiology (4th ed.). Churchill Livingstone, New York. 1975, 356.
6. Frederick PM, Westlake DWS. Degradation of Aromatics and Saturates in Crude Oil by Soil Enrichment. Water, Air and Soil Pollution. 1981; 16:367-375.
7. Gutrick DL, Rosenberg E. Oil Tankers and Pollution: A Microbiological Approach. Annual Reviews in Microbiology. 1977; 31:379-396.
8. Jasmine J, Mukherji I. Evaluation of Bioaugmentation and Biostimulation Effects on the Treatment of Refinery oily Sludge Using 2n full factorial design," *Environ. Sci.: Processes Impacts.* 2014; 16:1889-1896.
9. Malik ZA, Ahmed S. Degradation of Petroleum Hydrocarbons by Oil Field Isolated Bacteria Consortium. African Journal of Biotechnology. 2012; 1:650-658.
10. Mulkins-Phillips GJ, Stewart JE. Effects of Environmental Parameters on Bacterial Degradation of Bunker Oil, Crude Oil and Hydrocarbons. Applied Microbiology. 1974; 28:915-922.
11. Odu CTI. Degradation and Weathering of Crude Oil under Tropical Conditions in the Petroleum Industry and the Nigerian Environment. Proceedings of International Seminar: NNPC, PTI., Warri, Nigeria. 1981, 143-153.
12. Okpokwasili GC, Okorie BB. Biodeterioration Potentials of Microorganisms Isolated from Car Lubricating Oil. Tribology International. 1988; 21(4):215-220.
13. Riskuwa-Shehu1 ML, Ijah UJJ. Enhanced Removal of Crude Oil in Soil by Mixed Culture of *Bacillus megaterium* UL05 and *Pseudomonas aeruginosa* UL07. International Journal of Environmental Bioremediation & Biodegradation. 2016; 4(1):8-12.
14. Satishkumar M, Banupriya AR, Baik S, Yun S. Biodegradation of Crude Oil by individual Bacterial Strains and a mixed Consortium Isolated from Hydrocarbon Contaminated Areas. Clean. 2008; 36:92-96.
15. Zajic JE, Suplison B. Emulsification and Degradation of Bunker Fuel Oil by Microoganisms. Biotechnology and Bioengineering. 1972; 14:331-343.
16. Zobell CE. Microbial Modification of Crude Oil in the Sea. In: Proceeding of the Joint Conference on Prevention and Control of Oil Spills (A. P.J and F.W. P. eds.). New York. 1969, 317-320.
17. Zobell CE. Microbial Degradation of Oil: Present Status, Problems and Perspectives. In: The Microbial Degradation of Oil Pollution (D. G. Aheam and S. P. Meyer eds.). Center for Wetland Resources, Louisiana State University, Baton Rouge. 1973, 3-16.