©IDOSR PUBLICATIONS
International Digital Organization for Scientific Research ISSN: 2550-7931
IDOSR JOURNAL OF APPLIED SCIENCES 3(1) 76-85, 2018.

Isolation of hydrocarbon degrading bacterial species from soil contaminated with lubricating oil in Enugu South East Nigeria.

Onwa Ndubuisi Collins[1], Onochie Charles Chimezie[2] and Nwadiogbu Ifeanyi[2]

[1]Department of Applied Microbiology, Ebonyi State University Abakaliki, Nigeria.
[2]Applied Microbiology and Brewing Department, Enugu State University of Science and Technology, Enugu, Nigeria.

ABSTRACT

The isolation of hydrocarbon degrading bacterial species from soil contaminated with lubricating oil in Enugu, South East Nigeria was carried out. Four bacterial isolates capable of utilizing hydrocarbon in used engine oil were isolated from auto-mechanic workshop at Coal Camp, Enugu State, Nigeria. The isolates were identified based on morphological and biochemical characteristics as *Bacillus subtilis, Pseudomonas aeruginosa, Acinetobacter* spp and *Flavbacterium* spp. The gravimetric analysis revealed that *Pseudomonas aeruginosa., Bacillus subtilis, Acinetobacter* spp, were capable of utilizing 61.44%, 62.65% and 59.03% of used engine oil, respectively. *Flavobacterium* spp., showed the lowest degradation potential of 49.39% under laboratory conditions at 30^{0}C and 120rpm at 420nm with modified mineral salts medium in a 10day period. Observations of turbidity showed that *Bacillus subtilis., Pseudomonas aeruginosa, Acinetobacter* spp., and *Flavobacterium* spp., exhibited the optical densities of 0.898, 0.797, 0.530 and 0.354, respectively. An increase in oil degradation was correlated to an increase in cell number indicating that the bacterial isolates were responsible for the oil degradation. The results obtained demonstrate the degradation potential of these species and are have potential use in oil bioremediation.
Keywords: Hydrocarbon, Bacteria species, Degradation,

INTRODUCTION

Engine oil is a complex mixture of hydrocarbons and other organic compounds, including some organometallic constituents [1]. It is used to lubricate the parts of an automobile engine in order to keep everything running smoothly [2]. The most important characteristics of the lubricating oil for automotive use is its viscosity. New motor oil contains a high percentage of fresh and lighter hydrocarbons that would be more of a concern for acute toxicity to organisms. Used motor oil contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic hazards including mutagenicity and carcinogenicity [3]. Prolonged exposure and high oil concentration may cause the development of liver or kidney disease, possible damage to bone morrow and an increased risk of cancer [4]. In addition, PAHs, have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment [5]. The illegal dumping of used motor oil is an environmental hazard with global ramifications [6] [7]. The release of oil into the environment causes environmental concern and attracts public attention [8].

The most widely distributed environmental pollution can be attributed to hydrocarbon contamination caused by oil tanker accidents, storage tank ruptures and transport accidents [9] [10]. The environmental pollution by hydrocarbons at petrol stations or factory sites is a serious problem. Not only does the pollution cause damages to the environment, but also the sales value of the land decreases significantly

depending on its use. Physical technologies, such as combustion and solidification, have been carried out to remove hydrocarbons from contaminated soil. Although physical techniques may shorten the work period with low cost, plants are not able to grow in these soils [11]. It is well known that microbial degradation of spilled hydrocarbons is a major technique in the natural decontamination process [12].

Petroleum, a complex mixture of hydrocarbon has been generally grouped into four classes according to their differential solubilities in organic solvents.

i. The saturates (n-and branched chain alkanes and cycloparaffins)
ii. The aromatics (mono, di, and polynuclear aromatic compounds containing alkly side chains and /or fused cycloparaffin rings).
iii. The resins (aggregates with multitude of building blocks such aspyridines, quinolines, carbazoles(and),
iv. The asphaltenes (aggregates of extended polyaromatics naphthenic acids, sulfides, polyhydric phenol, fatty acids and metallopophyrins) [13].

Petroleum is made in nature by transforming biomass under high temperature and high pressure [14]. It is composed of aliphatic, alicyclic and aromatic hydrocarbons and has been the demand of today's activities [15].

Large amounts of lubrication oils, containing long-chain saturated hydrocarbons (base oil) and additives are used in car engines. The main components of the base oil are cyclic alkenes (c-alkanes). Long-chain hydrocarbon and c-alkenes are known as recalcitrant to microbial degradation. The base oil entrains C_{16}-C_{36} hydrocarbons and more than 75% c-alkenes. The rings number of c-alkenes in the base oil is from 1 to 3 and any ring contains 5 or 6 members. Most of the c-alkenes in the base oil have long alkyl side chain [16].

Since the degradation of long chain hydrocarbons which are solid at temperatures less than 10^0C, is hindered by their limited bioavailability, the waste oil is slowly degraded by microorganisms in nature [17]. Susceptibility of a hydrocarbon to microbial degradation varies with type and size of the hydrocarbon molecule. Alkanes of intermediate chain length (C_{10}-C_{24}) are often degraded rapidly, while very long chain alkanes are increasingly resistant to microbial degradation [18].

Although pesticides are hydrocarbon pollutants of the soils, the main sources of hydrocarbon pollution are the spills and leaks of petroleum products [19] The Exxon Valdexz poil spill in South Central Alaska is an example [20]. In Nigeria, the exploration and exploitation practices and the breaking of oil pipes lead to incessant pollution especially in Niger Delta Area and Southern part of Nigeria [21]. These spills have the largest and economic impact as they cause harm to a large extent in the ecosystem more than just the isolated location. In many spills involving tankers or offshore oil wells, some of the spills catch fire and consequently their combustion results in emission of large quantities of toxic ash which is detrimental to human health [22].

LITERATURE REVIEW

Motor oil or engine oil, is oil used for lubrication of various internal combustion engines [23]. While the main function is to lubricate moving parts, motor oil also cleans, inhibits corrosion, improves sealing and cools the engine by carrying heat away from the moving parts. Motor oil are derived from petroleum and non-petroleum synthetic oil. Motor oil mostly constituents of hydrocarbons, organic compounds, including some organ metallic constituents [24]. Most motor oils are made from a heavy, thick petroleum hydrocarbon base stock derived from crude oil with additives to improve certain properties [25]. The bulk of typical motor oil consists of hydrocarbon with between 18 and 34 carbon atoms per molecule. One of the most important properties of motor oil in maintaining a lubricating film between moving parts is its viscosity [25].

Hydrocarbons including PAHs have been long recognized as substrates supporting

microbial growth [26]. Bioremediation makes use of indigenous oil consuming microorganisms, called petrophiles, by enhancing and fertilizing them in their natura habits. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize them as a nutrient source [27]. Microorganisms degrade these compounds by using enzymes in their metabolism and can be useful in cleaning up contaminated sites [28].

Microbial remediation of a hydrocarbon contaminated site is accomplished with the help of a diverse group of microorganisms present in the soil. The microorganisms can degrade a wide range of target constituents present in oily sludge. [29]. A large number of *Pseudomonas* strains capable of degrading PAHs have been isolated from soil and aquifers [30] [31]. Other petroleum hydrocarbons degraders include *Yokenella* spp., *Alcaligenesd* spp., *Roseomonas* spp., *Stenotrophomonas* spp., *Streptococcus* spp., *Providencia* spp., *Sphingobacterium* spp., *Acinetobacter* spp., *Flavobacter* spp., *Corynebacterium* spp.,*Capnocytophage* spp., *Moraxella* spp and *Bacillus* spp [32], [33]. Other organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent. However, they take longer periods of time to grow as compared to their bacterial counterparts [34].

In recent times, an increasing amount of microbiological research has been devoted bioremediation of oil contaminated sites using various microbial spices. Notable among them were the species of *Arthrobacter, Flavobacterium, Sphingomoans* [35], *Pseudomonas* ssp. [36] and *Acinetobacter* [37]. Fungal genera such as *Trichoderma* and *Phanerochaete* [38] have been implication in hydrocarbon biodegradation.

[39] reported that three bacterial isolates namely *Flavobacterium* spp., *Acinetobacter* spp., and *Pseudomonas aeruginosa,* were isolated from engine oil contaminated soil. An increase in oil degradation corresponded to an increase in cell number during the degradation processes demonstrating the ability of utilizing engine oil as the energy source. All three isolates also demonstrated the ability of degrading n-paraffin with higher rates.

[40] reported the role of *Acinetobacter baumannii* in hydrocarbon bioremediation. *A. baumanii* can remove or degrade a wide range of organic materials such as phenol, toluene and inorganic compounds such as phosphates and metals [41]. Species of *Acinetobacter* have been attracting increasing attention in both environmental and biotechnological application [42].

MATERIALS AND METHODS

Collection of Samples

Soil samples were collected from auto-mechanic workshops that had a heavy spillage of used engine oil at Coal Camp, Enugu State, Nigeria. They were placed into sterile bottle and transported immediately to the laboratory. The hydrocarbon (spent engine oil) collected directly from the engine of a vehicle in a sterile container was purchased from auto-mechanic workshop at Coal Camp, Enugu State, Nigeria. Thereafter it was transported to the laboratory.

Isolation and identification of engine oil degraders

Engine oil degrading bacteria were isolated from soil sample by enrichment culture on mineral salt agar as described by [4]. The medium composition and preparation are presented Appendix II).

The engine oil contaminated soil samples were homogenously mixed. Ten (10) grams of the soil samples was added to 90ml of sterile distilled water contained in 250ml Erlnmeyer flask and shaked vigorously. A serial dilution of the soil samples was prepared. A mineral salt medium containing 1% (V/V) of used engine oil was prepared in 250ml Erlenmeyer flash. About 0.01% fungal inhibitor (mycostatin) was added in the medium. Bacteriological agar was also added to produce a solidified medium.

Characterization of the Degradation Potential of Isolates

A mineral salt medium was prepared as described by [8]. The medium composition and preparation are presented (Appendix I) 100ml of mineral salt medium supplemented with 1ml of used engine oil were measured into 250ml Erlenmeyer flasks. The preparation was autoclaved at 15psi and 121^{0}C for 15 minutes and allowed to cool to 45^{0}C. A loopful of the test isolated were aseotically inoculated into the 250ml Erlenmeyer flasks containing mineral salt medium in which 1% of the used engine oil had been introduced. Uninoculated control flasks were also setup. The flasks were incubated in a rotary shaker. During the period of incubation, the pH, viable count and optical density (O.D) of each bacterial isolate were determined. Turbidity of the broth compared with the uninoculated control was used as indicator of growth of the culture.

The pH was determined aseptically by pipetting 2ml sample from the 250ml Erlnmeyer flask at every 48 hours of incubation and measuring with standardized pH meter (Digital pH meter, MAC-552, MAC Anderson, England).

The viable count was measured by pipetting aseptically 1ml sample from the flask, serial diluted and cultured on nutrient agar plate at 30^{0}C. The counts were taken at every 48 hours to determine the colony forming unit, using a colony counter (Gallenkamp colony counter).

The optical density (O.D) of the isolates was determined after every 48 hours of incubation using spectrophotometer operation set at 420mm. This measured the level of turbidity of the isolates in the growth medium.

Determination of Residual Hydrocarbon and used Engine Oil Biodegradation

The level of used engine oil was determined using the gravimetric analysis [31] [32]. The percentage engine oil remaining was calculated and compared to the control.

For each bacterial isolate, a set of three bottles each containing 10ml of mineral salt medium was autoclaved at 121^{0}C for 15 minutes. The engine oil (50ml) was also autoclaved separately at 121^{0}C for 15 minutes and allowed to cool. 1ml of the sterilized engine oil was aseptically transferred to the bottles containing the mineral salt medium. One percent of a turbid suspension of the cells contained Erlenmeyer flask was inoculated in triplicate into the bottles containing sterilized mineral salt medium and spent engine oil. Uninoculated set of the bottles was also prepared as control. The whole preparations were then incubated in a rotary shaker at 30^{0}C for 10 days.

After incubation, the content of the bottles were transferred to separating funnels having filter paper (12.5cm Whatman No. 1) and the residual hydrocarbons extracted with 30ml toluene. The filtrates (toluene and hydrocarbon mixture) were collected into a preweighed breaker and the toluene was evaporated in a hot air oven at 70^{0}C and the beaker allowed to cool and reweighed.

The percentage (%) degradation was calculated as follows [33].

Weight of residual hydrocarbon = Weight of beaker containing extracted engine oil − Weight of empty beaker

Amount of engine oil degraded = Weight of engine added in the media − Weight of residual hydrocarbons

$$\% \text{ Degradation} = \frac{\text{Amount of engine oil degraded}}{\text{Amount of engine oil added in the media}} \times \frac{100}{1}$$

RESULTS

Table 1

Bacterial Isolates Colony Morphology	A Blue green pigment, smooth round	B White, large irregular edge	C Cream, smooth, mucoid	D Yellow-orange pigment, flat edges
Gram staining	-R	+R	-	-R
Spore staining	-	+	-	-
Motility	+	+	-	-
Methyl Red (MR)	-	+	-	-
Voges Proskauer (VP)	-	+	-	-
Starch hydrolysis	-	+	-	-
Oxidase	+	-	-	+
Catalase	+	+	+	+
Indole	-	-	-	+
Glucose	A/G	-	A	A
Sucrose	-	A	-	-
Lactose	-	-	A	-
Maltose	-	A	-	-
Fructose	-	A/G	-	-
Mannitol	-	-	-	-
Probable Organism	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Acinetobacter* spp	*Flavobacterium*

Key
+ = Position reaction A = Acid
- = Negative reaction A/G = Acid and Gas

Table 2: Variation of pH, optical density (OD) and total viable count values with time during the degradation of used engine oil with *Bacillus subtilis*

Period of incubation (days)	pH	O.D (420mm)	Total bacterial count ($x10^6$) (cfu/ml)
0	6.48	0.015	5.6
2	6.51	0.252	7.0
4	6.62	0.316	9.0
6	6.68	0.460	9.8
8	6.84	0.660	10.2
10	7.05	0.898	10.8

Table 3: Variation of pH, optical density (OD) and total viable count values with time during the degradation of used engine oil with *Pseudiomonas aeruginosa*

Period of incubation (days)	pH	O.D (420mm)	Total bacterial count ($x10^6$) (cfu/ml)
0	6.50	0.012	6.0
2	6.61	0.065	9.5
4	6.68	0.251	10.2
6	6.72	0.440	10.5
8	7.07	0.583	10.9
10	7.32	0.797	11.0

Table 4: Variation of pH, optical density (OD) and total viable count values with time during the degradation of used engine oil with *acinetobacter* spp.

Period of incubation (days)	pH	O.D (420mm)	Total bacterial count ($x10^6$) (cfu/ml)
0	6.50	0.005	6.8
2	6.55	0.075	9.8
4	6.59	0.284	7.0
6	6.86	0.315	7.5
8	6.95	0.472	8.0
10	7.12	0.530	8.5

Table 5: Variation of pH, optical density (OD) and total viable count values with time during the degradation of used engine oil with *Flavobacterium* spp.

Period of incubation (days)	pH	O.D (420mm)	Total bacterial count ($x10^6$) (cfu/ml)
0	6.50	0.005	4.0
2	6.55	0.167	6.1
4	6.57	0.219	6.5
6	6.67	0.278	7.0
8	6.96	0.328	7.4
10	7.21	0.354	7.6

Table 8: Used Engine Oil Biodegradation Values

W.t of empty beaker (g)	W.t beaker hydrocarbon extract (g)	W.t residual hydrocarbon (g)	Of amount hydrocarbon degraded (g)	% percentage degradation	Bacterial isolates
40.86	41.17	0.31	0.52	62.65	*Bacillus subitilis*
39.50	39.82	0.32	0.51	61.44	*Pseudomonas aeruginosa*
37.30	37.64	0.35	0.49	59.03	*Acinetobacteria* spp
42.55	42.97	0.42	0.41	49.39	*Flabobacterium* spp

DISCUSSION

In this study, hydrocarbon degrading bacteria were isolated from engine oil polluted soil based on their ability on used engine oil as a result of the stimulated effect of additional carbon and energy source in the form of lubricating oil. Four bacterial isolates, *Pseudomonas aeruginosa,, Bacillus subtilis, Acinetobacter* spp and *Flavobacterium* spp. were isolated using mineral salt medium (appendix 1). These isolates were identified based on their morphological and biochemical technique using taxonomic scheme of Bergey's Manual of Determination Bacteriology. Variation in pH, optical density, total viable count and degradation potential of these bacterial isolates were determined over of

Pseudomonas aeruginosa Bacillus subtilis from used engine oil contaminated soil. [34] reported *Pseudomonas aeruginosa* as a common bacterium capable of degrading hydrocarbon. *Pseudomonas aeruginosa* was found in a consortium of bacteria from the soil that can degrade hydrocarbon in light fuel oil [35]. [36] reported *Bacillus subtilis* as being the predominant isolate of the crude oil utilizing bacteria characterized from highly polluted soil sample. It was postulated that *Bacillus subtilis* was more tolerant to high levels of hydrocarbons in the soil due to their resistant endospores [37].

Acinetobacter spp was reported to assimilate saturated and aromatic hydrocarbon in the soil [38] and inorganic compounds such as phosphate [39]. *Acintobacter* spp was among the best hydrocarbon degrading bacteria reported in this work degrading up to 59.03% of the use engine oil, over a 10 day period (Table 6).

The pH values showed increase in pH from 6.50 to 7.32 for *pseudomonas aeruginosa.,* 6.48 to7.05 for *Bacillus subtilis.,* 6.5 to 7.12 for *Acinetobacter* spp and 6.50 to 7.12 *Flavobacterium* spp., over a 10 day period. This pH values fell within the range of the optimum value for an oil degradation as reported [40]. This signifies that the isolated bacteria increased the pH of the medium from acidic to slightly alkaline and that bioremediation of oil polluted soil may be best achieved at slightly alkaline pH. Although this result was not in agreement with the research done by [41] who observed a decreased in pH from 6.70 to 6.52 over a 10 day period with *Bacillus subtilis* during the degradation of crude oil. [42] observed a pH range of 6.65 to 7.15 during the degradation of oil contaminated soil with *P.* tuber-regium. [19] found optimum activity for microbial degradation at a pH 4.5 to 8.5

Total plate count of the isolates showed increased growth in cell number from 5.6x 10^6 to 10.8 x 10^6 cfu/ml, 6.0 x 10^6 to 11.0 x 10^6 cfu/ml, 4.8 x 10^6 to 8.5 x 10^6 for *Bacillus subtilis., Pseudomonas aeruginosa., Acinetibacter* spp., respectively over a 10 day period of incubation. Counts of *Flavobacterium* spp, increased from 4.0 x 10^6 to 7.6 x 106 cfu/ml (Tables 4) over the same laboratory condition.

In this study, *bacillus subtilis,* has the highest degradation potential of 62.65% and highest optical density (O.D) of 0.898. *Pseudomonas aeruginosa* has the degradation potential of 61.44% with optical density of 0.797, followed of *Acinetobacter* spp., with degradation potential of 59.03% (O.D; 0.530). *Flavobaterium* spp, had degradation potential of 49.39% with optical density of 0.354 (Tables 5). These findings indicate that an increase in oil degradation was corresponding to an increase in cell number during the degradation process demonstrating the ability of utilizing engine oil as carbon substrate. This observation was not in agreement with the research done by [12], for which they reported that the ability of microorganisms to produce turbidity in culture media does not necessarily indicate efficient hydrocarbon-degradation. They reasoned that such capability is determined by the ability of the organism to elaborate the vital enzymes required for decomposition of the recalcitrant components of the hydrocarbons rather than being nutritionally fastcitrant. Moreover, among the bacterial isolates, *Bacillus subtilis* exhibited the highest degradation potential. This may be due to their resistant endospores as reported by [23]. This shows that *Bacilluss subtilis* are actively involved in the natural degradation of oil-polluted environment. Although this was not in agreement with the reports of [8] who observed that spore-forming bacteria in general have negligible role in oil biodegradation. The result was, however, supported by the works of [7]; [8], [9], who observed that *Bacillus subtilis* have a good biodegrading potential.

CONCLUSION

This study showed that *Bacillus subtilis, Pseudomonas aeruginosa, Actnetobacter* spp and *Flavobacterium* spp., were isolated from soil samples contained with

used engine oil in auto-mechanic workshops at Coal Camp, Enugu State, Nigeria. *Bacillus subitis* achieved the highest degradation potential of 62.65%, followed by *Pseudomonas aeruginosa.,* 61.44%, *Acinetobacter* spp. 59.03% while *Flavobacterium* spp. showed the lowest degradation potential of 49.38% at 30^{0}C and 120rpm with mineral salt medium in a 10 day period. The results obtained demonstrated the potential for oil bioremediation of these isolates.

REFERENCES

1. Adenipekun, C. O (2008). Bioremediation of engine-oil polluted soil by *Pleurotus tuber-regium,* a Nigeria white-rot fungus. *Afri. J. of Biotechnol.* **7**(1): 55-58.
2. Alexander, M (1999). Biodegradation and bioremediation. 2nd edition. Academic Press, San Diego. Pp 43-48.
3. Andrea, R. C., Tania, A. A and Lucia, R. D (2001). Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. *Brazillan Journal of Microbiol,* **32**(4): 1517-1519.
4. Antai, S. P (1990). Biodegradation of Bonny light crude oil by *Bacillus* spp. and *Pseudomonas* spp. *Waste Mgt.* **10:** 61-64.
5. Aoshima, H., Hirase, T., Tada, T., Ichimura, N., Yamaguchi, H., Taguchi, M and Myoenzono, T (2006). Improvement of heavy oil degradation by *Rhodococcu erythropolis* C2.J. *Environ Biotecnol.***5**(2): 107-109.
6. Atlas, R. M and Bartha, R (1998). Microbial ecology: fundamentals and applications. 4th edition. Addison Wesley Longman, Inc., California, USA. PP 380-397.
7. Barathi, S. and Vasudevean, N (2001). Utilization of petroleum hydrocarbons by *Pseudomonas fluorescents* isolated from petroleum contaminated soil. *Environ Int.*b **26:** 416-416.
8. Blodgett, W. C (2008). Water-soluble mutagen production during the bioremediation of oil – contaminated soil. *Florida Scientist.* **60**(1): 28-36.
9. Boonchan, S., Britz, M. L and Stanley, G. A (2002). Degradation and mineralization of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal- bacterial consulters. *Appl. Environ.* **66**(3): 1007-1019.
10. Butler, C. S. and Mason, J. R (1997). Structure-function analysis of the bacterial aromatic ring-hydroxylation deoxygenates. *Advanced Microbiol Physiology,* **42:** 243-251.
11. Chaineau, C. H., Morel, J., Dupont, J., Bury, E and Oudot, J (2009). Comparison of the fuel oil biodegradation potential of hydrocarbon-assimilating microorganism isolated from a temperate agricultural soil. *Science Total Environmental International,***227:** 237-247.
12. Chang, R (1998). Chemistry. 6th edition. Mc Graw-Hill Companiesd, Inc. **24:** 962-963.
13. Collins, C (2007). Implementing phytoremediation of petroleum hydrocarbons. *Methods in Biotechnol.* **23:** 99-108.
14. Esin, A. A and Antai, S. P (2002). Biodegradation of the major components of Bonny light crude oil by *Bacillus subtilis. Global J. Pure Applied Sci.***8:** 215-222.
15. George-Okafor, U. O., Tasie, F. O and Nwankwo, J. I (2005). Degradation activities of bacterial flora resident at remote and recent hydrocarbon contamination soils located within Enugu metropolis. *J. Appl. Sci.* **8**(2): 4780-4791.
16. Gough, M. A and Rowland, S. J (1990). Characterization of unresolved Complex mixture of hydrocarbons in petroleum. *Nature.* **344:** 648-650.
17. Hagwell, I. S., Delfino, L. M and Rao, J. J (1992). Partitioning of polycyclic aromatic hydrocarbons form oil into water. *Environ. Sci. Technol.* **26:** 2104-2110.

18. Hardar, E (2004). Bioremediation of engine oil. Little Flower Academy, Dallas, Texas. **3:** 56-62.
19. Hawle-Ambrosch, E., Riepe, W., Dornmayr-Pfaffenhuemer, M., Radax, C., Holzinger, A and Stan-Lotter, H (2007). Biodegradation of fuel oil by hydrocarbons by a mixed bacterial consortium in sandy and loamy soil. *Biotechnology Journal,* **2:** 1564-1568.
20. Ijah, U. J and Antai, S. P (2003). Removal of Nigeria light crude oil in soil over a 12-month period Int. *Biodeterior. Biodegrad.* **51:** 93-99.
21. Itah, A. Y and Essien, J. P (2005). Growth profile and hydrocarbonoclastic potential of microorganisms isolated from tarballs in the Bight of Bonny, Nigeria. *World J. Microbiol. Biotechnol.,* **21:** 1317-1322.
22. Johnson, K., Anderson, S and Jacobson, C.S (1996). Phenotypic and genotypic characterization of phenathrene-degading fluorescent *Pseudomonas biovars. Appl. Environ,. NMicrobiol* **62:** 3818-3825.
23. Kasther, M., Breuer-Jammali, M an Mahro, B (1994). Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAHs). *Appl. Microbiol Biotechnol,* **41:** 267-273.
24. Kishore, D and Ashis, M. K (2007). Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India Bioresour. Technolo. **98:** 1339-1345.
25. Kiyohara, H., Takizawa, N and Nagoa, K (1992). Natural distribution of bacteria smetabolizing many kinds of polyaromatuc hydrocarbons. *J. Ferment. Bioeng,* **74:** 49-51.
26. Koma, D. M., Sakashita, Y., Kubota, K., Fujii, Y., Hasumi, F., Chung, S. Y and Kubo, M (2003). Degradation of car engine oil by *Rhodococus* spp. NSKK48 and *Gordnia* spp. NDKY 76A. *Biosci. Biotchnol. Biochem,* **67:** 15902-15903.
27. Leavy, J. G and Colwell, R. R (1990). Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* **54:** 305-315.
28. Lloyd, C. A and Cackette, T. A (2001). Diesel engines: Environmental Impact and Control. Air and waste Management Association. *Appl. Environ Microbiol.* **68:** 2509-2518.
29. Leung, S. T., Cassidy, M. B., Shaw. And, K. W., Lee, H., Trevors, J. T., Lohmeler-Vogel, E. M. and Vogel, H. J (1997). Pentachlorophenol Biodegradation by *Pseudomonas* spp. UG25 and UG30. *World J. of Microbiol,* 13: 305-313.
30. Mandri, T and Lin, J (2007). Isolation and characterization of engine oil degrading indigenous microorganism: In Kwazulu Natal, South Africa. *Afr. J. Biotchnol,***6**(1): 23-27.
31. Marquez-Rocha, F. j., Hernandez-Redriquez, V and Lamella, M. T (2001). Biodegradation of engine and diesel oil in soil by a microbial consortium. *Water Air and Soil Pollut.* **128:** 313-320.
32. Oloke, J. K and Glick, M. R (2005). Production of Bioemulsifer by an unusual isolate of salmon/red melanin containing *Rhodotorula glutinis. Afr. J. Biotechnol,* **4**(2): 164-171.
33. Potter, T. L (1993). Analysis of petroleum contaminated soil and water: An overview. In: Calabrese, E. J., Kostecki, P. T (Eds.). Principles and practices for petroleum contaminated soils. Lewis Publishers, Chelsea, London. Pp 11-14.
34. Prince, R. C (1993). Petroleum spill bioremediation in marine experiments. *Microbiol Rev.* **19:** 217-242.
35. Prenafeta-Boldu, X. F., Kuhn, A., Dmam, L., Anke, H and Bont, J. D (2001). Isolation and Characterization of fungi growing

on volatile aromatic hydrocarbons as their sole carbon and energy source. *Mycol. Res.* **4:** 477-484.

36. Pritchard, P. H., Muller, J. G., Kremer, F. V and Glaser, J. A (1992). Oil spill bioremediation: Experiences, lessons and results from the Exxon Valdez spill n Alaska. *Biodegradation,* **3:** 315-335.
37. Roling, W. F. M., Milner, M. G., Jones, D. M., Lee, K., Daniel, F., Swannell, R. J. P and Head, I. M (2002). Robust hydrocarbon degradation and hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. *Appl. Environ. Microbiol.* **68**(11): 5537-5548.
38. Sepahi, A. A., Golpasha, I. D., Emanmi, M and Nakhoda, A. M (2008). Isolation and characterization of crude oil degrading *Bacillus* spp. *Iran. J. Environ, Health. Sci. Eng,* **5**(3): 149-154.
39. Speight, J. G (1991). The chemistry and technology of petroleum. Lewis Publishers, Marcel Dekker, New York. PP. 30.
40. Troungson, P (2005). Hydrocarbon biodegration bacteria –in the search for potential species. *Environmental International,* **31:** 155-161.
41. Van Hamme, J. D., Odumeru, J. A and Ward, O. P (2000). Community dynamics of a mixed-bacterial culture growing on petroleum physrocarbons in batch culture. *Can J. Microbiol,* **46**(5): 441-450.
42. Vidali, M (2001). Bioremediation. An overview. *Pure Appl. Chem.* **739**(7): 1172.