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Full Length Research Paper

Removal of crude petroleum hydrocarbons by heterotrophic bacteria in soils amended with nitrogenous fertilizer plant effluents

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Nitrogenous fertilizer (NPK) plant effluents from NAFCON were used in amending plots of land experimentally polluted with crude oil. Counts of heterotrophic bacteria (THBC) and fungi (TF), and of petroleum utilizing bacteria (PUB) and fungi (PUF) were monitored during an 8 weeks period. Counts obtained showed that NPK served as a good supplement for the growth of the petroleum degrading/utilizing bacteria in oil-polluted soils. Crude oil disappearance in plots TSP ranged between 8.70 and 34.80% and 20.90 and 60.50% for TST; cumulative loss was 73.0%. The disappearance was influenced by the N/P ratio in the supplementing fertilizer effluent. A total of ten genera of petroleum degraders were isolated, namely, *Micrococcus*, *Pseudomonas*, *Acinetobacter*, *Proteus*, *Bacillus*, *Actinomyces*, *Corynebacterium*, *Enterobacter*, *Brevibacteria* and *Citrobacter*. Crops grown on the experimental plots at the end of the study period for soil recovery studies indicated good soil recovery.

Key words: Removal, petroleum, heterotrophic, bacteria, amendment, fertilizer, effluents.

INTRODUCTION

The growing demand and supply of fuel oil and new chemicals by the industrialized society of the twenty first century has placed increasingly higher stress on the natural environment (Jaffe, 1991). Large amounts of diverse chemicals enter the environment via industrial discharges and other anthropogenic activities. Of particular concern are the hydrophobic organic compounds, because of their toxicological characteristics and their ability to accumulate in the environment.

Soil and water represent the first lines of recipients of oil pollution. Ground water contamination by crude oil therefore is becoming an increasing sensitive issue in Nigeria because most of the water supply is derived from shallow and unconfined aquifers. Furthermore, contamination of land is of paramount importance of man in that it is on this portion of the earth that the anvil of man's existence and activities lie. The oil mineral producing areas in Nigeria are in danger because the land is damaged and made infertile due to oil spill and this prevents growth of crops for varying periods of time. The damaging effects are due to suffocation and toxicity of the crude oil (Plice, 1948). Apart from this, it has been reported that Nitrate formation was reduced. Even 0.1% (v/w) of oil when mixed with soil practically checked nit-rate formation and this is inimical to soil fertility (Murphy, 1929).

Currently, physical and chemical methods are the most widely used procedure for clean-up which are not simple or favourable to the environment, hence mankind. For example, the use of skimming, crude slick, chemical introduction of poisonous sorbent and dispersants are all regarded as fail-safe because they further introduce poisonous contaminants to the environment (Stevens, 1991).

Bacteria, which exist ubiquitously in the environment, have a great potential to degrade crude oil (Lee and Levy, 1991). Bioremediation is a new intervention method for post clean up whereby the natural biodegradable capabilities of the soil are enhanced by nutrient addition and/or cultured microorganisms with advantages as cost

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effectiveness and without causing any environmental damage. Odu (1978) reported an increase in bioremediation of oil-polluted soil after supplying paraffin supplemented nitrogenous fertilizer (PSF) to the soil.

Basically, nitrogen and phosphorous are the most limiting nutrients to oil degrading bacteria (Odu, 1978, Atlasand Bartha, 1973c). Thus indigenous soil bacteria in the presence of nitrogenous fertilizer plant effluent for bioremediation would be interesting to study in order to determine their relative importance. Generally, the concept of fitness of microorganisms as agents of geochemical transformation depends on three factors such as ubiquity of microorganisms, their metabolic potential and as well as their metabolic versatility which enables them to degrade a vast variety of naturally occurring organic materials.

Sufficient supply of nutrients and their influence on the ability of petroleum oxidizers to degrade crude oil products have remained controversial since crude oil is a mixture of hydrogen and carbon. Therefore, a spill in an area will result in an imbalance in the carbon – nitrogen ratio since more of the carbon will be added from the oil. Basically, bacteria require about 10 parts of carbon to 1 part of nitrogen for efficient growth (Jobson et al., 1974). If the ratio becomes greater as a result of oil spillage for instance 100:1, or 1000:1, growth of bacteria and utilization of carbon source will be retarded and also there will be nitrogen deficiency in such oil-soaked soil. Atlas and Bartha (1972) reported that nutrients are the main factors limiting the occurrence of petroleum degrading microorganisms. The availability of nitrogen and phosphorous in both seawater and soils were limiting to the occurrence of microorganisms that utilize petroleum and their subsequent degradation of crude oil (Odu, 1978; Floodgate, 1972; Athrendy, 1973). In contrast, Kinney et al. (1969) and Atlas (1981) observed that nitrogen and phosphorrous were not limiting factors to petroleum degrading microorganisms in oil contaminated soil. It was concluded that nitrogen and phosphorous are both limiting only with respect to rates of hydrocarbon degradation considering solubility, and that they are in no way limiting factors in the sense that the solubility of hydrocarbon is so low as to preclude establishment of any unfavorable carbon/nitrogen or carbon/phosphorous ratio.

The objectives of this study were to evaluate the potential for use of indigenous petroleum oxidizers/utilizers in bio-remediation of crude oil polluted soils and potential enhancement of crude oil removal from soils by controled addition of nitrogenous fertilizer factory plant effluents.

MATERIALS AND METHODS

Sources of materials

The crude oil used was “Bonny Light type”. The nitrogenous fertilizer plant effluent (NPK 15:15:15) was obtained from National Fertilizer Company Nigeria (NAFCON), Onne, Rivers State. All chemical reagents used in this study were of analytical grade.

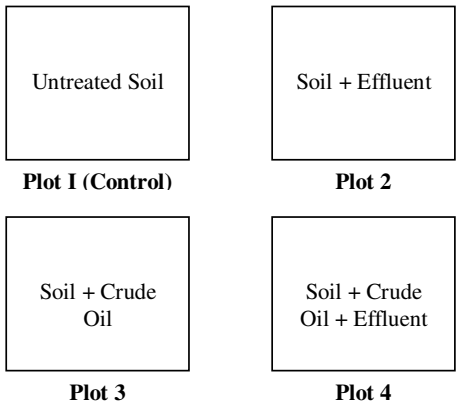

Figure 1. Test plot outlay.

Location of experimental plot

The experimental plots were located at the front entrance of Institute of Pollution Studies (IPS), Rivers State University of Science and Technology Nkpolu, Port Harcourt. It is an area characterized by typical grassland vegetation. The area was cleared and mapped out into four (4) plots of equal dimension (1 m x 1 m). The edges were elevated up to about 15 cm above the soil level to ensure proper demarcation and also to prevent run-off when it rains. The textural class of the soil is sandy, loamy (75% sand, 14% silt and 11% clay).

Experimental design

The work involved field experiment and laboratory simulation. Experiment was designed to involve a laboratory stage of isolation. Field-testing or experiment lasted for about five (5) months and employing three (3) separate treatment options and a control, each aimed at assessing the enhancement of bioremediation of crude oil polluted soils. After each treatment, the following parameters were determined: pH, moisture content, total organic carbon, total nitrogen and available phosphorous. The levels of exchangeable cation (potassium, calcium, magnesium and sodium), total organic carbon/nitrogen ‘C/N’ ratio, enumeration and identification of total aerobic heterotrophic bacteria/fungi count, total hydrocarbon utilizeing bacteria/fungi count, and Total hydrocarbon (THC) – (crude oil) level were also determined.

The second stage of the investigation involved characterization of petroleum degraders isolated from the treated soil and the final stage included 2 steps A and B stage including:

a) Simulating field experiment in the laboratory using conical flask under good aerating pumping system and also monitoring growth on refined petroleum such as diesel, engine oil kerosene and premier motor spirit (Petrol).

b) Observing recovery of the soil as indicated by growth of farm crops (monitoring growth parameters).

The test plot out lay is as shown below (Figure 1). The treated soil plots 3 and 4 were polluted with three litres of crude oil each and plots 2 and 4 was sprayed with (2) two litres of nitrogenous fertilizer plant effluent every other day for one month. Samples were then collected on appropriate days for testing in the laboratory.

Soil sample collection

Top soil samples (0 – 3 cm) deep were collected at random from each test plot into a labeled sterile plastic bag using sterile auger. It was mixed very well (bulked) for uniformity. The samples were then

taken to the laboratory immediately for microbiological analysis. The rest were left in open air (room temperature) to air dry for physico-chemical analysis. Sample collection was done weekly.

Microbiological analysis of soil samples

Total heterotrophic aerobic bacteria and fungi, petroleum utilizing bacteria and fungi were enumerated as outlined by Adoki et al. (1999).

Measurement of Total Hydrocarbon (THC) in the soil samples

The residual hydrocarbon remaining in soil after experimental period was determined according to the method described by Odu et al. (1985).

Aerated flasks biodegradation tests

From plot 4 petroleum utilizers and/or degrading bacteria were isolated using appropriate media. A mixture of the organisms was then prepared by washing surface of plates with strile physiological saline and subsequently used for seeding aerated biodegradation flasks. Seven 500 ml aeration flasks containing fertilizer plant effluent and 0.5% v/v Bonny medium crude were set up and each representing each day (one-week interval for the first four and two week interval for the last two flasks). Six of the flasks were inoculated with 1 ml of the bacterial suspension. The seventh flask was not inoculated and served as control. The microbial load and residual crude oil level (THC) were measured respectively. Using the same setup, the growth of the petroleum degrading bacteria on different hydrocarbons (refined products e.g. diesel, engine oil, kerosene and petrol) was also determined.

RESULTS AND DISCUSSION

Physicochemical characteristics of soils

Physicochemical parameters are of the nitrogenous fertilizer plant effluent are presented in Table 1. The physico-chemical characteristics of the untreated and crude oil treated soil supplemented with fertilizer effluent are as shown in Table 2. The pH status of the untreated soils did not vary remarkably from the treated. It ranged from 4.80 to 6.80 in the first four weeks (T_1 to T_2) and at the end of investigation 'T_3' decreased to 5.90. Exchangeable cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) increased in all except potassium 'K' which fluctuated at the initial stage for oil treated soil and at the final stage increased. The value of sodium at the onset in the Plot 1 (control) was 0.27 mg 100 g^{-1} soil, this rose to 0.30 mg 100 g^{-1} soil, 4.06 mg 100 g^{-1} soil and 4.35 mg 100 g^{-1} soil at the end of investigation 'T_3' in Plots 2, 3 and 4 respectively.

Phosphorus level initially increased but at the end of investigation decreased remarkably in plot 3, the oil polluted soil ranging 19.47 to 7.02 ppm. Plot 4 ranged from 19.77 - 12.28 ppm while plot 2 ranged from 20.19 – 19.18 ppm. The organic carbon increased in all treated soil with plot 3 recording the highest at the end of the study (T_3) with value of 3.80, 43.30 and 21.57%. However, a high reduction of nitrogen was observed in all comparing the values at the onset to those recorded at the end of the study period. Plot 3 had a calculated 45% reduction (ranging from 0.11 to 0.06%), 4.50% reduction (ranging from 0.16 to 0.08%) and 2 7.1% (ranging from 0.14 to 0.13%). Carbon to nitrogen (C/N) ratio gave a wider range for plot 3 than plot 4, (63.33 and 41.25) respectively. The moisture content of the soil is presented in Table 3. The value varied remarkably during the study (Day 0 to 56 days) when compared with pre treatment value with plot 4 recording the highest 48% on day 28. Plot 3 and 2 had 24.5 and 30.5%, respectively.

Table 1. Physico-chemical characteristics of fertilizer plant effluent.

Parameter	Value
pH	8.4
Conductivity	1.5 µs cm^{-1}
Ol; 1+/	4.0 NTU
8 + 9 urbidity	
Salinity	0.1%
Dissolved Oxygen (DO)	7.8 mg l^{-1}
NO_3	1.7 mg l^{-1}
PO_4	5.2 mg l^{-1}
SO_4	97.5 mg l^{-1}

Estimation of viable heterotrophic and petroleum degrading bacteria and fungi

Flooding of nitrogenous fertilizer plant effluent on oil polluted soil offers an interesting possibility of enhancing decontamination of the soil by indigenous soil microorganisms after few weeks. Counts at the end of the investigation revealed that plot 4 the oil treated and effluent supplemented soil supported more bacteria/fungi growth than the control. The counts are in order of Plot 4 > 3 > 2 > 1.

The corresponding petroleum degrading bacteria/fungi in the test plots followed the same trend. There was a progressive increase in counts until the 5th week when the organisms entered the decline phase due depletion of available nutrient and/or introduction of toxic metabolites in the system. In the investigation, it was observed that the counts of hydrocarbon utilizers were low compared with total viable heterotrophic bacteria and fungi; their presence at all in pre-treatment soil confirms the conclusions reached by Odokuma and Okpokwasili (1993), of their enduring presence in the Niger Delta ecosystems following oil exploration and production activities. An increase in counts of petroleum utilizing bacteria and fungi was accompanied by exponential increases in counts of viable heterotrophic bacteria. The observation agrees with those of Sexton and Atlas (1977), Odu and Isinguzo (1979), Atlas (1981), and Amanchukwu et al. (1989) who reported a corresponding increase in counts of hetero-

Table 2. Chemical content of untreated and oil treated soil.

Sampling Week	Treatment	pH	Exchangeable cations (Meg 100g^{-1}soil)				Available PO_4 (ppm)	%		C/N
			Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}		Org C*	TN*	
T_1	Control (Untreated)	4.80	2.05	0.01	0.04	0.27	18.60	1.53	0.12	12.75
	Effluent only	5.00	2.30	0.35	1.13	0.35	20.19	1.71	0.14	12.21
	Crude Oil only	5.40	2.15	0.45	0.02	2.43	19.47	3.90	0.11	35.45
	Crude Oil + Effluent	5.60	3.10	0.40	0.03	2.55	19.77	3.86	0.16	24.13
T_2	Control (Untreated)	4.80	1.90	0.05	0.04	0.21	18.60	1.50	0.12	12.50
	Effluent only	5.20	2.45	0.10	0.08	0.30	19.18	1.57	0.13	12.07
	Crude Oil only	5.00	2.15	0.35	0.05	4.06	7.02	3.80	0.06	63.33
	Crude Oil + Effluent	5.90	2.25	0.45	0.06	4.35	12.28	3.30	0.08	41.25

Note: T1 = First week of treatment; T2 = eight week of treatment.

Table 3. Moisture content (%) of fresh soil samples.

Days	Plot 1	Plot 2	Plot 3	Plot 4
Pre-Treatment	10.5			
0	10.5	12.0	10.5	14.0
14	10.5	20.5	17.5	20.0
28	12.5	30.5	24.5	42.0
56	12.0	25.0	15.0	30.0

Result represents mean of duplicate soil samples.

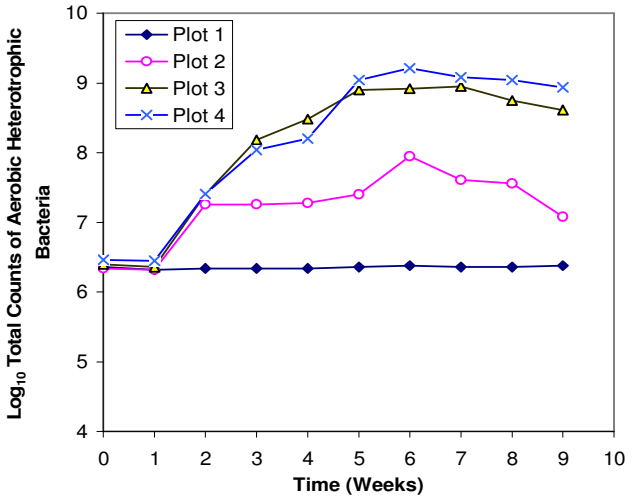

Figure 2. Viable counts of total aerobic heterotrophic bacteria in experimental plots.

trophic and petroleum degrading bacteria during hydrocarbon degradation. Low counts of petroleum deg-raders compared to the heterotrophs may be attributed to one or several of the factors of acclimatization, hitherto intolerant species and presence of products of mineralization from degradative activities of hydrocarbon utilizing population. The observation of rapid increased counts of PUB and PUF in plot 4 compared with 3 with the progress of the experiment indicated enrichment of the hydrocarbon-utilizing microbes by NPK effluent in the treatment options (Figures 2 - 5).

However, optimum growth was obtained for PUB at the 5th week for plot 4 and 6th week for plot 3. For PUF the optimum growth was at week 4 for both plots 4 and 3. It means that at the 6th week and 4th after flooding with effluents, petroleum utilizing microorganisms will reach their peak growth and so in terms of numbers, such a period is sufficient for these organisms to complete their biodegradation activity. The percentage ratio of degra-ders to viable heterotrophs is 29.5, 19.7 and 10.0% for bacteria and 18.7, 13.6 and 9.1% for fungi in plots 4, 3 and 2 respectively. This showed that a combination of the added nutrient (NPK effluent) and crude oil supplied the necessary substrates for the organisms. Result obtained for plot 3 although relatively low compared with those obtained for plot 4 showed that natural biodegradation albeit low was still possible, although it will take a longer time. Jones and Greenfield (1991) and Lee and levy (1991) showed in their studies that clean up of crude oil at low concentration may be left to nature and time.

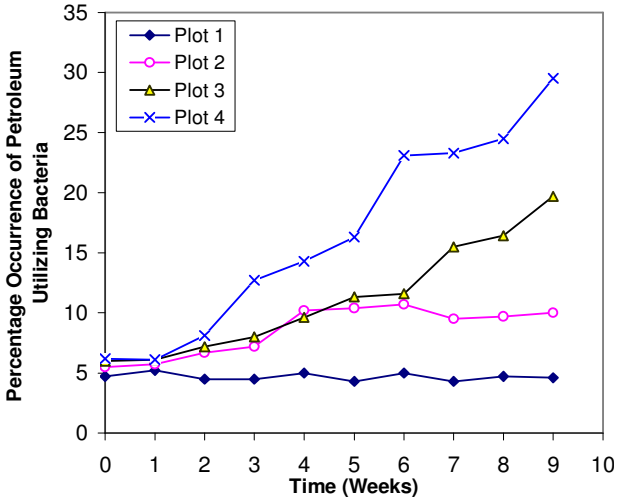

Figure 3. Relative (%) occurrence of petroleum utilizing bacteria in experimental plots

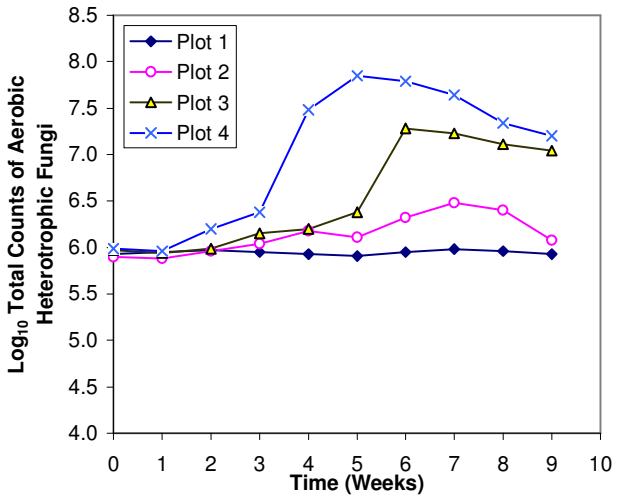

Figure 4. Total viable counts of petroleum utilizing bacteria in experimental plots.

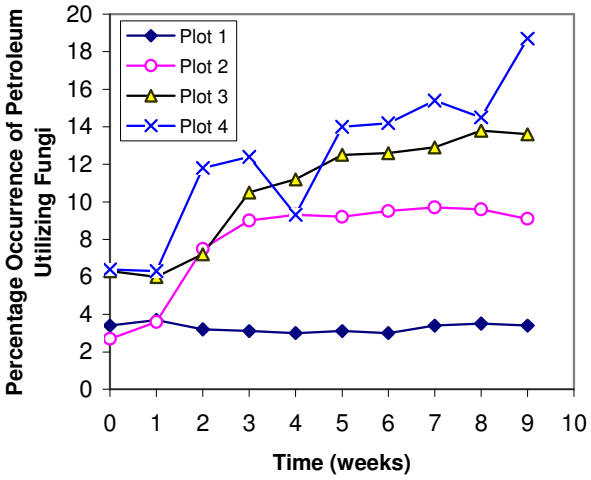

Figure 5. Relative (%) occurrence of petroleum utilizing fungi in experimental plots.

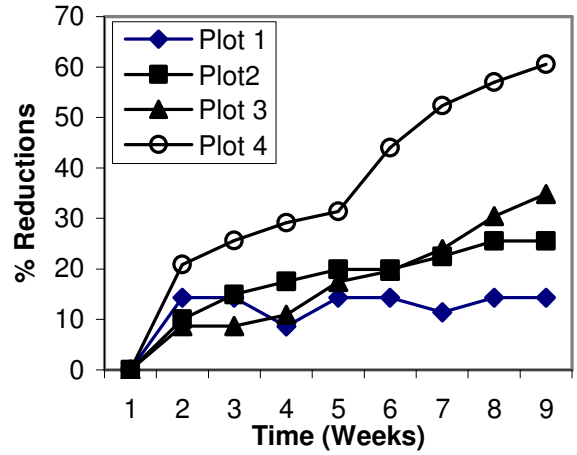

Figure 6. Hydrocarbon reductions by bacteria growing in soils amended

Isolation of hydrocarbon utilizing bacteria

The bacterial species isolated are similar to those reported by Odu (1972), Colwell and Walker (1977), Atlas et al. (1978), Amadi (1980) and Stephen et al. (1980). *Pseudomonas* sp. and *Bacillus* sp. were the most predominant in the test soil. Stephen et al. (1980) also reported *Pseudomonas* sp. to be predominant in petroleum hydrocarbon contaminated soils. The species isolated are assumed to be able to utilize petroleum hydrocarbon as sole source of carbon (Colwell and Walker, 1977). However, petroleum degraders have been reported to be endowed with enzyme systems and nutritional capabilities, which enable them to withstand adverse environmental conditions. Catabolic pathways responsible for the degradation of petroleum hydrocarbons have been extensively studied and found to be located on large catabolic plasmids mainly seen or found in *Pseudomonas* spp. (Lloyd and Lau, 1997). A detoxifying enzyme – glutathione transferase (GST) has been found to be present in *Proteus*, *Pseudomnas flavobacterium* and *Escherichia coli* (Lloyd and Lau, 1997). The organisms isolated are usually found in all crude oil polluted soil.

Microbial degradation of petroleum hydrocarbon in the soil

The residual hydrocarbon in the treated and untreated soil as shown by spectrophotometric analysis is as presented Figure 6. There was a gradual decrease in THC from week 1 to week 8 in all treated soils. The actual degradation or cumulative hydrocarbon degraded calcu-

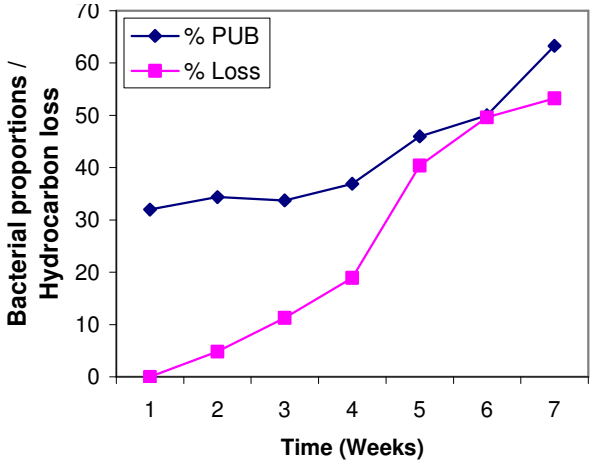

Figure 7. Growth proportions and reductions in hydrocarbon content by petroleum utilizing bacteria in liquid medium.

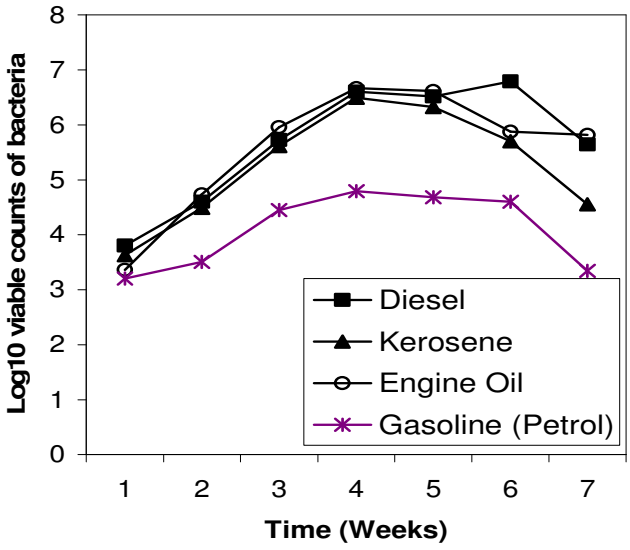

Figure 8. Growth profiles of bacteria utilizing different hydrocarbon fractions in liquid medium.

lated as plot 4 – [(plot 2 – plot 1) + plot 3] ranged from 118.35 to 439.74 µg g^{-1} with total percentage loss (% loss) as 73%. The disappearance of crude oil expressed as percentages loss in each treated soil range from 8.7 to 34.8% and 20.9 to 60.5% for plots 3 and 4 respectively. From the above, the treatment had proved effective because a resultant removal of 60% was achieved for plot 4 compared with 34.8% reduction obtained from plot 3 in the non-nutrient supplemented polluted soil. This on the other hand showed that it would take natural degradation about 161 days to achieve the result so obtained in 56 days. Although several investigators Olivieri et al. (1976); and Westlake et al. (1978) have indicated the effectiveness of fertilizers to enhance bioremediation, the comparatively low values of total losses noticeable in plot 4 where NPK fertilizer effluent was used might be due to the fertilizer type or residual nutrient left in the effluent. These workers used fertilizers with higher nitrogen to phosphorus ratio to achieve a higher removal of hydrocarbon. This therefore might suggest that there is need to develop bioremediation technology suitable for the effluent employed in this study. The overall results in terms of percentage degradation achieved were comparable to works of Chianelli et al. (1991), Glasser et al. (1991), Jones and Greenfield (1991), and Song and Bartha (1990). Although there was no total removal of hydrocarbon, the fraction undegraded might thus be due to duration of study (56 days), effluent type and quantity used as against an average of 90 days and pure fertilizers used by the above workers to achieve greater reduction.

In comparison with the field isolates in the biodegradation of hydrocarbons, the laboratory simulation experiment also showed some encouraging results. Figure 7 shows approximately 53% percent reduction in hydrocarbon levels.

Bacterial growth on different refined petroleum products

Figure 8 shows the growth of mixed bacterial cultures on different refined petroleum products as sole sources of carbon and energy. The result showed that bacterial growth was most supported by engine oil, followed by diesel, kerosene supported moderate growth while gasoline (petrol) supported a minimal amount of growth. The reason in part might be attributed to the more complex chemical composition of engine oil than those of petrol and kerosene. The heavier fraction was more susceptible to bacterial degradation, as it was readily available to bacterial attack (Nuzzi, 1973).

Generally, refined petroleum products such as petrol and kerosene contain short chain hydrocarbons of 5 – 14 carbon atoms in length while those of engine oil and diesel are higher, closer to pure crude oil. The hydrocarbon range of (C_4 – C_9) of petrol is less likely to support growth, they are very volatile and may not be readily available to bacteria and also, the lead anti-knock additives (tetraethyl lead) in gasoline (petrol) are probably inhibitory – toxic to microbes (Hill, 1984).

REFERENCES

Adoki A, Sokari TG, Ibiebele DD (1999). Effects of amendments on biodegradation of crude petroleum by sediment bacteria from Bonny River Estuary. Afr. J. Ecol. 37(3): 258-266.

Athrendy (1973). Conserving the Biodegradation of Oil in Natural Water Appl. Microbiol. 16(4): 17–24.

Atlas RM (1981). Microbial Degradation of Petroleum Hydrocarbons. An Environmental Perspective. Microbiol. Rev. 45: 180–209.

Atlas RM, Bartha R (1972). Biodegradation of Petroleum in Sea Water at Low Temperature, Dam J. Microbiol. pp. 1851–1855.

Atlas RM, Bartha R (1973c). Degradation and mineralization of Petro-

leum in Sea Water: Limitations by nitrogen and Phosphorous. Biotechnol. Bioeng. 14: 309–317.
Atlas RM, Sextone AP, Gustin O, Miller O, Linkins P, Everett K (1978). Biodegradation of crude oil by Tundra soil microorganisms Proceedings of the 4th International Biodegradation Symposium. Applied Science Publs. Ltd. pp. 21-26.
Colwell RR, Walker JD (1977). Ecological Aspects of Microbial Degradation of Petroleum in the Marine Environment. CRC Critical Rev. Microbiol. 5(4): 423–445.
Floodgate GD (1972). Biodegradation of oil in marine environments. Pollut. Bull. 3: 41–43.
Jaffe R (1991). Fate of hydrophobic organic pollutants in the aquatic environment. A review. Environ. Pollut. 69: 237-257.
Jobson AM, McLaugnlin FD, Cook FD, Westlake DWS (1974). Effects of Amendments on the Microbial Utilization of Oil Applied to Soil. Appl. Microbiol. 27: 166–171.
Jones MA, Greenfield JH (1991). In stitu comparison of Bioremediation Methods for a Number 6 Residual Fuel Oil Spill-in Lee County, Florida. Proceedings of the 1991 Oil Spill Conference. American Petroleum Institute, Washington, D. C.
Kinney PJ, Button DK, Schell DM (1969). Kinetics of Dissipation and Biodegradation of Crude Oil in Alaska's Cook Inlet. In: API/FWPCA Conference on Prevention and Control of Oil Spills, American Petroleum Institute Washington, D.C., pp. 333.
Lee K, Levy EM (1991). Bioremediation: Waxy Crude Oils Stranded on Low-Energy Shorelines. In: Proceedings of The 1991 Oil Spill Conference. American Petroleum Institute, Washington D. C.
Lloyd GJ, Lau PCK (1997). Gluthion S – Transferase Encoding Gene as Potential Probe for Environmental Bacterial Isolates Capable of Degrading Polycyclic Aromatic Hydrocarbons. Appl. Environ. Microbiol. 63(8): 3286–3290.
Murphy HF (1929). Some effects of crude petroleum on nitrate production. seed germination and Growth. Soil Sci. 27: 117–120.
Odokuma LO, Okpokwasili GC (1993). Seasonal Ecology of Hydrocarbon Utilizing Microbes in the Surface Waters of a River. Environ. Monit. Assess. 27: 175–191.
Odu CTI (1972). Microbiology of Soils Contaminated with Petroleum. Hydrocarbon Extent of Contamination and some Soil and Microbial Properties Affected after Contamination. J. Inst. Pet. London 58: 201–208.
Odu CTI (1978). The effects of Nutrient application and Aeration on Oil Degradation in Soil, Environ. Pollut. 15: 235–240.
Odu CTI, Isinguzo OSN (1979). Oil Spillage and Use of Instant Organisms for Enhanced Biodegradation. The Petroleum Industry and the Nigerian Environment. Proceedings of 1987. I'ntl. Seminar, NNPC, pp. 133–138.
Odu CTI, Esuruoso OF, Nwoboshi LC, Ogunwale JA (1985). Environmental Study of the Nigerian. Agip Oil Company, Operational Area. Soil and Fresh Water Vegetation Union Graft Publs. Milan pp. 21–25.
Plice MJ (1948). Some Effects of Crude Petroleum on Soil Fertilizer. Soil Sci. Soc. Amer. Proceedings 13: 413–416.
Stephen IH, Faw GM, Sizemore (1980). The bacterial Community Composition of an active Oil Field in the North – Western Gulf of Mexico. Mar. Poll. Bull. 11: 153–155.
Stevens S (1991). Selection of Nutrients to Enhance Biodegradation for the Remediation of Oil Spilled on Beaches. Proceedings of the 1991 Oil Spill Conference. American Petroleum Institute, Washington D. C.