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Effect of organic nutrient on microbial utilization of hydrocarbons on crude oil contaminated soil

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The effect of organic nutrient (poultry manure) on biodegradation of soil (5 kg) contaminated with crude oil (50 g) was investigated for seven weeks. Four different test options were prepared namely; (i) 100 g of contaminated soil + 30 g of poultry manure; (ii) 100 g of contaminated soil + 60 g of poultry manure; (iii) 100 g of contaminated soil + 90 g of poultry manure; (iv) 100 g of contaminated soil only (control). The microbial degradation was monitored by the measurement of total heterotrophic count (THC), hydrocarbon utilizing bacterial count (HUB) and gravimetric loss of the crude oil with time. The cumulative THC of 6.9×10^7, 9.0×10^7, 1.03×10^8 and 3.1×10^7 cfu/g were recorded for test options (i), (ii), (iii) and (iv), respectively. The hydrocarbon utilizing bacterial counts (HUB) were 1.68×10^5, 1.63×10^5, 1.9×10^5 and 4.8×10^4 cfu/g for tests options (i), (ii), (iii) and (iv), respectively. There was a corresponding gravimetric hydrocarbon loss of 40.0, 45.26, 49.47 and 29.47% for test conditions (i), (ii), (iii), and (iv), respectively. The results of the study suggest that addition of organic nutrient (especially 90 g poultry manure) will further enhance microbial utilization of hydrocarbons.

Key words: Biodegradation, crude oil, poultry manure.

INTRODUCTION

The petroleum industry has created economic boom for Nigeria and at the same time led to environmental and socio-economic problems. According to Ifeadi and Nwankwo (1989), statistics shows that the highest incident of oil spills occur in the mangrove swamp zones and near offshore areas of the Niger Delta of Nigeria.

Microorganisms are the major agents in the degradation of petroleum hydrocarbons. The organisms include bacteria, yeast, filamentous fungi and algae (Prince et al., 1993; Atlas, 1981). The principal bacteria and fungi genera responsible for oil degradation in both soils and aquatic environment have been identified as comprising mainly *Pseudomonas, Achrobacter, Bacillus, Micrococcus, Nocardia, Trichoderma,*

Penicillium, Aspergillus and *Morteilla* (Atlas, 1981; Bossert and Bartha, 1984; Okpokwasili and Amanchukwu, 1988; Ezeji et al., 2005).

This study reports the effects of organic nutrients (poultry manure) on the microbial utilization of petroleum hydrocarbon on polluted soil. Different concentrations of crude oil were added to different quantities of polluted soil samples in order to determine the nutrient ratio that gives the best performance for remediation purposes.

MATERIALS AND METHODS

Sample collection and Preparation

Garden topsoil (0-15 cm) with no previous history of crude oil contamination was collected from the Federal University of Technology (FUTO), Owerri, Nigeria. The crude oil (Bonny light) was obtained from Nigeria Agip Oil Company, Port Harcourt. The poultry manure used for this study was obtained from the School

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Table 1. A summary of test conditions and sample treatment employed in the microbial utilization of petroleum hydrocarbons in contaminated soil.

Trmt.	Condition	Description of condition
(i)	Nutrient added	100 g of contaminated soil + 30 g poultry manure
(ii)	Nutrient added	100 g of contaminated soil + 60 g poultry manure
(iii)	Nutrient added	100 g of contaminated soil + 90 g poultry manure
(iv)	No nutrient added	100 g of contaminated soil only (control)
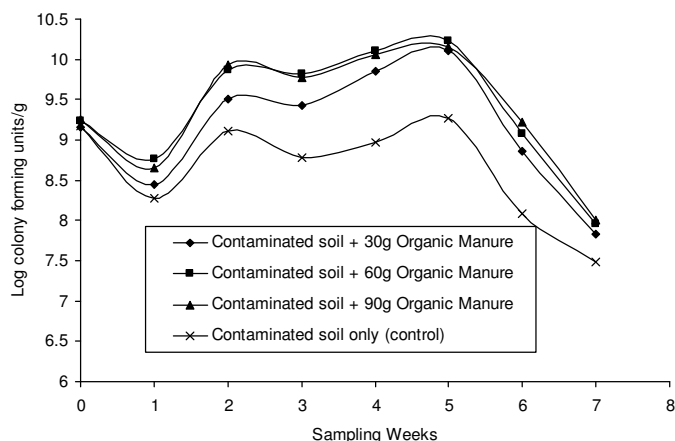
Figure 1. Total heterotrophic microbial population in crude oil contaminated soil with increasing addition of organic manure.

of Agriculture and Agricultural Technology, FUTO.

5 kg of the soil samples contained in plastic bags were contaminated with crude oil at the ratio of 10 g per kilogram (10 g/kg). The experimental samples were set up as shown in Table 1 and monitored for a period of seven weeks.

Heterotrophic bacterial count

The mean total aerobic bacteria present in the samples at the beginning of the experiment (day zero) and at weekly intervals for each of the treatment options were estimated using spread plate method with nutrient agar as medium. A ten-fold dilution using physiological saline was prepared and 0.1 ml of appropriate dilution was plated in duplicates and incubated for 18 – 24 h at room temperature after which the colonies were counted.

Enumeration of hydrocarbon utilizing bacteria

Aliquots (0.1 ml) of appropriate dilutions of soil samples were plated on to modified mineral salts medium of Mills et al. (1978) containing the following in g/l: NaCl, 10.0; $MgSO_4.7H_2O$, 0.42; KCl, 0.29; KH_2PO_4, 0.53; NH_4NO_3, 0.42; agar, 15.0 and distilled water. The vapour phase transfer technique of Okpokwasili (1988) was adopted, which employs the use of sterile filter paper soaked in crude oil, which served as the as carbon and energy source. The soaked sterile filter papers were then aseptically placed onto covers of the inoculated inverted plates and incubated for 5 to 7 days at room temperature. Average mean counts of colonies from duplicate

plates were recorded and used for the calculation of colony forming units per gram (cfu/g) of soil.

Measurement of crude oil utilization using gravimetric method

Residual crude oil was extracted from the soil samples using a modified method of Abu and Ogiji (1996). Soil samples were air dried to constant weights and 5 g were placed in chemically clean conical flasks and 10 ml of chloroform was added into each flask. Residual oil was extracted by gently shaking the flasks for 3 min. Each extract was filtered through cotton wool in a funnel and collected in a clean test tube, closed immediately and stored at 4 – 6°C for hydrocarbon concentration analysis. Quantitative determination of the crude oil extracts was carried out as described by Udeme and Antai (1988). A standard curve of absorbance (520 nm) against varying concentrations of bonny light crude oil (1 to 5%) in chloroform was drawn after taking readings from a PYE UNICAM SP6-550 UV/VS spectrophotometer. The hydrocarbon concentrations were calculated from the standard curve after multiplying by the appropriate dilution factor.

RESULTS AND DISCUSSION

Figure 1 shows the total heterotrophic bacterial population (THC) during the seven-week test period. The total heterotrophic count showed an increase in microbial population. This is in line with the works of Dibble and Bartha (1979), Atlas et al. (1978) and Buckley (1980). General soil fertility and biological activity has been known to increase in oiled soils as a result of the application of organic nutrients (Jobson et al., 1974; Holiday and Deuel, 1994). This increase might be as a result of the combined effort of the presence of hydrocarbon and nutrient supplementation since microbes with the ability to degrade crude oil components respond quite rapidly to the presence of petroleum (Baker, 1989; Lee and Levy, 1991; Fought and Westlake, 1992; Abu and Ogiji, 1996).

The result showed an increase in the population size of microbes in the soils amended with poultry manure compared to the one without the manure (control). This is due to the fact that poultry manure on its own contains a diversity of organisms in addition to being a nutrient source. At the end of week 7, the total heterotrophic count (THC) values were 6.9×10^7, 9.0×10^7, 1.10×10^8 and 3.1×10^7 cfu/g for treatment options (i), (ii), (iii) and (iv), respectively. This shows that the soil with 90 g nutrient supplement gave the highest heterotrophic bact-

Table 2. Percentage hydrocarbon utilizing bacterial population in crude oil contaminated soil with increasing addition of organic manure.

| *Trts/	Wks							
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
(i)	0.0001	0.0023	0.0049	0.0163	0.0101	0.0171	0.0589	0.2434
(ii)	0.0002	0.0011	0.0035	0.0035	0.0063	0.0132	0.0433	0.1811
(iii)	0.0003	0.0016	0.0034	0.0039	0.0068	0.0200	0.0416	0.1844
(iv)	0.0001	0.0035	0.0035	0.0058	0.0067	0.0084	0.1158	0.1548

*See Table 1 for details of the treatments i, ii, iii and iv.

Table 3. Loss of crude oil from the crude oil contaminated soil samples for each treatment option after seven weeks of treatment with increasing addition of organic manure.

Trt No.	Treatment	Conc. of residual Crude oil(mg/ml)	Total percentage loss in crude oil[a]	Net per. loss due to [b]Treatment
(i)	30 g nutrient	0.0057	40.00	10.53
(ii)	60 g nutrient	0.0052	45.26	15.79
(iii)	90 g nutrient	0.0048	49.47	20.00
(iv)	Control	0.0067	29.47	N/A
0[c]	N/A	0.0095	N/A	N/A

N/A = Not applicable
[a]Total percentage loss in crude oil = [conc. of crude oil (0[c]) - conc. of crude oil (treatment) x 100]/conc. of crude oil (0[c]).
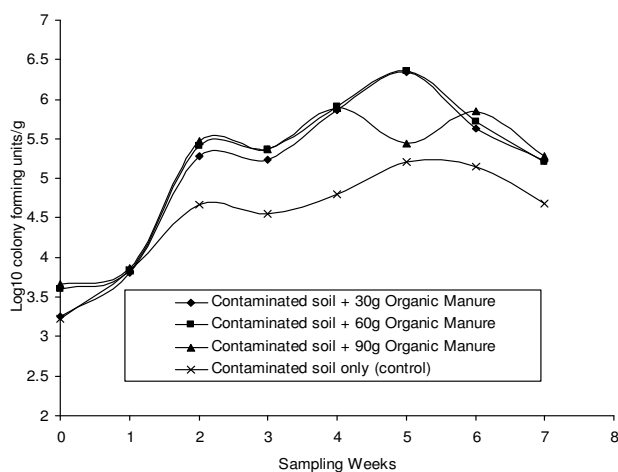
Figure 2. Total hydrocarbon utilizing bacteria in crude oil contaminated soil with increasing addition of organic manure.

erial count.

The initial drop in the total heterotrophic counts in the contaminated soil sample in the first week can be attributed to selective inhibition of members of the microbial community as a result of the toxic components of petroleum and also as a result of reduced aeration and upsets in carbon/inorganic nutrient balance for the indigenous population caused by the presence of petroleum (Atlas, 1984).

The ANOVA between treatments and between weeks were found to be highly significant at 99.9% confidence interval and a positive correlation (r=0.072) exists between total heterotrophic bacteria and time.

Figure 2 shows the total hydrocarbon-utilizing bacteria in all the treatment options. The hydrocarbon utilizing microbial count showed an overall increase in the contaminated soil samples. This is in line with the works of Baker (1989), Lee and Levy (1991), Fought and Westlake (1992), Abu and Ogiji (1996) which showed that microbes with the ability to degrade crude oil components respond quite rapidly to the presence of petroleum. Also in support of the above, Atlas (1984) recorded that the most widely documented response of microbial communities to exposure to oil is a rapid increase in size of hydrocarbon utilizing component of the community. The highest number (1.9×10^5cfu/g) of hydrocarbon degraders was recorded in the soil supplemented with 90 g organic fertilizer. The variation between the treatments was found to be significant at 99.9% confidence interval. There also exists a positive correlation (r=+0.40) between time and hydrocarbon utilizing bacterial counts.

Table 2 summarizes the percentage hydrocarbon utilizing bacterial population for all the treatment options. The percentage hydrocarbon-utilizing bacteria at the end of seven weeks treatment were 0.2434, 0.1811, 0.1844 and 0.1548% for test options (i), (ii), (iii), and (iv), respectively. This shows that the sample supplemented with 90 g of poultry manure gave the highest percentage hydrocarbon-utilizing bacteria.

The potential of the treatment option was shown by the percentage reduction of oil in the soil samples. Table 3 shows the gravimetric loss in crude oil at the end of the treatment period. The highest percentage oil reduction of 49.47% was observed in the sample supplemented with 90 g poultry manure. This was followed by the treatment with 60 g poultry manure (45.26%) and 30 g poultry manure (40.00%). The sample without any poultry manure had an oil reduction of 29.47%. The oil reduction achieved due the addition of the various concentrations of poultry manure ranged from 10.52 to 20.00%. This result shows that nutrient supplementation enhances biodegradation rate, which is in agreement with the works of Henry et al. (1991), Mark and Jeffrey (1991) and Abu and Ogiji (1996).

From this study it was discovered that organic manure enhances microbial utilization of petroleum hydrocarbon. The 100 g soil samples supplemented with 90 g organic manure gave the highest percentage loss of hydrocarbon. This suggests that bioremediation of polluted soil can best be carried out by treating the soil with organic manure.

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