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Bioremediation of Crude Oil Contaminated Soil using Wood Ash

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The efficiency of two surfactants; wood ash (a domestic/agricultural by-product) and Tween 80 (a chemically synthesized surfactant) was investigated during the bioremediation of crude oil contaminated soil. The soil samples were contaminated with crude oil at 5% and 10% levels and inoculated with hydrocarbonoclastic organisms. Microbial anaylsis of the soil amended with wood ash (2.5%) gave a range of 9.50 x10^3 Cfu/g to 1.80 x10^5 Cfu/g and 8.00 x10^3 Cfu/g to 1.70 x10^5 Cfu/g while those amended with Tween 80 (2.5%) was 6.00 x10^3 Cfu/g to 2.10 x10^5 Cfu/g and 1.00 x10^4 Cfu/g to 8.50 x10^4 Cfu/g for 10% and 5% levels of contamination respectively. The pH values for all soils were observed to increase from acidity (5.63) to near neutrality (averagely 6.75). Other soil physicochemical parameters also showed variations during the period. After 90 days, the soils' Total Residual Hydrocarbon Content (TRHC) revealed differing reductions as aided by the surfactants. Soil samples with 10% crude oil contamination had background crude oil level of 9,751 mg/Kg, and at the end of the period, soil amended with wood ash recorded a TRHC of 887.22 mg/Kg while that amended with Tween 80 recorded 1,450 mg/Kg representing 90.90% and 85.12% reductions respectively. Soils with 5% levels of contamination had higher reduction rates as wood ash recorded 94.54% while Tween 80 had 90.28% from a background contamination level of 8226 mg/Kg. Wood ash enhanced the degradation of the crude components C_8 to C_{28} more than that achieved by Tween 80.

Key words: Bioremediation, Surfactants, Wood ash, Tween 80, Hydrocarbonoclastic organisms.

The utilization of petroleum for its products and services throughout the world has increased to monumental levels. Notable oil spills around the world[1,2] and in the Niger Delta Region has highlighted huge negative environmental damage from mishaps in handling petroleum, as spillage releases large amount of organic compounds into the environment[3]. Molecules like saturates, aromatics, asphaltenes, and resins are released into the environment, being constituents of Crude oil[4]. All these molecules like any hydrophobic compounds cover the surfaces of nearby rivers, stream or even oceans, since they are less dense than water. The additional layer they form on water reduces the amount of sunlight, oxygen and other necessary diffusible nutrients reaching organisms on land and in water[5]. So, constituents of spilled oil are harmful to many of the organisms in various zones of the aquatic system and soil environment[6].

The removal of these spills from the environment, with the least concomitant ecological, economical and health damage is therefore imperative[7]. Among the several clean-up techniques available to remove petroleum hydrocarbons from soil and groundwater, bioremediation processes are gaining grounds due to their simplicity, higher efficiency and cost-effectiveness[8] when compared to other technologies[1]. These processes rely on the natural ability of biological agents to carry out the mineralization of organic compounds, leading

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ultimately to the formation of CO_2, H_2O and microbial biomass[9].

Surfactants are known to increase the bioavailability of hydrocarbon resulting in enhanced growth and degradation of contaminants by hydrocarbon-degrading microorganisms present in the polluted soil[10, 11]. Soberon-Chavez and Maier[12], in their review stated that biosurfactants act by accumulating at the interface between two immiscible fluids or between a fluid and a solid. By reducing surface (liquid-air) and interfacial (liquid-liquid) tension they reduce the repulsive forces between two dissimilar phases and allow these two phases to mix more easily. This leads to increased mobility and bioavailability of the hydrocarbon contaminants to microbial attack. Biosurfactants produced from domestic/ agricultural wastes allows for the ultilization of waste substrates and reduces their polluting effect at the same time[13]. Researches utilizing wastes to enhance the bioremediation of crude oil polluted soils include but not limited to: the amendment of polluted soils with wood ash, compost and sawdust[14]; use chicken droppings[15]; amendment of contaminated soil samples with grounded yellow and white melon shells[16]; and use of cow dung[17]. Studies on biosurfactants have reported them as being more eco-friendly, cheap and effective in the reclaimation of polluted soils in comparison with chemically synthesized surfactants[10,18].

This study compares the effectiveness of wood ash (agricultural/domestic waste by-product) and Tween 80 (chemical surfactant) in the bioremediation of soil samples.

MATERIALS AND METHODS

Collection and preparation of samples

The soil samples were collected following the protocol of Okop, *et al.,*[19] with modifications. Five to ten (5-10) centimeters deep topsoil was collected with shovel into clean bucket from a site at the Pharmacy farm and transported to the Microbiology Postgraduate laboratory, University of Uyo Town campus. Bonny light crude oil was sourced from Elf Petroleum, Nigeria. The wood ash was obtained from charcoal residue. The wood lump was chopped and ashed in the oven at 100°C. The char residue was grounded, sieved and stored in airtight containers at room temperature. Tween 80 (a chemical surfactant, which served as control) was bought from Ariaria market Aba, Abia State, Nigeria.

Screening for hydrocarbonoclastic bacteria

Bacterial isolates obtained from crude oil contaminated soil were cultured on mineral basal medium supplemented with 1 % v/v crude oil. The solid mineral basal medium was composed of $(NH_4)_2SO_4$, 1.0 g; KH_2PO_4, 1.0 g; K_2HPO_4, 1.0 g; $MgSO_4$, 0.2 g; $CaCl_2$, 0.02 g; $FeCl_3 \cdot 6H_2O$; 0.004 g, Agar, 15g and water 1 litre and adjusted to pH, 7 ± 0.2. Microbial growth and production of zone of clearing around the colonies on the medium were regarded as evidence of growth and utilization of oil as the sole carbon sole. The organisms' oil utilization potential were graded as strong (+++), moderate (++), weak (+) and no (") degrading potential[20].

Processing of soil samples

The soil sample was autoclaved to rid it of indigenous organisms. After cooling, it was divided into two portions, one portion received 50ml of crude oil per kg of soil (5% contamination) while the second received 100ml of crude oil (10% contamination). Screened hydrocarbonoclastic bacteria (*Pseudomonas, Bacillus, Corynebacterium* and *Alcaligenes* spp) previously isolated from contaminated soil were inoculated as a consortium into the sterilized soil at 5×10^7 cfu/ kg. The soil samples were subdivided to receive 2.5% surfactant amendment (wood ash and Tween 80) and left in perforated wooden boxes (14cm x14cm x 6cm) microcosms. Microbial analyses of the contaminated soils were carried out at two weeks interval for 90 days. The soil was moistened with 30ml of sterile water every 3-4 days to maintain adequate moisture level. The microcosms were analysed in triplicates.

Determination of the physicochemical properties

pH, electrical conductivity, total residual hydrocarbon content, concentration of organic matter, organic carbon, total nitrogen, available phosphorus and the following metals; Cu, Ni, Cd, Fe, and Pb were determined using standard methods[21,22]. Other parameters were the residual hydrocarbon content and petroleum oil fraction analysis.

Enumeration of bacteria in the microcosms

This was carried out using the pour plate method on Nutrient Agar. The plates were incubated at 30°C for 24 hours. The bacterial density was reported as mean of triplicate determination and recorded as Cfu/g of soil.

Determination of Residual Hydrocarbon Content

One gramme of the contaminated soil sample was disolved in 10ml of hexane and shaken for ten minutes using a mechanical shaker. The solution was filtered using Whatman No 1 filter paper and the filtrate diluted by taking 1ml of the extract into 50ml hexane. The absorbance of the solution was read at 460nm with Mamotte 701 Spectrophotometer using *n*-hexane as blank[23].

Chromatographic analysis

The hydrocarbon fractions were determined using the methods of ASTDM 3921 and USEPA 8270B[24]. The Total Residual Petroleum Hydrocarbons (TPH) and Polycyclic Aromatic Hydrocarbons (PAHs) were extracted from the soil samples and quantified using Gas Chromatograph-Mass Spectrometer HP 5890.

RESULTS AND DISCUSSION

Physicochemical results

The physicochemistry revealed that wood ash as being slightly alkaline (pH 7.8). This provided a buffering effect in the soil samples during the mineralization of crude oil. Wood ash also had a high carbon content of 82.4 mg/kg but lower Nitrogen and Phosphorus contents (0.002 Cmol/kg and 8.12 mg.kg respectively). These elemental contents of wood ash are suspected to aid the growth of the test bacteria with that already in the soil sample. This assertion is supported by the work of Gallego *et al.,*[25] who demonstrated these nutrients as important factors that enhance soil bioremediation. With the exception of nitrogen, wood ash had higher chemical component value than Tween 80 as presented in Table 1. The unamended soil was acidic (pH of 5.63) before contamination with Total Nitrogen content, Organic matter content and available phosphorus of 0.74 $CmolKg^{-1}$, 0.706mg/Kg and 9.28% respectively. The metals concentrations were within acceptable limits (Table 1)[26]. The 10% crude oil contaminated soil amended with wood ash had a pH of 6.71 while Tween 80 had 6.60. Soils with 5% crude oil contamination had pH values of 6.80 and 6.78 for wood ash and Tween 80 amendments, respectively. The addition of crude oil into the soils resulted in reduction in their phosphorus contents which

Table 1. Physicochemical properties of unamended, contaminated - amended soils and biosurfactant

Parameters	Unamended	Soils with 10% crude oil amendment with		Soils with 5% crude oil amendment with		Surfactants	
		wood ash	Tween 80	Wood ash	Tween 80	Wood ash	Tween 80
pH	5.63	6.71	6.60	6.80	6.73	7.8	4.8
Texture (%)	35	20	22	28	34	-	-
Nitrogen(Cmol/Kg)	0.74	0.48	0.56	0.74	0.59	0.002	0.22
Phosphorus (mg/Kg)	0.706	0.720	0.481	0.508	0.511	8.12	6.15
Organic matter(%)	9.28	9.15	5.48	9.05	7.49	82.4	56.3
Electrical conductivity (dS/m)	12	9	13	20	18	ND	ND
Exchangeable ions (Cmol/Kg)	12.27	15.12	9.51	10.53	19.17	ND	ND
Exchangeable acids (Cmol/Kg)	6.5	5.7	3.8	6.0	4.2	ND	ND
Cu (mg/L)	0.094	0.176	0.122	0.148	0.156	ND	ND
Ni(mg/L)	0.426	0.276	0.350	0.281	0.263	ND	ND
Cd(mg/L)	0.059	0.085	0.086	0.090	0.096	ND	ND
Pb (mg/L)	0.362	0.210	0.216	0.389	0.366	ND	ND
Fe (mg/L)	120.41	227.45	200.12	211.56	253.18	ND	ND

Table 2. Crude oil degrading potentials of bacterial isolates

Isolates	Degrading capability
Pseudomonas aeruginosa	+++
Corynebacterium sp	++
Alcaligenes sp	++
Bacillus sp	+++

Key: - = No Growth, + = weak, ++ = moderate, +++ = strong

Table 3. Total residual hydrocarbon content of soils

Sample	10% crude oil contamination with amendment		5% crude oil contamination with amendment	
	Wood ash	Tween 80	Wood ash	Tween 80
Initial concentration	9,751mg/Kg	9,751mg/Kg	8226mg/Kg	8226mg/Kg
Final concentration	887.22mg/Kg	1,450mg/Kg	449.28mg/Kg	799.41mg/Kg
% Reductions	90.90%	85.12%	94.54%	90.28%

increased with amendments (Table 1). These findings corroborate the earlier work of Osuji and Nwoye [22] who appraised the impact of petroleum hydrocrbons on soil fertility in Owaza, Delta State Nigeria. While the exchangeable acids in wood ash amended soils reduced, soils with Tween 80 amendments had higher values. The Electrical conductivity of the 5% contaminated soil amended with wood ash increased from 12dS/m to 20dS/m after 90 days. This indicates an increase in the amount of ions present in the soil[27]. Also the soil organic matter in all the microcosms reduced from the initial 9.28% to averagely 7.91%. The concentrations of the metals throughout the period were found to be low compared to the common ranges in soils as reported by Lindsay[27]. Fuller[28], while discussing the relatively high mobility of heavy metals with regard to pH, considered that in acid soils (pH 4.2-6.6) the elements Cd and Ni are highly mobile and Cu and Pb are practically immobile, and in neutral to alkaline (pH 6.7-7.8), Cd is moderately mobile while Ni is immobile. The 'near neutrality' condition of the microcosms could be the reason for increments in Cu, Cd, Pb and Fe and reductions in Ni concentrations in the microcosms.

The ability of the pure bacterial strains isolated from a contaminated soil to degrade and utilize crude oil for biomass was indicated by zones of clearing on the mineral salt medium supplemented with 1%v/v crude oil. *Pseudomonas, Bacillus, Corynebacterium* and *Alcaligenes* spp have been implicated in several other bioremediation studies[29-32] and their capabilities is presented on Table 2.

The bacterial counts, taken at two weeks interval, showed an initial reduction in bacterial load after inoculation into the sterile soil. This could be attributed to time required for the organisms to acclimate with the new environment and stress from the contaminant (Figs. 1 and 2). Atlas[33] and Amadi and Odu[34] concluded that hydrocarbonoclastic microorganisms have low initial counts when oil is introduced into an environment, but their counts increase tremendously only to revert to about the same counts after the oil had been degraded.

The Total Residual Hydrocarbon Content in soils is presented in Table 3, it is a measure of the level of crude oil in soil samples. The soils with 10% crude oil contamination level had initial total hydrocarbon content of 9,751mg/Kg which reduced to 887.22 mg/Kg with wood ash amendment and 1,450mg/Kg with Tween 80 amendment representing 90.90% and 85.12% reductions respectively. In comparison, the wood ash aided

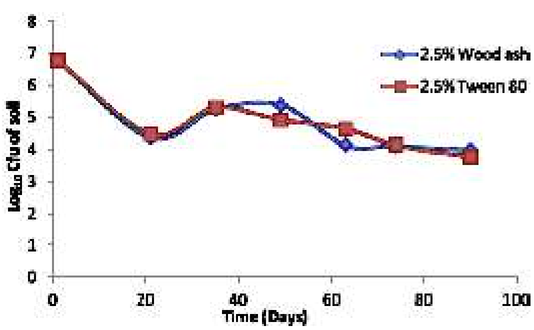

Fig. 1. Total Heterotrophic baterial count in soil contaminated with 10% crude oil

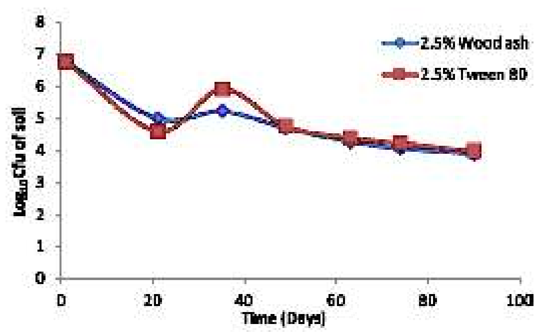

Fig. 2. Total Heterotrophic baterial count in soil contaminated with 5% crude oil

the removal of more crude oil compontents in the contaminated soil than Tween 80. Wood ash amended soil having 5% crude oil contamination reduced from 8,226mg/Kg to 449.28mg/Kg (94.54% reduction) been the highest recorded in the study after 90 days while 90.28% Total Residual Hydrocarbon Content was recorded for Tween 80 amended soil having reduced from 8,226mg/Kg to 799.41mg/Kg within the same time. The reduction rates were higher in soil samples with 5% contamination level, but in all, the Wood ash helped remove more crude oil components than Tween 80.

The Chromatograms showing the crude oil fractions and their abundance in the soil for the 10% and 5% contaminations levels are presented in Figures 3 to 8. This study showed that the crude oil components were degraded by the consortium but to different extents, in 10% crude oil contaminated soil amended with wood ash, components C_8 to C_{15} were completely mineralized except for C_{11} which had appreciable reduced peaks as components C_{16} to C_{28} in the same soil. With Tween 80 amendment, components C_8 to C_{14} were completely mineralized while components C_{15} to C_{28} showed drastic reductions in the peaks. In both amendments components C_{29} to C_{37} did not show significant reduction at the end of the 90 days. Soils with 5% crude oil contamination showed much reduced peak frequencies compared to those contaminated at 10% level (Fiqures 6 to 8). This could be due to the smaller quantity of crude in the 5% level. In soil amended with wood ash, Components C_8 to C_{15} were completely mineralized

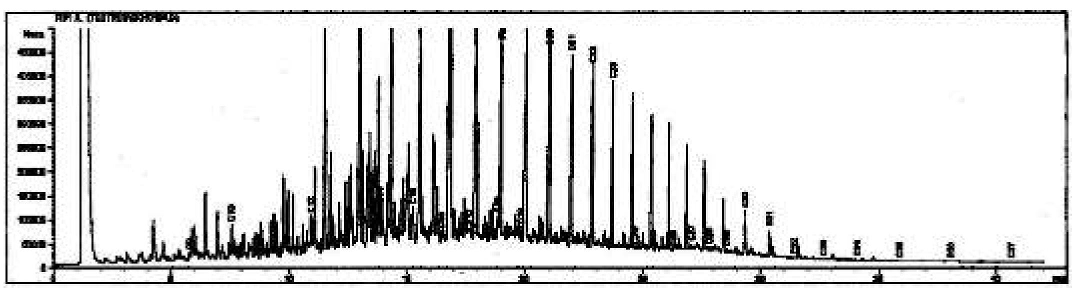

Fig. 3. Chromatogram of 10% crude oil contaminated soil (unamended)

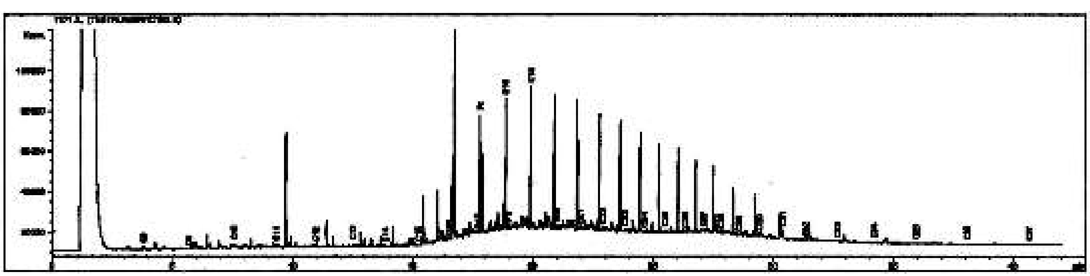

Fig. 4. Chromatogram of 10% crude oil contaminated soil amended with wood ash after 90 days

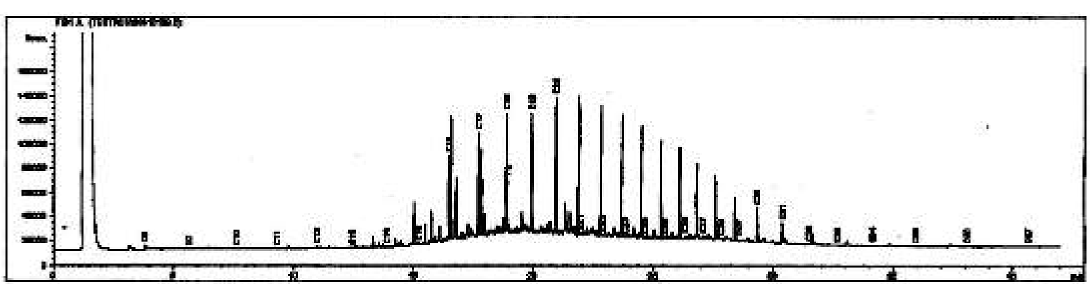

Fig. 5. Chromatogram of 10% crude oil contaminated soil amended with Tween 80 after 90 days

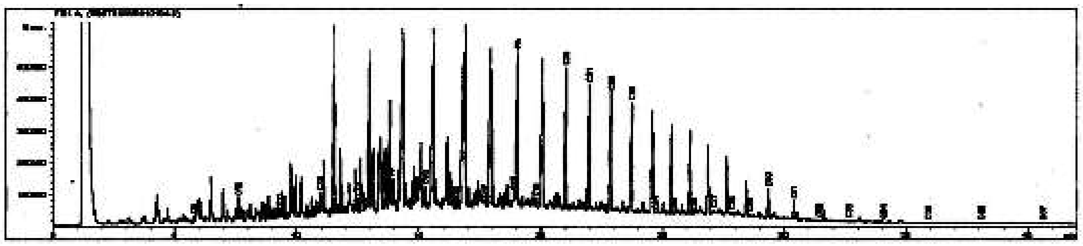

Fig. 6. Chromatogram of 5% crude oil contaminated soil (unamended)

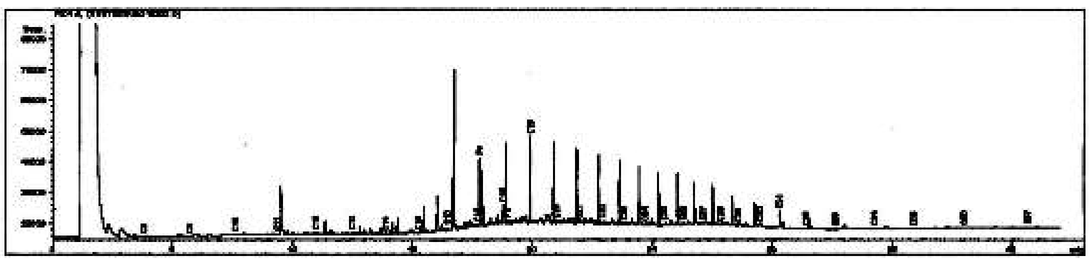

Fig. 7. Chromatogram of 5% crude oil contaiminated soil amended with wood ash after 90 days

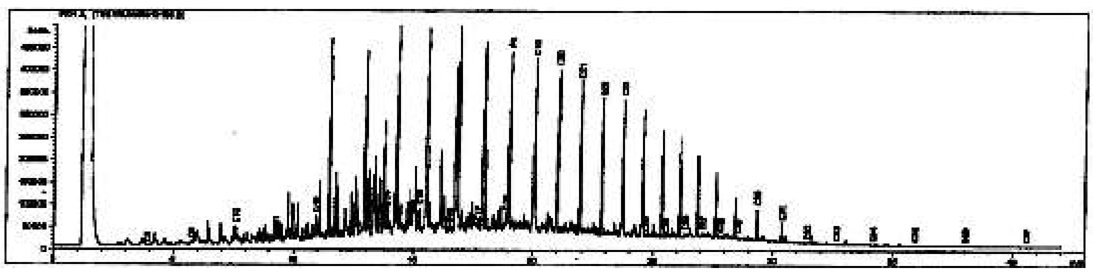

Fig. 8. Chromatogram of 5% crude oil contaminated soil amended with Tween 80 after 90 days

except also for component C_{11} while components C_{16} to C_{30} were seen to reduce drastically to minimal levels. The reductions in wood ash amended soil were higher as compared to soil amended with Tween 80 which still retained high appreciable crude oil fractions.

CONCLUSION

In this study, Wood ash showed a higher degradability of crude oil and biostimulated the soil with needed nutrients compared to Tween 80. With the 2015 projection of 15.63 x10^6 m^3 day^{-1} world petroleum consumption by the United States Energy Information Administration [35] high rate of oil spills are inevitable. Therefore, a cheap, eco-friendly and economical means of cleaning up oil spills should be developed and encouraged.

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