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Efficiency of cassava steep liquor for bioremediation of diesel oil-contaminated tropical agricultural soil

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Abstract Soil artificially contaminated with diesel oil, treated with cassava steep liquor (CSL) and designated EXPS. Similar polluted soil without CSL amendment (CSS1) and uncontaminated soil (CSS2) served as controls. There were dramatic changes in the physico-chemistry of systems EXPS and CSS1 with utilization of the inorganic nutrients to near-depletion in the former than the latter. In contrast, the properties of CSS2 remained relatively stable throughout the investigated period. Similarly, the population densities of microflora in the polluted soils showed an initial decrease between days 0 and 5 before assuming an increasing trend with percent hydrocarbon-utilizers ranging significantly ($P < 0.05$) from 0.56 to 6.6, 0.1 to 2.46 and 0.56 to 0.26, respectively for EXPS, CSS1, and CSS2. In EXPS, the residual oil decreased from 98,045 to 1,102.3 mg/kg soil at day 35 representing about 98.88% degradation. The corresponding value for CSS1 was 98,106.1 to 52,110 mg/kg soil, amounting to 46.88% oil disappearance. The GC fingerprints of alkane fractions of the recovered oil reduced significantly by day 15 for EXPS with near-similar results of CSS1. However, by day 35, there was complete disappearance of all peaks including the pristane and phytane molecules in the former whereas in CSS1, there were no observable changes. The germination and growth profiles of maize seed plants as evidence of recovery of oil-impacted soils were poor in CSS1 (10%) with pronounced abnormal morphology when compared with the data obtained for EXPS (74%) and CSS2 (80%). These results suggest that CSL could be an indispensable tool in bioremediation of environments contaminated with hydrocarbons. The technology of application is simple, rapid and cost-effective and may be appropriate for use in developing countries to ameliorate the problems of petroleum pollution.

Keywords Bioremediation · Cassava steep liquor · Diesel oil · Indigenous microflora · Maize seeds

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1 Introduction

Increasing petroleum exploration, refining and other associated industrial activities particularly, in the Niger Delta region of Nigeria have led to the wide scale contamination of most of its creeks, swamps, rivers, and streams with hydrocarbons and dispersant products (Okpokwasili and Odokuma 1990; Odokuma and Okpokwasili 1993). The contamination of these habitats constitutes a major public health and socio-economic hazards and most often results in violent protestations between the oil companies and their host communities. This incessant spillage and indiscriminate discharge of oil into the environment have called for more studies into oil pollution problems. Remediation of polluted systems could be achieved by mechanical, chemical or biological methods. However, mechanical cleaning of spilled oil in some Niger Delta communities is nearly impossible because of the difficult terrain and the nature of the ecosystem. Most importantly, the attendant negative consequences and negative public perception of the physico-chemical methods make the biological alternative or bioremediation more attractive and indispensable as the most natural technique to eliminate the bulk of

petroleum contaminants from the environment. The biological method exploits the diverse degradation abilities of microorganisms to convert the complex chemical components of crude petroleum into harmless products by mineralization (Adams and Jackson 1996; Mentzer and Ebere 1996; Adebusoye et al. 2007).

Since the proportion of hydrocarbon-utilizers implicated in crude oil degradation is naturally very low in soil and aquatic environments (Amund et al. 1987; Amund and Igiri 1990; Adebusoye et al. 2007), crude oil contaminant can persist in environments undegraded for many years (Atlas 1992; Bulich 1996; MacNaughton et al. 1999; Solano-Serena et al. 2000; Hamamura et al. 2006). Therefore, bioremediation protocols involving application of exogenous competent organisms as a supplement to those naturally present can improve the rate of recovery of polluted environments. But despite the apparent simplicity of bioaugmentation, there have been many failures (Vogel and Walter 2001; Wagner-Dobler 2003). Some of these failures have been attributable to pH and redox factors, the absence of key co-substrates (Barriault and Sylvestre 1993; Harms and Zehnder 1995; Thompson et al. 2005). Besides, the Exxon Valdez bioremediation experience, in particular, has been viewed by many researchers as a general rule that bioaugmentation is ineffective in petroleum and other biodegradation processes (Bragg et al. 1994; Van Hamme et al. 2003; McKew et al. 2007). An alternative approach to bioaugmentation is nutrients or biosurfactants addition to contaminated systems to stimulate the indigenous strains expressing hydrocarbon phenotypes or to solubilize the hydrophobic contaminant. While the use of biosurfactants to enhance biodegradation of crude oil has been reported (Kumar et al. 2006), however, effect of these surface-active agents on degradation is less straight forward. There are also evidences that biosurfactants may interfere with the interaction between biosurfactant-dispersed substrate and microbial cells resulting in decreased biodegradation (Falatko and Novak 1992).

While the use of microorganisms as pure or mixed cultures to degrade crude oil pollutant or the use of nutrient supplements to stimulate degraders in polluted matrices has been extensively studied (Amund and Igiri 1990; Morgan and Watkinson 1989; MacNaughton et al. 1999; Nwachukwu 2001; Roling et al. 2002; Van Hamme et al. 2003), no study has focused on liquid wastes emanating from food-based industries as potential agents for bioremediation. Therefore, the goal of this study was to determine if recovery rate of a tropical agricultural soil ecosystem contaminated with diesel oil could be accelerated through the addition of cassava steep liquor (CSL). CSL is a waste generated from cassava processing plants that are widespread in the country. Cassava-based foods are Nigeria's staples. Indiscriminate discharge of this waste into the environment is a public health concern. The use of CSL for diesel oil detoxification purposes will help arrest this ecological disaster and will further lower the cost of oil spill cleanup since the waste is readily available nationwide and at no extra cost.

Although literature is replete with biodegradation of diesel oil, a fraction of crude petroleum by microorganisms (Geerdink et al. 1996; Bicca et al. 1999; Margesin and Schinner 1997a, b), information is scanty on bioremediation of tropical African soils polluted with this complex petroleum cut. Diesel oil, a complex, petroleum hydrocarbon mixture consists mostly of linear and branched alkanes. It consists of a variety of paraffins (n-alkanes, branched alkanes, cycloalkanes), olefins, naphtha, phenolics, aromatic, and polycyclic aromatic compounds. The molecular weight of the hydrocarbons present in diesel is also variable, with molecules containing from 9 to 20 carbon atoms. The oil is one of the major pollutants of soil and groundwater near petrol stations and many industrial plants in Nigeria where heavy duty power generators are run on diesel oil across the country as a consequence of incessant and protracted electricity failure.

2 Materials and methods

2.1 Preparation of cassava steep liquor

Healthy cassava tubers (*Manihot esculenta*) were obtained from a farm produce store in Lagos, Nigeria. The tubers were peeled, washed thoroughly with tap water and subsequently cut into smaller pieces. These were soaked in tap water in a very clean plastic bucket and kept at room temperature (29 ± 2.0°C). After 5 days of steeping, the resulting cassava mash was mixed thoroughly and strained in a domestic sieve. Samples of the steep liquor were collected and subsequently used for microbiological and physico-chemical analyses. The CSL was used immediately for the bioremediation study.

2.2 Bioremediation protocols

Samples of soil were freshly collected from the upper 20 cm of an agricultural soil in the University of Lagos biological garden. The biological garden soil is dark brown in color and consists of fine, medium coarse silty sand with traces of plant roots. Specifically, it has the following particle sizes: Sand 68%, Silt 31%, and traces of fine gravel. In addition, it is non plastic (0 plasticity index) and has a coefficient of permeability of 3.14×10^{-5} m/s. The soil has no known history of contamination with diesel or petroleum related products. The soil was sieved (4 mm) and used without air-drying. Two kilogram of the soil

contained in open trays, 6.32 cm × 20.5 cm × 6.5 cm (internal dimension) was contaminated with 200 g of diesel oil, to give approximately 10% (v/w) pollution. This soil was supplemented with 200 ml of CSL, thoroughly mixed and was subsequently designated EXPS. Two controls were similarly setup namely, CSS1 containing all materials in EXPS but without CSL fortification and CSS2 containing 2 kg of uncontaminated soil only. Setup CSS1 was designed to determine the contribution made by organisms indigenous to the soil while CSS2 was meant to serve as the overall control to monitor physico-chemical and biological dynamics of the soil under unstressed condition. Both CSS1 and CSS2 were flooded with 200 ml of sterile distilled water to maintain relatively similar moisture level with EXPS. The three experimental designs were setup in three replicates and kept in the laboratory at room temperature (29 ± 2°C) throughout the investigation periods. They were watered weekly (with sterile distilled water) to maintain a moisture level of 25%. Samples were taken at 5-day interval for analysis.

2.3 Physico-chemical properties

The soil physico-chemistry was evaluated using standard analytical protocols described elsewhere (AOAC 1990; Chopra and Kanwar 1998; Singh et al. 1999). Dissolved oxygen (DO) was monitored by dissolved oxygen meter (Jenway) and pH by a pH meter pH meter (Jenway) according to Nwachukwu (2001).

2.4 Enumeration and characterization of soil microflora

The population densities of the soil microorganisms were determined by standard plate count techniques. Total heterotrophic counts of bacteria were performed on nutrient agar plates while that of fungi was evaluated on potato dextrose agar (PDA) plates fortified with streptomycin (0.125 g/l), incubation was carried out at room temperature for 24–72 h. The population densities of hydrocarbon-utilizing organisms were determined by plating on mineral salts (MS) medium previously defined by Kästner et al. (1994) and solidified by purified bacteriological agar. For hydrocarbon-utilizing bacteria, the medium was adjusted to pH 7.2 while for fungi, it was adjusted to pH 4.5 and further fortified with streptomycin to inhibit bacterial growth. In both cases, diesel oil was supplied as the sole carbon and energy source through vapor phase transfer previously described by Raymond et al. (1976). Microbial colonies were screened, counted, and identified based on the taxonomic schemes and descriptions of Bergey's Manual of Determinative Bacteriology (Holt et al. 1994), Barnett and Pankhurst (1974) and O'Donnell (1979).

2.5 Diesel oil analysis

The residual diesel oil was extracted thrice from the soil sample (5 g) using n-hexane: dichloromethane solvent system (1:1) and quantified gravimetrically as described by Nwachukwu (2001) and chromatographically as recently described by Adebusoye et al. (2007). Briefly, 5 g of soil sample was randomly taken from each replicate at surface, middle, and bottom and mixed thoroughly before analysis. The oil was extracted by mixing the soil with 30 ml volume of the solvent system and stirred for 5 min. The procedure was repeated thrice and extracts pooled and dried. The oil extract was subsequently analyzed by GC (Hewlett Packard 5890 Series II) fitted with flame ionization detector, and AJ & W scientific DB-1 fused silica 15 m long column (internal diameter, 0.32 mm; film thickness, 1.0 μm).

2.6 Statistical analysis

Statistical analysis including mean generation time, specific growth rate were calculated using non-linear regression analysis of growth curves. Regression, correlation, and analysis of variance were all performed using the Prism version 5 (GraphPad Software, San Diego, CA, USA).

3 Results

Preliminary studies conducted on the CSL used for this study indicated the presence of a relatively high proportion of hydrocarbon-utilizers (~1.2%). Representative organisms identified were *Candida* sp., *Saccharimyces* spp., *Bacillus* spp., *Lactobacillus* spp., *Corynebacterium* spp., and *Geotrichum candidum*. The physico-chemistry of the CSL sample is illustrated in Table 1. The pH of the liquor was 6.01. As depicted in Table 1, the CSL contained a relatively significant amount of nutrient sources needed for microbial growth. For example, the mean value of crude protein obtained was 20.3 mg/l, while soluble starch and phosphate were 33.4 and 0.765 mg/l, respectively.

Table 1 Physico-chemistry of cassava steep liquor

Parameter	Mean determination
pH	6.01
Total acidity (mg/l)	0.14
Ash content (mg/l)	0.62
Crude protein (mg/l)	20.3
Soluble starch (mg/l)	33.4
Phosphate (mg/l)	0.765

The efficacy of the CSL as a bioremediation candidate was tested by treating an artificially contaminated garden soil ecosystem simulated in the laboratory with the liquor as described in the "Materials and methods". The recovery of this ecosystem was compared with compartment CSS1 containing similar materials in EXPS but without CSL supplementation. Bioremediation indices monitored periodically included dynamics of the soil physico-chemistry, microbial population densities as well as reduction in the diesel oil contaminant analyzed both gravimetrically and gas chromatographically. It is noteworthy that our preliminary investigation on the possible effects of CSL on the soil indigenous microflora showed no significant change in population especially at the onset of study (data not shown).

A summary of the dynamics of physico-chemistry of the experimental and control setups is presented in Table 2. Generally, the trends observed for these variables were much more remarkable in EXPS compared with CSS1 and CSS2, with almost stable pH and DO in the latter. The results indicate that the levels of the inorganic nutrient sources namely, N, P, and K reduced consistently and were almost completely depleted in both EXPS and CSS1 but more so in the former. These decreases were very much significant between days 15 and 35 which coincidentally, were periods of dramatic microbial population changes and significant drop in residual oil content (see Figs. 2, 3). This would suggest that the increases in microflora were key factor responsible for the degradation rates observed and the reduction in the inorganic nutrient sources was as a result of their utilization for growth by the microorganisms. As illustrated in Table 2, the organic matter content was the only parameter that showed increasing trend in the two polluted setups during the bioremediation study; apparently due to the application of diesel oil and further fortification of compartment EXPS with CSL. For instance, while the values for CSS2 were relatively stable that of EXPS increased consistently from 9.5% to peak at 21.81% at day 30. These physico-chemical factors were found to differ significantly with the exception of phosphorus and nitrogen contents at 0.05% level of confidence when the three soil compartments were subjected to variance analysis.

The population dynamics of the different microbial groups present in the three soil compartments are illustrated in Figs. 1, 2. The microbial population densities in compartments EXPS and CSS1 showed an initial decrease between days 0 and 5, apparently suggesting toxicity of diesel oil to the indigenous microflora. Subsequently, the population assumed an increasing trend, an observation that was persistent until day 30 for EXPS but lasted the study period in CSS1. In the case of CSS2, populations of bacteria and fungi were relatively stable. The hydrocarbon-utilizers were found to be higher in EXPS compared with CSS1 and CSS2 not fortified with CSL. Not surprisingly, the percent hydrocarbon-utilizers determined for the three compartments relative to the total heterotrophic organisms was significantly different at $P < 0.05$ level of significance. The patterns of diesel oil disappearance in the two contaminated setups as displayed in Fig. 3 are reflective of the population profiles of the soil indigenous microorganisms. The results revealed that the magnitude of loss in residual oil concentrations was much more significant in EXPS compared with CSS1. The concentration of oil recovered in EXPS at day 20 was 22.17 g/kg soil from an initial concentration of 98.05 g/kg soil, thus giving a percent degradation of 77.39 at which time the corresponding value obtained for CSS1 was 32.33%. By the end of the treatability study, nearly (98.88%) all of the diesel added had been depleted whereas more than 53% of the oil was recovered in the CSL untreated soil giving an overall disappearance rate of approximately 2.77 and 1.31 g/kg soil/day, respectively for EXPS and CSS1. Interestingly, this 35-day study period was subjected to variance analysis and was found to be very highly significant at 0.05 confidence limit.

Table 2 Dynamics of physico-chemical composition of polluted and unpolluted soil systems

EXPS							CSS1						CSS2					
Time (day)	K	DO	OM	P	N	pH	K	DO	OM	P	N	pH	K	DO	OM	P	N	pH
0	3.19	7.08	9.50	3.19	5.80	6.47	2.87	7.20	8.70	2.16	2.90	6.87	2.90	7.31	7.70	2.2	2.30	6.96
5	2.15	5.53	10.36	2.42	6.20	6.33	1.28	6.58	11.68	1.59	2.80	7.06	2.65	7.48	9.32	2.10	2.20	6.98
10	1.46	3.0	13.56	2.21	2.80	6.20	1.07	5.55	11.98	1.11	2.40	7.0	2.15	7.09	8.30	2.36	2.30	7.10
15	0.46	1.96	13.61	1.09	1.30	6.0	0.61	4.37	13.01	1.36	2.20	6.66	2.22	6.55	8.20	1.98	1.90	7.10
20	0.33	1.60	15.61	0.99	1.00	5.80	0.92	3.90	14.01	0.66	0.10	6.66	1.58	6.0	10.01	1.56	1.80	6.81
25	0.26	0.28	18.20	0.29	0.90	5.50	0.87	3.01	19.10	0.23	1.20	6.40	1.33	7.41	9.88	1.87	1.90	6.99
30	0.22	1.20	21.81	0.19	1.40	6.01	0.66	3.35	18.10	1.23	1.30	6.30	2.01	7.01	8.56	2.01	2.10	7.02
35	0.32	3.57	13.75	0.29	2.40	6.55	0.76	2.53	18.01	0.87	1.10	6.30	2.11	6.91	8.01	1.96	2.10	7.19

K potassium content (mg/kg), *DO* dissolved oxygen (mg/kg), *P* available phosphorus (mg/kg), *N* total nitrogen (mg/kg), *OM* organic matter (%). All data presented are means of triplicate determinations

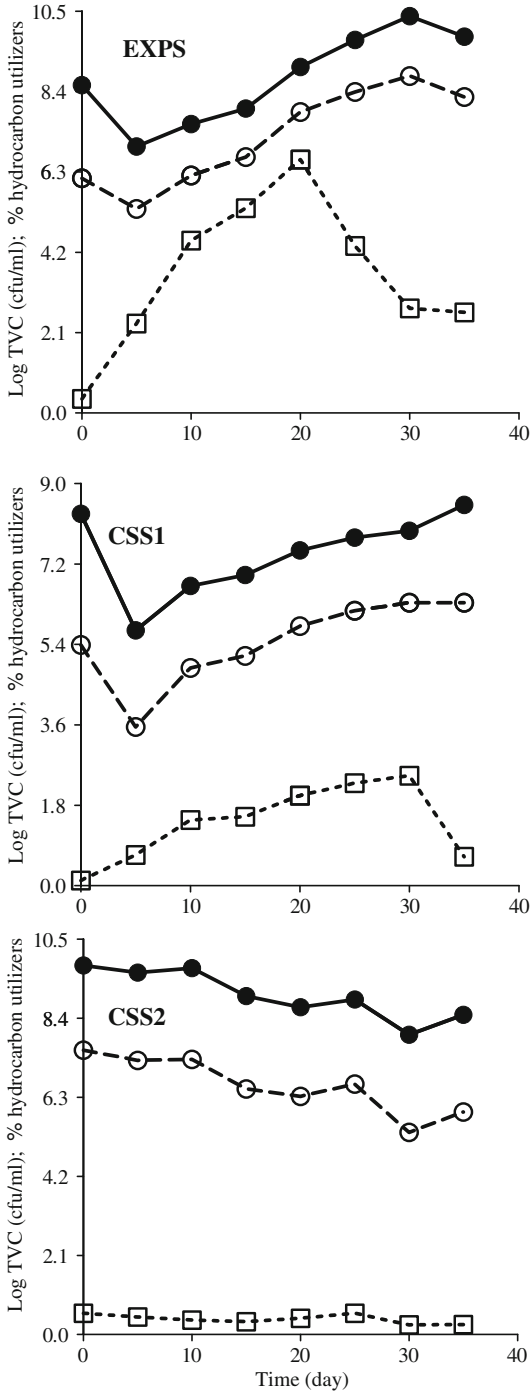

Fig. 1 Population dynamics of the soil heterotrophic bacteria (●), hydrocarbon-utilizing bacteria (○), and percent hydrocarbon-utilizers (□). Data presented are the averages of triplicate determinations

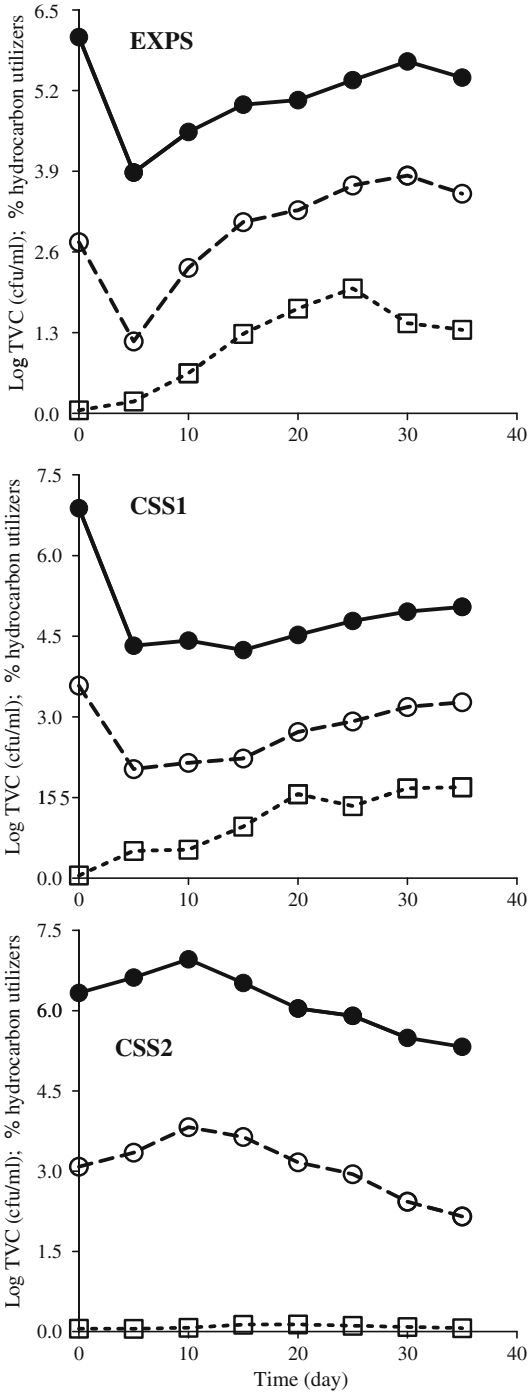

Fig. 2 Population dynamics of the soil heterotrophic fungi (●), hydrocarbon-utilizing fungi (○), and percent hydrocarbon-utilizers (□). Data presented are the averages of triplicate determinations

Qualitative changes in the diesel oil components in EXPS and CSS1 for days 0, 15 and 35 are depicted in Figs. 4, 5, respectively. Both systems displayed similar GC fingerprints for week 0 (Figs. 4a, 5a) when treatments were given just before the commencement of biodegradation. The sharp peaks represent the aliphatics and the peaks between them comprise the naphthenes and aromatics having similar molecular weights to the adjacent aliphatics. By comparing the chromatograms, there was a significant reduction in the aliphatic peaks during the first 15 days in both systems EXPS and CSS1, although more extensive in the former than the latter. However, by day 35, there was

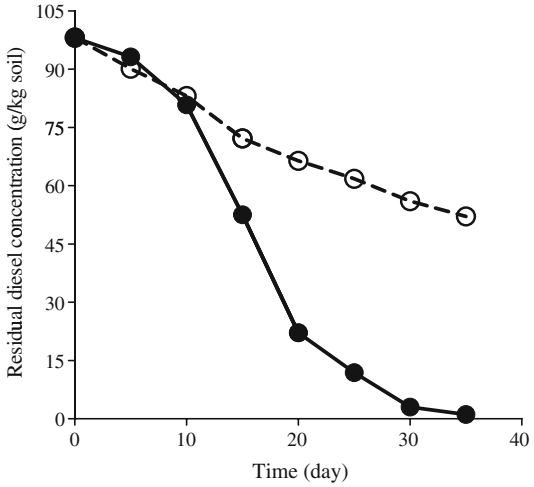

Fig. 3 Recovered diesel oil from contaminated soils EXPS (●) and CSS1 (○). Data presented are the averages of triplicate determinations. The residual diesel oil was extracted thrice using n-hexane:dichloromethane solvent system (1:1)

complete disappearance of all the aliphatic fractions and possibly including all the naphthenic and aromatic components as well in EXPS (see Fig. 4c) while amounts recovered from CSS1 were very significant only showing minor peak reductions compared with the GC tracings obtained for day 15 (Fig. 5d, e). Furthermore, the ratios of nC17/pristane and nC18/phytane obtained from the GC fingerprints decreased consistently from the onset to the end of the investigation (Table 3). Quite surprisingly, the reduction of these biomarkers in CSS1 was much more pronounced than EXPS (Table 3) at day 15. However, by day 35, all of these isoprenoids molecules were completely degraded in EXPS while there was no significant change in CSS1.

The kinetics of growth summarized in Table 4 showed that the mean generation times for the various physiological microbial groups were generally low in EXPS while corresponding higher values were calculated for CSS1 and the undisturbed soil, CSS2. Moreover, the specific growth rate obtained for the bacterial communities was quite higher than the fungi components of the ecosystem. A Pearson's correlation coefficient between hydrocarbon-utilizing bacteria and residual diesel concentration was high but negative (−0.91) in EXPS, the corresponding value for CSS1 was low (−0.65). These analyses suggest that the more rapid reduction in oil content observed for EXPS could be owing to the activities of the bacteria whose growth was profusely stimulated by the addition of CSL. This is further reinforced by the fact that the depletion rate of diesel was the fastest between days 10 and 25 which interestingly were the periods of remarkable population increases most especially for hydrocarbon-utilizing bacteria.

Given the consistent depletion rate of the oil, it would take at least an additionally 34 days from day 35 for the total disappearance of the oil pollutant from CSS1 and ~2 days for EXPS, suggesting that at least a total of 69 days would elapse before complete recovery of system CSS1. Nevertheless, the setups were examined 35 days after the bioremediation treatment were given for biodegradation of the diesel oil contaminant, and hence the recovery of the soils from the oil impact. This was achieved by planting maize (*Zea mays*) grains and comparing the results obtained with those of the undisturbed soil. Whereas seeds in EXPS and CSS2 germinated between 3 and 5 days, it took additional 3 days or more for CSS1. The percent seed germination in EXPS was almost 74%. The equivalent values for CSS1 and CSS2 were 10 and 80%, respectively. Generally, the growth profiles of seedlings were poor in CSS1 with pronounced abnormal morphology such as stunted growth and chlorosis of the leaves and stem when compared with the results obtained for CSS2 and EXPS.

The predominant organisms encountered in systems EXPS and CSS1, but with higher species diversities in the former included the species of *Lactobacillus*, *Clostridium*, *Streptomyces*, *Pseudomonas*, *Bacillus, Flavobacterium*, *Corynebacterium*, *Nocardia*, *Candida*, *Saccharomyces, Rhodotorula, Aspergillus*, *Penicilium*, and *Geotrichum*. Interestingly, all of these isolates (with the exception of the first two and the last three which were not tested) grew extensively in MS medium supplemented with diesel as the sole source of carbon and energy (data not shown).

4 Discussion

Contamination of the environment with hazardous and toxic chemicals is one of the major problems facing industrialized nations today. The problems become compounded in oil producing countries particularly, in Nigeria where influx of these pollutants is largely under-regulated and spillage, when it occurs is often not reported and/or investigated. The petroleum industry is responsible for the generation of high amounts of hydrocarbons and their derivatives as well as for the pollution of air, soils, rivers, seas, and underground water. Of paramount interest is the need to find an acceptable remedial technology for sites polluted with these chemicals. An increasingly important strategy involves enhancing the indigenous microbial population in soil to degrade the contaminant by a process referred to as in situ bioremediation. The success of this approach largely depends on the number and catabolic versatility of indigenous hydrocarbon-degraders in such systems. In this study, we have demonstrated that the use of

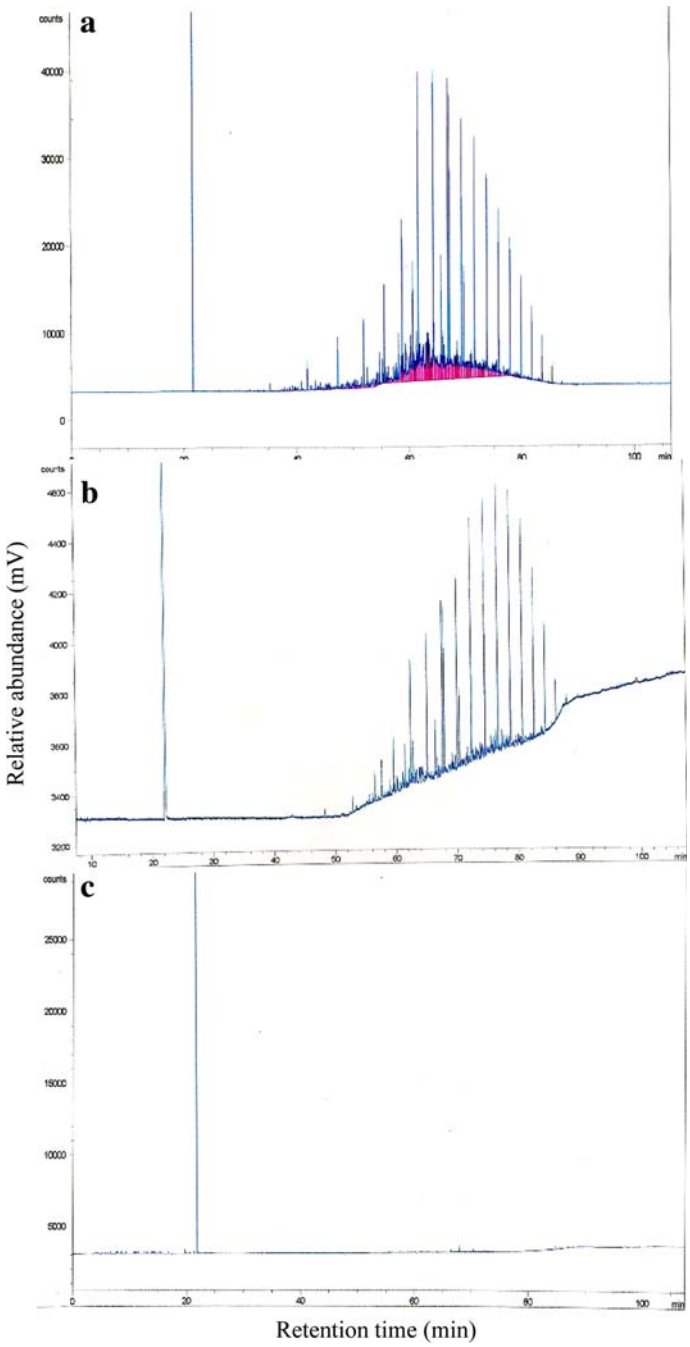

Fig. 4 Gas chromatogram of diesel recovered at day 0 (**a**), day 15 (**b**), and day 35 (**c**) from contaminated system EXPS treated with cassava steep liquor. Note the complete disappearance of hydrocarbon peaks at day 35

CSL can effectively accelerate bioremediation of agricultural soil contaminated with diesel oil.

Both systems EXPS and CSS1 showed dramatic physico-chemical and biological changes upon contamination, with diesel oil. The amount of oil used to contaminate the two ecosystems was perhaps, adequate enough to cause sterility of the soils. As a result, the initial drop in the microbial populations of these soils following pollution as observed on day 5 (Figs. 1, 2) is a confirmation of the toxicity of petroleum and associated products to the biota (Atlas 1991; Teal et al. 1992; Yveline et al. 1997; Nwachukwu 2001). Although, the impact of hydrocarbons on microorganisms may not be directly related to their toxicity, as several workers have reported destruction of inorganic nutrient sources that are essential for microbial growth and catabolic activities due to the ability of hydrocarbons to react and form complexes with nitrates, sulfates, and phosphates; thus making them unavailable to the soil organisms (Teal et al. 1992; Andrew and Jackson 1996).

The rapid decrease in oil concentration in the experimental setups with CSL amendment when compared with

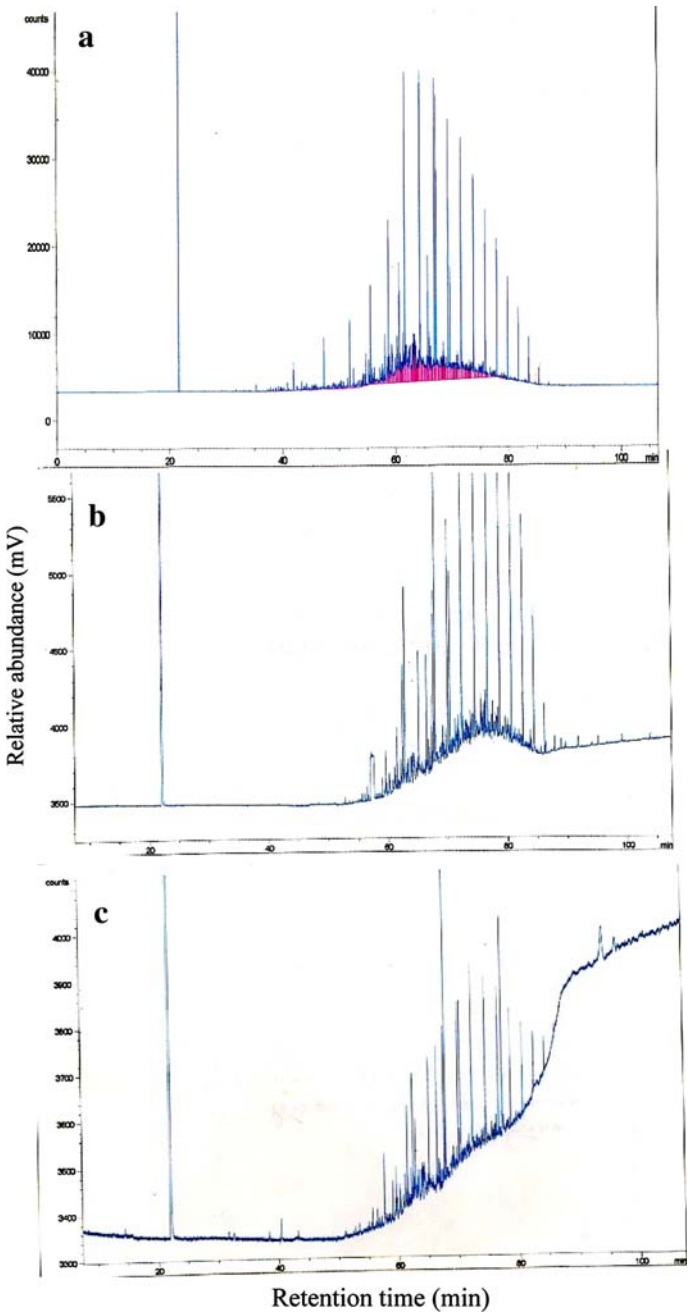

Fig. 5 Gas chromatogram of diesel recovered at day 0 (**a**), day 15 (**b**), and day 35 (**c**) from contaminated system CSS1. There was no significant degradation between days 15 and 35

Table 3 n-C17/pristane and n-C18/phytane ratios of recovered diesel oil from contaminated soil microcosms

Sample	Day 15		Day 35	
	n-C17/ pristane	n-C18/ phytane	n-C17/ pristane	n-C18/ phytane
EXPS	1.008 (4.36)	1.116 (31.15)	0 (100)	0 (100)
CSS1	0.918 (12.93)	1.070 (44.29)	0.849 (15.3)	0.997 (48.08)

Percent reductions of ratios are written in parentheses and have been calculated with reference to the amount obtained from day 0. Data presented are means of triplicate determinations

the oil level in CSS1 could be attributed to the presence of growth factors and essential nutrient sources inherent in CSL that were able to stimulate rapid development of microflora relevant to oil degradation, particularly, the hydrocarbon-utilizers. Although, CSL also has sizeable proportion of hydrocarbon-utilizers, these also could have contributed to the overall recovery of EXPS by complementing the metabolic activities of the indigenous organisms. Critical examination of the growth dynamics of microflora in EXPS showed a surge in population densities between days 10 and 20 with proportional increases in

Table 4 Growth kinetics of hydrocarbon-utilizing microorganisms

Sample	Bacterial populations				Fungal populations				% HC degradation	Rate of HC utilization[a]
	Heterotrophs		HC-utilizers		Heterotrophs		HC-utilizers			
	T_g	μ	T_g	μ	T_g	μ	T_g	μ		
EXPS	2.39	0.290	3.34	0.207	5.28	0.131	5.86	0.118	97.86	2,769.79
CSS1	6.08	0.114	5.43	0.128	8.60	0.081	5.76	0.120	44.85	1,314.17
CSS2	7.35	0.094	6.79	0.102	10.36	0.067	14.85	0.047	N/A	N/A

T_g mean generation time (d), μ specific growth rate (d^{-1}). All values are means for triplicate determinations

[a] Determined as mg/kg of soil/day

hydrocarbon-utilizers (4.5–6.6%, bacteria; 0.65–1.69, fungi). Interestingly, this coincided with periods of extensive changes in the soil physico-chemistry coupled with rapid oil disappearance. Degradation of hydrocarbons is largely an aerobic process (although anaerobic degradation has also been reported) requiring a relatively high oxygen tension. This may have accounted for the decrease in DO concentrations which is particularly significant in EXPS than CSS1. In the former where CSL supplementation resulted in over 98% (Fig. 3) degradation of the oil pollutant, there was near-depletion of inorganic nutrient sources. The loss of these nutrients from the polluted system is evidence of degradation by microbial action. Previously, Ward et al. (1984) had remarked that rapid activity of microorganisms in response to environmental perturbation may result in alteration of the environment such as depletion or lowered nutrient levels. It is therefore, not surprising that the DO, pH, nitrogen, phosphorus, and potassium contents in the undisturbed system, CSS2 remained relatively stable all through the period of study.

The data obtained from qualitative changes in the oil pollutant as revealed by gas chromatographic analysis reaffirm that the bioremediation treatments for the oil-impacted agricultural soil were effective in the elimination of the contaminant. The GC fingerprints of EXPS and CSS1 showed significant reduction in the linear alkanes during the first 15 days (Figs. 4, 5). The most degradation was noted for the low molecular weight n-alkanes. Metabolism of the branched alkanes, pristane, and phytane was presumably slower. These results are in agreement with results obtained by many investigators (Diaz et al. 2002; Gouda et al. 2007). Ratios of nC17/pristane and nC18/phytane were relatively similar in EXPS and CSS1. However, progressive biodegradative removal was suggested by decreasing ratios and outright depletion in EXPS by day 35. Pristane and phytane are low molecular-weight isoprenoid molecules that are recalcitrant to biodegradation. The decreasing trend in the ratios of normal alkane nC17 and nC18 to that of pristane and phytane, respectively is an indication that biodegradation has taken place. The rate of decrease is an indication of the severity of biodegradation. The biodegradation potentials observed in this study indicate that certain changes in the chemical distribution of the hydrocarbons in the oil may be attributed to microbial action and with respect to EXPS, efficacy of CSL.

Microbial communities within contaminated ecosystems tend to be dominated by those organisms capable of utilizing and/or surviving toxic contamination. As a result, these communities are typically less diverse than those in unstressed systems (MacNaughton et al. 1999). Results obtained from the examination of systems EXPS, CSS1, and CSS2 for their microbial compositions are comparable with these findings. Our analysis revealed a variety of bacterial and fungal genera with EXPS however, exhibiting more species diversity than the other two systems thus, suggesting recovery of this soil from oil impact. It is worthy of note that most of the strains isolated belonged to the genera known for their capacity to degrade hydrocarbons (Atlas 1992; Van Hamme et al. 2003). Data from the seed germination experiment reinforce the recovery of EXPS and further illustrate the phytotoxicity impacts of hydrocarbons. Plaza et al. (2005) had reported that bioassays using plants were sensitive indicators of soil quality and can be used to evaluate the quality of bioremediated soil. In this study, the level of oil in CSS1 (52,110 mg/kg soil) was very high and was probably responsible for the observed poor germination and the general abnormality in the morphology of the seedlings. The devastating effects of chemical pollutants inducing significant variation in the normal morphology of several sensitive plants was also demonstrated previously (Anderson et al. 1997; Saquib and Khan 1999; Nwachukwu 2001). The morphology of seedlings grown in the EXPS was comparable with those propagated on CSS2. These indicate that treatment of oil, contaminated soil with CSL as a bioremediation agent does produce soil which is capable of growing healthier plants than where bioremediation has not taken place.

It is apparent from this study that the use of CSL has effective ability to enhance recovery of oil-impacted soil without additional substrate supplementation or augmentation with capable microorganisms. This could prove a

more environmentally friendly approach to bioremediation which would on the long run enhance sustainable development rather than the use of exotic microbial strains, chemicals, or more commonly, nutrient amendments. Overall, the benefit of this technology in addition to its environmental compatibility lies in its simplicity and cost-effectiveness.

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