IJAMBR 5 (2017) 27-39 ISSN 2053-1818

International Journal of Applied Microbiology and Biotechnology Research

www.bluepenjournals.org/ijambr

Efficacy of brewery spent grain and rabbit droppings on enhanced *ex situ* bioremediation of an aged crude oil contaminated soil

Chimezie Jason Ogugbue[1]*, Chiaka Mbakwem-Aniebo[1] and Leera Solomon[2]

[1]Department of Microbiology, Faculty of Science, University of Port Harcourt, East-West Road, P. M. B. 5323, Choba, 500004 Port Harcourt, Nigeria.

[2]Department of Science Laboratory Technology, School of Science and Technology, Port Harcourt Polytechnic, Rumuola, P. M. B. 5936, Port Harcourt, Nigeria.

Article History

Received 12 March, 2017
Received in revised form 30 March, 2017
Accepted 30 March, 2017

Keywords:
Contaminated soil, *Ex situ* bioremediation, Brewery spent grain, Rabbit droppings, Biostimulation efficacy.

Article Type:
Full Length Research Article

ABSTRACT

Brewery spent grain (BSG) and rabbit droppings (RD) were investigated to determine their potentials as biostimulation agents for enhanced *ex situ* bioremediation of an aged crude oil contaminated soil (COCS). Samples were collected from a fresh oil spill site and 300 g of soil was amended with 150 g each of RD and BSG. The control (CT) contained 300 g of COCS without amendment. Monitoring of various physico-chemical parameters including total petroleum hydrocarbon (TPH) content of COCS was done for 5 weeks at 7-days intervals. Hydrocarbon utilizing bacterial isolates identified include *Corynebacterium*, *Staphylococcus*, *Pseudomonas*, *Bacillus* and *Flavobacterium* genera. In RD amended treatment, the total heterotrophic bacterial/hydrocarbon-utilizing bacterial (THB/HUB) and the total heterotrophic fungal/hydrocarbon-utilizing fungal (THF/HUF) ratios were 2.45 and 2.32 respectively, as against 1.46 and 1.23 obtained for BSG. The ratios in the control were 0.75 (THB/HUB) and 0.94 (THF/HUF) thus, indicating growth stimulation of hydrocarbon degrading microorganisms as a result of the amendments. The TPH content of COCS amended with RD and BSG decreased by 57.9 and 39.6%, respectively whereas, the CT reduced by only 0.59%. There was a significant ($p<0.05$) reduction in TPH after 35 days. The order of biostimulation efficacy (RD<BSG) showed that RD enhanced the removal of TPH from soil during remediation better than BSG. Data from this study indicate the biotechnological potentials of these waste materials for remediation of COCS by enhanced natural attenuation.

INTRODUCTION

A critical impact of the petroleum industry is the pollution of the environment by crude oil and other related products which are highly toxic and exhibit molecular recalcitrance (Yveline et al., 1997; Odokuma, 2012). Oil spills, oil transportation, drilling operations, refineries and local fuel filling stations are some of the factors responsible for hydrocarbon contamination.

They persist in nature for long periods of time and cause hazardous effects on flora and fauna of terrestrial and aquatic ecosystems due to their complexity and variability (Schafer et al., 2009; Dorn et al., 1998). Oil diminishes the total microbial diversity in soil, destroys infrastructure and contaminates landscape (USEPA, 2011).

When soil is polluted, the ecosystem is altered and

*Corresponding author. E-mail: ceejay55us@yahoo.com; jason.ogugbue@uniport.edu.ng. Tel: +2348108643429.

agricultural activities are affected due to poor aeration, immobilization of soil nutrients, loss of water holding capacity, lowering of soil pH, reduction in soil catalase enzyme activity (Atuanya, 1987; Achuba and Peretiamo–Clarke, 2006), as well as inhibitory effect on the nitrate reductase activities of plants (Odjegba and Atebe, 2007; Ekpo et al., 2012). Many techniques of remediation of contaminated soil such as physical, chemical and photo-degradation have been developed (UNEP, 2011).

However, most of these methods have some draw backs in completely remediating crude oil contaminated soil (Alvim-Ferraz et al., 2006). Some of these methods leave behind daughter compounds which are more toxic to the environment than the parent compounds (Sims and Sims, 1991; Aislabie et al., 1998).

More so, current chemical methods typically recover no more than 10–15 percent of the crude after a major spill and almost always leave the receiving environment in worse conditions, as the resultant products are in most cases toxic in the soil environment (Abu and Ogiji, 1996). Organic matter is often scarce in contaminated soil resulting generally in low microbial activity in such soils. Low microbial activity may also be attributed to some of the contaminating hydrocarbons (especially cyclic hydrocarbons) which are toxic to bacterial membranes (Sikkema et al., 1995; Demirbas and Demirbas, 2007; Agarry and Ogunleye, 2012).

Despite these unfavourable phenomena, a broad spectrum of microbial genera still survives in hydrocarbon-contaminated soil (Kampfer et al., 1991; Lunch et al., 2004) with a diverse range reported to degrade hydrocarbons (Atlas, 1981; Rosenberg, 1992). Although the bacterial community in such environments is adapted to the presence of the contaminant, other environmental conditions such as nutrient availability and oxygen concentration may be limiting thus, resulting in the slow degradation of the contaminants by microbes *in situ*.

However, the activities of the microbes and the specific degrading microorganisms can be enhanced by deploying composting technologies such as the addition of an organic matrix to contaminated soil. The mineralization of organic matter enhances nutrient concentrations that can alleviate the nutrient limitation of the hydrocarbon degrading bacteria leading to greater biodegradation of hydrocarbons.

The re-mineralization of organic matter significantly increases the availability of inorganic nutrients. The negative impacts of crude oil pollution on agricultural soil can be contained using a more holistic approach of bioremediation (Wackett and Ellis, 1999). Bioremediation technology can be broadly classified as *ex situ* or *in situ*. *Ex situ* technologies are those treatment strategies which involve physical removal of the contaminated material to another location for treatment. On the other hand, *in situ* strategies involve treatment of contaminated material in place (Okpokwasili, 2006; Ibiene et al., 2011).

Selecting the most appropriate bioremediation technology can be guided by considering three fundamental principles: (a) The amenability of the pollutant to be biologically transformed to less toxic products (biochemistry), (b) The accessibility of the pollutant to microorganisms (bioavailability), and (c) The opportunity for optimization of biological activity (bioactivity).

Traditional approaches for enhancing biodegradation processes rely on the addition of one or more nutrients (nitrogen, phosphorus, trace minerals and vitamins) which provides microorganisms with essential elements to reproduce and thrive (Aislabie et al., 1998; Liebeg and Cutright, 1999). Such nutrients should be non-toxic and sufficiently reactive to support the desired chemical or biological reaction at rates that meet treatment objectives (Adriano et al., 1998; Dawson et al., 2007). Nutrient supplementation facilitates crude oil metabolism in soil environment by hydrocarbonoclastic microorganisms, thereby reducing the negative impacts of crude oil pollution on the ecosystem (Semple et al., 2001; USEPA, 2010).

Hence, it is imperative to give adequate attention to enhanced bioremediation of crude oil contaminated soil using low-cost materials as supplements. This study was therefore carried out to investigate the bioremediation potentials of an indigenous microbial population in an aged crude oil contaminated soil following nutrient amendment with brewery spent grain and rabbit droppings.

MATERIALS AND METHODS

Sample collection

COCS was excavated from an oil spill site in Yorla in Khana Local Government Area (LGA), Rivers State. The sampling site had been inundated with an oil spill over the years before sample collection. The topsoil (15 cm depth) was collected using a manual soil auger into clean polythene bags. Brewer's spent grain, a waste product from malted barley was collected from a brewery in clean polythene bags while rabbit droppings was collected from the University Demonstration Farm and composted following the method of Semple et al. (2001) before use. Samples were transported to the Microbiology Laboratory in an ice box at 4°C.

Bioremediation treatment design

The experimental set-up consisted of three treatment options designated as:

Set A: COCS (300g) + BSG (150g) +Tilling
Set B: COCS (300g) + RM (150g) +Tilling

Set C: COCS (300g) + Tilling

One liter of water was added at interval of two days and tilled to mix nutrients with polluted soil, enhance aeration and microbial metabolism. The experiments were carried out in duplicates.

Determination of hydrocarbon-utilizing bacteria and fungi

Soil slurry was prepared by mixing 1 g of wet soil from treatment set up with 9 ml of sterile saline suspension. The soil suspension was then subjected to a 10-fold serial dilution before plating out on the respective media. Each dilution was plated in triplicate. The hydrocarbon-utilizing bacteria were enumerated as described by Hamamura et al. (2006) using mineral salts medium of Mill et al. (1978) with crude oil supplied by vapour phase transfer.

For hydrocarbon-utilizing fungi, the same procedure was followed except that 1 ml of lactic acid was added to Sabouraud dextrose agar to inhibit the growth of bacteria. Control plates were incubated without any carbon source.

Determination of total heterotrophic bacteria and total fungi

The total heterotrophic bacterial count was determined using the spread plate method on nutrient agar as described by Hussemann (1993). Soil suspensions were prepared by serial ten-fold dilutions with 1 g of soil and dilutions were spread plated in triplicates. Culture plates were then incubated at room temperature (28±2°C) for 24 h. For total fungi, serially diluted soil suspension above was spread-plated on Sabouraud dextrose agar in triplicates and incubated at room temperature (28±2°C) for 5–7 days.

Purification and characterization of bacterial and fungal isolates

Bacterial colonies that developed on the plates after incubation were purified by sub culturing and identified based on their colonial, microscopic and biochemical characteristics with reference to the Bergey's Manual of Systematic Bacteriology (Holt et al., 1994).

Colonies were isolated from nutrient agar plates with the highest dilutions. These were anticipated to represent the most numerous culturable bacteria. For fungi each purified isolate was placed on clean and grease-free slide and a drop of lactophenol added. The preparation was covered with a cover slip and slide observed under x10 and x40 objective lenses.

Determination of physicochemical parameters of soil samples

The physicochemical parameters (pH, phosphate content nitrate content, moisture content, and total organic carbon) of soil samples from the various treatment set ups were determined using standard methods. The pH was determined using pH meter (Jenway 3015 method), total organic carbon (TOC) and moisture content by methods adopted from Lyman (1990), while the phosphate, sulphate and nitrate contents were determined spectrophotometrically using the method of American Public Health Association (APHA, 1995).

Extraction and gas chromatography

To determine the residual total petroleum hydrocarbon composition of the soil samples, a modified EPA 8015 technique (Adeniyi and Afolabi, 2002) was adopted. The petroleum hydrocarbons in soil sample were extracted using n-hexane: dichloromethane solvent system (1:1) and an aliquot of the extract injected into a gas chromatograph (HP 5890, Hewlett Packard, PA, USA) equipped with a flame ionization detector (FID).

This was quantified by gravimetric method (Sims and Sims, 1991; Yveline et al., 1997). The chromatograms were examined only visually and were only used for qualitative interpretation. Quantization or fractionation on the silica gel column into aliphatic and aromatic components was not performed.

RESULTS AND DISCUSSION

Figures 1 to 4 show the changes in population of the different physiological groups during enhanced *ex situ* bioremediation of COCS. In the treatment amended with BSG, the total heterotrophic bacterial count (THBC) increased from 1.76×10^5 CFU/g at the onset of experiment to 4.82×10^5 CFU/g by the 3rd week (Figure 1) while the hydrocarbon-utilizing bacterial count (HUBC) also increased from 1.93×10^3 to 6.83×10^4 CFU/g within 3 weeks (Figure 2).

The total fungi (TF) and hydrocarbon-utilizing fungi (HUF) also increased from 1.54×10^4 and 1.43×10^3 CFU/g in week zero to 4.08×10^5 and 5.14×10^4 CFU/g respectively (Figures 3 and 4) by the 3rd week of experiment. Thereafter, the microbial populations decreased steadily until week 5 of monitoring.

The initial relative increase in microbial numbers following BSG addition to the COCS when compared to the control experiment (Figure 1) could be attributed to the utilization of nutrients contained in the BSG by the microbes thus, suggesting that BSG could stimulate microbial growth in the soil.

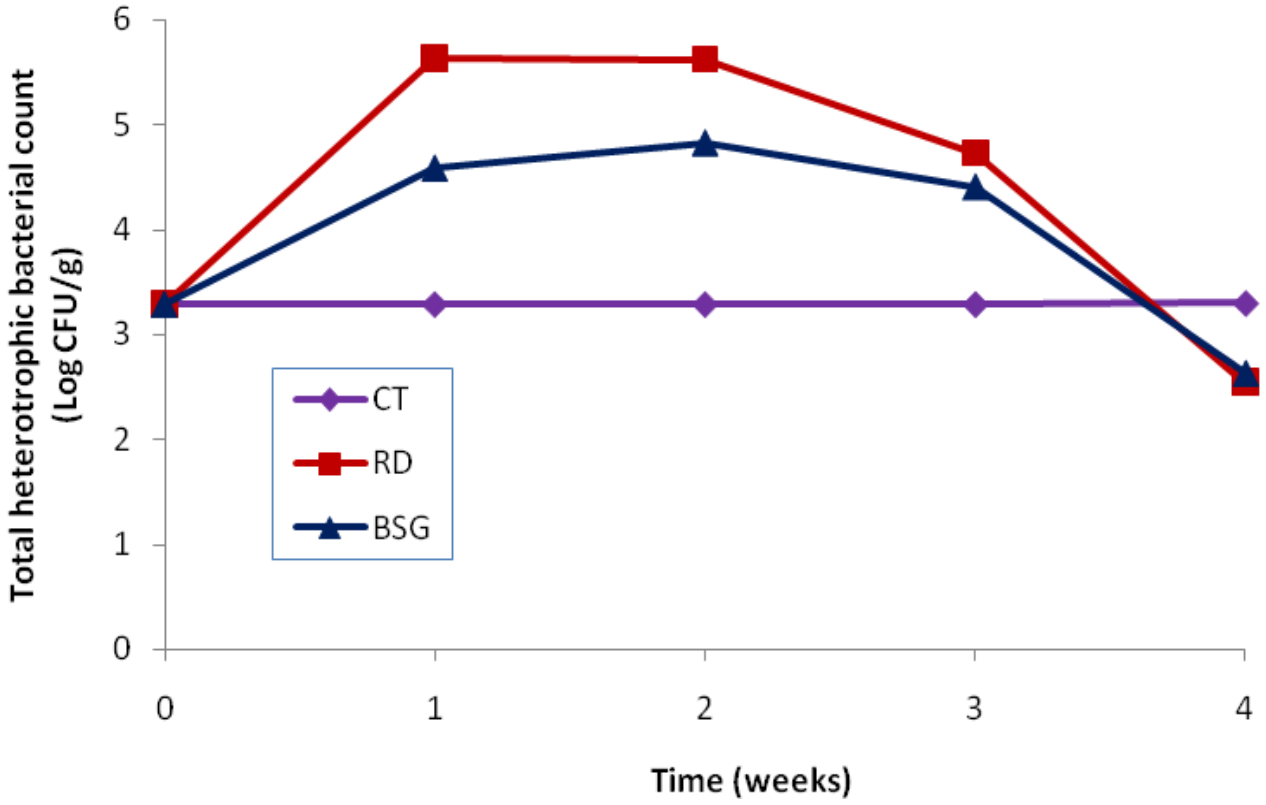

Figure 1. Changes in total heterotrophic bacterial counts of COCS amended with RD and BSG during the study period.

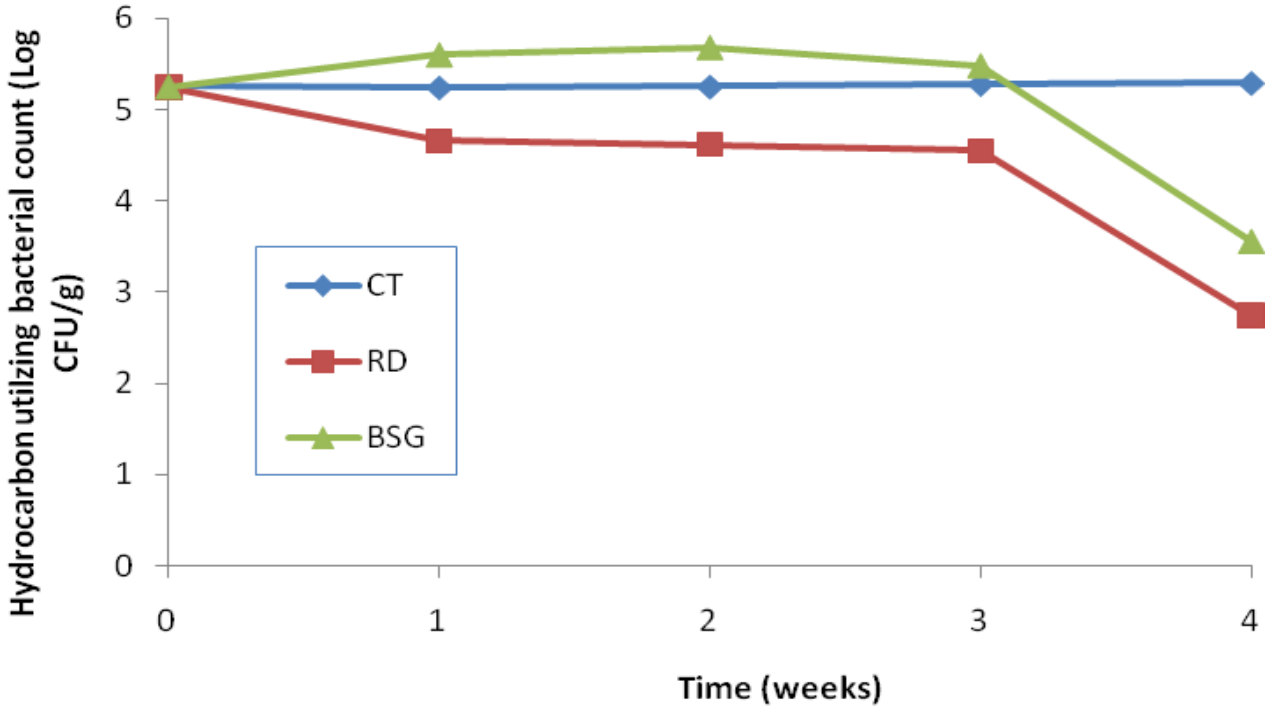

Figure 2. Changes in hydrocarbon utilizing bacterial counts of COCS amended with RD and BSG during the study period.

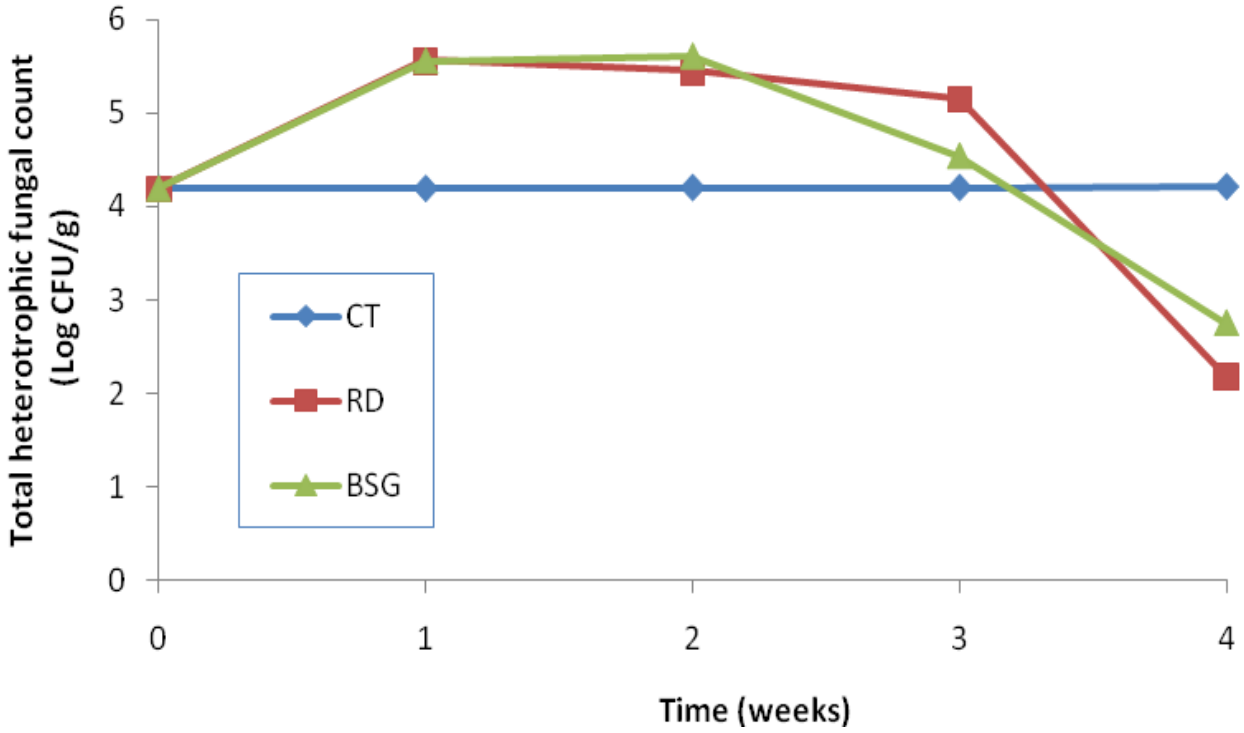

Figure 3. Changes in total heterotrophic fungal counts of COCS amended with RD and BSG during the study period.

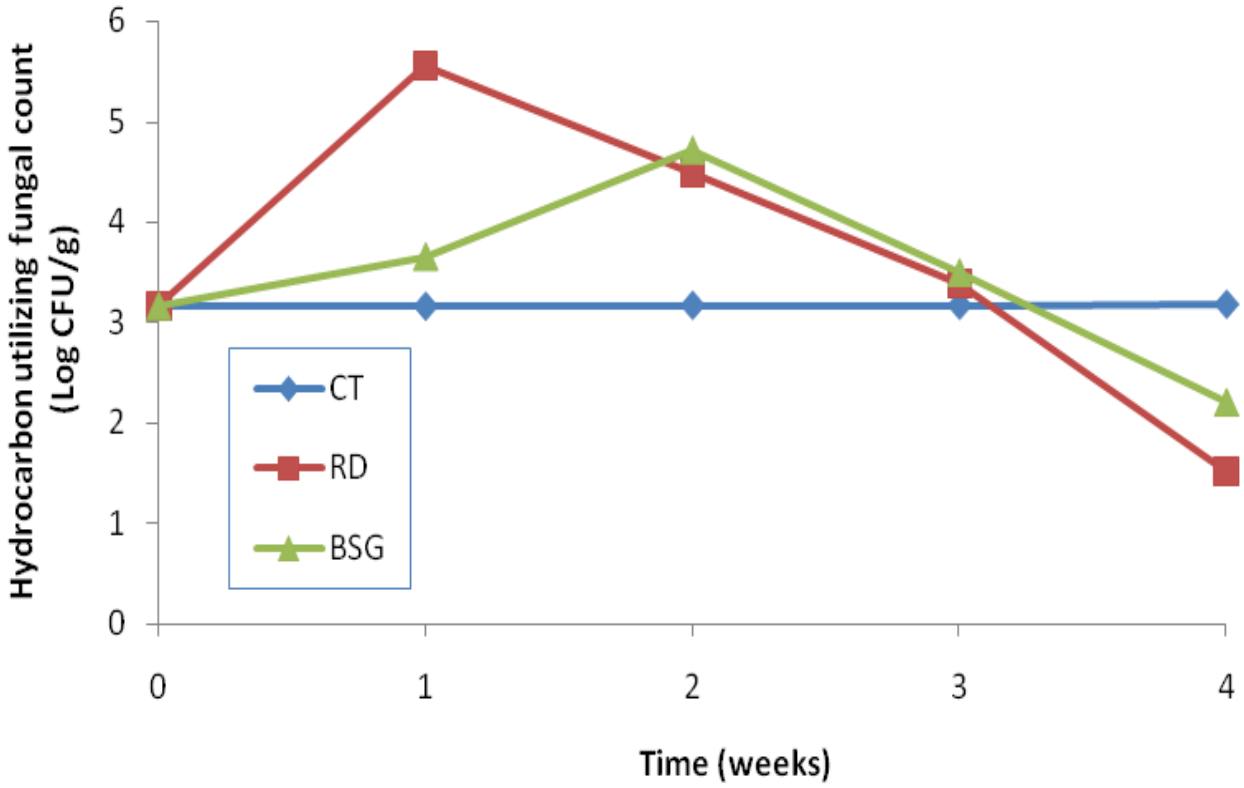

Figure 4. Changes in hydrocarbon utilizing fungal counts of COCS amended with RD and BSG during the study period.

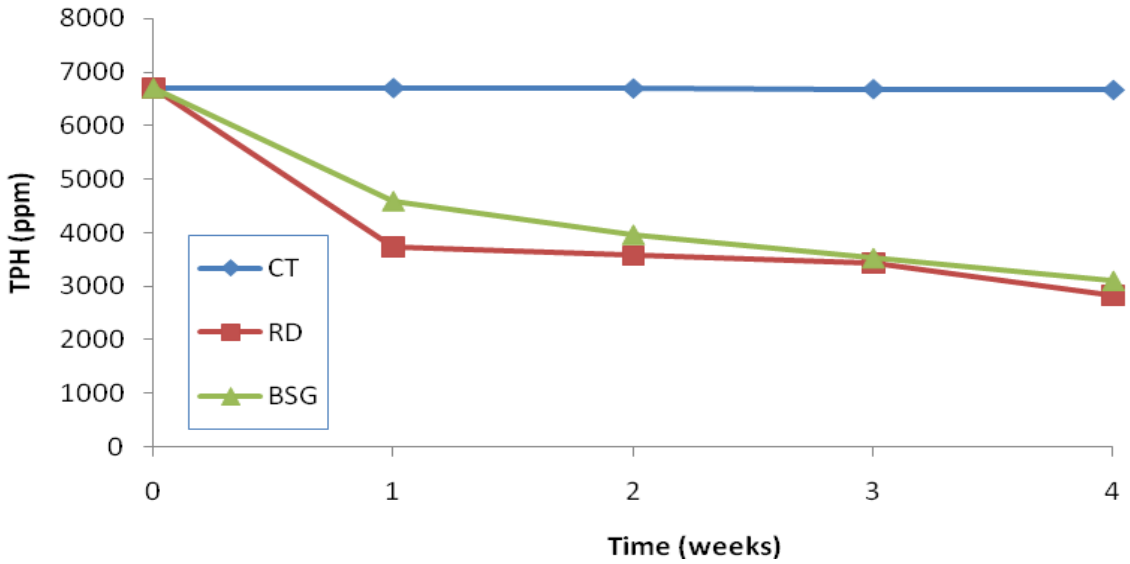

Figure 5. Changes in total petroleum hydrocarbon content of COCS amended with RD and BSG.

However, the decrease in microbial numbers obtained after 3 weeks of enhanced activity may be attributed to the depletion of the BSG nutrients in the COCS by the resident microorganisms. In the treatment amended with rabbit droppings, the HUB population increased from 1.76×10^4 to 4.13×10^4 CFU/g by week 3 and subsequently dropped to 0.55×10^3 CFU/g after week 5.

Likewise, the THBC increased from 1.93×10^3 to 4.25×10^5 CFU/g by week 3, before decreasing to 0.35×10^3 CFU/g by the 5th week. The total heterotrophic fungal (THF) and hydrocarbon-utilizing fungal (HUF) counts followed the same trend and increased from 1.54×10^4 CFU/g and 1.43×10^3 CFU/g respectively to 2.75×10^5 and 3.05×10^4 CFU/g by the 3rd week before a decrease to 0.15×10^3 CFU/g (THF) and 0.32×10^2 CFU/g (HUF).

Generally, there was a significant ($p<0.05$) reduction in the different physiological groups of microorganisms after week 3 indicating the initial increase in microbial populations in the second and third week was due to nutrient supplementation whereas, the decrease after the 3rd week could be attributed to nutrient limitation or consumption by the microbes (Liebeg and Cutright, 1999; Delille and Coulon, 2008). The results obtained for the different physiological groups collaborates the findings of Chikere et al. (2009) who in a tropical crude oil polluted soil undergoing bioremediation observed that the use of NPK fertilizer, urea fertilizer and poultry droppings effectively stimulated bacterial species into utilization of crude oil, thus, increasing their population.

The positive effect of BSG obtained from malted barley had been reported by other workers (Aliyu and Muntari, 2011; Huige, 1994; Thomas and Rahman, 2006). The ratios of HUB to THB in treatments containing RD and BSG were 2.45 and 1.46 respectively while 2.32 and 1.23 were obtained as ratios of HUF to TF in RD and BSG treatments respectively (Figure 5). The control had ratio of 0.75 (THB/HUB) and 0.94 (THF/HUF).

The proliferation of the HUB and HUF in RD and BSG amended COCS is an indication of their enhanced growth and activity as a result of the amendments. It has been previously reported that the exposure of soil to crude oil results in an immediate change in bacterial community structure, increasing abundance of hydrocarbon-degrading microorganisms and a rapid rate of oil degradation, which suggests the presence of a pre-adapted oil-degrading microbial community (Hamamura et al., 2006).

Exploration and production activities may have encouraged the adaptation of crude oil degrading microorganisms in the soil where such activities prevail. Previous studies had reported that as oil-utilizing microorganisms become adaptive to the environment, they make use of the residual crude oil in the soil as energy source and replicate faster (Ting, 1999; Obiukwu and Abu, 2003; Ebuehi et al., 2005). However, the increase in microbial abundance is facilitated by the introduction of nutrients (in this case, RD and BSG) into the soil which enhances the growth of the hydrocarbon-degrading microbes and stimulates the production of enzymes required for the breakdown of the complex hydrocarbon compounds.

Figure 6 shows the reduction in TPH concentration of COCS amended with BSG and RD including the control experiment. In the treatment amended with BSG, the TPH reduced from 6706.76 to 3102.96 ppm after week 5. This corresponds to a percentage TPH reduction of 57.7.

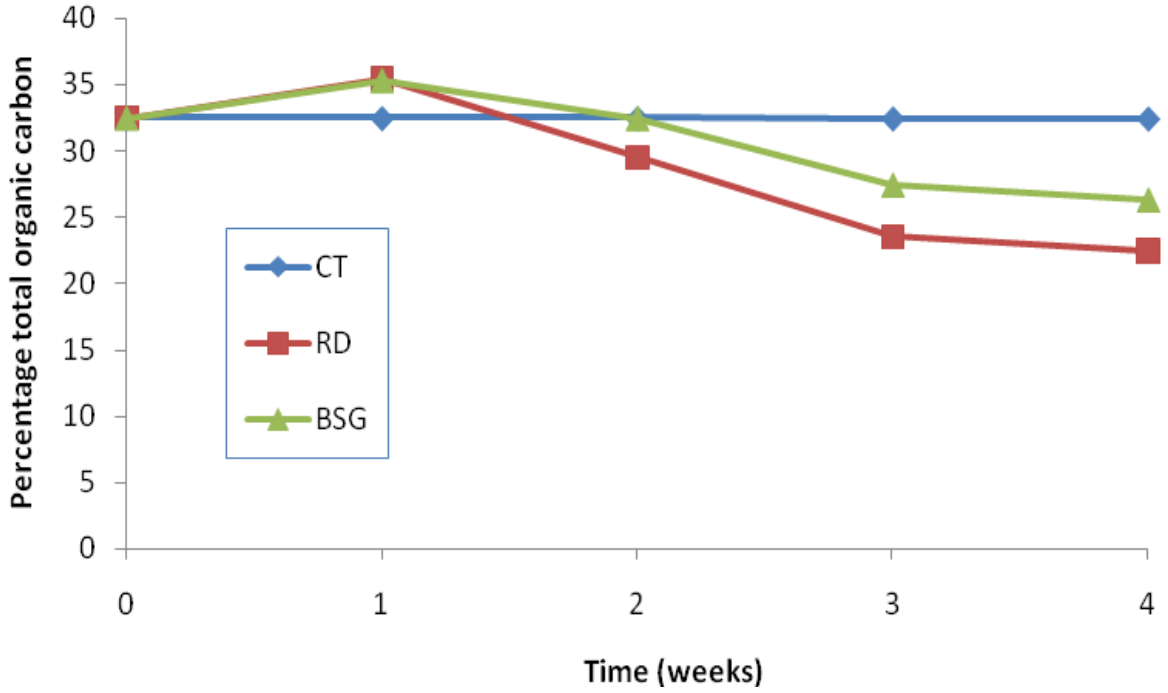

Figure 6. Changes in total organic carbon of COCS amended with RD and BSG during the study period.

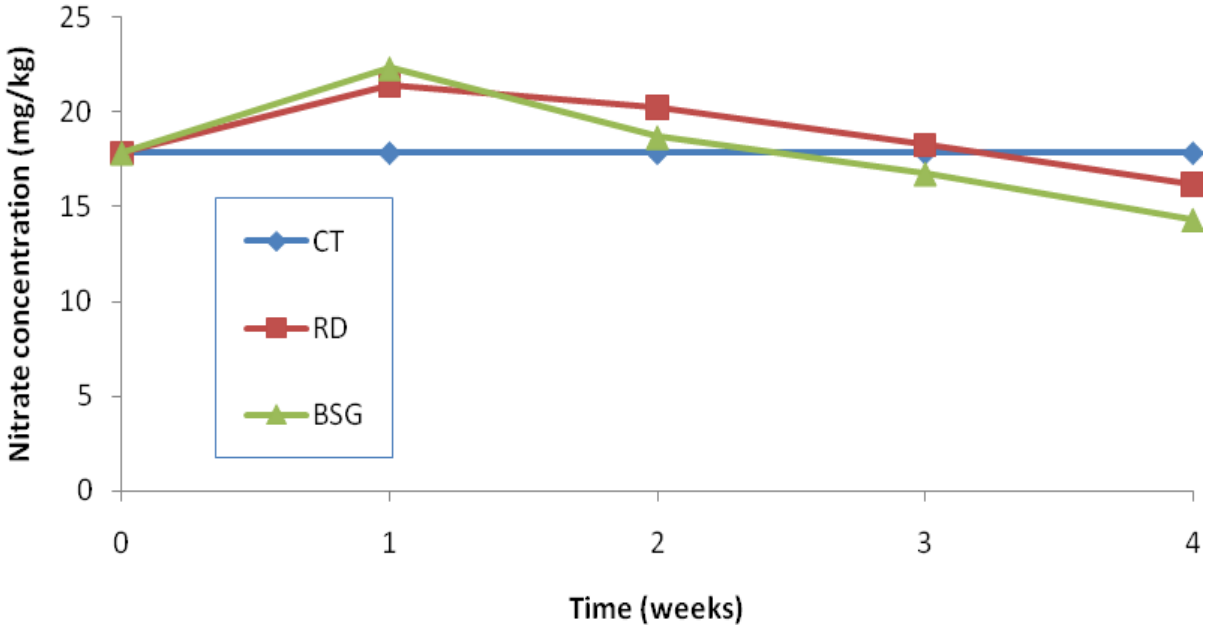

Figure 7. Changes in nitrate content of COCS amended with RD and BSG during the study period.

The RD amended treatment showed a reduction of TPH from 6706.76 to 2818.42 ppm, indicating a 57.9% reduction in total petroleum hydrocarbons of the COCS in 5 weeks whereas, in the control experiment, TPH reduction was from 6706.76 to 6666.76 ppm indicating a reduction in TPH of only 0.59% (Figure 7).

The significant ($p<0.05$) reduction in TPH concentration of the COCS after 5 weeks of treatment with the amendments suggests that the microbes in the COCS have the capability to utilize the crude oil as a source of

carbon and energy and that the RD and BSG had stimulating effects on crude oil utilization by these indigenous microbes in the COCS. This suggests that RD and BSG amendments were effective in enhancing remediation of COCS.

This inference is in agreement with that of other researchers (Amanchukwu et al., 1989; Delille and Coulon, 2008; Chikere et al., 2011) who reported the biostimulation efficiency of organic nutrients for enhanced bioremediation of total petroleum hydrocarbons contaminated environments. Other workers (Alvim-Ferraz et al., 2006; Abioye et al., 2009; Seklemora et al., 2001) had also reported the positive effect the addition of organic wastes had on the bioremediation of used motor oil contaminated soil.

The bacteria isolated from the COCS included members of the following genera: *Corynebacterium*, *Staphylococcus*, *Pseudomonas*, *Achromobacter*, *Serratia*, *Bacillus*, *Proteus*, *Micrococcus*, *Clostridium*, *Acinetobacter*, *Flavobacterium*, *Citrobacter* and *Alcaligenes*.

The abundance of these hydrocarbon-utilizing genera in the COCS could be attributed to the increased nitrogen and phosphorus content of the soil mediated by the addition of the organic materials which enhanced microbial growth and the catabolism of the petroleum hydrocarbon contaminants (Wackett and Ellis, 1999; Watanabe, 2001; Nweke and Okpokwasili, 2004).

The fungal isolates included the following: *Candida*, *Cryptococcus*, *Rhodotorula*, *Saccharomyces*, *Trichosporium*, *Geotrichum*, *Aspergillus*, *Penicillium*, *Mucor*, *Fusarium*, *Cladosporium*, *Cephalosporium*, *Monosporium*, *Neurospora*, *Rhizopus* and *Microsporium*.

These fungal genera have been isolated by other workers and their biodegradation capability previously evaluated (Okpokwasili and Amanchukwu, 1988; Macnaughton et al., 1999; Onifade and Abubaker, 2007; Khidzir et al., 2010). The natural microbial community in soil is usually able to degrade oil hydrocarbons, since many of the bacterial species known to degrade hydrocarbons are commonly found in soil. Thus, it is more important to create suitable conditions for these indigenous bacteria than to introduce new species.

Figures 8 to 12 show the changes in physicochemical parameters in the RD and BSG amended COCS during the study period. In the RD amended treatment, the % total organic carbon, nitrate content (mg/kg), and phosphate content (mg/kg) decreased from 32.48 to 22.41; 17.82 to 16.14 and 24.12 to 14.71, respectively.

The pH also reduced from 5.34 to 4.74 by the 5th week (Figure 11). In the BSG amended COCS (Figures 8 to 12), there was also a significant ($p<0.05$) decrease in its TOC, nitrate and phosphate contents. Decrease in TOC and nitrate content from 32.48 to 26.30 mg/kg and from 17.82 to 14.28 mg/kg respectively were also obtained after the 5th week. The decrease in pH of soil may be attributed to the release of acidic intermediates or end products of catabolic metabolism.

The gas chromatograms of samples obtained during the bioremediation of the COCS are as presented in Figures 10 to 12. The gas chromatograms show the extent of the different carbon fractions in the amended and un-amended COCS during the study period.

Weathering and microbial activities prior to sample collection may have been responsible for the low amounts of C_2-C_{16} fractions of the crude as the gas chromatograms of the hydrocarbons present in the soil did not resemble the chromatograms of fresh products (Figure 10). Evaporation of volatile compounds may also have taken place during the initial excavation, transport and sieving of the soil. However, the hydrocarbons left in the soil were degraded during the study period when organic amendments were introduced.

In COCS amended with BSG and RD, there was enhanced attenuation of the peaks within the region of C_{12}–C_{23} fractions when compared to the control (un-amended) indicating that the isolates were stimulated to degrade the short chain hydrocarbon fractions within that range. The ability of these microbes to utilize these carbon fractions may be attributed to the possession and activation of special enzyme (dehydrogenases and oxygenases) systems which can break down the crude oil contaminants, bringing about their mineralization to carbon (IV) oxide and water and thus, reducing levels of total petroleum hydrocarbon (TPH) contaminant in the soil. The changes in peak height obtained in the chromatograms did not follow the trend observed for changes in TPH content in COCS as certain peaks increased in height after initial peak attenuation during the study period (Figures 10 to 12).

This seemingly unusual trend in peak height attenuation with respect to degradation of the TPH may be due to the metabolic activities of the hydrocarbon-utilizing microbes which mediate changing substrate conditions in a stressed environment as smaller molecules are used to build larger ones and complex molecules broken down into smaller ones.

Nevertheless, significant attenuation of chromatographic peak heights was obtained after 5 weeks in amended COCS which suggests that enhanced bioremediation of oil spill sites can be successfully prosecuted using RD and BSG as biostimulants in order to restore polluted soils to their natural state. Hence, it seems that it is possible to obtain lower end point concentrations with aged contaminants just like with freshly gasoline-spiked soil.

The exact nature of the residual fractions in the soil after the study period is not known, but it is likely that it consists of branched-chain alkanes, multi-ring saturates (naphtenes) and aromatics, each of which may have alkyl side chains attached to their core ring structure. For aged contaminations, these residual fractions which are

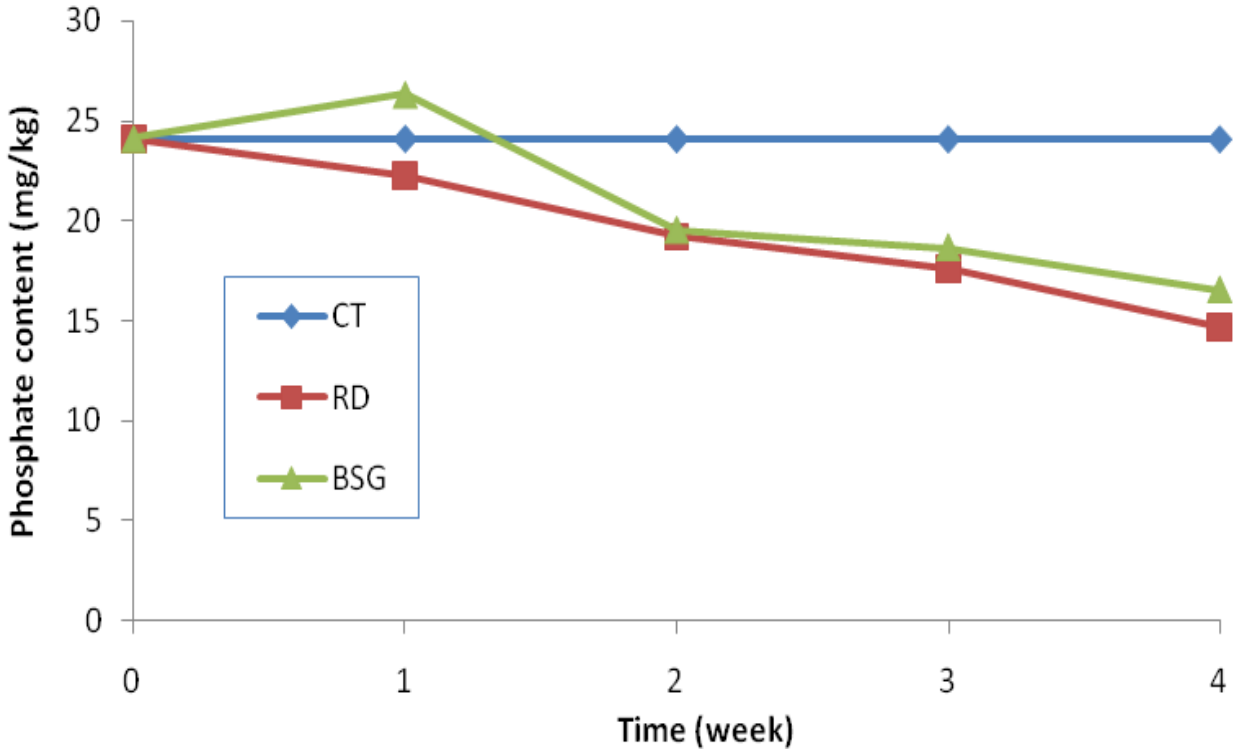

Figure 8. Changes in phosphate content of COCS amended with RD and BSG during the study period.

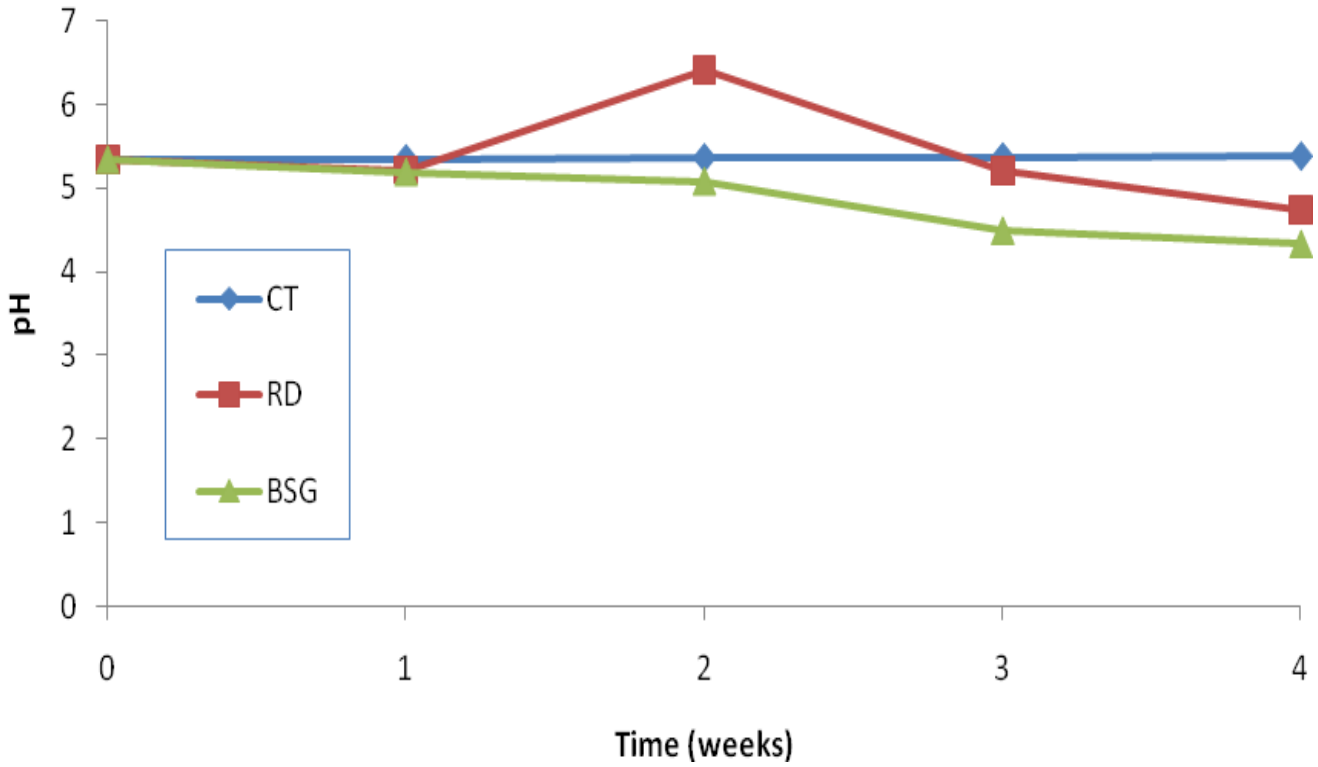

Figure 9. Changes in pH of COCS amended with RD and BSG during the study period.

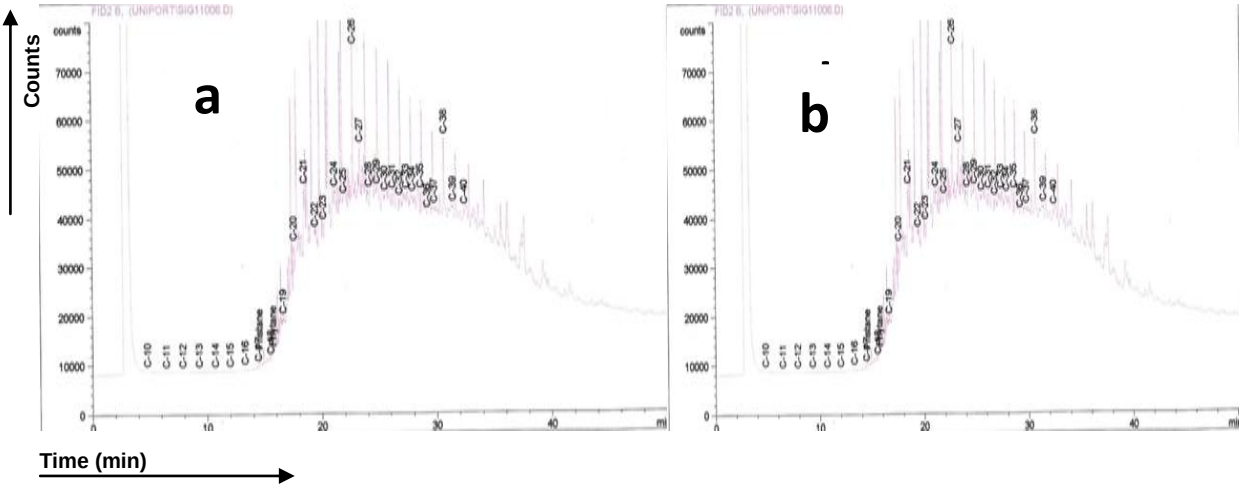

Figure 10. The chromatogram of un-amended COCS (control) showing the extent of the different carbon fractions of the crude oil after week zero (a) and week 4 (d) of experiment.

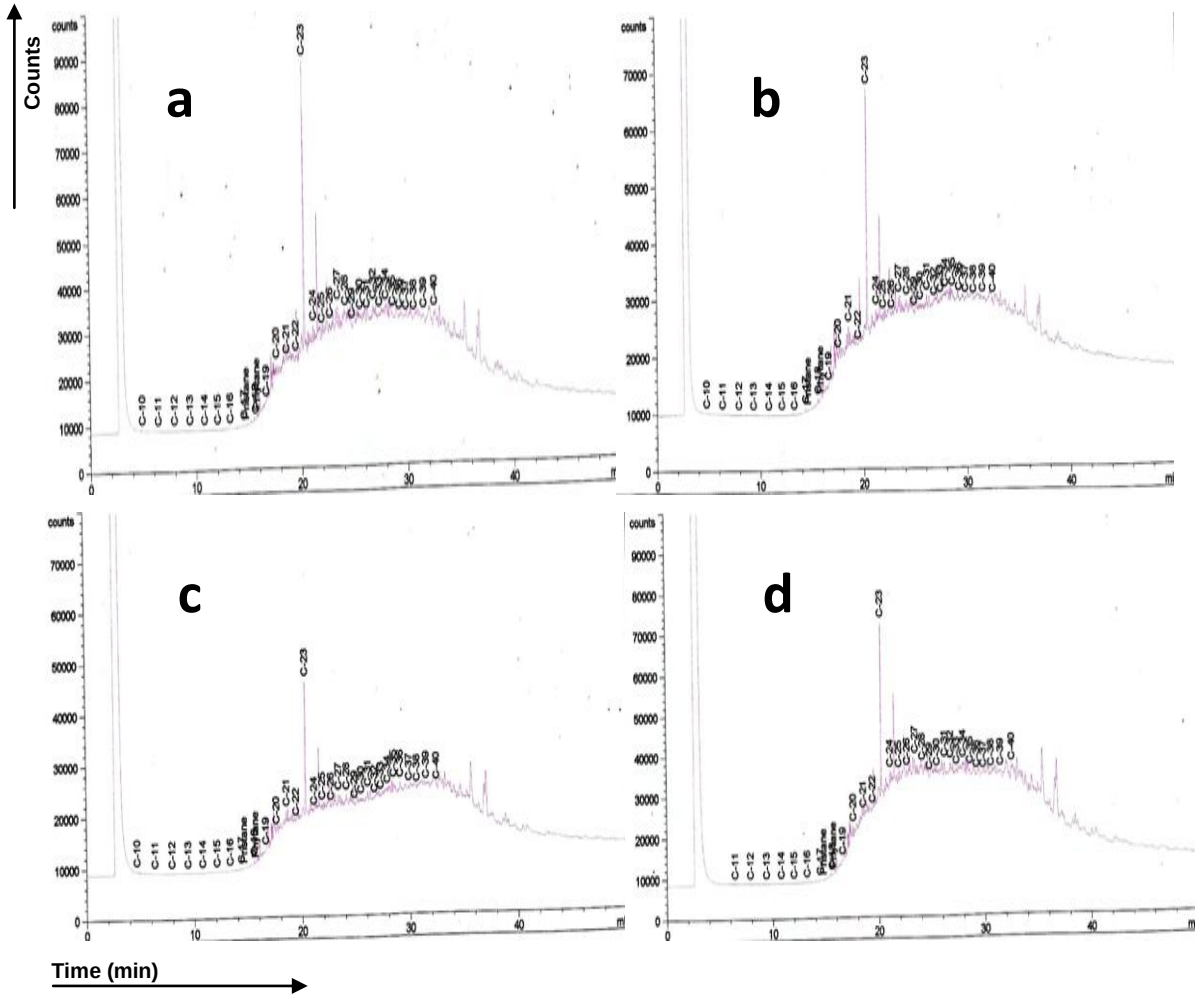

Figure 11. The chromatogram of COCS amended with RD showing the extent of the different carbon fractions of the crude oil after weeks 1 (a), 2 (b), 3(c) and 4 (d) of treatment.

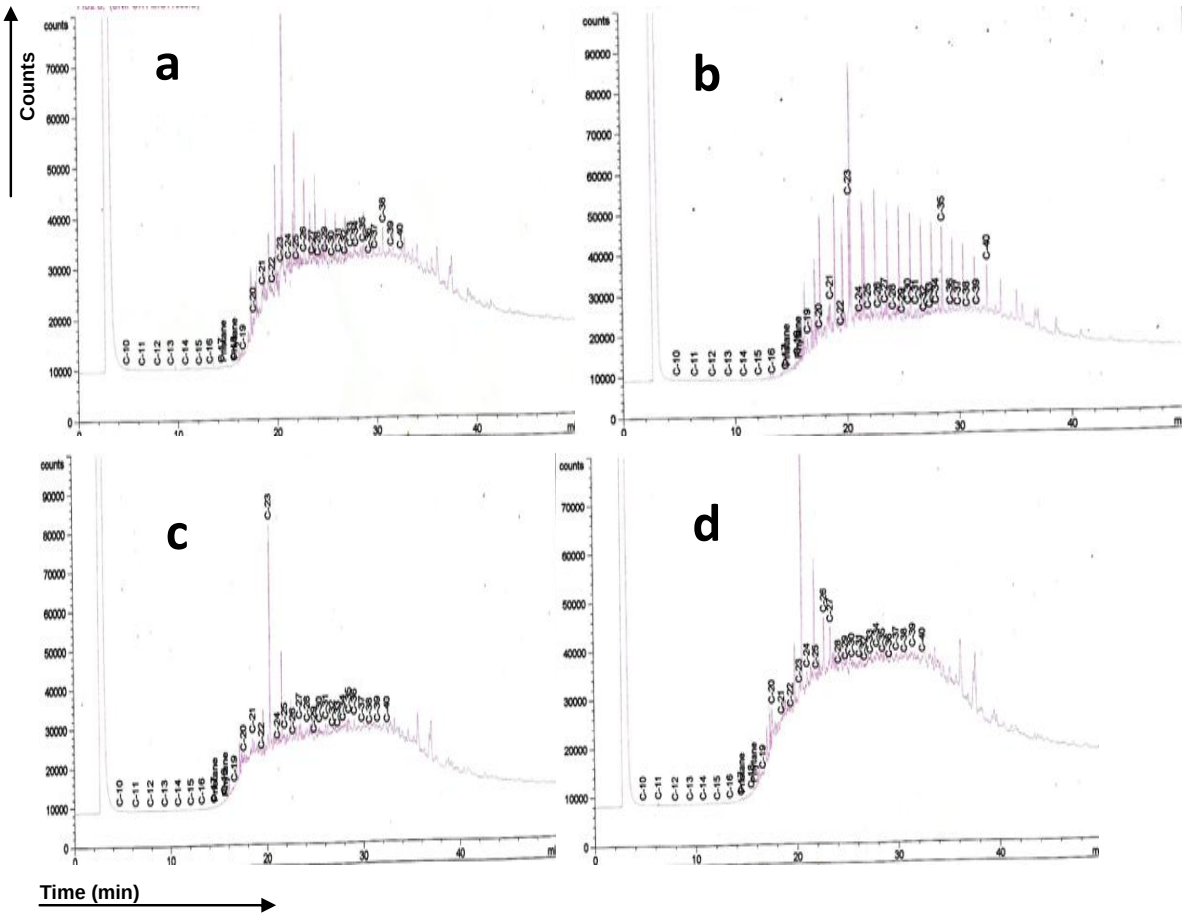

Figure 12. Chromatogram of COCS amended with BSG showing the extent of the different carbon fractions of the crude oil after weeks 1(a), 2(b), 3(c) and 4(d) of treatment.

degraded slowly are usually not bioavailable since they are `locked up' in particle pores, though it cannot be excluded that the residual fraction is inherently recalcitrant (Huesemann, 1997). Moreover, the aged status of the stimulated microorganisms at the end of the bioremediation event may also explain the non-removal of the residual fractions since the vigour of degrading the contaminant may have been weakened.

Thus, our results indicate that the application of organic substrates (brewery spent grain and rabbit droppings) to aged crude oil contaminated soils could serve as a low-cost remediation strategy for returning such polluted soil ecosystems to their natural state and as an alternative to the current practice of applying large quantities of inorganic fertilizers.

Conclusion

Enhanced *ex situ* bioremediation of a COCS using BSG and RD as amendments was achieved in this study using indigenous soil microorganisms. Rapid increase in microbial populations of the COCS was obtained in treatments containing the two amendments albeit at varying rates. Decrease in TPH of COCS was also enhanced in treatments containing the amendments when compared to the un-amended COCS (control) with a relatively higher TPH removal obtained in RD amended COCS.

Evidence of mineralization of the hydrocarbons in the COCS by the indigenous hydrocarbon-utilizing microbes was presented in the gas chromatograms obtained before and after the experiments which suggests that RD and BSG can be used as efficient bio-stimulants during bioremediation of crude oil polluted soil. However, RD was more effective in stimulating the growth of intrinsic microbial species and enhancing crude oil biodegradation in the COCS than BSG.

ACKNOWLEDGEMENT

The authors are grateful to the Staff of Anal Concept Nigeria

Ltd, Elimgbu, Port Harcourt for the gas chromatography (GC) analyses of COCS soil samples.

REFERENCES

Abioye P. O., A. Abdul Aziz & P. Agamuthu (2009). Enhanced biodegradation of used engine oil in soil amended with organic wastes. Water, Air and Soil Pollution, 209:173-179.

Abu G. O. & Ogiji P. O. (1996). Initial test of a bioremediation scheme for the cleanup of an oil polluted water body in a rural community in Nigeria. Bioresour. Technol. 58:7-12.

Achuba F. I. & Peretiamo–Clarke B. O. (2006). Effect of spent engine oil on soil catalase and dehydrogenase activities. Int. Agrophysics. 22:2-4.

Adeniyi A. A. & Afolabi J. A. (2002). Determination of total petroleum hydrocarbons and heavy metals in the soil within the vicinity of facilities handling refined products in Lagos Metropolis. Environ Int. 28:79-82.

Adriano D. C., Chlopecka A. & Kaplan K. I. (1998). Role of soil chemistry in soil and ecosystem conservation. Soil Sci. Soc. America. Pp. 361-386.

Agarry S. E. & Ogunleye O. (2012). Box enhanced design application to study enhanced bioremediation of soil artificially contaminated with spent engine oil using biostimulation strategy. Int. J. Energy & Environ. Eng. Pp. 3-31.

Aislabie J., McLeod M. & Fraser R. (1998). Potential for biodegradation of hydrocarbons in soil from the Ross Dependency, Antarctica. Appl. Microbiol. Biotechnol. 49:210-214.

Aliyu S. & B. Muntari (2011). Brewer's spent grain: A review of its potentials and applications. Afr. J. Biotechnol. 10(3):324-331.

Alvim-Ferraz M. C. M., Albergaria J. & Delerue-Matos C. (2006). Soil remediation time to achieve clean-up goals II: influence of natural organic matter and water contents. Chemosphere 64:817-825.

Amanchukwu S. C., Obafemi A. & Okpokwasili G. C. (1989). Hydro-carbon degradation and tilization by Palm wine Isolates. FEMS Microbiol. Lett. 57:151-154.

APHA (1995). Standard methods for the examination of water and wastewater. 19th Ed. APHA-AWWA-WPCF. Washington D.C. Pp. 4-85.

Atlas R. M. (1981). Microbial degradation of petroleum hydrocarbons: An environmental perspective. Microbiol. Rev. 45:180–209.

Atuanya E. J. (1987). Effect of oil pollution on physical and chemical properties of soil: A case study of waste oil contaminated delta soil in Bendel State. J. Appl. Sci. 55:156-176.

Chikere C. B., Okpokwasili G. C. & Chikere B. O. (2009). Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremedia-tion. Afr. J. Biotech. 8(11):2535-2540.

Chikere C. B., Okpokwasili G. C. & Chikere B. O. (2011). Monitoring of microbial hydrocarbon remediation in the soil. 3 Biotech. 1(3):117-138.

Dawson J. J., Godsiffe E. J., Thompson I. P., Ralebiso-Senior T. K., Killham K. S. & Paton G. I. (2007). Application of biological indicators to assess recovery of hydrocarbon impacted soil. Biol. Biochem. 39:164-177.

Delille D. & Coulon F. (2008). Comparative mesocosm study of biostimulation Efficiency in two different oil-amended sub-Antartic soils. Microb. Ecol. 56:243-252.

Demirbas A. H. & Demirbas I. (2007). Importance of rural bioenergy for developing countries. Energ. Convers. Manage. 48: 2386.

Dorn P. B., Vipond T. E., Salanitro J. P. & Wisniewski H. L. (1998). Assessment of the acute toxicity of crude oils in soils using earthworms, Microtox (R) and plants. Chemosphere. 37:845-860.

Ebuehi O. A. T., Abibo I. B., Shekwolo P. D., Sigismund K. I., Adoki A. & Okoro I. C. (2005). Remediation of crude oil contaminated soil by enhanced natural attenuation technique. J. Appl. Sci. Environ. Manage. 9(1):103-106.

Ekpo I. A., Agbor R. B., Okpako E. C. & Ekanem E. B. (2012). Effect of crude oil polluted soil on germination and growth of soybean (Glycerine max). Ann. Biol. Res. J. 6:3049-3054.

Hamamura N., Olson S. H., Ward D. M. & Inskeep W. P. (2006). Microbial population dynamics associated with crude oil biodegradation in diverse soils. Appl. Environ. Microbiol. 72:6316-6324.

Huesemann M. H. (1997). Incomplete hydrocarbon biodegradation in contaminated soils: Limitations in bioavailability or inherent recalcitrance. Bioremed. J. 1:27–39.

Huige N. J. (1994). Brewery by-products and effluents. In: Hardirick, W. A. (Ed), Handbook of Breweing. Marcel Dekker, New York. Pp. 501-550.

Hussemann M. H. W. (1993). General guidelines for bioremediation of petroleum hydrocarbon contaminated soils. Technical Progress Report. WRC 285-92, Shell Development Westhollow Research Centre, Houston.

Ibiene A. A., Orji F. A., Ezidi C. O. & Ngwobia C. L. (2011). Bioremediation of hydrocarbon contaminated soil in the Niger Delta using spent mushroom compost and other organic wastes. Nig. J. Agric. Food & Environ. 7(3):1-7.

Holt J. G., Krieg N. R., Sneath P. H. A., Stanley J. T. & Williams S. T. (1994). Bergey's manual of systematic bacteriology, 9th Ed. Williams & Wilkins Co. Baltimore, Maryland. 786p.

Kämpfer P., M. Steiof & W. Dott (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. Microb. Ecol. 21:227.

Khidzir K. M., Noorlidah A. & Agamuthu P. (2010). Brewery spent grain: Chemical characteristics and utilization as an Enzyme substrate. Malaysian Journal Science 29(1):41-51.

Liebeg E. W. & Cutright T. J. (1999). The investigation of enhanced bioremediation through the addition of macro and micro nutrients in a PAH contaminated soil. Int. Biodeter. Biodegrad. 44:55-64.

Lyman W. J. (1990). Handbook of chemical property estimation methods. Washington DC. American Chem. Soc. 3: 4-29.

Lynch J. M., Benedetti A., Insam H., Nuti M. P., Smalla K., Torvik V. & Nannipieri P. (2004). Microbial diversity in soil: ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biology and Fertility of Soils. 40:363-385.

Macnaughton S. J., Stephen J. R., Venosa A. O., Davis G. A., Chang Y. J. & White D. C. (1999). Microbial population changes during bioremediation of an experimental oil spill. Appl. Environ. Microbiol. 65:3566-3574.

Mills A. L., Breulland C. & Colwell R. R. (1978). Enumeration of Petroleum degrading marine and estuarine microorganisms by most probable number method. Can. J. Microbiol. 12:234-248.

Nweke C. O. & Okpokwasili G. C. (2004). Effects of bioremediation treatments on the bacterial populations of soil at different depths. Nig. J. Microbiol. 18:163-372.

Obiukwu C. & Abu G. O. (2003). Utilization of phenol by bacteria isolated from petroleum refinery effluent. Nig. J. Microbiol. 17(1):7-11.

Odjeba V. I. & Atebe J. O. (2007). The effect of used engine oil on carbohydrate, mineral content and nitrate reductase activity of leafy vegetable (*Amaranthus hybridus*). J. Appl. Sci. Environ. Manage. 11(2):191-196.

Odokuma L. O. (2012). The genius in the microbes: An indispensable tool for the management of xenobiotic mediated environmental flux. University of Port Harcourt Inaugural Lecture Series, NO. 87.

Okpokwasili G. C. & Amanchukwu S. C. (1988). Petroleum hydrocarbon degradation by *Candida* sp. Environ. Int. 14:243-247.

Okpokwasili G. C. (2006). Microbes and the environmental challenge. An inaugural lecture series, University of Port Harcourt. No. 53.

Onifade A. K. & Abubakar F. A. (2007). Characterization of hydrocarbon degrading microorganisms isolated from crude oil contaminated soil and remediation of the soil by enhanced natural attenuation. Res. J. Biol. Sci. 2(1):149-155.

Rosenberg E. (1992). The hydrocarbon-oxidizing bacteria. In: A. Balows et al. (Ed.). The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification and applications. Springer Verlag, Heidelberg, Germany. Pp 446-459.

Schafer A. N., Snape I. & Siciliano S. D. (2009). Influence of liquid water

and soil temperature on petroleum hydrocarbon toxicity in Antartic soil. Environ. Toxicol. Chem. 28:1409-1415.
Seklemora E., Pavlova A. & Kovacheva K. (2001). Biostimulation-based bioremediation of diesel fuel: Field demonstration. Biodegradation. 12:311-316.
Sikkema, J., J. A. de Bont & B. Poolman (1995). Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 59:201–222.
Semple K. T., Reid B. J. & Femor T. R. (2001). Impact of composing strategies on the treatment of soils contaminated with organic pollutants. Environ. Pollut. 112:269-283.
Sims R. C. & Sims J. L. (1991). Remediation techniques for contaminated soils. EPA seminar publication: Site characterization for subsurface remediation, EPA/625/4-91/026.
Thomas K. R. & Rahman P. K. S. M. (2006). Brewery wastes. strategies for sustainability. A review. Aspects of Applied Biology. 80:147-153.
Ting Y. P., Hu H. I. & Tan H. M. (1999). Bioremediation of petroleum hydrocarbons in soil microcosms. Resour. Environ Biotech. 2:197-218.
United Nations Environmental Programme (UNEP) (2011). Environmental Assessment of Ogoniland. 1: 65-66. www.unep.org/nigeria.
USEPA (2010). EPA requirement for quality assurance project plans. US Environmental Protection Agency, Office of Environmental Information, Washington DC.
USEPA (2011). U.S. Environmental Protection Agency, land revitalization and reuse (2007). Technological Partners.
Wackett L. P. & Ellis L. B. M. (1999). Predicting biodegradation. Environ. Microbiol. 1(2):119-124.
Watanabe K. (2001). Microorganisms relevant to bioremediation, Curr. Opin. Biotech. 12(3):237-241.
Yveline I. D., Frederic J., Pierre D., Michael G., Jean C. B. & Gilbert M. (1997). Hydrocarbon balance of a site which had been highly and chronically contaminated by petroleum waste of a refinery from 1956–1992. Mar. Pollut. Bull. 34(6): 456-468.