Effect of Feedstock Type on Biostimulation Efficiency and Microbial Community Structure during Biochar-Facilitated Remediation of Petroleum Contaminated Soil

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ABSTRACT

This study investigated the effect of plant feedstock– and animal feedstock–derived biochar at 10%w/w and 15%w/w amendment levels on the biostimulation efficiency and the cultivable microbial community in the soil during biochar-facilitated remediation of petroleum contaminated soil using standard techniques. Biostimulation was most effective with the animal-based biochar (ABB) treatment while total petroleum hydrocarbon (TPH) removal was greatest in the plant-based biochar (PBB) amended soil. Observed mean TPH levels on Day 60 ranged from about 7000 mg/kg – 7800 mg/kg for the PBB and 11000 mg/kg – 14000 mg/kg for ABB representing removal levels of roughly 51.0%, 57.7%, 72.4% and 73.7% in 10% ABB, 15% ABB, 10% PBB and 15% PBB amended contaminated soils respectively. The cultivable bacterial diversity for both feedstock types shifted from the combination of Firmicutes, Actinobacteria and Proteobacteria phyla at the onset of the study to predominantly Proteobacteria by the end of the study with a distinct reduction in diversity observed with increasing contact time. The dominant cultivable heterotrophic bacterial isolates were *Bacillus* spp., *Pseudomonas* spp. and *Staphylococcus aureus* for ABB and *Pseudomonas* spp., *Klebsiella pneumoniae* and *Bacillus* spp. for PBB. Amongst the cultivable hydrocarbon utilising bacteria obtained, *Klebsiella pneumoniae*, *Pseudomonas* spp. and *Enterobacter* spp. dominated. There were significant differences in TCHB and CHUB abundance and TPH removal efficiency between PBB and ABB amendments at 95% confidence interval. The study established that application of biochar effectively manages petroleum pollutants in soil by stimulating the proliferation and activities of relevant degradative species.

INTRODUCTION

Petroleum may contaminate terrestrial systems by varying means that could be natural, accidental, deliberate or due to negligence. Contamination by petroleum hydrocarbons is currently one of the most vivid examples of the negative impacts of anthropogenic activities on the environment. The impact of these compounds on terrestrial ecosystems is of grave concern as their presence has far-reaching implications for food and water security, environmental sustainability and human health. The impact of spillage is often interminable with the effects still palpable even long after the spillage incident. The continued onslaught on terrestrial systems results in rapid land degradation which impacts negatively on agricultural activities and, in turn, has unfavourable socioeconomic knock-on effects [1,2].

In a bid to restore impacted ecosystems, several technologies have been employed in the management of petroleum spills. These technologies utilise physical, chemical and biological means to delimit, immobilise and ultimately remove these contaminants with each method having its inherent merits and demerits. The technologies are of varying efficiencies, costs and functionality in the field. The management of pollution using biological systems, often termed bioremediation, is gaining increasing relevance due to its environmentally benign nature, relatively low cost and readily available materials. Petroleum hydrocarbon fractions are not readily degraded by most soil microorganisms [3], however, application of protocols like biostimulation enhance the biodegradation of these pollutants. Biostimulation in petroleum contamination management is a bioremediation technique designed to encourage the rapid growth and proliferation of autochthonous hydrocarbon degrading

microorganisms mainly by boosting their nutrient and/or oxygen supply. The increased density of microbial groups that are able to utilise petroleum as a carbon source enhances the competitive advantage of these specialised species and increases the rate of biodegradation of the polluting hydrocarbon compounds [4].

Biochar, also known as bio-charcoal, is produced by pyrolysis of organic biomass in the presence of limited oxygen. Biochar may be produced from a wide variety of organic matter and the feedstock could be of plant or animal origin. Plant-derived biochar will often include parts of plants or field crop by-products like husks while animal-derived biochar may utilise animal waste/ droppings or carcasses [5]. Biochar is loaded with nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium and labile organic carbon; all of which support the growth of plants and microorganisms [6]. The application of biochar during soil remediation has several eco-friendly advantages. Researchers maintain that biochar significantly improves soil fertility and plant yield in agricultural soil mostly by boosting soil organic content and water holding capacity and reducing soil acidity; all of which make soil more conducive for plants and soil microbes. Biochar production often utilises end-of-life agricultural biomass that would otherwise present a waste management challenge. The production method of pyrolysis is not of environmental concern unlike the incineration method sometimes used to dispose of these "waste" materials. The conversion of waste into biochar for various purposes promotes a circular economy and drives sustainability [7,8].

The biostimulation capacity of biochar with regards to petroleum pollution management has not been explored extensively. The suitability of biochar for any specific purpose including pollutant management depends upon its physicochemical properties and these properties will often differ based on the feedstock used and the conditions of the thermal degradation process. Nartey and Zhao [9] stated that biochar is able to adsorb most organic and inorganic compounds from both soil and water and stressed that the feedstock type and production conditions play a vital role here. Ultimately, however, the effectiveness of bioremediation is a function of the microbial community and how it can be enriched and maintained. This study looked to investigate the potentials of plant feedstock– and animal feedstock–derived biochar as biostimulation agents and their effect on the soil microbial community during biochar-facilitated remediation of petroleum contaminated soil.

MATERIALS AND METHODS

Collection of Samples
The soil used in this study was collected from the Botanical Gardens of University of Port Harcourt, Nigeria. Soil was collected from top to 15 cm depth using a hand trowel and composited in the field into a sterile sampling bag. The composited soil was immediately transported to the laboratory where it was sieved using a 2 mm mesh screen to exclude debris and homogenise the soil sample. The white corn cobs were gathered from sellers in the local market while the long bones from cows (White Fulani cattle variant) were collected from a randomly selected local abattoir in Port Harcourt, Nigeria.

Production of Biochar
The washed and dried corn cobs and cow bones were subjected to slow pyrolysis under anoxic conditions in a muffle furnace (SIOMM, model SXL 1700C, Shanghai, China). Pyrolysis was at 500 °C for 2 h. No external fuel source was used. The charred marrow in the bones was used. The biochar produced was reduced to nanoscale particles before application. The biochar produced was sterilised by autoclaving at 121 °C and 15 psi for 30 minutes.

Characterisation of Biochar

Microscopic Characterisation
The biochar obtained was characterised morphologically using scanning electron microscopy (Quanta FEG 450, Apollo X – EDAX) using Au/Pd film on 0.5 g of sample.

Physicochemical Characterisation
The methods prescribed by APHA [10] were employed in the characterisation of the synthesised biochar as follows:
Determination of yield from the biochar samples
The yield was calculated as below:

$$\text{Yield (\%)} = \frac{W_f}{W_i} \ \text{x} \ 100$$

Where
W_i – Weight of organic feedstock (g)
W_f – Weight of biochar produced after pyrolysis (g)

Determination of moisture content
About 2 g of sample was weighed into a crucible of known weight and then placed in a hot air oven (DHG-9023A, Hinotek, China) at 105°C for 2 h, cooled in a desiccator and weighed again to determine water loss in the sample. Drying was done until a constant weight was achieved.

Determination of elemental content
The carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) contents of biochar samples were determined by dry combustion using a CHNS/O analyser while the oxygen (O) content was calculated by mass difference [11].

Determination of ash content
For ash content, the biochar sample was reduced to ash in a muffle furnace (SIOMM, model SXL 1700C, Shanghai, China) at 550 °C for 6 hours. After cooling in a desiccator, the ash content was ascertained based on the weight obtained [12].

Determination of electrical conductivity and pH
Electrical conductivity (EC) was determined using a benchtop combination meter while a pH meter (Wintab digital pH meter, Germany) was used for determination of pH of samples. The EC and pH of the biochar were measured in deionized water at 1:5 biochar/ water suspension ratio after thorough mixing.

Determination of calcium carbonate (CaCO₃) content
The calcium carbonate, $CaCO_3$, was calculated from the alkalinity levels, based on the principle that the neutralization of 1 cmol H^+ requires 0.5 g $CaCO_3$. The alkalinity of the biochar samples was determined using the modified titration method of Yuan *et al*. [13].

Determination of cation exchange capacity
The modified barium chloride compulsive exchange method was used to determine the cation exchange capacity (CEC) of the biochar samples [14,15].

Experimental Design
The laboratory set up was as outlined in **Table 1**. Treatments consisted of microcosms of 1000 g soil spiked with Bonny Light crude oil to a heavy pollution concentration of about 10 % w/v. The two types of biochar differentiated based on feedstock (animal-based biochar and plant-based biochar) were used at two

different treatment levels. All treatments were set up in two replicates and incubated at room temperature. Deionized water was added regularly to maintain the moisture content at 60 % water holding capacity. Soil pH, TPH levels, microbial abundance and diversity were evaluated at regular intervals after a 7-day resting period.

Table 1. The experimental set-up.

Treatment	Description
Group 1 (Unpolluted Control)	Soil alone
Group 2 (Oiled Control)	1 kg Crude Oil Contaminated Soil alone
Group 3	1 kg Contaminated Soil + 10% w/w ABB
Group 4	1 kg Contaminated Soil + 15% w/w ABB
Group 5	1 kg Contaminated Soil + 10% w/w PBB
Group 6	1 kg Contaminated Soil + 15% w/w PBB

Note: ABB – Animal-Based Biochar (from cow bones);
PBB – Plant-Based Biochar (from corn cobs)

Determination of Total Petroleum Hydrocarbon (TPH) Levels

The TPH levels were ascertained using a gas chromatograph equipped with a flame ionisation detector (GC-FID) (Agilent 6890N, USA) in a capillary column. The soil sample was dehydrated using anhydrous sodium sulphate. Extraction was then carried out using 30 mL dichloromethane added to 10 g of soil sample in an amber glass bottle with shaking for around 6 h at room temperature. The mixture was filtered and then allowed to concentrate to 1 mL by evaporation in a fume cupboard. The sample was eluted using pentane as solvent.

The TPH removal efficiency was determined using the formula:

$$\text{Removal Efficiency (\%)} = \frac{W_0 - W_t}{W_0} \times 100$$

Where
W_0 – Initial TPH concentration (mg kg^{-1}),
W_t – Residual TPH concentration at time t (mg kg^{-1}),
t – Remediation time (days).

Enumeration and Characterisation of Soil Microorganisms
Enumeration and Characterisation of Total Cultivable Heterotrophic Bacteria (TCHB)

Isolation of various bacterial species in soil was done using nutrient agar while plate count agar was used for enumeration of TCHB using the dilution plate technique. Ten grams of the relevant soil sample was first suspended in 90ml of sterile normal saline; after vigorous agitation, a ten-fold serial dilution was carried out. About 0.1 mL aliquots of the serially diluted samples were plated out in triplicates on oxoid nutrient agar (Merck, Germany) and incubated at 37 °C for 24 hours. Only plates with counts of 30 – 300 colonies were selected for determination of count [16]. Isolates were purified by streaking onto fresh nutrient media. Pure cultures were preserved on relevant media slants until required for further investigation. Representative isolates were characterised as described by Holt *et al.* [17]. The bacterial isolates obtained from the study were identified on the basis of their macroscopic, microscopic and biochemical characteristics as recommended by [16].

Enumeration and Characterisation of Cultivable Hydrocarbon Utilising Bacteria (CHUB)

Isolation of the CHUB employed the enrichment method described by [10] using nystatin-amended mineral salt medium (MSM) containing 1 % crude oil as the sole carbon source. Approximately 0.1 mL aliquots of serially diluted samples (dilution 10^{-4}) were plated out on the MSM with incubation at 30°C. Discrete colonies that developed on MSM were purified and preserved on slants for subsequent microscopic and biochemical characterisation tests. For enumeration of the CHUB, the vapour phase method was employed. About 1ml aliquots of the relevant dilutions were inoculated unto separate agar plates containing sterile nystatin-amended MSM. Sterile Whatmann No.1 filter papers were then saturated with crude oil and aseptically placed into the lids of the inverted agar plates. The plates were incubated in the inverted position at 30 °C for up to 7 days. Plates with visible colonies ranging from 30 – 300 were enumerated and expressed as colony forming units per gram of soil sample. The percentage of CHUB to TCHB was determined.

Statistical Analysis

The data was analysed by two-way Analysis of Variance, ANOVA, to ascertain whether the TPH removal efficiency and the biostimulation efficiency based on bacterial counts differed significantly from one feedstock type to the other and from one concentration level to the other. Statistical significance of data sets was determined at $p < 0.05$ using Microsoft Excel® 2013.

RESULTS AND DISCUSSION

Characteristics of the Biochar Samples

The physicochemical and morphological characteristics (SEM, x 1000 magnification) of the two types of biochar produced are shown in Table 2 and Figure 1 respectively. Both biochar types had relatively similar proximate and elemental contents however, ABB generally had higher yield, electrical conductivity and pH. The observed pH values for both products were between 8.0 and 10.0.

Table 2. Physicochemical characteristics of biochar samples.

Physicochemical Parameter	Animal-based Biochar	Plant-based Biochar
Yield (%)	33.50	18.40
pH	9.84	8.43
Electrical Conductivity (μS/cm)	872.85	522.03
Ash Content (%)	4.33	5.68
Moisture Content (%)	3.84	1.72
Total Nitrogen (%)	1.39	2.24
Sulphur (%)	1.22	0.78
Carbon (%)	73.65	75.43
Hydrogen (%)	2.98	1.60
Nitrogen (%)	0.40	0.62
Oxygen (%)	19.46	10.40
Calcium carbonate, CaCO$_3$ (%)	7.70	5.49
H/C ratio	0.0404	0.0212
O/C ratio	0.260	0.140
H/C$_{org}$	0.33	0.40
CEC (cmol/kg)	115.40	189.10

Note; CEC – cation exchange capacity; Corg – organic carbon content

The two types of biochar used in this study met the recommendations of the International Biochar Initiative and the European Biochar Certificate Program for pyrogenic matter to be classed as biochar [18,19]. The samples may also be considered stable as they meet the criteria of $> 65\%$ organic carbon and ≤ 0.7 H/C$_{org}$ ratio highlighted by Joseph *et al.* [20]. The stability is further underscored by the O/C ratio of ≤ 0.4 which depicts that both the plant derived biochar and the animal derived biochar would keep well in the soil. A pH range of 7.1 – 10.5 has been reported for biochar from different sources by other researchers [21,22]. The CEC is usually dependent on the type of feedstock used in biochar production.
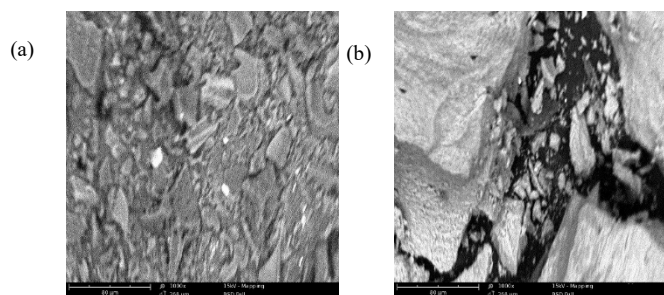
Fig. 1. Scanning electron micrograph of biochar from corn cobs (a) and cow bones (b)

The CEC value for plant-based biochar in this study is higher than the values of 5 – 162 cmol/kg considered by Guo *et al*. [6] to be typical of biochar. The observed higher CEC values in the PBB is buttressed by Wang *et al*. [23] who stated that plant derived biochar will normally have much higher CEC than biochar from other sources. Yang *et al*. [24] likewise confirmed that biochar with higher ash content will normally have higher CEC levels. Cely *et al*. [25] recorded relatively low CEC of 32.7 cmol/kg and 81.4 cmol/kg for pig and chicken manures respectively; both produced at 500°C while a CEC value of 122 cmol/kg was obtained for sugarcane bagasse [26]. The calcium carbonate, $CaCO_3$, equivalence of biochar characterises its liming potential. From the results in the current study, the biochar from cow bones is shown to have a better liming potential than its plant derived counterpart.
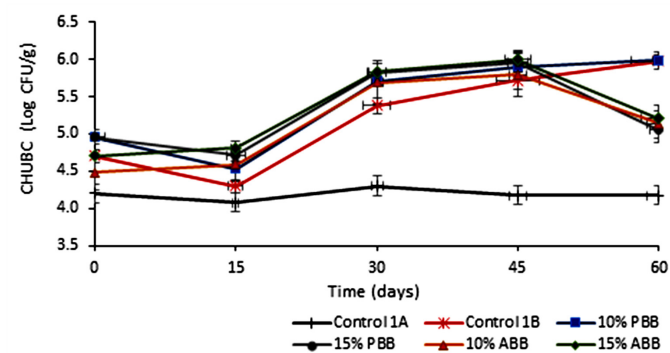
Biostimulation Efficiency of Biochar Samples – Microbial Abundance and Diversity

The variations in abundance of the total cultivable heterotrophic bacteria (TCHB) and cultivable hydrocarbon utilising bacteria (CHUB) in the soil during the 60-day study period are illustrated in Figure 2. The proliferation of soil bacteria was best stimulated with the 15% w/w animal-based biochar while the greatest CHUB counts were obtained in the oiled control and the 10% w/w PBB amended soil (Figure 3).

Peak microbial counts were obtained between days 30 and 45 displaying increases in TCHB abundance of 318.46%, 369.84%, 264.91% and 348.0% for 10% ABB, 15% ABB, 10% PBB and 15% PBB treatments respectively. For CHUB, much greater spikes in population of 2000%, 1800%, 988.89% and 900.0% were obtained for 10% ABB, 15% ABB, 10% PBB and 15% PBB amended treatments respectively. Mean peak microbial counts obtained for total cultivable heterotrophic bacteria (TCHB) were 6.318 logCFU/ ml and 6.350 logCFU/ ml for 10 % and 15 % PBB respectively while greater values of 6.435 logCFU/ ml and 6.471 logCFU/ ml were obtained for 10 % and 15 % ABB respectively.
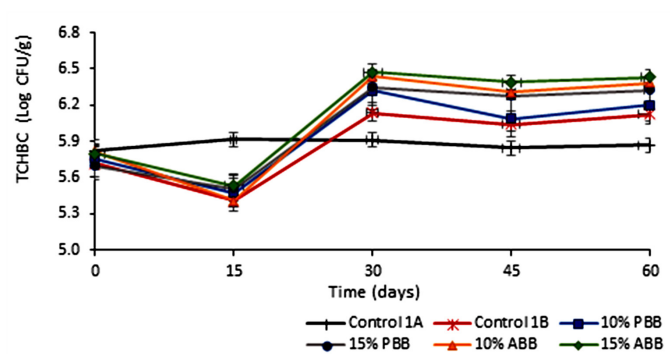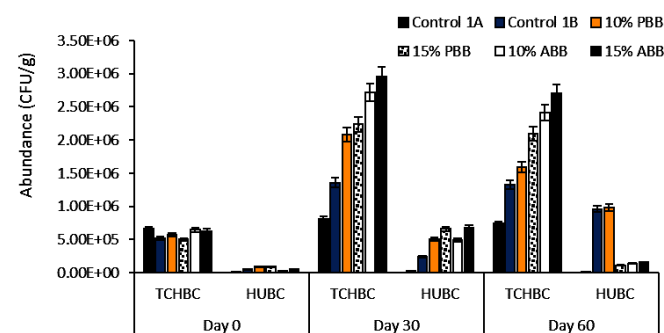

(i)

(ii)

Fig. 2. Biostimulation efficiency of the biochar amendments on total cultivable heterotrophic bacteria (i) and cultivable hydrocarbon utilising bacteria (ii) in crude oil contaminated soil.
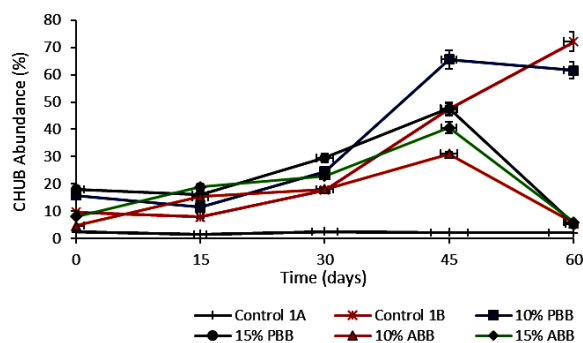Bars represent standard error.

Fig. 3. Abundance of cultivable heterotrophic bacteria and hydrocarbon utilisers in biochar-amended crude oil contaminated soil samples. Bars represent standard deviation from the mean.

The variations in the percentage abundance of the CHUB relative to the total cultivable heterotrophic bacteria in the soil systems are shown in Figure 4. Predictably, the abundance of hydrocarbon utilising bacteria in the soil increased to a peak on day 45 following the pollutant spike and subsequently declined as the study progressed. The only exception was seen in the oiled control (which had no treatment applied); here, the CHUB continued to rise steadily achieving levels of around 72% of soil TCHB compared to less than 2% seen in the unpolluted control. Elevated levels of CHUB in hydrocarbon polluted environmental matrices are anticipated; these numbers typically decline over time as contaminant levels fall as observed in the current study but will scarcely return to the pre-pollution levels [31].

Fig. 4. Variations in percentage abundance of cultivable hydrocarbon utilising bacteria in various biochar-amended crude oil contaminated soil samples. Bars represent standard error.

Biostimulation efficiency in this study is considered a measure of the enhanced abundance of both heterotrophic bacteria and the hydrocarbon utilising bacteria alongside the residual TPH content at the end of the study. The 15% ABB amendment produced the strongest biostimulation results for both the cultivable heterotrophic bacteria and the cultivable hydrocarbon utilisers. All the biochar amended soils showed better stimulation of heterotrophic bacteria and cultivable hydrocarbon utilising bacteria than the unamended controls. Generally, the PBB provided better TPH removal whereas ABB better stimulated the populations of the tested categories of soil bacteria without commensurate TPH removal, when compared to PBB. The physicochemical properties of biochar may account for its biostimulatory effect.

Biochar has been known to provide nutrients like potassium and phosphorus which are essential for microbial growth [22]. The abundance of the cultivable bacteria and hydrocarbon utilising bacteria in the present study exhibited a gradual increase with counts peaking around day 30 for TCHB and day 45 for CHUB; this was followed by a steady decline in microbial volume. This decline coincided with the drop in TPH levels in the soil. The simulated petroleum spike resulted in rapid proliferation of CHUB; however, as the pollutant levels declined, these numbers dropped. The percentage content of CHUB relative to the TCHB in the soil, interestingly, returned to pre-pollutant levels by day 60.

Following introduction of the crude oil, a distinct acclimatisation period was observed. During this period, there was a drop in soil microbial abundance for both TCHB and CHUB. This initial drop in microbial abundance could be indicative of the cytotoxicity of crude oil on some bacteria. Exposure of such groups to crude oil will generally inhibit bacterial growth and metabolic activity often resulting in cell lysis [28]. The response by the petroleum sensitive groups is

often accompanied by a period of adaptation by more resilient select groups with the capacity to utilise petroleum as a carbon source. The bacteria adjust to the unfamiliar stimulus by synthesising relevant catabolic enzymes triggered by the activation of relevant genes. The post-acclimatisation period is, therefore, characterised by increased numbers of bacteria with the genetic disposition to degrade petroleum hydrocarbons as observed in the current study. This rapid proliferation also corroborates several related studies [29–31]. The period of acclimatisation has been linked to the biodegradation efficiency of the microorganisms and the microbial growth cycle is deemed to be indicative of the biodegradation rate [32,33]. After the acclimatisation period, microbial abundance and, by implication, biodegradation rates; increased quite rapidly, peaked and then slowly declined in response to declining TPH levels in the soil. The purported relationship between the decrease in microbial abundance and the reduction in TPH concentration is evidenced in the absence of a similar drop in the oiled control (Control 1B) where TPH levels remain relatively the same throughout the study so that a progressive rise in the abundance of CHUB in seen up till day 60.

Similar to the observations in the current study, several other studies have reported an increase in microbial abundance in soils amended with biochar [34 – 36]. Likewise, comparable to the current study, Zhang et al. [2] in their bioremediation study using biochar reported an initial boost in the counts and diversity of TPHs-degrading bacteria followed by a decline after 40 days. Ameloot et al. [37] reported a 29% increase in microbial biomass in soil amended with biochar derived from willow wood. The feedstock for the biochar has been highlighted as an important factor in the impact of biochar on soil microbial abundance and diversity [22,27]. A study by [38] using corn cob-derived biochar, similar to the current study albeit much lower, observed a 12% – 37% increase in bacterial diversity after a 96-day period. The impact of biochar amendments on the soil bacterial community is likely due to its impact on soil nutrient ratios.

Effect on Soil Microbial Community

The diversity and occurrence of the total cultivable heterotrophic bacteria and the hydrocarbon utilising bacterial isolates obtained before (day 0), during (day 30) and at the end of the 60-day study are shown in Figure 5 while phyla and classes of the isolates are outlined in Figure 6. For the TCHB across the two types of biochar, there seemed to be a shift in abundance from Gram positive bacteria to Gram negative bacteria and a distinct reduction in diversity as the study proceeded. The represented phyla shifted from a combination of Firmicutes, Actinobacteria and Proteobacteria phyla at the onset of the study (day 0) to Firmicutes and Proteobacteria during the study to mostly Proteobacteria by the end of the study (day 60). The dominant cultivable heterotrophic bacterial isolates were *Bacillus* spp., *Pseudomonas* spp. and *Staphylococcus aureus* for ABB and *Pseudomonas* spp., *Klebsiella pneumoniae* and *Bacillus* spp. for PBB. Amongst the cultivable hydrocarbon utilising bacteria obtained, *Klebsiella pneumoniae*, *Pseudomonas* spp. and *Enterobacter* spp. dominated.

The conclusions of a study on the impact of biochar on the microbial community structure in a Fir plantation in China are comparable with the present study. The authors found that application of biochar to soil resulted in marked shifts in microbial community composition [39]. A number of studies have shown that only a small percentage of bacteria are able to utilise hydrocarbon compounds as their sole carbon source [40] and that petroleum contamination will, therefore, always result in
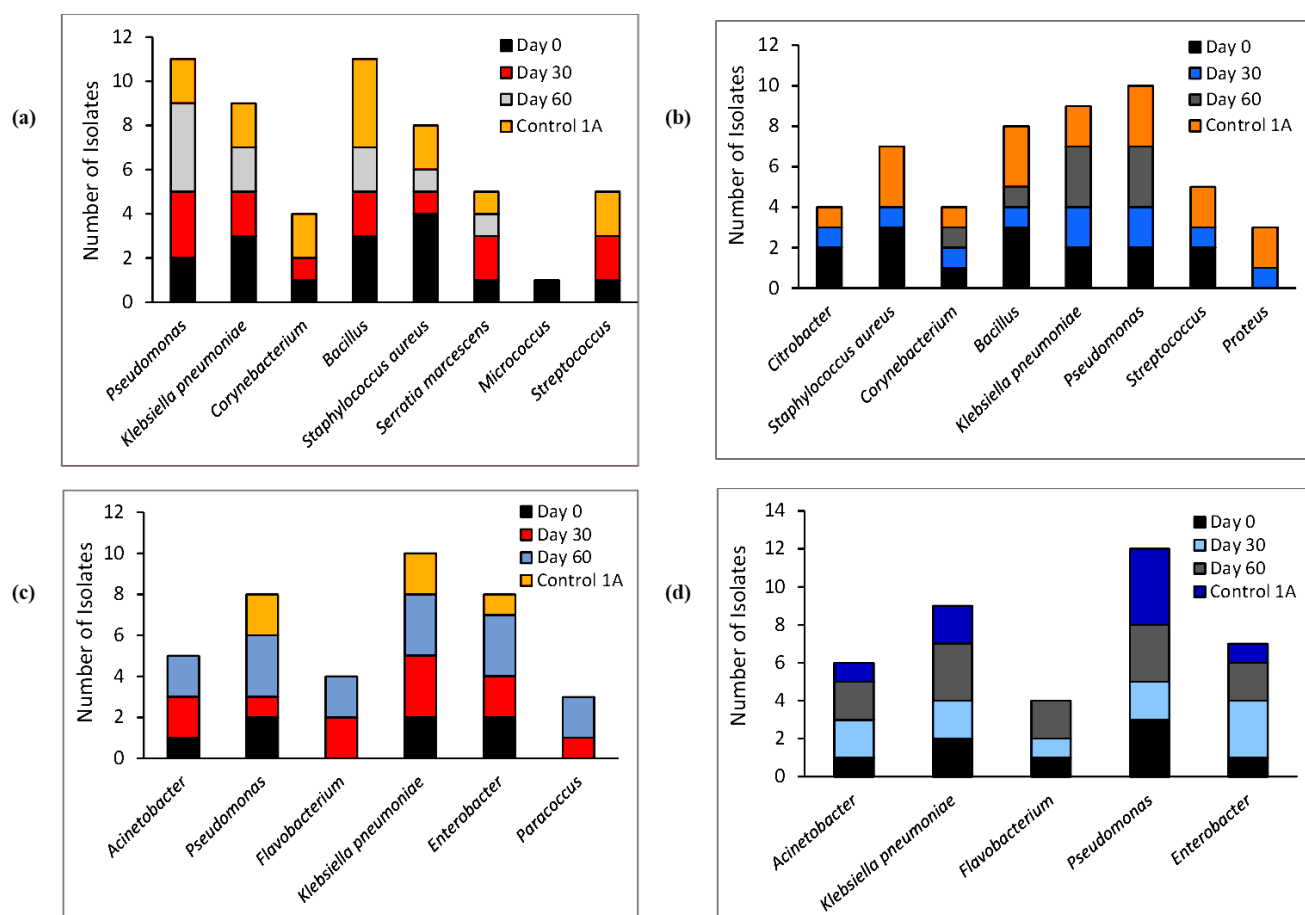
Fig. 5. Diversity and occurrence of cultivable heterotrophic bacteria in the biochar-amended soils during the study (a) and (b) show TCHB for animal- and plant-based biochar respectively; (c) and (d) show results for cultivable hydrocarbon utilising bacteria in ABB and PBB, respectively.
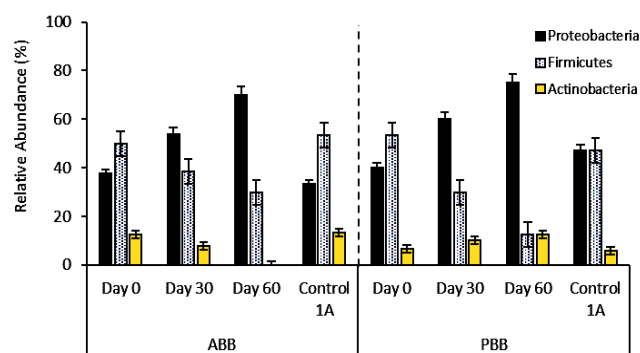
Fig. 6. Distribution of cultivable heterotrophic bacteria at phylum level. Bars represent standard deviation from the mean.

The dominance of Gram negative bacteria among the cultivable hydrocarbon utilising groups corresponds with the findings of Shahi et al. [42] and may be attributed to their cell membrane structure compared with their Gram positive counterparts. The reports of several researchers buttress the dominance of members of Proteobacteria following a spike in soil petroleum concentration [43–45]. They further confirm the presence of the phylum Firmicutes in addition to Proteobacteria during remediation of petroleum compromised soils. At the genus level, the occurrence of certain groups was impacted by the presence of the hydrocarbon pollutant and the addition of biochar. The presence of nine cultivable genera (amongst the TCHB) at the beginning of the current study gave way to eight genera as the study proceeded and then only five genera by day 60 of the study. The emergence of *Bacillus* species as the most abundant genus may be attributed to its spore-forming character which allows it to survive the presence of environmental stressors. Moreover, as in the present study, *Pseudomonas* has been frequently highlighted for its role in hydrocarbon degradation [29,40,46]. Solomon *et al.* [47], likewise, highlighted the dominant role of *Pseudomonas* spp. during bioremediation of petroleum hydrocarbons in the presence of plant feedstock-derived biostimulants. No clear trend was observed in the abundance and diversity of soil bacteria with regards to feedstock type.

Variations in Soil pH and TPH Removal Efficiency during the Bioremediation Study

The pH levels in the soil reached on day 60 of the study were within 6.8 – 6.9 for all the four treatments while the unpolluted control and the oiled control had pH levels of 5.8 and 6.1 respectively on day 60. The levels obtained for the treatments represent increases of 0.6 – 0.7 from day 0 of the study. In the controls, the observed variations in pH during remediation were in the range of 0.1 – 0.2. The pH levels in the soil from the start day only varied slightly until Day 60, similar to the reports of Ducey et al. [27] who monitored the impact of biochar applied to coastal plain soil on the indigenous microbial community. The observed changes in pH during the course of the current study could be attributed to the by-products of the enzymatic degradation of the hydrocarbons. As metabolites are released into the system, the pH will vary.

The initial organic acids characteristic of hydrocarbon biodegradation may have been further broken down by soil microorganisms resulting in the production of more alkaline secondary metabolites. Furthermore, the biochar samples were ash and calcium carbonate–rich which define the liming factor; this liming factor likely provided a buffer effect against the acidity of the organic acids produced during the biodegradation of the hydrocarbon pollutants. The CEC property of the biochar further supports this effect. TPH removal from soil at the end of the study was most efficient with the 15% w/w plant-based biochar treatment as depicted in Figure 7. The least removal efficiency occurred in ABB-amended soils. Mean TPH levels at the end of the 60-day study ranged from about 7000 mg/kg – 7800 mg/kg for the PBB and approximately 11000 mg/kg – 14000 mg/kg for ABB.
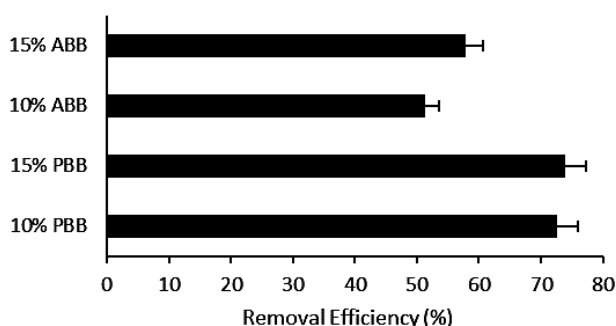

Fig. 7. TPH removal efficiency in biochar-amended crude oil contaminated soil samples. Bars represent standard deviation from the mean.

The application of 15% PBB resulted in the greatest TPH removal from the artificially contaminated soil systems. The micropore characteristics and structure of the two types of biochar are considered the most probable reasons for the observed performance. Tomczyk et al. [22] maintain that the efficiency of pollutant removal by biochar is a function of both its CEC and its specific surface area. The CEC allows for bonding between the biochar and pollutant molecules. Guo et al. [6] state that the pore sizes of biochar make it a more effective immobilisation agent for microorganisms than other materials. This could account for the better removal efficiency of the plant derived biochar as they have been shown to have a higher abundance of micropores and, thus, more specific surface area along with more uniform pore size distribution [48]. The enhanced surface area and micropore distribution in the PBB

highlighted by [48] translate to greater pollutant adsorption potential. Biochar forms a p–p electronic bond with the pollutant strengthening the stability of the adsorption effect and making it somewhat irreversible [49,50]. This, in turn, increases the availability of the hydrocarbon pollutant to microorganisms for degradation. The rough surface of the biochar pores not only supports adsorption of the pollutant molecules but could provide surface attachment (and by extension a facilitated access to nutrients and the pollutant) for microbial biofilms as well. In this study, the advantage of possible increased micropores on PBB for adsorption and colonization proved to supersede the enhanced biostimulatory effects on microbes observed with the ABB with regards to removal of TPH from the polluted soil.

Statistical Relationships

There were significant differences in TCHB and CHUB abundance and TPH removal efficiency between PBB and ABB amendments. The observed counts in ABB and PBB amendments differed significantly from the Control set-ups at 95% confidence interval. Bacterial abundance and TPH removal, however, did not differ significantly between the two amendment levels of 10% w/w and 15% w/w for both ABB and PBB ($p<0.05$).

CONCLUSION

The study established that application of biochar effectively manages petroleum pollutants in soil. Even though the animal derived biochar proved to be a better bacterial growth stimulant achieving increases of up to about 370% in total cultivable heterotrophic bacteria and 2000% in cultivable hydrocarbon utilising bacteria; the plant derived biochar was more effective in the removal of the hydrocarbon pollutant from the soil. Observed mean TPH levels at the end of the 60-day study ranged from about 7000 mg/kg – 7800 mg/kg for the PBB and roughly 11000 mg/kg – 14000 mg/kg for ABB representing removal levels of approximately 51.0%, 57.7%, 72.4% and 73.7% in 10% ABB, 15% ABB, 10% PBB and 15% PBB amended soils respectively. The cultivable bacterial diversity shifted from the combination of Firmicutes, Actinobacteria and Proteobacteria phyla at the onset of the study to mostly Proteobacteria by the end of the study and a distinct reduction in diversity with increasing contact time. Members of the Proteobacteria phyla dominated amongst the cultivable hydrocarbon utilising bacteria as well. The dominant heterotrophic bacterial isolates were *Bacillus* spp., *Pseudomonas* spp. and *Staphylococcus aureus* for ABB and *Pseudomonas* spp., *Klebsiella pneumoniae* and *Bacillus* spp. for PBB. Amongst the cultivable hydrocarbon utilising bacteria obtained, *Klebsiella pneumoniae*, *Pseudomonas* spp. and *Enterobacter* spp. dominated. Considering the already well-known advantages of biochar in soil, its incipient role in hydrocarbon pollution management is encouraging.

DECLARATION

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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