ENHANCEMENT OF MICROBIAL DEGRADATION OF DIESEL OIL BY BIOCHAR IN AN OXISOL

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Abstract
The remediation of oil-contaminated soils is a major challenge in oxisols (soils with low pH) because degradation process is very slow. Application of biochar to soils is currently gaining global interest due to its multiple benefits, such as liming effect. The present study was carried out to investigate the effectiveness of two biochar types in enhancing microbial degradation of diesel oil in an acid soil, Ankasa series (Plinthic acrudox). The acid soil was contaminated with diesel oil at 100 mL/kg soil. The biochar types, rice straw (RB) and saw dust (SB), were applied to the contaminated soils at 0, 65, 130, 195 and 260 Mg/ha. The treated soils were incubated in the laboratory under room temperature and sampled for analysis of changes in hydrocarbon utilizing bacteria (HUB) population and soil pH, and the amount of oil degraded. In another experiment, the soil was contaminated with diesel oil at 100 mL/kg soil, amended with RB at 195 Mg/ha and fertilized with Nitrogen (N) and/or Phosphorus (P) in the form of ammonium nitrate and single super phosphate, respectively at 60 kg/ha and incubated for 40 days. Cowpea was then grown after the incubation period to test for efficiency of the remediation. The cowpea plants were harvested 6 weeks after planting for the determination of nodule number, shoot and root dry weights. In the first experiment, results showed that all the biochar treatments significantly ($p < 0.05$) increased the amount of diesel oil degraded, HUB populations and soil pH. Fertilizing RB (195 Mg/ha) treated soils with N and/or P significantly ($p < 0.05$) increased the amount of diesel oil degraded and HUB population. N and/or P fertilization also enhanced shoots and roots dry weights of cowpea. Application of biochar alone, biochar plus P, and P alone treatments enhanced nodulation, however, inhibitory effect was observed in nodule formation when N was applied. The enhanced oil degradation was attributed to the liming effect of biochar as a result of increased pH, improved soil microbiology and other chemical properties. The study suggests that biochar could be used to remediate oil contaminated oxisol, however, effective remediation for crop production could be done by applying it with plant nutrients such as N and P.

Keywords
Biochar, degradation, nitrogen, oil, phosphorus

Introduction

The application of liming materials to oxisols to create favourable conditions for plant growth has a long history. Different soil conditioners had been used to serve as liming agents and their liming requirements, composition and benefits had been investigated intensively by researchers. Recently, biochar produced by the conversion of biomass in a low or no oxygen environment at high temperatures has received much attention because of its multiple benefits. According to Downie et al. (2009), biochar applications have been shown to increase soil pH, improve nutrient storage, effective cation exchange capacity (ECEC), increase soil carbon content and water holding capacity, decrease aluminum toxicity and tensile strength, change microbiology of the soil, decrease greenhouse gases (N_2O and CH_4) emissions from the soil, improve soil conditions for earthworm populations and improve fertilizer use efficiency. Most literature have documented that, the greatest positive effects of biochar are seen on acid soils where pH values are raised. Literature have also shown that biochar can stimulate soil microbial activities (Lehmann et al., 2011).

Many published articles have documented the potentials of native microorganisms to degrade oil both in the laboratory (Lawson et al., 2012) and in field trials (Bragg et al., 1994), however, these potentials are to a large extent curtailed in unfavourable soil conditions. For example, oxisols which are acidic in nature create such unfavourable conditions for microbial degradation that, there is about near zero degradation in the soil (Walworth et al., 2005). In the Western Region of Ghana, where most of the oil and gas production activities take place, the soils are classified as oxisols with low pH because of the leaching of basic cations as a result of high rainfall. These soils are not suitable for the cultivation of some crops, especially grain legumes because nodule initiation and formation, nitrogen fixation and growth are adversely affected due to low pH and very low available phosphorus (Attar et al., 2012).

The present study seeks to rely on the numerous benefits of biochar to acid soils, to create a favourable condition for the microorganisms involved in crude oil degradation as well as provide favourable soil condition for crops like grain legumes that are sensitive to low pH and oil contamination to grow. Although the use of biochar to enhance crude oil degradation is not a new development, specific attention of its use in oil contaminated soils with low pH has received little attention. It is against this background that the present study is aimed at investigating the effectiveness of biochar, as a liming agent, in enhancing microbial degradation of oil in an acid soil as well as creating favourable condition for plant growth.

Materials and Methods

Soil and biochar types

The Oxisol used in this study is the Ankasa series (Plinthic Acrudox). It was sampled (0–20 cm depth) from the Ankasa Wildlife Protected Area (05°12.922'N and 02°39.031'W) in the Western Region of Ghana, which has no oil contamination history. The mean annual temperature of the area is about 25°C and mean annual rainfall in the range of 1500 to 2000 mm. The sampled soil was passed through 2 mm sieve and characterized. Some of the properties of the soil are shown in Table1.

Table 1. Some physical and chemical properties of Ankasa series

Soil properties	Values
Clay (%)	6.0
Silt (%)	21.1
Sand (%)	72.9
Soil Texture	Sandy loam
Bulk density (Mg/m^3)	1.3
pH (H_2O)	4.7
Electrical conductivity (dS/m)	0.42
OC (%)	1.09
Na (cmol/kg)	0.06
K (cmol/kg)	0.09
Mg (cmol/kg)	0.43
Ca (cmol/kg)	0.70
TEB (cmol/kg)	1.28
Base Saturation (%)	45.23
Exchangeable Acidity (cmol/kg)	1.55
ECEC (cmol/kg)	2.43
Total N (%)	0.12
Available P (mg/kg)	2.00

TEB = Total exchangeable bases, ECEC = Effective cation exchange capacity

Rice straw and saw dust were collected from the Soil and Irrigation Research Centre of the University of Ghana at Kpong and a Carpentry shop in Kumasi, respectively. The feedstocks were charred at 500°C pyrolysis temperature at Soil Research Institute of Council for Scientific and Industrial Research Institutes at Kwadaso. The prepared biochar types, rice straw (RB) and saw dust (SB) biochar were passed through 2 m sieve and characterized. The RB had pH (1:5) 10.5, ash content 4%, carbon 49%, nitrogen 0.16%, phosphorus 0.26%, potassium 0.97%, calcium 0.74%, magnesium 0.44% and sodium 0.18%. The X-ray diffraction analysis showed that RB contained large quantities of silica (SiO_2), calcite ($CaCO_3$), calcium differate oxide ($CaFe_2O_4$), potassium chloride (KCl), siloxene (H_2OSi_2) and periclase (MgO) as shown in Fig. 1.

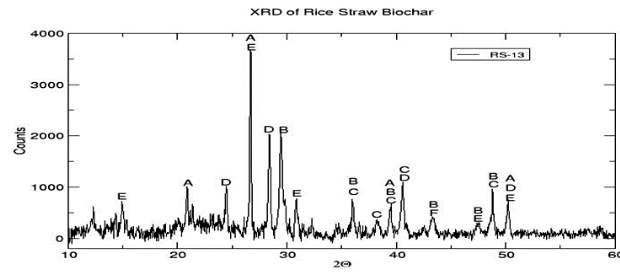

Figure 1. X-Ray diffractogram of rice straw biochar (RB) *A= Silica (SiO_2), B= Calcite ($CaCO_3$), C= Calcium differate oxide ($CaFe_2O_4$), D= Potassium chloride (KCl), E= Siloxene (H_2OSi_2), F= Periclase (MgO), G= Magnesium oxide (MgO), L= Iron oxide (FeO), M= Graphite, N= Magnesium silicate (Mg_2SiO_4) O= Iron magnesium oxide ($FeMgO_4$).*

The SB had pH (1:5) 7.5, ash content 2%, carbon 48%, nitrogen 0.2%, phosphorus 0.03%, potassium 0.21%, calcium 0.67%, magnesium 0.15% and sodium 0.14%. SB also contained large quantities of silica (SiO_2), calcite ($CaCO_3$), Iron oxide (FeO) and graphite, as well as minute quantities of magnesium oxide (MgO), magnesium silicate (Mg_2SiO_4) and iron magnesium oxide ($FeMgO_4$) as shown in Fig. 2.

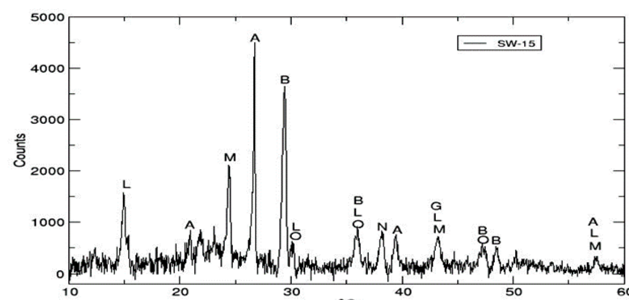

Figure 2. X-Ray diffractogram of saw dust biochar (SB)

Diesel oil degradation

Two (2) kg of the sieved soil was weighed into an experimental pot and contaminated with diesel oil at 100 mL oil/kg of soil. The contaminated soils were then amended with rice straw (RB) and saw dust (SB) biochar types at 0, 65, 130, 195 and 260 Mg/ha as documented by (Chintala et al., 2014). The treatments were maintained under same moisture content (15%; wt:wt basis) and replicated four times. The set up was incubated in the laboratory at ambient temperature (mean temperature of 29°C) and samples were taken at an interval of 10 days for the determination of hydrocarbon utilizing bacteria, quantity of diesel oil degraded and changes in some chemical properties. In a second experiment, two (2) kg of the sieved soil was contaminated with diesel oil at 100 mL oil/kg of soil. The contaminated soils were amended with Nitrogen (N) alone, Phosphorus (P) alone, N + P, RB alone, RB + N, RB + P, RB + N + P. Ammonium nitrate was used as source of nitrogen (N) at 60 kg/ha, single superphosphate as source of phosphorous (P) at 60 kg P2O5/ha and RB applied at 195 Mg/ha. The treatments were also incubated at ambient temperature (mean temperature of 29°C) and samples were taken at an interval of 10 days for the determination of hydrocarbon utilizing bacteria and quantity of diesel oil degraded.

The hydrocarbon utilizing bacteria count was estimated using the mineral salts agar medium of Mills et al. (1978) and modified vapour phase transfer technique of O'hara et al. (1988). The modified vapour transfer technique involves spreading 0.5 mL of diesel (serving as carbon source) onto the mineral salts agar medium after setting and allowed the diesel oil to diffuse into the agar medium for about 1 hr before incubated at room temperature for 5 to 7 days. The number of colonies formed was used to estimate the hydrocarbon utilizing bacterial population. Residual diesel oil in the contaminated soils was extracted using a modified method of Abu and Ogiji (1996) and the amount of oil degraded determined. Quantitative determination of diesel oil extracts was employed as described by Udeme and Antai (1988).

Plant culture

Cowpea (*Vigna unguiculata*) seeds were sterilized in 70% ethanol and sown into the treated soils of the second experiment described above 40 days after incubation. The seeds were sown at 5 seeds per pot and thinned to 2 seedlings 5 days after sowing. The plants were harvested 6 weeks after sowing to determine nodule number, shoot and root dry weights. The shoots and roots were oven dried at 70°C for 24 hours.

Data analyses

The data collected were subjected to analysis of variance (ANOVA) and the LSD at 5% was calculated to compare treatment means.

Results and Discussions

Changes in some chemical properties

Both biochar types significantly ($p<0.05$) increased pH, organic carbon (OC), available P, total exchangeable bases (TEB), base saturation (BS) and effective cation exchange capacity (ECEC) of the treated soils 40 days after incubation (DAI) as shown in Table 2.

The increase in these properties was higher in the RB treatments than the SB treatments. The increase in pH could be attributed to the liming effect of these biochar types because they contained large quantities of carbonates and oxides. This result is in conformity with works done by Lehmann and Rondon (2006), who suggested that biochar can serve as a liming agent, resulting in increased pH for a number of different soil types. The very high carbonates and oxides present in RB could attribute to its ability to raise pH more than SB and this was also suggested by Wang et al. (2009). The increased organic carbon came from the biochar as documented by Downie et al. (2009). The increase in available P by these biochar types could be due to the increased soil pH. The high amounts of basic cations in the biochar and the associate effects of increased pH could be the reason for the increased TEB and BS. The increased ECEC by these biochar types could be attributed to the explanation documented by Liang et al. (2006) who stated that biochar has great ability to adsorb and retain cations in the exchangeable form due to its greater surface area, and negative surface charge. The increase in ECEC could also be due to the increased negative charges arising from the increased exposed carboxyl groups of the biochar with time as documented by Novak et al. (2009).

Results also showed that the two biochar types generally decreased total nitrogen and exchangeable acidity of the soils. For total nitrogen, significant ($p<0.05$) differences were observed between 130 and 260 Mg/ha for RB and between 195 and 260 Mg/ha for SB. The decrease in nitrogen could be due to immobilization of nitrogen by the microorganisms as result of increase in carbon from the biochar amendment raising the C:N ratio. Exchangeable acidity significantly ($p<0.05$) decreased with increase in biochar application rate. The decrease in exchangeable acidity could be attributed to the alkaline nature of the biochar used. Novak et al. (2009) documented that when Ca, Mg, and K oxides and carbonates in biochar are released into the soil environment, they react with the H^+ and monomeric Al species, raise the soil pH, and thereby decreasing exchangeable acidity.

Hydrocarbon utilizing bacteria population

The growth of hydrocarbon utilizing bacteria (HUB) in the contaminated soils amended with biochar in the first experiment is presented in Fig. 3a. The initial HUB population in the soil was 1.6 x 10^5 cfu/g but it increased sharply 10 DAI in all treatments. The peak HUB populations were observed at 20 DAI for all biochar treatments. RB_{200} had the highest peak value (1.42×10^7 cfu/g) but not significantly ($p>0.05$) different from RB_{150} (1.38×10^7 cfu/g). These two peak values were significantly ($p<0.05$) higher than the corresponding peak values of SB applications (SB_{200}, 8.5×10^6 cfu/g and SB_{150}, 8.0×10^6 cfu/g).

There was decline in HUB populations after 20 DAI for all biochar treatments. The control treatment on the other hand continued increasing but at a lower value as compared to the biochar treatments. At the end of the experiment (40 DAI), the HUB populations of the biochar treated soils were significantly ($p<0.05$) higher than the control value. In the second experiment, the HUB population also increased sharply 10 DAI (Fig. 3b) for all amended treatments and declined after peaking. Similar growth pattern exhibited by the HUB in the soils was observed by Lawson et al. (2012).

At 40 DAI, biochar + N + P, biochar + N, and biochar + P treatments contained HUB populations that were significantly ($p<0.05$) higher than the other treatments. The highest HUB population was observed in biochar + N + P treatment. The amendment of the contaminated soil with biochar alone had significant ($p<0.05$) number of HUB more than N alone, P alone and N+P treatments.

Table 2. Some chemical properties of the treated soils 40 days after incubation

Treatment/ Property	pH	OC %	TN %	Av. P mg/kg	TEB cmol/kg	BS %	ECEC cmol/kg	Exc. Acidity cmol/kg
Control	4.80	1.56	0.11	1.99	1.11	42.21	2.63	1.52
RB_{65}	6.09	3.12	0.09	2.11	3.13	98.12	3.19	0.07
RB_{130}	6.49	3.89	0.08	2.12	4.05	98.54	4.11	0.06
RB_{195}	6.88	4.36	0.08	2.15	5.17	99.04	5.22	0.05
RB_{260}	7.19	4.50	0.07	2.16	5.67	99.13	5.72	0.05
SB_{65}	5.12	3.04	0.10	2.03	2.78	97.54	2.85	0.09
SB_{130}	5.55	3.26	0.10	2.03	3.29	98.21	3.35	0.08
SB_{195}	5.90	3.68	0.09	2.05	4.20	98.59	4.26	0.08
SB_{260}	6.19	3.87	0.08	2.05	4.64	98.72	4.70	0.07
Lsd (5%)	0.38	0.16	0.02	0.06	0.20	0.10	0.02	0.03

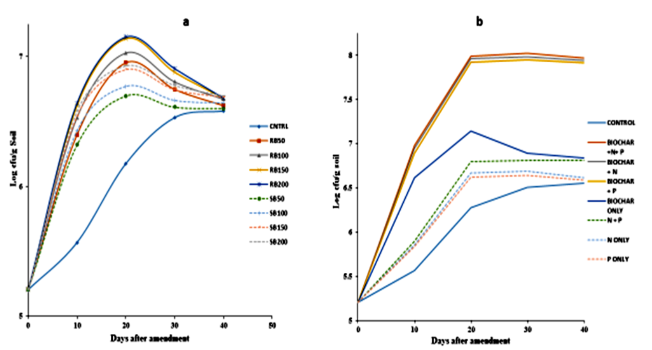

Figure 3. A graph of HUB population of the contaminated soils amended with biochar, N and P

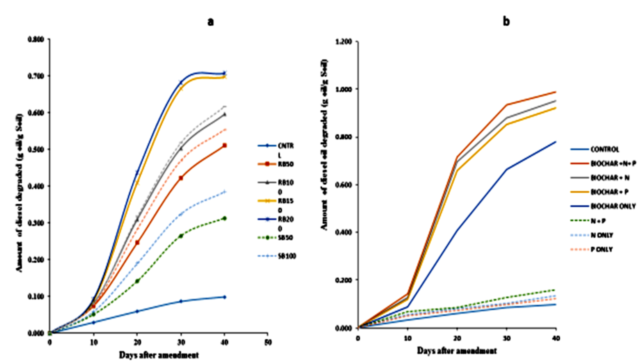

Figure 4. Amount of diesel oil degraded in the contaminated soils amended with biochar, N and P.

Amount of oil degraded

In the first experiment, there was significant ($p<0.05$) increase in cumulative diesel oil degraded when the two biochar types were added (Fig. 4a). From 0 to 10 DAI, there was a gradual increase in the amount of oil degraded for all the biochar treatments but between 10 and 30 DAI the increment was sharp.

Between 30 and 40 DAI the amount of oil degraded for RB_{150} and RB_{200} plateaued but the other biochar treatments continued increasing. At 40 DAI, the highest amount of oil degraded was recorded in RB_{200} (0.708g oil/kg soil), followed by RB_{150} (0.698g oil/kg soil) but there was no significant ($p>0.05$) difference between these two treatments. Similar degradation pattern exhibited in the soils was observed by Lawson et al. (2012).

In experiment 2, there was significant ($p<0.05$) increase in cumulative diesel oil degraded as a result of combining biochar with N and/or P (Fig. 4b). From 0 to 10 DAI, there was a gradual increase in the amount of oil degraded for all the treatments. A sharp increase in the amount of oil degraded was observed between 10 and 30 DAI for all biochar amended treatments but a steady increase for treatments without biochar. At the end of the experiment, combining biochar with N and/or P significantly ($p<0.05$) increased the amount of oil degraded when compared to the control. The highest amount of oil degraded was observed in biochar + N + P (0.989g oil/kg soil) followed by biochar + N (0.951g oil/kg soil) but the difference between these treatments was not significant ($p>0.05$). Calculations indicate that the amount of oil remained in the non-biochar treatments was in the range of 89 to 93% while that of biochar alone, biochar + P, biochar + N and biochar + N + P treatments had low amounts of 36.8, 25.1, 22.2 and 20.2%, respectively, 40 days after incubation.

Growth and nodulation of cowpea

The germination response of the test crop to biochar with or without N and P is shown in Fig. 5. Compared to the control, all treatments containing biochar significantly ($p<0.05$) enhanced germination of cowpea.

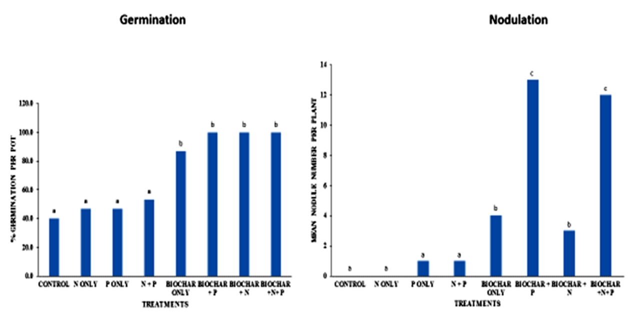

Figure 5. Germination and nodulation in the contaminated soils amended with biochar, N and P. Treatments with same alphabet(s) on top of bar are not significantly different

Amending the soils with N or P alone increased percent germination from 40 to 46.7%. Application of biochar alone resulted in 86.7% germination of cowpea, however, there was 100% when biochar was combined with N and/or P. The low percent germination in the control and soils amended with N or P alone might be due to the presence of high amount of oil in these soils before planting the cowpea. Similar results were reported by Gallegos Martínez et al. (2000) who found a reduction in germination between 30–90% in the tropical native Mexican species subjected to soil contamination with crude oil. According to Ogbo et al. (2009) small hydrocarbon molecules of oil that penetrate plants can be phytotoxic, explaining the decrease in seed germination.

Results showed that no nodule was formed in the control and soils amended with N alone (Fig. 5). The addition of P alone and combining P with N enhanced nodulation in cowpea, but the differences were not significant ($p>0.05$) from the control. However, the application of biochar with or without N and/or P significantly ($p<0.05$) increased nodule number, with the highest number of nodules formed on the biochar + P treatment. Inhibitory effect of N was observed when biochar, and biochar + P were combined with N. The poor nodulation in the control and N alone treatments could be attributed to high amounts of oil and low soil pH of these soils. This finding agrees with a study by John et al. (2011) which revealed that symbiotic nitrogen fixing bacteria associated with legume are very sensitive to crude oil. Addition of biochar and P enhanced nodulation. Apart from raising of pH by the two biochar types, they also increased available P of the soils. It is documented that phosphorus addition enhances the growth and survival of rhizobia (O'hara et al., 1988), nodule formation (Drevon and Hartwig, 1997) and nodule functioning (Tang et al., 2001). The inhibitory effect of nitrogen on nodulation has been reported by many researchers. For example, Ohyama et al. (2011) observed inhibitory effect of nitrogen on nodulation of soybean.

The shoot and root dry weights of the cowpea grown in the various soils are shown in Fig. 6. The dry weights of cowpea plants grown in soils amended with N and P alone were not significantly ($p>0.05$) higher than that of the control treatment. This shows that the high content of oil in these treatments might have inhibited the growth of cowpea. The toxicity of petroleum hydrocarbons has been linked to displacement of nutrients and nutrient uptake (Amadi et al., 1993) and reduction in available phosphorus and total nitrogen (Benka-Coker and Ekundayo, 1995). Significant ($p<0.05$) increases in dry matter were observed when the soils were amended with biochar, biochar plus N and/or P. The increase in dry matter might be attributed to presence of low oil concentration due to increased oil degradation in these treatments explained earlier. Agbogidi et al. (2007) also found increase in dry matter yield of crops at lower concentrations of oil.

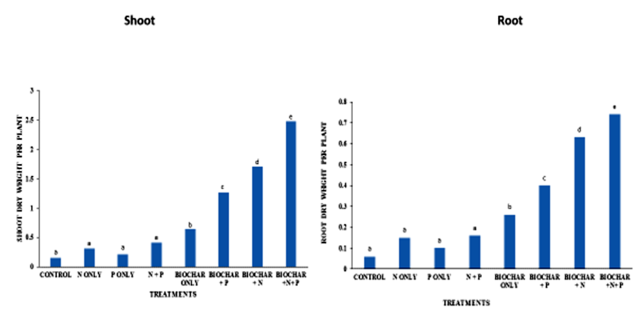

Figure 6. Shoot and root development in the contaminated soil amended with biochar, N and P. Treatments with same alphabet(s) on top of bar are not significantly different

Conclusion

The two biochar types used in the present study were effective in enhancing microbial degradation of diesel oil in the acid soil. Combining biochar with N and/or P was effective in oil degradation and it removed toxicity of the oil to germination. Application of biochar, biochar + P enhanced nodulation in cowpea, however, addition of N was inhibitory.

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