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Biochar Application in Crude Oil Impacted Soil Stimulates the Growth of Autotrophic Nitrifiers

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Abbreviations: AOB – Ammonium Oxidizing Bacteria, NOB – Nitrite Oxidizing Bacteria, Pb – Lead, As -Arsenic and Cd– Cadmium, SPSS - Statistical Package for Social Sciences

ABSTRACT

The effect of biochar on the growth of autotrophic nitrifiers in crude oil contaminated soil was investigated at different levels of crude oil contamination and with different concentrations of biochar in the laboratory. Three levels of crude oil (5 %, 10 % and 15 %) were considered at five (5) concentrations of biochar (2 %, 4 %, 6 %, 8 % and 10 %). The samples were incubated for thirty five (35) days and subsamples drawn at seven days interval to evaluate the population of culturable Ammonium Oxidising Bacteria (AOB) and culturable Nitrite Oxidising Bacteria (NOB) as well as changes in soil pH. Soil samples contaminated with 15 % crude oil exhibited the least cell count of AOB (2.07×10^5cfu/g) and NOB (2.60×10^5cfu/g) in the absence of biochar. Addition of 10 % biochar to the soil samples contaminated with 15 % crude oil significantly ($P < 0.05$) increased the growth of the organisms. Addition of biochar to the soil samples also increased the soil pH in a manner proportional to the concentrations of biochar and crude oil contamination level. When 10 % of biochar was added to soil contaminated with 15 % of crude oil, the soil pH increased from 4.37 to 6.39. However, when 10 % of biochar was added to the control (no crude oil) and 5 % crude oil contaminated soil, the pH increased from 5.69 in the control to 8.51 and 7.66 respectively. There was no significant effect ($P > 0.05$) on pH when 2 % of biochar was added to soil samples contaminated with 15 % of crude oil. Adding 6 % biochar to soil samples with 5 % w/w of crude oil exhibited the highest cell count of both AOB and NOB. In conclusion, crude oil contamination inhibited the growth of AOB, NOB. However, the inhibitory effects were overcome by addition of biochar in concentration dependent manner.

INTRODUCTION

The Niger Delta ecosystem is continuously subjected to man-induced changes and obviously threatened by increasing environmental issues. The aquatic and terrestrial ecosystems of the region face increasing ecological problems from the release of crude oil related pollutants [1]. Crude oil is a naturally occurring, viscous liquid consisting of hydrocarbons of various molecular weights and other liquid organic compounds found in geologic formations beneath the Earth's surface [2]. It is recovered principally through oil drilling. Crude oil is most times called the "Mother of all Commodities" because of it is used in the manufacture of a wide range of materials [3; 4].

Sensitivity of soil nitrifiers to petroleum hydrocarbons depends on the quantity and quality of oil released and previous exposure of indigenous soil microbial population to crude oil and its products [5]. The principal factors influencing microbial community composition after exposure to crude oil include (i) contaminant mixture type(ii) nature of soil (i.e., physical, chemical, and biological characteristics of soils), and(iii) time [6]. The effect of crude oil spillage on soil reduces the oxygen concentration of the soil. This is because crude oil decreases soil porosity. This also may lead to a decrease in nitrifier population and activities since oxygen is vital in the physiology of nitrifying bacteria [1]. Oxygen affects nitrification rates because of its roles as a substrate for the ammonia monooxygenase enzyme and as the terminal electron acceptor from cytochrome c oxidases [7].

Biochar is the dark grey residue consisting of carbon, and any remaining ash, obtained by removing water and other volatile components from animal and vegetation substances. The addition of biochar to crude oil impacted soil have the potential to change soil nitrifier populations [8]. The abundant beneficial properties associated with biochar additions to soil may function alone or in combination with other processes in order to influence nutrient transformations. The effects of biochar range from improving water-holding capacity and porosity, enhancing cation-exchange capacity (in combination with organic material), increasing levels of beneficial bacteria, providing a refuge from predation for mycorrhizal fungi, and enhancing beneficial soil-fauna such as earthworm populations [9; 10]. The liming effect of biochar on acidic soils is achieved because the alkaline substances in biochar are more easily released into the soil compared with its feedstock when biochar samples are incubated with the soil. When biochar with higher pH value was applied to the soil, the amended soil generally became less acidic [11;12]. Biochar has also been found to sorb a variety of heavy metals, including lead (Pb), arsenic (As) and cadmium (Cd) [13]. The aim of this current study was to determine the optimum concentration of biochar needed to stimulate the growth of AOB and NOB in soil impacted with varying concentrations of crude oil.

MATERIALS AND METHODS

Sample Collection

Three samples were used in the study which includes soil, crude oil and Biochar.

Soil Sample Collection and Analysis

A measured amount of five Kilogram (**5Kg**) of subsoil was collected from ten locations in Etche Local Government Area of Rivers State. The soil was collected with a stainless steel soil auger 1.25m in Length and 150 mm diameter. The subsoil was carefully gotten from 5 – 10 cm deep with the soil auger after clearing the surface vegetation and removing the topsoil [14]. The soil samples gotten from the Ten (10) locations were then mixed thoroughly to form a composite sample. The composite sample was passed through a 4 mm sieve to achieve even texture and remove stones [15]. Baseline analysis for physicochemical, heavy metals and microbiological parameters was done. Ammonium Oxidising Bacteria and Nitrite Oxidising Bacteria were analysed using a method adopted in [1].

Crude Oil Sample Collection and Analysis

The crude oil sample classified as Bonny Light Crude was obtained from the Agbada flow station. A facility owned by Shell Petroleum Development Company (SPDC) of Nigeria. The sample was carried in plastic (4 litres) containers and stored in a refrigerator. The crude oil sample used for the experiment had API gravity of 34.5, sulphur content of 0.06 % and pour point of + 85.

Biochar Sample Collection and Analysis

Biochar was produced from an indigenous rain forest shrub *Alcornia cordifolia*in a pyrolysis furnace. The resulting biochar mass was blended to achieve a particle size > 2mm [16]. The biochar had pH of 9.01 (1:10 ratio in distilled water), crude oil adsorption capacity 0.4g/h and cation exchange capacity (CEC) of 32 $cmol_c g^{-1}$.

Preparation of Soil Sample

The soil sample for this study was passed through a 4 mm sieve to remove stones and other objects that might interfere with the actual weight of the soil sample [17] after which 100g of the soil was measured out using a digital weight scale (Camry Emperors scale manufactured by Dial Spring Scale, China). The measured samples were transferred into clean plastic containers and were used for all experiments [18].

Contamination of Soil Sample with Crude Oil

Varying concentrations of crude oil (5 g (5% w/w), 10 g (10% w/w) and 15 g (15% w/w)) was measured with the aid of a 10 mL glass beaker using a digital weight scale (Camry Emperors scale manufactured by Dial Spring Scale, China) and added to 100 g of soil in plastic containers [1]. This was done in triplicates [18]. Nine (9) of the soil samples were left uncontaminated to serve as control experiments. The different concentrations of crude oil were thoroughly mixed with the soil sample in three replicates and clearly labeled [19].

Soil amendment with Biochar

Varying concentrations (2.00 g, 4.00 g, 6.00 g, 8.00 g, 10.00 g) of biochar were measured and transferred to plastic containers containing 100 g of soil already contaminated with 5 %, 10 % and 15 % w/w of crude oil in replicates [20;21]. Two milliliters (2 mL) of a 50 % v/v AOB medium/distilled water was then added to serve as starter fertilizer and to irrigate the sample [22; 23]. The contents of the plastic container were mixed thoroughly to ensure uniformity. The different concentrations were clearly labeled and kept in the same section of the laboratory. Two milliliters (2mL) of distilled water was dropped on the surface of all the samples at twenty-four [24] hour interval to irrigate the samples to irrigate the samples [22]. The experimental set up was allowed for 35 days.

Enumeration of microorganisms

Four different groups of microorganisms were studied. They include AOB and NOB. The two groups of microorganisms were enumerated using the pour plate method. The results were read as colony forming units (CFU) after seven days of aerobic incubation at room temperature [23].

Enumeration of Ammonia Oxidising Bacteria (AOB)

In brief, Five gram (5.0 g) of homogenized soil was transferred using a stainless steel spatula into a sterile test tube containing 45 mL of the diluents [15]. AOB were enumerated by soil dilution plate count technique with sterile physiological saline (0.85 % w/w sodium chloride) as diluent. One milliliter (1.0 mL) aliquot of 10^4 of each soil sample was removed with a sterile pipette and separately pour plated with AOB medium formulated as in [24].

Enumeration of Nitrite Oxidising Bacteria (NOB)

The NOB was also assayed using the soil dilution plate count technique. The method employed in enumerating NOB was similar with the method already discussed for AOB. However, sodium nitrate ($NaNO_2$) replaced ammonium sulphate ($(NH_4)_2SO_4$) in the medium formulated [24].

Measurement of soil pH

Five gram (5g) of the soil samples were mixed with 5 ml of distilled water and stirred very well. This was allowed to stand for 30 minutes. The electrode of an Extech pH meter (D0700) was put into slurry of the soil-water mixture and the pH of the soil was read off [25]. This was repeated every seven days across all concentrations of crude oil and biochar.

Data Analysis

All experimental setups were performed in three replicates. Readings were taken at seven days interval. Means and standard errors were calculated for pooled results in all experiments for each test. Analysis of variance was performed on the data obtained to determine significant differences among the means on the basis of 5% level of significance using Statistical Package for Social Sciences (SPSS)[26].

RESULTS

3.1 Effect of crude oil on nitrifiers

3.1.1 Growth response of AOB to crude oil contamination

Figure 1 shows the effect of crude oil on the concentration of AOB in the absence of biochar. The result indicated that, there was an observed decrease in cell concentration with increasing levels of crude oil. The highest growth inhibition was exhibited by 15 % crude oil on day 35 (2.30 x 10^5Cfu/g) while the least concentration (5 %) of crude oil recorded the highest number of viable cells (4.03 x 10^5Cfu/g) on day 21. Crude oil level of 5 % and the control (0% crude oil) had their maximum cell count at day 14 and day 21 respectively after which there was a steady and significant ($P < 0.05$) decline in the cell count to day 35 (3.30 x 10^5Cfu/g to 2.40 x 10^5Cfu/g and 4.03 x 10^5Cfu/g to 3.30 x 10^5Cfu/g, respectively). There was a continuous and significant decline in cell count for 10 % crude oil from day 7 till day 28, after which a noticeable increase from day 28 to day 35. The maximum cell concentration for both 10 % and 15 % was recorded at day 7 (3.06 x 10^5Cfu/g and 3.03 x 10^5 Cfu/g respectively). In comparison with the control (no crude oil), contamination with 5% crude oil results in growth stimulation while 10% and 15% crude oil contamination resulted in inhibition of AOB growth.

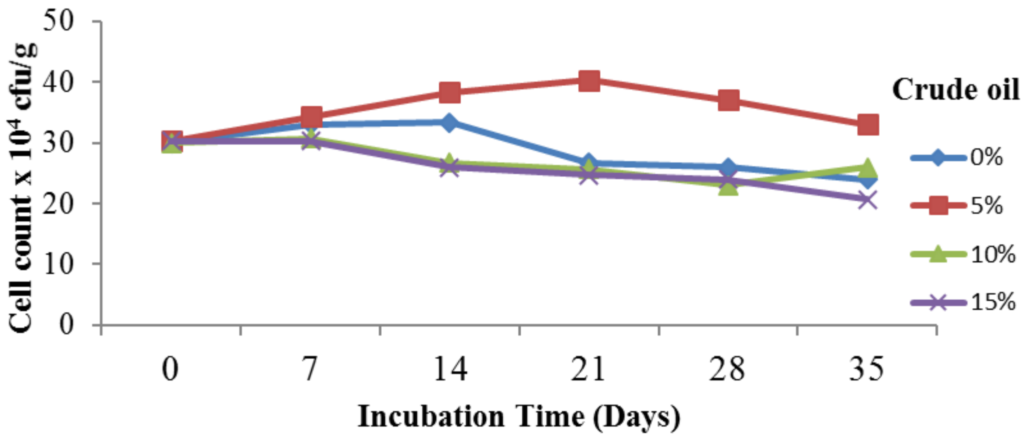

Figure 1. Effect of Crude Oil on Cell Population of AOB

Growth response of NOB to crude oil contamination

Figure 2 shows the effect of different concentrations of crude oil on the cell concentration of NOB in the absence of biochar. The highest growth inhibition (2.60 x 10^5Cfu/g) was exhibited by the most contaminated sample (15 % crude oil) on day 35 of the incubation period. The highest number of cell count (9.0 x 10^5Cfu/g) was achieved by the least treatment concentration (5 % of crude oil) on day 21. There was a relatively steady rise in the cell count of the control (0 % of crude oil) from day 0 to day 14 and started decreasing from day 14 to 35. The result also revealed a steady and significant decrease in cell concentration from day 0 to day 28 for 10% of crude oil and a noticeable increase to day 35. There was also a continuous and significant decrease in the cell count of NOB from day 0 to day 35 when 15%

of crude oil was added to the soil without biochar. As in the case of AOB 5% crude oil contamination stimulated the growth of AOB but higher concentrations of 10 and 15% inhibited the growth of NOB.

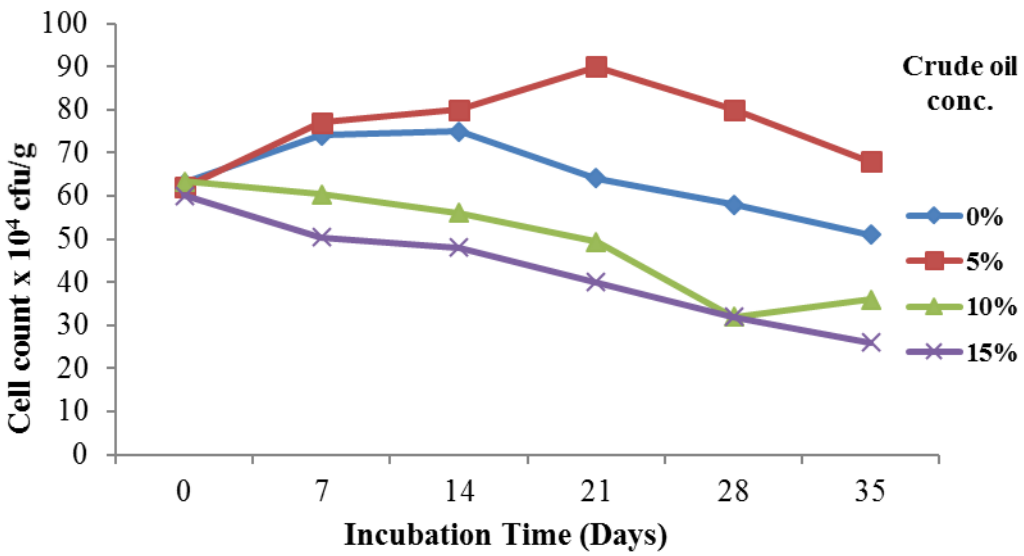

Figure 2. Effect of Crude Oil on Cell Population of NOB

Effect of crude oil on soil pH

Figure 3 shows the effect of crude oil contamination on soil pH. The reduction of pH by the crude oil contamination was concentration dependent (15% > 10% > 5% > 0%). The lowest soil pH (4.37) was exhibited by 15% of crude oil followed closely by 10 % crude oil (4.47) at day 7 of the experiment (5.61 on day 0) after which the pH started increasing steadily towards day 35. The pH at 15% crude oil contamination was significantly lower (pH < 0.05) than the pH of the control. There was no significant difference (P > 0.05) in soil pH between soil contamination with 5 % crude oil and the control on day 0, 7, 14 and 35. However, the pH of the control (0% crude oil) remained fairly constant throughout the experiment.

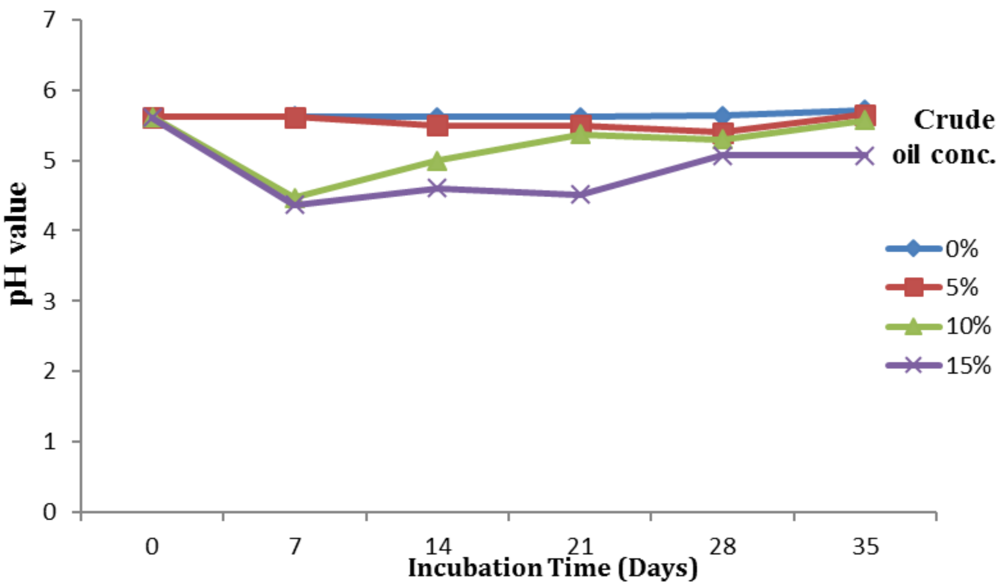

Figure 3. Effect of Crude Oil on soil pH

Effect of biochar on AOB and NOB at three (3) levels of crude oil

Figures 4 – 9 depict the effect of biochar on the growth of AOB and NOB at three levels of crude oil. The effect of biochar on AOB and NOB was investigated using 2 %, 4 %, 6 %, 8 % and 10 % of biochar in 100 g of soil contaminated with 5 %, 10 % and 15 % of crude oil. Triplicate samples without biochar and crude oil served as the control.

Effect of biochar on the growth of AOB at 5 % crude oil concentration

Figure 4 shows the concentration of AOB at 5 % concentration of crude oil. Six percent (6 %) of biochar exhibited the highest cell concentration (7.10 x 10^5Cfu/g) of AOB. In the control (0% biochar) experiment, the AOB increased to 4.03 x 10^5Cfu/g on day 14 and thereafter decreased to 3.3 x 10^5cfu/g on day 35.

Ten percent (10%) biochar in soil at 5% crude oil contamination level exhibited less cell count than all other concentrations of biochar from day 1 to day 21 but obtained higher concentration than 2% biochar from day 21 to 35 to reach its maximum concentration (6.03 x 10^5Cfu/g) on day 35.

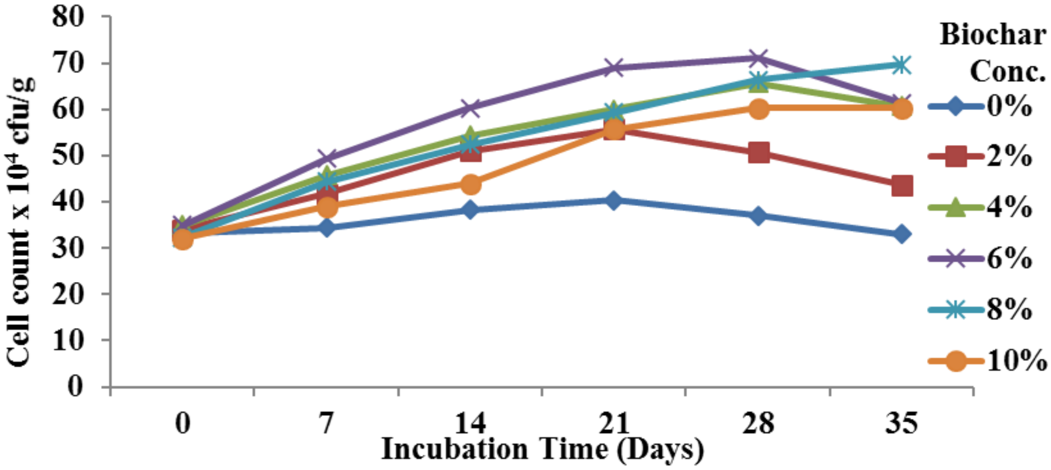

Figure 4. Effect of Biochar on cell growth of AOB at 5 % of Crude oil

Figure 5 represents the cell concentration of NOB at 5 % concentration of crude oil. The highest cell concentration of NOB (1.18 x 10^5Cfu/g) was exhibited by 6 % of biochar. This was significantly ($P < 0.05$) greater than the cell concentration of the control at the same time of incubation. In the absence of biochar (control experiment), the NOB concentration increased steadily from day 1 to day 21 (9.0 x 10^5Cfu/g) and thereafter decreased to day 35.

It was also observed that at 5% crude oil contamination, 10% biochar exhibited a steady decrease in cell growth from day 1 to day 35 and also had the least concentration (5.8 x 10^5cfu/g) of NOB in the experiment.

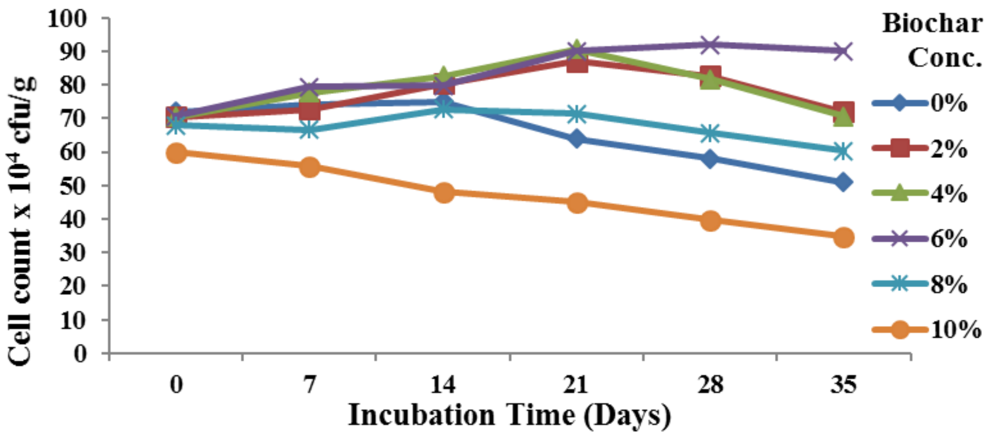

Figure 5. Effect of Biochar on cell growth of NOB at 5 % of Crude oil

3.6.3 Effect of biochar at 10 % crude oil concentration

Figure 6 shows the effect of biochar on the growth of AOB at 10 % crude oil concentration. In the absence of biochar, the result showed a significant ($P < 0.05$) and steady decrease in cell concentration of AOB from day 0 to day 28, then an increase from day 28 to day 35. The highest cell count was exhibited by 8% biochar throughout the duration of the experiment, followed by 10% and 6% as can be seen from figure 6.

Generally, higher concentrations of biochar (10%, 8% and 6 %) exhibited higher cell count than the others (4%, 2% and the control).

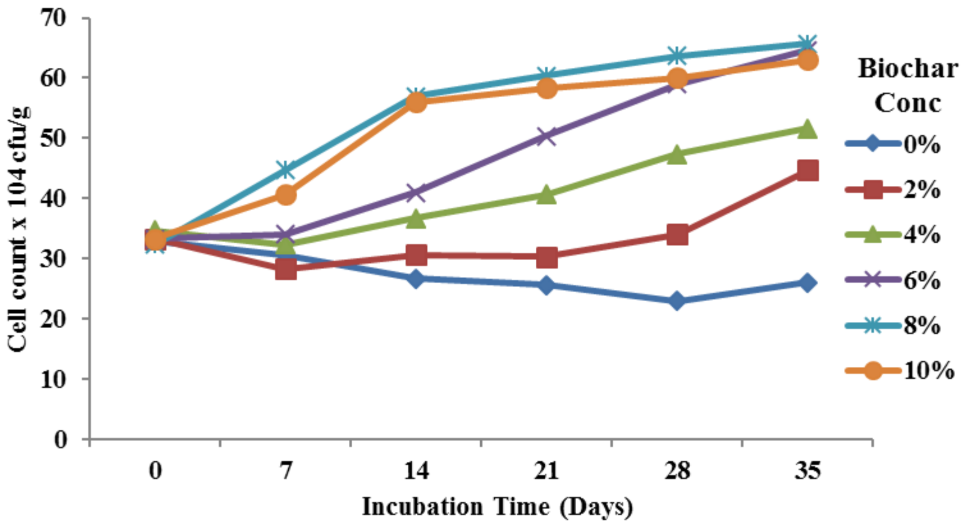

Figure 6. Effect of Biochar on cell population of AOB at 10 % of Crude oil

Fig. 7shows the effect of biochar on the growth of NOB at 10 % crude oil concentration. NOB cell concentration at 0% and 2 % of biochar decreased steadily from day 0 to day 28 after which it increased on day 35. The lowest NOB cell count (3.20×10^5cfu/g) was exhibited in the control (0% biochar) on day 28 while the highest cell concentration in the experiment (9.83×10^5Cfu/g) was exhibited by 10% biochar on day 35. Six percent (6 %) biochar maintained a steady increase in cell concentration from day 7 to day 28 for NOB after which the cell counts decrease on day 35.

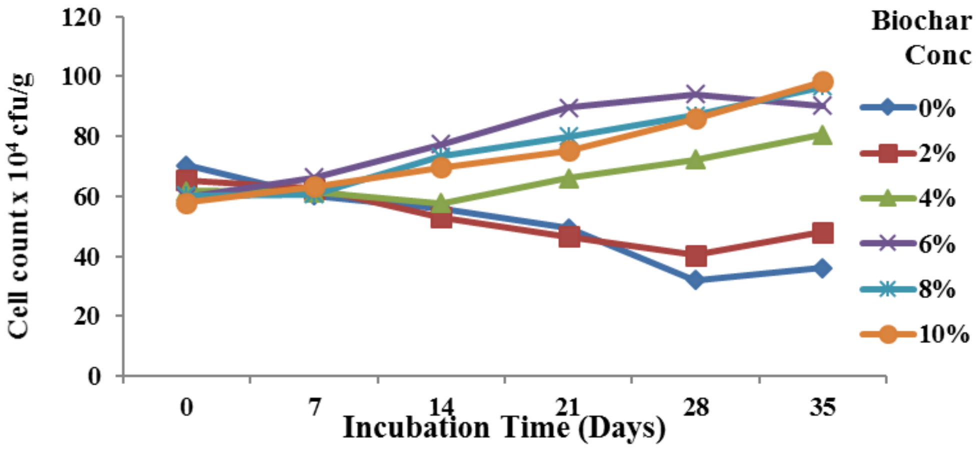

Figure 7. Effect of Biochar on cell population of NOB at 10 % of Crude oil

Effect of biochar on AOB and NOB at 15 % crude oil concentration

Figure 8 and figure 9 represents the cell count of AOB and NOB at the highest level of crude oil (15 %). The control and 2 % biochar exhibited a steady and significant decrease in cell concentration from day 1 to day 21 followed by an increase in day 28 for the control and day 35 for 2% (figure 8). The highest cell count in the experiment (6.07 x 10^5Cfu/g) was exhibited by 10% biochar. This was followed closely by 8% (6.03 x 10^5Cfu/g). The study also revealed that at 8, 6 and 4 % of biochar, there was a steady and significant increase in cell count from day 7 to day 35 for AOB.

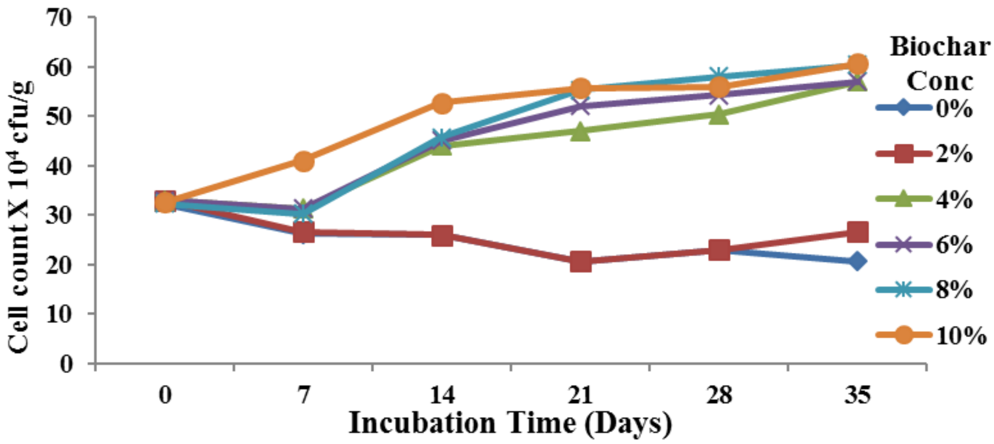

Figure 8. Effect of Biochar the growth of AOB at 15 % of Crude oil

Figure 9 showed that the highest cell concentration of NOB(8.10 x 10^5) cfu/g was obtained by 10% biochar. There was also, a steady and significant rise in the cell counts when 8 and 10% biochar was added to the contaminated soil sample when compared with the control.

In the absence of biochar, there was a steady and significant decrease in cell counts from day 0 to day 21 of the experiment. There was no significant difference between the growth pattern exhibited by the control and 2% biochar between day 0 and day 28 of the incubation period. The control and 2% biochar exhibited the least cell counts throughout the experiment (2.60 x 10^5 cfu/g).

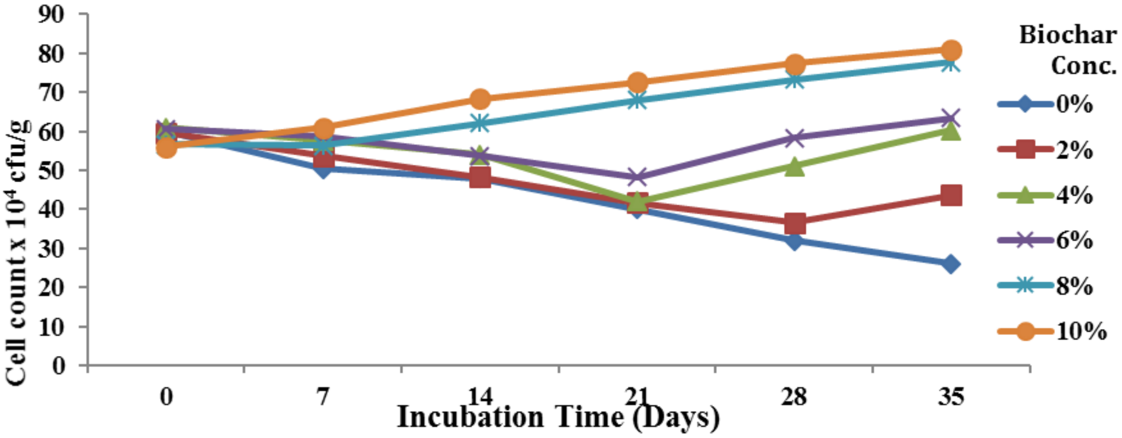

Figure 9: Effect of Biochar on NOB at 15 % of Crude oil

Effect of biochar on the growth of AOB and NOB at day 21 of the experiment

The growth response of AOB and NOB to all the contamination levels of crude oil (0%, 5%, 10% and 15%) across all levels of biochar (0, 2%, 4%, 6% 8% and 10%) was studied at day 21 of the experiment.

Figure 10 shows the growth response of AOB across all levels of crude oil contamination at day 21. It was observed that there is no significant difference ($P > 0.05$) in cell counts of AOB when 2% of biochar is added to soil contaminated with 15% crude oil from the control (0% biochar). The least cell count in the experiment (2.067 x 105 cfu/g) was exhibited by 15% crude oil contamination level at 2% biochar and the control. The highest cell count in the experiment (6.9 x 105 cfu/g) was exhibited by 5% crude oil contamination level when 6% biochar was added. This was higher than the cell count exhibited at 8% and 10% biochar at the same crude oil contamination (5%) level. Addition of biochar stimulates the growth of AOB at all the levels of crude oil contamination. It was also observed that at 15% crude oil contamination, the increase in the cell count of AOB was directly proportional to the concentration of biochar (above 2% biochar) added (4% < 6% < 8% < 10%).

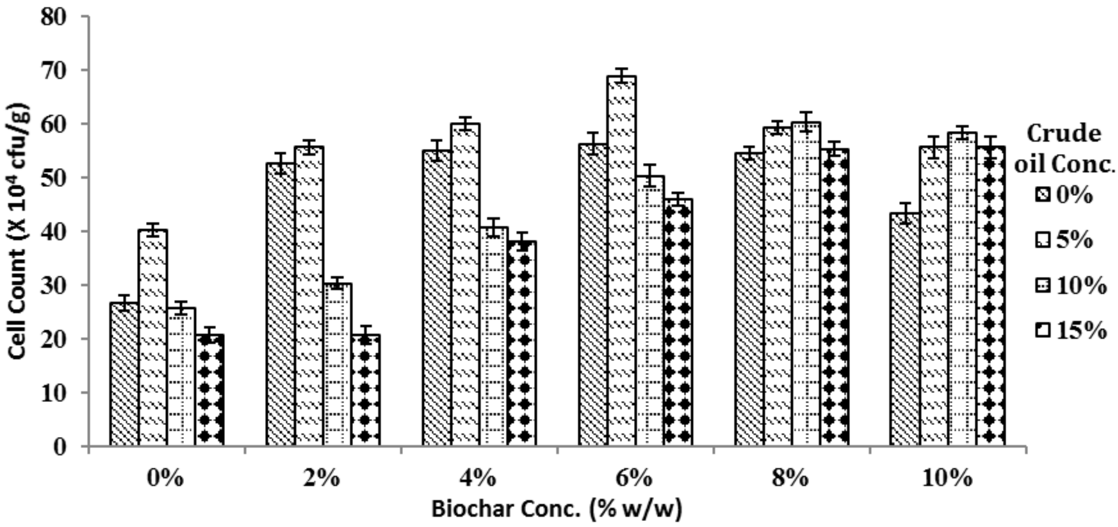

Figure 10. The effect of biochar on the growth of AOB at day 21 of the experiment

The growth response of NOB to all concentrations of crude oil and biochar is shown in figure 11. It was observed that 15% had the lowest cell count (4.0 x 10^5cfu/g) of NOB. There was no significant difference in cell count of NOB when 0%, 2% and 4% of biochar were added to 15% crude oil in soil. When 4%, 6%, 8% and 10% biochar were added to the soil contaminated with 15% crude oil, the cell concentration of NOB increased with increase in the concentration of biochar. The highest cell count (1.10 x 10^5cfu/g) at day 21 was exhibited when 6% of biochar was added to 5% of crude oil. For 5% and 10% of crude oil, the optimum biochar concentration was 6%, however, for 15% crude oil concentration, the growth continued to increase up to 10% biochar.

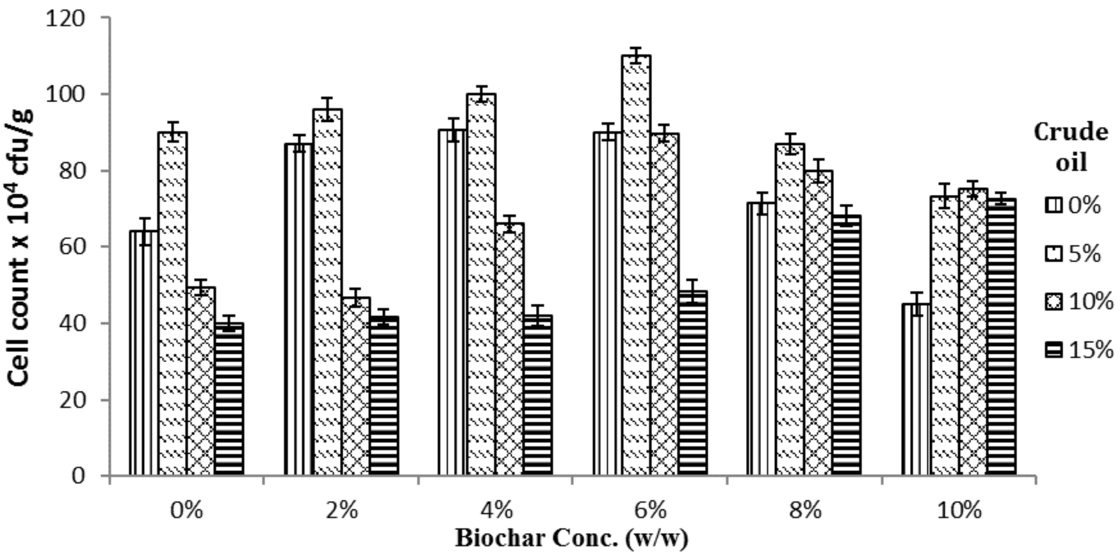

Figure 11: The effect of biochar on the growth NOB at day 21 of the experiment

Effect of biochar on the pH of soil contaminated with crude oil.

The effect of pH on soil contaminated with crude oil was investigated using five concentrations of biochar (2%, 4%, 6%, 8% and 10%) across three crude oil contamination levels (5%, 10% and 15%). The effect of biochar on the pH of the contaminated soil was compared with the control.

At 2% biochar, 15% crude oil exhibited the lowest soil pH (5.47). This was significantly less than the pH of the control (5.70) at that time of the experiment. It was also observed that 2% of biochar exhibited a significant ($P < 0.05$) increase in soil pH at 5% crude oil and the control. Generally, 10% and 15% crude oil contamination levels exhibited a lower pH than 5% and the control.

The effect of biochar on the pH of the soil contaminated with crude oil was investigated across three contamination levels (5%, 10% and 15%). When the soil was contaminated with 10% and 15% crude oil, the pH of the soil increased (from 4% biochar) in a manner proportional to the concentration of biochar (4% < 6% < 8% < 10% biochar). Higher concentrations of biochar (6% - 10%) in the control (0% crude oil), exhibited pH changes significantly higher than the pH of the control at day 0.

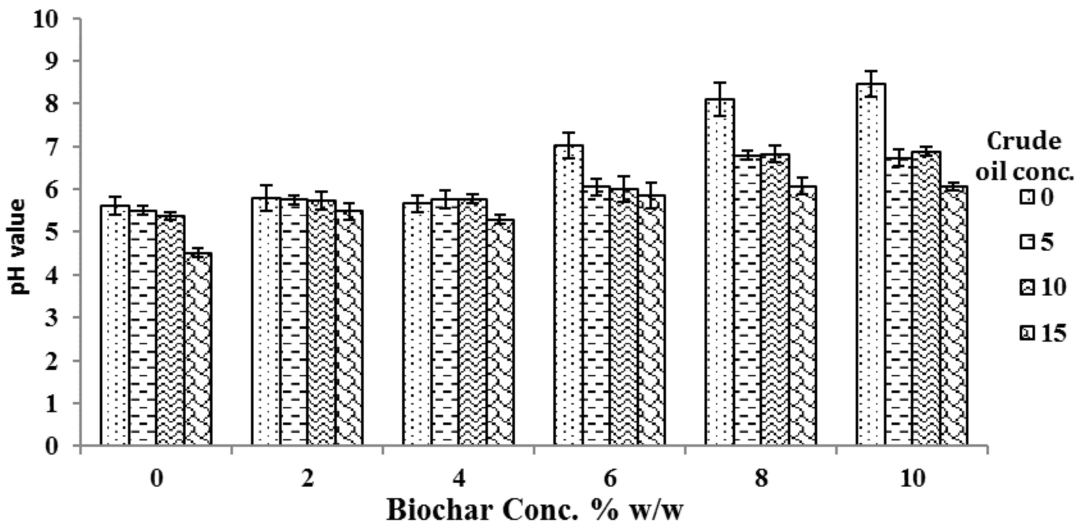

Figure 12. The effect of biochar on the pH of soil contaminated with crude oil at day 2

DISCUSSION

Generally, addition of biochar (charcoal for application to soils) increased the population of both AOB and NOB across all levels of crude oil contamination when compared with the control. Addition of biochar to the soil contaminated with crude oil also increased the pH of the soil in a manner related to the concentration of biochar. Oxygen affects the survival and proliferation of autotrophic nitrifiers through its roles as a substrate for the ammonia monooxygenase enzyme and as the terminal electron acceptor from cytochrome c oxidases [7]. Oxygen availability in the soil is controlled by the interaction of oxygen consumption and diffusion from the surface through air filled pores [27]. So any condition that will limit oxygen supply in the soil will affect the growth and proliferation of autotrophic nitrifiers [14;8].

It had been suggested that biochar stimulates the growth of both AOB and NOB,[28]. This response is likely due to its roles in the liming of the soil, with subsequent decrease in the acidity of the contaminated soil [29;30], this is because biochar is rich in basic cations and will induce an effective cation exchange capacity thereby increasing the soil pH [31]. The adsorption of toxic compounds to its surface [32] and increased soil porosity for better aeration [33]. This is in agreement with previous works by [34;35] which support the idea that biochar addition to acidic soil favors nitrification by adsorbing the inhibitory compounds in the soil to its surface thereby reducing their effect on the microbial cells.

The shift in the time of highest cell count by the addition of 2 % biochar at 0% and 5% crude oil contamination could be attributed to the nutrient retention and supplementation characteristic of biochar [17]. The increase in cell population by the addition of biochar could also be linked to the role of biochar in the sorption of microbial signaling compounds [36]. It could be inferred that the inability of 2 % of biochar to display high cell population of both AOB and NOB at 10 and 15 % of crude oil before day 21 was because of inefficient soil amendment by biochar at this level of crude oil contamination [37; 38].

Six percent (6 %) of biochar could not effectively stimulate microbial growth at 15 % of crude oil. This agrees with previous report on the role of biochar on biodegradation of petroleum hydrocarbon by hydrocarbon utilizing bacteria [39]. This could be associated to the inhibiting effect of crude oil at such high concentrations even in the presence of 6 % biochar [40; 41]. The quantity of biochar at this level is not enough to effectively aerate the clogged soil sample using its porous structure in the soil already clogged by crude oil. This anaerobic condition created as a result of the smoldering effect of crude oil at 15 % does not favour the proliferation of the autotrophic nitrifiers who depend largely on oxygen for growth [14]. It can also be inferred that the inability of 6 % of biochar to effectively stimulate the growth of the nitrifiers when 15% crude oil was added is because 6% could not effectively reduce the acidic condition created by 15 % crude oil to a safe level for the optimal growth of autotrophic nitrifiers. This is because the nitrifiers are known to have specific growth range of pH [42]. This inefficient amendment is because the impact of biochar is dependent on the amount applied at a certain crude oil contamination level [37; 39].

It can also be deduced that higher levels of crude oil (10 and 15 %) need higher concentrations of biochar for effective stimulation of microbial growth. This is probably because at such levels of crude oil contamination, the soil loses its water retention capacity because of the hydrophobic nature of the oil. Thus, more biochar is needed at this level of soil contamination for microbial activities to progress since it has been established from previous studies that the nitrifying bacterial cell count was moisture-limited [43]. Biochar attracts and retain water and oxygen because of its porous structure and large surface area [39]. This is important because biodegradation of crude oil by the oil degraders take place in the contact zone between the oil and water [44].

The highest growth of both AOB and NOB exhibited by high concentrations of crude oil (10 % and 15) was noticed on day 35 of the incubation period. This could be as a result of natural attenuation in form of microbial degradation of the crude oil by Hydrocarbon Utilizing Bacteria (HUB) and Heterotrophic Bacteria [40;11] and photooxidation. Photooxidation of crude oil through the process of photolysis increases the biodegradability of petroleum hydrocarbon by breaking the aromatic ring structures thus increasing its bioavailability and enhancing microbial activities towards the later part of the experiment [45].

In the study of the effect of crude oil on nitrifiers, it was discovered that 5 % of crude oil had more cell concentration than the control (0 % of crude oil) and hence could be said that crude oil was stimulatory on both AOB and NOB at that concentration. This finding is in line with previous studies [24;1] which reported that low levels of crude oil in soil tend to stimulate soil biochemical processes such as nitrification and geochemical cycling of elements which thereafter increase the number and activities of the organisms.

Meanwhile, the higher concentrations of crude oil (10 % and 15 %) had lower cell growth than the control in the absence of biochar. The lowest cell growth (highest growth inhibition) was exhibited by 15 % of crude oil. It could be reasoned that the higher levels of crude oil were inhibitory on AOB and NOB. It has been suggested from previous studies that crude oil decreased the population of nitrifiers at a rate proportional to the level of contamination [4:1]. This is likely because of the toxicity of the hydrocarbons resulting mainly from their chemical structure because the toxicity of crude oil increases with the number of carbon atoms in the compound [46;14].

CONCLUSION

The following conclusion can be drawn from the study;

- Crude oil contamination in soil reduces the population of autotrophic nitrifiers in a concentration dependent manner.
- The addition of biochar to crude oil contaminated soil will encourage the growth of autotrophic nitrifiers.
- Six percent (6%) weight by weight of biochar is best suited to stimulate the growth of autotrophic nitrifiers in soil moderately contaminated with crude oil (5% w/w),
- Crude oil contamination in soil reduces the soil pH towards increasing acidity.
- Addition of a particular quantity of biochar to the soil increases the pH of the soil in a rate related to the concentration of the crude oil.

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