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A LABORATORY STUDY ON THE ENHANCED BIOREMEDIATION OF PYRENE IN SOIL USING ACTIVATED CARBON

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ABSTRACT

Contamination of the environment by petroleum products such as polycyclic aromatic hydrocarbons (PAHs) is inevitable due to oil production, transportation and distribution activities. The potentials of activated carbon as a bioremediation alternative for soils contaminated with pyrene which is a PAH was studied. The rate of biodegradation of pyrene was studied for a period of 28 days under laboratory condition. The result of the microbial counts for soils spiked with 200 mg/kg pyrene was a total heterotrophic bacteria (THB) count in soil amended with commercial activated carbon ranging from 2.97±0.22 to 7.03±0.24 x 10^6 CFU/g. Unamended control soil had THB count ranging from 1.54±0.12 to 1.70±0.18 x 10^6 CFU/g while THB count in unamended autoclaved control soil ranged from 1.15±0.02 to 1.21±0.01 x 10^3 CFU/g. The count of total hydrocarbon-utilizing bacteria (THUB) in activated carbon amended soil ranged from 1.70± 0.11 to 5.10±0.18 x 10^5 CFU/g while unamended control soil had THUB ranging from 7.10±0.12 to 7.90±0.14 ×10^4 CFU/g and THUB count in unamended autoclaved control soil ranged from 5.50±0.01 x 10^1 to 5.80±0.04 x 10^3 CFU/g. The percentage pyrene removal in activated carbon amended soil was 62.2%, the percentage pyrene removal in unamended control soil was 7.70% while the percentage pyrene removal in unamended autoclaved control soil was 2.80% after 28 days. Evaluation of the first order kinetic model resulted in biodegradation rate constant of 0.196 day^{-1} and half-life of 3.54 days for activated carbon amendment of 30 g after 28 days of treatment while unamended control resulted in biodegradation rate constant of 0.012 day^{-1} and half-life of 57.76 days and unamended autoclaved control resulted in biodegradation rate constant of 0.001 day^{-1} and half-life of 69.31 days. The results suggest that activated carbon supplementation would be effective in the remediation of pyrene polluted soils.

KEYWORDS: PAH, Bioremediation, Biiodegradation, Pyrene, Bacteria, Activated Carbon, THB, THUB

INTRODUCTION

Soil contamination as a result of natural and anthropogenic activities is a serious health and environmental issue. Vast amount of financial and human resources are being devoted to the mitigation and cleanup of such soil contamination. One of the major contaminants of soil is Polycyclic Aromatic Hydrocarbons (PAHs) according to Lau *et al*. (2009); Ghaly, *et al*.(2013). PAHs are organic compounds that consist of two or more fused rings of benzene (aromatic ring) that are arranged in various structural configurations. Polycyclic Aromatic Hydrocarbons are highly hydrophobic and this has resulted in their large concentration in the environment. They are also characterized by their resistance to natural degradation and their toxic, carcinogenic, mutagenic and teratogenic properties (Irwin *et al*., 1997), therefore, their removal from the environment without introducing secondary contamination is highly imperative

(European Commission, 2002).

Polycyclic Aromatic Hydrocarbons are generally introduced into the environment through two major means; naturally or artificially by human activities. In the natural way, PAHs occur in fossil fuels such as coal, petroleum and natural processes like volcanic eruption and carbonization and forest fires but are formed majorly by incomplete combustion of organic materials like coal, wood, crude oil, and vegetation (WHO, 2000). Accidental spillage, misguided disposal of petroleum, intensive combustion of fossil fuels, coal, wood preserving products and leakages from underground tanks are examples of human activities that introduce PAHs into the environment (Dyke *et al*., 2003).

Pyrene is one of the sixteen priority pollutant PAHs on the US EPA list. It consists of four fused benzene rings and it is one of the common mutagenic PAHs present in various environments (Liu *et al.,* 2011, Choi *et al.*, 2014). The chemical formula is $C_{16}H_{10.}$ This colorless solid is the smallest peri-fused PAH (one where the rings are fused through more than one face). Like most PAHs, pyrene is used to make dyes, plastics and pesticides. It has also been used to make another PAH called benzo (a) pyrene (Faust, 1991). Pyrene is released by incomplete combustion processes originating from industries, domestic sources including cigarette smoke and motor vehicle exhaust as well as natural events such as forest fires and volcanic eruptions (Osagie and Owabor, 2015). Exposure to pyrene can occur by eating foods grown in contaminated soil or by eating meat or other food that is grilled. Grilling and charring food actually increases the amount of PAHs in the food. Exposure to pyrene can also occur by eating smoked fish or meats. Pyrene has been been detected in coal tar, so working at a business that makes or uses coal tar could also lead to exposure to pyrene and other PAHs (ATSDR, 1990).

Bioremediation is the application of biodegradation to decrease pollutant concentrations (Olson *et al*., 2003). The process relies upon microbial enzymatic activities to transform or degrade the contaminants from the environment (Philips *et al.,* 2005). However, lack of sufficient carbon and nutrient sources to sustain the growth of biodegrading microorganisms may affect bioremediation success (Odokuma and Dickson, 2003; Onuoha*et al*., 2014). Nutrient and carbon additions can enhance microbial activities which may promote cometabolism (Ward and Singh, 2004), this is biostimulation. In most soil bioremediation studies, inorganic chemical fertilizers have been widely used as biostimulating agent, however, it is relatively costly as well as not sufficient for agriculture due to high demand, let alone for cleaning oil spills (Agarry*et al*., 2010; Danjuma*et al*., 2012; Agarry and Jimoda, 2013). Therefore, the search for cheaper and environmentally friendly options of enhancing petroleum hydrocarbon degradation through biostimulation has been the focus of research in recent times (Agarry*et al*., 2010; Danjuma*et al*., 2012; Nyankanga*et al*., 2012). One of such option is the use of activated carbon which may help overcome the toxicity of organicpollutants to microbes and plants during soil bioremediation (Vasilyeva, 2006). A few researchers such as Kim *et al*. (2003); Vasilyeva*et al*. (2006); Hilber and Bucheli (2010) and Agarry*et al*. (2013) have investigated the potential use of activated carbon derived from different sources as biostimulating agents in the clean-up of soil contaminated with petroleum hydrocarbons and were found to show positive influence on petroleum hydrocarbon biodegradation in a polluted environment. Nevertheless, the search for cost effective and environmentally friendly methods of enhancing petroleum hydrocarbon biodegradation in soil still needs to be further investigated.

MATERIALS AND METHODS

Collection of Samples

The soil sample used for the study was collected from the top surface soil (0 – 15cm) of the Teaching and Reseach

farm of LadokeAkintola university of Technology (LAUTECH), Ogbomoso, Nigeria. The soil samples were air dried, homogenized, passed through a 2 mm (pore size) sieve and stored in a polyethylene bag and kept in the laboratory prior to use. The pyrene (manufactured by Sigma-Aldrich, St. Louis, MO, USA) was of analytical grade.

Characterization of Soil Sample

The soil sample was characterized for total carbon (TOC), total nitrogen (N), total phosphorus, moisture content, and pH according to standard methods. Total nitrogen was determined by kjedahl digestion and steam distillation method of Bremner and Mulvaney, (1982). Available phosphorus was determined through the method used by Olsen and Sommers (1982). Available potassium was determined using the flame photometer (Chapman and Pratt, 1978). Available micro nutrientswere determined by the DTPA (diethylenetriaminepentaacetic acid) micronutrient extraction method, developed by Lindsay *et al.*(1978), Total Heterotrophic Bacteria (THB) and Total Hydrogen Utilizing Bacteria (THUB) present in the soil were determined according to the methods of Odokuma and Okpokwasili (1993); Odokuma and Ibor (2002); Amanchukwu*et al.*(1989) and Mills *et al*. (1978). The pH was determined according to the modified method of McLean (1982); total organic carbon was determined by the modified wet combustion method (Nelson and Sommers, 1982) and moisture content was determined by the dry weight method. The physicochemical characterized parameters are presented in Table 1.

Table 1: Soil Sample and Activated Carbon Physicochemical and Microbiological Analysis

Parameter	Soil
pH	6.8±0.1
Organic Carbon (%)	1.15±0.02
Total Nitrogen %)	0.75±0.02
Phosphorus (%)	0.06±0.01
Potassium (%)	0.09±0.01
Moisture Content (%)	10.41±0.2
Residual Pyrene (mg/kg)	0.25±0.02
Sand (%)	14.2±0.2
Silt (%)	78.2±0.2
Clay (%)	7.6±0.2
THUB	0.68×10^5±0.2
THB	14.8×10^5±0.1

Data presented are means of triplicate determination ±standard deviation.

Preparation of Contaminated Soil

200 mg of pyrene was dissolved in 50 ml of ether and added to 1 kg of soil present in a plastic bucket. After capping for 24 h, the cap was opened and evaporated for 24 h in a hood. The final concentration of the soil was then 200.25 mg /kg, which is in the concentration range found in contaminated sites (Zemanek*et al*., 1997; Fung*et al*., 2010).

Biodegradation Experiments

One kilogram (1kg) of soil contaminated with 200 mg of pyrene was put into reactors labeled A1 to A5. Varying quantities of activated carbon (10, 15, 20, 25 and 30 g) were added to the contaminated soil as shown in Tables 2. The moisture content was adjusted to 50% water holding capacity by the addition of sterile distilled water and incubated at room temperature (28±2°C). The content of reactor was tilled twice a week for aeration and the moisture content was maintained at 50% water holding capacity. The soil in reactors A6 and A7 served as the controls. The soil in reactor A6

had no amendment while the soil in reactor A7 was autoclaved at 121^{o}C for 30 min before contamination with pyrene. The set-up is represented in Table 2. The experiment was set up in triplicate. In total, 21 microcosms were settled and incubated for 28 days. Periodic sampling from each container was carried out at 7 day intervals for 28 days to determine the THB count, THUB count and percentage pyrene reduction respectively.

Table 2: Activated Carbon Amendment in Different Soil Microcosms

Reactor Number	Activated carbon Treatment
A1	1 kg of soil + 200 mg of pyrene + 10 g of activated carbon
A2	1 kg of soil + 200 mg of pyrene + 15 g of activated carbon
A3	1 kg of soil + 200 mg of pyrene + 20 g of activated carbon
A4	1 kg of soil + 200 mg of pyrene + 25 g of activated carbon
A5	1 kg of soil + 200 mg of pyrene + 30 g of activated carbon
A6 (Control 1)	1 kg of soil + 200 mg of pyrene
A7 (Control 2)	1 kg of autoclaved soil + 200 mg of pyrene

Enumeration and Identification of Bacteria in Soil

Three replicate samples from the pyrene polluted soil were withdrawn prio to contamination and after contamination at the stipulated days for enumeration of total heterotrophic bacteria (THB) count. Serially diluted samples (0.1ml) of dilutions that produce colony counts of between 30- 300 colonies of soil suspension in sterile water formed from 1.0g of soil in 1L of sterile water on nutrient agar plates using the spread plate technique (Odokuma and Okpokwasili, 1993; Odokuma and Ibor, 2002) were enumerated. Bacteria colonies were enumerated after 48 h of incubation at 30°C. Total hydrocarbon utilizing bacteria (THUB) in the soil samples were enumerated using modified mineral salt medium of Mills *et al*, (1978) 1.8g K_2HPO_4, 4.0g NH_4Cl, 0.2g $MgS0_4.7H_2O$, 1.2g KH_2PO_4, 0.01g $FeS0_4.7H_2O$, 0.1g NaCl, 20g agar, in 1000ml distilled water, pH 7.4). The vapour phase transfer method (Amanchukwu*et al*., 1989) was used. A filter paper saturated with pyrene was aseptically placed on the inside of the inverted Petri dishes and the culture plates were incubated at (28±2°C) for 7 days (Odokuma and Okpokwasili, 1993; Odokuma and Ibor, 2002). Plates yielding 30 - 300 colonies were enumerated. Colonies of different hydrocarbon utilizing bacteria were randomly picked and pure isolates were obtained by repeated sub-culturing on nutrient agar. The bacteria isolates were characterized using microscopic techniques and biochemical tests. The identities of the isolates were determined by comparing their characteristics with those of known taxa as described by Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1994).

Determination of Residual Pyrene in Soil Sample

Samples were taken before contamination and after contamination at the stipulated days from each of the experimental runs. The residual pyrene content in the pyrene polluted soil during the study period was determined gravimetrically by toluene cold extraction method of Adesodun and Mbagwu (2008). Soil samples (10 g) were weighed into 50 ml flask and 20ml of toluene was added to extract the pyrene in the soil. After shaking for 30 min, the mixture was allowed to stand for 10 min and it was then filtered through whatman No1 filter paper. The liquid phase of the extract was measured at 420 nm absorbance using a spectrophotometer (Model 6100 PYE UNICAM Instrument England). The pyrene content in the soil was estimated with reference to standard curve derived from fresh pyrene diluted with toluene. The total pyrene content data obtained was fitted to the first order kinetic model of Yeung*et al*., (1997).

$$Y = ae^{-kt} \quad \textbf{(1)}$$

Where Y = residual PAH content in soil (mg/kg)

a = initial PAH content in soil (mg/kg)

k = biodegradation rate constant (day^{-1})

t = time (day)

The model estimated the biodegradation rate and half-life of the PAH in soil relative to treatments applied. Half-life was calculated from the model of Yeung*et al.*, 1997 as

$$\text{Half-life} = \frac{\ln(2)}{k} \quad \textbf{(2)}$$

The model was based on the assumption that the degradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in soil (Yeung*et al*, 1997).

Bioremediation Kinetics

Kinetic analysis is a key factor for understanding biodegradation process, bioremediation speed measurement and development of efficient clean up for a crude oil contaminated environment. The information on the kinetics of soil bioremediation is of great importance because it characterizes the concentration of the contaminant remaining at any time and permits prediction of the level likely to be present at some future time. Biodegradability of crude oil is usually explained by first order kinetics (Pala *et al*., 2006; Agarry*et al*., 2010; Zahed*et al*., 2011; Agarry and Jimoda, 2013) and this is given in Equation 1. The biological half-life is the time taken for a substance to lose half of its amount. Biodegradation half-lives are needed for many applications such as chemical screening (Arosone*t al*., 2006), environmental fate modeling (Sinkkonen and Paasivirta, 2000) and describing the transformation of pollutants (Dimitrov*et al*., 2007; Matthies *et al*., 2008). Biodegradation half-life times ($t_{1/2}$) are calculated by Equation 2 (Yeung *et al*., 1997; Zahed*et al*., 2011; Agarry*et al*., 2013; Onuoha*et al*., 2014).

RESULTS AND DISCUSSIONS

Changes in THB Count

The changes in THB count for a period of 28 days using various doses of activated carbon ranging between 10 g and 30 g are shown in Figure 1. The THB count increased from $2.97x10^6$ cfu/g for 10 g dose of activated carboni.e soil microcosm A1 to $5.32x10^6$cfu/g for 30 g dose i.e. soil microcosm A5 at day 7. Thus, THB increased with increasing activated carbon dose. This is similar to the changes in THB count observed at 14, 21 and 28 days with the values of $3.33x10^6$ cfu/g, $3.70x10^6$cfu/g, $4.27x10^6$cfu/g for 10 g and $5.51x10^6$cfu/g, $5.92x10^6$cfu/g, $7.03x10^6$cfu/g for 30 g, respectively. Whereas control A6 which consists of contaminated soil with no activated carbon amendment showed an increase from $1.54x10^6$cfu/g at day 7 to $1.70x10^6$ cfu/g at day 28 while control A7 which consists of contaminated autoclaved soil with no activated carbon amendment showed an increase from $1.15x10^1$cfu/g at day 7 to $1.21x10^3$cfu/g at day 28.

The changes in THB with respect to time is similar to the changes in THB with respect to dosage rate of activated carbon as also shown in Figure 1 where it can be observed that THB also increased with time. At 10 g dose of activated

carbon, THB increased from 2.97x10^6 cfu/g at 7days to 4.27x10^{6}cfu/g at 28 days which is the same trend other dosage levels followed.

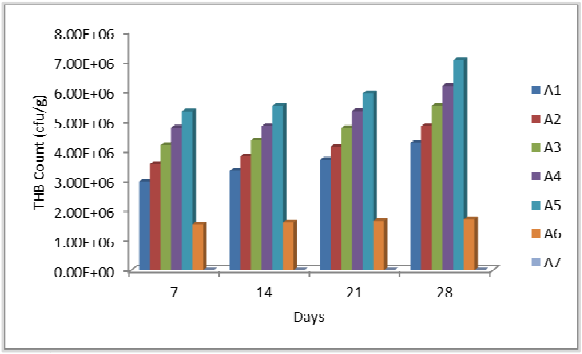

Figure 1: Effect of Activated carbon on THB Count in Pyrene Contaminated Soil

These results indicate that amendment with the activated carbon enhanced the microbial growth rates in the contaminated soil which accounted for the higher microbial counts observed in all the amended soil microcosms than the unamended soil microcosms. This is due to the due to the hydrophobic nature of activated carbon, its high specific surface (8,001,200 m^2/g) and microporous structure affordingit to be a co-substrate that can play the role of alternate carbon source and thus promote the proliferation and activity of the microbial community as reported by Wong *et al*. (2002); Lee *et al*. (2003); Hameed (2009); Llado*et al*. (2009). Activated carbon also acts as mass transfer agent between the liquid phase of the soil and the solid matrix (Carmichael and Pfaender, 1997; Huang *et al*., 2004; Mellendorf *et al*., 2010). Activated carbon therefore serves diverse roles in soil bioremediation.

Changes in THUB Count

The effect of activated carbon amendments of 10 g to 30 g for 28 days on THUB count in the contaminated soil microcosms is shown in Figure 2. The THUB count increased from 1.70x10^{5}cfu/g for the minimum treatment of 10 g (A1) at 7 days to 3.30x10^{5}cfu/g for the maximum treatment of 30 g (A5) at 7 days. Thus, the THUB increased with increasing activated carbon dose. This same trend was also observed at 14, 21 and 28 days with THUB counts increasing from 2.1x10^{5}cfu/g, 2.40x10^{5}cfu/g and 2.80x10^{5}cfu/g for 10g activated carbon treatment to 3.9x10^{5}cfu/g, 4.30 x10^{5}cfu/g and 5.10x10^{5}cfu/g for 30g activated carbon treatment respectively. THUB count also increased with time as shown in Figure 2 where it increased from 1.70x10^{5}cfu/g at 7days and 10g activated carbon treatment to 5.10x10^{5}cfu/g at 28 days and 30 g activated carbon treatment. Control A6 which consists of contaminated soil with no activated carbon increased from 7.1x10^{4}cfu/g at day 7 to 7.9x10^4 cfu/g at day 28 while control A7 which consists of contaminated autoclaved soil with no activated carbon amendment increased from 5.5x10^{1}cfu/g at day 7 to 5.8x10^{3}cfu/g at day 28.

The graph indicates that the THUB counts in all the contaminated soils increased with increasing level of activated carbon amendment and also with time but at different rates and at a higher rate compared to the controls which do not contain any amendment. This meant that the indigenous hydrocarbon utilizing bacteria were able to utilize the activated carbon as a nutrient source as discussed in the changes in THB previously. This is in agreement with the findings of Zimmerman *et al.* (2004); Tang and Weber (2006); Agarry and Jimoda (2013) who all reported higher increase in activity in the hydrocarbon degraders using activated carbon amendment in petroleum hydrocarbon contaminated samples studied compared to unamended samples. The increase in the THUB count can also be attributed to the fact that the use of

activated carbon may help overcome the toxicity of organic pollutants to microbes and plants during soil bioremediation as reported by Vasilyeva*et al.* (2006).

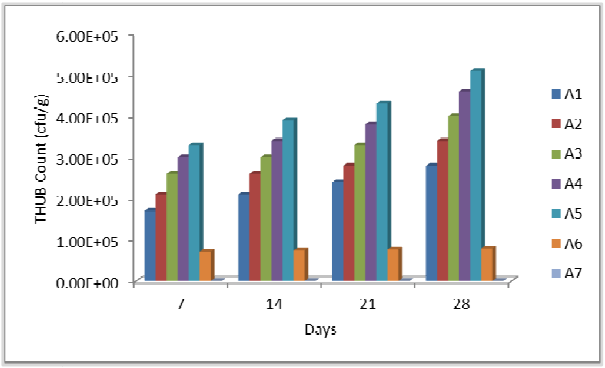

Figure 2: Effect of Activated Carbon on THUB Count in Pyrene Contaminated Soil

Changes in Percentage Pyrene Reduction

The change in percentage pyrene concentration in the contaminated soil for a period of 28 days using activated carbon (10-30 g) as the nutrient amendment is shown in Figure 3. The graph indicates that the extent of degradation increased with respect to increase in the amendment level and also with time. Amending with 10 g of activated carbon (A1) yielded a percentage reduction of 37.20% after 7 days and amending with 30 g activated carbon (A5) yield a percentage reduction of 42.20% after 7 days respectively. This indicates clearly that percentage pyrene reduction increased with the level or dosage of activated carbon amendment. It can also be observed from Figure 3 that the percentage pyrene reduction increased with time where 10 g activated carbon amendment gave 42% reduction after 7 days and 50.8% reduction after 28 days. Also, amending with 30 g of activated carbon increased the percentage pyrene reduction from 45% after 7 days to 62.20% after 28 days. Control A6 which consists of contaminated soil with no activated carbon resulted in 5.20%, 6.4%, 7.0% and 7.1% percentage concentration reduction after 7, 14, 21 and 28 days respectively while control A7 which consists of contaminated autoclaved soil with no activated carbon yielded 0.07%, 1.40%, 1.80% and 2.80% percentage concentration reduction after 7, 14, 21 and 28 days respectively.

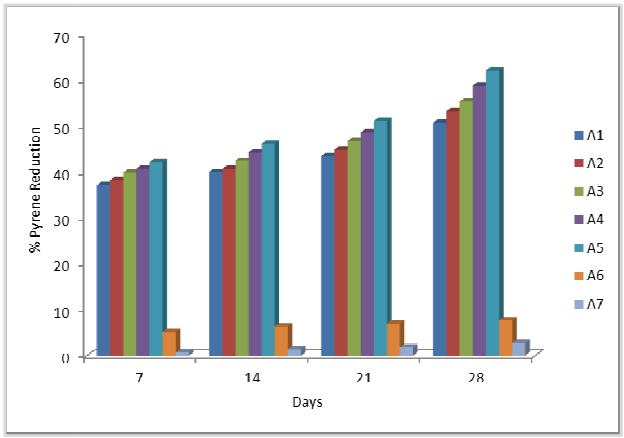

Figure 3: Effect of Activated Carbon on % Reduction of Pyrene

These results indicate that the percentage reductions in all the contaminated soils increased with increasing level of activated carbon amendment and also with time with the amendment of 30 g after 28 days yielding the highest

percentage reduction of 62.2%. It was also evident in this study that there was a marked relationship between the THB and THUB counts on one hand and the percentage PAH concentration reduction on the other hand. The higher the THB or THUB count, the higher the degree of degradation evident in the percentage reduction. Previous studies have also shown the percentage degradation of petroleum hydrocarbons increasing with increase in dosage level of the activated carbon. Vasilyeva*et al*. (2001); Vasilyeva*et al.* (2002) and Xu*et al*. (2011), reported that the percentage degradation of 2, 6-DCP in soil increased with the increase in the activated carbon dosage. This can be attributed to the beneficial effect of the activated carbon on the soil matrix as a result of increase in dosage.

Workers such as Kim *et al*. (2003); Murillo *et al.* (2004); Mohan *et al.* (2007); Ademiluyi*et a*l. (2009); Cao *et al*. (2009) and Uchimiya, *et al.* (2010) have also reported beneficial effects of activated carbon during soil bioremediation. This is because nutrient leaching can also be reduced by activated carbon application to soil (Lehmann *et al.,* 2003; Major *et al.,* 2009; Novak *et al.,* 2009; Singh *et al.*, 2010). Further potential benefits of adding activated carbon to soil have also been reported, these include the adsorption of dissolved organic carbon, increases in soil pH and key soil macro-elements, and reductions in trace metals in leachates (Pietikainen*et al.,* 2000; Novak *et al*., 2009).

Unlike other soil amendments, activated carbon longevity in soil reduces the possibility of heavy metal accumulation associated with repeated applications of other amendments (Lehmann and Joseph, 2009) such as sewage sludge. Activated carbon has also been shown to have a very high affinity and capacity for sorbing organic compounds (Cornelissen and Gustafsson, 2005; Lohmann, *et al*., 2005; Oen*et al.*, 2006 and Brandli*et al.,* 2008). Therefore, activated carbon amendment has proven to be a promising option for the reclamation of such contaminated sites not only for organic but also for inorganic pollutants (Bes and Mench, 2008).

At the end of 28 days and activated carbon amendment of 30 g, pyrene concentration in the contaminated soil dropped from 200.17 mg/kg to 75.6 mg/kg. For control A_6, for control M_6, pyrene concentration reduced to 184.8 mg/kg and for control M_7, pyrene concentration became 194.5 mg/kg.

Biodegradation Rate Constant and Half-Life

The biodegradation of pyrene in the various treatments was evaluated using first–order kinetic model of Yeung *et al,* (1997). Kinetic analysis is a key factor for understanding biodegradation process, bioremediation speed measurement and development of efficient clean up for a crude oil contaminated environment. The information on the kinetics of soil bioremediation is of great importance because it characterizes the concentration of the contaminant remaining at any time and permits prediction of the level likely to be present at some future time. Biodegradability of crude oil is usually explained by first order kinetics (Pala *et al*., 2006; Agarry *et al*., 2010; Zahed *et al*., 2011; Agarry and Jimoda, 2013) and this is given in Equation 1. The biological half-life is the time taken for a substance to lose half of its amount. Biodegradation half-lives are needed for many applications such as chemical screening (Arosone*t al*., 2006), environmental fate modeling (Sinkkonen and Paasivirta, 2000) and describing the transformation of pollutants (Dimitrov *et al*., 2007; Matthies *et al*., 2008). Biodegradation half-life times ($t_{1/2}$) are calculated by Equation 2 (Yeung *et al*., 1997; Zahed *et al*., 2011; Agarry *et al*., 2013; Onuoha *et al*., 2014). Table 3 shows the biodegradation rate constant (K) and half-life (t½) for the different treatments within the period of study. Data for the sampling periods were combined before this model could be used.

Table 3: Biodegradation Rate and Half-Life of Pyrene in Polluted Soil

Reactor Number	Activated Carbon Treatment	Biodegradation Constant (k) day^{-1}	Half-life ($t_{1/2}$) day
A1	1 kg of soil + 200 mg of pyrene + 10 g of activated carbon	0.114	6.08
A2	1 kg of soil + 200 mg of pyrene + 15 g of activated carbon	0.130	5.33
A3	1 kg of soil + 200 mg of pyrene + 20 g of activated carbon	0.140	4.95
A4	1 kg of soil + 200 mg of pyrene + 25 g of activated carbon	0.168	4.13
A5	1 kg of soil + 200 mg of pyrene + 30 g of activated carbon	0.196	3.54
A6 (Control 1)	1 kg of soil + 200 mg of pyrene	0.012	57.76
A7 (Control 2)	1 kg of autoclaved soil + 200 mg of pyrene	0.010	69.31

It can be observed that the higher the biodegradation rate constants, the faster is the rate of biodegradation and consequently the lower is the half-life times. It could be seen from Table 3 that among the soil microcosms amended with 10, 15, 20, 25, 30 g of activated carbon, the soil microcosm amended with 30 g of activated carbon (A5) had the highest biodegradation rate constant k(0.196 day-1) and the lowest half-life time ($t_{1/2}$=3.54 days). The biodegradation rate constant (k) and half-life time ($t_{1/2}$) for the unamended soil microcosms (A6 and A7) were correspondingly found to be 0.012 day^{-1}and 57.76 days and 0.010 day^{-1} and 69.31 days respectively. Thus, the biodegradation rate constants obtained for the different pyrene contaminated soil microcosms amended with activated carbon were higher and had lower half-life times when compared with those of the unamended soil microcosms. Thus, the addition of activated carbon enhanced pyrene reduction as the dosage of the activated carbon increased.

CONCLUSIONS

This research was carried out to determine the potential of activated carbon in enhancing biodegradation of pyrene in contaminated soil. The results obtained confirm that the use of activated carbon enhanced the rate of pyrene biodegradation in contaminated soil microcosms. The biodegradation rate constant obtained from the application of first order kinetics described the rate of pyrene biodegradation with and without activated carbon. The rate constant (k) ranged between 0.114 day^{-1}and 0.196 day^{-1} for amended soil microcosms, for unamended soil microcosms, the biodegradation rate (k) values obtained were 0.012 day^{-1} and 0.010 day^{-1}. Half-life times ($t_{1/2}$) of 57.76 days and 69.31 days were obtained for biodegradation of pyrene in soil not amended with activated carbon (A6) and biodegradation of pyrene in autoclaved soil not amended with activated carbon (A7) respectively. This was reduced to between 6.08 and 3.54 days with the use of activated carbon in the range 10 – 30 g after 28 days of treatment. The amendment of activated carbon for soils contaminated with pyrerne and other petroleum hydrocarbons could be suitable in field due to its low costs and the low environmental risk associated with volatile hydrocarbon losses. The large increase in microbial population in the amended soils suggests that the supplementation with activated carbon may enhance degradation of petroleum hydrocarbon in nutrient poor soils.

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