A pilot study on the biodegradation of hydrocarbon and its kinetics on kerosene simulated soil

Akpoveta O.V.1, Egharevba F.2, Medjor O.W.3
Department of Chemistry, Ambrose Ali University, P.M.B 14, Ekpoma, Edo state, Nigeria.
akpovin2@yahoo.com

doi:10.6088/ijes.00202010006

ABSTRACT

A pilot study was carried out on soil from the Niger Delta region of Southern Nigeria, contaminated with kerosene by 10% artificial simulation to determine the attendant effect associated with the soil physicochemical properties and microbiological composition. Biodegradation of the contaminant using soil microbes biostimulated with a blend of animal wastes and the kinetics of such process was also investigated. Soil parameters such as pH, electrical conductivity, total organic carbon and matter, total nitrogen and phosphorus, texture, heavy metals (Cd, Pb, Ni, V and Cr) and total petroleum hydrocarbon (TPH) were characterized using standard analytical methods. Trend in growth phase of soil heterotrophic and hydrocarbon utilizing microbes were investigated. Kerosene contamination was seen to affect certain soil properties as a reduction in pH, conductivity, total phosphorus and heterotrophic microbial population was observed, while an increase in the concentration of heavy metals such as Nickel, Vanadium and Chromium were recorded. Other soil properties were unaffected by the impact of kerosene. The rate of microbial degradation was found to be dependent on pH and nutrient source. Effective degradation and increased microbial growth occurred between pH 6.0 and 9.5 but recorded reduced microbial growth and biodegradation rate at much higher pH thereby defining a suitable pH condition for the process. The method was found to be very effective and efficient as an impressive 82.24 % remediation efficiency was achieved on the sixth week. Kinetic evaluation of the biodegradation process shows that the degradation pattern followed first order with a rate constant of 0.034day\(^{-1}\). A determination of the biodegradation isotherm \(K_d\) gave negative value of unity showing the opposing trend between the concentration of the contaminant in the soil \((C_s)\) and the concentration degraded by the microbes \((C_d)\); which explains that as \(C_s\) is decreasing with time, \(C_d\) is increasing.

Keywords: Biodegradation, Kerosene, Biodegradation isotherm, Kinetics, Total petroleum hydrocarbon, Simulation.

1. Introduction

Contamination of the environment is frequently associated with hydrocarbon pollution because of the increasing global demand for petroleum hydrocarbons and its products. The growing demand for kerosene as the major energy source of domestic cooking and lighting in Nigeria has evidently led to disturbing cases of kerosene spillages. Kerosene is a Complex mixture of hydrocarbons consisting of paraffins, cycloparaffins, aromatic and olefinic hydrocarbons with carbon numbers predominantly in the C9 to C16 range. The components of kerosene as a fraction of petroleum could pose serious environmental problems when it directly or indirectly enters into the environment because of their chemical nature. Contamination of the soil with petroleum hydrocarbon affects soil microflora and
microfauna, underground water and plants, depending on the degree and magnitude of contamination, such soil may remain unsuitable for crop growth for a very long time.

The environmental consequences of soil pollution include adverse effect on the soil microflora all of which assist in soil fertility (Torstensson et al., 1998). Microbial presence in the soil is of great importance in maintaining soil fertility as it was reported by Torstensson et al., (1998) that soils which maintain a high level of microbial biomass are capable of not only storing more nutrients, but also of cycling more nutrients through the system. The sustainability of soil fertility, quality and productivity is of immense interest and concern to man because of the attendant detriments of hydrocarbon contamination on soil, since there is direct reliance and dependence of man’s existence on the soil. Therefore indices that serve as indicators for assessing soil quality and fertility must be continually maintained and monitored. This brings out the importance of carrying out a pilot study on kerosene simulated soil to determine the effects of kerosene pollution on the physical, chemical and biological components of the soil.

Hydrocarbon pollutants in contaminated soils can potentially be degraded by microbial activity. The potentiality of microbes as agents of degradation of several compounds thus indicates biological treatment as the major promising alternative to attenuate environmental impact caused by pollutants (Nwaake and Okpokwasili, 2003). Microbial breakdown of hydrocarbon pollutants is generally a very slow process but it could be optimized to enable the rate of microbial transformation proceed more rapidly. Optimum biodegradation can only occur if the right environmental conditions such as pH, temperature, nutrients and relevant microbial consortia are present. Conditions such as temperature and microbial composition cannot be influenced in real practical bioremediation situations except on ex-situ bioremediation programs. However, conditions such as pH and nutrient availability can be optimized to enhance speedy indigenous microbial breakdown of contaminants if these conditions are predetermined. Conditions suitable for effective biodegradation processes vary from place to place because soils vary in their physical, chemical and biological composition and properties. Knowledge of such conditions that could be influenced will enhance the ordinarily slow natural attenuation process towards speedy bio-remediation. The study is therefore aimed at providing information on the potentials of exploiting soil indigenous microbes with hydrocarbon utilizing capabilities and determining suitable conditions for optimum biodegradation of kerosene in soil of the Niger Delta environment of southern Nigeria. Determining the kinetics of the process will also help in understanding the rate at which microbial degradation proceeds thereby extending the scope of this research towards an evaluation of the kinetics of biodegradation.

2. Materials and Method

2.1 Samples Collection

Soil samples were collected with a soil auger at surface depth (0-15cm) from a virgin fallow land in the forest area of Agbor, Delta State in southern Nigeria, having no pollution history and devoid of hydrocarbon contamination. DPK-Domestic petroleum kerosene (kerosene) samples with specific gravity of 0.817g/cm$^3$ was obtained from a petroleum marketing station in Agbor, Delta state. Cow dung was collected from a cow farm situated along Lagos-Asaba road in Agbor, Delta State. While pig and poultry droppings were respectively collected from the piggery and poultry house in the Agric unit of the College of Education Agbor, Delta State.
2.2 Sample Preparation, Simulation and Amendment

Soil was air dried for a period of one week in a clean well-ventilated laboratory, homogenised by grinding, and filtered by passing through a 2mm mesh sieve. 1kg of soil was each measured into two clean dry plastic containers and moistened to 20% water holding capacity with distilled water to ensure proper mixing with the contaminant. Simulation of the soil samples was done by measuring 100g of Kerosene corresponding to 122.4ml kerosene from gravimetric measurement into the two containers containing 1kg of soil each. The individual mixtures were thoroughly mixed to achieve a 10% artificial contamination. 10% spiking was adopted to achieve severe contamination because beyond 3% concentration, oil has been reported to be increasingly deleterious to soil biota and crop growth (Osuji et al. 2005). The manure samples were sun dried for one week after which they were grinded, thoroughly mixed, sieved through a 2mm sieve to achieve uniform particle size and stored in neat polythene bag for use. 1kg of the mixed manure was added to one of the containers containing 1kg of crude oil simulated soil in a 1:1 ratio and thoroughly mixed to obtain homogeneity. The second container containing 1kg of crude oil simulated soil served as the control.

2.3 Microbiological Analysis of Fungi and Bacteria Utilizing Microbes

The indigenous soil microbes with hydrocarbon utilizing abilities were isolated, identified and their microbial population determined before and within intervals of the treatment process using selective enrichment techniques and standard bacteriological methods (Bergey and Breed, 1957; Anon., 2010) so as to monitor the progress of the bioremediation process. In order to isolate and enumerate both heterotrophic and hydrocarbon utilizing bacteria, bacteria enrichment process using modified mineral salt medium of Mills et al. (1978) was carried out. Gram staining reaction method of stewart and Beswick (1977) was adopted for the characterization of isolates. Citrate utilization test, oxidase test, indole production test and Urease hydrolysis test were all performed using methods as described by Cruickshank et. al (1975). Isolation and enumeration of fungal isolates were carried out with Sabraund dextrose agar using the spread plate technique as described by ALPHA, (1998). Fungal isolates were identified using the method of Harrigan and McCane (1976).

2.4 Soil Characterisation/ Physicochemical Analysis

Soil physicochemical characteristics such as soil texture, pH, total organic carbon, total organic matter, Carbon/Nitrogen ratio, total nitrogen, total phosphorus, soil conductivity and heavy metals (V, Pb Ni, Cd, Cr) were determined before contamination, one week after contamination and one week after the bioremediation process. Soil pH was determined electrometrically following the procedure outlined by Mylavarapus and Kannelly (2002). Particle size analysis was done using bouyoucos hydrometer method (Bouyoucos, 1951). Total organic carbon and matter were determined by the wet dichromate acid oxidation method (Nelson and Sommers,1982). Total Nitrogen was determined using the method of Radojevic and Bashkin(1999). Total Phosphorus was determined by Bray and Kurtz method (1965). Electrical conductivity was carried out as described by Chopra and Kanzer(1988). Heavy metals were determined by digesting the samples with concentrated mixtures of hydrofluoric, nitric and perchloric acid (AOAC, 1970) so as to convert all the metals present in the sample into such a form that they can be analyzed by the atomic absorption spectrophotometer.
2.5 Determination of Total petroleum Hydrocarbon (TPH)

1g of the soil sample was dissolved in 10ml of hexane and shaken for ten minutes using a mechanical shaker. The solution was filtered using a whatman filter paper and the filtrate diluted by taking 1ml of the extract into 50ml of hexane. The absorbance of this solution was read at 460nm with HACH DR/2010 Spectrophotometer using n-hexane as blank. Total petroleum hydrocarbon was determined at weekly intervals for six weeks.

2.6 Quality Control

Procedural blanks and standard solutions were prepared and included to ensure analytical quality control so as to assure the accuracy and reproducibility of the results. Replicate analyses were carried out on the determination of TPH to yield a mean which will be used to determine trueness and also standard deviation of the mean to measure precision (Stanton, 1966; Valcarcel., 2000).

3. Results and Discussion

The physicochemical characteristics of the soil influenced by the impact of kerosene as shown in table 1 are substantiated below. A reduction in pH, conductivity and total phosphorus were observed on simulation of the soil with kerosene from 5.1 to 4.5, 191.776µS/cm to 134.76µS/cm and 6.1mg/kg to 5.4mg/kg respectively; while a significant increase in total petroleum hydrocarbon (TPH) from 8.64mg/kg in the control soil to 1894.87mg/kg in the kerosene simulated soil was recorded as seen in table 1. The weak acidity observed in the control soil is common with reduced anaerobic soils and sediments in the Niger Delta (Odu et al. 1985; Isirimah, 1987). The pH for the unpolluted soil fell within the pH range of between 5-7 which is suitable for most good agricultural soils since Ritter (2006) reported that most good agricultural soils have a pH between 5 and 7. Increased acidity occasioned by the presence of kerosene is a problem for agricultural soil because very low pH values, indicative of acidity, are associated with adverse soil conditions including reduced microbial activity, increased availability and toxicity of heavy metals and reduced availability of plant nutrients. Conductivity value recorded in the control soil is due to the presence of soluble polar mobile solutes in the soil. The resulting decrease on contamination is due to the effect of kerosene which provides a non polar environment for the soil ions retarding their movement and immobilising them resulting in reduced ionic mobility, velocity and consequently bringing about reduced conductivity. Presence of hydrocarbon in soil reduces available forms of phosphorus as has been shown by Dimitrow and Markow(2000), Okolo et al. (2005), Okonokhua (2007). The observed reduction in pH and conductivity was similar to the findings of Osuji and Nwoye (2007). After the bioremediation process, an increase in pH (4.5 to 6.9), conductivity (134.76 to 162.8µS/cm) and total phosphorus(5.4 to 5.51mg/kg) were observed. Substantial reduction in hydrocarbon concentration thereby providing a polar environment for the soil ions accounted for the increased conductivity. Introduction of exogenous nutrients such as phosphorus, nitrogen and other cations from the animal waste used in the bioremediation process possibly explains the observed increase in pH and total phosphorus content. Soil properties such as total nitrogen(0.15 to 0.17 to 0.31mg/kg), organic carbon(2.34to 5.34 to 5.85%) and organic matter(4.03 to 9.21 to 10.09%) increased on addition of the hydrocarbon to the soil and subsequently increased after the bioremediation process as seen in table 1.
A pilot study on the biodegradation of hydrocarbon and its kinetics on kerosene simulated soil

Table 1: Results of nutrient analysis; soil physicochemical properties and heavy metals before, one week after pollution and after remediation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal waste</th>
<th>Soil</th>
<th>Soil+ kerosene</th>
<th>Remediated soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.9</td>
<td>5.1</td>
<td>4.5</td>
<td>6.9</td>
</tr>
<tr>
<td>Conductivity(µs/cm)</td>
<td>191.7</td>
<td>134.76</td>
<td>162.8</td>
<td></td>
</tr>
<tr>
<td>Nitrogen(mg/kg)</td>
<td>0.51</td>
<td>0.15</td>
<td>0.17</td>
<td>0.31</td>
</tr>
<tr>
<td>Phosphorus(mg/kg)</td>
<td>0.111</td>
<td>6.1</td>
<td>5.4</td>
<td>5.507</td>
</tr>
<tr>
<td>Organic Carbon(%)</td>
<td>7.53</td>
<td>2.34</td>
<td>5.34</td>
<td>5.85</td>
</tr>
<tr>
<td>Organic Matter(%)</td>
<td>12.98</td>
<td>4.03</td>
<td>9.21</td>
<td>10.09</td>
</tr>
<tr>
<td>C/N Ratio</td>
<td>14.76:1</td>
<td>15.6:1</td>
<td>31.41:1</td>
<td>18.87:1</td>
</tr>
<tr>
<td>Total Petroleum Hydrocarbon (mg/kg)</td>
<td>8.64</td>
<td>1894.87</td>
<td>336.60</td>
<td></td>
</tr>
<tr>
<td>Lead(mg/kg)</td>
<td>0.382</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cadmium(mg/kg)</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>&lt;0.001</td>
<td>0.015</td>
</tr>
<tr>
<td>Nickel(mg/kg)</td>
<td>0.446</td>
<td>0.419</td>
<td>0.456</td>
<td>0.528</td>
</tr>
<tr>
<td>Vanadium(mg/kg)</td>
<td>0.812</td>
<td>0.792</td>
<td>0.912</td>
<td>0.998</td>
</tr>
<tr>
<td>Chromium(mg/kg)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.069</td>
<td>0.043</td>
</tr>
<tr>
<td>% Sand</td>
<td>83.31</td>
<td>83.10</td>
<td>83.13</td>
<td></td>
</tr>
<tr>
<td>% Silt</td>
<td>1.22</td>
<td>1.44</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td>% Clay</td>
<td>15.47</td>
<td>15.46</td>
<td>15.47</td>
<td></td>
</tr>
</tbody>
</table>

Although kerosene contamination positively influenced these properties since they are critically important in maintaining soil fertility, it comes at a very high price. Kerosene is a product of crude oil which is highly carbonaceous and contains some proportion of nitrogen since crude oil contains varying proportions of nitrogenous substances, thus accounting for the increased levels of carbon and nitrogen in the kerosene simulated soil. Result of the animal waste analysis as seen in table 1 shows high levels of total nitrogen (0.51mg/kg), organic carbon (7.53mg/kg) and organic matter (12.98%) as against values in the control and simulated soils which accounts for their observed increase in the soil after the bioremediation process. The observed values of carbon to nitrogen ratio in the simulated and treated soil follows similar changes in organic carbon and total nitrogen as seen in table 1. Particle size distribution of the soil was unaffected as the sand (83.10-83.31%), silt(1.22-1.44%) and clay(15.46-15.47%) fractions were all in the same range for the control, contaminated and bio-remediated soils. A classification of the soil based on the USDA textural class (USDA,
2002) shows that the soil is loamy sand (coarse textured soil) and its classification according to the soil taxonomy classes shows that it is typic paleudit.

The results of heavy metal analysis gave elevated values of Nickel and vanadium in the kerosene simulated soil (Ni=0.456mg/kg, V=0.912mg/kg) and bio-remediated soil (Ni=0.528mg/kg, V=0.998mg/kg) when compared to the control soil (Ni=0.419mg/kg, V=0.792mg/kg). The increase in metal concentrations in the kerosene simulated soil could be due to contribution of the metals from kerosene since petroleum products have been shown to contain heavy metals (Akpoveta and Osakwe, 2010). Subsequent increase in the bioremediated soil could be on account of contributions of these metals from the animal waste used since heavy metal characterisation of the waste revealed high levels of Ni(0.446mg/kg) and V(0.812mg/kg). Lead recorded a negligible concentration of less than 0.001 in the control, simulated and treated soils showing absence of available form of this metal. Cadmium gave a concentration of 0.025mg/kg in the control soil, less than 0.001mg/kg in the simulated soil and 0.015mg/kg in the bioremediated soil suggesting no anthropogenic input of the metal from the hydrocarbon. Chromium had an increased concentration of 0.062mg/kg in the kerosene simulated soil and 0.043mg/kg in the bioremediated soil as against the control soil with a value of less than 0.001mg/kg. The increased concentration in the contaminated soil could be due to anthropogenic input of the metal from kerosene contaminant. The reduced concentration of 0.043mg/kg in the bioremediated soil as against the kerosene contaminated soil(0.069mg/kg) could be due to the metal been present in fractions that are less bio-available leading to reduced availability of the total metal content after the biodegradation process.

Table 2: Bacterial, Fungal and Total Viable Count for the bioremediation of kerosene simulated soil amended with animal wastes

<table>
<thead>
<tr>
<th>Time(days)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>820x10^5</td>
<td>930x10^5</td>
<td>4.6x10^4</td>
<td>2.67x10^4</td>
<td>4.5</td>
</tr>
<tr>
<td>7</td>
<td>134x10^5</td>
<td>1.21x10^5</td>
<td>7.2x10^3</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>98x10^5</td>
<td>18.9x10^4</td>
<td>7.2x10^3</td>
<td>8.97</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>46x10^5</td>
<td>0.202x10^4</td>
<td>3.2x10^3</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>6.7x10^5</td>
<td>5.2x10^4</td>
<td>2.1x10^4</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>3.6x10^5</td>
<td>3.1x10^4</td>
<td>2.9x10^4</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>4.1x10^5</td>
<td>1.6x10^4</td>
<td>2.3x10^4</td>
<td>9.8</td>
<td></td>
</tr>
</tbody>
</table>

UP : Unpolluted soil sample + Amendment(Animal waste) only

TVCk : Total viable count for soil sample polluted with kerosene+ amendment(Animal waste).

THUBk : Total hydrocarbon utilizing bacteria for Soil sample polluted with kerosene+ Animal waste.

TFk : Total fungal count for soil sample polluted with kerosene+ amendment(Animal waste).

CFU/g: Colony formation unit per gram.

A characterization of the soil microbial composition shows that a total of twelve heterotrophic bacteria are present, of which eight are hydrocarbon utilizers. The heterotrophic bacteria are Alcaligen spp, Bacillus spp, Micrococcus spp, Chromobacterium spp,
Corynebacterium spp, Serratia spp, Pseudomonas spp, Cellulomonas spp, Proteus spp, Flavobacterium spp, Norcardia spp, and Alcaligen spp. The eight hydrocarbon degrading bacteria are Alcaligen spp, Bacillus spp, Chromobacterium spp, Corynebacterium spp, Pseusomonas spp, Aeromonas spp, Serratia spp and Flavobacterium spp. Five hydrocarbon degrading fungi were also isolated and identified, they are: tricodema spp, penicillium spp, Rhizopus spp, fusarium spp and Aspergillus. The concentration of kerosene introduced into the soil drastically affected the growth phase of the soil microbes as seen in table 2 where the population of total heterotrophic microorganisms initially increased from 820x10^5 cfu/g in the control soil to 930x10^5 cfu/g in the kerosene polluted soil on the first day which negates the view that an initial reduction is expected due to the inability of some microbes to thrive under the new hydrocarbon environment; but subsequently decrease from 930x10^5 cfu/g to 134x10^5 cfu/g on the seventh day and thereafter to 4.1x10^5 cfu/g on the last day. The only plausible explanation and justification for this puzzling increase observed on the first day could be that the kerosene instantaneously supported the growth of some microbes before the others which could not adapt to the hydrocarbon environment started dying. The further decrease in microbial population indicates the inability of some heterotrophic microbes to survive the strange kerosene environment. A fluctuating trend was observed in the microbial count for the hydrocarbon utilizing bacteria in the first four weeks (twenty eight days) as seen in table 2, recording both increase and decrease in population at one point or the other. This is suggestive of the inability of some species to adapt properly. While some species are effectively making judicious use of the nutrient and carbon source to multiply, the growth of others are probably been retarded leading to their death due to the high hydrocarbon concentration and the production of toxic metabolites from the hydrocarbon mineralization. A progressive decrease in population was thereafter observed in the last two weeks (twenty eight to forty two days) which could be attributed to unavailability of nutrients, reduced hydrocarbon concentration and extremely high alkaline pH. The inconsistent trend in population of the hydrocarbon utilizing bacteria seems to be associated with the fluctuating alkaline pH suggesting that their growth rate is not favoured at extremely high alkaline pH range. An increase in population of the hydrocarbon utilizing fungi was recorded in the first one week but thereafter decreased progressively as evident in table 2. Availability of sufficient nutrient and carbon source explains the observed increase but a decline in hydrocarbon concentration and lack of nutrients for the build up of microbial cells accounts for the subsequent decrease in fungi population. pH analysis carried out to observe the trend in pH with time within the bioremediation period shows an excessive increase in pH to alkalinity which is due to the high concentration of animal waste supplement used. The change in pH to alkalinity was similar to the findings of Okiemen and okiemen (2005) where change in pH from 6.82 to 8.41 upon application of sludge as nutrient source was also reported. This observation also supports the findings of Naramabuye and Haynes (2006) where the effect of applications of manures to acid soils is an increase in soil pH and the liming-effect of manures was primarily attributable to their high pH and high Ca and Mg carbonate content. Several researchers have shown that the addition of animal manures to acid soils increased pH (Whalen et al. 2000; Materechera and Mkhabela, 2002; Mokolobate and Haynes, 2002a; 2002b; Parham et al., 2002) and these corroborates the observation herein. The pH influence towards alkalinity brought about by the presence of animal waste favours the biodegradation process as seen from the results, but alkaline pH of above 9.5 seems not to favour the growth and multiplication of the hydrocarbon utilizers. Substantial growth and multiplication was noticed at a pH of 8.97 corresponding to a population of 18.9x10^5 cfu/g from 4.6x10^4 cfu/g for the hydrocarbon utilizing bacteria and a pH of 9.12 corresponding to a population of 19x10^4 cfu/g from 2.67x10^3 cfu/g for the hydrocarbon utilizing fungi.
The six weeks (fourty two days) study on the bioremediation of kerosene contaminated soil showed a T.P.H reduction of 82.24 % at the end from an initial T.P.H concentration of 1894.87ppm obtained from 10% spiking of the soil with kerosene. The rate of biodegradation was faster within the first four weeks (twenty eight days) as evident in table 3 recording 73.59% reduction in T.P.H but significantly reduced within the last two weeks (twenty eight to forty two days) . The rate of biodegradation reduces as pH drifted towards very high alkalinity, as microbial population declines and as the hydrocarbon concentration diminishes. It could therefore be inferred from the findings that the suitable pH range for optimum and efficient biodegradation of kerosene in the Niger Delta soil must average between 6.0 and 9.5 as seen from the pH analysis of this study. pH values above 9.5 will not favour rapid biodegradation and microbial growth. The increased rate of biodegradation within the first twenty eight days was also due to high presence of animal waste which provided the required nutrients for the microbes.

Table 4: Concentration of total petroleum hydrocarbon (TPH) in soil(Cs) and TPH biodegraded(Cd) for the diesel simulated soil

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Cs</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1894.87</td>
<td>718.79</td>
<td>648.45</td>
<td>585.93</td>
<td>500.49</td>
<td>373.15</td>
<td>336.60</td>
</tr>
<tr>
<td>7</td>
<td>1176.06</td>
<td>1246.42</td>
<td>1308.94</td>
<td>1394.38</td>
<td>1521.72</td>
<td>1558.27</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Plot of % decrease (weight loss) of TPH in the soil against time for the bioremediation of kerosene simulated soil.

Figure 2: Plot of ln C (TPH) against time for the bioremediation of kerosene simulated soil. Rate constant k equals 0.034 since –k=-0.034.
Figure 3: First order profile for the bioremediation of kerosene contaminated soil

Figure 4: Linear degradation isotherm for the biodegradation of kerosene contaminated soil.
3.1 Kinetics of the biodegradation process

The percentage decrease in total petroleum hydrocarbon (T.P.H) was plotted against time for the biodegradation of kerosene simulated soils as shown in figure 1. The points were linearly related. Correlation analysis of $R^2$ was found to be 0.984 for the biodegradation of kerosene which indicates positive correlation for the reduction in T.P.H with respect to time. Concentration of total petroleum hydrocarbon (TPH) in the soil and ln of TPH concentrations were also plotted against time as shown in figure 2 and 3 in order to analyse the kinetics for the biodegradation processes. The biodegradation process followed first order kinetics with a rate constant of 0.034day$^{-1}$ since plot of TPH concentration in soil against time gave an exponential curve and ln of TPH concentration against time was linear. The degradation pattern was similar to those reported by Peijun et al (2004) and Maletic et al (2009). Correlation analysis of $R^2$ for the biodegradation kinetics process was found to be 0.835, indicating linearity and positive correlations for the decrease in concentration as a function of time. Theoretical predictions using statistical forecast on the existing data’s reveals a 99.8% T.P.H reduction to be achieved on the 10th week (70th day) for the biodegradation of kerosene contaminated soil.

3.2 Kerosene Degradation isotherm for the bioremediation study

Kerosene degradation isotherm at ambient temperature for the bioremediation study of the kerosene polluted soil was computed from the concentrations of total petroleum hydrocarbon degraded by the indigenous microbes and the residual concentration of total petroleum hydrocarbon left in the soil at each time. Concentration of total petroleum hydrocarbon (TPH) degraded by the indigenous microbes was computed by subtracting the amount of residual TPH left after degradation from the initial concentration. Concentrations of residual TPH($C_s$) and TPH degraded($C_d$) are presented in table 4. There are two concentration values representing the concentration of TPH degraded by the indigenous microbes($C_d$) and the residual concentration left in the soil after degradation($C_s$) at each time interval for the bioremediation process. A plot of $C_s$ versus $C_d$ gave a straight line graph with the values of $C_s$ and $C_d$ linearly related. The line plotted through the points is known as the linear biodegradation isotherm and it is giving by $K_d = C_s/C_d$. The biodegradation isotherm for the biodegradation of the kerosene simulated soil is shown in figure 4. The biodegradation isotherms were plotted to the same scale for comparison, and the $K_d$ values were calculated using a linear regression analysis as shown in the linear regression equations. The negative value of unity for $K_d$ shows the opposite trend between $C_s$ and $C_d$, which explains that as the concentration of the contaminant in the soil($C_s$) is decreasing with time, the concentration degraded by the microbes($C_d$) is increasing. The correlation coefficients ($R^2$) computed from the biodegradation isotherm indicates a very positive correlation between the degraded contaminant and the parent contaminant left in the soil during the degradation process at each time for the biodegradation experiment.

4. Conclusion

Kerosene contamination affected soil physicochemical properties such as pH, conductivity, total phosphorus, microbial biomass and heavy metal content which are important indicators for assessing soil quality, fertility and productivity. Conditions that will ensure effective biodegradation must be satisfied for optimum, effective and efficient clean up to occur. It was seen from the findings of this study that the rate of microbial growth and hydrocarbon breakdown were pH dependent. Sufficient nutrient availability also favoured high
degradation rate at the early stage of the process. Nutrient availability and pH must therefore be considered as very important conditions to be satisfied for an effective and efficient biodegradation process to occur within a short time.

5. References


