Modelling Phytoremediation Augmented Bioremediation based on Biomass and Yield Kinetics

Musa, A.S.¹, Oyoh, K.B.², Osoka, E.C.³, Onyelucheya, O.E.⁴
¹Department of Petroleum Resources, Abuja, NIGERIA
²,³,⁴Department of Chemical Engineering, Federal University of Technology, OWERRI

ABSTRACT

Bioremediation and phytoremediation of crude oil contaminated soils has been studied based Total Petroleum Hydrocarbon content, using Locoweed and Sunflower as the plants for phytoremediation. The data was fit to models based on a biomass growth rate and yield coefficient. The results reveal that bioremediation and phytoremediation augmented bioremediation are based on the same mechanism. During both processes the biomass grows according to the logistic model (with inhibition as the amount of substrate is depleted) and the rate of production of biomass per unit substrate consumed can be considered constant. Locoweed shows higher effectiveness in enhancing the remediation process in comparison to Sunflower. Bioremediation reduces the contaminant concentration by about 65%, while when augmented with Phytoremediation, the contaminant concentration is reduced by 69% and 88% for Sunflower and Locoweed respectively.

Keywords----- Bioremediation, Phytoremediation, hydrocarbon, substrate, contaminated, model

I. INTRODUCTION

Petroleum hydrocarbons represent a complex mixture of organic compounds mainly grouped into four fractions: alkenes, aromatics, resins and asphaltenes (Ruijuan et al, 2013). Hydrocarbon pollution of the environment has remained a major challenge for man over the years and has been escalating in proportions with increase in industrial activities. Such pollutions are usually occasioned by human error, equipment failure, vandalism, wars and natural disasters. Prominent among the deleterious effects of such pollutions on land is the destruction of natural flora and fauna thereby ultimately reducing the capacity of the ecosystem to support life. Several techniques have been developed over the years to combat this menace. These techniques are grouped broadly into two namely; In-situ methods (such as leaching or washing, isolation and containment, volatilization, bioremediation and passive bioremediation) and Ex-situ methods (such as incineration, solidification and stabilization, soil washing, and land farming) (Das and Mukherjee, 2007). Bioremediation is a term that describes the deliberate use of organisms to remove or reduce man-made pollution. Bioremediation is the use of biological methods in restoring contaminated land, principally by the addition of bacteria and other micro-organisms that consume or neutralize contaminants in the soil (Gibson and Salyer, 1992). Microorganisms have been known to degrade hazardous compounds considered recalcitrant and resistant to biodegradation. Advantages of biological bioremediation compared to other treatment methods include destruction rather than transfer of contaminants to another medium, minimal exposure of workers to contaminants, longtime protection of public health and possible reduction in the duration of the remediation process (Okoh and Trejo-Hernandez, 2006; Machin-Ramitez et al, 2008). The natural bioremediation process usually needs to be enhanced because hydrocarbon biodegradation in soil can be limited by many factors such as nutrients, pH, temperature, moisture, oxygen, soil properties and contaminant presence (Atagana, 2008). Most of these limiting factors can be controlled but metals concentration poses more difficulty as mobility of microbes’ enzyme degradation is impaired. This limiting factor and moisture content can be minimized through the employment of phytoremediation technology. Phytoremediation is the use of living green plants for reduction and/or removal of contaminants from polluted soil, water and air. Phytoremediation is based upon the basic physiological mechanisms taking place in higher plants and associated microorganisms such as transpiration, photosynthesis, metabolism and mineral nutrition.
analyses indicated a decrease in the level of hydrocarbons present after phytoremediation. There was equally, a significant reduction in growth parameters of the plant such as plant height, leaf number, tiller number and total dry weight, compared to the control. Anatomical studies of sections of the plants, stems did not reveal the presence of accumulated oil within the tissues but rather denatured internal parenchyma cells traction ways observed. Bacteria capable of degrading hydrocarbon were isolated from the rhizosphere of the grass. The isolates include Arthrobacter sp, Bacillus pumilus, Bacillus sphaericus and Serratiamarcescens. Growth in mineral salts medium supplemented with 0.5% crude oil for 21 days resulted in 95.9%, 95.6%, 98.3% and 96.7% degradation of oil for Arthrobacter sp., B. pumolus, S. Macescens and B. Sphaericus respectively. A soil microcosm set up with the consortium of the isolates resulted in 87.70% degradation of crude oil in 45 days.

Ezonu (2013) studied the decomposition of used motor oil in soil as influenced by plant treatment. In their work, soil was contaminated with used motor oil to a concentration of 1.5% w/w. The contaminated soil was seeded with soyabean (Glycine max)/green bean (Phaseolus vulgaris); sunflower (Helianthus annus)/Indian mustard (Brassica juncea); mixed grasses/maize (zea mays); and mixed clover (red clover, Trifoliumpratense/ladino clover, Trifoliumrepens) and incubated Soxhlet-extractable oil and grease remaining in the soil was monitored after 100 and 150 days. After 150days in the clover treatment, the added oil was no longer detected. A total of 67% of the oil was removed in sunflower/mustard, and with addition of NPK fertilizer, the oil was completely removed. The grass/maize treatment resulted in a 38% oil reduction, which increased to 67% with fertilizer application. The control treatment reduced oil in the soil by 82% when fertilizer was added. At 150 days the sunflower/mustard and wheat/oats treatments produced the greatest biomass in the presence of used oil. Gas chromatograph/Mass spectroscopy (GC/MS) spectra of oil/grease extracts revealed the presence of new peaks associated with hydrocarbon decomposition. The presence of new hydrocarbons was corroborated by changes in Fourier- transformed infrared spectroscopy (FTIR) spectra. Fertilizer addition during treatments resulted in negligible changes to FTIR bands. Based on oil/grease residues and biomass results, the clover and sunflower/mustard are considered superior to the other plant treatments in terms of overall phytodegradation of used oil hydrocarbons.

Ajoy et al (2012) evaluated the use of phytoremediation to clean up soils contaminated with weathered curded oil. Alkane’s Total Petroleum Hydrocarbon (TPH) and polycyclic Aromatic Hydrocarbon (PAH) degradation level in the crude-oil contaminated soil was significantly higher in rhizosphere soil as compared to bulk soil. Results from their study showed that TPH levels
after 6 months were significantly lower in vegetated fertilized plots than in non-vegetated, non-fertilized plots. Vegetation establishment and fertilizer addition result in increased bacterial and fungal degradation levels.

Microbial Growth: The mathematical description of the rate of growth of a microbial culture frequently makes use of an exponential growth pattern. This is based on the premise that the growth rate is directly proportional to the existing population and the proportionality constant is a function of the organism type. Malthus’ law gives exponential growth as:

$$\frac{dX}{dt} = \mu X$$

On integration it gives:

$$X = X_0 e^{\mu t}$$

This growth, however, cannot be sustained indefinitely and for one reason or another will lead to a stationary phase. The exponential growth equation was modified by adding a further term to account for ‘inhibition’ at high biomass concentration:

$$\frac{dX}{dt} = \mu X - \mu \gamma X^2$$

On integration it gives:

$$X = \frac{X_0 e^{\mu t}}{1 - \gamma X_0 (1-e^{\mu t})}$$

This is the ‘logistic equation’.

Substrate degradation and yield coefficient: The growth of a microbial culture, consuming substrate for energy purposes, for incorporation into its own cellular material, or for synthesis of a product, gives rise to the concept of yield. Yield is ratio of mass of product obtained to that of reactant consumed and is expected to be constant for given reaction conditions. In more sensitive experiments the yield appears not to be a constant quantity, but a function of time as well as the physico-chemical environment. This is the result of the changing composition of the microbial cell and the phenomenon of adaptation.

When the yield is considered constant we have:

$$Y = \frac{\Delta X}{\Delta S}$$

A material balance for the consumption of substrate gives

$$\frac{dS}{dt} = \frac{1}{Y_G} \frac{dX}{dt} + mX$$

Substrate consumed for growth is usually much larger than that consumed for maintenance, such that equation (6) can be simplified thus:

$$\frac{dS}{dt} = \frac{1}{Y_G} \frac{dX}{dt}$$

Modification of Yield coefficient: Oyoh and Osoka (2007), in their study of NPK fertilizer enhanced bioremediation, proposed a new definition for the yield coefficient at times when it is not a constant quantity. In this definition averages of mass of product obtained (change in biomass concentration) and average of reactant consumed (change in substrate concentration) are used instead. They theorized that the use of averages will normalize the variation of yield with time, so we have:

$$Y_m = \frac{\Delta X / X}{-\Delta S / S}$$

Equation (7) will take the form:

$$\frac{1}{S} \frac{dS}{dt} = \frac{1}{Y_{mg}} \frac{1}{X} \frac{dX}{dt}$$

This may be written in the form:

$$\frac{d(\ln S)}{dt} = \frac{1}{Y_{mg}} \frac{d(\ln X)}{dt}$$

On Integration it gives:

$$S = S_o \left( \frac{e}{\gamma} \right)^{Y_{mg}}$$

Equations (7) and (11) can be applied for either exponential growth model or logistic model.

If microbial growth rate is exponential in nature and yield is constant we have:

$$S = S_o + \frac{X_o}{Y_G} \left[ 1 - e^{\mu t} \right]$$

If microbial growth rate is exponential in nature with the yield not being constant we have:

$$S = S_o \exp \left( \mu t \right) \frac{1}{Y_{mg}}$$

If microbial growth rate is logistic in nature with the yield being constant we have:

$$S = S_o + \frac{X_o}{Y_G} \left[ 1 - \frac{e^{\mu t}}{1 - \gamma X_0 (1-e^{\mu t})} \right]$$

If microbial growth rate is logistic in nature with the yield not being constant we have:

$$S = S_o \left( \frac{\exp \left( \mu t \right)}{1 - \gamma X_0 (1-\exp \left( \mu t \right))} \right)^{Y_{mg}}$$

If death sets into microbial growth, it can be represented with the model below (Musa, 2015)

$$x = x_o \exp \left( \mu t (k - t/2) \right)$$

Equation (7) which relates substrate concentration and biomass concentration at constant yield can be integrated and represented thus:

$$S = S_o + \frac{1}{Y_{mg}} (x_o - x)$$
Substituting equation (16) -which represents a situation when death of biomass has set in – into equation (17), we have;

\[ S = S_o + \frac{x_o}{Y_o} (1 - \exp (\mu t \times (k - t/2))) \]  

(18)

For situations where there is death of biomass and yield is not constant, we substitute equation (16) into equation (11) to obtain;

\[ S = S_o \exp (\mu t \times (k - t/2))^{\frac{1}{Y_o}} \]  

(19)

The above equations can be used to fit experimental data in order to obtain the appropriate rate model for the degradation of the substrate through bioremediation.

II. METHODOLOGY

Soil samples were collected from Erema town of Ogba-Egbema-Andoni LGA of Rivers State, Nigeria on 4° 55' 55"N and 6° 32' 48"E. Experimentation was carried out in microbiology and Chemical/Petrochemical Engineering laboratories in Rivers State University of Science and Technology, Port Harcourt. Mud auger were used at 0-30cm depth to collect muddy polluted soil. Glass containers fitted with plastic lids were used. The microbiology laboratory was used to isolate existing native microbes present in each sample. Microbes so isolated were cultured and identified. The soil samples were inoculated with the cultured microbes and nutrients addition was controlled to allow for increase in microbial population and hence increase in degradation rate of carbon source. This involved the application of nitrogen and phosphorous fertilizers. Sunflower and Locoweed Plantlets were sought for and planted in two of the polluted soil samples and studied with aim of examining the effect of flora and fauna applications in bioremediation, while the third soil sample had nothing planted on it.

Residual oil and its fraction were determined by adding 10g of anhydrous sodium sulphates to 10g of air-dried soil samples. The hydrocarbons were Soxhlet extracted and evaporated in a pre-weighed dish and the amount of the total petroleum hydrocarbons (TPHs) was determined and the loss (%) of TPH was calculated. This was done in two weeks intervals for twenty weeks and recorded.

III. RESULTS AND ANALYSIS

The following graphs show the graphical fits for the bioremediation based on the nature of biomass growth.
It can be observed from Fig. 1 to Fig. 6 that the model fit to experimental data is poor for Fig. 1, Fig. 2, Fig. 5 and Fig. 6 but good for Fig. 3 and Fig. 4. The numerical fit results based on the Adjusted-$R^2$ show that the bioremediation process fit most to the model for Logistic growth with constant yield. It thus suggests that the biomass during bioremediation grow according to the logistic model, with inhibition as the amount of substrate is depleted and the rate of production of biomass per unit substrate consumed is constant, which is, the constant yield idea.

The following graphs show the fit for the Sunflower assisted phytoremediation based on the nature of biomass growth.

<table>
<thead>
<tr>
<th>Rate Model equation</th>
<th>$\frac{S_0}{Y_G}$ or $Y_mG$</th>
<th>$\mu$</th>
<th>$\gamma X_o$ or $k$</th>
<th>$R^2$</th>
<th>Adj-$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S = S_o \frac{X_o}{Y_G} [1 - e^{\mu t}]$</td>
<td>723.5</td>
<td>5.972e-5</td>
<td>-</td>
<td>0.1910</td>
<td>0.1012</td>
</tr>
<tr>
<td>$S = S_o \exp (\mu t)^\gamma G$</td>
<td>10.03</td>
<td>0.8162</td>
<td>-</td>
<td>0.6823</td>
<td>0.6471</td>
</tr>
<tr>
<td>$S = S_o + \frac{X_o}{Y_G} \left[1 - \frac{e^{\mu t}}{1 - \gamma X_o [1 - e^{\mu t}]}\right]$</td>
<td>0.09935</td>
<td>0.7036</td>
<td>0.137</td>
<td>0.9948</td>
<td>0.9935</td>
</tr>
<tr>
<td>$S = S_o \left(\frac{\exp (\mu t)}{1 - \gamma X_o (1 - \exp (\mu t))}\right)^\gamma m_G$</td>
<td>16.76</td>
<td>2.268</td>
<td>8.46e-8</td>
<td>0.9907</td>
<td>0.9897</td>
</tr>
<tr>
<td>$S = S_o + \frac{X_o}{Y_G} (1 - \exp (\mu t (k - t/2)))$</td>
<td>332.7</td>
<td>3.061e-5</td>
<td>11.8</td>
<td>0.7708</td>
<td>0.7135</td>
</tr>
<tr>
<td>$S = S_o \exp (\mu t (k - t/2))^{\gamma m_G}$</td>
<td>42.51</td>
<td>0.499</td>
<td>14.27</td>
<td>0.9670</td>
<td>0.9588</td>
</tr>
</tbody>
</table>
It can be observed from Fig. 7 to Fig. 11 that the model fit to experimental data is poor for Fig. 7, Fig. 8, Fig. 11 and Fig. 12 but good for Fig. 9 and Fig. 10. The numerical fit results, based on the Adjusted-$R^2$ show that the phytoremediation augmented bioremediation process using Sunflower fit most to the model for Logistic growth with constant yield. It thus suggests that the biomass during phytoremediation augmented bioremediation process grows according to the logistic model, with inhibition as the amount of substrate is depleted and the rate of production of biomass per unit substrate consumed is constant, which is, the constant yield idea.

The numerical fit result summary for the Phytoremediation with Sunflower based on the nature of biomass growth is given in Table 2 below.

<table>
<thead>
<tr>
<th>Rate Model equation</th>
<th>$X_a/Y_G$ or $Y_{mg}$</th>
<th>$\mu$</th>
<th>$\gamma X_a$ or $k$</th>
<th>$R^2$</th>
<th>Adj-$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S = S_o + \frac{X_a}{Y_G} [1 - e^{\mu t}]$</td>
<td>1164</td>
<td>3.93e-5</td>
<td>-</td>
<td>0.2756</td>
<td>0.1951</td>
</tr>
<tr>
<td>$S = S_o \exp (\mu t) \frac{1}{Y_{mg}}$</td>
<td>8.973</td>
<td>0.8162</td>
<td>-</td>
<td>0.7655</td>
<td>0.7394</td>
</tr>
<tr>
<td>$S = S_o + \frac{X_a}{Y_G} \left[ 1 - \frac{e^{\mu t}}{1 - \gamma X_a \left( 1 - e^{\mu t} \right)} \right]$</td>
<td>0.1160</td>
<td>0.6386</td>
<td>0.1483</td>
<td>0.9971</td>
<td>0.9963</td>
</tr>
<tr>
<td>$S = S_o \left( \frac{\exp (\mu t)}{1 - \gamma X_a (1 - \exp (\mu t))} \right) \frac{1}{Y_{mg}}$</td>
<td>0.1160</td>
<td>0.6386</td>
<td>0.1483</td>
<td>0.9971</td>
<td>0.9963</td>
</tr>
<tr>
<td>$S = S_o + \frac{X_a}{Y_G} (1 - \exp (\mu t (k - t/2)))$</td>
<td>250.9</td>
<td>3.93e-5</td>
<td>12.27</td>
<td>0.8552</td>
<td>0.8190</td>
</tr>
</tbody>
</table>
The graphical results for the locoweed assisted phyto-remediation based on the nature of the biomass growth are given in the following graphs.

Table 3: Numerical Fit Results for the Locoweed assisted Phytoremediation Biomass growth

<table>
<thead>
<tr>
<th>Rate Model equation</th>
<th>$\frac{X_o}{Y_G}$ or $Y_{mg}$</th>
<th>$\mu$</th>
<th>$\gamma X_o$ or $k$</th>
<th>$R^2$</th>
<th>Adj-$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S = S_o + \frac{X_o}{Y_G} \left[1 - e^{\mu t}\right]$</td>
<td>660</td>
<td>7.973e-5</td>
<td>-</td>
<td>0.6872</td>
<td>0.6524</td>
</tr>
<tr>
<td>$S = S_o \exp\left(\mu t \frac{1}{Y_G}\right)$</td>
<td>7.319</td>
<td>0.8162</td>
<td>-</td>
<td>0.9585</td>
<td>0.9538</td>
</tr>
</tbody>
</table>
from North-East India”, Bioresource Technology, 98: 1339 – 1345.

REFERENCES