RATE MODEL FOR BIOREMEDIATION BASED ON TOTAL HYDROCARBON CONTENT

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ABSTRACT
Three bioremediation options, including a control, were studied based on reduction in Total Hydrocarbon Content, and hydrocarbon utilizing bacteria (HUB) count, among other factors, for eight weeks, with measurements taken at two-week intervals, using Rumuekpe community of Rivers State Nigeria as a case study.
Models were developed for the microbial (HUB) growth rate and reduction in Total hydrocarbon content (THC) and fit to the experimental data.
Results reveal that the treatment options improve the percentage reduction in Total Hydrocarbon content (THC) from 2% (for the control) to as much as 89%. The microbial growth rate follows the exponential growth curve for all treatment options that involve addition of NPK fertilizer, but follow the logistic growth curve for the treatment option where NPK fertilizer was not added**.**
For all cases where NPK fertilizer was added, the substrate (crude oil) degradation rate curve revealed that the yield was not constant, but for the control, without addition of NPK fertilizer, the substrate (crude oil) degradation rate curve showed that the yield remains constant.

Key words: Bioremediation, Biodegradation, yield, logistic, exponential.

1 INTRODUCTION

When oil spills occur, oil-degrading bacteria responds to the elevated supply of carbon by undergoing exponential growth, as they feed on and secrete enzymes that degrade the pollutant. As the more susceptible components are degraded, the microbial population selectively adapt themselves to the changing composition of the remaining oil (Atlas et al, 1991; atlas and Bartha, 1993: Atlas, 1998).

This natural process by which microorganisms break down or degrade petroleum hydrocarbons into products such as CO_2 and H_2O and partially oxidized biologically inert by-products is called biodegradation (Onwurah, 2000). Bioremediation is the optimization of biodegradation. This is necessary because appropriate specific pollutant degrading microorganisms are always present at target sites but however, at low concentrations (Bossert and Bartha, 1984).

Bioremediation causes less damage to the environment, and many results from such clean up technology show that this procedure is effective, safe to humans and environmentally friendly. It can also be done at reduced cost, less time and with less risk of exposure to hazardous wastes for workers and those who live near the sites (Onwurah, 2000). However, Rittman (1993) reports that bioremediation is still associated with mystery and controversy because of its multidisciplinary nature, unpredictability of bioremediation in the field and lack of understanding on how to combine molecular biology with existing engineering practice.

Gibb et al (2001) studied comparatively the Biodegradation of Alberta Sweet Mix crude oil at 5^0C and at ambient temperatures (21^0C) by using a gas chromatography to determine CO_2 production rates.

The result of the experiment suggested that temperature only affected the biodegradation rates of crude oil in the initial phase of the biodegradation process. After approximately three months, the degradation rates of crude oil at 5^0C and 21^0C were similar.

Kashir et al (2007) studied the kinetics of remediation of soils contaminated with hydrocarbons by low-temperature oxidation (LTO).

Their work revealed that the mechanism of LTO could be divided into four groups: Original maltenes comprised of slow-reacting and volatile maltenes. Slow-reacting maltenes react with oxygen to produce alphaltenes and water, alphaltenes undergo polymerization and dehydrogenation to form coke, product-volatile maltenes, and hydrogen; the volatile maltenes oxidize to form carbon oxides and water.

Rate of bioremediation vary with the soil, kind of environment, compound to be degraded, its concentration in the environment and microbial population ecology. A wide variety of non-linear models have been developed for the description of patterns of biodegradation of organic compounds that would occur in a host of different environmental circumstances including.

This work seeks to develop a model for rate of bioremediation of polluted soils under selected treatments, including addition of nutrients to enhance growth of indigenous bacteria.

2 MODEL DEVELOPMENT

2.1 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:

$$\frac{dX}{dt} = \mu X \tag{1}$$

On integration it gives:

$$X = X_0 e^{\mu t} \tag{2}$$

This growth, however, cannot be sustained indefinitely and for one reason or another will lead to a stationary phase. Pearl and Reed [9] modified the exponential growth equation by adding a further term to account for 'inhibition' at high biomass concentration:

$$\frac{dX}{dt} = \mu X - \mu\gamma X^2 \tag{3}$$

On integration it gives:

$$X = \frac{X_0 e^{\mu t}}{1 - \gamma X_0 (1 - e^{\mu t})} \tag{4}$$

Which is the 'logistic equation'.

2.2 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:

$$Y = \frac{\Delta X}{-\Delta S} \tag{5}$$

A material balance for the consumption of substrate gives

$$\frac{dS}{dt} = \frac{1}{Y_G}\frac{dX}{dt} + mX \tag{6}$$

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

$$\frac{dS}{dt} = \frac{1}{Y_G}\frac{dX}{dt} \tag{7}$$

2.3 Modification of Yield coefficient

For the purpose of this study, a new definition is proposed 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. The use of averages will normalize the variation of yield with time, so we have:

$$Y_m = \frac{\Delta X / X}{-\Delta S / S} \tag{8}$$

Equation (7) will take the form:

$$\frac{1}{S}\frac{dS}{dt} = \frac{1}{Y_{mG}}\frac{1}{X}\frac{dX}{dt} \tag{9}$$

Which may be written in the form:

$$\frac{d(\ln S)}{dt} = \frac{1}{Y_{mG}}\frac{d(\ln X)}{dt} \tag{10}$$

On Integration it gives.

$$S = S_0\left(\frac{X}{X_0}\right)^{\frac{1}{Y_{mG}}} \tag{11}$$

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

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

$$S = S_0 + \frac{X_0}{Y_G}\left[1 - e^{\mu t}\right] \tag{12}$$

If microbial growth rate is exponential but yield is not constant we have:

$$S = S_0\left(e^{\mu t}\right)^{\frac{1}{Y_G}} \tag{13}$$

If microbial growth rate has inhibition with yield being constant we have:

$$S = S_0 + \frac{X_0}{Y_G}\left[1 - \frac{e^{\mu t}}{1 - \gamma X_0\left(1 - e^{\mu t}\right)}\right] \tag{14}$$

If microbial growth rate has inhibition and the yield is not constant we have:

$$S = S_0\left(\frac{e^{\mu t}}{1-\gamma X_0(1-e^{\mu t})}\right)^{\frac{1}{Y_G}} \quad (15)$$

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.

3 SAMPLING METHOD

Soil samples were collected by the aid of soil auger at a depth to at least 30cm. This was done before the commencement of treatment to enable the comparison on efficiency of treatment options. Samples were collected at four sampling points including a control. Each sample point measured $5m^2$

Schematic representation of treatment options carried out on the samples in the presence of indigenous hydrocarbons utilizing bacteria (HUB) are:

(1) Hot water washing + fertilizer application + tilling
(2) Soil mixing (contaminated + uncontaminated soils) + fertilizer application + tilling
(3) Fertilizer application + tilling and (4) Control

Fertilizer was applied to achieve optimum loading of nutrients; NPK fertilizer (15:15:15) was used. Physicochemical analysis was performed on the samples to determine the Moisture content, pH, Total hydrocarbon content, Organic carbon content, Hydrocarbon utilizing bacteria count among others, immediately after treatment and at two-week intervals for a period of eight weeks and records taken.

4 DISCUSSION OF RESULTS

The pH readings from Figure 1 shows a slight decrease in pH value – increase in soil acidity - for the first few weeks followed by an increase, except for the treatment 4 (control) which shows a consistent decrease in pH value.

Decrease in pH value can be due to local decomposition of organic residues to acid or CO_2 evolution, while the increase in pH value may be a result of bicarbonate accumulation during biodegradation of petroleum compounds by microorganisms (Rangaswani and Bagyaraj, 1993).

There was significant increase in the moisture contents (Figure 2) of soils for all options of remediation treatment. The increase in moisture content indicates that the microorganisms degraded hydrocarbons in the soil and water (one of the two products of biodegradation, the other being carbon dioxide) was produced.

Figure 3 shows the total hydrocarbon content only degraded by two percent (2%) for treatment 4 (control), while treatment 3 (which involves tilling plus fertilizer application) gave the highest total hydrocarbon degradation (89%), in eight weeks.

Figure 4 shows that the carbon to nitrogen (C/N) ratio reduces for all treatment options except for treatment 4 that shows slight increase. The control (treatment 4) follows a different mechanism from the other options and causes an increase in the amount of organic carbon.

Figure 5 shows that the Hydrocarbon Utilizing Bacteria (HUB) increased only slightly for treatment 4 (control) in comparison with other treatment options. This indicates that the NPK fertilizer enhanced bioremediation treatment and is effective in degrading hydrocarbon in petroleum polluted soils.

From Tables 1,it can be observed that treatment option 4 has the highest initial Hydrocarbon Utilizing bacteria (HUB) count. This means that the process of tilling before fertilizer application has a negative effect on the initial HUB count.

The numerical fit results of Tables 1 show that for all treatment options involving the addition of NPK fertilizer, the Hydrocarbon Utilizing Bacteria (HUB) grows exponentially, without inhibition, while the treatment 4 (control) has its Hydrocarbon Utilizing Bacteria (HUB) growing according to the Logistic growth curve (indicating the presence of inhibition).

Treatment 2 (which involves soil mixing plus tilling plus fertilizer application) has the highest specific growth rate for the hydrocarbon-utilizing bacteria, followed by treatment 3 (which involves tilling plus fertilizer application).

The numerical fit results in Tables 2 shows that for treatments 1,2 and 3, which involve the application of fertilizer, the yield is not constant. The experimental data for the three treatment options thus fit the rate model based on the modified yield coefficient for the substrate consumption rate while treatment 4 (control) follows the rate model based on the assumption of a constant yield coefficient.

5 CONCLUSION

Biodegradation occurs naturally without any treatment but at a relatively low pace (two percent THC reduction in eight weeks), which calls for enhanced bioremediation with nutrient supplementation.

The microbial growth rate follows the exponential growth curve for all treatment options that involve addition of NPK fertilizer, but follows the logistic growth curve for the treatment option where NPK fertilizer was not added.

For all cases where NPK fertilizer was added, the substrate (crude oil) degradation rate curve followed a modified equation for situations where yield cannot be assumed constant: $S = S_0\left(e^{\mu t}\right)^{\frac{1}{Y_G}}$.

For the case of treatment 4 (control), the substrate (crude oil) degradation rate curve followed the equation for situations where yield can be considered as being constant: $S = S_0 + \frac{X_0}{Y_G}\left[1-\frac{e^{\mu t}}{1-\gamma X_0\left(1-e^{\mu t}\right)}\right]$

The best bioremediation option is that involving tilling plus fertilizer application (without soil mixing or hot water washing) – treatment 3.

This work can be used to optimize remediation of polluted sites, especially those of Rumuekpe community, and as a basis for study in other communities.

6 NOMENCLATURE

6.1 List of Symbols

X	hydrocarbon utilizing bacteria (HUB) count	(cfu/g)
S	substrate concentration	(ppm)
Y	yield	(cfu/g.ppm)
m	maintenance coefficient	(g.ppm/cfu.week)
t	time	(weeks)
μ	specific growth rate	$(week)^{-1}$
γ	reciprocal of final HUB count	(g/cfu)

6.2 Subscripts

G	value used for microbial growth
O	initial values
m	maximum value
MG	value used for microbial growth for modified yield

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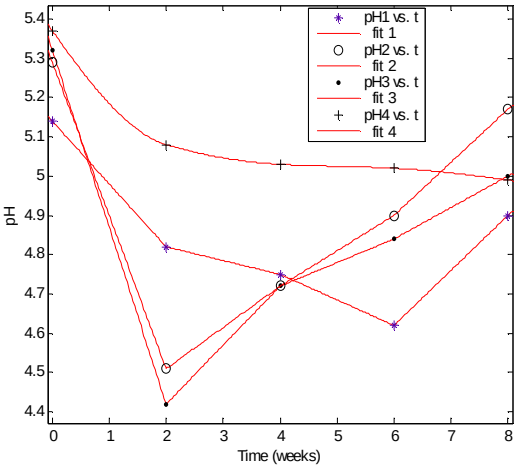

Figure 1:Plot of pH versus time

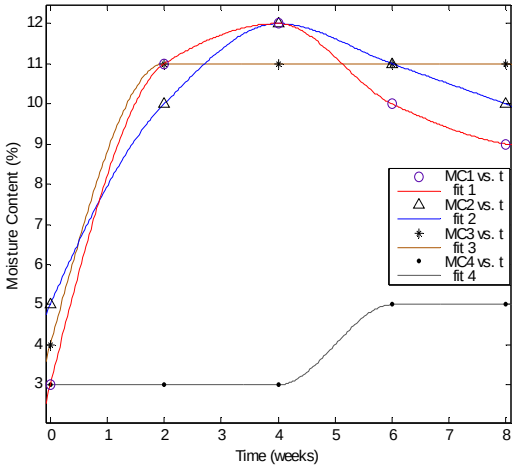

Figure 2: Plot of Moisture content versus time

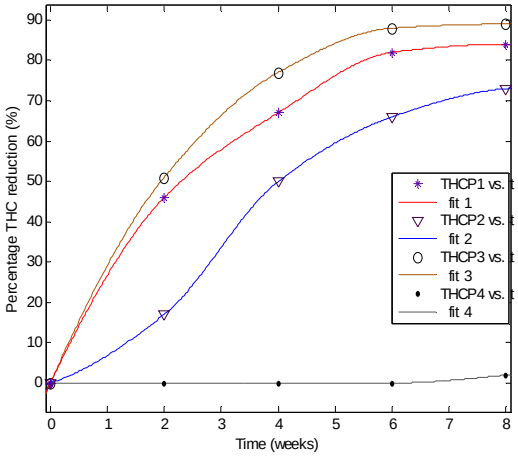

Figure 3: Plot of percentage THC versus time

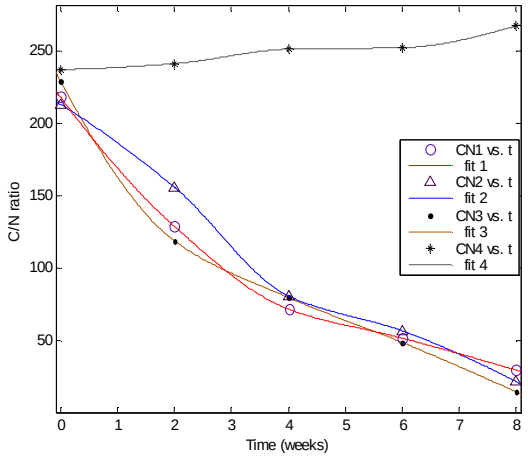

Figure 4: Plot of C/N ratio versus time

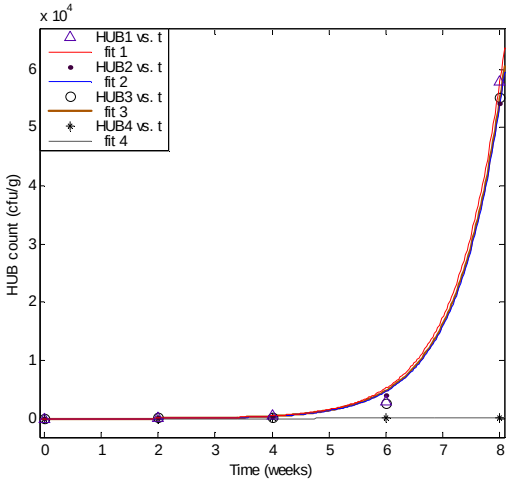

Figure 5: Plot of HUB count versus time

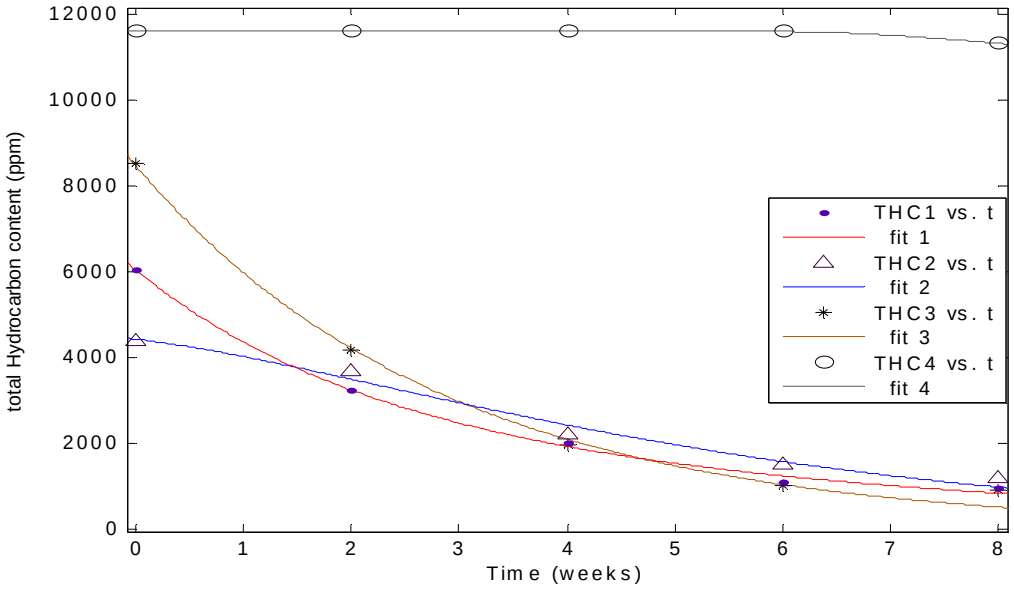

Figure 6: Plot of THC vs. time

Table1: Numerical fit Results For Growth of Bacteria

Model Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4(control)
Xo (cfu/g)	3.90	3.00	3.30	6.30
μ (Week^{-1})	1.201	1.225	1.215	0.6513
γ (g/cfu)	0	-	-	0.0191608
R^2	0.9979	0.9997	0.9977	0.9131

Table 2: Numerical fit Results For Substrate Consumption

Model Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4(control)
Y_G (cfu/g ppm)	-	-	-	1901
Y_{MG} (cfu/g ppm)	4.2955	7.6511	3.4892	-
γ (g/cfu)	-	-	-	-7.637e-6
R^2	0.9920	0.9645	0.9959	0.9999