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Kinetic Model for Bioremediation of Crude Oil Polluted Soil Using *Pseudomonas aeruginosas* as Biodegrader

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Abstract: Theoretical models for biodegradation of crude oil spill have been developed. The model was formulated on specific rate of biodegradation of the crude oil for both single and multiple microbial catalyzed reactions. The formulated model was tested for biodegradation of the diesel oil with experimental data obtained. The result of the simulation showed that the models are suitable for predicting the rates of biodegradation of hydrocarbon mixtures.

Key words: Biodegradation, *Pseudomonas aeruginosa*, modelling

INTRODUCTION

One of the most serious forms of water pollution is oil spill and it has often been used in connection with losses of crude oil or petroleum products to the marine environment (Atlas, 1995). About 10% of the oil spilling into the sea comes from tanker accidents. A typical example is the wreck of Exxon Valdez in 1989 that spilled 240,000 barrels (30,000 tons) of crude oil into Prince William Sound in Alaska (Jordan and Payne, 1999). The frequency of large-scale spillage of petroleum and its products in the last two decades has been alarming and the pollution it has caused has resulted in adverse ecological effects (Amadi *et al*., 1993).

Thus, considerable research interests have been focused not only on the way of delivering rapid clean-up operations for combating this pollution concern but also on the study of the weathering process of petroleum hydrocarbon with a view to eliminating the contaminants. Various clean-up methods by mechanical and chemical means are in vogue as well as natural weathering processes. Similarly, the degradation of this product by microorganisms is well documented (Bragg *et al*., 1994; Atlas, 1995; Wami and Ogoni, 1997; Sani and Ajisebutu, 2003; Olu-Arotiowa *et al*., 2007).

Microorganisms convert the hydrocarbons into carbon IV oxide and water with the release of energy and cell mass, which are essential to the microbial growth, development and activities (Rozas *et al*., 2000). The activities of hydrocarbon degraders have attracted the attention of researchers in recent years. Investigation into this area is aimed at improving the rate of degradation of petroleum products so as to ameliorate both terrestrial and marine environments.

Over the years, a lot of studies have been reported on studies of petroleum hydrocarbon degraders (Abowei and Susu, 1989) and in particular the inadequacies in studying the kinetics of petroleum hydrocarbon degradation. Some of the areas in which kinetic models have been developed include: Bioremediation of polluted environment. Biotreatment of industrial effluent and Microbial enhanced oil recovery.

A lot of works have been done on kinetics of biotechnology in the petroleum industry, particularly enhanced oil recovery (Onwioduokit, 1993). These detailed and embracing works on bio-kinetics have neglected environmental aspects of the industry, an area that is now a major concern to environmentalists. Nwanchukwu *et al*. (1998) in their report noted that few studies have addressed the issue of bioremediation potential of crude oil waste.

Although there is no comprehensive and conclusive report on the kinetics of biodegradation of crude oil (Rockne *et al*., 2000), various kinetic models have been developed to predict the biodegradation of petroleum hydrocarbon components or associated components (Rozas *et al*., 2000). Hence a lot of deficiencies exist on the kinetics of petroleum hydrocarbon mixtures. This research is geared towards the development of theoretical kinetic models for biodegradation of diesel oil in a single and multiple microbial catalyzed reactions.

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Model formulation: The mathematical formulations for the following conditions were developed:

- A reaction catalyzed by a single microorganism.
- A reaction catalyzed by multiple microorganisms.

Single microbe catalyzed reaction: Generally, a microbial catalyzed reaction of a given substrate can be presented since the rate of product formation depends on the dissociation of product forming complex. The rate of reaction can be written as:

$$V^* = K_P[EH] \quad (1)$$

A material balance equation for the distribution of the total microorganisms in the reacting system can be expressed as:

$$[E_T] = [E]+[EH] \quad (2)$$

Combining Eq. 1 and 2 gives

$$\frac{V^*}{[E^T]} = \frac{K_P[EH]}{[E]+[EH]} \quad (3)$$

The dissociation constant K_S can be expressed as the ratio of the reverse rate constant k_{-1} to the forward rate constant k_1 i.e.,

$$K_S = \frac{k_{-1}}{k_1} = \frac{[E][H]}{[EH]} \quad (4)$$

$$[EH] = \frac{[E][H]}{K_S} \quad (5)$$

Substituting Eq. 5 into Eq. 3 gives

$$\frac{V^S}{[E_T]} = \frac{K_P\frac{[E][H]}{K_S}}{[E]+[E][H]} = \frac{K_P\frac{[H][E]}{K_S}}{[E]+\frac{[H][E]}{K_S}} \quad (6)$$

The reaction rate will be maximum when the total microbes form a complex, hence

$$V_{S\,max} = K_P[E_T] \quad (7)$$

Combining Eq. 6 and 7 gives

$$\frac{V^S}{V^S{}_{max}} = \frac{[H]}{K_S+[H]} \quad (8)$$

Hence,

$$V^S = V^S{}_{max}\frac{[H]}{K_S+[H]} \quad (9)$$

Equation 9 is the usual form of Henry Michael's equation. With petroleum hydrocarbon mixture as the growth limiting substrate, assuming H_1, H_2, H_3,……H_n are the hydrocarbons present, Eq. 9 can be expressed as:

$$V^S = V^S{}_{max}\left[\frac{[H_1]}{K_{S1}+[H_1]}+\frac{[H_2]}{K_{S2}+[H_2]}+\frac{[H_3]}{K_{S3}+[H_3]}+.....\frac{[H_n]}{K_{Sn}+[H_n]}\right] \quad (10)$$

But the rate of hydrocarbon degradation is

$$V^S = \frac{dH}{dt} \quad (11)$$

Thus combining Eq. 10 and 11 gives

$$V^S{}_{max} = \left[\frac{K_{S1}+[H_1]}{H_1]}+\frac{K_{S2}+[H_2]}{[H_2]}+\frac{K_{S3}+[H_3]}{[H_3]}+.....\frac{K_{Sn}+[H_n]}{[H_n]}\right]dt \quad (12)$$

Equation 12 be expressed as:

$$V^S{}_{max}dt = \sum_{i=1}^{n}\left[\frac{KS_i+[H_i]}{[H_i]}\right]dH$$

Or

$$V^S{}_{max}dt = \sum_{i=1}^{n}\left[1+\frac{K_{Si}}{H_i}\right]dH \quad (13)$$

For a single microbial system,

$$V^S{}_{max}dt = \left[\frac{K_S}{H}+1\right]dH \quad (14)$$

On integration,

$$V^{S}_{max}dt = K_S \ln\frac{H}{H_O} + (H_O - H)$$
$$V^{S}_{max}t = K_S \ln\gamma + (1-\gamma) \quad (15)$$

where, $\gamma = H/H_O$ in Eq. 15 represents the model for a single microbe catalyzed reaction and can be applied in the computation of maximum reaction rate (Abowei and Susu, 1989).

Reaction catalyzed by multiple microbes: Given that

$$V^{M} = V^{M}_{max1}\frac{H_1}{K_{S1}+[H_1]} + V^{M}_{max2}\frac{H_2}{K_{S2}+[H_2]} + \ldots\ldots V^{M}_{maxn}\frac{H_n}{K_{Sn}+[H_n]} \quad (16)$$

Combining Eq. 11 and 16 gives

$$\frac{dH}{dt} = V^{M}_{max1}\frac{H_1}{K_{S1}+[H_1]} + V^{M}_{max2}\frac{H_2}{K_{S2}+[H_2]} + \ldots\ldots V^{M}_{maxn}\frac{H_n}{K_{Sn}+[H_n]} \quad (17)$$

Therefore,

$$\frac{dH}{dt} = \sum_{i=1}^{n} {}^{M}_{max\,i}\frac{[H_i]}{K_{S1}+[H_i]}$$

or

$$\frac{dH}{dt} = V^{M*}_{max}\sum_{i=1}^{n}\frac{[H_i]}{K_{Si}+[H_i]} \quad (18)$$

Where:

$$V^{M*}_{max} = \sum_{i=1}^{n} V^{M}_{max\,i}$$

Therefore,

$$V^{M*}_{max}dt = \sum_{i=1}^{n}\frac{K_{S1}+[H_i]}{[H_i]}$$

Separating the variables and integrating gives

$$V^{M*}_{max}t = \sum_{i=1}^{n}K_{Si}\sum_{i=1}^{n}\ln\left[\frac{H}{H_O}\right] + \sum_{i=1}^{n}\left[\frac{H_O - H}{H}\right]$$

i.e.,

$$V^{M*}_{max}t = \sum_{i=1}^{n}K_{Si}\sum_{i=1}^{n}\ln\gamma_i + \sum_{i=1}^{n}\gamma_i \quad (19)$$

Equation 19 represents the model equation for the rate of reaction being catalyzed by multiple microbes (Wami and Ogoni 1997).

Model testing: The only requirement for testing the developed kinetic models is the knowledge of the concentrations of the petroleum hydrocarbons in a degrading environment. To achieve this, the crude oil sample subjected to a biodegradation reaction was analyzed for changes in the concentrations of the various hydrocarbon groups.

MATERIALS AND METHODS

The main experimental set up were two basins made of glass (100×60×50 cm) designed and filled up with sand. With 5 outlets for inoculums, oxygen supply, temperature sensor and outlet. Also provided were oxygen cylinder, thermometer and manometer.

The hydrocarbon degraders used were stocked culture of *Pseudomonas aerations* (NCIB950) and *Pseudomonas fluorescence* (NCIB3756). These are known to be potential and active degraders of hydrocarbons. These microorganisms were obtained from the Microbiology Department, Obafemi Awolowo University, Nigeria.

The crude oil sample used was purchased from Nigerian Petroleum Company (NNPC). The system was initially sterilized with steam for 5 h and allowed to cool. A ratio of 99: 1 sterilized distilled water to petroleum hydrocarbon mixture was charged into the bioreactor with recipe salts.

Sea water was pumped from a tank inorder to stimulate tide cycles (4 tide cycles per 24 h). Both basins were added with oleophilic fertilizers and aerated by using land farming techniques for oxygen supply, while one basin was inoculated with the micro organisms, the other was not. Temperature and flow rate were monitored throughout the experimental period.

Samples were withdrawn from the experiment with sterilized automatic pipette and immediately transferred into sterilized screw cap bottles. This was repeated at an interval of 5 days for 20 days. Each of these samples was analyzed for the hydrocarbon groups using a gas chromatography 3600 with Flame ionization Detector (FD).

RESULTS AND DISCUSSION

Table 1 shows the results of the analysis for the metabolites of the various samples. The analysis of sample "A", which is the undegraded crude oil shows that the hydrocarbons present were C_4 to C_{12+}, with higher

Table 1: Results of analysis of unweathered crude

		Sample B (After 10 days)		Sample C (After 15 Days)	
Component	Sample A	Concentration	Degraded (%)	Concentration	Degraded (%)
$1C_4$	0.01	0.00	100.00	0.00	100.00
NC_4	0.00	0.00	0.00	0.00	00.00
$1C_5$	0.01	0.01	100.00	0.00	100.00
NC_5	0.01	0.01	100.00	0.00	100.00
C_6	0.07	0.04	42.86	0.03	57.14
C_8	2.47	1.89	23.48	1.65	33.20
C_9	7.30	6.90	5.50	6.40	12.33
C_{10}	12.31	12.20	0.89	11.34	7.88
C_{11}	13.09	13.50	-	13.20	-
C_{12}	64.31	65.27	-	67.22	-

Table 2: Table of the maximum specific rate and the dissociation constant for the various components

Parameter	Component				
V_{Max}	C_6	C_7	C_8	C_9	C_{10}
K_S	0.006	0.007	0.90	0.11	0.36

Table 3: Specific rate of biodegradation

Component	Substrate concentration	Specific rate of biodegradation	Theoretical values
C_6	0.026	0.0026	0.00242
C_7	0.154	0.0038	0.0039
C_8	1.890	0.0510	0.0421
C_9	6.900	0.0560	0.0157
C_{10}	12.200	0.0440	0.0512

Table 4: Overall specificity rate of degradation for multiple microbial catalyzed reactions

Substrate concentration	Specific rate of biodegradation
22.37	0.180
19.85	0.170
15.50	0.150

molecular hydrocarbons C_{11} to C_{12+} predominating. C_4 to C_{10} hydrocarbons constituted less than 22.4% of all the hydrocarbons present while C_{11} to C_{12+} were about 77.6%.

For the weathered sample, there were decreases in the concentration of C_4 to C_{10} while those of C_{11} to C_{12+} showed increase over their concentrations in the free sample. This because free radicals of short chain H-C combines together to produce long chain H-C as shown below:

$$C_5H_{11}^{+}+C_5H_{11}^{+} = C_{10}H_{22}$$

Table 2 shows the specific rates of degradation while the values of V_{max} and K_S were determined from the Line Weaver-Burke plot as computed in Table 3. The values of the maximum specific rate for each of the degraded petroleum hydrocarbons were computed for both single microbial and multiple microbial reactions using the experimental data generated and presented in Table 1-3.

The maximum specific rate for each of the degraded petroleum hydrocarbon was computed using the formulated model and Table 1. The results are presented in Table 2. The specific rate of biodegradation was also computed, the results are shown in Table 3. Similarly, the maximum specific rate and specific rates of biodegradation for multiple microbial catalyzed reactions were computed using the formulated kinetic models and the results are shown in Table 4.

The result of the analysis indicates a gradual change in the concentration of the individual petroleum hydrocarbon as the time increased for the period of reaction. The rate of change of the concentration varied with time and is different for different hydrocarbons. It was also observed that the specific rate of reaction decreased with time and concentration, which is in line with previous reports (Margesin and Schinner, 1997). The result also showed that degradation rate increased with increase in molecular weight up to C_{10}.

The results obtained from the theoretically developed models show a slight discrepancy with the experimental results. This could be attributed to possible experimental factors affecting the determination of various constants for the kinetic model. However, despite the discrepancies, the results as obtained from the computation using the formulated models showed similar patterns with those obtained from the experimental procedure. A comparison of the experimentally determined specific rates and those computed from the model equations is presented in Table 4.

CONCLUSION

Kinetic models for biodegradation of oil spill were developed for both single and multiple microbial catalyzed systems. The developed models were tested and showed that they could be useful for the following applications:

- Estimating the period of biodegradation of petroleum hydrocarbon based industrial effluent.
- Determining the residence time for the design of biotreatment reactors.
- Monitoring of bioremediation of pollution emanating from oil spillage on land and aquatic environments.

NOMENCLATURE

[E]	:	Free Microbe.
[ET]	:	Total Microbe.
$[H_{1, 2,3....n}]$	:	Substrate Concentration.
$[EH_{1,2,3..n}]$	:	Enzyme Substrate Complex.
$[P_{1,2,3....n}]$	:	Product Concentration.
K_1, K_{-1}	:	Rate constant for forward and backward reactions.
K_P	:	Rate constant for the breakdown of substrate complex.
K_S	:	Dissociation constant of the microbial-substrate complex.
V^S	:	Specific rate of reaction for single microbial catalyzed reaction.
V^S_{max}	:	Maximum specific rate for single catalyzed reaction.
t	:	Time.
V^M	:	Specific rate of reaction for multiple microbial catalyzed reaction.
V^M_{max}	:	Maximum specific rate of reaction for multiple microbial catalyzed reaction.
H-C	:	Hydrocarbon.

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