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MATHEMATICAL MODELLING OF THE MICROBIAL GROWTH AND DECAY RATE OF PSEUDOMONAS SPECIES ON BIODEGRADATION OF BONNY LIGHT CRUDE OIL

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ABSTRACT

Mathematical models were developed to simulate the microbial growth and decay rate of pseudomonas species in biodegradation of bonny light crude oil in an ambient temperature of 25-35°C (mesophilic temperature). The developed models were used to predict the growth and decay rate of pseudomonas species. Experimental investigation for the microbial growth and decay rate were measured by a standard plate count technique using difco plate count agar. The models were simulated with the aid of visual C^{++} programme software and it was found that the growth rate increase with time at the progressive phase and decay rate decreases with time at the death or decline phase. Form the experimental result, it was observed that the maximum growth rate of the pseudomonas species were experienced at the progressive phase with the pH ranges from 7.48 to 6.87, turbidity = 230 to 226FTY, and hardness Ca^{2+} = 100.8 to 98.4mg/l, mg^{2+} = 30.20 to 34.24mg/l. The death or decline phase was observed after the substrate had been consumed by the microbes, at this point the temperature of the effluent was equal to the ambient temperature with the pH value dropping to 6.92. The developed models were found useful in predicting and monitoring the degradation rate of bonny light crude by pseudomonas species.

KEYWORDS: Mathematical modeling, microbial growth, decay rate, pseudomonas species, Bonny light crude

INTRODUCTION

The activities of the petroleum industry on the environment have attracted the attention of environmentalists on the effluent discharge into the environment emanating from exploration, production, transportation, refining and utilization. Recent investigations carried out by various research groups reveal that due to the high level of petroleum activities in Nigeria, there is a high concentration of the petroleum hydrocarbon contaminants traceable to both upstream and downstream sectors of the economy (Gandy and Gandy, 1988). This has resulted in environmental pollution, which affects the ecological system (John 1999). There are several microorganism that degrade petroleum products. The degradation activities of the microbes on petroleum and its products are well documented (Amadi *et al.* 1991,1992).

Studies conducted by various research groups reveal that the microbial activities are beneficial in several ways, (Beckman, 1926, Premuzic *et al.* 1990). Results of the investigation attracted the attention of scientific groups, as microbes are now used to enhance remediation of contaminants present in the marine and terrestrial environments. It may be difficult to make a conclusive and comprehensive report on bioremediation of petroleum hydrocarbon contaminated environment, except a design model on the basis of appropriate approach to bioremediation development.

MATHEMATICAL MODEL FORMULATION

Biomass Growth Rate Model

When an organic waste (petroleum hydrocarbon) is discharged into the environment, the organic content of the effluent undergoes biochemical reactions as reported by (Zhang *et al.* 1992). The rate of biodegradation is influenced by the concentration of the substrates and the product inhibition (Tenneman, 1989).

The biodegradation is generally an aerobic process, and results in the production of new biomass, carbon dioxide, water and product.

For dynamic studies, the general conservation equation for a steady state must be modified to give the following unsteady-state mass balance;

$\frac{d}{dt}$ (biomass in the system) = (rate of addition of microorganism to system) – (rate of removal of microorganism from system) + (rate of production within system) (1)

Assuming the rate of addition and removal of microorganism from the system is equal. Therefore equation (1)

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Therefore,

$$\phi = -0.04055 \quad (27)$$

Substituting equation (26) and (27) into equation (20) we have

$$\mu_{D(t)} = \mu_o \ell^{(-0.4055)t} \quad (28)$$

$$\Rightarrow \mu_{D(t)} = \mu_o \ell^{-0.4055t} \quad (29)$$

Equation (29) is known as the Decay Rate in Biomass per unit weight at the death or decline phase.

Enzyme Kinetics Model

Recalling, Henri's enzyme kinetic studies by Ronald, (1992) that the initial rate of a bio-reaction is directly proportional to the concentration of enzyme but increases in a linear manner with increasing substrate concentration upto a limiting maximum rate.[10] The overall reaction can be represent as follows:

$$[E] + [S] \underset{K_2}{\overset{K_1}{\rightleftharpoons}} [ES] \underset{K_p}{\overset{K_o}{\rightleftharpoons}} [P] + [E] \quad (30)$$

Assuming normal first order steady state reactions with respect to each entity as presented by Ronald,[10] the following equation describes the mass balance [ES]

$$\frac{d[ES]}{dt} = K_2[E][S] - K_1[ES] + K_o[E][P] - K_p[ES] \quad (31)$$

At steady state condition $\frac{d[ES]}{dt} = 0$, therefore equation (31) reduces to

$$K_2[E][S] - K_1[ES] + K_o[E][P] - K_p[ES] = 0 \quad (32)$$

The rate of formation of [ES] from product and enzymes is normally negligible i.e.; $K_o \approx 0$, Ronald (1992), therefore equation (32) becomes,

$$K_2[E][S] - K_1[ES] - K_p[ES] = 0 \quad (33)$$

The overall velocity of the reaction depends on the rate of product formation as presented by Ronald, (1992).

$$V = \frac{d[P]}{dt} = K_p[ES] \quad (34)$$

The following mass balance also applied at any time Ronald (1992).

$$[E]_o = [E] + [ES] \quad (35)$$

Rearranging equation (35) yields

$$[E] = [E]_o - [ES] \quad (36)$$

Substituting equation (36) into equation (33) yields

$$K_2([E]_o - [ES])[S] - K_1[ES] - K_p[ES] = 0 \quad (37)$$

Rearranging equation (37) and making [ES] the subject of the formular, yields

$$K_2[E]_o[S] - K_2[ES][S] - K_1[ES] - K_p[ES] = 0 \quad (38)$$

$$[ES](K_2[S] + K_1 + K_p) = K_2[E]_o[S] \quad (39)$$

$$[ES] = \frac{K_2[E]_o[S]}{K_2[S] + K_1 + K_p} \quad (40)$$

Dividing the RHS of equation (40) by K_2, it becomes

$$[ES] = \frac{[E]_o[S]}{K + [S]} \quad (41)$$

Therefore equation (6) becomes

$$\mu_{(t)} = \mu_o \ell^{(In Y_S)t} \quad (12)$$

Thus $\mu_{(t)} = \mu_o \ell^{0.4055t} \Rightarrow$ biomass growth rate (13)

where

$$\mu_{(t)} = n\mu_o \quad (14)$$

n = microbial number in terms of weight, thus

$$n\mu_o = N_o \ell^{0.4055t} \quad (15)$$

Decay Rate Model

Applying the differential equation, the decay rate model is presented as below:

$$\frac{d\mu_D}{dt} = -\phi\mu_D \quad (16)$$

On rearranging equation (16) gives

$$\frac{d\mu_D}{dt} + \phi\mu_D = 0 \quad (17)$$

Considering the necessary boundary conditions such as:

$$t = 0, \mu_{D(0)} = \mu_{Do} \quad (18)$$

Therefore application of laplace transform to equation (17) and substituting the boundary condition yields

$$\mu_{D(S)} = \frac{\mu_{Do}}{S + \phi} \quad (19)$$

Therefore inversion of equation (19) yields

$$\mu_{D(t)} = \mu_{Do} \ell^{-\phi t} \quad (20)$$

The value of ϕ is negative because biomass in the death or decline phase decreases with time.

At t = 1 day, equation (20) becomes

$$\mu_{D(1)} = \mu_{Do} \ell^{-\phi(1)} \quad (21)$$

$$\Rightarrow \mu_{D(1)} = \mu_{Do} \ell^{-\phi} \quad (22)$$

from the investigation it was observed that at t = 1day,

$\mu_{D(1)} = \frac{3}{2}\mu_{Do}$.

Therefore equation (22) becomes

$$\frac{3}{2}\mu_{Do} = \mu_{Do} \ell^{-\phi} \quad (23)$$

Equation (23) is defined only at t = 1day and rearranging, it becomes

$$\ell^{-\phi} = \frac{3}{2} \quad (24)$$

Simplifying equation (24) yields

$$-\phi = In\frac{3}{2} \quad (25)$$

$$\Rightarrow \phi = -In\frac{3}{2} \quad (26)$$

Assuming $-\frac{\psi}{Y} = 1$, at the equilibrium stage of the reaction, therefore equation (51) becomes

$$\mu = \frac{\mu_{max}[S]}{K + [S]} \tag{52}$$

Equation (52) is known as the Michaelis – Menten equation (Ronald, 1992). It was originally developed by them to describe enzyme kinetics but has been found to apply to many other types of reactions. The equation (52) is identical to the Monod equation for microbial kinetic which relates the specific growth rate of the microorganism and the limiting component is presented (Alkinson, 1983).

To determine the constant μ_{max} and k, equation (52) is rearranged to give the expression that will lead to obtain the Monod equation in terms of $1/\mu$ and $1/S$. Therefore equation (52) becomes

$$\frac{1}{\mu} = \frac{K}{\mu_{max}} \bullet \frac{1}{[S]} + \frac{1}{\mu_{max}} \tag{53}$$

Equation (54) is use in illustrating the Line Weaver-Burk plot as presented (Ronald, 1992); from where the constant μ_{max} and K will be determined.

Equation (12) and (13) are the rate of biomass increase and optimal biomass increase respectively at the progressive phase. Similarly equation (28) and (29) are the rate of biomass decrease and optimal biomass decrease respectively at the death or decline phase.

EXPERIMENTAL METHOD

Microbial Culture

Crude oil sample from production terminal in Niger Delta area was collected. The microbes from the crude oil were isolated and identified as pseudomonas species according to the methods of Buchaman (1974) and Gerbardt (1981). A microbial culture was then prepared (*pseudomonas* species) for the research investigation.

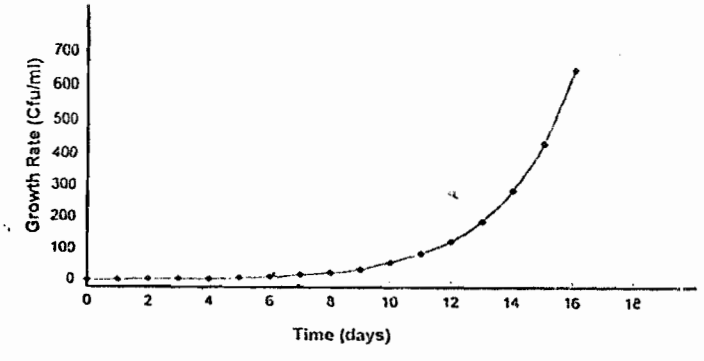

Figure 1: Growth Rate of Microorganism for Uncatalysed Reaction at the Progressive Phase

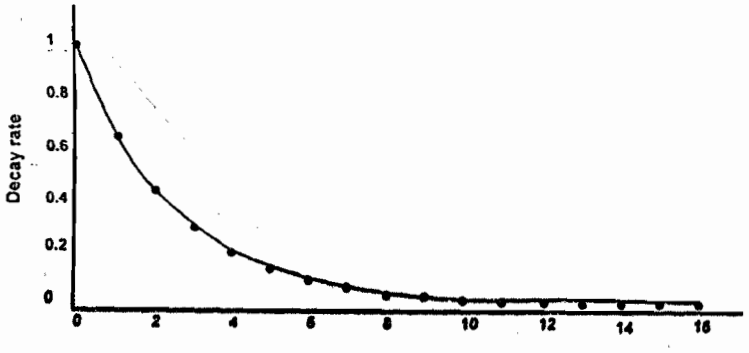

Figure 2: Decay Rate of Microorganism for Uncatalysed Reaction at the Death or Decline Phase

where,

$$K = \frac{K_1 + K_p}{K_2}$$

Substituting equation (41) into equation (34) yields

$$V = \frac{K_p[E]_o[S]}{K + [S]} \quad (42)$$

The maximum rate of reaction (V_{max}) will occur when [ES] = $[E]_o$, Ronald (1992) Therefore,

$$V = K_p[E]_o = V_{max} \quad (43)$$

Substituting equation (43) into equation (42) results in

$$V = \frac{V_{max}[S]}{K + [S]} \quad (44)$$

The rate of substrate removal is proportional to the rate of biomass growth, therefore

$$\frac{d[S]}{dt} \alpha - \frac{d\mu}{dt}$$

$$\frac{d[S]}{dt} = -\frac{1}{y}\frac{d\mu}{dt} \quad (45)$$

The velocity of the reaction in this case is presented by James and David (1977) as:

$$V = \frac{d[S]}{dt} \quad (46)$$

Substituting equation (46) into equation (42) gives

$$\frac{d[S]}{dt} = \frac{K_p[E]_o[S]}{K + [S]} \quad (47)$$

Substituting equation (45) into equation (47) yield

$$-\frac{1}{Y}\frac{d\mu}{dt} = \frac{K_p[E]_o[S]}{K + [S]} \quad (48)$$

Substituting equation (3) into equation (48) yields

$$\frac{-\psi}{Y}\mu = \frac{K_p[E]_o[S]}{K + [S]} \quad (49)$$

The maximum growth rate of biomass (μ_{max}) will occur when [ES] = $[E]_o$, as reported by James and David (1977). Therefore

$$\mu = K_p[E]_o = \mu_{max} \quad (50)$$

Substituting equation (50) into equation (49) yields

$$-\frac{\psi}{Y}\mu = \frac{\mu_{max}[S]}{K + [S]} \quad (51)$$

Table 1: Microbial population for the uncatalysed reaction at the lag progressive phase

Sample	Time (days)	Microbial Number (μ) Cfu/ml Pseudomonas sp			
		Lag phase	Progressive phase	Stationary phase	Death or decline phase
A	1	5.0×10^3	-	-	-
B	2	5.0×10^3	-	-	-
C	3	5.0×10^3	-	-	-
D	4	-	6.59×10^3	-	-
E	5	-	2.31×10^4	-	-
F	6	-	6.02×10^4	-	-
G	7	-	2.88×10^5	-	-
H	8	-	2.85×10^6	-	-
I	9	-	2.36×10^7	-	-
J	10	-	-	2.36×10^7	-
K	11	-	-	2.36×10^7	-
L	12	-	-	-	2.0×10^7
M	13	-	-	-	2.80×10^7
N	14	-	-	-	2.70×10^6
O	15	-	-	-	2.90×10^5
P	16	-	-	-	1.60×10^4

Table 2: Physical and chemical composition of Bonny Light crude sample at different days of analysis

Sample	Concentration of the Parameters								
	Turbidity (FTY)	pH	Dissolved oxygen (mg/l)		Total Dissolved solid (g/l)	Alkalinity (mg/l)		Hardness (mg/l)	
			So_4^{2-}	No_3^{2-}		CO_3^{2-}	HCO_3	Ca^{2+}	Mg^{2+}
1	235.9	7.68	17.00	8.00	4.7	218	3537.5	121.1	28.74
2	231.6	7.66	31.00	10.00	3.7	441.84	3038.2	115.6	29.10
3	230.9	7.48	35.00	21.60	3.7	198.7	2996.2	100.8	30.24
4	230.0	736	33.00	25.30	5.3	148.8	2769.4	100.2	31.43
5	229.4	7.27	36.00	26.70	5.5	146.0	2678.6	99.8	31.92
6	228.7	7.22	36.00	26.91	5.8	121.64	2330.1	99.1	32.85
7	227.8	7.00	36.00	30.00	6.3	117.05	2010.7	98.9	33.33
8	227.4	6.98	37.00	34.00	6.9	102.01	1986.0	98.7	33.86
9	226.0	6.87	40.00	36.52	6.9	137.66	1235.1	98.4	34.24
10	221.3	6.955	38.00	26.02	7.01	137.00	986.4	96.2	36.63
11	106.5	6.93	38.00	26.00	7.03	127.46	917.0	84.0	38.17
12	98.2	6.89	38.00	25.89	7.03	120.16	622.7	78.6	40.54
13	76.9	6.88	27.00	12.00	7.03	105.00	503.3	62.1	41.02
14	76.8	6.88	25.00	7.53	7.03	100.71	333.6	53.7	41.92
15	74.0	6.87	20.00	6.61	7.03	100.71	301.8	46.0	50.31
16	70.6	6.87	10.00	6.00	7.03	100.71	301.8	32.0	56.74

one day increase in number of biomass of a particular microbial species leads to the development of biomass growth and decay rate model. At this point, it was assumed that the initial microbial number (μ_0) = 5000cfu/ml, since at 72hours (3days) the same 5000cfu/ml were recorded. When sample D_4 was analysed the result shows an increase in biomass upto 150% $\frac{3}{2}\mu_o$. Increase in biomass was observed for E_5, F_6, G_7, H_8, I_9, J_{10} and K_{11}. But at J and K the biomass growth rate was the same (stationary phase). The death and decline phase was observed after K_{11}. Similarly the death or decline phase of the microbes increased in the following order L_{12}, M_{13}, N_{14}, O_{15} and P_{16}.

RESULTS AND DISCUSSION

The results obtained from samples A_1 to C_3 (lag phase), D_4 to I_9 (progressive phase) J_{10} to K_{11} (stationary phase) and L_{12} to P_{16} (death or decline phase) was used in the determination of the microbial number (μ) cfu/ml at each phase. It was observed that for the lag phase there was a decrease in the initial biomass number and increase in biomass at the progressive phase as shown in Table 1.

As shown above in table 1, it was discovered, from analysis, that growth rate of *pseudomonas* species at the progressive phase increased linearly until the whole substrate concentration was reduced to the minimum level when the process became stagnant (stationary phase). Similarly the decay rate increase as the substrate consumption decreased.

Results obtained as reflected in figures 1 and 2 indicated that the growth rate of the *Pseudomonas* species increased with increase in time until the stationary phase was attained at t = 216 hours (9 days). Similarly the decay rate of the pseudomonas species increased with increase in time until the whole substrate concentration was reduced to the minimum level at t = 288 hours (12 days).

In modeling, the rate of microbial growth and decay of *Pseudomonas* species in degradating bonny light crude was influenced by the degree of environmental factors such as temperature, the substrate concentration, pH, turbidity, hardness, total dissolved solid, dissolved oxygen and alkalinity as shown in Table 2.

The extent of the growth rate was observed to increase with increase in time. The initial number of the pseudomonas species introduced in the system was 6800cfu/ml. It was observed that about 1800cfu/ml of the species died off at the lag phase remaining 5000cfu/ml which was used as the initial number of the *pseudomonas* species in the progressive phase, as determined from experimental results.

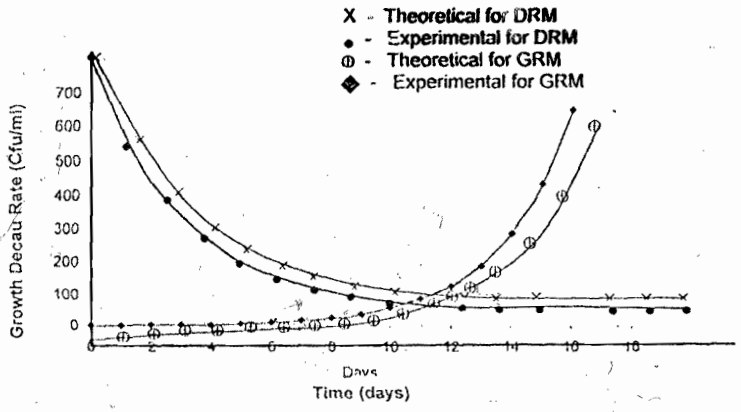

Figure 3: Comparison of the Theoretical and Experimental Results
DRM - Decay Rate of Microorganism
GRM - Growth Rate of Microorganism

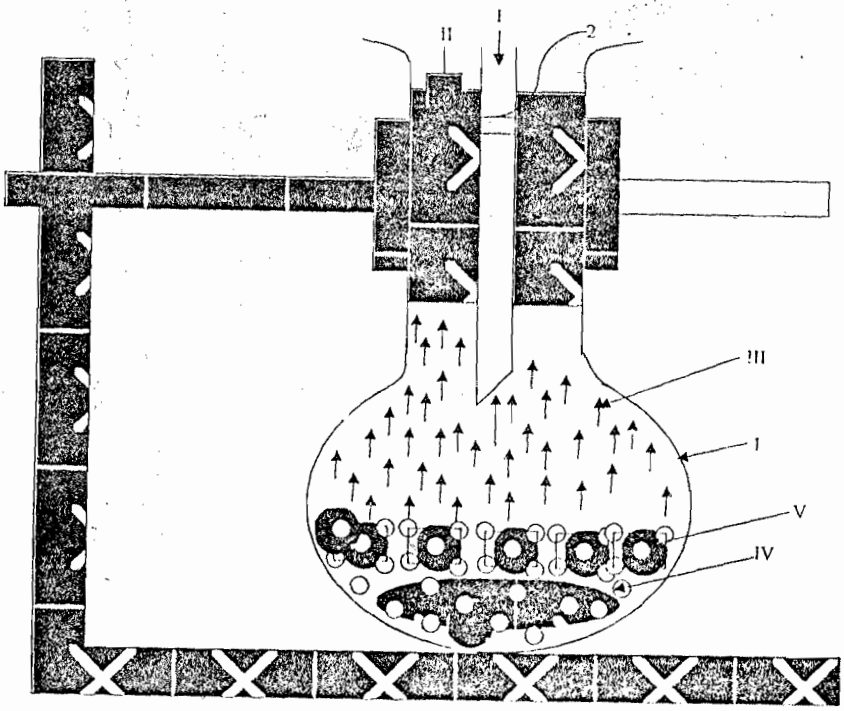

Figure 4: Main experimental set up (labeled A-P)

1 – Reactor, 2-filter, I-point of sample introduced; II – inoculation point, III-Gases liberated; IV – crude oil mixture with water; V – mixture of crude, water and microbes.

Determination of Microbial Population

Sixteen experimental units A to P were setup to generate the required data as shown in Figure 4. The population of individual petroleum degrading species in each of the unit was determined by counting the colony forming units/milliliter (cfu/ml) in a pour plate of mineral salt agar medium containing 0.5% crude oil.

Prior to this the different microbial isolates (species) had been inoculated aseptically into different experimental unit.

Microbial Sample

Total microbial counts were measured by a standard plate count technique using difco plate count agar (APHA, 1998).

COMPUTATIONAL PROCEDURE

Equation (12) and (13) represents the biomass growth rate model. The model was developed to determine the maximum specific growth rate of a microbial species (*Pseudomonas* sp) in an uncatalysed reaction. Similarly equation (28) and (29) represents the decay rate model of biomass. From the investigation considering an interval of

In this investigation, comprehensive models for microbial growth and decay of *pseudomonas* species were developed for uncatalysed reaction.

The mathematical models developed in this investigation are presented in equations (13) and (29). The mathematical models were developed only for uncatalysed reaction of bonny light crude oil degradation on the areas of microbial growth and microbial decay. The comparison of the theoretical and experimental result is shown in figure 3, the result shown a good match.

CONCLUSION

The developed models were tested for both biomass growth and decay rate. Therefore the application of these models could be used in monitoring the rate of degradation of the bonny light crude oil and their products. It could also help to understand the mechanism of biodegradation, estimating the period of biodegradation for bonny light crude oil, predicting the performance crude bonny light crude oil utilized, estimating the residence time for each hydrocarbon component in the design of bio-treatment reactor, and also serve as a guide in monitoring bioremediation process and to quality.

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