Journal of the Nigerian Association of Mathematical Physics
Volume **16 (May, 2010), pp 317 - 324**

Development of a transport model for the microbial degradation of polycyclic aromatic hydrocarbons in a saturated porous medium.

Owabor, C.N[a]., Agarry, S.E[b] and Azeez, T.O[a].

[a] Department of Chemical Engineering, University of Benin, Benin City, Edo State.
[b] Department of Chemical Engineering, Ladoke Akintola University, Ogbomoso, Osun State
[a]Corresponding author: email owabor4you@yahoo.com, Tel: +2348034101218

Abstract

A mathematical model for first order reaction rate under isothermal condition was developed for predicting the diffusivity and transport rate of anthracene and pyrene during biodegradation using two microbial strains (corynebacteria spp and pseudomonas putida) in a heterogeneous porous medium. The formulation followed the conservation laws and employed the concepts from the correlations of Fick's law of diffusion, Malthus equation and Monod kinetics.

Experimental results on reaction rate constant, transport rate for the contaminant solutes as well as the saturation constant, yield coefficient and maximum specific growth rate of the microbial strains were used to characterize the biodegradation process. The results showed that corynebacteria spp was more effective for the degradation of anthracene and pseudomonas putida more suitable for pyrene utilization. Studies further showed a decrease in the effective diffusivity with increasing degree of penetration, decreasing solute concentration and increasing microbial mass, and affirm that the interactions between microbial species in a pure culture are significant for the prediction of biodegradation kinetics.

In the light of limitations arising from the expensive and cumbersome nature of experimental studies, the developed model has been demonstrated to be adequate in effectively providing insight into an appropriate methodology of classifying microbes in order of their preference for contaminant solutes.

Keywords: Conservation laws, kinetics, porosity, convective and diffusive transfer, degradation

1.0 Introduction

Environmental pollution due to polycyclic aromatic hydrocarbons (PAHs) spill is a major challenge confronting both scientists and engineers ([1] and [11]). These groups of organic chemicals are classified into primary, principal and natural sources which include ([2] and [19]).

Biodegradation plays a major role as monitored natural attenuation for depletion of PAHs in the environment ([4] and [14]). Though, biodegradation has been in existence as a clean up technique since the discovery of crude oil and with the increased input of PAHs into the environment due to the increased daily activities within the petroleum industries, the method has however, not been commercially viable, due to the limitations arising from the inability to access quantitative information on the effects of mass transfer and heterogeneous nature of most porous medium on biodegradation rate.

Some of the most overwhelming literature on biodegradation studies are based strictly on experimental works involving the use of specific microorganism with specific reducing culture for degradation of PAHs, metabolic pathways of the PAHs, patterns of disappearance of PAHs and growth of microbial mass either under aerobic and /or anaerobic conditions ([15] and [16]). Research works have also been directed to the effect of biosurfactants ([6], [7], [13], [23] and [25]), bioavailability of contaminants ([3] and [22]), the effect of nutrient concentration on bioremediation ([18] and [21) as well as the determination of kinetic and biokinetic parameters during biodegradation ([20], [22] and [24]). These researches were unable to perform optimally in relation to determining the concentration and effective diffusivity during biodegradation in a saturated porous medium.

The objective of this study therefore is to obtain quantitative insight into diffusion behaviour and hence the reaction rate constants using anthracene and pyrene as model contaminant solutes during biodegradation in a saturated porous medium. This is with a view to ensuring as quickly as possible, the elimination of both long and short term effects of contaminants that compromise the integrity of the environment.

2.0 Theoretical Framework

This study focuses on the development of a suitable model for predicting the diffusivity of PAHs during biodegradation in a saturated porous medium, based on the following theoretical concepts:

i the movement of the PAHs in a porous medium under isothermal condition is by molecular diffusion assumed to be governed by Fick's law ([5], [8] and [10]).

ii the theory of convective and diffusive transfer of solutes in the direction of flow [9]. Convection is due to fluid flow while diffusion is due to solute transport.

iii the biodegradation of PAHs is limited to the growth of microbial mass following the concept of Malthus correlation first order reaction rate, size and shape of a rectangular slab. The slab represents a sampling zone selected at random.

iv one-dimensional flow, constant porosities and heterogeneous nature of the porous medium.

3.0 Model Development

The rate of mass transfer of PAHs in the subsurface soil from the bulk liquid to the surface of microbial mass is given as:

$$J = D\ \frac{dC}{dz} \tag{3.1}$$

The microbial degradation process is described by the stoichiometric equation

$$C + M \rightarrow M + P \tag{3.2}$$

The rate of reaction of equation (3.2) for first order reaction under isothermal condition (Levenspiel, 2002) is:

$$-R = kC \tag{3.3}$$

$$-\frac{dC}{dt} = kC \tag{3.4}$$

Subject to the initial and boundary conditions:

$$C = C_o \ at\ t = 0, \tag{3.5}$$

$$C = C\ at\ t = t \tag{3.6}$$

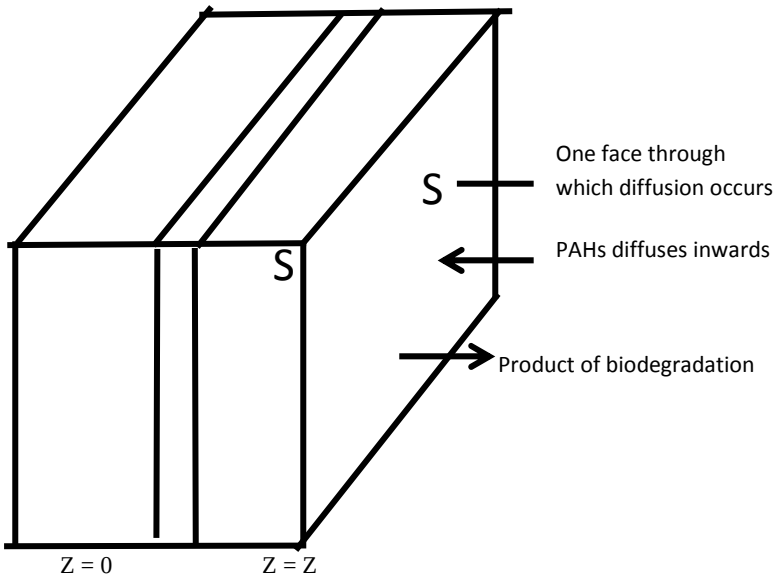
Integrating equation (3.4) using the initial and boundary conditions yields:

$$\ln \frac{C_o}{C} = kt \tag{3.7}$$

Due to the fact that biodegradation of PAHs occur within the active site of the microbial mass in the porous medium, an idealized geometric shape of a rectangular form shown below in Figure 1 was conceptualized and considered as the porous medium.

Invoking the law of conservation of materials yields:

Rate of mass transfer of PAHs at the surface z	-	Rate of mass transfer of PAHs at a distance z + Δz	+	Rate of production of PAHs over the distance Δz bv	= 0



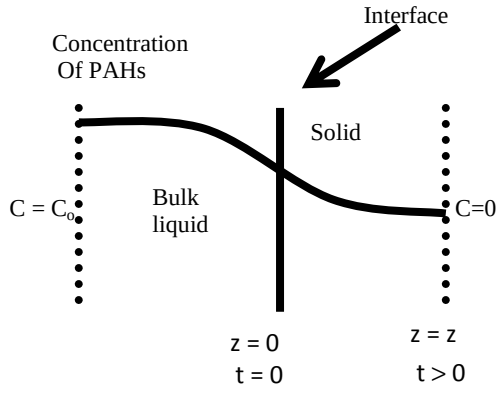


Figure 2: Concentration gradient of PAHs between the bulk liquid-solid phases

Fig.1: A schematic representation of the rectangular slab model of a porous medium.

Invoking the law of conservation of materials yields:

$$J, _z\Big|_z - J, _z\Big|_{z+\Delta z} - R\Delta Z = 0 \tag{3.8}$$

Taking the limit of $\Delta Z \longrightarrow 0$

$$\frac{dJ}{dz} = kC \tag{3.9}$$

Integration of equation (3.7) with boundary conditions

For inlet: $C = C_o, J, _z = 0\ at\ Z = 0$ (3.10)

For outlet: $C = C,\ J_{,z} = J\ at\ Z = Z$ (3.11)

$$\int_0^J dJ_Z = kC\int_0^z dz \tag{3.12}$$

Thus equation (3.12) becomes

$$J_{,z} = kCZ \tag{3.13}$$

Combining equations (3.1) and (3.13) since the rate of mass transfer of PAHs per unit area is the same as the rate of consumption of PAHs gives;

$$-\frac{dC}{dz} = \frac{kCZ}{D} \tag{3.14}$$

Setting equation (3.14) as a function of time i.e. unsteady state condition we multiply both sides of equation (3.14) by $\frac{dz}{dt}$ and obtain

$$-\frac{dC}{dt} = \frac{kCZ}{D}\,dz \tag{3.15}$$

Applying the Malthus correlation, the rate of consumption of PAHs by microbial mass implies the growth of microbial mass and this is given as;

$$R = \frac{dx}{dt} = \mu X \tag{3.16}$$

But $$\mu = \frac{\mu_m C}{k_s + C} \tag{3.17}$$

and $$\frac{dx}{dt} = -Y\,\frac{dC}{dt} \tag{3.18}$$

The negative sign (-) indicates that an increase in concentration of microbial mass is accomplished by a decrease in PAHs concentration. Hence multiplying both sides of equation (3.17) by dt yields:

$$dx = -YdC \tag{3.19}$$

On integration,

$$X = X_o\ at\ C = C_o \tag{3.20}$$

$$X = X\ at\ C = C \tag{3.21}$$

Thus, $$X - X_o = Y\left(C_o - C\right) \tag{3.22}$$

Combining equations (3.16) and (3.18) yields:

$$\frac{dx}{dt} = -Y\frac{dC}{dt} = \mu X \tag{3.23}$$

and,

$$-\frac{dC}{dt} = \frac{\mu X}{Y} \tag{3.24}$$

Therefore, substituting the value of μ into equation (3.24) gives:

$$-\frac{dC}{dt} = \frac{\mu_m CX}{Y\left(k_s + C\right)} \tag{3.25}$$

Equation (3.25) describes the rate of biodegradation as a function of microbial yield, concentration of the microbial mass, PAH concentration, maximum specific growth rate, saturation constant, time and distance covered by PAHs in the direction of flow.

Equations (3.15) and (3.25) both show the rate of consumption of PAHs and hence equating them yields:

$$Zdz = \frac{D\mu_m X}{kY\left(k_s + C\right)}dt \tag{3.26}$$

Integrating equation (3.26) with initial and boundary conditions

Inlet: $z = 0\ at\ t \le 0$ (3.27)

Outlet: $z = z\ at\ t = t$ (3.28)

Therefore,

$$Z^2 = \frac{D\mu_m X}{kY\left(k_s + C\right)}t \tag{3.29}$$

$$Z = \phi\sqrt{t} \tag{3.30}$$

Where $$\phi = \sqrt{\frac{D\mu_m X}{kY(k_s + C)}} \quad (3.31)$$

Equation (3.31) is a measure of the transport parameter of the PAHs in the microbial culture environment as the contaminant PAHs diffuse over the distance z with time and become converted into metabolic products.

From the linear plot of equation (3.26), the gradient gives the value of ϕ which provides a means of predicting the effective diffusivity of the PAHs within the subsurface soil.

Hence**,**

$$D = \frac{k\phi Y(k_s + C)}{\mu_m X} \quad (3.32)$$

4.1 Results

The model was validated using experimental data from previous work [17]. The simulation results presented in this paper clearly demonstrates the efficacy of using a theoretical model to describe and predict mass transfer effects occurring during the biodegradation of anthracene and pyrene which served as homologous substrates for a system of microbial culture. The microbial growth and substrate consumption rates were determined from the results of the batch experiments on substrate limiting microbial degradation using the Monod kinetics performance equation. The biodegradation kinetics of the contaminant solutes and the growth kinetics of the microbes are presented below in Figures 3 to 6 and Table 1.

4.2 Discussions

The pure microbial strains used in this work exhibited a high level of metabolic activity in the presence of the contaminant PAHs. These findings led to the development of the transport model, in which the effective diffusivity of the PAHs as a function of the key parameters of degradation and growth kinetics of the strains was shown to have a strong influence on the overall removal of the contaminant PAHs.

The mineralization of the solutions of crystalline anthracene and pyrene resulted in increased biomass concentration as indicated by the batch growth curve shown in Figures 3 to 6. There was no observed induction period. This is expected and can be attributed to the fact that the microbes used were from a secondary culture and as such were already adapted to the substrate environment as good carbon and energy sources. Limiting substrate, increased temperature as a result of increased cell yield, may have been responsible for the observed unpronounced stationary phase. The sharp and rather steep decrease in concentration which characterized the decay phase demonstrated by *Pseudomonas putida* is predicated on the incidence of substrate exhaustion due to increase in cell crowding. From Figures 3 and 4, the concentration of anthracene decreased from 100.00mg/l and 100.00mg/l to 0.163mg/l and 0.158mg/l during an experimental period of 90hours and 96hours using *corynebacteria spp* and *pseudomonas putida* respectively. Thereafter, the concentration remained unchanged throughout the remaining hours of the experiment. Similarly, the mass of the *corynebacteria spp* and *pseudomonas putida* was found to increase from 0.016mg/l and 0.012mg/l to 0.105mg/l and 0.098mg/l, an indication of an exponential growth (increased microbial biomass). Both microbes however, maintained their stationary phases between 84hours to 90hours and 96hours to 102hours and this was closely followed by a decrease in their biomass, characterized by the death of the microbes. Following the result above, the yield coefficient of *Pseudomonas putida* on anthracene was
lower than that of *corynebacteria spp* with values as summarized in Table 1.The distance covered by anthracene in the subsurface soil in the presence of *corynebacteria spp* and *pseudomonas putida* increased from 0cm at the onset of the experiment to 10cm and 9.4cm respectively at the end of the experiment.

Results in Figures 5 and 6 showed a decreased concentration of pyrene from 100.00mg/l and 100.00mg/l to 5.52mg/l and 1.89mg/l respectively for the same period of 84hours. The concentration became constant for the remaining hours of
the experiment. The microbial mass was again observed to increase but with the *pseudomonas putida* having a higher mass.

The stationary phases of *corynebacteria spp* and *pseudomonas putida* were identified to be between 84hours to 90hours and 90hours to 96hours respectively. As was observed in the case of anthracene, a subsequent decrease (0.097mg/l and 0.080mg/l) in the concentration of the microbial biomass; served as a useful tool for ascertaining the death phases of the growth pattern for the organisms. Thus the yield coefficient of *pseudomonas putida* on pyrene was found to be higher than that of *corynebacteria spp*. Again, the distance covered by pyrene in the presence of *corynebacteria spp* and *pseudomonas putida* increased from 0cm to 7.4cm and 7.2cm respectively.

The success of the transport model presented in this work was exploited in the estimation of the transport parameter ϕ) presented in Figures 7 to 10. A comparison of the result for the contaminant solutes showed that on the one hand, the transport parameter was higher for anthracene in the presence of *corynebacteria spp* while on the other; it was higher for pyrene using *pseudomonas putida*. This can be attributed to such effects as density, molecular weight, resistance to transfer and soil kinetics.

The value of the saturation constant (k_s) obtained for anthracene was lower when *corynebacteria spp* was used while that of pyrene was lower when *pseudomonas putida* was used. The saturation constant measures the metabolic ability of the organism to utilize the contaminant solute as carbon and energy sources. A high saturation constant is indicative of a low affinity for the solute by the microbe.

The result of the biodegradation experiment with anthracene and pyrene are shown in Table 1. Anthracene was consumed faster using *corynebacteria spp* while the biodegradation of pyrene was faster in the presence of *pseudomonas putida*. The values of the reaction rate constants are as given in Table 1. The reaction rate approximates the fraction of the solute present that is converted to product per small increment of time. The implication of these results is that the biodegradation of anthracene and pyrene in *pseudomonas putida* and *corynebacteria spp* media, are to a large extent limited by the very slow transport of these chemicals into the active site of the microbial cells since their membranes have over time due to exposure become adapted to the hydrophobic solutes pyrene and anthracene respectively.

Interestingly, the effective diffusivity of anthracene and pyrene obtained (see Tables 2 to 5) following the independent estimation of the parameters in equation (32) increased with decreasing contaminant concentration, increasing degree of penetration inwards into subsurface soil and at increased concentration of the microbial mass. This maybe attributed to the rate controlling, slow desorptive step since diffusion into pores or biologically active sites lowers the contaminant concentration. The implication of this result is significant as it affirms that the biodegradation of the contaminant solutes is a function of transport parameters and hence their effective diffusivity. In addition to Fick's first law of diffusion, which does not put into account the frictional force between PAHs and microbial mass but consider the concentration gradient as the only driving force, the developed model considers the microbial mass concentration as a frictional force to the flow of PAHs.

Conclusion

This study is focused on the potential application of bioremediation as a means of effective clean up of contaminated soils in regions where oil and gas activities are prevalent. The experimental results suggest that *corynebacteria spp* is a more effective and suitable microorganism for degrading anthracene and *pseudomonas putida* for the removal of pyrene in a sandy subsurface soil supplemented with microbes.

The estimated ratio of the effective diffusivity to the concentration of the contaminant solutes serves as an alternative option in the selection of microbes capable of facilitating the restoration of PAH contaminated sites while the simulated transport parameter forms the basis for predicting the bioavailability and mobility of the contaminants in the soil.

The mathematical formulations and solution approach adopted in this study differ significantly from previous attempts at the study of the kinetics of the biodegradation of polycyclic aromatic hydrocarbons. The developed model is a viable option/management strategy to maintaining the integrity of the environment as it obviates the need for experimental measurements over relatively long distances or spatial and temporal scales and/or time periods.

The quantitative parameters (measured, calculated and estimated) represent a valuable pool of information to the Environmental regulatory and control agencies in their functions and duties with a view to containing and remediating contaminated sites.

Notation

X = Concentration of microbial mass (mg/l)

μ = Specific growth rate of microbial mass (hr^{-1})

μ_m = Maximum specific growth rate of microbial mass (hr^{-1})

k_s = Monod kinetic Constant (kg/m^3)

Y = Yield coefficient of microbial mass on substrate (kg)

$J_{,z}$ = Rate of mass transfer of PAHs over a distance z per unit area ($kg\ hr^{-1}cm^{-2}$)

D = Effective diffusivity (cm^2/hr)

C = Concentration of PAHs (mg/l) at any time t

C_o = Concentration of PAHs (mg/l) at time t = 0

Z = Distance covered by PAHs (cm)

M = Microbial mass

P = Product of microbial degradation

k = Reaction rate constant (hr^{-1})

R = Rate of product formation (mg/l. hr)

ϕ = Transport parameter ($cm\ hr^{-½}$)

t = Time (hr)

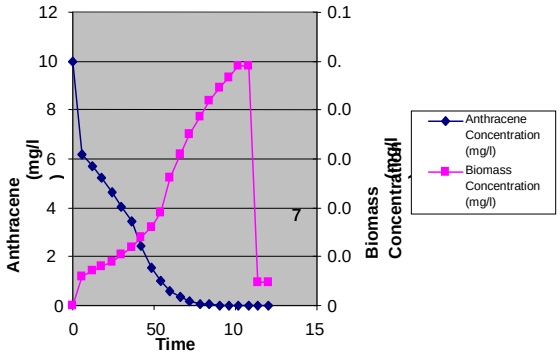


Fig.3: Mineralization of Anthracene *Pseudomona putid*

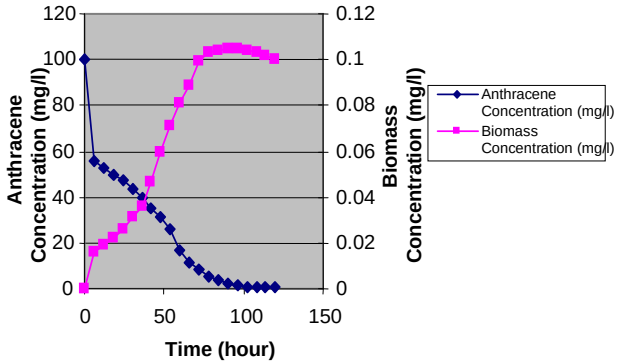


Fig.4: Mineralization of Anthracene by *Corynebacteria spp*

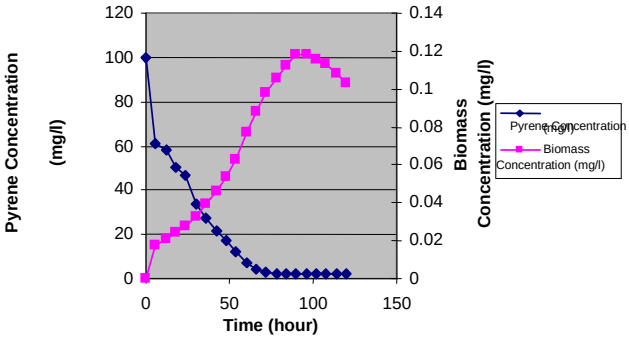


Fig.5: Mineralization of Pyrene by *Pseudomonas putida*

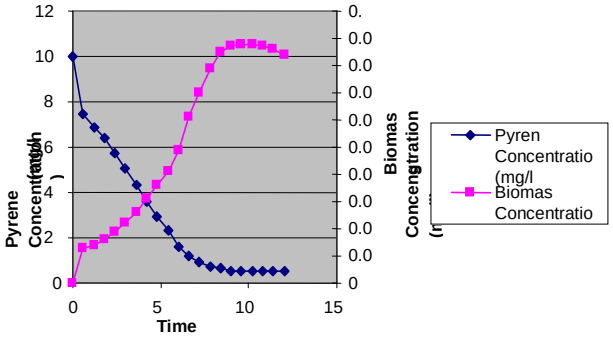
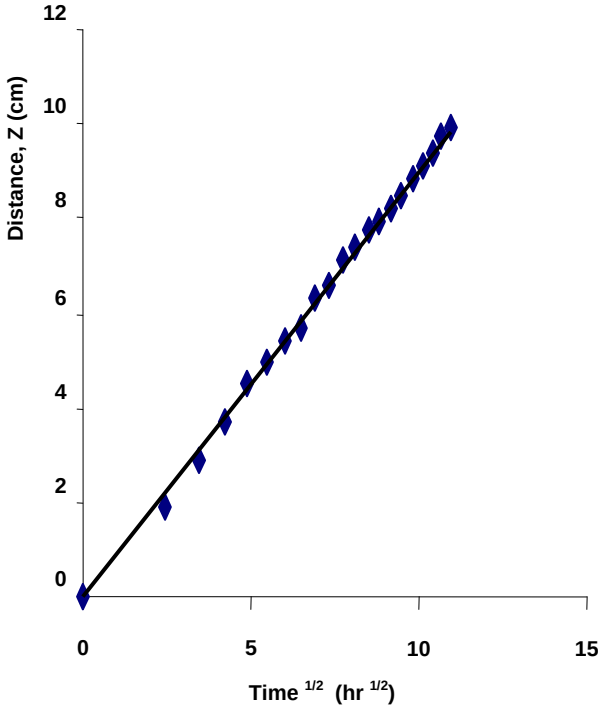


Fig.6: Mineralization of Pyrene *Corynebacteria*



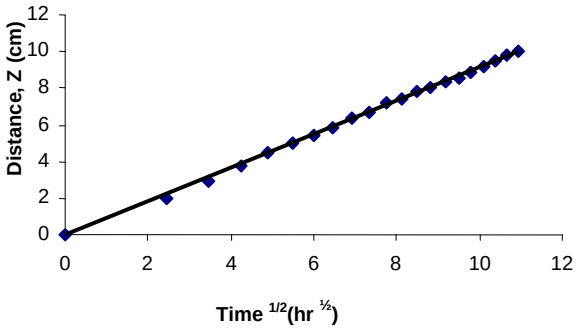


Figure 7: Simulated transport paramter for anthracene by corynebacteria spp as a function of distance and time

Figure 8 Simulated transport paramter for

Anthracene by Pseudomonas putida as a function of distance and time.

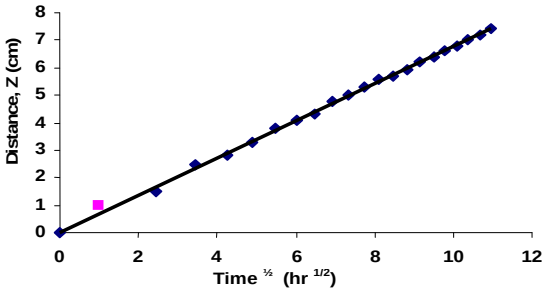


Figure 9: Simulated transport paramter for pyrene by corynebacteria spp as a function of distance and time.

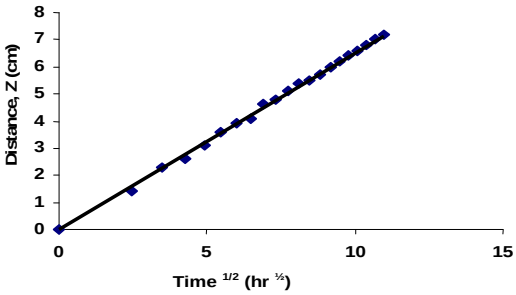


Figure 10: Simulated transport paramter for pyrene by pseudomonas putida as a function of distance and time .

Table 1: Estimated for reaction and transport parameters during biodegradation

Microbial mass on selected PAH	Reaction rate constant $k \ \left(hr^{-1}\right)$	Yield coefficient Y	Monod kinetic constant $\left(k_s\right)$	Maximum specific growth rate $\left(\mu_m\right)$	Transport parameter φ
Corynebacteria spp on Anthracene	0.0551	0.0014	0.3703	0.0760	0.9108
Pseudomonas putida on Anthracene	0.0368	0.0013	1.158	0.0263	0.9015
Corynebacteria spp on Pyrene	0.0261	0.0012	11.026	0.0308	0.6526
Pseudomonas putida on Pyrene	0.0345	0.001	6.572	0.0456	0.6764

Table 2: Evaluated effective diffusivity of anthracene using *Corynebacteria spp*

Time (hr)	Biomass concentration (mg/l)	Concentration of anthracene (mg/l)	Effective diffusivity (cm^2/hr)
0	0.016	62.007	0.00194
6	0.019	56.972	0.00212
12	0.022	52.291	0.00226
18	0.026	46.546	0.00237
24	0.031	40.163	0.00245
30	0.036	34.56	0.00246
36	0.047	24.418	0.00227
42	0.06	15.553	0.00186
48	0.071	10.092	0.00145
54	0.081	6.192	0.00104
60	0.089	3.78	0.00072
66	0.099	1.794	0.000418
72	0.103	0.518	0.000178
78	0.104	0.376	0.000151
84	0.105	0.234	0.000123
90	0.105	0.163	0.000109
96	0.104	0.163	0.000108
102	0.103	0.163	0.000107
108	0.102	0.163	0.000106
114	0.1	0.163	0.000104
120	0.097	0.163	0.000101

Table 3: Evaluated effective diffusivity of anthracene using *Pseudomonas putida*

Time (hr)	Biomass concentration (mg/l)	Concentration of anthracene (mg/l)	Effective diffusivity (cm^2/hr)
0	0.012	55.624	0.00109
6	0.014	52.645	0.00119
12	0.016	49.809	0.00129
18	0.018	47.043	0.00138
24	0.021	43.213	0.00148
30	0.024	39.667	0.00156
36	0.028	35.34	0.00163
42	0.032	31.369	0.00166
48	0.038	26.121	0.00165
54	0.052	16.475	0.00146
60	0.062	11.369	0.00124
66	0.07	8.106	0.00103
72	0.077	5.695	0.00084
78	0.084	3.709	0.00065
84	0.089	2.503	0.00052
90	0.093	1.582	0.00041
96	0.098	0.587	0.00027
102	0.098	0.587	0.00027
108	0.097	0.587	0.00027
114	0.095	0.587	0.00026
120	0.093	0.587	0.00025

Table 4: Evaluated effective diffusivity of pyrene using *Corynebacteria spp*

Time (hr)	Biomass concentration (mg/l)	Concentration of pyrene (mg/l)	Effective diffusivity (cm^2/hr)
0	0.013	74.443	0.000283
6	0.014	68.939	0.000292
12	0.016	63.839	0.000293
18	0.019	57.597	0.000325
24	0.022	50.685	0.000335
30	0.026	43.436	0.00034
36	0.031	36.255	0.00034
42	0.036	29.543	0.000326
48	0.041	23.436	0.000314
54	0.049	16.255	0.000293
60	0.061	12.228	0.00264
66	0.07	9.409	0.00025
72	0.08	7.597	0.000241
78	0.085	6.456	0.00024
84	0.087	5.517	0.00024
90	0.088	5.517	0.000238
96	0.088	5.517	0.000238
102	0.087	5.517	0.000235
108	0.086	5.517	0.000233

Table 5: Evaluated effective diffusivity of pyrene using *Pseudomonas putida*

120	0.08	5.517	0.000217

Time (hr)	Biomass concentration (mg/l)	Concentration of pyrene (mg/l)	Effective diffusivity (cm^2/hr)
0	0.018	60.886	0.000716
6	0.021	58.201	0.000781
12	0.024	50.336	0.000836
18	0.028	46.524	0.000893
24	0.033	40.617	0.000947
30	0.039	33.906	0.000988
36	0.046	27.06	0.00101
42	0.054	21.557	0.00102
48	0.063	17.195	0.00101
54	0.077	11.959	0.000977
60	0.088	6.859	0.000952
66	0.098	4.51	0.000932
72	0.106	2.899	0.000918
78	0.112	2.094	0.00091
84	0.118	1.892	0.000908
90	0.118	1.826	0.000908
96	0.116	1.826	0.000893
102	0.113	1.826	0.000869
108	0.108	1.826	0.000831
114	0.103	1.826	0.000793
120	0.097	1.826	0.000747

References

[1] Akpofure, E.A., Efere, M.L. and Ayawei, P. (2007). *Integrated grass root post-impact assessment of acute damaging effects of continuous oil spills in the Niger Delta January 1998-January 2000,* in: Oil spillage in Nigeria's Niger Delta, Urhobo Historical Society.

[2] Allan, I. J.; Semple, K. T.; Hare, R. and Reid, B. J. (2007). *"Cyclodextrin Enhanced Biodegradation of PAHsand Phenols in Contaminated Soil Slurries"*. Environmental Science and Technology. Vol.41 (15), 5498-5504.

[3] Alexander, M. (2000). *"Ageing, bioavailability and overestimation of risk from environmental pollutants."* Environmental science and technology. Vol. 34, 4259-4265.

[4] Ayotamuno, M.J., Okparanma, R.N., Nweneka, E.K., Ogaji, S.O.J. and Probert, S.D. (2007). *"Bioremediation of a sludge containing hydrocarbons"*. Applied Energy, Vol. 84, 936-943.

[5] Bird, R.B., Stewart, W.E. and Lightfoot, E.N. (2005). Transport Phenomena. John Wileyand sons, New York, 521 – 543.

[6] Brown, D.G., Guha, S. and Jaffe, P.R. (1999). *"Surfactant-enhanced biodegradation of a PAH in a soil slurry reactor."* Bioremediation J. Vol. 3, 269-283.

[7] Brown, D.G. (2007). *"Relationship between micellar and hemi-cellar processes and the bioavailability of surfactant-solubilized hydrophobic organic compounds."* Environ. Sci. Technol. Vol. 41(4), 1194-1199.

[8] Coulson, J.M and Richardson, J.F. (2000). Chemical Engineering, John Wiley and Sons, New York, 6th edition, l, 626-642.

[9] Couteliers, F.A., Burganos, V.N. and Payatakes, A.C. (2004). *"Model of adsorption-reaction-desorption in a swarm of spheroidal particles."* AICHE J. Vol. 50, 779-785.

[10] Fogler, H.S. (2006). Elements of Chemical Reaction Engineering. Prentice-Hall of India Private Limited, New Delhi, 3rd edition, 383-386, 581-616.

[11] Karthikeyan, R. and Bhandari, A. (2001).*"Anaerobic biotransformation contamination in soil and groundwater."* Chemistry and Industry. Vol. 4. 784-789.

[12] Levenspiel, O. (2002). Chemical Reaction Engineering, John Wiley and Sons Inc. New York, 3rd edition, 13-65, 67-641.

[13] Makkar, R. S. and Rockne, K. J. (2003). *"Comparison of Synthetic Surfactants and Biosurfactants in enhancing Biodegradation of PAHs"*. Environ. Toxicol.and Chemistry. Vol. 22 (10), 2280-2292.

[14] Nwachukwu, S.C.U. (2001). *"Bioremediation of sterile agricultural soils polluted with crude petroleum by application of the soil bacterium, pseudomonas putida, with inorganic nutrient supplementation"*. Current Microbiology. Vol. 42, 231-236.

[15] Oboh, B.O., Ilori, M.O., Akinyemi, J.O. and Adebusoye, S.A. (2006). *"Hydrocarbon degrading potentials of bacteria isolated from a Nigerian bitumen (Tarsand) deposit"*. Nature and Science. Vol. 4 (3) 51-57.

[16] Odokuma, L.O. and Dickson, A.A. (2003). *"Bioremediation of crude oil polluted tropical rain forest soil."* Global J. of Environmental Sciences. Vol. 2, 29-40.

[17] Ofeimu, K.E and Salami, O. (2006). Biodegradation kinetics of polycyclic aromatic hydrocarbons by pure fungal isolates. B.Eng Thesis, University of Benin, Benin City.

[18] Oh, Y.S., Sim, D.S. and Kim, S.J. (2001). *"Effects of nutrients on crude oil biodegradation in the upper interstitial zone."* Mar. Pollut. Bull. Vol. 42, 1367-1372.

[19] Ohnuwa, T.; Suto, K. and Inoue, C. (2007). *"Degradation of PAHs by bacteria isolated from light oil polluted soils"*. American Institute of Physics. Vol. 898, 235-238.

[20] Oleszczuk, P. and Baran, S. (2003). *"Degradation of individual polycyclic aromatic hydrocarbons (PAHs) in soil polluted with aircraft fuel."* Polish J. Environ. Studies. Vol. 12, 431-437.

[21] Owabor, C.N. and Irheren, M.O. (2006). "Effect of nutrient and moisture content variation on the bioremediation of anthracene contaminated soil". Nigerian J. of Biomedical Engineering. Vol. 4(1), 9-14.

[22] Owabor, C.N. (2007). Substrate bioavailability and biodegradation of naphthalene, anthracene and pyrene on contaminated aqueous-soil matrix. PhD Thesis. University of Lagos, Lagos Nigeria.

[23] Pignatello, J.J. and Li, J. (2006). *"Facilitated bioavailability of PAHs to native soil bacteria promoted by nutrient addition."* American Geophysical Union, Fall meeting, H331-05.

[24] Shor, L.M., Kosson, D.S., Rockne, K.J., Young, L.Y. and Taghon, G.L. (2004). *"Combined effects of contaminant desorption and toxicity on risk from PAH contaminated sediments."* Risk Analysis. Vol. 24, 1109-1119.

[25] Zhu, L., Chen, B. and Tao, S. (2004). *"Sorption behaviour of polycyclic aromatic hydrocarbons in soil-water system containing non-ionic surfactant."* Environ. Eng. Sci. Vol. 21 (2), 263-272.