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Model Simulation of Biodegradation of Polycyclic aromatic Hydrocarbon in a Microcosm

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ABSTRACT: The solutions of mathematical models for the estimation of the kinetic, and biokinetic parameters of naphthalene, anthracene and pyrene during degradation in surface and subsurface soils are presented in this work. The models were developed using the twin concepts of rate-determining step and steady-state approximation method. They described the biodegradation of single and a mixture of polycyclic aromatic hydrocarbons. Prediction of the concentration of the reactive PAHs with time was aided by fitting the models to the experimental data obtained from a soil microcosm reactor. Given an initial concentration of 100mg/L, approximately 2.9%, 1.9% and 1.4% of naphthalene, pyrene and anthracene present in the microcosm reactor at zero time were found to be utilized in a minute when the velocity of the reaction remained constant for the period.The rate-determining step model gave a better fit as its reaction rate constant (k) closely fitted the experimental values. Prediction by the steady state approximation model was not feasible as a comparative analysis of both single and multisubstrate results showed that the steady state approximation overestimates the biodegradation rates.Using the relative error method, results indicated that the rate-determining step model showed a deviation of 7.5%. The rate-determining step model was chosen because the differences in the model fits were small and its prediction of mixture experiment was more enhanced.

Key words: PAH, Degradation, Mathematical models, Solution method, Twin concept

INTRODUCTION

Large amounts of petroleum and petroleum products are discharged into the environments as a result of exploration, production, transportation, refining and utilization. Despite careful handling and containment, some may spill into the soil through blowouts, accidents; rupture of oil pipelines and from domestic waste. In Nigeria, as a result of increasing daily activities within the petroleum industry, polycyclic aromatic hydrocarbons input into the environment have increased greatly (Asuquo *et al.*, 2004; Akpofure *et al.*, 2007). It has therefore become imperative and very desirable to decontaminate locations that have had loading of petroleum or petroleum products.

Concerted efforts have been made to understand the reactions of these groups of compounds in aqueous-solids/sediments matrix. Bioremediation has become a huge success in harnessing the natural activity of microorganisms for the performance of beneficial functions that have greatly enhanced our standard of living (Bhatt, 2002; Oleszczuk and Baran, 2003; Janikowski *et al.*, 2004; Xu and Obbard, 2004). The microorganisms exposed to these anthropogenic substances have sometimes responded by acquiring new genes for degradation of these compounds, either for detoxification or to enable the microbe use the contaminant as a source of energy to meet metabolic needs (Boochan et al, 2000; Chunga and King, 2001; Reardon *et al;* 2002; de Lucas *et al.*, 2005). There are dearth of reports on the rate and metabolism of this group of compounds in experimental systems developed to model natural degradation. The use of the microbes as catalysts in bioremediation, particularly in Nigeria has been to essentially increase the rate of degradation so as to eliminate as quickly as possible, both long and short-term effects of these contaminants that compromise the integrity of the environments (Nwachukwu, 2001; Ebuehi *et al.*, 2005; Oboh *et al.*, 2006; Ojo, 2006).

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In this study, the use of a microcosm reactor is advanced. The microcosm was set up to provide acclimated soil for the microbiota. The investigation was to determine the rates of mineralization of the polycyclic aromatic hydrocarbons and assess the level of microbial activity using oxygen uptake and carbon dioxide evolution data as indicators. A mathematical model was also developed. It incorporates interaction between molecular diffusion and kinetics of the process which will help to validate the data obtained from experiments.

MATERIALS & METHODS

The polycyclic aromatic hydrocarbons used (naphthalene, anthracene and pyrene) were purchased from Harrison and Harrison Laboratories Co. Ltd in Lagos. The culture media used were Potato Dextrose Agar and Nutrient Agar. The Minimal salts medium consisted of the following K_2HPO_4, KH_2PO_4, $MgSO_4$, NaCl, $CaCl_2$ and NH_4NO_3. Trace elements solution was prepared using MgO, $CaCO_3$, $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$, $MgSO_4.4H_2O$, $CuSO_4.5H_2O$, H_3BO_3, and HCl. Other chemicals used were: potassium hydroxide, calcium hydroxide, pyrogallol, and mercuric chloride. All reagents used were of analytical grade. Distilled water was used for solution, sample preparation and dilution.

Soil sample collected from unimpacted zones at field 17 in the Nigerian Institute For oil Palm Research (NIFOR), near Benin City, Edo State, Nigeria was used. The sampling was done around a sampling point to a depth of 0-15cm.The bulked composite soil samples were put in a sterile black polyethylene bag sealed and stored in a refrigerator prior to analysis.The contaminant hydrocarbons used for the study include naphthalene, anthracene and pyrene.1 kg unimpacted surface and subsurface soils were excavated and placed inside the microcosm. The soil was spiked with a mixture of the contaminant hydrocarbons (200mg each) dispersed in 2 liters of water containing a 0.02% surfactant sodium hexametaphosphate, SHMP (Koeppel *et al.*, 1997) and nutrients (straw, saw dust, and poultry dung) as prescribed by the organization for economic cooperation and development (OECD). Another reactor was also set up and used as a control. A constant flow rate of oxygen was then sent into the two reactors. The temperature and pressure of the microcosm reactors were monitored throughout the period of experimentation using a digital multimeter and pressure gauge.

Thereafter, samples were taken on a weekly basis and analyzed using solvent extraction and gas chromatography methods to determine the concentration of contaminants. The oxygen uptake and carbon (iv) oxide evolution measurements were also carried out.

The absorbent (100mL) in the absorption bottle consisted of one volume of a 1% (w/v) aqueous solution of pyrogallol with three volumes of a 30% (w/v) aqueous solution of potassium hydroxide. Opening the stopcock connecting the absorption bottle to the microcosm absorbed oxygen, sample gas was drawn into the absorbent solution and the amount of oxygen not consumed was determined colorimetrically using a UV/V spectrophotometer (Spectronic 21D), at a wavelength of 605nm. Sampling was done every 5days.

Carbon dioxide evolved was carried out using orsat gas analyzer. The absorption vessel was charged with 100mL calcium hydroxide as the absorbent. The leveling bottle was filled with a confining liquid (5% sulphuric acid solution containing a few drops of methyl orange indicator). The leveling bottle was raised to the top of the analyzer, with the 3-way cock at the end of the manifold opened. The burette was filled with water up to the capillary tube and air was removed from the connecting tube using a rubber bellows pump. With the 3-way cock suitably set, sample gas was drawn into the burette by lowering the leveling bottle until the water meniscus reaches the lowest graduation mark of the burette. The stopcock connecting the burette to the absorption vessel containing calcium hydroxide was opened and the level bottle rose. The level bottle was lowered again and the gas brought into the burette until the absorbent in the vessel reaches the mark just above the top of the vessel. The operation was repeated until absorption was complete as was evidenced when the meniscus in the level bottle was at the level with that in the burette. The burette reading was recorded. Sampling was done every 10 days.Kinetic models for the biodegradation of contaminants at the gas-liquid and liquid-solid interface film were developed using the concepts of rate-determining step (RDS) and steady state approximation (SSA), with molecular diffusion governed by Fick's law inclusive. The kinetic model equations for both a single and multisubstrate catalyzed reactions, are given below using the equations described by Owabor *et al.*, 2002.

a. RDS:

(i) Single Substrate Reaction, n = 1

$$C = \frac{1}{2}\left[\frac{C_o e^L}{e^L - 1} + C_o\right] - \left[\frac{C_o e^x}{2\left(e^L - 1\right)}\right] \qquad (1)$$

(ii) Multi - Substrate Reaction, n > 1

$$C = \left[\frac{C_o e^L}{e^L - 1} + C_o\right]\left[\frac{C_o e^x}{2\left(e^L - 1\right)}\right] + \frac{n-1}{x} \quad (2)$$

b. SSA:

(iii) Single substrate Reaction, n =1

$$C = \left(\frac{V_{\max}\left[C_o\right]}{K_m + \left[C_o\right]}\right)\frac{x}{D} \quad (3)$$

(iv) Multi substrate Reaction, n >1

$$C = \frac{V_{\max}\sum\left[C_i\right]}{K_m + \sum\left[C_i\right]}\frac{x}{D} \quad (4)$$

The initial condition:

C (z, t) = f(z) at t = 0 and ze" 0 (5)

Boundary conditions:

C (z, t) = 1 at z = 0 and t d" 0 (6)

C (z, t) = 0 at z = 1 and t e" 0 (7)

RESULTS & DISCUSSION

The experiments conducted in this study provide a deep understanding of the degradation kinetics of both single and a mixture of naphthalene, anthracene and pyrene as model polycyclic aromatic hydrocarbons (PAHs) in the surface and subsurface aqueous-soil matrix.The main objective of the microcosm study was to provide an insight on the activity of the micro-organisms as well as a source of acclimated soils. Results of the physicochemical properties of the soil are as shown in Table 1.

The salient characteristics of the uncontaminated soil used showed that the soil was highly porous with a particle size distribution in the ratio 85%: 14%: 1% for sand, clay and silt respectively. The microbial activities which would have been impeded as a result of the low moisture content (5%) of the soil were enhanced by the addition of water which provided an aqueous environment suitable for the degradation process. The cation exchange capacity of the soil was within the recommended value of 5 cmol/kg for sandy soil low in organic matter. This is especially significant because decaying organic matter shrinks and swells and encourages micro fauna which create or widen openings in the soil thus improving infiltration and aeration. The level of exchangeable acidity as reflected by the pH implied that the soil was very acidic. This, therefore, portends a reducing environment. The redox condition necessary to enhance microbial activity and hence the degradation of the PAHs in the soil was achieved through the use of oxygen which functioned as electron donor. The low nitrogen and low-to- moderate available phosphorus in the soil accounted for the low nutrient level which was however, supplemented with straw, sawdust and poultry dung as prescribed by the organization for economic cooperation and development (OECD).

Table 1. Soil Characteristics

Soil moisture (%)	**5.027**
Bulk density	1.25
Total porosity	0.481
Particle size (%)	
Sand	85
Silt	1
Clay	14
pH (1:1) H_2O	3.98
Organic Carbon (%)	2.44
Organic Matter (%)	4.19
Total Nitrogen (%)	0.114
Available Phosphorus (mg/Kg)	6.65
Exchangeable Bases (cmol/kg)	
Ca^{++}	0.65
Mg^{++}	0.14
K^{+}	0.12
Na^{+}	0.08
Exchangeable Acidity ($Al^{3+} + H^{+}$)	2.66
CEC (cmol/kg)	3.65

As a means of assessing the level of microbial activities in relation to biodegradation kinetics in the soil, the soil samples in the microcosm reactor was used as a source of acclimated micro biota for measuring oxygen uptake by respiration to determine biodegradation kinetics and carbon (iv) oxide evolution kinetics. The objective was to ascertain the actual oxygen uptake and carbon (iv) oxide evolved as a result of the mineralization of the PAHs in the absence of soil organic carbon. Fig. 1 showed the net cumulative car

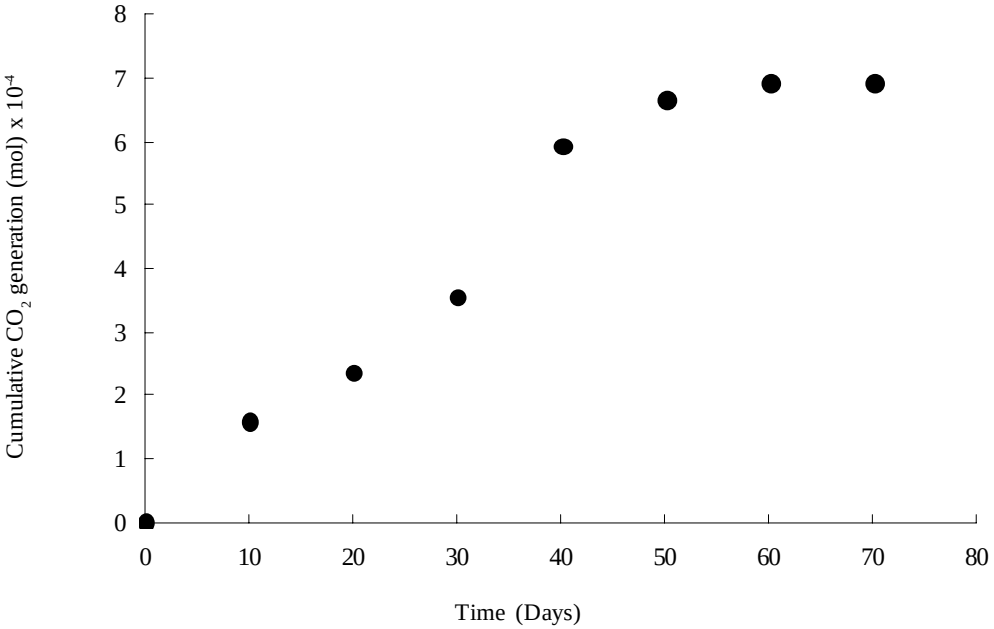

Fig. 1. Net Cumulative carbon dioxide evolution from the reactor spiked with a mixture of Naphthalene, Anthracene and Pyrene

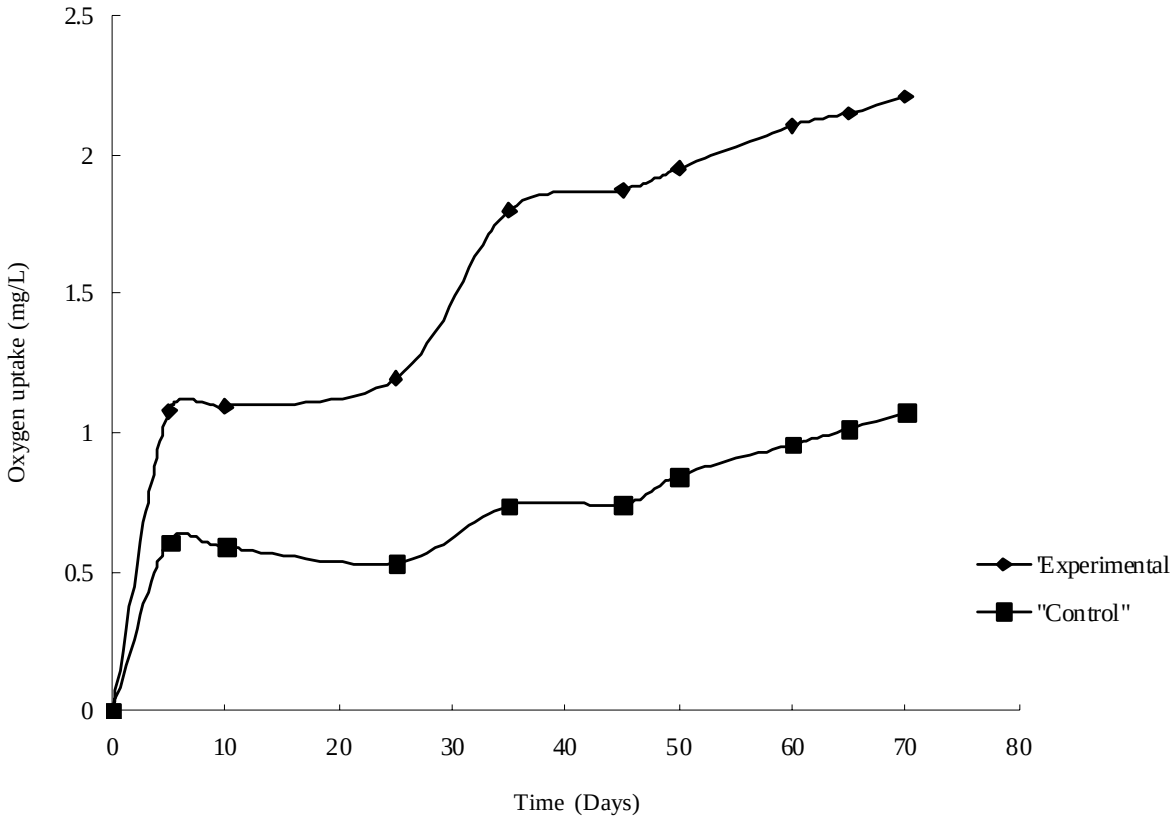

Fig. 2. Oxygen uptake in Microcosm reactor

bon (iv) oxide evolution, i.e., actual carbon (iv) oxide evolution from microcosm minus the carbon (iv) oxide evolution from the control reactor spiked with only the OECD nutrients. The results showed that after impacting the soil with a mixture of PAHs, the net cumulative carbon (iv) oxide evolution increased and attained a plateau concentration with a carbon (iv) oxide limit of 6.925×10^{-4} moles within 60days of contact time. The oxygen uptake curve is depicted by Fig. 2 while its net cumulative uptake curve in Fig. 3 similarly showed an equilibrium time of 60days. These results were validated by the result of the GC/FID analysis of the soil core sample, which did not detect any contaminant from the 64th day of the study as shown in Fig. 4. This suggested that a reasonable degree of PAH acclimation was achieved in the soil microcosm within the exposure time.Representative experimental biodegradation kinetics data for naphthalene, anthracene and pyrene is shown in Fig. 4.

Approximately 98.4% of the naphthalene, 82.98% anthracene and 89.67% pyrene contained in the microcosm had been degraded within 33 days of exposure. The extent of degradation of the PAHs tested was found to be dependent on the molecular weight, solubility and diffusivity in water as given by Zander (1983), Perry and Green, (1998) Oleszczuk and Baran, (2003). The increasing order of molecular weight would have suggested a progressive decrease in the degradation rate but their diffusivity/transfer rate and solubility in water present some degree of variation, which explains the observed preferential decay of one PAH over the other. Anthracene exhibits a lower solubility in water and hence it was not readily available for utilization by the indigenous microbes present in the soil. The experimental data from the soil microcosm reactor was used to simulate the change in PAH concentration in contaminated soil as a function of time, for both single and multisubstrate catalysis. Figs 5 and 6 show the simulated concentration plots for single and multisubstrate catalysis using both rate-determining step and steady state approximation methods.

The typical hockey–stick curves obtained is an indication of an initial, rapid decrease in the concentration of the PAHs, followed by a significantly slower rate of degradation. This may be attributed to oxygen diffusion limitation in the microcosm. At the onset, oxygen diffuses rapidly in the upper section of the microcosm and thereafter, a slower rate in the lower section. In addition, the incidence of refraction of the contaminants may also have been responsible for the observed exponential decay pattern.

The effects of these limitations are reflected in the estimated kinetic and biokinetic parameters calculated based on all measured quantities as shown in Tables 2 and 3. The reaction rate constant k approximates the fraction of the substrate present that is converted to product per small increment of time. The results imply that approximately 2.9%, 1.9% and 1.4% of naphthalene, pyrene and anthracene present in the microcosm reactor at zero time would be utilized in a minute if the velocity of the reaction remained constant for the period.

Table 2. Kinetic Constants for Contaminant PAHs with model fits

	k/min
Naphthalene	0.029
Anthracene	0.014
Pyrene	0.019
Single Substrate RDS Model	0.06
Multi Substrate RDS Model	0.069
Single Substrate Steady State Approximation Model	51.487
Multi Substrate Steady State Approximation Model	12.05

The objective of the simulation was to obtain the attainable reaction rate constant (k) of the biodegradation process. From the results obtained, the RDS model gave a better fit as its k value closely fitted the experimental values. Prediction by the SSA model was not feasible as its k values for both single and multisubstrate catalysis deviated widely from experimental data. Comparing the simulated and experimental results show that the SSA overestimates the biodegradation rates. This observation can be attributed to the existence of two types of intermediates at steady state usually present at such small concentrations that its rate of change in the mixture can be assumed to be zero or negligible. The RDS model was therefore chosen because the differences in the model fits were small and its prediction of mixture experiments were more enhanced.

The biokinetic parameters for each of the three PAHs estimated using the celebrated Michaelis-Menten kinetics equation via the Lineweaver-Burk plot for rate against concentration of contaminant are shown below in Table 3. The Lineweaver-Burk reciprocal plot is simply a linear transformation of the basic velocity of the Michaelis-Menten kinetic equation (Levenspiel, 1999).

From the results, using the numerical values of V_{max} and K_m the following observations were adduced;

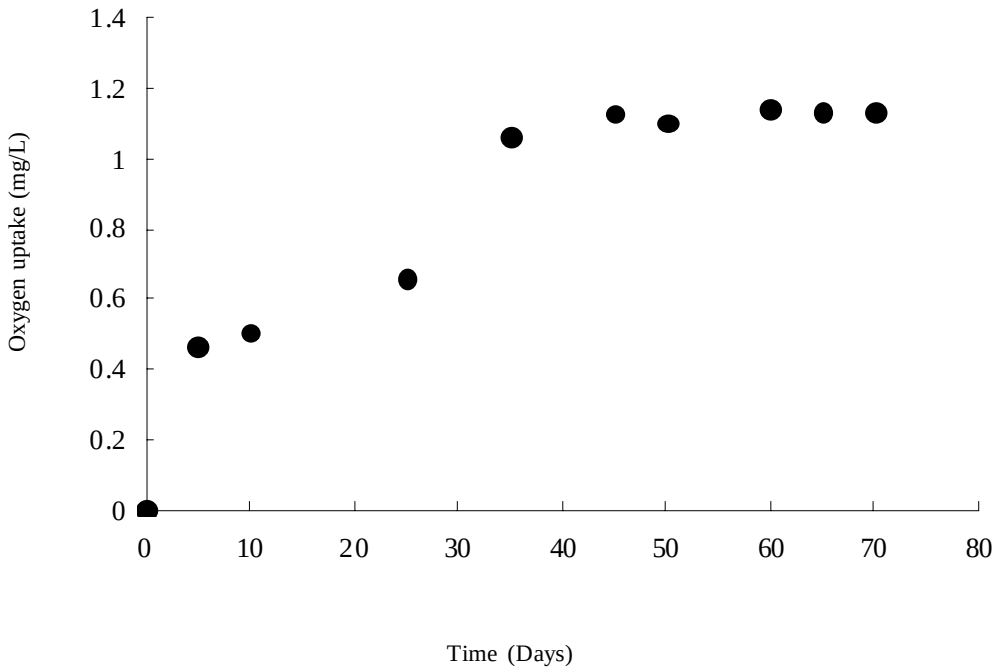

Fig. 3. Net Cumulative oxygen uptake in Microcosm reactor

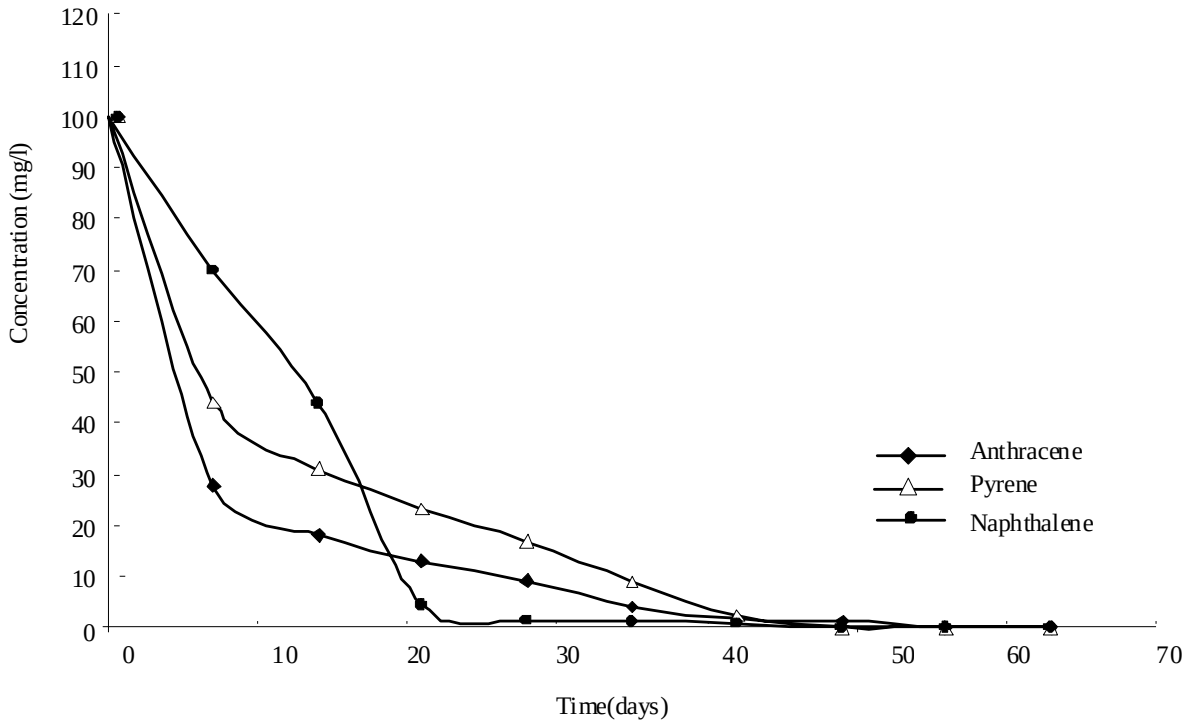

Fig. 4. Variation of concentration of PAHs against time

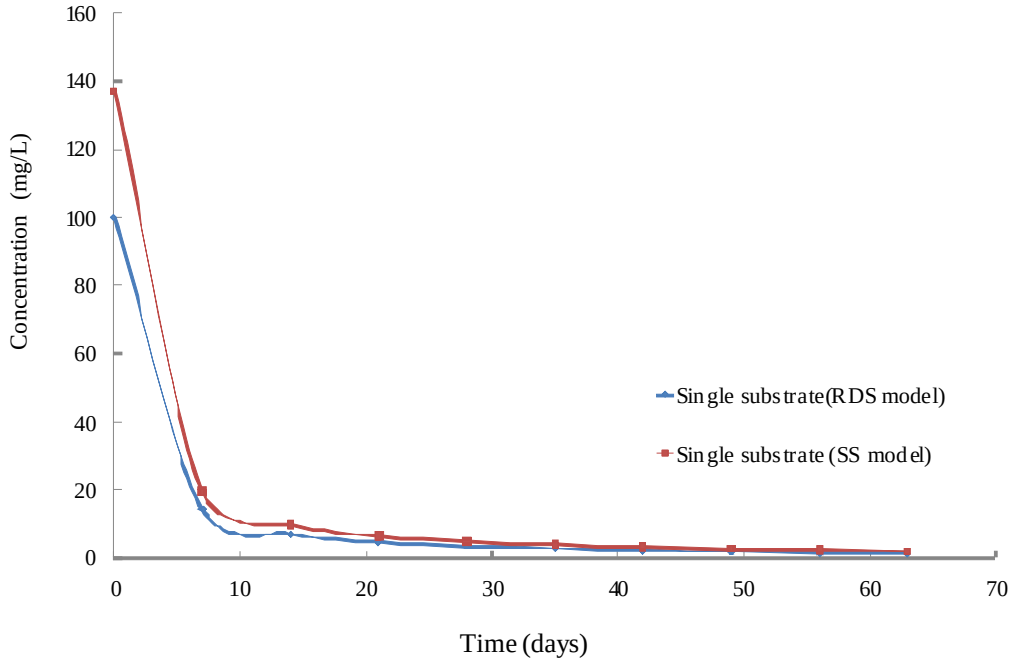

Fig. 5. Simulated concentration of single substrate model against time

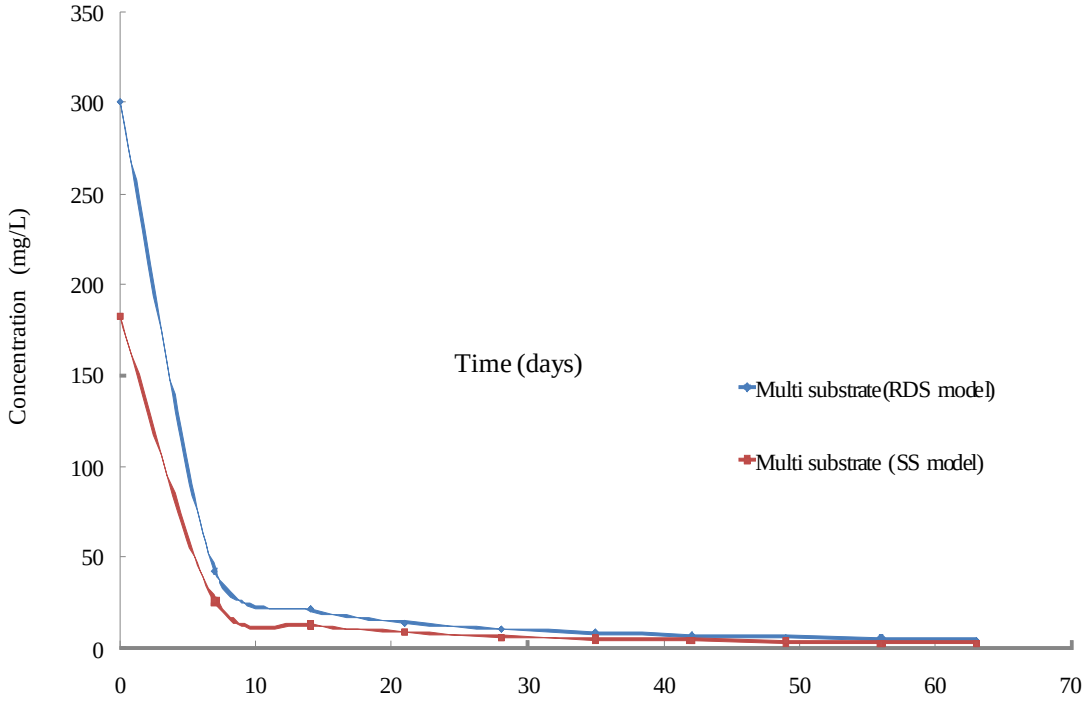

Fig. 6 . Simulated concentration of Multisubstrate model against time

Table 3. Biokinetic Constants for Contaminant PAHs

Constants	Naphthalene	Anthracene	Pyrene
V_{max} (moles/l.min)	0.0028	0.015	0.0023
K_m (M)	0.00314	0.0468	0.0035

Table 4. Relative Error between the two Models and Experimental data

Rate Determing Step		Steady State Approximation	
Single substrate	Multi substrate	Single substrate	Multi substrate
0.0651	0.065	0.133	0.1364

that the enzyme catalyzing the degradation process were specific, the enzymes catalyzing the breakdown of naphthalene and pyrene had very close attributes and that there was a weak binding between anthracene and the enzymes catalyzing its breakdown as indicated by the K_m value.

The K_m which is Michaelis constant is a pseudo-equilibrium constant expressing the relationship between the actual steady state concentration rather than the equilibrium concentrations. It measures the strength of the ES complex. Low indicates strong binding while a high indicates weak binding. V_{max} is not a constant of the enzyme in the reaction, but defines the maximal velocity that would be observed when the entire enzyme is present as an intermediate ES.

4. 0 Model validation

In the validation of the kinetic model, two sets of model simulation were carried out. The simulated effectiveness factor of the rate-determining step and steady state approximation model was depicted using a relative error (RRE) method to show the goodness of fit between the experimental data and the model. The result is presented in Table 4.

$$RRE = \frac{\sqrt{\sum_{i=1}^{m}\left[\mathrm{mod}(t_i) - Exp(t_i)\right]^2}}{mExp(t_i)}$$

Where:

Mod (t_i) = value determined from the model at time t_i

Exp () = experimental value at time

m = number of experimental points

The RRE show the deviation of the simulated results from the experimental data. The result of the validation of the model gave a 6.5% and 13.5% deviation for the rate-determining step and steady state approximation method respectively. This implied that prediction by the rate-determining step closely approximates the data from experiment.

CONCLUSION

In this work, reasonable fit was obtained between the rate-determining step model and the experimental biodegradation kinetics data. The agreement achieved clearly reflects the efficiency of the RDS model in the prediction of the concentration of the contaminants under steady state conditions. Prediction by the steady-state model was found to be very poor as it deviated widely from experimental data (about 13.3%) and overestimates biodegradation rates. From the results of the study, it was ascertained that the enzymes catalyzing the degradation of the PAHs were specific. Anthracene had the lowest rate constant which suggested the effects of some limitation to its degradation considering that it has a lower molecular weight when compared with pyrene. The observed values further imply that there is a weak ES complex between anthracene and the enzymes catalyzing the degradation. Finally, naphthalene was more readily metabolized and used as energy sources by the microbes as indicated by the estimated rate constant k.

Nomenclature

C_o Concentration at t = 0

C_i Concentration of contaminant in the external pellet surface i.e. opening of the Pores (mg/L)

D Diffusivity of contaminant m^2/s
L Length of reactor (m)
k Reaction rate constant (min^{-1})
X Distance in the direction of flow (m)
RRE Relative Error

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