Biodegradation (2008) 19:223–233
DOI 10.1007/s10532-007-9129-3

ORIGINAL PAPER

Biodegradation kinetics of select polycyclic aromatic hydrocarbon (PAH) mixtures by *Sphingomonas paucimobilis* EPA505

Anuradha M. Desai · Robin L. Autenrieth · Petros Dimitriou-Christidis · Thomas J. McDonald

Received: 25 September 2006 / Accepted: 24 April 2007 / Published online: 30 May 2007

Abstract Many contaminated sites commonly have complex mixtures of polycyclic aromatic hydrocarbons (PAHs) whose individual microbial biodegradation may be altered in mixtures. Biodegradation kinetics for fluorene, naphthalene, 1,5-dimethylnaphthalene and 1-methylfluorene were evaluated in sole substrate, binary and ternary systems using *Sphingomonas paucimobilis* EPA505. The first order rate constants for fluorene, naphthalene, 1,5-dimethylnaphthalene, and 1-methylfluorene were comparable; yet Monod parameters were significantly different for the tested PAHs. *S. paucimobilis* completely degraded all the components in binary and ternary mixtures; however, the initial degradation rates of individual components decreased in the presence of competitive PAHs. Results from the mixture experiments indicate competitive interactions, demonstrated mathematically. The generated model appropriately predicted the biodegradation kinetics in mixtures using parameter estimates from the sole substrate experiments, validating the hypothesis of a common rate-determining step. Biodegradation kinetics in mixtures were affected by the affinity coefficients of the co-occurring PAHs and mixture composition. Experiments with equal concentrations of substrates demonstrated the effect of concentration on competitive inhibition. Ternary experiments with naphthalene, 1,5-dimethylnaphthalene and 1-methylfluorene revealed delayed degradation, where depletion of naphthalene and 1,5-dimethylnapthalene occurred rapidly only after the complete removal of 1-methylfluorene. The substrate interactions observed in mixtures require a multisubstrate model to account for simultaneous degradation of substrates. PAH contaminated sites are far more complex than even ternary mixtures; however these studies clearly demonstrate the effect that interactions can have on individual chemical kinetics. Consequently, predicting natural or enhanced degradation of PAHs cannot be based on single compound kinetics as this assumption would likely overestimate the rate of disappearance.

A. M. Desai · R. L. Autenrieth (✉) ·
P. Dimitriou-Christidis
Department of Civil Engineering, Texas A&M University, College Station, TX 77843-3136, USA
e-mail: r-autenrieth@tamu.edu

Present Address:
A. M. Desai
Department of Civil and Environmental Engineering, University of Houston, Houston, TX 77204-4003, USA
e-mail: amdesai@mail.uh.edu

Present Address:
P. Dimitriou-Christidis
Carollo Engineers, 376 E Warm Springs Road Suite 250, Las Vegas, NV 89119, USA
e-mail: pdimitriou@carollo.com

T. J. McDonald
Health Science Center, School of Rural Public Health, Texas A&M University, College Station, TX 77802-1266, USA

Keywords PAHs · Biodegradation kinetics · Mixtures · Substrate interactions · Monod parameters · Multisubstrate

Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent a large family of organic compounds that are considered environmental contaminants. These compounds are widespread in the environment and can be present in quantities that threaten environmental and human health. PAHs are of concern because some PAHs constitute a significant group of chemical carcinogens (Dabestani and Ivanov 1999; Sherma 1993). Microbial degradation can be the dominant process in the removal of PAHs in the environment, but photo-oxidation and volatilization may be competitive removal mechanisms (Mueller et al. 1989) for low molecular weight (LMW) PAHs such as naphthalene and fluorene. PAHs that typically consist of 2–3 rings are considered LMW, and those that have 4–7 rings are high molecular weight (HMW). Biodegradation kinetics of individual PAH compounds by pure and mixed microbial communities have been reported by several researchers (Boldrin et al. 1993; Cerniglia 1992; Heitkamp and Cerniglia 1988; Kanaly and Harayama 2000; Ye et al. 1996). However, contaminated sites are commonly polluted by complex mixtures of PAHs (Bauer and Capone 1988; Guha et al. 1998; Guha et al. 1999; Leblond et al. 2001). Bioremediation is a remediation approach in over 135 Superfund and Underground Storage Tank (UST) sites, as well as many other sites contaminated with complex mixtures of PAHs (USEPA 1989). Yet, the efficacy of bioremediation still remains challenged (Mohan et al. 2006).

For bioremediation of PAHs to be successfully implemented as a remediation technology, it is essential to understand biodegradation in mixtures of PAHs. Interactions between PAHs are possible which can alter the rate and extent of biodegradation within a mixture (Beckles et al. 1998; Guha et al. 1999; Kelley and Cerniglia 1995; Knightes 2000). Rarely can biodegradation patterns of single PAHs be extended to degradation patterns of their mixtures (Beckles et al. 1998). Yet most of the studies have focused either on individual PAH biodegradation or reported substrate interactions in PAH mixtures on a qualitative basis without integrating kinetic modeling. Competitive inhibition of phenanthrene biodegradation in the presence of naphthalene, methylnaphthalene and fluorene was mathematically described by a competitive inhibition model indicating that similar enzyme systems are being employed by two pseudomonads (Stringfellow and Aitken 1995). A few studies applied a multisubstrate model to predict biodegradation kinetics in simple and complex mixtures using parameters obtained from sole substrate experiments (Guha et al. 1999; Knightes 2000).

Sphingomonas paucimobilis EPA505 was isolated from a creosote facility (Mueller et al. 1989) and has the ability to utilize fluoranthene, a HMW PAH as a sole carbon and energy source (Mueller et al. 1990). Ye et al. (1996) reported that the bacterium has broad substrate specificity, since fluoranthene is able to induce enzyme(s) that can catalyze the degradation of a variety of PAHs (Mueller et al. 1990). A few studies investigated biodegradation kinetics and substrate interactions in PAH mixtures by *S. paucimobilis* (Lantz et al. 1997; Luning Prak and Pritchard 2002; Ye et al. 1996). These studies considered biodegradability of PAH mixtures with PAH concentrations above their aqueous solubility and used a surfactant to enhance solubilization of PAHs. Biodegradation kinetics accounted for additional effects due to dissolution processes and they observed a correlation between biodegradation and rate of dissolution (Ye et al. 1996). In addition, none of these studies modeled the biodegradation kinetics in the presence of multiple substrates. Although kinetic models of simple mixtures do not mimic environmental conditions, they provide a starting point in understanding mixed-substrate kinetics (Kovárová-Kovar and Egli 1998).

The objective of this study was to evaluate the aqueous phase biodegradation kinetics of PAHs for sole substrates, binary and ternary systems using *S. paucimobilis*. Polycyclic aromatic hydrocarbon concentrations were below their aqueous solubilities to minimize bioavailability effects so that biodegradation could emerge as the process controlling the fate of PAHs. The PAHs evaluated were fluorene (FLE), naphthalene (NAP), 1,5-dimethylnaphthalene (15DMN), and 1-methylfluorene (1MFLE). The Monod model was fitted to the experimental data to generate the biokinetic parameters of maximum specific substrate utilization rate (q_{max}) and affinity coefficient (K_s). The data obtained from the multi-substrate experiments were evaluated with two

models, competitive inhibition and sole substrate Monod. The competitive inhibition model assumes that PAHs exhibit a common rate-limiting step (Segel 1975). The underlying hypothesis of this research was that biodegradation of the selected PAHs in mixtures would result in competition kinetics.

Materials and methods

Chemicals and chemical analysis

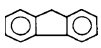
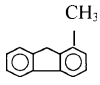
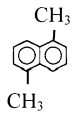
FLE was purchased from Sigma Chemical Co. (St Louis, MO). NAP, 15DMN, and 1MFLE were purchased from Alfa Aesar (Ward Hill, MA), ND Avocado Research Chemicals (Heysham, England) and Ultra Scientific (North Kingstown, RI), respectively. The physical and chemical properties of the test compounds are provided in Table 1. The purity of all chemicals was greater than 95%. Tween 80 was purchased from Sigma Chemical Co. (St Louis, MO). The protein assay kit with Bovine Serum Albumin (BSA) as standard was purchased from Bio-Rad (Hercules, CA). The internal standard used for PAH quantitation via gas chromatography-mass spectrometry (GC/MS) consisted of 1:1 mixture of 20016 and GRH-IS standards purchased from Absolute Standards, Inc. (Hamden, CT) and Accustandard, Inc. (New Haven, CT), respectively.

A HP 5890 Series II GC/MS coupled with a HP 5972 mass selective detector was used for quantification of the PAH compounds. The column for the GC-MS was a HP 5MS ((5%- Phenyl)-Methylpolysiloxane, 0.25 mm × 30 m × 0.25 μm, J & W Scientific). The operating conditions were: flow rate of 0.63 ml/min, temperature program: 60°C, 8.0°C/min for 30 min to 300°C. The mass spectrometer was operated in the selective ion mode (SIM). The detection limit on the GC/MS was 1 ppb.

Bacterial strain, culture conditions, and inocula preparation

S. paucimobilis DSMZ 7526 (strain EPA505) was purchased from DSMZ—Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). A protocol similar to Dimitriou-Christidis (2005) was followed. Reconstituted cells were grown in nutrient broth for 5 days. Subsequently, the cells were added to a sterile mineral salts base (MSB) medium containing 100 mg/L fluoranthene and 200 mg/L Tween 80 (Mueller et al. 1990). The solution was incubated for 72 hours at 30°C in the dark with constant stirring on a horizontal shaker operating at 160 rpm (Ye et al. 1996). Following the incubation period, 10% glycerol solution was added and stored in cryopreservation vials at −80°C. The

Table 1 Physical and chemical properties of test PAHs[a]

PAH	Structure	Molecular Weight (g)	Aqueous Solubility (mg/L)	Log K_{ow}
Naphthalene		128	31	3.37
Fluorene		166	1.89	4.18
1-Methylfluorene		180	1.09	4.97
1,5-Dimethylnaphthalene		156	3.19	4.38

[a] Compiled from Mackay et al. (1992)

biomass for the experiments was prepared by adding 7 ml of the culture pregrown on the MSF medium to 800 ml sterilized nutrient broth containing 0.4 g glucose (Ye et al. 1996). The solution was incubated in the dark at 30°C on a horizontal shaker at 160 rpm for a period of 24 h. Following the incubation period, the cells were centrifuged (6653 × g, 10 min) and washed with Bushnell-Haas medium three times. After centrifugation, the supernatant was discarded and the concentrated cells were resuspended in 45 ml of Bushnell-Haas media.

Biodegradation experiments

Binary and ternary mixture experiments were designed with combinations of NAP, FLE, and 15DMN (Table 2) in addition to being tested individually. All experiments were conducted under conditions of extant kinetics (Grady et al. 1996). The time required for depletion of the individual compounds and the mixtures were initially determined to set sampling times. Further experiments using 15DMN, NAP, and 1MFLE (Table 2) were then performed.

Stock solutions of individual compounds were prepared in hexane. The aqueous solutions of the PAH mixtures were prepared by adding the appropriate volume of the stock solution of the individual PAH compounds to 800 ml of Bushnell-Haas medium taking care that the concentration of any one of the PAHs in the mixture would not exceed its solubility. The initial concentrations of compounds were determined such that the substrate concentration was greater than the affinity coefficient, $C >> K_s$ (Dimitriou-Christidis 2005). This allows the biokinetic parameter estimates to be determined independently (Ellis et al. 1996). The aqueous solutions were kept in the dark for three days prior to the experiment to allow complete solubilization in the aqueous solution. The experiments were conducted in duplicate 250 ml amber serum bottle reactors containing 150 ml of the aqueous PAH solution. The reactors were placed on a horizontal shaker throughout the experiment. The use of BSA measurements for cell biomass quantification is an accepted method in such studies (Guha et al. 1999; Knightes and Peters 2000). Required quantity of the concentrated suspension of cells was added to each reactor based on an absorbance from the BSA analysis of 0.25 at 595 nm. The biomass concentration was quantified at the beginning of the experiment as protein by the method of Bradford (1976) using BSA as a standard. Ten 7 ml samples from each reactor were added to sampling vials containing 3 ml of dichloromethane (DCM), at predetermined sampling times. Reactors containing only the aqueous PAH mixture solution without any biomass represented controls for the experiments. The sampling vials were placed on a rotary shaker for 12 h to allow complete transfer of the PAHs into the DCM phase. PAHs extracted from the DCM phase were analyzed using gas chromatography/mass spectrometry (GC/MS).

Table 2 Experimental design for multisubstrate biodegradation

Expt.	FLE mg/L	15DMN mg/L	NAP mg/L	1MFLE mg/L	X mg protein /L
A	0.36	–	–	–	4.06
B	–	0.17	–	–	4.06
C	–	–	0.79	–	3.61
D	0.40	0.23	–	–	5.21
E	0.33	–	0.74	–	4.80
F	–	0.17	0.66	–	4.75
G	0.27	0.14	0.57	–	4.86
I	–	–	–	0.09	2.93
J	–	0.26	0.53	0.11	2.90

Sole substrate and multisubstrate modeling

Estimation of biokinetic parameters for individual PAHs

The sole substrate parameters were estimated for NAP, 15DMN, FLE, and 1MFLE. The mass balance equation representing the sole substrate system (Monod 1949) is

$$\frac{dC}{dt} = \frac{q_{max} C X}{K_s + C} \quad (1)$$

where C is the aqueous phase concentration of the PAH measured over time, X is initial biomass concentration (a constant in Eq. 1) and t is the sampling time. The fourth order Runge–Kutta method was used for fitting the Monod equation to the experimental data to generate q_{max} and K_s. The uncertainty in the fitting parameters expressed as 95%

confidence interval was determined using the method described by Smith et al. (1998).

Multisubstrate parameter estimation

The parameters obtained from the sole substrate experiments formed a basis for modeling multicomponent systems. The experimental data from the mixture experiments was evaluated using two models. The first model was the sole substrate Monod model which can be described as a no-interaction model. It represents the case where a compound in a mixture behaves as if it were the only compound present not accounting for the effects resulting from other existing substrates. The second model was the competitive inhibition model. The equation for a mixture of substrates exhibiting competitive inhibition kinetics (Segel 1975) is represented as

$$\frac{dC_i}{dt} = \frac{q_{maxi} C_i X}{K_{si} + \sum_{j=1}^{n} \frac{K_{si}}{K_{sj}} C_j} \quad (2)$$

where C_i is the concentration of substrate *i*, C_j is the concentration of substrate *j* present in the mixture, K_{si} is the affinity constant for substrate *i*, and K_{sj} is the affinity constant for substrate *j*. Equation 2 can be extended for any number of components provided the compounds exhibit competitive inhibition kinetics. Thus, in Eq. 2, K_{sj} represented a constant, while K_{si}, q_{maxit} and C_i were fitting parameters.

Results

Sole substrate experiments

Substrate depletion data were generated for the individual PAHs (FLE, 15DMN, and NAP), binary mixtures of these compounds and the ternary mixture. Biodegradation studies for anthracene were attempted, but due to its low solubility and the analytical methods used changes in concentration could not be detected. The time dependent depletion of individual PAHs is illustrated in Fig. 1. Duplicate reactors for each PAH experiment with the same biomass are treated as independent data sets. Duplicates were run at the same time in a single run with the same biomass. However, data presented here are representative of the performance of the duplicate reactors and are obtained from independent reactors. The experiments were highly reproducible since standard deviation between the data sets varied from 0.1 to 7% (data not shown). The biokinetic parameters (q_{max} and K_s) obtained for any of the PAHs were not statistically different since 95% confidence intervals overlapped for duplicate sets only varying between ±2% and ±11%. The Monod model was fitted to the sole substrate experimental data to yield biokinetic parameters q_{max} and K_s (Table 3). The Monod model adequately simulated the experimental data (Fig. 1). The 95% confidence intervals are summarized in Table 3.

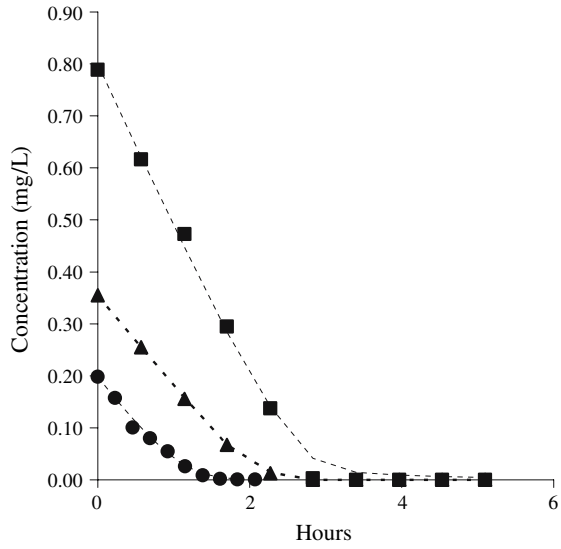


Fig. 1 Sole substrate degradation of naphthalene (■), fluorene (▲), and 1,5-dimethylnaphthalene (●). Closed symbols represent experimental observations. Dashed lines are generated by the Monod model

Table 3 Estimated parameters for degradation of individual PAHs

Parameters	FLE	15DMN	NAP
q_{max} (mg substrate/mg protein/h)	0.04 ± 0.00	0.05 ± 0.00	0.10 ± 0.00
K_s (mg/L)	0.02 ± 0.00	0.04 ± 0.00	0.08 ± 0.01
q_{max}/K_s (h^{-1}/mg protein/L)	2.60 ± 0.19	1.19 ± 0.09	1.33 ± 0.10
$C_{t=0}$ (mg/L)	0.35 ± 0.01	0.18 ± 0.01	0.78 ± 0.03

Standard deviation from the mean of duplicate measurements is indicated as ±

The ratio of the specific first-order rate constants, q_{max}/K_s, for 15DMN and NAP were comparable, but q_{max} and K_s were significantly different (Table 3). Based on the 95% confidence interval, the maximum substrate utilization rates for FLE and 15DMN were statistically different. FLE exhibited a K_s less than twice that of 15DMN, indicating its greater binding strength for an enzyme(s). NAP exhibited an affinity coefficient four times greater than FLE suggesting a low binding strength. Since the K_s values for all three PAHs were below the initial concentrations used in the experiments, these parameters are considered to be independent of the initial substrate concentration.

The specific first order rate constants, also known as the specific affinity (q_{max}/K_s), were compared to evaluate the extent of degradation. All three PAHs exhibited similar but statistically different specific affinities. Knightes and Peters (2000) reported values of 1.11 and 0.026 per hour per mg protein/L for NAP and FLE, respectively. The value for NAP reported by Knightes and Peters (2000) is comparable to that obtained from this study; however there is a two order magnitude difference for FLE. The pure culture of *S. paucimobilis* used in this study was highly favorable in PAH degradation. Knightes and Peters (2000) used a mixed microbial community which may not have been as effective in FLE degradation. The culture difference can account for the FLE specific affinity differences.

Multisubstrate experiments

Binary and ternary experiments were performed with combinations of FLE, 15DMN, and NAP. The substrate depletion curves are compared in Fig. 2. The biodegradation kinetics of a single PAH (always represented by closed symbols) were altered in the presence of other substrates. Enhanced degradation resulting from the presence of multiple substrates was not observed. However, enhancement would be unlikely under the extant conditions in which there is no biomass growth. No significant changes were observed in the biomass concentrations at the end of the experiment. The results are indicative of substrate interactions. Substrate interactions for the binary mixtures were also predicted by the competitive inhibition model for NAP, FLE, and 15DMN in

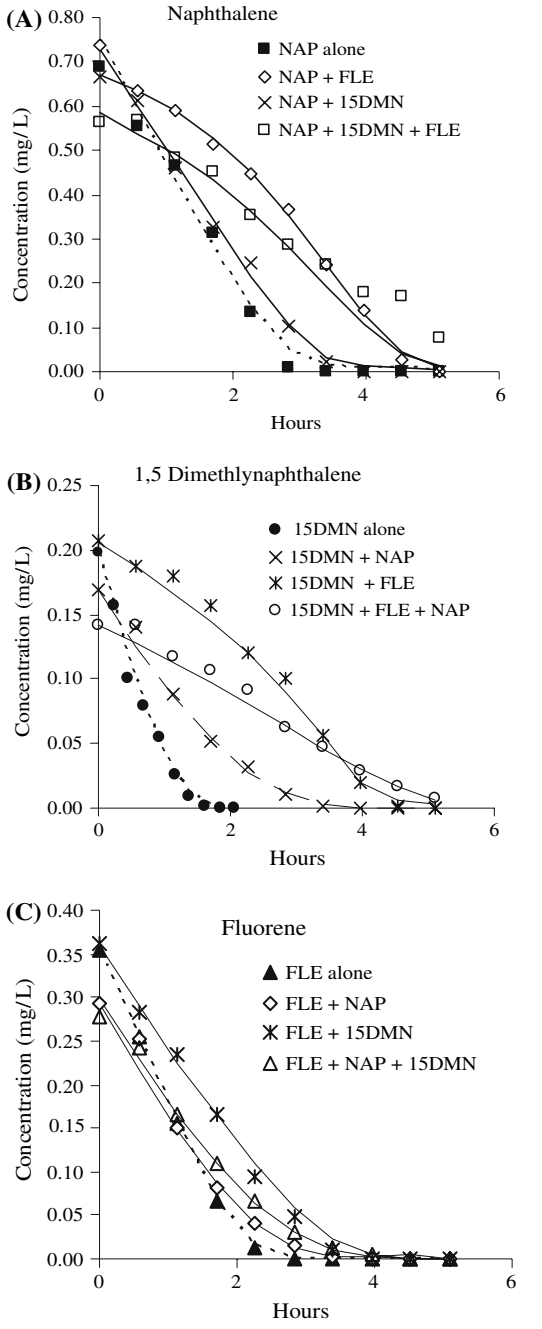


Fig. 2 (**A**) Naphthalene degradation in sole substrate, binary and ternary systems; (**B**) 1,5-Dimethylnaphthalene degradation in sole substrate, binary and ternary systems; (**C**) Fluorene degradation in sole substrate, binary and ternary systems. Symbols represent experimental observations and solid lines represent simulation by the competitive inhibition model. The dashed line represents the Monod model

binary and ternary mixtures. In some cases, the kinetics in mixtures converged to no-interaction kinetics since estimated parameters were not different from sole substrate estimates. However, in ternary mixtures there was a significant difference in the biodegradation kinetics as compared to individual degradation, appropriately predicted by the competitive inhibition model.

Discussion

The chemical class of PAHs is highly diverse and microorganisms will have variable metabolic versatility in their degradation. By selecting a metabolically diverse bacterium, *S. paucimobilis*, for these studies there is an inherent bias to generate kinetic values that represent an upper end in the rates of chemical transformation. However, it was necessary to use a metabolically diverse organism to make it possible to evaluate the interaction effects of PAH mixtures. These PAHs (Table 1) were selected because of the similarities in their chemical structures. Using NAP as the simplest PAH, the other PAHs represent increasing chemical complexity yet similarity. Chemical parameters such as aqueous solubility and K_{ow} (Table 1) values have been used to predict environmental fate and the selected PAHs demonstrate more than an order of magnitude difference in the ranges in these values. The chemical structure similarities enhance the likelihood of common enzyme systems and, if true, then there is the potential for interaction effects. Extant kinetic studies eliminate cell growth as a parameter and allow isolation of substrate effects, expressed as K_s and q_{max}. While these parameters may not represent the performance of the rate-limiting enzyme or the combined effect of several, they do provide insight to the relative biodegradation of these select PAHs.

Evidence for the activity of common degradative enzymes for these PAHs is indicated by the competitive inhibition model applied to the binary systems. In applying the model, a common enzyme was assumed to interact with the tested PAHs. The Monod (no-interaction) model did not adequately simulate the multisubstrate data since it did not yield a stable solution. Assuming that a single enzyme is responsible for competitive inhibition is unlikely, but the success of the approach indicates commonality in the degradative process.

Once competitive inhibition was determined, the fractional velocity rate equation (Segel 1975) was used to estimate the inhibition of multiple substrates on a single substrate. For the binary system of a substrate C and an inhibitor I, the equation is

$$V_i/V_0 = \frac{K_s + C}{K_s\{1 + \frac{I}{K_I}\} + C} \quad (3)$$

Or

$$i = 1 - \frac{V_i}{V_0} \quad (4)$$

where V_0 is the catalytic rate in the absence of an inhibitor, V_i is the catalytic rate in the presence of an inhibitor, K_s is the affinity coefficient for substrate C (mg/L) and K_I is the affinity coefficient for the inhibitor I (mg/L). The equation can be extended for a ternary system. Equation 4 was used to estimate proportion of inhibition (i) in the presence of other substrates (Table 4).

NAP degradation was impacted by the presence of the other PAHs in binary and ternary mixtures (Fig. 2A). Initial degradation rates of NAP in binary mixtures were slower than its degradation individually, as observed from the fractional velocities (Table 4). The effect of competitive inhibition in the presence of FLE is illustrated by the high degree of slope change in the initial portion of the curve captured by the (K_sS_1/K_{s1}) term in the denominator (Eq. 3) where S_1 represents I and Ks_1 represents K_I. The biodegradation kinetics of NAP in the presence of 15DMN was not significantly different from its individual degradation. The differences in the two

Table 4 Fractional velocities for binary and ternary mixtures

Mixture	V_i/V_0		
	NAP	15DMN	FLE
FLE + NAP	0.24 ± 0.01	–	0.73 ± 0.01
FLE + 15 DMN	–	0.18 ± 0.01	0.84 ± 0.03
15DMN + NAP	0.65 ± 0.00	0.36 ± 0.05	–
Ternary mixture	0.20 ± 0.04	0.12 ± 0.00	0.71 ± 0.01

Standard deviation from the mean of duplicate measurements is indicated as ±

curves, one in the presence of FLE and the other with 15DMN, were due to the relative magnitude of substrate affinities represented by the value for K_sS_1/K_{s1}. NAP degradation proceeded slowest in the ternary mixture compared to single compound behavior (V_i/V_0 = 20%).

The fractional velocities (Table 4) reveal that degradation of 15DMN was affected by the presence of other substrates (Fig. 2B). The observations suggest that competitive inhibition became pronounced when (affinity coefficient of alternate substrate) K_{s1} << (affinity coefficient of substrate C) K_s, $C_1 >> K_{s1}$ and $C_1 >> C$. The competitive inhibition model for binary and ternary mixtures appropriately described biodegradation kinetics in mixtures based on parameters estimated from the sole substrate experiments, indicating that sole substrate parameters are also representative of multisubstrate systems consistent with the assumptions of the model.

FLE degradation in binary and ternary mixtures was comparable to its degradation individually as shown by fitting of the competitive inhibition model (Fig. 2C). Biodegradation kinetics of FLE in binary and ternary mixtures resembled sole substrate kinetics. The biokinetic estimates obtained from the competitive inhibition model were not significantly different from sole substrate parameters indicating that the presence of NAP and 15DMN did not significantly affect the kinetics of FLE biodegradation.

An experiment with equal concentrations of all three substrates was performed to examine the effect of substrate concentrations on competitive inhibition (Fig. 3). Competitive inhibition was dramatic for NAP (V_i/V_0 = 12%) while for 15DMN V_i/V_0 was 28%. This effect was demonstrated by the term K_s ($C_1/K_{s1} + C_2/K_{s2}$), indicating that competitive inhibition is more prominent with the combined effects of $1/K_{s(15DMN)} << 1/K_{s(FLE)}$, $C_{(NAP)} >> K_{s(NAP)}$, $C_{(FLE)} >> K_{s(FLE)}$.

In all the binary mixtures, the presence of one PAH retarded the biodegradation of the co-occurring PAH with the exception of FLE since degradation of FLE in sole substrate system was comparable to its degradation in binary and ternary systems. On the basis of the absence of substrate interactions in binary experiments where substrate concentrations were just below their solubility, Guha et al. (1999) concluded that substrate interactions in binary mixtures may not be important in contaminated environments.

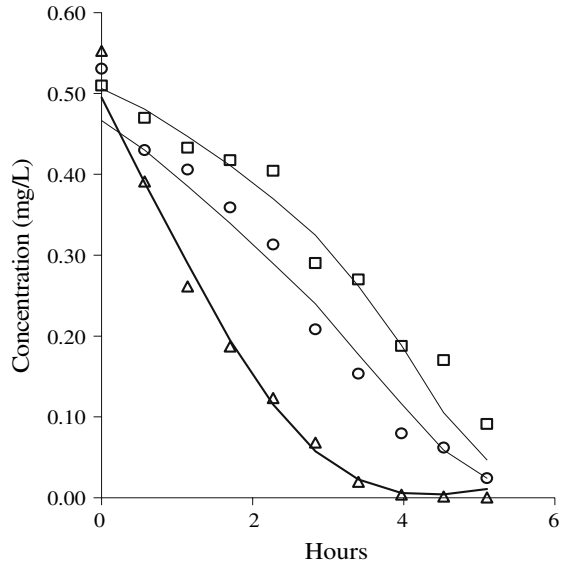


Fig. 3 Degradation of a ternary system consisting of equitable concentrations of naphthalene (□), fluorene (△), and 1,5-dimethylnaphthalene (○). Open symbols denote experimental observations. Solid line represents degradation predicted by the competitive inhibition model. Presented data were obtained from duplicate batch reactors

However, this research demonstrates that competitive inhibition was considerable in binary experiments where the concentrations of substrates were below their aqueous solubilities.

S. paucimobilis completely transformed all the components in binary and ternary mixtures; however, the initial degradation rates of individual components decreased in the presence of competitive substrates. The effect of the presence of multiple substrates on a single substrate was expressed in the form of a fractional velocity (Eq. 3). Decreased degradation rates in the presence of multiple substrates can be due to toxicity (Bouchez et al 1995), formation of toxic metabolites, and competitive inhibition (Stringfellow and Aitken 1995). In the present study, mixture biodegradation kinetics were governed by competitive inhibition which was demonstrated experimentally and mathematically by the competitive inhibition model (Figs. 2, 3) for NAP, FLE, and 15DMN. The extent of inhibition depended upon the number, affinities and concentration of the co-occurring substrates. For example, NAP degradation in mixtures followed a pattern in the order of: degradation individually (V_0) > degradation in the presence of 15DMN (70% V_0) > degradation in the presence of

FLE (31% V_0) > degradation in ternary mixture (20% V_0). Thus, competitive inhibition is a function of affinity of the alternate substrates (K_{s1}) and the relative magnitude of S_1/K_{s1} and becomes significant under conditions when $K_{s1} << K_s$, $C_1 >> K_{s1}$ and $C_1 >> C$. NAP and 15DMN did not produce dramatic effects on FLE degradation; consequently the degradation of FLE in binary and ternary mixtures was comparable as indicated by the V_i/V_0 ratios. This is because FLE had the greatest substrate affinity (K_s = 0.017 mg/L) among the tested PAHs and was present in concentrations in the range of 0.27–0.36 mg/L ($C >> K_s$).

The second set of multisubstrate biodegradation experiments consisted of a combination of 1MFLE, NAP, 15DMN. Sole substrate depletion curves were generated for 1MFLE (Fig. 4A). The Monod model was fitted to the experimental data to yield q_{max} = 0.105 ± 0.022 mg substrate/mg protein/h and K_s = 0.157 ± 0.016 mg/L. The highest affinity coefficient resulted for 1MFLE among the tested compounds.

Substrate interactions were evident in the ternary mixture of NAP, 15DMN and 1MFLE. Substrate depletion curves (Fig. 4A and B) illustrate reduced degradation rates of NAP and 15DMN compared to their degradation individually which is indicative of antagonistic effects. Biokinetic parameters for NAP and 15DMN in the ternary system with 1MFLE could not be evaluated since neither the competitive inhibition nor the no-interaction model predicted the biodegradation kinetics of the two compounds. However, 1MFLE exhibited degradation comparable to its degradation individually (q_{max} = 0.082 ± 0.004 mg substrate/mg protein/h; K_s = 0.160 ± 0.009 mg/L). Consequently, 1MFLE behaved as if it were the only compound present and its degradation was not affected by the presence of NAP and 15DMN. This behavior was validated by the no-interaction (Monod) model which adequately captured the data of 1MFLE in the ternary mixture (Fig. 4A). A case of preferential degradation of substrates could not be uniquely established in this ternary system, where degradation of NAP and 15DMN slowed down in the presence of 1MFLE. The competitive inhibition model did not validate the substrate interactions for either NAP or 15DMN in the ternary system. The fact that competitive inhibition was not evident does not exclude the possibility that other interactions may be occurring. The competitive inhibition model may not be valid because 1MFLE may not be a competitive substrate to NAP or 15DMN indicating that 1MFLE does not compete for the active site of the enzyme with the other substrates. Mechanisms other than competitive inhibition may govern the degradation of PAH mixtures. The results indicate that kinetics in PAH mixtures may not be governed by pure competitive inhibition and that there is a need to test alternative interaction models.

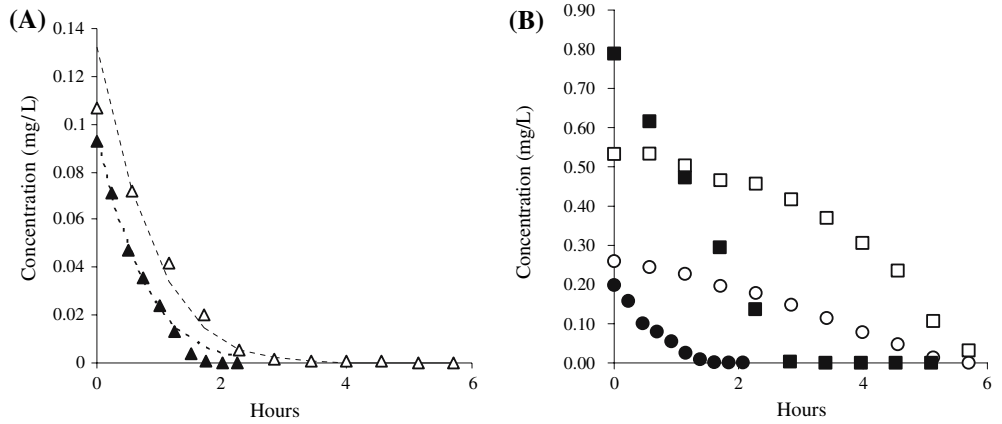


Fig. 4 Comparison of sole substrate degradation to ternary system degradation for 1-methylfluorene (▲,△) (**A**), naphthalene (■,□) and 1,5-dimethylnaphthalene (●,○) (**B**). Open symbols denote experimental observations in the ternary system. Closed symbols denote experimental observations in the sole substrate system. Dashed lines represent degradation predicted by the Monod model

Competitive inhibition establishes that substrates compete for the same active site of an enzyme revealing the presence of common enzyme systems; however, it does not disclose any information about the nature of this enzyme. Another possible mechanism other than enzymatic transformation is cell membrane penetration. Transport and degradation kinetics are not differentiated in these models. Yet these compounds likely compete with each other for movement across membranes and competitive inhibition requires that compounds share a common rate limiting step. In addition, these compounds exhibit medium to low q_{max} values which may support a substrate uptake limitation rather than a biodegradation effect (Kovárová-Kovar and Egli 1998). The possibility that competitive inhibition is related to a common membrane transport mechanism cannot be proven with these results because governing biodegradation kinetics would be consistent with the observed results. The separation of transport from biodegradation kinetics for hydrophobic compounds was not definitively possible.

Conclusions

The binary and ternary experiments indicate that the potential for substrate interactions exists for simple component systems where concentrations of PAHs are below aqueous solubilities. The complexity of interactions is related, but not limited, to the substrate affinities, concentrations of co-occurring substrates and number of components in the mixture. Biodegradation kinetics in PAH mixtures may be governed by pure competitive inhibition kinetics; however, interactions other than competitive inhibition cannot be ignored in systems where the chemical and structural diversity is far more complex than these simple systems. Substrate interactions in mixtures require multisubstrate models that account for simultaneous degradation of substrates. However, simultaneous degradation may not always be the mechanism for degradation of mixtures as observed from the ternary mixture of NAP, 15DMN, and 1MFLE. Interactions between LMW and HMW PAHs where preferential degradation of LMW PAHs can delay degradation of HMW PAHs is another means of predicting behavior of such complex mixtures (Molina et al. 1999). Although the binary and ternary systems do not mimic the complexity innate to mixtures, they indicate that interactions may become increasingly complex with the mixture composition.

Acknowledgements We thank the Superfund Basic Research Program at Texas A&M University that supported this work.

References

Bauer JE, Capone DG (1988) Effects of co-occurring aromatic hydrocarbons on degradation of individual aromatic hydrocarbons in marine sediment slurries. Appl Environ Microbiol 54:1649–1655

Beckles DM, Ward CH, Hughes JB (1998) Effects of mixtures of PAHs and sediments on fluoranthene biodegradation patterns. Environ Toxicol Chem 17:1246–1251

Boldrin B, Tiehm A, Fritzche C (1993) Degradation of phenanthrene, fluorene, fluoranthene, and pyrene by a *Mycobacterium* sp. Appl Environ Microbiol 59:1927–1930

Bouchez M, Blanchet D, Vandecasteele J-P (1995) Degradation of polycyclic hydrocarbons by pure strains and by defined strain associations: Inhibition phenomena and cometabolism. Appl Microbiol Biotechnol 43:156–164

Bradford MM (1976) A rapid & sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cerniglia CE (1992) Biodegradation of PAHs. Biodegradation 3:351–368

Dabestani R, Ivanov IN (1999) A compilation of physical, spectroscopic and photophysical properties of PAHs. Photochem Photobiol 70:10–34

Dimitriou-Christidis P (2005) Modeling the biodegradability and physicochemical properties of PAHs. Dissertation, Texas A&M University, College Station

Ellis TG, Barbeau DS, Smets BF, Grady CPL (1996) Respirometric technique for determination of extant kinetic parameters describing biodegradation. Water Environ Res 68:917–926

Grady CPL, Smets BF, Barbeau DS (1996) Variability in kinetic parameter estimates: A review of possible causes and a proposed terminology. Wat Res 30:742–748

Guha S, Peters CA, Jaffé PR (1999) Multisubstrate biodegradation kinetics of naphthalene, phenanthrene, and pyrene mixtures. Biotechnol Bioeng 65:491–499

Guha S, Jaffé PR, Peters CA (1998) Solubilization of PAH mixtures by a nonionic surfactant. Environ Sci Technol 32:930–935

Heitkamp MA, Cerniglia CE (1988) Mineralization of PAHs by a bacterium isolated from sediment below an oil field. Appl Environ Microbiol 54:1612–1614

Kanaly RA, Harayama S (2000) Biodegradation of high-molecular-weight PAHs by bacteria. J Bacteriol 182: 2059–2067

Kelley I, Cerniglia CE (1995) Degradation of a mixture of high-molecular-weight polycyclic aromatic hydrocarbons by a *Mycobacterium* strain PYR-1. J Soil Contam 4:77–91

Knightes CD (2000) Mechanisms governing sole-substrate and multi-substrate biodegradation kinetics of polycyclic

aromatic hydrocarbons. Dissertation, Princeton University, Princeton

Knightes CD, Peters CA (2000) Aqueous phase biodegradation kinetics of 10 PAH compounds. Environ Eng Sci 20:207–217

Kovárová-Kovar K, Egli T (1998) Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. Microbiol Mol Biol Rev 62:646–666

Lantz SE, Montgomery MT, Schultz WW, Pritchard PH, Spargo BJ, Mueller JG (1997) Constituents of an organic wood preservative that inhibit the fluoranthene-degrading activity of *Sphingomonas paucimobilis* strain EPA505. Environ Sci Technol 31:3573–3580

Leblond JD, Schultz TW, Sayler GS (2001) Observations on the preferential biodegradation of selected components of polyaromatic hydrocarbon mixtures. Chemosphere 42:333–343

Luning Prak DJ, Pritchard PH (2002) Degradation of PAHs dissolved in Tween 80 surfactant solutions by *Sphingomonas paucimobilis* EPA505. Can J Microbiol 48:151–158

Mackay D, Shiu YW, Ma KC (1992) Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Lewis Publishers, Chelsea, MI

Mohan SV, Kisa T, Ohkuma T, Robert AK, Shimizu Y (2006) Bioremediation technologies for treatment of PAH-contaminated soil and strategies to enhance process efficiency. Rev Environ Sci Biotechnol 5:347–374

Molina M, Araujo R, Hodson RE (1999) Cross-induction of pyrene and phenanthrene in a *Mycobacterium* sp. isolated from PAHs contaminated river sediments. Can J Microbiol 45:520–529

Monod J (1949) The growth of bacterial cultures. Annu Rev Microbiol 3:371–394

Mueller JG, Chapman PJ, Blattmann BO, Pritchard PH (1989) Action of a fluoranthene-utilizing bacterial community on PAH components of creosote. Appl Environ Microbiol 55:3085–3090

Mueller JG, Chapman PJ, Blattmann BO, Pritchard PH (1990) Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. Appl Environ Microbiol 56:1079–1086

Segel IH (1975) Enzyme kinetics. John Wiley & Sons, New York

Sherma J (1993) Handbook of chromatography. CRC Press, Inc., Boca Raton, FL

Smith LH, McCarty PL, Kitanidis PK (1998) Spreadsheet method for evaluation of biochemical reaction rate coefficients and their uncertainties by weighted nonlinear least-squares analysis of the integrated Monod equation. Appl Environ Microbiol 64:2044–2050

Stringfellow WT, Aitken MD (1995) Competitive metabolism of naphthalene, methylnaphthalenes, and fluorene by phenanthrene-degrading Pseudomonads. Appl Environ Microbiol 61:357–362

U.S. Environmental Protection Agency (1989) The superfund innovative technology programme: Technology profiles. EPA Report No. 540/5–89/01. US EPA Risk Reduction Engineering Laboratory Office of Research and Development, Cincinnati, OH. In: Wilson SC, Jones KC (1993) Bioremediation of soil contaminated with PAHs: A review. Environ. Pollution 81:229–249

Ye D, Siddiqi MA, Maccubbin AE, Kumar S, Sikka HC (1996) Degradation of PAHs by *Sphingomonas paucimobilis*. Environ Sci Technol 30:136–142