Aqueous Phase Biodegradation Kinetics of 10 PAH Compounds

Christopher D. Knightes1 and Catherine A. Peters*

Department of Civil and Environmental Engineering
Princeton University
Princeton, NJ 08544

ABSTRACT

Biodegradation kinetics were individually studied for 10 polycyclic aromatic hydrocarbons (PAHs): naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2-ethyl-naphthalene, acenaphthene, fluoranthene, phenanthrene, anthracene, fluoranthene, and pyrene. Experiments consisted of aerobic, continuously mixed, batch aqueous systems inoculated with a PAH-degrading consortium. The design of the experiments and the mathematical interpretation of the data isolated the kinetics of biotransformation. Other physical-chemical processes that may affect bioavailability were eliminated or independently measured. Model parameters were estimated using the maximum likelihood method for a bivariate data set of substrate and biomass concentration over time. For four of the PAHs, naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, and 2-ethyl naphthalene, there was sufficient growth to estimate biomass yield coefficients. For two of the PAHs, naphthalene and 1-methylnaphthalene, Monod parameters $q_{max}$ and $K_s$ were estimated. For the remaining compounds, only the first-order rate coefficients were estimated. The consortium was able to degrade all the PAHs except acenaphthene. The observed biodegradation rate coefficients did not correlate with molecular weight or any other chemical property. The biomass-normalized first-order biodegradation rate coefficients spanned a little less than two orders of magnitude, from 0.0255 to 1.11 h$^{-1}$ (mg protein)$^{-1}$. This is much less variation than is typically observed for PAHs in soils and sediments. The suggests that in field samples differences in biodegradation rates are not governed by differences in the kinetics of biotransformation. Rather, the differences in biodegradation rates in the field are primarily governed by the physical-chemical processes that control bioavailability, which differ significantly for different PAHs.

Key words: PAH; PAHs; biodegradation; bioavailability; kinetics

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental contaminants because many are suspected carcinogens (U.S. EPA, 1993). In the environment, PAHs may occur in hydrocarbon mixtures such as coal tar, creosote, and diesel fuel (Peters et al., 1999; Mahjoub et al., 2000), as semisolubile mixtures associated with lampblack (Burks and Hanson, 2001), as dissolved and colloid-associated solutes in groundwater (Vilholth, 1999; Mackay and Gschwend, 2001; Yang et al., 2001), and as sediment contaminants.

1Corresponding author: Department of Civil and Environmental Engineering, Princeton University, Princeton, NJ 08544. Phone: 609-258-5645; Fax: 609-258-2799; E-mail: cap@princeton.edu
2Present address: U.S. EPA, National Exposure Research Laboratory, Ecosystems Research Division, 960 College Station Road, Athens, GA 30615.
in surface waters (Araya et al., 2001; Rockne et al., 2002). PAHs are known to be biodegradable, particularly under aerobic conditions (Cermiglia, 1992). Previous studies of PAH biodegradation kinetics in soils or sediments have reported that different PAHs have very different biodegradation rates (Ruessel and Bartha, 1996; Heukamp and Cermiglia, 1987; Keck et al., 1989; Park et al., 1990; Kelley and Cermiglia, 1995; Wilcox et al., 1996; Bousman et al., 1997). Significant research has been performed to examine the relationship between PAH desorption, dissolution, and bioavailability (Manittel and Alexander, 1991; Guerin and Boyd, 1992; Weissenschild et al., 1992; Erickson et al., 1993; Mihelic et al., 1993; Volkening et al., 1993; Comelissen et al., 1998; Zhang et al., 1998). It is currently unclear to what extent the observed differences in biodegradation rates of PAHs are attributable to differences in the physical-chemical properties governing bioavailability or to the molecular features that govern biochemical transformation. Observed biodegradation rates of PAHs in soils and sediments are generally found to be inversely related to molecular weight. Hydrophobicity of PAHs is inversely related to molecular weight, which largely explains why soil studies have found that biodegradation rate is inversely related to molecular weight. This leads to the hypothesis that biodegradation rates of PAHs in aqueous systems (in the absence of bioavailability-limiting processes) are less variable and may not be related to hydrophobicity metrics.

The goal of this research was to study the biodegradation kinetics of a suite of PAHs by a mixed culture in aqueous systems. Through careful experimental design and mathematical inference, we isolated the microbial biodegradation process, independent of other rate-limiting processes. We then examined the relationship to various features of molecular structure. With the exception of Leblond et al. (2001), who measured biodegradation rates for PAHs in mixtures in aqueous systems, to our knowledge there has been previously no study of the biodegradation rates of a range of PAHs in aqueous systems. The experiments in our study were designed to examine each PAH individually, to eliminate any possible substrate interaction effects that may be operative when PAHs co-occur.

Ten PAHs were selected for study: naphthalene (NPH), 1-methylnaphthalene (1MN), 2-methylnaphthalene (2MN), 2-ethyl-naphthalene (2EN), acenaphthene (ACE), fluorene (FLE), phenanthrene (PHN), anthracene (ANTH), fluoranthene (FLN), and pyrene (PYR). Much of the previous research on the biodegradation kinetics of individual PAHs has focused on naphthalene and phenanthrene (Guerin and Boyd, 1992; Volkening et al., 1993; Guba and Jaffe, 1996; Ghoshal and Luthy, 1998; Ahn et al., 1998; Guba et al., 1999). There has been some research on the biodegradation kinetics of 2-methylnaphthalene, anthracene, fluorene, fluoranthene, and pyrene (Bouwer et al., 1992; Boudin et al., 1993; Stringfellow and Atkhen, 1995; Jimenez and Bartha, 1996; Bouchez et al., 1997). To our knowledge, biodegradation kinetic parameters for 1-methylnaphthalene and 2-ethylnaphthalene have not been previously reported. As shown in Table 1, the compounds selected span a wide range of properties such as aqueous solubility, hydrophobicity (as indicated by log Kow), molecular weight, and aqueous diffusivity. The compounds also represent a variety of molecular structural features such as: the size of the molecule, the arrangement of the aromatic rings in the molecule, the presence of alkyl-functional groups, and the presence and location of a five-membered ring.

The challenge in measuring biodegradation rates of dissolved PAHs derives from the small amounts of total substrate due to the low aqueous solubilities of these compounds. This precludes the use of standard growth kinetic measurements to infer substrate biodegradation rates. For all of the compounds studied, we measured both substrate depletion and biomass growth. For these compounds for which it was possible, we inferred Monod kinetic parameters and yield coefficients. For the compounds that were present at concentrations that were too small to cause growth, we inferred first-order kinetic parameters from substrate depletion data alone. In all cases, it was extremely important that the experimental systems were carefully modeled to account for the unique starting conditions for each PAH and as well as for the abiotic processes (e.g., volatilization and sorption to the apparatus) that affected the kinetics in the experiments. This made it possible to confidently infer biodegradation kinetic parameters and draw comparisons across the PAHs in this study.

MATERIALS AND METHODS

Chemicals

Polycyclic aromatic hydrocarbons were purchased from Aldrich Chemical Co. (Milwaukee, WI) and had purities of 98% or greater, except for anthracene with a purity of 97%. Methanol and acetonitrile were of optima quality and purified from Fisher Scientific Co. (Fairlawn, NJ). Water was deionized and ultrapureified using HYDRO Pico systems Plus (HYDRO services, Durham, NC).

Microbial consortium and biomass analysis

The enrichment culture used in these experiments was originally obtained from a petroleum-contaminated soil sample. It has been used in several studies to study biodegradation and bioavailability of phenanthrene.
### Table 1. Structures and physical-chemical properties of PAHs selected for this study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbrev</th>
<th>Structure</th>
<th>Molecular weight</th>
<th>log Kow</th>
<th>Aqueous solubility 25°C (mg/L)</th>
<th>Aqueous diffusivity (10⁻⁹ m²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>NPH</td>
<td><img src="image1" alt="Structure" /></td>
<td>128</td>
<td>3.36</td>
<td>31</td>
<td>7.35</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>1MN</td>
<td><img src="image2" alt="Structure" /></td>
<td>142</td>
<td>3.87</td>
<td>28</td>
<td>6.13</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>2MN</td>
<td><img src="image3" alt="Structure" /></td>
<td>142</td>
<td>3.86</td>
<td>24.6</td>
<td>6.12</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>ACE</td>
<td><img src="image4" alt="Structure" /></td>
<td>154</td>
<td>4.03</td>
<td>3.8</td>
<td>5.98</td>
</tr>
<tr>
<td>2-Ethylacenaphthene</td>
<td>2EN</td>
<td><img src="image5" alt="Structure" /></td>
<td>156</td>
<td>4.38</td>
<td>8.0</td>
<td>4.97</td>
</tr>
<tr>
<td>Fluorene</td>
<td>FLR</td>
<td><img src="image6" alt="Structure" /></td>
<td>166</td>
<td>4.18</td>
<td>1.9</td>
<td>5.36</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>PHN</td>
<td><img src="image7" alt="Structure" /></td>
<td>178</td>
<td>4.57</td>
<td>1.1</td>
<td>4.97</td>
</tr>
<tr>
<td>Anthracene</td>
<td>ANTH</td>
<td><img src="image8" alt="Structure" /></td>
<td>178</td>
<td>4.54</td>
<td>0.05</td>
<td>5.04</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>FLN</td>
<td><img src="image9" alt="Structure" /></td>
<td>202</td>
<td>5.22</td>
<td>0.26</td>
<td>4.44</td>
</tr>
<tr>
<td>Pyrene</td>
<td>PVR</td>
<td><img src="image10" alt="Structure" /></td>
<td>202</td>
<td>5.18</td>
<td>0.13</td>
<td>4.52</td>
</tr>
</tbody>
</table>

Sources: log Kow and aqueous solubilities (Mackay et al., 1992), diffusivities (Gustafson and Dickhut, 1994).

(Guba and Jaffé, 1996; Brown et al., 1999). In a separate investigation using 16S rRNA analysis, at least three distinct species in the consortium have been identified as *Sphingomonas yanoikuyae* (21 out of 24 samples), an un-matched *Pseudomonas* sp. (1/24), and *Methyllobacter capsulatus* (1/24) (unpublished study). One cloned sample could not be identified.

For several years the consortium has been grown on the 10 PAHs involved in this study. Every 5 days the consortium medium was centrifuged at 1404 × g for 20 min. Half of the concentrated biomass was resuspended in fresh PAH nutrient buffer stock solution. This solution was prepared by adding a methanol solution containing the PAHs to a BOD nutrient buffer solution made of KH₂PO₄, K₂HPO₄, CaCl₂, MgSO₄, Na₂HPO₄, NH₄Cl, FeCl₃ (HACH Co., Loveland, CO). The resulting PAH concentrations in the PAH nutrient buffer stock solution were 52 mg/L, NPH, 20 mg/L, 1MN, 20 mg/L, 2MN, 24 mg/L, ACE, 9.6 mg/L, FLR, 20 mg/L, 2EN, 9.6 mg/L, PHN, 0.08 mg/L, ANTH, 0.4 mg/L, FLN, and 0.48 mg/L, PVR. Some PAHs were initially present in excess of their solubility limits, but over the 5-day growth period all except a did dissolve. The remaining half of the biomass was available for use in experiments.

Polycyclic aromatic hydrocarbons sorb to biomass and the extracellular material produced by the biomass. Before each experiment, it was necessary to wash the biomass clean of PAHs. The washing protocol involved two washings with a solution of 500 mg/L. Tween 80 (ICI Americas, obtained from Aldrich Co., Milwaukee, WI), two washings with a solution of 250 mg/L Tween 80, and three washings with BOD nutrient buffer. Each step consisted of 20 min of continuous mixing, followed by 20 min of centrifugation at 1404 × g. The washed biomass was resuspended in 50 mL of BOD nutrient buffer in the absence of growth substrate for 24 h. Toluene extraction of the washed biomass demonstrated that all PAH concentrations were below detection limits, and viability tests verified that the washing procedure did not have toxic effects (Knight, 2000).

Biomass concentrations were measured using the Bio-Rad protein assay, based on the Bradford method (Bio-Rad Laboratories, Hercules, CA). Polycyclic aromatic hydrocarbons do not interfere with this assay. To lyse the cells, 2 mL of 0.075 N NaOH were added to 2 mL of sample. Bio-Rad dye was added and after 30 min absorbance at 595 nm was measured using a Spectronic 20 Genesys Spectrophotometer (Spectronic Instruments, Rochester, NY). The calibration curve was constructed using a bovine gamma globulin protein standard.

**Biodegradation experiments**

For each PAH, the biodegradation experiment was comprised of two parallel series of reactor vessels, one series with biomass ("biotic") and one series without biomass ("abiotic"), each with 15 35-mL serum bottles. The PAH was dissolved in 1 to 2 mL of methanol and equilibrated with 1 L of BOD nutrient buffer. The resulting solution had a PAH concentration slightly below aqueous solubility, and the resulting methanol concentration was less than 0.2% by volume. [A biomass growth experiment with methanol present as the only substrate resulted in no appreciable growth of the consortium, validating the assumption that the methanol present in the reactor vessel did not interfere with interpretation of biomass growth on...
PAHs. Furthermore, methanol at these concentrations has been found to not impact microbial activity (Burns and Speece, 1991). To each reactor vessel, 25 mL of this solution were added. Each reactor vessel in the biotic series was inoculated with 1 mL of the washed consortium. The biomass concentration of the inoculum was measured and the initial biomass concentration in the reactor vessel, C₀, was inferred using the 1:26 dilution factor. These values, along with the initial substrate concentrations, Cₛ, are listed in Table 2. The range of variation in the starting biomass concentrations was due to the compounding of losses due to the series of washing, rinsing, and decanting procedures.

All vessels were triplicated with a Teflon-coated septum, covered with aluminum foil, and continuously mixed using a Teflon-coated magnetic stir bar. The reactor vessels were maintained at the laboratory temperature of 23 ± 2°C. The parallel series of abiotic reactor vessels were used to independently measure sorption to the apparatus and any abiotic losses that might have occurred over time.

The experiment was conducted by sacrificing one reactor vessel from each of the biotic and abiotic series at each observation time. This ensures that the observations have statistically independent errors. Samples taken from the biotic reactor vessels were filtered through a 0.1-μm Anopore 10 filter. Within 3 min following filtration, the dissolved oxygen concentration was periodically measured using CHEMetrics Dissolved Oxygen Kit K-512 (CHEMetrics, Calverton, VA), and found not to deviate significantly from the original 6 to 8 mg/L.

Table 2. Initial substrate and biomass concentrations in the biodegradation experiments.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cₛ (mg/L)</th>
<th>Xₑ (mg protein/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>19.4</td>
<td>0.053</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>11.1</td>
<td>0.053</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>8.34</td>
<td>0.053</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>1.67</td>
<td>2.39</td>
</tr>
<tr>
<td>2-Ethynaphthalene</td>
<td>2.24</td>
<td>0.388</td>
</tr>
<tr>
<td>Fluorene</td>
<td>1.31</td>
<td>2.38</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.522</td>
<td>1.58</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.041</td>
<td>0.994</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>0.0829</td>
<td>0.401</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.0236</td>
<td>0.401</td>
</tr>
</tbody>
</table>

*average concentration of all observations.

Chemical analysis

Concentrations of PAHs were analyzed using a Hewlett Packard Series 1050 High-Pressure Liquid Chromatography (HPLC) equipment with an HP Spherisorb ODS 2 column (5 μm, 125 × 4 mm), operated at 28°C. Samples with higher concentrations were detected using a 20-μL injection and ultraviolet (UV) detection. In the intermediate concentration range, a 2-μL injection volume was used followed by fluorescence detection (FLD). For the lowest concentrations, a 20-μL injection volume was used with an identical FLD method. The compounds ANTH, TLM, and PYR, were detected using only FLD. The mobile phase was 72.5-27.5 (acetoniitrile:water) for NPH, 1MN, and 2MN, 80:20 for ACE, FLR, 2EN, and PHN, and 85:15 for ANTH, TLM, and PYR. The flow rate was 1.0 mL/min for all methods. The detection technique was optimized for each PAH using the strongest ultraviolet absorbance wavelength, and fluorescence excitation and emission wavelength (Knightes, 2000). These methods allowed quantification well below the aqueous solubilities of each PAH. The detection limit for NPH, 1MN, PHN, and PYR was 0.005 mg/L. The detection limit for 2MN, ACE, FLR, 2EN, and FLN was 0.001 mg/L. The detection limit of ANTH was 0.0005 mg/L.

Endogenous decay

The reduction in growth rate due to cell maintenance and cell death was modeled as first-order biomass decay, where k is the endogenous decay coefficient (h⁻¹). This coefficient was estimated using an independent substrate-free experiment with a high starting biomass concentration (3.29 mg protein/L). The batch reactor was covered with aluminum foil, had a foam stopper to maintain oxygen supply, and was continually mixed. From this reactor, three replicate biomass samples were taken at four times over a period of 140 h. Least-squares linear regression was used to estimate k as 0.0042 ± 0.0019 h⁻¹ (95% confidence level). This result agrees with reported values of 0.002-0.004 h⁻¹ (Gilani and Jaffé, 1996; Ghoshal and Luther, 1998; Gilani et al., 1999) and typical values of 0.001-0.003 h⁻¹ (Thoburn and Spikes, 1991).

MODELING AND PARAMETER ESTIMATION

Biodegradation kinetics

For PAHs present at concentrations high enough to cause measurable growth, the Monod model was used to describe the substrate biodegradation kinetics [Equation (1)] and the associated biomass growth [Equation (2)]. The coupled differential equations are:
AQUEOUS PHASE BIODEGRADATION KINETICS OF 10 PAHs

\[
\frac{dC}{dt} = -q_{\text{max}} \frac{C}{K_C + C} \quad (1)
\]

\[
\frac{dX}{dt} = \gamma q_{\text{max}} \frac{C}{K_C + C} \quad (2)
\]

where the variables are substrate concentration, \(C\) (mg/L), biomass concentration, \(X\) (mg protein/L), and time, \(t\) (h).

The parameters are the maximum substrate utilization rate per unit biomass, \(q_{\text{max}}\) (mg substrate/mg protein/h), the half-saturation coefficient, \(K_C\) (mg/L), and the yield coefficient, \(\gamma\) (mg protein/mg substrate).

For PAHs present at concentrations high enough to cause some growth, but too low for Monod behavior, the following model described the kinetics:

\[
\frac{dC}{dt} = -\frac{q_{\text{max}} CX}{K_S} \quad (3)
\]

\[
\frac{dX}{dt} = \frac{\gamma q_{\text{max}} CX}{K_S} \quad (4)
\]

This model has two separable parameters, \(q_{\text{max}}K_S\) and \(\gamma\). Finally, for PAHs present at concentrations too low to cause growth, a first-order model (equivalent to Equation (3)) described substrate biodegradation kinetics. In this case, \(X\) is constant and \(C\) is the only variable. The only parameter is \(q_{\text{max}}K_S\).

Processes other than biodegradation

Additional governing processes were modeled to accurately describe the changes in aqueous phase PAH concentrations. In each abiotic experiment, we observed a quick initial drop in the PAH concentration in the aqueous phase. This happened on a time scale of a few minutes, which is very fast compared to the hours-to-days time scale of the biodegradation kinetics. For some of the PAHs, this initial drop was followed by slow continuous decreases in concentration possibly due to volatilization or slow sorption to reactor vessel surfaces. Based on these observations, we modeled both instantaneous equilibrium partitioning to the reactor vessel as well as time-dependent abiotic losses. The extent of equilibrium partitioning to the apparatus was estimated as an abiotic partition coefficient, \(K_p\), as

\[
K_p = \frac{C_F - C_{S, F}}{C_{S, F}} \quad (5)
\]

where \(C_F\) is the initial mass of substrate present in the system divided by the aqueous phase volume, and \(C_{S, F}\) is the aqueous phase concentration in the abiotic system at \(t = 15\) min. The time-dependent abiotic loss was modeled using a first-order rate model. The abiotic loss rate coefficient, \(k_a\) (h\(^{-1}\)), was estimated by fitting a first-order rate law to the concentration measurements in the abiotic reactor vessels after the initial drop. For naphthalene and 2-methylphenanthrene, \(k_a\) values were significantly different from zero at the 95% confidence level. For each of the remaining compounds, \(k_a\) was not found to be significantly different from zero.

The sorption of PAHs to biomass was modeled as instantaneous equilibrium partitioning. We define \(K_B\) (mg protein) as the biomass partition coefficient, such that the product of \(K_B\) and \(X\) is the effective concentration of substrate sorbed to the biomass. The extent of partitioning to biomass was estimated from early concentration measurements in the bioreactor vessels. Part of the initial drop in concentration in the biotic reactor vessels is due to abiotic partitioning processes. \(K_B\) was estimated by accounting for that portion of the change in concentration that was not explained by \(K_p\), that is,

\[
K_B = \frac{1}{X} \left( \frac{C_{F} - K_p C_{S, F}}{C_{F, X}} - C_{S, F} \right) \quad (6)
\]

where \(C_{F, X}\) is the aqueous phase concentration in the biotic system at \(t = 15\) min. The estimated parameters \(K_p\), \(K_B\), \(\gamma\), and \(q_{\text{max}}\) for each PAH are listed in Table 3.

System model and parameter estimation

The experimental systems were modeled using overall mass balance equations that combined the biodegradation/growth models (Equations (1) and (2), Equations (3) and (4), or Equation (3) alone) with the expressions for endogenous decay, genetically controlled abiotic loss, and reversible equilibrium partitioning to the apparatus and biomass. For example, for the PAHs that caused Monod growth, the coupled equations are

\[
\frac{dC}{dt} = -\frac{q_{\text{max}} C}{K_C + C} X - k_C C - K_p C \frac{dX}{dt} \quad (7)
\]

\[
\frac{dX}{dt} = \gamma q_{\text{max}} \frac{C}{K_C + C} X - bX \quad (8)
\]

These equations were simultaneously fit to the data from the biotic experimental series for a given PAH, comprising of 10 to 15 observations of \(X\) and \(C\) over time. The combination of \(C_t\) and \(X_t\) data provides redundant kinetic information and maximizes the potential for statistical inference about growth kinetic parameters, as has been discussed in a previous publication (Knights and Peters, 200). The parameters, \(K_p\) in \(K_B\), \(\gamma\), and \(q_{\text{max}}\), were estimated by minimizing an objective function derived from the maximum likelihood equation. This approach accommodated the larger errors in the measurements of \(X\) relative to the errors in the measurements of \(C\), and the need statistically give more weight to the substrate concentration data.

For all the PAHs, an attempt was made to fit the three-parameter model (Equations (7) and (8)) but it was not al-
ways possible. The inability to find a unique minimum to the objective function in the \( \eta_{\text{max}} - K_S \) space served to indicate that the data were insufficient to provide resolution on these two parameters. Rather, the minimization revealed a unique value of the ratio of \( \eta_{\text{max}} \) and \( K_S \). For the PAHs for which this was the case, the minimization did reveal a unique estimate of \( Y \). This was equivalent to fitting the data with a two-parameter kinetic model [Equations (3) and (4)]. This was the case for 2-naphthaldehyde, for which the statistical analysis was presented in a previous publication (Knights and Peters, 2000).

If the parameter estimation routine failed to estimate both \( \eta_{\text{max}}/K_S \) and \( Y \), the attempt to use both \( C \) and \( X \) as variables was abandoned. This occurred for PAHs that did not cause measurable growth. In this case, the mass balance equation that described the system is

\[
(1 + K_S + K_X) \frac{dC}{dt} = -\Delta n_{\text{max}} C - \eta_0 C
\]  

(9)

This equation was fit to the \( C(t) \) data alone, and the observations of the biomass concentration were averaged and assumed constant over the course of the experiment. For these PAHs, the \( X_0 \) reported in Table 2 is the average value, rather than the first measurement. A single parameter, \( \Delta n_{\text{max}}/K_X \), was estimated, which is a biomass-normalized first-order biodegradation rate coefficient.

RESULTS AND DISCUSSION

Parameter estimation results

The substrate concentration data from the 10 experiments and the modeling results are presented in Fig. 1. The concentration axes are presented in log-space, which is consistent with the lognormally-distributed errors of the PAH concentration measurements (Knights and Peters, 2000). Error bars (representing 1 standard deviation, determined from replicate sample measurements and error from the calibration regression) are smaller than the symbols. We show all 10 plus with the same scale on the concentration axis to emphasize the large range in the starting substrate conditions for the different PAHs. These differences make it nearly impossible to qualitatively compare biodegradation rates simply by visual inspection, which illustrates the need for careful system modeling and data interpretation. The model calibrations (solid curves) fit the experimental data quite well, which provides supporting evidence to validate the various model formulations used. The biomass concentration data are not included because for many of the PAHs there was negligible growth. For two of the compounds, naphthalene and 2-naphthaldehyde, for which there was sufficient growth to use the \( x(t) \) data in the regression, the biomass concentration data have been published previously (Knights and Peters, 2000).

The estimated parameters with their confidence values are presented in Table 4. For naphthalene and 1-methylnaphthalene, the substrate concentrations were high enough and there was sufficient biomass growth to estimate all three parameters, \( \eta_{\text{max}}, K_S \), and \( Y \). The two Monod parameters \( \eta_{\text{max}} \) and \( K_X \), were estimated in lognormal space because this transformation helped to linearize the error function (Knights and Peters, 2000). As a result, the estimated errors for these parameters are relative errors, rather than absolute. To our knowledge, this is the first study to report biodegradation kinetic parameters for 1-methylnaphthalene. It is interesting to note that the maximum specific growth rates on naphthalene and 1-methylnaphthalene are nearly equal, but there is an order of magnitude higher affinity for naphthalene than there is for 1-methylnaphthalene.

For 2-methylnaphthalene and 2-ethyl-naphthalene, there

<table>
<thead>
<tr>
<th>Compound</th>
<th>( K_S ) (( \mu \text{g} \text{protein} ))</th>
<th>( K_X ) (( \mu \text{g} \text{protein} ))</th>
<th>( \eta_{\text{max}} ) (( \mu \text{g} \text{C} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.877</td>
<td>1.36</td>
<td>0.0148</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>0.225</td>
<td>0.453</td>
<td>0.03</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>0.199</td>
<td>0.291</td>
<td>0.0095</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.353</td>
<td>0.691</td>
<td>0.04</td>
</tr>
<tr>
<td>Fluorene</td>
<td>0.470</td>
<td>0.361</td>
<td>0.03</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.408</td>
<td>2.07</td>
<td>0.02</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.777</td>
<td>0.340</td>
<td>0.02</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>0.427</td>
<td>3.38</td>
<td>0.02</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.177</td>
<td>11.4</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Not statistically different from zero at the 95% confidence level.
was sufficient biomass growth to estimate a statistically significant value of $\gamma$ but only the ratio, $q_{max}/K_s$ (mg protein/L) $^{-1}$, could be uniquely estimated. To our knowledge, this is the first study to report a biodegradation kinetic parameter for 2-ethyl-naphthalene. Its biodegradation rate is comparable to that of 2-methyl-naphthalene.

For naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, and 2-ethyl-naphthalene, the yield coefficient, $\gamma$, was estimated as mg protein/mg PAH substrate and converted to mg C cell/mg C substrate. These values are reported in Table 4. All of these values fall within the typically accepted range of 0.4-0.8 for aerobic heterotrophs.
Table 4. Parameter estimation results.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \frac{\text{q}_{\text{w}}}{\text{mg protein/L}} )</th>
<th>( K_{\text{S}} ) (mg/L)</th>
<th>( \text{Y}^{\prime} ) (mg C substrate)</th>
<th>( \frac{\text{q}<em>{\text{w}}}{\text{K}</em>{\text{S}}} ) (mg protein/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.636 (2%)</td>
<td>0.572 (0.4%)</td>
<td>0.41 ± 0.08</td>
<td>1.11 (2%)</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>0.614 (3%)</td>
<td>5.33 (1.9%)</td>
<td>0.58 ± 0.02</td>
<td>0.115 (0.2%)</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>nd</td>
<td>nd</td>
<td>0.38 ± 0.09</td>
<td>0.193 (4%)</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>nd</td>
<td>nd</td>
<td>0.5 ± 0.4</td>
<td>0.21 (56%)</td>
</tr>
<tr>
<td>Fluorene</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.026 (28%)</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.27 (17%)</td>
</tr>
<tr>
<td>Anthracene</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.24 (17%)</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.32 (29%)</td>
</tr>
<tr>
<td>Pyrene</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.35 (32%)</td>
</tr>
</tbody>
</table>

*Conversion factor: roughly 50% of a cell is comprised of protein (Brook et al., 1979) and roughly 50% of the cell is carbon.

Relative errors (95% confidence) are listed in parentheses, and the absolute errors for \( \text{Y}^{\prime} \) (95% confidence) are given as ±. nd = not determined.

as reported by Metcalf and Eddy, Inc. (Tchobanoglous and Burton, 1991).

For the remaining compounds, there was not sufficient biomass growth to reliably estimate yield coefficients. For one of the compounds, acenaphthene, there was no observed degradation. Fluorene, phenanthrene, anthracene, fluoranthene, and pyrene all followed first-order kinetics. This is evident from the linearity of the log concentration plots in Fig. 1. For these compounds, estimates of \( \frac{\text{q}_{\text{w}}}{\text{K}_{\text{S}}} \) are reported in Table 4.

Comparison across PAHs

To compare the biodegradation rates across the PAHs in this study, we examine the biomass-normalized first-order biodegradation rate coefficients, \( \frac{\text{q}_{\text{w}}}{\text{K}_{\text{S}}} \) (mg protein/L)\(^{-1}\). Even though separate Monod constants were estimated for naphthalene and 1-methylnaphthalene, it is meaningful to compare all the PAHs using a first-order rate coefficient because the operative reactions rates in the environment are likely to be first order due to low concentrations. Furthermore, the biomass-normalized form of the first-order biodegradation rate coefficient is independent of biomass concentration, a system-specific variable.

Ranking the PAHs in order of magnitude of the biomass-normalized first-order rate coefficient shows:

\[ \text{NPH} > \text{PYR} > \text{FLN} = \text{PHN} = \text{ANTH} = \text{2EN} = \text{2MN} = \text{1 MN} > \text{FLR} > \text{ACE} \]

An analysis conducted to examine correlation of the rate coefficients with the physical-chemical properties of molecular weight, aqueous solubility, aqueous diffusion, and log \( K_{\text{oc}} \) revealed no trends. This suggests that the differences across the PAHs are related to features of molecular structure that determine biochemical interactions rather than size-dependent molecular properties. Because these experiments involved a bacterial consortium acclimated to a mixture of PAHs, it is possible that the differences in biodegradation rates are a function of the relative abundance of individual species, or the availability of specific enzymes. On the other hand, there is compelling evidence that bacterial enzymes responsible for PAH biodegradation have broad functionality (Stringfellow and Aiiken, 1995; Chen and Aiiken, 1999). The PAH biodegradation in our experiments may have been mediated by a common enzyme system. We recognize that this issue is unresolved, but for purposes of postulation the following discussion is based on the premise that the differences do not derive from microbiological differences but from differences in PAH molecular structures.

In our study, the largest PAH (pyrene) degraded almost as fast as the smallest PAH (naphthalene). The two three-ring compounds, anthracene and phenanthrene, degraded at similar rates (both significantly slower than naphthalene and pyrene) despite the different arrangements of the three aromatic rings. Bossert and Bartha (1986) reported that phenanthrene was removed faster than anthracene in soil systems. They suggested that the likely reason for the slower degradation of anthracene is that its aqueous solubility is two orders of magnitude lower than that of phenanthrene. The finding that in our study phenanthrene and anthracene were found to have almost identical biodegradation rates provides additional evidence that once bioavailability constraints are re-
moved these two molecules respond similarly to biodegradation from bacterially mediated processes. The presence of an alkyl functional group on naphthalene showed biodegradation by factors of five (2-methyl-naphthalene) and 10 (1-methyl-naphthalene). The alkyl-naphthalenes degraded at rates of similar order as phenanthrene, anthracene, and fluoranthene. Stringfellow and Atken (1995) point out that the ability to metabolize methyl-naphthalenes varies greatly across organisms. They found that for two phenanthrene-degrading bacteria the biotransformation rates of 1-methyl-naphthalene, 2-methyl-naphthalene, and naphthalene were comparable. Others have reported evidence of slower degradation of 2-methyl-naphthalene relative to naphthalene in spiked soil systems (Heitkamp and Cermiglia, 1987, 1989). (In soils, the difference could be explained by the slightly greater hydrophobicity of 2-methyl-naphthalene.) Comparing the biodegradation rate coefficients for 1-methyl-naphthalene and 2-methyl-naphthalene in the present study suggests that the location of the alkyl group is important, for 2-methyl-naphthalene degraded twice as fast as 1-methyl-naphthalene. Leblond et al. (2001) similarly found that in a mixture, the first-order biodegradation rate coefficient of naphthalene was larger than that of 2-methyl-naphthalene, which was larger than that of 1-methyl-naphthalene. These differences may be related to the multiple biochemical pathways possible for degradation of these compounds (Mahajan et al., 1994; Dutta et al., 1998). From our study, it is difficult to assess the importance of alkyl chain length on biodegradation rate because of the lack of confidence in the 2-ethyl-naphthalene parameter estimate. In summary, our findings contribute to the growing body of evidence that alkyla tion significantly affects PAH biodegradation.

Three of the PAHs studied have a nonaromatic ring:acenaphthene, fluorene, and fluoranthene. Fluorene, which is similar in size to phenanthrene and anthracene, has a biodegradation rate coefficient that is an order of magnitude smaller than those of phenanthrene and anthracene. Fluoranthene, a relatively large molecule, has a biodegradation rate similar to phenanthrene and anthracene. The similarity in biodegradation rate of these compounds has been explained by the potential similarity in their biodegradation reaction pathways (Weisensel et al., 1991). The consortium was unable to observably biodegrade acenaphthene. One biodegradation pathway proposed for acenaphthene involves oxidation of the aliphatic ring resulting in nonproductive dead-end products (Schocken and Gibson, 1984; Cermiglia, 1992). It may be the case that the consortium used in these experiments does not have the necessary enzymes to carry out this initial reaction or that the genes for this enzyme are not induced. The structure of acenaphthene includes a naphthoic moiety, which one would imagine could be a site of attack by the dioxygenase enzyme, but attack via dioxygenase at this site has not been documented. This suggests that the five-membered ring attached to naphthalene may inhibit a dioxygenase attack on the naphthoic moiety. We conclude that PAH molecules that include a nonaromatic ring structure biodegrade more slowly than purely benzoic structures of similar molecular weight.

Comparison with biodegradation rates in soils and sediments

The final issue we address is the amount of variation in the biodegradation rate coefficients and the extent to which this variation explains the variations that are reported in the literature for PAH biodegradation in soils. The rate coefficients from this study span less than two orders of magnitude. The rate coefficients for six of the PAHs lie within a narrower range of 0.115 to 0.32 h⁻¹ (mg protein)⁻¹. This similarity in biodegradation rates agrees with a recent study by Chen and Atken (1999), who presented biodegradation rates for low and high molecular weight PAHs, identified by salicylate. The biodegradation rates for three- and four-ring PAHs (phenanthrene, fluoranthene, pyrene, benzo[α]anthracene, and chrysene) fell within a range comparable to our study, while the uninduced removal rates were similar to killed controls.

We next compare the rate coefficients in the present study to those determined by Park et al. (1996). For 14 compounds, they inferred first-order biodegradation rate coefficients from experiments using two different soil types spiked with PAHs. Their rate coefficients are plotted on a log scale against PAH molecular weight in Fig. 2 along with the biomass-normalized rate coefficients from this study. Their values were not reported as biomass-normalized rate coefficients, but the biomass content was reported to be comparable and roughly constant in all the experiments. Consequently, their rate coefficients can be compared to values from the present study, not in magnitude but in relative terms. A linear regression shows that there is a rough inverse correlation with molecular weight for the rate coefficients in soils. The rate coefficients from our studies show no such trend. This supports the conjecture that the variation in the properties governing bioavailability may largely, if not solely, explain the variation of observed biodegradation rates in soils.

Heitkamp and Cermiglia (1987, 1989) studied PAH mineralization in sediment/water systems and found that the biodegradation rates for naphthalene, phenanthrene, and pyrene were inversely related to molecular weight. The same investigators used a Mycobacterium sp. isolated from PAH-contaminated soil to study PAH biodegradation.

ENVIRON ENG SCI VOL. 20, NO. 3, 2003
tion in an aqueous medium, and found that fluoranthene and pyrene biodegraded faster than phenanthrene or naphthalene (Heitkamp and Cerniglia, 1988). This result is partially consistent with our findings that fluoranthene and pyrene are biodegraded faster than phenanthrene and other intermediate-size PAHs. Heitkamp and Cerniglia concluded that the difference in the ranking of the biodegradation rates was due to differences in the organisms present in the two experimental systems. It is also possible that by removing the sediment sorption process, bioavailability limitations were removed and hydrophobicity was no longer the governing metric.

SUMMARY AND CONCLUSIONS

This study was designed to combine experimental measurement with mathematical modeling to infer biodegradation kinetic parameters for PAHs dissolved in aqueous systems. To achieve this it was necessary to (1) independently measure and mathematically model the abiotic processes that may have affected the kinetics in the reactor vessels, and (2) account for the unique starting conditions for each PAH experiment. To the extent possible, both substrate depletion data as well as biomass growth data were used to provide kinetic information. However, biomass growth data were used in the parameter estimation for only four of the PAHs. For the remaining compounds, there was insufficient biomass growth to provide statistically useful information. For two of the PAHs, naphthalene and 1-methylnaphthalene, Monod kinetic parameters were estimated. Biomass-normalized first-order biodegradation rate coefficients were estimated for all but one of the PAHs studied. Acmethylnaphthalene did not degrade under the conditions of this study.

The value of measuring biodegradation kinetics in aqueous systems is that it provides information about the kinetics of biotransformation of these compounds, in the absence of any physical-chemical processes that may affect kinetics by limiting bioavailability. We have found that the 10 PAHs studied (from naphthalene up to pyrene) have quite comparable biodegradation rates despite the vast differences in molecular sizes and chemical properties. This finding is quite different from what is observed when PAH biodegradation rates are studied in soils and sediments where biodegradation is usually much slower for the higher molecular weight compounds. This implies that the biodegradation rates observed in field samples are largely, if not entirely, governed by rate-limiting physical-chemical, not biological, processes.

ACKNOWLEDGMENTS

This research was supported by a grant from the National Science Foundation, project number EAR-9708406. The authors acknowledge Dr. Jerome J. Kaluz
AQUEOUS PHASE BIODEGRADATION KINETICS OF 10 PAHs

(Rutgers University) and Ms. Li Li (Princeton University) for assistance in efforts to characterize the microbial consortium, and Ms. Kristine Wammer (Princeton University) for her biodegradation and growth studies of anthrathol. The authors also acknowledge the anonymous reviewers whose careful reviews of this manuscript aided in its final preparation.

REFERENCES


KECK, J., SIMS, R.C., COOVER, M., PARK, M., and

ENVIRON ENG SCI, VOL. 20, NO. 3, 2003


