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Intra-Aggregate Mass Transport-Limited Bioavailability of Polycyclic Aromatic Hydrocarbons to *Mycobacterium* Strain PC01

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Biodegradation kinetics for three- and four-ring PAHs by *Mycobacterium* sp. strain PC01 were measured in whole and density-fractionated estuarine sediments and in a system without intra-aggregate mass transport limitations. The biokinetic data in the systems with and without intra-aggregate mass transport limitations were compared with abiotic PAH desorption kinetics. The results indicate that intra-aggregate mass transport limitations, and not the intrinsic bacterial PAH utilization capacity, were most important in controlling the rate of biodegradation of sediment-sorbed PAHs. Achievable extent of biodegradation could be predicted by the independently measured fraction of desorbable PAHs in the fast-diffusion regime of a two-domain intra-aggregate mass transport model. A closed-form mathematical model was developed to describe sediment-pore water partitioning and rapid aqueous-phase diffusion of PAHs through the macropore and mesopore network of sediment aggregates, followed by first-order biodegradation of desorbed PAHs in the bulk aqueous domain. The model effectively predicted independent biodegradation kinetics of PAHs field-aged in two estuarine sediments. Despite low aqueous solubility of PAHs, macropore and mesopore diffusion may be an important mechanism controlling intra-aggregate mass transport and bioavailability of the most readily and extensively desorbed PAHs in sediments.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of common sediment contaminants characterized by low aqueous solubility and high octanol−water partition coefficients (*1*). In aquatic environments PAHs tend to partition into organic-matter rich sediments where they may accumulate over long periods of time. Such "aged" contaminants, however, tend to be less extractable and less bioavailable (*2*−*5*), and also may pose a diminished toxicity risk (*6*). Methods to measure and predict bioavailability of aged hydrophobic organic contaminants (HOCs) are needed to optimize remediation processes including biodegradation, to improve the accuracy of risk assessments, and to make more informed sediment management decisions.

Contaminant desorption from the solid phase has been shown to control bioavailability in a variety of natural and model systems (*7*−*11*). In other cases, additional factors including oxygen limitations have been shown to contribute to the recalcitrance of soil-sorbed HOCs (*12*). Although complete dissolution is not a prerequisite of bioavailability (*13*−*17*), certainly HOCs must be accessible to microorganisms before they can be biodegraded (*18*). One strategy for deconvoluting the complex interactions involved in limiting bioavailability of aged contaminants in natural systems is to de-couple the physical and chemical factors controlling HOC intra-aggregate mass transport from the biological factors controlling HOC biodegradation.

The objective of this study was to establish the influence of intra-aggregate mass transport limitations on the bioavailability of field-aged PAHs from two whole estuarine sediments and sediment density fractions, and to predict the rate and extent of PAH biodegradation in these sediments. Examining field-aged PAHs provides a better measure of contaminant behavior in chronically impacted sediments, but complexity and heterogeneity of natural materials makes elucidation of governing mechanisms difficult. Previous reports successfully modeling intra-aggregate mass transport and biodegradation kinetics have focused on contaminants amended to model (*9*) or natural materials (*19*) or have adjusted parameters in the model to fit the biodegradation data (*7*). To our knowledge, no prior study has successfully predicted independent bacterial utilization kinetics of any persistent compound that was field-aged in natural soil or sediments.

Our strategy was to systematically build complexity to establish the physical, chemical, and biological factors controlling bioavailability. First, PAH-contaminated sediments from two study sites were fractionated by size and density, and extensively characterized for physical and chemical factors likely to affect PAH desorption and bioavailability (*20*). Next, we measured compound- and sediment fraction-specific abiotic desorption kinetics (*20*, *21*) and developed a mass transport model to describe intra-aggregate diffusion of PAHs in sediments (*21*). Then, utilization kinetics of a mixture of PAHs derived from field-aged sediment were measured in a system without intra-aggregate mass transport limitations to determine a *Mycobacterium* strain's inherent capacity to degrade various PAHs. Biodegradation kinetics of PAHs in sediments were compared with sediment-free biodegradation kinetics, and with abiotic desorption kinetics for the same compounds from the same sediment fractions. Finally, a closed-form mathematical model solution was developed that describes fast-domain aqueous diffusion of PAHs through the macropore and mesopore network of sediment aggregates followed by first-order PAH biodegradation in the bulk or interstitial aqueous domain. The model successfully predicts the independently measured rate and extent of biodegradation of field-aged PAHs from weathered sediments.

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Materials and Methods

Sediments. Sediments were collected from Piles Creek (PC), New Jersey (40°36.53′ N, 74°13.60′ W) and Newtown Creek (NC), New York (40°44.28′ N, 73°56.75′ W) in the New York/New Jersey Harbor estuary. PC is a tidal stream, a tributary of Arthur Kill, and runs through a region rich in the emergent vegetation *Spartina alterniflora* and *Phragmites australis*. NC is a subtidal waterway, a tributary of the East River, and runs through a heavily industrialized area with no emergent vegetation. The sediments were collected, homogenized, and separated into density fractions by equilibrium settling in a saturated solution of cesium chloride (approximate density 1.7 g mL^{-1}), as described previously (*20*). The physical and chemical properties of the study sediments and the effect of those properties on sequestration and desorption of PAHs have been described (*20*, *21*).

Culture Conditions and Microorganism. Biodegradation experiments were done in solutions of mineral salt basal medium (MSB or Stanier's medium (*22*)) adjusted to 25‰ salinity with 0.675 g L^{-1} of $MgCl_2 \cdot 6\,H_2O$, 0.075 g L^{-1} of $CaCl_2$, and 20 g L^{-1} of NaCl. Prior to use, the medium was sterilized by autoclaving for 20 min at 120 °C. The bacterial strain used in the biodegradation experiments was a *Mycobacterium* strain, designated PC01, isolated from PC sediment as previously described (*23*, *24*). *Mycobacterium* sp. strain PC01 is a rod-shaped, aerobic, gram-positive organism with a broad substrate range and is able to utilize phenanthrene, fluoranthene, or pyrene as a sole carbon and energy source. Anthracene and benz[*a*]anthracene can also be metabolized in sediment containing a mixture of PAHs (*23*). According to the bacterial adhesion to hydrocarbons (BATH) method (*25*), *Mycobacterium* sp. strain PC01 has a hydrophobic cell surface (*23*).

For biodegradation experiments, strain PC01 was pregrown in MSB medium (15‰ salinity) containing 0.018 g L^{-1} yeast extract and incubated at 30 °C for 5 days with rotary shaking at 200 rpm. Cells were collected and washed twice in MSB medium to remove residual yeast via centrifugation at 3200*g* for 15 min (Beckman GS-6, Beckman Instruments, Palo Alto, CA). Cell biomass was quantified by protein using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL) and compared with bovine serum albumin standards (New England Biolabs, Beverly, MA).

Biodegradation of Mixed PAH Sediment Extract. The purpose of this experiment was to quantify the biodegradation rate of mixed PAHs in quantities and proportions similar to those of the PAHs contained in the sediments, along with other unknown but potentially biodegradable or inhibitory compounds that may also be present in the sediment, but without intra-aggregate mass transfer limitations. The initial content of PAHs in the batch microcosms is given in Table 1, along with a comparison of the biomass-normalized PAH content in the sediment extract experiment with the biomass-normalized content of PAH in the sediment biodegradation experiments, which are described below. Biodegradation rates measured in this way were thus intended to represent the "intrinsic" PAH biodegradation rate that would occur in the sediment biodegradation experiments without the diffusion limitations in desorption from the solid matrix.

To prepare the mixed extract, PAHs were extracted from whole PC sediment by sonication in hot (80 °C) acetonitrile (HPLC-grade VWR International, West Chester, PA) for 2 h. The supernatant was separated from the sediment by centrifugation (5600*g*, 30 min), and then concentrated by heating (60 °C) under a gentle N_2 stream. Aliquots of the concentrated sediment extract were added to sterile 30-mL glass serum bottles. Each serum bottle was covered loosely with aluminum foil, and the solvent was evaporated overnight at room temperature. A yellow residue of PAHs and other unidentified compounds coated the bottoms of the serum bottles. To each coated serum bottle were added 7 mL of modified MSB media containing 0.15 mg protein of pregrown and washed *Mycobacterium* cells. Bottles were sealed with Teflon-lined septa and aluminum-crimp closures. Then, 10 mL of headspace was replaced through the septum of each serum bottle with pure O_2, a volume predetermined to ensure that biodegradation was not oxygen-limited. Serum bottles were incubated upright on a rotary shaker for up to 5 days (200 rpm, 30 °C). Controls were prepared identically except no bacteria were added in the background controls, and 0.5 mL of 37% formaldehyde was added to the sterile controls. The controls established that there was no measurable indigenous PAH degradation activity or abiotic losses. Given their low aqueous solubility and high hydrophobicity, PAHs in the batch microcosms resided primarily in the nonaqueous coating and rapidly and reversibly partitioned into the aqueous phase where they were available to microorganisms.

TABLE 1. Initial Content of PAHs in Sediment-Free and Sediment Biodegradation Experiments for Phenanthrene (Phen), Anthracene (Anth), Fluoranthene (Flan), Pyrene (Pyr), and Benz[*a*]Anthracene (Benz-*a*) and Values of Specific First-Order Biodegradation Rate Constants.

	phen	anth	flan	pyr	benz-*a*
Initial PAH Content [μg PAH]					
PAH extract experiment	0.67	0.56	3.4	3.4	1.2
PC sediment experiment	1.3	1.9	6.1	10	4.4
NC sediment experiment	3.1	2.0	7.5	11	6.3
Biomass-Normalized Initial PAH Content [μg PAH (mg protein)$^{-1}$]					
PAH extract experiment	4.5	3.8	23	23	8.0
PC sediment experiment	2.6	3.8	12	20	8.9
NC sediment experiment	6.3	4.1	15	22	13
Specific First-Order Biodegradation Rates, k_b [1 h^{-1} (mg protein)$^{-1}$]					
k_b	0.41	0.32	0.18	0.13	0.008
$\pm\, k_b$	0.023	0.018	0.012	0.0062	0.005
error %	6%	6%	7%	5%	64%

At each sampling point, triplicate serum bottles were sacrificed. From each bottle, 0.2 mL was removed for protein analysis, and then 0.5 mL of a 37% formaldehyde solution was added to stop biodegradation. Sacrificed serum bottles were re-sealed with new septa and stored in the dark at 4 °C pending quantification of remaining PAHs. The quantity of PAHs remaining in each serum bottle was determined by liquid−liquid extraction. Hexane (5 mL; Optima Grade, VWR International, West Chester, PA) was added to each serum bottle, then the bottles were immediately re-sealed and shaken upright in the dark (150 rpm, 22 °C) for 1 week. The volume of hexane lost by evaporation was determined gravimetrically by weighing each bottle before and after the extraction period. (On average, 7% of the hexane evaporated in the week of extraction.) Next, the hexane phase of each bottle was sampled with a disposable plastic syringe and passed through a 0.2-μm-pore-diameter nylon Acrodisc HPLC syringe filter (Pall Gelman, Ann Arbor, MI). Finally, the concentration of PAHs in the hexane was quantified by HPLC−UV absorbance as described previously (*20*) and PAHs were identified by comparison of UV absorbance spectra with spectra of known PAH standards. The total PAH content was computed by multiplying the concentration of PAHs in the sampled hexane by the exact hexane volume remaining after extraction in each microcosm.

Biodegradation kinetics were modeled by a first-order kinetics rate law:

$$-\frac{dC}{dt} = k_b C \qquad (1)$$

where C [mg L^{-1}] is the PAH concentration, t [h] is time, and k_b [h^{-1}] is the first-order biodegradation rate constant. Biodegradation rates were computed by finding the best-fit slope of a plot of $\ln[C(t)/C_o]$ versus time (only including data from the first 26 h, before depletion began to occur) by least-squares linear regression according to the integrated form of eq 1:

$$\ln\frac{C(t)}{C_o} = -k_b t \tag{2}$$

where C_o is the initial content of PAHs in replicate batch microcosms. Biodegradation rate constants were then normalized to the measured biomass quantified as total milligrams of protein. Growth was neglected because biomass (measured in triplicate at every sampling point) did not change over the course of the experiment.

Biodegradation of PAHs in Sediments. Biotransformation of phenanthrene, anthracene, fluoranthene, pyrene, and benz[*a*]anthracene by *Mycobacterium* sp. strain PC01 was examined for field-aged estuarine sediments and for density-fractionated sediments. Details of the biodegradation experiment were presented elsewhere (*23*, *24*). Briefly, whole sediments, and sediments fractionated by density separation, were collected, homogenized, characterized, and fractionated as previously described (*20*) except that additional sediment washing after density separation was performed to prevent toxic response from the CsCl solution. The washing procedure was shown to adequately prevent CsCl toxicity to the microorganism (*24*). Each 50-mL serum bottle contained 1.5 g of sediment in 4 mL of filter-sterilized seawater and 12 mL of MSB medium containing 0.5 mg of PC01 protein. Biodegradation of PAHs in sediments was measured using the same experimental design as that used for the sediment-free PAH biodegradation experiments, including using identically prepared bacterial cultures, in the same media with excess of oxygen, and incubation at the same temperature and shake rate, to facilitate comparison with the sediment-free experiments. At time intervals, 0.5 mL of the culture slurry was sampled using a disposable syringe with a 20-gauge needle, and the sediment was extracted and remaining PAHs were quantified as described previously (*20*). As with the sediment-free biodegradation experiments, background and sterile controls showed no indigenous PAH degradation activity or abiotic losses.

Abiotic PAH Desorption Experiments. Abiotic desorption of PAHs from sediments were measured for up to one year for split samples of homogenized sediments also used in the biodegradation experiments by a batch "infinite sink" Tenax desorption method as reported elsewhere (*20*). Mass transport modeling has also established for the compound- and sediment-specific intra-aggregate mass transport limitations on desorption from the sediments (*21*).

Model Development

Desorption kinetics of three- and four-ring PAHs (including all of the biodegradable compounds considered here) from the sediments used in this study for time periods of several months are best described by a two-domain intra-aggregate mass transport model (*21*). In the two-domain model, a fraction of each PAH was considered to behave by a "fast-domain" release mechanism and the remainder behaved according to a "slow-domain" release mechanism. The underlying mechanism for each domain is uncertain, but the fast-domain mechanism may be consistent with rapid diffusion of PAHs initially residing in, or available to reversibly partition into, the water-filled macro- and meso-pore network of sediment aggregates. Fast-domain release can be represented by diffusion from a spherical particle with observed diffusivities on the order of 5×10^{-10} cm^2 s^{-1}. Thus, approximately 90% of the PAH fraction controlled by fast-domain release is desorbed within the first 8 h. In contrast, the "slow-domain" release was represented by diffusion-controlled release from a spherical particle with observed diffusivities on the order of 1×10^{-13} cm^2 s^{-1}. Thus, less than 8% of this fraction was released over the same period of time. Therefore, for the purposes of modeling processes occurring over a few days, it is appropriate to use a single, fast-domain intra-aggregate mass transport model.

Fick's second law of diffusion in spherical coordinates is

$$\frac{\partial C}{\partial t} = D_{obs}\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \tag{3}$$

where the parameters are C [mg kg^{-1}], the PAH concentration in the solid phase; t [s], time; D_{obs} [cm^2 s^{-1}], the observed diffusion coefficient; and r [cm], the radial distance from the particle center. By making the substitution $u = Cr$, and for the boundary conditions $u = 0$ for $r = 0$ and $t > 0$; $u = 0$ for $r = a$ and $t > 0$; $u = C_I \cdot r$ for $0 < r < a$ and $t = 0$ (where C_I is the initial PAH concentration distribution, and a is the particle radius), an analytical solution is known (*26*):

$$\frac{M(t)}{M(\infty)} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp(-D_{obs\,f}n^2\pi^2 t/\bar{a}^2) \tag{4}$$

where $M(t)/M(\infty)$ [dimensionless] is the mass fraction of desorbable PAHs that are desorbed at time, t [s], and $D_{obs\,f}$ is the fast-domain diffusivity. For heterogeneous sediments, $\bar{a}$ [cm], is defined as the volume-weighted average sediment radius computed from measured sediment size distribution data (*20*) according to the method of Rao et al. (*27*). The model formulation and analytical solution relies on several simplifying assumptions: (i) PAHs were initially uniformly distributed throughout spherical sediment aggregates; (ii) either by virtue of the abiotic desorption experimental conditions, or by uptake by microorganisms in the biodegradation experiments, the PAH concentration at the exterior sediment/water interface is maintained effectively at zero; (iii) it is reasonable to neglect an exterior boundary layer (*28*); and (iv) PAH partitioning at sediment/pore water interfaces is rapid and reversible.

Although it is possible to employ many other formulations to describe intra-aggregate mass transport (that also rely on many additional measurable and adjustable parameters, and are described by greater mathematical complexity), one of our goals was to show the influence of intraparticle diffusion on bioavailability using a reasonable model for which (1) all parameters could be established a priori and (2) a useful, closed-form analytical solution could be found.

Fast-domain desorption of three- and four-ring PAHs may occur via a macropore and mesopore aqueous diffusion mechanism (*21*). Such fast-domain diffusivity can be predicted by

$$D_{obs\,f} = \frac{D_{mol}/\tau}{1 + \frac{\rho}{\epsilon}K_{sed-pw}} \tag{5}$$

where D_{mol} [cm^2 s^{-1}] is the free aqueous diffusivity of the PAH, which is tabulated for some compounds or can be estimated by applying empirical relations (*29*). The sediment tortuosity, τ [dimensionless] is computed following an approximation of Millington and Quirk (*30*) where $\tau = \epsilon^{-1/3}$ as described previously (*21*), giving a value of tortuosity consistent with those of other reports (e.g., ref *9*). Sediment intra-aggregate porosity, ϵ [dimensionless] (internal pores with diameters greater than 2 nm), and density, ρ [g mL^{-1}], for these sediments were measured and reported previously

TABLE 2. Input Parameters for the Prediction of Biodegradation by the Fast-domain Macropore and Mesopore Diffusion-Limited Biodegradation Model (eq 8), Including the Initial PAH Concentration of the Sediment, C_s, and the First-Order Biodegradation Rate Constant, k_b [a]

	C_s [mg kg^{-1}]	k_b [h^{-1} mg protein^{-1}]	f	K_{sed-pw} [L kg^{-1}]	D_{mol} [cm^2 s^{-1}]	$D_{obs\ f}$ [cm^2 s^{-1}]
			Whole PC Sediment			
phenanthrene	0.86	0.41	0.62	6.5×10^3	7.0×10^{-6}	3.1×10^{-10}
anthracene	1.3	0.32	0.79	2.0×10^4	7.1×10^{-6}	1.0×10^{-10}
pyrene	6.7	0.13	0.80	3.2×10^4	6.7×10^{-6}	6.1×10^{-11}
			Whole NC Sediment			
phenanthrene	2.1	0.41	0.17	1.2×10^5	7.0×10^{-6}	3.0×10^{-11}
anthracene	1.4	0.32	0.42	4.6×10^5	7.1×10^{-6}	7.9×10^{-12}
pyrene	7.4	0.13	0.80	5.3×10^5	6.7×10^{-6}	6.5×10^{-12}

	$\bar{a}$ [cm]	ϵ [%]	ρ [L kg^{-1}]
whole PC sediment	0.0066	43	1.1
whole NC sediment	0.0028	48	0.74

[a] Other parameters were reported previously (*21*), and are included here for clarity, including the fraction of total PAH in fast intra-aggregate mass transport domain, f, the sediment-pore water equilibrium partition coefficient, K_{sed-pw}, the free aqueous PAH diffusivity, D_{mol}, and sediment properties including volume-weighted average particle radius, $\bar{a}$, intra-aggregate porosity, ϵ, and density, ρ. From these parameters the fast-domain diffusivity, $D_{obs\ f}$, is also computed (see eq 5).

TABLE 3. Comparison of Extent of Biodegradation and Desorption, and Fraction, f, of Desorbable PAHs Determined to Be in the Fast Domain of a Two-Domain Intra-Aggregate Mass Transport Model (*21*)[a]

	whole NC			whole PC		
	% biodeg	% desorb	f	% biodeg	% desorb[c]	f
phenanthrene	19 ± 4	5 ± 1	0.17	47 ± 16	41 ± 7	0.62
anthracene	31 ± 2	14 ± 0	0.42	67 ± 6	51 ± 2	0.79
fluoranthene	75 ± 0	nd[e]	0.73	87 ± 7	nd	0.69
pyrene	72 ± 0	61 ± 2	0.80	83 ± 4	88 ± 2	0.80
benz[*a*]anthracene	43 ± 2	30 ± 4	0.68	64 ± 5	64 ± 2	0.82

	low-density NC			high-density NC		
	% biodeg	% desorb	f	% biodeg	% desorb[c]	f
phenanthrene	15 ± 9	5 ± 1	0.39	1 ± 9[d]	nd	0.26
anthracene	22 ± 5[d]	6 ± 1	0.34	18 ± 7	14 ± 1	0.54
fluoranthene	15 ± 8	nd	0.57	70 ± 1	nd	0.74
pyrene	16 ± 8	20 ± 1	0.70	72 ± 1	47 ± 3	0.79
benz[*a*]anthracene	6 ± 2[d]	16 ± 1	0.66	25 ± 8	38 ± 2	0.82

	low-density PC	high-density PC
	% biodeg	% biodeg
phenanthrene	32 ± 13	NA[f]
anthracene	25 ± 9[d]	51 ± 2
fluoranthene	22 ± 12	84 ± 2
pyrene	24 ± 13	84 ± 3
benz[*a*]anthracene	17 ± 12[d]	15 ± 11[d]

[a] Extent of biodegradation at 5 days (average and standard deviation of triplicates) and extent of desorption at 5 days[b] (average and range of duplicates) are expressed as a percentage of total sediment PAH concentration. [b] Standard deviation or range was computed from replicate measurements of percent biodegraded or desorbed at 5 days versus the average value for starting PAH concentration. [c] Percent desorption at 5 days estimated by interpolation. [d] Decrease in sediment concentration after 5 days not statistically significant ($p > 0.1$). [e] nd: Not determined due to insufficient desorption data. [f] NA: Not applicable; average sediment concentration increased after 5 day incubation.

(*20*). K_{sed-pw} [mL g^{-1}], the sediment-pore water equilibrium-partitioning coefficient, was also reported previously (*21*). All parameter values also are reproduced here in Table 2.

Substituting eq 5 into eq 4 and setting $M(\infty)$ equal to the product of C_s [mg kg^{-1}], the total initial sediment PAH concentration, and f, the fraction of sediment PAHs in the fast intra-aggregate mass transport domain, the change in sediment PAH concentration by fast-domain desorption is

$$C(t) = C_s - C_s f\left(1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{D_{mol}/\epsilon^{-1/3}}{1+\frac{\rho}{\epsilon}K_{sed-pw}}n^2\pi^2 t/\bar{a}^2\right)\right) \quad (6)$$

The fraction f for each compound desorbing from each sediment fraction was previously determined and reported (*21*). In that work, the best value for the fast and slow domain diffusivities, $D_{obs\ f}$ and $D_{obs\ s}$, and the fraction of desorbable PAHs in the fast intra-aggregate mass transport domain, f, were simultaneously determined by minimizing the sum of squared errors between a two-domain intra-aggregate mass transport model and the measured desorption kinetics. However, as also stated in the previous work, the fast-desorption, readily biodegradable fraction can also be approximated by the percent of desorbable PAHs that are desorbed in the first 24 h.

In addition to the analytical solution for *abiotic* fast-domain intra-aggregate mass transport (eq 6), we also

developed a solution for a second case, where compounds diffused from sediments under the driving force of extra-aggregate biodegradation. In formulating this model, the following assumptions are invoked (in addition to all previous assumptions): (v) biodegradation occurs outside sediment particles based on previous theoretical (*18*), and experimental (*9*, *19*, *31*) reports; (vi) biodegradation of mixed PAHs by *Mycobacterium* strain PC01 occurs simultaneously according to first-order kinetics. (Support for the last assumption is provided in the Results and Discussion section.)

The model case with first-order biodegradation occurring outside sediment aggregates is also based on the previous governing equation (eq 3) with the same initial condition, but with a different boundary condition:

$$-D_{obs}\frac{\partial C}{\partial r} = k_b(C_o - C_o') \text{ for } r = a \text{ and } t > 0 \qquad (7)$$

where $\partial C/\partial r$ is the radial concentration gradient and C_o and C_o' are the actual and equilibrium surface concentrations of PAHs, respectively. This set of equations has been solved numerically (*9*). However, if one assumes that the rate of biodegradation is constrained by the rate of intra-aggregate diffusion (an appropriate assumption given Φ, the dimensionless group for comparing rates of intraparticle diffusion and biodegradation (*9*), is >1), the changing sediment PAH concentration, $C(t)$, by desorption-limited biodegradation can be modeled by approximating first-order biodegradation as a difference equation for a small time interval, Δt, between the current period (t) and the previous period ($t - \Delta t$), and assuming that the total PAH mass available for biodegradation can be approximated by the mass desorbed (according to eqs 4 and 5) but not degraded in the previous time interval plus additional PAHs desorbed in the current interval:

$$C(t) = C_s\left\{1 - f\frac{M(t-\Delta t)}{M(\infty)} + f\sum_{j=1}^{t-1}\left[(1-k_b\Delta t)^{t-j}\left(\frac{M(t)-M(t-\Delta t)}{M(\infty)}\right)\right]\right\} \qquad (8)$$

where

$$\frac{M(t)}{M(\infty)} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left(-\frac{D_{mol}/\epsilon^{-1/3}}{1+\frac{\rho}{\epsilon}K_{sed-pw}}n^2\pi^2 t/\bar{a}^2\right)$$

All parameters are as described and were determined a priori.

Results and Discussion

PAHs in Mixtures Were Degraded Simultaneously by First-Order Kinetics. Sediment-free utilization of PAHs in the mixed sediment extract was rapid and extensive for phenanthrene, anthracene, fluoranthene, and pyrene (Figure 1). The data also established that phenanthrene, anthracene, fluoranthene, and pyrene were degraded simultaneously by a first order rate law (Figure 1B). The degradation of benz[*a*]anthracene was limited, and occurred only after a lag phase of more than 1 day. The rate of degradation varied by compound, with phenanthrene and anthracene being degraded more rapidly than fluoranthene and pyrene in the liquid-only system. The magnitude of k_b [h^{-1} mg $protein^{-1}$] for PAHs in the mixed PC sediment extract was 0.41 for phenanthrene, 0.32 for anthracene, 0.18 for fluoranthene, 0.13 for pyrene, and 0.008 for benz[*a*]anthracene (Table 1). Subsequently, these biokinetic parameters were used to predict bacterial utilization of sediment-sorbed PAHs.

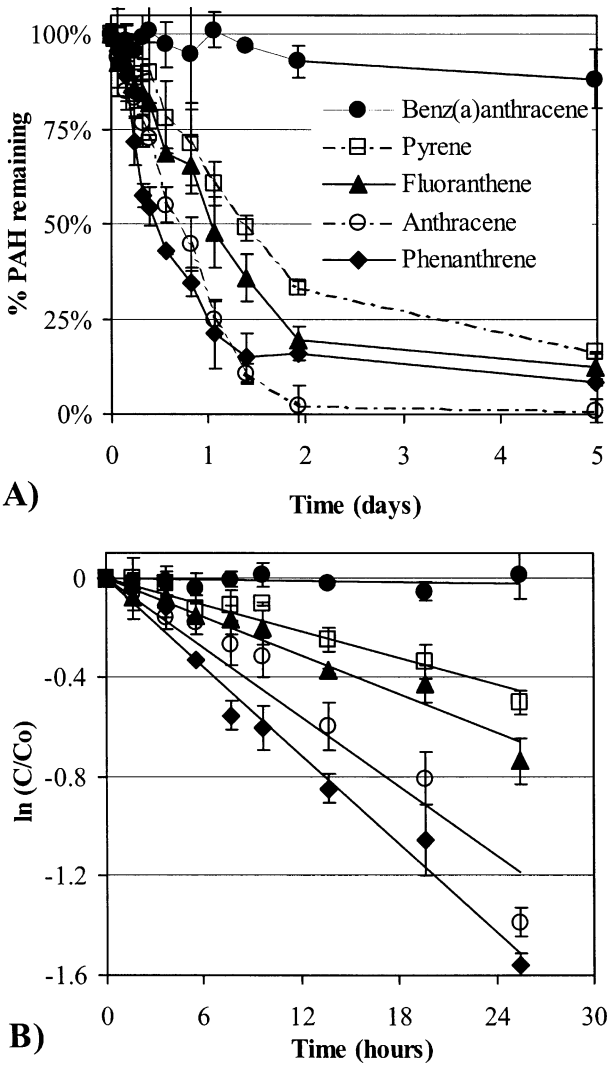

FIGURE 1. Sediment-free utilization of PAHs chemically extracted from whole PC sediment and amended to interior surface of glass batch microcosms by liquid cultures of *Mycobacterium* sp. strain PC01. Data reflect mean and standard deviation of triplicates. Data are presented as (A) percent PAH mass remaining and (B) natural log of mass fraction remaining (see eq 2) where the negative slope of each series equals the first-order biodegradation rate constant, k_b. Initial PAH content and values of k_b are given in Table 1.

Most-Bioavailable PAHs in Sediment Were Pyrene and Fluoranthene. Biodegradation of sediment-sorbed fluoranthene and pyrene was rapid and extensive for both PC and NC whole sediments (Figure 2). For example, in PC sediment, about 70% of fluoranthene and 40% of pyrene were utilized in the first 12 h. The rapid loss of fluoranthene and pyrene indicates that a large portion of these compounds was readily bioavailable to strain PC01 even though the PAHs had resided in the field for an undetermined period of time. In contrast, metabolism of benz[*a*]anthracene in both whole sediments occurred slowly throughout the 120 h of incubation, whereas utilization of phenanthrene and anthracene occurred only within the first few hours of incubation (Figure 2).

After 5 days of incubation, more than 70% of the pyrene and fluoranthene were utilized by strain PC01 in both whole sediments (Table 3). Compared with fluoranthene and pyrene, a much smaller fraction of total phenanthrene and anthracene in whole PC and NC sediment was degraded after 5 days of incubation (Table 3). Among the five biodegradable PAHs in both PC and NC whole sediments, the extents of biodegradation of fluoranthene and pyrene

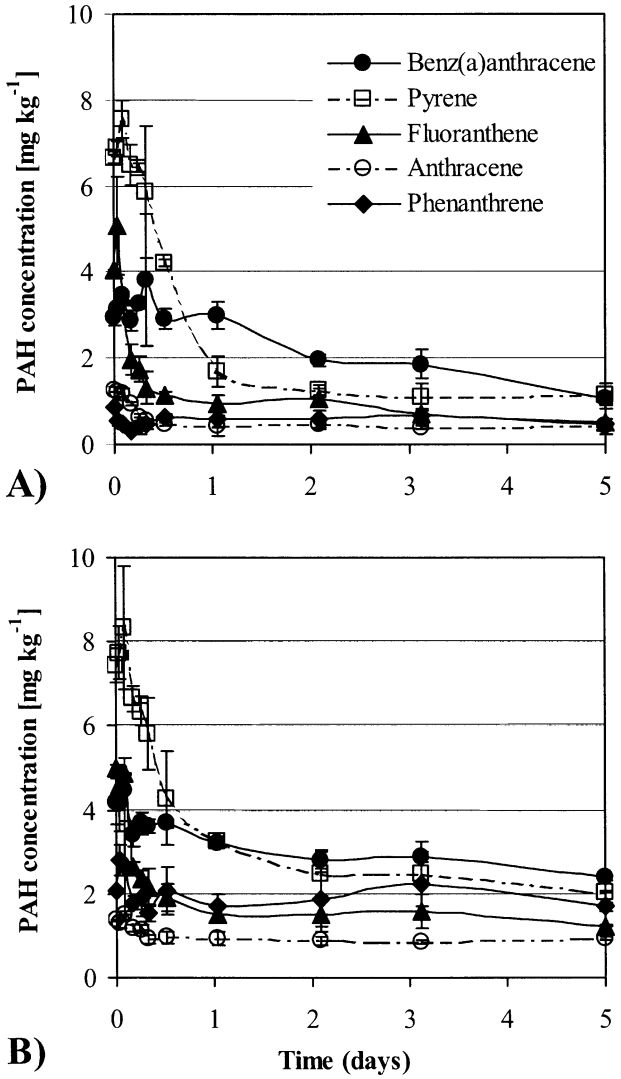

FIGURE 2. Loss of PAHs in (A) whole PC and (B) whole NC sediment incubated with *Mycobacterium* sp. strain PC01. Data reflect mean and standard deviation of triplicate measurements.

were the highest and the extent of biodegradation of phenanthrene was the lowest. Metabolism of PAHs in the high-density sediment fractions was very similar to the results observed for the corresponding whole sediments. For example, in PC and NC high-density sediment fractions about 50−80% of fluoranthene and pyrene were utilized within the first 12.5 h by strain PC01 (*23*). In addition, after 5 days incubation pyrene was the most extensively degraded PAH in both high-density sediment fractions (Table 3).

The concentrations of PAHs in the organic-matter-rich low-density fractions of both PC and NC sediments were about 10 to 100 times higher than those in the corresponding high-density fractions and whole sediments (*20*). However, PAHs were degraded at a slower rate and to a lesser extent in the low-density fractions relative to degradation in corresponding high-density fractions or whole sediments (*23*). For several of the PAHs in low-density NC and PC sediment, the PAH concentrations after 5 days incubation were not statistically different from the starting concentrations ($p > 0.1$) (Table 3). Degradation of phenanthrene, fluoranthene, and pyrene in the PC low-density sediment fraction was statistically significant ($p < 0.05$), but the rate and extent were much lower than for the corresponding whole or high-density fractions.

Intra-Aggregate Desorption Limits Rate and Extent of Biodegradation. The relative magnitudes of the first-order pyrene decay rate constant by *Mycobacterium* sp. strain PC01 in the different field-aged sediment fractions were high-density > whole > low-density for both PC and NC sediments (*23*). These relative rankings in biodegradation rate for pyrene were consistent with the relative rankings of diffusivity fit to desorption kinetics data for pyrene among the same sediment fractions (*21*). Similarly, the relative rankings in rate of biodegradation by compound (pyrene ≈ fluoranthene > phenanthrene ≈ anthracene (*23*)) were also consistent with the relative rankings of diffusivity among PAHs from various sediment fractions (*21*). However, in the sediment-free PAH biodegradation experiment, the relative rankings of PAH biodegradation rate followed a different trend: anthracene and phenanthrene were degraded significantly faster than fluoranthene and pyrene. These results suggest that the intra-aggregate mass transport limitations control the rate of biodegradation of PAHs in these sediments.

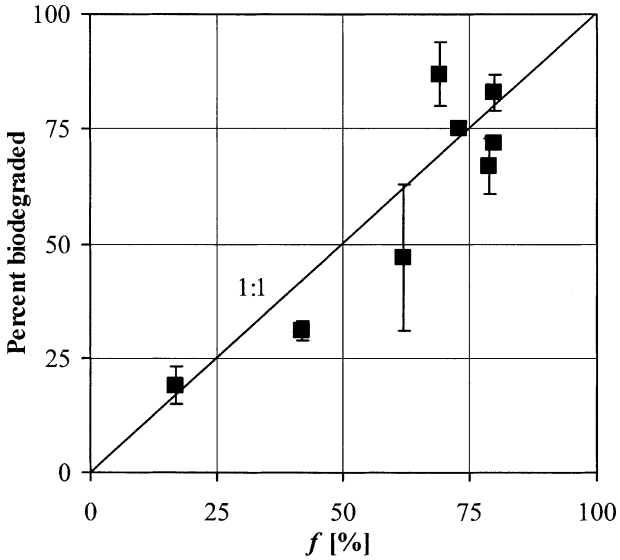

FIGURE 3. Comparison between percent of PAHs in fast intra-aggregate mass transport regime, *f*, versus percent biodegraded in 5 days for phenanthrene, anthracene, fluoranthene, and pyrene from whole NC and PC sediments. Error bars show standard deviation of triplicate biodegradation measurements.

The statistically significant correlation between percent biodegraded and percent desorbed at 5 days among all PAHs in whole sediments ($p = 0.009$, slope = 0.8, data shown in Table 1) indicates intra-aggregate mass transport limitations also control the *extent* of biodegradation. Interestingly, the parameter *f* (the fraction of total PAHs in the fast-diffusion domain), previously determined from fitting the desorption data to a two-domain intra-aggregate mass transport model (*21*), was closely correlated with extent of biodegradation ($p = 0.03$, slope = 1) in both whole sediments (Figure 3). These results are consistent with a previous report that found the amount of PAHs in the rapidly desorbing fraction was a good predictor for extent of PAH biodegradation (*32*). Here, we extend those results to *Mycobacteria* and two estuarine sediments.

Rapid Macropore and Mesopore Diffusion Predicts PAH Biodegradation Rate. The changing sediment PAH concentration during incubation with *Mycobacterium* sp. strain PC01 was simulated by aqueous-phase biodegradation of PAHs made available by fast-domain desorption (eq 8). These model predictions were compared with the experimentally determined utilization data for phenanthrene, anthracene, and pyrene from whole PC and NC sediments (Figure 4). The biodegradation model prediction was consistent with loss of PAHs from the sediments. However, the results for phenanthrene were somewhat inconclusive given the large range of

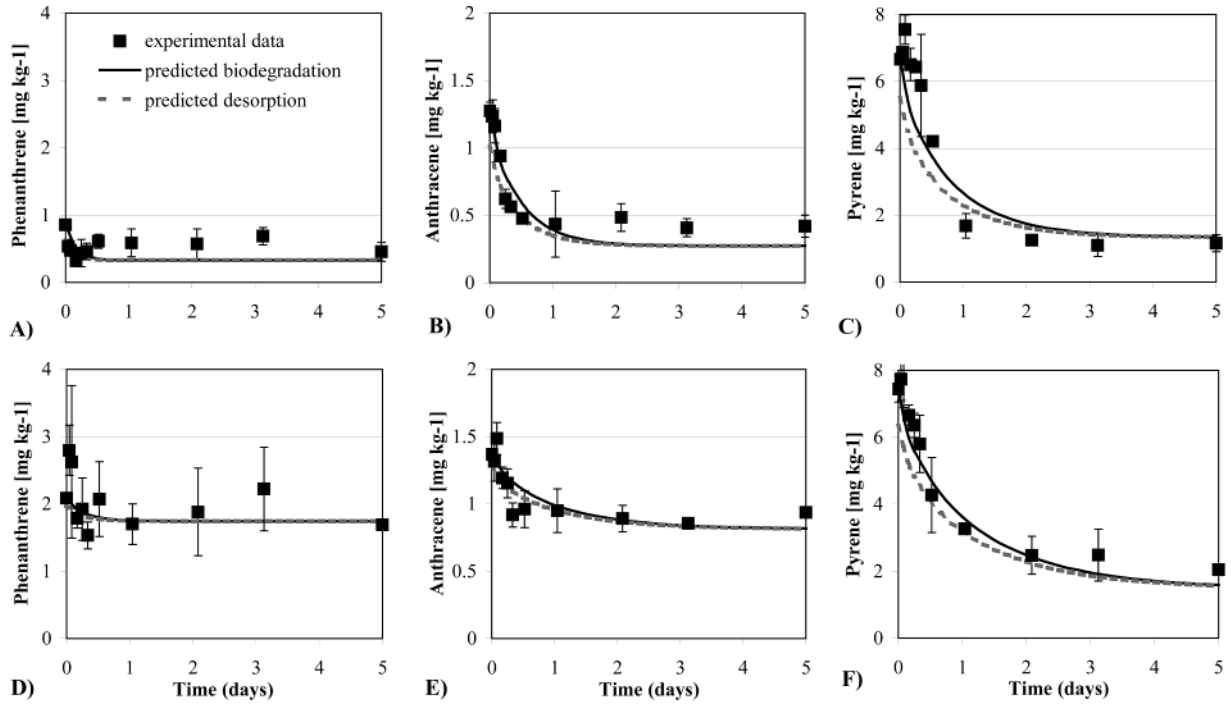

FIGURE 4. Predicted biodegradation (eq 8) of PAHs made available in solution by macropore and mesopore diffusion (eq 6) versus independently measured PAH loss (mean and standard deviation of triplicates) for phenanthrene, anthracene, and pyrene from whole estuarine sediments: PC (panels A−C) and NC (panels D−F).

data. Also, the predicted biodegradation of pyrene from PC sediment somewhat over-estimates the measured biodegradation in the first few hours. In most cases, but particularly for anthracene in both sediments, and for pyrene in NC sediment, the biodegradation model prediction was within the range of the experimentally determined data.

Our results suggest that the biodegradation rate was controlled by fast-domain intra-aggregate mass transport. As previously discussed (*21*), the mechanism for fast-domain intra-aggregate mass transport may be rapid diffusion of aqueous-phase PAHs (either freely dissolved, or complexed with dissolved organic carbon, or DOC) through large water-filled pores in the sediment. Therefore, despite the low aqueous solubility and large carbon-normalized sediment/water partition coefficients (K_{oc}) reported for PAHs (*1*), macropore and mesopore diffusion may be a dominant mechanism controlling the biodegradation rate of PAHs in porous estuarine sediments during short time frames.

Extent of Bioavailability Is Controlled by Extent of Fast-Domain Desorption. Overall, the macropore and mesopore diffusion-limited biodegradation model (eq 8) accurately represents the ultimate extent of biodegradation measured at 5 days. In each case, the predicted extent of biodegradation was within a few percent of the measured extent of biodegradation (Figure 4). According to the model formulation, extent of desorption—and thus biodegradation—is constrained by the product of the starting PAH concentration in the sediment and the fraction *f* of PAHs in the fast-diffusion regime. The fraction in the fast-diffusion regime was the only parameter in the model that was not a readily obtainable parameter and was determined from desorption kinetics data as described previously (*21*).

The correlation between *f* and percent biodegraded was apparent for many PAHs in both whole sediments (Table 3). However, the correlation between *f* and percent biodegraded in the high- and low-density fractions of both sediments was not as apparent. This may be the result of inadvertent dilution of the pore water DOC content during repeated sediment washing necessary for density separation and removal of residual CsCl. Given the importance of DOC on apparent solubility of PAHs in these pore waters (*33*), and the importance of pore diffusion on PAH intra-aggregate mass transport (*21*), future studies investigating the mechanisms of desorption and bioavailability should be careful to avoid altering the quantity or quality of DOC, and should explicitly consider DOC concentration and PAH−DOC interactions.

In this study, rate and extent of biodegradation of field-aged PAHs in whole, weathered sediments was successfully predicted by a simple closed-form difference equation incorporating fast-domain macropore and mesopore diffusion. The model and experimental data indicate that biodegradation by *Mycobacterium* strain PC01 was limited by intra-aggregate desorption. Given that PAH biodegradation rates by strain PC01 are relatively slow compared to many aerobic PAH-degrading bacteria (*23*), intra-aggregate mass transport limitations to PAH biodegradation would likely be observed in similar experiments using other microorganisms. The model is fully defined by independently measured or calculated parameters, and can be easily evaluated using commercially available mathematical software packages. However, the model is applicable only for biodegradation in short time frames in well-mixed systems. In-situ biodegradation may proceed much more slowly, and bioavailability may be limited by other mechanisms. In addition, although all the parameters to capture rate of desorption are readily measured, the critical parameter of the model describing the fraction of total PAH content available for biodegradation, *f*, is more difficult to establish a priori. Although this fraction can be established by analysis of measured desorption kinetics, as we have done, and can also be approximated by the fraction of desorbable PAHs that are desorbed in the first 24 h, additional research on predicting the bioavailable fraction is needed.

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Literature Cited

(1) Mackay, D.; Shiu, W. Y.; Ma, K. C. *Illustrated Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals: Volume II Polynuclear Aromatic Hydrocarbons, Polychlorinated Dioxins, and Dibenzofurans*; Lewis Publishers: Chelsea, MI, 1992.
(2) Nash, R. G.; Woolson, E. A. *Science* **1967**, *157*, 924−927.
(3) Hatzinger, P. B.; Alexander, M. *Environ. Sci. Technol.* **1995**, *29*, 537−545.
(4) MacLeod, C. J. A.; Semple, K. T. *Environ. Sci. Technol.* **2000**, *34*, 4952−4957.
(5) Northcott, G. L.; Jones, K. C. *Environ. Sci. Technol.* **2001**, *35*, 1103−1110.
(6) Alexander, M. *Environ. Sci. Technol.* **2000**, *34*, 4259−4265.
(7) Rijnaarts, H. H. M.; Bachmann, A.; Jumelet, J. C.; Zehnder, A. J. B. *Environ. Sci. Technol.* **1990**, *24*, 1349−1354.
(8) Scow, K. M.; Alexander, M. *Soil Sci. Soc. Am. J.* **1992**, *56*, 128−134.
(9) Chung, G.-Y.; McCoy, B. J.; Scow, K. M. *Biotechnol. Bioeng.* **1993**, *41*, 625−632.
(10) White, J. C.; Alexander, M. *Environ. Toxicol. Chem.* **1996**, *15*, 1973−1978.
(11) Nam, K.; Alexander, M. *Environ. Sci. Technol.* **1998**, *32*, 71−74.
(12) Madsen, E. L.; Mann, C. L.; Bilotta, S. E. *Environ. Toxicol. Chem.* **1996**, *15*, 1876−1882.
(13) Calvillo, Y. M.; Alexander, M. *Appl. Microbiol. Biotechnol.* **1996**, *45*, 383−390.
(14) Guerin, W. F.; Boyd, S. A. *Appl. Environ. Microbiol.* **1992**, *58*, 1142−1152.
(15) Manilal, V. B.; Alexander, M. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 401−405.
(16) Ortega-Calvo, J.-J.; Alexander, M. *Appl. Environ. Microbiol.* **1994**, *60*, 2643−2646.
(17) Thomas, J. M.; Yordy, J. R.; Amador, J. A.; Alexander, M. *Appl. Environ. Microbiol.* **1986**, *52*, 290−296.
(18) Shor, L. M.; Kosson, D. S. In *Bioremediation*; Valdes, J. J., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000; pp 15−43.
(19) Karapanagioti, H. K.; Gossard, C. M.; Strevett, K. A.; Kolar, R. L.; Sabatini, D. A. *J. Contam. Hydrol.* **2001**, *48*, 1−21.
(20) Rockne, K. J.; Shor, L. M.; Young, L. Y.; Taghon, G. L.; Kosson, D. S. *Environ. Sci. Technol.* **2002**, *36*, 2636−2644.
(21) Shor, L. M.; Rockne, K. J.; Taghon, G. L.; Young, L. Y.; Kosson, D. S. *Environ. Sci. Technol.* **2002**, *37*, 1535−1544.
(22) Stanier, R. Y.; Palleroni, N. J.; Doudoroff, M. *J. Gen. Microbiol.* **1966**, *43*, 159−271.
(23) Liang, W. Ph.D. Dissertation, Rutgers, The State University of New Jersey, 2002.
(24) Rockne, K. J.; Liang, W. L.; Young, L. Y.; Taghon, G. L. *FEMS Microbiol. Ecol.* **2003**, *43*, 185−189.
(25) Rosenberg, M.; Gutnick, D.; Rosenberg, E. *FEMS Microbiol. Lett.* **1980**, *9*, 29−33.
(26) Crank, J. *The Mathematics of Diffusion*; Oxford University Press: Oxford, U.K., 1975.
(27) Rao, P. S. C.; Jessup, R. E.; Addiscott, T. M. *Soil Sci.* **1982**, *133*, 342−349.
(28) Pedit, J. A.; Miller, C. T. *Environ. Sci. Technol.* **1995**, *29*, 1766−1772.
(29) Wilke, C. R.; Chang, P. *AIChE J.* **1955**, *1*, 264−270.
(30) Millington, R. J.; Quirk, J. P. *Trans. Faraday Soc.* **1961**, *57*, 1200−1207.
(31) Ramaswami, A.; Luthy, R. G. *Environ. Sci. Technol.* **1997**, *31*, 2260−2267.
(32) Cornelissen, G.; Rigterink, H.; Ferdinandy, M. M. A.; van Noort, P. C. M. *Environ. Sci. Technol.* **1998**, *32*, 966−970.
(33) Shor, L. M. Ph.D. Dissertation, Rutgers, The State University of New Jersey, 2002.

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