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Chapter 3

Enhanced kinetics of solid-phase microextraction and biodegradation of polycyclic aromatic hydrocarbons in the presence of dissolved organic matter

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Abstract

The uptake kinetics of fluorene, phenanthrene, fluoranthene, pyrene, and benzo[*e*]pyrene by solid-phase microextraction fibers was studied in the presence of dissolved organic matter (DOM) obtained from sediment pore water and resulted in increased fiber absorption and desorption rate coefficients. Compared to the control without DOM, these rate coefficients were increased at a DOM concentration of 36.62 mg C/L by a factor of 1.27 to 2.21 and 1.31 to 2.10 for fluorene and benzo[*e*]pyrene, respectively. The calculated values for the fiber absorption and desorption rate coefficients show that diffusion through an unstirred boundary layer (UBL) surrounding the fiber probably forms the rate-limiting step of the process. The mineralization of aqueous-phase phenanthrene and pyrene by a representative polycyclic aromatic hydrocarbon (PAH)–degrading bacterium (*Mycobacterium gilvum* VM552) also was found to be enhanced by DOM. The initial degradation rates of phenanthrene (9.03 μg/L) and pyrene (1.96 μg/L) were significantly higher compared to the control values and were enhanced by a factor of 1.32 and 1.26 at a DOM concentration of 43.14 and 42.15 mg C/L, respectively. We suggest that such an enhancement results from the combination of faster uptake kinetics of the water-dissolved compounds in the UBL surrounding microbial cells and direct access of the bacteria to DOM-associated PAHs. These enhanced kinetic effects of DOM may have strong implications in sediment processes like desorption, nonequilibrium exposure, and biodegradation.

Introduction

Dissolved organic matter (DOM) in the aqueous phase (or pore water) of sediments polluted by hydrophobic organic contaminants (HOCs) can significantly affect the fate of these pollutants in the sediment bed. For example, DOM is able to enhance the apparent water solubility [1] and to promote the transport and desorption of HOCs in sediment pore waters by acting as a carrier [2, 3]. In addition, DOM generally is considered to decrease the bioavailability of HOCs, because their binding to DOM often results in a decrease of freely dissolved concentrations [4].

An effect of natural and synthetic DOM in enhancing the absorption kinetics (or diffusive conductivity) of HOCs toward passive samplers has been observed in a number of studies focused on HOC sorption to DOM and the determination of freely dissolved concentrations through solid-phase microextraction (SPME) [5–9]. This effect occurs when diffusion of HOCs through an unstirred boundary layer (UBL) is the rate-limiting step in the absorption by passive samplers [7]. In this case, absorption rate coefficients will be constant, and desorption rate coefficients will decrease, with an increase in hydrophobicity of HOCs [10, 11]. This effect has preferentially been called the diffusion layer effect to prevent confusion with other types of matrix effects (*e.g.*, fouling of passive samplers or salinity effects) [12]. Diffusion layer effects have been observed in SPME fiber absorption experiments that showed an increase in uptake of lindane and polychlorinated biphenyls (PCBs) as a function of protein concentration [7]. Furthermore, an enhancement of the kinetics of SPME was observed in humic acid (HA) solutions for fluoranthene [5] and for 2,5-dichlorophenol [6]. Other organic matrices also caused an enhanced SPME of 2,4,6-trichlorophenol with and without liposomes in a dialysis experiment [8], and bovine serum albumin caused the enhancement of octylphenol extraction [9]. The occurrence of a diffusion layer effect for 4-chloro-3-methylphenol, estradiol, PCB-77, and 4-quinolones in the presence of defined organic matrices (bovine serum albumin and HA from Sigma-Aldrich [referred to hereafter as Aldrich HA], Zwijndrecht, The Netherlands), however, was not observed in a number of SPME studies [12–15]. The absence of any effect can occur when the rate-limiting step is diffusion into the fiber rather than through the UBL [13, 15–17] or when DOM concentrations are relatively low [14]. The occurrence of this effect in the presence of DOM from sediment pore water remains relatively unexplored, possibly as a result of the complexities caused by the extraction procedure for DOM [18].

The enhanced diffusive flux of HOCs through UBLs caused by DOM was investigated by Mayer *et al*. [19, 20]. One of their studies [19] described the effect of Aldrich HA, sodium dodecyl sulphate, and hydroxypropyl-β-cyclodextrin on the diffusive mass transfer of fluoranthene between two silicone disks through a UBL of 100 μm. Relative conductivities for diffusive mass transfer were calculated from fitted rate constants, resulting in maximum relative conductivities (as compared to that of water) of 8, 39, and 34 for HA (4 g/L), sodium dodecyl sulphate (~10 g/L), and cyclodextrin (~140 g/L), respectively. In another study [20], the work was extended to 12 polycyclic aromatic hydrocarbons (PAHs), to different artificial and natural aqueous solutions, and to lower concentrations of the medium constituent. These authors

suggested that enhanced diffusive mass transfer through a UBL may increase uptake by sediment organisms in dynamic systems, such as those performing biodegradation. It may not, however, affect equilibrium partitioning phenomena.

A number of studies that focused mainly on humic fractions dissolved in the aqueous phase [21, 22], added to soils [23–27], and present in clay suspensions [28, 29] have found contrasting results for the effect of DOM on biodegradation of HOCs. Depending on the type of compound under study, these results show either suppressed degradation in the case of chlorinated phenolic compounds [21, 22] or enhanced degradation for PCBs [23] and PAHs [24–26, 28–31]. The addition of DOM to model or contaminated soils caused enhanced degradation of PCBs and PAHs, probably as a result of the enhanced desorption of these compounds from soils [23–26]. Other studies have shown no change in the degradation rate of PAHs in the presence of DOM [30, 32].

A few of these studies have addressed cell surface–related phenomena in explaining the effects of DOM on biodegradation of HOCs. A combination of HA (10–100 mg/L) extracted from soils and montmorillonite clay (1–10 g/L) stimulated the degradation of low concentrations (1 μg/ml) of water-dissolved phenanthrene by *Pseudomonas fluorescens*. Rates and extents of phenanthrene mineralization were significantly higher in the presence of HA sorbed to clay compared to suspensions that contained fulvic acid sorbed to clay. The addition of separate HA or clay did not, however, produce a significant stimulation under comparable conditions. The increased biodegradation was thought to be occurring by physical association of bacteria with HA and montmorillonite particles, therefore providing direct access to the sorbed carbon source [28]. Recently, the capability to degrade phenanthrene sorbed to Aldrich HA was used to select for phenanthrene-degrading strains from soil of coal-gasification plants [32]. No differences were observed in these strains between the extent and rate of mineralization of nonsorbed phenanthrene and HA-sorbed phenanthrene at levels above its aqueous solubility, indicating that sorption by HA did not significantly slow the mineralization process. Isolates obtained via the conventional enrichment with solid phenanthrene were not able to degrade HA-sorbed phenanthrene. Vacca *et al*. [32] hypothesized that uptake by competent strains occurred from a layer of surfactant micelles that formed on the cell surface.

The present research is an integrated study regarding the effect of DOM on the kinetics of SPME and mineralization of PAHs initially equilibrated in DOM solutions. We employed natural DOM preparations obtained from sediment pore water through a procedure that minimized the disturbance of their components. The uptake kinetics by SPME fibers and mineralization by a representative PAH-degrading bacterium (*Mycobacterium gilvum* VM552) were followed in the presence of DOM under controlled conditions. Our main objectives were to determine whether DOM affects the kinetics of fiber uptake and mineralization and, if so, to determine the possible mechanisms involved.

Materials and methods

Chemicals

Fluorene, phenanthrene, fluoranthene, pyrene, benzo[*e*]pyrene, and sodium azide (NaN_3) were purchased from Sigma-Aldrich. The radiolabeled PAHs [9-^{14}C]phenanthrene and [4,5,9,10-^{14}C]pyrene (radiochemical purity, >98%; specific activity, 13.3 and 58.7 mCi/mmol, respectively) also were obtained from Sigma-Aldrich. Salts used for medium preparation were obtained from Merck (Darmstadt, Germany) or Panreac (Barcelona, Spain), and solvents (analysis quality) were obtained from Rathburn (Walkerburn, Scotland) or Biosolve (Valkenswaard, The Netherlands).

Extraction of DOM from sediment

Sediments were sampled at three different locations: Two freshwater lakes in eastern Finland (Lake Kontiolampi [KON] and Lake Mekrijärvi [MEK]), and the Rhine River sedimentation area in The Netherlands (Lake Ketelmeer [KET]). The sediments from KON (62º43'46"N, 29º51'18"E), MEK (62º45'60"N, 30º57'42"E), and KET (52º36'N, 5º45'E) have organic carbon contents of 55.3, 21.03, and 5.51% dry weight, respectively. The small, brown-water KON was chosen because DOM from this lake efficiently reduces the bioavailability of HOCs [4]. Characteristics of MEK and KET sediments have been published elsewhere [33]. The sediments were stored in the dark at 4ºC before use.

Extraction of DOM from sediments was performed by shaking wet sediment and medium at a sediment to water ratio of 1:2 (w/w) for KON and 4:1 (w/w) for MEK and KET for 1 h on a rotary shaker operating at 200 rpm. For the extraction of DOM from KON, medium of low ionic strength (pH 7) was used that contained the following salts: $CaCl_2 \cdot 2H_2O$ (58.8 mg/L), $MgSO_4 \cdot 7H_2O$ (24.7 mg/L), $NaHCO_3$ (13.0 mg/L), and KCl (1.2 mg/L) dissolved in Nanopure water (18.3 MΩ; Barnstead International, Dubuque, Iowa, USA). Sodium azide was added in a concentration of 65.0 mg/L to prevent microbial growth in sterile treatments [34]. The sediments of MEK and KET were extracted with medium that also was used in mineralization experiments (pH 5.8; composition described below). The suspension was centrifuged for 1 h at 7,400 *g* to separate the aqueous and solid phases. Subsequently, the supernatant was centrifuged for 3 h at 31,000 *g* to obtain a solution that includes the organic matter that potentially is present in sediment pore water. The bulk DOM solutions were diluted to obtain duplicate series with a total volume of 50 ml. Medium without DOM was used as a control in all measurements. The procedure of centrifugation is preferred here to the conventional procedure of filtration over 0.45 μm, because filtration artefacts occurred as a result of selective removal of aromatic components of DOM by membrane fouling. The pH of the DOM solutions was adjusted to pH 7 for experiments employing SPME fibers (KON) and to pH 5.8 for mineralization experiments (MEK and KET). The pH remained constant for the duration of the experiments. Concentrations of DOM were estimated as dissolved organic carbon determined by high-temperature

combustion with either a TOC-V Combustion Standalone High Sensitivity or a TOC-5000 (Shimadzu Benelux, 's-Hertogenbosch, The Netherlands).

Effect of DOM on the kinetics of SPME

Coated silicon fibers with a core diameter of 110 μm and a coating of 28.5 μm of polydimethylsiloxane (PDMS; 12.4 μl/m) were cut into pieces of 2 cm and thermally cleaned under a He flow (±10 ml/min) for 2 h at 250ºC. Duplicate fibers were exposed to 50 ml of control or DOM solutions in 50-ml flasks provided with Teflon®-lined stoppers. The solutions were spiked with 20 μl of an acetonitrile solution that contained a mixture of fluorene (final concentration, 12.70 μg/L), phenanthrene (25.69 μg/L), fluoranthene (9.56 μg/L), pyrene (5.15 μg/L), and benzo[*e*]pyrene (1.66 μg/L). Samples were shaken in the dark on a rotary shaker (80 rpm) kept at 20ºC, and after specific time intervals, the fibers were sampled and gently blotted dry with a tissue. The PAHs were desorbed from the fibers in 150 μl of an acetonitrile solution containing 500 μg/L of benzo[*a*]anthracene as injection standard. The PAHs were analyzed with reversed-phase, high-performance liquid chromatography by injecting 1 μl on a Lichrospher column (length, 125 mm; inner diameter, 2.0 mm; RP18; Phenomenex, Torrance, CA, USA) with 3-μm pore size maintained at a temperature of 36ºC. The flow composition was held constant at 45% water and 55% acetonitrile (0.40 ml/min), and the PAHs were detected by fluorescence. The excitation/emission wavelengths were 280/330, 250/385, 288/462, 335/383, 284/399, and 283/394 nm for fluorene, phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene, and benzo[*e*]pyrene, respectively. Chromatograms were analyzed using ChromStar (Ver 5.03; SCPA, Weyhe-Leeste, Germany). Solutions containing known concentrations of PAHs served as calibration standards.

In the system of water and fiber, a two-compartment model with first-order kinetics was assumed [13]:

$$c_{\mathrm{PDMS},t} = \frac{k_1}{k_1 \frac{V_{\mathrm{PDMS}}}{V_{\mathrm{water}}} + k_2} c_{\mathrm{total},0} \left(1 - \mathrm{e}^{-[k_1 (V_{\mathrm{PDMS}}/V_{\mathrm{water}}) + k_2]t}\right)$$

where $c_{\mathrm{PDMS},t}$ is the PDMS fiber concentration at time t; $c_{\mathrm{total},0}$ is the total aqueous PAH concentration at $t = 0$; V_{PDMS} and V_{water} are the PDMS fiber and water volumes, respectively; and k_1 and k_2 are the absorption and desorption rate coefficients, respectively, of PAHs from water to fiber and vice versa. At different DOM concentrations, the initial total concentration available for absorption by the fiber at $t = 0$ ($c_{\mathrm{total},0}$) was assumed to be the sum of fiber and freely dissolved concentrations determined at the last sampling time (t = 503.1 h). Fiber concentrations were calculated from calibration solutions, and freely dissolved concentrations were calculated by dividing fiber concentrations by the PDMS to water partition coefficient fitted ($K_{\mathrm{PDMS}} = k_1/k_2$)

when no DOM was present. The parameters (k_1 and k_2) were fitted with nonlinear regression performed using GraphPad Prism (Ver 3.02; GraphPad Software, San Diego, CA, USA).

Bacteria, media, and cultivation

Cultures of a PAH-degrading bacterium (*M. gilvum* VM552 [35]) were incubated on a rotary shaker (300 rpm at 30ºC) with crystalline pyrene (2.5 g/L) as a sole source of carbon and energy. The growth medium with pH 7 contained the salts K_2HPO_4 (3.244 g/L), $NaH_2PO_4 \cdot H_2O$ (1.0 g/L), and NH_4Cl (2.0 g/L) as well as the micronutrients $MgSO_4 \cdot 7H_2O$ (0.2 g/L), $FeSO_4 \cdot 7H_2O$ (0.012 g/L), $MnSO_4 \cdot H_2O$ (0.003 g/L), $ZnSO_4 \cdot 7H_2O$ (0.003 g/L), $CoSO_4 \cdot 7H_2O$ (0.001 g/L), and nitrilotriacetic acid (0.1 g/L). After approximately 10 d, crystalline pyrene was filtered out of the incubated solution, and the culture was shaken overnight to degrade the residual pyrene. Subsequently, bacteria were concentrated by centrifugation (20 min at 8,000 rpm) and washed twice with fresh medium. After centrifugation, the pellet was resuspended in 10 to 15 ml of medium before inoculation of 0.5 ml into the experimental system (final density, $2.2–2.5 \times 10^6$ bacteria/ml).

Mineralization experiments

To measure mineralization of PAHs, experiments were performed with sterile CO_2-evading medium buffered to pH 5.8 and prepared as the above-described growth medium, with the exception that the concentrations of K_2HPO_4 and $NaH_2PO_4 \cdot H_2O$ were 0.495 and 3.7 g/L, respectively. Flasks of 100 ml were filled with 50 ml of medium or DOM from MEK or KET and spiked with 20 µl of an acetone solution containing ^{14}C-labeled phenanthrene (final concentration 9.03 µg/L) or pyrene (1.96 µg/L). The solutions were allowed to equilibrate in the dark on a rotary shaker (80 rpm) at room temperature (±17ºC) for 1 d. Before mineralization experiments with DOM from MEK, fibers also were added to the DOM and control solutions. The ^{14}C-labeled PAHs were desorbed from the fibers in a vial containing 200 µl of toluene. Subsequently, the content of the vial was shaken with 5 ml of scintillation cocktail (Ready Organic™; Beckman Instruments, Fullerton, CA, USA) and measured by liquid scintillation counting (model LS6500; Beckman Instruments). Freely dissolved concentrations were calculated as described above. Because of the reduced volume of the fibers, this procedure leads to the removal of a minimal amount (5.0–10.3% for pyrene) of PAHs before addition of the bacteria.

After inoculation, the flasks were sealed with a Teflon-lined stopper and incubated on a rotary shaker at 80 rpm. Mineralization of PAHs was monitored by $^{14}CO_2$ production appearing in an alkali trap suspended from the stopper containing 0.5 M NaOH. At specific time intervals, the NaOH solution was sampled and shaken with 5 ml of scintillation cocktail (Ready Safe™; Beckman Instruments), and fresh NaOH solution was directly added into the alkali trap. The samples were placed in the dark for approximately 8 h to dissipate chemiluminescence, and radioactivity was measured by liquid scintillation counting. Total concentrations of parallel treatments containing nonlabeled PAHs were measured by liquid–liquid extraction with pentane

and high-performance liquid chromatography at the beginning and end of the biodegradation experiments to calculate the residual amount after degradation (only for MEK).

Mass balances were calculated at the end of the experimental period by adding the percentage of ^{14}C mineralized to the percentages of ^{14}C recovered directly from the suspensions. It is not expected that photochemical oxidation of DOM or PAHs occurred, because the experiments were performed in the dark. Also, the cultures were not deprived of nutrients during the relatively fast degradation experiments. The continuous-shaking conditions guaranteed the homogenization of the suspensions and prevented the aggregation of DOM and bacterial cells, as evidenced by macroscopic observations.

Results and discussion

Effect of DOM on absorption of PAHs into PDMS fibers

The absorption of pyrene into fibers during the first 100 h became faster in the presence of increasing DOM concentrations, resulting in lower fiber as well as freely dissolved concentrations and earlier establishment of equilibrium between fiber and water (Fig. 1). Absorption and desorption rate coefficients were calculated with the above equation for fluorene, phenanthrene, fluoranthene, pyrene, and benzo[*e*]pyrene (Table 1), in which the total initial concentration was assumed to be the sum of the fiber and freely dissolved concentrations as determined at the last sampling time. The ratios of these rate coefficients show constant PDMS to water partition coefficients (K_{PDMS}) (Table 1). At a DOM concentration of 36.62 mg/L, k_1 and k_2 are enhanced by a factor of 1.27 to 2.21 and 1.31 to 2.10 for fluorene and benzo[*e*]pyrene, respectively, compared to the control. This effect is more pronounced for higher DOM concentrations and increasing hydrophobicity of the PAHs. No significant correlation, however, could be found for k_1 and k_2 as a function of either the freely dissolved or DOM-sorbed fractions.

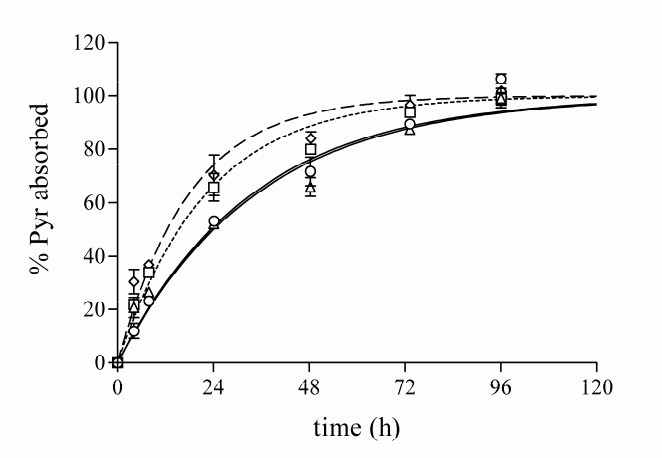

Figure 1. Percentages of pyrene (Pyr) absorbed to solid-phase microextraction fibers at increasing dissolved organic matter concentrations (○ control; △ 9.15 mg C/L; □ 18.31 mg C/L; ◊ 36.62 mg C/L) fitted with a two-compartment model. Dissolved organic matter was extracted from Lake Kontiolampi (Finland) sediment.

Table 1 shows that in the absence of DOM, fiber absorption rate coefficients (k_1) are nearly constant, and fiber desorption (k_2) rate coefficients are decreasing, when the PDMS partition coefficient (K_{PDMS}) becomes higher. This suggests that sorption of PAHs to the fiber is controlled by diffusion through the UBL [10, 11, 36] and could be affected by the presence of DOM, as evidenced by higher k_1 and k_2 at similar log K_{PDMS}. The effect of a matrix in solution (*e.g.*, HA, proteins, etc.) on the absorption kinetics of a polymeric phase has been shown previously [7, 12]. Our results extend those findings by indicating that faster kinetics of SPME can occur with natural DOM samples also containing DOM fractions other than the humic and protein fractions.

The matrix affects the extraction process by increasing the diffusive conductivity of the UBL around the SPME fiber, thus increasing k_1 and k_2 and decreasing the equilibration time. The effect occurs when the rate-limiting step is diffusion into the UBL surrounding the fiber and desorption from the matrix is fast compared to diffusion through the UBL. Because compounds are depleted in the UBL by absorption into the SPME fiber, desorption of compounds from the matrix will follow the imposed concentration gradient of the compound between fiber and UBL. Subsequently, desorption is driven by the relative amount of sorbing phase or matrix in the UBL and the hydrophobicity or complexing ability of the compounds to the matrix [7, 9].

Table 1. Concentrations of dissolved organic matter ([DOM]) and polycyclic aromatic hydrocarbons of fiber-exposed solutions (c_{free}) and calculated values of absorption and desorption rate coefficients (k_1 and k_2, respectively) and logarithmic values of the fiber to water partition coefficient (log K_{PDMS}) for fluorene, phenanthrene, fluoranthene, pyrene, and benzo[*e*]pyrene in the presence and absence of DOM extracted from Lake Kontiolampi (Finland) sediment[a]

Compound	[DOM][a] (mg C/L)	c_{free} (µg/L)[b]	k_1 (1/h)	k_2 (1/h)	log K_{PDMS}[c]
Fluorene	0.00	11.00	746.7±46.99	0.1871±0.0126	3.601±0.040
	9.15	9.57	952.8±59.08	0.2406±0.0158	3.598±0.039
	18.31	8.48	956.2±43.89	0.2392±0.0116	3.602±0.029
	36.62	7.41	950.9±59.65	0.2450±0.0163	3.589±0.040
Phenanthrene	0.00	22.10	774.7±40.60	0.1081±0.0060	3.855±0.022
	9.15	16.03	879.6±53.84	0.1272±0.0083	3.840±0.026
	18.31	12.49	1002±31.15	0.1441±0.0048	3.842±0.014
	36.62	9.05	1048±51.20	0.1515±0.0079	3.840±0.021
Fluoranthene	0.00	9.54	586.1±46.45	0.0327±0.0030	4.253±0.047
	9.15	3.23	637.2±59.10	0.0351±0.0038	4.259±0.053
	18.31	2.12	899.3±82.94	0.0524±0.0054	4.235±0.042
	36.62	1.23	1440±180.4	0.0805±0.0109	4.253±0.054
Pyrene	0.00	4.49	599.7±41.34	0.0259±0.0021	4.364±0.031
	9.15	1.40	592.5±45.90	0.0268±0.0025	4.345±0.034
	18.31	0.84	896.3±56.17	0.0408±0.0029	4.342±0.027
	36.62	0.49	1109±90.87	0.0506±0.0047	4.341±0.035
Benzo[*e*]pyrene	0.00	1.16	640.0±78.98	0.0051±0.0010	5.099±0.099
	9.15	0.03	823.3±71.92	0.0067±0.0009	5.093±0.069
	18.31	0.02	1082±84.06	0.0087±0.0010	5.094±0.060
	36.62	0.01	1415±130.4	0.0107±0.0014	5.123±0.071

[a] Values for k_1, k_2, and log K_{PDMS} are presented as the mean ± standard error; [b] $c_{free} = c_{PDMS}/K_{PDMS}$, where K_{PDMS} is calculated using a two-compartment model without DOM. PDMS = polydimethylsiloxane; [c] $K_{PDMS} = k_1/k_2$.

Diffusive exchange of organic compounds occurs mostly at boundaries in many environmental processes under nonequilibrium conditions. In the present study, DOM has been shown to be important in absorption by SPME fibers of PAHs. Transport of PAHs through the pore water of sediments can be facilitated by DOM [2]. Also, it has been suggested that the presence of DOM in pore water might be responsible for enhancing desorption of PAHs from sediment. In sediment column experiments, an enhanced desorption occurred for anthracene and benzo[*a*]anthracene when passing river and soil HA through columns with low organic carbon sediments [2]. The more hydrophobic benzo[*a*]anthracene and soil HA showed the largest effect, implying that both hydrophobicity of sorbate and complexing ability of sorbent play a key role in desorption over boundary layers. Therefore, processes like desorption can be enhanced when diffusion occurs at an aqueous boundary layer and the relative proportion of DOM-bound PAHs is high.

Effect of DOM on mineralization of PAHs

The mineralization curves for 1.96 μg/L of pyrene at 0, 10.8, and 43.1 mg C/L of DOM from MEK sediment are shown in Fig. 2. Before inoculation with pyrene-degrading bacteria, SPME fibers were added to the solutions and sampled after an equilibration time of 10 d. The measurement of freely dissolved pyrene concentrations yielded values of 1.71, 1.20, and 0.63 μg/L at 0, 10.8, and 43.1 mg C/L of DOM, respectively. Mineralization of pyrene occurred rapidly after inoculation (in <12 h) and at a higher rate when DOM was present (Fig. 2). In particular, DOM induced statistically significant differences in the initial rates of mineralization (0.209 and 0.187 ng/ml/h) in the first 2 h at 10.8 and 43.1 mg C/L, respectively (Table 2), compared to the control (0.148 ng/ml/h), but not in the final extents of degradation. The lower DOM concentration of 10.8 mg C/L even resulted in a higher mineralization rate than the higher DOM concentration of 43.1 mg C/L. These results suggest that pyrene mineralization in the presence of DOM was not controlled by the freely dissolved concentration initially measured. Another mineralization experiment was performed in flasks containing 3.17 μg/L of [^{12}C]pyrene, and the same concentrations of DOM from MEK as described above were incubated under similar conditions to determine possible differences in the residual pyrene concentrations when the mineralization plateau (50 h) was reached. Final concentrations measured in these nonlabeled treatments were not statistically different, independent of the presence of DOM. The low average concentration measured (0.13 μg/L) confirmed that the fraction of pyrene biodegraded in the presence of DOM included the amount initially present in the aqueous phase as a result of the sorption equilibrium, but also a major part of the compound sorbed initially to DOM.

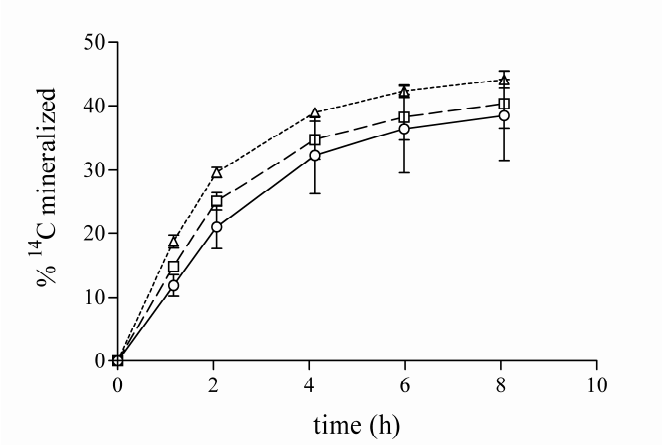

Figure 2. Mineralization of pyrene over time, expressed as cumulative percentage of ^{14}C mineralized, at increasing dissolved organic matter concentrations (○ Control; △ 10.8 mg C/L; □ 43.1 mg C/L). Results are given as the average ± standard deviation as calculated from duplicate samples. Dissolved organic matter was extracted from Lake Mekrijärvi (Finland) sediment.

Experiments performed with DOM from KET also showed a significant enhancement of maximum mineralization rates for 9.03 µg/L of phenanthrene at 10.54, 21.08, and 42.15 mg C/L of DOM compared to the control (Table 2). Differences in extents were only evident with the highest DOM concentration used (42.15 mg C/L). Initial mineralization rates and extents of pyrene (1.96 µg/L) often were higher than the control at the same DOM concentrations, but the differences were not statistically significant (Table 2). No significant compound losses occurred during the course of the mineralization experiments, as determined by mass balances calculated at the end of the experimental period (Table 2). The losses in treatments containing DOM were not statistically different compared to the control. No residual concentrations of nonlabeled compounds were determined in these experiments with DOM from KET.

Table 2. Rates and extents of mineralization of phenanthrene and pyrene in the presence and absence of dissolved organic matter (DOM) extracted from Lake Mekrijärvi (MEK; Finland) and Lake Ketelmeer (KET; The Netherlands) sediments

Treatment[a]	[DOM] (mg C/L)	Max rate (ng/ml/h)[b]	n[c]	Extent (%)[d]	Mass balance (%)[e]
MEK – Pyr	0.00	0.148 ± 0.005	4	41.3	72.2
	10.79	*0.209 ± 0.009*	4	46.9	80.7
	43.14	*0.187 ± 0.004*	4	42.9	79.2
KET – Phe	0.00	0.208 ± 0.009	6	> 20.0	91.2
	10.54	*0.275 ± 0.020*	6	> 24.6	100.5
	21.08	*0.257 ± 0.017*	6	> 19.6	91.1
	42.15	*0.275 ± 0.016*	6	> 29.6	95.0
KET – Pyr	0.00	0.164 ± 0.020	6	42.1	64.7
	10.54	0.179 ± 0.015	6	38.9	63.1
	21.08	0.210 ± 0.009	6	49.9	74.2
	42.15	0.194 ± 0.011	6	48.1	74.2

[a] Pyr and Phe denote treatments with pyrene (1.96 µg/L) and phenanthrene (9.03 µg/L), respectively;
[b] Max. rate = Maximum rate of mineralization. Values are presented as the mean ± standard error as determined by linear regression, and values in italics are significantly different from the control value ($p < 0.05$);
[c] n = number of data points (time points in duplicate) used in determining the maximum rate of mineralization;
[d] Extent = extent of mineralization, presented as a percentage of ^{14}C mineralized at the end of the experiment;
[e] Calculated as the cumulative amount recovered divided by the total amount added initially, expressed as a percentage.

The observed enhancement of PAH biodegradation by DOM could not be explained through an enhancement of PAH solubility [27], because under the conditions used, all PAHs were present at concentrations lower than their maximum water solubility. Obviously, direct access to sorbed PAHs by bacteria attached to surfaces, which has explained previous observations regarding enhancement of biodegradation by mineral-associated HAs [28, 29], also can be excluded, because the procedure for DOM isolation eliminated the colloidal-associated surfaces. It is possible that the kinetics of uptake by bacterial cells of the freely dissolved compound was enhanced in the presence of DOM in a way analogous to that observed during the present study at similar DOM concentrations for the absorption of PAHs into PDMS fibers. Studies concerning the influence of diffusion on substrate uptake by phytoplankton [37] and bacteria [38, 39] have determined that microbial cells often experience reduced substrate concentrations at their immediate surfaces as a result of the differences in the rate of uptake and metabolism and the rate of mass transfer or diffusion through the UBL surrounding the cells. The thickness of this UBL, in which the substrate concentration is 90% of the ambient concentration or less, is approximately 10 µm for bacterial cells of 1 µm in diameter, assuming that their shape is spherical [38–40]. It is difficult to attribute the observed enhancement of net mineralization rates solely to increased uptake kinetics in the UBL, however, given the significant reduction in freely dissolved pyrene concentrations caused by DOM (as evidenced by SPME). The mechanism of the enhancing effects of DOM on the transformation can be better understood by postulating a direct access to DOM-sorbed pyrene by bacterial cells. The precise mechanism is unknown, but it may involve a direct contact of cell surfaces with DOM components, thus causing an

increased concentration of PAHs in the vicinity of bacterial cells, in a way analogous to what has been observed for the biodegradation of phenanthrene sorbed to humic fractions [28, 32].

Conclusion

We observed enhanced absorption kinetics of PAHs into SPME fibers and mineralization of these HOCs by bacteria in the presence of environmentally relevant concentrations of DOM obtained from sediment pore water. These two effects may be explained by different mechanisms: Whereas the faster uptake during SPME can be attributed to an increased diffusive conductivity in the UBL surrounding the fibers, the enhancement in net biodegradation rates can be explained by the combination of faster uptake kinetics of the freely dissolved PAHs in the UBL surrounding the cells and direct microbial access to DOM-associated PAHs. These processes may have strong environmental implications, such as in processes involving desorption, nonequilibrium exposure, and biodegradation.

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