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Enhanced Mobilization of Field Contaminated Soil-bound PAHs to the Aqueous Phase under Anaerobic Conditions

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Abstract Although microbially-mediated redox environments can alter the characteristics of soil/sediment organic matter (SOM) and its interactions with persistent hydrophobic organic contaminants (HOCs) bound to soils and sediments, the nature of their effects has not been adequately addressed. In this study, a field soil collected from a manufacturer gas plant site and contaminated historically with creosotes was incubated under aerobic and anoxic/anaerobic conditions along with various amendments (extra carbon and enrichment minerals) for stimulating microbial activities. Anaerobic conditions stimulated significant fractions of bound polycyclic aromatic hydrocarbons (PAHs) encompassing naphthalene through benzo[g,h,i]perylene to be mobilized to the aqueous phase, leaving their aqueous phase concentrations far in excess of solubility (increases in their apparent aqueous phase concentrations by factors as high as 62.8 relative to their initial aqueous phase concentrations). Such effects became more evident for high molecular weight PAHs. Dissolved organic matter exhibiting a high affinity for PAHs was liberated from soils during the anaerobic soil incubations. Feasibility of this concept for field applications was evaluated with a lab-scale continuous flow system composed of an anaerobic soil column followed by an aerobic bioreactor inoculated with PAH-degrading microbes. High quantities of PAHs exceeding their aqueous solubilities were eluted from the anaerobic soil column and those mobilized PAHs were readily bioavailable in the secondary aerobic bioreactor. This study may offer a potential method for cost-effective and performance-efficient ex situ remediation technologies (or in situ if appropriate hydrological control available in the contaminated field site) and risk assessment for the HOC-contaminated soils/sediments.

Keywords Soil/sediment organic matter · PAH mobilization · Anaerobic soil incubation · Redox condition

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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are representative toxic, carcinogenic, and persistent hydrophobic organic contaminants (HOCs) found ubiquitously in geo-environments (Menzie et al. 1992; Mueller et al. 1989). These compounds reach soils or sediments via accidental spillage of creosote, coal tar, crude oil, petroleum products, incomplete combustion emission, industrial discharges, and/or natural release (volcanoes,

forest fires, etc., Mueller et al. 1996). One of the biggest concerns in remediation practices for PAH-contaminated geo-environments is very low bioavailabilities of PAHs given by their highly hydrophobic character and low aqueous solubilities and the high sorption capacities that most soils and sediments exhibit for such contaminants (Kim and Weber 2005; Ehlers and Luthy 2003; Stucki and Alexander 1997).

The interaction of PAHs with soil or sediment components typically plays a primary role in determining their overall bioavailability. In particular, PAH bindings with soil organic matter (SOM) determine their aqueous and solid (soil and sediment) phase concentrations so that they have significant impacts on reactivity, transport, and ultimate fate of PAHs in the saturated subsurface (Chiou et al. 1986; Cho et al. 2002; Gamst et al. 2007; Johnson and Amy 1995; Mackay and Gschwend 2001; Rutherford et al. 1992). Similarly, other studies (Gauthier et al. 1987; Grathwohl 1990) have demonstrated that variations in PAH-SOM binding characteristics can be envisioned with physico-chemical properties of SOM or geological background of SOM origin (i.e., diagenetic history of SOM).

Most previous studies related to HOC-SOM interactions have, in fact, been carried out under the controlled environments (e.g., microbial activity-controlled conditions, per se, microbially-inactive conditions), which cannot realistically describe the field conditions where indigenous geo-microbes are alive. Microorganisms are known to actively participate in SOM evolution and primarily control the redox condition of geo-environments (Aiken et al. 1985; Almendros and Dorado 1999; Coates et al. 2000; Filip and Alberts 1994; Hertkorn et al. 2002; Kalbitz et al. 2003; Krom and Sholkovitz 1977). Only several studies have investigated the microbial impacts on SOM properties and resulting HOC-SOM interactions. Pravecek et al. (2005) discovered that mobilization of bound PAHs from the aged soils to the aqueous phase can be stimulated under anaerobic conditions. Under elevated pH/reduced conditions created by reduction of sulfate as an anaerobic final electron acceptor and subsequent hydrogen ion consumption, more dissolution (release) of SOM occurred, resulting in enhanced binding of PAHs with the dissolved organic matter (DOM). Kim and Pfaender (2005) also demonstrated that the physicochemical properties of SOM can be modified in response to microbially-mediated redox conditions and the DOM released from soil incubated under anaerobic conditions became large, more condensed, and highly aromatic, exhibiting high sorption capacity for PAHs.

In this study we expand the aforementioned findings with respect to PAH mobilization from the field-contaminated soil to the associated aqueous phase. Thus, we examined our hypothesis that imposing anaerobic conditions on contaminated soils stimulate the release of bound PAHs to the aqueous phase and make them bioavailable for degradation. A field soil that has been highly contaminated with various HOCs and creosote for many years was incubated in batch type microcosms under anaerobic conditions and the behaviors of PAHs as well as characteristic changes of SOM were monitored. In a subsequent experiment, the mobilization of PAHs from the contaminated soil and the bioavailability of the mobilized PAHs were assessed using a continuous flow system consisting of an anaerobic soil column and a secondary aerobic bioreactor. This investigation is expected to provide significant implications for the fate and transport of bound HOCs in contaminated subsurface systems by stressing roles of geo-microorganisms in association with the distribution of such contaminants. It is also our expectation that this concept can be used to establish a cost-effective and new remediation technique for the saturated contaminated sites of which redox environments are commonly known anaerobic.

2 Materials and Methods

2.1 Materials

The soil employed in this study was collected from a depth of 15 cm at a manufactured gas plant field site in Kentucky, USA (referred to as KY soil) that has been contaminated for more than 50 years with wood-treating wastes containing high levels of PAHs. This sandy loam soil consisting of 67% of sand, 30% of silt, and 3% of clay (by weight) contained 1.97% of organic carbon, 0.51% of nitrogen, and 1.64% of hydrogen (by weight, determined by Huffman Laboratories, Golden, CO, USA). A mixture of the 16 US EPA Priority Pollutant PAHs employed as a standard for our PAH analysis was purchased from Sigma-Aldrich (Supelco EPA 610 PAH mixture, St. Louis, MO, USA).

Dichloromethane, methanol, and acetonitrile (high pressure liquid chromatography (HPLC) analysis grade, purity >99%) were purchased from Fisher Scientific (Chicago, IL, USA). An inorganic mineral (IM) medium was used as microbial enrichment medium, consisting of 6 g of $Na_2HPO_4(3H_2O)$, 3 g of KH_2PO_4, 0.25 g of $MgSO_4(7H_2O)$, 0.5 g of NaCl, 1 g of NH_4Cl, and 0.01 g of $CaCl_2(2H_2O)$, and 1 ml of trace mineral nutrient stock solution per liter of distilled water at a pH of 7. The trace mineral nutrient stock solution contained 1 g of $FeSO_4(7H_2O)$, 1 g of $MnSO_4(H_2O)$, 0.25 g of $(NH_4)_6Mo_7O_2(4H_2O)$, 0.25 g of $CuCl_2(2H_2O)$, 0.25 g of $ZnCl_2$, 0.1 g of NH_4VO_3, 2.5 g of $Ca(NO_3)_2$, 1 g of $C_6H_9NO_6$, and 0.25 g of $CoCl_2$ per liter of distilled water. The mineral chemicals used in the IM medium were supplied from Sigma-Aldrich. The IM medium used for anaerobic soil incubations was sterilized by autoclaving at 121°C and 15 psi for 30 min and then cooled down by pre-purging with $N_{2\ (g)}$ aseptically using a sterilized 0.2-mm filter (Millipore, Burlington, MA, USA) overnight to remove oxygen prior to use.

2.2 Soil Incubation in Batch Type Microcosms

The KY soil was incubated for 100 days under aerobic and anaerobic conditions in 25-ml borosilicate microcosm serum bottles (no additional microbial inoculation) at 25±0.5°C in the dark. Each microcosm received 5 g of KY soil and 10 ml of IM medium. The KY soil was passed through 2-mm sieve to remove plant debris. The IM medium was supplemented with sugar-based Molasses (0.3% by volume, 1 ml≅1 mg of sugar, purchased from a local grocery) as a carbon additive to stimulate anaerobic microbial growth and sterilized by autoclaving prior to use. Molasses has been used as a co-substrate for the biodegradation of soil-bound 2,4,6-trinitrophenylmethylnitramine in the previous study (Boopathy and Manning 1998). The soils and media were loaded into microcosms in an anaerobic glove chamber (Coy Laboratory Incorporation, Great Lake, MI, USA) filled with 96% of N_2 and 4% of H_2 (by volume). The aerobic microcosms were capped with sponge stoppers and purged with water-saturated air once in a day to maintain aerobic conditions. The microcosms used for anaerobic incubations were capped with butyl rubber stoppers and aluminum crimp tops. To promote activities of anaerobic microbes that use nitrate and sulfate as their final electron acceptors, anaerobic microcosms were amended with 0.5 mM of $NaNO_3$ and 0.5 mM of Na_2SO_4, respectively. The anaerobic gas mixtures (N_2:$CO_{2\ (g)}$ and H_2:$CO_{2\ (g)}$, a ratio of 8:2 by volume) were injected to the anaerobic microcosms (initial pressure of 14.5±0.5 psi) aseptically to evaluate the effect of anaerobic headspace gases. The microcosm setup is summarized in Table 1. Since the sterilization of soil by thermal or radiation processes or chemical inhibitors can result in unexpected and significant changes in physical and chemical properties of SOM, aerobic microcosms were employed as an incubation control for the anaerobic incubations instead of abiotic control. Three replicate microcosms for each incubation set were sacrificed for sampling at day 1, 50, and 100, respectively. At each time point, each microcosm was centrifuged at 1,500×*g* for 30 min and the supernatant decanted and passed through a glass fiber membrane (0.2–0.6 μm cutoff, Millipore). The DOM released from the soil was analyzed quantitatively and qualitatively as described in later section (Section 2.4). The aqueous phase PAH concentrations of the filtrates were determined by HPLC. Among the 16 US EPA Priority Pollutant PAHs, 14 PAHs were selectively monitored in this study since acenaphthylene and indeno[1,2,3-c,d] pyrene were not detected by our analytical system. Initial levels of PAHs in the KY soil are provided in Table 2. The headspace gas pressure, oxidation-reduction potential (ORP), and pH were also monitored during incubations.

2.3 Continuous Flow System

A continuous flow system composed of an anaerobic soil column and an aerobic bioreactor was operated to evaluate the liberation of PAHs bound to the KY soil and bioavailability of the liberated PAHs. A schematic diagram of this system is provided in Fig. 1. The first-stage anaerobic soil column consisted of a cylindrical borosilicate glass column (inner diameter of 5.7 and 65.8 cm of effective bed length, equipped with a water jacket). The column temperature was controlled at 25±0.5°C by a water bath circulator (Fisher Scientific). Prior to the soil column operation, the KY soil (passed through 2-mm sieve) was added to the column in a stepwise way (repetition of 100 g of soil addition and rigorous hand-shaking of column) to ensure the homogenous soil packing. At each time of soil loadings, approximately 100 ml of sterilized IM

Table 1 Summary of microcosm setup

Microcosm	Final electron acceptor process	Headspace gas
Microcosm 1	Oxygen (aerobic)	Air
Microcosm 2	Nitrate (nitrate-reducing)	N_2/CO_2 (8:2 *v*/*v*)
Microcosm 3	Nitrate (nitrate-reducing)	H_2/CO_2 (8:2 *v*/*v*)
Microcosm 4	Sulfate (sulfidogenic)	N_2/CO_2 (8:2 *v*/*v*)

medium (pre-purged with N_2 and no Molasses) contained in a 5-l glass reservoir (LabGlass Chromware, HPLC reservoir, Fisher Scientific, continuously purged with $N_{2(g)}$ to maintain anoxic state) was injected from the bottom of soil column to saturate the added soil so that the column received 1,070 g of KY soil and 1,057 ml of IM medium. The feed reservoir was stored in the refrigerator (~4°C) to minimize potential microbial growth. The soil column was pre-flushed with 4 l of the sterilized anoxic IM medium and the effluent was used as an initial liquid loading for the secondary aerobic bioreactor. The top outlet of the soil column was connected to a 7-l stainless steel cylindrical aerobic bioreactor by a stainless steel tube (1/8 in. of inner diameter) to minimize losses of the PAHs liberated from the soil column. The water-saturated air was supplied to the bottom of the reactor at 0.3 ml/min. The effluent was continuously removed from the surface of liquid using a tubing pump (Masterflex variable speed pump, Cole-Parmer, Vernon Hills, IL, USA) to maintain 4 l of liquid during the reactor run. A mixed culture of soil microbes was eluted from a creosote-contaminated site soil in which active microbial PAH-degrading activities have been reported (Carmichael and Pfaender 1997; Roper and Pfaender 2001) and inoculated to the reactor (initial biomass concentration of 0.12 mg-protein/ml). The liquid in the reactor was continuously agitated by a magnetic stirrer. In the initial run, the IM medium containing 50 mM of sodium nitrate (as an electron acceptor) and Molasses (as a co-substrate, 0.3% by volume) was continuously supplied at 0.1 ml/min to the bottom inlet of column (upflow, retention time of 7.34 days) for the first 80 days using an HPLC pump (Waters 510 HPLC pump, Milford, MA, USA). Then, sodium nitrate was removed from the feed solution at day 80 and the feed solution was allowed to flow through the column until day 190 at the same flow rate to stimulate activities of strict anaerobic methanogenic microbes. At day 190, the flow rate was reduced to 0.015 ml/min to provide a longer retention time of approximately 50 days in the soil column. The aqueous phase samples were collected at the top outlet of the anaerobic soil column and directly from the secondary aerobic bioreactor. Levels of concentrations of PAHs quantified by HPLC, pH, and ORP in the aqueous samples were monitored during the reactor run. Microbial growth in the secondary aerobic bioreactor was also monitored by biomass concentrations in the reactor.

2.4 Dissolved Organic Matter Characterization

Organic carbon contents in the aqueous DOM obtained from each of incubated microcosms were quantified as total organic carbon (TOC) concentrations. The ratio of spectroscopic absorptivities at wavelengths of 465 and 665 nm for DOM (E_4/E_6 ratio) were employed for the general molecular composition, chemical structure, and diagenetic characteristics of DOM (Aiken et al. 1985; Chen et al. 1977). Specific ultra-violet absorbance (SUVA) values measured at wavelengths at 254 and 280 nm were also used for the aromatic property of DOM (Aiken et al. 1985; Frimmel 2001). Relative size and weight of DOM were estimated using high-pressure size exclusion chromatography (HPSEC) as described in a prior study (Zhou et al. 2000). Relative weigh-average molecular weight (M_w), relative number-average molecular weight (M_n), and polydispersivity (ρ) were determined using the following equations:

$$M_w = \frac{\sum_{i=1}^{N} w_i M_{w,i}}{\sum_{i=1}^{N} w_i} = \frac{\sum_{i=1}^{N} w_i M_{w,i}^2}{\sum_{i=1}^{N} f_i M_{w,i}} \quad (1)$$

$$M_n = \frac{\sum_{i=1}^{N} f_i M_{w,i}}{\sum_{i=1}^{N} f_i} \quad (2)$$

$$\rho = \frac{M_w}{M_n} \quad (3)$$

Table 2 Levels of PAHs in the KY soil and their concentrations in the aqueous phase of soil slurries during soil incubations (batch microcosm system)

Compound	Soil phase concentration[a] (mg/kg)	Aqueous phase concentration (μg/l)								
		Initial level[b]	Microcosm 1 (aerobic)		Microcosm 2 (nitrate-reducing with N_2/CO_2 headspace)		Microcosm 3 (nitrate-reducing with H_2/CO_2 headspace)		Microcosm 4 (sulfidogenic)	
			Incubation period (days)							
			50	100	50	100	50	100	50	100
Naphthalene	529.8 (11.6)	585.2 (31.3)	ND	13.2 (0.7)	967.6 (54.7)	525.6 (43.5)	818.0 (51.5)	573.7 (68.8)	833.4 (75.4)	949.0 (64.1)
Acenaphthene	1,142.8 (27.9)	442.6 (15.5)	ND	121.3 (7.4)	768.5 (55.5)	635.2 (55.3)	1,457.1 (95.4)	527.3 (35.4)	233.0 (25.1)	1,153.7 (72.3)
Fluorene	1,345.9 (38.1)	271.5 (23.6)	ND	14.0 (1.2)	629.5 (28.4)	317.1 (17.7)	633.8 (54.5)	488.3 (78.5)	298.6 (21.5)	1,137.0 (59.5)
Phenanthrene	4,739.8 (23.1)	420.7 (10.5)	ND	1.8 (0.1)	1,465.2 (98.5)	888.8 (50.3)	1,505.1 (151.4)	1,493.7 (110.2)	403.5 (30.4)	3,819.3 (123.5)
Anthracene	463.1 (15.0)	26.9 (4.3)	ND	18.5 (1.1)	860.1 (45.1)	347.0 (15.2)	783.3 (34.5)	244.0 (31.2)	58.6 (12.4)	639.0 (31.1)
Fluoranthene	3,244.3 (71.5)	135.8 (15.2)	7.2 (0.9)	104.4 (4.2)	2,609.2 (151.3)	1,151.3 (34.1)	2,829.7 (124.6)	964.0 (51.5)	140.7 (11.1)	2,829.7 (110.1)
Pyrene	1,130.4 (40.9)	89.6 (7.9)	56.6 (4.5)	100.6 (3.9)	1,379.2 (88.2)	771.4 (34.2)	1,269.8 (78.5)	719.0 (54.6)	105.8 (9.8)	1,816.0 (99.8)
Benzo[a]anthracene	512.3 (15.3)	16.9 (1.5)	ND	17.1 (1.1)	446.9 (18.5)	259.3 (15.3)	551.3 (31.5)	164.0 (25.3)	20.1 (1.2)	565.0 (45.5)
Chrysene	890.8 (27.3)	3.5 (0.1)	6.9 (1.2)	12.6 (0.8)	143.4 (8.4)	62.1 (3.3)	131.8 (12.3)	34.3 (3.0)	3.0 (0.1)	59.7 (3.6)
Benzo[b]fluoranthene	392.5 (16.9)	13.8 (2.1)	3.5 (0.2)	25.3 (5.5)	375.2 (15.1)	157.7 (4.5)	298.4 (18.5)	130.0 (7.7)	12.3 (2.1)	301.3 (16.8)
Benzo[k] fluoroanthene	182.8 (28.7)	4.1 (0.5)	2.4 (0.1)	12.0 (1.2)	192.0 (15.3)	74.2 (7.7)	181.4 (10.9)	48.3 (4.3)	5.4 (0.2)	132.3 (10.7)
Benzo[a]pyrene	449.5 (18.0)	9.5 (0.1)	7.3 (0.9)	24.5 (3.7)	468.6 (35.6)	155.0 (9.5)	405.1 (38.2)	128.0 (5.8)	12.9 (1.7)	290.7 (15.4)
Dibenzo[a,h] anthracene	26.5 (1.2)	0.2 (0.0)	ND	1.5 (0.1)	6.2 (0.2)	ND	6.8 (0.2)	ND	1.5 (0.1)	5.2 (1.1)
Benzo[g,h,i]perylene	248.8 (28.7)	5.0 (0.8)	ND	15.4 (2.8)	290.2 (21.7)	55.2 (8.2)	190.0 (11.4)	47.3 (10.4)	8.3 (0.9)	316.0 (31.5)
Total PAHs[c]	15,299.3	2,025.5	83.9	482.2	10,601.8	7,569.8	11,061.4	5,562.0	2,136.9	14,013.9

Values in the parentheses denote standard deviation of triplicate samples.

ND: not detected

[a] Determined prior to incubation.

[b] Determined 1 day after the beginning of incubation.

[c] 12PAHs (naphthalene through benzo[g,h,i]perylene) analyzed in this study

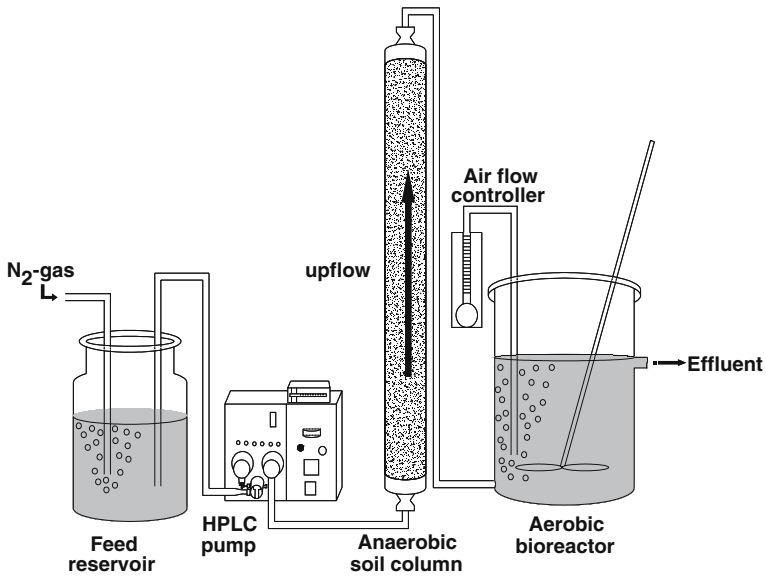

Fig. 1 Schematic diagram of continuous flow system composed of anaerobic soil column and aerobic bioreactor

where w_i is the weight of a characteristic molecular weight of $M_{w,\ i}$; $M_{w,\ i}$ is the characteristic molecular weight of i fraction; f_i is the frequency of a characteristic molecular weight $M_{w,\ i}$; and, N is the number of fractions of molecules according to molecular weight.

2.5 Analytical Procedures

PAH concentrations were determined by a reverse-phase HPLC (Waters HPLC system, Waters, Bellefonte, PA, USA) equipped with a fluorescence detection system (Waters 470 Scanning Fluorescence, Waters, Bellefonte, PA, USA). Separation of PAHs was accomplished on a Supelcosil LC-PAH column (250×4.6 mm, 5 μm, Supelco, St. Louis, MO, USA) with a mobile phase consisting of acetonitrile and water (6:4 by volume at the start and 100% acetonitrile at the end) at 1.5 ml/min. Concentrations of nitrite, nitrate, and sulfate were determined by an ion-chromatography (Dionex) equipped with an IonPac AS4A-SC column (125×3.2 mm, Dionex) and a conductivity meter (Dionex, Model CDM-2) in a mobile phase consisting of 0.191 g of Na_2CO_3 and 0.143 g of $NaHCO_3$ per liter of distilled water at 1 ml/min. TOC analysis was conducted by a TOC analyzer (TOC-5000 analyzer, Shimadzu, Japan) using potassium hydrogen phthalate as an external standard. E_4/E_6 ratios and SUVA values were obtained by a spectrophotometer (Hitachi, Model U-3300, Japan). An HPSEC system consisting of a HPLC system (Hewlett Packard, HP series 1050, Palo Alto, CA, USA), a SEC column (Protein Pak 125 SEC column, Waters), and a photodiode array detector (Hewlett Packard, HP 1050) for the detection at a wavelength of 280 nm in a mobile phase consisting of 0.348 g of K_2HPO_4, and 0.272 g of KH_2PO_4, and 5.844 g of NaCl per liter of distilled water at 1 ml/min was employed to determine the relative molecular weights and polydispersivity of DOM. Salicylic acid and sodium polystyrene sulfonate polymers obtained from Sigma-Aldrich and Polyscience, Inc. (Warrington, PA, USA), respectively, were used as reference molecular weight standard compounds and their average molecular weights were 138.1, 1,800, 4,600, 8,000, 18,000, and 35,000 Da. Biomass concentration was determined by protein measurement using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA) and a spectrophotometer (Hitachi) at an absorbance of 595 nm.

3 Results and Discussion

3.1 Mobilization of Bound PAHs to the Aqueous Phase in Batch-Type Microcosms

Redox potential was monitored during soil incubations in batch-type microcosms. While the ORP values for aerobic incubations (microcosm 1) were ranging from +86 to +195 mV, those values for anaerobic incubations (microcosms 2, 3, and 4) decreased markedly as low as −118 to −220 mV. The redox potentials for aerobic

incubation (microcosm 1) and sulfidogenic incubation (microcosm 4) correspond well with those representing redox environments mediated by soil microbes (Cookson 1995). The values for microcosms 2 and 3 (nitrate-reducing) were lower than those values known for the typical nitrate-reducing subsurface perhaps due to the complete consumption of nitrate as a microbial electron acceptor available and subsequent change of redox conditions to further reduced states. It may also be notable that the soil incubations in the absence of oxygen may have reached methanogenic conditions since no other inorganic dissolved electron acceptors became available. These were supported by that nitrite, nitrate, and sulfate were not detected at 50 and 100 days of incubations. Although we did not perform the analysis of metallic electron acceptors such as oxidized iron or manganese, these metallic compounds should have been favored thermodynamically by geo-microbes over sulfate, and the observed redox potentials were indeed lower than those values for iron or manganese reductions (Cookson 1995).

The aqueous phase concentrations of PAHs released from the KY soil were monitored during microbial incubations under various redox conditions and the results are summarized in Table 2. Note that the aqueous phase concentrations are the apparent concentrations (C_{app}) of solutes (i.e., sum of concentrations of free aqueous phase solutes (C_{free}) and those of DOM associated solutes (C_{DOM}, i.e., $C_{app}=C_{free}+C_{DOM}$). Except low molecular weight PAHs (LMWPAHs, e.g., naphthalene, acenaphthene, and fluorene, comprised of two benzene rings), the initial C_{app} levels of high molecular weight PAHs (HMWPAHs, composed of three or more benzene rings) in the aqueous phase were apparently higher than their aqueous solubilities, indicating that PAHs were readily released to the aqueous phase and present in a DOM-associated form. Values of C_{app} for PAHs in the aerobic microcosms were mostly ranging lower than their initial values during soil incubations, which may have been attributed to microbial degradation of PAHs under aerobic conditions. In contrast, the C_{app} values increased markedly and they were far in excess of their initial levels by factors of 1.4 to 62.8 over the course of anaerobic incubations. No notable differences between microcosms 2 and 3 were observed with respect to the behavior of C_{app} values for PAHs, indicating that hydrogen gas initially present in the headspace did not appear to be effective to stimulate the activities of H_2-utilizing anaerobes. The most marked mobilization of the soil-bound PAHs to the aqueous phase took place at 50 and 100 days of soil incubation under nitrate-reducing (microcosms 2 and 3) and sulfate-reducing (microcosm 4), respectively. This may be attributed to longer acclimation time and slower growth pattern of sulfate-reducers compared with those of nitrate-reducers.

Effect of physical and chemical properties of PAHs on the mobilization was examined by correlating the ratios of $C_{app}/C_{initial}$ obtained from anaerobic microcosms with their molecular weights and octanol-water partitioning coefficients (K_{ow}) that represent the hydrophobicities of PAHs as depicted in Fig. 2. Since PAHs could have been microbially degraded under aerobic conditions, their aqueous phase concentrations during the aerobic soil incubations (microcosm 1) were not included in the analysis. The mobilization effects become far more evident as the molecular weight and hydrophobicity of PAH increase, supporting that DOM incubated under anaerobic conditions interacts more favorably with heavier and more hydrophobic PAHs. Previous studies (Grathwohl 1990; Rutherford et al. 1992) demonstrated similar results that the association capacities of HOCs with organic matter originated from soils and sediments increase with hydrophobicities of HOCs due to their favorable chemical affinities for SOM.

Recently, it has been discovered that microbial soil incubation under anaerobic conditions facilitated the solubilization and release of organic matter associated on/within the soil matrix as a result of elevated pH (~9.5) with subsequent release of bound HMWPAHs to the aqueous phase (Pravecek et al. 2005). It is to be noted, however, that pH would seldom reach as high as the level at which SOM can be dissolved effectively in most lab-scale experiments and field-scale practices owing to the sufficient pH buffer capacity in natural systems. In our soil incubations, pH values were also varying in a pretty much neutral range of 6.2 to 8.1 (data not illustrated), suggesting that the enhanced dissolution of SOM induced by basic surrounding water cannot explain the increase of C_{app} values for PAHs in our anaerobic soil incubations.

3.2 Characteristic Changes of DOM during Soil Incubation

The physical and chemical properties of DOM released from the soils incubated under various redox

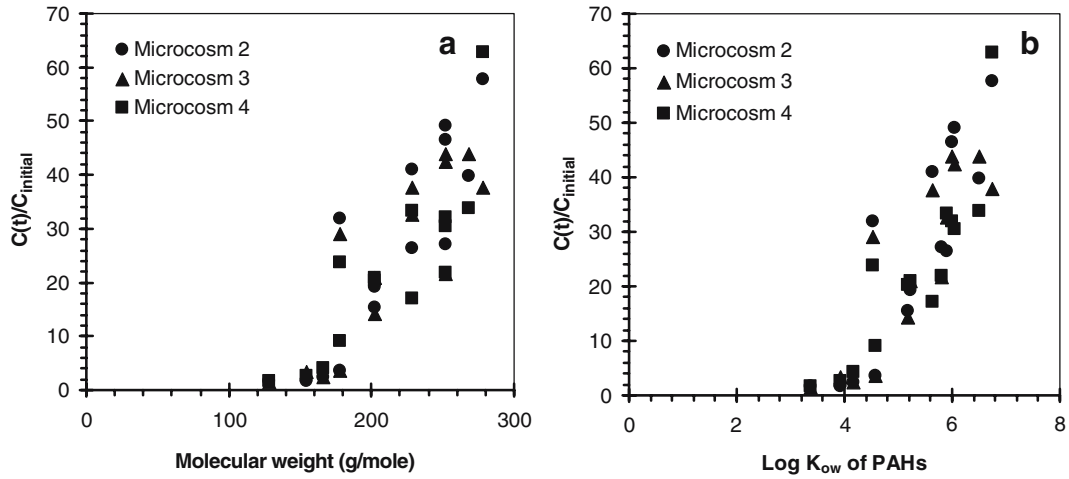

Fig. 2 Correspondence of the apparent aqueous phase concentrations for PAHs (C_{app} for PAHs) to their molecular weights (**a**) and hydrophobicities (**b**)

conditions were analyzed and summarized in Table 3. Quantities of DOM mobilized to the aqueous phase (determined by TOC concentrations) were apparently different compared to its initial level depending on incubating redox conditions (p=0.00005–0.02): e.g., microcosm 3 > microcosm2 >> microcosm 4 (≅initial level) > microcosm 1. This order in terms of DOM quantity does not correlate the order of the C_{app} values for PAHs mobilized, supporting that the mobilization of PAH was not stimulated solely by the enhanced solubilization of SOM as suggested by Pravecek et al. (2005). Instead, Kim and Pfaender (2005) demonstrated that the microbially-mediated redox conditions can lead to the modification of physical and chemical properties of SOM similar to the SOM diagenesis with respect to physical and chemical properties of SOM and the DOM incubated under highly reduced anaerobic conditions exhibited far more favorable binding characteristics for HOCs compared to aerobically incubated DOM. In the same manner chemical and physical properties of the released DOM in this study were examined by SUVA, E_4/E_6 ratio, relative molecular size, and polydispersivity. SUVA values and E_4/E_6 ratio have been known to provide information for aromatic property and humification degree of SOM (Aiken et al. 1985; Chen et al. 1977). SUVA values and E_4/E_6 ratios for the DOM samples incubated under anaerobic conditions were higher and lower, respectively, than those values for the initial DOM and aerobically incubated DOM, supporting that the anaerobically incubated DOM became more aromatic and humified than that for the initial and aerobically incubated DOM samples. The size of DOM liberated from the soil during incubations was compared using its relative molecular weight determined by HPSEC. Figure 3 presents the HPSEC chromatograms of DOM samples collected from the incubated microcosms that showed the most evident PAH mobilization, co-substrate Molasses, and initial DOM. Responses corresponding to high molecular weight increased remarkably in the chromatograms for the DOM liberated from the anaerobically incubated soils relative to DOM from the aerobically incubated SOM, initial DOM, and Molasses. Such changes are quantitatively evaluated by relative molecular weights (M_w and M_n) as summarized in Table 3. The larger the M_w and M_n values, the higher the C_{app} values for PAHs. In addition, as polydispersivity of DOM increased, the PAH mobilization also became more significant. These results are in close accordance with Kim and Pfaender (2005) in terms of increases of DOM-PAH binding affinity as a result of physical and chemical characteristic changes of DOM.

3.3 Release of Bound PAHs and Their Bioavailabilities in the Continuous Flow System

The enhanced mobilization of bound PAHs to the aqueous phase was examined again in the continuous flow system to evaluate the effects of the mobilization on the fate and transport of bound contaminants in the saturated subsurface. In the first 80 days of nitrate-

Table 3 Summary of DOM characterization – total organic carbon (TOC) content, specific UV absorbance at 254 and 280 nm ($SUVA_{254}$ and $SUVA_{280}$), E_4/E_6 ratio, weight-average molecular weight (M_w), number-average molecular weight (M_n), and polydispersivity (ρ)

	Incubation period (days)	TOC (mg/l)	$SUVA_{254}$ (l/mg C-m)	$SUVA_{280}$ (l/mg C-m)	E_4/E_6	M_w (Da)	M_n (Da)	ρ
Initial DOM[a]		330.2 (13.7)	2.12	1.31	6.26	760.6	497.3	1.53
Microcosm 1	50	210.5 (11.2)	2.03	1.12	7.28	1,614.0	424.8	3.80
	100	110.2 (9.5)	1.91	0.92	8.11	1,712.5	432.6	3.96
Microcosm 2	50	830.9 (22.8)	2.17	1.35	4.96	4,293.0	990.5	4.33
	100	580.5 (10.8)	2.01	1.12	5.05	4,115.2	988.5	4.16
Microcosm 3	50	1,410.5 (54.8)	2.07	1.21	5.23	3,756.0	1,020.0	3.68
	100	830.1 (35.4)	1.98	1.05	5.07	3,815.9	1,025.4	3.72
Microcosm 4	50	250.2 (12.1)	2.35	1.34	5.91	1,235.2	628.2	1.97
	100	373.0 (13.8)	2.81	1.82	4.16	4,916.4	1000.1	4.92

Values in the parentheses denote standard deviation of triplicate samples.

[a] Determined 1 day after the beginning of incubation.

reducing condition under which the ORP values were varying at around +40 mV, HWMPAHs (fluoranthene through benzo[g,h,i]perlyene) bound to the KY soil were released at the levels in excess of their aqueous solubilities by factors of 1.1 to 74.5. Values of C_{app} for the mobilized PAHs decreased in the later period perhaps because of fast flow that may have resulted in wash-out of DOM released from soil.

After the supply of nitrate ended, ORP value was dropped to a value less than −250 mV. As presented in Fig. 4, the PAH mobilization resumed, supported by rapid increases of C_{app} values for PAHs, and they reached the highest values at day 110. The C_{app} values for PAHs then slowly decreased, which we attributed to slow growth of strictly anaerobic microbes (e.g., sulfate reducers and perhaps mainly methanogens) compared to the fast flow rate. In other words, the hydraulic retention time in the soil column did not appear to provide a sufficient period for creating anaerobic conditions, stimulating modification, and subsequent release of SOM that can serve as a PAH mobilization facilitator to the aqueous phase. The bound PAHs were mobilized markedly after the reduction of flow rate at day 190, resulting in substantial increases of C_{app} values for HMWPAHs. Their levels at the end of soil column were 2.6–334.8

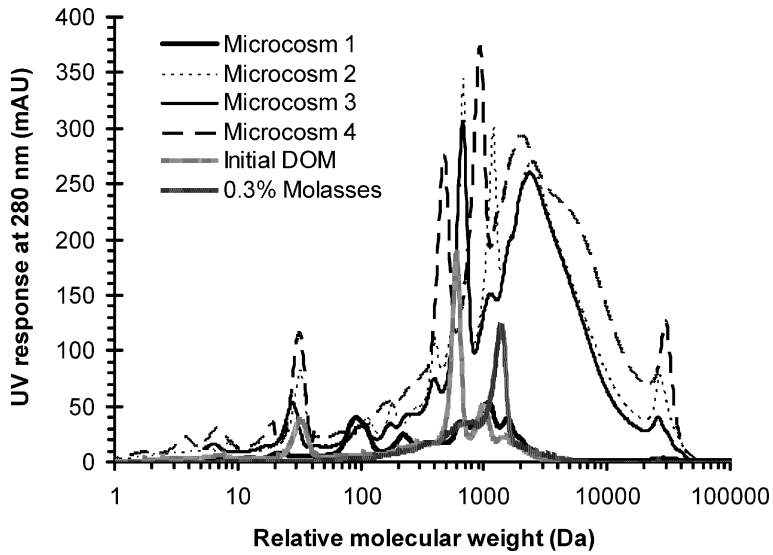

Fig. 3 HPSEC chromatograms of DOM samples obtained during soil incubations and a carbon additive (molasses). Samples for microcosms 1, 2, and 3 were collected at day 50 of incubation and sample for microcosm 4 at day 100. Each sample contained 200 mg/l of organic carbon

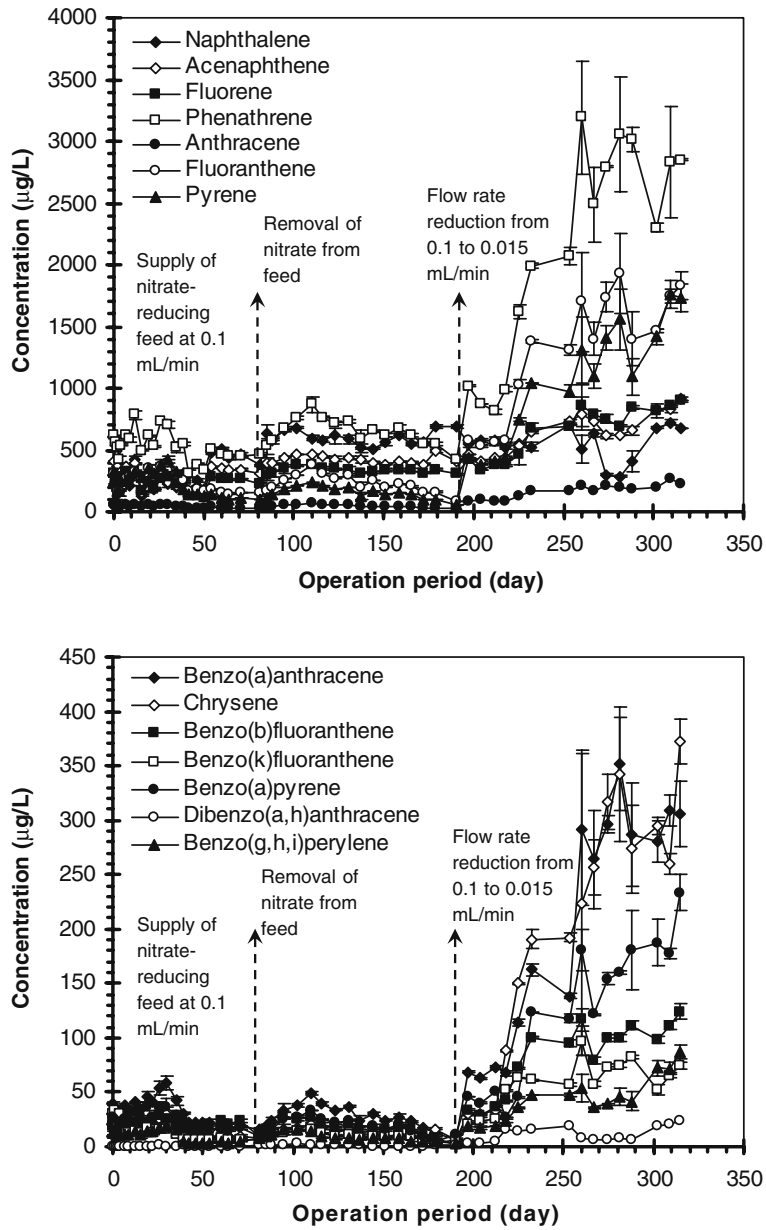

Fig. 4 Behaviors of C_{app} values for PAHs released from the anaerobic soil column. *Error bars* denote standard deviation of triplicate samples

times higher than their aqueous solubilities. As observed previously in the batch microcosm systems, the PAH mobilization effects became far more pronounced as the molecular weight increased.

Chiou et al. (1986) was among the first to suggest the role of DOM in the transport of organic contaminants. Cho et al. (2002) has presented the surfactant-like effects of DOM. Relevant to this study, Mackay and Gschwend (2001) have implicated DOM colloids in the transport of coal-tar PAHs in groundwater. Localized anaerobiosis may be an explanation for the formation of these colloids. Mitra and Dickhut (1999) have stressed that increased PAH pore water concentrations are the result of PAH-DOM associations. Under anaerobic conditions, this importance appears to carry over into the bulk water phase.

Bioavailability of the PAHs mobilized from the anaerobic soil column was examined by the biodegradation test in the secondary aerobic bioreactor. Biodegradability of each PAH compound was evalu-

ated by its removal rate (C_{out}/C_{in}) as summarized in Table 4. The PAH degrading microbes eluted from a creosote contaminated soil effectively removed all PAHs from the beginning of reactor operation. When the flow rate was maintained at 0.1 ml/min (until day 190), relatively lighter PAHs (e.g., naphthalene through pyrene) exhibited very high removal rate (≥99%), and the removal rates for the remaining heavier PAHs except chrysene were also fairly high (≥77%). The biodegradability of those PAHs was greatly enhanced when a longer retention time was provided to the reactor. All PAHs were degraded at the removal rate higher than 95% except dibenzo[a,h] anthracene and benzo[g,h,i]perylene, these two compounds being degraded still at fairly high removal rates (>85%). Meanwhile, biomass including PAH-degrading microbes in the aerobic reactor did not change markedly (ranging from 0.032 to 0.038 mg-protein/ml), supporting that organic matter released from the anaerobic soil column did not appear to stimulate the growth of microbes in the secondary reactor significantly. These results support that the PAHs mobilized from the anaerobic soil incubations were readily bioavailable to aerobic PAH-degrading microbes.

3.4 Research Significance

It has been demonstrated that the mobility and bioavailability of bound HOCs in the soil and sediment matrix depend on the degree and rate of their transfer from the solid-associated phase to the aqueous phase (Crawford and Crawford 1996; Johnson and Amy 1995). The physico-chemical nature of SOM has been believed as a principal determinant of the chemical distribution of hydrophobic solutes, and the HOC-SOM interactions have been extensively examined with respect to the property of SOM (Chin et al. 1997; Cho et al. 2002; Grathwohl 1990). No previous work has, however, addressed HOC-SOM interactions and subsequent transport, bioavailability, and ultimate fate of bound HOCs under live (i.e., microbially-active) conditions, which can more realistically describe the real field conditions. The findings addressed in this study are expected to provide important and more realistic information for the ultimate fate of PAHs in microbially-active subsurface systems. Meanwhile, the release of PAH from the aged contaminated soil into the surrounding water is a biomodal process and the second phase is associated with very slow sorption and

Table 4 Removal rate for PAHs mobilized from the anaerobic soil column in the aerobic bioreactor

Compound	Operation period			
	Initial[a] Removal rate (%)	80 days[b]	190 days[c]	315 days[d]
Naphthalene	100[e]	100	100	100.0
Acenaphthene	48.6	98.1	100	100.0
Fluorene	67.4	99.4	100	100.0
Phenanthrene	48.3	99.9	100	100.0
Anthracene	59.0	96.1	100	99.7
Fluoranthene	61.2	98.0	99.8	100.0
Pyrene	67.8	97.2	99.9	99.9
Benzo(a)anthracene	0	10.2	91.6	97.1
Chrysene	0	0	59.3	96.8
Benzo(b)fluoranthene	0	9.5	81.0	95.3
Benzo(k)fluoroanthene	95.2	7.1	83.3	95.4
Benzo(a)pyrene	0	0	77.2	95.5
Dibenzo(a,h)anthracene	0	21.1	77.7	86.5
Benzo(g,h,i)perylene	40.2	14.5	82.1	90.7

[a] Determined 1 day after the beginning of system operation.

[b] Nitrate was removed from feed solution to change the redox condition in the soil column at a flow rate of 0.1 ml/min.

[c] Change of flow rate from 0.1 to 0.015 ml/min.

[d] End of system operation.

[e] 100% removal denotes that this compound was not detected in the effluent of the aerobic bioreactor.

desorption rates (Weber and Kim 2005). This may impede bioremediation efforts by extending the length of time required and challenge the current understanding of clean-up standards and risks associated with exposure (Alexander 2000; Luthy et al. 1997). Therefore, a major soil remediation goal is the transfer of aged HOCs from the soil into the phase in which microbial degradation takes place. In this study, we found that the imposing anaerobic conditions on contaminated soils can stimulate HOCs that have been present in the soil for many years to be effectively mobilized to the aqueous phase as well as bioavailable for degradation. Improved understanding for the enhanced mobilization of bound HOCs under anaerobic conditions as demonstrated in this study could lead to new strategies for their remediation in contaminated geo-materials. Note that our results may be useful for the remediation schemes for other types of organic as well as inorganic contaminants that interact with SOM such as chlorinated persistent organic compounds, heavy metals, arsenics, etc. It should be also noted that while potentially allowing for more enhanced remediation of aged PAHs, it could increase the risk associated with the contaminant exposure. Thus, we also expect that our results provide new perspectives for exposure risk assessment for the bound contaminants. In addition, a temperature range in the geo-environment is typically lower than that at which the results obtained in this study, which should be taken into account for the application in the contaminated field sites.

4 Conclusions

Anaerobic conditions primarily mediated by soil microbes and their final electron acceptor processes stimulated the alteration and release of soil-bound organic matter to the aqueous phase. This dissolved organic matter tended to interact favorably with PAHs, resulting in increased binding capacity and subsequent mobilization of PAHs bound to the historically contaminated soil to the aqueous phase. Larger (macro) and more aromatic dissolved organic matter exhibiting high association affinity for hydrophobic PAHs was released or produced under anaerobic conditions. Such PAH mobilization was confirmed by remarkable increases of the apparent concentrations of PAHs in the aqueous phase during anaerobic soil incubations, and such phenomena are far more evident for high molecular weight PAHs. The continuous flow system experiment demonstrated that the aged bound PAHs can be effectively liberated to the aqueous phase under the highly anaerobic environment and the mobilized PAHs are readily bioavailable to the PAH-degrading soil microbes. This study provides very important implications for the remediation and risk assessment of HOC-contaminated subsurface systems such as soils, sediments, lagooned deposits, etc.

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