Bull. Environ. Contam. Toxicol. (2001) 67:898–905

DOI: 10.1007/s00128-001-0206-8

Bulletin of Environmental Contamination and Toxicology

Degradation of Phenanthrene in River Sediment Under Nitrate-Reducing Conditions

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Received: 27 March 2001/Accepted: 25 September 2001

Sediment contamination by petroleum hydrocarbons from ships, oil spills and surface runoff is of great concern. There is concern about polycyclic aromatic hydrocarbons (PAHs) due to their potential hazardous properties, recalcitrance, and prevalence in the environment. They are produced by natural and anthropogenic processes such as by the incomplete combustion of fossil fuels and as by product of coke production and petroleum refining (Sims and Overcash 1983). Due to their hydrophobicity, they partition strongly into nonaqueous phases which decreases their general availability and rate of degradation (Mihelcic et al. 1993). Aerobic degradation of PAHs in sediments have been extensively documented (Cullen et al. 1994; Geiselbrecht et al. 1996). Although evidence for anaerobic degradation of PAHs under nitrate-reducing conditions has been presented (Bregnard et al. 1996; Langenhoff et al. 1996), information on the degradation of phenanthrene under nitrate-reducing conditions is scarce.

In previous study we measured aerobic degradation rate constants and half-lives for phenanthrene in sediment samples collected at five sites along the Keelung River in northern Taiwan ranged from 0.12 to 1.13 1/day and 0.61 to 5.78 day, respectively (Yuan et al. 2001). We suggested that anaerobic microorganisms might have greater potential for organic-pollutant detoxification in the environment. For the present study, river sediments collected from the same five sampling sites were used to investigate the effects of factors such as various reducing conditions, the presence of other PAHs, the addition of electron donors or acceptors on phenanthrene degradation under nitrate-reducing conditions.

MATERIALS AND METHODS

Polycyclic aromatic compounds (phenanthrene, acenaphthene, anthracene, fluorene and pyrene) with 99.0% analytical standards were purchased from Aldrich Chemical Co. (Milwaukee, WI). Solvents were purchased from Mallinckrodt, Inc. (Paris, KY). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions of individual PAHs dissolved in dimethyl sulfoxide were initially established at a concentration of 10,000 mg/L and diluted to 500 mg/L before use.

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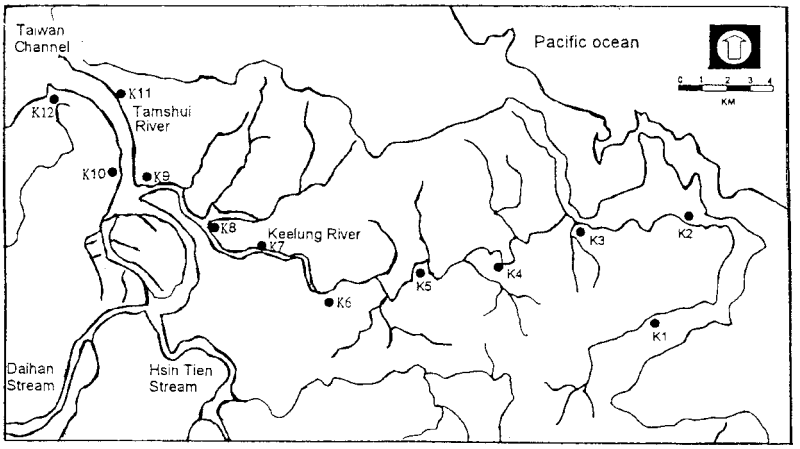

Figure 1. Sediment sampling sites along the Keelung River.
K1 to K3, upriver; K4 to K6, midriver; K7 to K12, downriver.

Figure 1 shows collection sites for sediment samples along the Keelung River--one of the most heavily contaminated rivers in northern Taiwan. Using an Ekman grab sampler, we collected surface sediment (1-10 cm) from five sites in the middle or lower portions of the Keelung in August, 1998. The sampling sites of K6 river sediment were middle stream of Keelung River, and the sites of K8, K10, K11 and K12 river sediments were down stream of Keelung River. The river is branch of Tamshui River also belong to heavily contaminated sites. In the K6, K8, K10, K11, and K12 samples, respective BOD values were 14, 52, 34, 17, and 27 mg/L, NH_3-N values were 4.8, 5.8, 5.3, 2.1, and 4.9 mg/L, pH value were 6.8, 6.5, 6.8, 7.0, and 6.8, and anaerobic microbial populations were 2.2×10^5, 3.9×10^6, 2.8×10^6, 1.7×10^5, and 4.6×10^6 cells/g of sediment (Yuan et al. 2001; Chang et al. 2001). Adaptation was performed by adding phenanthrene (1 mg/kg) to 500 g of sediment at 7-day intervals under static incubation at 30℃ without light for six months.

Experiments were performed using 125 mL serum bottles containing 45 mL medium and 5 g river sediment to which 2 μg/g phenanthrene was added. Our experimental medium consisted of (all concentrations in g/L): NH_4Cl, 2.7; $MgCl_2.6H_2O$, 0.1; $CaCl_2.2H_2O$, 0.1; $FeCl_2.4H_2O$, 0.02; K_2HP0_4, 0.27; KH_2P0_4, 0.35; yeast extract, 2.0; resazurin, 0.001 and sodium nitrate, 20 mM. Medium pH value was adjusted to 7.0 following autoclaving; 0.9 mM titanium citrate was added as a reducing reagent. The following factors were manipulated to investigate their effects on phenanthrene biodegradation: culture with sediment or sediment-free culture; sampling site (K6, K8, K10, K11 or K12); electron donors or acceptors (sodium acetate, 20 mM; sodium pyruvate, 20 mM; sodium lactate, 20 mM; manganese dioxide, 50 mM; ferric chloride, 50 mM); sulfate-reducing conditions (20 mM sodium sulfate), nitrate-reducing conditions (20 mM sodium nitrate), and methanogenic conditions (20 mM sodium hydrogen carbonate); and inoculated control (no sodium sulfate, sodium nitrate or sodium hydrogen

carbonate added); and the presence of additional phenanthrene or other PAHs (acenaphthene, pyrene, fluorene or anthracene at 2 μ g/g). Sediment-free culture was created by adding 1 g sediment to 9 mL of our experimental medium in a culture tube; after shaking, sediment particles were allowed to settle for approximately 1 min prior to separation. Sterile controls were produced via autoclaving at 121℃ for 3 h. Each treatment was performed in duplicate.

Bottles were capped with butyl rubber stoppers and wrapped in aluminum foil to prevent photolysis, then incubated without shaking at 30℃ in darkness. All experiments were conducted in an anaerobic glove box (Forma Scientific, model 1025 S/N) filled with 85% N_2, 10% H_2, and 5% CO_2 gases. Samples were periodically taken from treatment bottles for the purpose of measuring residual PAH concentration, oxidation-reduction potential (ORP), and pH values.

PAH was extracted three times by addition of hexane, each followed by centrifugation for 10 min at 12,000 g and filtering through 0.45 μ m filters (Lida Corp.). Extracts were analyzed with a high performance liquid chromatograph equipped with a fluorescence detector (model FL-1), pump (model 125), autosampler (model 502) and system gold (all from Beckman), plus a polymetric-bound silica column (Phenomenex). Fluorescence detector excitation and emission levels were set at 254 nm and 390 nm, respectively. The mobile phase employed a 40:60 mixture of acetonitrile and water. Detection limits for phenanthrene, acenaphthene, fluorene, anthracene and pyrene were set at 0.1, 0.5, 0.5, 0.01, 0.1 and 0.1 mg/L, respectively. Degradation rates were determined from the time course of PAH disappearance, using 2 to 4 points in the linear portion of graphs that related PAH concentration to time. Remaining percentage was calculated as the PAH residue concentration divided by PAH original concentration. Significant differences were calculated with a standard variance F-test.

RESULTS AND DISCUSSION

As shown in Table 1, remaining amounts of phenanthrene in our sterile control at the end of 56 days of incubation were 90.2 and 92.0% in the K8 and K10 samples, respectively. As also shown in Table 1, phenanthrene was completely degraded by day 56 in K8 and remained 43.2% in K10 sediment-containing samples. In comparison, phenanthrene was completely degraded by day 30 in K8 and remained 32.0% in K10 sediment-free culture samples -- a significantly faster rate. This is strong evidence in support of the argument that phenanthrene anaerobic degradation in river sediment is the result of microbial action, and that such action is enhanced by sediment-free culture samples. One possible explanation for this significant difference is that phenanthrene's tendency to adsorb to sediment particles may reduce the degrading effectiveness of microorganisms or the bioavailability of phenanthrene, thus retarding the process.

Table 1. Phenanthrene remaining percentage in sterile control, sediment-containing, and sediment-free culture within 56 days incubation.

Treatment	Remaining percentage (%)[a]	
	K8 sediment sample	K10 sediment sample
Sterile control[b]	90.2 ± 1.9	92.0 ± 1.2
Sediment-free culture	—[c]	32.0 ± 1.9[e]
Sediment-containing culture	—[d]	43.2 ± 0.9[e]

[a] Values are means ± standard deviations.

[b] Sediment was present in the culture.

[c] Phenanthrene was completely degraded within 30 days.

[d] Phenanthrene was completely degraded within 56 days.

[e] Significant difference were found at P = 0.05.

We compared phenanthrene remaining percentage and degradation rates under nitrate-reducing conditions in sediment samples collected from five Keelung River sites (Table 2). We found that phenanthrene degradation rates were 0.024, 0.036, 0.031, 0.028, 0.034 μ g/g/day for K6, K8, K10, K11 and K12 sample sites. Phenanthrene remaining percentages were 33.3, non-detectable level (< 0.5 mg/L), 13.2, 23.1 and 3.8% for K6, K8, K10, K11 and K12 sample sites within 56 days. The results showed that the order of biodegradation rates (high to low) being K8 → K12 → K10 → K11 → K6. In investigation of Tamshui River sediments (of which the Keelung River is one branch), Liu et al. (2000) found 16 PAHs at concentrations ranging from 0.01 to 3.06 μ g/g dry weight, with highest concentrations found for fluorene, pyrene and phenanthrene. Four of our sampling sites (K8, K10, K11 and K12) were downstream from the Tamshui River. Results from these sampling sites appear to indicate that microorganisms adapt to site-specific conditions, resulting in varied degradation capacity. K8 sediment sample had a higher degradation rate than other sampling sites. We will therefore restrict our discussion to results from the K8 sediment under nitrate-reducing conditions in the next experiment.

Table 2. Phenanthrene remaining percentage and degradation rates under nitrate-reducing conditions in river samples collected from five Keelung River sites within 56 days incubation.

Sampling sites	Remaining percentage [a] (%)	Degradation rates [a] (μ g/g/day)
K6	33.3 ± 1.9	0.024 ± 0.005
K8	—[b]	0.036 ± 0.012
K10	13.2 ± 1.1	0.031 ± 0.007
K11	23.1 ± 1.3	0.028 ± 0.009
K12	3.8 ± 0.9	0.034 ± 0.010

[a] Values are means ± standard deviations.

[b] Phenanthrene was completely degraded within 56 days.

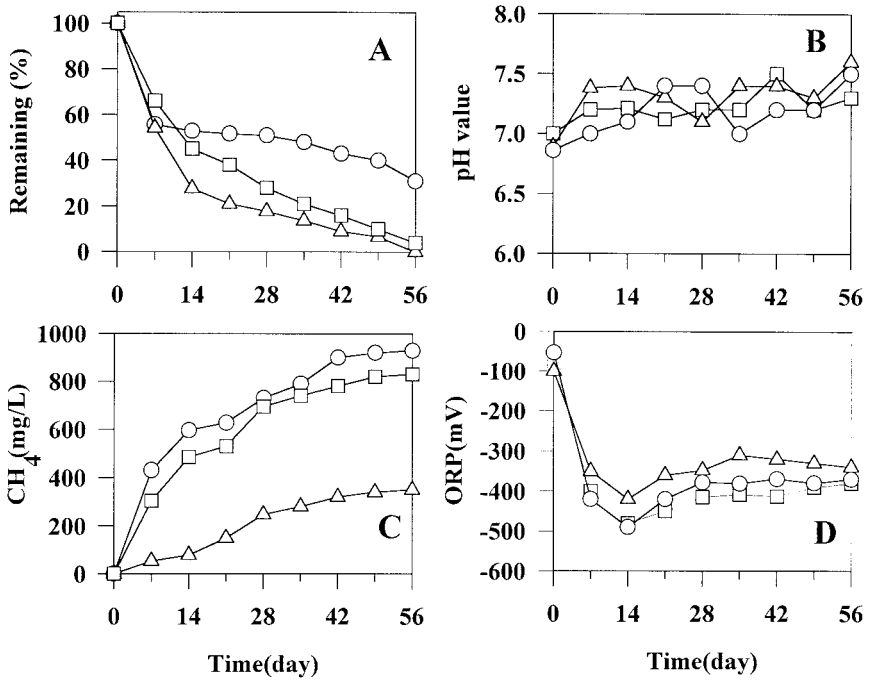

Figure 2. Comparison of three reducing conditions on phenanthrene degradation (A), pH value (B), methane production (C), and ORP value (D) in K8 sediment samples. Symbols:○, methanogenic conditions; □, sulfate-reducing conditions; △, nitrate-reducing conditions.

Comparison of phenanthrene degradation, methane production, pH values and ORP values under three reducing conditions are presented in Figures 2. We found the order of phenanthrene remaining percentage for K8 sediment sample are nitrate-reducing conditions > sulfate-reducing conditions > methanogenic conditions (Figure 2a). Under three reducing conditions, pH values were measured from 6.8 to 7.7 within 56 days incubations (Figure 2b). In a previous study we reported that the optimal pH value for anaerobic degradation between 7.4 and 7.8 (Chang et al. 1998). The orders of methane production were methanogenic conditions > sulfate-reducing conditions > nitrate-reducing conditions (Figure 2c). The maximun methane production was 930, 830, 350 mg/L for methanogenic conditions, sulfate-reducing conditions and nitrate-reducing conditions, respectively within 56 days incubations.

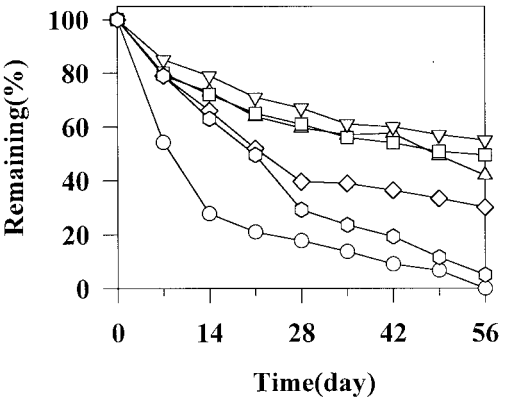

Figure 3. Effects of various electron donors or acceptors on phenanthrene degradation in the K8 sediment sample. Symbols: ○, inoculated control; □, lactate; △, acetate; ▽, pyruvate; ◇, $FeCl_3$; ⬡, MnO_2.

Figure 2d shows that under three reducing conditions ORP values started within a range of -25 to -100 mV; decreased to a range of -400 to -500 mV from day 7 to day 14 under methanogenic conditions and sulfate-reducing conditions, ORP values decreased to a range of –200 to -390 mV from day 7 to day 14 under nitrate-reducing conditions; then increased to a range of -300 to -380 mV within 56 days incubations under three reducing conditions. It appears that ORP value under nitrate-reducing conditions is lower than under methanogenic and sulfate-reducing conditions, and no relationship exists between changes in ORP values and phenanthrene degradation. It is possible the observed changes in ORP values were due to the growth of our experimental anaerobic consortium. These results match those reported in our previous study (Chang et al. 1998).

Figure 3 presents addition of various electron donors or acceptors on phenanthrene degradation under nitrate-reducing conditions in the K8 sediment sample. We found phenanthrene degradation was delayed by addition of electron donors or acceptors such as pyruvate, lactate, acetate, manganese dioxide and ferric chloride in comparison with our inoculated control. Addition of acetate, lactate or pyruvate promote the growth of methanogen, thus leading to an increased rate of degradation (Oremland 1988; Chang et al. 1998). In this experiment, methanogen may not play a role on phenanthrene degradation, and found inhibition by addition of acetate, lactate or pyruvate. The addition of ferric chloride or manganese dioxide was also found to inhibit degradation. It may be that ferric (III) and manganese (IV) serve as electron acceptors for ferric- and manganese-reducing bacteria under anaerobic conditions, and then inhibited denitrifying bacteria's growth (Lovley et al. 1995). Our previous study on aerobic degradation using samples collected from same sampling sites, showed that phenanthrene aerobic degradation was not significantly influenced by the addition

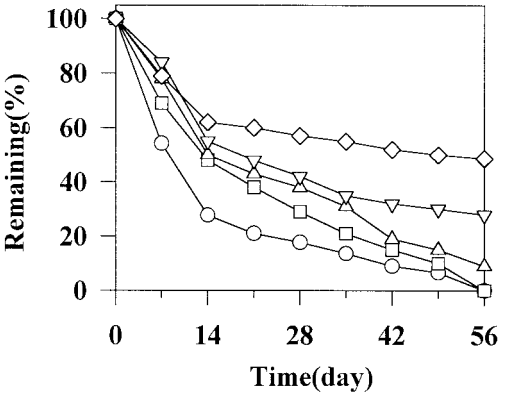

Figure 4. Degradation of five PAHs individually in K8 river sediment samples. Symbols:○, phenanthrene; □, acenaphthene; △, pyrene; ▽, fluorene; ◇, anthracene.

of carbon sources such as acetate, pyruvate, and yeast extract (Yuan et al. 2001). These results shown those different microorganisms are involved in aerobic or anaerobic degradation.

Figure 4 presents degradation of five PAHs individually in K8 river sediment samples. We found that additional acenaphthene and phenanthrene was completly degraded within 56 days incubation, respectively. But pyrene, fluorene, and anthracene were degraded 4.0, 28.0 and 48.7 % within a 56 days incubations period. High to low degradation rates were phenanthrene → acenaphthene → pyrene → fluorene → anthracene. We added phenanthrene in adaptation process for six months, thus phenanthrene showed higher degradationrate than other PAHs in river sediment sample. Bauer and Capone (1988) have suggested that enzymes with broad substrates are responsible for PAH biodegradation. PAHs with higher molecular weights are more resistant to biotransformation than those in complex waste mixtures; in contrast, PAHs with lower molecular weights transform more rapidly as pure compounds. According to Walton and Anderson (1988), PAH bioavailability and biodegradability depend primarily on the complexity of their chemical structures and corresponding physicochemical properties.

We then tried to isolate phenanthrene-degrading organisms from our experimental mixed cultures, and in fact were able to isolate five strains after 1 to 2 weeks of incubation. However, we found that the degradation power of these strains was very weak; it appears that very close synthrophic relationships exist among the various organisms found in this culture.

Acknowledgments. This research was supported by the National Science Council, Republic of China (NSC90-2621-B-031-003).

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