Anaerobic, Sulfate-Dependent Degradation of Polycyclic Aromatic Hydrocarbons in Petroleum-Contaminated Harbor Sediment

MARY M. POTHERMICH, LORI A. HAYES, AND DEREK R. LOVLEY*
Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003

It has previously been demonstrated that [14C]-labeled polycyclic aromatic hydrocarbons (PAHs) can be oxidized to 14CO2 in anoxic, PAH-contaminated, marine harbor sediments in which sulfate reduction is the terminal electron-accepting process. However, it has not previously been determined whether this degradation of [14C]-PAHs accurately reflects the degradation of the in situ pools of contaminant PAHs. In coal tar-contaminated sediments from Boston Harbor, [14C]-naphthalene was readily oxidized to 14CO2, but, after 95 d of incubation under anaerobic conditions, there was no significant decrease in the detectable pool of in situ naphthalene in these sediments. Therefore, to better evaluate the anaerobic biodegradation of the in situ PAH pools, the concentrations of these contaminants were monitored for ca. 1 year during which the sediments were incubated under conditions that mimicked those found in situ. There was loss of all of the PAHs that were monitored (2–5 ring congeners), including high molecular weight PAHs, such as benzo[ap]yrene, that have not previously been shown to be degraded under anaerobic conditions. There was no significant change in the PAH levels in the sediments amended with molybdate to inhibit sulfate-reducing bacteria or in sediments in which all microorganisms had been killed with glutaraldehyde. In some instances, over half of the detectable pools of in situ 2–3 ring PAHs were degraded. In general, the smaller PAHs were degraded more rapidly than the larger PAHs. A distinct exception was the Boston Harbor sediment was naphthalene which was degraded very slowly at a rate comparable to the larger PAHs. In a similar study of fuel-contaminated sediments from Liepaja Harbor, Latvia, there was no decline in PAH levels in samples that were sulfate-depleted. However, when the Latvia sediments were supplemented with sufficient sodium sulfate or gypsum to elevate pore water levels of sulfate to approximately 14–25 mM there was a 90% decline in the naphthalene and a 60% decline in the 2-methylnaphthalene pool within 90 days. These studies demonstrate for the first time that degradation by anaerobic microorganisms can significantly impact the in situ pools of PAHs in petroleum-contaminated, anoxic, sulfate-reducing harbor sediments and suggest that the self-purification capacity of contaminated harbor sediments is greater than previously considered.

Introduction

Marine harbor sediments are frequently polluted with hydrocarbon contaminants derived from fuel spills, industrial waste, shipping activities, runoff, soot, and creosote-treated pilings. Although the aliphatic and monoaromatic components of these contaminants are often readily degraded or volatilized, the PAHs tend to accumulate in the sediment due to their low aqueous solubilities, low volatility, and high affinity for particulate matter (1, 2). PAHs accumulate in aquatic animals and are associated with cancerous lesions in fish (3). Some PAHs are acutely toxic and/or implicated as possible or probable carcinogens in humans (3). Thus, there is a need to understand the fate of PAHs in harbor sediments.

Aerobic degradation of low molecular weight (LMW) PAHs (2–3 rings) has been well documented (4–7), and reports of aerobic degradation of high molecular weight (HMW) PAHs (>3 rings) have been accumulating over the past decade (8). However, with the exception of the most surficial layer, the bulk of organic matter-rich marine sediments are expected to be anoxic (9). Up until the late 1980s it was assumed that microorganisms required molecular oxygen to degrade PAHs and that PAHs in anoxic sediments were recalcitrant to biodegradation. Since then, however, evidence has been accumulating to suggest that there is potential for anaerobic degradation of these compounds under nitrate-, Fe(III)-, and sulfate-reducing conditions (10–23). Of these anaerobic processes, PAH degradation coupled to sulfate reduction is the most relevant to marine harbor sediments because sulfate is abundant in seawater, whereas nitrate concentrations are typically low and Fe(III) is often only sparingly available, especially in heavily contaminated sediments (24).

Previous studies have demonstrated that harbor sediments that are heavily contaminated with petroleum products such as jet fuel or bunker fuel have the potential to anaerobically oxidize [14C]-naphthalene and [14C]-phenanthrene as well as [14C]-fluoranthene (one of the higher molecular weight congeners) to 14CO2 (12, 16, 17, 24). Sediments that were not heavily contaminated with PAHs did not have significant potential for anaerobic [14C]-PAH oxidation. Inhibition of the activity of sulfate-reducing microorganisms, either by adding the specific inhibitor molybdate (25) or by depleting the sediments of sulfate, prevented oxidation of the [14C]-PAHs, indicating that sulfate-reducers were involved in this process. Culturing (17) and molecular studies (26) have indicated that the increased potential for anaerobic PAH degradation in contaminated sediments is associated with an increase in the number of PAH-degrading sulfate-reducers. The results of these studies suggest that the microbial communities of harbor sediments respond to PAH contamination and that this response could potentially result in significant, natural attenuation of PAH contamination.

Although these various lines of evidence demonstrate that sulfate-reducers are capable of metabolizing some PAH congeners added to (i.e., spiked into) sediments, it has yet to be proven that the potential for degradation demonstrated by mineralization of traces of added-in [14C]-PAHs reflects the potential for degradation of the long-standing PAHs found in situ in contaminated harbors. Studies with aerobic soils and sediments have suggested that [14C]-PAHs added to

* Corresponding author phone: (413)545-9651; fax: (413)545-1578; e-mail: dlovley@microbio.umass.edu.

10.1021/es0200241 CCC: $22.00 © 2002 American Chemical Society
Published on Web 10/10/2002 Vol. 36, No. 22, 2002 / ENVIRONMENTAL SCIENCE & TECHNOLOGY • 4811
sediments may be more available for microbial degradation than PAHs that have been long present in sediments as a result of contamination (27–30). The tendency for PAHs to bind strongly to both mineral and organic particulate matter such as soot (31, 32) or to become occluded in tight pore spaces can limit microbial degradation. Thus, rates of PAH degradation measured with [13C]-PAHs or by loss of PAHs that have been added-in to a matrix may greatly overestimate the true potential for in situ PAH degradation in contaminated sites and cannot confirm that natural attenuation will occur in complex environments. Furthermore, it has yet to be determined if any of the high molecular weight PAHs, such as benz[a]anthracene or benzo[a]pyrene, are degraded under sulfate-reducing conditions.

The purpose of this study was to investigate the possibility of significant microbial degradation of the PAH component of petroleum-contaminated harbor sediments under sulfate-reducing conditions by directly examining the degradation of the PAHs occurring in that sediment as a consequence of the contamination. The results demonstrate that, although the mineralization of [13C]-PAHs did not provide a reliable estimate of the rate that the in situ contaminant PAHs were degraded, in situ PAHs in these sediments, including high molecular weight PAHs, were degraded to a significant extent when microbial sulfate respiration was possible.

Materials and Methods

Sediment Collection and Incubation. Boston Harbor sediments were acquired from the marine harbor immediately adjacent to a former coal-tar processing facility in an area of Everett, MA referred to as Island End. Coal-tar works had been in production in this area from the late 1800s to about 1960. The sediment used for this study overlaid the site of a leaking underground storage tank that had been removed in the 1980s after an oil sheen was observed on the surface of the water. Disturbance of the sediment for the grab samples acquired for this study also produced an oil sheen on the surface. The surficial sediment, to a depth of about 20 cm, was collected from under 2–3 m of water with a grab sampler as previously described (12, 17, 33). The top 1 cm of light brown oxic sediment was discarded, and the remaining sulfide-blackened anoxic sediment was packed into quart brown oxic sediment was discarded, and the remaining

PAHs were degraded, in situ PAHs in these sediments, including high molecular weight PAHs, were degraded to a significant extent when microbial sulfate respiration was possible.

Measurement of Metabolism of [14C]-PAHs. The metabolism of [14C]-PAHs was measured as described previously (17). Briefly, approximately 10 g of wet sediment were incubated in anaerobic culture tubes (18 × 150 mm; Bellco Glass, Vineland, NJ) under anaerobic conditions. [14C]-PAHs were delivered to each tube by use of an uncontaminated sediment pellet as a vehicle as previously described. [U-14C]-naphthalene (18.6 mCi/mmol, radiochemical purity 99.6%) or [14C]-phenanthrene (13.3 mCi/mmol, radiochemical purity 100%) in methanol (1–2 μL) was added to dried, sterile pellets of sediment to provide 1 μCi per pellet; the methanol on the pellet was volatilized (ca. 30 s). A pellet was added to each culture tube under a stream of N2/CO2 (93/7). The tubes were then resealed, vortexed, and incubated at 16 °C. [14C]-PAH was also added to a separate control pellet at the same time as those used to deliver the PAH to the experimental tubes in order to determine the amount of radiolabel added to each tube. The amount of radiolabel on the control pellet was determined in Ecolume scintillation fluid (Fisher Scientific, Pittsburgh, PA) on a scintillation counter. The final amount of [14C]-PAH added per tube was 0.0268–0.0356 μmol of naphthalene or 0.0373–0.0498 μmol of phenanthrene. When noted, molybdate inhibited was added from a concentrated anoxic stock to provide a final concentration of 20 mM. The production of 14CH4 and 14CO2 was monitored with a gas proportional counter as previously described (33).

Degradation of in Situ PAH Pools. For the short term (90-day) study of Boston Harbor sediments that was done parallel to the [14C] studies, samples (ca. 125 mL) were incubated in 160-mL serum bottles. For the long-term Boston Harbor study, 800–900 mL of sediment was incubated in 1-L Pyrex bottles; these sediments were overlaid with approximately 300 mL of filter-sterilized seawater from the sampling site that had been held for ca. 2 h at approximately 98 °C in a steam chamber and subsequently bubbled with N2 to remove dissolved oxygen. The bottles were then sealed with thick butyl rubber stoppers after the anoxic seawater transferred to the bottles had been further bubbled with N2/CO2 (93/7) for about 30 min. At each sampling time, the overlying surface seawater was siphoned off so that sediment could be removed without mixing with the surface water. After the sediment aliquots were removed, the remaining sediment in the bottles was again overlaid with fresh, filtered, anoxic seawater which was briefly and gently mixed with the seawater replenishments were the only source of sulfate and were sufficient to maintain pore water sulfate concentrations at 10–20 mM sulfate, depending on the time elapsed since the replenishment. The seawater was also replaced once between sampling dates. Molybdate-inhibited control bottles were maintained with sufficient NaMoO4 to achieve a concentration of 20 mM in the total porewater + surface water volume. Duplicate killed control bottles were prepared by mixing a sufficient amount of 70% glutaraldehyde syrip into the sediment to achieve a final concentration of 3%.

Latvian Sediment and Gypsum Addition. Latvian sediments were homogenized and distributed to 1-L Pyrex bottles in the manner described above except that only 400 mL of sediment was distributed to each bottle because of the limited amount of this sediment that was available to us. And, because natural water from the site was not available, sediment in the incubation bottles was overlaid with anoxic artificial brackish water (ca. 500 mL each) with or without 14 mM sodium sulfate as described in the Results. Composition (per L) of this water was as follows: 11.39 g of NaCl, 0.36 g of KCl, 0.042 g of NaBr, 0.015 g of NaHCO3, 0.0135 g of H3BO3, 0.135 g of NH4Cl, 0.044 g of NaHPO4·2H2O, 0.0135 g of NaF, 0.65 g of TaPSO4·6H2O, pH adjusted to 7.6; 5.59 g of MgCl2·6H2O, 0.73 g of CaCl2·2H2O, 0.012 g of SrCl2·H2O, 0.001 g of FeCl3·6H2O (last 4 ingredients added aseptically after autoclaving). The total lack of sulfate in these sediments allowed for a different experimental design than that utilized with the Boston Harbor sediments. In this
case, controls were left depleted of sulfate rather than poisoned with glutaraldehyde or chemically inhibited with molybdate to prevent sulfate reduction by sulfate-reducing bacteria. Test treatments for sulfate additions consisted of 1) sodium sulfate-amended (14 mM) artificial brackish water or 2) gypsum supplementation of 4.34 g of gypsum (CaSO4·2H2O) per bottle.

**PAH Analysis.** To measure PAHs in the sediments, approximately 3–3 1/2 g of wet sediment were placed in 45-mL screw top, heat and pressure-resistant Corex tubes (Corning Co., Corning, NY) that had the screw threads wrapped with PTFE tape. The exact weight of the sediment samples was recorded, and 35 mL of acetonitrile was added to each tube. The samples were disrupted with the 1/8-in. microrip of an ultrasonic probe (Fisherbrand Dismembranator, Fisher Scientific, Pittsburgh, PA) for 2 min, pulsed. The ultrasonication step was adapted from EPA method 3550A for extraction of high concentrations of PAH from solid matrices. Samples were additionally subjected to heat and pressure-accelerated extraction in the following manner: A small stir bar was placed in the tube, which was then sealed with a PTFE-lined phenolic resin cap. Samples were stirred and heated at 110 °C in a silicon oil bath for 30 min. After cooling, the samples were centrifuged at 2300 rpm for 10 min, and the supernatant was decanted, filtered through a PTFE 0.45 µm syringe filter, and stored in glass vials with PTFE-wrapped threads and sealed with PTFE-lined caps. The sediment pellets were drained of any residual acetonitrile and re-extracted two more times by heat and agitation as described above. This solvent extraction method was compared to room temperature (16) or 30 °C (34) mechanical shaking methods for extraction of PAHs from sediment or soil and produced an approximate 10-fold increase in recovery (data not shown). Furthermore, the effectiveness of ultrasonication has been shown to compare favorably to classic Soxhlet extraction by various workers (35, 36).

PAH concentrations in the extracts were determined by high-pressure liquid chromatography (HPLC) using a Supelcosil LC–PAH column, 15 cm × 4.6 mm, (Supelco, Bellefonte, PA) maintained at 30 °C. The mobile phase was a water/acetonitrile mixture pumped according to a gradient program ranging from 50 to 100% acetonitrile in 17.2 min at an average flow rate of 2 mL min⁻¹. The PAHs were detected with ultraviolet (UV) absorption at 220 nm, and fluorescence spectroscopy at excitation at 260 nm and emission at 350 and 420 nm, and identified and quantified by comparison of retention times and integrated peak areas to known standards. Cleanup and concentration steps were not necessary due to the extremely high levels of PAHs in these coal tar or bunker fuel-contaminated sediments and the extremely sensitive and selective detection of PAHs possible with optimized UV and fluorescence detection (37–39).

**Results**

**Comparison of Mineralization of [14C]-Naphthalene and [14C]-Phenanthrene with Degradation of Total in situ Pools.** As previously observed (17), [14C]-naphthalene and [14C]-phenanthrene were readily mineralized with no lag period in the Boston Harbor sediments (Figure 1A). Over 60% of the radiolabeled naphthalene and over 30% of the radiolabeled phenanthrene were mineralized to 14CO2 within 45 days (Figure 1A). In contrast, although 35% of the detectable phenanthrene pool was degraded over the course of 95 days, there was no apparent decline in the detectable pool of naphthalene over that same period of time (Figure 1B). Both [14C]-PAH mineralization and the degradation of the detectable phenanthrene pool were completely inhibited when molybdate was added to inhibit sulfate reduction. These results demonstrated that the correspondence between the rates of oxidation of added [14C]-PAHs and the degradation of the in situ PAH pools was not consistent, as the rate of degradation of the detectable naphthalene pool was grossly overestimated by the [14C]-naphthalene mineralization results, whereas the rate of detectable phenanthrene degradation was, in this case, similar to the rate of [14C]-phenanthrene mineralization.

**Long-Term Study of Degradation of PAHs in Boston Harbor Sediments.** To better evaluate the anaerobic degradation of the in situ contaminant PAHs, the detectable concentrations of in situ PAHs in the Boston Harbor sediments were monitored for ca. 1 year under larger scale and more in situ-like conditions than those described above. Initially, the distribution of PAHs was characteristic of the typical signature of asphaltenic petroleum products, i.e., very high levels of fluoranthene and pyrene, and considerably more parent material than substituted constituents (e.g. naphthalene vs methylated naphthalenes) (Figure 2).

In unamended sediments, there was, within a year, a detectable loss of all of the PAHs that were monitored (Figure 2; Table 1). There was no significant change in the PAH levels of the sediments amended with molybdate or in which all microorganisms had been killed with glutaraldehyde (Figure 2). In some instances, over half of the individual detectable in situ PAHs were degraded within a year (Table 1). In general, the smaller PAHs were degraded more rapidly than the larger PAHs. The clear exception to this trend was naphthalene which was only slowly degraded at a rate comparable to the larger PAHs. For several of the smaller PAHs, the rate of PAH degradation was...
loss slowed as the concentration of the in situ pool declined (Table 1). For most of the larger PAHs, such as benz[a]anthracene, fluoranthene, benzo[k or b]fluoranthene, and pyrene there was no apparent degradation over the first 105 days of incubation (Table 1), but thereafter there was steady loss of these PAHs with continued incubation.

**Degradation of PAH in Latvia Sediments.** The concentrations of PAHs in the sediment from Latvia were ca. 10-fold higher than in the Boston Harbor sediments (Figure 3). The distribution of PAHs in the Latvia sediment was characteristic of the typical signature of diesel or bunker fuel-type petroleum products, i.e., very high levels of anthracene, and relatively high levels of substituted constituents relative to parent compound (e.g. 2-methylnaphthalene vs naphthalene) (Figure 3). This sediment was completely depleted of sulfate at the time of receipt at the laboratory.

Over the course of a 90-day incubation, there was no significant decline in the PAHs of the unamended sediment

<table>
<thead>
<tr>
<th>PAH</th>
<th>Initial PAH level (µmol/Kg dry sediment)</th>
<th>PAH levels and % decline over ca. 1 yr (µmol PAH / Kg dry sediment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>after 105 days</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>436 ± 29</td>
<td>398 ± 24 8%</td>
</tr>
<tr>
<td>1-methylnaphthalene</td>
<td>54 ± 11</td>
<td>30 ± 6 45%</td>
</tr>
<tr>
<td>2-methylnaphthalene</td>
<td>99 ± 10</td>
<td>77 ± 6 22%</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>132 ± 14</td>
<td>35 ± 2 74%</td>
</tr>
<tr>
<td>Fluorene</td>
<td>112 ± 19</td>
<td>51 ± 5 54%</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>231 ± 30</td>
<td>131 ± 13 43%</td>
</tr>
<tr>
<td>Anthracene</td>
<td>293 ± 55</td>
<td>269 ± 16 8%</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>970 ± 70</td>
<td>986 ± 69 0%</td>
</tr>
<tr>
<td>Pyrene</td>
<td>1,015 ± 112</td>
<td>1,097 ± 70 0%</td>
</tr>
<tr>
<td>Benz[a]-anthracene</td>
<td>301 ± 63</td>
<td>325 ± 23 0%</td>
</tr>
<tr>
<td>Chrysene</td>
<td>385 ± 41</td>
<td>389 ± 27 0%</td>
</tr>
<tr>
<td>Benzo[b]-fluoranthene</td>
<td>258 ± 19</td>
<td>260 ± 19 0%</td>
</tr>
<tr>
<td>Benzo[k]-fluoranthene</td>
<td>203 ± 17</td>
<td>206 ± 10 0%</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>330 ± 27</td>
<td>302 ± 22 8%</td>
</tr>
</tbody>
</table>

TABLE 1. Incremental Decline (over 11 Months) of in Situ PAHs in Microcosms of Anoxic, Coal Tar-Contaminated Boston Harbor Sediment Maintained with Adequate Sulfate To Support Respiration in Sulfate-Reducing Bacteria.
samples. However, there was a marked loss of naphthalene and 2-methylnaphthalene when the sediments were supplemented with sufficient gypsum to elevate pore water levels to approximately 25 mM sulfate or with artificial brackish water with 14 mM sodium sulfate (Figure 3). Ninety percent (90%) of the naphthalene pool and 60% of the 2-methylnaphthalene pool were depleted by 90 days when these provisions for terminal electron acceptor were made.

Discussion
This study demonstrates for the first time that in situ pools of PAHs in highly petroleum-contaminated marine sediments can be degraded under in situ-like, sulfate-reducing conditions. This study also provides the first evidence for the degradation of large molecular weight PAHs, such as chrysene and benzo[a]pyrene, under strict anaerobic, sulfate-reducing conditions. These results suggest that anoxic contaminated harbor sediments—which are environments where sulfate reduction is the predominant form of microbial activity—have a much greater potential for self-purification (when sulfate is available) than was previously considered.

Failure of Studies with [14C]-PAHs To Accurately Predict Degradation of in situ Pools. Earlier studies with [14C]-PAHs were instrumental in first demonstrating the potential for
the anaerobic oxidation of PAHs coupled to sulfate reduction in contaminated harbor sediments (12, 16, 17). Measuring the conversion of [14C]-PAHs to 14CO2 has several technical advantages over measuring the loss of in situ PAH pools over time. Analysis of 14CO2 production is easier than extracting and measuring in situ PAHs, and it is possible to measure even low levels of PAH mineralization with [14C]-PAHs because of the high sensitivity in detecting 14CO2. However, the results presented here demonstrate that although measuring the conversion of [14C]-PAHs to 14CO2 may be an acceptable technique for determining whether sediments have the potential to anaerobically degrade PAHs, this technique does not provide a reliable estimate of the rate that in situ pools are being degraded. In the Boston Harbor sediments the [14C]-PAH technique greatly overestimated the rate that the detectable in situ naphthalene pool was being degraded, whereas, at least in this study, the rate of [14C]-phenanthrene mineralization was roughly similar to the rate of detectable in situ phenanthrene degradation.

There are several potential reasons why the rates of degradation of the added [14C]-PAHs might not correspond with the rate of degradation of in situ pools. Studies with aerobic soils and sediments have suggested that [14C]-PAHs added to sediments may be more available for microbial degradation than the PAHs that had been previously introduced into the sediments as the result of contamination (27–30, 40–42). This may be because the added [14C]-PAHs do not equilibrate with the PAH pools that are sorbed onto particles or occluded in tight pore spaces (27).

Degradation of in situ Contaminant PAHs. The results suggest that the in situ pools of PAHs in coal tar-contaminated Boston Harbor can be degraded under the conditions found within the sediments. In these studies it was necessary to rely on laboratory incubations in order to monitor a discrete sample of sediment over time. However, the sediments were maintained under conditions that represented those found in situ. The sediment was not diluted, slurried, or agitated during the incubation, and the incubation temperature was maintained at 16°C, an ordinary in situ temperature during mild weather in Boston Harbor. Natural seawater was the only source of sulfate. The only PAHs in the sediments were those that were present at the time of collection. Other potential strategies for measuring the in situ degradation of PAHs, such as diagenetic modeling of PAH distributions with depth in the sediment, were not possible because of large uncertainties about the historical rate of PAH inputs into these sediments.

As has previously been observed in studies on aerobic degradation of PAHs (7, 8), in general, the rate of sulfate-dependent PAH degradation in this study was related to the size of the PAHs. Smaller PAHs were degraded more rapidly than larger PAHs. This could be due to preferential metabolism of small congeners in a mixture first (43, 44)—degradation of most of the HMW PAHs did not begin until after 100 days which was concurrent with a slowing of the rate of the LMW PAHs (Table 1). The only exception was naphthalene (in the Boston Harbor sediment) which was degraded more slowly than all but the largest PAHs. This result is surprising as, extrapolating from studies on aerobic degradation (4, 7, 45, 46), it might be expected that naphthalene would be degraded the most rapidly. Furthermore, naphthalene was the most rapidly degraded PAH in the sulfate-amended Latvia sediments and [14C]-naphthalene was degraded faster in the Boston Harbor sediments than was [14C]-phenanthrene. The reasons for the relatively slow degradation of the in situ naphthalene pool in Boston Harbor sediments are not clear and require further investigation. Obviously, structural simplicity and solubility (e.g., 32 mg L−1 for naphthalene; 1.3 mg L−1 for phenanthrene) do not alone determine which substrates are most readily available to microorganisms in this complex environment. The striking difference in the vulnerability of naphthalene to rapid degradation between the Boston sediment and the Latvia sediment may be due to a variety of factors that could include organic matter sorption (amount as well as type), particle size (i.e. sand, silt, or clay), petroleum fraction (asphaltene vs diesel) of the contaminants, soot content, length of time of exposure, etc. The total organic matter content of the Boston Harbor site (15%) was considerably higher than that of the Liepaja Harbor site (2.5%). The naphthalene that was detected in the Boston Harbor sediment could represent the fraction of naphthalene that was most powerfully sorbed to organic matter after the more labile fraction had long since been degraded in this old-contamination site. Boston and Liepaja Harbors were apparently contaminated by different petroleum fractions (asphaltene vs diesel) as suggested by historical information as well as the signatures of the PAH congener distributions. These results emphasize that with the current level of understanding it is difficult to predict the rate that PAHs will be anaerobically degraded in sediments.

Stimulation of PAH Degradation in Sulfate-Depleted Sediments. The studies with the Latvia sediments suggested that in cases of extreme contamination, the addition of sulfate to the sediments may enhance anaerobic PAH degradation. The supply of sulfate to marine sediments is limited by the rate of sulfate diffusion from the overlying water. In organic-rich marine sediments with high rates of organic matter degradation the rate of sulfate consumption via sulfate reduction may exceed the rate of sulfate supply (47). Under such conditions methane production becomes the predominant terminal electron-accepting process (TEAP). This phenomenon was apparent in the sulfate-depleted Latvia sediments which had high concentrations of organic contamination. (The headspace gases of the nonsulfate-amended control bottles of Liepaja Harbor sediments were as much as 5% methane in contrast to less than 0.2% in the sodium sulfate or gypsum amended treatment bottles [data not shown]).

Previous studies on the anaerobic degradation of benzene have suggested that the degradation of aromatic hydrocarbons is slower under methanogenic conditions than when sulfate reduction is the TEAP (48, 49). The addition of sulfate to the methanogenic zone of a petroleum-contaminated aquifer greatly stimulated the rate of anaerobic benzene degradation (48). In a similar manner, the addition of sulfate to the methanogenic Latvia sediments greatly stimulated the loss of naphthalene and 2-methyl-naphthalene. A major difference in remediating aquifer and marine sediments with sulfate is that sulfate can be added in a dissolved form to groundwater, whereas it is preferable to add sulfate to marine sediments in a less soluble form, such as gypsum, which will specifically sediment the sediments and then be released via dissolution into the sediments over time.

Longer term incubation studies will be necessary to determine whether continued sulfate additions to the Latvia sediments will eventually stimulate the degradation of other PAHs in the sediments. However, the results to date clearly show the potential benefits of adding sulfate to stimulate anaerobic degradation of the PAH parent compounds in heavily contaminated sediments. Such sulfate additions may also be important for potential anaerobic treatment of PAH-contaminated dredge spoils. Dredged sediments are removed from the source of sulfate from the overlying seawater. Maintaining sufficient sulfate levels in dredged sediments might promote anaerobic degradation of PAHs and minimize the toxicity of these wastes.

Implications for Remediation of PAH-Contaminated Harbor Sediments. These studies demonstrate that once petroleum-related inputs of PAHs are stopped, microorganisms naturally living in harbor sediments may remove...
significant quantities of the PAH contamination under anoxic conditions. Even large PAHs, such as chrysene and benzo(a)pyrene, appear to be slowly degraded. However, long-term incubations on the order of several years will be necessary in order to fully assess the rate and extent of anaerobic degradation of some of the larger PAHs in Boston Harbor sediments as well as naphthalene which was only slowly degraded. Similar studies should be extended to other harbor sediments, as even a superficial comparison of the Boston Harbor and Latvia sediments suggests that rates of anaerobic, sulfate-dependent PAH degradation may vary greatly based upon sediment type and the source of the PAHs. Further studies should also be conducted to consider the possible formation of toxic or dead-end products from incomplete PAH metabolism (50, 51) and to determine whether well-weathered (e.g. soot-bound) and/or lower concentrations of PAHs can be degraded. Although such studies are laborious and time-consuming, at present they provide the best estimate of the rate of sediment self-purification of PAH contamination.

Acknowledgments

We would like to thank Alia Black and Erika Condon for their considerable assistance with that “laborious and time consuming” work that made this study possible and the Office of Naval Research for funding of this project as well as provision of the sediments from Liepaja Harbor, Latvia.

Literature Cited