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Rates of Microbial Transformation of Polycyclic Aromatic Hydrocarbons in Water and Sediments in the Vicinity of a Coal-Coking Wastewater Discharge†

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To facilitate predictions of the transport and fate of contaminants at future coal conversion facilities, rates of microbial transformation of polycyclic aromatic hydrocarbons were measured in stream water and sediment samples collected in the vicinity of a coal-coking treated wastewater discharge from November 1977 through August 1979. Six radiolabeled polycyclic aromatic hydrocarbons were incubated with sediment and water samples; $^{14}CO_2$, cell-bound ^{14}C, and polar transformation products were isolated and quantified. Whereas $^{14}CO_2$ and bound ^{14}C were major transformation products in sediment assays, soluble polar ^{14}C dominated transformation in water samples. Mean rate constants (measured at 20°C) in sediments collected downstream from the effluent outfall were 7.8×10^{-2} h^{-1} (naphthalene), 1.6×10^{-2} h^{-1} (anthracene), and 3.3×10^{-3} h^{-1} [benz(a)anthracene], which corresponded to turnover times of 13, 62, and 300 h, respectively. No unequivocal evidence for transformation of benzo(a)pyrene or dibenz(a,h)anthracene was obtained. Only naphthalene and anthracene transformations were observed in water samples; rate constants were consistently 5- and 20-fold lower, respectively, than in the corresponding sediment samples. The measured rate constants for anthracene transformation in July 1978 sediment samples were not related to total heterotroph numbers. In late July 1978, the effluent was diverted from the primary study area; however, no differences were observed either in transformation rate constants or in the downstream/upstream sediment rate constant ratio. These results are consistent with the hypothesis that continuous inputs of polycyclic aromatic hydrocarbons result in an increased ability within a microbial community to utilize certain polycyclic aromatic hydrocarbons. However, because transformation rates remained elevated for more than 1 year after removal of the polycyclic aromatic hydrocarbon source, microbial communities may shift only slowly in response to changes in polycyclic aromatic hydrocarbon concentrations.

Commercial production of synthetic fuels from coal has been proposed as one means of alleviating the present shortage of liquid hydrocarbon fuels in the United States. A large-scale coal conversion facility would almost certainly cause some degree of contamination of local surface waters due to site runoff, product spillage, or discharge of treated effluents. One class of contaminants produced during coal conversion which is anticipated to be of major concern is the polycyclic aromatic hydrocarbons (PAH) (9). Several PAH are potent carcinogens (4), and their continuous presence at trace levels in surface waters may constitute a chronic human health hazard. PAH are found in effluents from such high-temperature industrial processes as coal coking and petroleum refining (1) and have been detected at microgram per liter concentrations in condensate samples from several pilot-scale coal conversion facilities (15, 21).

Any assessment of the potential hazards to humans from waterborne PAH resulting from coal conversion facilities requires knowledge concerning the transport and fate of PAH in surface waters downstream from such facilities. An environmental process which may be of importance in determining the fate of PAH is microbial transformation, both in sediments and in the water column. The ability of some microorganisms to degrade PAH has been known for 30 years (22), and there have been reports which have quantitated degradation of PAH by enriched cultures (23, 24). However, few measurements of PAH transformation rates in sediments or natural waters have been reported. Moreover, PAH transformation rates in a relatively highly PAH-contaminated environment may differ

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substantially from rates measured in less contaminated systems (7).

Because untreated coal-coking wastewater is qualitatively and quantitatively similar to untreated coal conversion wastewater (17) and because PAH have been found in elevated concentrations in sediments downstream from coal-coking facilities (2), a coke plant was selected as being the most likely existing surrogate for a future coal conversion facility. Therefore, the objectives of this study were (i) to measure rates of microbial transformation of representative PAH in water and sediments in the environment receiving effluents from a coal-coking plant, and (ii) to assess correlations of PAH levels with rates of PAH transformation.

MATERIALS AND METHODS

Site description. More than 70 by-product coke plants were evaluated to find a suitable surrogate for a coal conversion facility; criteria included effluent volume, efficacy of wastewater treatment, length of continuous discharge, and hydrological simplicity and water quality of the receiving river (6). The site selected was the Bethlehem Steel Corp. coke plant in Bethlehem, Pa., which is one of the five largest coke plants in the United States (14). Approximately 9×10^5 liters of highly contaminated wastewater per day is treated by an activated sludge unit which removes phenols and cyanide before discharge. This unit was one of the first of its kind and it has been operating continuously with high efficiency since the early 1960s (12).

Until late July 1978, the treated coke effluent was diluted and discharged at a rate of 0.25 m^3/s into Saucon Creek, a shallow, rapidly flowing stream about 15 m wide and 30 cm deep (average flow rate, approximately 2.5 m^3/s) which originates as groundwater 15 km upstream. A transient outfall of the Bethlehem Municipal Wastewater Treatment Plant is located 1.2 km below the coke outfall. A blast furnace slag pile approximately 50 m high lines the eastern bank 50 to 100 m from the stream. With the exception of a small town (Hellertown) 3 km upstream from the outfall, the region upstream is rural. Water quality in Saucon Creek above the effluent discharge is generally high, although zinc concentrations are elevated as a result of zinc mine drainage 15 km upstream (3). Sediments are predominantly gravel and coarse sand, with pockets of silt.

On 28 July 1978, a diversion pipeline parallel to Saucon Creek was completed by the Bethlehem Steel Corp., and the entire flow of the coking effluent was diverted from the creek and discharged directly into Lehigh River 10 m east of the Saucon Creek-Lehigh River confluence.

Sample collection. Sediment samples were collected on the following seven dates: 10 October 1977, 30 January 1978, 25 April 1978, 18 July 1978, 29 August 1978, 28 November 1978, and 31 July 1979. Water samples were obtained on the last five dates. Samples were collected at an upstream control site (Fig. 1, site 1) and at three downstream locations (sites 4, 6, and 7). Sediments were collected by scraping the upper 2 cm into acetone- and water-washed, 200-ml, glass, wide-mouthed bottles. Each sample was sieved to remove objects larger than 6.25 mm and was held at 4°C. Water samples were collected directly in 200-ml bottles. Samples were shipped in a refrigerated container to Oak Ridge National Laboratory by air express and were processed within 48 h of collection. Additional sediment samples (200 g) were frozen (−78°C) for later PAH analysis; 3.8- or 7.6-liter water samples for PAH measurements were sterilized by adding chloroform (40 ml) and were held at 4°C during shipment to Oak Ridge National Laboratory.

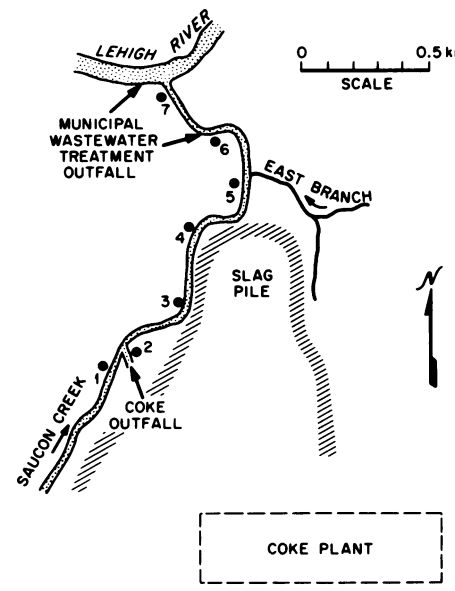

FIG. 1. *Coking plant study area in Bethlehem, Pa., showing sampling locations 1 through 7.*

PAH analysis. The procedures used for PAH analysis have been described in detail elsewhere (5). Sediment portions (40 g) were thawed, spiked with 0.05 μg of ^{14}C-labeled benzo(a)pyrene, and extracted for 48 h with acetone in a Soxhlet apparatus. Acetone was replaced with cyclohexane under nitrogen, and the extract was purified by florasil and alumina column chromatography. Concentrations of PAH were determined by gas chromatography, using a Perkin-Elmer 3920 instrument equipped with a flame ionization detector and a 3% Dexsil 400 column (3 m; 80/100-mesh HP Chromosorb G support; 110 to 320°C; 2°C/min). Compounds were identified by cochromatography and gas chromatography-mass spectrometry. Water samples were spiked with 10^4 dpm of [^{14}C]benzo(a)pyrene and were extracted twice with 200-ml portions of methylene chloride, which were reduced in volume and purified similarly by column chromatography; PAH were quantitated by gas chromatography. Measured PAH concentrations were corrected for recovery of [^{14}C]benzo(a)pyrene, which generally exceeded 80% in sediments and 60% in water samples.

Microbial transformation rate assays. The radiolabeled PAH used were obtained commercially

(American Radiochemical and Amersham Corp.) and included [1-^{14}C]naphthalene (specific activity, 2.00 × 10^8 Bq/mmol), [9-^{14}C]anthracene (1.25 × 10^9 Bq/mmol), [5,6-^{14}C]benz(a)anthracene (BA) (2.22 × 10^9 Bq/mmol), [7,10-^{14}C]benzo(a)pyrene (BP) (9.8 × 10^8 Bq/mmol), [7-^{14}C]dibenz(a,h)anthracene (DBA) (1.06 × 10^9 Bq/mmol), and [12-^{14}C]-7,12-dimethylbenz(a)anthracene (DMBA) (8.2 × 10^8 Bq/mmol). Each PAH was dissolved in several milliliters of acetone; each stock solution (except [^{14}C]naphthalene) was purified at intervals of several months by thin-layer chromatography on silica gel, using toluene as the eluant. All work was performed under gold fluorescent lighting (cutoff, 450 nm) to reduce photolytic decomposition of the PAH.

Sediment samples were assayed for PAH transformation rates as described previously (19). Each sediment sample was mixed well with a glass rod; portions (0.5 ± 0.05 g) were weighed and placed into acetone- and water-rinsed 20-ml glass scintillation vials. Selected vials were autoclaved at 122°C for 30 min to serve as controls. Sufficient radiolabeled PAH dissolved in acetone were added to result in total activities of approximately 5 × 10^4 dpm per vial (final PAH concentrations, approximately 1 μg/g of sediment); no more than 2 μl of acetone solution per vial was required. Glass cups containing KOH were added, and vials were tightly sealed and held in a constant temperature incubator. The incubation temperature was 21 ± 1°C for all samples except those collected in April 1978, which were incubated at 15°C.

The kinetics of transformation of anthracene and BA were measured in October 1977 sediment samples from sites 1, 2, and 6 by incubation of replicate vials for 1, 2, 3, 5, and 24 h; replicate sterilized control vials were incubated for 1, 2, and 24 h. In all other studies two samples and two sterilized control vials were sacrificed after incubations of 4 h (naphthalene), 24 h (anthracene), or 72 or 96 h (BA, BP, DBA, and DMBA). Incubation times were selected from preliminary studies so that they resulted in transformation of 5 to 30% of the ^{14}C-labeled PAH added.

At each termination time, microbial activity was halted by adding 5 ml of acetone; $^{14}CO_2$ was liberated by adding HNO_3, trapped in KOH, and quantitated by liquid scintillation counting. Each sediment sample was extracted with acetone in a micro-Soxhlet unit; bound ^{14}C in sediments was measured by combustion of the sediment residues (Packard Tri-Carb Sample Oxidizer) after extraction. Extracts were concentrated under nitrogen and applied to silica gel thin-layer plates, which were developed in toluene; fractions at the origin and solvent front were isolated by scraping, and ^{14}C was quantitated by liquid scintillation. Preliminary studies (19) demonstrated that essentially all of the ^{14}C-labeled transformation products remained at the origin, whereas ^{14}C-labeled PAH eluted near the solvent front. For each fraction ($^{14}CO_2$, bound ^{14}C, polar ^{14}C, and unaltered PAH), the amount of ^{14}C was expressed as a percentage of the amount of ^{14}C initially added as ^{14}C-labeled PAH.

To test the efficacy of thin-layer chromatograms in separation of ^{14}C-labeled BP transformation products, acetone extracts of sterile and nonsterile sediments from site 4 (July 1978) were reduced in volume under nitrogen, and acetone was replaced with methylene chloride. The radiolabeled compounds in the extracts were separated by reverse-phase high-performance liquid chromatography, using established techniques (20); fractions of column eluant were collected, and ^{14}C levels were determined by liquid scintillation counting.

The procedures for measuring transformation rates in water samples were similar to those used in earlier studies with batch cultures (8). Portions (5 ml) of a water sample (or sterilized control) were added to acetone- and distilled water-rinsed scintillation vials. Approximately 5 × 10^4 dpm of ^{14}C-labeled PAH stock solution was added to each vial with gentle mixing, a KOH trap was added, and the vial was sealed tightly. After 24 h (naphthalene), 48 h (anthracene), or 96 h (all other PAH) microbial activity was halted by adding 0.1 ml of 1 N HNO_3 to two nonsterile and two sterile vials, which were then capped tightly for 1 h before KOH traps were removed. Each water layer was filtered (Reeve-Angel 984 H glass fiber filter; nominal particle retention diameter, 0.4 μm); the filtrate was extracted twice with ethyl acetate, and the ^{14}C remaining in the water was quantified by liquid scintillation. Each filter was rinsed twice with ethanol; the ^{14}C bound in cells was measured by direct liquid scintillation counting of the filter in a dioxane-based scintillation cocktail. Ethanol and ethyl acetate extracts were combined and replaced with heptane, which was reduced to 2 ml under nitrogen; the amount of ^{14}C in a 0.4-ml portion was determined by liquid scintillation counting. The remainder was applied to a silica gel thin-layer plate, which was developed in toluene, and the ^{14}C at the origin and the solvent front was quantitated as described above for the sediment rate assays. Bound ^{14}C, $^{14}CO_2$, water-soluble ^{14}C, and thin-layer chromatography-separable polar ^{14}C fractions were expressed as percentages of the ^{14}C added initially as PAH. Tests with sterile sediment and water samples amended with ^{14}C-labeled PAH and $NaH^{14}CO_3$ demonstrated the completeness of the solvent extraction and $^{14}CO_2$ trapping procedures, respectively.

Microbial transformations of [^{14}C]anthracene were measured at all sites samples. Transformations of all other PAH compounds were measured only in site 4 samples.

Enumeration of microbial populations. Sediment samples were also collected by G. S. Sayler (Department of Microbiology, University of Tennessee) by scraping to a 2-cm depth at three locations on a transect at each of sites 1, 3, and 7 on 18 July 1978. The number of total viable colony-forming units was determined by plating serial dilutions of a sediment suspension on yeast extract peptone glucose agar and counting colonies after 14 to 28 days of incubation at 25°C. Phenanthrene-utilizing microorganisms were enumerated similarly after serial dilutions on silica gel plates containing 10% reagent-grade phenanthrene (18). Plates containing between 30 and 300 colonies were counted; at least three plates were counted for each transect location.

RESULTS

PAH concentrations in water and sediments. Table 1 shows the levels of representative PAH in sediment and water samples col-

lected during July 1978 from several sites in Saucon Creek. Similar concentrations were observed in April 1978 sediment and water samples. No PAH were detected in water samples from sites 1, 4, and 7 in either August 1978 or November 1978 (detection limit, approximately 0.03 μg/liter). Low PAH concentrations in site 1 sediment were attributable to background levels (possibly from atmospheric deposition), whereas concentrations at sites 4, 6, and 7 were elevated, apparently due to the coke plant wastewater discharge. Sediment concentrations at all sites were somewhat lower in August and November samples, but variability among samples was large, possibly due to differing sediment organic carbon contents and particle size distributions. In July 1978, concentrations in sediments downstream from the effluent outfall exceeded waterborne concentrations by factors of 10^4 to 10^5.

Transformations of added ^{14}C-labeled PAH in sediments. Mean recoveries of added ^{14}C ranged from 88% (naphthalene) to 103% (DBA) in sediment assays. Mean recoveries for sterile controls did not differ significantly from recoveries for nonsterile samples.

Nonextractable bound ^{14}C was the major transformation product of anthracene in sediments, averaging 90% of the total transformed anthracene in 18 assays. The quantities of $^{14}CO_2$ and bound ^{14}C were approximately equal in most assays of naphthalene and BA. In the assays where transformation products were detected in BP, DBA, and DMBA, bound ^{14}C usually exceeded $^{14}CO_2$ evolution.

As observed previously (19), the accumulation of ^{14}C-labeled polar metabolites separable by thin-layer chromatography was not quantitatively significant in anthracene or BA assays. Polar transformation products of BP and DMBA varied widely and sometimes were quantitatively less in nonsterile sediments than in sterile controls, apparently due to PAH oxidation on the thin-layer plates (10). Separations of extracts of sterile and nonsterile sediments incubated with BP by using high-performance liquid chromatography confirmed the absence of polar metabolites produced during incubation, although several polar compound peaks present in the chromatogram of the sterile control extract appeared to be reduced in size in the chromatogram of the nonsterile sample (Fig. 2). Thus, calculated sediment transformation rate constants were based on formation of $^{14}CO_2$ and bound ^{14}C only.

As observed previously in petroleum-contaminated sediments (19), the appearance of transformation products in October 1977 sediments from sites 1, 2, and 6 occurred without a lag period, and the accumulation of these products continued at a linear rate for 24 h (Fig. 3). Transformation rate constants were calculated for all sampling periods with the assumption that degradation proceeded as a first-order process without a lag period.

Correlations among relative quantities of bound ^{14}C, polar ^{14}C, and $^{14}CO_2$ were tested for each compound for all sediments assayed. Bound ^{14}C and $^{14}CO_2$ were significantly correlated for naphthalene ($r = 0.803$, $n = 6$) and BA ($r = 0.905$, $n = 7$), but not for anthracene ($r = 0.382$, $n = 23$; critical $r_{0.05} = 0.404$). Polar ^{14}C did not correlate significantly with either bound ^{14}C or $^{14}CO_2$ for any compound.

The sediment rate constants for site 4 are shown in Fig. 4. The mean rate constants calculated before and after the diversion did not differ significantly for any of the four compounds. The geometric means of the rate con-

TABLE 1. *Mean concentrations of replicate determinations of several PAH in water and sediment samples collected from Saucon Creek in July 1978*

PAH	Concn in:			
	Water (μg/liter)		Sediment (μg/g, dry wt)	
	Site 1	Sites 4, 6, and 7	Site 1	Sites 4, 6, and 7
Anthracene	BD[a]	BD	0.14 (0.15)[b]	3.4 (2.1)
Fluoranthene	BD	0.58 (0.09)	3.0 (3.6)	19 (17)
Pyrene	BD	0.39 (0.08)	2.3 (2.9)	12 (11)
BA	BD	0.11 (0.08)	1.4 (1.5)	6.9 (7.2)
BP	BD	0.04 (0.02)	0.33 (0.23)	4.8 (5.7)
Dibenzanthracenes	BD	BD	BD	12 (10)

[a] Below detection limit (0.03 μg/liter of water or 0.03 μg/kg of sediment).

[b] Numbers in parentheses are standard deviations.

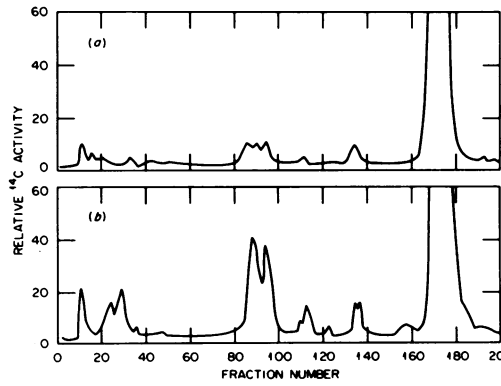

FIG. 2. *High-performance liquid chromatographic elution profiles of extracts of ^{14}C-labeled BP incubated for 96 h with nonsterile sediment (a) and sterile sediment (b) (site 4). The major peak at fractions 170 to 180 corresponds to unaltered BP.*

stants for all sample periods were as follows: naphthalene, 7.8×10^{-2} h^{-1}; anthracene, 1.6×10^{-2} h^{-1}; BA, 3.3×10^{-3} h^{-1}; BP, 3.4×10^{-4} h^{-1}. The calculated mean turnover times (1/rate constant) for the four compounds were as follows: naphthalene, 13 h; anthracene, 62 h; BA, 300 h; BP, 2,900 h (approximately 120 days). Of the four rate constants measured for DBA (January, April, August, and November 1978), only one (1.2×10^{-4} h^{-1}, August 1978) differed significantly from zero; the turnover time for that rate constant was 5,800 h (240 days). The only DMBA rate constant measured (July 1978) corresponded to a turnover time of 3,600 h (150 days). Although the rate constant patterns at site 4 appeared to be similar (Fig. 4) for naphthalene, anthracene, and BA, only naphthalene and anthracene were significantly correlated ($r = 0.900$, $n = 6$, $P < 0.01$).

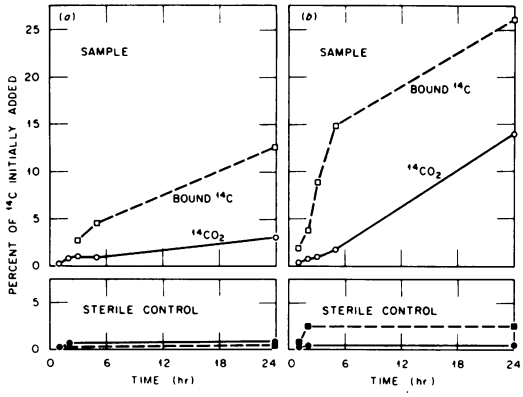

FIG. 3. *Appearance of $^{14}CO_2$ (○ and ●) and bound ^{14}C (□ and ■) from [^{14}C]anthracene incubated with sterile and nonsterile sediments from site 1 (a) and site 2 (b) (October 1977) at 21°C.*

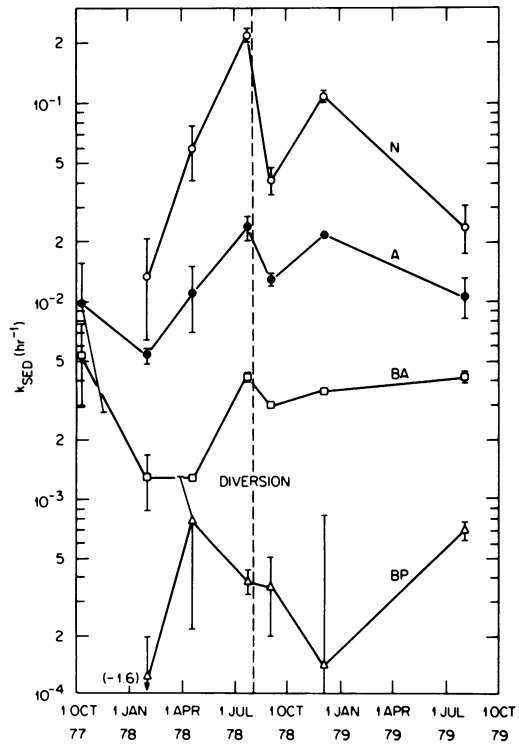

FIG. 4. *Sediment rate constants (k_{SED}) for transformation of ^{14}C-labeled PAH in sediments collected downstream from the coking outfall. All sediments were from site 4, except the October 1977 and January 1978 samples, which were from site 6. Vertical bars represent the standard errors of replicate determinations. A, Anthracene; N, naphthalene.*

The rate constants for anthracene transformation at sites 1, 4, 6, and 7 for all sample periods are shown in Fig. 5. To examine the effect of the outfall diversion in late July 1978 on the rate of anthracene degradation in sediments, the rate data were subdivided into prediversion (October 1977 and January, April, and July 1978) values and postdiversion (August and November 1978 and August 1979) values. Downstream sites 4, 6, and 7 were assumed to be replicates at each sample time to permit estimation of within-site variance. The within-site variance was assumed to be independent of the season and the presence or absence of the outfall and was assumed to be equal at all sites. After logarithmic transformation of the rate constants to equalize variances, the within-site variance was estimated as the pooled mean of the weighted variances for sites 4, 6, and 7 for the April 1978, July 1978, August 1978, and August 1979 sampling periods ($s^2 = 0.0270$). Two-sided t tests with 5 degrees of freedom were then used to compare the rate constants from site 1 with the means of the rate constants from sites 4, 6, and 7 before and after diversion. Three comparisons were tested, with the following results. (i) Upstream and downstream rate constants differed significantly before diversion ($t = 5.09$, $P < 0.01$). (ii) Upstream and downstream rate constants differed significantly after diversion ($t = 4.76$, $P < 0.01$). And (iii) The difference between upstream and downstream rate constants before diversion was statistically the same as after diversion ($t = 0.28$). Mean downstream values of the rate constant for anthracene were 4.1 and 3.7 times greater than the upstream values of this rate constant before and after diversion, respectively.

Transformation of added ^{14}C-labeled PAH in water samples. Recoveries of ^{14}C were lower for each compound in water samples than they were in sediment assays; mean recoveries ranged from 52 to 87%. The large difference in mean recoveries of naphthalene between samples (52%) and sterile controls (69%) suggested

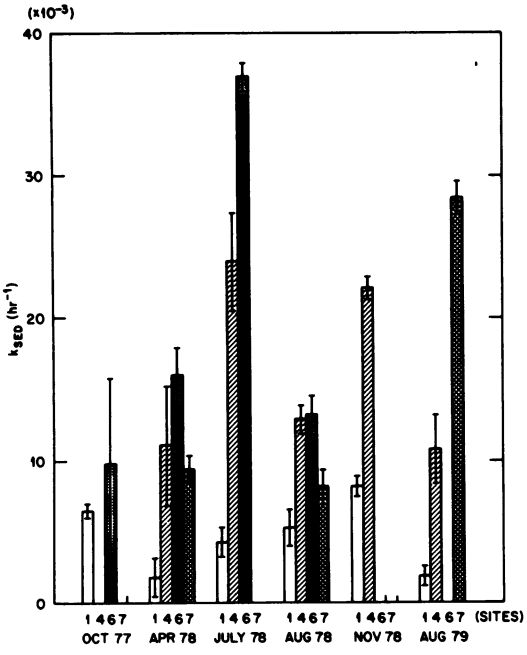

FIG. 5. *Sediment rate constants (k_{SED}) for [^{14}C]anthracene transformation in Saucon Creek sediments. The vertical bars represent the standard errors of replicate determinations. Where bars are not drawn, no rate constants were determined.*

that incomplete recovery of transformation products may have occurred in addition to volatilization (and perhaps sorption) losses.

In contrast to sediment assays, polar ^{14}C was the dominant anthracene transformation product fraction in water samples and averaged 82% of the total transformed ^{14}C. Despite the relatively large variability in controls, the quantity of polar ^{14}C-labeled metabolites was significantly greater than zero in 10 anthracene assays; however, neither the quantity of polar ^{14}C nor the quantity of total transformed ^{14}C was different from zero in any BA, BP, DBA, or DMBA assay.

All three transformation product fractions (polar ^{14}C was not measured due to the volatility of ^{14}C from thin-layer plates) in the five naphthalene assays were significantly correlated; the correlation coefficients for filter-bound ^{14}C and $^{14}CO_2$, filter-bound ^{14}C and water-soluble ^{14}C, and water-soluble ^{14}C and $^{14}CO_2$ were 0.964, 0.954, and 0.995, respectively. In anthracene assays only water-soluble ^{14}C and thin-layer-separable polar ^{14}C were significantly correlated ($r = 0.631$, $n = 20$).

In water samples, transformation of naphthalene was observed at all sampling times at site 4; the mean of log-transformed rate constants was 3.2×10^{-3} h^{-1}, resulting in a turnover time of 310 h (13 days). However, because some losses of transformation products apparently occurred, the calculated rate constants for naphthalene may have underestimated true values. At no time did the transformation rate constants for anthracene in water samples from site 1 differ significantly from zero. The log-transformed mean for sites 4, 6, and 7 over all sampling periods was 2.0×10^{-3} h^{-1}, corresponding to a turnover time of 500 h (21 days). Transformations of BA, BP, DBA, and DMBA were not detectable.

Before the effluent diversion, similar patterns of anthracene transformation activity in Saucon Creek water samples were observed in April and July 1978 (Fig. 6). Rate constants were essentially zero both immediately upstream and downstream from the effluent, reaching maxima 1.2 to 1.5 km below the outfall. Rate constants measured in July 1978 were two- to sixfold greater at each site than in April 1978. After the diversion, only five downstream (sites 4, 6, and 7) rate constants for anthracene transformation were measured; two of these were significantly nonzero, compared with five of six nonzero rate constants before the diversion. The means of log-transformed rate constants for sites 4, 6, and 7 before and after diversion did not differ significantly ($t = 1.73$, df = 9). Similarly, log-transformed mean rate constants for naphthalene degradation at site 4 did not differ significantly before and after diversion ($t = 0.24$, df = 3).

The rate constants for naphthalene and anthracene in site 4 water samples were strongly correlated ($r = 0.999$, df = 8, $P < 0.01$). If water rate constants for naphthalene were in fact underestimated by a constant factor, the correlation between naphthalene and anthracene would remain the same.

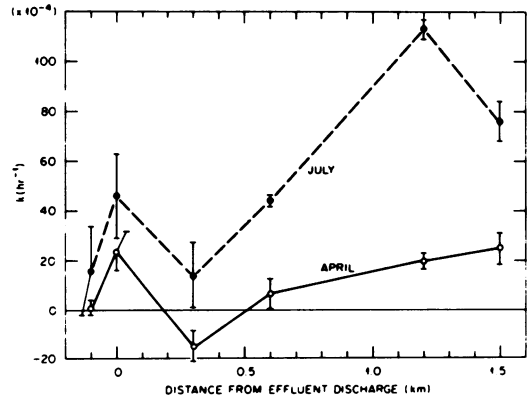

FIG. 6. *Rate constants (k) for [^{14}C]anthracene transformation in Saucon Creek water samples collected in April and July 1978. The vertical bars represent the standard errors of replicate determinations.*

Interrelationships between water and sediment rate constants. Rate constants for naphthalene in water and sediment assays were paired by sampling date; the correlation was significant ($r = 0.868$, $P < 0.05$). A similar pairing of anthracene rate constants by sampling date and location (sites 1, 4, 6, and 7) produced a similar relationship ($r = 0.840$, $P < 0.01$).

Microbial population densities in sediment samples. Means and standard deviations (in parentheses) of triplicate samples for the logarithm of the total viable colony-forming units per gram (dry weight) of sediment during the July 1978 sample period were as follows: site 1, 7.37 (0.43); site 3, 6.22 (0.28); and site 7, 6.31 (0.38). The corresponding values for phenanthrene-utilizing colony-forming units per gram (dry weight) of sediment were as follows: site 1, 5.60 (0.13); site 3, 5.60 (0.25); and site 7, 5.78 (0.67). One-way analyses of variance were used to test for significant differences among log total viable colony-forming unit values and among log phenanthrene-utilizing colony-forming unit values. The $F_{2,6}$ values for these analyses were 9.87 and 0.20, respectively; the former is significant ($P < 0.05$), whereas the latter is not. To determine whether upstream and downstream values for log total viable colony-forming units differed significantly, the mean of sites 3 and 7 was compared with the log total viable colony-forming unit value at site 1. The upstream and downstream values differed significantly ($t = 4.26$, df $= 6$, $P < 0.01$).

DISCUSSION

The relationship between PAH molecular size and transformation rates in both water and sediment was similar to the relationship observed previously in petroleum-contaminated sediment (7); there was a consistent decrease in the rate constant with increasing molecular size. In this previous work, Herbes and Schwall speculated that contamination of sediments with certain PAH may favor development of microorganism populations capable of utilizing those compounds at more rapid rates. The sediment concentrations of the larger PAH were consistently higher downstream from the coke effluent in the present study than in the petroleum-contaminated sediments analyzed previously (7); transformation rate constants for anthracene and BA were also greater by more than 10-fold. Similarly, the anthracene concentration at site 4 in the July 1978 sample exceeded that at site 1 by 1 order of magnitude; the transformation rate constant at site 4 was more than fivefold higher. Therefore, the anthracene and BA concentration and transformation rate data for the July 1978 sediment and the previously analyzed petroleum-contaminated sediments are consistent with the hypothesis that increased levels of PAH contamination in sediments cause enrichment of the microbial community with strains which are capable of transforming the PAH at a relatively rapid rate.

In contrast to PAH levels, which appear to be related to sediment transformation rates, the limited amount of data suggests that sediment microbial population estimates are not related to transformation rates. Whereas the [^{14}C]anthracene transformation rate constants in the July 1978 sediment samples were sevenfold higher at the two downstream locations (sites 4 and 6) than at site 1, phenanthrene-utilizing populations (which presumably utilize anthracene also) were the same size both above and below the outfall. At the same time, total heterotroph numbers were more than 1 order of magnitude greater above the outfall than at the downstream sites. More rapid downstream transformation rates may be due to elevated nutrient levels downstream from the effluent, which contains relatively high concentrations of phosphorus and ammonia; hydrocarbon degradation in lake water has been shown previously to be increased 5- to 20-fold by nutrient addition (25).

Transformation of the five-ring PAH in sediments did not relate directly to sediment concentrations, in contrast to transformation of anthracene and BA. Although $^{14}CO_2$ and bound ^{14}C were formed during site 4 sediment incubations with BP in the present study, the disappearance of ^{14}C-labeled impurities observed in the high-performance liquid chromatogram (Fig. 2) suggests that the impurities, rather than BP, were degraded. Thus, no unambiguous evidence exists for BP transformation in the site 4 sediment, although the BP concentration in July 1978 exceeded the concentration in the petroleum-contaminated sediment analyzed previously by 100-fold. Similarly, virtually no transformation of DBA was observed, although in the July 1978 site 4 sediment the dibenzanthracene concentration (albeit mixed isomers) was similar to the anthracene and BA concentrations. McKenna and Heath (13) have reported cometabolism of these compounds in the presence of naphthalene, which suggests that there are bacteria which can transform them. Khesina et al. (11) found degradation of up to 50% of the BP in soils over a 3-month period, with the most extensive degradation in highly contaminated soil from a refinery. Because in the latter study the BP concentration of the refinery soil was about 10-fold higher than the site 4 July 1978 sediment BP concentration in the present study, the possibility exists that higher concentrations are re-

quired to induce degradation of BP. An alternative explanation for the lack of BP or DBA transformation in sediment is that the five-ring PAH partition onto sediment particles to such an extent that only an extremely small fraction is present in the interstitial water, and thus virtually none is available for uptake and transformation by microorganisms (26, 27).

Although sedimentary and waterborne PAH concentrations downstream from the coking outfall appeared to decrease substantially after the effluent diversion, little effect on microbial PAH transformation rate constants was observed. Perhaps the lack of observable effects was due to slowness of population shifts; although Pierce et al. (16) observed a rapid increase in naphthalene-degrading ability upon exposure of bacteria to naphthalenic hydrocarbons, more than 1 year was required for the proportion of naphthalene utilizers to decline to pre-contamination levels, even though the concentration of aromatics declined by 75% within 8 days. Alternatively, the lack of effect of the diversion may indicate that residual PAH concentrations were sufficiently high to maintain degradative enzyme activities at prediversion levels.

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