APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1997, p. 4759–4764 Vol. 63, No. 12
0099-2240/97/$04.00+0

Carboxylation as an Initial Reaction in the Anaerobic Metabolism of Naphthalene and Phenanthrene by Sulfidogenic Consortia

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Received 7 July 1997/Accepted 22 September 1997

The anaerobic biodegradation of naphthalene (NAP) and phenanthrene (PHE) was investigated by using sediment collected from the Arthur Kill in New York/New Jersey harbor. The initial cultures were composed of 10% sediment and 90% mineral medium containing 20 mM sulfate. Complete loss of NAP and PHE (150 to 200 μM) was observed after 150 days of incubation. Upon refeeding, NAP and PHE were utilized within 14 days. The utilization of both compounds was inhibited in the presence of 20 mM molybdate. [^{14}C]NAP and [^{14}C]PHE were mineralized to $^{14}CO_2$. The activities could be maintained and propagated by subculturing in mineral medium. In the presence of halogenated analogs, 2-naphthoate was detected in NAP-utilizing enrichments. The mass spectrum of the derivatized 2-naphthoate from the enrichment supplemented with both [^{13}C]bicarbonate and NAP indicates the incorporation of $^{13}CO_2$ into NAP. In the PHE-utilizing enrichment, a metabolite was detected by both high-pressure liquid chromatography and gas chromatography-mass spectrometry analyses. The molecular ion and fragmentation pattern of its mass spectrum indicate that it was phenanthrenecarboxylic acid. The results obtained with [^{13}C]bicarbonate indicate that $^{13}CO_2$ was incorporated into PHE. It appears, therefore, that carboxylation is an initial key reaction for the anaerobic metabolism of NAP and PHE. To our knowledge, this is the first report providing evidence for intermediates of PAH degradation under anaerobic conditions.

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment through their presence in coal and petroleum and through their formation during combustion. Some PAHs are mutagenic and/or carcinogenic (3) and are considered priority pollutants; these include naphthalene (NAP), phenanthrene (PHE), and pyrene (23). One of the limitations of PAH biodegradation is the low water solubility of these compounds, e.g., 32, 1, and 0.1 ppm for NAP, PHE, and pyrene, respectively, hence limiting their bioavailability. Many aerobic bacteria capable of PAH degradation have been isolated and characterized (3). Their biochemistry and genes coding for key reactions are actively being investigated (11, 19). Much less is known, however, about their fate in the absence of oxygen. Their presence in anoxic estuarine sediments (18) suggests that little if any degradation takes place.

There is an increasing number of investigations of anaerobic degradation of PAHs, mainly the two-ring PAH NAP. NAP loss was observed under nitrate-reducing conditions (1, 2, 6, 21, 24), under methanogenic conditions (7, 26), and under sulfate-reducing conditions (30). Langenhoff et al. (21) and Coates et al. (4, 5) recently reported that tracer amounts of [^{14}C]NAP were mineralized in sediment columns and sulfate-fed microcosms, respectively. Only the latter study, however, also provided evidence that degradation is coupled to sulfate reduction. At present, no anaerobic transferable enrichment has been reported, nor has an anaerobic PAH-metabolizing pure culture been isolated, and there are no reports indicating possible intermediates or proposed pathways of anaerobic biodegradation of PAHs.

In this report, we describe two highly enriched transferable consortia capable of mineralizing 100 to 200 μM NAP or PHE under sulfate-reducing conditions. We also show that the utilization of NAP and PHE is coupled to sulfate reduction and that carboxylation is an initial key reaction of NAP and PHE metabolism in the absence of oxygen.

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MATERIALS AND METHODS

Culture preparation and PAH stocks. The sediment used in this study was collected from Arthur Kill (AK), in the New York/New Jersey Harbor Estuary, which is heavily contaminated with PAHs and other components due to the large volume of chemical and petroleum commerce within the region (12, 18). To collect the sediment, a gravity core sampler supplied with plastic core liners was used. The cores were then capped with butyl rubber stoppers. They were transported on ice and refrigerated until used.

To prepare the PAH substrate stock, each individual PAH was weighed and dissolved in 2 ml of pentane. The PAH solution was then mixed with weighed and dried AK sediment to reach a final theoretical concentration of 40 μmol per g of dry sediment. The actual concentration in the stock varied somewhat due to loss during preparation. The pentane in the mixture was evaporated under vacuum. The dry sediment containing the sorbed PAH was used as the PAH stock.

The sulfate-reducing medium used for this study has been described previously (27) and was used with minor modifications. The medium contained, per liter of water, 1.0 g of $MgCl_2 \cdot 6H_2O$, 0.11 g of $CaCl_2$, 1.2 g of KCl, 0.27 g of NH_4Cl, 0.20 g of KH_2PO_4, 0.5 mg of resazurin, 2.84 g of Na_2SO_4, 4.2 g of $NaHCO_3$, 18 g of NaCl, 1 ml of the trace element solution, 2 ml of the vitamin solution, and 0.29 g of $Na_2S \cdot 9H_2O$. The trace element solution contained, per liter of water, 3.5 g of nitrilotriacetic acid, 0.062 g of H_3BO_3, 0.098 g of $MnCl_2 \cdot 4H_2O$, 1.5 g of $FeCl_2 \cdot 4H_2O$, 0.12 g of $CoCl_2 \cdot 6H_2O$, 0.24 g of $NiCl_2 \cdot 6H_2O$, 0.13 g of $CuCl_2 \cdot 2H_2O$, 0.068 g of $ZnCl_2$, and 0.024 g of $Na_2MoO_4 \cdot 2H_2O$. The vitamin solution contained, per liter of water, 20 mg of biotin, 20 mg of folic acid, 100 mg of pyridoxine HCl, 50 mg of riboflavin, 50 mg of thiamine, 50 mg of nicotinic acid, 50 mg of pantothenic acid (hemicalcium salt), 1 mg of cyanocobalamin (vitamin B_{12}), 50 mg of *p*-aminobenzoic acid, and 50 mg of thiotic acid. The medium was buffered at pH 7.2 by the addition of 50 mM bicarbonate, which was equilibrated with 30% CO_2–70% N_2. A total of 10% (wet weight) of the sediment was used as the inoculum, and mineral medium made up the remaining volume. Then 0.5 g of the PAH stock and 100 ml of the medium slurry were transferred to serum bottles (160 ml), which were used as culture vessels. The bottles were sealed with Teflon-coated rubber stoppers and aluminum crimp-seals. The cultures were incubated without shaking in the dark at 30°C. Strict anaerobic techniques were used throughout all steps of the culture preparation.

Later subcultures and transfers were made into the same medium with 10% clean sediment collected from a local pond on campus. The medium was steril-

ized by autoclaving for 30 min at 120°C on two consecutive days prior to use. When transfers were made, 30 to 50% of the enrichments was used.

PAH mineralization. [^{14}C]NAP (uniformly labeled) and [^{14}C]PHE (labeled in the 9 position) (Sigma Chemical Co., St. Louis, Mo.) were solubilized and diluted in 100% methanol. The slurry from an active culture was subdivided into six aliquots of 30 ml each in 60-ml serum bottles. Nonradiolabeled PAH was added to a final concentration of 150 μM to all the bottles. A set of three bottles was injected with 4 μl of the [^{14}C]PAH in methanol. The other set of three bottles received 4 μl of methanol and was monitored for the loss of the unlabeled PAH. The added concentrations of [^{14}C]NAP and [^{14}C]PHE were 0.03 and 0.12 μM, respectively. The slurry samples were incubated at room temperature (24 ± 2°C), and incubation was terminated when the nonradiolabeled PAH was reduced to less than 1%.

$^{14}CO_2$ produced from radiolabeled PAH was trapped in the scintillation cocktail (Oxosol C^{14}; National Diagnostics, Atlanta, Ga.) through a CO_2-trapping system (22). The gas phase of a sample bottle was vented through a needle to which tubing was attached and connected to a series of three vials containing the scintillation cocktail. Collection of $^{14}CO_2$ was initiated by the injection of 3 ml of 6 N HCl to the sample bottle prior to flushing with nitrogen gas. The bottle was shaken periodically during nitrogen flushing, which lasted for 20 min. A 0.5-ml aliquot of sediment slurry was then withdrawn and injected into a scintillation vial contained 10 ml of Ready Safe liquid scintillation cocktail for determination of the radioactivity remaining in the sample slurry (Beckman, Fullerton, Calif.).

[^{13}C]bicarbonate test. Under anaerobic conditions, a 60-ml active enrichment was washed with a phosphate-buffered medium (20 mM and pH 6.8) to deplete the HCO_3^- and CO_2 in the original medium. The cells and sediment were centrifuged (GS-6 centrifuge; Beckman) for 10 min at 2,500 rpm. After the supernatant was removed, the phosphate-buffered medium was added to suspend the precipitated sediment by vortexing and hand-shaking. The gas phase was replaced by nitrogen gas (100%). The washing cycle was repeated three times with 100, 100, and 50 ml of the phosphate-buffered medium. The washed slurry culture was then divided into four aliquots of 12 ml of slurry each. Into two of the bottles, [^{13}C]bicarbonate (^{13}C, 99%; Cambridge Isotope Laboratories, Inc., Andover, Mass.) solution was injected to reach a final concentration of 6.3 mM. Into the other two bottles, unlabeled bicarbonate solution was added to the same final concentration of 6.3 mM. One bottle from each set of duplicates was autoclaved for 30 min at 120°C. All the bottles were incubated for 2 days at 30°C, at which time high-pressure liquid chromatography (HPLC) revealed the accumulation of 2-naphthoate (2-NA) in the active cultures. The HPLC detection limit for 2-NA was 0.2 μM.

GC analysis. For routine determination of the PAHs, a 0.75-ml sample of slurry from a well-mixed bottle was pipetted into a 2-ml vial containing 0.75 ml of hexane with 0.4 mM biphenyl as an internal standard. The culture vessel was flushed with anaerobic gas before being resealed. The 2-ml vials containing the samples were placed on their sides and shaken overnight on a shaker. The sample vials were then centrifuged and analyzed by gas chromatography (GC) (5890 series II chromatography apparatus; Hewlett-Packard, Wilmington, Del.) with a flame ionization detector and an autosampler (Leap Technologies, Chapel Hill, N.C.). The GC column was 30 m long and 0.32 mm in inner diameter with phase DBVAX film thickness of 0.25 μm (J&W Scientific, Folsom, Calif.). A 2-μl hexane sample was injected automatically. The injector temperature was 280°C, and the column temperature started at 160°C, increased to 250°C at 25°C/min, and was held at 250°C for 5 min.

HPLC analysis. For the analysis of polar compounds and metabolites, HPLC was performed in an HPLC apparatus (Beckman) with an autosampler (Gilson Medical Electronics, Inc., Middleton, Wis.). A well-mixed 0.5-ml slurry sample was taken and mixed with 0.5 ml of 95% ethanol in a microcentrifuge tube. After vortexing and centrifugation, the supernatant was used for analysis. Methanol, water, and acetic acid in a ratio of either 80:19:1 or 60:38:2 were components of the eluting solvent flowing through a reverse-phase C_{18} column (Ultrasphere C-18 column; Beckman) and UV detector (280 nm).

Metabolite analysis by GC-MS. A slurry culture was centrifuged, and the supernatant was transferred to a 60-ml serum bottle and acidified with 1 ml of 6 N HCl. Then 12 ml of pentane was added to the bottle, which was shaken and centrifuged. The pentane phase was transferred to a 13-ml serum bottle and evaporated under vacuum. The pentane extraction process was then repeated. The crystallized extract was solubilized in 1 ml of pentane, transferred to a 2-ml vial, and dried under vacuum. The extract in the vial was solubilized in either 0.1 ml of pyridine or 0.1 ml of methylene chloride and derivatized with 0.1 ml of BSTFA–0.1% TMS (Sigma Chemical Co.) at 60°C for 30 min. The derivatized sample of 1 to 2 μl was then subjected to GC-mass spectrometry (MS) by being injected into a Hewlett-Packard 5890 series II GC coupled with a mass-selective detector (Hewlett-Packard 591 series). The column for the GC-MS analysis was 30 m by 0.25 mm (inner diameter) with phase DB-5MS film thickness of 0.25 μm (J & W Scientific). The injector temperature was 280°C, and the column temperature started at 80°C for 1 min, increased to 300°C at 10°C/min for the metabolite from NAP or 15°C/min for the metabolite from PHE, and was held at 300°C for 2 min.

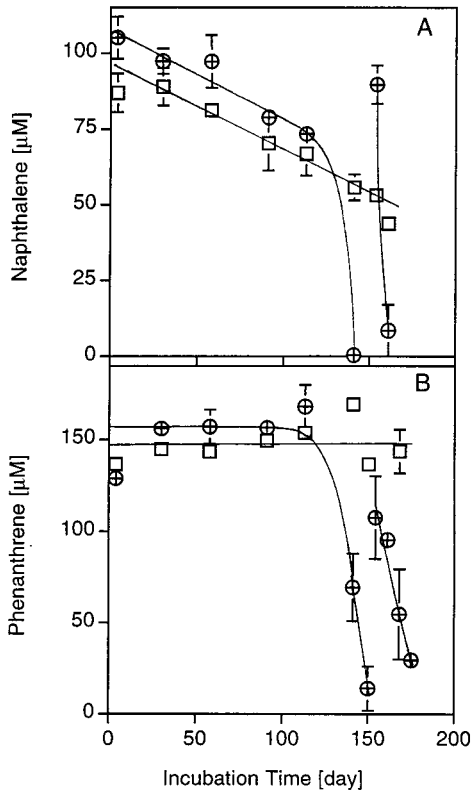

FIG. 1. Initial degradation of NAP (A) and PHE (B) in 10% AK sediment-inoculated, sulfate-reducing enrichments. The slow decline of the NAP concentration in the autoclave controls is due to volatile loss during sampling. Data points represent the means of three replicates for active cultures (⊕) and the means of two replicates for autoclaved controls (□).

RESULTS

Enrichments capable of NAP and PHE degradation were established by inoculating 10% of the AK sediment into sulfidogenic media. Initial loss of NAP or PHE was observed between 120 and 150 days of incubation (Fig. 1). After refeeding, the PAHs were utilized without a lag in less than 14 days (Fig. 1), indicating the establishment of a microbiologically mediated process. Although only one refeeding is illustrated in Fig. 1 for clarity, this could be repeated numerous times in these cultures. The slight loss of NAP in the sterile controls is due to volatilization and loss of NAP vapor during sampling. Routinely, when 30 to 50% of the enrichments was transferred into new mineral medium, every 2 weeks for NAP and every 4 weeks for PHE, activity was maintained.

As shown in Fig. 2, when molybdate, a specific inhibitor of sulfate reduction (25, 29), was amended to a final concentration of 20 mM in the enrichment cultures, metabolism of NAP and PHE was inhibited. NAP and PHE, however, were degraded in less than 20 days in the active cultures without molybdate. Furthermore, in the absence of sulfate, no degradation takes place. As shown in Fig. 3, almost complete loss of NAP was observed after 20 h of incubation in the cultures supplemented with 20 mM sulfate whereas no detectable loss of NAP occurred in the parallel cultures in which sulfate was absent. Metabolism of NAP and PHE, therefore, is coupled to and dependent on sulfate reduction in these mixed consortia.

Mineralization and conversion of NAP and PHE was determined with ^{14}C-radiolabeled substrates. [^{14}C]NAP and [^{14}C]PHE were added to the corresponding enrichments along with

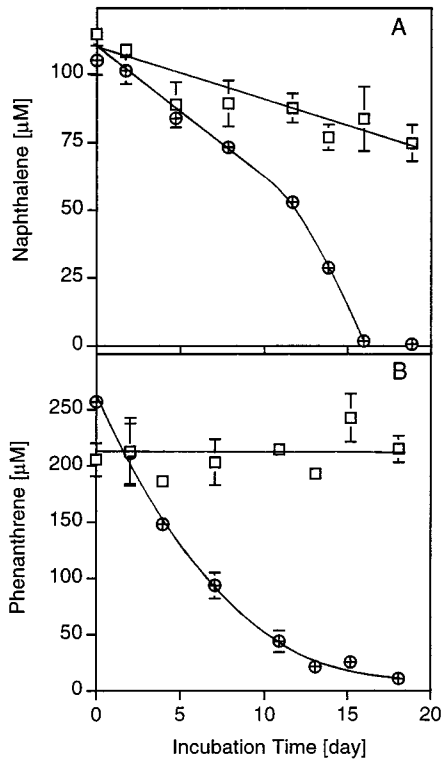

FIG. 2. Effect of molybdate on the degradation of NAP (A) and PHE (B) in active NAP- and PHE-degrading consortia, respectively. The data are means of duplicate samples. Symbols: □, 20 mM molybdate added; ⊕, molybdate not added.

the respective unlabeled PAHs (150 μM). Parallel enrichments fed only unlabeled PAHs (150 μM) were monitored for substrate loss, and the incubation (24°C) was stopped when the concentrations decreased to less than 1%. As seen in Table 1 most of the radioactivity (89 and 92%, for NAP and PHE, respectively) was recovered as carbon dioxide after the slurry samples were acidified and flushed with nitrogen gas. A small portion remained in the enrichments (4.1 and 5.3% for added

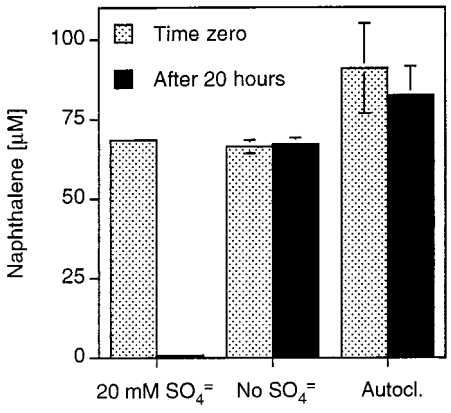

FIG. 3. Utilization of NAP by NAP-degrading consortia in the presence and absence of sulfate. All the consortia were washed three times with sulfate-free medium and then supplemented with sulfate-amended or sulfate-free medium. Results for autoclaved replicates are also shown. Bar graphs represent the means of duplicate samples.

TABLE 1. Mineralization of [^{14}C]PAHs in acclimated, sulfidogenic consortia[a]

PAH tested	Total added radioactivity (dpm)[b]	Amt of radioactivity (dpm):		Total radioactivity recovered (%)
		Recovered as $^{14}CO_2$	Left in slurry	
NAP	107,170	95,671 (89.3%)	4,350 (4.1%)	93.3
PHE	529,076	487,057 (92.1%)	27,709 (5.3%)	97.3

[a] For each PAH, six replicate samples were established and 150 μM unlabeled PAH was added. Radiolabeled PAH was then added to three of the six replicates. Samples without radioactive PAH were analyzed to monitor the progress of the PAH degradation. Incubation lasted 24 and 42 days for NAP and PHE, respectively, at room temperature (24 ± 2°C).

[b] Standard deviations of the reported numbers were within a 2% range (n = 3).

NAP and PHE, respectively) and may be attributed to undegraded substrate or conversion to cell biomass.

To enhance the accumulation of the intermediate(s) of NAP metabolism, a series of NAP analogs was tested in the presence of NAP. As noted in Table 2, three fluorinated benzoates and 1-fluoro-NAP enhanced the accumulation of an unknown compound, which was later identified as 2-NA by GC-MS. The concentration of 2-NA ranged from 0.5 to 6.1 μM. The retention times for 1-NA and 2-NA are 10.16 and 10.41 min, respectively. Other compounds shown in Table 2, as well as 1-chloro-NAP, 1-bromo-NAP, and 2-bromo-NAP, did not lead to the accumulation of any metabolites. The unknown peak was identified as 2-NA by its GC retention time and by comparison to trimethylsilyl (TMS)-derivatized standards. When deuterated NAP was amended in NAP-degrading culture, deuterated 2-NA was formed and identified by GC-MS (data not shown). The rapid and almost complete utilization of 2-NA by NAP-utilizing cultures (Table 2), although not constituting proof, is consistent with its role as an intermediate of NAP metabolism. 1-Naphthol, 2-naphthol, and 1-NA were not readily utilized (Table 2) and were not detected in NAP-degrading cultures.

The formation of 2-NA from NAP strongly suggests that a carboxylation takes place. To confirm this, [^{13}C]bicarbonate was used after removal of the bicarbonate buffer. Figure 4 shows three mass spectra: Fig. 4A is the derivatized 2-NA standard; Fig. 4B is the derivatized 2-NA from the culture supplemented with NAP and [^{12}C]bicarbonate and illustrates the same mass spectrum as the standard; Fig. 4C is from the

TABLE 2. Effect of fluorinated analogues and naphthalene derivatives[a]

Analog	Analog loss (%)	2-NA accumulation
Benzoate	100	−
2-Fluorobenzoate	2	+
3-Fluorobenzoate	0	+
4-Fluorobenzoate	0	+
1-Fluoronaphthalene	0	+
1-Naphthol	7	−
2-Naphthol	4	−
1-Naphthoate	0	−
2-Naphthoate	96	−
NAP	100	−

[a] The concentration of the tested analogs was 100 μM and the NAP concentration was 150 μM. Incubations lasted for 5 to 10 days, at which time NAP in the enrichment cultures fed only NAP was completely metabolized.

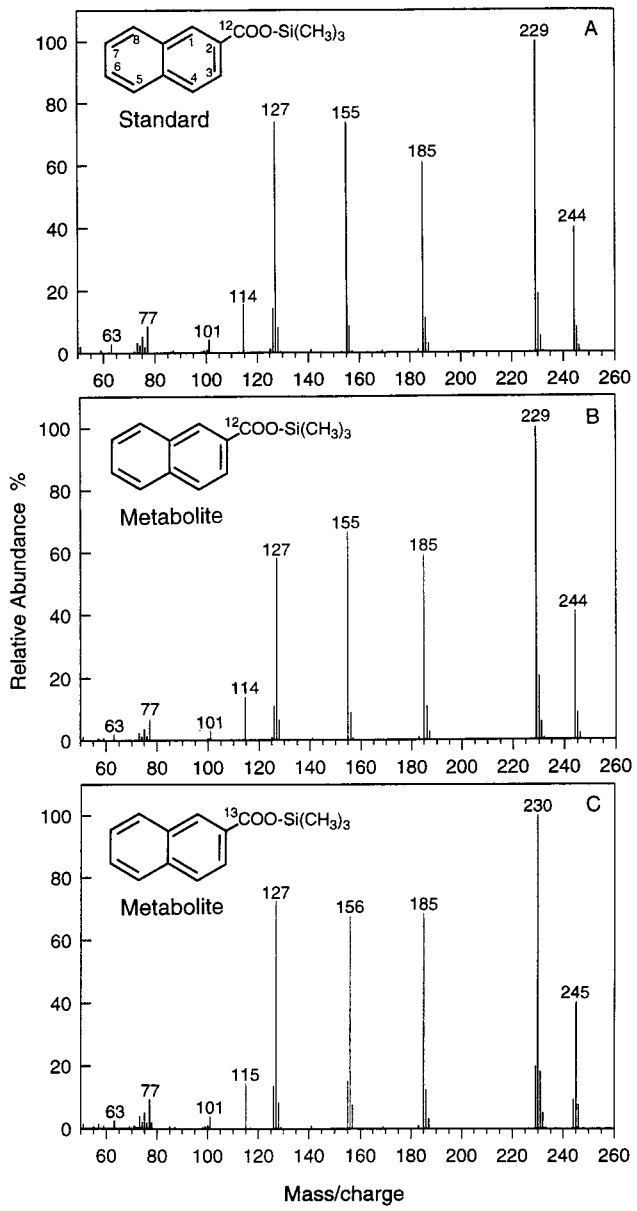

FIG. 4. Mass spectra of trimethylsilyl derivatives of a 2-NA standard (A) and 2-NA extracted from the sample supplemented with NAP and either [^{12}C]bicarbonate (B) or [^{13}C]bicarbonate (C). The mass spectrum of the 2-NA standard (A) contains five major peaks: *m/e* 244, 229, 185, 155, and 127. The *m/e* 244 peak represents the molecular ion. The fragmentation ion of *m/e* 229 is the result of the loss of a $-CH_3$ group (244 − 15 = 229); the fragmentation ion of *m/e* 185 is from the loss of a $-CH_3$ and a $-COO$ group (244 − 59 = 185); the fragmentation ion of *m/e* 155 is from the loss of an $-OSi(CH_3)_3$ group (244 − 89 = 155); and the fragmentation ion of *m/e* 127 is from the loss of a $-COOSi(CH_3)_3$ group (244 − 117 = 127) (28). The identification of the 2-NA metabolites in panels B and C is based on comparison of the GC retention time of the derivatized standard for 2-NA (10.41 min).

culture with NAP and [^{13}C]bicarbonate. As noted in Fig. 4C, the molecular ion of *m/e* 245 and the ions of *m/e* 230 and 156 were all 1 mass unit larger than the corresponding ions of the 2-NA standard (Fig. 4A) and the 2-NA metabolite (Fig. 4B), indicating that the $^{13}CO_2$ was incorporated into NAP. The mass/charge, however, of the ions of *m/e* 185 and 127 are the same in all three panels because the $-^{13}COO$ and $-^{12}COO$ units have been removed from these fragmentation ions (28). The derivatized 2-NA was not detected in the sterilized sample supplemented with NAP plus either [^{12}C]bicarbonate or [^{13}C]bicarbonate, supporting the conclusion that the incorporation of CO_2 into NAP was microbiologically mediated. Some 2-NA with incorporated $^{12}CO_2$ was also detected in the mass spectrum of the derivatized 2-NA from the sample with added [^{13}C]bicarbonate (*m/e* 244, 229, and 166) (Fig. 4C) and is partially due to CO_2-HCO_3^- carryover from the original slurry.

During routine incubation of the PHE-degrading enrichment cultures, a transient peak was detected by HPLC. GC-MS analysis of the derivatized extract from the culture identified the peak as a PHE carboxylic acid (PHE-CA). The standard available at the time was 9-PHE-CA, which had a GC retention time of 19.30 min. For the PHE-CA metabolite identified in the culture, the GC retention time was 19.86 min. Hence, although the metabolite is a PHE-CA and has been confirmed by GC-MS, it is not carboxylated in the 9 position.

Figure 5 illustrates mass spectral data for [^{12}C]PHE-CA (Fig. 5A) and [^{13}C]PHE-CA (Fig. 5B). The derivatized sample extracted from the culture with added [^{13}C]bicarbonate (Fig. 5B) showed a peak with a molecular ion of *m/e* 295 and two fragmentation ions of *m/e* 280 and 206, 1 mass unit larger than those in the mass spectrum of the sample with added [^{12}C]bicarbonate (Fig. 5A). The fragmentation ions of *m/e* 235 and 177 are the same in both mass spectra due to the loss of the $-COO$ moiety during fragmentation. In the autoclaved sample supplemented with [^{13}C]bicarbonate, no carboxylated PHE was observed.

DISCUSSION

NAP and PHE are largely insoluble in water. To provide the substrate to the cultures uniformly and to improve the access of the substrate to the microbes, NAP and PHE were distributed onto dry sediment, which was then used as the PAH stocks. The individual PAH was added to 150 or 200 μM, which is an order of magnitude higher than the concentration used in other studies. Although more than 99% of added PAH was sorbed onto the sediment material, which made up 10% of the active culture, the large surface area on which the PAH was distributed may have contributed to the successful cultivation of these PAH-degrading enrichments.

Several lines of evidence for anaerobic biodegradation of the PAHs are presented. (i) Although the initial lag before detectable PAH loss was 120 to 150 days of incubation, the acclimated cultures degraded the PAHs without a lag upon refeeding and could be subcultured without loss of the degradation activities. (ii) Sterile controls showed no activity. (iii) Inhibition of the activities by molybdate and dependence on the presence of sulfate indicate that sulfate-reducing bacteria are important for degradation. (iv) The 89 and 92% conversion to $^{14}CO_2$ of [^{14}C]NAP and [^{14}C]PHE, respectively, confirms the mineralization of the compounds. (v) Cultures can be repeatedly subcultured with their activity being maintained.

The results of the [^{13}C]bicarbonate experiment provide strong evidence for the carboxylation of NAP. The product of the carboxylation was identified as 2-NA by the GC retention time of the 2-NA standard. 2-NA was also readily metabolized without a lag by NAP-adapted enrichments (Table 2). This is consistent with 2-NA being an intermediate. On the other hand, 1-NA was neither detected nor utilized in the NAP-adapted enrichments. Carboxylation of NAP therefore appears to be specific for the 2-position.

It should be noted that for NAP, the net atomic charge of the carbon in the 2-position is the most negative (−0.0579) of

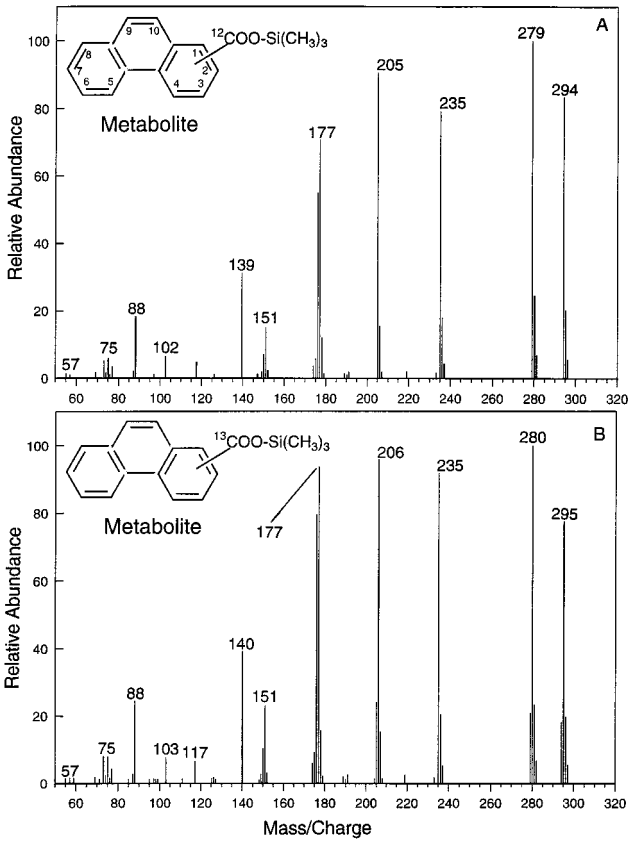

FIG. 5. Mass spectra of TMS derivatives of PHE-CA extracted from a PHE-degrading culture supplemented with PHE and either [^{12}C]bicarbonate (A) or [^{13}C]bicarbonate (B). At this time, the location of the carboxyl group is unclear. It is, however, not at the C-9 position, since the GC retention times of the derivatized 9-PHE-CA standards (19.30 min) is different from that detected in the cultures with added PHE (19.86 min).

all the carbons in NAP (e.g., carbon-1, −0.0414; carbon-3, essentially equivalent to carbon-2, −0.0576; and the two inner carbons, −0.0382 and −0.0386) (17). This may help explain why the 2-position is the most susceptible to attack by a carboxyl group. If the same reasoning is applied to PHE, carboxylation is likely to occur in the 2- or 3-position, since these carbons are the most negatively charged (−0.0568 and 0.0582, respectively). Our data are consistent with this hypothesis. The GC retention times for the PHE-CA metabolite is different from that of the standard (9-PHE-CA), suggesting that carboxylation does not occur at the carbon-9 position.

Based on the results we presented here, the metabolism of NAP and PHE is summarized in Fig. 6. NAP is first carboxylated to 2-NA, while PHE is most likely carboxylated on one of the outer ring carbons. Subsequent steps, which are unknown at this time, further metabolize the compounds to carbon dioxide.

To our knowledge, there are no reports of the anaerobically catalyzed carboxylation of PAHs. On the other hand, other aromatic compounds, such as, phenol, catechol, hydroquinone, and resorcinol, can be carboxylated by anaerobic bacteria (8–10, 14, 15, 31, 32). Two kinds of carboxylations have been reported: (i) reversible decarboxylation of 4-hydroxybenzoate and 3,4-dihydroxybenzoate, which is not affected by avidin, an inhibitor of biotin-requiring carboxylation (2, 14, 15, 30, 31); and (ii) carboxylation which requires the consumption of energy, as reported in one study in which phenol is carboxylated to 4-hydroxybenzoate through a phenol phosphorylation (20).

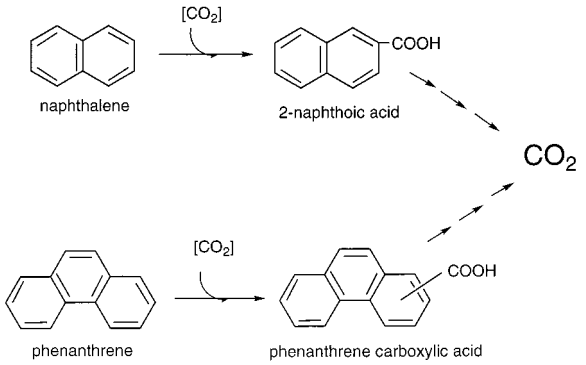

FIG. 6. Proposed summary pathways for the anaerobic metabolism of NAP and PHE in the sulfidogenic enrichments.

Carboxylation of PAHs leads to the formation of benzoate-like analogs, which are ideal substrates for further activation by coenzyme A ligation and subsequent aromatic ring reduction (13, 16). PAH carboxylation supports the notion that reductive hydrogenation is possible only after the aromatic ring is destabilized or activated by some reaction such as carboxylation. Whether the carboxylated PAHs undergo similar sequential ring reduction and ring fission to that seen with benzoate under anaerobic conditions (13, 16) remains to be seen.

In summary, this report describes the establishment of two highly enriched, sulfidogenic consortia capable of mineralization of NAP and PHE, respectively. To our knowledge, this is the first documentation presenting evidence for the identification of intermediates of the anaerobic biodegradation of PAHs. This work furthers our understanding of the anaerobic microbial mechanisms mediating PAH metabolism. Successful isolation of the responsible microorganisms would advance this effort.

ACKNOWLEDGMENTS

We thank Beau Ranheim and the crew of the *Osprey*, NYC-DEP, for helping us collect Arthur Kill sediment; S. Kallesser for laboratory assistance; M. Togna for technical support; and M. Häggblom for editorial help.

This research was supported in part by grants from DARPA (N0001492J1888) and ONR (N00149311008).

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