APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2000, p. 2743–2747 Vol. 66, No. 7
0099-2240/00/$04.00+0

Anaerobic Naphthalene Degradation by a Sulfate-Reducing Enrichment Culture†

RAINER U. MECKENSTOCK,[1]* EVA ANNWEILER,[2] WALTER MICHAELIS,[2] HANS H. RICHNOW,[2]‡ AND BERNHARD SCHINK[1]

Department of Biology, University of Konstanz, D-78457 Konstanz,[1] and Institute of Biogeochemistry and Marine Chemistry, University of Hamburg, D-20146 Hamburg,[2] Germany

Received 23 December 1999/Accepted 11 April 2000

Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture was studied by substrate utilization tests and identification of metabolites by gas chromatography-mass spectrometry. In substrate utilization tests, the culture was able to oxidize naphthalene, 2-methylnaphthalene, 1- and 2-naphthoic acids, phenylacetic acid, benzoic acid, cyclohexanecarboxylic acid, and cyclohex-1-ene-carboxylic acid with sulfate as the electron acceptor. Neither hydroxylated 1- or 2-naphthoic acid derivatives and 1- or 2-naphthol nor the monoaromatic compounds *ortho*-phthalic acid, 2-carboxy-1-phenylacetic acid, and salicylic acid were utilized by the culture within 100 days. 2-Naphthoic acid accumulated in all naphthalene-grown cultures. Reduced 2-naphthoic acid derivatives could be identified by comparison of mass spectra and coelution with commercial reference compounds such as 1,2,3,4-tetrahydro-2-naphthoic acid and chemically synthesized decahydro-2-naphthoic acid. 5,6,7,8-Tetrahydro-2-naphthoic acid and octahydro-2-naphthoic acid were tentatively identified by their mass spectra. The metabolites identified suggest a stepwise reduction of the aromatic ring system before ring cleavage. In degradation experiments with [1-^{13}C]naphthalene or deuterated D_8-naphthalene, all metabolites mentioned derived from the introduced labeled naphthalene. When a [^{13}C]bicarbonate-buffered growth medium was used in conjunction with unlabeled naphthalene, ^{13}C incorporation into the carboxylic group of 2-naphthoic acid was shown, indicating that activation of naphthalene by carboxylation was the initial degradation step. No ring fission products were identified.

Polycyclic aromatic hydrocarbons (PAH) are hazardous compounds which are found on various contaminated sites such as former gas plant sites or mineral oil refineries. Due to their obvious persistence in anoxic environments, PAH have been considered to be recalcitrant under anoxic conditions. However, anaerobic degradation of naphthalene, methylnaphthalene, phenanthrene, and a few more PAH has been demonstrated in microcosm experiments converting trace amounts of radioactively labeled substrates to CO_2 (8, 9, 20, 23, 26). Nitrate, sulfate, or ferric iron served as the terminal electron acceptor. Whereas anaerobic degradation of toluene and ethylbenzene has been investigated with several pure cultures (1, 4, 10, 11, 15, 21, 22, 24), attempts to cultivate anaerobic PAH-degrading bacteria have failed for a long time (18). A sulfate-reducing enrichment culture of marine origin growing with naphthalene as the sole carbon and energy source has been reported recently (31). 2-Naphthoic acid and phenanthroic acid were identified as metabolites of naphthalene and phenanthrene degradation, respectively. [^{13}C]bicarbonate was incorporated into the carboxylic group of 2-naphthoic acid, and it was assumed that a carboxylation reaction was the initial step. Since the culture could also use this compound as a carbon source, it was suggested to be the first intermediate in anaerobic naphthalene degradation by sulfate-reducing bacteria. The same authors reported later on reduced naphthoic acid derivatives as further metabolites in the degradation pathway (X. Zhang and L. Y. Young, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. Q-281, p. 467, 1998). Other authors observed naphthol as a side product in a sulfate-reducing, naphthalene-degrading culture and suggested that the first step in anaerobic naphthalene degradation might be a hydroxylation reaction (2). It was not reported which naphthol isomer was generated and if the organisms could grow with it as a carbon source. A marine sulfate-reducing pure culture and denitrifying naphthalene-degrading pure cultures have been reported recently (14, 25).

Here we report on a sulfate-reducing, naphthalene-degrading freshwater culture which was enriched from a contaminated aquifer. Identification of metabolites and substrate utilization tests were performed to obtain initial information on the pathway of anaerobic naphthalene degradation by sulfate-reducing bacteria.

MATERIALS AND METHODS

Organisms and growth conditions. A sulfate-reducing culture was enriched from soil material of a contaminated aquifer near Stuttgart, Germany, with naphthalene as the sole carbon and energy source in the presence of the solid adsorber resin Amberlite-XAD7 (Fluka, Buchs, Switzerland). XAD7 served as a substrate buffer providing the cultures with sufficient amounts of hydrocarbons and keeping the concentration at a constantly low level of about 50 μM. XAD7 was carefully washed five times with ethanol (99.8%) and five times with distilled water. Traces of ethanol were removed by drying for 2 to 3 days at 90°C. A 0.3-g portion of XAD7 was autoclaved in an empty 100-ml serum bottle, and the bottle was filled with 50 ml of bicarbonate-buffered freshwater medium, pH 7.2 to 7.4, reduced with 1 mM sulfide (29, 30). Subcultures were inoculated with a 10% volume of the liquid phase at 4-week intervals with 10 mM sulfate as the electron acceptor. The bottles were flushed with N_2–CO_2 (80:20), closed with Viton rubber stoppers (Maag Technik, Dübendorf, Switzerland), and incubated at 30°C in the dark. After five to six transfers the culture was also able to grow in the absence of XAD7. Naphthalene and other polycyclic aromatic compounds were added as liquids or as solid crystals (2 to 4 mg/50 ml), and monoaromatic

* Corresponding author. Mailing address: Department of Biology, University of Konstanz, Universitätstr. 10, D-78457 Konstanz, Germany. Phone: 49-7531-884541. Fax: 49-7531-882966. E-mail: rainer.meckenstock@uni-konstanz.de.

† Publication 88 of Deutsche Forschungsgemeinschaft priority program 546, Geochemical Processes with Long-Term Effects in Anthropogenically Affected Seepage and Groundwater.

‡ Present address: UFZ Leipzig-Halle GmbH, D-04318 Leipzig, Germany.

water-soluble substrates from 1 M stock solutions were added to final concentrations of 0.5 to 1 mM. [1-^{13}C]naphthalene was synthesized as reported elsewhere (28) and added to the culture medium as solid crystals (2 to 4 mg per 50 ml). Decahydro-2-naphthoic acid (decalin-2-carboxylic acid) was synthesized by hydration of 2-naphthoic acid with molecular hydrogen in the presence of platinium charcoal as the catalyst. The chemical structure was proven by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy, and chemical purity was proven by gas chromatography-mass spectrometry (GC-MS) analysis. The GC chromatogram revealed a racemic mixture of four isomers which had identical mass spectra in GC-MS analysis. Deuterated naphthalene-D_8 (Aldrich, Steinheim, Germany) was added in crystal form. To study incorporation of [^{13}C]bicarbonate into naphthoic acid, 10 ml of freshwater medium was supplemented with 20 mM Na-[^{13}C]bicarbonate (Sigma, St. Louis, Mo.) and 1 mM sodium sulfide, and the pH was adjusted to 7.4. The medium was filter sterilized into 50-ml serum bottles, flushed with N_2–CO_2 (80:20), and inoculated with 10 ml of a dense naphthalene-degrading culture with a syringe through the stopper.

Substrate utilization was monitored as sulfide production (7) or as substrate depletion by high-performance liquid chromatography (HPLC) analysis on a Beckman System Gold equipped with a C_{18} reversed-phase column and UV detection at 206 nm. The eluent was isocratic acetonitrile–50 mM ammonium phosphate buffer, pH 3.5 (70:30). Samples of 250 μl were taken with a syringe through the stopper, mixed with 1 ml of ethanol (99.8%), and subjected to HPLC analysis after removal of precipitates by centrifugation (for 5 min at 15,000 × *g*).

Analysis of metabolites. Culture growth was stopped routinely in the exponential-growth phase with 100 mM NaOH, and samples were stored at −20°C until metabolite analysis. The remaining naphthalene was extracted with hexane, and the water phase was acidified with 6 M hydrochloric acid to pH 2.0. Carboxylic acids and aromatic alcohols were extracted three times with dichloromethane. The combined dichloromethane extracts were concentrated to 1 ml by vacuum evaporation and were dried over anhydrous sodium sulfate. A 0.5-ml volume of ethereal diazomethane solution was added to methylate carboxylic acids and aromatic hydroxyl groups (12). The solvent was removed by a gentle stream of nitrogen, and products were exchanged into hexane and analyzed by GC-MS. GC analysis was performed with a Carlo Erba Fractovap 4160, equipped with a 60-m capillary column (DB-5; inner diameter, 0.32 mm; film thickness, 0.25 μm; J & W Scientific) and a flame ionization detector (FID). Hydrogen was used as the carrier gas, and the temperature program was 80°C (5 min isothermal), 80 to 310°C (4°C/min), 310°C (10 min isothermal). GC-MS measurements were performed with a Hewlett-Packard 6890 gas chromatograph coupled with a Quattro II mass spectrometer (Micromass, Attrincham, United Kingdom). Helium was used as the carrier gas, and GC conditions were the same as those described above. The following MS conditions were used: ionization mode, EI^+; ionization energy, 70 eV; emission current, 200 μA; source temperature, 180°C; mass range, *m/z* 50 to 400. For identification of metabolites, instrumental library searches applying the NIST/NIH/EPA mass spectral database (National Institute of Standards and Technology/National Institutes of Health/U.S. Environmental Protection Agency), comparison with published mass spectra, and coinjection with available authentic reference compounds were used.

RESULTS

Substrate utilization tests. A naphthalene-degrading, sulfate-reducing culture was tested for growth with different monoaromatic, polycyclic, and alicyclic compounds as the sole carbon and energy source. The culture could utilize 2-methylnaphthalene without a significant lag phase, as indicated by increasing sulfide concentrations (Fig. 1A). 2-Naphthoic acid was readily used as a carbon source, whereas 1-naphthoic acid was utilized only after a lag phase of 40 days (Fig. 1A). The culture could not grow with 1-methylnaphthalene. Other polycyclic compounds such as 2-naphthylacetic acid and 1-naphthylacetic acid or the hydroxylated compounds 1-naphthol, 2-naphthol, 1-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, and 2-hydroxy-1-naphthoic acid could not be utilized by the culture. Among the tested monoaromatic compounds, only benzoic acid (Fig. 1B) and phenylacetic acid served as substrates, whereas benzene, *o*-phthalic acid, 2-carboxy-1-phenylacetic acid (homophthalic acid), and salicylic acid were not utilized within the first 100 days.

The totally or partially reduced substrates cyclohexanecarboxylic acid and cyclohex-1-ene-carboxylic acid were utilized after a lag phase of 25 or 40 days, respectively (Fig. 1B). Cyclohexane, 1,2,3,4-tetrahydro-2-naphthoic acid (tetralin-2-carboxylic acid), cyclohexane-1,2-trans-dicarboxylic acid, decahydro-2-naphthoic acid (decalin-2-carboxylic acid), and cyclohexaneacetic acid could not be used.

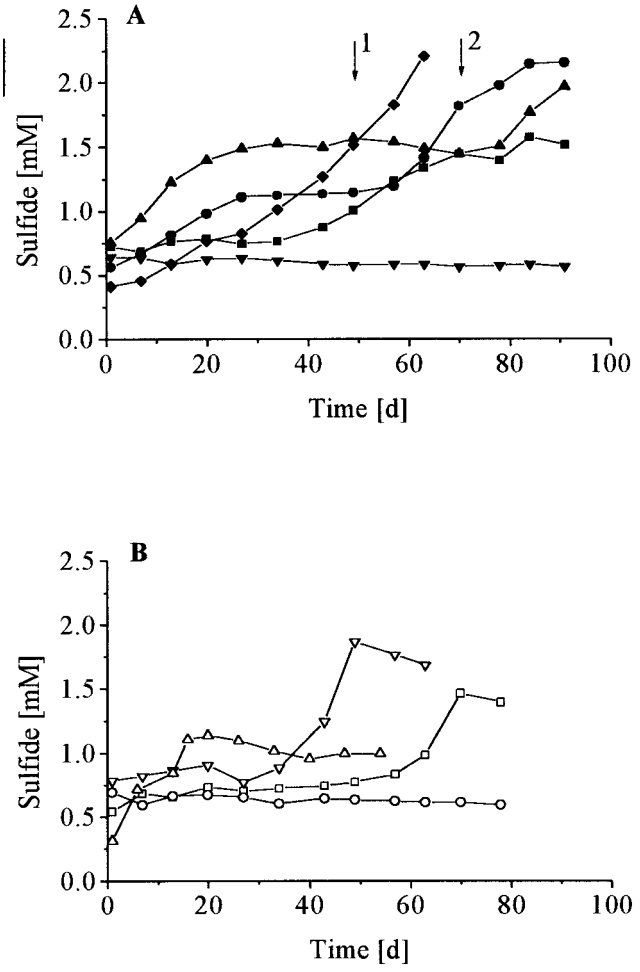

FIG. 1. Sulfide formation by a sulfate-reducing culture with various substrates. Results of representative experiments from three parallels are shown for each compound. (A) Sulfide production with naphthalene (◆), 1-methylnaphthalene (▼), 2-methylnaphthalene (▲), 1-naphthoic acid (■), and 2-naphthoic acid (●). Arrows indicate second additions of 2-naphthoic acid (arrow 1) and 2-methylnaphthalene (arrow 2). (B) Sulfide production with benzoic acid (△), cyclohexanecarboxylic acid (▽), cyclohex-1-ene-carboxylic acid (□), and *o*-phthalic acid (○).

The cultures were examined microscopically after growth, and in all cases short, oval rods were dominant.

Identification of metabolites of naphthalene degradation. Naphthalene-degrading cultures were extracted and analyzed for potential metabolites by GC-MS. One prominent metabolite in all naphthalene-degrading cultures investigated was 2-naphthoic acid (Fig. 2A). The compound was identified by the reference mass spectrum and by coelution with purchased 2-naphthoic acid. [^{13}C]2-naphthoic acid was found when the culture was grown with [1-^{13}C]naphthalene, which proved that naphthalene was the precursor (Fig. 2B).

Two tetrahydro-2-naphthoic acid derivatives were identified. 1,2,3,4-Tetrahydro-2-naphthoic acid could be identified by the mass spectrum and by coelution with the commercial reference compound (Fig. 3A). It was present only in small amounts, but growth experiments with [1-^{13}C]naphthalene proved that it originated from naphthalene degradation. 5,6,7,8-Tetrahydro-2-naphthoic acid was tentatively identified by the mass spec-

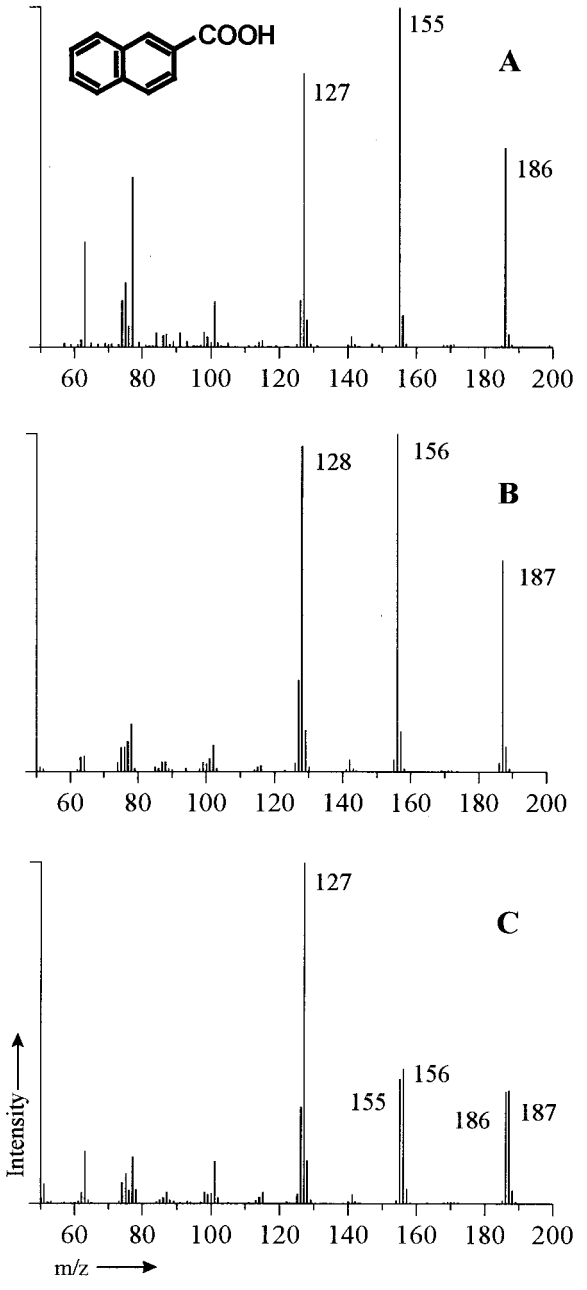

FIG. 2. Mass spectra of 2-naphthoic acids extracted from a sulfate-reducing culture grown with differently labeled naphthalenes (as methyl esters). Shown are mass spectra of 2-naphthoic acid as confirmed with the commercial reference compound (A), [1-^{13}C]2-naphthoic acid extracted from [1-^{13}C]naphthalene-grown cultures (B), and a mixture of unlabeled 2-naphthoic acid and [^{13}C]2-naphthoic acid extracted from a culture grown with unlabeled naphthalene and [^{13}C]bicarbonate (C).

trum and was found at high concentrations in every culture (Fig. 3B). Moreover, one octahydro-2-naphthoic acid was tentatively identified (Fig. 3C).

Decahydro-2-naphthoic acid isomers (decalin-2-carboxylic acid) were identified as further metabolites (Fig. 3D). The GC-MS chromatogram of the chemically synthesized reference compound decahydro-2-naphthoic acid revealed four isomers of unknown absolute configuration which are separated in the GC but have identical mass spectra. Coelution experiments with culture extracts showed that two of those were present in the anaerobic naphthalene degradation experiments (data not shown).

All metabolites were identified as well in [1-^{13}C]naphthalene-degrading cultures, which proved that they were formed from introduced naphthalene. In addition, cultures grown with naphthalene-D_8 produced the same GC-MS spectra of metabolites carrying a D_7 label. So far, we were not able to detect naphthalene ring fission products or monoaromatic metabolites, and no hydroxylated derivatives of naphthalene or naphthoic acid could be identified.

Formation of 2-naphthoic acid. The origin of the carboxyl group of 2-naphthoic acid was investigated using [^{13}C]bicarbonate as a medium buffer. After 25 days of growth, the cultures were stopped with 100 mM NaOH and subjected to metabolite screening by GC-MS analysis. Again, 2-naphthoic acid was identified and the mass spectrum clearly indicated an incorporation of the ^{13}C label into the carboxyl group of 2-naphthoic acid (Fig. 2C). Due to carryover of [^{12}C]bicarbonate from the inoculum, the mass spectrum depicts a mixed label of ^{12}C and ^{13}C in the carboxyl group of 2-naphthoic acid, resulting in a double peak at masses 186 and 187 and at 155 and 156. The mass peak at 127 represents the aromatic ring system and therefore does not appear as a double peak.

DISCUSSION

In the present study, we report on anaerobic naphthalene degradation by a sulfate-reducing enrichment culture from a freshwater source. Putative intermediates of the naphthalene degradation pathway were tested as growth substrates, and naphthalene degradation products were identified by GC-MS using unlabeled, ^{13}C-labeled, and fully deuterated naphthalene-D_8 as well as [^{13}C]bicarbonate.

In an earlier study, 2-naphthoic acid was reported to be an intermediate of anaerobic naphthalene degradation in a marine sulfate-reducing enrichment culture (31). The authors stated that 2-naphthoic acid is a product of naphthalene carboxylation, as shown by incorporation of [^{13}C]bicarbonate. In our culture also, 2-naphthoic acid was the major metabolite which appeared in the growth medium. Furthermore, incorporation of [^{13}C]bicarbonate into the carboxylic group of 2-naphthoic acid was observed, which could indicate that naphthalene is activated through addition of a C_1 compound. Experiments with ^{13}C-labeled and deuterated naphthalene-D_8 proved the formation of 2-naphthoic acid from naphthalene. Since the culture could readily grow with 2-naphthoic acid, it is likely that 2-naphthoic acid is an intermediate in naphthalene degradation. The culture could also oxidize 1-naphthoic acid after a lag phase, perhaps due to some unspecificity of the pathway. 1-Naphthoic acid could not be identified as a metabolite of naphthalene degradation.

We could also identify a number of further naphthalene-derived metabolites as shown by labeling experiments with [1-^{13}C]naphthalene and naphthalene-D_8 (Zhang and Young, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., 1998). Decahydro-2-naphthoic acid and trace amounts of 1,2,3,4-tetrahydro-2-naphthoic acid were identified by their mass spectra and by coelution with reference compounds. Two further 2-naphthoic acid derivatives were tentatively identified as 5,6,7,8-tetrahydro-2-naphthoic acid and an octahydro-2-naphthoic acid as deduced from their mass spectra. The metabolites identified point to a stepwise reduction of 2-naphthoic acid to tetralin-2-carboxylic acid and subsequently to decahydro-2-naphthoic acid. Reduction of 2-naphthoic acid prior to ring fission would be analogous to the benzoyl coenzyme A (CoA) degradation

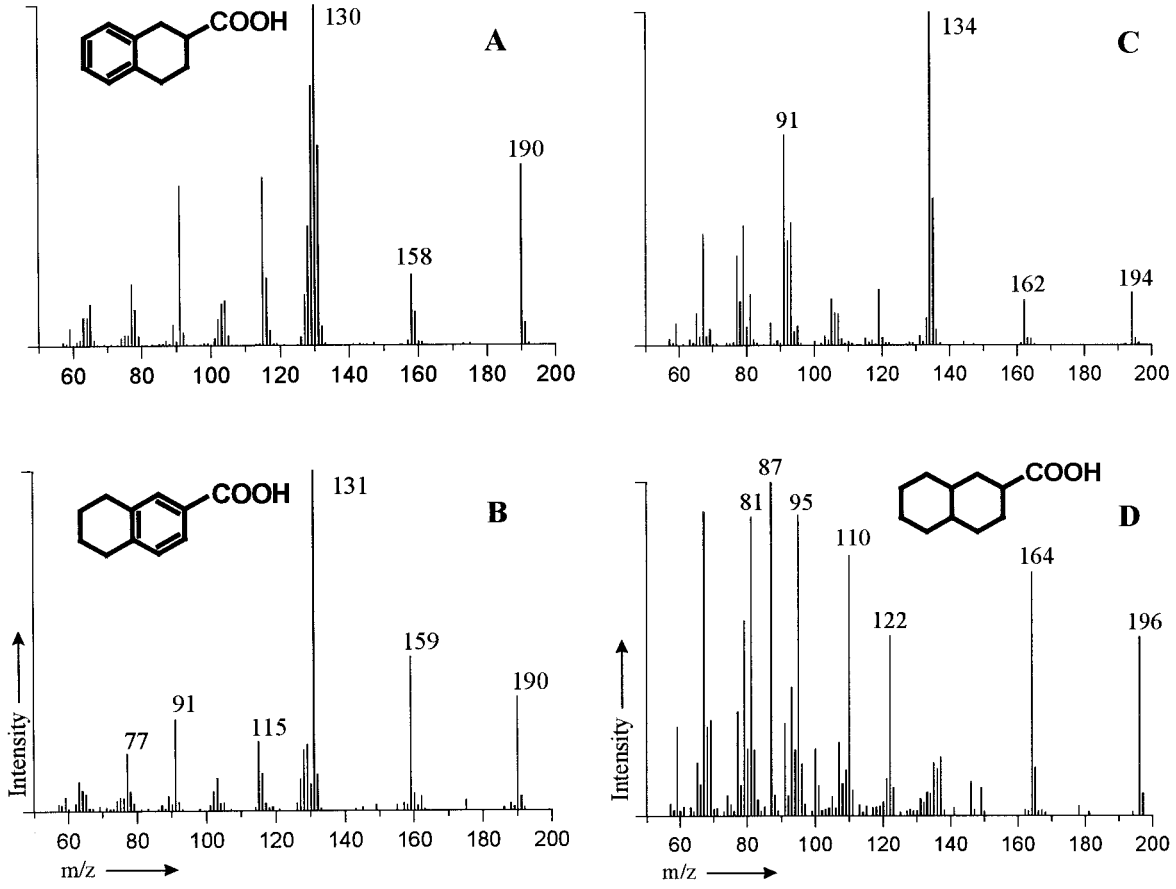

FIG. 3. Mass spectra of reduced metabolites extracted from a naphthalene-grown, sulfate-reducing culture (as methyl esters). Shown are mass spectra of 1,2,3,4-tetrahydro-2-naphthoic acid as confirmed by the commercial reference compound (A), tentative 5,6,7,8-tetrahydro-2-naphthoic acid (B), tentative octahydro-2-naphthoic acid (C), and decahydro-2-naphthoic acid as confirmed by the synthesized reference compound (D).

pathway which has been studied with nonsulfur purple bacteria and denitrifying bacteria of the genera *Thauera* and *Azoarcus* (16, 17). However, in both variants of the benzoyl-CoA pathway, the partially reduced ring system is hydroxylated by the addition of water to a double bond after the first or the second two-electron reduction step, respectively. The aromatic ring is not completely reduced, and in vitro measurements of benzoyl-CoA reductase with cell extracts have shown cyclohex-1-ene-carboxylic acid as the most reduced compound (19).

The pathway of reductive 2-naphthoic acid degradation might differ from benzoyl-CoA degradation with respect to the pattern of water addition, because hydroxylated intermediates of anaerobic naphthalene degradation have not been identified. In addition, the culture was not able to grow with one of the three hydroxylated naphthoic acid derivatives 1-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, and 2-hydroxy-1-naphthoic acid. No ring fission products were found by GC-MS, which neither supports nor excludes a ring fission reaction analogous to the benzoyl-CoA pathway. Nevertheless, the culture was able to grow with the reduced substrates cyclohexanecarboxylic acid and cyclohex-1-ene-carboxylic acid, which could theoretically derive from a decahydro-2-naphthoic acid. However, cyclohexanecarboxylic acid supports the growth of most sulfate-reducing bacteria that can degrade aromatic compounds and is therefore not a specific feature of anaerobic naphthalene degradation. The same holds true for growth of the naphthalene-degrading culture with benzoate.

The fact that the culture could not grow with 1,2,3,4-tetralin-2-carboxylic acid and decahydro-2-naphthoic acid does not necessarily indicate that these compounds are not intermediates of anaerobic naphthalene degradation, as the organisms might lack appropriate uptake mechanisms. Nevertheless, the reduced 2-naphthoic acid derivatives could be dead-end metabolites as well.

Other authors have suggested that naphthol is an intermediate in anaerobic naphthalene degradation by a sulfidogenic sediment (2). Hydroxylation as the initial attack in anaerobic naphthalene degradation is unlikely in the present culture, as hydroxylated intermediates were not identified by GC-MS and the culture could not grow with 1- or 2-naphthol. The incorporation of [^{13}C]bicarbonate into the carboxyl group of 2-naphthoic acid rather supports carboxylation of naphthalene as proposed by Zhang and Young (31; Abstr. 98th Gen. Meet. Am. Soc. Microbiol., 1998). Addition of fumarate to the [2-C] atom of naphthalene, similar to the radical mechanism in anaerobic toluene degradation, is therefore also unlikely (3, 5, 6, 13, 18, 27).

ACKNOWLEDGMENTS

This work was partly financed by the Deutsche Forschungsgemeinschaft.

We thank Christian Garms and Wittko Franke for chemical synthesis of decahydro-2-naphthoic acid.

REFERENCES

1. **Ball, H. A., H. A. Johnson, M. Reinhard, and A. M. Spormann.** 1996. Initial reactions in anaerobic ethylbenzene oxidation by a denitrifying bacterium, strain EB1. J. Bacteriol. **178:**5755–5761.
2. **Bedessem, M. E., N. G. Swoboda-Colberg, and P. J. S. Colberg.** 1997. Naphthalene mineralization coupled to sulfate reduction in aquifer-derived enrichments. FEMS Microbiol. Lett. **152:**213–218.
3. **Beller, H. R., and A. M. Spormann.** 1997. Benzylsuccinate formation as a means of anaerobic toluene activation by sulfate-reducing strain PRTOL1. Appl. Environ. Microbiol. **63:**3729–3731.
4. **Beller, H. R., and A. M. Spormann.** 1997. Anaerobic activation of toluene and *o*-xylene by addition to fumarate in denitrifying strain T. J. Bacteriol. **179:**670–676.
5. **Biegert, T., G. Fuchs, and J. Heider.** 1996. Evidence that anaerobic oxidation of toluene in the denitrifying bacterium *Thauera aromatica* is initiated by formation of benzylsuccinate from toluene and fumarate. Eur. J. Biochem. **238:**661–668.
6. **Chee-Sanford, J. C., J. W. Frost, M. R. Fries, J. Zhou, and J. M. Tiedje.** 1996. Evidence for acetyl coenzyme A and cinnamoyl coenzyme A in the anaerobic toluene mineralization pathway in *Azoarcus tolulyticus* Tol-4. Appl. Environ. Microbiol. **62:**964–973.
7. **Cline, J. D.** 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. **14:**454–458.
8. **Coates, J. D., R. T. Anderson, and D. R. Lovley.** 1996. Oxidation of polycyclic aromatic hydrocarbons under sulfate-reducing conditions. Appl. Environ. Microbiol. **62:**1099–1101.
9. **Coates, J. D., J. Woodward, J. Allen, P. Philip, and D. R. Lovley.** 1997. Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. Appl. Environ. Microbiol. **63:**3589–3593.
10. **Dolfing, J., J. Zeyer, P. Binder-Eicher, and R. P. Schwarzenbach.** 1990. Isolation and characterization of a bacterium that mineralizes toluene in the absence of molecular oxygen. Arch. Microbiol. **154:**336–341.
11. **Evans, P. J., D. T. Mang, K. S. Kim, and L. Y. Young.** 1991. Anaerobic degradation of toluene by a denitrifying bacterium. Appl. Environ. Microbiol. **57:**1139–1145.
12. **Falews, H. M., T. M. Jaouni, and J. F. Babashak.** 1973. Simple method for preparing ethereal diazomethane without resorting to codistillation. Anal. Chem. **45:**2302–2303.
13. **Frazer, A. C., W. Ling, and L. Y. Young.** 1993. Substrate induction and metabolite accumulation during anaerobic toluene utilization by the denitrifying strain T1. Appl. Environ. Microbiol. **59:**3157–3160.
14. **Galushko, A., D. Minz, B. Schink, and F. Widdel.** 1999. Anaerobic degradation of naphthalene by a pure culture of a novel type of marine sulphate-reducing bacterium. Environ. Microbiol. **1:**415–420.
15. **Harms, G., K. Zengler, R. Rabus, F. Aeckersberg, D. Minz, R. Rossello-Mora, and F. Widdel.** 1999. Anaerobic oxidation of *o*-xylene, *m*-xylene, and homologous alkylbenzenes by new types of sulfate-reducing bacteria. Appl. Environ. Microbiol. **65:**999–1004.
16. **Harwood, C. S., G. Burchardt, H. Herrmann, and G. Fuchs.** 1999. Anaerobic metabolism of aromatic compounds via the benzoyl-CoA pathway. FEMS Microbiol. Rev. **22:**439–458.
17. **Heider, J., and G. Fuchs.** 1997. Anaerobic metabolism of aromatic compounds. Eur. J. Biochem. **243:**577–596.
18. **Heider, J., A. M. Spormann, H. R. Beller, and F. Widdel.** 1999. Anaerobic bacterial metabolism of hydrocarbons. FEMS Microbiol. Rev. **22:**459–473.
19. **Koch, J., and G. Fuchs.** 1992. Enzymatic reduction of benzoyl-CoA to alicyclic compounds, a key reaction in anaerobic aromatic metabolism. Eur. J. Biochem. **205:**195–202.
20. **Langenhoff, A. A. M., A. J. B. Zehnder, and G. Schraa.** 1996. Behaviour of toluene, benzene and naphthalene under anaerobic conditions in sediment columns. Biodegradation **7:**267–274.
21. **Lovley, D. R., and D. J. Lonergan.** 1990. Anaerobic oxidation of toluene, phenol, and *p*-cresol by the dissimilatory iron-reducing organism, GS-15. Appl. Environ. Microbiol. **56:**1858–1864.
22. **Meckenstock, R. U.** 1999. Fermentative toluene degradation in anaerobic defined syntrophic cocultures. FEMS Microbiol. Lett. **177:**67–73.
23. **Milhelcic, J. R., and R. G. Luthy.** 1988. Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. Appl. Environ. Microbiol. **54:**1182–1187.
24. **Rabus, R., R. Nordhaus, W. Ludwig, and F. Widdel.** 1993. Complete oxidation of toluene under strictly anoxic conditions by a new sulfate-reducing bacterium. Appl. Environ. Microbiol. **59:**1444–1451.
25. **Rockne, K. J., J. C. Chee-Sanford, R. A. Sanford, B. P. Hedlund, J. T. Staley, and S. E. Strand.** 2000. Anaerobic naphthalene degradation by microbial pure cultures under nitrate-reducing conditions. Appl. Environ. Microbiol. **66:**1595–1601.
26. **Rockne, K. J., and S. E. Strand.** 1998. Biodegradation of bicyclic and polycyclic aromatic hydrocarbons in anaerobic enrichments. Environ. Sci. Technol. **32:**3962–3967.
27. **Seyfried, B., G. Glod, R. Schocher, A. Tschech, and J. Zeyer.** 1994. Initial reactions in the anaerobic oxidation of toluene and *m*-xylene by denitrifying bacteria. Appl. Environ. Microbiol. **60:**4047–4052.
28. **Staab, H. A., and M. Haenel.** 1970. [1-^{13}C]-Naphthalin: Synthese, NMR-Spektrum, ESR-Spektrum des Radikalanions und Automerisierungsversuche. Chem. Ber. **103:**1095–1100.
29. **Widdel, F., G. W. Kohring, and F. Mayer.** 1983. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov., and *Desulfonema magnum* sp. nov. Arch. Microbiol. **134:**286–294.
30. **Widdel, F., and F. Bak.** 1992. Gram-negative mesophilic sulfate-reducing bacteria, p. 3352–3378. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The Prokaryotes, vol. 4. Springer, New York, N.Y.
31. **Zhang, X., and L. Y. Young.** 1997. Carboxylation as an initial reaction in the anaerobic metabolism of naphthalene and phenanthrene by sulfidogenic consortia. Appl. Environ. Microbiol. **63:**4759–4764.