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Microbial Hydroxylation of Quinoline in Contaminated Groundwater: Evidence for Incorporation of the Oxygen Atom of Water

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Studies conducted in an aquifer contaminated by creosote suggest that quinoline is converted to 2(1H)quinolinone by an indigenous consortium of microorganisms. Laboratory microbial experiments using $H_2{}^{18}O$ indicate that water is the source of the oxygen atom for this hydroxylation reaction under aerobic and anaerobic conditions.

Groundwater contamination by hazardous organic compounds is a relatively common problem in certain industrialized regions of the United States. Many of these contaminants are aromatic or heterocyclic compounds that potentially are hazardous to human health and the environment. Some of these compounds also are carcinogenic or mutagenic at extremely low concentrations. Because of the general anaerobic environment (7) and the slow movement of groundwater, many of these aromatic compounds are refractory and persist in contaminated aquifers for relatively long periods of time (7, 11).

Under certain hydrologic conditions, such as infiltration of precipitation, anaerobic zones in shallow aquifers may become aerobic as a result of the rise or the decline of the water table, as influenced by the capillary fringe. Because of possible alternating oxidizing and reducing conditions, it is reasonable to assume that both aerobic and anaerobic microorganisms in these zones of contaminated aquifers have adapted enzyme systems capable of metabolizing organic compounds, thus influencing their fate and transport in the subsurface.

Investigations conducted at sites contaminated by creosote and coal tar have indicated that groundwater at these sites is contaminated by polynuclear-azaheterocyclic compounds or azaarenes (10, 11). These compounds potentially are hazardous to human health. They are combustion products formed during the conversion of fossil fuels, such as coal and oil shale, into synthetic fuels (15). It is not surprising that these compounds also have been identified in contaminated groundwater near an underground coal gasification site (17) and near a back-flooded in situ oil shale-retorting facility (8).

Because of the widespread occurrence of azaarenes in environmental samples associated with the processing and use of fossil fuels and related products and because of the potential for groundwater contamination, it is essential, from a health perspective, to understand the fate and movement of these compounds in contaminated aquifers.

Studies conducted at an abandoned wood treatment facility near Pensacola, Fla., indicated that groundwater was contaminated by azaarenes derived from creosote (12, 13). Groundwater samples collected from wells near the source of contamination contained relatively high concentrations of quinoline and isoquinoline and of their respective oxygenated analogs 2(1H)quinolinone and 1(2H)isoquinolinone. However, groundwater samples from wells at more-distant down-gradient sites contained 2(1H)quinolinone and 1(2H) isoquinolinone but no detectable concentrations of quinoline or isoquinoline. The presence of oxygenated azaarenes in both the aerobic and the anaerobic zones of the aquifer suggested that these compounds might be microbial transformation products of quinoline and isoquinoline.

Bennett et al. (1) demonstrated that four species of pseudomonads isolated from creosote-contaminated soil from the Pensacola site converted quinoline to 2(1H)quinolinone under aerobic conditions. 2(1H)quinolinone was degraded rapidly, probably by a mechanism involving ring cleavage, to unknown products. These quinoline-degrading pseudomonads were unable to degrade 2(1H)quinolinone under anaerobic conditions.

Updegraff et al. (D. M. Updegraff, J. L. Bennett, W. E. Pereira, and C. E. Rostad, submitted for publication) and Pereira et al. (12) reported that quinoline, isoquinoline, and 4-methylquinoline were converted to oxygenated derivatives in anaerobic laboratory cultures by methanogenic consortia. These oxygenated azaarenes were partially transformed into N-methyl, C-methyl, and O-methyl derivatives. Oxygenated and alkylated azaarenes were not degraded further. Similar transformation products were identified in groundwater from anaerobic zones in the contaminated aquifer, which suggests anaerobic microbial activity.

Under aerobic conditions, molecular oxygen is required as a terminal electron acceptor during respiration and also for hydroxylation of aromatic compounds before ring cleavage. These reactions are mediated by oxygenases. However, in the absence of molecular oxygen, aromatic compounds may be degraded by methanogenic consortia or in the presence of other electron acceptors such as nitrate or sulfate (19).

Previous investigators have demonstrated the incorporation of the oxygen atom of water into aromatic compounds such as benzene and toluene (4, 18). More recently, it was reported that indole was converted to oxindole under methanogenic conditions (2).

Because the source of the oxygen for the initial oxidation of quinoline to 2(1H)quinolinone under aerobic and anaerobic conditions was unknown, laboratory experiments were

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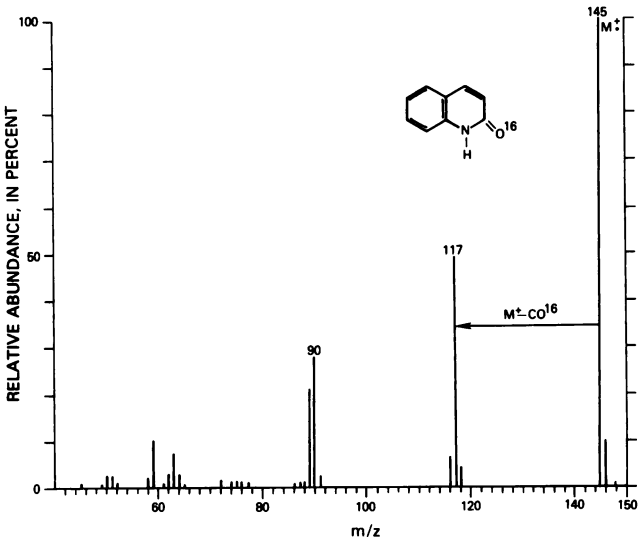

FIG. 1. Low-resolution mass spectrum of unlabeled 2(1H)quinolinone isolated from a microbial culture.

conducted with $H_2{}^{18}O$ in an attempt to elucidate the mechanism of this hydroxylation reaction.

A methanogenic consortium previously obtained from sewage sludge (Updegraff et al., submitted) was cultivated under strictly anaerobic conditions by the method of Owen et al. (9). The cultures were grown in 6-ml serum bottles under a nitrogen-carbon dioxide mixture (70:30, vol/vol). A 1.0-ml portion of the basal medium (9) was added to each of six serum bottles, and the water was evaporated off at 30°C. Then the following additions were made to the bottles under a continuous stream of the nitrogen-carbon dioxide mixture: (i) 0.04 ml of acetate-propionate solution, (ii) 0.96 ml of distilled water (either $H_2{}^{16}O$ or $H_2{}^{18}O$), (iii) 0.9 ml of solution S5, (iv) 0.9 ml of solution S6, (v) 0.1 ml of inoculum culture, and (vi) 0.2 μl of quinoline (see reference 9 for the compositions of these solutions). The inoculum was from a transfer from a culture (number 3) used in a previous study (Updegraff et al., submitted). Cultures remained anaerobic throughout the incubation period, as shown by complete reduction of the resazurin indicator.

Enrichment cultures for the aerobic experiment were obtained from creosote-contaminated soil from the Pensacola site (1). The culture was derived from a set of several transfers of the mixed culture to reduce organic contamination from the soil inoculum. Aerobic cultures were prepared in screw-cap culture tubes (12 by 100 mm) containing 1 ml of culture and 8 ml of air, the latter of which contained far more oxygen than was required for complete oxidation of the substrate to CO_2 and H_2O. Sterile Stanier solution (1 ml) (16) was evaporated to dryness at 30°C. $H_2{}^{18}O$ or $H_2{}^{16}O$ (1 ml) and 246 μg of quinoline were added to the dry residue in the screw-cap culture tubes. The solution then was inoculated with the mixed culture, and it was grown aerobically at 30°C with frequent agitation. When the solution became turbid (24 h), indicating bacterial growth, the experiment was terminated. Because of the high cost of $H_2{}^{18}O$, sterile controls were conducted only on samples dissolved in $H_2{}^{16}O$. In both the aerobic and anaerobic experiments, these controls indicated that 2(1H)quinolinone was not formed, indicating that the hydroxylations were microbially mediated.

Cultures from the aerobic and anaerobic experiments were centrifuged and filtered through 0.45-μm-pore-size filters. The filtrates were extracted twice with purified diethyl ether (2 × 2 ml). After the addition of benzene (100 μl), the organic extract was evaporated under nitrogen to a volume of 100 μl. The extracts were analyzed by gas chromatography–low-resolution mass spectrometry and gas chromatography–high-resolution mass spectrometry.

Low-resolution mass spectra were obtained on a Finnigan OWA or MAT TSQ 46-B gas chromatograph-mass spectrometer system. The gas chromatograph was equipped with a fused silica capillary column (30 m [length] by 0.26 mm [inside diameter]) with 0.25-μm DB-5 bonded film (J & W Scientific). Accurate mass measurements were made on a VG 7070HS high-resolution mass spectrometer at a resolution set statically at 4,000. The gas chromatograph contained a cross-linked column (25 m by 0.2 mm [inside diameter]; Hewlett-Packard Co.) with 5% phenylmethylsilicone. The linear velocity of helium through the gas chromatograph column was 26 cm/s. Splitless injections of 1 μl of culture filtrate extract were made at 280°C. The gas chromatograph oven temperature was held at 50°C for 4 min, and the temperature was then increased at 6°C/min to a maximum of 300°C. The oxygenated metabolite of unlabeled quinoline was confirmed by comparison of its retention index and mass spectrum with those of an authentic standard.

A low-resolution mass spectrum of unlabeled 2(1H)quinolinone isolated from a microbial culture is shown in Fig. 1. Low-resolution electron impact mass spectra of 2(1H)quinolinone isolated from the aerobic and anaerobic cultures in the presence of $H_2{}^{18}O$ are shown in Fig. 2. These spectra demonstrate incorporation of ^{18}O from $H_2{}^{18}O$, as evidenced by the molecular ion at *m/z* 147. Aerobic and anaerobic control experiments conducted in the presence of $H_2{}^{16}O$ indicated a molecular ion at *m/z* 145. The presence of a molecular ion at *m/z* 145 in the mass spectrum from the anaerobic culture was due to the addition of the inoculum containing 2(1H)quinolinone.

Fragment ions at *m/z* 117 in both spectra indicated loss of $C^{18}O$, confirming the incorporation of ^{18}O into the molecule. Further confirmation of these results was obtained by conducting accurate mass measurements on a high-resolution mass spectrometer for ions at *m/z* 117, *m/z* 145, and *m/z* 147 obtained from the anaerobic cultures; the results are presented in Table 1. These results conclusively demonstrate incorporation of the oxygen atom of water, as hydroxyl, into position 2 of the quinoline ring without participation of atmospheric oxygen under aerobic and anaerobic conditions. The lactam 2(1H)quinolinone is the more stable tautomer. Hydroxylation in position 2 of the quinoline ring was confirmed by comparing Kovats and Lee retention indices (14) and mass spectra of an authentic standard with those of the oxygenated metabolite produced in the microbial experiments. The Kovats and Lee retention indices of 2(1H)quinolinone were 1752.21 and 293.97, respectively;

TABLE 1. Accurate mass measurements for 2(1H) quinolinone in the anaerobic experiment

m/z			Elemental composition				
Theoretical exact mass	Observed exact mass	Δ[a]	C	H	^{16}O	^{18}O	N
117.0578	117.0566	1.2	8	7	0	0	1
145.0527	145.0527	0.0	9	7	1	0	1
147.0570	147.0575	−0.5	9	7	0	1	1

[a] Values indicate error (in millimass units) between observed and theoretical exact masses.

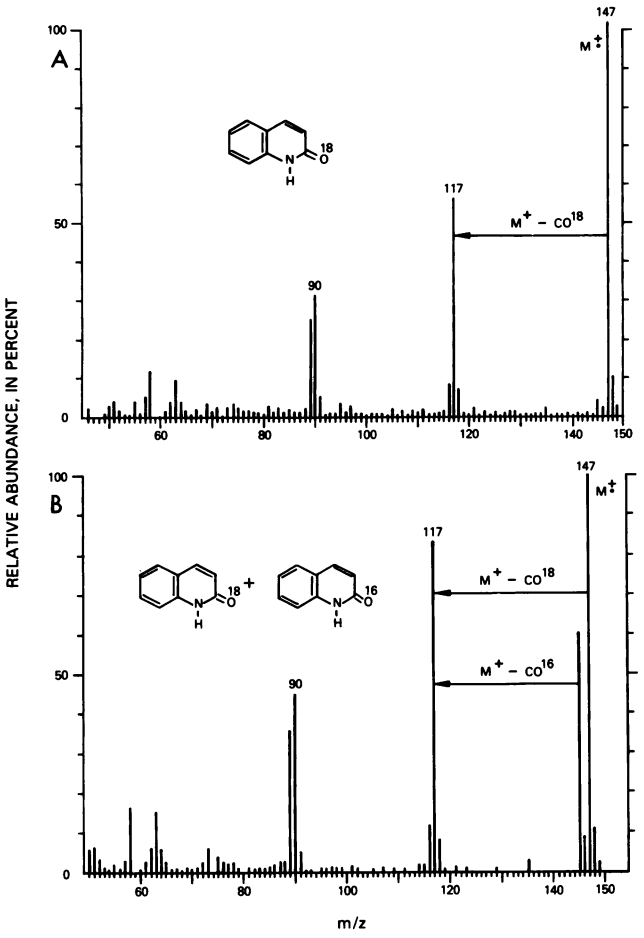

FIG. 2. Low-resolution electron impact mass spectra of 2(1H) quinolinone isolated from aerobic (A) and anaerobic (B) cultures in the presence of $H_2{}^{18}O$.

4-hydroxyquinoline had a Kovats retention index of 2010.69 and a Lee retention index of 331.17.

Microbial enzyme systems that hydroxylate aromatic compounds under anaerobic conditions (4, 18) and ring-substituted heterocycles under aerobic and anaerobic conditions, without involvement of molecular oxygen, have been reported (3, 5, 6); however, this study demonstrates for the first time the incorporation of an oxygen atom derived from water into an unsubstituted nitrogen heterocycle.

Although it is difficult to demonstrate microbial degradation of organic contaminants in subsurface environments, laboratory studies provide evidence for microbially mediated transformations of nitrogen heterocycles in the absence of molecular oxygen in contaminated aquifers. Whether ring hydroxylation of nitrogen heterocycles is a prerequisite to ring fission by microbial consortia is not known. However, laboratory experiments and field observations suggest that oxygenated azaarenes are relatively more water soluble, more mobile, and more persistent in contaminated aquifers than their unoxygenated analogs are. The environmental significance of hydroxylations involving water warrants further investigation.

LITERATURE CITED

1. **Bennett, J. L., D. M. Updegraff, W. E. Pereira, and C. E. Rostad.** 1985. Isolation and identification of four species of quinoline degrading pseudomonads from a creosote-contaminated site at Pensacola, Fla. Microbios Lett. **29**:147–154.
2. **Berry, D. F., E. L. Madsen, and J.-M. Bollag.** 1987. Conversion of indole to oxindole under methanogenic conditions. Appl. Environ. Microbiol. **53**:180–182.
3. **Dagley, S., and P. A. Johnson.** 1963. Microbial oxidation of kynurenic, xanthurenic, and picolinic acids. Biochim. Biophys. Acta **78**:577–587.
4. **Grbić-Galić, D, and T. M. Vogel.** 1987. Transformation of toluene and benzene by mixed methanogenic cultures. Appl. Environ. Microbiol. **53**:254–260.
5. **Hayaishi, O., and A. Kornberg.** 1952. Metabolism of cytosine, thymine, uracil, and barbituric acid by bacterial enzymes. J. Biol. Chem. **197**:717–732.
6. **Hirschberg, R., and J. C. Ensign.** 1971. Oxidation of nicotinic acid by a *Bacillus* species: source of oxygen atoms for the hydroxylation of nicotinic acid and 6-hydroxynicotinic acid. J. Bacteriol. **108**:757–759.
7. **Josephson, J.** 1980. Safeguards for groundwater. Environ. Sci. Technol. **14**:38–44.
8. **Leenheer, J. A., and T. I. Noyes.** 1985. Effects of organic wastes on water quality from processing oil shale from the Green River Formation, Colorado, Utah, and Wyoming, p. 1–56. U.S. Geological Survey professional paper no. 1338. U.S. Geological Survey, Denver.
9. **Owen, W. F., D. C. Stuckey, J. B. Healy, Jr., L. Y. Young, and P. L. McCarty.** 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res. **13**: 485–492.
10. **Pereira, W. E., and C. E. Rostad.** 1986. Investigations of organic contaminants derived from wood-treatment processes in a sand and gravel aquifer near Pensacola, Florida, p. 65–80. U.S. Geological Survey water-supply paper no. 2290. U.S. Geological Survey, Denver.
11. **Pereira, W. E., C. E. Rostad, J. R. Garbarino, and M. F. Hult.** 1983. Groundwater contamination by organic bases derived from coal-tar wastes. Environ. Toxicol. Chem. **2**:283–294.
12. **Pereira, W. E., C. E. Rostad, D. M. Updegraff, and J. L. Bennett.** 1987. Fate and movement of azaarenes in an aquifer contaminated by wood-treatment chemicals. Environ. Toxicol. Chem. **6**:163–176.
13. **Pereira, W. E., C. E. Rostad, D. M. Updegraff, and J. L. Bennett.** 1987. Anaerobic microbial transformations of azaarenes in ground water at hazardous waste sites, p. 111–123. *In* R. C. Averett and D. M. McKnight (ed.), Chemical quality of water and the hydrologic cycle. Lewis Publishers, Inc., Chelsea, Mich.
14. **Rostad, C. E., and W. E. Pereira.** 1986. Kovats and Lee retention indices determined by gas chromatography/mass spectrometry for organic compounds of environmental interest. J. High Resolut. Chromatogr. Chromatogr. Commun. **9**:328–334.
15. **Santodonato, J., and P. H. Howard.** 1981. Azaarenes: sources, distribution, environmental impact and health effects, p. 421–438. *In* J. Saxena and F. Fisher (ed.), Hazard assessment of chemicals, vol. 1. Academic Press, Inc., New York.
16. **Stanier, R. Y., M. Doudoroff, and E. A. Adelberg.** 1957. The microbial world, 2nd ed., p. 308. Prentice-Hall, Inc., Englewood Cliffs, N.J.
17. **Stuermer, D. H., D. J. Ng, and C. J. Morris.** 1982. Organic contaminants in ground water near an underground coal gasification site in northeastern Wyoming. Environ. Sci. Technol. **16**:582–587.
18. **Vogel, T. M., and D. Grbić-Galić.** 1986. Incorporation of oxygen from water into toluene and benzene during anaerobic fermentative transformation. Appl. Environ. Microbiol. **52**:200–202.
19. **Young, L. Y.** 1984. Anaerobic degradation of aromatic compounds, p. 487–523. *In* D. T. Gibson (ed.), Microbial degradation of organic compounds. Marcel Dekker, Inc., New York.