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Biotransformation of quinoline and methylquinolines in anoxic freshwater sediment

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Abstract

Quinoline (Q) and some isomers of methylquinoline (MQ) were transformed to hydroxylated products in freshwater sediment slurries incubated under methanogenic conditions at 25 °C. Methylquinoline transformation was not affected by a methyl group on the C–3 or C–4 carbon atom of the pyridine ring; 2-MQ, however, was not transformed. All isomers of dimethylquinoline (DMQ) tested (2,4-, 2,6-, 2,7-, and 2,8-DMQ) with a methyl group at the number 2 carbon also persisted in sediments after anaerobic incubation for one year at 25 °C.

In most experiments, quinoline initially was transformed to 2-hydroxyquinoline (2-OH-Q), which was further metabolized to unidentified products. A second product, 4-CH_3–2-OH-Q, was detected in some experiments. This product accumulated and was not further transformed. 6-, 7-, and 8-Methylquinoline (6-, 7-, 8-MQ) were hydroxylated to form the respective 2-OH-MQ products. These hydroxylated products accumulated and were not further transformed. Hydroxylation of Q and 6-, 7- and 8-MQ at the 2-carbon position was confirmed by GC/FTIR and GC/MS analyses. The transformations of Q and MQs were pH dependent with an optimal pH of 7–8.

The results of this study suggest that two pathways may exist for the anaerobic transformation of quinoline; one pathway leads to the formation of a hydroxylated intermediate and the other to a methylated and hydroxylated intermediate. In addition, our results suggest that a methyl substituent on the number 2 carbon inhibits the anaerobic transformation of quinoline derivatives.

Abbreviations: GC – gas chromatography, GC/FTIR – gas chromatography/Fourier transform infrared spectrometry, GC/MS – gas chromatography/mass spectrometry, HPLC – high performance liquid chromatography, MQ – methylquinoline, Q – quinoline

Introduction

Aromatic nitrogen-heterocyclic compounds, including quinoline (Q) and methylquinolines (MQs), are found in the aqueous effluents of wood-treating processes as well as oil-shale and coal-mining operations (Bollag & Kaiser 1991; Francis & Wobber 1982; Gangual 1981; Godsy et al. 1992; Hanson et al. 1979; Leenheer et al. 1982; Neufield & Spinola 1978; Pereira et al. 1987a). Quinoline, MQs and other N-heterocycles are more polar than their homocyclic analogues and characteristically are more water soluble and have a lower tendency to sorb to organic constituents of soil and aquifer materials. Thus, N-heterocyclic pollutants may be transported through soils, sediments, and aquifer materials and contaminate groundwater supplies. In fact, Q has been detected in groundwater proximate to wood-treating and underground coal gasification sites (Godsy et al. 1992; Pereira et al. 1983; Stuermer et al. 1982). Because Q and MQ derivatives have toxic, mutagenic, and carcinogenic properties (Reinhardt & Britteli 1981), and because current and potential releases of these compounds are significant, there is a need to understand their transport and transforma-

tions to determine their impact on the environment and human health.

Although several studies have demonstrated the microbial transformation of Q and MQs in aerobic systems, few investigations have been performed to assess the metabolism of N-heterocyclic compounds under anaerobic conditions (Pereira et al. 1987a,b; Wang et al. 1984). Wang et al.(1984) reported the anaerobic transformation of Q and indole in a granular, activated-carbon anaerobic digester but did not find evidence for transformation of MQ. Recently, Godsy et al. (1992) reported field evidence and corroborative laboratory data indicating the microbial degradation of Q and selected methylphenols in contaminated, anoxic ground-water ecosystems. The research presented here partially addresses the question of the fate of N-heterocyclics by describing the transformation of Q and MQs in freshwater sediments incubated under methanogenic conditions (alternative electron acceptors such as nitrate and sulfate were excluded). In addition, the effects of methyl substitution on the homocyclic ring and the heterocyclic ring are discussed.

Materials and methods

Chemicals

Quinoline, 6-methylquinoline (6-MQ), 7-methylquinoline (7-MQ), and 8-methylquinoline (8-MQ) were obtained from Sigma Chemical Co. (St. Louis, MO). 2-Hydroxyquinoline (2-OH-Q), 4-hydroxyquinoline (4-OH-Q), 5-hydroxyquinoline (5-OH-Q), 8-hydroxyquinoline (8-OH-Q), 2,4-dimethylquinoline (2,4-DMQ), 2,6-dimethylquinoline (2,6-DMQ), 2,7-dimethylquinoline (2,7-DMQ), 2,8-dimethylquinoline (2,8-DMQ), 2-hydroxy–4-methylquinoline (2-OH–4-CH_3-Q), and 4-hydroxy–2-methylquinoline (4-OH–2-CH_3-Q) were purchased from Aldrich Chemical Co. (Milwaukee, WI). 2-Methylquinoline (2-MQ), 3-methylquinoline (3-MQ), and 4-methylquinoline (4-MQ) were obtained from American Tokyo Kasei, Inc. (Portland, OR). Methylene chloride and chloroform were obtained from American Burdick and Jackson (Muskegon, MI). All chemicals and solvents were of analytical or HPLC grade.

Sampling

Samples of sediment and overlying site water were collected from a freshwater pond (Logan's Pond, near Athens, GA) contaminated with small particles of asphalt. The sediment was characterized as reducing because of its organic carbon content (25 mg/g TOC), dark color, and faint hydrogen sulfide odor. Samples were collected to avoid atmospheric oxygen contact by completely filling jars at the sediment/water interface with sediment (top 10 cm) or site-water and immediately sealing. Upon arrival in the lab, samples were flushed with a gas mixture of N_2:H_2 (19:1) and were stored in an anaerobic chamber (Coy Laboratory Products, Inc., Ann Arbor, MI).

Anaerobic transformation studies

The fate of Q and MQs was investigated in sediment slurries maintained under anaerobic conditions. Sediment slurries were prepared in an anaerobic chamber as described by Hale et al. (1990). Sieved sediment (1 mm mesh) was diluted to 10% solids with anoxic (N_2-flushed) site water and 15 mL of slurry were dispensed into sterile serum bottles or Bellco culture tubes (Bellco Glass, Vineland, NJ) which subsequently were capped with butyl rubber stoppers and secured with aluminum crimp seals. Following their removal from the anaerobic chamber, samples were flushed with a filter-sterilized gas mixture of N_2:H_2 (19:1) and amended with sodium sulfide (Na_2S) and the appropriate test compound. Aliquots of a concentrated Na_2S solution were added to sediment slurries to yield a final concentration of 0.5 g/L. Sterile controls were prepared by autoclaving slurries at 121 °C for 30 min for 3 consecutive days prior to the addition of test compounds. Stock solutions (10–20 g/L) of test chemicals (Q and MQs) were prepared by adding the chemicals to acetonitrile (ACN). Aliquots (less than 10 uL) of the stock solutions were added to experimental cultures and sterile controls to a final concentration range of 10 to 20 mg/L. The final ACN concentration was always less than 0.1% (v/v) solvent. Additional control sediment slurries were amended with ACN only. Samples were incubated as stationary cultures in the dark at 23 to 25 °C. All experiments were performed in duplicate unless otherwise stated. At specified times, the experimental microcosm vials were vigorously mixed and samples were aseptically removed using a sterile syringe and needle. These subsamples were mixed with an equal volume of acetonitrile to stop microbiological activity and to facilitate solubilization of sorbed aromatics.

In some experiments, inorganic nutrients (Shelton & Tiedje 1984) were added as a concentrated stock

solution to sediment slurries to a final concentration (mg/L) of: NH_4Cl, 530; $CaCl_2$–$2H_2O$, 75; $MgCl_2$–$6H_2O$, 100; $FeCl_2$–$4H_2O$, 20; $MnCl_2$–$4H_2O$, 0.5; H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl_2$, 0.03; Na_2MoO_4–$2H_2O$, 0.01; $CoCl_2$–$6H_2O$, 0.5; $NiCl_2$–$6H_2O$, 0.05, and Na_2SeO_4, 0.05. In addition, $NaHCO_3$ was added separately to a final concentration of 1.2 g/L (Shelton & Tiedje 1984). Before substrate addition, vials were flushed with a gas mixture consisting of N_2:CO_2 (19:1) followed by addition of H_2 (5% v/v).

To determine the pH range of quinoline and MQ transformation, sediment slurries were adjusted to pH 5.0–9.0 within the anaerobic chamber by addition of anoxic solutions of monobasic and dibasic potassium phosphate (20 mM final concentration). Na_2S was added as a concentrated stock solution (neutral pH) after the sediment slurries were pH-adjusted and the vials were stoppered to prevent loss of volatile sulfides.

Analytical methods

High performance liquid chromatography (HPLC) was used for the quantitation of Q and the MQs and for the preliminary identification of transformation products. The HPLC apparatus consisted of a Rabbit HP pump (Ranin; Woburn, MA), an analytical polymeric C–18 column (4.6 × 150 mm; 10 um particle size; International Chemical Inc., Mountain View, CA), an LC 600 autosampler (Perkin-Elmer; Norwalk, CT), a model–757 UV absorbance detector (Applied Biosystem; Ramsey, NJ) set at 310 nm, and a model–746 data module (Waters; Milford, MA). The mobile phase consisted of a mixture of ACN, water, and triethanolamine (60:39.85:0.15, v/v/v) adjusted to pH 8.3 and operated at a flow rate of 0.75 ml/min. Before analysis, samples of the sediment slurry (plus ACN) were vortexed, centrifuged, and filtered (0.22 um) according to the procedures of Hale et al. (1990). Quantitation of test chemicals was carried out by the external standards method at a wavelength of 310 nm.

Identification of products

Gas chromatography/mass spectrometry (GC/MS) and gas chromatography/Fourier transform infrared spectrometry (GC/FTIR) were used in the identification of products of Q and MQ biotransformation. To facilitate identification, the products were partially purified by solvent extraction and preparative HPLC. Transformation products resulting from the degradation of Q, 6-MQ, 7-MQ and 8-MQ were partially purified as follows. Samples (20 ml) of substrate-adapted sediments were centrifuged at 3300 × g for 20 min and the supernatant fluid was extracted twice with 18 ml aliquots of methylene chloride. The extract was then evaporated to dryness using a rotary evaporator and resuspended with 0.5 mL of acetonitrile. Transformation products present in the acetonitrile extract were purified by preparative HPLC using a Spherisorb C–18 column (25 cm × 2.5 cm; Metachem Technologies, Redondo Beach, CA). The mobile phase was the same as that used for analytical liquid chromatography but the flow rate was adjusted to 2 ml/min. Fractions from preparative chromatography containing transformation products were diluted four-fold with 0.1 M phosphate buffer (pH 7.0) and applied to a methanol conditioned C_{18} adsorbent cartridge (Analytichem International, Harbor City, California). The cartridge was washed with one bed volume of 0.1 M phosphate buffer (pH 7.0) followed by product elution with 15 mL of chloroform. GC/MS and GC/FTIR analyses were performed using the chloroform eluant.

GC/MS analyses were performed with a Hewlett-Packard 5890 series II gas chromatograph coupled to a HP 5970 mass selective detector. Separation of components was achieved using a DB–5 (J and W Scientific, Folsom, CA), fused-silica capillary column (30 m × 0.25 mm I.D.; 0.33 um film thickness). The column was operated using a temperature program of 40 °C to 275 °C at 10 °C/min after an initial temperature of 40 °C for 2 min. The final temperature of 275 °C was held for 5 min. The detector was programmed to scan over a mass range of 25 to 375 mass units at 1.5 scans/sec. Standard solutions of Q, 6-MQ, 7-MQ, 8-MQ, and 2-OH-Q were analyzed as reference compounds.

GC/FTIR analyses were performed with a Hewlett-Packard model 5890 gas chromatograph coupled to a Digilab model FTS–60 spectrometer and a Digilab model 3200 workstation. The FTIR spectrometer was equipped with a model GC/C 32 light-pipe-based interface and a narrow band, mercury-cadmium-telluride detector. Generated spectra had 8 cm^{-1} resolution with a useful range of 4000 to 700 cm^{-1}, and were subjected to a standard Fourier domain-smoothing algorithm. Sample components were separated on a DB–5 (J and W Scientific) megabore column (30 m × 0.52 um I.D.; 1.5 um film thickness) using the same temperature program as described for GC/MS experiments. Helium was used as the carrier gas at a linear velocity of 22 cm/s. The FTIR interface was held at 250 °C for the light pipe and the transfer lines. Spectral database

matching was accomplished with the Stadtler gas phase library. Spectra for hydroxyquinolines (4-OH-Q, 5-OH-Q, and 8-OH-Q) and hydroxymethylquinolines (4-CH_3–2-OH-Q and 2-CH_3–4-OH-Q) were determined by analysis of authentic standards.

Results

Biotransformation of Q and MQS

Quinoline, 6-MQ, 7-MQ, and 8-MQ (initial concentration, 15 mg/L) were transformed to intermediate products in freshwater sediment slurries after anaerobic incubation for 40, 68, 80, and 68 days, respectively (Figs. 1a, b). No appreciable lag periods were noted for Q and MQ isomer biotransformation. Further, little or no significant differences were noted in the initial rates of biotransformation of Q and MQ isomers based on the profile of substrate loss. Transformation products were produced concomitant with the disappearance of the test compounds (Fig. 1a-b). Low-resolution GC/MS spectra of the transformation products of Q and MQs resulted in mass spectrometry data base matches to monohydroxylated Q and monohydroxylated 6-, 7-, and 8-MQ (Fig. 2a and 3a). GC/FTIR spectra exhibited excellent agreement between the sample and specific IR library spectra; the hydroxyl group was located at the C–2 carbon of Q and isomers of MQs (Fig. 2b and 3b).

A slightly faster rate of transformation of Q compared to the MQs (Fig. 1b) was indicated by the differences in the formation rates of 2-hydroxyquinoline (2-OH-Q) and 6-, 7-, and 8-methyl–2-hydroxyquinolines (6-CH_3–2-OH-Q, 7-CH_3–2-OH-Q and 8-CH_3–2-OH-Q). The concentration of 2-OH-Q resulting from Q transformation peaked after incubation for 40–50 days and was reduced to less than detectable levels by day 68. No apparent decreases in the concentrations of 6-, 7-, or 8-CH_3–2-OH-Q were detected during the 68 day incubation period.

In autoclaved-sediment slurries, small but significant losses of Q and MQ isomers were evident (Fig. 1a). Approximately 30% of the initial concentrations of Q and of the 6-, 7- and 8-MQs were depleted after anaerobic incubation for 68 days; no transformation products were detected in any of the autoclaved-control experiments amended with Q, MQ, or DMQ isomers.

Sediment slurries reamended with Q following its depletion (below 1 mg/L) exhibited an apparent enhanced rate of Q biotransformation. Additions of

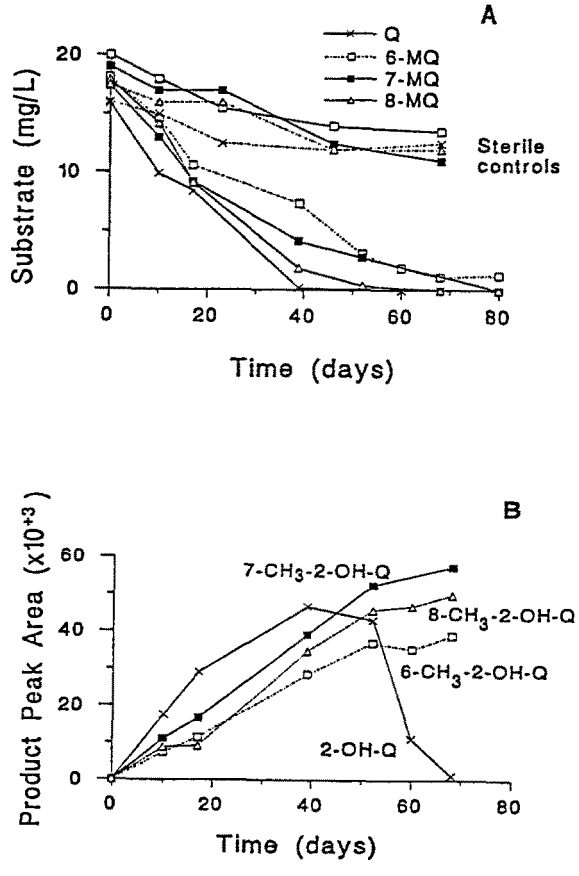

Fig. 1. (a) Profile of the anaerobic biotransformation of quinoline (Q) and isomers of methylquinoline (MQ) in freshwater sediment incubated under methanogenic conditions at room temperature. (b) Formation of 2-OH-Q and 2-OH derivatives of 6-, 7-, and 8-MQ as intermediate products of Q and 6-, 7-, and 8-MQ biotransformation, respectively, in methanogenic freshwater sediment.

quinoline at days 60 and 92 (Fig. 4) resulted in Q biotransformation without a lag period and at rates equal to or greater than the initial biotransformation rate. The maximum detected concentration of the transformation product (2-OH-Q) was less following the second and third additions of Q compared to the initial Q addition. Further, the total time required for complete loss of the transformation product was significantly less (25–30 days compared to 40–50 days) after the second and third additions of Q. These sediment slurries were considered to be adapted to degrade Q.

Similar experiments also were performed using 6-, 7-, and 8-MQ acclimated sediments. The rate of biotransformation of 6-MQ increased after sequential additions of 6-MQ in a fashion analogous to transformation of quinoline (Fig. 4). Similar results were

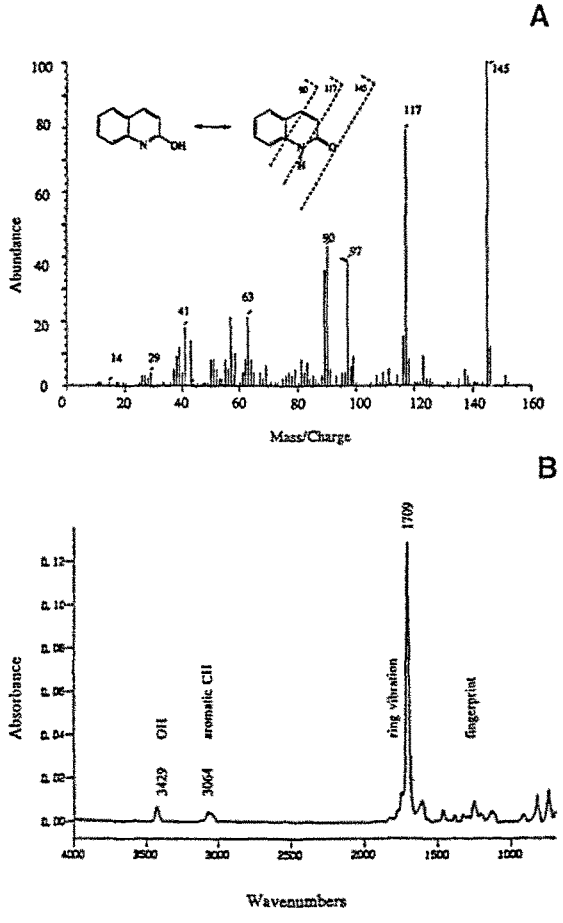

Fig. 2. (a) Mass spectrum of the Q biotransformation product identified as 2-OH-Q. (b) FTIR spectrum of the Q biotransformation product.

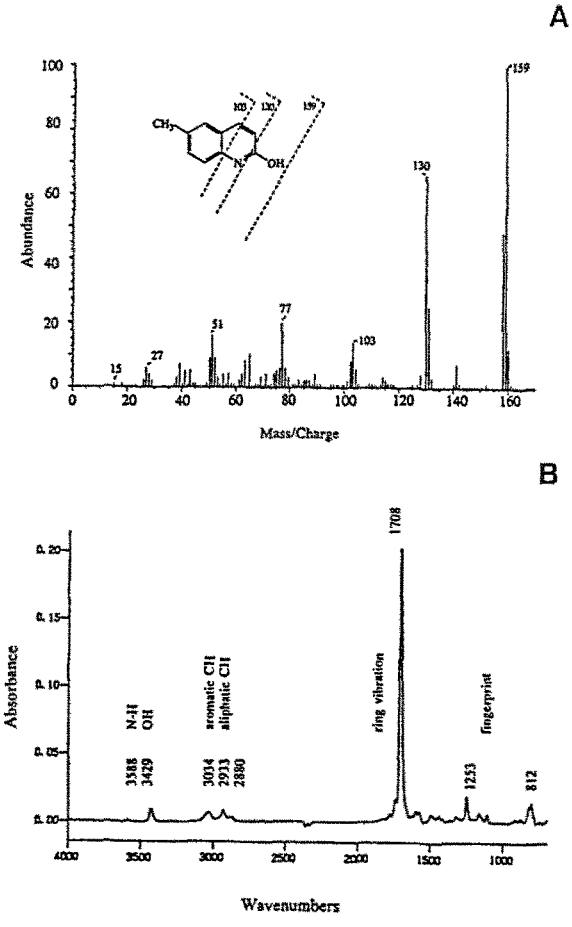

Fig. 3. (a) GC/MS spectrum of the 6-MQ biotransformation product identified as 6-methyl–2-OH-Q. (b) GC/FTIR spectrum of the 6-MQ biotransformation product. The respective 2-OH-methylquinoline isomers were observed as products in experiments amended with 7- and 8-MQ.

observed in experiments in which 7- and 8-MQ were added as the parent quinoline derivative. In contrast to quinoline amended experiments, the product of 6-MQ transformation (6-CH_3–2-OH-Q) accumulated in the adapted sediment slurries after several reamendments of 6-MQ and remained at a relatively constant level for the duration of the experiment (total of 130 days). The lack of further accumulation of the product suggests either the simultaneous production and consumption of 6-CH_3–2-OH-Q or our inability to accurately quantitate this product at high concentration (using only peak integration values). Because a standard solution of the transformation product was unavailable, the actual product concentration was not determined.

The biotransformation of several MQ isomers, having a methyl group on the heterocyclic ring of Q, also

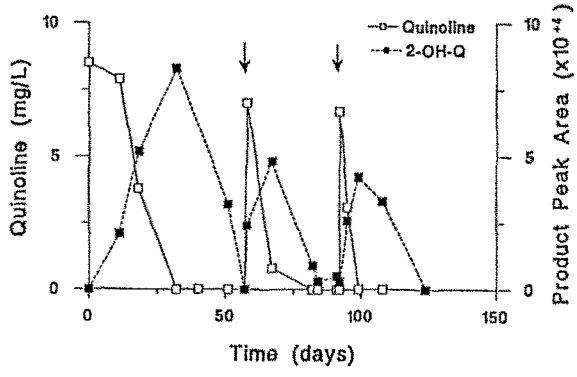

Fig. 4. Biotransformation of quinoline and its metabolic product, 2-OH-Q, in methanogenic freshwater sediment following multiple additions of quinoline. Arrows indicate the times of quinoline addition.

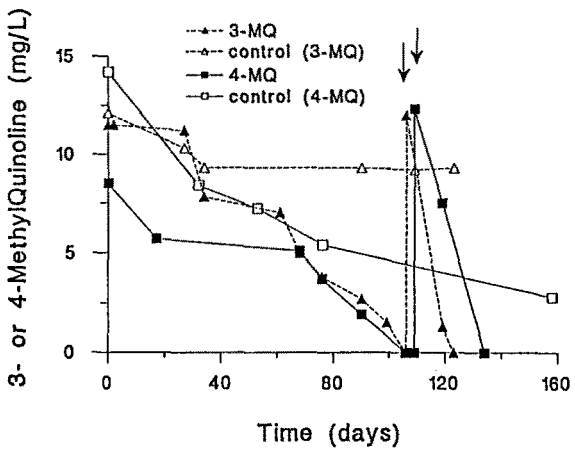

Fig. 5. Profile of 3- and 4-MQ biotransformation in methanogenic freshwater sediment. Arrows indicate times of readdition of substrate.

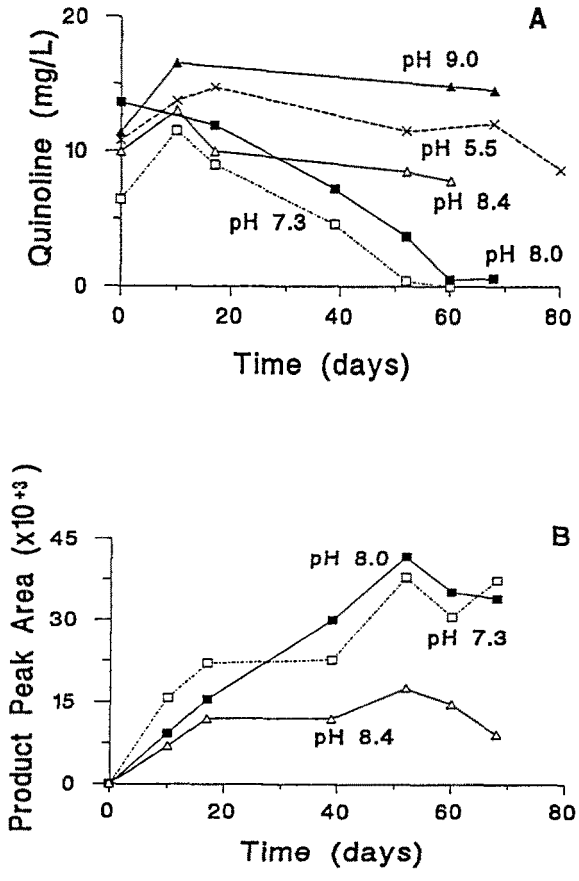

Fig. 6. (a) Effect of pH on Q biotransformation in methanogenic freshwater sediment. The pH effect was similar in experiments amended with 6-, 7-, and 8-MQ. (b) Profile of appearance of the Q transformation product (identified as 2-OH-Q) at various pH.

was examined. Sediment slurries transformed 3- and 4-MQ after anaerobic incubation for 106 days at 25 °C (Fig. 5). The unidentified transformation products of 3- and 4-MQ accumulated and were not further degraded. The rates of biotransformation of the 3- and 4-MQs increased dramatically after reamendment of the respective MQs. In concurrent experiments, however, 2-MQ was not transformed after anaerobic incubation for 4 months. Further, all isomers of dimethylquinoline (DMQ) tested (2,4-, 2,6-, 2,7- and 2,8-DMQ) with a methyl group at the number 2 carbon were persistent after incubation for one year at 25 °C.

Effects of pH and inorganic nutrients on Q and MQ transformation

The rates of disappearance of Q and MQs and the rates of appearance of their respective transformation products were pH dependent. The effects of pH on quinoline transformation and quinoline product formation are presented in Figs. 6a and b. The highest transformation activity was observed at pH values of 7.3 to 8.0 and little or no transformation occurred at pH values less than 5.5 or greater than 9.0. Results for transformation of 6-, 7-, and 8-MQ were similar (data not shown).

Addition of inorganic nutrients or a mix of bicarbonate plus carbon dioxide to sediment slurries did not enhance the rates of transformation of Q or the MQs compared to unamended sediments. In fact, the rates of transformation of Q and 8-MQ were approximately 25% lower in nutrient-amended sediments. Furthermore, 2-OH-Q accumulated to a greater extent before its subsequent transformation in sediments to which inorganic nutrients were added. In multiple experiments in which inorganic nutrients were added, two transformation products of Q metabolism were detected which were subsequently identified from GC/FTIR spectra as 4-CH_3–2-OH-Q and 2-OH-Q. Neither product was metabolized after 90 days incubation.

When added to either Q-adapted or unadapted sediment slurries, 4-CH_3–2-OH-Q was not transformed after incubation for 150 days. In contrast, 2-OH-Q was transformed without a lag period when added to both unadapted and Q-adapted sediment slurries. Some loss of 2-OH-Q was observed in autoclaved control experiments. A minimum of 65% of the initially added

concentration of 2-OH-Q remained in the autoclaved control sediments after incubation for 140 days.

Discussion

The premise that biological activity was primarily responsible for the observed transformation of Q and MQs in methanogenic sediment slurries is based on several specific observations. First, Q and MQs were transformed at a faster rate in sediment slurries than in sterile (autoclaved) controls. Second, transformation rates increased after repeated additions of Q and MQs to sediment slurries. Third, transformation rates were pH-dependent and the optimal pH was near neutrality (between pH 7 and 8).

Analysis of GC/MS and GC/FTIR spectra confirmed that hydroxylation was the initial transformation reaction of Q, 6-MQ, 7-MQ, and 8-MQ in sediment slurries under methanogenic conditions. Hydroxylation occurred at the C–2 carbon of the heterocyclic ring. Transformation of 3- and 4-MQ also was evident although transformation products were not positively identified. Based on HPLC retention times and comparison to HPLC profiles of other hydroxylated Q and MQ transformation products, however, it is likely that hydroxylated derivatives of 3- and 4-MQ were produced. The 2-MQ isomer and the isomers of DMQs (2,4-, 2,6-, 2,7-, and 2,8-) tested were not transformed in sediment slurries. Apparently, by blocking the 2-carbon with a methyl substituent, the hydroxylation of the quinoline derivative is prevented, thus accounting for the persistence of this particular chemical in the environment.

The hydroxylation of the quinoline ring is not unique to anaerobic systems. Aislabie et al. (1990) reported that *Pseudomonas aeruginosa* QP hydroxylated Q to 2-OH-Q aerobically. They also observed the formation of monohydroxymethylquinolines from 6-MQ, 7-MQ, and 8-MQ but not from 2-MQ, 4-MQ, or 2,6-DMQ and were unable to identify the specific sites of hydroxylation. When the organism was added to the heterocyclic-nitrogen fraction of an oil shale, Q, 6-MQ/7-MQ (co-chromatographed), and 8-MQ decreased in concentration but the levels of 2-MQ, isoquinoline, and methyl-isoquinolines were unaffected. The authors also reported that *P. aeruginosa* QP was unable to transform MQs or DMQs under denitrifying conditions. Shukla (1986) reported that a species of *Pseudomonas* isolated from sewage was able to convert Q to 2-OH-Q; the isolate further converted the 2-OH-Q to 8-hydroxycoumarin. Pereira et al. (1988), using ^{18}O-labelled compounds, demonstrated that water was the source of the oxygen atom, as hydroxyl, for Q hydroxylation under aerobic and anaerobic conditions. The detailed mechanism of microbial hydroxylases which catalyze the transformation of complex N-heterocyclic compounds under anoxic conditions awaits further investigation.

In one of our experiments (inorganic nutrient amended sediments, pH 8.5), both 4-CH_3–2-OH-Q and 2-OH-Q accumulated as the transformation products of quinoline degradation. In a separate study, Pereira et al. (1987b) also identified 4-CH_3–2-OH-Q, as well as 1,4-dimethyl–2-OH-Q, as minor transformation products. In the same report, these authors postulated that transformation of 4-MQ proceeded through 4-CH_3–2-OH-Q and was further transformed through different pathways to 2-CH_3O–4-CH_3-Q and 1,4-dimethyl–2-OH-Q. These latter methylations represent the formation of carbon-oxygen and carbon-nitrogen bonds whereas the methylation of 2-OH-Q results in the formation of a carbon-carbon bond.

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