

Humic Acids as Electron Acceptors for Anaerobic Microbial Oxidation of Vinyl Chloride and Dichloroethene

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Anaerobic oxidation of [1,2-¹⁴C]vinyl chloride and [1,2-¹⁴C]dichloroethene to ¹⁴CO₂ under humic acid-reducing conditions was demonstrated. The results indicate that waterborne contaminants can be oxidized by using humic acid compounds as electron acceptors and suggest that natural aquatic systems have a much larger capacity for contaminant oxidation than previously thought.

The chlorinated solvents tetrachloroethene (PCE) and trichloroethene (TCE) are common groundwater contaminants and are observed to biodegrade to dichloroethene (DCE) and vinyl chloride (VC) under anoxic conditions. The production of VC, an Environmental Protection Agency priority pollutant, is especially problematic because it is a potent carcinogen (5, 15, 23, 25) and tends to accumulate in solution under highly reducing conditions (5, 12, 13, 21, 23, 25, 28, 29). Biodegradation of VC under reducing conditions is thought to be restricted to reductive dechlorination of VC to ethene or ethane (2–5, 9, 12, 13, 16, 21, 22, 28). Reductive dechlorination of VC requires a surplus of electron donors (12, 13), generally occurs at a low rate (2–5, 9, 12, 13, 16, 21, 22, 28), and characteristically does not result in significant mineralization to CO₂ (12, 13, 16, 21, 22).

However, in organic compound-rich bed sediments collected from a blackwater stream, we recently observed significant mineralization of VC under methanogenic conditions (7). In these experiments, up to 45% of the [1,2-¹⁴C]VC radiolabel was recovered as ¹⁴CO₂ after 37 days (7). Ethene and ethane were observed in trace quantities but did not accumulate over time (7). The magnitude of the observed VC mineralization (7), the fact that significant VC mineralization under methanogenic conditions has been reported only once previously (28), and the recent laboratory evidence indicating that methanogens are not necessarily involved in VC degradation (2, 13, 21, 22, 27) suggested that the VC mineralization we observed under methanogenic conditions was not, in fact, coupled to methanogenesis.

To test the hypothesis that the mineralization of VC previously observed in streambed sediment microcosms under methanogenic conditions was not coupled to methane production, the effects of 20 mM BES (2-bromoethanesulfonic acid, an inhibitor of methanogenesis) on methane production and VC mineralization were investigated in microcosms containing bed sediment material collected from the same black-water stream at Naval Air Station Cecil Field, Jacksonville, Fla. (7). The study site and the general methods for microcosm preparation and monitoring have been described in detail previously (7). Anaerobic microcosms, prepared with a helium headspace and amended with [1,2-¹⁴C]VC, demonstrated extensive methane production (Fig. 1a) and about 15% mineralization of

[1,2-¹⁴C]VC radiolabel to ¹⁴CO₂ (Fig. 1b) within 23 days. The lack of dissolved oxygen (<0.5 μM), nitrate (<0.2 μM), sulfate (<0.2 μM), iron(II) (<0.3 μM), and sulfide (<0.3 μM) in these microcosms indicated that O₂, NO₃, Fe(III), and SO₄ reductions were insignificant under these culture conditions. Addition of 20 mM BES completely inhibited methane production (Fig. 1a) without significantly impacting VC mineralization (Fig. 1b). These results unequivocally demonstrate that the VC mineralization observed under methanogenic conditions was not coupled to methanogenesis. Moreover, the results indicate that VC mineralization involved a metabolic pathway other than O₂, NO₃, Fe(III), or SO₄ reduction.

Because the sediment samples used in this study were collected from a streambed environment characterized by a high content of natural organic material (2.5% dry mass organic content) and saturated with humic acid-laden black water, and because humic acids are capable of serving as electron acceptors for the oxidation of naturally occurring organic acids (19), we hypothesized that the presence of naturally occurring humic acids may stimulate the anaerobic mineralization of VC. To test this hypothesis, a microcosm experiment was initiated with fresh, bed sediment material in order to compare the mineralization of [1,2-¹⁴C]VC under anaerobic, humic acid-amended conditions with [1,2-¹⁴C]VC mineralization under unamended, aerobic and unamended, anaerobic conditions. Rapid mineralization of [1,2-¹⁴C]VC (Fig. 2) was observed under aerobic (no humic acids added) conditions (92% ± 11% [mean ± standard deviation] recovery as ¹⁴CO₂ in 15 days). This observation is consistent with numerous, previous reports of rapid aerobic oxidation of VC (6, 11, 17, 18, 20, 24)). In unamended (no humic acids added) anaerobic microcosms, mineralization of [1,2-¹⁴C]VC was approximately linear over the first 15 days (29% ± 7% recovery of ¹⁴CO₂) and subsequently leveled off with a final ¹⁴CO₂ recovery of 39% ± 3% in 50 days (Fig. 2). As indicated previously (see Fig. 1a), extensive production of methane was observed in these microcosms (data not shown). Addition of humic acids (Aldrich Chemical Co., St. Louis, Mo.) significantly stimulated both the rate of [1,2-¹⁴C]VC mineralization and the final recovery of ¹⁴CO₂ (91% ± 9% in 50 days) (Fig. 2). Addition of humic acids decreased methane production by about 80% (data not shown). The fact that humic acid addition stimulated [1,2-¹⁴C]VC mineralization (Fig. 2) but inhibited methane production corroborates the conclusion that mineralization was not coupled to methanogenesis and strongly suggests that naturally occurring humic acid compounds play a significant role in the mineralization of VC under these conditions.

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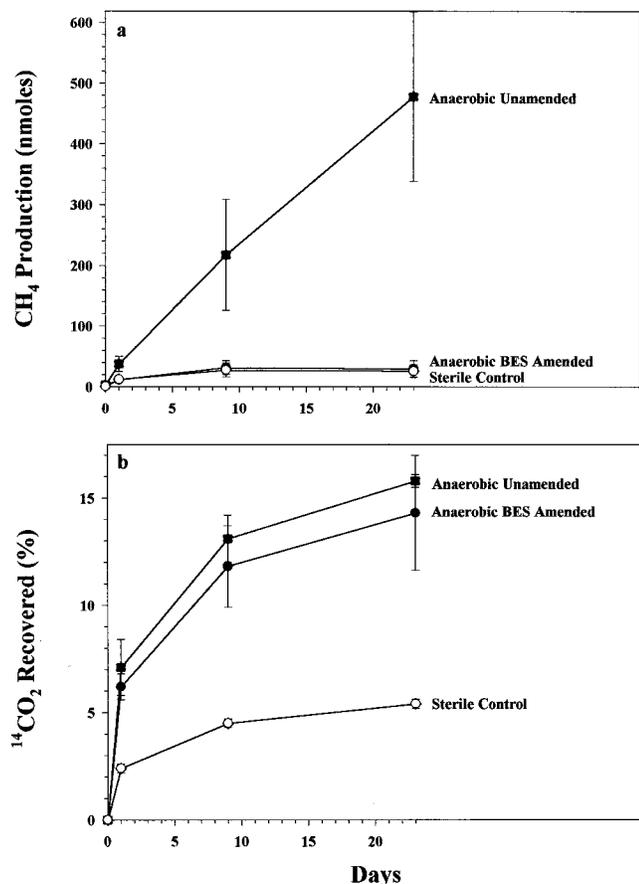


FIG. 1. Effects of 20 mM BES amendment on methanogenesis (a) and percent mineralization of [1,2-¹⁴C]VC to ¹⁴CO₂ (b). Microcosms were prepared in 30-ml serum vials with 20 g of fresh, creek bed sediment (15 g dry weight), an atmosphere of helium, and approximately 10⁵ dpm of [1,2-¹⁴C]VC (5 μM initial dissolved concentration). Methane production was measured by gas chromatography-flame ionization detection and quantified as nanomoles per 0.5-ml injection. Injection volume was replaced with helium. ¹⁴CO₂ was collected in 3 M KOH and quantified by liquid scintillation counting as described previously (7). Data are means ± standard deviation (SD) for triplicate microcosms. Recovery as ¹⁴CO₂ was confirmed in select microcosms by barium hydroxide precipitation as described previously (11). Sterile controls were prepared as described above and autoclaved for 1 h at 120°C and 15 lb/in². [1,2-¹⁴C]VC was obtained from NEN Dupont and had a radiochemical purity of 97%.

The mechanism for VC degradation observed in this study is not consistent with that described previously for VC degradation under methanogenic conditions (2–5, 9, 12, 13, 16, 21, 22, 28). Previous reports describe reductive dechlorination of VC to ethene or ethane (2–5, 9, 12, 13, 16, 21, 22, 28) without significant mineralization to CO₂ (12, 13, 16, 21). In contrast, ¹⁴CO₂ was the only product of [1,2-¹⁴C]VC degradation detected in this study, with 39% ± 3% recovery under unamended, anaerobic conditions and about 100% recovery (in 50 days) under humic-acid-amended conditions (Fig. 2). Gas chromatographic analysis of the headspace indicated no detectable accumulation of ethene or ethane in this study (detection limit of 50 nmol/liter of headspace or 2% of the initial, maximum headspace VC concentration). Thus, while degradation of VC in previous investigations involved a net reduction, in this study VC degradation involved a net oxidation. The stoichiometric conversion of [1,2-¹⁴C]VC to ¹⁴CO₂ under humic acid-amended conditions, the apparent absence of reduced intermediate products, and the comparable magnitudes

of VC mineralization under aerobic and humic acid-amended anaerobic conditions observed in the present study are consistent with net oxidation of VC to CO₂ in the presence of humic acids. The ability of humic acid-type compounds to stimulate the oxidation (19) or reduction (10, 14, 26) of organic compounds is known and generally attributed to the presence of quinone-hydroquinone moieties (10, 14, 19, 26). To our knowledge, however, this is the first demonstration of the ability of humic acids to stimulate the oxidation of contaminant compounds.

To determine if the stimulatory effect of humic acid amendment on VC oxidation could be attributed to humic acids serving as electron acceptors, we examined VC mineralization in liquid cultures (second transfer, 10⁻⁴ final dilution of original bed sediment slurry inoculum) containing 2,6-anthraquinone disulfonate (AQDS, a model humic acids compound) (10, 19) as sole electron acceptor and [1,2-¹⁴C]VC as sole electron donor (Fig. 3). Cultures were maintained on sterile, minimal medium consisting of 98% phosphate buffer (8.3 mM; pH 7.2), 1% Wolfe's mineral solution (1), 1% Wolfe's vitamin solution (1), 4 mM AQDS, and [1,2-¹⁴C]VC. Mineralization of [1,2-¹⁴C]VC (10⁵ dpm or 5 μM initial concentration) to ¹⁴CO₂ was monitored over time as described previously (7). Significant mineralization of [1,2-¹⁴C]VC to ¹⁴CO₂ (14% ± 2% in 24 days) was observed in AQDS-amended, live treatments but not in AQDS-amended, sterile controls, AQDS-amended, cell-free controls, or live treatments lacking AQDS (Fig. 3b). VC oxidation in AQDS-amended live treatments was associated with accumulation of reduced AQDS (2,6-anthrahydroquinone disulfonate [AHD]) as evidenced by the orange color formation and increased absorbance (450 nm) of the culture medium (Fig. 3a). The fact that introduction of oxygen at the end of the incubation resulted in immediate loss of color and decreased absorbance confirmed that AQDS was reduced during the incubation (Fig. 3a). The lack of significant mineralization in unamended microcosms, AQDS-amended cell-free control microcosms, and sterile control microcosms demonstrated that active microorganisms and concomitant reduction of AQDS were required for significant oxidation of VC to occur (Fig. 3b). The results demonstrate that AQDS can serve as an electron acceptor for microbial oxidation of VC and indicate that the VC oxidation observed under humic acid-amended conditions was coupled to humic acid reduction.

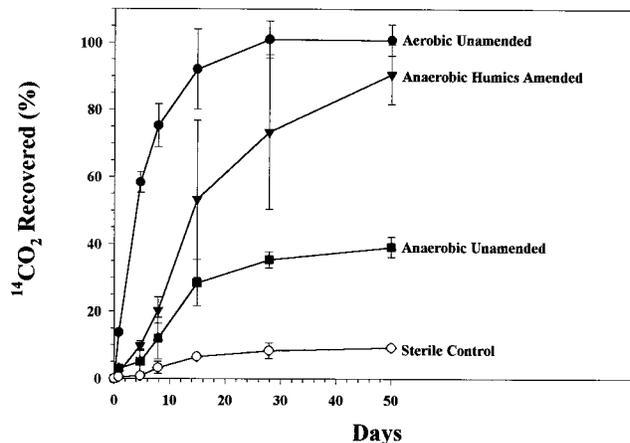


FIG. 2. Percent mineralization of [1,2-¹⁴C]VC to ¹⁴CO₂ in aerobic and anaerobic microcosms and in anaerobic microcosms amended with 2 mg of humic acids/liter. Microcosms were prepared as described in the legend for Fig. 1. Data are means ± SD for triplicate microcosms.

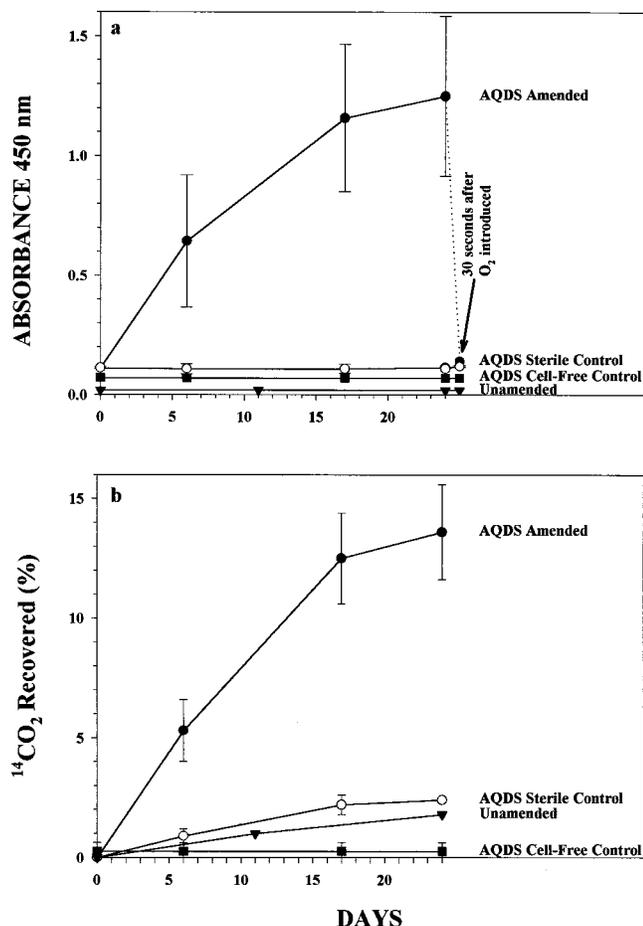


FIG. 3. Reduction of AQDS (a) and mineralization of $[1,2-^{14}\text{C}]\text{VC}$ to $^{14}\text{CO}_2$ (b) in anaerobic culture tubes containing minimal medium, 10^5 dpm of $[1,2-^{14}\text{C}]\text{VC}$ ($5\ \mu\text{M}$ initial dissolved concentration), and $4\ \text{mM}$ AQDS. The unamended microcosm was prepared in the same manner without AQDS. Reduction of AQDS was quantified spectrophotometrically as the increase in absorbance at $450\ \text{nm}$. For each treatment, data are means \pm SD for duplicate microcosms.

Accumulation of VC in the environment is affected by the production as well as the degradation of VC. In the majority of chlorinated ethene-contaminated sites, the immediate precursor of VC during reductive dechlorination of PCE and TCE is DCE. To test the hypothesis that humic acids may also reduce the environmental risk associated with VC by stimulating efficient degradation of DCE, a microcosm experiment was initiated with fresh, bed sediment material in order to compare the mineralization of $[1,2-^{14}\text{C}]\text{DCE}$ under anaerobic, humic acid-amended conditions with $[1,2-^{14}\text{C}]\text{DCE}$ mineralization under unamended, aerobic and unamended, anaerobic conditions. Rapid mineralization of $[1,2-^{14}\text{C}]\text{DCE}$ (Fig. 4) was observed under aerobic (no humic acids added) conditions ($67\% \pm 11\%$ recovery as $^{14}\text{CO}_2$ in 50 days). Aerobic oxidation of DCE has been reported previously for these sediments (8). In unamended (no humic acids added) anaerobic microcosms, low but statistically significant mineralization of $[1,2-^{14}\text{C}]\text{DCE}$ ($7\% \pm 1\%$ in 50 days) was observed (Fig. 4). Trace amounts of VC ($10\ \text{nmol/liter}$) and of ethene and ethane (both at the detection limit of $50\ \text{nmol/liter}$) were observed in the headspace for unamended, anaerobic microcosms (data not shown). Addition of humic acids significantly stimulated $[1,2-^{14}\text{C}]\text{DCE}$ mineralization ($25\% \pm 6\%$ in 50 days) (Fig. 4). No VC, ethene, or

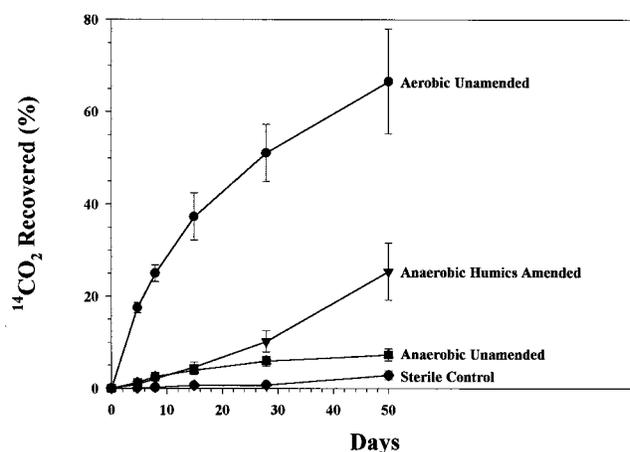


FIG. 4. Percent mineralization of $[1,2-^{14}\text{C}]\text{DCE}$ to $^{14}\text{CO}_2$ in aerobic and anaerobic microcosms and in anaerobic microcosms amended with $2\ \text{mg}$ of humic acids/liter. Data are means \pm SD for triplicate microcosms. DCE mineralization was evaluated by using a neat mixture of $[1,2-^{14}\text{C}]\text{DCE}$ (10^5 dpm or $15\ \mu\text{M}$ initial dissolved concentration; Moravak Biochemicals, Inc., Brea, Calif.). The $[1,2-^{14}\text{C}]\text{DCE}$ used in this study was a mixture of 29% *trans* and 71% *cis* isomers and had a radiochemical purity of $\geq 99.9\%$.

ethane was detected under humic acid-amended conditions (data not shown). These results indicate that humic acids can reduce the environmental risk associated with chlorinated ethene contamination by stimulating efficient degradation of DCE without accumulation of VC.

This investigation is, to our knowledge, the first report that humic acid compounds can serve as electron acceptors for anaerobic oxidation of organic contaminants. The results indicate that, in the presence of humic acids, efficient mineralization of VC and DCE without accumulation of reduced intermediates can occur even under methanogenic conditions. Thus, the results have important implications for bioremediation of anaerobic sites contaminated with chlorinated ethenes.

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