

Biodegradability of Trifluoroacetic Acid

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ABSTRACT

Trifluoroacetic acid (TFA) is a product of the atmospheric oxidation of several man-made fluorine-containing organic compounds. The environmental fate of trifluoroacetic acid (TFA) is poorly understood. There are no known abiotic sinks for TFA, and there is a controversy on its biological fate. To provide insight into the environmental fate of TFA, a long-term (90-week) study was conducted to assess its biodegradability in an engineered anaerobic reactor. Trifluoroacetic acid was found to be cometabolically degradable in an anaerobic environment. Biodegradation is a potential sink for trifluoroacetic acid, and may limit its accumulation in the environment.

Key words: trifluoroacetic acid; anaerobic degradation; dehalogenation; co-metabolic degradation; hydrofluorocarbons; hydrochlorofluorocarbons; refrigerant

INTRODUCTION

RECOGNITION OF THE ADVERSE EFFECT of chlorofluorocarbon (CFC) release into the atmosphere on tropospheric ozone has led to an international effort to replace CFCs with environmentally acceptable alternatives. Hydrofluorocarbons (HFCs) and hydrochlorofluorocarbons (HCFCs) are classes of compounds developed to replace CFCs in applications such as refrigeration fluids and cleaning agents for electronic equipment. It is well known that trifluoroacetic acid (TFA), CF_3COOH , is produced when certain HFCs and HCFCs, for example, $\text{CF}_3\text{CCl}_2\text{H}$ (HCFC-123) and CF_3CFH_2 (HFC-134a),

are degraded in the atmosphere (Wallington *et al.*, 1994). Concern has been expressed that TFA from such sources may accumulate in seasonal wetlands with adverse environmental consequences (Tromp *et al.*, 1995; Likens *et al.*, 1997). TFA is a strong organic acid with a pK_a of 0.23, is miscible with water, has a low Henry's law constant ($0.112 \text{ atm cm}^3/\text{mol}$) (Bowden *et al.*, 1996), partitions into aqueous compartments of the environment, and is mildly phytotoxic (Ingle, 1988). The trifluoroacetate ion, CF_3COO^- , is stable in the aqueous phase. No significant abiotic loss processes of the trifluoroacetate ion (e.g., hydrolysis, photolysis, or precipitation) have been identified. Unless subject to

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biodegradation, it appears that TFA will accumulate in the environment.

There are conflicting reports of TFA degradation under anaerobic conditions. Visscher *et al.* (1994) observed that natural sediments reduced TFA. However, even though this work was performed in replicate, these experimenters and others were unable to reproduce it in subsequent studies using anaerobic digester sludge, rumen fluid, freshwater sediments, and marine sediments (Emptage *et al.*, 1997). Potential biological sinks for TFA in the environment have been investigated. Standley and Bott (1998) found that a small amount of TFA was metabolically incorporated into the tissues of exposed aquatic organisms. Richey *et al.* (1997) reported that some soils with high organic content or high iron and aluminum content exhibited strong retention of TFA. However, Richey *et al.* (1997) considered TFA to be a relatively mobile organic compound in soils because at the majority of sites investigated the soils did not retain much TFA. At the present time there is no clear answer to the question: "Is TFA biologically degradable?"

TFA has obvious structural similarities to trichloroacetic acid (TCA). TCA is known to be degraded cometabolically through anaerobic reductive dehalogenation (Weightman *et al.*, 1992). At relatively high concentrations TCA (Lakowski and Broadbent, 1970; Itickey *et al.*, 1987) and TFA (Visscher *et al.*, 1994) exhibit a similar inhibitory behavior on methane production. It is conceivable that TFA could be degraded in a similar fashion to TCA.

Previous studies of the biodegradability of TFA (Visscher *et al.*, 1994; Emptage *et al.*, 1997) were based on relatively short-term batch incubation experiments that can be heavily dependent on initial conditions and may not be reproducible. To assess the biodegradability of TFA we have conducted long-term (90-week) continuous-flow experiments using laboratory-scale anaerobic reactors. The length of the experiment was equivalent to approximately 30 turnovers of micro-organisms. The results are reported herein.

MATERIALS AND METHODS

Materials

TFA (97%, Fisher Scientific, Fair Lawn, NJ), TCA (99%, Fisher Scientific), and ethanol (100%, Aaper Alcohol and Chemical Co., Shelbyville, KY) were used to make up feed solutions for the anaerobic reactors. Additional chemicals, difluoroacetic acid (DFA) (98%, Aldrich Chemical Co.), dichloroacetic acid (DCA) (99%, Aldrich Chemical Co., Inc.), monofluoroacetic acid (MFA) (95%, Aldrich Chemical Co., Inc., Milwaukee,

WI), and monochloroacetic acid (MCA) (99%, Fisher Scientific) were used to prepare standard solutions for chromatographic analysis. A standard solution of sodium fluoride and sodium chloride (Dionex Corp., Sunnyvale, CA) was used for ion chromatographic analysis of fluoride and chloride ions.

Two nutrient solutions were supplied to the bioreactors: one solution with several inorganic salts in deionized water and the other solution with vitamins only (Cheng *et al.*, 1996). Amounts of the salts and vitamins were selected to satisfy the nutritional requirements of the micro-organisms. The pH of the salt solution was <1 to ensure complete dissolution of the salts. A buffer solution was also prepared by dissolving sodium sulfide, sodium carbonate, and sodium hydroxide in deionized water. Amounts of the chemicals were selected so that the pH of the reactor contents was maintained at 7.2.

Aqueous effluent samples were taken periodically from bioreactors, filtered with 0.22- μ m or 0.45- μ m nylon filters (MSI, Westboro, MA), immediately acidified with two drops of 85% phosphoric acid to about pH 2, and analyzed using gas and liquid chromatography. A portion of each sample was refrigerated for subsequent analysis for fluoride and chloride ions. Gaseous effluent samples were taken from gas reservoirs using a 10-mL syringe (Poopper & Sons, Inc., New Hyde Park, NY) and analyzed for composition using gas chromatography.

Analytical methods

Fluoroacetic acids (TFA, DFA, and MFA) were analyzed using a liquid chromatograph (LC) (Waters Model 501, Milford, MA) equipped with a conductivity detector and an IC Pak anion HC column (4.6 $\times$ 150 mm, with 10- μ m particles) (Waters). A mixture of borate, acetonitrile, and butyl alcohol was used as eluant. Chloroacetic acids (TCA, DCA, and MCA) were analyzed using a Hewlett Packard LC equipped with a diode array UV detector and a column (Accubond C-18, 150 $\times$ 4.6 mm, J&W Scientific, Folsom, CA).

Fluoride and chloride ions were analyzed by an ion chromatograph (Dionex Model DX 120, Dionex Corp., Sunnyvale, CA) equipped with a conductivity detector and a column (IonPac AS14, Dionex Corp.). A solution of 3.5 mM Na₂CO₃ and 1 mM NaHCO₃ was used as eluant. The concentration of fluoride ion in the influent was negligible, whereas that of chloride ion was about 1 mg/L excluding the contribution from the salt solution.

Concentrations of alcohols (methanol, ethanol, and isopropanol) and volatile fatty acids (acetic acid and propionic acid) in the aqueous effluent samples were measured by a gas chromatograph (GC) (Hewlett Packard Model 5890, Series II, San Fernando, CA) equipped with a flame

ionization detector (FID) and a column ($1.83 \text{ m} \times 2 \text{ mm}$) packed with 5% Carbowax on a 60/80 Carbowax B-DA (Supelco, Inc., Bellefonte, PA). Nitrogen was used as a carrier gas for the analysis.

Gas composition was determined using a GC (Hewlett Packard 5890 Series II) equipped with a thermal conductivity detector and a column ($3 \text{ m} \times 3.2 \text{ mm}$) packed with 45/60 molecular sieve (Hewlett-Packard Company, San Fernando, CA). Argon was used as a carrier gas. The daily gas production rate was measured with a wet tip gas meter (Environmental and Water Research Engineering, Nashville, TN).

Experimental methods

Two 10-L stainless steel reactors were operated as chemostats and were initially seeded with an anaerobic mixed culture capable of degrading ethanol. The contents of the reactors were completely mixed with magnetically coupled mixers. The influent consisted of a substrate solution, two nutrient solutions, and a buffer solution. Each reactor was fed at a flow rate of 0.5 L/day, resulting in a hydraulic retention time of 20 days. The pH and temperature of the contents of both reactors were maintained at 7.2 and 35°C , respectively.

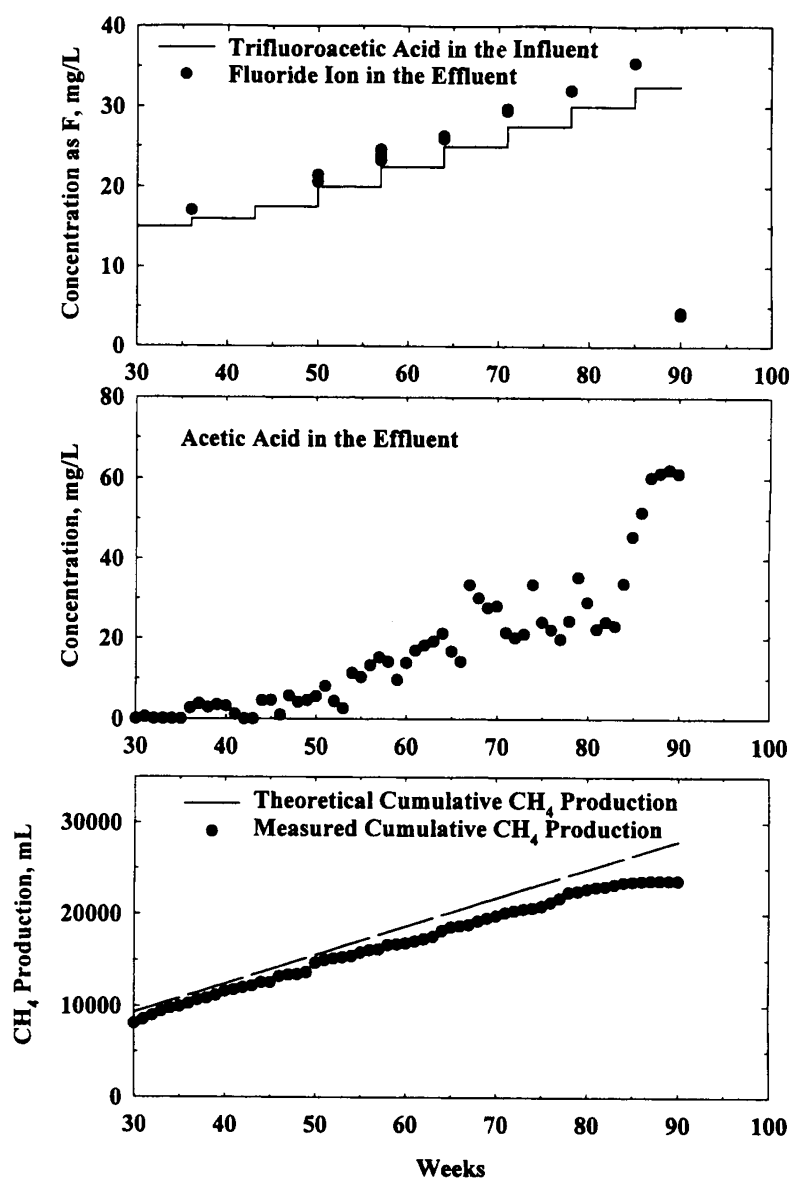


Figure 1. Anaerobic degradation of trifluoroacetic acid.

For the first 20 to 30 weeks the substrate solution was doped only with ethanol to ensure that micro-organisms in the reactors were viable. During this period, almost all of the ethanol introduced into the reactors was converted to methane. TFA was then added to the substrate solution fed to one reactor while TCA was added to the solution fed to the other reactor. The TFA concentration in the substrate solution was increased incrementally in eight steps over a time period of 60 weeks (see top panel in Fig. 1) while the ethanol concentration was maintained at 100 mg/L. TCA was added to the substrate solution

fed to the other reactor in a similar fashion (see top panel of Fig. 2). In control experiments performed using a sterile substrate solution in the absence of micro-organisms there was no discernable loss of TFA or TCA.

RESULTS AND DISCUSSION

Biodegradability of trifluoroacetic acid

Figure 1 shows the TFA concentration in the influent, F^- concentration in the effluent, acetic acid concentra-

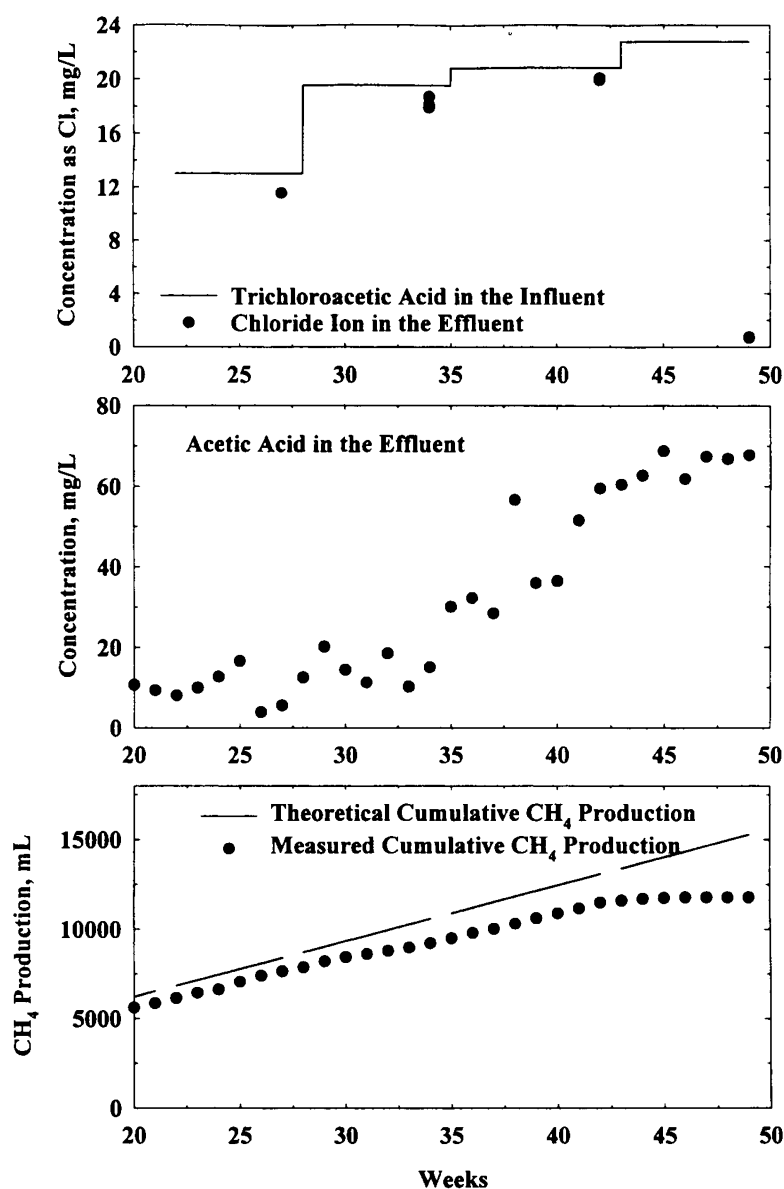


Figure 2. Anaerobic degradation of trichloroacetic acid.

tion in the effluent, and cumulative CH_4 production during the experiment. In the top panel, the TFA concentration is expressed as F to compare with the fluoride-ion concentration in the effluent. There was no detectable F in the influent. Except for the last loading increase when the influent TFA concentration was increased to 65 mg/L (32.5 mg/L as F), no TFA, DFA (difluoroacetic acid), or MFA (monofluoroacetic acid) were found in the effluent. As seen from Fig. 1, there is excellent agreement between the concentration of fluoride ion measured in the effluent and that originally in the form of trifluoroacetic acid in the influent. We conclude that under the low TFA loading conditions there was complete anaerobic degradation of TFA, and no appreciable amounts of TFA were present in the reactor. During the last loading increase, the anaerobic degradation virtually stopped. At this point, the effluent was found to contain TFA, DFA, and MFA at 51, 8.3, and 6.2 mg/L, respectively. The TFA concentration represents about 80% of the influent concentration. In addition, about 60% of the ethanol fed was not degraded. The inhibition by TFA was also evidenced by a gradual increase (rapid during the last loading period) of acetic acid concentration (middle panel) and the eventual cessation of methane production (bottom panel). It is clear that high concentrations of TFA inhibit methane formation. This inhibition is not consistent with the findings by Emptage *et al.* (1997), who reported no inhibition of methane production by TFA for concentrations of TFA up to 10 mM (1,140 mg/L), which is much higher than the highest TFA concentration observed in this study (51 mg/L). In contrast, Visscher *et al.* (1994) observed an inhibitory effect of TFA on methanogenic activity at $>1 \mu\text{M}$ (0.1 mg/L). The observation of DFA and MFA in the present work is consistent with the biodegradation of TFA occurring via step-wise successive defluorination.

Biodegradability of trichloroacetic acid

Figure 2 shows the results obtained in the anaerobic degradation study of trichloroacetic acid. As was the case for TFA, all of the TCA introduced into the reactor was degraded except for the last loading increase when the influent TCA concentration was 35 mg/L (22.8 mg/L as Cl). No TCA, DCA, and MCA were found in the effluent except for the last loading during which TCA, DCA, and MCA were found in the effluent at 28, 10, and 0 mg/L, respectively. Inhibition by TCA has been reported previously (Laskowski and Broadbent, 1970; Hickey *et al.*, 1987), and occurs at a loading lower than for TFA. The facile biodegradation of TCA observed in the present work is entirely consistent with expectations based upon previous work (Laskowski and Broadbent, 1970; Hickey *et al.*, 1987).

CONCLUSIONS

The goal of the present study was to address the question "Is TFA biologically degradable?" The results in Fig. 1 show that the answer to this question is "Yes." As shown in the top panel of Fig. 1, the microbial community established in the anaerobic reactor system was able to degrade all of the TFA fed to the system for a period of more than 1 year. TFA and TCA have a similar chemical structure. TCA degrades cometabolically through anaerobic reductive dehalogenation (Weightman *et al.*, 1992); it seems reasonable to conclude that TFA also undergoes cometabolically degradation. Finally, we need to consider the implications of the present work in terms of the environmental fate of TFA. It should be noted that the present experimental study was conducted under laboratory conditions, not in the natural environment. Practical considerations precluded us from conducting experiments in the natural environment. However, the mixed culture used in this study contains naturally occurring micro-organisms that are commonly found natural water systems. Their composition and growth rates may vary depending on environmental conditions, such as pH, temperature, and substrate concentrations. Therefore, the main question is not whether or not these organisms are present in the natural environment, but how fast the TFA degradation occurs. In this paper, it was intended to show whether or not TFA is biodegradable, not how fast TFA could be degraded in the nature. The results presented herein clearly show that anaerobic degradation is a potential sink for TFA in freshwater sediments, and may limit the accumulation of this compound in the environment.

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