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Anaerobic transformation of 1,1,1-trichloroethane by municipal digester sludge

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Abstract

Anaerobic transformations of 1,1,1-trichloroethane (TCA), 1,1-dichloroethane (DCA), and chloroethane (CA) were studied with sludge from a lab-scale, municipal wastewater sludge digester. TCA was biologically transformed to DCA and CA and further to ethane by reductive dechlorination. TCA was also converted to acetic acid and 1,1-dichloroethene (11DCE) by cell-free extract. 11DCE was further biologically converted to ethene. This pathway was confirmed by transformation tests of TCA, DCA and CA, by tests with cell-free extract, and by chloride release during TCA degradation. With cell-free extract, acetic acid accounted for approximately 90% of the TCA transformed; tests with live cells indicate that the fraction of TCA transformed by this pathway decreased with lower biomass. The dechlorination of DCA to CA and CA to ethane was not stoichiometric. A high rate of TCA removal was observed under the experimental conditions. The results indicate that removal of TCA in anaerobic digestion should be complete, but DCA and CA could persist in a normally operating digester.

Introduction

1,1,1-Trichloroethane (TCA) is introduced into the environment through its wide use as a solvent, degreasing agent or as a chemical process intermediate. In 1991, TCA was the 8th largest chemical released to the environment in the United States, including point and nonpoint air emissions, surface water discharges, underground injection and releases to land (USEPA, 1991). It is one of the most common contaminants found in groundwater used for drinking water supply in the US (Westrick et al., 1984). TCA also has frequently been found in municipal wastewater and is hydrophobic enough to partition to wastewater solids (Ballapragada et al., 1998). TCA, like other highly chlorinated aliphatic compounds, is more readily transformed under anaerobic than aerobic conditions (McCarty and Semprini, 1994). Thus its fate in anaerobic digestion is of interest.

TCA can be degraded by biological and chemical transformations, primarily under anaerobic or reducing conditions (e.g. Bouwer et al., 1983; Parson et al., 1985). McCarty and co-workers extensively studied TCA transformations, and as early as 1987, Vogel and McCarty (1987a) proposed pathways of TCA degradation under methanogenic conditions. TCA is biologically converted to 1,1-dichloroethane (DCA), then to chloroethane (CA), which may be hydrolyzed abiotically to ethanol. Abiotic transformations of TCA result in the formation of acetic acid through hydrolysis and 1,1-dichloroethene (11DCE) by dehydrochlorination, which can further be biologically converted to vinyl chloride (VC). Finally, ethanol and acetic acid can be anaerobically tranformed via methanogenesis and VC by reductive dehalogenation to ethene. These compounds can also be aerobically oxidized to CO_2.

Not all the proposed pathways have been confirmed experimentally in methanogenic systems with TCA.

DCA has often been observed as a product of anaerobic degradation of TCA, while CA has occasionally been seen. Egli et al. (1983) reported the reductive dehalogenation of TCA to DCA by a pure culture of *Desulfobacterium* sp. without seeing other intermediates in batch tests. Parsons et al. (1985) showed that TCA underwent reductive dehalogenation under anaerobic conditions in batch bottle tests. Vogel and McCarty (1987a) investigated transformations of TCA in methanogenic biofilm reactors. TCA was rapidly converted to DCA at a 6 day retention time with acetate as a primary substrate. In other tests, CA was the main product. Galli and McCarty (1989) reported biological transformation of TCA by a *Clostridium* sp. isolated from the effluent of an anaerobic suspended growth bioreactor. With the presence of tryptone, yeast extract and trace metals, TCA was degraded to DCA with traces of CA and to acetic acid with other unidentified products. van Eekert et al. (1999) have also shown conversion by unacclimated methanogenic granular sludge, primarily to DCA and CA. Biological reductive dehalogenation has been shown to transform TCA to DCA and to CA. The anaerobic transformation of CA, however, has not been shown in experimental studies.

There are several reports of chemical transformations of TCA. Jeffers et al. (1989) measured the hydrolysis rate for TCA, and Cline and Delfino (1989) reported conversion of TCA to 11DCE by dehydrochlorination. Jeffers et al. summarized the range of estimated half-lives at room temperature to be from 0.5 to 3 years. Vogel and McCarty (1987b) reported abiotic transformation of TCA to 11DCE via an elimination reaction (dehydrochlorination) and also possibly to acetic acid (not measured) in groundwater. Klecka et al. (1990) reported the abiotic transformations of TCA to 11DCE and to a polar metabolite (either acetic acid or CO_2 or both) in subsurface solids and groundwater in batch bottle tests. Although the chemical pathway has been demonstrated, the rates are slow and the role of biochemicals in the reactions has not been shown. Therefore, the confirmation and evaluation of a TCA transformation biopathway under methanogenic conditions, with live cells and with cell-free extract, are of interest in this study.

We report experimental studies, using anaerobic digester sludge, that are intended to further elucidate the biological and chemical reactions, the pathways and extent of chloroethane transformation. The role of biochemicals in chemical transformations is demonstrated using cell-free extracts of the anaerobic culture. The transformation of DCA and CA is also studied to investigate transformations that are difficult to see with TCA as parent compound. The study is intended to provide an improved understanding of the degradation of TCA in anaerobic sludge digestion, including its toxicity and persistent metabolites.

Materials and methods

Chemicals

The compounds used in this study were TCA (99%), DCA (99%), CA (99.9%) (Aldrich Chemical Company, Inc. Milwaukee, Wisconsin); 1,1-DCE, observed as a product, was obtained from Aldrich Chemical Company for standardization at 99% purity. Standard gases included CO_2 and CH_4 (19.6% nitrogen, 29.2% CO_2 and balance CH_4, Airco Special Gases, Vancouver, Washington), ethane (Scotty Mini-Mix, Can Mix 366, 0.994% ethane in nitrogen, Scott Specialty Gases), and ethene (Matheson, East Rutherford, New Jersey). Vinyl chloride was obtained as a standard solution in methanol from Supelco Inc. (Supelco Park, Bellefonte, PA).

Culture media

Reduced anaerobic mineral medium (RAMM) (Shelton and Tiedje, 1984) was used in all bottles except for chloride release tests. The medium was autoclaved and subsequently boiled while being purged with oxygen-free N_2. $NaHCO_3$ and $Na_2S{\cdot}9H_2O$ were added to the media after cooling. Resazurin was used as a redox indicator to make sure the medium was reduced under all conditions. Serum bottles with volumes of 35, 120 and 160 mL were used for bottle tests.

A modified low chloride RAMM medium was prepared with the same procedures and used in chloride release tests. It consisted of KH_2PO_4 (0.41 g/L), $Na_2HPO_4.2H_2O$ (0.53 g/L), NH_4HCO_3 (0.42 g/L), $CaHPO_4$ (0.088 g/L), MgO (0.53 g/L), $NaHCO_3$ (4.3 g/L), trace minerals, and $Na_2S.9H_2O$ (5 mg/L).

Source of biomass

Anaerobic sludge from a laboratory-scale digester (Ballapragada et al., 1995) was used as the seed. This

digester had been fed with primary and waste activated sludge from Renton Wastewater Treatment Plant (WWTP), Washington, along with a mixture of chlorinated compounds, including PCBs (Aroclor 1254), pentachlorophenol, 1,2,4-trichlorobenzene, perchloroethylene and carbon tetrachloride, but not including TCA or other chloroethanes. The digester was operated at 35 °C with a retention time of 25 days for 21 months with suspended solid (SS) and volatile suspended solids (VSS) concentrations of 20 g/L and 11 g/L, respectively. The VSS concentration in bottle tests to study the effect of biomass on TCA transformation varied from 55 to 1100 mg/L. The VSS concentration in all other bottle tests was 1.5–2.5 g/L.

Analytical methods

Chlorinated compounds were analyzed in liquid samples by purge and trap (Tekmar ALS-LSC), followed by gas chromatography (Perkin-Elmer 8700) with an electrolytic conductivity (Model 1000 Hall) detector. The column was held isothermal for 5 minutes at 35 °C, followed by a ramp rate of 8 °C/min to 199 °C. Ethane and ethene were measured using a gas chromatograph equipped with a HayeSep Q (Supelco) packed column and a flame ionization detector (FID). All compounds had detection limits below 0.5 μmol/L.

Gas production was measured by displacement using a glass syringe at atmospheric pressure. Gas composition (CH_4 and CO_2) was analyzed by gas chromatography, using a Hayesep Q (Supelco) 6 packed column and a thermal conductivity detector (TCD).

Acetic acid was measured by gas chromatography, using a 60/80 Carbopack column, and FID. The detection limit was approximately 0.2 mmol/L.

Inorganic chloride was analyzed using a specific ion electrode (Orion Research Inc. Lab, Boston, Massachusetts). The electrode was calibrated using NaCl solutions as external standards. Chloride could be measured at concentrations above 50 μmol/L. Initial values were subtracted from those measured during the experiment to determine the chloride released.

Total quantities of chlorinated compounds in liquid phase samples in bottles were calculated using the volumes of the liquid and gas phases and the Henry's law constant for each compound. Dimensionless Henry's constants for the compounds used are as follows (Gossett, 1987): TCA = 0.987, DCA = 0.241, CA = 0.651, 11DCE = 1.541. Ethene and ethane were measured in the gas phase only.

TCA toxicity

Serum bottles were set up as batch bioassays. Digester sludge was dispensed into the bottles and purged with oxygen-free N_2. Bottles were closed with butyl rubber stoppers and sealed with aluminum crimp tops. Raw sludge (1 mL) from Renton WWTP and TCA from saturated aqueous solution (1500 mg/L) were added using disposable sterile microsyringes. Initial liquid concentrations of TCA were 0, 0.25, 0.5, 0.75, 1.0, 3.0, 5.0, 10 and 20 mg/L (0, 1.9, 3.8, 5.6, 7.5, 22, 38, 75 and 150 μmol/L). All bottles were incubated on a shaker (150 rpm) at 35 °C. Gas production and gas composition were measured every day during the first two weeks and every other day after that to determine the inhibitory effects of TCA on methane production. The concentrations of chlorinated compounds were analyzed at day 0, 14 and 25.

Transformation of TCA, DCA and CA

To understand the biological pathways of TCA degradation, batch bottle assays were conducted with repeated refeeding of electron donor and respikings of TCA, DCA and CA. Bottle bioassay procedures were adopted from those described by Shelton and Tiedje (1984) and Owen et al. (1979) and those used in our laboratory (Perkins et al., 1994).

Lactate was the sole electron donor and carbon source and was fed once a week. The lactate was fed to a concentration of 720 μmol/L. Serum bottles of 160 mL with 120 mL liquid were used. Twenty mL digester sludge and 100 mL RAMM were fed to each bottle. Autoclaved killed controls were also set up.

The bottles were purged with oxygen-free N_2 and CO_2 before each refeeding. TCA and DCA were fed with saturated stock solution. CA was added as pure gas. The initial pH was 7. All bottles were incubated on a shaker (150 rpm) at 35 °C; liquid samples were removed for analysis at intervals ranging from every other day to every other week, depending on the compound and the rate of the transformations.

The effect of biomass on TCA transformation

To study the biomass effect on the transformations of TCA, digested sludge was diluted with RAMM to 1100, 550, 110, and 55 mg VSS/L, and then spiked with TCA to about 100 μmol/L. Other conditions, including lactate feeding, were as described above for TCA transformation experiments.

Cell-free extract test

In order to understand abiotic transformations of TCA under anaerobic conditions, reduced cell free extracts were prepared. A sonicator (8893-MT Sonicator, Cole-Parmer Instrument Cop., Chicago, Illinois) was used to disrupt the cells of the laboratory digester culture with a VSS of 1.1 g/L. The cells were sonicated for 20 min and centrifuged at 5,000 rpm and 5 °C for 20 min. The supernatant was then further filtered through 0.45 μm membrane filter (Millipore). Procedures were carried out in an anaerobic glove box, except for centrifugation which was carried out in bottles gassed with oxygen-free N_2. The cell free extract was dispensed to replicate bottles containing RAMM along with 500 mg/L Na_2S or 2 mmol/L titanium (III) citrate as reducing agent. One set of bottles contained no reducing agent. Finally TCA stock solution was added to each bottle. All bottles were shaken at 35 °C. Controls without cell-free extract were prepared with water only and with 500 mg/L Na_2S, and controls with live cells from the same source were also prepared.

A high concentration of TCA (approximately 2600 μmol/L) was used to study its conversion to acetic acid at concentrations that could be detected by the GC-FID. Liquid samples for chlorinated compound analysis and acetic acid analysis were taken initially and at five times during the 27 day experiment.

Chloride release

Tests were set up in order to measure the chloride release during the transformation of TCA. They were carried out in 2.2 L bottles with 1.3 L of low chloride medium (modified RAMM) and 400 mL of digester sludge, which was washed three times with degassed, deionized water. An autoclaved killed control was set up under similar conditions. Lactate was the sole carbon and electron source and was added on several occasions during the experiment to support continuing gas production. Chlorinated compounds and inorganic chloride were measured at approximate weekly intervals, using sample volumes of approximately 100 mL. Both bottles had background inorganic chloride concentrations of approximately 200 μmol/L. Background chloride was subtracted from chloride measured during the test.

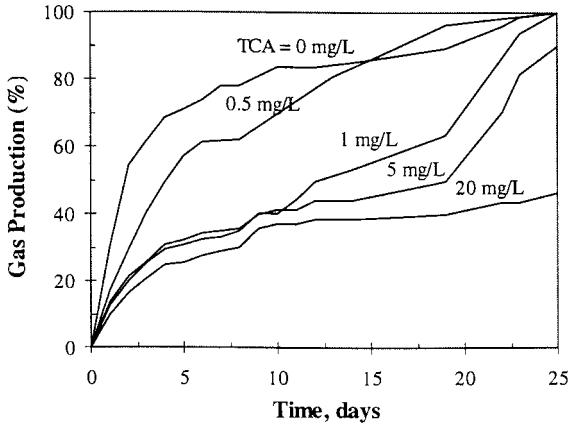

Figure 1. Effect of TCA concentration on gas production (Gas production is normalized to gas production in control bottle at day 25).

Results

Effect of TCA on biogas production

Figures 1 and 2 show the effect of TCA concentration on biogas production. Total biogas yield was quite similar in all bottles, except with the highest TCA feeding (20 mg/L). Methane production followed the same pattern as total gas production, constituting 70% or so of the produced gas (results not shown). The control bottle, which did not receive any TCA, produced 62 mL of biogas over a period of 25 days; about 55% of the total gas was produced within the first two days, after which the daily gas production declined to a steady endogenous rate. The addition of 0.5 mg/L (3.8 μmol/L) of TCA did not have a significant effect on gas production as the pattern of gas production overlapped with the control bottle. TCA at 1.0 mg/L (7.5 μmol/L) or higher inhibited gas production (Figure 1). The inhibition increased with increasing TCA concentrations. However, in most bottles the inhibition decreased with time, as TCA was removed and transformed to DCA and CA. For example, at a TCA concentration of 5 mg/L (38 μmol/L), daily gas production declined after an initial burst, but recovered after 18 days when TCA had been degraded and partially converted to DCA and CA (Figure 2).

Transformation of TCA, DCA and CA

TCA was removed quickly by the digester sludge culture without lag (see Figure 3 for typical results). Most of the TCA removal (80%) was observed in the first 2 days, while only modest amounts of DCA were

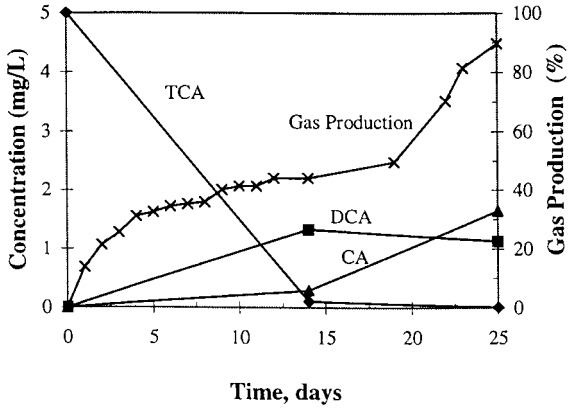

Figure 2. Gas productuion with TCA transformations (Initial TCA concentration = 37.5 μmol/L. Gas production is normalized to gas production in control bottle at day 25).

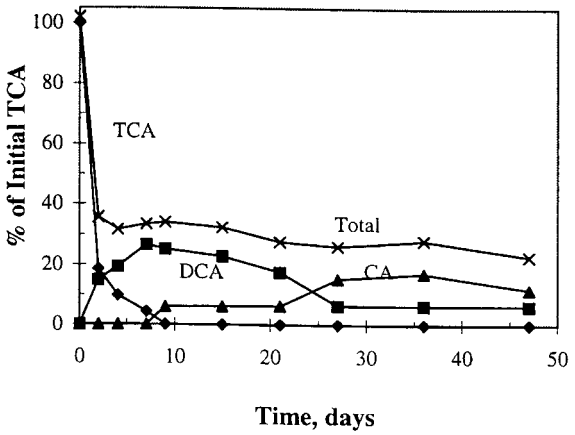

Figure 3. TCA transformations under anaerobic conditions (Initial TCA concentration = 31.6 μmol/L), showing DCA, CA and the total of the chloroethanes.

formed. The remaining TCA was gradually removed with simultaneous formation of DCA. Before TCA removal was complete, DCA reached its highest concentration (about 25% of initial TCA). Then, DCA decreased slowly with accumulation of CA, which reached about 20% of initial TCA. Only a slight decrease in CA concentration was observed during the experimental period. Traces of ethane and ethene were measured in the gas phase (total about 5% of initial TCA).

A stoichiometric balance was not obtained, as formation of DCA, CA, ethane and ethene, accounted for less than 30% of the TCA removal. Killed controls did not show any of these transformations (results not shown). Small amounts of 11DCE, however, were detected in killed controls. Acetic acid was

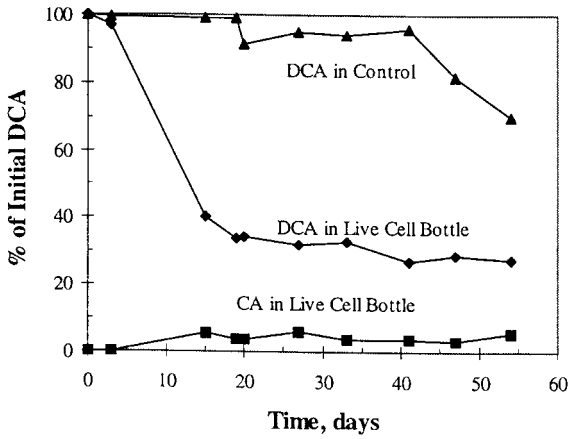

Figure 4. DCA degradation under anaerobic conditions (Initial DCA concentration = 195 μmol/L).

not analyzed because the low amounts, which could potentially have formed, were below the detection limit of the method used. Results after TCA respiking with lactate refeeding were similar to those for the initial test; neither the pattern nor the kinetics of TCA transformation changed.

In Figure 4 the transformation of DCA added to a live culture and a killed control is shown. DCA was partially degraded in the bottle with live culture. More than 70% removal of DCA was observed with the formation of CA, mostly during the first two weeks. Traces of ethane were detected during the tests (results not shown). The transformation of DCA to CA and ethane was not stoichiometric. 60–70% of initial DCA was removed after two weeks with about 10% conversion to CA and less than 3% to ethane. Some losses were observed in killed controls (30% of total DCA at day 54), but no formation of lower chlorinated ethanes was detected.

CA was also degraded by the digesting sludge, resulting in the formation of ethane, although ethane accounted for less than 1% of CA added (Figure 5). Therefore most of the CA does not appear to be reductively dehalogenated, but instead undergoes other transformations, possibly to nonchlorinated compounds. Similar to the transformation of DCA, most of the CA removal (50% of initial CA added) took place during the first two weeks. In the killed control bottle CA was lost at a constant low rate during the 54 day incubation with losses totaling 30% of the initial CA added.

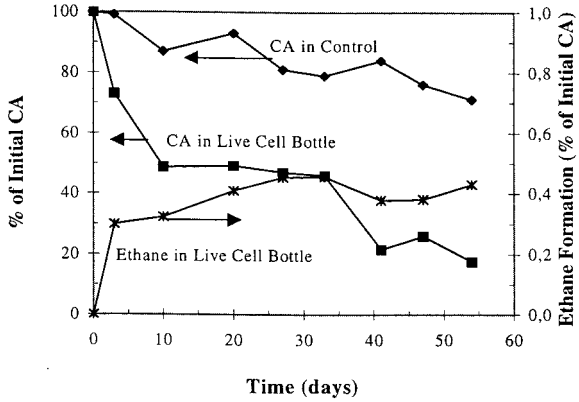

Figure 5. Chloroethane degradation under anaerobic conditions (Initial chloroethane concentration = 149 μmol/L).

Effect of biomass on TCA transformation

When different sludge concentrations were used, the most rapid removal of TCA was observed at the highest concentration, which was 1100 mg/L (see Figure 6a). In contrast, at the lowest concentration of 55 mg/L, the removal rate was slower and lagged by about 10 days. The lag may have been caused by insufficient initial biomass to develop the strongly reducing environment necessary for the transformations. No such lag phases were observed at intermediate biomass concentrations of 110 and 550 mg/L.

Along with the removal of TCA, DCA concentrations increased in each test (Figure 6b). At higher biomass concentrations (1100, and 550 mg/L), DCA decreased after about 10 days, when TCA was nearly depleted. For lower biomass concentrations, DCA accumulated slowly during the test period. At the end of the experiment DCA formation represented 9, 17, 12 and 8% of the TCA removed for 1100, 550, 110 and 55 mg/L biomass concentrations, respectively.

The reductive dechlorination of TCA to DCA occurred to a significant degree but the conversion of DCA to CA could not be detected. Traces of 11DCE were observed after about two weeks in all bottles.

A substantial portion of the TCA that was removed was not accounted for as DCA or 11DCE. This portion was calculated to be 88, 80, 65 and 47% of the initial TCA added for the biosolids of 1100, 550, 110 and 55 mg/L, respectively, and clearly depended directly on the biosolids.

TCA transformations in cell-free extract

Acetic acid can be a major product of TCA transformation. Cell free extract was used to avoid possible

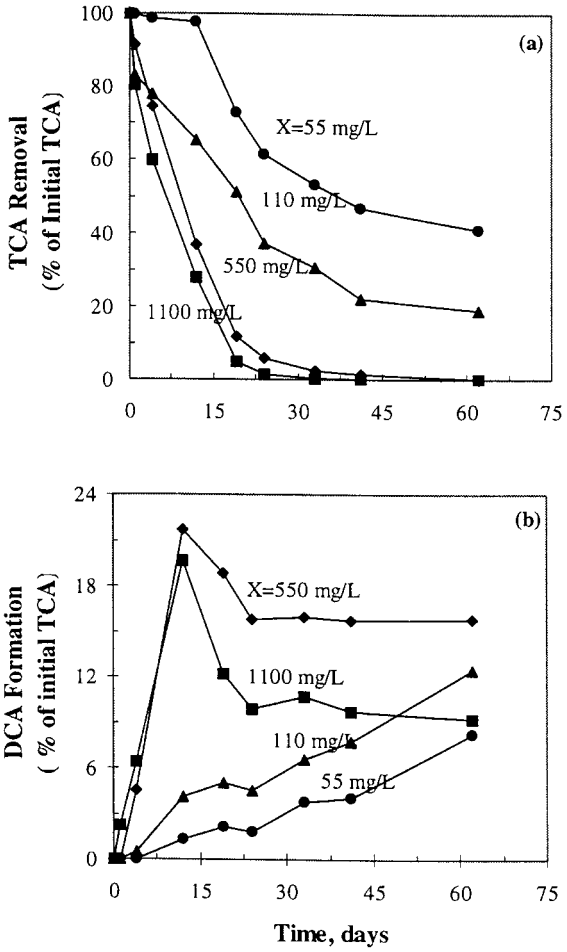

Figure 6. Effect of biomass on TCA transformation: (a) TCA removal; (b) DCA formation (Biomass concentrations: ● 55 mg/L; ▲ 110 mg/L; ♦ 550 mg/L; ■ 1100 mg/L. Initial TCA concentration of 120 μmol/L).

metabolic conversion of products, such as acetic acid. Transformation of TCA occurred with cell free extract in modified RAMM medium reduced with titanium (III) citrate (Figure 7) and with Na_2S (results not shown). TCA removal was rapid during the first week, after which it slowed down. The bottles were fed with high concentrations of TCA in order to detect acetic acid and 11DCE, which were the only products observed and which accounted stoichiometrically for all of the TCA removal. Of the initial TCA, 65% of TCA was transformed after 27 days, resulting in 57% yields of acetic acid and 5% 11DCE. Similar 11DCE formation was observed in reduced control bottles without cell-free extract (results not shown). No transformation was observed in unreduced control bottles or with unreduced cell-free extract.

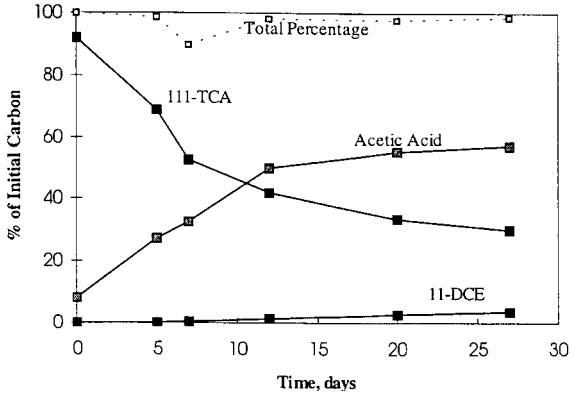

Figure 7. TCA transforamtion with reduced cell-free extract (Initial TCA concentration = 2600 μmol/L).

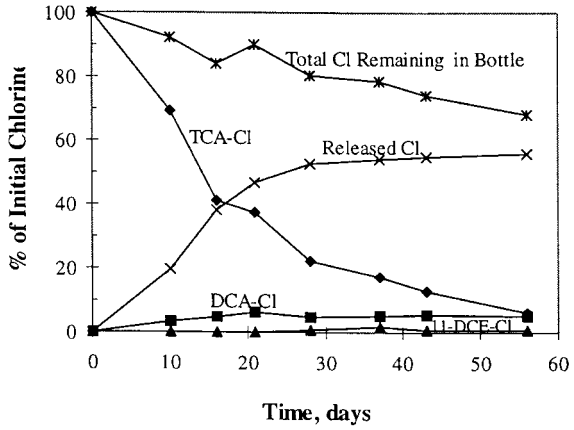

Figure 8. Chloride release during TCA degradation (Initial TCA concentration = 2590 μmol/L).

Chloride release during TCA degradation

Chloride release tests were conducted to show the overall chloride balance during TCA transformation with the low chloride medium (Figure 8). Results are presented based on the chlorine remaining in the bottle, based on remaining liquid volume and measured concentrations, normalized for chlorine of the initial TCA concentration. During the test, approximately 30% of the solution was removed as liquid samples.

Most of the TCA was transformed during the test, and inorganic chloride accounted for over 90% the chlorine associated with transformed TCA. DCA accounted for only 5% of the chlorine. 11DCE was the only other chlorinated compound detected, and it occurred in trace quantities. 11DCE was also detected in trace quantities in a killed control (not shown). Chloride release was not detectable in the killed control.

Discussion

Chloroethane toxicity

Inhibition of biogas production was seen at TCA concentrations of 7.5 μmol/L and higher, but often gas production recovered during the test as TCA was removed and partially converted to DCA and CA. Blum and Speece (1991) reported IC_{50} values (inhibit culture by 50% compared to uninhibited control) for methanogenesis to be 3.75 μmol/L for TCA and 62.6 μmol/L for DCA. Thus the amounts of DCA (and CA) formed from TCA are likely to be non-inhibitory to methane formation in all tests, and the inhibition seen with TCA is consistent with the literature.

TCA transformations with cell-free extract

This study showed the formation of acetic acid and 11DCE from TCA when incubated with reduced cell free extract (Figure 7). With autoclaved cells, little conversion of TCA occurred, but small amounts of 11DCE were seen after long incubations. Dehydrochlorination of TCA occurs under reducing conditions, resulting in the formation of 11DCE, and hydrolysis occurs (Jeffers, et al., 1989), leading to acetic acid, but rates are very slow. The rapid transformation of TCA to acetic acid, requiring reducing conditions and biological molecules that can be inactivated by autoclaving, may contribute to the significant initial removal of TCA observed under methanogenic conditions (Figure 3).

TCA Transformations with live cells

Reductive dechlorination is a biological transformation that is not observed in the absence of live cells and is a common TCA transformation pathway under anaerobic conditions. Reductive dechlorination of TCA to DCA was observed; further dechlorinations of DCA to CA and ethane occurred in varying amounts and only with live cells. The maximum amount of DCA and CA formation was about 30% of initial TCA, and both tended to be removed with further incubation.

Further transformation of 11DCE was observed in some experiments with live cells. The reductive dechlorination of 11DCE might be the precursor of the ethene which was also detected in some tests, although vinyl chloride, a likely intermediate in this path, was not detected.

DCA and CA transformations

The municipal digester culture partially transformed DCA and CA. Incomplete degradation of DCA and CA in these tests is consistent with their persistence the TCA degradation tests. About 75% of DCA was removed, forming only about 8% CA and 2% ethane during the experimental period (52 days). Mechanisms other than reductive dechlorination were responsible for the bulk of the DCA transformation, but the products were not measured.

Similarly, 80% CA removal was observed in two months with only 0.5% ethane produced. Both DCA and CA transformations showed unaccounted losses, similar to that of TCA, which may have been the result of hydrolysis mediated by biochemicals. Tests for DCA and CA transformation with cell-free extract were not conducted during this study and may be a topic of further research.

TCA biopathway

Based on this study, the pathway for TCA transformation under methanogenic conditions is shown in Figure 9. TCA was degraded via three pathways. Reductive dechlorination of TCA in live cultures resulted in the formation of DCA, CA, which was partially converted to ethane, all with yields below 30%. Reductive dechlorination was not enriched by repeated refeeding and respiking. Abiotic dechlorination of TCA led to the formation of small amounts of 11DCE in killed controls and reduced, uninoculated controls, as well as live cultures. 11DCE may have been biologically dechlorinated to ethene, which was seen only in live cultures. Acetic acid was shown to be the predominant product of tranformation by reduced, cell-free extract. In live cultures, it presumably is also formed in large amounts and may be consumed by aceticlastic methanogens. This hydrolysis is analogous to similar reactions seen in abiotic systems (Jeffers et al., 1989), but occurs much more rapidly with the biological molecules present in the cell-free extract. It is likely that similar reactions are responsible for the unaccounted for removal of DCA and CA in tests with live cells.

TCA was removed rapidly and completely by diluted municipal sludge digester culture that had been exposed to chlorinated compounds, but not to chloroethanes. For digesters operating at normal solids retention times and solids concentrations, TCA should be completely removed with a major fraction mineralized, probably via hydrolysis to acetic acid and metabolism to methane and CO_2. However, the slow and

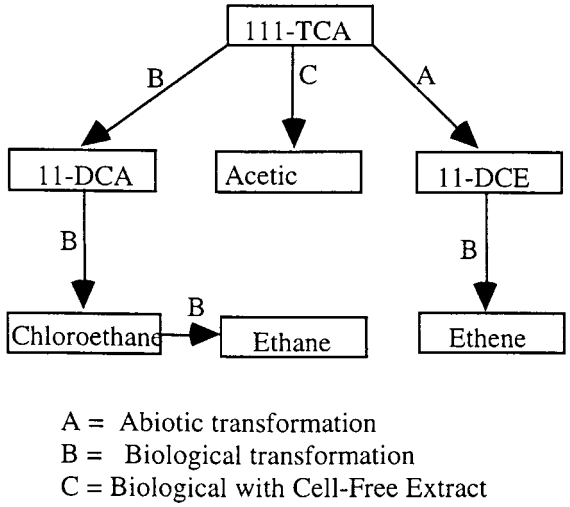

Figure 9. TCA transformations by municipal digester sludge under methanogenic conditions.

incomplete conversion of DCA and CA and 11DCE indicate that low concentrations of these could persist in sludge digestion.

Shock exposure of TCA is moderately inhibitory to methanogenesis, but apparently less so to TCA transformations. When TCA is removed, inhibition is eased. DCA and CA are reported to be much less inhibitory to methanogenesis.

The role of acclimation of the sludge to chlorinated compounds was not determined in this study, since our digester sludge had been exposed to several chlorinated compounds, but not to TCA. Repeated refeeding and respiking had no apparent effect on TCA transformation in our tests, but it is possible that specific populations of reductively dechlorinating microorganisms are involved in tranformation of TCA or other compounds involved in TCA transformation pathway.

Acknowledgment

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