Electrolytic Model System for Reductive Dehalogenation in Aqueous Environments

Craig S. Criddle* and Perry L. McCarty

Environmental Engineering and Science, Stanford University, Stanford, California 94305

Introduction

Reductive dehalogenation by microorganisms may find useful application for the removal of many halogenated contaminants in either in situ or aboveground treatment systems. Unfortunately, the products of reductive dehalogenation are often toxic and, in some cases, may be more harmful to human health than the parent compound. Free radicals are frequently generated in the course of these transformations, and such species can react in a somewhat indiscriminate manner with constituents of the surrounding milieu. Of course, the ability to control the product distribution is critical to the planning and operation of any new treatment system, whether biotic or abiotic. By understanding the causes of unwanted transformations, it may be possible to engineer systems that prevent or minimize their occurrence. Because of the complexity of biological systems, however, determining cause and effect in these systems can be difficult. One potentially useful tool for the investigation of reductive dehalogenation in aqueous systems is electrolysis. Controlled electrolysis in water solutions permits study of the underlying principles of reductive dehalogenation in the absence of many of the confounding and complicating factors observed in even the best controlled microbial processes. In particular, electrolysis permits examination of reductive transformations without the possibility of subsequent oxidative transformations.

A good model compound for the study of reductive transformations is tetrachloromethane (CT) since it is reducible under conditions that are not too extreme, and it is known to undergo transformation by a multiplicity of pathways in microbial and mammalian systems. In the following article, the known biotic and abiotic transformations of CT are briefly outlined, and the use of an electrolytic model system for the study of these transformations is demonstrated. The value of electrolytic studies in water is also demonstrated for 1,1,1-trichloroethane (TCA).

Transformations of Tetrachloromethane

Figure 1 provides a synthesis of known and proposed pathways of CT transformation. Although CT may undergo direct hydrolysis, most researchers agree that the first step is the one-electron reduction of CT to give a trichloromethane radical and a chloride ion (1-5). Depending upon environmental or experimental conditions, transformations of the radical proceed by one of five principal routes: dimerization to hexachloroethane (2) (6, 7); sequential reduction to trichloromethane (8-10) and, in sufficiently reduced environments, to dichloromethane (3) (12, 20, 21); addition to molecular oxygen (4) (22-26); formation of a carbenoid or a chloromethyl complex (5) (27-33); and, last, covalent binding to cell material (6) (34-38). The products and intermediates of these routes may be hydrolyzed or oxidized, or they can enter the pathways of one-carbon metabolism. Typically, several pathways operate simultaneously and competitively in both microbial and abiotic systems.

Microbial Transformations.

While there is evidence that most of the pathways in Figure 1 can occur in microbial systems, this article focuses on pathways 3 and 5. Hydrogenolysis of CT to trichloromethane (CF) occurs in a variety of microbial systems (12-15). As the number of chlorine substituents decreases, removal of additional chlorine substituents becomes energetically and kinetically more difficult (21). Consequently, the CF that is produced from CT can be further reduced to dichloromethane, but this transformation requires a more reduced environment to achieve equivalent rates (11, 20, 38). For microbial systems transforming CT to CF, fastest rates are observed in the most reduced environment (11, 39). No transformations were observed in aerobic mixed cultures (39).

Certain microorganisms can convert CT to carbon dioxide under anaerobic conditions (11, 15, 40). Usually, both CF and CO₂ are produced. CF is one of the most difficult to degrade of the one-carbon alkyl halides, and because of its persistence (hydrolysis half-life of 1850 years at 25 °C; ref 41), toxicity, and possible carcinogenicity, it is one of the least desirable metabolites of CT. Bouwer and Wright (11) decreased CF formation and increased CO₂ production by increasing the fluid residence time in their column studies of a mixed culture under sulfate-respiring conditions. Egli et al. (15) were the first to demonstrate that parallel pathways for CT transformation were operative in a single organism. They hypothesized that the acetyl-CoA pathway for carbon dioxide fixation was related to CT metabolism, on the basis of their observation that ¹⁴CO₂ derived from [¹⁴C]-CT was converted to acetate by Acetobacterium woodii and their observation of CT-degrading activity in other organisms possessing the acetogenic pathway for CO₂ fixation. Most pure culture denitrifiers tested to date do not transform CT (15, 42, 43).

* Present affiliation: Department of Civil and Environmental Engineering and the NSF Center for Microbial Ecology, Michigan State University, East Lansing, MI 48824.
Figure 1. Known abiotic and biotic transformations of CT. Products and intermediates that have been reported in the literature are shown in boxes.

An exception is *Pseudomonas* strain KC, a denitrifying organism that can rapidly transform CT without production of CF (43).

The conversion of CT to CO₂ represents a net hydrolysis and requires no net change in the formal +4 oxidation state of the carbon atom. Theoretically, this outcome could be accomplished by a straightforward hydrolysis, substituting OH for Cl, followed by hydrolytic decomposition of the product, trichloromethanol. Alternatively, it can be done indirectly, by coupling one or more reductive steps, in which the formal oxidation state of the carbon atom is first decreased, with an equivalent number of oxidation steps, so that the final oxidation state is restored to its initial value. At present, the mechanism for the uncatalyzed hydrolysis of CT is unknown (41, 44). This leaves open the possibility of biotic or abiotic catalysis. Hydrolytic catalysis would be valuable for detoxification of CT because simple hydrolysis does not depend upon the continual input of reducing power. To date, however, only one pathway is well established for the formation of CO₂ from CT in aqueous environments—the addition of molecular oxygen to the trichloromethyl radical (pathway 4, Figure 1). This pathway may be operative in the transformation of CT to carbon dioxide under low oxygen conditions by *Escherichia coli* k-12 (42). However, the formation of carbon dioxide by anaerobic microorganisms remains a mystery. One possibility is that the CT is reduced to formate or CO and subsequently oxidized to carbon dioxide, but to date there have been no reports of formate production from CT in aqueous environments.

**Theoretical Considerations.** As shown in Figure 1 pathway 5, formate could arise from CT by the two-electron reduction of CT to form dichlorocarbene. Thermodynamic considerations suggest that a two-electron re-duction would be competitive with the one-electron re-
duction of CT. This is illustrated in Figure 2. Here, the reduction potential of the electron donor needed to produce a trichloromethyl radical is compared with the potential required for the two-electron reduction that produces a singlet dichlorocarbene radical. As illustrated, the half-reaction for dichlorocarbene production becomes thermodynamically favorable when coupled with the oxidation of an electron donor that has a potential of +0.2 V or less. By comparison, the half-reaction for formation of the trichloromethyl radical is not favorable unless it is coupled with oxidation of an electron donor that has a potential of -0.15 V or less. It should be emphasized that these calculations assume unit activity for the radical species, and these relationships could vary greatly depending upon possible scavenging of the radicals. In any case though, these two reactions may be competitive in those environments in which both one- and two-electron transfers can occur.
Table I. Characteristics of the Electrolysis Cell

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimensions</td>
<td></td>
</tr>
<tr>
<td>Liquid volume, mL</td>
<td>170</td>
</tr>
<tr>
<td>Gas volume, mL</td>
<td>184</td>
</tr>
<tr>
<td>Silver cathode surface area, cm²</td>
<td>9</td>
</tr>
<tr>
<td>Platinum anode surface area, cm²</td>
<td>15</td>
</tr>
<tr>
<td>Nafion membrane surface area, cm²</td>
<td>4.5</td>
</tr>
<tr>
<td>Buffer</td>
<td></td>
</tr>
<tr>
<td>NaH₂PO₄, M</td>
<td>0.10</td>
</tr>
<tr>
<td>NaOH, M</td>
<td>0.03</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 (without adjustment)</td>
</tr>
</tbody>
</table>

Utility of an Electrolytic Model System. The preceding discussion indicates that CT is transformed by parallel pathways in microbial systems, and that competing electron-transfer reactions are one possible explanation. In complex biological systems, the causes for parallel pathways are difficult to determine since oxidative and reductive reactions coexist. In a two-compartment electrolytic cell, reduced products can accumulate in the cathode compartment without possibility of a subsequent oxidative transformation. Tests to evaluate such a system are described in the following sections.

Experimental Section

Electrolysis Cell. The electrolysis cell was a glass vessel (Belco Catalog No. 1972-00100, Belco Glass, Inc., Vineland, NJ) consisting of vigorously stirred anode and cathode compartments, separated by a proton-permeable membrane (Nafion 117, Du Pont, Wilmington, DE). Water is oxidized at a platinum anode releasing molecular oxygen, electrons, and protons. Electrons traverse an external circuit to a silver cathode, where they engage in reductive reactions. Protons pass through the membrane, balancing the negative charge transferred via the external circuit. The reduction potential at the cathode is maintained constant with a PC-controlled potentiostat (Model EP-301, Summitech, San Jose, CA). The voltage between a reference electrode (Ag/AgCl with 1 M Na₂SO₄ filling solution) and the silver cathode is maintained at a set point reference voltage, and the voltage between the anode and the cathode is adjusted to maintain the set point reference potential. Current was determined by measuring the voltage drop across a 1-kΩ resistor placed in series with the external circuit. The reference electrode tip was positioned within a few millimeters of the cathode to prevent voltage drops that might result from solution resistance. A large impedance (>2 MΩ) between the reference electrode and the cathode prevented significant current from passing between these two electrodes. The lid of each glass compartment was clamped to the corresponding glass base, and sealed with high-vacuum grease. Cell characteristics are summarized in Table I.

General Procedures. The cathode compartment was deoxygenated by purging with argon or zero-grade helium while a low potential (~0.1 V vs Ag/AgCl/Na₂SO₄, reference electrode: ~0.38 V vs SHE) was applied to the cathode to remove traces of oxygen. When the current had fallen to less than 5 μA, 2 μL of the halogenated aliphatic compound of interest was injected into the liquid phase of the cathode compartment and allowed to equilibrate with the gas phase. Estimated mass-transfer rates (~2.4 h⁻¹) were approximately 10 times the maximum rates of reductive transformation, indicating that equilibrium between the gas and liquid phases could reasonably be assumed in subsequent analyses. When the gas-phase concentration leveled off at a stable value, the reference potential was increased to the desired set point. The voltage between the cathode and the anode varied from 2.5 to 3.0 V.

A control cell of similar construction, but lacking electrodes, served as a control for physical processes, such as sorption or diffusion through the Nafion membrane.

Gas-phase (200-μL) samples were obtained with a Precision gas-tight syringe for analysis by gas chromatography. External standards were prepared by injecting known volumes of alkyl halide standards prepared in isooctane into large glass vessels (volumes of 2.14 or 2.16 L) that were sealed with Teflon Mininert valves and maintained at 21 °C where the liquids evaporated. A carbon monoxide standard was prepared by injecting 200 μL of 100% carbon monoxide into one of the sealed glass vessels. External standards were analyzed periodically during the course of each experiment.

To identify volatile unknowns, gas samples (200 μL) were injected directly onto a Hewlett-Packard 5890 gas chromatograph equipped with a Hewlett-Packard Model 5970B mass selective detector and a DB 5 capillary column (60 m × 0.53 mm, J&W Scientific, Folsom, CA). Gas samples were also injected onto a reduced gas analyzer (Trace analytical model RGD2, Menlo Park, CA), with air as the carrier, and a detector temperature of 280 °C to measure hydrogen and carbon monoxide. At the conclusion of an experiment, chloride was analyzed with a combination chloride electrode (Orion Model 9617B, Boston, MA). Henry’s constants of 1.23 for CT and 1.34 for TCA at 25 °C (20) were used together with measured gas and liquid volumes to compute the total number of moles in the cathode compartment.

Experiments with Tetrachloromethane. Two experiments were conducted to assess the effect of reduction potential on CT transformation. In the first, a reference potential of ~0.93 V was applied (~0.71 V vs SHE), and the gas phase was monitored with a Packard 437 A gas chromatograph equipped with an electron capture detector, a Model 681 compact flow unit, and a Carbopack B packed column (3% SP-1500, 3 ft × 1/8 in. in 80/120, Supelco, Inc., Bellefonte, PA). In the second experiment, the reference potential was made more negative at ~1.15 V, and products were analyzed on a Hewlett-Packard gas chromatograph, Model 5890, equipped with a Hall electrolytic conductivity detector (Model 4420) and a megabore capillary column (30 m × 0.53 mm, DB-624, J&W Scientific, Folsom, CA), with oven temperature maintained at 25 °C. Formate was quantified by direct injection of an aqueous sample onto a Dionex Series 4000 ion chromatograph using a hydroxide buffer (5 mM, 2 mL/min) and equipped with a Dionex ion-exchange column (HPIC A54A).

External standards were prepared using 105 μL of CT (99+%, Aldrich Chemical Co., Milwaukee, WI), 18 μL of CP (99.5%, Photrex, J. T. Baker, Phillipsburg, NJ), and 6 μL of dichloromethane (MC; 99.8%, Baker Analyzed, J. T. Baker) in 2 mL of isooctane. This standard was used to prepare secondary gas standards, as described previously.

Experiment with 1,1,1-Trichloroethane. External standards were prepared using 99 μL of TCA (99+%, Aldrich), 18 μL of 1,1-dichloroethane (DCA; 98%, Chem Service, West Chester, PA), and 2 μL 1,1-dichloroethene (99%, Aldrich) in 2 mL of isooctane. This standard was used to prepare secondary gas standards, as described previously.

Results and Discussion

Tetrachloromethane. Figure 4 illustrates the transformation of CT to CF at a potential of ~0.93 V vs Ag/AgCl/Na₂SO₄ reference electrode (~0.71 V vs SHE) over a 6-h period. In this experiment, CT was reduced to CF and to trace amounts of carbon monoxide. The change...
in current over this period is shown in Figure 5. After another 6 h of electrolysis, CF was measured and the sampling discontinued. The current shown in Figure 5 was obtained by subtracting out the background current (0.33 ± 0.03 mA) determined by operating the cell at the same applied potential in the absence of CT. This background current was attributed to hydrogen-forming reactions, as indicated by the measured increases in hydrogen in the cathode compartment during the experiment. Consequently, the hatched area under the curve in Figure 5 represents charge transfer that can be attributed to the reduction of CT.

In a second experiment with CT, the reference potential was made more reducing at -1.15 V (-0.93 V vs SHE). In this case, CT underwent hydrogenolysis beyond CF to MC, as illustrated in Figure 6. Carbon monoxide was also detected in this experiment, but it was present at low concentrations and was not quantified. After ~5 h of electrolysis, an additional 1.2 mL of CT was introduced into the active cell in an effort to increase the concentration of intermediates.

The experiment with CT confirmed the hypothesis that the extent of hydrogenolysis is related to the applied reference voltage. No MC was detected at a reference voltage of -0.93 V (-0.71 V vs SHE), and the CF that was produced appeared to persist. At the more reduced potential of -1.15 V (-0.93 V), formation of MC was observed. Another important observation concerns the total charge transferred in these experiments. As shown in Figure 5, the charge associated with CT transformation can be estimated by integrating the hatched area under the curve.

Since Faraday's constant $F$ gives the moles of electrons per coulomb of charge, the moles of electrons transferred for CT transformation can be computed:

$$q = \int_{0}^{t} i \, dt$$  \hspace{1cm} (7)

where $i$ is the current (amps), $q$ is the charge transferred up to time $t$ (coulombs), and $t$ is time in seconds.

$$n = q / F$$  \hspace{1cm} (8)

where $n$ is moles of electron transferred. The area under the curve in Figure 5 (2.33 C) was computed by fitting a polynomial to the data and integrating over the 6-h test period. This amount of charge indicates that 24.1 μmol of electrons were used to remove 12.4 μmol of CT, giving a ratio of 1.9 mol of electrons/mol of CT removed. This value is close to the value of 2.0 that would be expected for the reduction of CT to CF or for the reduction of CT to CO or formate.

Another significant observation is the detection of carbon monoxide as a trace product of CT transformation. Formation of 1 mol of CO requires 2 mol of electrons and 1 mol of water per mole of CT removed. The formation of CO is significant because it suggests that CT could be converted to carbon dioxide anaerobically via carbon monoxide.

Table II gives a mass balance on chloride after 12 h of electrolysis at a reference potential of -0.93 V (-0.71 V vs SHE). Column A gives the amount of chloride that would be released by complete dechlorination of all the CT that was removed. However, complete dechlorination did not occur since some of the CT that was removed was converted to CF (~15%). The amount of chloride associated with this CF is given in column B. The difference between columns A and B gives the amount of chloride that would be released by complete dechlorination of all the CT that was removed. However, complete dechlorination did not occur since some of the CT that was removed was converted to CF (~15%). The amount of chloride associated with this CF is given in column B. Column C gives the amount of chloride that would be released by complete dechlorination of all the CT that was removed.
experiment at -1.15 V (-0.93 V vs SHE), samples were taken to aid in identification of the non-CF product. Chloride and formate measurements were both made. After 16 h of electrolysis, 23 μmol of CT were removed, yielding 1.4 μmol of CF, 100 μmol of chloride, and 17 μmol of formate. Thus, in this experiment, reduction to CF accounted for only ~6% of the CT transformation, while reduction to formate accounted for ~75%.

1,1,1-Trichloroethane. Figure 7 illustrates the transformation of TCA to DCA at a reference potential of -1.15 V (-0.93 V vs SHE) over a 6-h period. At the end of the 6-h period, the liquid phase was sampled for chloride measurement. Reduction of TCA resulted in the formation of DCA, but other fully dechlorinated products were also produced. This is analogous to the reduction of CT to products other than CF in previous experiments. After 6 h of electrolysis, only ~7% of the TCA that was removed appeared as DCA. Table III gives a chloride balance for the electrolysis of TCA. As indicated by comparing the difference between columns A and B with the measured amount of free chloride, the products of TCA dechlorination were fully dechlorinated.

Another interesting aspect of TCA dechlorination is the change in current over the time, as shown in Figure 8. Once again, the current values shown here were obtained by subtracting the background current due to hydrogen production from the total measured current. This background value (0.4 mA at -1.15 V) was determined by electrolysis of a blank cell in the absence of TCA. Accordingly, the hatched area under the curve in Figure 8 is the charge associated with electron transfers to TCA or its derivatives.

The area under the curve in Figure 8 (2.84 C) was computed by fitting a polynomial to the data and integrating over the 6-h test period. A value of 2.2-2.5 mol of electrons were consumed per mole of TCA removed. The uncertainty in the estimate of the moles of electrons used per mole of TCA removed is due to some doubt about the exact quantity of TCA removed. Chloride data (Table IV) and calculations based on the 2 μL volume injected suggest that somewhat more TCA may have been removed than suggested by Figure 7. This would be consistent with the possibility that some sorption may have occurred prior to the initiation of electrolysis. Desorption from a solid phase would feed additional TCA into the system, resulting in gas-phase concentrations somewhat higher than the expected values in the absence of sorption. In this situation, measurements of changes in gas-phase concentrations would tend to underestimate the extent of removal. Nevertheless, the ratio of moles of electrons transferred to moles of CT removed does give some idea about the degree of reduction of the non-DCA products and suggests that a possible candidate would be acetaldehyde.

Summary

A significant finding of these simple electrolytic studies is the observation that CT can undergo a kind of hydrolytic reduction under certain conditions. Although such a transformation was previously recognized as theoretically possible, evidence for its occurrence is scarce. The identification of trichloromethane and dichloromethane, as well as carbon monoxide and formate, demonstrates that CT does undergo parallel transformation by hydrogenolysis and by hydrolytic reduction in aqueous solutions. From a treatment viewpoint, hydrolytic reductions are preferable to hydrogenolysis reactions because the products are oxygenated, making them more biodegradable. As indicated by the half-reaction reduction potentials listed in Table IV (20, 45), hydrolytic reductions are energetically quite favorable. For these reactions to occur naturally would require the near-simultaneous transfer of two electrons to a target molecule in an aqueous environment. In microbial systems, the environment is partially aqueous and partially organic in nature. Consequently, hydrolytic reduction could conceivably occur if a reactive two-electron donor were present. The production of formate could explain the transformation of CT to carbon dioxide in anaerobic environments, including mixed culture methanogenic systems (46), denitrifying systems (4), fermenting cultures of E. coli k-12 (42), and possibly in
denitrifying cultures of _Pseudomonas_ sp. strain KC (43). Formate undergoes oxidation to carbon dioxide in many of these systems. If this occurred, CT would function as both electron acceptor and electron donor. On the other hand, CT could not serve as the sole substrate, since reducing power is required for cell synthesis, and no extra electrons are released by these proposed transformations. Reduction followed by oxidation might also explain the observed formation of acetate from TCA by microorganisms (12). If TCA were hydrolytically reduced to acetaldehyde, oxidation of acetaldehyde would yield acetate.

For both CT and TCA, the observation of parallel and competing pathways in electrolytic and microbial systems suggests that similar phenomena may underlie the rate and extent of reduction and the products of transformation in these systems. Beland et al. (46) made a similar comparison between the reductive dehalogenation of lindane in anaerobic sewage sludge and its transformation at a mercury-coated cathode in dimethyl sulfoxide. The aqueous system described in this work, however, should be even more useful as a tool for elucidating reductive pathways in aqueous systems.

Once factors governing the product distribution and pathways of reductive dehalogenation are better understood, modeling and control of degradation can be accomplished in a more rational way. In addition to the value of aqueous electrolysis as a research tool for investigating reductive dehalogenation, it may also find direct application as a new treatment technology. Schmal et al. (47) have demonstrated the use of graphite fiber cathodes for electrolytic reduction of a wide range of halogenated compounds in aqueous environments. Their work and the findings reported here indicate that electrolysis could provide total treatment or pretreatment for a subsequent biological process.

Acknowledgments

We thank Dr. René Galli for his invaluable assistance in the design, preparation, and testing of several prototypes of the electrolysis cell used in this research.

Note Added in Proof. Since submission of this paper, identification of formate as a major product of CT reduction in aqueous systems has been confirmed by HPLC/UV analysis of a cathode compartment solution using the method of Stevens et al. (48).

**Literature Cited**


Received for review May 15, 1990. Revised manuscript received December 19, 1990. Accepted January 2, 1991. This research was based upon work supported by the U.S. National Science Foundation Grant ECE-8519243.