APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1983, p. 1286–1294
0099-2240/83/041286-09$02.00/0

Vol. 45, No. 4

Transformations of 1- and 2-Carbon Halogenated Aliphatic Organic Compounds Under Methanogenic Conditions

EDWARD J. BOUWER†* AND PERRY L. McCARTY

Department of Civil Engineering, Environmental Engineering and Science, Stanford University, Stanford, California 94305

Received 25 August 1982/Accepted 4 January 1983

Several 1- and 2-carbon halogenated aliphatic organic compounds present at low concentrations (<100 μg/liter) were degraded under methanogenic conditions in batch bacterial cultures and in a continuous-flow methanogenic fixed-film laboratory-scale column. Greater than 90% degradation was observed within a 2-day detention time under continuous-flow methanogenic conditions with acetate as a primary substrate. Carbon-14 measurements indicated that chloroform, carbon tetrachloride, and 1,2-dichloroethane were almost completely oxidized to carbon dioxide, confirming removal by biooxidation. The initial step in the transformations of tetrachloroethylene and 1,1,2,2-tetrachloroethane to nonchlorinated end products appeared to be reductive dechlorination to trichloroethylene and 1,1,2-trichloroethane, respectively. Transformations of the brominated aliphatic compounds appear to be the result of both biological and chemical processes. The data suggest that transformations of halogenated aliphatic compounds can occur under methanogenic conditions in the environment.

The presence of synthetic organic compounds in the environment poses a threat to public health. Of particular concern is the presence of trihalomethanes and other low-molecular-weight halogenated organic compounds in groundwater, drinking water, and wastewater. Numerous incidents of well contamination by compounds such as chloroform, 1,1,1-trichloroethane, and tetrachloroethylene have been documented (7, 12, 22, 26, 32, 33). Many of these compounds are commonly used for industrial and domestic purposes, and their presence in the environment appears due to inadequate disposal techniques and accidental spillage, causing contamination of water and soil. The production of trihalomethanes from the chlorination of water containing organic materials is another source of organic contamination (3, 13, 16, 35). Many of the halogenated aliphatic organic compounds have been designated by the U.S. Environmental Protection Agency as priority pollutants (17) and are known or suspected carcinogens or mutagens (16).

Evidence suggests that 1- and 2-carbon halogenated aliphatic compounds are quite persistent in the environment as they are transported easily through soil by groundwater movement (30), break through rapidly in granular activated carbon beds (21), and do not appear to be degraded under aerobic conditions (4, 6, 29). However, we have previously provided evidence that transformations of trihalomethanes can occur under anaerobic conditions (6). Lang et al. (R. R. Lang, P. R. Wood, R. A. Parsons, J. Demarco, H. J. Harween, I. L. Payan, L. M. Meyer, M. D. Ruiz, and E. D. Ravelo, Annu. Meet. Am. Water Works Assoc., 1981) also reported on the biotransformation of some haloaliphatic compounds and postulated that this occurs through a series of reductive dechlorination reactions. Field evidence for the long-term transformation of some of the halogenated compounds was obtained at the Palo Alto groundwater recharge project (31) and during the rapid infiltration of secondary wastewater at the Phoenix 23rd Avenue Project (5). This paper discusses additional batch laboratory experiments that confirm these observations and reports on the biotransformation of haloaliphatic compounds at low concentrations (10 to 30 μg/liter) in a continuous-flow methanogenic column. We present evidence that the transformation of the chloroaliphatic compounds is the result of biological action, whereas a combination of biological and chemical processes appears responsible for the transformations of bromoaliphatic compounds under reducing conditions. The end products of the observed transformations are also described.

† Present address: Environmental Engineering Program, Department of Civil Engineering, University of Houston, Houston, TX 77004.

MATERIALS AND METHODS

Chemicals and radioisotopes. The halogenated organic compounds used were reagent-grade chloroform (CF), carbon tetrachloride (CT; Mallinckrodt Chemical Co., St. Louis, Mo.), 1,1,1-trichloroethane (1,1,1-TCE), trichloroethylene (TCE), 1,1,2,2-tetrachloroethane (1,1,2,2-TECE), tetrachloroethylene (TECE), 1,2-dibromoethane (1,2-DBE; Matheson Chemical Co., Norwood, Ohio), dibromochloromethane (DBCM; Columbia Organic, Columbia, S.C.), bromodichloromethane (BDCM), bromoform (BF), and 1,2-dichloroethane (1,2-DCE; Aldrich Chemical Co., Milwaukee, Wis.). The following radiochemicals were used: sodium [1-^{14}C]acetate (4.8 mCi/mmol; ICN Chemical and Radioisotope Div., Irvine, Calif.), [^{14}C]CF (5.4 mCi/mmol; New England Nuclear Corp., Boston, Mass.), [^{14}C]CT (22 mCi/mmol; Amersham Corp., Arlington Heights, Ill.), and 1,2-dichloro[*U*-^{14}C]ethane (23 mCi/mmol; Amersham Corp.).

Batch transformation experiments. Three batch transformation studies were conducted under methanogenic conditions. The first was conducted with CT, 1,1,1-TCE, TCE, and TECE at initial concentrations of about 200 μg/liter. Sterile 60-ml serum bottles were purged with N_2 gas and completely filled by anaerobic pipette with deoxygenated anaerobic medium (25). Except for sterile controls, the medium was seeded with a methanogenic mixed culture (10 ml/liter) grown in a laboratory-scale digester fed waste-activated sludge, xylan, and cellulose and operated at 35°C. Each compound in dilute aqueous solution was added to a series of bottles, and the bottles were immediately sealed without head space with a Teflon disk and an aluminum cap. Each aqueous solution added contained 200 μg of an organic compound per ml and was prepared by diluting a stock solution in methanol (5 to 10 mg/ml of methanol) with deionized water from a four-cartridge MILLI-Q water purification system (Millipore Corp., Bedford, Mass.). The bottles were incubated in the dark at 35°C in an inverted position to minimize gas leakage.

In the second batch experiment, a mixture of CF, BDCM, DBCM, BF, TCE, and TECE was added in two series of bottles to give concentrations of about 10 and 30 μg of each compound per liter. After complete transformation of some of the compounds had occurred, the remaining bottles were spiked with 10 or 30 μg of each compound initially present per liter, and the concentrations were further monitored with time to determine the degree of organism acclimation to the organic compounds.

A third similar batch experiment with carbon-14–labeled CF, CT, and 1,2-DCE in separate seeded cultures was conducted to study the end products of transformations. Here, 160-ml serum bottles were used, and a sample of a methanol solution containing the chlorinated organic compound (1.6 mg/ml) and carbon-14 tracer (~2 μCi/ml) was added directly to each bottle before sealing.

Identification of transformation products by radioisotopes. In the third batch experiment, samples were extracted after incubation at low, neutral, and high pH with either 1.0 ml of pentane or 1.0 ml of methylene chloride in sealed vials for 30 min on a shaker table. Samples (1 ml) of the aqueous phase before and after extraction and 0.5 ml of the organic phase, taken with a syringe, were counted for carbon-14 activity. In other cases, two drops of concentrated HCl or 6 N NaOH were added to the glass counting vial, 1.0-ml samples were ejected from the syringe into the acid or base, and 10 ml of liquid scintillation counting solution (ACS; Amersham Corp.) was added. In addition, other samples at low and high pH were bubbled with nitrogen (50 ml/min) for 5 min to strip volatile components before counting. Finally, samples stripped at low and high pH were made basic or acidic, respectively, and stripped again before counting. The stripping procedures were employed as a presumptive test for the production of $^{14}CO_2$, which would not be stripped at high pH, but would be at low pH. Also, if no volatile organic end product was produced, then the fraction of CF, CT, or 1,2-DCE remaining in the samples could be determined, since these compounds are volatile at any pH and are easily removed by stripping. Samples were also counted before and after filtration through a 0.45-μm membrane filter to determine the fraction of radioactivity incorporated into cells or onto particulates.

The production of $^{14}CO_2$ was confirmed by the addition of 2.0 g of $Ba(NO_3)_2$ to a filtered (0.45-μm membrane filter) 20-ml sample at high pH, mixing for 30 min, and assaying the radioactivity in solution before and after membrane filtration of the barium carbonate precipitate formed (27).

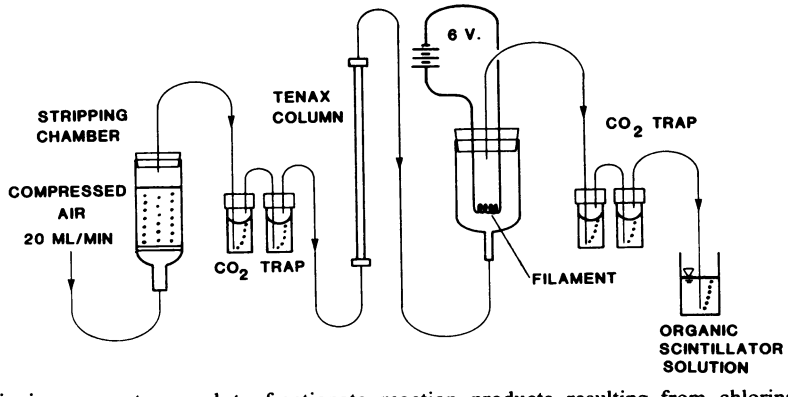

FIG. 1. Stripping apparatus used to fractionate reaction products resulting from chlorinated aliphatic transformations.

Where activity was removed by stripping at high pH, an apparatus (Fig. 1) was used to differentiate the activity of remaining compounds from that of other volatilized compounds. A 20-ml sample was stripped at low or high pH for 45 min at room temperature with a compressed air flow of 20 ml/min. The air was then passed through a pair of CO_2 traps, a stainless steel column (0.2 cm [inside diameter] by 28 cm) filled with Tenax to remove halogenated organic compounds (24), a glass chamber with hot filament (18 W) to combust organic compounds to CO_2, a second pair of CO_2 traps, and finally bubbled into 10 ml of organic scintillator solution to collect any remaining organic compounds. Each CO_2 trap was a 5-ml septum screw-cap vial containing 3 ml of 1.0 N NaOH. Sample activity before and after stripping and the activity recovered in each CO_2 trap and organic scintillator solution were measured. The activity retained by the Tenax column was determined by heating the column to 180°C for 20 min and continuing the air flow so that desorbed compounds were combusted to CO_2 and then absorbed in NaOH for counting. This procedure was tested with standard solutions and allowed the determination of the fraction of sample activity associated with the remaining chloroorganic compounds, the production of CO_2, and the production of a volatile non-halogenated organic compound which was presumed to be methane.

Continuous-flow fixed-film studies with methanogenesis. Two upflow glass columns (2.5 cm [inside diameter] by 22 cm) filled with 3-mm-diameter glass beads were connected in series (Fig. 2) to achieve anaerobic conditions. A sterile solution of the medium used in the batch experiments, containing 1,000 mg of sodium acetate per liter, was continuously applied to the lead column with a syringe pump equipped with a 60-ml plastic syringe. This column was initially seeded with primary sewage effluent and produced an anoxic effluent (dissolved oxygen by the Winkler method was below the detection limit of 0.5 mg/liter) that became the influent to the second anaerobic column. A bacterial inoculum from a laboratory-scale methanogenic anaerobic filter treating rum distillery wastewater was used to seed the second column. A concentrated solution of CF, BDCM, DBCM, BF, 1,1,1-TCE, and TECE, each at about 160 μg/liter, with additional acetate as the primary substrate (500 mg/liter), was pumped to the second-column influent feed from a 10-ml gas-tight syringe at a rate of 1.0 ml/4.4 ml of medium flow. The total feed rate to the second column was 20 ml/day (hydraulic loading of 4 cm/day), resulting in an actual liquid detention time in the column of 2 days. The tubing, fittings, and sample vials used were made of Teflon or glass to minimize sorptive interactions. The columns were covered with aluminum foil to prevent the growth of photosynthetic organisms, and the system was operated at room temperature (22 to 23°C) for 19 months.

After 12 months of operation, the acetate concentration in the feed to the lead column was decreased to 100 mg/liter. At the same time, acetate additions to the influent of the methanogenic column were stopped for 1 month and increased to 100 mg/liter for the remaining 6 months. CT, 1,2-DCE, and 1,1,2,2-TECE were included in the influent to the methanogenic column during the last 4 months of operation.

Methanogenic-column effluent was collected in a 20-ml glass syringe barrel with a tight-fitting Teflon float to prevent volatilization losses and to allow the recovery of the gas produced. Extractions of organic compounds were performed directly on samples in the syringe. Concentrations entering the methanogenic-column feed were taken to equal those in the concentrated solutions divided by the dilution factor of 5.4.

Short-term experiments with carbon-14–labeled CF, CT, and 1,2-DCE were performed to characterize the end products of transformations and to confirm removal by a biological mechanism. Tracers were added to the methanogenic column from the concentrated feed solution. After at least three detention times to allow the establishment of quasi-steady-state conditions, samples were taken for analyses and the assay of radioactivity.

Determination of organic concentrations and radioactivity. The halogenated aliphatic compounds were determined with a lower detection limit of 0.1 μg/liter by gas chromatography (GC) (15), in which samples were sealed in 60- or 20-ml vials and extracted with 1 ml of pentane. A 5-μl sample of the extract was injected into a packed column GC (10% squalane on Chromosorb W/AW) equipped with a linearized ^{63}Ni detector (28). 1,2-DBE was used as an internal standard. Response factors ranged between 0.1 and 2.0 for the compounds except for 1,2-DCE, which had a value of 111. Samples were also analyzed for organic intermediates by GC/mass spectrometry.

An external standard GC procedure was used to determine acetate concentrations in the methanogenic-

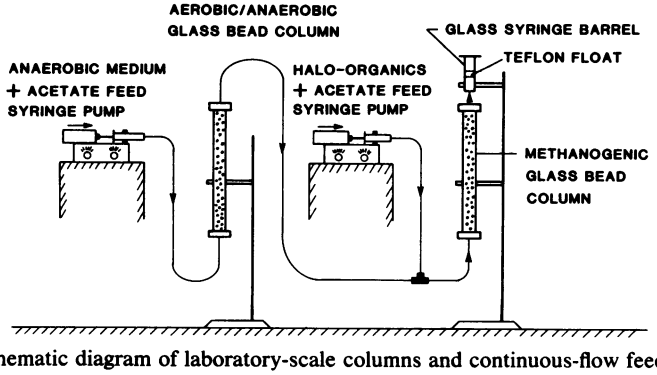

FIG. 2. Schematic diagram of laboratory-scale columns and continuous-flow feeding system.

column influent and effluent when the feed concentration was about 600 mg/liter (34). At the lower acetate feed concentration of 100 mg/liter, removal was monitored with sodium [1-^{14}C]acetate and liquid scintillation counting.

Carbon-14 activity was assayed by mixing samples with 10 ml of ACS (Amersham Corp.) and counting with a Tri-Carb model 3330 liquid scintillation spectrometer (Packard Instrument Co.). The gain was 10%, with a red channel window of 50 to 100 and a green channel window of 50 to 1,000. Counts were corrected for counting efficiency by the channels ratio method (2). For the counting conditions employed, the minimum detectable activity at the 97.5% confidence level was 3.0 dpm (1).

RESULTS

Batch experiments with trihalomethanes. With CF in the second batch study, an acclimation period was observed before it was degraded in seeded cultures at initial concentrations of 15 and 40 μg/liter (Fig. 3). However, CF was immediately transformed without a noticeable lag period after the spiked addition, which suggests that the removal was the result of biotransformation. The rapid transformation of BDCM was observed in seeded cultures at initial concentrations of 12 and 37 μg/liter (Fig. 4). After the spiked additions, BDCM was even more rapidly degraded, nearly to the detection limit. Here, a small decline in the sterile control concentrations was measured with time. The results of DBCM and BF transformation were similar to those for BDCM. These results are consistent with our previous finding (6) that the brominated trihalomethanes were transformed in both anaerobic sterile controls and seeded cultures, suggesting that chemical transformation may also be significant. However, the decomposition rate increased in the presence of microbial activity and was faster after the reinjection of the compounds.

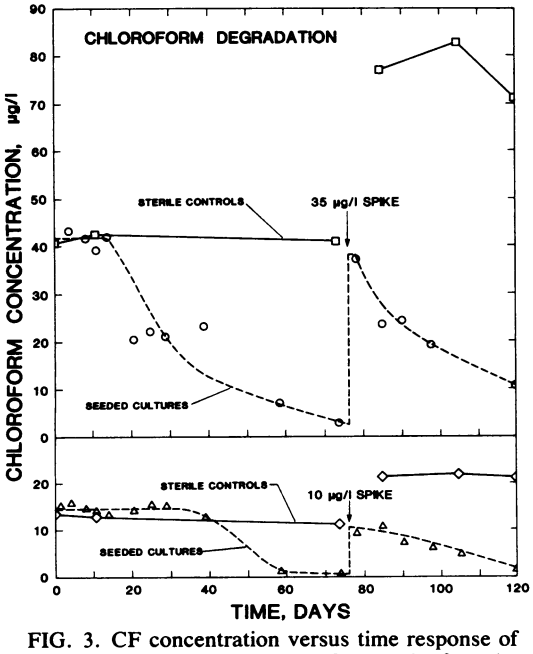

FIG. 3. CF concentration versus time response of methanogenic batch cultures before and after the spiked addition of CF.

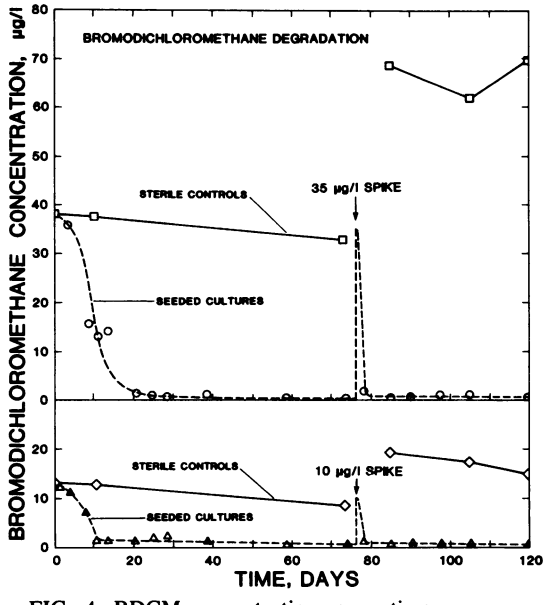

FIG. 4. BDCM concentration versus time response of methanogenic batch cultures before and after the spiked addition of BDCM.

Batch experiments with CT, 1,1,1-TCE, TCE, and TECE. Table 1 shows the change in concentration of CT, 1,1,1-TCE, TCE, and TECE over an 8-week period in the presence of methanogenic bacteria. No significant concentration change occurred in sterile controls, but seeded cultures showed a significant reduction in concentrations. CT was degraded most rapidly to below the detection limit after 16 days. A nearly complete transformation of 1,1,1-TCE and TECE occurred after 8 weeks. TCE was removed most slowly, with a reduction of 40% after 8 weeks. The seeded cultures incubated with TECE contained 91 μg of TCE per liter after 8 weeks, suggesting that TCE was an intermediate of TECE transformation.

Products of CF, CT, and 1,2-DCE transformation. In the third batch study, 1,2-DCE was found to be transformed as were CF and CT. No other haloorganic intermediates from the transformations of these compounds could be detected by GC/mass spectrometry. No significant extractable (in pentane and methylene chloride) product(s) was obtained at low, neutral, or high

TABLE 1. Degradation of chlorinated hydrocarbons under methanogenic conditions

Compound	Time (days)	Concn[a] (μg/liter)	
		Sterile controls	Seeded cultures
CT	0	149 ± 15	ND[b]
	16	ND	<0.1
	54	145 ± 15	<0.1
1,1,1-TCE	0	229 ± 23	ND
	16	ND	35 ± 4
	54	227 ± 23	0.3 ± 0.3
TCE	0	178 ± 18	ND
	19	ND	171 ± 17
	57	180 ± 18	107 ± 11
TECE	0	152 ± 15	ND
	19	ND	160 ± 16
	57	162 ± 16	<0.1[c]

[a] One standard deviation of mean values is given.
[b] ND, Not determined.
[c] Contained 91 μg of TCE per liter.

pH. Some radioactivity was measured in the organic phase after each extraction, but it corresponded to the remaining substrate as measured by GC. Most of the activity in the seeded cultures incubated with CF and CT could not be stripped at high pH, but was completely stripped at low pH, suggesting the production of CO_2. This was confirmed by the precipitation of inorganic carbon with barium, which removed the activity in solution at high pH, and this activity was recovered in the $BaCO_3$ formed. In some samples, some loss of activity occurred at high pH after stripping, which represented nondegraded CF or CT or volatile end product(s) or both.

A nearly complete transformation of CF and CT was detected after 3 weeks of incubation. The transformation of 1,2-DCE proceeded at a slower rate, with a concentration reduction of 63% after 25 weeks. No loss of tracer activity from the sterile controls and seeded cultures occurred during the incubation times indicated. The stripping apparatus (Fig. 1) was used to characterize the radioactivity from CF, CT, and 1,2-DCE transformations in methanogenic batch cultures. The activity in seeded cultures could be completely stripped from solution at low pH, and the component fractions are shown in Table 2. The primary transformation product from CF, CT, and 1,2-DCE degradation was CO_2. Nine percent of the activity from CF degradation appeared to be methane, but methane was not apparently produced from the degradation of CT or 1,2-DCE. With 1,2-DCE, the fraction of the radioactivity recovered by the Tenax column was greater than that explained by the measured concentration of 1,2-DCE remaining. This difference may have been due to the formation of a halogenated compound not detected by the GC procedure but adsorbed by Tenax or the result of inaccuracies in the GC method for 1,2-DCE because of poor detector response and interference from background noise. In any event, these results confirm a biological mechanism for the transformations of CF, CT, and 1,2-DCE.

Continuous-flow column experiments. The continuous-flow column results were in agreement with the batch results (Table 3). Almost no acclimation period was required for the transformations of the brominated trihalomethanes to below the detection limit. Significant removals of CF, 1,1,1-TCE, and TECE resulted after 10 weeks of acclimation. The presence of TCE in the methanogenic column effluent and not in the feed supported the batch experiment results, indicating that TCE was an intermediate of TECE degradation. Several milliliters of gas were produced each day from the decomposition of acetate.

During the period when the feed acetate concentration to the lead column was decreased to 100 mg/liter and was eliminated to the methanogenic column, the acetate concentration in the methanogenic column influent was below 10

TABLE 2. Characterization of products from CF, CT, and 1,2-DCE transformation in methanogenic batch cultures[a]

Substrate (incubation time [wk])	Seeded culture concn (μg/liter)	% Radioactivity		Fraction recovered during Tenax desorption	% Total recovery
		$^{14}CO_2$ recovered in CO_2 trap[b]	Activity passing Tenax column and combusted to $^{14}CO_2$[c]		
CF (3)	0.5 ± 0.1	88 ± 2	9 ± 2	2 ± 2	99 ± 3
CT (3)	<0.1	99 ± 2	0 ± 2	1 ± 2	100 ± 3
1,2-DCE (25)	65 ± 10	50 ± 5	0 ± 2	48 ± 5	98 ± 7

[a] Initial concentrations for CF, CT, and 1,2-DCE were 30 ± 4, 45 ± 5, and 174 ± 18 μg/liter, respectively. One standard deviation of mean values is given.
[b] The production of CO_2 was confirmed by precipitation with barium.
[c] The volatile component was presumed to be methane.

TABLE 3. Average organic concentrations in methanogenic-column influent and effluent after acclimation

Compound	Acclimation period (wk)	Column influent[a] (μg/liter)	Column effluent[a] (μg/liter)	% Steady-state removal
CF	10	33 ± 7	1.2 ± 0.6	96 ± 2
BDCM	0	30 ± 4	<0.1	>99
DBCM	0	34 ± 5	<0.1	>99
BF	0	34 ± 4	<0.1	>99
CT[b]	0	17 ± 1	<0.1	>99
1,2-DCE[b]	>16	22 ± 3	24 ± 3	−1 ± 20
1,1,1-TCE	10	25 ± 3	0.55 ± 0.3	98 ± 1
1,1,2-TCE[c]	—[d]	0	2.5 ± 1.1	—
TCE[e]	—	0	1.2 ± 0.6	—
1,1,2,2,-TECE	0	27 ± 1	0.90 ± 0.7	97 ± 3
TECE	10	18 ± 3	2.6 ± 1.3	86 ± 7
Acetate (initial 12 mo)	0	600 ± 60 mg/liter	42 ± 6 mg/liter	93 ± 2
Acetate (after 13 mo)	0	100 ± 5 mg/liter	37 ± 3 mg/liter	63 ± 4

[a] One standard deviation of the mean values is given (24 samples).
[b] The compound was added to the column feed after 15 months of operation.
[c] The compound was detected in the effluent after the addition of 1,1,2,2-TECE to the column feed.
[d] —, None.
[e] The compound was detected in the effluent after 10 weeks of acclimation.

mg/liter. However, no change in the removal efficiencies of the halogenated hydrocarbons resulted, suggesting that the removals were more a function of organism concentration than of the quantity of the primary substrate being removed.

After the influent acetate concentration was reduced from 600 to 100 mg/liter, CT, 1,2-DCE, and 1,1,2,2-TECE were added to the feed. The 4 months of data obtained are shown in Table 3. No acclimation period was required for the transformation of CT and 1,1,2,2-TECE. Apparently, the acclimation of the biofilm culture to CF, 1,1,1-TCE, and TECE led to simultaneous acclimation to CT and 1,1,2,2-TECE. After the new additions, 1,1,2-TCE was detected in the methanogenic-column effluent, suggesting that it was an intermediate of 1,1,2,2-TECE transformation. Significant transformation of 1,2-DCE, however, did not occur even after 4 months. This was contrary to the batch results, but the 2-day detention time in the column may have been too short for the acclimation and transformation of 1,2-DCE.

Acetate was the primary organic substrate present to support bacterial growth, and CO_2 was the only electron acceptor available (O_2, NO_3^-, and SO_4^{2-} were not present in the feed); therefore, an enrichment culture of methanogens capable of utilizing acetate developed in the column. Epifluorescence and scanning electron microscopy indicated a predominance of an organism that resembled the fat rod of *Methanothrix soehngenii* as described by Zehnder et al. (36). Attempts were made to inhibit bacterial activity with 2-bromoethanesulfonic acid (BESA) and 2-chloroethanesulfonic acid (CESA) (14) to test the effect on the removal of the halogenated hydrocarbons. At the concentrations of BESA (6×10^{-4} M) and CESA (5.4×10^{-4} M) used, acetate (influent concentration of 100 mg/liter) utilization was reduced only 41%. The extent of halogenated compounds removed was not affected. Either the acetate decarboxylating methanogen was not involved in the transformation or else the reduction in acetate utilization is not a factor affecting the ability to transform chlorinated hydrocarbons.

Results from short-term carbon-14 tracer experiments with the methanogenic column are summarized in Table 4. As with the batch cultures, the primary product of CF and CT transformations was CO_2. CF transformation also appeared to result in the production of a small fraction of methane. The less than 100% recovery of feed activity in the effluent probably resulted from losses to the gas phase and may have represented the loss of $^{14}CH_4$ from solution. No significant activity could be detected in cells or particulates during the transformation of CF or CT. The tracer results for 1,2-DCE indicated that a small percentage was transformed to $^{14}CO_2$, but most remained intact through the column. With carbon-14–labeled acetate in the influent, the effluent activity was comprised of nondegraded acetate and $^{14}CO_2$. The acetate radioisotope was labeled only on the carboxyl carbon; therefore, tracer methane would not be produced from methanogenic decomposition (36). Hence, the value for $^{14}CH_4$ from acetate is zero in Table 4. The poor recovery of activity in the column effluent with carbon-14 acetate tracer was probably due to losses in the gas produced.

TABLE 4. Methanogenic-column effluent tracer activity after addition of carbon-14 tracers to influent

Substrate	% Feed activity in effluent	% Effluent activity		
		Original ^{14}C-substrate	$^{14}CO_2$	$^{14}CH_4$
Influent acetate = 605 mg/liter				
CF (feed activity = 903 ± 10 dpm/ml)	88 ± 2	1 ± 2	97 ± 2	2 ± 2
Influent acetate = 100 mg/liter				
CF (feed activity = 401 ± 6 dpm/ml)	98 ± 2	1 ± 2	98 ± 2	1 ± 2
CT (feed activity = 194 ± 5 dpm/ml)	95 ± 2	1 ± 2	99 ± 2	0 ± 2
1,2-DCE (feed activity = 900 ± 10 dpm/ml)	100 ± 2	94 ± 2	6 ± 2	0 ± 2
Acetate (feed activity = 765 ± 20 dpm/ml)	65 ± 2	56 ± 2	44 ± 2	0 ± 2

DISCUSSION

The degradation of halogenated aliphatic compounds was not found to occur under aerobic conditions (6). However, this study demonstrated that many low-molecular-weight haloaliphatic compounds at trace concentrations can be transformed by a methanogenic mixed culture with acetate as a primary substrate. Similar transformations occurred in a continuous-flow fixed-film system where all of the compounds, except 1,2-DCE, were nearly completely transformed within a 2-day actual detention time. Batch experiments with the reinjection of halogenated compounds showed that the cultures could acclimate to both CF and the brominated trihalomethanes, indicating removal by biotransformation. The biooxidation of CF, CT, and 1,2-DCE were confirmed with carbon-14 tracers in methanogenic-batch and continuous-flow studies as evidenced by the production of $^{14}CO_2$.

Transformations in batch cultures of brominated trihalomethanes in both the sterile controls and seeded methanogenic cultures suggested chemical removal (6). The lack of a detectable lag period for the transformation of the brominated organic compounds in a continuous-flow methanogenic fixed-film column was additional evidence for this hypothesis. Some reduction of bromoaliphatic compounds occurred in sterile controls, suggesting partial chemical transformation, but transformation was much more rapid in the presence of active microorganisms, indicating that the biological mechanism predominated.

A possible mechanism for some of the observed transformations of halogenated aliphatic compounds is reductive dehalogenation (11, 18, 19, 23) or the replacement of a halogen with a hydrogen atom. This involves the transfer of electrons from reduced organic compounds during oxidation via microorganisms or a nonliving mediator, such as inorganic ions (Fe^{2+}, HS^-) or biological products (NADH, NADPH, flavin, flavoprotein, hemoprotein, porphyrins, chlorophyll, cytochromes, and glutathione). Reductive dehalogenation reactions are known to be mediated by microorganisms capable of degrading organochlorine insecticidal chemicals, such as dichlorodiphenyltrichloroethane to dichlorodiphenyldichloroethane (11, 19). Lindane, heptachlor, and dieldrin also can be transformed by reductive dechlorination, which appears to involve discrete enzymes and electron-carrying proteins (19).

Lang et al. reported that almost all chlorinated methane, ethane, and ethene compounds are susceptible to transformation by reductive dechlorination reactions in anaerobic environments (Lang et al., Annu. Meet. Am. Water Works Assoc., 1981). Evidence in the study reported here for reductive dehalogenation was the production of TCE in batch methanogenic cultures incubated with TECE and the presence of TCE and 1,1,2-TCE in the effluent of the methanogenic fixed-film column resulting from the degradation of TECE and 1,1,2,2-TECE, respectively.

Reductive dehalogenation, however, does not explain the production of $^{14}CO_2$ observed in the carbon-14 tracer experiments. The final end products identified in the transformations of CF, CT, and 1,2-DCE were thus probably the consequence of biological oxidations. The theoretical stoichiometric reactions describing the complete biodecomposition of these three compounds under methanogenic conditions are (20):

$$CHCl_3 + 1.5H_2O \rightleftarrows 0.75CO_2 + 0.25CH_4 + 3H^+ + 3Cl^- \quad \Delta G°(w) = -381{,}000 \text{ J/mol} \quad (1)$$

$$CCl_4 + 2H_2O \rightleftarrows CO_2 + 4H^+ + 4Cl^- \quad \Delta G°(w) = -523{,}000 \text{ J/mol} \quad (2)$$

$$C_2H_4Cl_2 + 1.5H_2O \rightleftarrows 0.75CO_2 + 1.25CH_4 + 2H^+ + 2Cl^- \quad \Delta G°(w) = -254{,}000 \text{ J/mol} \quad (3)$$

(Free energy of formation values ($\Delta G_f°$) for the compounds were obtained from *Lange's Handbook of Chemistry* (8) and converted to aqueous conditions at 25°C. $[H^+] = 10^{-7}$ M.) All of the standard free energy changes at pH 7 and 25°C are negative and thus are energetically favorable.

From the above, the methanogenic decomposition of CF would yield 75% of the carbon as

CO_2 and 25% as CH_4. CT is transformed to CO_2 by hydrolysis and not by oxidation-reduction; therefore, CT would not be expected to serve as a sole source of carbon and energy for heterotrophic bacterial growth. Since CF was apparently biotransformed to CO_2, and CF has been proposed as an intermediate of CT transformation (Lang et al., Annu. Meet. Am. Water Works Assoc., 1981), a possible pathway might have been the reductive dechlorination of CT to CF with the subsequent biooxidation of the CF as indicated above.

For 1,2-DCE, 38% of the carbon would go to CO_2, and the remainder would evolve as CH_4. The carbon-14 experiments indicated that nearly all of the carbon was transformed to CO_2. As with CF, this differed from the theoretical equations and suggests that the electrons generated in the oxidation to CO_2 of at least some of the chlorinated compounds were coupled with the reduction of another organic compound, such as acetate. A similar phenomenon has been demonstrated with *Pseudomonas oxalaticus* grown on a mixture of acetate and formate in a continuous culture (9). When small amounts of formate were added to an acetate-limited culture, formate served only as an energy source for acetate assimilation. Similar results were reported for the same organism grown on a mixture of oxalate and formate (10). In the methanogenic fixed-film column, the halogenated compounds were at trace concentrations when compared with acetate, the primary substrate. Thus, their complete oxidation solely for energy would have had little overall effect on acetate utilization.

Whether acetate-decarboxylating methanogens were, at least in part, responsible for the transformations was not determined. The experiment with BESA and CESA was inconclusive as BESA or CESA at the concentrations employed partially inhibited acetate utilization and did not affect the extent of the transformations of the halogenated organic compounds. The fact that acetate was the only primary substrate used in the column studies and also that the extent of the decomposition of the haloorganic compounds was similar to that of acetate suggest that the acetoclastic bacteria play a role. Further study of the organisms involved in the transformations is clearly needed.

In summary, this study has shown that several potentially hazardous halogenated organic compounds of importance in drinking water and groundwater can be transformed even when present at very low concentrations. Some of the chloroaliphatics were nearly completely oxidized to CO_2 under methanogenic conditions, confirming removal by biooxidation. Biological processes also appeared to be most significant in transforming the brominated compounds, although chemical processes also played a role. Transformations were observed within a 2-day detention time under continuous-flow methanogenic conditions requiring an active population of microorganisms supported by acetate as a primary substrate. A better understanding of the organisms involved, the conditions required for transformations, and reaction mechanisms is desirable to help explain and predict the fate of these chemicals under environmental conditions.

ACKNOWLEDGMENTS

Steven C. Young assisted with the laboratory studies.

This work was supported in part by grant no. EPA-R-808034010, Office of Research and Development, U.S. Environmental Protection Agency and by a National Science Foundation Graduate Fellowship.

LITERATURE CITED

1. **Altshuler, B., and B. Pasternack.** 1963. Statistical measurement of the lower limits of detection of a radioactivity counter. Health Phys. **9**:293–298.
2. **Bell, G. G., and F. N. Hayes.** 1958. Liquid scintillation counting. Pergamon Press, Inc., New York.
3. **Bellar, T. A., J. J. Lichtenberg, and R. C. Kroner.** 1974. The occurrence of organohalides in chlorinated drinking waters. J. Am. Water Works Assoc. **66**:703.
4. **Bouwer, E. J., and P. L. McCarty.** 1981. Biofilm degradation of trace chlorinated organics, p. 196–202. *In* Proceedings of the ASCE Environmental Engineering Division Specialty Conference, Atlanta, Ga., July 8–10. American Society of Civil Engineers, New York.
5. **Bouwer, E. J., M. Reinhard, P. L. McCarty, H. Bouwer, and R. C. Rice.** 1982. Organic contaminant behavior during rapid infiltration of secondary wastewater at the Phoenix 23rd Avenue Project. Technical report no. 264, Department of Civil Engineering, Stanford University, Stanford, Calif.
6. **Bouwer, E. J., B. E. Rittmann, and P. L. McCarty.** 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. Environ. Sci. Technol. **15**:596–599.
7. **Davis, J. A., and K. Speicher (ed.).** 1980. Groundwater protection. Water quality management report. U.S. Environmental Protection Agency, Washington, D.C.
8. **Dean, J. A. (ed.).** 1979. Lange's handbook of chemistry, 12th ed. McGraw-Hill Book Co., New York.
9. **Dijkhuizen, L., and W. Harder.** 1979. Regulation of autotrophic and heterotrophic metabolism in *Pseudomonas oxalaticus* OX1: growth on mixtures of acetate and formate in continuous culture. Arch. Microbiol. **123**:47–53.
10. **Dijkhuizen, L., and W. Harder.** 1979. Regulation of autotrophic and heterotrophic metabolism in *Pseudomonas oxalaticus* OX1: growth on mixtures of oxalate and formate in continuous culture. Arch. Microbiol. **123**:55–63.
11. **Esaac, E. G., and F. Matsumura.** 1980. Metabolism of insecticides by reductive systems. Pharm. Ther. **9**:1–26.
12. **Giger, W., and E. Molner-Kubica.** 1977. Tetrachloroethylene in contaminated ground and drinking waters. Bull. Environ. Contam. Toxicol. **19**:475–580.
13. **Glaze, W. H., and J. E. Henderson IV.** 1975. Formation of organochlorine compounds from the chlorination of municipal secondary effluent. J. Water Pollut. Control Fed. **47**:2511–2515.
14. **Gunsalus, R., D. Eirich, J. Romesser, W. Balch, S. Shapiro, and R. S. Wolfe.** 1976. Methyl transfer and methane formation, p. 191–198. *In* G. Schlegel, G. Gottschalk, and D. Pfennig (ed.), Microbial production and utilization of gases (H_2, CH_4, CO). Goltze, Göttingen, Germany.
15. **Henderson, J. E., G. R. Peyton, and W. H. Glaze.** 1976. A

convenient liquid-liquid extraction method for the determination of halomethanes in water at the parts-per-billion level, p. 105–112. *In* L. H. Keith (ed.), Identification and analysis of organic pollutants in water. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.

16. **Jolley, R. L., H. Gorchev, and D. H. Hamilton, Jr. (ed.).** 1978. Water chlorination environmental impact and health effects, vol. 2. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.
17. **Keith, L. H., and W. A. Telliard.** 1979. Priority pollutants I—a perspective view. Environ. Sci. Technol. **13**:416–423.
18. **Kobayashi, H., and B. E. Rittmann.** 1982. Microbial removal of hazardous organic compounds. Environ. Sci. Technol. **16**:170–183.
19. **Lal, R., and D. M. Saxena.** 1982. Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. Microbiol. Rev. **46**:95–127.
20. **McCarty, P. L.** 1971. Energetics and bacterial growth, p. 495–531. *In* S. J. Faust and J. V. Hunter (ed.), Organic compounds in aquatic environments. Marcel Dekker, Inc., New York.
21. **McCarty, P. L., D. G. Argo, and M. Reinhard.** 1979. Operational experiences with activated carbon adsorbers at Water Factory 21. J. Am. Water Works Assoc. **71**:683–689.
22. **Morrison, A.** 1981. If your city's well water has chemical pollutants, then what? Civ. Eng. **51**:65–67.
23. **Morrison, R. T., and R. N. Boyd.** 1974. Organic chemistry, p. 452–491. Allyn and Bacon, Boston.
24. **Otson, R., D. T. Williams, and P. D. Bothwell.** 1979. A comparison of dynamic and static head space and solvent extraction techniques for the determination of THM's in water. Environ. Sci. Technol. **13**:936–939.
25. **Owen, W. F., D. C. Stuckey, J. B. Healy, Jr., L. Y. Young, and P. L. McCarty.** 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res. **13**:485–492.
26. **Petura, J. C.** 1981. Trichloroethylene and methyl chloroform in groundwater: a problem assessment. J. Am. Water Works Assoc. **73**:200–205.
27. **Rapkin, E.** 1962. Measurement of $^{14}CO_2$ by scintillation techniques. Packard technical bulletin no. 7. Packard Instrument Co., La Grange, Ill.
28. **Reinhard, M., E. T. Everhart, J. E. Schreiner, and J. W. Graydon.** 1979. Specific compound analysis by gas chromatography and mass spectrometry. NATO/CCMS Conference on Adsorption Techniques, Washington, D.C.
29. **Rittmann, B. E., E. J. Bouwer, J. E. Schreiner, and P. L. McCarty.** 1980. Biodegradation of trace organic compounds in ground water systems. Technical report no. 255. Department of Civil Engineering, Stanford University, Stanford, Calif.
30. **Roberts, P. V., P. L. McCarty, and W. M. Roman.** 1978. Direct injection of reclaimed water into an aquifer. J. Environ. Eng. Div. (Am. Soc. Civ. Eng.). **104**:933–949.
31. **Roberts, P. V., J. E. Schreiner, and G. D. Hopkins.** 1982. Field study of organic water quality changes during groundwater recharge in the Palo Alto baylands. Water Res. **16**:1025–1035.
32. **Roux, P. H., and W. F. Althoff.** 1980. Investigation of organic contamination of ground water in South Brunswick Township, New Jersey. Ground Water **18**:464–471.
33. **Seraglitz, J. J., and D. W. Miller.** 1978. Status of groundwater contamination in the U.S. J. Am. Water Works Assoc. **70**:162–166.
34. **Supelco, Inc.** 1975. Analysis of VFAs from anaerobic fermentation. Bulletin 748E. Supelco, Inc., Bellefonte, Pa.
35. **Trussell, R. R., and M. D. Umphres.** 1978. The formation of trihalomethanes. J. Am. Water Works Assoc. **70**:604–612.
36. **Zehnder, A. J. B., B. A. Huser, T. D. Brock, and K. Wuhrmann.** 1980. Characterization of an acetate-decarboxylating non-hydrogen-oxidizing methane bacterium. Arch. Microbiol. **124**:1–11.