First publ. in: Applied and Environmental Microbiology 54 (1988), 11, pp. 2819-2824

Transformation of Tetrachloromethane to Dichloromethane and Carbon Dioxide by *Acetobacterium woodii*

CHRISTINE EGLI, THOMAS TSCHAN, RUDOLF SCHOLTZ, ALASDAIR M. COOK, AND THOMAS LEISINGER*

Department of Microbiology, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zurich, Switzerland

Five anaerobic bacteria were tested for their abilities to transform tetrachloromethane so that information about enzymes involved in reductive dehalogenations of polychloromethanes could be obtained. Cultures of the sulfate reducer *Desulfobacterium autotrophicum* transformed some 80 μM tetrachloromethane to trichloromethane and a small amount of dichloromethane in 18 days under conditions of heterotrophic growth. The acetogens *Acetobacterium woodii* and *Clostridium thermoaceticum* in fructose-salts and glucose-salts media, respectively, degraded some 80 μM tetrachloromethane completely within 3 days. Trichloromethane accumulated as a transient intermediate, but the only chlorinated methanes recovered at the end of the incubation were 8 μM dichloromethane and traces of chloromethane. *Desulfobacter hydrogenophilus* and an autotrophic, nitrate-reducing bacterium were unable to transform tetrachloromethane. Reduction of chlorinated methanes was thus observed only in the organisms with the acetyl-coenzyme A pathway. Experiments with [^{14}C]tetrachloromethane were done to determine the fate of this compound in the acetogen *A. woodii*. Radioactivity in an 11-day heterotrophic culture was largely (67%) recovered in CO_2, acetate, pyruvate, and cell material. In experiments with cell suspensions to which [^{14}C]tetrachloromethane was added, $^{14}CO_2$ appeared within 20 s as the major transformation product. *A. woodii* thus catalyzes reductive dechlorinations and transforms tetrachloromethane to CO_2 by a series of unknown reactions.

Chlorinated aliphatic hydrocarbons, some of which are carcinogens or mutagens, are common water pollutants. Several mono- and dihalogenated hydrocarbons are utilized by aerobic microorganisms as carbon and energy sources for growth, and some trihaloalkanes are subject to aerobic transformations. Microbial transformation of the industrially relevant polychlorinated hydrocarbons tetrachloromethane, tetrachloroethylene, and 1,1,1-trichloroethane, however, is catalyzed only under anaerobic conditions by undefined dehalogenation mechanisms (10, 30).

Until recently, the dehalogenation of highly chlorinated hydrocarbons had been observed only in mixed cultures of anaerobic bacteria (10, 24, 30). Results from several laboratories now indicate that pure cultures of laboratory strains of the strictly anaerobic methanogenic and sulfate-reducing bacteria, none of which had previous exposure to chlorinated solvents, can transform polyhalogenated compounds. A *Methanosarcina* sp. reductively dechlorinates tetrachloroethene to trichloroethene (16), and pure cultures of several methanogens produce ethane, ethene, and ethyne from halogenated hydrocarbons (3). We confirmed some of those reactions independently and observed a different specificity of reductive dehalogenation in *Desulfobacterium autotrophicum*, which reduced tetrachloromethane quantitatively to trichloromethane and dichloromethane and converted 1,1,1-trichloroethane to 1,1-dichloroethane (15).

We have compared dehalogenation of tetrachloromethane by representatives of two types of sulfate-reducing bacteria, by acetogenic bacteria (17, 23, 33), and by a culture of an autotrophic, nitrate-reducing bacterium to examine the involvement of different metabolic sequences in anaerobic dehalogenation. We now report the results of this comparison and a novel conversion of tetrachloromethane to carbon dioxide by acetogenic bacteria.

* Corresponding author.

MATERIALS AND METHODS

Materials. [U-^{14}C]acetate (2.07 TBq/mol), [2-^{14}C]acetate (1.96 TBq/mol), and [^{14}C]tetrachloromethane (2.3 TBq/mol; 95% [contained lower chlorinated chloromethanes]) were obtained from Amersham (Buckinghamshire, England). Sodium [^{14}C]bicarbonate (0.25 TBq/mol) was obtained from Sigma Chemical Co. (St. Louis, Mo.). 4-Phenyl-phenacylacetate was synthesized according to Durst et al. (14). Viton (15) and butyl rubber septa were used. Scintillation fluid (Ready-Solv HP) was obtained from Beckman (Galway, Ireland). Other chemicals were of the highest quality from Fluka (Buchs, Switzerland) or from Merck-Schuchardt (Munich, Federal Republic of Germany).

Values for concentrations of halomethanes in cultures were calculated as if they were entirely in the aqueous phase (15, 31). The SI units becquerel (Bq; per second) and katal (kat; moles per second) were used.

Organisms. *Desulfobacterium autotrophicum* HRM2 (DSM 3382), *Acetobacterium woodii* (DSM 1030), and *Desulfobacter hydrogenophilus* (DSM 3380) were kindly supplied by G. Fuchs (Universität Ulm, Ulm, Federal Republic of Germany). *Clostridium thermoaceticum* (DSM 512) was obtained from Deutsche Sammlung von Mikroorganismen (Braunschweig, Federal Republic of Germany). Hydrogen-oxidizing, autotrophic, nitrate-reducing bacteria were obtained from a commercial groundwater-treatment plant. The population was homogeneous as judged by microscopy and by plating on salts medium solidified with 1.5% agar under a H_2-CO_2 atmosphere.

Growth conditions. All cultures were grown in 100-ml portions in 250-ml screw-cap bottles pressurized to 150 kPa with a mixture of H_2 and CO_2 (80:20, vol/vol). The inoculum (5 ml) was from a culture in mid-exponential phase. All cultures were incubated in the dark and shaken once per day.

Desulfobacterium autotrophicum was grown at 28°C in sulfate-containing medium either autotrophically or with 5

mM lactate as the carbon source (7). *Desulfobacter hydrogenophilus* was grown at 28°C in sulfate-containing medium with 20 mM acetate as the carbon source (32). *A. woodii* was grown at 30°C either autotrophically or with 8 mM fructose as the carbon source (2). *C. thermoaceticum* was grown at 55°C with 10 mM glucose as the carbon source (13). Hydrogen-oxidizing, autotrophic, nitrate-reducing bacteria were grown at 15.5°C. The mineral medium (pH 7.4) comprised 20 mM Na_2HPO_4, 11 mM KH_2PO_4, 20 mM $NaHCO_3$, 25 mM $NaNO_3$, 2 mM NH_4Cl, 0.8 mM $MgSO_4$, 70 μM $CaCl_2$, 20 μM $FeCl_3$, and trace elements solution (9) (2.5 ml/liter).

Degradation studies. Experiments with unlabeled chlorinated hydrocarbons in growing cultures or suspensions of nongrowing cells were done in 25 ml of medium in 130-ml screw-cap bottles with viton septa. Chlorinated hydrocarbons (1 or 2 μmol per bottle) were added as colloidal solutions in sterile water (15) or in gas form (chloromethane). Degradation of chlorinated compounds was monitored by gas chromatographic (GC) analyses of samples (0.3 ml) of headspace. Optical density or protein was measured in samples of the culture fluid.

Experiments with nongrowing cells were done with cell material from 100 ml of a heterotrophically grown, late-exponential-phase culture harvested under anaerobic conditions (35 min, 2,000 × *g*, 4°C). The pellet was washed once in anaerobic buffer (140 mM $NaHCO_3$, 2.5 mM KH_2PO_4, 2.7 mM K_2HPO_4, 2.5 mM cysteine hydrochloride, 1 mM Na_2S, 0.4 mM $MgSO_4$, pH 7.0), suspended in 5 ml of the same buffer, and used to start the reaction in a screw-cap bottle. Prereaction mixtures were incubated for 30 min at 30°C to ensure equilibration of the halohydrocarbons between the liquid and gas phases. Samples (about six) of the gas phase were taken at intervals for analysis of chlorohydrocarbons, and biomass was quantified as protein. Experiments were done in duplicate.

Degradation of [^{14}C]tetrachloromethane. Degradation of $^{14}CCl_4$ by growing cells of *A. woodii* was followed in 130-ml screw-cap bottles containing 25 ml of medium. The culture was incubated until an optical density of 0.1 was attained, when 1 μmol (74 kBq) of labeled CCl_4 was added. Transformation was followed by GC and flame ionization detection. After 11 days of incubation, the reaction was stopped by bringing the pH to 2.5 with perchloric acid (3 M). Chlorinated compounds and the CO_2 released by acidification were fractionated by GC and trapped individually in scintillation fluid, and the radioactivity was measured in a liquid scintillation counter. Nonvolatile material was separated by high-pressure liquid chromatography (HPLC).

Pulse experiments with nongrowing cells were done in 13.5-ml flasks sealed with rubber stoppers. Reaction mixtures contained a washed-cell suspension (3 ml; see above), anaerobic buffer (9 ml), 67.5 nmol of sodium acetate, and 300 nmol of unlabeled CCl_4. The reaction to give radiochemical products was started by the addition of $^{14}CCl_4$ stock solution (60 μl [220 kBq] in ethanol). Samples of headspace (0.3 ml, for halomethanes) and of the liquid phase (1.5 ml for CO_2, acetate, and nonvolatiles) were taken at intervals. Data from samples of the liquid phase were used without adjustment, and data from samples of the gas phase were adjusted for the altered volume.

Apparatus and analytical methods. The following GC models were used: 5830A (equipped with a thermal conductivity detector [TCD]), 5840A (equipped with a flame ionization detector), 5890 (equipped with a mass spectrometer [MS; MSD 5970]). All were obtained from Hewlett-Packard, Avondale, Pa. HPLC (LKB, Bromma, Sweden), liquid scintillation counting (model LS 1801; Beckman Instruments, Inc., Fullerton, Calif.), and spectrophotometric analyses (Uvikon 820; Kontron, Zurich, Switzerland) were performed with the standard equipment indicated.

GC. Chlorinated hydrocarbons and methane were routinely determined by GC and flame ionization detection after separation on a Porapak P column (15); cochromatography with authentic material on other columns was used to confirm the identity of products (15). Standards were prepared in culture bottles containing the appropriate sterile growth medium. Acetic acid was separated for GC-MS analysis on a methyl silicone capillary column (HP1; 12 m by 530 μm).

CO_2 in buffer (0.5 ml) was placed in a 1.2-ml septum vial and released to the gas phase by the addition of concentrated H_2SO_4 (20 μl). CO_2 in the headspace (0.3 ml) was determined (by GC-TCD) after separation on a Carbosieve B column (60/80 mesh, 300 by 0.3 cm). The carrier gas was He (29 ml/min), and injector, oven, and detector temperatures were set at 150, 100, and 200°C, respectively.

Radiolabeled volatile compounds were separated on Carbosieve B (CO_2; oven temperature, 100°C) or Porapak P ($CHCl_3$ and CCl_4; oven temperature, 135°C) columns attached to a TCD, similar to the determination of CO_2. The gas stream from the TCD was piped in viton tubing (45 by 0.3 cm) to scintillation fluid, and volatiles in peaks were trapped in that fluid (35). Unlabeled CCl_4 and $CHCl_3$ were added to radiolabeled gas samples prior to analysis of these compounds to give measurable peaks. Standard scintillation fluid (10 ml) was adequate to trap the halomethanes; the fluid was supplemented with 2 ml of methanol and 2 ml of phenethamine to trap CO_2 (35). The system was tested with $^{14}CCl_4$ and sodium [^{14}C]bicarbonate standards.

HPLC analysis. Samples were acidified (3 M perchloric acid) to pH 2.5 and stored on ice for 10 min to eliminate radioactive CO_2. Cells were then removed (10,000 × g for 10 min at 4°C), and the supernatant fluid was used directly for analyses. Acetate, as acetic acid, was separated on reversed-phase columns with 100 mM potassium phosphate buffer (pH 2.2; 1 ml/min) as the mobile phase (20) and quantified in a UV detector set at 195 nm. The standard curve was linear in the range from 0.5 to 50 mM. Total radioactivity in growth medium was measured by an adaptation of this method. After 7 min of isocratic elution, a linear gradient (0 to 50% of the second eluent [80% (vol/vol) methanol in 10 mM potassium phosphate, pH 2.2] over 20 min) was started, and the final condition was maintained for 10 min. The column eluate was collected in portions (0.3 ml) in vials containing 20 μl of 1 N NaOH and monitored for radioactivity.

Isolation, derivatization, and degradation of [^{14}C]acetate. Acetate was separated by distillation (4) from other compounds in the growth medium after acidification of the sample (to pH 2.5 with 3 M perchloric acid) and buffered with 1 M citrate (pH 3.0; 50 μl/ml of sample).

The pH of the distillate was brought to 8.0 with 0.1 mM KOH, and water was removed in a rotary evaporator. Dry potassium acetate was alkylated with a surplus of 4-phenylphenacylbromide (14). The product, 4-phenyl-phenacylacetate, was isolated by flash chromatography (29) on Silica Gel 60 (Fluka, Buchs) (230/400 mesh) with dichloromethane as the solvent. The dry product was redissolved in toluene, and the radioactivity was measured. Unlabeled 4-phenylphenacylacetate was used as a standard.

Acetic acid obtained by distillation was oxidized in the Schmidt degradation (18, 28). $^{14}CO_2$ was trapped in 1 N NaOH, and radioactivity in portions (100 to 200 μl) was

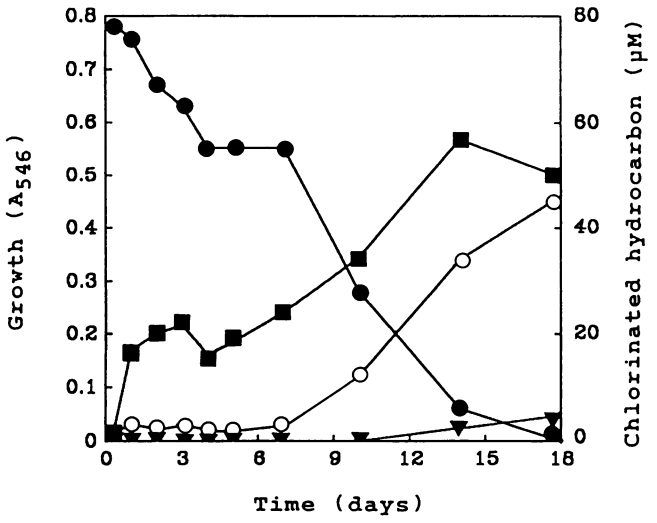

FIG. 1. Anaerobic degradation of CCl_4 by *Desulfobacterium autotrophicum* during heterotrophic growth in lactate-salts medium. Symbols: ●, CCl_4; ■, $CHCl_3$; ▼, CH_2Cl_2; ○, growth.

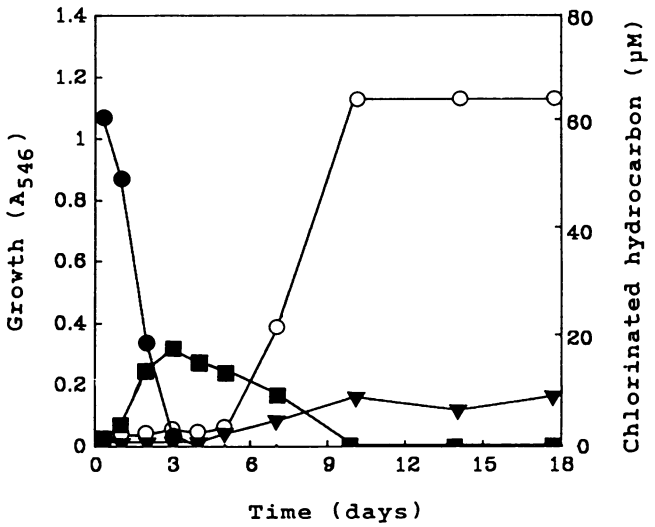

FIG. 2. Anaerobic degradation of CCl_4 by *A. woodii* during heterotrophic growth in fructose-salts medium. Symbols: ●, CCl_4; ■, $CHCl_3$; ▼, CH_2Cl_2; ○, growth.

measured. As controls, [U-^{14}C]acetate and [2-^{14}C]acetate were degraded in parallel reactions.

Protein in whole cells was measured by a Lowry method (9). Optical density was measured in a spectrophotometer at 546 nm in 1-cm-light-path cuvettes.

RESULTS

Degradation of tetrachloromethane under growth conditions. CCl_4 (80 μM) added at the time of inoculation to heterotrophic cultures of *Desulfobacterium autotrophicum*, *A. woodii*, and *C. thermoaceticum* disappeared and gave rise to trichloromethane and dichloromethane, which were conclusively identified. No transformation of CCl_4 was observed in a culture of *Desulfobacter hydrogenophilus*, whose heterotrophic and autotrophic growth was completely inhibited by the compound, or in a culture of the nitrate-reducing bacterium, which was not affected by CCl_4. The inability of *Desulfobacter hydrogenophilus* to metabolize CCl_4 was confirmed in an experiment with resting cells, in which no transformation of the chlorinated compound was observed after 2 days of incubation; *Desulfobacterium autotrophicum*, *A. woodii*, and *C. thermoaceticum* totally transformed CCl_4 within 5 h under these conditions (data not shown). The three organisms that are known to possess the acetyl coenzyme A (acetyl-CoA) pathway (17, 23, 33) thus were able to transform CCl_4, whereas *Desulfobacter hydrogenophilus*, which has all enzymes of a complete citric acid cycle (26), and the nitrate-reducing bacterium, which presumably assimilated CO_2 by the Calvin cycle (22), were unable to metabolize CCl_4.

Striking differences were observed when the interactions of growing cultures of *Desulfobacterium autotrophicum* and *A. woodii* with CCl_4 were examined more closely. *Desulfobacterium autotrophicum* reduced CCl_4 largely to trichloromethane and a small amount of dichloromethane (Fig. 1). Complete reduction of CCl_4 required 18 days of incubation, and growth was observed only when most of the CCl_4 had been transformed. After prolonged incubation, the amount of trichloromethane in the culture decreased with a concomitant increase in the concentration of dichloromethane (data not shown). *A. woodii* was able to degrade 80 μM CCl_4 completely within 3 days (Fig. 2). Trichloromethane accumulated as a transient intermediate, but the only chlorinated methanes recovered at the end of the incubation period were 8 μM dichloromethane and traces of chloromethane. Thus, 90% of the CCl_4 was degraded to unknown products. Similar results were obtained with *C. thermoaceticum* (data not shown). The small amounts of methane typically formed by sulfate-reducing bacteria and a *Clostridium* sp. (25, 26) were also observed in our work. *A. woodii* and *Desulfobacterium autotrophicum* formed 61 and 16 nmol of methane per 25-ml culture, respectively, during growth. In the presence of 80 μM CCl_4, methane formation was reduced by about 50% in both organisms, so CCl_4 was not reduced to methane. Some pathway other than reduction must be present in the acetogens.

Autotrophic growth of *Desulfobacterium autotrophicum* and *A. woodii* was inhibited by 80 μM CCl_4, but both organisms formed the same transformation products as during heterotrophic growth, though at considerably lower rates.

Degradation rates of chlorohydrocarbons in resting cells. The degradation rates of CCl_4 in resting cells of *Desulfobacterium autotrophicum* and *A. woodii* were 0.29 mkat/kg of protein and 0.12 mkat/kg of protein, respectively. The highest degradation rates in both organisms were observed with cells harvested in the exponential growth phase.

Transformation rates in resting cells of *A. woodii* for a number of chlorohydrocarbons, together with the chlorinated degradation products observed, are given in Table 1. The presence of fructose stimulated the reductive dehalogenation of CCl_4 by threefold, of trichloromethane by fourfold, and of 1,1,1-trichloroethane by ninefold. The reduction of dichloromethane, chloromethane, and tetrachloroethylene occurred only when fructose was present in the reaction medium. 1,1-Dichloroethane was not degraded. In general, the degradation rate decreased with a decrease in the level of chlorination, as predicted by Vogel et al. (30).

Products formed from [^{14}C]tetrachloromethane by *A. woodii*. Growing cultures of *A. woodii* converted 92% of the added $^{14}CCl_4$ (40 μM) to nonhalogenated products. Much of the

TABLE 1. Degradation of chlorinated hydrocarbons by cell suspensions of *A. woodii*[a]

Halogenated compound (amt present [in nmol per test] after 48 h)[b]	Degradation rate (μkat/kg of protein)	Chlorinated products formed (amt present [in nmol per test] after 48 h)[c]
CCl_4 (0)	350	$CHCl_3$ (0)[d] CH_2Cl_2 (150) CH_3Cl (40)
$CHCl_3$ (0)	14	CH_2Cl_2 (570) CH_3Cl (50)
CH_2Cl_2 (920)	<1	CH_3Cl (40)
CH_3Cl (0)	4	ND[e]
CCl_3—CH_3 (0)	39	$CHCl_2$—CH_3 (910)
CCl_2=CCl_2 (960)	<1	CHCl=CCl_2 (20)

[a] Fructose-grown cells were incubated with chlorinated hydrocarbons (40 μM) in anaerobic buffer containing 5 mM fructose as described in Materials and Methods.
[b] 1,000 nmol of each compound was used per test.
[c] Neither substrate disappearance nor formation of products was observed in controls containing autoclaved cultures.
[d] $CHCl_3$ was a transient intermediate (≤320 nmol per test).
[e] ND, No product detected.

initial radioactivity (67%) was recovered as CO_2, acetate, pyruvate, and cell material; the remainder comprised an unknown, hydrophobic material and CH_2Cl_2 (Table 2).

CO_2 was identified as a compound which dissolved in alkali, volatilized on acidification, and cochromatographed by GC-TCD with authentic material; GC-MS analyses confirmed this identification. Pyruvate was tentatively identified by cochromatography in HPLC with authentic material.

Radioactivity which cochromatographed in HPLC with the acetate peak was confirmed to be labeled acetate after distillation, repetition of the cochromatography, and derivatization to 4-phenyl-phenacylacetate, whereby 92% of the radioactivity added as acetate was recovered in a product with the correct melting point (111°C [27]) and mass spectrum (data not shown). GC-MS analyses confirmed the identity of acetic acid.

The distribution of radioactivity in the acetate produced from $^{14}CCl_4$ by *A. woodii* was determined by Schmidt

TABLE 2. Products from [^{14}C]tetrachloromethane degradation by a growing culture of *A. woodii*[a]

Radioactivity recovered in following fraction	% of total radioactivity
CO_2	13
Acetate	38
Pyruvate	10
Fraction A[b]	14
Cells	6
CH_2Cl_2	8
Hydrophobic material[c]	10
Total	99

[a] Organisms were grown in fructose-salts medium, and the products were examined after 11 days. The amount of radioactivity added to this representative experiment was 74 kBq.
[b] An unidentified peak (retention time, 11 min) in HPLC chromatograms. Acetate and pyruvate eluted at 6.1 and 4.4 min, respectively.
[c] All material eluted from the HPLC column after fraction A (some 17 peaks).

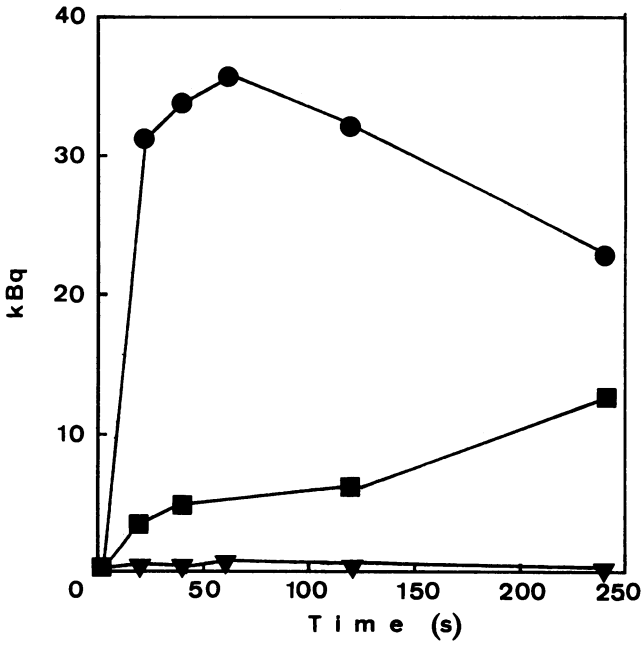

FIG. 3. Early transformation products formed from 40 μM $^{14}CCl_4$ by resting cells of *A. woodii*. Symbols ●, CO_2; ■, acetate; ▼, $CHCl_3$.

degradation. A total of 69% of the radioactivity was recovered in the carboxyl group, and 24% was recovered in the methyl group. The recovery of radioactivity in control experiments with [2-^{14}C]acetate and [U-^{14}C]acetate was 92 to 95%.

CO_2 as the first product from tetrachloromethane. A pulse-labeling experiment was done to determine whether the radioactivity introduced into acetate from $^{14}CCl_4$ proceeded through CO_2 or through more reduced C1 compounds. After 1 min, eight times more label was found in CO_2 than in acetate; there was little radioactivity in trichloromethane (Fig. 3). Thereafter, the amounts of radioactive acetate and trichloromethane increased, whereas the amount of labeled CO_2 decreased. At 30 min, the following distribution of the radioactivity introduced as CCl_4 was found: trichloromethane, 15%; chloromethane, 4%; acetate, 5%; CO_2, 5%; pyruvate, 3%. We presume that CO_2 is the first detectable intermediate of CCl_4 degradation in *A. woodii* and that this CO_2 is converted to acetate via the acetyl-CoA pathway.

DISCUSSION

The distribution of reductive dechlorination in different organisms in this study leads us to correlate the acetyl-CoA pathway (17, 23, 33) with the dechlorination, and this idea is supported by the data published on pure cultures, all of which have this pathway (3, 15, 16; also see references 17 and 26). If the hypothesis is correct and exclusive, mixed cultures transforming haloalkanes under denitrifying conditions (5) are active due to contaminants containing enzymes of the acetyl-CoA pathway.

A. woodii, *C. thermoaceticum*, and *Desulfobacterium autotrophicum* contain cobamides, whereas *Desulfobacter hydrogenophilus* does not (11). The reactivity of halomethanes with cobamides (19, 34) and an apparent dehalogenation of methyl iodide by cobamide (21) suggest that cobamides could be involved in the dehalogenation of CCl_4. The

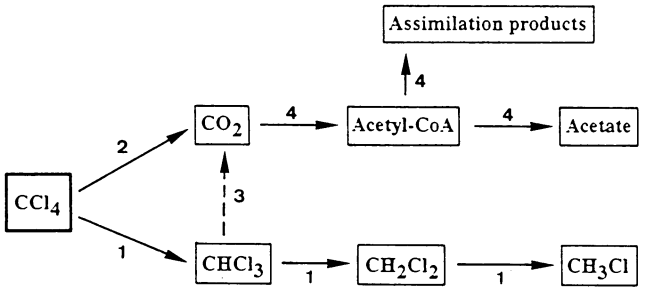

FIG. 4. Proposed pathway of CCl_4 degradation in bacteria which utilize the acetyl-CoA pathway. 1, Sequential reductive dehalogenation; 2, substitutive transformation (see text); 3, oxidative transformation (see text); 4, CO_2 assimilation by the acetyl-CoA pathway. Type 1 reactions were observed in *Desulfobacterium autotrophicum*; type 1, 2, and 4 reactions were observed in *A. woodii*, which in addition may catalyze type 3 reactions.

inhibition of the formation of trace amounts of methane ("mini-methane" [17, 26]) by CCl_4 may indicate that some of the cobamide protein, presumed to be involved in the acetyl-CoA pathway (17), reacted with chlorinated methanes to catalyze reductive dehalogenation. The reaction rate for reduction of the chlorinated methanes generally decreases with decreasing chlorination (Table 1). This may be explained by the increased carbon-halogen bond energy at each level of reduction (6) and the corresponding difficulty in cleaving a C—Cl bond. The reduction of the C—Cl bond at iron centers is also known (1, 8).

Whereas the reductive dehalogenation of CCl_4 to trichloromethane and dichloromethane is the only pathway of polychloromethane degradation observed in *Desulfobacterium autotrophicum* (Fig. 1) (15), *A. woodii* seemingly degraded only a fraction of the CCl_4 via this sequence (Fig. 2), transforming some 90% of the CCl_4 to nonchlorinated products (e.g., CO_2 and acetate) (Table 2 and Fig. 3). This latter transformation is concomitant with acetogenesis in our limited study. The conversion of CCl_4 to CO_2 has precedents in work on mixed cultures under methanogenic (30), denitrifying, and sulfate-reducing (5, 6) conditions.

Our data suggest that CCl_4 metabolism by *A. woodii* comprises at least two sequences (Fig. 4). First, there is the reductive branch putatively catalyzed by corrinoid enzymes and leading to trichloromethane, dichloromethane, and chloromethane. Second, the substitutive branch transforms CCl_4 into CO_2 by a series of unknown reactions, which do not cause a net change in the oxidation state of the carbon atom. It is not clear whether intermediates of the reductive pathway, e.g., trichloromethane, are also subject to transformation to CO_2 (Fig. 4).

The mechanism of transformation of CCl_4 to CO_2 is unknown, but there is an example of the anaerobic introduction of an oxygen atom into CCl_4 with the formation of CO. This has been observed in anaerobic rat liver microsomal cytochrome P-450 (1), and a cytochrome P-450–Fe^{2+} dichlorocarbene complex is the proposed intermediate; the reaction is some 50-fold slower than the degradation of CCl_4 by *A. woodii*. *A. woodii* is not known to contain cytochromes (11), so an analogous reaction would have to be cytochrome independent.

The pathway leading from CCl_4 to CO_2 could provide a carbon source for autotrophic growth of *A. woodii*. Assuming the concentration of CCl_4 in the medium can be kept below the threshold causing growth inhibition, it should be possible to culture the organism with a mixture of CCl_4 and H_2.

In the reductive pathway, CCl_4 and the other chlorinated methanes serve as electron acceptors. A similar reaction, the reductive dechlorination of 3-chlorobenzoate to benzoate by bacterium DCB-1, is postulated to provide the organism with energy (12). It is not known whether the reductive removal of chlorine substituents from polychloromethanes provides the organisms which we examined with energy for growth; this will be difficult to assess because only low concentrations of chlorinated methanes are tolerated by growing organisms.

ACKNOWLEDGMENTS

We are grateful to A. Dutly for GC-MS analyses and to S. Raillard for synthesizing 4-phenyl-phenacylacetate.

This work was supported by a grant from the Swiss Federal Institute of Technology, Zurich, Switzerland.

LITERATURE CITED

1. **Ahr, H. J., L. J. King, W. Nastainczyk, and V. Ullrich.** 1980. The mechanism of chloroform and carbon monoxide formation from tetrachloromethane by microsomal cytochrome P-450. Biochem. Pharmacol. **29**:2855–2861.
2. **Balch, W. E., S. Schoberth, R. S. Tanner, and R. S. Wolfe.** 1977. *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. Int. J. Syst. Bacteriol. **27:** 355–361.
3. **Belay, N., and L. Daniels.** 1987. Production of ethane, ethylene, and acetylene from halogenated hydrocarbons by methanogenic bacteria. Appl. Environ. Microbiol. **53**:1604–1610.
4. **Bergmeyer, H. U., and H. Möllering.** 1974. Acetat: Bestimmung mit vorgeschalteter Indikatorreaktion, p. 1566–1574. *In* H. U. Bergmeyer (ed.), Methoden der enzymatischen Analyse, 3rd ed., vol II. Verlag Chemie, Weinheim, Federal Republic of Germany.
5. **Bouwer, E. J., and P. L. McCarty.** 1983. Transformations of halogenated organic compounds under denitrification conditions. Appl. Environ. Microbiol. **45**:1295–1299.
6. **Bouwer, E. J., and J. P. Wright.** 1988. Transformations of trace halogenated aliphatics in anoxic biofilm columns. J. Contam. Hydrol. **2**:155–169.
7. **Brysch, K., C. Schneider, G. Fuchs, and F. Widdel.** 1987. Lithoautotrophic growth of sulfate-reducing bacteria, and description of *Desulfobacterium autotrophicum* gen. nov., sp. nov. Arch. Microbiol. **148**:264–274.
8. **Castro, C. E., R. S. Wade, and N. O. Belser.** 1985. Biodehalogenation: reactions of cytochrome P-450 with polyhalomethanes. Biochemistry **24**:204–210.
9. **Cook, A. M., and R. Hütter.** 1981. *s*-Triazines as nitrogen sources for bacteria. J. Agric. Food Chem. **29**:1135–1143.
10. **Cook, A. M., R. Scholtz, and T. Leisinger.** 1988. Mikrobieller Abbau von halogenierten aliphatischen Verbindungen. GWF Gas Wasserfach: Wasser Abwasser **129**:61–69.
11. **Dangel, W., H. Schulz, G. Diekert, H. König, and G. Fuchs.** 1987. Occurrence of corrinoid-containing membrane proteins in anaerobic bacteria. Arch. Microbiol. **148**:52–56.
12. **Dolfing, J., and J. M. Tiedje.** 1987. Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic coculture. Arch. Microbiol. **149**:102–105.
13. **Drake, H. L., S. I. Hu, and H. G. Wood.** 1980. Purification of carbon monoxide dehydrogenase, a nickel enzyme from *Clostridium thermoaceticum*. J. Biol. Chem. **255**:7174–7180.
14. **Durst, H. D., M. Milano, E. J. Kikta, Jr., S. A. Connelly, and E. Grushka.** 1975. Phenacyl esters of fatty acids via crown ether catalysis for enhanced ultraviolet detection in liquid chromatography. Anal. Chem. **47**:1797–1801.
15. **Egli, C., R. Scholtz, A. M. Cook, and T. Leisinger.** 1987. Anaerobic dechlorination of tetrachloromethane and 1,2-dichloroethane to degradable products by pure cultures of *Desulfobacterium* sp. and *Methanobacterium* sp. FEMS Microbiol.

Lett. **43**:257–261.
16. **Fathepure, B. Z., and S. A. Boyd.** 1988. Reductive dechlorination of perchloroethylene and the role of methanogens. FEMS Microbiol. Lett. **49**:149–156.
17. **Fuchs, G.** 1986. CO_2 fixation in acetogenic bacteria: variation on a theme. FEMS Microbiol. Rev. **39**:181–213.
18. **Fuchs, G., E. Stupperich, and G. Eden.** 1980. Autotrophic CO_2 fixation in *Chlorobium limicola*. Evidence for the operation of a reductive tricarboxylic acid cycle in growing cells. Arch. Microbiol. **128**:64–71.
19. **Ghambeer, R. K., H. G. Wood, M. Schulman, and L. Ljungdahl.** 1971. Total synthesis of acetate from CO_2. Arch. Biochem. Biophys. **143**:471–484.
20. **Grossenbacher, H., T. Thurnheer, D. Zürrer, and A. M. Cook.** 1986. Determination of sulphonated azo dyestuffs and their bacterial metabolites by high-performance liquid chromatography. J. Chromatogr. **360**:219–223.
21. **Holder, U., D.-E. Schmidt, E. Stupperich, and G. Fuchs.** 1985. Autotrophic synthesis of activated acetic acid from two CO_2 in *Methanobacterium thermoautotrophicum*. III. Evidence for common one-carbon precursor pool and the role of corrinoid. Arch. Microbiol. **141**:229–238.
22. **Kornberg, H. L., J. F. Collins, and D. Bigley.** 1960. The influence of growth substrates on metabolic pathways in *Micrococcus denitrificans*. Biochim. Biophys. Acta **39**:9–24.
23. **Ljungdahl, L. G.** 1986. The autotrophic pathway of acetate synthesis in acetogenic bacteria. Annu. Rev. Microbiol. **40**:415–450.
24. **Parsons, F., and G. B. Lage.** 1985. Chlorinated organics in simulated groundwater environments. J. Am. Water Works Assoc. **77**:52–59.
25. **Postgate, J. R.** 1969. Methane as a minor product of pyruvate metabolism by sulphate-reducing and other bacteria. J. Gen. Microbiol. **57**:293–302.
26. **Schauder R., B. Eikmanns, R. K. Thauer, F. Widdel, and G. Fuchs.** 1986. Acetate oxidation to CO_2 in anaerobic bacteria via a novel pathway not involving reactions of the citric acid cycle. Arch. Microbiol. **145**:162–172.
27. **Schwetlick, K. (ed.)** 1977. Organikum. Organisch-chemisches Grundpraktikum, 15th ed., p. 766. VEB Deutscher Verlag der Wissenschaften, Berlin.
28. **Simon, H., and H. G. Floss.** 1967. Anwendung von Isotopen in der Organischen Chemie und Biochemie, vol. 1, p. 23–27. Springer-Verlag KG, Berlin.
29. **Still, W. C., M. Kahn, and A. Mitra.** 1978. Rapid chromatographic technique for preparative separations with moderate resolution. J. Org. Chem. **43**:2923–2925.
30. **Vogel, T. M., C. S. Criddle, and P. L. McCarty.** 1987. Transformations of halogenated aliphatic compounds. Environ. Sci. Technol. **21**:722–736.
31. **Wackett, L. P., and D. T. Gibson.** 1988. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida* F1. Appl. Environ. Microbiol. **54**:1703–1708.
32. **Widdel, F.** 1987. New types of acetate-oxidizing, sulfate-reducing *Desulfobacter* species, *D. hydrogenophilus* sp. nov., *D. latus* sp. nov., and *D. curvatus* sp. nov. Arch. Microbiol. **148**: 286–291.
33. **Wood, H. G., S. W. Ragsdale, and E. Pezacka.** 1986. The acetyl-CoA pathway of autotrophic growth. FEMS Microbiol. Rev. **39**:345–362.
34. **Wood, J. M., F. S. Kennedy, and R. S. Wolfe.** 1968. The reaction of multihalogenated hydrocarbons with free and bound reduced vitamin B_{12}. Biochemistry **7**:1707–1713.
35. **Zehnder, A. J. B., and T. D. Brock.** 1979. Methane formation and methane oxidation by methanogenic bacteria. J. Bacteriol. **137**:420–432.