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Syed A. Hashsham
Michigan State University

David L. Freedman
Clemson University, dfreedm@clemson.edu

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Enhanced Biotransformation of Carbon Tetrachloride by *Acetobacterium woodii* upon Addition of Hydroxocobalamin and Fructose

SYED A. HASHSHAM[1]* AND DAVID L. FREEDMAN[2]

Center for Microbial Ecology and Department of Civil and Environmental Engineering, Michigan State University, E. Lansing, Michigan 48824,[1] and Department of Environmental Engineering and Science, Clemson University, Clemson, South Carolina 29631[2]

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The objective of this study was to evaluate the effect of hydroxocobalamin (OH-Cbl) on transformation of high concentrations of carbon tetrachloride (CT) by *Acetobacterium woodii* (ATCC 29683). Complete transformation of 470 μM (72 mg/liter [aqueous]) CT was achieved by *A. woodii* within 2.5 days, when 10 μM OH-Cbl was added along with 25.2 mM fructose. This was approximately 30 times faster than *A. woodii* cultures (live or autoclaved) and medium that did not receive OH-Cbl and 5 times faster than those controls that did receive OH-Cbl, but either live *A. woodii* or fructose was missing. CT transformation in treatments with only OH-Cbl was indicative of the important contribution of nonenzymatic reactions. Besides increasing the rate of CT transformation, addition of fructose and OH-Cbl to live cultures increased the percentage of [^{14}C]CT transformed to $^{14}CO_2$ (up to 31%) and ^{14}C-labeled soluble materials (principally L-lactate and acetate), while decreasing the percentage of CT reduced to chloroform and abiotically transformed to carbon disulfide. $^{14}CS_2$ represented more than 35% of the [^{14}C]CT in the presence of reduced medium and OH-Cbl. Conversion of CT to CO was a predominant pathway in formation of CO_2 in the presence of live cells and added fructose and OH-Cbl. These results indicate that the rate and distribution of products during cometabolic transformation of CT by *A. woodii* can be improved by the addition of fructose and OH-Cbl.

Metallocoenzymes like corrinoids, cytochrome P-450, and iron(II) porphyrins are known to play a major role in biotransformation and detoxification of carbon tetrachloride (CT) (22). The homoacetogen *Acetobacterium woodii* (1) is one of many organisms that transform CT at relatively high rates due in part to its use of the acetyl coenzyme A (CoA) pathway and correspondingly high levels of corrinoids (25). CT is transformed by fructose-grown *A. woodii* to mainly chloroform (CF), dichloromethane (DCM), and several soluble nonchlorinated products (9, 10, 24). Autoclaved *A. woodii* cultures also transform CT at an appreciable rate, perhaps indicating the heat stability of the enzymes involved. CT is also transformed by *Shewanella alga* BrY, an organism lacking the acetyl-CoA pathway (28). BrY reduces vitamin B_{12}, which subsequently transforms CT primarily to carbon monoxide (CO).

These and other studies suggest that the role of microorganisms in dechlorinating CT may be limited to reduction of corrinoids or other cofactors, which can be accomplished equally well with chemical reducing agents (5, 17). However, previous results with a mixed culture grown on DCM and amended with cyanocobalamin indicates that an active microbial population may play a bigger role in CT transformation than simply providing reducing power (2, 14). Addition of cyanocobalamin significantly increases the rate of CT transformation and decreases CF accumulation, in comparison to autoclaved culture or sulfide-reduced basal medium supplemented with B_{12}. Avoiding CF accumulation is a major concern with in situ bioremediation of CT.

The objective of this study was to determine the role of live *A. woodii* cells in CT transformation when provided with fructose and hydroxocobalamin (OH-Cbl). Based on results from a methanogenic enrichment culture (14), we hypothesized that a pure culture of *A. woodii* supplemented with fructose and OH-Cbl would transform CT faster than autoclaved cells with the same amount of OH-Cbl and faster than OH-Cbl present in sulfide-reduced basal medium. In addition, we expected the presence of live cells supplemented with fructose and OH-Cbl to shift the product distribution from CT away from CF and DCM and towards CO and CO_2. The results indicate that *A. woodii* recycles supplemental corrinoid to its reduced form, increases the rate of CT transformation to CO versus carbon disulfide (CS_2), and drives metabolism of CO to CO_2 and fermentation products.

(Some preliminary results of this study were presented at the 96th Annual Meeting of the American Society for Microbiology, New Orleans, La., 19 to 23 May 1996.)

MATERIALS AND METHODS

Chemicals. CT, CF, and DCM (all ≥99.9%) and acetate, lactate, fumarate, and isobutyrate were obtained from Aldrich Chemical Co.; CS_2 was from EM Industries, Inc. OH-Cbl (98%) and cyanocobalamin (99%) were purchased from Sigma Chemical Co. [^{14}C]CT was obtained from Dupont NEN Products and diluted to 1.9×10^7 dpm/ml with distilled deionized water (0.54 mM CT). The radiochemical purity of this [^{14}C]CT stock solution was evaluated to be 99.6% ± 0.11% (± 1 standard deviation for duplicate bottles) by using the procedures described under "Analysis of ^{14}C products." EcoScint (Baker Diagnostics, Inc.) was used as liquid scintillation cocktail for all ^{14}C radioactivity measurements.

Growth conditions. *A. woodii* (ATCC 29683) was grown on fructose in a basal medium described by Freedman and Gossett (11), modified as follows: the Fe^{2+} concentration was reduced to 1.2 mg/liter, and 10 ml of a vitamin solution per liter was added (27). Fructose was added as a sterile stock solution (2.8 or 25.2 mM).

A. woodii was grown in 160-ml serum bottles that were modified by connecting a 1-cm inside-diameter test tube at a right angle to the side of the bottles near the base, resulting in a final bottle volume of 173 ml. These modified serum bottles resemble culture flasks with a side arm (e.g., Bellco Biotechnology or Ace Glass), making it possible to monitor growth of *A. woodii* by optical density at 620 nm

* Corresponding author. Present address: Department of Civil and Environmental Engineering, A126 Research Complex—Engineering, Michigan State University, East Lansing, MI 48824. Phone: (517) 355-8241. Fax: (517) 355-0250. E-mail: hashsham@pilot.msu.edu.

(Bausch and Lomb Spectronic 20 spectrophotometer). A correlation was developed between optical density and dry weight. Cells were harvested by centrifugation and resuspended in the basal medium. The suspension was filtered through a glass fiber filter (Whatman, 21 mm), dried at 105°C overnight, and weighed. An optical density of 0.1 using a 1-cm light path corresponded to 45 mg of cells per liter.

Basal medium was distributed to serum bottles in an anaerobic glove box (Coy Laboratory products, Inc.). The headspace of the bottles was then purged with an N_2-CO_2 gas mixture (70%/30% [vol/vol]) to remove hydrogen and equilibrate the bicarbonate in the basal medium with CO_2 (resulting in a pH of 7.0). The serum bottles were sealed with slotted gray butyl rubber septa, covered with aluminum foil, and incubated at 35°C on a gyratory shaker table, with the liquid in contact with the septum.

Volatile organic product analyses. CT, CF, DCM, CH_4, and CS_2 were measured by gas chromatographic analysis (Perkin-Elmer model 9000) of 0.5-ml samples taken from the 65-ml headspace of the serum bottles. A Carbopack 1% SP-1000 column was used along with a flame ionization detector, as previously described (11). Although flame ionization is not the most suitable detector for measurement of CS_2, a linear response was obtained for concentrations less than 50 μmol per bottle. Detection limits (nanomoles per bottle) were 60 for CH_4, 20 for DCM, 100 for CS_2, 20 for CF, and 4 for CT. CO was analyzed isothermally (90°C) on a gas chromatograph (GOW-MAC Instruments) equipped with a thermal conductivity detector (150°C), a 3.2-mm by 2.44-m stainless steel 80/100 mesh, Molecular Sieve 5A column (GOW-MAC Instruments), and helium as the carrier gas (30 ml/min). The detection limit for CO was approximately 1 μmol per bottle.

All of the CT transformation products reported in this study are in micromoles per bottle. Aqueous phase concentrations of the volatile compounds can be obtained by using their Henry's constant (mol-m^{-3} gas concentration/mol-m^{-3} aqueous concentration) at 35°C, as reported previously (14), and the liquid and headspace volumes in the serum bottles. The percentages of each compound found in the aqueous phase (108 ml) were 4.8% for CH_4, 3.4% for CO, 93% for DCM, 56% for CS_2, 88% for CF, and 47% for CT.

CT transformation studies. Experiments were initiated by adding 2 ml of a log-phase culture of *A. woodii* growing on fructose to 106 ml of basal medium in serum bottles. Initially 2.8 mM fructose was added, to grow the culture to a dry cell mass of 12.2 ± 0.44 mg/bottle. During this period, the bottles were not shaken. After growth was complete (i.e., no further increase in optical density) by day 3, 11 treatments were set up, using duplicate serum bottles for each treatment. CT (100 μmol per bottle), additional fructose (25.2 mM), and OH-Cbl (108 nmol per bottle) was then added to appropriate bottles, which were then monitored for optical density and CT transformation products. Treatments 1 to 7 consisted of sulfide-reduced basal medium (2.1 mM sulfide) with combinations of live or autoclaved *A. woodii*, OH-Cbl, and fructose. Water controls (treatment 8) consisted of 160-ml serum bottles with 100 ml of autoclaved deionized water and 100 μmol of CT, to evaluate losses through the septum and measure the radiochemical purity of the [^{14}C]CT. Treatments 9 through 11 were included to check growth of *A. woodii* on fructose and/or OH-Cbl.

Analysis of ^{14}C products. [^{14}C]CT was added (approximately 1.9×10^6 dpm) along with unlabeled CT to bottles representing treatments 1 to 8 (see Table 1). Treatment 1 (live *A. woodii* culture plus [^{14}C]CT plus OH-Cbl plus fructose) included two identical sets; one analyzed for ^{14}C transformation products after 2.5 days and the other after 13 days (along with all of the other treatments) prior to analysis of ^{14}C products.

The amount of ^{14}C activity associated with volatile products was determined by gas chromatographic separation and combustion tube analysis of headspace samples (11). The efficiency of product recovery measured as trapped CO_2 after passing through the combustion tube was 95% ± 2.7% for CT, CF, and DCM and 98% ± 0.01% for methane. $^{14}CO_2$ was determined by stripping acidified samples with N_2 and trapping in NaOH, as previously described (11).

Analysis of ^{14}C nonvolatile products was carried out by acidifying a 20-ml portion of the liquid sample with HCl and sparging with N_2 for 30 min. ^{14}C not purged at acid pH corresponded to nonstrippable residue (NSR). ^{14}C-labeled NSR retained by a 0.22-μm-pore-size filter (Whatman, Inc.) was presumed to be cell-associated. ^{14}C-labeled NSR filtrate (i.e., soluble NSR) was either concentrated 50- to 100-fold in a vacuum centrifuge before high-performance liquid chromatography (HPLC) analysis or injected directly onto the HPLC (10 to 250 μl). Separation was achieved on a 300-mm-diameter HPX-87H ion exclusion column (Bio-Rad Laboratories) connected to a UV-visible light absorbance detector (model 486; Millipore Corp.) set at 210 nm. Three different mobile phase conditions (0.013 N H_2SO_4, 0.7 ml/min, 30°C; 0.013 N H_2SO_4 with 5% acetonitrile, 0.5 ml/min, 27°C; and 0.007 N H_2SO_4 with 10.8% acetonitrile, 0.5 ml/min, 27°C) were used in order to confirm the identity of the compounds by shifts in retention times. The presence of acetate and butyrate was also confirmed by gas chromatographic analysis (Hewlett Packard 5890A series II) with a 4-mm inside diameter and 183-cm-long glass column (GP 10% SP-1200–1% H_3PO_4 on 80/100 Chromosorb W AW; Supelco, Inc.).

To quantify which of the peaks from the HPX-87H column contained ^{14}C activity, fractions were collected in 0.5- to 1-min intervals, mixed with 15 ml of scintillation cocktail, and counted on a liquid scintillation counter. The identity of ^{14}C labeled L-lactate was also confirmed by its reactivity with lactate dehydrogenase. The average percent recovery during the HPLC analysis [Σ(^{14}C in all fractions)/^{14}C injected] was 89% ± 5.0%.

The recovery efficiency during the ^{14}C analyses was defined as the total disintegrations per minute recovered in all components (CT + CF + DCM + CS_2 CO + CO_2 + pyruvate + L-lactate + acetate + isobutyrate + unidentified soluble NSR + cell associated fraction) divided by the total disintegrations per minute present at the time of analysis. The total disintegrations per minute present in a bottle at the time of ^{14}C analysis was the sum of 0.5-ml headspace sample disintegrations per minute and 100-μl aqueous phase sample disintegrations per minute measured by direct injection into scintillation cocktail. For bottles with low CS_2 levels (i.e., ≤22%), the recovery based on ^{14}C remaining was 96% ± 3.7%; for bottles with high CS_2 levels, the recovery was 80.8% ± 1.2%. All ^{14}C transformation products are expressed as percent of disintegrations per minute initially injected without correcting for losses through the septum during the incubation period. CT transformation rates are obtained by dividing the difference of initial and final aqueous CT concentrations by the number of days it took for the transformation, adjusted for losses in the water controls.

RESULTS AND DISCUSSION

Rate enhancement due to OH-Cbl addition. The focus of this research was to determine if the rate of CT biotransformation by *A. woodii* can be enhanced by biochemical amendments, especially at high CT concentrations. OH-Cbl was selected for this purpose because cobalamins are one of the main coenzymes in the acetyl-CoA pathway implicated in the cometabolic transformation of CT by *A. woodii* (10), and they are also known to transform CT in abiotic systems (18).

Results presented in Fig. 1a demonstrate that adding OH-Cbl and fructose to *A. woodii* caused a 30-fold increase in the rate of CT biotransformation with respect to controls that did not receive additional OH-Cbl (26 mg/liter/day for treatment 1 versus 0.83 mg/liter/day for treatment 5). When fructose or *A. woodii* cells were omitted or *A. woodii* cells were killed (treatments 2, 3, and 4), the addition of OH-Cbl still increased the rate fivefold, indicating the importance of abiotic sulfide-mediated CT transformations. The difference in rate between the treatment that contained *A. woodii* with fructose and OH-Cbl and all others that received only OH-Cbl indicates that *A. woodii* cells needed fructose to take advantage of the added OH-Cbl for CT transformation. The rate of CT transformation by *A. woodii* receiving only fructose was similar to previously reported rates (0.8 to 1.7 mg/liter/day) by Stromeyer et al. (24) and Egli et al. (10). Biomass concentrations estimated from protein and optical density data in all three studies were comparable (300 to 500 mg/liter). The amount of OH-Cbl (1 μmol/bottle) added in this study was only 1% of the initial CT, but it substantially increased the total amount of corrinoids present. Based on a corrinoid content of 0.52 nmol/mg of dry cell mass (7) and a cell mass of 40 mg/bottle, the corrinoid contributed by *A. woodii* cells was only 2% of the added OH-Cbl.

When OH-Cbl was replaced with an equimolar amount of cyanocobalamin in the live culture with fructose, there was only a marginal enhancement in the rate of CT transformation over live cells alone (data not shown), perhaps due to the toxicity of cyanide to *A. woodii* (20). Cyanocobalamin was successful in improving the rate of CT and CF transformation, as reported previously in a DCM-grown enrichment culture (2, 14) as well as in a mixed culture (15).

Since biomass concentration typically has a significant effect on the rate of CT transformation, growth was also monitored in all treatments involving *A. woodii*. The presence of CT inhibited growth of *A. woodii* (Fig. 1b). This was expected, since the aqueous CT concentration used was much higher than in most previous studies (72 versus <2 mg/liter). However, once CT was consumed and most of the accumulated CF was also transformed, (treatment 1; Fig. 1a and c), *A. woodii* grew to a cell density that was slightly higher than the maximum cell density for the controls with no CT. The absence of

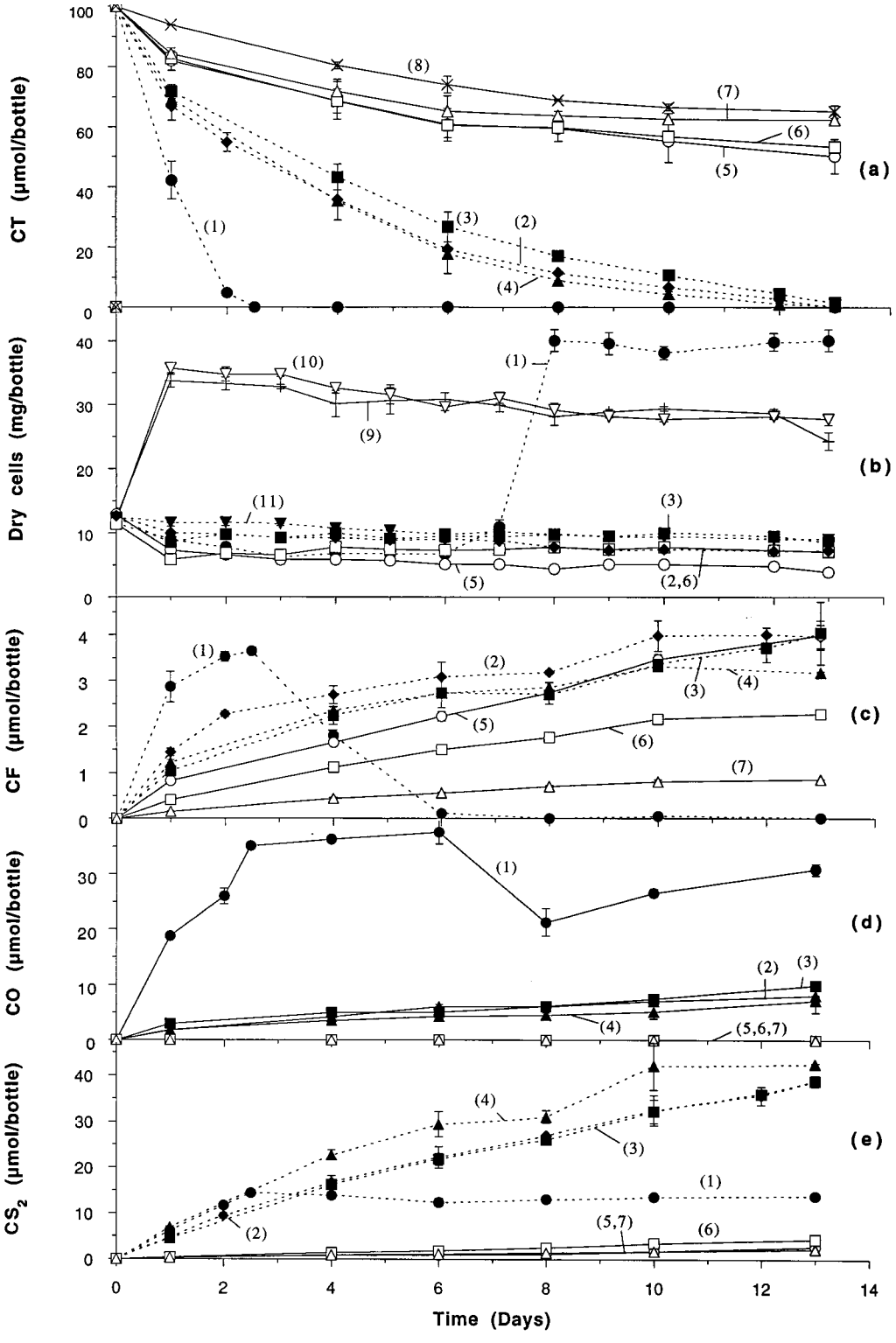

FIG. 1. Transformation of CT (a), cell growth (b), and accumulation of chloroform (c), CO (d), and CS_2 (e) by *A. woodii* and basal medium under various treatment conditions. 1, live *A. woodii* with CT, OH-Cbl, and fructose (●); 2, live *A. woodii* with CT and OH-Cbl but no fructose (◆); 3, autoclaved *A. woodii* with CT and OH-Cbl (■); 4, autoclaved medium with CT and OH-Cbl (▲); 5, live *A. woodii* with CT and fructose but no OH-Cbl (○); 6, autoclaved *A. woodii* with CT but no OH-Cbl (□); 7, autoclaved medium with CT but no OH-Cbl (△); 8, autoclaved water with CT but no OH-Cbl (×); 9, *A. woodii* seed control with fructose but no CT and no OH-Cbl (+); 10, *A. woodii* seed control with OH-Cbl and fructose but no CT (▽); and 11, *A. woodii* seed control with OH-Cbl but no fructose and no CT (▼). Vertical bars represent 1 standard deviation for duplicate bottles.

TABLE 1. Distribution of [^{14}C]CT transformation products

Product	% of injected dpm by treatment[a]								
	1[b]		2	3	4	5	6	7	8
	Live *A. woodii* + OH-Cbl (2.5 days)	Live *A. woodii* + OH-Cbl (13 days)	(live *A. woodii* + OH-Cbl, no fructose)	(autoclaved *A. woodii* + OH-Cbl)	(autoclaved medium + OH-Cbl)	(live *A. woodii*)	(autoclaved *A. woodii*)	(autoclaved medium)	(autoclaved water)
CT	0.62 ± 0.76	0	0	1.7 ± 0.41	0.52 ± 0.20	51 ± 0.29	48 ± 0.25	53 ± 0.28	65 ± 2.0
CF	3.4 ± 0.01	0	4.0 ± 0.29	4.1 ± 0.71	3.1 ± 0.21	4.1 ± 0.33	2.3 ± 0.04	0.88 ± 0.00	0
CS_2	18 ± 1.3	13 ± 0.57	31 ± 1.8	30 ± 0.02	35 ± 0.12	1.9 ± 0.17	3.5 ± 0.15	2.1 ± 0.14	0
CO	25 ± 1.9	0.15 ± 0.06	8.6 ± 0.15	10 ± 0.05	6.5 ± 0.32	0.31 ± 0.21	0.77 ± 0.02	0	0
CO_2	21 ± 0.44	31 ± 0.53	8.4 ± 0.38	5.0 ± 0.09	4.8 ± 0.64	2.4 ± 0.05	2.6 ± 0.23	1.6 ± 0.01	0.28 ± 0.06
Pyruvate	3.5 ± 0.09	0.98 ± 0.08	2.1 ± 0.19	1.5 ± 0.12	1.6 ± 0.14				
L-Lactate	2.5 ± 0.04	18 ± 0.31	1.8 ± 0.08	0	0				
Acetate	2.8 ± 0.13	6.5 ± 0.08	0.53 ± 0.08	1.9 ± 0.05	1.8 ± 0.11				
Isobutyrate	1.0 ± 0.03	3.6 ± 0.05	0.65 ± 0.05	0.93 ± 0.15	0.57 ± 0.05				
Other soluble NSR[c]	0.2 ± 0.04	1.3 ± 0.11	0.06 ± 0.02	0.31 ± 0.05	1.5 ± 0.07	2.1 ± 0.05	1.8 ± 0.15	1.1 ± 0.06	
Cell associated	4.3 ± 0.84	4.5 ± 0.28	2.2 ± 0.09	2.1 ± 0.04	1.4 ± 0.07	1.3 ± 0.08	0.95 ± 0.02	0.48 ± 0.05	

[a] Values represent the mean ± 1 standard deviation for duplicate bottles.
[b] Two sets in duplicate.
[c] For treatments 5 through 8, NSR was not analyzed for components. The soluble fraction of treatment 1 (live *A. woodii* plus OH-Cbl, 13 days) also included ethanol (2.3% ± 0.05%).

growth with fructose and OH-Cbl until nearly all of the CF was consumed (day 8, Fig. 1c) may be indicative of the activity of *A. woodii* towards toxicity reduction. There was no indication of OH-Cbl being used as a carbon source for growth in the presence or absence of fructose during the period of this study (Fig. 1b, treatments 9, 10, and 11).

Effect of OH-Cbl addition on volatile product distribution. The distribution of CT transformation products is significantly influenced by experimental conditions, including the type of reducing environment, organisms present, and concentration of CT and coenzymes (5, 12, 16, 17, 26). The major intermediates observed during CT transformation are shown in Fig. 2. Egli et al. (10) presented a similar diagram based on studies with *A. woodii*, although it did not include CO or CS_2. CO formation from CT is known to be catalyzed by corrinoids under reduced conditions. Further biotransformation of CO yields nonhazardous CO_2 and organic acids. CS_2 is produced from CT mainly under sulfide-mediated reducing conditions. Although it is a neurotoxin (13), its presence in drinking water is not currently regulated. CF is the most common undesirable transformation product of CT under various anaerobic conditions. DCM may also accumulate to some extent, via reduction of CF. Strategies that minimize accumulation of CF, DCM, and CS_2 are of interest for application of in situ bioremediation.

Addition of OH-Cbl promoted accumulation of CO (Fig. 1d, treatment 1). Without OH-Cbl, there was no detectable CO (below 1 μmol/bottle in treatments 5, 6, and 7). The increase in CO beyond day 8 corresponded to an increase in cells in bottles with *A. woodii*, OH-Cbl, and fructose (Fig. 1b), following depletion of the accumulated CF (Fig. 1c). Addition of OH-Cbl also promoted the accumulation of CS_2 (Fig. 1e). In the bottles with live culture plus OH-Cbl and fructose (treatment 1), the CS_2 peaked after 2.5 days and remained nearly constant thereafter, since all of the CT was consumed. CS_2 formation from CT has been observed in other microbial studies carried out in sulfide-reduced media (6, 14). However, neither Stromeyer et al. (24) nor Egli et al. (10) reported any CS_2 formation in their studies with *A. woodii* in a sulfide-reduced medium.

OH-Cbl addition also had a significant effect on accumulation of CF. In other studies with *A. woodii* (9, 10, 24) and several anaerobic pure cultures (4, 19) without supplemental cobalamins, CF and DCM were among the major metabolites that accumulated during CT transformation. In this study also, accumulation of CF occurred in all of the treatments that received CT, but to a lower extent when OH-Cbl and fructose were also present with *A. woodii*.

The fastest rate of CT transformation and the highest level of CO accumulation occurred with the fructose and OH-Cbl amended live *A. woodii* (treatment 1), with a correspondingly lower level of CS_2 (compared to treatments 2, 3, and 4) and CF accumulation. Thus, active metabolism of an electron donor by *A. woodii* in the presence of supplemental OH-Cbl shifted the transformation of CT in favor of CO and away from CS_2 and CF. The combination of live cells, metabolism of the electron donor, and OH-Cbl was necessary to affect this shift. CT transformation still occurred at a high rate with sulfide-reduced OH-Cbl, but in the absence of fructose and active cells, CS_2 became the predominant product.

Product distribution based on [^{14}C]CT. Use of [^{14}C]CT made it possible to determine the distribution of soluble products and CO_2 originating from CT and to confirm the distribution of volatile products obtained by gas chromatographic analysis of headspace samples (Fig. 1). Table 1 shows the distribution of products from ^{14}C[CT] for each of the treatment conditions. The amount of ^{14}C lost during incubation was proportional to the total amount of CT remaining, as indicated by CT losses from the water controls (treatment 8). The maximum percentage of $^{14}CO_2$ we observed when fructose and OH-Cbl were added to live *A. woodii* cells (31%) was significantly higher than those reported in other pure culture studies, including *A. woodii* grown on fructose (9, 10, 24). Although oxidation of ^{14}CO appears to be the principal mode of $^{14}CO_2$ formation in the presence of live cells, small amounts of $^{14}CO_2$ (<9%) did form with autoclaved cells and with basal medium alone (treatments 6 and 7); somewhat higher levels formed in the presence of supplemental OH-Cbl (treatments 3 and 4). How CT is converted to CO_2 in reduced medium without cells is not well established. One possibility is hydrolysis of CS_2 (17), which is favored at alkaline pH. With the OH-Cbl and fructose-supplemented active cultures, oxidation of CF may have contributed to CO_2 formation (2, 10). However, the extent of

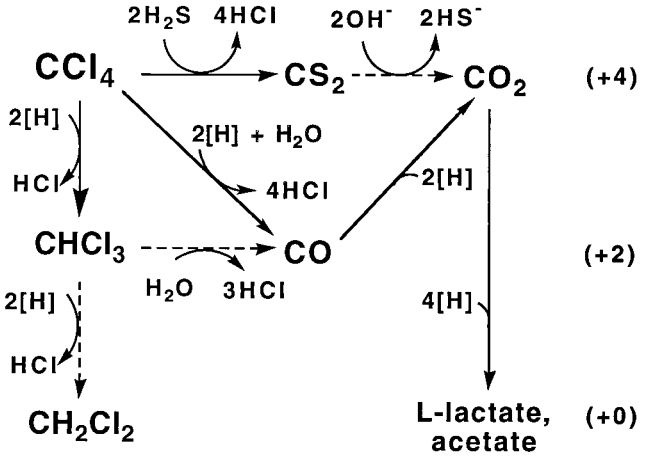

FIG. 2. Pathways for biotransformation of CT by *A. woodii* in sulfide-reduced basal medium. Solid arrows represent major processes observed in this study; dashed arrows represent pathways reported elsewhere, but were minor in this study. Further reduction of DCM to chloromethane is possible, but occurs at very slow rates. Numbers in parentheses represent oxidation states.

CF accumulation was small relative to direct conversion of CT to CO. It should be noted that although CO was detected in treatment 1 by gas chromatographic analysis on day 13 (Fig. 1d), virtually none of it was ^{14}C labeled at this point. The decrease in ^{14}CO between days 2.5 and 13 in treatment 1 was accompanied by increases in $^{14}CO_2$ and ^{14}C-labeled L-lactate, acetate, ethanol, and isobutyrate. This is consistent with involvement of the acetyl-CoA pathway in CT transformation as proposed by Egli et al. (10); i.e., oxidation of CO to CO_2 and formation of fermentation products. *A. woodii* typically produces 3 mol of acetate per mol of fructose under nonlimiting conditions, although formation of other compounds has been observed under stressed conditions (3). In this study, the stress was most likely due to CT and CF. Vitamin B_{12} and carbon monoxide dehydrogenase, two key enzymes in the acetyl-CoA pathway, are adversely affected by CT (8). This effect may have been partially mitigated by the addition of supplemental OH-Cbl.

The results of this study are in agreement with previous experiments that observed enhanced CT transformation by a methanogenic enrichment culture supplemented with cyanocobalamin (14). When provided with fructose and supplemental OH-Cbl, *A. woodii* plays a bigger role in CT transformation than simply supplying coenzymes that carry out nonenzymatic transformations. Metabolically active cells are needed to realize the highest transformation rates and most favorable product distribution. The mechanism by which this occurs needs further investigation. For example, it is not yet known if the added OH-Cbl acts extracellularly, (as shown with vitamin B_{12} and *Shewanella alga* BrY [28]), intracellularly, or by some combination of the two.

Supplemental addition of cobalamin along with an electron donor offers the prospect of accelerating the rate of in situ anaerobic transformation of CT in contaminated groundwater, as well as minimizing formation of hazardous metabolites like CF. The relatively high cost of cobalamins may be more than offset by a reduction in the time required for remediation, as well as minimization of hazardous products. One negative aspect of this approach is the formation of CS_2 in the presence of sulfides, although it can be removed under aerobic conditions, if necessary (23). Adsorption of cobalamin to aquifer material has also been found to be low, with a retardation factor of 2.1 in sand (15). This is in the same range as for CT (21), suggesting that distribution of cobalamin along with an electron donor throughout a contaminated area is a feasible process.

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