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Single-Carbon Catabolism in Acetogens: Analysis of Carbon Flow in *Acetobacterium woodii* and *Butyribacterium methylotrophicum* by Fermentation and ^{13}C Nuclear Magnetic Resonance Measurement

R. KERBY,[1] W. NIEMCZURA,[2] AND J. G. ZEIKUS[1]*

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706[1] and Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822[2]

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The catabolism of methanol, formate, or carbon monoxide to acetate or butyrate or both was examined in two acetogenic bacteria. *Butyribacterium methylotrophicum* simultaneously transformed methanol and formate mainly to butyrate with concomitant H_2 and CO_2 production and consumption. In contrast, methanol plus CO was primarily converted to acetate, and only slight amounts of CO_2 were produced. In vivo ^{13}C nuclear magnetic resonance analysis of [^{13}C]methanol transformation by *B. methylotrophicum* indicated that methanol was predominantly incorporated into the methyl of acetate, $^{13}CO_2$ was produced and then consumed, and butyrate was formed from the condensation of two acetate precursors. The analysis of the position of acetate labeled by a given ^{13}C single-carbon substrate when *B. methylotrophicum* or *Acetobacterium woodii* was grown in the presence of a second one-carbon substrate indicated two trends: when methanol was consumed, CO, CO_2, or formate predominantly labeled the acetate carboxyl; when CO was consumed, CO_2 and formate were principally funneled into the acetate methyl group, and CO remained a better carboxyl precursor. These data suggest a model of acetate synthesis via the combined operation of two readily reversible single-carbon pathways which are linked by CO_2.

Anaerobic acetate-producing bacteria are generally referred to as acetogens (20). Besides using saccharides, most of the known acetogens also ferment single-carbon compounds to acetate, and under certain conditions, some produce butyrate (14, 41) and caproate (14). Bacterial growth on single-carbon substrates as both carbon and energy sources has been termed unicarbonotrophy (40).

Most of the information on acetogen single-carbon transformations derives from analysis of the glucose catabolism of *Clostridium thermoaceticum* (20). In this anaerobe, approximately one of every three acetates originates from two pyruvate carboxyl moieties. One pyruvate carboxyl yields the methyl group of acetate via CO_2 and its subsequent reduction through free formate (2, 33), tetrahydrofolate (THF) derivatives (3, 24), and finally corrinoid one-carbon carriers (19, 37). The acetate carboxyl arises from the second pyruvate carboxyl group directly, without the involvement of free CO_2 (29).

Recent results of Drake et al. (12) and Hu et al. (15) indicate that CO also serves as an acetate carboxyl precursor in *C. thermoaceticum* cell extracts, and these workers purified several protein fractions which converted methyl-THF and CO or pyruvate to acetyl-CoA or acetyl phosphate (11, 12). One fraction included a nickel-containing carbon monoxide dehydrogenase (10) activity and catalyzed both the oxidation of CO to CO_2 and the exchange of CO with the carboxyl of acetyl-CoA.

Hu et al. (15) described a model of CO catabolism by acetogens based on the single-carbon transformations elucidated using glucose-grown *C. thermoaceticum*. In this model, CO is transformed through a formate derivative to the acetate carboxyl and methyl groups. The potential of this sequence of single-carbon transformations is supported by the recent finding of CO and H_2-CO_2 catabolism by *C. thermoaceticum* (16).

In addition to CO and H_2-CO_2, methanol and formate serve as substrates for some acetogens (40). *Butyribacterium rettgeri*, now speciated as *Eubacterium limosum* (23), was shown in early studies to convert CO_2 into both positions of acetate when grown on glucose (26), and later work by Hamlett and Blaylock (N. V. Hamlett

and B. A. Blaylock, Bacteriol. Proc., p. 149, 1969) indicated its utilization of methanol. These investigators hypothesized the occurrence of two acetate synthesis pathways in *B. rettgeri*, one catalyzing acetate synthesis from CO_2, and a second catalyzing acetate formation from methanol as a methyl precursor and formate or CO_2 as a carboxyl precursor. More recently, *E. limosum* has been shown to utilize H_2-CO_2, methanol-CO_2, and CO for growth (13, 14). Formate can replace CO_2 when the organism is grown on methanol (14).

Butyribacterium methylotrophicum has a one-carbon growth substrate range similar to that of *E. limosum* (21, 22, 41). Depending on the single-carbon substrate present, *B. methylotrophicum* forms either acetic acid, butyric acid, or a mixture of the two. For example, when grown on methanol and CO_2 in the presence of high acetate concentrations, approximately three butyric acids were formed per 10 methanols and two carbon dioxides consumed (22), whereas on H_2-CO_2 or carbon monoxide alone, acetic acid is the major product formed (21, 22). *Acetobacterium woodii* possesses a single-carbon substrate range similar to that of *B. methylotrophicum* and *E. limosum* but does not produce butyric acid (4, 5). All three organisms contain high levels of corrinoids (25, 31, 41).

B. methylotrophicum extracts display hydrogenase, CO dehydrogenase, and formate dehydrogenase activities. Curiously, the level of CO dehydrogenase in *B. methylotrophicum* was higher in cell extracts prepared from methanol than CO-grown cultures (21).

In this report, unicarbonotrophy in *B. methylotrophicum* and *A. woodii* is examined in an attempt to answer several questions. (i) How do carbon monoxide and formate influence the methanol metabolism of *B. methylotrophicum*? (ii) Are mixtures of single-carbon growth substrates simultaneously metabolized during the growth of acetogens? (iii) Are the various single-carbon compounds catabolized by a single system or by discrete mechanisms? (iv) What single-carbon intermediates link acetate carboxyl and methyl group synthesis? (v) How is unicarbonotrophy in these species related to the CO catabolism model proposed by Hu et al. (15)?

MATERIALS AND METHODS

Gases, chemicals, and isotopes. Medium components and other chemicals were reagent grade. The *dl*-THF (77%) used in formate determinations was purchased from Sigma Chemical Co. (St. Louis, Mo.) and was anaerobically dissolved (5 mg/ml) in 1 M β-mercaptoethanol at pH 7.0 in black-tape-wrapped sealed vials. Nitrogen (>99.998% pure), helium (>99.995% pure), CO (>99.99% pure), CO_2 (>99.8% pure), and H_2 (>99.9% pure) were purchased from Matheson Gas Co. (Joliet, Ill.). All gases except CO and CO_2 were passed through heated copper columns to remove O_2 before use. [^{13}C]methanol, $NaHCO_3$, C-1 acetate, and C-2 acetate (all 90% ^{13}C-enriched) were purchased from Stohler Isotope Chemicals (Waltham, Mass.). [^{13}C]sodium formate and CO (both also 90% ^{13}C-enriched) were purchased from K. O. R. Inc., Cambridge, Mass.

Organisms and growth conditions. *A. woodii* was obtained from N. Pfennig, University of Konstanz, West Germany, and was maintained on the medium described below containing 100 mM formate. A second stock culture of the organism was maintained on CO. Two strains of *B. methylotrophicum* were used, the Marburg strain (41) and a strain adapted to grow on 100% CO, the CO strain (21).

Both acetogenic species were cultured on phosphate-buffered (PB) medium containing (per 950 ml of distilled water): NaCl, 0.45 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; NH_4Cl, 1.0 g; yeast extract (Difco Laboratories, Detroit, Mich.), 0.5 g; trace mineral solution (38), 10 ml; vitamin solution (38), 5 ml; resazurin (0.2% solution), 1 ml. The pH was adjusted to 7.2 to 7.4 with NaOH, and the medium was heated to boiling while being gassed with either N_2 or He (if CO was to be measured). The cooled medium was dispensed into serum bottles (total stoppered volume, 158 ml; Wheaton Scientific, Millville, N.J.) or pressure tubes (total stoppered volume, 26.4 ml; Bellco Glass, Inc., Vineland, N.J.) under constant gassing as described previously (21). When CO was to be a substrate, the vessel headspace was flushed in a hood with CO for several minutes and the vessels were inverted during autoclaving to prevent the loss of CO through the black butyl rubber bung. Sterilized solutions of phosphate buffer (15% KH_2PO_4, 29% $K_2HPO_4 \cdot 3H_2O$ [pH 7.0]) and 2.5% sodium sulfide were added (0.25 ml each) per 10 ml of autoclaved medium, using disposable syringes.

For growth curves of *B. methylotrophicum* on methanol-acetate-formate, each serum bottle contained 60 ml of PB medium under N_2. After the addition of phosphate and sodium sulfide, 1.5 ml of sterile 4 M methanol–1 M sodium acetate solution and 1.5 ml of sterile 1 M sodium formate solution were added. When the substrates were methanol-acetate-CO, 40 ml of PB medium was autoclaved in each CO (100%, 1 atmosphere [ca. 101.3 kPa] at 25°C)-containing serum bottle, and 1 ml each of phosphate buffer, sodium sulfide, and methanol-acetate mixture was added. Cultures were transferred twice on medium identical to that used in the experiment before inoculation with a 1% inoculum. Inoculated tubes and bottles were incubated horizontally with shaking (160 rpm) at 37°C for *B. methylotrophicum*. *A woodii* was grown at 30°C, but with the vessels arranged vertically to allow a more gentle swirling motion. The growth of *A. woodii* was inihibited by constant vigorous shaking, an effect previously reported (5).

Quantification of fermentation substrate-product transformations and growth. During growth curves, 0.8-ml liquid samples were aseptically withdrawn with sterile N_2- or He-flushed 1-ml syringes, and the absorbance at 660 nm (A_{660}) and pH were determined. Samples were frozen until analyzed for soluble metabolites. For methanol analysis, additional liquid samples (0.5 ml) were removed and frozen immediately at −70°C to prevent substrate vaporization. Gas samples

(0.4 ml) were withdrawn using glass syringes with pressure-lock valves (Mininert; Anspec Co., Ann Arbor, Mich.).

Hydrogen was measured on a Packard 800 gas chromatograph equipped with a thermal conductivity detector and a stainless steel column (0.32 by 182.9 cm) packed with Poropak N (80/100 mesh) (Waters Associates, Inc., Milford, Mass.). The column was operated at room temperature with a N_2 carrier gas flow rate of 30 cm^3/min. CO and CO_2 were measured as previously described (21). The total amounts of gas (i.e., headspace, dissolved, and ionized) were determined using Bunsen coefficients (1), Henry's Law, and a knowledge of the influence of the medium pH on the amount of ionized CO_2. Acetate and butyrate were measured in acidified samples by gas chromatography, using a flame ionization detector coupled to a glass column (182.9 cm by 2 mm [inside diameter]) containing Chromosorb 101 (80/100 mesh) (Supelco Inc., Bellefonte, Pa.). Nitrogen carrier gas passed through the column at 36 cm^3/min. The column, detector, and injector temperatures were 155, 240, and 220°C, respectively. Methanol was determined either with this gas chromatograph or another instrument equipped with a glass column (182.9 cm by 2 mm [inside diameter]) containing Super Q packing (80/100 mesh) (Alltech Associates, Inc., Deerfield, Ill.) connected to a flame ionization detector. Helium carrier gas flowed through the column at 100 cm^3/min, and the column, detector, and injection port temperatures were 170, 220, and 200°C, respectively.

Formate was measured using the formyl-THF synthetase activity of *A. woodii* cell extracts (27). A standard curve was prepared each time the assay was performed and was linear from 0 to 300 μM formate. The results were measured spectrophotometrically at 350 nm, and calculations based on the standard curve deviated from results obtained using the extinction coefficient for methenyl-THF by less than 6%.

The initial liquid volumes of 66.5 ml (when methanol and formate were substrates) and 43.5 ml (when methanol and CO were substrates) were used in calculating the amounts of nongaseous substrates and products from their measured concentrations. Thus, decreases in the amounts of soluble substrates do not reflect the decreasing vessel liquid volumes over the time course. The amounts of gases, however, reflect the headspace and liquid volumes present at a given time. At the end of each fermentation time course, 10-ml samples were filtered through 0.4-μm polycarbonate membrane filters (Bio-Rad Laboratories, Richmond, Calif.), rinsed with 10 ml of phosphate buffer solution (pH 7.0), and dried to constant weight over $CaSO_4$ at 60°C. The micromoles of cell C were calculated by multiplying the cell yield by the percent carbon (0.451 μg of C/μg of cells) (21) and the molar conversion factor (1 μmol of C/12 μg of C). The fermentation balances are based on the initial and final substrate-per-product amounts. These balances indicate the completeness of the substrate and product analysis, but may not accurately reflect growth yields since the relative amounts, and even types, of substrates changed in these batch cultures during fermentation (18, 32).

NMR methods. To determine ^{13}C-label fractionation at the end of a fermentation, both *A. woodii* and *B. methylotrophicum* were grown in pressure tubes on PB medium under either He or N_2 headspaces. Substrate solutions (2 M methanol, 0.5 M $NaHCO_3$, 2 M sodium formate) were prepared with either ^{12}C- or ^{13}C-labeled compounds and sterilized by autoclaving. When CO was a substrate, 500 μmol of ^{12}CO or ^{13}CO was injected before autoclaving the (inverted) tubes. *B. methylotrophicum* cultures contained 26 μmol of [^{12}C]acetate when methanol was a substrate. Sterile phosphate buffer and Na_2S were added to the medium as indicated above along with substrates in the following amounts: methanol, 440 μmol; formate, 500 μmol when in combination with another substrate, 1,000 μmol when the sole substrate; bicarbonate, 220 μmol when methanol was a cosubstrate and (if added) 450 μmol when CO was the substrate. The final medium volume was approximately 11 ml/tube. When a large amount of ^{12}C-labeled gas (CO or CO_2) was used in a dilution experiment, the incubation was done in the 158-ml sealed serum bottles with 5,400 μmol of gas in the headspace and a 22-ml liquid volume with substrate concentrations equivalent to those used in the tube experiments. The inoculated tubes and bottles were incubated as described above.

After growth of the organisms (*B. methylotrophicum* A_{660} = 0.6 to 1.0; *A. woodii* A_{660} = 0.2 to 0.4), the tubes were cooled, the cells were pelleted, and the decanted broth was made basic (pH approximately 10) with NaOH and evaporated to dryness under an air stream. The resulting salts were suspended in 2 ml of double-distilled H_2O and 1 ml of D_2O (Aldrich Chemical Co., Milwaukee, Wis.). The samples were placed in 12-mm (outside diameter) nuclear magnetic resonance (NMR) tubes (Wilmad Glass Co., Inc., Buena, N.J.), and 360 to 460 proton-decoupled ^{13}C spectra accumulated in the Fourier transformation mode on a Nicolet NT-200 spectrometer at 50.3 MHz. The spectrum width was ±5,620 Hz, using quadrature detection with a pulse duration of 4 μs (approximately 30° tip angle) and a 4- or 5-s relaxation delay between pulses. The spectra were plotted, and the peaks were integrated. Peak identities were determined by comparing chemical shifts with those of reference compounds (CH_3OH, $NaHCO_3$, sodium formate, C-1 sodium acetate, and C-2 sodium acetate) determined under similar conditions and by comparison of spectra with published values (7).

Two factors presented difficulties in the use of ^{13}C NMR spectra to compare the amounts of label incorporated at each position in a molecule. With acetate, a combination of a carboxyl group relaxation time longer than that of the methyl group and the enhancement of the methyl group signal by the nuclear Overhauser effect (17) resulted in a stronger signal from a ^{13}C-methyl group than from a ^{13}C-carboxyl group. Using a short pulse width and long delays minimized the intensity distortions of the carboxyl group. Furthermore, the use of highly ^{13}C-enriched substrates ensured that a portion of the acetate was labeled in both carbons with the resulting ^{13}C spin-spin coupling causing a splitting of the carboxyl and methyl signals. By measuring the areas of the carboxyl and methyl signals of [1,2-^{13}C]acetate (which ideally would be equal), correction factors for each spectrum indicating the intensity bias were determined. These factors ranged from 1.04 to 1.78. Then the carboxyl signals of [1,2-^{13}C]acetate and [1-^{13}C]acetate in a given spectrum were augmented by multiplication of the signal area by the correction factor and the relative amount of ^{13}C at

each carbon position determined.

As an internal control, the areas of the signal of the C-3 and C-4 of butyrate were measured when butyrate was synthesized. Although complex splitting patterns prevented an analysis of the four butyrate carbons comparable to that performed for acetate, the ratio of the butyrate C-3 to C-4 signal areas closely correlated with the ratio of the acetate C-1 (corrected) to C-2 signal areas (data not shown).

For the in vivo $^{13}CH_3OH$ consumption experiment, two serum bottles containing 50 ml of *B. methylotrophicum* culture were harvested in mid-log phase (A_{660} = 0.58 and 0.66) by centrifugation in a Sorvall GSA rotor for 25 min at 1,500 × *g*. The pelleted cells were carefully transferred, on ice, to an anaerobic glove bag (Coy Laboratory Products, Ann Arbor, Mich.). The supernatant of each bottle was removed, and each pellet was suspended in prereduced PB medium (final total volume, 21 ml) containing 10 mM sodium acetate and 25 mM $NaHCO_3$. This suspension was transferred to an anaerobic flat-bottom 20-mm (outside diameter) NMR tube, which was stoppered and placed on ice for 4 h.

When the spectrometer was ready, 39 μl of $^{13}CH_3OH$ (to 40 mM) was injected into the cell suspension. The tube was placed in a probe maintained at 37°C. No internal lock was used, nor was the sample spun. Proton-decoupled spectra were serially obtained in the FT mode in sets of 1,000 accumulations. A 10-μs pulse width (approximately 35° flip angle), 1.3-s pulse interval, and ±8772-Hz spectrum width (quadrature detection) were used with a total acquisition time per set of 23.7 min. The tube was removed from the probe after the second and fourth sets of accumulations and shaken before being reinserted.

RESULTS

Fermentation time courses illustrating the influence of CO and formate on the methanol metabolism of *B. methylotrophicum* are shown in Fig. 1 and 2. Both formate and CO served as cosubstrates and were simultaneously consumed with methanol. When methanol and formate were substrates, both hydrogen and carbon

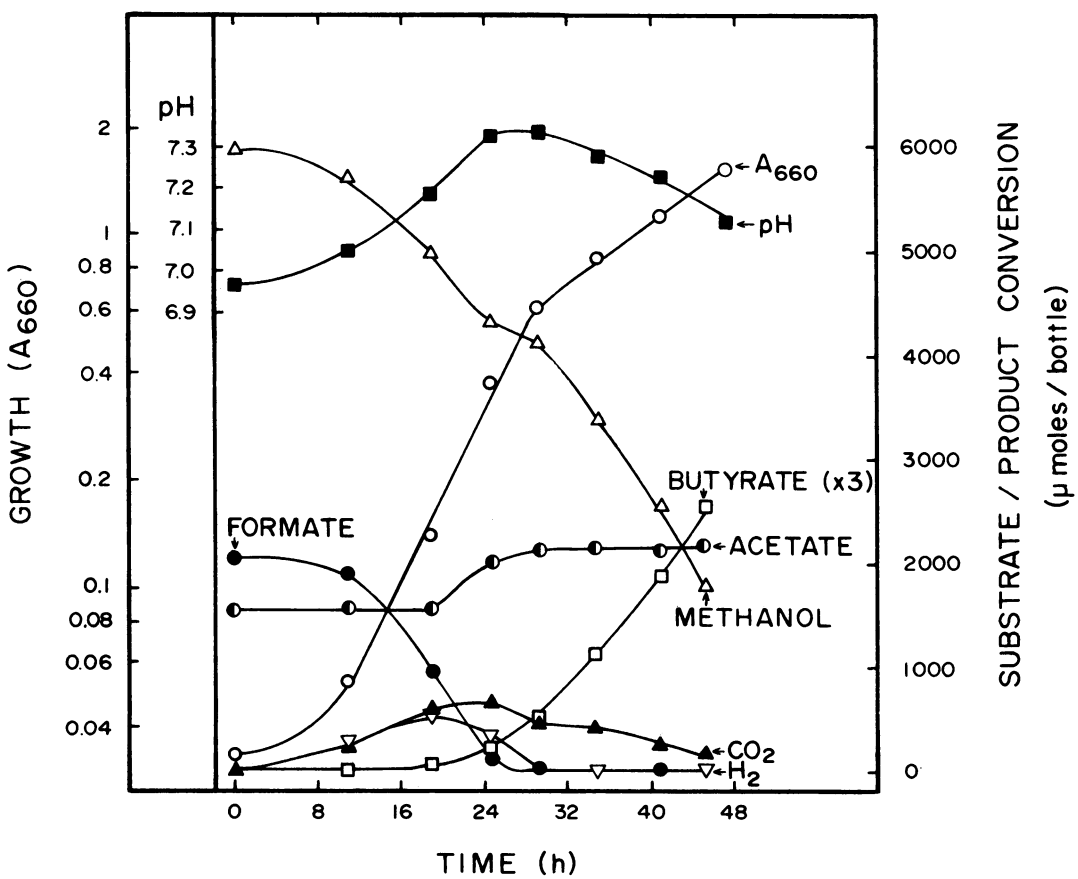

FIG. 1. Simultaneous fermentation of methanol and formate by *B. methylotrophicum*. *B. methylotrophicum* (Marburg strain) was grown with shaking at 37°C in 158-ml serum bottles containing a N_2 headspace and 66.5 ml of PB medium and additions. The plotted results represent the averages of four separate bottles, and the numbers in parentheses are the factors by which the data shown on the *y*-axis should be divided to give the actual results. For example, the amount of butyrate present at 29 h was 510/3 or 170 μmol/bottle.

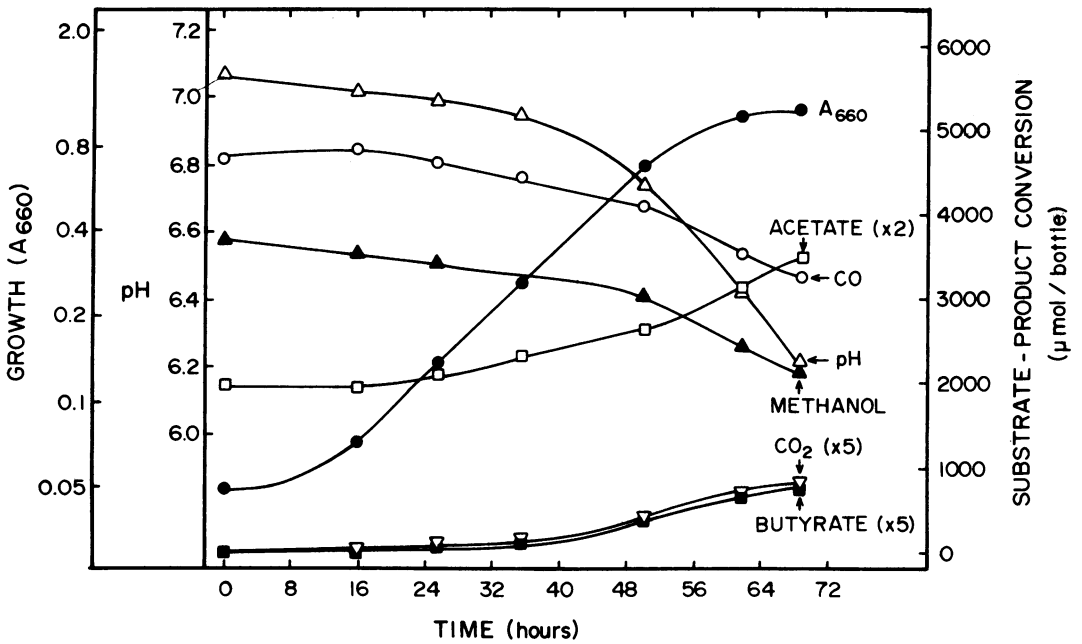

FIG. 2. Simultaneous fermentation of methanol and carbon monoxide by *B. methylotrophicum*. The organism was grown with shaking at 37°C in 158-ml serum bottles containing a CO (100%) headspace and 43.5 ml of PB medium and additions. The plotted results represent the averages of four separate bottles, and the numbers in parentheses are the factors by which the data shown on the *y*-axis should be divided to give the actual results.

dioxide were produced and then consumed (Fig. 1). Complete hydrogen consumption coincided with formate depletion, whereas net CO_2 consumption occurred at a slower rate. The pH initially increased to 7.34, a result of sodium formate utilization, and then decreased to pH 7.12. Rapid growth occurred while formate was being consumed, but the doubling time of 5 h increased threefold upon formate depletion. The fermentation balance calculated was (in micromoles per vessel):

$$4{,}200CH_3OH + 2{,}056HCOOH \rightarrow 854 \text{ butyrate} + 643 \text{ acetate} + 162CO_2 + 2H_2 + 1{,}031 \text{ cell C}$$

This reaction stoichiometry accounted for carbon and electron recoveries of 94 and 91%, respectively.

The fermentation of methanol and carbon monoxide by *B. methylotrophicum* is illustrated in Fig. 2. The initial transfer of the Marburg strain into medium vessels containing 1 atmosphere (ca. 101.3 kPa) of 100% CO was marked by an extended lag before the onset of growth. Subsequent transfers readily demonstrated growth under these conditions. Notably, acetate synthesis predominated, and only minor amounts of butyrate were formed. Also, continuous CO_2 synthesis was observed instead of the net production and consumption fluxes seen on CH_3OH and formate. Formate and hydrogen were detected throughout the time course, but neither exceeded 2 μmol/bottle during growth. The doubling time during log growth remained constant at 11 h. Methanol and CO were consumed in nearly equimolar amounts, and the calculated fermentation balance (in micromoles per vessel) was:

$$1{,}573CH_3OH + 1{,}417CO \rightarrow 164CO_2 + 153 \text{ butyrate} + 747 \text{ acetate} + 515 \text{ cell C}$$

This reaction stoichiometry accounted for carbon and electron recoveries of 93 and 91%, respectively.

^{13}C NMR studies were initiated to examine the route of methanol transformation to acetate and butyrate. Figure 3 illustrates the time-dependent substrate-to-product conversions observed when whole cells were incubated with [^{13}C]methanol and subjected to in vivo NMR monitoring. By the end of the first spectrum, methanol (49.3 ppm) consumption had started, and the acetate methyl group (23.3 ppm) and bicarbonate (160.1 ppm) were slightly labeled. The acetate methyl and the bicarbonate were even more strongly labeled in the second spectrum, and the label was beginning to appear in the C-4 (13.3 ppm) and C-2 (39.6 ppm) of butyrate, consistent with their precursor being the acetate methyl group. In the third, fourth, and fifth spectra, methanol was further consumed,

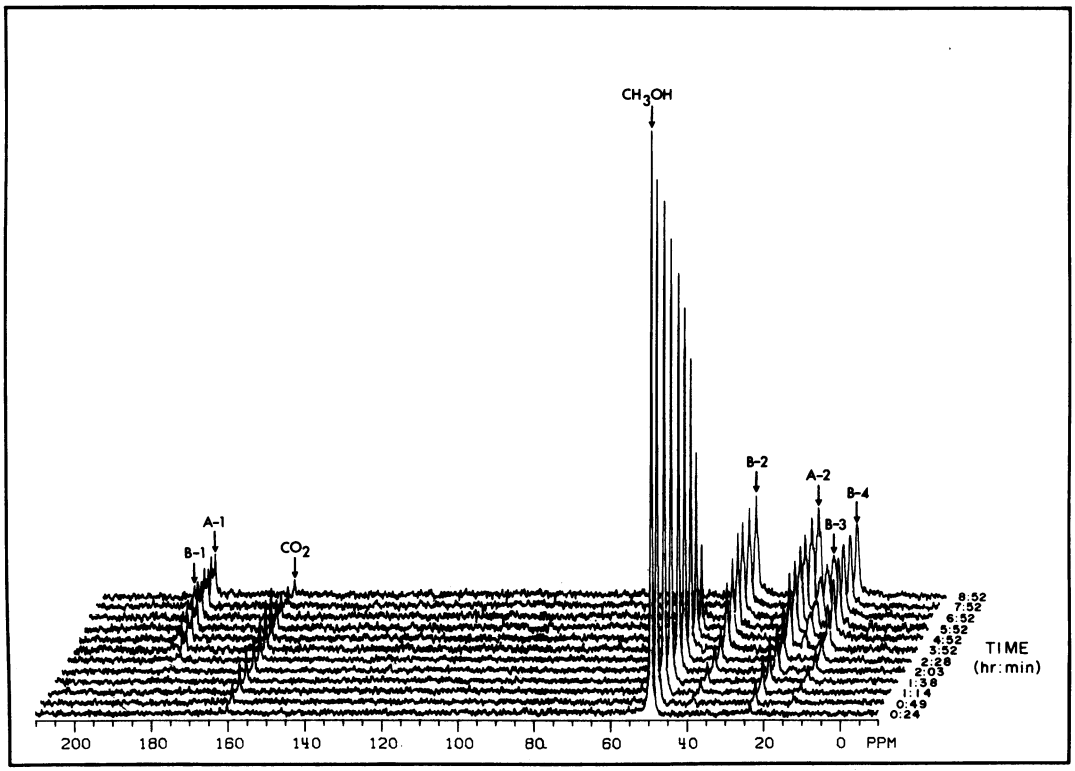

FIG. 3. In vivo monitoring of $^{13}CH_3OH$ utilization by a cell suspension of *B. methylotrophicum*. Each spectrum represents 23.7 min of accumulation and is the average of 1,000 scans. CO_2 and CH_3OH are indicated; A-1 and A-2 correspond to the C-1 and C-2 of acetate; and B-1, B-2, B-3, and B-4 correspond to the C-1, C-2, C-3, and C-4 of butyrate. The *x*-axis is the chemical shift (relative to A-2 at 23.3 ppm) in parts per million, and the completion time of each set of accumulations is noted adjacent to the spectrum.

and the product signals present in the first two spectra progressively increased. Only in the sixth spectrum were the acetate carboxyl (180.2 ppm) and butyrate C-3 (19.2 ppm) labeled. By the seventh spectrum, the butyrate C-1 position was labeled (183.8 ppm). The signals of each position increased in the subsequent spectra with the exception of methanol and CO_2, both of which decreased. A careful inspection of Fig. 3 shows a net production followed by a net consumption of $^{13}CO_2$. Methanol was completely consumed, and a final spectrum (4,000 accumulations [data not shown]) obtained 12 h after the initiation of the experiment revealed only previously identified signals. As determined in a separate experiment, the signal arising from [^{13}C]sodium formate occurred at 170.1 ppm, easily differentiated from that of the bicarbonate (160.1 ppm) and carbonate (168.2 ppm) anions.

The amounts of ^{13}C label incorporated into the methyl and carboxyl positions of acetate after the growth of *B. methylotrophicum* and *A. woodii* on various single-carbon substrates were also determined. Table 1 presents the results of methanol fermentation by *B. methylotrophicum*. With methanol and carbon dioxide as substrates, the ^{13}C of methanol was predominantly incorporated into the methyl of acetate, whereas $^{13}CO_2$ was predominantly incorporated into the acetate carboxyl. When excess $^{13}CO_2$ was present, label from methanol was exclusively found in the acetate methyl group. In the control experiments, where both substrates were labeled, 85% of the acetate produced was labeled in both carbons, with the remaining fraction of ^{13}C divided between the methyl and carboxyl groups of singly labeled acetate. That doubly labeled acetate was not the sole product was not surprising since each substrate was enriched to 90% ^{13}C, giving a 0.81 (0.9 × 0.9) possibility for the labeling of both acetate carbons.

With methanol and carbon monoxide as cosubstrates, methanol was again preferentially incorporated into the acetate methyl group (Table 1). ^{13}CO predominantly labeled the acetate carboxyl. Excess CO, like excess CO_2, prevented the transfer of methanol label into the acetate carboxyl group. A similar pattern was found with alternately labeled methanol and formate: methanol was mainly incorporated into the ace-

TABLE 1. Influence of single-carbon substrates on the distribution of ^{13}C atoms found in acetate after growth of *B. methylotrophicum* on methanol[a]

Substrate label position	Fractional distribution of ^{13}C atoms in acetate				
	$^{13}CH_3$-COOH	CH_3-^{13}COOH	$^{13}CH_3$-^{13}COOH	$\Sigma^{13}CH_3$-	Σ-^{13}COOH
$^{13}CH_3OH$-CO_2	0.38	0.08	0.54	0.64	0.36
CH_3OH-$^{13}CO_2$	0.10	0.71	0.20	0.19	0.81
$^{13}CH_3OH$-$^{13}CO_2$	0.07	0.08	0.85	0.50	0.50
$^{13}CH_3OH$-CO_2 (excess)	1.0	0	0	1.0	0
$^{13}CH_3OH$-CO	0.64	0.07	0.29	0.78	0.22
CH_3OH-^{13}CO	0.08	0.56	0.35	0.26	0.74
$^{13}CH_3OH$-^{13}CO	0.06	0.08	0.86	0.49	0.51
$^{13}CH_3OH$-CO (excess)	1.0	0	0	1.0	0
$^{13}CH_3OH$-HCOOH	0.48	0.08	0.44	0.70	0.30
CH_3OH-H^{13}COOH	0.08	0.54	0.39	0.28	0.74
$^{13}CH_3OH$-H^{13}COOH	0.07	0.08	0.84	0.49	0.50

[a] The Marburg strain was grown at 37°C with shaking in 26.4-ml anaerobic tubes containing 11 ml of PB medium. When excess CO_2 or CO was used, the experiments were performed in 158-ml serum bottles containing 22 ml of medium. The standard deviation of acetate label distribution was ≤0.05 with the exception of the results for $^{13}CH_3OH$-CO, where standard deviations of ±0.11 and ±0.12 were found for $^{13}CH_3$-COOH and $^{13}CH_3$-^{13}COOH, respectively. Each determination was performed in duplicate or in triplicate.

tate methyl group, and formate was mainly incorporated into the carboxyl moiety (Table 1).

Table 2 compares the ^{13}C single-carbon transformations into acetate when *A. woodii* and *B. methylotrophicum* fermented CO or formate. These experiments were performed because formate, CO, and CO_2 were all similarly incorporated into acetate when methanol was catabolized. Each organism was adapted to grow on carbon monoxide by repeated transfers on PB medium with 100% CO in the headspace before the initiation of these experiments. Curiously, in both organisms the more highly oxidized one-carbon compound (i.e., CO_2) was predominantly incorporated into the acetate methyl position, whereas CO was preferentially incorporated into the acetate carboxyl position. *A. woodii* also catabolized CO and formate differentially (Table 2). Carbon monoxide was preferentially incorporated into the acetate carboxyl, whereas formate was a better methyl precursor.

DISCUSSION

The data presented on single-carbon fermentations and ^{13}C incorporation patterns support the catabolic carbon flow model shown in Fig. 4. This schematic model predicts several interesting points about the one-carbon metabolism of acetogens. First, single-carbon substrates enter

TABLE 2. Influence of single-carbon substrates on the distribution of ^{13}C atoms found in acetate after growth of acetogenic bacteria on carbon monoxide or formate[a]

Organism, substrate label position	Fractional distribution of ^{13}C atoms in acetate				
	$^{13}CH_3$-COOH	CH_3-^{13}COOH	$^{13}CH_3$-^{13}COOH	$\Sigma^{13}CH_3$-	Σ-^{13}COOH
B. methylotrophicum					
^{13}CO	0.06	0.10	0.84	0.48	0.52
^{13}CO-CO_2	0.14	0.46	0.41	0.34	0.66
CO-$^{13}CO_2$	0.48	0.16	0.36	0.66	0.34
^{13}CO-$^{13}CO_2$	0.04	0.07	0.88	0.48	0.51
A. woodii					
^{13}CO	0.05	0.07	0.88	0.49	0.51
^{13}CO-CO_2	0.14	0.47	0.39	0.34	0.67
CO-$^{13}CO_2$	0.54	0.14	0.33	0.71	0.31
^{13}CO-$^{13}CO_2$	0.06	0.05	0.89	0.51	0.50
^{13}CO-HCOOH	0.16	0.50	0.35	0.33	0.67
CO-H^{13}COOH	0.60	0.08	0.32	0.76	0.24
^{13}CO-H^{13}COOH	0.06	0.06	0.88	0.50	0.50
H^{13}COOH	0.07	0.07	0.86	0.50	0.50

[a] The procedures used were the same as those described in Table 1, footnote *a*, except that *A. woodii* was grown at 30°C. A CO-adapted strain of each organism was used. The standard deviation of acetate label distribution was ≤0.06 with the exception of the results for CO-$^{13}CO_2$, with *B. methylotrophicum*, where a standard deviation of ±0.08 for $^{13}CH_3$-COOH was found, and those for ^{13}CO, CO-$^{13}CO_2$, and ^{13}CO-$^{13}CO_2$ with *A. woodii*, where a single determination was performed for each condition.

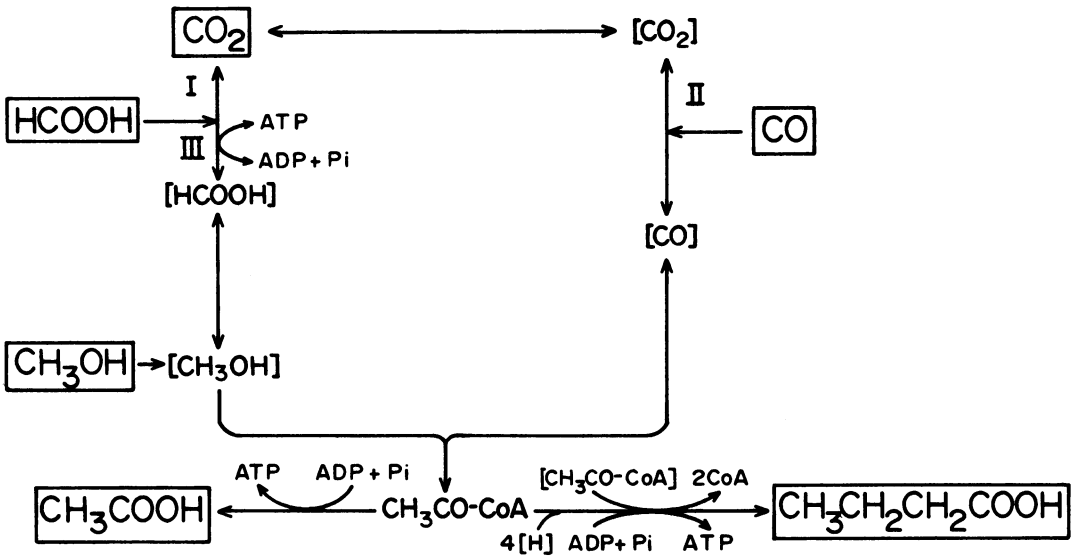

FIG. 4. Single-carbon catabolism flow model proposed for acetogenic bacteria that synthesize acetate or butyrate from single-carbon compounds. Acetyl-CoA is the direct precursor for acetic or butyric acid. [CH_3OH] and [CO] represent the immediate methyl and carbonyl precursors for acetyl-CoA synthesis and are precursors for the synthesis of CO_2. This scheme predicts that two distinct formyl-level intermediates, [HCOOH] and [CO], are linked by formate, CO_2, and a carboxyl intermediate [CO_2]. The Roman numerals indicate the following enzymatic activities: I, formate dehydrogenase; II, CO dehydrogenase; and III, formyl-THF synthetase.

a common metabolic route at distinct points and are differentially transformed to acetyl-CoA. Although the reduction state of a substrate would not necessarily correlate with its distribution into the acetate product (e.g., CO_2 is a better methyl precursor than CO), the site of entry of the substrates would. Second, single-carbon catabolism is regulated such that mixtures of single-carbon substrates can be simultaneously transformed. Third, the entry of substrates on the methyl side of the pathway (i.e., methanol, formate) would require CO_2 as an intermediate in acetate synthesis, whereas an alternative supply of carboxyl carbons (e.g., CO) would eliminate the need for CO_2. Fourth, the model supports the need for formate dehydrogenase, CO dehydrogenase, and formyl-THF synthetase activities in single-carbon catabolism.

The model presented in Fig. 4 agrees, for the most part, with the theoretical scheme depicting the catabolism of CO by acetogens as presented by Hu et al. (15) and extends their scheme by including routes of methanol, formate, and CO_2 catabolism. The schemes differ, however, in the mechanism of interconversion between the two formyl-oxidation level intermediates. In their model, conversion between the acetate carboxyl precursor ([HCOOH]) and the acetate methyl precursor (formyl-THF) occurs directly. As they (15) and others (12, 21) have suggested, direct conversion during CO catabolism would be energetically advantageous since the ATP-utilizing (28) transformation of formate to formyl-THF could be circumvented. According to our model, the conversions between the two formyl-oxidation level intermediates ([CO] and [HCOOH] as the acetate carboxyl and methyl group precursors, respectively) proceed via formate, CO_2, and a carboxyl moiety [CO_2]. The inclusion of [CO_2] is in accordance with the role of the pyruvate carboxyl group in glucose catabolism by *C. thermoaceticum* (29).

NMR spectroscopy (30) is a powerful tool for analyzing the incorporation routes of single-carbon substrates into acetate because the technique allows the determination of the proportions of [1,2-^{13}C]acetate, [1-^{13}C]acetate, and [2-^{13}C]acetate. Procedures using ^{14}C indicate label position but not its configuration. Wood (39) used mass spectroscopy to analyze acetate synthesis by *C. thermoaceticum* for this reason.

When methanol is one of the substrates, the cosubstrate predominantly passes to the acetate carboxyl group, whereas methanol most strongly labels the methyl group. Hamlett and Blaylock (Hamlett and Blaylock, Bacteriol. Proc., p. 149, 1969) reported a similar result, using [^{14}C]methanol and *B. rettgeri*. That some methanol label appears in the carboxyl group and some cosubstrate label appears in the methyl group indicates linkage of the methyl and carboxyl group synthesis mechanisms.

The existence of a single formyl (or any more reduced)-level intermediate linking methyl and carboxyl group synthesis seems unlikely. In *A. woodii*, formate is a better methyl group precursor, whereas CO is the better carboxyl precursor. A single common intermediate would cause randomization and equal dispersion of label between the acetate positions, as occurs in *Clostridium acidiurici* in which methylene-THF is the precursor of both acetate carbons (36). Although *A. woodii* can catabolize formate (4; Table 2) or CO individually (B. R. S. Genthner, announced at the Annual Meeting of the American Society for Microbiology, 1982, Atlanta, Ga.; Table 2), the sequential use of these two substrates is precluded by the presence of singly labeled acetate: sequential use would result solely in doubly labeled acetate formation followed by unlabeled acetate formation, or vice versa, depending on the label source and the order of utilization. Likewise, the dissimilar utilization of CO and CO_2 by *A. woodii* and *B. methylotrophicum* (Table 2) is consistent with the bifurcated acetate synthesis model presented here, and these data suggest that both *B. methylotrophicum* and *A. woodii* transform CO in an analogous manner. Thus, it is proposed that both organisms have a similar biochemical mechanism for acetyl-CoA synthesis from single-carbon substrates that is probably common to acetogenic species which utilize the methyl corrinoid pathway (20).

These results, although incompatible with the function of a single common intermediate other than CO_2, do not preclude multiple intermediates of similar oxidation levels or their direct (without oxidation or reduction) interconversion. When the substrates are methanol and CO_2 in the presence of acetate (22), *B. methylotrophicum* consumes approximately one CO_2 per five methanols used. Similar results have been reported for *E. limosum* (14). *A. woodii* consumes one CO_2 per two methanols (4), but does not utilize acetyl-CoA as an electron acceptor and produces only acetate, therefore not requiring the additional reducing equivalents from methanol oxidation for acetate reduction to butyrate. The occurrence of indirect transformations between the methyl and carboxyl synthesis pathways (oxidation and reductions occur with CO_2 as an intermediate) is thermodynamically unnecessary and is not obvious from the reported growth stoichiometries.

One indication of CO_2 production from methanol during net CO_2 consumption came from the in vivo ^{13}C NMR experiment (Fig. 3). With methanol as the label source and unlabeled CO_2 as an electron acceptor, the first amounts of acetate produced are labeled in the methyl group only with label simultaneously appearing in CO_2. One would expect doubly labeled acetate to be formed if any of the intermediates of methanol oxidation (CO_2 excluded) could exchange with [CO]. The carboxyl groups of acetate and butyrate and the C-3 methylene of butyrate (derived from the C-1 of acetate) become labeled, however, only after the CO_2 pool is sufficiently labeled (a large $^{12}CO_2$ pool was initially present). The simultaneous appearance of label in the carboxyl groups and the butyrate C-3 methylene indicates that the previous absence of label in the carboxyl groups is not a result of the inherent lower signal intensity of nonprotonated carbons and provides evidence that butyrate synthesis occurs by the condensation of acetyl-CoA.

It must be emphasized that these data do not unambiguously support one model over the other. For example, the ^{13}C in vivo labeling experiment (Fig. 3) and the apparent dilution by CO and CO_2 of methanol label transformation into the carboxyl of acetate (Table 1) do not make it clear whether methanol oxidation occurs via these compounds or via intermediates (e.g., [HCOOH], [CO]) with which they readily exchange. Likewise, the data which indicate that CO_2 is a better acetate methyl group precursor when CO is the sole energy source (Table 2) do not differentiate between the conversion of CO to the methyl group via CO_2 or a direct conversion of CO to [HCOOH] followed by a rapid CO_2-[HCOOH] exchange.

Since *B. methylotrophicum* co-catabolizes methanol and formate, with formate utilization paralleling CO_2 utilization (Table 1), we could observe the postulated intermediary role of CO_2 by replacing it with formate as an alternative electron acceptor. The growth curve results (Fig. 1) are reminiscent of the in vivo ^{13}C NMR experiment and are consistent with Fig. 4. To synthesize acetate carboxyl groups and reducing equivalents for butyrate synthesis, the organism converts some of the substrate to CO_2 and H_2. The net consumption of CO_2 occurs after formate depletion. Hydrogen may also be a required intermediate, playing a role similar to that which it plays in lactate and pyruvate oxidation by certain sulfate-reducing bacteria (35), or perhaps it is a by-product of CO_2 production.

The lack of similar patterns of carbon transformations when another formyl-oxidation-level substrate, carbon monoxide, replaces formate is consistent with Fig. 4 as CO_2 production from CO is not required to form [CO] under these conditions. The dramatic shift in product formation may be associated with inhibitory effects of CO (e.g., inhibition of hydrogenase [21]), as well as with its mechanistic (Fig. 4) and thermodynamic dissimilarities with formate. The following data are from reference 34.

$$H_2O + CO \rightarrow\!\!\!\!\!\rightarrow H_2 + CO_2\ \Delta G^{\circ\prime} = -2.8\ \text{kcal/mol}\ (-11.7\ \text{kJ/mol})$$

$$H^+ + HCO_2^- \rightarrow\!\!\!\!\!\rightarrow H_2 + CO_2\ \Delta G^{\circ\prime} = 1.2\ \text{kcal/mol}\ (5.02\ \text{kJ/mol})$$

The model (Fig. 4) predicts an additional site of substrate-level phosphorylation (formyl-THF to formate) (8) when methanol is a substrate and may explain the relatively high yields of these organisms when grown on methanol. *B. methylotrophicum* (22) and *A. woodii* (4) synthesize 8.1 and 8.2 g of cells per mol of methanol, respectively, when they are grown in medium containing 0.05% yeast extract. No net ATP synthesis by substrate-level phosphorylation can be envisioned with this model, however, when H_2 and CO_2 or CO or HCOOH are the substrates. In each case, one ATP is consumed by formyl-THF synthetase per acetyl-CoA produced. The oxidation of CO coupled with the reduction of formaldehyde to methanol-oxidation level intermediates (21, 34) or acetate to butyrate (9) is sufficiently exergonic to allow ATP synthesis by electron transport phosphorylation, and similarly exergonic reactions with H_2 and CO_2 or formate as electron donors can be written. The formaldehyde-to-methanol level reduction as an electron-accepting couple in electron transport phosphorylation seems more likely since *A. woodii* does not synthesize butyrate and *B. methylotrophicum* produces essentially no butyrate when grown on CO (21), H_2 and CO_2 (22), or formate (R. Kerby, unpublished data).

More work is needed before the exact route(s) of unicarbonotrophy in *B. methylotrophicum* and *A. woodii* can be defined. Results indicating high levels of THF enzymes, including formyl-THF synthetase, in *Clostridium thermoautotrophicum* (6) when grown on methanol and CO_2 or H_2 and CO_2 and in *A. woodii* (31) when grown on H_2 and CO_2 support the operation in these acetogens of a catabolic mechanism similar to that of Fig. 4.

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ADDENDUM

While this manuscript was being revised, Diekert and Ritter (FEMS Microbiol. Lett. 17:299–302, 1983) published results which also provide in vivo evidence for the preferential incorporation of CO into the carboxyl group of acetate during the catabolism of H_2 and CO_2 by *A. woodii*.

ADDENDUM IN PROOF

Studies of CO dehydrogenase purified from *C. thermoaceticum* also suggest that CO is transformed to the methyl group of acetate via CO_2 and formate (S. W. Ragsdale, J. E. Clark, L. G. Ljungdahl, L. L. Lundie, and H. L. Drake. J. Biol. Chem. 258:2364–2369, 1983).

LITERATURE CITED

1. **American Gas Association.** 1965. General tables and charts, p. 1–19. In C. George Segeler (ed.), Gas engineers handbook. Industrial Press, New York.
2. **Andreesen, J. R., and L. G. Ljungdahl.** 1974. Nicotinamide adenine dinucleotide phosphate-dependent formate dehydrogenase from *Clostridium thermoaceticum*: purification and properties. J. Bacteriol. 120:6–14.
3. **Andreesen, J. R., A. Schaupp, C. Neurauter, A. Brown, and L. G. Ljungdahl.** 1973. Fermentation of glucose, fructose, and xylose by *Clostridium thermoaceticum*: effect of metals on growth yield, enzymes, and the synthesis of acetate from CO_2. J. Bacteriol. 114:743–751.
4. **Bache, R., and N. Pfennig.** 1981. Selective isolation of *Acetobacterium woodii* on methyoxylated aromatic acids and determination of growth yields. Arch. Microbiol. 130:255–261.
5. **Balch, W. E., S. Schoberth, R. S. Tanner, and R. S. Wolfe.** 1977. *Acetobacterium*, a new genus of hydrogen-oxidizing carbon dioxide-reducing anaerobes, and characterization of *Acetobacterium woodii* sp. nov. Int. J. Syst. Bacteriol. 27:355–361.
6. **Clark, J. E., S. W. Ragsdale, L. G. Ljungdahl, and J. Wiegel.** 1982. Levels of enzymes involved in the synthesis of acetate from CO_2 in *Clostridium thermoautotrophicum*. J. Bacteriol. 151:507–509.
7. **Counotte, G. H. M., R. A. Prins, R. H. A. M. Janssen, and M. J. A. deBie.** 1981. Role of *Megasphaera elsdenii* in the fermentation of *dl*-[2-^{13}C]lactate in the rumen of dairy cattle. Appl. Environ. Microbiol. 42:649–655.
8. **Curthoys, N., and J. C. Rabinowitz.** 1972. Formyltetrahydrofolate synthetase. Binding of folate substrates and kinetics of the reverse reaction. J. Biol. Chem. 247:1965–1971.
9. **Decker, K., K. Jungermann, and R. K. Thauer.** 1970. Energy production in anaerobic organisms. Angew. Chem. Int. Ed. Engl. 9:138–158.
10. **Drake, H. L.** 1982. Occurrence of nickel in carbon monoxide dehydrogenase from *Clostridium pasteurianum* and *Clostridium thermoaceticum*. J. Bacteriol. 149:561–566.
11. **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.
12. **Drake, H. L., S.-I. Hu, and H. G. Wood.** 1981. Purification of five components from *Clostridium thermoaceticum* which catalyze synthesis of acetate from pyruvate and methyltetrahydrofolate. J. Biol. Chem. 256:11137–11144.
13. **Genthner, B. R. S., and M. P. Bryant.** 1982. Growth of *Eubacterium limosum* with carbon monoxide as the energy source. Appl. Environ. Microbiol. 43:70–74.
14. **Genthner, B. R. S., C. L. Davis, and M. P. Bryant.** 1981. Features of rumen and sewage sludge strains of *Eubacterium limosum*, a methanol- and H_2-CO_2-utilizing species. Appl. Environ. Microbiol. 42:12–19.
15. **Hu, S.-I., H. L. Drake, and H. G. Wood.** 1982. Synthesis of acetyl coenzyme A from carbon monoxide, methyltetrahydrofolate, and coenzyme A by enzymes from *Clostridium thermoaceticum*. J. Bacteriol. 149:440–448.
16. **Kerby, R., and J. G. Zeikus.** 1983. Growth of *Clostridium*

thermoaceticum on H_2/CO_2 or CO as energy source. Curr. Microbiol. **8**:27–30.
17. **Levy, G. C., R. L. Lichter, and G. L. Nelson.** 1980. Carbon-13 nuclear magnetic resonance spectroscopy, 2nd ed. John Wiley & Sons, Inc., New York.
18. **Linton, J. D., and R. J. Stephenson.** 1978. A preliminary study on growth yields in relation to the carbon and energy content of various organic growth substrates. FEMS Microbiol. Lett. **3**:95–98.
19. **Ljungdahl, L., E. Irion, and H. G. Wood.** 1965. Total synthesis of acetate from CO_2. I. Co-methylcobyric acid and Co-(methyl)-5-methoxybenzimidazolylcobamide as intermediates with *Clostridium thermoaceticum*. Biochemistry **4**:2771–2780.
20. **Ljungdahl, L. G., and H. G. Wood.** 1982. Acetate biosynthesis, p. 165–202. *In* D. Dolphin (ed.), B_{12}, vol. 2. John Wiley & Sons, Inc., New York.
21. **Lynd, L., R. Kerby, and J. G. Zeikus.** 1982. Carbon monoxide metabolism of the methylotrophic acidogen *Butyribacterium methylotrophicum*. J. Bacteriol. **149**:255–263.
22. **Lynd, L. H., and J. G. Zeikus.** 1983. Metabolism of H_2-CO_2, methanol, and glucose by *Butyribacterium methylotrophicum*. J. Bacteriol. **153**:1415–1423.
23. **Moore, W., and E. Cato.** 1965. Synonomy of *Eubacterium limosum* and *Butyribacterium rettgeri*. Int. Bull. Bacteriol. Nomen. Taxon. **15**:69–80.
24. **Parker, D. J., T.-F. Wu, and H. G. Wood.** 1971. Total synthesis of acetate from CO_2: methyltetrahydrofolate, an intermediate, and a procedure for separation of the folates. J. Bacteriol. **108**:770–776.
25. **Perlman, D., and J. B. Semar.** 1963. Production of cobamides by *Butyribacterium rettgeri*. Biotechnol. Bioeng. **5**:21–25.
26. **Pine, L., and H. A. Barker.** 1954. Tracer experiments on the mechanism of acetate formation from carbon dioxide by *Butyribacterium rettgeri*. J. Bacteriol. **68**:216–226.
27. **Rabinowitz, J. C., and W. E. Pricer, Jr.** 1965. Formate, p. 308–312. *In* H. Bergmeyer (ed.), Methods of enzymatic analysis. Academic Press, Inc., New York.
28. **Rader, J. I., and F. M. Huennekens.** 1973. Folate coenzyme-mediated transfer of one-carbon groups, p. 197–223. *In* P. D. Boyer (ed.), The enzymes, 3rd ed., vol. 9, part B. Academic Press, Inc., New York.
29. **Schulman, M., R. K. Ghambeer, L. G. Ljungdahl, and H. G. Wood.** 1973. Total synthesis of acetate from CO_2. VII. Evidence with *Clostridium thermoaceticum* that the carboxyl of acetate is derived from the carboxyl of pyruvate by transcarboxylation and not by fixation of CO_2. J. Biol. Chem. **248**:6255–6261.
30. **Shulman, R. G., T. R. Brown, K. Ugurbil, S. Ogawa, S. M. Cohen, and J. A. den Hollander.** 1979. Cellular applications of ^{31}P and ^{13}C nuclear magnetic resonance. Science **205**:160–166.
31. **Tanner, R. S., R. S. Wolfe, and L. G. Ljungdahl.** 1978. Tetrahydrofolate enzyme levels in *Acetobacterium woodii* and their implication in the synthesis of acetate from CO_2. J. Bacteriol. **134**:668–670.
32. **Tempest, D. W.** 1978. The biochemical significance of microbial growth yields: a reassessment. TIBS **3**:180–184.
33. **Thauer, R. K.** 1972. CO_2-reduction to formate by NADPH. The initial step in the total synthesis of acetate from CO_2 in *Clostridium thermoaceticum*. FEBS Lett. **27**:111–115.
34. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100–180.
35. **Traore, A. S., C. E. Hatchikian, J.-P. Belaich, and J. Le Gall.** 1981. Microcalorimetric studies of the growth of sulfate-reducing bacteria: energetics of *Desulfovibrio vulgaris* growth. J. Bacteriol. **145**:191–199.
36. **Waber, L. J., and H. G. Wood.** 1979. Mechanism of acetate synthesis from CO_2 by *Clostridium acidiurici*. J. Bacteriol. **140**:468–478.
37. **Welty, F. K., and H. G. Wood.** 1978. Purification of the "corrinoid" enzyme involved in the synthesis of acetate by *Clostridium thermoaceticum*. J. Biol. Chem. **253**:5832–5838.
38. **Wolin, E. A., M. J. Wolin, and R. S. Wolfe.** 1963. Formation of methane by bacterial extracts. J. Biol. Chem. **238**:2882–2886.
39. **Wood, H. G.** 1952. A study of carbon dioxide fixation by mass determination of the types of ^{13}C-acetate. J. Biol. Chem. **194**:905–931.
40. **Zeikus, J. G.** 1983. Metabolism of one-carbon compounds by chemotrophic anaerobes. Adv. Microb. Physiol. **24**:215–299.
41. **Zeikus, J. G., L. H. Lynd, T. E. Thompson, J. A. Krzycki, P. J. Weimer, and P. W. Hegge.** 1980. Isolation and characterization of a new, methylotrophic, acidogenic anaerobe, the Marburg strain. Curr. Microbiol. **3**:381–386.