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Additional Characteristics of One-Carbon-Compound Utilization by *Eubacterium limosum* and *Acetobacterium woodii*

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Growth characteristics of *Eubacterium limosum* and *Acetobacterium woodii* during one-carbon-compound utilization were investigated. *E. limosum* RF grew with formate as the sole energy source. Formate also replaced a requirement for CO_2 during growth with methanol. Growth with methanol required either rumen fluid, yeast extract, or acetate, but their effects were not additive. Cultures were adapted to grow in concentrations of methanol of up to 494 mM. Growth occurred with methanol in the presence of elevated levels of Na^+ (576 mM). The pH optima for growth with methanol, H_2-CO_2, and carbon monoxide were similar (7.0 to 7.2). Growth occurred with glucose at a pH of 4.7, but not at 4.0. The apparent K_m values for methanol and hydrogen were 2.7 and 0.34 mM, respectively. The apparent V_{max} values for methanol and hydrogen were 1.7 and 0.11 μmol/mg of protein · min^{-1}, respectively. The K_s value for CO was estimated to be less than 75 μM. Cellular growth yields were 70.5, 7.1, 3.38, and 0.84 g (dry weight) per mol utilized for glucose, methanol, CO, and hydrogen (in H_2-CO_2), respectively. *E. limosum* was also able to grow with methoxylated aromatic compounds as energy sources. Glucose apparently repressed the ability of *E. limosum* to use methanol, hydrogen, or isoleucine but not CO. Growth with mixtures of methanol, H_2, CO, or isoleucine was not diauxic. The results, especially the relatively high apparent K_m values for H_2 and methanol, may indicate why *E. limosum* does not usually compete with rumen methanogens for these energy sources. However, its versatility in using many other energy sources plus its tolerance of relatively high osmolarity may allow it to compete in the rumen under unusual conditions. We also found that *A. woodii* grew with CO as the sole energy source with a doubling time of 13 h. Acetate was the only volatile fatty acid detected.

Previous reports on *Eubacterium limosum* indicate that it uses one-carbon compounds including methanol, H_2-CO_2, and CO as energy sources (4, 5) in the production of acetate and longer-chain fatty acids. *E. limosum* is similar in most respects to an organism called "*Butyribacterium methylotrophicum*" (7, 10–12, 18) and similar in some characteristics to *Acetobacterium woodii* (2, 16). Since *E. limosum* may compete with methanogens for H_2 and methanol in the rumen only under certain very special conditions, e.g., with a mainly liquid molasses diet (5), it was of interest to determine other growth characteristics when this organism used one-carbon and other compounds as energy sources. We also report the ability of *A. woodii* to grow with carbon monoxide as the sole energy source.

MATERIALS AND METHODS

Bacterial strains. *E. limosum* RF was isolated from the rumen fluid of a sheep fed a liquid molasses diet (5). *A. woodii* ATCC 29683 (2) was generously donated by R. S. Wolfe, University of Illinois.

Media and culture techniques. The anaerobic techniques and the basal medium used for the cultivation of *E. limosum* have been previously described (4, 5). Unless otherwise indicated, all media contained 5% rumen fluid and were inoculated with 0.05 ml of culture, dispensed into rubber-stoppered culture tubes (18 by 150 mm), and incubated at 35°C. Media to be pressurized with gases were dispensed into serum culture tubes (18 by 150 mm). The medium for growth of *A. woodii* was similar to the basal medium previously described (4, 5), except that calcium-D-pantothenate was the only vitamin added, and the final pH was 6.8 under 2.5 atm (254 kPa) of N_2-CO_2 *A. woodii* cultures were incubated at 30°C in a horizontal position without shaking. Each datum point is the mean of triplicate cultures, unless otherwise stated. Inocula were grown with the substrate under study, unless otherwise indicated.

Growth with methoxylated aromatic compounds. Ferulic, syringic, and vanillic acids were dissolved in 80% ethanol under N_2-CO_2 and added via syringe to sterile tubes of reduced medium (5-ml samples), resulting in 0.1 and 0.2% (wt/vol) final concentrations. Control media were also prepared which contained *p*-hydroxybenzoic acid, cinnamic acid, or ethanol (27.4 mM; equivalent to the amount of ethanol added when adding the ethanol solutions of aromatic compounds). Cultures were incubated at 39°C.

Growth yields. Growth yields were determined for glucose and methanol in the basal medium at 39°C. Glucose or methanol cultures (75 ml) were grown in 125-ml serum bottles under N_2-CO_2. Filter-sterilized glucose was made anaerobic and added to sterile medium. Triplicate 20-ml samples of the glucose or methanol cultures were collected after maximum growth was reached, and the samples were filtered through dried (12 h, 60°C) preweighed membrane filters (0.45-μm pore size; Millipore Corp.), rinsed twice with 10 ml of sterile 0.8% NaCl, and placed in a desiccator to cool before being weighed. Cell dry weight was calculated from the mean value of triplicate samples from quadruplicate bottles. Growth yields for H_2-CO_2 or CO were determined in a similar manner, except that the culture volume was 25 ml and seven bottles were inoculated. Bottles were either pressurized to 2 atm (203 kPa) with 100% H_2 or were pressurized to 2 atm with 100% N_2 after 50 ml of CO was added. One 20-ml samples was collected from each bottle,

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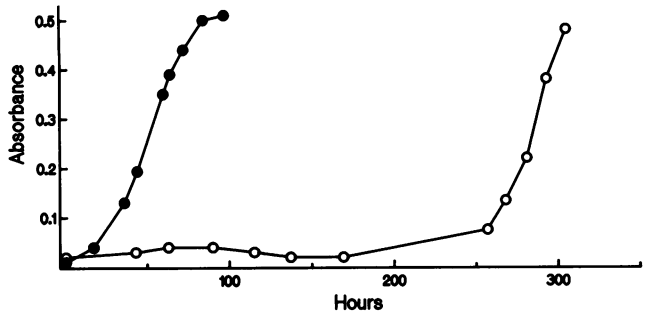

FIG. 1. Growth of *E. limosum* in basal medium with 147 mM sodium formate. Each point is the mean of duplicate cultures. Symbols: ●, H_2-CO_2-grown inoculum; ○, methanol-grown inoculum.

and dry weight was determined as described above. The cell yield for formate was estimated from the absorbance obtained with formate (147 mM) (see equation in Table 2, footnote *e*). It was assumed that the absorbances obtained in formate and methanol media were directly comparable in terms of cell yield, i.e., grams (dry weight) per mole of substrate utilized.

Growth with formate. Basal medium containing sodium formate (147 mM) as the only energy source was used to determine growth with formate. Formate medium (7 ml) was dispensed into rubber-stoppered tubes (18 by 150 mm). A modified basal medium containing 74 mM methanol and 14.7 mM sodium formate was prepared to determine the ability of formate to replace carbon dioxide during growth with methanol. The bicarbonate buffer in this medium was replaced by a phosphate buffer (50 mM, pH 7.2), and the N_2-CO_2 (80:20) gas phase was replaced by 100% N_2. The medium was dispensed (3.5-ml samples) into rubber-stoppered tubes (13 by 100 mm). Control media included the modified basal medium without sodium formate, basal methanol medium, and basal formate medium. All media were inoculated with a methanol-grown culture not previously grown with formate.

pH optimum. Basal medium in which the amount of sodium bicarbonate added was adjusted to obtain the desired pH was used. To adjust the pH to less than 6.0, sodium bicarbonate was deleted and sterile HCl was added. Inocula were grown with the energy source under investigation. All media were prepared in serum culture tubes (18 by 150 mm) and dispensed in 7-ml amounts. Anaerobic methanol was added via syringe to sterile medium to a final concentration of 49.4 mM before inoculation. Hydrogen (843 μmol) and CO (169 μmol) were added after inoculation. After the addition of CO, cultures were pressurized to 2 atm with 100% N_2. Cultures were incubated at 37°C. The initial pH was determined on duplicate tubes of uninoculated medium.

Methanol and Na^+ tolerance. Media containing 49.4 to 494 mM methanol were prepared to determine tolerance to methanol. Cultures were passaged three times in these media. Defined (24 mM sodium acetate) methanol (49.4 mM) media containing 48 to 576 mM NaCl were prepared to determine tolerance to Na^+. These media were inoculated with 0.01 ml of a methanol-grown culture.

Substrate affinity (K_m) and maximum velocity (V_{max}) for methanol and H_2. The apparent K_m and V_{max} values for methanol and H_2 were determined by measuring the rate of substrate disappearance as a function of initial substrate concentration on suspensions of intact cells. Initial velocities were generated by linear regression analysis of the uptake data. Apparent K_m and V_{max} values were estimated from a Lineweaver-Burk plot of the data thus obtained. Values were the means of duplicate experiments.

Triplicate serum bottles (120 ml) containing 50 ml of methanol medium (0.5 to 20 mM methanol) were inoculated with 25 ml of a methanol-grown culture. Samples (1 ml) were removed before the cultures were incubated, to determine the initial concentration of methanol. Cultures were incubated at 39°C, and thereafter, samples were removed at intervals of 15 to 60 min depending on the initial concentration of methanol. Samples were held at 4°C and then centrifuged at 10,000 × *g* for 5 min in a Microfuge 11 (Beckman Instruments, Inc., Fullerton, Calif.) (4°C). The supernatant fluid was collected and stored frozen until analyzed.

Triplicate bottles (100 ml) of basal medium (25 ml) were inoculated with 7.5 ml of a H_2-CO_2-grown culture before 90.4 to 752.9 μmol of H_2 was added. Cultures were shaken during incubation and vigorously shaken before sampling. Samples (100 μl) of the gas phase were removed with a gas-tight, Levr tip syringe (The Hamilton Co., Reno, Nev.) and analyzed. Percent H_2 was determined, and dissolved H_2 was calculated after correction for temperature and pressure.

Wet weight of cells was determined on inoculum and on cultures after the last sample was collected. Cell protein was determined by the method of Lowry et al. (9) after pelleted cells were treated with 0.1 M NaOH for 45 min.

The K_s value for CO was estimated by measuring the growth rate of *E. limosum* as a function of CO concentration.

Analytical methods. Methanol and hydrogen were determined on a model 5830A gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with a model 1850A integrator. Methanol was detected by flame ionization with an SP-1000 column (10% SP-1000, 1% H_3PO_4; 100/120-mesh Chromosorb W; 1.8 m by 2 mm; Supelco, Inc., Bellefonte, Pa.) at 150°C with argon as the carrier gas (17 ml/min). Hydrogen was detected by thermal conductivity with a Carbosieve B column (120/140-mesh; 3.0 m by 2 mm; Supelco) at 140°C, with argon as the carrier gas (7 ml/min). Methanol solutions of known concentrations and 100% hydrogen were used as standards to quantify samples. Free fatty acids were determined by the acidification method previously described (5). Glucose was determined by the Glucostat assay (Sigma Chemical Co., St. Louis, Mo.), and lactic acid was determined by the method of Barker and Summerson (3). Growth was estimated by measuring the A_{600} with a Spectronic 70 (Bausch & Lomb, Inc., Rochester, N.Y.). Generation times were calculated from the straight portion of the growth curve when the curve was plotted on semilogarithmic paper.

RESULTS

Additional C_1 energy sources of *E. limosum*. After formate (147 mM) medium was inoculated with a methanol-grown culture, 260 h elapsed before significant growth was evident (Fig. 1). This lag phase was not observed with a second passage in formate medium. The doubling time with formate was 22 h after three serial transfers in formate medium (data not shown). Formate medium inoculated with a H_2-CO_2-grown culture showed immediate growth (Fig. 1). In addition, formate replaced the CO_2 requirement for growth with methanol (Fig. 2). Growth with methanol did not occur with the modified basal medium containing 100% N_2, but the addition of formate to this medium restored growth. In this experiment, growth did not occur in the formate medium at 210 h.

Growth occurred with the methoxylated aromatic compounds vanillate, syringate, and ferulate, but not with the nonmethoxylated compounds *p*-hydroxybenzoic and cinnamic acids (Table 1). Growth observed in cultures grown with syringate was twice that of cultures grown with vanillate, if the data were corrected to equal molarity. Increasing the concentration of vanillate or syringate increased growth proportionately. The doubling time at 39°C with syringate or vanillate was 8.2 h. The doubling time of methanol cultures grown in the presence of 27.4 mM ethanol was 8.6 h. Maximum growth (A_{600}) with methanol was not affected by the presence of ethanol, nor did growth occur with ethanol alone. Initially, cultures grown with ferulate showed a lag of 44 h before growth was apparent, but the lag phase disappeared with subsequent passage through the medium. The doubling time with ferulate was 11.8 h, but the growth yield was similar to that with equimolar amounts of vanillate.

Additional growth characteristics of *E. limosum* with C_1 compounds. Growth did not occur in a medium containing methanol but lacking rumen fluid. The addition of yeast extract (0.1%) or sodium acetate (chemically defined medium) restored growth in this medium. The doubling time in both media was 7.5 h. The doubling time in the presence of both yeast extract and sodium acetate did not change. Little growth occurred in the medium in the absence of methanol (data not shown).

The optimum pH for maximum growth with methanol, H_2-CO_2, or CO was between 7.0 and 7.2. In methanol medium, a lag phase appeared, and the growth rate and maximum growth decreased at pH 6.9 or less. Growth did not occur at pH 4.2. Similar results were obtained for H_2-CO_2, CO, and glucose. The final pHs of cultures were found to be 0.9 pH units below the initial pHs (data not shown).

Increasing the methanol concentration to 148 mM had little effect on growth, but at 198 mM, the doubling time increased to 24 h and continued to increase, reaching 64 h at 494 mM methanol. In cultures with greater than 198 mM methanol, a lag phase of 79 h occurred, but serial transfer in these media resulted in decreased lag phase and doubling time (data not shown).

Growth occurred in chemically defined methanol medium containing additional NaCl (48 to 576 mM), but maximum growth was poor at more than 405 mM Na^+. At 215 mM Na^+, the doubling time increased to 16.7 h, and it was 70.8 h at 405 mM Na^+ (data not shown).

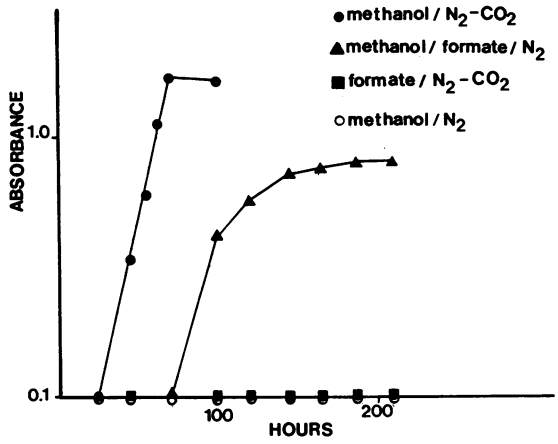

FIG. 2. Carbon dioxide requirement replaced by sodium formate during growth of *E. limosum* with methanol. See Materials and Methods for composition of media.

TABLE 1. Growth of *E. limosum* with limiting amounts of methoxylated and nonmethoxylated aromatic compounds in basal (5% rumen fluid) medium[a]

Compound	Concn (mM)		Growth[b]
	In medium	Of methoxyl groups	
p-Hydroxybenzoate	0.72	0.0	0.0
Vanillate	0.60	0.6	0.24
	1.20	1.2	0.46
Syringate	0.50	1.0	0.39
	1.00	2.0	0.72
Cinnamate	0.68	0.0	0.0
Ferulate	0.50	0.5	0.19
	1.00	1.0	0.41

[a] Tubes (18 by 150 mm) of medium (5 ml) were inoculated with 0.1 ml of a methanol (49.4 mM)-grown culture.

[b] Mean value (A_{600}) of triplicate cultures during passage 3 through medium indicated. Corrected for growth without added energy source (i.e., A_{600} = 0.08 without energy source).

The apparent K_m values for methanol and H_2 were 2.7 mM and 0.34 mM, respectively. The apparent V_{max} values were 1.7 and 0.11 μmol/mg of protein · min^{-1} for methanol and H_2, respectively. Protein content (7.25 to 7.5 mg of protein per liter) changed very little during the course of the experiment. The K_s (substrate affinity for growth) value for CO was estimated to be less than 75 μM, because the doubling time with 5% CO (equivalent to 75 μM dissolved CO) was the same as that of cultures grown with 40% CO, indicating that CO was utilized at maximum velocity at the lowest CO concentration tested (data not shown).

Growth yields (grams [dry weight] per mole of substrate utilized) obtained are shown in Table 2. The absorbance obtained with 147 mM formate was equivalent to the absorbance obtained with 18.5 mM methanol. Using the formula in Table 2, we estimated the cellular yield for formate to be 0.89 g (dry weight) per mol.

Gluose effect. A diauxic growth curve was observed when growth-limiting glucose (1.95 mM) and nonlimiting methanol, H_2, or isoleucine were present in the same medium. This kind of growth was not true of glucose plus CO. The doubling time in glucose medium lacking rumen fluid (defined) was 5.8 h (Fig. 3A), and the maximum growth was 0.64 (A_{600}). In defined medium with glucose and methanol (75 mM), the initial doubling time was 6.1 h followed by a lag of

TABLE 2. Growth yields of *E. limosum* in basal medium with growth-limiting amounts of energy source added

Energy source (amt added)	Growth yield[a]
Glucose (5 mmol/liter)	70.5 ± 1.8
Methanol (49.4 mmol/liter)	7.09 ± 0.39
CO (2.48 mmol/liter)	3.38 ± 0.18
H_2-CO_2 (3.8 mmol)	0.84 ± 0.04[b]
Formate (147 mmol)	0.89[c]

[a] Growth yield (grams[dry weight] per mole of energy source utilized) was determined as described in text. Values are means of duplicate determinations ± the standard deviation, except methanol, which is the mean of five determinations.

[b] Value is per mole of H_2 utilized. Value calculated for CO_2 was 1.68 g/mol of CO_2 utilized.

[c] Estimated from A_{600} obtained with 147 mM formate (equivalent to that obtained with 18.5 mM methanol) as follows: (18.5 mM CH_3OH/147 mM CHOOH) × (7.09 mg/mmol of CH_3OH).

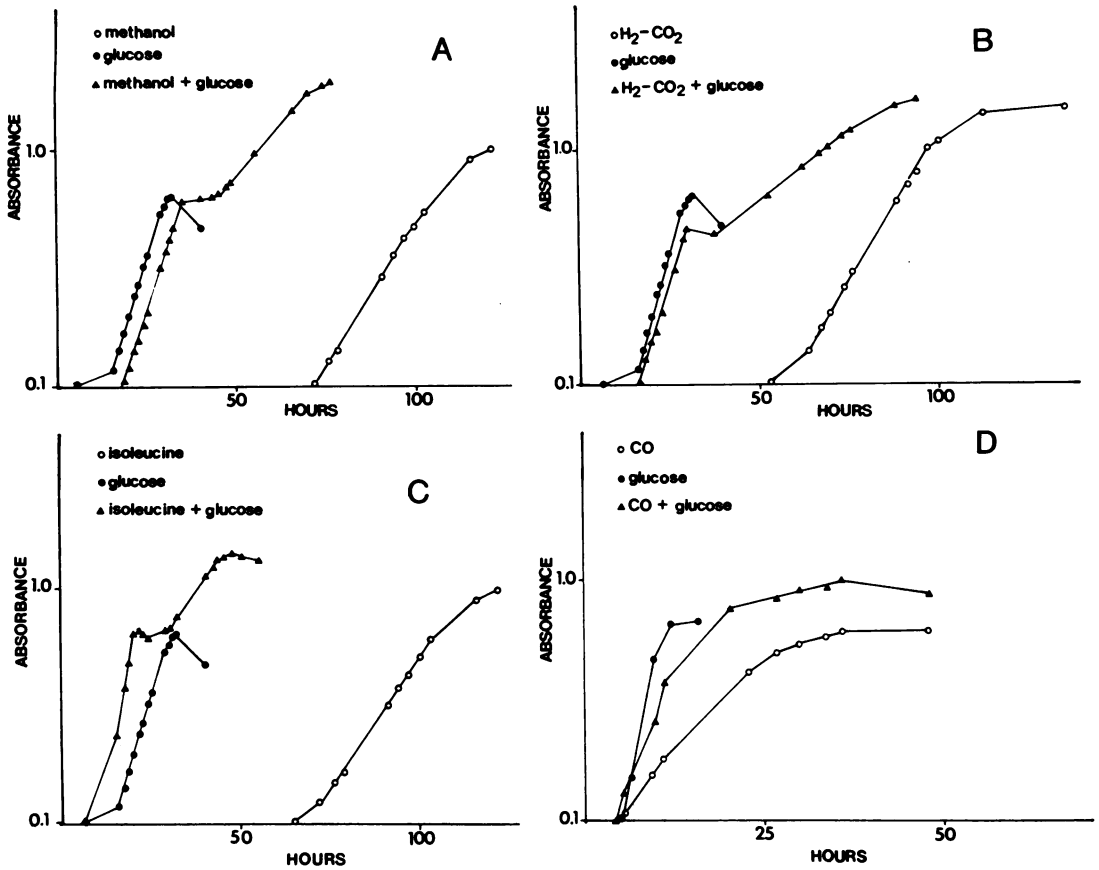

FIG. 3. Growth of *E. limosum* in defined (24 mM sodium acetate) medium containing a growth-limiting amount of glucose (1.95 mM) in combination with unlimited (A) methanol (75 mM), (B) H_2 (843 μmol), (C) isoleucine (20 mM), or (D) CO (169 μmol). Serum tubes of medium (7 ml) were inoculated with 0.1 ml of culture. Methanol, isoleucine, and H_2 were added to defined glucose medium (24 mM sodium acetate), and CO was added to glucose medium containing 5% rumen fluid. Media to which H_2 or CO was added were pressurized to 2 atm.

8 h, after which growth resumed at a slower rate (Fig. 3A). The presence of H_2 in the gas phase of defined glucose medium increased the doubling time (7.1 h) and decreased the maximum growth during the initial growth phase to 0.47 (Fig. 3B). After a lag of 6.5 h, growth resumed at a slower rate. The observed effect of H_2 was not seen in subsequent experiments, and lactic acid, a suspected end product, was not detected. The doubling time of the initial growth phase in defined isoleucine (20 mM)-and-glucose medium was decreased to 3.4 h, but maximum growth was not altered (Fig. 3C). The doubling time in defined isoleucine medium was 8.8 h.

Nondiauxic growth. A diauxic growth curve was not observed in basal medium containing glucose and 10% CO (Fig. 3D) or in medium containing isoleucine plus methanol, H_2, or CO (data not shown). Maximum growth obtained was higher in the presence of both substrates than in the presence of the individual energy sources. There was a lag in the initial growth with isoleucine when the medium was inoculated with methanol-, H_2-CO_2-, or CO-grown cultures (24, 35, and 45 h, respectively). There was a slight decrease in doubling time when methanol and H_2 or CO were present in the same medium, but diauxic growth was not observed. Growth curves indicated that H_2-CO_2- or CO-grown cells retain their ability to grow with methanol without requiring readaptation (data not shown).

Growth of *A. woodii* with CO. Growth of *A. woodii* with CO as the sole energy source was proportional to the initial amount of CO added. Maximum growth (A_{600}) with 10, 20, and 30% CO (0.21, 0.42, and 0.63 mmol) was 0.43, 0.93, and 1.24, respectively. An adaptation period was not required for the growth of *A. woodii* with CO. Yeast extract replaced rumen fluid in supporting the growth of *A. woodii* with CO, but growth could not be sustained in defined CO medium. The doubling time at 30°C was 13 h (data not shown). Acetate was the only volatile fatty acid detected.

DISCUSSION

E. limosum grew with methoxylated aromatic compounds (Table 1). Growth was proportional to the number of moles of methoxyl groups present, which suggested that they were the energy source. The doubling time with vanillate or syringate was 8.2 h. This doubling time was slower than that with methanol (7 h), but similar to the doubling time of cultures grown with methanol in the presence of 27.4 mM ethanol (8.6 h), indicating that the presence of ethanol slowed growth.

Bache and Pfenning (1) reported that *A. woodii* grew with methanol. We were unable to grow *A. woodii* in our methanol medium. Apparently, the concentration must be kept very low for *A. woodii* to grow with methanol. They also found that *A. woodii* grew with methoxylated aromatic

compounds and that the growth yields were proportional to the methoxyl groups available. Thus, their data support our conclusion that methoxyl groups are equivalent to methanol. They also reported that the growth yield of *A. woodii* with ferulate, which contains a double bond, was higher than the yield with vanillate. The product formed from ferulate was hydrocaffeic acid, which contains a saturated side chain and lacks a methoxyl group (16). It was concluded that *A. woodii* reduced the double bond present in the side chain and in so doing obtained additional energy. In contrast to *A. woodii*, *E. limosum* was found to grow to the same maximum absorbance on equimolar amounts of vanillate and ferulate (Table 1). The lag in growth that was seen with ferulate and the longer doubling time indicate that ferulate was not used as effectively by *E. limosum* as by *A. woodii*. The lag phase disappeared upon subsequent passage with this energy source, but the doubling time and growth yields did not change. Thus, *E. limosum* may be unable to obtain more energy from ferulate than from vanillate.

As was found with *E. limosum* (4), *A. woodii* used CO as the sole energy source and formed acetate. *A. woodii*, which is maintained on H_2-CO_2, did not require an adaptation period to grow with CO.

Formate replaced the CO_2 requirement of *E. limosum* (Fig. 2) during growth with methanol. Growth in the methanol-formate-N_2 medium was not a result of utilization of formate alone, since growth had not occurred in the formate control medium at 210 h. It was reported that formate or carbon monoxide replaced the CO_2 requirement for growth with methanol of "*B. methylotrophicum*" (7).

In the present study, formate as the sole energy source was also found to support the growth of *E. limosum*, but, as with H_2-CO_2 (5) or CO (data not shown), a long adaptation period was required when a methanol culture was the inoculum. In contrast, H_2-CO_2-grown cultures readily grew with formate (Fig. 1), CO, or methanol (data not shown). In addition, cultures which were adapted to H_2-CO_2 and were passaged three times in methanol medium showed no lag in growth on reintroduction into H_2-CO_2 medium (data not shown). These data indicate that the alteration which allowed growth with H_2-CO_2, CO, or formate may have been a permanent alteration and did not prevent subsequent utilization of methanol.

The phenomenon of adaptation may be the result of enzyme induction or of the selection of a mutant population capable of growing with H_2-CO_2, CO, or formate. Two characteristics of the adaptation suggest that a mutant population capable of growing with the more oxidized one-carbon compounds was being selected. First, the time required for adaptation (260 h) was long for enzyme induction. Second, after being adapted to H_2-CO_2, methanol-grown cultures no longer showed a lag phase when reintroduced into medium containing H_2-CO_2, CO, or formate as the energy source.

The pathway proposed by Wood et al. (17) explains the ability of the nonadapted methanol strain to utilize CO_2 or formate in conjunction with methanol even though it cannot grow with them as a sole energy source. The critical step in adaptation may be the tetrahydrofolate enzymes (17) involved. These enzymes could function to generate or accept electrons depending on the energy source involved. A mutant capable of growing on all of the one-carbon compounds may contain tetrahydrofolate enzymes capable of functioning in either direction, while those in the original methanol strain may function only to generate electrons from reduced methanol. It is also possible that the mutant population has an altered protein that becomes capable of interacting with the more oxidized substrates.

The apparent K_m values of *E. limosum* for methanol and H_2 were 2.7 and 0.34 mM, respectively. The apparent K_m value we obtained for H_2 may be an overestimate due to rate-limiting diffusion across the liquid interface (13). However, it should not be a gross overestimate since the bacterial concentration we used (approximately 1.2×10^8 CFU/ml [8]) was equal to or less (8) than the biomass concentration found to be critical for accurate K_m determination (13). The estimated K_s value for CO (<75 μM) was apparently less than that for either methanol or H_2. Lynd et al. (10) estimated a K_s value of 28 to 56 μM for CO utilization by "*B. methylotrophicum*" from growth rate data. The relative order in growth yields (Table 2) obtained for *E. limosum* with methanol, CO, H_2-CO_2, or formate was expected from the standard changes in free energy (i.e., −55.2, −41.4, −26.4, and −25.1 kJ, respectively) during formation of acetate from the particular one-carbon energy source (15). Actually, *E. limosum* should obtain slightly more energy from methanol because of its ability to form butyrate (i.e., −59.8 kJ/mol of methanol used) when grown with methanol. The growth yields obtained for *E. limosum* with methanol and CO are similar to those reported for "*B. methylotrophicum*" (11). In contrast, the standard changes in free energy, i.e. growth yield, in forming methane from these one-carbon compounds (−78.7, −49.1, −33.9, and −32.6 kJ for methanol, CO, H_2, and formate, respectively [15]) are greater, and the K_m of methanogens for H_2 is lower (2.5 to 12 μM) than that of *E. limosum* (6, 13, 14).

These data suggest that, in addition to the ability of glucose to repress its utilization of one-carbon substrates, there are other factors that could prevent *E. limosum* from competing well in the rumen for normal methanogenic substrates (i.e., H_2, methanol, or formate). Unfavorable conditions in the rumen may allow *E. limosum* to compete with methanogens, because it is a hardy anaerobe which tolerates high osmolarity (i.e., sodium and methanol tolerance), low pH, and greater oxygen concentrations (5) and is more versatile in energy source utilization than are methanogens.

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