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Metabolism of Formate in *Methanobacterium formicicum*

N. L. SCHAUER AND J. G. FERRY*

Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Methanobacterium formicicum strain JF-1 was cultured with formate as the sole energy source in a pH-stat fermentor. Growth was exponential, and both methane production and formate consumption were linear functions of the growth rate. Hydrogen was produced in only trace amounts, and the dissolved H_2 concentration of the culture medium was below 1 μM. The effect of temperature or pH on the rate of methane formation was studied with a single fermentor culture in mid-log phase that was grown with formate under standard conditions at 37°C and pH 7.6. Methane formation from formate occurred over the pH range from 6.5 to 8.6, with a maximum at pH 8.0. The maximum temperature of methanogenesis was 56°C. H_2 production increased at higher temperatures. Hydrogen and formate were consumed throughout growth when both were present in saturating concentrations. The molar growth yields were 1.2 ± 0.06 g (dry weight) per mol of formate and 4.8 ± 0.24 g (dry weight) per mol of methane. Characteristics were compared for cultures grown with either formate or H_2-CO_2 as the sole energy source at 37°C and pH 7.6; the molar growth yield for methane of formate cultures was 4.8 g (dry weight) per mol, and that of H_2-CO_2 cultures was 3.5 g (dry weight) per mol. Both formate and H_2-CO_2 cultures had low efficiencies of electron transport phosphorylation; formate-cultured cells had greater specific activities of coenzyme F_{420} than did H_2-CO_2-grown cultures. Hydrogenase, formate dehydrogenase, chromophoric factor F_{342}, and low levels of formyltetrahydrofolate synthetase were present in cells cultured with either substrate. Methyl viologen-dependent formate dehydrogenase was found in the soluble fraction from broken cells.

All described strains of methanogenic bacteria utilize H_2 as an electron donor for methanogenesis and growth. Formate is an electron donor for approximately one-half of all described strains (3). Little is known about the metabolism of this substrate (12, 13, 22). Studies have been confined to investigations of formate dehydrogenase in several species. *Methanobacterium ruminantium* and *Methanospirillum hungatii* possess a formate dehydrogenase that is linked to coenzyme F_{420} as the first low-molecular-weight anionic electron transfer coenzyme (9, 22). Formate-dependent growth of *Methanococcus vanneilii* is stimulated by the addition of selenium and tungsten to a mineral salts medium (13). The selenium-dependent formate dehydrogenase of *M. vanneilii* contains selenocysteine (11).

The work presented here was initiated to investigate the growth and biochemical characteristics of *Methanobacterium formicicum* strain JF-1 cultured with formate as the sole energy source.

(This work was reported in part at the 78th Annual Meeting of the American Society for Microbiology, Las Vegas, Nev., 14-19 May 1978.)

MATERIALS AND METHODS

Organism and culture media. The organism used throughout this study was isolated from a benzoate-degrading consortium and was identified as *M. formicicum* strain JF-1 (8). The basal medium used in all studies contained the following constituents (in final percent compositions by weight): KH_2PO_4, 0.021; K_2HPO_4, 0.021; $(NH_4)_2SO_4$, 0.021; NaCl, 0.042; $MgSO_4 \cdot 7H_2O$, 0.0084; $CaCl_2 \cdot 2H_2O$, 0.015; cysteine hydrochloride, 0.025; $Na_2S \cdot 9H_2O$, 0.025; resazurin, 0.001; yeast extract (Difco Laboratories), 0.05; and Trypticase (BBL Microbiology Systems), 0.05. In addition, vitamin and trace mineral solutions (both at 1%, vol/vol) were added (25). Basal medium supplemented with 0.4% (wt/wt) Na_2CO_3 was used for cultivation with 80% H_2-20% CO_2. Basal medium supplemented with 0.5% (wt/wt) sodium formate was used for cultivation with formate.

Cultural procedures. All studies were performed in a model MF-105 fermentor (New Brunswick Scientific) with a working volume of 3.5 liters. Growth with H_2-CO_2 was as previously described (5). Growth with formate as the sole energy source was performed in a fermentor fitted with reference and measuring electrodes (Leeds and Northrup). The electrodes were connected to a model 132 pH controller (New Brunswick Scientific) that contained a reservoir of 25 N formic acid. The formic acid was delivered through

Tygon tubing by a peristaltic pump housed in the pH controller. The Tygon tubing was connected to a section of glass tubing that extended through an addition port in the fermentor head plate. The fermentation vessel with medium and electrodes in place was autoclaved and then sparged with N_2 (30 ml/min per liter of culture) while cooling to 37°C. Traces of O_2 were removed from the N_2 by passage through a column (2 by 10 cm) of reduced copper filings maintained at 350°C. Sodium sulfide was added after temperature equilibration. The pH after autoclaving and addition of sodium sulfide was 7.3. It was not necessary to sterilize the formic acid reservoir and Tygon tubing. Inocula (300 ml) were grown with H_2-CO_2 and formate (0.5%) in shake flasks as previously described (5). The fermentor stir rate was 400 rpm. As the culture metabolized formate, the pH increased to 7.6, after which the pH was maintained at 7.6 ± 0.05 by automatic addition of formic acid with the pH controller.

Analytical methods. Formate was determined by the formyltetrahydrofolate synthetase method, using enzyme from *Clostridium cylindrosporum*, which was a gift from J. Rabinowitz (18).

Analyses of H_2 and CH_4 were made with a Dohrmann model 15C3 gas chromatograph equipped with a Poropak QS column (6 feet [ca. 1.83 m]) connected to a thermal conductivity detector. The column was operated at 25°C. Nitrogen at 30 ml/min was the carrier gas.

Dissolved H_2 in the medium was estimated by adapting a procedure previously described (R. F. Strayer, J. A. Robinson, and J. M. Tiedje, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, N27, p. 166). Culture fluid (20 ml) and 30 ml of H_2-free CO_2 were withdrawn aseptically into a 50-ml syringe, and the contents were shaken to strip the dissolved H_2. The 30-ml gas phase, which contained CO_2 and stripped H_2, was then slowly injected through a serum stopper fitted into the bottom of a glass column (0.6 by 20 cm) which contained 1 M NaCl and 5 M NaOH. After absorption of CO_2, the trapped gas bubble (1 ml) was sampled with a gas-tight syringe through a serum stopper fitted into the top of the column and analyzed by gas chromatography. Before sampling, the fluid level was readjusted in a side arm attached to the column. Based on Henry's law constants, recoveries of more than 85% were obtained with distilled water samples equilibrated with 0.001 atmosphere of H_2 at 25°C. The coefficient of variation was 9.2% ($n = 6$).

Growth studies. The total amount of methane produced during exponential growth was calculated from the graph obtained with the least-squares fit of $\log y = mt + b$, where y is the micromoles of CH_4 per minute at time t (in minutes), m is the slope, and b is the intercept. The y values were determined several times during exponential growth by measuring the CH_4 (micromoles per milliliter) in the exit gas and multiplying by the flow rate (milliliters per minute). The total amount of methane produced from t_1 to t_2 (T_{methane}) is represented by the equation:

$$T_{\text{methane}} = \int_{t_1}^{t_2} e^{2.303(mt+b)}\, dt$$

In the integrated form, this equation becomes

$$T_{\text{methane}} = \frac{y_{t_2} - y_{t_1}}{2.303m}$$

Cell dry weight was determined by filtering 10- to 20-ml culture samples through preweighed membrane filters (0.45 μm; Millipore Corp.) and then drying them to a constant weight at 95°C. The increase in weight was taken as the dry weight. Growth was followed at 550 nm with a Spectronic 20 colorimeter. One optical density unit corresponds to a cell mass of 0.75 g (dry weight) per liter. Formate consumption was determined by monitoring the formic acid reservoir and formate levels in the culture medium during growth.

Enzyme assays. A crude cell-free extract of *M. formicicum* was prepared anaerobically by passing a suspension of 15 g (wet weight) of packed cells in 15 ml of 50 mM O_2-free potassium phosphate buffer (pH 7.5) through a French pressure cell at 20,000 lb/in^2. The cell lysate was collected in serum-stoppered Corex tubes (15 ml) and centrifuged at 10,000 × *g* for 20 min in a Dupont-Sorvall RC-5B centrifuge at 4°C. The dark brown supernatant solution was collected anaerobically and stored in liquid N_2. Anaerobic conditions were maintained by flushing the French pressure cell with O_2-free N_2 while filling. The atmosphere in each centrifuge tube was evacuated and replaced with O_2-free N_2 before the addition of lysate. All solutions were made O_2-free by vacuum degassing and replacement with H_2 or N_2. Traces of O_2 were removed from the gases by passage through a column (3 by 70 cm) of reduced BASF catalyst (Chemical Dynamics Corp., Plainfield, N.J.). All transfers were made with Hamilton gas-tight syringes previously rinsed with O_2-free distilled water. Protein was determined by the Bio-Rad protein assay, using bovine serum albumin (Sigma Chemical Co.) as a standard (4).

Hydrogenase was assayed spectrophotometrically by following the reduction of methyl viologen (Sigma) ($\varepsilon = 11.3 \times 10^3\ M^{-1}\ cm^{-1}$) at 604 nm in a Beckman Acta M6 recording spectrophotometer. The reaction mixture (0.75 ml) contained (in micromoles): 2-mercaptoethanol, 1.5; potassium phosphate buffer (pH 7.5), 45; and methyl viologen, 15. The reaction mixture was made O_2-free and transferred to a serum-stoppered anaerobic cuvette which contained O_2-free H_2. The reaction was initiated by adding crude cell-free extract (5 μg of protein).

The assay for formate dehydrogenase was the same as that for hydrogenase except that O_2-free N_2 replaced H_2 in the cuvette and 7.5 μmol of sodium formate was also included in the reaction mixture (0.75 ml). The reaction was initiated by adding crude cell-free extract (0.25 mg of protein).

Formyltetrahydrofolate synthetase was assayed as previously described (7).

Coenzymes. Coenzyme F_{420} was separated from the other soluble components of the crude cell-free extract with a DEAE-cellulose (Whatman DE-52) column (1.5 by 20 cm) which had been equilibrated previously with 50 mM potassium phosphate buffer (pH 7.3). The column was developed with 200 ml of a 0 to 2 M linear NaCl gradient at a flow rate of 15 ml/

h. The peak of 420-nm-absorbing material eluted at 0.8 M NaCl. Coenzyme F_{420} was quantitated by using an extinction coefficient at 420 nm of 51.5 for a 1-mg/ml solution at pH 8.0 (6).

Chromophoric factor F_{342} was partially purified by first subjecting crude extract to ultracentrifugation for 2 h at 150,000 × *g*. The particulate-free supernatant solution was then applied to an anaerobic column of Sephadex G-75 (2.5 by 30 cm) that was equilibrated with 50 mM potassium phosphate buffer (pH 7.5) containing 2 mM sodium dithionite and 2 mM 2-mercaptoethanol. The column was eluted with the same buffer at a flow rate of 0.5 ml/min. Fractions were collected anaerobically. A Beckman Acta M6 recording spectrophotometer was used to obtain UV-VIS spectra.

RESULTS

Mass culture. An initial attempt to mass culture with formate by conventional batch methods resulted in wide fluctuations in pH and formate concentration during growth in accordance with the following equation:

$$4HCOONa + 5H_2O \rightarrow 3HCO_3^- + 3H^+ + CH_4 + 4NaOH \quad (1)$$

The use of a pH controller for the addition of formic acid resulted in a constant pH (pH 7.6 ± 0.05), and the formate concentration was maintained in a narrow range from 130 to 110 mM without strongly buffered medium. Figure 1 indicates that formate was an adequate energy source for the mass culture of *M. formicicum*. Growth was exponential, with a doubling time of 11 h and a specific growth rate constant of 0.06 h^{-1}. Rates of methane production and formate utilization were linear functions of the specific growth rate constant, which indicates that energy-yielding metabolism and energy-consuming reactions were coupled throughout growth. These conditions allowed reliable estimates of growth parameters. Maximum cell yields of 30 g (wet weight) per 12-liter fermentor were obtained, which were equivalent to yields of H_2–CO_2-grown cultures. The molar growth yield for formate ($Y_{formate}$) was 1.2 ± 0.06 g (dry weight) per mol, and $Y_{methane}$ was 4.8 ± 0.24 g (dry weight) per mol, as calculated from the stoichiometry of four formates consumed per CH_4 produced. The data were obtained with eight cultures, and measurements were made three to six times during exponential growth (data not shown). Similar results were observed with *M. hungatii* in a complex medium (data not shown).

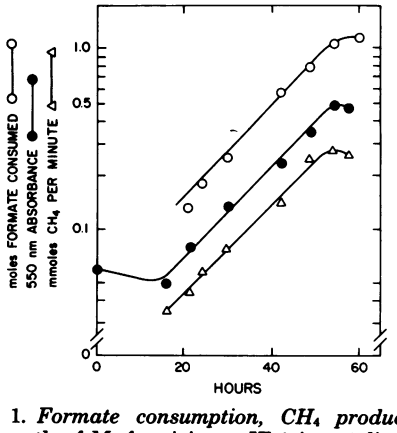

FIG. 1. *Formate consumption, CH_4 production, and growth of M. formicicum JF-1 in a 5-liter fermentor with formate as the sole energy source and sparging with N_2.*

Influence of temperature and pH. Figures 2 and 3 show the effects of temperature and pH on methane formation. The data in each figure were obtained with a single formate-grown culture, as described above (37°C and pH 7.6). When a culture reached log phase, the fermentor was adjusted to the range of temperature or pH indicated. The new rate of methane formation at each temperature or pH was recorded when the rate became constant, which was 5 to 10 min after adjustment. The increase in cell mass during the experiment was not significant. No attempt was made to determine the effect of temperature or pH on the growth of *M. formicicum*.

In the experiment shown in Fig. 2 the temperature of the culture (absorbance at 550 nm, 0.18) was decreased to 22°C and then increased in increments to 60°C. Rapid sparging with N_2 minimized the effect of temperature on the release of dissolved gases. The maximum rate of methane formation occurred at 56°C. Hydrogen became apparent in the exit gas between 49 and 63°C.

In the experiment shown in Fig. 3 the pH of the culture (absorbance at 550 nm, 0.21) was decreased to 6.9 by adding sterile 0.1 N HCl and then increased in increments from pH 6.9 to 8.8 by adding sterile 0.1 N KOH. The pH was maintained at each level by adding formic acid from the pH controller. Data obtained by reverse titration from pH 8.8 to 7.0 with formic acid are also included. Methane formation from formate with *M. formicicum* occurred over a wide pH range (from pH 6.5 to 8.5), with a broad optimum near pH 7.9. In contrast to the effect of temperature, only traces of H_2 were detected in the exit gas over the pH range tested.

Hydrogen metabolism. Data from three separate cultures grown at 37°C and pH 7.6 resulted in a mean ratio of formate utilized to methane produced of 4.05 ± 0.25, which is in close agreement with the expected value of 4.0.

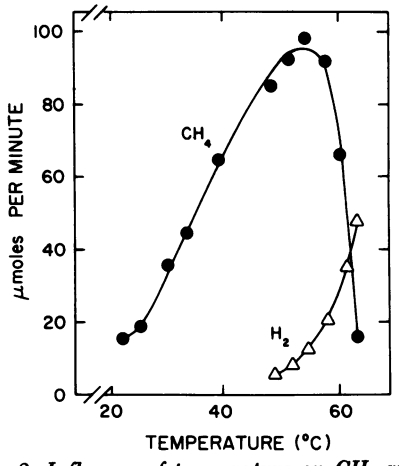

FIG. 2. *Influence of temperature on CH_4 and H_2 production from formate with a formate-grown culture of M. formicicum JF-1. Data were obtained by varying the temperature in a single log-phase culture (absorbance at 550 nm, 0.18) grown with formate at 37°C and pH 7.6.*

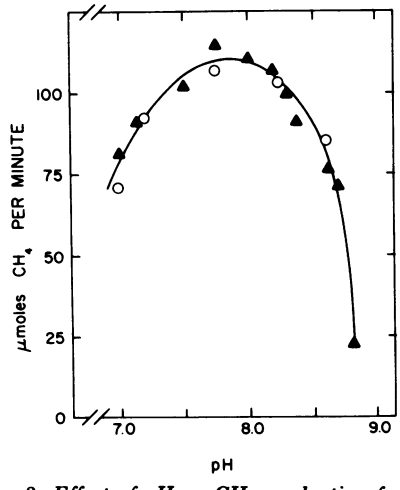

FIG. 3. *Effect of pH on CH_4 production from formate with a formate-grown culture of M. formicicum JF-1. Forward titration with 0.1 N KOH (○) and reverse titration with formic acid (●) (see text). Data were obtained by varying the pH in a single log-phase culture (absorbance at 550 nm, 0.21) grown with formate at 37°C and pH 7.6.*

The excellent agreement between experimental and expected stoichiometries suggests little or no loss of H_2 during growth with formate at 37°C and pH 7.6. Table 1 shows that H_2 in the exit gas never exceeded 10^{-4} atmospheres or 0.1 μM H_2, as calculated from Henry's constant in water at 25°C. Direct measurements revealed that the dissolved H_2 ranged between 0.1 and 0.33 μM throughout exponential growth at 40°C and a specific growth rate constant of 0.06 h^{-1} (Table 1). There is no explanation for the apparent increase in dissolved H_2 at 23 h. Above 50°C increasing amounts of H_2 were produced in response to decreased rates of methane formation (Fig. 2).

The influence of H_2 on formate utilization was determined with formate-grown cultures as described above except that 80% H_2–20% CO_2 replaced N_2 as the sparging gas. The growth curve (data not shown) was similar to that obtained with an N_2 atmosphere (Fig. 1). Formate was consumed throughout growth; however, the $Y_{formate}$ (1.6 ± 0.1 g [dry weight] per mol) with an 80% H_2–20% CO_2 atmosphere (data not shown) was significantly greater than that obtained with an N_2 atmosphere (1.2 ± 0.06 g [dry weight] per mol), which suggests that H_2 also contributed as a substrate throughout growth.

Growth parameters of H_2-CO_2 and formate. It was of interest to determine growth parameters of H_2-CO_2 and formate with the same organism under similar growth conditions since the energy available in H_2 and the energy available in formate are equal, as shown by the following equations:

$$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O \quad (\Delta G^{o\prime} = -135.6 \text{ kJ/reaction}) \quad (2)$$

$$4HCOO^- + 4H^+ + H_2O \rightarrow 3HCO_3^+ + 3H^+ + CH_4 \quad (\Delta G^{o\prime} = -130.4 \text{ kJ/reaction}) \quad (3)$$

Growth with 80% H_2–20% CO_2 was exponential, and CH_4 was a linear function of the growth rate (Fig. 4). The growth parameters of H_2–CO_2- and formate-grown cultures are shown in Table 2. The same temperature (40°C), pH (7.2), and stir rate (400 rpm) were maintained in both the H_2–CO_2- and formate-grown cultures. The growth rate constant in formate-grown cultures was only slightly less than that in H_2–CO_2-grown cultures. Greater differences between substrates were ap-

TABLE 1. *Partial pressures of CH_4 and H_2 in the exit gas and concentration of dissolved H_2 in the medium of M. formicicum JF-1 cultured with formate*

Time (h)[a]	CH_4 partial pressure ($\times 10^{-3}$ atm)[b]	H_2 partial pressure ($\times 10^{-6}$ atm)	H_2 concn (μM)
19	11.3	53	0.095
22	ND[c]	ND	0.102
23	14.9	54	0.334
26	16.6	39	0.108
27	16.8	54	0.118

[a] Time after inoculation. Measurements were taken during exponential growth.

[b] atm, Atmospheres; 1 atmosphere = 101.3 kPa.

[c] ND, Not determined.

parent with all other parameters measured. Most significant was the greater $Y_{methane}$ obtained with formate compared with that obtained with H_2-CO_2. This was substantiated by the decreased specific rate of CH_4 production ($q_{methane}$) in formate cultures compared with that in H_2-CO_2 cultures.

The ATP/CH_4 values shown in Table 2 were calculated by assuming an arbitrary Y_{ATP} value of 10.5 g (dry weight). Only 0.34 mol of ATP was synthesized per mol of CH_4 derived from H_2-CO_2, and 0.45 mol of ATP was synthesized per mol of CH_4 when the cultures were grown with formate. From the $\Delta G^{o\prime}$ values (equations 2 and 3) it would be expected that the ATP/CH_4 ratio would be approximately 2.5 for each substrate if it is assumed that 50.2 kJ/mol is required to synthesize ATP. The ATP/CH_4 ratios obtained represent efficiencies of only 14 and 18% for H_2-CO_2 and formate cultures, respectively.

Biochemical comparison of H_2-CO_2 and formate. The blue-green fluorescence of oxidized cell paste under long-wave UV light was strikingly greater in formate-grown cells than in H_2-CO_2-grown cells. An examination of cell-free extracts revealed that absorbance at 420 nm per milligram of protein was significantly greater in formate-grown cells. Table 3 shows that a two-fold-greater amount of coenzyme F_{420} was partially purified from the crude cell-free extract of formate-grown cells compared with the extract of H_2-CO_2-grown cells. The UV-VIS spectrum of coenzyme F_{420} (data not shown) was similar to the spectra obtained from other species of methanogenic bacteria (6, 9). Formate dehydrogenase and hydrogenase were present in both formate- and H_2-CO_2-grown cells. K_m values of formate dehydrogenase were 0.29 and 0.24 mM formate in H_2-CO_2- and formate-grown cells, respectively (data not shown). Washed formate-grown cell suspensions produced CH_4 from 80% H_2-20% CO_2 at a specific rate ($q_{methane}$) of 4.0 mmol of CH_4 per h per g (dry weight), and washed H_2-CO_2-grown cell suspensions produced CH_4 at a specific rate of 6.0 mmol of CH_4 per h per g (dry weight) from 50 mM formate (data not shown).

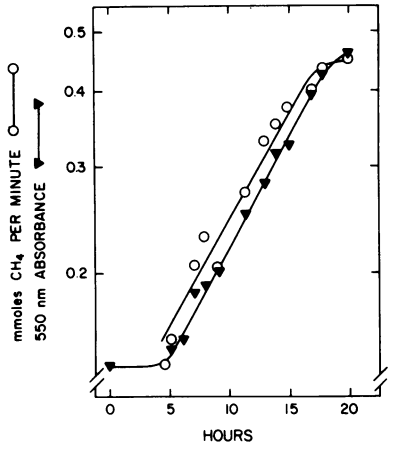

FIG. 4. *Growth and CH_4 formation of an M. formicicum JF-1 culture sparged with 80% H_2-20% CO_2 as the sole energy source.*

Low levels of formyltetrahydrofolate synthetase were found in both H_2-CO_2- and formate-grown cells. This confirms earlier work which concluded that the tetrahydrofolate pathway is not a major metabolic pathway in the methanogenic bacteria (7).

A low-molecular-weight chromophore similar to factor F_{342} (10) was present in both formate- and H_2-CO_2-grown cells. The chromophore isolated from formate-grown cells of *M. formicicum* was free of protein and exhibited major absorption peaks at 342 and 272 nm (Fig. 5). The chromophore from *M. formicicum* also exhibited a blue fluorescence under long-wave UV light. The chromophores from H_2-CO_2- and formate-grown cells were identical in every characteristic.

TABLE 2. *Comparison of growth parameters for H_2-CO_2-grown and formate-grown cultures of M. formicicum JF-1*[a]

Growth substrate	Specific growth rate constant (h^{-1})	$q_{substrate}$ (mol of substrate consumed/h per g [dry wt])	$q_{methane}$ (mol of CH_4 produced/h per g [dry wt])	$Y_{substrate}$ (g [dry wt]/mol of substrate consumed)	$Y_{methane}$ (g [dry wt]/mol of CH_4 produced)	ATP/substrate[b]	ATP/CH_4[c]
H_2-CO_2	0.082 (0.049–0.115)	ND[d]	0.027 (0.021–0.033)	ND	3.5 (2.9–4.2)	ND	0.33 (0.27–0.39)
Formate	0.055 (0.041–0.069)	0.050 (0.036–0.064)	0.012 (0.006–0.018)	1.17 (0.71–1.63)	4.8 (4.0–4.8)	0.111 (0.066–0.156)	0.46 (0.44–0.48)

[a] Mean of three replicate cultures. Values in parentheses are the 95% confidence intervals.

[b] Moles of ATP produced per mole of substrate consumed, calculated with an assumed Y_{ATP} of 10.5 g (dry weight) per mol.

[c] Moles of ATP produced per mole of CH_4 produced, calculated with an assumed Y_{ATP} of 10.5 g (dry weight) per ml.

[d] ND, Not determined.

TABLE 3. *Comparison of coenzyme F_{420} and enzyme activities in H_2-CO_2- and formate-cultured cells of M. formicicum JF-1*[a]

Growth substrate	Activity of:			
	Coenzyme F_{420} (μg/mg of protein from extract)[b]	Formate dehydrogenase (μmol of product/min per mg of protein)[c]	Hydrogenase (μmol of product/min per mg of protein)[c]	Formyltetrahydrofolate synthetase (μmol of product/min per mg of protein)[c]
H_2-CO_2	10.0 ± 2.7	0.42 ± 0.11	9.5 ± 0.11	0.003 ± 0.001
Formate	20.6 ± 4.2	0.75 ± 0.18	11.0 ± 1.5	0.004 ± 0.001

[a] Values are means ± standard deviations of three fermentor cultures.
[b] Partially purified from a crude cell-free extract.
[c] The protein was from a crude cell-free extract.

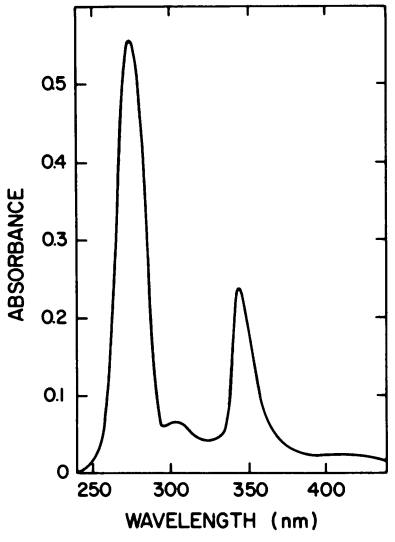

FIG. 5. *Absorption spectrum of the chromophoric factor isolated from formate-cultured cells of M. formicicum JF-1. The fraction from the Sephadex G-75 column that exhibited a strong blue fluorescnece was oxidized with air. The sample and reference buffer was 50 mM phosphate, pH 7.5.*

This compound was also observed in H_2-CO_2- and formate-grown cells of *M. hungatii*. No attempt was made to quantify the compound in either organism.

We performed experiments to determine the cellular location of formate dehydrogenase in formate-grown cells. Crude cell-free extract (9 ml) was centrifuged anaerobically at 150,000 × *g* for 2 h. The supernatant solution was removed under anaerobic conditions, and the pellet was suspended in O_2-free buffer (1 ml) which contained 2 mM sodium dithionite and 2 mM 2-mercaptoethanol. Almost all methyl viologen-dependent formate dehydrogenase activity was found in the supernatant solution (data not shown). Very little formate dehydrogenase activity was observed with whole cells or cell suspensions subjected to osmotic shock under anaerobic conditions (17). The lysate of shocked cells retained almost all of the activity observed in the cell-free extract of untreated cells, which shows that formate dehydrogenase was retained during osmotic shock.

DISCUSSION

It has not been possible to obtain accurate growth parameters with formate as the sole energy source or to compare H_2-CO_2- and formate-grown cells biochemically with the cultural methods used previously. The results presented here show that when substrate was supplied as a function of growth in a pH-stat, formate was comparable to H_2-CO_2 for the growth of methanogenic bacteria. The techniques used here should apply to the culture of acetate-utilizing methanogenic bacteria as well.

Our results show little loss of H_2 during growth with formate below 40°C, and the dissolved H_2 of the medium (0.1 to 0.3 μM) was well below the K_m of 2.0 μM H_2 (unpublished data) for H_2-CO_2-grown *M. formicicum*. Assuming that the intracellular H_2 is in equilibration with the medium, then formate-grown cultures would be substrate limited if H_2 were an obligatory intermediate. Growth rates with saturating H_2 (Fig. 4) were comparable to those in formate-grown cultures (Fig. 1), which suggests that H_2 is not an intermediate. Above 40°C the rate of methane formation decreased and H_2 increased. In *M. vaniellii* H_2 is produced in amounts up to 40% of the total gas as the pH increases (20). Further experimentation is needed before any conclusions can be drawn regarding H_2 as an intermediate during formate metabolism.

The greater $Y_{formate}$ in H_2-CO_2-sparged cultures compared with N_2-sparged formate-grown cultures indicates that both H_2 and formate are utilized when they are present in saturating amounts. It was not possible to measure H_2 uptake with the large background of 1.0 atmosphere of H_2; however, it has been confirmed with lower H_2 levels that formate and H_2 are con-

sumed concurrently (manuscript in preparation). Our results show that diauxie does not occur with H_2 and formate.

The $Y_{methane}$ values reported for other species grown with H_2-CO_2 are as follows: *Methanobacterium* strain M.o.H., 2.3 g (19); *Methanobacterium thermoautotrophicum*, 1.6 g (21); and *Methanosarcina barkerii*, 6.4 g (23). Molar growth yields with formate-grown cultures other than *M. formicicum* are not available. The results reported here and elsewhere point out the extreme inefficiency of electron transport phosphorylation, which is less than 20%.

It is interesting to compare the molar growth yields of the methanogenic bacteria with the values obtained for H_2- and formate-oxidizing sulfate-reducing bacteria. The sulfate reducers are similar in that ATP must be synthesized by electron transport phosphorylation during H_2 and formate metabolism and that the available energy is comparable to the energy obtained from the reduction of CO_2 to CH_4. The maximum $Y_{sulfate}$ values reported for sulfate-reducing bacteria grown with H_2 are as follows: *Desulfovibrio vulgaris* (Madison strain), 5.4 g (dry weight) per mol (2); and *D. vulgaris* (Marburg strain), 8.3 g (dry weight) per mol (1). The $Y_{sulfate}$ reported for *D. vulgaris* grown with formate is 11.6 g (dry weight) per mol (15). The mean value of $Y_{sulfate}$ is greater than $Y_{methane}$, which indicates a greater efficiency of electron transport phosphorylation in the sulfate-reducing bacteria, assuming that Y_{ATP} values are similar to those in the methanogenic bacteria. The abundance of cytochromes in the sulfate reducers and the inability to demonstrate functional cytochromes in methanogenic bacteria lend provisional support for this hypothesis. This difference in efficiencies may explain why sulfate reducers compete more effectively than methanogenic bacteria for H_2 in marine environments (16). Additional growth yield data with continuous cultures under various conditions of growth are needed before any conclusions can be made.

It has been shown that the formate dehydrogenase and the fumarate reductase of *Vibrio succinogenes* are located on the outer and inner aspects of the cytoplasmic membrane, respectively (14). It was proposed that this orientation of electron donor and acceptor functions to generate a proton gradient which drives ATP synthesis and transport. This principle has been extended to the methanogenic bacteria as well (24). Methyl viologen at 10^{-5} M inhibits methanogenesis from H_2-CO_2 and formate in *M. formicicum* (unpublished data), which indicates that methyl viologen penetrates the cell wall and is likely to interact with components of the cell membrane. The inability to demonstrate methyl viologen-dependent formate dehydrogenase in whole cells suggests that this activity is not located on the outer aspect of the cytoplasmic membrane. These results also suggest that formate dehydrogenase is either soluble or loosely bound to the particulate fraction. Additional experiments are needed before any conclusions can be made regarding the location of formate dehydrogenase.

The observed increase in coenzyme F_{420} in formate-grown cells compared with H_2-CO_2-grown cells indicates that formate metabolism may be dependent on coenzyme F_{420}, as suggested by previous studies (9, 22). Chromophoric factor F_{342}, for which there is no known function, was first reported in *M. thermoautotrophicum* (10). The presence of factor F_{342} can now be extended to include formate- and H_2-CO_2-grown cells of *M. formicicum* and *M. hungatii*.

It has been shown that selenium and tungsten stimulate growth of *M. vanneilii* with formate in a mineral salts medium (13). We were not able to improve the growth rate of *M. formicicum* with formate when these elements (at 1, 10, and 100 μm) and molybdenum were added singly or in combination. Sufficient amounts of these trace elements may be supplied in the yeast extract and Trypticase present in the medium.

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