Relationship of Intracellular Coenzyme F₄₂₀ Content to Growth and Metabolic Activity of *Methanobacterium bryantii* and *Methanosarcina barkeri*

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The use of F₄₂₀ as a parameter for growth or metabolic activity of methanogenic bacteria was investigated. Two representative species of methanogens were grown in batch culture: *Methanobacterium bryantii* (strain M.o.H.G.) on H₂ and CO₂, and *Methanosarcina barkeri* (strain Fusaro) on methanol or acetate. The total intracellular content of coenzyme F₄₂₀ was followed by high-resolution fluorescence spectroscopy. F₄₂₀ concentration in *M. bryantii* ranged from 1.84 to 3.65 $\mu\text{mol} \cdot \text{g}$ of protein⁻¹; and in *M. barkeri* grown with methanol it ranged from 0.84 to 1.54 $\mu\text{mol} \cdot \text{g}^{-1}$ depending on growth conditions. The content of F₄₂₀ in *M. barkeri* was influenced by a factor of 2 depending on the composition of the medium (minimal or complex) and by a factor of 3 to 4 depending on whether methanol or acetate was used as the carbon source. A comparison of F₄₂₀ content with protein, cell dry weight, optical density, and specific methane production rate showed that the intracellular content of F₄₂₀ approximately followed the increase in biomass in both strains. In contrast, no correlation was found between specific methane production rate and intracellular F₄₂₀ content. However, qCH₄(F₄₂₀), calculated by dividing the methane production rate by the coenzyme F₄₂₀ concentration, almost paralleled qCH₄(protein). These results suggest that F₄₂₀ may be used as a specific parameter for estimating the biomass, but not the metabolic activity, of methanogens; hence qCH₄(F₄₂₀) determined in mixed populations with complex carbon substrates must be considered as measure of the actual methanogenic activity and not as a measure of potential activity.

At least three different metabolic groups of microorganisms are involved in anaerobic digestion of organic material to methane and carbon dioxide (biogas): the fermentative, the acetogenic, and the methanogenic bacteria (see, e.g., reference 4). Process stability often depends on the methanogens (14, 38), which generally are considered to be the most sensitive organisms of the population (33). Therefore, an effective control of anaerobic digestion demands a selective quantification of biomass or metabolic activity of the methanogenic bacteria. Delafontaine et al. (7) were the first researchers to propose using coenzyme F₄₂₀ to assess the potential methanogenic activity [qCH₄(F₄₂₀)] in mixed populations. Coenzyme F₄₂₀ is present in all methanogens (16) and is restricted to this group of bacteria in anaerobic methanogenic environments. Only traces of F₄₂₀ or closely related compounds were found in some proactinomyces and euactinomyces, in several halophilic and acidophilic archaeobacteria (16), as well as in *Anacystis nidulans* and *Scenedesmus acutus* (for references, see reference 15). In its oxidized state, coenzyme F₄₂₀ exhibits a specific blue-green fluorescence (5) enabling its selective determination (e.g., in cell extracts). When cells are exposed to oxygen, coenzyme F₄₂₀ is degraded (26). This occurs concomitant with formation of two derivatives (F₃₉₀-A and F₃₉₀-G) which, however, are absent from cells protected from oxygen (11a). F₄₂₀ may be also used to identify methanogens by fluorescence microscopy (8, 19).

Coenzyme F₄₂₀ is a key electron carrier in metabolism (15). However, little is known about the relationship of coenzyme F₄₂₀ to biomass and metabolic activity of methanogens. Several investigators studied this relationship in

complex microbial populations (9, 20, 36; W. J. de Zeeuw, Ph.D. thesis, University of Wageningen, Wageningen, The Netherlands, 1984). Yet, under these conditions a selective determination of the methanogenic biomass is not possible. Only few data on comparable studies in pure cultures of methanogens are available. Taya et al. (29) showed that culture fluorescence paralleled cell concentration for *Methanobacterium thermoautotrophicum* and *Methanobrevibacter arboriphilus*. However, only relative fluorescence was determined and not absolute amounts of F₄₂₀. In addition, the results of these researchers do not show how the metabolic activity (qCH₄) was related to coenzyme F₄₂₀ concentration. Chua and Robinson (6) studied *Methanobacterium formicicum* in formate-limited chemostat culture. According to their data, the level of coenzyme F₄₂₀ does not vary significantly with increasing specific methanogenic activity in *Methanobacterium formicicum*.

This prompted us to study the relationship among growth, methane production, and coenzyme F₄₂₀ content in two representative species of methanogenic bacteria, *Methanobacterium bryantii* and *Methanosarcina barkeri*. These organisms utilize different metabolic pathways, involving coenzyme F₄₂₀ in different ways (13, 15). Growing only on H₂ and CO₂, *M. bryantii* is extremely specialized, representing the lithotrophic metabolic type widespread among methanogenic bacteria (1). *M. barkeri* was studied as a representative of the organotrophic metabolic type able to use compounds containing methyl groups (acetate, methanol, methylamines; in addition, *M. barkeri* also grows on H₂ and CO₂).

MATERIALS AND METHODS

Organisms and culture media. *M. barkeri* Fusaro (DSM 804) and *M. bryantii* M.o.H.G. (DSM 862) were obtained

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from the German Collection of Microorganisms, Göttingen, Federal Republic of Germany. A modified Hungate technique (3) in combination with the serum bottle technique as modified by Scherer and Sahm (24) was used for medium preparation and cultivation under strictly anaerobic conditions.

To study the relationship of coenzyme F₄₂₀ to biomass and metabolic activity, we cultivated *M. barkeri* in the complex medium described by Hippe et al. (12) with 10 ml of trace element solution S1, using methanol (200 mM) as the substrate. The final pH was 6.3 to 6.4. Trace element solution S1 (21) was modified and contained (per liter): nitrilotriacetic acid, 0.5 g; 0.5 N NaOH, 10 ml; ZnSO₄ · 7H₂O, 30.2 mg; MnCl₂ · 4H₂O, 8.9 mg; H₃BO₃, 90.0 mg; CoCl₂ · 6H₂O, 60.0 mg; CuCl₂ · 2H₂O, 3.1 mg; NiCl₂ · 6H₂O, 6.0 mg; Na₂MoO₄ · 2H₂O, 9.0 mg. *M. bryantii* was grown in a complex medium containing (per liter): K₂HPO₄ · 3H₂O, 1.44 mmol; KH₂PO₄, 1.84 mmol; (NH₄)₂SO₄, 1.89 mmol; NaCl, 8.56 mmol; MgSO₄ · 7H₂O, 1.13 mmol; CaCl₂ · 2H₂O, 423 μmol; yeast extract, 2 g; tryptone, 2 g; mineral solution (containing [per liter]: Na₂SeO₃ · 5H₂O, 2.6 mg; Na₂WO₄ · 2H₂O, 2.5 mg; NiCl₂ · 6H₂O, 121.2 mg) (34), 11 ml; vitamin solution (containing 2 mg of cyanocobalamin per liter) (34), 11 ml; sodium acetate, 1.08 mmol; Na₂CO₃, 11.90 mmol; resazurin, 4.4 μmol; antifoam solution (2% [vol/vol] polypropylene in ethanol, for fermentor cultures only), 222 μl; reducing agent solution (containing [per ml]: NaOH, 16.4 mg; cysteine hydrochloride, 29.5 mg; Na₂S · 9H₂O, 29.5 mg), 16.7 ml. The pH of the complex medium was adjusted to 7.0 to 7.1 with anaerobic, sterile 2 N HCl.

To investigate the influence of the carbon and energy source on the coenzyme F₄₂₀ content, we grew *M. barkeri* in a minimal medium containing the following constituents (per liter): CaCl₂ · 2H₂O, 1 mmol; MgCl₂ · 6H₂O, 2 mmol; NH₄Cl, 10 mmol; KCl, 5 mmol; NaCl, 38.5 mmol; trace element solution S2, 10 ml (differences from S1 [per liter]: NiCl₂ · 6H₂O, 121.2 mg; Na₂MoO₄ · 2H₂O, 12.1 mg; in addition, Na₂SeO₃ · 5H₂O, 2.6 mg); vitamin solution (containing 2 mg of cyanocobalamin per liter) (34), 10 ml; resazurin, 4.4 μmol; NaHCO₃, 10.12 mmol; (NH₄)₂Fe(SO₄)₂, 100 μmol; potassium phosphate buffer (pH 6.7; KH₂PO₄-K₂HPO₄ · 3H₂O), 4 mmol; cysteine hydrochloride, 850 μmol; Na₂S · 9H₂O, 625 μmol. CH₃COONa · 3H₂O (100 mM) was added as carbon source. When methanol (200 mM) was used as the substrate, potassium phosphate buffer was replaced with 2 mM sodium phosphate buffer (pH 7.0; Na₂HPO₄ · 2H₂O-NaH₂PO₄ · H₂O) and the final concentrations of cysteine hydrochloride and Na₂S · 9H₂O were 1.71 and 1.25 mM, respectively. The final pH of the minimal medium was 6.5 to 6.6.

Culture procedures. The growth temperature was 37°C. For studies on the relationship of coenzyme F₄₂₀ to biomass and metabolic activity, *M. barkeri* was mass cultured in 20-liter carboys with 19 liters of complex methanol medium by the method of Fiebig (Ph.D. thesis, University of Göttingen, Göttingen, Federal Republic of Germany, 1981). *M. bryantii* was cultivated in a 12-liter fermentor. Just after inoculation with 4.4% (vol/vol) of an actively growing cell suspension, the culture was gassed with H₂ and CO₂ (80:20, vol/vol) at a rate of 215 ml · min⁻¹ and stirred at 10 rpm. When the culture became visibly turbid, stirring was gradually increased to 500 rpm and the gassing rate was adjusted to 260 ml · min⁻¹. During cultivation, sulfide was periodically replenished. To investigate the influence of the substrate, we grew *M. barkeri* in sealed spherical 1.8-liter vials containing 500 ml of minimal medium. An actively gassing

cell suspension (5% [vol/vol]) served as the inoculum. Purity was checked by phase-contrast and fluorescence microscopy (Zeiss photomicroscope with the following fluorescence equipment: exciter filter BP 390-440, beam splitter FT 460, and barrier filter LP 470).

Coenzyme F₄₂₀ determination. To prevent degradation and interconversion of coenzyme F₄₂₀ (11a), all samples were anaerobically removed from the cultures, immediately cooled on ice, harvested by centrifugation at 16,000 × g for 30 min at 4°C, and washed twice with 0.9% (wt/vol) NaCl at 4°C. After the samples were frozen, they could be stored at -20°C under air without loss of coenzyme F₄₂₀ caused by oxidative degradation, as already indicated by Schönheit et al. (26). This was further substantiated by excitation and emission spectroscopy of cell extracts (see below).

A 110- to 230-mg (wet weight) sample of these cells was suspended in 3 ml of extraction buffer (Na₂HPO₄ · 2H₂O, KH₂PO₄, 50 mM each, pH 5.7). Extracts were prepared by keeping these suspensions in a boiling-water bath for 15 min under reflux condensers with magnetic stirring. After cooling in ice for 10 min, samples were centrifuged at 48,000 × g for 20 min at 2°C. Subsequently, 3.5-ml samples of the supernatant were vigorously mixed with 3.5 ml of isopropanol precooled to -20°C and centrifuged again (20 min; 48,000 × g; 2°C). The supernatants were stored under air at -20°C (14 to 19 h). Excitation and emission spectra of these preparations showed no peak or shoulder at λ_{ex} = 390 nm or λ_{em} = 450 nm, respectively, which would have indicated the presence of F₃₉₀-A and F₃₉₀-G (see above).

After thawing, samples were kept at 4°C. For dilution, they were brought to room temperature and were analyzed within 7 h. The whole period between extract preparation and measurement was long enough to convert all coenzyme F₄₂₀ molecules into their oxidized form since the fluorescence of coenzyme F₄₂₀ did not change significantly within 9 days, as was also found by van Beelen et al. (31). The fluorescence of these extracts was measured in quartz cuvettes (diameter, 1 cm) with a Perkin-Elmer model 650-10S spectrofluorimeter under the following operating conditions: excitation wavelength, 407 nm; emission wavelength, 470 nm; bandpass, 5 nm; response time, 2 s; double-beam mode; cuvette volume, 2 to 3 ml, cell holder thermostatted at 20°C. Both the wavelengths of excitation (407 nm) and emission (470 nm) as well as the method of extraction were found to be optimal for coenzyme F₄₂₀ determination under our conditions. Esculin in isopropanol was used as an external standard to quantify the fluorescence of extracts. These values were used to determine the coenzyme F₄₂₀ concentration in the extracts by using a calibration curve relating concentrations of esculin in isopropanol and of pure coenzyme F₄₂₀ (a generous gift of G. D. Vogels, Nijmegen) in extraction buffer-isopropanol (1:1, vol/vol). Each determination was repeated three times.

Analytical methods. In fermentor cultures, total gas production was measured continuously with a 1-liter wet test gas meter (Ritter, Bochum, Federal Republic of Germany) or periodically with a eudiometer. Total gas in sealed vials was measured either manometrically by the method of Scherer and Sahm (24) or with hypodermic syringes. Methane was determined on a Packard model 428 gas chromatograph equipped with a flame ionization detector. A glass column (2 m by 2 mm) with Porapak QS (80 to 100 mesh; Werner Günther Analysentechnik, Düsseldorf, Federal Republic of Germany) was employed. Gas samples of 5 μl each were injected. The optical density of cell suspensions was measured in glass cuvettes (diameter, 1 cm) with an Eppendorf

FIG. 1. Growth and concentration of coenzyme F_{420} in cultures of *M. bryantii*. The cells were cultivated with H_2 and CO_2 (complex medium). Symbols: $\circ$, cell protein; $\square$, dry weight; Δ , optical density at 600 nm; $\bullet$, coenzyme F_{420} ; arrow indicates addition of H_2S .

photometer (1101 M) at 623 nm with culture medium as the reference.

Cell dry weight was determined by filtering 10- to 250-ml culture samples through washed, dried (at 100°C for 24 h), and preweighed membrane filters (0.45- μm pore size for *M. barkeri*; 0.22- μm pore size for *M. bryantii*). The cells were washed twice with 0.9% (wt/vol) NaCl containing 0.05% (vol/vol) formaldehyde and after deposition were dried to a constant weight at 100°C. Cell protein was determined by the method of Schmidt et al. (25).

RESULTS

Relationship of coenzyme F_{420} to growth and metabolic activity. Figures 1 and 2 show that for both *M. bryantii* and *M. barkeri*, the respective growth curves (cell protein, dry weight, optical density) almost paralleled each other. For *M. bryantii*, the doubling time of cell protein was 8.8 h, the doubling time of dry weight was 10.6 h, and the doubling time of optical density was 9.4 h. For *M. barkeri*, the doubling time of cell protein was 7.1 h and that of dry weight was 7.0 h. The doubling time of coenzyme F_{420} in *M. bryantii* was 7.5 h and that in *M. barkeri* was 6.5 h.

To compare coenzyme F_{420} content and metabolic activity, we normalized methane production rates and coenzyme F_{420} concentrations to cell protein. Both quotients changed in a different way (Fig. 3 and 4). The specific methane

FIG. 2. Growth and concentration of coenzyme F_{420} in cultures of *M. barkeri*. The cells were cultivated with methanol (complex medium). Symbols: $\circ$, cell protein; $\square$, dry weight; $\bullet$, coenzyme F_{420} .

production rate, $qCH_4(\text{protein})$, had already reached its maximum at the beginning of exponential growth, and after remaining constant for a short period, it decreased, as did the methane production rate, $qCH_4(F_{420})$ (Fig. 3 and 4), even while exponential growth (cell protein, dry weight, optical density) was still going on (compare with Fig. 1 and 2). $qCH_4(\text{protein})$ reached a constant level in the fed-batch

FIG. 3. Coenzyme F_{420} content and specific methane production rates $qCH_4(\text{protein})$ and $qCH_4(F_{420})$ during growth of *M. bryantii*. The values were calculated from those of Fig. 1. Symbols: Δ , intracellular coenzyme F_{420} ; $\circ$, $qCH_4(\text{protein})$; $\square$, $qCH_4(F_{420})$.

cultures of *M. bryantii* (Fig. 3), whereas in the batch cultures of *M. barkeri*, the specific methane production rate dropped at the beginning of the stationary phase (Fig. 4) owing to limitation of methanol. In contrast, the intracellular coenzyme F₄₂₀ content did not reach its maximum until the end of exponential growth. In the stationary phase, the coenzyme F₄₂₀ content of both species decreased slightly. These results indicate that coenzyme F₄₂₀ could be used as a biomass parameter instead of cell protein, but not as a measure of metabolic activity. According to this conclusion, a specific methane production rate, $qCH_4(F_{420})$, calculated with coenzyme F₄₂₀ as the biomass parameter, changed roughly in proportion to $qCH_4(\text{protein})$ (Fig. 3 and 4).

Influence of substrate and medium composition on intracellular coenzyme F₄₂₀ content. The coenzyme F₄₂₀ content in *M. bryantii* grown with H₂ and CO₂ was higher by a factor of 2 than the coenzyme F₄₂₀ content in *M. barkeri* grown with methanol (Fig. 1 to 4). This might have been due to strain differences (11), but could also indicate an influence of the different growth substrates. To study this, we cultured *M. barkeri* in minimal medium containing 100 mM acetate or 200 mM methanol. Growth was followed by measuring total gas production. There was only a small difference in the coenzyme F₄₂₀ content of cells in the late exponential and stationary phases (Table 1; Fig. 3 and 4). However, the influence of the substrate was considerable: the intracellular coenzyme F₄₂₀ content of cells grown on methanol was three to four times higher than the coenzyme F₄₂₀ content of cells grown on acetate. The growth rate increased by a factor of 1.7. Furthermore, the coenzyme F₄₂₀ content was influenced by the composition of the culture medium (Table 1); in a minimal medium, the coenzyme F₄₂₀ content of *M. barkeri* growing on methanol was only half of that obtained in a complex medium.

DISCUSSION

It was shown here that during batch growth of both *M. bryantii* and *M. barkeri*, the intracellular concentration of coenzyme F₄₂₀ changed in accordance with the biomass parameters, i.e., cellular protein, dry weight, and optical density. The correlation was best in *M. barkeri* during

FIG. 4. Coenzyme F₄₂₀ content and specific methane production rates $qCH_4(\text{protein})$ and $qCH_4(F_{420})$ during growth of *M. barkeri*. The values were calculated from those of Fig. 2. Symbols: Δ , intracellular coenzyme F₄₂₀; $\circ$, $qCH_4(\text{protein})$; $\square$, $qCH_4(F_{420})$.

TABLE 1. Influence of substrate and medium composition on the intracellular coenzyme F₄₂₀ content of *M. barkeri*

Substrate	Medium	Growth rate (day ⁻¹)	Coenzyme F ₄₂₀ (μmol · g of protein ⁻¹) ^a
Acetate	Minimal	0.58	0.15 ± 0.004*
			0.165 ± 0.005**
Methanol	Minimal	1.01	0.648 ± 0.001*
			0.5 ± 0.014**
Methanol	Complex	2.34	1.422*
			1.220**

^a For each substrate, cultures were harvested in the late exponential (*) and in the stationary (**) phase (see text). When indicated (by their deviation from the mean), duplicate samples from two different cultures each were used. For conditions of growth, see Materials and Methods. Cultures of 500 ml (minimal) or 19 liters (complex) were used.

exponential growth (Fig. 1 and 2). In *M. bryantii*, the increase in F₄₂₀ was somewhat steeper compared with the other growth parameters. This leaves optical density, dry weight, and cellular protein as the most accurate parameters for estimating the biomass of methanogens in pure cultures. However, in both organisms the ratio of F₄₂₀ to cellular protein varied at most by a factor of 2 or 1.8, respectively (see coenzyme F₄₂₀ in Fig. 3 and 4). Thus, under conditions which do not allow a specific determination of dry weight or protein of methanogenic bacteria (mixed cultures in natural sediments or bioreactors), fluorimetric determination of coenzyme F₄₂₀ may be used to estimate methanogens. This is also suggested by findings of Taya et al. (29). Using *Methanobacterium thermoautotrophicum* and *Methanobrevibacter arboriphilus* grown with H₂ and CO₂, these investigators showed that the specific fluorescence change rate of acetone-treated cells (at $\lambda_{\text{ex}} = 404$ nm, $\lambda_{\text{em}} = 470$ nm) was nearly equal to the specific growth rate.

However, we found no correlation between $qCH_4(\text{protein})$ and the intracellular concentration of F₄₂₀ (Fig. 3 and 4). For example, in *M. barkeri* the specific methane formation, qCH_4 , dropped from a maximum of 23 mmol · h⁻¹ · g of protein⁻¹ to virtually zero (Fig. 4). In contrast, the F₄₂₀ content remained almost constant within the same time span (between 16 and 20 μg · g of protein⁻¹). This was also found by Chua and Robinson (6) with *Methanobacterium formicum* in formate-limited chemostat culture. Upon increasing the dilution rate, the specific methane production rate increased, without a significant change of the coenzyme F₄₂₀ content (6). Consequently, it is not a reliable parameter with which to measure the activity of the methanogens as was assumed by Melchior et al. (18) and Whitmore et al. (32). The method of fluorimetric determination of F₄₂₀ (as also applied in this paper) yields the total concentration of this coenzyme. Recently, it was suggested that the methanogenic activity should be assessed by measuring only the oxidized part of F₄₂₀ (22).

By using coenzyme F₄₂₀ as the biomass parameter, it is possible to define a specific methane production rate, $qCH_4(F_{420})$ (7). For *M. bryantii* and *M. barkeri*, $qCH_4(F_{420})$ changed roughly in proportion to $qCH_4(\text{protein})$ (Fig. 3 and 4). These results indicate that it may be used to follow the actual metabolic activity of the methanogens in complex microbial communities. This was also suggested by de Zeeuw (Ph.D. thesis, Agricultural University of Wageningen, 1984), who studied anaerobic treatment of mixtures of propionic and acetic acids.

When using coenzyme F₄₂₀ as a biomass parameter, the influence of growth conditions and strain differences on the

intracellular concentration of the coenzyme should be considered. Investigations by several researchers (2, 6, 11, 23, 26–28, 30, 31, 35, 39) show that the content of F_{420} in methanogens may vary widely (to facilitate the following discussion, values of F_{420} concentration were normalized to micromoles per gram of cell protein when necessary, assuming a molecular weight of $860.7 \text{ g} \cdot \text{mol}^{-1}$ [10], a protein content of 0.5 g [dry weight], and a dry weight/wet weight ratio of 0.2): the range is from $0.008 \text{ } \mu\text{mol} \cdot \text{g}$ of protein $^{-1}$ in *M. barkeri* 227 grown on acetate (2) to $7.2 \text{ } \mu\text{mol} \cdot \text{g}$ of protein $^{-1}$ in *Methanobacterium formicicum* grown on formate (6). Since these studies were done in different laboratories with different strains under different growth conditions and analytical procedures, it is difficult to estimate the extent to which growth conditions and/or strains used influenced the results. Our results show that within one and the same strain the concentration of F_{420} varied by a factor of 2 depending on medium composition (Table 1, methanol-minimal medium versus methanol-complex medium) or depending on the growth phase (Fig. 3 and 4). However, the type of substrate exerted the strongest influence. In *M. barkeri* grown on acetate in minimal medium, the coenzyme F_{420} level was one-third to one-fourth of that present in cells grown on methanol (Table 1).

There are only few comparable data from other investigators which show the influence of the substrate alone, having been evaluated with one organism and by the same analytical procedures. However, these investigations give contradictory results. As in our studies with *M. barkeri*, Schauer and Ferry (23) observed with *Methanobacterium formicicum* an influence of the substrate. They reported that the coenzyme F_{420} content was twice as high when this organism was cultivated on formate as it was during growth with H_2 and CO_2 . On the other hand, Baresi and Wolfe (2) found nearly identical levels of coenzyme F_{420} in cells of *M. barkeri* 227 grown on acetate ($0.0076 \text{ } \mu\text{mol} \cdot \text{g}$ of protein $^{-1}$), methanol ($0.007 \text{ } \mu\text{mol} \cdot \text{g}$ of protein $^{-1}$), or H_2 and CO_2 ($0.0073 \text{ } \mu\text{mol} \cdot \text{g}$ of protein $^{-1}$). Yet, their values are very low when compared with the findings of this study (Table 1; Fig. 4). The higher values we obtained during growth of *M. barkeri* Fusaro with methanol or acetate were more comparable with the data of van Beelen et al. (30) (*M. barkeri*, mixotrophic, H_2 - CO_2 and methanol: $0.32 \text{ } \mu\text{mol}$ of $F_{420} \cdot \text{g}$ of protein $^{-1}$) and Eirich et al. (11) (*M. barkeri* MS, mixotrophic, H_2 - CO_2 and acetate: $0.18 \text{ } \mu\text{mol}$ of $F_{420} \cdot \text{g}$ of protein $^{-1}$). Furthermore, even taking strain differences into account, the coenzyme F_{420} content of *M. bryantii* grown under lithotrophic conditions was significantly higher than that of *M. barkeri* Fusaro grown under organotrophic conditions (Fig. 1 to 4 and Table 1). The coenzyme F_{420} content of *M. bryantii* presented in this study was in good agreement with the data of Eirich et al. (11), while other researchers published lower values (2). It should be noted that in our study, losses of coenzyme F_{420} owing to formation of the derivatives F_{390} -A and F_{390} -G under oxygen exposure (11a) could be minimized (see Materials and Methods).

Despite the influence of strain differences, substrates, growth conditions, and analytical procedures, a comparison of the coenzyme F_{420} content as presented in this paper (Fig. 3 and 4; Table 1) and by several other researchers (2, 6, 11, 23, 26–28, 30, 31, 35, 39) indicates that the intracellular coenzyme F_{420} concentration in methanogens may decrease, depending on the substrate, in the order: formate or H_2 - CO_2 > methanol > acetate. As already shown for the NAD(H) content of several nonmethanogenic microorganisms (17), the coenzyme F_{420} content of methanogens may vary with

the metabolic pathway utilized for a special substrate. This merits further investigation.

In conclusion, caution has to be exercised when the methods of fluorimetric analysis of cell extracts dealt with in this paper are used to quantitate methanogenic activity. The influence of substrates, growth conditions, and strains used on the coenzyme F_{420} content is considerable. This may be even more so with mixed populations in a digester, as also discussed by de Zeeuw (Ph.D. thesis) with respect to H_2 - CO_2 and acetate. This method will give reliable results only if the relative proportions of the different trophic groups of methanogens involved in degradation of a given complex waste remain rather constant. That this condition may in fact be met in some cases is suggested by a comparison of colony counts of methanogenic bacteria during continuous fermentation of green and ensiled crops with varying loading rates and retention times and different plant materials used as substrates (37). In addition, in a reactor continuously fed with wastewater from pectin production, the coenzyme F_{420} level was found to be independent of the substrate concentration (36). Generally, however, the composition of the waste material added to a digester remains a critical factor which may cause population shifts toward a different trophic group (9, 20, 36).

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