CURRENT MICROBIOLOGY Vol. 38 (1999), pp. 285–289

Current Microbiology
An International Journal

Transient Production of Formate During Chemolithotrophic Growth of Anaerobic Microorganisms on Hydrogen

Verena Peters, Peter H. Janssen,* Ralf Conrad

Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Strasse, D-35043 Marburg, Germany

Received: 24 November 1998 / Accepted: 30 December 1998

Abstract. The homoacetogenic bacteria *Acetobacterium woodii*, *A. carbinolicum*, *Sporomusa ovata*, and *Eubacterium limosum*, the methanogenic archaeon *Methanobacterium formicicum*, and the sulfate-reducing bacterium *Desulfotomaculum orientis* all produced formate as an intermediate when they were growing chemolithoautotrophically with H_2 and CO_2 as sources of energy, electrons, and carbon. The sulfate-reducing bacterium *Desulfovibrio vulgaris* grew chemolithoheterotrophically with H_2 and CO_2 using acetate as carbon source, but also produced formate when growth was limited by sulfate. All these bacteria were also able to grow on formate as energy source. Formate accumulated transiently while H_2 was consumed. The maximum formate concentrations measured in cultures of *A. woodii* and *A. carbinolicum* were proportional to the initial H_2 partial pressure, giving a ratio of about 0.5 mM formate per 10 kPa H_2. The methanogen *Methanobacterium bryantii*, on the other hand, was unable to grow on formate and did not produce formate during chemolithoautotrophic growth on H_2. The results indicate that the ability to utilize formate, that is, to possess a formate dehydrogenase, was the precondition for the production of formate during chemolithotrophic growth on H_2.

Formate is a typical product of mixed acid fermentation in enterobacteria and is formed by the pyruvate formate lyase [19]. *Escherichia coli* and related enterobacteria possess a formate-hydrogen lyase enzyme system that catalyzes the reversible conversion of formate to H_2 plus CO_2 [4, 29]. Formate is also known to be a product when homoacetogenic bacteria are grown on hexoses, for example, *Clostridium aceticum* [16], *Clostridium formicoaceticum*, [1] and *Peptostreptococcus productus* [27]. Formate instead of H_2 is often produced when syntrophic bacteria grow on organic acids and alcohols, and then replaces H_2 in the so-called interspecies-formate-transfer to methanogenic archaea [5, 13, 34]. Many methanogenic archaea are able to utilize formate. They apparently route the formate into the metabolism via a formate-hydrogen lyase system consisting of formate dehydrogenase and hydrogenase [2, 38]. Indeed, these methanogens were shown to produce formate from H_2/CO_2 and, vice versa, H_2 from formate [3, 38]. In digestive tracts in which significant activities of homoacetogenic formation of acetate from H_2 and CO_2 were detected, an H_2-dependent formation of formate was also found [7, 11, 26, 28]. Elevated H_2 concentrations in anoxic sediments and paddy soil give rise to elevated formate concentrations [9, 10, 25]. There is also a rapid isotopic exchange between formate and CO_2 [10]. Hence, one may hypothesize that anaerobic chemolithotrophic bacteria that are able to utilize alternatively H_2/CO_2 or formate may be responsible for the formate production from H_2/CO_2 observed in anoxic environments.

Therefore, we systematically tested different anaerobic bacteria, that is, homoacetogens, sulfate reducers, and methanogens, that were able to grow on formate and on H_2/CO_2 to determine whether they produced formate as an intermediate during growth on H_2/CO_2, and found that all of them did.

Materials and Methods

Acetobacterium woodii WB1 (DSM 1030), *Acetobacterium carbinolicum* WoProp1 (DSM 2925), *Sporomusa ovata* H1 (DSM 2662), *Eubacterium limosum* (DSM 20543), *Desulfotomaculum orientis* Singapore1 (DSM 765), *Desulfovibrio vulgaris* Marburg (DSM 2119),

* *Present address:* Department of Microbiology and Immunology, University of Melbourne, Parkville, Victoria 3052, Australia.

Correspondence to: R. Conrad

Methanobacterium formicicum MF (DSM 1535), and *M. bryantii* M.o.H. (DSM 863) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). The bacteria were grown on the bicarbonate-buffered, sulfide-reduced medium FM described by Janssen et al. [22] at pH 7.2 and 25°C. The medium contained trace element solution [35], vitamin solution 1 [22] and a selenite/tungstate solution [35]. The bacteria were routinely cultured in 125-ml serum bottles containing 50 ml mineral medium under a gas phase of $H_2 + CO_2$ (4:1) for chemolithoautotrophic growth and under gas phase of $N_2 + CO_2$ (4:1) for heterotrophic growth with 10 mM sodium formate as substrate. For growth of sulfate-reducing bacteria, the medium was supplemented with 0.5–10 mM sodium sulfate. For growth of *D. vulgaris*, the medium was supplemented with 5 mM sodium acetate as carbon source. Formate production was assessed after the microorganisms were inoculated into new media with $N_2 + CO_2$ (4:1) gas phase, followed by injection of H_2 to give initial partial pressure of 0.5–50 kPa.

Gas samples (0.2 ml) were taken from the headspace of the culture vessel with gas-tight syringes (Dynatech, Baton Rouge, La.) and analyzed for H_2 and CH_4 by gas chromatography [31]. Fatty acids were analyzed in membrane-filtered (regenerated cellulose, 0.2 µm; Sartorius, Göttingen, Germany) liquid samples (0.1 ml) by ion exclusion high-pressure chromatography [24]. The detection limit for formate was 5 µM. Growth of bacteria was followed by measuring the optical density at 400 nm.

The activity of formate dehydrogenase (EC 1.2.99.−) was measured in cell-free extracts of *D. vulgaris* after chemolithotrophic growth on H_2 and heterotrophic growth on formate with the assay of Janssen and Morgan [21] with methylviologen as electron acceptor. The cells were harvested in the early stationary phase by centrifugation, suspended in 100 mM Tris-HCl (pH 7.5), chilled on ice, and broken by ultrasonication (Branson Sonifier 250) for 2–3 min. Whole cells and large cell debris were removed by centrifugation at 8000 *g* for 10 min. Protein concentration was determined as described by Bradford [6].

Results

All four homoacetogenic bacteria tested (*Acetobacterium woodii*; *A. carbinolicum*; *Sporomusa ovata*; *Eubacterium limosum*) produced small amounts of formate as an intermediate when growing on H_2/CO_2 as energy and carbon source. As an example, a typical experiment with *A. carbinolicum* is shown in Fig. 1A. Formate started to accumulate with the onset of H_2 consumption, reached a maximum at about 2.0 mM, and then decreased again during the late phase of H_2 consumption. Simultaneously, acetate was produced and the turbidity of the culture increased (Fig. 1A). Similar growth curves were obtained with the other homoacetogens. The growth yields were in a range of 0.5 to 1.7 gram dry weight per mol H_2 and thus similar to those reported in the literature [12, 15, 35]. Similar patterns of transient formate production were also observed with the methanogen *M. formicicum* (Fig. 1B) and the sulfate reducers *D. orientis* (Fig. 1C), but not with the methanogen *M. bryantii*, which showed no formate production (Fig. 1D). In contrast to the other microorganisms tested, *M. bryantii* is able to grow only on H_2/CO_2, but not on formate.

The maximum formate concentrations that were reached in the different bacterial cultures at different initial H_2 partial pressures are summarized in Fig. 2. Linear regression of the data obtained with *A. woodii* and *A. carbinolicum* resulted in a significant correlation ($r = 0.86$; $P < 0.01$) of the maximum formate concentrations with initial H_2 partial pressures at a ratio of approximately 0.5 mM per 10 kPa H_2. The data obtained with *S. ovata* and *M. formicicum* showed almost the same relationship, whereas *E. limosum* and *D. orientis* showed a lower ratio of formate to H_2.

In *D. vulgaris*, formate production (up to 2.6 mM) was observed only when sulfate concentrations were limiting for growth, that is, at 0.5 mM sulfate, but not at 10 mM sulfate. *D. vulgaris* exhibited activity of formate dehydrogenase after chemolithotrophic growth on H_2 (0.23 µmol min^{-1} [mg protein]$^{-1}$) as well as after heterotrophic growth on formate (0.25 µmol min^{-1} [mg protein]$^{-1}$).

Discussion

Many methanogenic, homoacetogenic and sulfate-reducing microorganisms are able to utilize both H_2/CO_2 or formate as energy and carbon source. Our results demonstrate that all of them produced formate as an intermediate during utilization of H_2/CO_2. This was the case even though formate plays different roles in the catabolism of H_2/CO_2 by the different anaerobes. Formate production by *A. woodii*, *A. carbinolicum*, *S. ovata*, and *M. formicicum* appeared to be proportional to the initial H_2 partial pressure. However, the maximum amount of formate found in the growth medium was less than that expected at thermodynamic equilibrium, which would be about 8.3 mM rather than 0.5 mM (calculated from the tables of Thauer and colleagues [33] using 10 kPa H_2, 20 kPa CO_2, pH 7.2, 25°C). The maximum amounts of formate produced by chemolithotrophically growing *E. limosum* and *D. orientis* were even lower. Probably, the observed formate concentration was the result of a dynamic equilibrium between production and consumption and thus was less than expected from the thermodynamic equilibrium. For example, formate may leak from the cells, with a much higher intracellular concentration, perhaps in equilibrium with the H_2 partial pressure, than is suggested by the levels measured in the medium.

In chemolithotrophic homoacetogenic bacteria with H_2/CO_2 as substrate, formate is an obligatory intermediate in the energy metabolism [14, 18, 37]. Reduction of CO_2 to formate production is the first step in the acetyl-CoA pathway. Formate is produced by formate dehydrogenase and is then converted to N^{10}-formyltetrahydrofolate by the N^{10}-formyltetrahydrofolate synthetase. Since free formate is formed by the formate

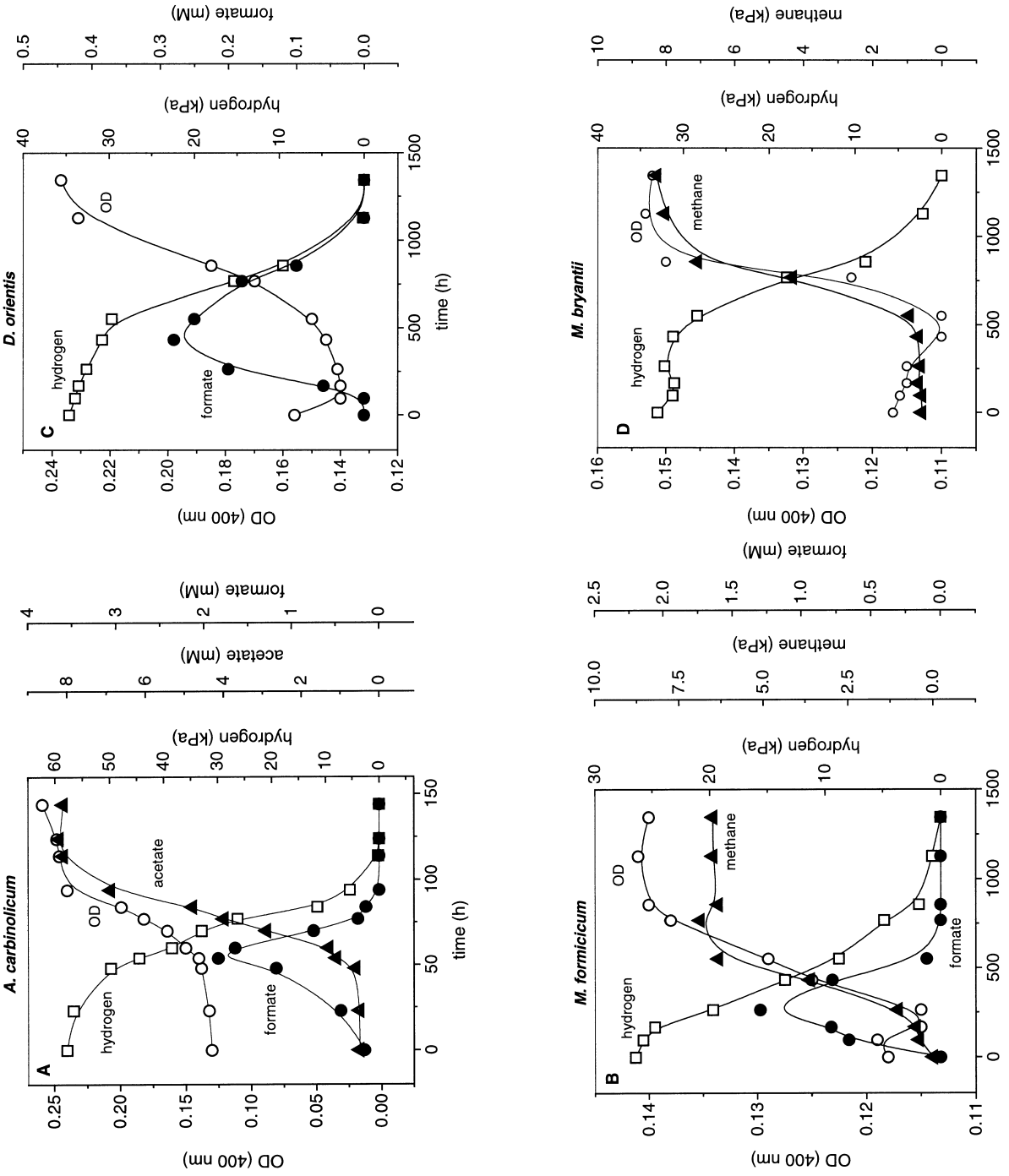

Fig. 1. Chemolithotrophic growth of (A) *Acetobacterium carbinolicum* on H_2/CO_2, (B) *Methanobacterium formicicum* on H_2/CO_2, (C) *Desulfotomaculum orientis* on H_2/CO_2/sulfate and (D) *M. bryantii* on H_2/CO_2.

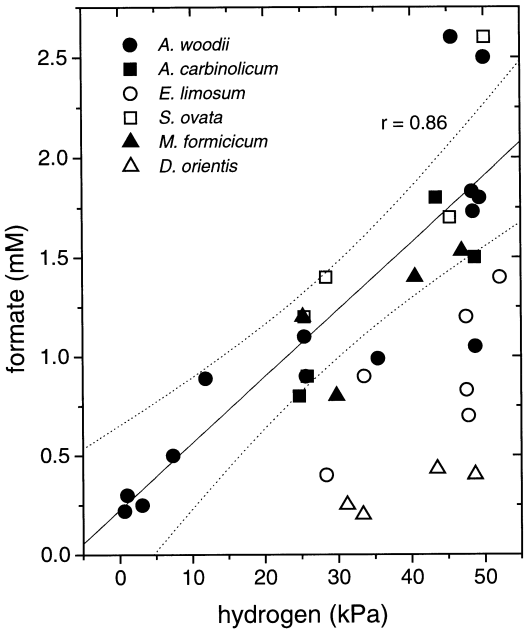

Fig. 2. Maximum concentration of formate as a function of the initial H_2 partial pressure during chemolithotrophic growth of different anaerobes on H_2/CO_2. The regression line (with 95% confidence limit) was calculated for the combined data of *A. woodii* and *A. carbinolicum*.

dehydrogenase, homoacetogens should generally be able to utilize formate as substrate, which indeed is the case [14, 23]. Interestingly, H_2 is formed as a transient intermediate in formate catabolism in *Butyribacterium methylotrophicum* [23]. Here, we show that the opposite is also true, that is, that formate is transiently formed during catabolism of H_2/CO_2 in various homoacetogens. We assume that the ATP-dependent synthesis of N^{10}-formyltetrahydrofolate constitutes a temporary bottleneck in the catabolism of H_2/CO_2. This conclusion is consistent with the observation by Yang and Drake [40] that formate was the major end-product in H_2-grown cultures of *Acetogenium kivui* impaired in energy metabolism.

Several sulfate-reducing bacteria, for example, *Desulfotomaculum orientis*, use the acetyl-CoA pathway for autotrophic synthesis of cell carbon [8]. Again, formate should be an intermediate during autotrophic growth on H_2/CO_2 and, indeed this was the result of our experiments. *Desulfovibrio vulgaris*, on the other hand, is unable to grow autotrophically and needs acetate for biosynthesis of biomass when growing with H_2/sulfate as energy source [36]. The acetyl-CoA pathway is not used for CO_2 fixation in this bacterium, and thus formate is not an intermediate in anabolism. However, *D. vulgaris* was able to grow on formate/sulfate and exhibited formate dehydrogenase activity. The existence of formate dehydrogenase in *D. vulgaris* is known from the literature [30, 39]. The presence of this enzyme probably allowed the production of formate during chemolithotrophic growth on H_2/sulfate in the presence of CO_2. *D. vulgaris* is obviously also able to produce H_2 from formate, since it can be grown on formate in a methanogenic co-culture with *Methanobacterium bryantii* that is able to utilize only H_2, but not formate [20].

In methanogens, formate is not a free intermediate in the catabolism of H_2/CO_2 to CH_4, but many methanogens are able to utilize formate as an energy source instead of H_2 [17]. In *Methanobacterium formicicum* and *Methanospirillum hungatei*, formate was shown to be routed into the metabolism by a formate–hydrogen lyase system consisting of formate dehydrogenase and hydrogenase [2, 38]. The formate–hydrogen lyase system is reversible, and H_2 was shown to be produced as an intermediate in formate catabolism and vice versa [38]. Here, we have shown that *M. bryantii*, which is unable to utilize formate, also does not produce formate as an intermediate during growth on H_2/CO_2. Similar observations were made for other methanogens that are either able or unable to utilize formate [3].

In conclusion, we assume that anaerobes that are unable to utilize formate also do not produce it as an intermediate during utilization of H_2/CO_2. Microorganisms able to utilize formate, on the other hand, also should produce it as an intermediate during growth on H_2/CO_2. This ability may be important to account for formate production in anoxic environments. In many anoxic environments formate is a significant intermediate in the carbon flow, where it is eventually converted to CH_4 by methanogens such as *M. formicicum* [32]. In some environments formate instead of H_2 may be produced by syntrophic acetogens that couple via interspecies-formate-transfer to formate-utilizing methanogens [13, 34]. However, as shown in this study, formate may also be produced in significant amounts during growth of various anaerobic chemolithotrophic bacteria on H_2/CO_2.

ACKNOWLEDGMENTS

This work was financially supported by the Fonds der Chemischen Industrie.

Literature Cited

1. Andreesen JR, Gottschalk G, Schlegel HG (1970) *Clostridium formicoaceticum* nov. spec. Isolation, description and distinction from *C. aceticum* and *C. thermoaceticum*. Arch Mikrobiol 72:154–174
2. Baron SF, Ferry JG (1989) Reconstitution and properties of a coenzyme F_{420}-mediated formate hydrogenlyase system in *Methanobacterium formicicum*. J Bacteriol 171:3854–3859
3. Bleicher K, Winter J (1994) Formate production and utilization by methanogens and by sewage sludge consortia—interference with the concept of interspecies formate transfer. Appl Microbiol Biotechnol 40:910–915

4. Böhm R, Sauter M, Böck A (1990) Nucleotide sequence and expression of an operon in *Escherichia coli* coding for formate hydrogenlyase components. Mol Microbiol 4:231–243
5. Boone DR, Johnson RL, Liu Y (1989) Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems and its implications in the measurement of K_m for H_2 or formate uptake. Appl Environ Microbiol 55:1735–1741
6. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:249–254
7. Breznak JA, Switzer JM (1986) Acetate synthesis from H_2 plus CO_2 by termite gut microbes. Appl Environ Microbiol 52:623–630
8. Brysch K, Schneider C, Fuchs G, Widdel F (1987) Lithoautotrophic growth of sulfate-reducing bacteria, and description of *Desulfobacterium autotrophicum* gen. nov., sp. nov. Arch Microbiol 148:264–274
9. Chin KJ, Conrad R (1995) Intermediary metabolism in methanogenic paddy soil and the influence of temperature. FEMS Microbiol Ecol 18:85–102
10. DeGraaf W, Cappenberg TE (1996) Evidence for isotopic exchange during metabolism of stable-isotope-labeled formate in a methanogenic sediment. Appl Environ Microbiol 62:3535–3537
11. DeGraeve KG, Grivet JP, Durand M, Beaumartin P, Cordelet C, Hannequart G, DeMeyer D (1994) Competition between reductive acetogenesis and methanogenesis in the pig large-intestinal flora. J Appl Bacteriol 76:55–61
12. Diekert G (1992) The acetogenic bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The Prokaryotes, vol 1. New York: Springer, pp 517–533
13. Dong XZ, Stams AJM (1995) Evidence for H_2 and formate formation during syntrophic butyrate and propionate degradation. Anaerobe 1:35–39
14. Drake HL (1994) Acetogenesis, acetogenic bacteria, and the acetyl-CoA ''Wood/Ljungdahl'' pathway: past and current perspectives. In: Drake HL (ed) Acetogenesis, New York: Chapman & Hall, pp 3–60
15. Eichler B, Schink B (1984) Oxidation of primary aliphatic alcohols by *Acetobacterium carbinolicum* sp. nov., a homoacetogenic anaerobe. Arch Microbiol 140:147–152
16. ElGazzawi E (1967) Neuisolierung von *Clostridium aceticum* Wieringa und stoffwechselphysiologische Untersuchungen. Arch Mikrobiol 57:1–19
17. Ferry JG, ed. (1993) Methanogenesis. Ecology, Physiology, Biochemistry and Genetics, New York: Chapman & Hall
18. Fuchs G (1986) CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol Rev 39:181–213
19. Gottschalk G (1986) Bacterial metabolism, 2nd edn. New York: Springer
20. Guyot J-P, Brauman A (1986) Methane production from formate by syntrophic association of *Methanobacterium bryantii* and *Desulfovibrio vulgaris* JJ. Appl Environ Microbiol 52:1436–1437
21. Janssen PH, Morgan HW (1992) Glucose catabolism by *Spirochaeta thermophila* RI19.B1. J Bacteriol 174:2449–2453
22. Janssen PH, Schuhmann A, Mörschel E, Rainey FA (1997) Novel anaerobic ultramicrobacteria belonging to the *Verrucomicrobiales* lineage of bacterial descent isolated by dilution culture from anoxic rice paddy soil. Appl Environ Microbiol 63:1382–1388
23. Kerby R, Zeikus JG (1987) Anaerobic catabolism of formate to acetate and CO_2 by *Butyribacterium methylotrophicum*. J Bacteriol 169:2063–2068
24. Krumböck M, Conrad R (1991) Metabolism of position-labelled glucose in anoxic methanogenic paddy soil and lake sediment. FEMS Microbiol Ecol 85:247–256
25. Krylova N, Conrad R (1998) Thermodynamics of propionate degradation in methanogenic paddy soil. FEMS Microbiol Ecol 26:281–288
26. Lajoie SF, Bank S, Miller TL, Wolin MJ (1988) Acetate production from hydrogen and [^{13}C]carbon dioxide by the microflora of human feces. Appl Environ Microbiol 54:2723–2727
27. Misoph M, Drake HL (1996) Effect of CO_2 on the fermentation capacities of the acetogen *Peptostreptococcus productus* U-1. J Bacteriol 178:3140–3145
28. Prins RA, Lankhorst A (1977) Synthesis of acetate from CO_2 in the cecum of some rodents. FEMS Microbiol Lett 1:255–258
29. Sauter M, Böhm R, Böck A (1992) Mutational analysis of the operon (*hyc*) determining hydrogenase 3 formation in *Escherichia coli*. Mol Microbiol 6:1523–1532
30. Sebban C, Blanchard L, Bruschi M, Guerlesquin F (1995) Purification and characterization of the formate dehydrogenase from *Desulfovibrio vulgaris* Hildenborough. FEMS Microbiol Lett 133: 143–149
31. Seitz HJ, Schink B, Pfennig N, Conrad R (1990) Energetics of syntrophic ethanol oxidation in defined chemostat cocultures. 1. Energy requirement for H_2 production and H_2 oxidation. Arch Microbiol 155:82–88
32. Stams AJM (1994) Metabolic interactions between anaerobic bacteria in methanogenic environments. Antonie van Leeuwenhoek 66:271–294
33. Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180
34. Thiele JH, Zeikus JG (1988) Control of interspecies electron flow during anaerobic digestion: significance of formate transfer versus hydrogen transfer during syntrophic methanogenesis in flocs. Appl Environ Microbiol 54:20–29
35. Tschech A, Pfennig N (1984) Growth yield increases linked to caffeate reduction in *Acetobacterium woodii*. Arch Microbiol 137:163–167
36. Widdel F, Hansen TA (1992) The dissimilatory sulfate- and sulfur-reducing bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The Prokaryotes, vol 1. New York: Springer, pp 583–624
37. Wood HG, Ragsdale SW, Pezacka E (1986) The acetyl-CoA pathway of autotrophic growth. FEMS Microbiol Rev 39:345–362
38. Wu WM, Hickey RF, Jain MK, Zeikus JG (1993) Energetics and regulations of formate and hydrogen metabolism by *Methanobacterium formicicum*. Arch Microbiol 159:57–65
39. Yagi T (1969) Formate:cytochrome oxidoreductase of *Desulfovibrio vulgaris*. J Biochem 66:473–478
40. Yang H, Drake HL (1990) Differential effects of sodium on hydrogen- and glucose-dependent growth of the acetogenic bacterium *Acetogenium kivui*. Appl Environ Microbiol 56:81–86