APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1987, p. 429–433
0099-2240/87/020429-05$02.00/0

Vol. 53, No. 2

Thermophilic Anaerobic Degradation of Butyrate by a Butyrate-Utilizing Bacterium in Coculture and Triculture with Methanogenic Bacteria

BIRGITTE K. AHRING†* AND PETER WESTERMANN

Department of General Microbiology, University of Copenhagen, Sølvgade 83 H, 1307 Copenhagen K, Denmark

Received 22 July 1986/Accepted 17 November 1986

We studied syntrophic butyrate degradation in thermophilic mixed cultures containing a butyrate-degrading bacterium isolated in coculture with *Methanobacterium thermoautotrophicum* or in triculture with *M. thermoautotrophicum* and the TAM organism, a thermophilic acetate-utilizing methanogenic bacterium. Butyrate was β-oxidized to acetate with protons as the electron acceptors. Acetate was used concurrently with its production in the triculture. We found a higher butyrate degradation rate in the triculture, in which both hydrogen and acetate were utilized, than in the coculture, in which acetate accumulated. Yeast extract, rumen fluid, and clarified digestor fluid stimulated butyrate degradation, while the effect of Trypticase was less pronounced. Penicillin G, D-cycloserine, and vancomycin caused complete inhibition of butyrate utilization by the cultures. No growth or degradation of butyrate occurred when 2-bromoethanesulfonic acid or chloroform, specific inhibitors of methanogenic bacteria, was added to the cultures and common electron acceptors such as sulfate, nitrate, and fumarate were not used with butyrate as the electron donor. Addition of hydrogen or oxygen to the gas phase immediately stopped growth and butyrate degradation by the cultures. Butyrate was, however, metabolized at approximately the same rate when hydrogen was removed from the cultures and was metabolized at a reduced rate in the cultures previously exposed to hydrogen.

At least three entirely different groups of bacteria are active during complete anaerobic conversion of complex organic molecules to carbon dioxide and methane (8, 9, 15, 24). The fermentative bacteria hydrolyze the polymers to mono- and oligomers by means of extracellular enzymes. The soluble products are then absorbed and fermented by the same group of bacteria to an array of products such as acetate and other short-chained fatty acids, alcohols, hydrogen, and carbon dioxide. The second metabolic group, the obligately hydrogen-producing acetogenic bacteria, oxidize propionate, butyrate, and other fatty acids and alcohols to acetate, hydrogen, and carbon dioxide. Owing to the unfavorable thermodynamics of these reactions, the metabolism of the acetogenic bacteria demands a low partial pressure of hydrogen. Terminally, the methanogenic bacteria utilize the acetate and hydrogen produced by the first two microbial groups, forming methane.

Normally, propionate and butyrate account for 20% of the methane produced in an anaerobic digestor (18). Several mesophilic fatty acid-utilizing acetogenic bacteria in coculture with either hydrogen-utilizing methanogenic bacteria or sulfate-reducing bacteria have been described. *Syntrophomonas wolfei* β-oxidizes even- and odd-numbered fatty acids to acetate and hydrogen or acetate, propionate, and hydrogen (19, 20). *Syntrophobacter wolinii* degrades propionate to acetate, hydrogen, and carbon dioxide (6). A number of sporeforming bacteria degrading butyrate in coculture with hydrogen-consuming bacteria have recently been isolated (21–23). The only thermophilic hydrogen-producing acetogenic bacterium previously described is a butyrate-degrading bacterium isolated in coculture with the hydrogen-utilizing *Methanobacterium thermoautotrophicum* (11).

In the present paper, we describe the isolation and metabolism of a thermophilic butyrate-degrading bacterium when grown in syntrophic association with *M. thermoautotrophicum* (the coculture) or *M. thermoautotrophicum* and the thermophilic acetate-utilizing methanogenic TAM organism (the triculture).

MATERIALS AND METHODS

Source of organisms. The initial enrichment was made from a thermophilic bench-scale digestor operating at 60°C on sewage sludge obtained from the sewage treatment plant in Usserød, Denmark.

Media and conditions for cultivation. Anaerobic techniques were used as described by Hungate (13) and modified by Bryant (7) and Balch and Wolfe (5). The basal medium used for enrichment and cultivation of the cocultures was basically the sulfate-free medium used for enrichment and isolation of the TAM organism (1, 2). The butyrate concentration was 10 mM, added as sodium butyrate, and the basal medium was usually supplemented with 0.1% yeast extract. Experiments were performed in serum vials (50 ml, containing 25 ml of medium) closed with butyl rubber stoppers and aluminum crimps (5). Unless stated otherwise, the gas phase in the vials was 80% N_2–20% CO_2 pressurized to 1 atm (101.3 kPa) overpressure and all incubations were made at 60°C in triplicate. The inocula used were 5% (vol/vol), and the triculture was obtained from a chemostat.

Enrichment and isolation. Enrichment of the butyrate-degrading cocultures was carried out in a 1-liter serum bottle containing 750 ml of butyrate basal medium inoculated with 10 ml of thermophilic digested sludge. The serum bottle was operated in a semicontinuous system as previously described for enrichment of the TAM organism (1), with the

* Corresponding author.
† Present address: Institute of Biotechnology, The Technical University of Denmark, 2800 Lyngby, Denmark.

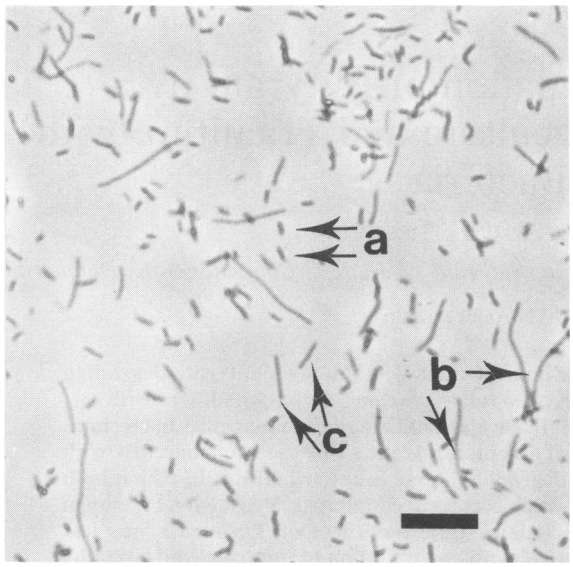

FIG. 1. Phase-contrast photomicrograph of the triculture. (A) Butyrate-utilizing bacteria; (B) *M. thermoautotrophicum*; (C) TAM organisms. Bar, 10 μM.

exception that butyrate basal medium was used instead of acetate basal medium.

After 8 months of continuous cultivation, isolation was performed by serial dilution of the enrichment culture in basal medium and inoculation of 0.5 ml of each dilution into agar (3%) roll tubes of medium with and without butyrate. Before the tubes were solified, half of them received 0.5 ml of a log-phase *M. thermoautotrophicum* previously isolated from the thermophilic sludge digestor (3).

Culture purity. Cultures were routinely checked for heterotrophic contaminants by microscopic examination and inoculation into thioglycolate medium (Difco Laboratories) and AC medium (Difco) incubated under anaerobic and aerobic conditions which allowed no growth of the cultures.

Growth and fermentation balance. Growth of the cultures was defined in terms of the specific butyrate consumption rate (μ_{but}), calculated as the slope of the linear part of the half-logarithmic graph of butyrate concentration versus time. The formation of fermentation products with time was monitored by inoculating vials with and without butyrate. Fermentation balances were determined from measurements at the beginning and end of the incubation period, and the values were corrected for the small amount of products produced in vials without substrate.

Analytic procedures. Acetate and butyrate were measured by gas chromatography with a flame ionization detector as previously described (1, 4). Gases were separated on Porapak Q; methane was detected by flame ionization as previously described (1), and hydrogen and carbon dioxide were detected by thermal conductivity with H_2 or N_2 as the carrier gas, respectively. When carbon dioxide was quantified, the measured values were corrected for the amount absorbed in the medium as determined by injection of different concentrations of CO_2 into vials of uninoculated medium. All gas injections were carried out with Pressure-lok gastight syringes (Precision Sampling Corp.).

Gases and chemicals. High-purity gases were used, and traces of oxygen were removed by passing the gases over heated copper (300°C). All chemicals were reagent grade.

RESULTS

Isolation and description of the cocultures. After several months of continuous enrichment in butyrate basal medium, a relatively stable consortium of long, rod-shaped bacteria together with a smaller number of curved rods developed. Only a few (10^3/ml) heterotrophic contaminants could be counted. Two colony types appeared in roll tubes that were made from the 10^{-6} dilution and contained butyrate. One colony type was approximately 2 mm in diameter, yellow to brownish, and irregular in shape, while the other type was smaller, whitish, and with an entire circumference. The latter type was the more numerous colony in the roll tubes to which a large inoculum of an actively growing culture of *M. thermoautotrophicum* was added before the tubes were solidified. Microscopic examination showed that this colony consisted of *M. thermoautotrophicum* and a slightly curved rod, while the first type was a three-membered colony also containing a straight, filamentous rod similar to the thermophilic acetate-utilizing methanogen, the TAM organism, previously described (1, 2). Individual colonies were picked and transferred to the same medium. A photomicrograph of the triculture is shown in Fig 1. The butyrate-degrading bacterium was found to be a nonmotile curved rod that had tapered to round ends and stained gram negative. The organism consisted of one or two cells with a mean dimension of 0.5 to 0.8 μm in width and 2 to 3 μm in length and contained no spores.

Metabolism of the cultures. Figure 2A and B shows the consumption of butyrate and production of metabolic products by the cultures. Acetate accumulated in the coculture but was used concurrently with its production in the triculture. Carbon dioxide was produced only in the triculture, and more methane was produced in this culture than in the coculture. Only low concentrations (10^{-3} to 10^{-4} atm; 101.3 to 10.13 Pa) of hydrogen could be detected in the headspaces during the exponential phase of the butyrate degradation. The specific butyrate consumption rates determined from the experiments shown in Fig. 2A and B were somewhat lower for the coculture (0.0201 h^{-1}) than for the triculture (0.0323 h^{-1}), indicating that acetate utilization influences the growth of the butyrate-degrading bacterium.

Table 1 shows the fermentation products formed by the cultures. Methane and carbon dioxide and methane and acetate were the principal end products of the triculture and the coculture, respectively, representing 94 and 96.3% of the available carbon from butyrate. The results supported the following stoichiometric reactions: $CH_3CH_2CH_2COO^- + 2H_2O \rightleftharpoons 2CH_3COO^- + H^+ + 2H_2$ in the coculture and $CH_3CH_2CH_2COO^- + 2H_2O \rightleftharpoons \frac{5}{2}CH_4 + \frac{1}{2}CO_2 + HCO_3^-$ in the triculture in which butyrate conversion to methane was complete.

Studies on the cocultures. Addition of clarified rumen fluid (3 to 5%), yeast extract (0.5 to 2 g/liter), and clarified digestor fluid (3 to 5%) had a pronounced stimulating effect on the specific rate of butyrate consumption, while the addition of Trypticase (BBL Microbiology Systems, Cockeysville, Md.) (0.5 to 2 g/liter) was not as effective (Table 2).

Complete inhibition of growth and butyrate consumption was found upon addition of penicillin G (1 g/liter), D-cycloserine (0.1 g/liter), and vancomycin (0.1 g/liter) to the cultures. Also, addition of specific inhibitors of methanogenic bacteria, 2 bromoethanesulfonic acid (2-BES) (2 mM) and chloroform (5 μM), was completely inhibitory. Electron acceptors such as fumarate, nitrate, and sulfate, added to give a final concentration of 1 mM, were not used by the

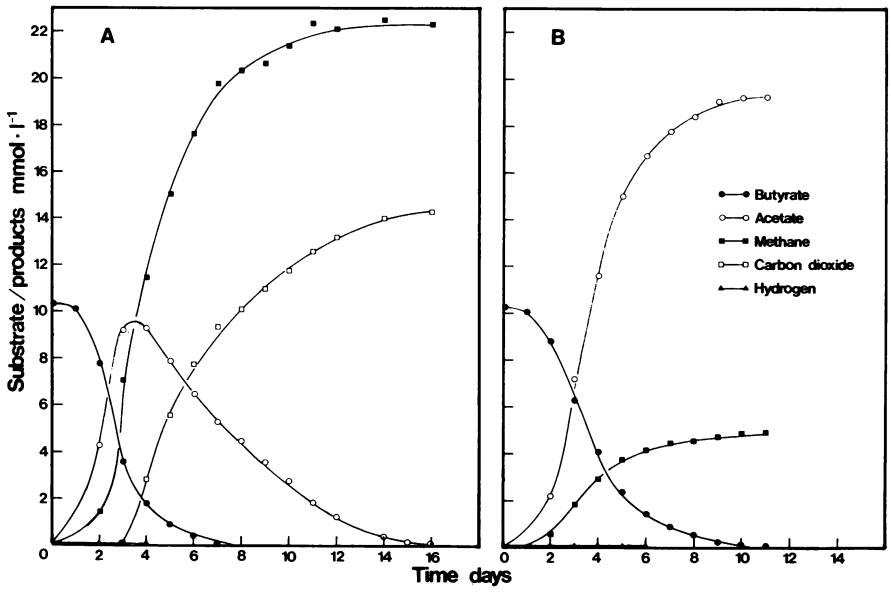

FIG. 2. Time course of butyrate degradation and product formation. (A) Triculture; (B) coculture.

cultures, not even under conditions for which the methanogenic bacteria were inhibited by 2-BES.

Addition of oxygen to the gas phase of exponentially growing cultures immediately stopped growth of and butyrate utilization by the cultures (Fig. 3). The butyrate consumption, however, was resumed afer a lag period of 1 to 2 days when the cells were reincubated under anaerobic conditions, but the rate was lower than the butyrate consumption rate obtained with untreated cocultures. Microscopic examination revealed various numbers of lysed cells in the cultures exposed to oxygen. Hydrogen in the gas phase at a concentration of more than 2×10^{-2} atm (2.026×10^3 Pa) was also found to be completely inhibitory to the consumption of butyrate by the cultures (Fig. 4). In contrast to the situation with oxygen, however, butyrate consumption continued at the same rate after a lag period, when hydrogen was eliminated from the cultures.

DISCUSSION

The isolation of cocultures containing a butyrate-degrading bacterium oxidizing butyrate at thermophilic temperatures in syntrophic association with the hydrogen-utilizing methanogenic bacterium *M. thermoautotrophicum* (11; this work) provides evidence for the existence of thermophilic obligately hydrogen-producing acetogenic bacteria similar to the bacteria isolated under mesophilic conditions (6, 9, 19–23). Our butyrate-degrading bacterium was morphologically similar to the thermophilic butyrate-degrading bacterium described by Henson and Smith (11), except that it was found both singly and in pairs, as was the mesophilic butyrate-degrading bacterium *Syntrophomonas wolfei* (19, 20). Furthermore, the acetate-utilizing bacterium in the butyrate enrichments and in the cultures isolated from butyrate enrichments by Henson and Smith was a *Methanosarcina* sp. rather than the TAM organism. Our thermophilic butyrate degrader was stimulated by a growth factor (factors) present in rumen fluid, yeast extract, and clarified digestor fluid, while the bacterium isolated by Henson and Smith (11) required the presence of supplement in clarified digestor fluid and, to some extent, in rumen fluid. Butyrate was utilized at a higher rate in our cultures when incubated with shaking, in contrast to the results obtained by

TABLE 1. Fermentation products produced from 10 mmol of butyrate liter^{-1} by the thermophilic butyrate-degrading coculture and triculture

Conditions	Products[a] (mmol liter^{-1})[b]			Carbon recovery (%)
	Acetate	CH_4	CO_2	
Coculture	19.3 ± 0.4	5.0 ± 0.2	−4.95[c]	96.3
Triculture	0	23.3 ± 0.8	14.3 ± 0.8	94.0

[a] Other fermentation products were not found.
[b] Values are means of triplicate vials ± standard deviations.
[c] Calculated as equal to the amount of methane.

TABLE 2. Specific butyrate consumption rate (μ_{but}) of the coculture and triculture in various media

Supplement[a]	μ_{but} (h^{-1})[b] for:	
	Coculture	Triculture
None	0.0121 ± 0.002	0.0156 ± 0.002
Clarified rumen fluid (3%, vol/vol)	0.0198 ± 0.005	0.0310 ± 0.008
Clarified rumen fluid (5%, vol/vol)	0.0201 ± 0.004	0.0345 ± 0.007
Clarified digestor fluid (3%, vol/vol)	0.0190 ± 0.006	0.0295 ± 0.009
Clarified digestor fluid (5%, vol/vol)	0.0192 ± 0.005	0.0311 ± 0.008
Yeast extract (0.5 g/liter)	0.0195 ± 0.007	0.0290 ± 0.008
Yeast extract (1.0 g/liter)	0.0210 ± 0.004	0.0325 ± 0.005
Yeast extract (2.0 g/liter)	0.0201 ± 0.005	0.0330 ± 0.006
Trypticase (0.5 g/liter)	0.0155 ± 0.008	0.0253 ± 0.009
Trypticase (2 g/liter)	0.0165 ± 0.006	0.0251 ± 0.008

[a] The butyrate basal medium was supplemented with the indicated growth factors.
[b] Values are means of triplicate vials ± standard deviations.

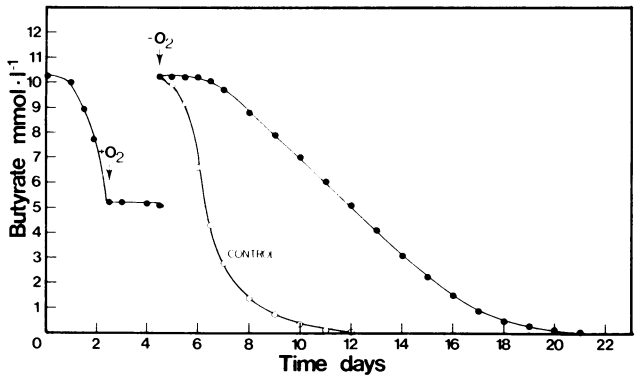

FIG. 3. Effect of oxygen on butyrate consumption by the triculture. Oxygen (10% O_2–70% N_2–20% CO_2) was introduced into the gas phase after 2.5 days of incubation, as indicated by an arrow. At day 4.5, the cells were reincubated under anaerobic conditions together with untreated tricultures (controls), as indicated by an arrow.

Henson and Smith (11), which showed that butyrate was consumed more quickly when the cocultures were incubated in static culture.

All fatty acid-oxidizing bacteria described previously were isolated in roll tubes by using a hydrogen-oxidizing bacterium (a sulfate reducer or a methanogen) as a scavanger for hydrogen (6, 11, 19–23). The isolation of a thermophilic butyrate-degrading bacterium described in this paper was possible without addition of a further inoculum of hydrogen-utilizing bacteria probably owing to the high numbers of *M. thermoautotrophicum* already present in the enrichment culture (approximately 75% of the bacteria present). Normally, colonies containing the butyrate-degrading bacterium together with both *M. thermoautotrophicum* and the TAM organism dominated in roll tubes made from the enrichment culture. However, if the roll tubes were supplemented with a large inoculum of *M. thermoautotrophicum*, colonies consisting of the butyrate-degrading bacterium together with *M. thermoautotrophicum* were the most numerous type. No differences were observed in either morphotypes or metabolism between cocultures and tricultures isolated with or without a lawn of *M. thermoautotrophicum*.

The products formed from the degradation of butyrate by the coculture were similar to products of *Syntrophomonas wolfei* (19, 20) and imply a β-oxidation mechanism with hydrogen as electron sink. In the triculture, the acetate formed was converted to equimolar amounts of methane and carbon dioxide, as shown for pure cultures of the TAM organism (1, 2). The products formed in this culture are in accordance with the stoichiometry proposed by Lawrence and McCarty (17).

The higher butyrate consumption rate found for the triculture utilizing acetate as well as hydrogen can be explained partially by product inhibition by acetate in the coculture in which acetate accumulated, since experiments have shown that acetate concentrations of 10 and 25 mM cause an inhibition of the butyrate consumption by the coculture of 6.9 and 33.5%, respectively (manuscript in preparation). Another reason could be that a higher degree of stability was found in the triculture, in which the degradation of butyrate was complete. Acetate metabolism has been shown to be the rate-limiting process for complete degradation of butyrate, and the numbers of acetate-utilizing methanogens tend to decrease when the coculture is cultivated in batch cultures by repeated transfers of inocula (4). Batch cultivation of the three-membered coculture over time resulted in a greater extent of acetate accumulation before its utilization and a butyrate consumption rate that approached the rate of that of the two-membered coculture.

Methanogenic bacteria have been shown not to be affected by antibiotics that have a specific effect on the cell wall polymers (1, 10, 12, 14). The sensitivity of the cultures to antibiotics known to act upon the cross-linking reactions of the cell wall shows that the thermophilic butyrate-degrading bacterium probably is a eubacterium containing a peptidoglycan wall, as shown for *Syntrophomonas wolfei* (19).

The inhibition of butyrate utilization by specific inhibitors of methanogenic bacteria, e.g., 2-BES and chloroform, shows that the butyrate-degrading bacteria are dependent upon the activity of the hydrogen-utilizing methanogenic bacteria. The effect of 2-BES was caused merely by an inhibition of the methanogenic bacteria, and growth and butyrate utilization were observed after a lag period in cocultures inhibited with 2-BES, when a large inoculum of a thermophilic hydrogen-oxidizing *Desulfovibrio* sp. was added. Common electron acceptors such as fumarate, sulfate, and nitrate could not be utilized instead of protons.

The degradation of butyrate by the cultures was possible only under anaerobic conditions. Oxygen reduced the butyrate consumption rate obtained when the cultures were reincubated under anaerobic conditions, which could be explained by the presence of the lysed cells in cocultures previously exposed to oxygen. Some methanogenic bacteria, including *M. thermoautotrophicum*, have been shown to be relatively tolerant to oxygen (14, 16). When exponentially growing cultures of the TAM organism were exposed to oxygen and afterwards treated as described for the cocultures, the same methane production rate as that for untreated cultures was obtained after a short lag period (data not shown). This indicates that the thermophilic butyrate-

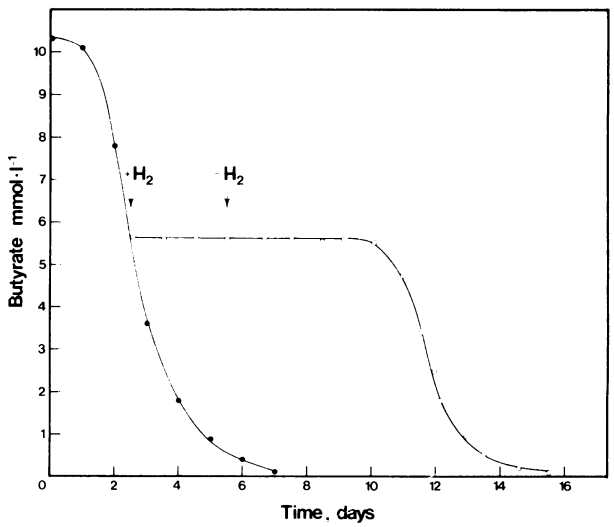

FIG. 4. Effect of hydrogen on butyrate consumption by the tricultures. Hydrogen (10% H_2–70% N_2–20% CO_2) was introduced into the gas phase after 2.5 days of incubation (indicated by an arrow) and was replaced twice a day until day 5.5, when the H_2 was removed (indicated by an arrow). Symbols: ●, tricultures incubated under 80% N_2–20% CO_2 for the entire experiment; ○, tricultures incubated with 10% H_2–70% N_2–20% CO_2 for 3 days and then reincubated under 80% N_2–20% CO_2.

degrading bacterium is extremely sensitive to oxygen and that oxygen has a toxic effect on this bacterium.

The presence of hydrogen in the gas phase inhibited butyrate utilization by the cultures as also shown for *Syntrophomonas wolfei* (19) and the thermophilic butyrate-degrading bacterium isolated by Henson and Smith (11). Butyrate utilization, however, resumed at the same rate when hydrogen was removed from the cultures, indicating that hydrogen merely caused a product inhibition of the metabolism instead of a toxic effect on the butyrate-degrading bacterium.

ACKNOWLEDGMENTS

We thank Karin Vestberg for excellent technical assistance.

This work was supported by grants 11-3888, 11-3982, and 11-5080 from the Danish Natural Science Research Council.

LITERATURE CITED

1. **Ahring, B. K., and P. Westermann.** 1984. Isolation and characterization of a thermophilic, acetate-utilizing methanogenic bacterium. FEMS Microbiol. Lett. **25**:47–52.
2. **Ahring, B. K., and P. Westermann.** 1985. Methanogenesis from acetate: physiology of a thermophilic, acetate-utilizing methanogenic bacterium. FEMS Microbiol. Lett. **28**:15–19.
3. **Ahring, B. K., and P. Westermann.** 1985. Sensitivity of thermophilic methanogenic bacteria to heavy metals. Curr. Microbiol. **12**:273–276.
4. **Ahring, B. K., and P. Westermann.** 1987. Kinetics of butyrate, acetate, and hydrogen metabolism in a thermophilic, anaerobic, butyrate-degrading coculture. Appl. Environ. Microbiol. **53**: 434–439.
5. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **32**:781–791.
6. **Boone, D. R., and M. P. Bryant.** 1980. Propionate-degrading bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from methanogenic ecosystems. Appl. Environ. Microbiol. **40**:626–632.
7. **Bryant, M. P.** 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. **25**:1324–1328.
8. **Bryant, M. P.** 1979. Microbial methane production-theoretical aspects. J. Anim. Sci. **48**:193–201.
9. **Bryant, M. P., E. A. Wolin, M. J. Wolin, and R. S. Wolfe.** 1967. *Methanobacillus omelianskii*, a symbiotic association of two species of bacteria. Arch. Microbiol. **59**:20–31.
10. **Godsy, E. M.** 1980. Isolation of *Methanobacterium bryantii* from a deep aquifier by using a novel broth-antibiotic disk method. Appl. Environ. Microbiol. **39**:1074–1075.
11. **Henson, J. M., and P. H. Smith.** 1985. Isolation of a butyrate-utilizing bacterium in coculture with *Methanobacterium thermoautotrophicum* from a thermophilic digester. Appl. Environ. Microbiol. **49**:1461–1466.
12. **Hilpert, R., J. Winther, W. Hammes, and O. Kandler.** 1981. Sensitivity of archaebacteria to antibiotics. Zentralbl. Bakteriol. Mikrobiol. Hyg., 1 Abt. Orig. C **2**:11–20.
13. **Hungate, R. E.** 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. **14**:1–49.
14. **Huser, B. A., K. Wuhrmann, and A. J. B. Zehnder.** 1982. *Methanothrix soehngenii* gen. nov. sp. nov., a new acetotrophic non-hydrogen-oxidizing methane bacterium. Arch. Microbiol. **132**:1–9.
15. **Kaspar, H. F., and K. Wuhrmann.** 1978. Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. Appl. Environ. Microbiol. **36**:1–7.
16. **Kiener, A., and T. Leisinger.** 1983. Oxygen sensitivity of methanogenic bacteria. Syst. Appl. Microbiol. **4**:305–312.
17. **Lawrence, A. W., and P. L. McCarty.** 1969. Kinetics of methane fermentation in anaerobic treatment. J. Water Pollut. Control Fed. **41**:RI–R17.
18. **Mackie, R. I., and M. P. Bryant.** 1981. Metabolic activity of fatty acid-oxidizing bacteria and the contribution of acetate, propionate, butyrate, and CO_2 to methanogenesis in cattle waste at 40 and 60°C. Appl. Environ. Microbiol. **41**:1363–1373.
19. **McInerney, M. J., M. P. Bryant, R. B. Hespell, and J. W. Costerton.** 1981. *Syntrophomonas wolfei* gen. nov. sp. nov., an anaerobic, syntrophic, fatty acid-oxidizing bacterium. Appl. Environ. Microbiol. **41**:1029–1039.
20. **McInerney, M. J., M. P. Bryant, and N. Pfennig.** 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch. Microbiol. **122**:129–135.
21. **Shelton, D. R., and J. M. Tiedje.** 1984. Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol. **48**:840–848.
22. **Stieb, M., and B. Schink.** 1985. Anaerobic oxidation of fatty acids by *Clostridium bryantii* sp. nov., a sporeforming obligately syntrophic bacterium. Arch. Microbiol. **140**:387–390.
23. **Tomei, F. A., J. S. Maki, and R. Mitchell.** 1985. Interactions in syntrophic associations of endospore-forming, butyrate-degrading bacteria and H_2-consuming bacteria. Appl. Environ. Microbiol. **50**:1244–1250.
24. **Zehnder, A. J. B., K. Ingvorsen, and T. Marti.** 1982. Microbiology of methane bacteria, p. 45–68. *In* D. E. Hughes (ed.), Anaerobic digestion 1981. Elsevier/North-Holland Biomedical Press, Amsterdam.