APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1981, p. 346-354
0099-2240/81/020346-09$02.00/0

Vol. 41, No. 2

Anaerobic Degradation of Lactate by Syntrophic Associations of *Methanosarcina barkeri* and *Desulfovibrio* Species and Effect of H_2 on Acetate Degradation

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When grown in the absence of added sulfate, cocultures of *Desulfovibrio desulfuricans* or *Desulfovibrio vulgaris* with *Methanobrevibacter smithii* (*Methanobacterium ruminantium*), which uses H_2 and CO_2 for methanogenesis, degraded lactate, with the production of acetate and CH_4. When *D. desulfuricans* or *D. vulgaris* was grown in the absence of added sulfate in coculture with *Methanosarcina barkeri* (type strain), which uses both H_2-CO_2 and acetate for methanogenesis, lactate was stoichiometrically degraded to CH_4 and presumably to CO_2. During the first 12 days of incubation of the *D. desulfuricans-M. barkeri* coculture, lactate was completely degraded, with almost stoichiometric production of acetate and CH_4. Later, acetate was degraded to CH_4 and presumably to CO_2. In experiments in which 20 mM acetate and 0 to 20 mM lactate were added to *D. desulfuricans-M. barkeri* cocultures, no detectable degradation of acetate occurred until the lactate was catabolized. The ultimate rate of acetate utilization for methanogenesis was greater for those cocultures receiving the highest levels of lactate. A small amount of H_2 was detected in cocultures which contained *D. desulfuricans* and *M. barkeri* until after all lactate was degraded. The addition of H_2, but not of lactate, to the growth medium inhibited acetate degradation by pure cultures of *M. barkeri*. Pure cultures of *M. barkeri* produced CH_4 from acetate at a rate equivalent to that observed for cocultures containing *M. barkeri*. Inocula of *M. barkeri* grown with H_2-CO_2 as the methanogenic substrate produced CH_4 from acetate at a rate equivalent to that observed for acetate-grown inocula when grown in a rumen fluid-vitamin-based medium but not when grown in a yeast extract-based medium. The results suggest that H_2 produced by the *Desulfovibrio* species during growth with lactate inhibited acetate degradation by *M. barkeri*.

Complete breakdown of biodegradable organic matter in anaerobic ecosystems without light or electron acceptors other than CO_2 involves at least three general metabolic groups of microbes whose metabolic interactions lead to the final products CO_2, methane, ammonia, and microbial cells (5, 9, 11). A complex group of anaerobes hydrolyzes and ferments the primary substrates, with major production of fatty acids, CO_2, H_2, and ammonia. The intermediate metabolic group, the obligate proton-reducing (H_2-forming) acetogenic bacteria, oxidize fatty acids to acetate, CO_2, and H_2 (3, 5, 7, 10-12). The terminal group, the methanogenic bacteria (1), use primarily H_2, formate, CO_2, or acetate as energy source and convert these to methane or methane and CO_2 (Table 1). The maintenance of a very low concentration of H_2 in the ecosystem by the methanogens is essential for the catabolism of fatty acids or alcohols such as ethanol by the obligate proton-reducing acetogenic bacteria; also, it allows the fermentative bacteria to produce more acetate, CO_2, and the electron sink product H_2 and less of the other electron sink products such as propionate, butyrate, ethanol, and lactate (5, 10). Acetate is a very important extracellular intermediate in that about 65% or more of the methane produced in the usual ecosystem is produced via acetate degradation by methanogens (5, 9-11, 17).

The obligate proton-reducing acetogenic bacteria so far documented include those that beta-oxidize short-chain fatty acids (12), decarboxylate propionate (3), and oxidize ethanol and similar alcohols (7). In addition, some species of the genus *Desulfovibrio*, which in pure culture utilize sulfate as the electron sink in their catabolism of energy sources such as ethanol or lactate to acetate (Table 1, equation A), will grow without the usual exogenous electron acceptors and produce H_2 as the electron sink product if grown

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TABLE 1. *Equations involving the degradation of lactate by pure cultures of Desulfovibrio spp. (with sulfate) or by syntrophic associations of Desulfovibrio spp. with M. barkeri (without sulfate)*[a]

	Equation	$\Delta G^{0'}$ (KJ reaction^{-1})
A	$2CH_3CH_2OHCOO^- + SO_4^{2-} \rightleftharpoons 2CH_3COO^- + HS^- + 2HCO_3^-$	−160.3
B	$2CH_3CH_2OHCOO^- + 2H_2O \rightleftharpoons 2CH_3COO^- + 2HCO_3^- + 2H^+ + 4H_2$	−8.4
C	$4H_2 + HCO_3^- + H^+ \rightleftharpoons CH_4 + 3H_2O$	−135.6
D	$2CH_3COO^- + 2H_2O \rightleftharpoons 2CH_4 + 2HCO_3^-$	−62.0
B + C	$2CH_3CH_2OHCOO^- + H_2O \rightleftharpoons 2CH_3COO^- + CH_4 + H^+ + HCO_3^-$	−144.0
B + C + D	$2CH_3CH_2OHCOO^- - 3H_2O \rightleftharpoons 3CH_4 + 3HCO_3^- + H^+$	−206.0

[a] Free-energy data are taken from Bryant et al. (6) and Thauer et al. (18). H_2 and CH_4 are in the gaseous state; all other substances are in aqueous solution at 1 mol of activity per kg.

in coculture (syntrophic association) with an H_2-utilizing methanogen (Table 1, equations B and B + C).

Only a few methanogens have been documented to utilize acetate (1, 11, 17). These include mesophilic methanogens such as *Methanosarcina barkeri*, which utilizes acetate (Table 1, equation D), methanol and methylamines, and, usually, H_2-CO_2 (Table 1, equation C) as energy sources, and *Methanobacterium soehngenii* (not yet obtained in pure culture), which appears to utilize only acetate (19, 25).

The present experiments were initiated to determine whether syntrophic cocultures of *Desulfovibrio* species and *M. barkeri* would catabolize lactate completely to methane and CO_2 (Table 1, equation B + C + D). Although experiments showing that *M. barkeri* strain MS grew with acetate as the energy source had been done (unpublished data), the more definitive studies of Mah and his students (10, 17) had not yet been published. We reasoned that determination of the amount of methane produced from a growth-limiting concentration of lactate by the cocultures, in which both acetate and H_2 produced by *Desulfovibrio* might be made available in more physiological levels for growth of *M. barkeri*, might be a good method to use for determination of acetate use by strains of *M. barkeri*. It was also reasoned that studies on the time course of the reactions might give data of ecological interest, although we surmised that other methanogens which do not utilize acetate would grow faster and, therefore, maintain a lower concentration of H_2 in cocultures compared with *M. barkeri*.

The results indicate that in some media, such as a rumen fluid-vitamins-based medium without H_2, *M. barkeri* type strain MS utilizes acetate as well in pure culture as in syntrophic coculture. Results also show that *M. barkeri* utilizes H_2-CO_2 in preference to acetate, and this is especially so in cultures which were previously grown with H_2-CO_2 as the energy source. A preliminary report was presented (M. J. McInerney and M. P. Bryant, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, I42, p. 88).

MATERIALS AND METHODS

Bacterial strains. *Methanobrevibacter smithii* (*Methanobacterium ruminantium*) type strain PS (German Collection of Microorganisms, DSM 861, 1), *M. barkeri* type strain MS (DSM 800), *Desulfovibrio desulfuricans* type strain Essex 6 (DSM 642), and *Desulfovibrio vulgaris* strain EC1 (6) were obtained from our culture collection (Department of Dairy Science, University of Illinois).

Media and conditions of cultivation. The anaerobic techniques for preparation and use of media were those of Hungate as modified by Bryant and co-workers (4, 12), except where otherwise indicated.

The basal RV medium, containing 5% (vol/vol) rumen fluid, minerals, B vitamins, cysteine-sulfide reducing agents, $NaHCO_3$, and an 80% N_2–20% CO_2 gas phase (final pH 7.2), was that of McInerney et al. (12). The yeast extract-based (YE) medium was identical to RV medium except that it contained 0.1% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.), nitrogen- and sulfur-free mineral solution 3 (24), and 10 mM NH_4Cl; the rumen fluid, B vitamins, and Pfennig's minerals and metals solution, present in the RV medium, were deleted. For maintaining slant cultures, 1% agar (Difco) was included. Each medium was dispensed in culture tubes (18 by 150 mm) as previously described (4, 12), except that no. 1 butyl rubber stoppers (Arthur Thomas Co., Philadelphia, Pa.) were used. For 50-ml inoculum cultures, the appropriate medium was anaerobically prepared and autoclaved in 125-ml Nephelo culture flasks (Belco Glass, Inc., Vineland, N.J.) fitted with no. 4 black rubber stoppers. For cultures grown in 125-ml bottles, the appropriate medium was anaerobically prepared in a 500-ml round-bottom flask. A 50-ml aliquot was then dispensed into a 125-ml bottle which had been previously gassed with 80% N_2–20% CO_2 for 2 min. The bottles were closed with no. 1 butyl rubber stoppers and autoclaved in an aluminum press (Belco Glass, Inc.). The appropriate amount of 5% $NaHCO_3$ and cysteine-Na_2S solutions were each added to the cooled, sterile medium in the 125-ml bottle or flask. Each medium was supplemented with sodium acetate, sodium DL-lactate (70%) syrup, or Na_2SO_4 as indicated by including these compounds in the medium before autoclaving.

The methanogens were maintained in slant cul-

tures, and liquid inoculum cultures were grown with an 80% H_2–20% CO_2 gas phase as previously described (7). An acetate-utilizing culture of *M. barkeri* was initiated by inoculating 5 ml of an H_2-grown culture into a tube containing an equal volume of RV medium plus 20 mM sodium acetate. The culture was maintained by weekly transfer of 50% of the volume into RV medium plus 20 mM sodium acetate. The *Desulfovibrio* species were maintained in slant cultures, and liquid inoculum cultures were grown in YE medium with 20 mM each sodium lactate and Na_2SO_4.

The cocultures of *D. desulfuricans* or *D. vulgaris* and *M. barkeri* or *M. smithii* were initiated by inoculating a tube containing the YE medium with 20 mM sodium lactate without Na_2SO_4 with 1 ml each of a liquid culture of the *Desulfovibrio* species and the methanogen. The coculture was maintained by transferring 2 ml of the culture into a tube containing 8 ml of the YE medium with 20 mM sodium lactate every 2 weeks. The coculture was transferred at least four times before being used as inoculum for an experiment.

All incubations were at 35°C with cultures held in a vertical position, except for those liquid cultures that had H_2 added to the gas phase. These were incubated in a slanted position on a reciprocal shaker (7). The gas phase of cultures, grown in 125-ml bottles, was replaced as indicated by inserting through the stopper a 22-gauge needle connected to the gassing apparatus (4), through which flowed the appropriate gas mixture. Another 22-gauge needle served as an exit port for the gas. The gas phase of the bottle was then flushed for 2 min.

Determination of growth. The optical density of a culture was determined at 600 nm, using a Bausch & Lomb Spectronic 70 spectrophotometer. The optical density of cultures grown in 125-ml bottles was estimated in 13- by 100-mm tubes after transferring a 3-ml sample with a syringe and needle. The tube contained an 80% N_2–20% CO_2 gas phase and was closed with a rubber stopper. All manipulations with syringe and needles were performed as described previously (13, 14).

Direct counts of cells preserved in 10% (vol/vol) formaldehyde–0.8% (wt/vol) NaCl solution were determined as described by Warner (21), using a Petroff-Hauser counting chamber.

Analytical methods. The concentrations of lactate and acetate were determined by the butylester-gas chromatographic method (15). The recoveries of 10 mM standard solutions of sodium acetate and lithium lactate (Sigma Chemical Co., St. Louis, Mo.) were 95.0 ± 5.0 and 92.0 ± 3.0%, respectively. The concentration of lactate in YE medium was within 97.4% of the value determined by the colorimetric method of Barker and Summerson (2). Liquid samples were obtained with a syringe and needle, centrifuged (12,000 × *g*, 10 min, 4°C), and frozen at −20°C until used for the analysis of acids. When substrates and products were estimated, a liquid sample was withdrawn at the start and end of the experiment, and the values were corrected for the small amounts of acetate, H_2, or methane (or all three) produced in the control medium without added substrate.

The gas volume of a culture was determined at the end of the experiment, using a manometer as previously described (7). During an experiment, the gas volume was determined with a needle and syringe as described by Paynter and Hungate (14). Methane, H_2, and CO_2 production were determined by gas chromatography (23). When CO_2 was estimated, a culture from which liquid samples had not been withdrawn was acidified by injecting 0.5 ml of 1 M H_3PO_4 solution with a syringe to convert the bicarbonate to CO_2. The amount of CO_2 (micromoles) produced per milliliter of culture during growth of *M. barkeri* with acetate was calculated by subtracting the total amount (micromoles) of CO_2 formed per milliliter in cultures without added acetate from that formed in cultures with added acetate. The amount of CH_4 (micromoles) formed per milliliter of culture during an experiment was determined as described by Mah et al. (10). The amount of CH_4 (micromoles) formed per milliliter of culture grown in 125-ml bottles was calculated by using the following equation: micromoles of CH_4 formed per milliliter = $\Sigma_1^n (M_n - M_{n-1}) \cdot V_n^{-1}$, where M_n and M_{n-1} are total micromoles of CH_4 formed at sampling times n and $n - 1$, respectively, and V_n is the volume (milliliters) of the culture at sampling time n. This calculation was necessary to correct for the decreasing volume of the culture due to samples taken for analysis of the acids.

RESULTS

Growth and methane formation. In cocultures grown on the YE medium with lactate as energy source and for long incubation times, *M. barkeri* plus either of the *Desulfovibrio* strains produced methane as expected, if all of the lactate was degraded by the *Desulfovibrio* strains and the acetate and H_2 produced was degraded with methane production by *M. barkeri*. Cocultures with *M. smithii* produced methane as expected if just the H_2 and some CO_2 produced during lactate degradation by the *Desulfovibrio* strains were used in methane production by *M. smithii* (Table 2). Although growth (optical density) estimates with cultures containing *M. barkeri* were not very accurate due to clumping of cells, the growth observed fit approximately with the observed amount of methane produced. Cocultures with *M. smithii* grew much faster than those with *M. barkeri*, which grows much slower on H_2-CO_2, but final growth was greater with the latter, presumably because it uses the acetate as well as H_2-CO_2.

As expected with substrates catabolized by syntrophic associations, little or none of the lactate was utilized for growth or gas production by the single strains of methanogens or *Desulfovibrio* spp. As in previous work (6), only a small amount of H_2 was produced by the *Desulfovibrio* strains alone, and no H_2 was detected at the end of incubation in cultures of methanogens or cocultures (Table 2).

When acetate was the substrate in YE medium, only the cultures containing *M. barkeri*

TABLE 2. *Gas production and growth by cocultures of Desulfovibrio spp. with M. barkeri or M. smithii in YE medium with lactate or acetate as energy source*[a]

Energy source	Culture[b]	Growth (OD)[c]	Gas (μmol ml^{-1})[d]		Expected CH_4 yield (μmol ml^{-1})[e]
			CH_4	H_2	
Lactate	MS + DD	0.35 (11)	36.7 ± 1.2	0	37.5
	MS + EC1	0.47 (22)	37.4 ± 1.7	0	37.5
	PS + DD	0.27 (5)	11.8 ± 1.2	0	12.5
	PS + EC1	0.38 (5)	12.2 ± 0.1	0	12.5
	MS	0.01 (25)	0	0	0
	PS	0	0	0	0
	DD	0.03 (2)	0	1.8 ± 0.1	0
	EC1	0.02 (2)	0	1.6 ± 0.1	0
Acetate	MS + DD	0.12 (15)	14.8 ± 0.4	0	20.0
	MS + EC1	0.13 (22)	22.4 ± 0.1	0	20.0
	PS + DD	0	0	0	0
	PS + EC1	0	0	0	0
	MS	0.01 (25)	5.7 ± 2.8	0	20.0
	PS	0	0	0	0
	DD	0.01 (2)	0	<0.1	0
	EC1	0	0	<0.1	0

[a] Each of three tubes containing 5 ml of YE medium with 25 mM sodium lactate, 20 mM sodium acetate, or no energy source was inoculated with 0.5 ml of the appropriate culture and incubated for 33 days before gaseous products were determined.

[b] MS, *M. barkeri* strain MS; PS, *M. smithii* strain PS; DD, *D. desulfuricans*; EC1, *D. vulgaris* strain EC1.

[c] Mean of the optical density (OD) of three individual cultures with the small amount of growth in YE medium without added energy sources subtracted. Numbers in parentheses are the days required to reach the maximum OD.

[d] Mean of culture ± the standard deviation of the values obtained from three individual cultures, which were corrected for the small amounts of gasses produced in medium without added energy source.

[e] Based on stoichiometry of reactions expected (Table 1).

grew or produced methane (Table 2). Growth and methanogenesis were not good, especially in the pure culture of *M. barkeri*, suggesting that cultures may need to be adapted to acetate in order to use it well or that the YE medium did not support growth very well, especially in pure cultures when acetate was the energy source.

From further results with cultures studied after various incubation times (Fig. 1), it was evident that RV medium was generally better than YE medium for *M. barkeri* strain MS and that this strain adapted very rapidly from use of H_2-CO_2 to use of acetate as the major energy source on RV medium compared with YE medium. Growth (data not shown) and methane production from acetate were about the same for the RV medium-grown pure cultures of *M. barkeri* or the cocultures with *Desulfovibrio* spp. whether cells grown with RV medium with acetate or with H_2-CO_2 as the energy source were used (Fig. 1A). When the same three inocula were used for the YE-acetate medium (Fig. 1B), growth and methane production was excellent with the acetate-grown *M. barkeri* inoculum, slightly less rapid for the acetate-grown coculture inoculum, and considerably less rapid with the H_2-CO_2-grown *M. barkeri* inoculum. Since inocula from RV-based media could carry over growth factors such as vitamins and minerals to the YE-based medium, the results were not definitive.

Fermentation products. The pure culture of *M. barkeri* grown on acetate in RV medium produced methane (96% of theoretical) and CO_2 (80% of theoretical); no other products such as H_2 or organic acids were detected.

The products of *M. barkeri-Desulfovibrio* sp. coculture grown on lactate included only methane, and those of *M. smithii-Desulfovibrio* sp. coculture included acetate and methane (Table 3) in amounts compatible with equations shown in Table 1; no H_2 or other organic acids were detected after 28 days of incubation. The *M. barkeri-Desulfovibrio* sp. coculture produced only methane from acetate, and the *M. smithii-Desulfovibrio* sp. coculture did not grow, use acetate, or produce any products under identical conditions (Table 3).

Time course of lactate and acetate degradation in *M. barkeri-D. desulfuricans* cocultures. Results in Fig. 2 show that lactate was completely degraded within 12 days, and this was accompanied by stoichiometric increases in the amounts of acetate and methane and pro-

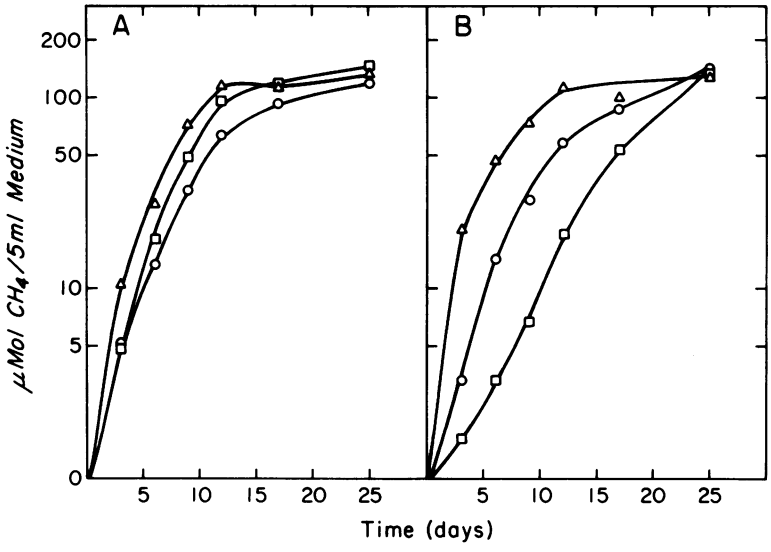

FIG. 1. *Methane produced from acetate in YE or RV medium by the coculture of M. barkeri with D. desulfuricans, previously grown on acetate, or by pure cultures of M. barkeri, previously grown with acetate or H_2-CO_2 as the methanogenic substrate. Tubes containing 5 ml of RV or YE medium with and without 30 mM sodium acetate were inoculated with 0.5 ml of culture. Each inoculum culture was grown in the RV medium with the respective energy source for four transfers. The values are means obtained from three individual cultures and are corrected for the small amount of CH_4 produced in medium without added acetate. (A) Cultures grown in RV medium; (B) cultures grown in YE medium. Symbols: ○, coculture of M. barkeri and D. desulfuricans previously grown with acetate as inoculum; △, M. barkeri previously grown with acetate as inoculum; □, M. barkeri previously grown with H_2-CO_2 as inoculum.*

TABLE 3. *Fermentation products formed by cocultures of D. desulfuricans (DD) with M. barkeri (MS) or M. smithii (PS) in YE medium with lactate or acetate as energy source*[a]

Product	mol/100 mol of substrate degraded[b]			
	Lactate		Acetate	
	MS + DD	PS + DD	MS + DD	PS + DD
Acetate	0	87 ± 15	—[c]	—
CH_4	131 ± 14	43 ± 9	108 ± 5	0

[a] Each of three tubes containing 5 ml of YE medium with or without the indicated energy source added at a concentration of 20 mM was inoculated with 1 ml of the appropriate culture and incubated for 28 days.

[b] Mean ± standard deviation of the values obtained from three individual cultures corrected for the small amount of acetate or CH_4 or both produced in the medium without added energy source.

[c] —, Not a product.

duction of H_2 to a partial pressure of about 0.02 atm (2.03 kPa) in the gas phase. These data indicated that lactate was degraded by *D. desulfuricans* to acetate, H_2, and, presumably, CO_2 and that *M. barkeri* produced methane from the reduction of CO_2 with H_2.

After 18 days, the partial pressure of H_2 decreased to <0.01 atm, and acetate was stoichiometrically degraded to methane and, presumably, CO_2. Acetate was degraded at a rate of about 1.4 mM per day, and methane was produced at a similar or slightly faster rate during this period.

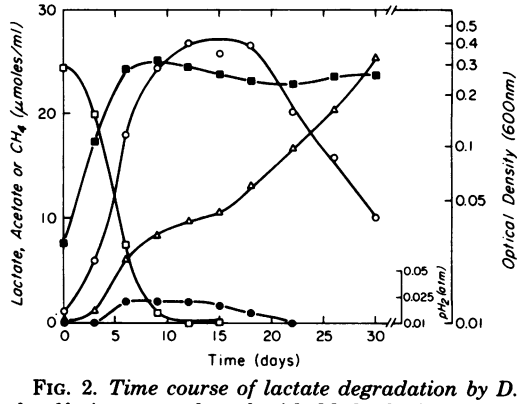

FIG. 2. *Time course of lactate degradation by D. desulfuricans cocultured with M. barkeri. Each of two 125-ml culture bottles containing 50 ml of YE medium with 25 mM sodium lactate was inoculated with 10 ml of a 50-ml YE-lactate medium inoculum culture. Values are means of the values obtained from these two cultures. Symbols: □, lactate; ○, acetate; △, CH_4; ●, pH_2 (partial pressure of H_2); ■, optical density.*

The growth (optical density) of the coculture was most rapid during the first 6 days of incu-

bation (Fig. 2), and concurrent with acetate degradation, there was a slight but reproducible increase in growth. Direct microscopic counts showed that *D. desulfuricans* numbers dramatically increased and then decreased after 9 days (data not shown); thus, the increase in optical density during acetate degradation was probably due to growth of *M. barkeri*. Direct counts of the latter (strain MS) are difficult because of cell aggregation.

Because of the static incubations (Fig. 2), there was probably a lag in time between the H_2 increase and decrease in concentration in the gas phase and its production and use in the culture fluid.

In a further experiment with both YE medium and RV medium with 20 mM acetate and 0, 5, and 20 mM lactate (Fig. 3), the results on acetate and H_2 production and degradation and on lactate degradation by the coculture were similar to those of the previous experiment (Fig. 2), except that the RV medium supported more rapid utilization of acetate and H_2 by *M. barkeri* than did the YE medium.

Effect of lactate and H_2 on acetate degradation by *M. barkeri*. Results indicated that lactate had no effect on acetate degradation by *M. barkeri* in pure culture. As part of the experiment in Fig. 3 (data not shown), the RV-based media were inoculated with the pure culture of *M. barkeri* that was grown in RV-acetate medium; acetate degradation and methane production in media with or without lactate were similar to those of the coculture without lactate (Fig. 3B), and lactate was not degraded.

When *M. barkeri* cultures previously grown in RV medium with only acetate as the energy source were shaken and given H_2 as an additional energy source they grew much more rapidly and produced more methane, but the acetate degradation rate did not increase (or decreased somewhat) during growth unless all of the H_2 was utilized (Fig. 4 and 5). Control cultures without H_2 utilized much more acetate, even though growth was much less, and this was especially so when H_2 was maintained in the experimental cultures for a longer period of time and more acetate was present (Fig. 4).

DISCUSSION

It was previously shown that *Desulfovibrio* species degraded little lactate or ethanol to ace-

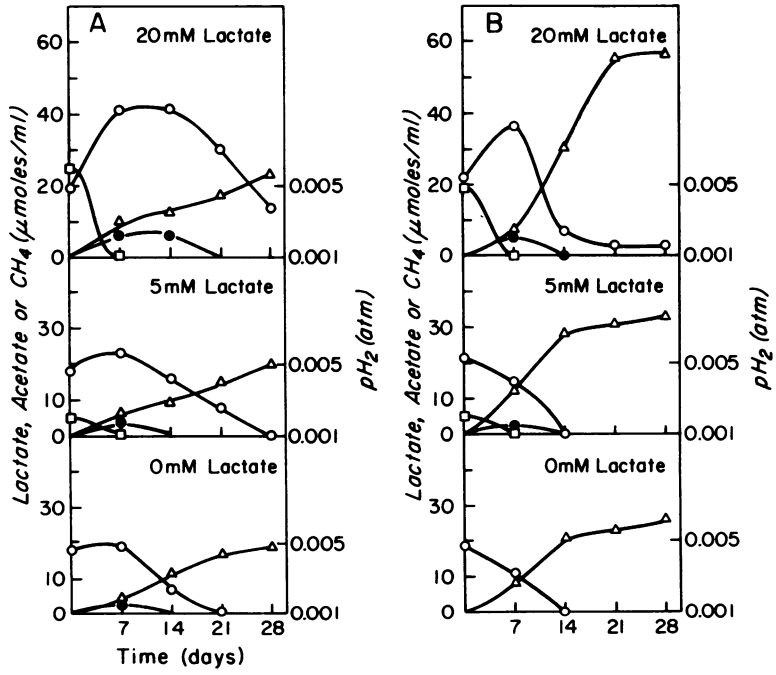

FIG. 3. *Effect of lactate concentration on acetate degradation and methanogenesis by the coculture of M. barkeri with D. desulfuricans. Each of four tubes containing 8 ml of YE (A) or RV (B) medium with 20 mM sodium acetate and the indicated concentration of sodium lactate was inoculated with 2 ml of a 50-ml inoculum culture grown in the YE- or RV-lactate medium. Two tubes of each medium were sampled to determine the concentration of lactate and acetate; the other two were sampled to quantitate the amount of CH_4 and H_2. The values are means of the values obtained from two individual cultures. Symbols are the same as for Fig. 2.*

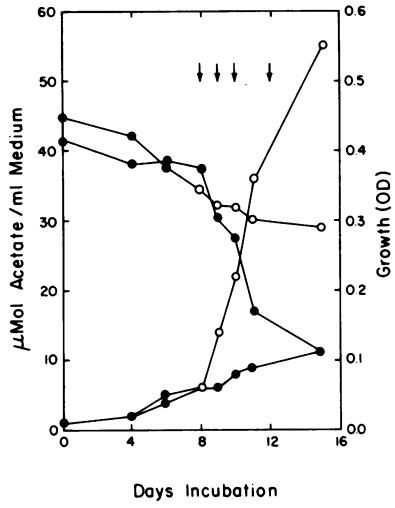

FIG. 4. *Effect of H_2 on acetate utilization and growth of M. barkeri grown in four 125-ml bottles containing 50 ml of RV medium with 40 mM acetate added. A 2% (vol/vol) inoculum from a 40 mM acetate-RV medium culture was used. The cultures were maintained on a shaker, and at 8 days of incubation, the two cultures showing the most rapid utilization of acetate were gassed once daily with 80% H_2–20% CO_2 on days 8, 9, 10, and 12 and the two control cultures were similarly gassed but with the usual 80% N_2–20% CO_2. Points represent mean values of two cultures. Symbols: ●, N_2-CO_2; ○, H_2-CO_2. Arrows refer to gas phase replacement. OD, Optical density.*

tate and H_2 (and CO_2 in the case of lactate) in the absence of sulfate unless H_2-using bacteria (such as H_2-using methanogens) were present to maintain a low concentration of H_2 in the culture environment, presumably due to the relatively positive change in free energy of the reactions unless H_2 concentration is maintained at a low level (6). This was confirmed in the present study with other methanogens: *M. smithii*, which utilizes only H_2 or formate in methanogenesis, and *M. barkeri*, which utilizes only H_2, acetate, and methanol or methylamines in methanogenesis (1). Growth of *Desulfovibrio* spp. on lactate in coculture with *M. smithii* was much more rapid than that with *M. barkeri* (Table 2; data not shown), presumably due to the ability of *M. smithii* to grow faster with H_2 as energy source compared with *M. barkeri*.

However, the present results indicated the complete anaerobic degradation of lactate to methane and CO_2 by syntrophic cocultures of *Desulfovibrio* spp. and *M. barkeri* in a medium in which each organism was essential for the growth of the other. It was previously shown (23) that a coculture of *Acetobacterium woodii* and *M. barkeri* carried out the complete anaerobic degradation of hexose; however, in this coculture, *M. barkeri* was not necessary for the degradation of 1 mol of hexose to 3 mol of acetate by *A. woodii*, and growth of *M. barkeri* occurred mainly in response to acetate and after catabolism of hexose by *A. woodii* was complete.

The present data showed that acetate degradation by the type strain of *M. barkeri* was regulated by H_2 or a product of H_2 metabolism. When growth of *M. barkeri* was due to acetate and H_2 produced from lactate by the *Desulfovibrio* sp. in coculture, little or no acetate was degraded (depending on whether YE or RV medium was used) until the H_2 was at a very low level. In pure culture, lactate had no effect on acetate use by *M. barkeri*, but when an acetate culture without H_2 was exposed to a high level of H_2 (80% H_2 in the gas phase), with resultant more rapid growth, the acetate degradation rate did not increase until after essentially all H_2 was

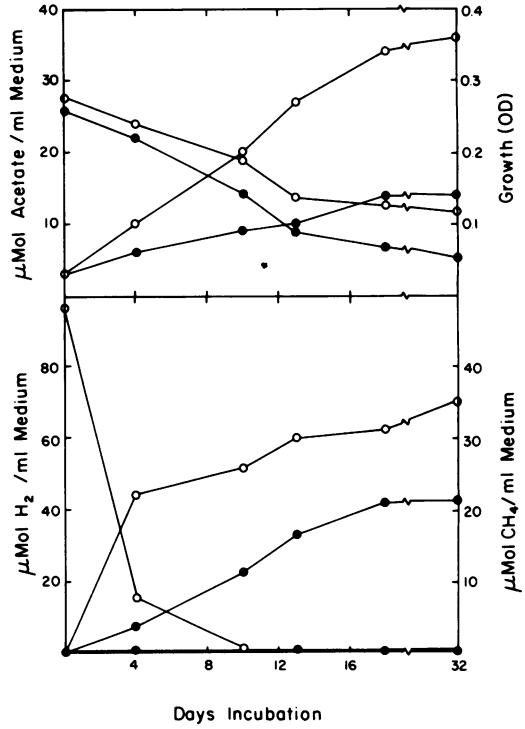

FIG. 5. *Effect of H_2 on acetate utilization, growth, and methane production of M. barkeri grown in culture tubes containing 5 ml of RV medium with 25 mM acetate added. A 20% (vol/vol) inoculum from a culture grown on the same medium was used. Cultures were maintained on a shaker, and at zero time half of the tubes were gassed with 80% H_2–20% CO_2 (○) rather than with the usual 80% N_2–20% CO_2 (●). Each point represents the mean value for two cultures. OD, Optical density.*

utilized. The results are consistent with the idea that H_2 has little effect on the acetate-degrading system previously biosynthesized during growth without H_2 but stops biosynthesis of one or more of the enzymes essential to acetate degradation until H_2 is at a very low level; however, other explanations are possible. The rapid increase in rate of acetate degradation after H_2 was gone, even though only a little further growth occurred, suggested that many of the cells produced during growth on H_2 retained the potential to produce the complete set of enzymes necessary for acetate degradation. The data support the suggestion (17) that acetate degradation is regulated in response to more rapidly metabolizable substrates such as H_2-CO_2 or methanol by a mechanism resembling catabolite repression, although different strains of *M. barkeri* were studied.

Study of the time course of acetate degradation during lactate (or ethanol) degradation by cocultures containing a non-acetate-utilizing methanogen that grows more rapidly on H_2 than *M. barkeri*, in addition to *Desulfovibrio* spp. and *M. barkeri*, would be of interest. This three-species coculture might more closely resemble the natural ecosystem and maintain acetate production and utilization during lactate degradation due to the maintenance of a lower concentration of H_2 in the culture environment. The use of continuous or semicontinuous cultures maintained with various dilution rates and combinations of species in these kinds of studies would also be of interest relative to acetate and H_2 production and utilization.

Short-term exposure to H_2 did not affect acetate degradation in digestor sludge (9) or in acetate enrichment cultures (19) that contain mainly a large rod presumed to be *M. soehngenii*, which does not utilize H_2 (25). Recently, a thermophilic strain of *Methanosarcina* (26) and other mesophilic acetate utilizers that have limited or no ability to utilize H_2 in reduction of CO_2 have been isolated. H_2 at 0.1 atm, however, completely inhibited methanogenesis in acetate-grown cells of the thermophilic *Methanosarcina* sp., although no H_2 was used (16). The roles that H_2 and other rapidly used substrates of methanogens play in regulating acetate degradation in open natural ecosystems need more study.

Several reports have indicated that long adaptation periods are required before H_2-CO_2- or methanol-grown cells of *M. barkeri* are able to use acetate for methanogenesis (22, 23). Smith and Mah (16) suggested that this may be partly due to the lysis of cells grown in medium containing more than 0.01% sulfide, since they observed that the adaptation period was shortened if lower concentrations of sulfide were used. Another possibility is that the population may be genetically heterogeneous with respect to its ability to use acetate as a methanogenic substrate and that procedures used for isolation and maintenance of *M. barkeri* may select against cells that have the potential to degrade acetate (10). Initially, we observed that a long adaptation period was required before *M. barkeri* strain MS used acetate for methanogenesis. However, after four transfers in medium with H_2-CO_2 as the main methanogenic substrate, this culture was able to produce CH_4 from acetate at a rate comparable to that of cultures that were maintained in medium with acetate as the main methanogenic substrate (Fig. 1). Nutritional factors also seem to be important since cells pregrown with H_2-CO_2 produced CH_4 from acetate at a slower rate in YE medium than in RV medium (Fig. 1). These observations indicate that a systematic study of the nutritional factors and other factors which may affect acetate degradation by *M. barkeri* needs to be done.

ACKNOWLEDGMENTS

We thank Jack Althaus for expert technical assistance and Dave Odelson for help in the quantitation of the acids. Helpful discussions with R. B. Hespell and C. J. Smith are acknowledged.

This research was supported by U.S. Department of Agriculture grant 35-331, National Science Foundation grant PCM 77-17542, and the Agricultural Experiment Station of the University of Illinois.

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