APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1985, p. 589–594
0099-2240/85/090589-06$02.00/0

Vol. 50, No. 3

Sulfate-Dependent Interspecies H_2 Transfer between *Methanosarcina barkeri* and *Desulfovibrio vulgaris* during Coculture Metabolism of Acetate or Methanol

T. J. PHELPS,[1] R. CONRAD,[1] AND J. G. ZEIKUS[2]*

Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin 53706,[1] and Michigan Biotechnology Institute and Departments of Biochemistry and Microbiology, Michigan State University, East Lansing, Michigan 48824[2]

Received 31 January 1985/Accepted 10 June 1985

We compared the metabolism of methanol and acetate when *Methanosarcina barkeri* was grown in the presence and absence of *Desulfovibrio vulgaris*. The sulfate reducer was not able to utilize methanol or acetate as the electron donor for energy metabolism in pure culture, but was able to grow in coculture. Pure cultures of *M. barkeri* produced up to 10 μmol of H_2 per liter in the culture headspace during growth on acetate or methanol. In coculture with *D. vulgaris*, the gaseous H_2 concentration was ≤2 μmol/liter. The fractions of $^{14}CO_2$ produced from [^{14}C]methanol and 2-[^{14}C]acetate increased from 0.26 and 0.16, respectively, in pure culture to 0.59 and 0.33, respectively, in coculture. Under these conditions, approximately 42% of the available electron equivalents derived from methanol or acetate were transferred and were utilized by *D. vulgaris* to reduce approximately 33 μmol of sulfate per 100 μmol of substrate consumed. As a direct consequence, methane formation in cocultures was two-thirds that observed in pure cultures. The addition of 5.0 mM sodium molybdate or exogenous H_2 decreased the effects of *D. vulgaris* on the metabolism of *M. barkeri*. An analysis of growth and carbon and electron flow patterns demonstrated that sulfate-dependent interspecies H_2 transfer from *M. barkeri* to *D. vulgaris* resulted in less methane production, increased CO_2 formation, and sulfide formation from substrates not directly utilized by the sulfate reducer as electron donors for energy metabolism and growth.

The transformation of H_2 is an important biochemical reaction performed by terminal bacterial trophic groups during the anoxic decomposition of organic matter. If H_2 accumulates in the environment, thermodynamically unfavorable conditions for organic acid and alcohol degradation may result (36). Three diverse bacterial groups which can consume H_2 as an energy source are the methanogens, the sulfate reducers, and the acetogens (37). Methanogens have been extensively studied for their ability to consume H_2 and participate in interspecies H_2 transfer (5, 24, 26, 34, 35) as the final electron acceptor. Both acetogenic and sulfate-reducing bacteria have been shown to function as net H_2 producers in syntrophic cultures with methanogens (4–6, 16, 24, 27, 35). Even acetate has been shown to be oxidized by syntrophic cultures with methane formed indirectly from H_2 and CO_2 (Zinder and Koch, Arch. Microbiol., in press). Sulfate reducers grown under sulfate-limiting conditions can oxidize ethanol or lactate in the presence of methanogens (4). Under these conditions, the methanogens act as the terminal electron acceptor for catabolic oxidation reactions performed by the sulfate reducers. Zhilina and Zavarzin (38) reported the presence of sulfate reducers in crude *Methanosarcina* enrichment cultures transferred on methanol and proposed that the methanogen may have produced H_2 which was subsequently utilized by the sulfate reducer. However, because the enrichment contained many types of bacteria, definitive conclusions were not possible. It was recently demonstrated that *Methanosarcina* species produce and consume H_2 during growth on various organic substrates, including acetate and methanol (11, 20; J. Krzycki, R. Conrad, and J. G. Zeikus, submitted for publication).

In anoxic freshwater sediments, sulfate reducers have been suggested to compete with methanogens for common energy sources, such as acetate and H_2 (12, 33). Sulfate reducers have been shown to possess more favorable kinetic properties than methanogens for metabolism of H_2 (8, 10, 14, 25). In previous Lake Mendota studies (33; Phelps and Zeikus, submitted for publication), dramatic increases in $^{14}CO_2$ production from 2-[^{14}C]acetate or [^{14}C]methanol were observed when sulfate concentrations were increased by lake turnover or after the addition of exogenous sulfate to stratified sediments. In addition, when *Desulfovibrio vulgaris* and sulfate were added to the stratified sediments, more $^{14}CO_2$ was produced from [^{14}C]methanol or 2-[^{14}C]acetate, suggesting that H_2 consumption by sulfate reducers could alter the sedimentary metabolism of methanogens. This was of particular interest because methanol- or acetate-degrading populations of sulfate or sulfur reducers were not detectable at significant levels in Lake Mendota sediments, which contain very low levels of electron acceptors, except during turnover.

The purpose of this study was to test the hypothesis that H_2 consumption by *D. vulgaris* could alter the internal carbon and electron flow of *Methanosarcina barkeri* grown on organic substrates which alone could not support the growth of the sulfate reducer. Sulfate-dependent interspecies H_2 transfer between a methanogen and a sulfate reducer provides a new physiological mechanism for ecological interactions between these anaerobic bacteria in freshwater ecosystems similar to Lake Mendota sediment (31–33).

MATERIALS AND METHODS

Gases, chemicals, and isotopes. The nitrogen, H_2, N_2-CO_2 (95:5), and H_2-CO_2 (80:20) used were more than 99.9% pure (Matheson Gas Co., Joliet, Ill.) and were passed over

* Corresponding author.

copper-filled Vycor furnaces (Sargent-Welch Scientific Co., Skokie, Ill.) to remove oxygen. All chemicals used were of reagent grade and were obtained from Mallinckrodt (Paris, Ky.) or Sigma Chemical Co. (St. Louis, Mo.). Radioactive 2-[^{14}C]acetate (56 mCi/mmol) was obtained from Amersham Corp. (Arlington Heights, Ill.) and was dissolved in water. Radioactive [^{14}C]methanol (50 mCi/mmol) dissolved in water was purchased from ICN (Irvine, Calif.). Radioactive [^{14}C]formate (55 mCi/mmol) in ethanol-water (7:3) and $^{14}HCO_3^-$ (46 mCi/mmol) in alkaline solution were obtained from New England Nuclear Corp. (Boston, Mass.).

Organisms and growth conditions. *M. barkeri* strain MS (9, 30) and *D. vulgaris* strain Madison (3) were grown on modified PBB medium (7, 15) supplemented with organic substrates and an N_2-CO_2 (95:5) gas phase or with 2.0 atmospheres (220 kPa) of H_2-CO_2 (80:20). Modifications to PBB medium included addition of 5.0 to 20.0 mM sodium bicarbonate, 0.15% MOPS [3-(*n*-morpholine)propanesulfonic acid] buffer, 20 to 30 mM sodium sulfate, 0.05% yeast extract, and 0.05% cysteine hydrochloride as medium reductant. Sulfide was removed from the vials containing the cysteine hydrochloride solution by repeatedly flushing and evacuating the vials with filter-sterilized N_2 gas. A titanium-containing reducing solution (17) was added dropwise as an additional reducing agent as required. The substrates used for growth included methanol (50 to 80 mM), sodium acetate (30 to 60 mM), and H_2-CO_2 (220 kPa). Experiments were conducted in 158-ml serum vials (Wheaton Scientific, Millville, N.J.) containing 75 ml of medium with a final pH of 6.88 to 7.02. Inoculated vials were incubated with shaking in the inverted position at 37°C.

M. barkeri inocula were grown in triplicate 11-liter carboys containing modified PBB medium and either ethanol plus acetate (40 mM each), acetate (50 mM), or H_2-CO_2 (pressurized to 100 kPa daily). Carboys containing methanogenic cultures grown on H_2-CO_2 were stirred with a magnetic stirrer. After 10 to 12 days of incubation, the cells were harvested by allowing the methanogenic clumps to settle to the bottom of the carboy and then displacing the spent medium by an overpressure of N_2. The final 50- to 100-ml dense suspension of *M. barkeri* was transferred into a sterile 158-ml serum vial which contained 0.5 ml of cysteine hydrochloride (2.5%) and 0.05 ml of the titanium reducing solution. Samples (approximately 0.2 ml) from each dense suspension (H_2-CO_2-methanol-, or acetate-grown cells) were added to serum vials by using a 1.0-ml glass syringe and a 27-gauge needle.

D. vulgaris inocula were grown on the same medium in serum vials containing H_2 as the electron donor. When growth reached 75% of maximum (change in optical density at 660 nm, 0.3), the pressurized vials were centrifuged and inverted, and the spent medium was expelled through venting needles. The cells were suspended in the basal medium and washed two times. Cell suspensions were prepared so that inocula of 0.2 to 1.0 ml corresponded to 0.78 to 30 mg (dry weight) of *D. vulgaris* per liter of medium.

Quantification of fermentation parameters. A 2.0- to 4.0-ml portion of culture broth was aseptically removed with a syringe at each sample time. Tubes containing 2.75 ml of anaerobic 0.04% zinc acetate received 0.25 ml of broth for the determination of sulfide (33). Another 0.25-ml sample and 25 μl of 1.0 N phosphoric acid were used for sulfate analyses (33). Fatty acid and alcohol determinations were conducted immediately on 0.1-ml samples, as described previously (7). Changes in optical density at 660 nm, pH, and dry weight were determined with 2.0-ml samples. Because *M. barkeri* cells formed large clumps which settled to the bottom of vessels, optical density measurements reflected the sulfate-reducing biomass and were not useful for measuring growth of the methanogen. Dry weight analyses were performed by using predried polycarbonate membrane filters (pore size, 0.2 μm; Nuclepore Corp., Pleasanton, Calif.) dried for 24 h at 103°C.

The growth of *D. vulgaris* in cocultures was determined by measuring the amounts of desulfoviridin (23) per milligram (dry weight) of cells in 1.0-ml samples. Cells were sedimented by centrifugation, suspended in 1.0 ml of 0.6 N NaOH to extract desulfoviridin, and centrifuged again. The alkaline supernatant was then assayed for desulfoviridin by using a spectrophotofluorometer (American Instrument Co., Silver Spring, Md.) with an excitation wavelength of 365 nm and an emission wavelength of 716 nm. The biomass of *D. vulgaris* in cocultures was determined from a standard curve of dry weight versus fluorescence intensity.

Samples for measuring gaseous end products were obtained by using 1.0-ml syringes equipped with Pressure-Lok valves (Minnert, Anspec Co., Ann Arbor, Mich.). Gaseous CH_4, $^{14}CH_4$, CO_2, and $^{14}CO_2$ were measured by using a gas chromatograph-gas proportional counting system as previously described (19), with total CO_2 determined from acidified controls and by using Bunsen coefficients and Henry's Law calculations. Lower methane concentrations were determined by using a flame ionization gas chromatograph (33). Trace amounts of H_2 in the headspaces of culture bottles were measured by the mercury oxide-to-mercury vapor technique (29), using a 1.0-ml gas sample obtained with a Gas-Tight syringe (Glenco, Houston, Tex.). The lower detection limit was 10 parts of H_2 per billion (10 nl of H_2 per liter). Gas samples greater than 5 ppm (5 μl/liter) were diluted before analysis with air made free of H_2 by passage over a metal oxide converter (Hopkalit; Drägerwerke, Lübeck, Federal Republic of Germany). The total amounts of H_2 present in the headspaces of the culture bottles were calculated from the H_2 mixing ratios, volumes, and pressures of the headspaces, as measured with a mechanical needle monometer.

The percentage of carbon recovery was calculated as follows: (micromoles of CO_2 + micromoles of CH_4/micromoles of substrate consumed × number of carbon positions in substrate) × 100. The percentage of electron equivalents recovered was calculated as follows: (total electron equivalents of products produced by one species/total electron equivalents observed in products) × 100.

TABLE 1. Comparison of H_2 concentrations during growth of *M. barkeri* on methanol or acetate in the absence and presence of *D. vulgaris*[a]

Substrate	Culture	H_2 concn (μmol/liter of gas headspace) after incubation for:		
		24 h	72 h	120 h
Methanol	Monoculture	4.83	5.42	9.75
	Coculture	2.96	4.83	2.17
Acetate	Monoculture	1.29	5.37	10.5
	Coculture	0.46	0.50	0.54

[a] Cultures were grown in 158-ml serum vials which contained 75 ml of PBB medium, substrate at a concentration of 100 mM, 20 mM bicarbonate, 20 mM phosphates, 0.05% yeast extract, and 0.05% MOPS buffer (pH 7.0). The initial inocula were 20 mg of *M. barkeri* per liter and 2.4 mg of *D. vulgaris* per liter. The H_2 concentration at zero time was 1.5 ± 0.2 μmol/liter.

TABLE 2. Comparison of methanol fermentation by *M. barkeri* in pure culture and in coculture with *D. vulgaris*[a]

Culture	Utilization of methanol (μmol)	Growth			Product formation (μmol/100 μmol of substrate consumed)				Fraction of $^{14}CO_2$ produced[b]	Carbon recovery (%)	Electron recovery (%)	% of electron equivalents converted by:	
		Change in optical density at 660 nm	Change in methanogen dry wt (mg/liter)	Change in sulfidogen dry wt (mg/liter)	CO_2	CH_4	H_2	HS^-				*M. barkeri*	*D. vulgaris*
Pure *M. barkeri*	712		56		28.1	65.4	0.039		0.26 ± 0.026	93	87	100	
Coculture with 1× *D. vulgaris*	550	0.13	43	22	33.5	59	0.016	18.6	0.32 ± 0.03	94	105	76	24
Coculture with 4× *D. vulgaris*	690	0.15	50	55	32	43.5	0.014	34.8	0.58 ± 0.12	86	101	58	42

[a] We used 158-ml serum bottles that contained 75 ml of PBB medium, 20 mM sodium sulfate, substrate at a concentration of 60 mM, 30 mM bicarbonate, 0.15% 3-(n-morpholine) propanesulfonic acid buffer, 30 mM phosphates, 0.05% yeast extract, and an N_2-CO_2 (95:5) gas phase. The pH remained between 6.9 and 7.2 in all vials. The inocula used were 25 mg (dry weight) of *M. barkeri* per liter, 7.8 mg (dry weight) of *D. vulgaris* per liter (1× *D. vulgaris*), and 30 mg (dry weight) of *D. vulgaris* per liter (4× *D. vulgaris*). The bottles were incubated for 120 h at 37°C.

[b] Calculated as follows: dpm of $^{14}CO_2$/(dpm of $^{14}CO_2$ + dpm of $^{14}CH_4$).

RESULTS

Fermentation analysis in pure culture. Initial studies in our laboratory demonstrated that during growth on acetate, *M. barkeri* produced H_2 as a trace gas during methanogenesis (20; J. Krzycki, R. Conrad, and J. G. Zeikus, submitted for publication). As shown in Table 1, *M. barkeri* produced trace amounts of H_2 during growth on either acetate or methanol. As expected, *D. vulgaris* could not utilize methanol or acetate as an electron donor for growth in this medium. Both *M. barkeri* and *D. vulgaris* utilized H_2 as an electron donor for growth in modified PBB medium. Under these conditions, the sulfate reducer exhibited greater H_2 consumption activity and a faster growth rate than the methanogen.

Fermentation analysis in coculture. Preliminary studies demonstrated that *D. vulgaris* could be repeatedly transferred as a culture partner when *M. barkeri* was grown in medium containing either methanol or acetate as a carbon and energy source. We performed experiments to examine the relationship among substrate consumption, growth, and production of methane, hydrogen, and sulfide in pure cultures and cocultures (Table 2). In pure culture, *M. barkeri* consumed 712 μmol of methanol and displayed a threefold increase in biomass during the 5-day experiment. Stoichiometrically, 25% of the carbon from methanol was expected to yield CO_2, and the fraction of $^{14}CO_2$ recovered from [^{14}C]methanol was 0.26. Similarly, for every 100 μmol of methanol consumed, 28, and 65 μmol of CO_2 and CH_4, respectively, were recovered. For every 1,000 μmol of methanol consumed, 0.4 μmol of H_2 was detected in the gas phase.

When *M. barkeri* was grown on methanol in a coculture with a *D. vulgaris* inoculum of 7.8 mg (dry weight) per liter, both populations grew, as evidenced by increases in dry weight and optical density at 660 nm. Because of clump formation by *M. barkeri*, only the growth of *D. vulgaris* could be estimated by light scattering and optical density. Also, a 20% increase in the fraction of $^{14}CO_2$ and CO_2 formed per mol of methanol consumed was observed. Less H_2 accumulated, less CH_4 was produced, and 18.6 μmol of sulfide was formed per 100 μmol of methanol consumed. *D. vulgaris* converted 24% of the available electron equivalents to sulfide, whereas the methanogen utilized 76% of the electron equivalents for methanogenesis. This fermentation exhibited balanced carbon and electron recovery.

When *M. barkeri* was grown on methanol in a coculture with a *D. vulgaris* inoculum of 30 mg (dry weight) per liter, higher levels of methanol oxidation to CO_2, sulfate reduction, and growth of the sulfate reducer were observed. Under these conditions, the fraction of $^{14}CO_2$ produced from methanol was twice that in the methanogenic pure culture, and only two-thirds as much methane was formed. Again, less H_2 was detected in the coculture than in the monoculture. *D. vulgaris* utilized 42% of the electron equivalents in methanol to produce 34.8 μmol of sulfide per 100 μmol of methanol consumed.

Table 3 shows the effects of *D. vulgaris* on acetate metabolism by *M. barkeri*. In a monoculture, *M. barkeri* produced 1 mol of CH_4 per mol of acetate consumed, and, as is typical of this strain, 16% of the methyl group of acetate was oxidized to CO_2 (9). Additionally, 0.29 μmol of H_2 was evolved per 100 μmol of acetate consumed. In coculture *D. vulgaris* increased its biomass, whereas the methanogen produced only two-thirds as much methane per unit of substrate consumed, and H_2 accumulation was an order of magnitude lower than H_2 accumulation in the monoculture. The fraction of $^{14}CO_2$ produced from 2-[^{14}C]acetate doubled in the coculture, and significantly more total CO_2 was formed; 30% of the electron equivalents available to the methanogen were utilized for the reduction of sulfate. At the elevated *D. vulgaris* inoculum levels 33% of the electron equivalents in acetate were used to reduce sulfate to sulfide.

We performed additional experiments in which [^{14}C]formate, [^{14}C]methanol, and 2-[^{14}C]acetate were added to cocultures after 1 day of incubation to evaluate whether these metabolites were passed from the methanogen to the sulfate reducer. If formate had been the intermediate substate transfered between the organisms, additions of [^{14}C]formate would have produced $^{14}CO_2$. Less than 5% of the [^{14}C]formate added to methanol- or acetate-grown cocultures produced $^{14}CO_2$, and the formate concentration remained at ≤100 μM (data not shown), indicating that

TABLE 3. Comparison of acetate fermentation by *M. barkeri* in pure culture and in coculture with *D. vulgaris*[a]

Culture	Utilization of acetate (μmol)	Growth			Product formation (μmol/100 μmol of substrate consumed)				Fraction of $^{14}CO_2$ produced[b]	Carbon recovery (%)	Electron recovery (%)	% of electron equivalents converted by:	
		Change in optical density at 660 nm	Change in methanogen dry wt (mg/liter)	Change in sulfidogen dry wt (mg/liter)	CO_2	CH_4	H_2	HS^-				*M. barkeri*	*D. vulgaris*
Pure *M. barkeri*	490		48		82	98	0.29		0.16 ± 0.017	90	98	100	
Coculture with 1× *D. vulgaris*	436	0.06	38	32	117	66	0.015	28.2	0.32 ± 0.06	91	93	70	30
Coculture with 4× *D. vulgaris*	500	0.10	38	20	110	66	0.010	33	0.43 ± 0.02	85	99	66	33

[a] For experimental conditions, see Table 2, footnote *a*.
[b] See Table 2, footnote *b*.

formate was not an intermediary metabolite utilized for sulfate reduction in the cocultures. When [^{14}C]methanol was added after 1 day of incubation to acetate-grown cocultures, it was not significantly utilized, nor was acetate significantly utilized in methanol-grown cocultures. Controls showed that inhibition of sulfate reduction resulted in typical methanogenic fermentation of methanol. When sodium molybdate (5.0 mM), a widely used inhibitor of sulfate reduction (13, 21), was added to *M. barkeri* cultures containing methanol and a 30-mg (dry weight) per liter *D. vulgaris* inoculum, the *D. vulgaris* biomass decreased 50% during the 5-day experiment (as estimated by desulfoviridin), and the fraction of $^{14}CO_2$ produced from [^{14}C]methanol was 0.3 ± 0.11. Only 5% of the electron equivalents of methanol were converted to sulfide. Similar to the methanogenic monoculture, for every 100 μmol of methanol consumed 70 μmol of CH_4, 30 μmol of CO_2, and less than 4 μmol of sulfide were formed (data not shown). In cocoulture experiments in which the organisms were grown on acetate, addition of molybdate also increased methane production and decreased sulfide formation.

In separate control experiments, the effects of H_2 addition on coculture acetate metabolism were examined. When 2-[^{14}C]acetate and 5% H_2 gas were added to 3-day-old cocultures growing on acetate, the fraction of $^{14}CO_2$ produced during the following 8 h decreased from 0.29 ± 0.03 to 0.18 ± 0.02. Hydrogen was consumed by *D. vulgaris*, and sulfate was reduced to sulfide. These data demonstrated that when excess H_2 was added to cocultures, the sulfate reducer consumed the H_2, produced sulfide, and no longer had an effect on acetate catabolism by *M. barkeri*. Similarly, the significant decrease in the H_2 pool size of cocultures (Table 1) was due to H_2 consumption by *D. vulgaris*.

DISCUSSION

Our data demonstrated that during coculture growth of *M. barkeri* with acetate or methanol as a carbon and energy source, *D. vulgaris* decreased methanogenesis as a consequence of linking interspecies hydrogen transfer to sulfate reduction. Hydrogen was judged to be the electron donor for sulfate reduction in cocultures because it was detected at higher levels in monocultures of *M. barkeri* than in cocultures and, when added to cocultures, it decreased $^{14}CO_2$ production from 2-[^{14}C]acetate. These results provide the first well-documented evidence that sulfate reducers can grow via interspecies H_2 transfer by utilizing reducing equivalents generated by methanogens grown on organic substrates. Thus, these results further extend the significance of competition between methanogens and sulfate reducers for hydrogen. Furthermore, these results provide a physiological mechanism for how higher sulfate levels in freshwater sediments can lead to increased oxidation of organic substrates to CO_2 in lieu of direct substrate utilization by the sulfate-reducing populations.

The trophic association of *D. vulgaris* with *M. barkeri* in crude methanol enrichment cultures was first established by Zhilina and Zavarzin (38). Sulfate is generally removed from basal culture media to aid in selective enrichment and to maintain culture purity of methanogens (36). Recently, Archer (2) reported that *Desulfovibrio* and other H_2-consuming bacteria were present in a methanogenic enrichment grown on a basal mineral medium containing acetate as the carbon and energy source. During growth on acetate or methanol in pure cultures, hydrogen is apparently produced by *M. barkeri* more readily than it is consumed, and hydrogen accumulates as a trace gas (20). However, the significance of hydrogen production from organic substrates during growth of *M. barkeri* is only clearly recognized in cocultures when *D. vulgaris* consumes the produced hydrogen and links this consumption to sulfate reduction and growth.

Classical reports on interspecies H_2 transfer involve displacing unfavorable thermodynamic equilibra by eliminating accumulation of H_2, which enables an organic compound-degrading species and an H_2-consuming species to grow in an obligately syntrophic relationship (4, 5). Our results do not describe a synergistic metabolic interaction or communication (37) between two partners per se, but rather an antagonistic or competitive behavior based on loss of a potential electron donor for the methanogen. Thus, in coculture, *D. vulgaris* appears to keep the hydrogen partial pressure low enough to shift the apparent equilibrium constant of the catabolic redox system of the methanogen such that considerable H_2 is produced and more substrate is oxidized to CO_2 in lieu of methane. This phenomenon

appears at first glance to be nonbeneficial for *M. barkeri* because of the potential loss of energy conservation that is coupled to methanogenesis. However, it is possible that under the coculture conditions, *M. barkeri* could transform more substrate per unit time, couple H_2 production with maintaining ionic or pH gradients, or conserve energy during the oxidation of the substrate.

H_2 competition between methanogens and sulfate reducers may be more environmentally significant than is generally believed (1, 8, 10, 13) because H_2 consumption by sulfate reducers results in less methanogenesis from organic methane precursors and H_2-CO_2. In neutral-pH, anoxic freshwater sediments, elevated levels of sulfate correlate with enhanced oxidation of 2-[^{14}C]acetate or [^{14}C]methanol (10, 13, 31, 33). This phenomenon has been generally accredited to sulfate reducers and methanogens which compete for acetate as an energy source (28, 31). Although acetate-utilizing sulfate or sulfur reducers have been isolated (22), their population levels and activities in freshwater sediments have not been established. Elsewhere we propose that the enhanced oxidation of methanol or acetate in the presence of high sulfate concentrations in Lake Mendota sediments is in part due to interspecies H_2 transfer processes rather than to direct competition between methanogens and sulfate reducers for organic substrates.

LITERATURE CITED

1. **Abram, J. W., and D. B. Nedwell.** 1978. Inhibition of methanogenesis by sulfate reducing bacteria competing for transferred hydrogen. Arch. Microbiol. **117**:89–92.
2. **Archer, D. B.** 1984. Hydrogen-using bacteria in a methanogenic acetate enrichment culture. J. Appl. Bacteriol. **56**:125–129.
3. **Badziong, W., R. K. Thauer, and J. G. Zeikus.** 1978. Isolation and characterization of *Desulfovibrio* growing on hydrogen plus sulfate as the sole energy source. Arch. Microbiol. **116**: 41–49.
4. **Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill.** 1977. Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl. Environ. Microbiol. **33**:1162–1169.
5. **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. Mikrobiol. **39**:20–31.
6. **Iannotti, E. L., D. Kafkewitz, M. J. Wolin, and M. P. Bryant.** 1973. Glucose fermentation products of *Ruminococcus albus* grown in continuous culture with *Vibrio succinogenes*: changes caused by interspecies transfer of H_2. J. Bacteriol. **114**: 1231–1240.
7. **Kerby, R., W. Niemczura, and J. G. Zeikus.** 1983. Single-carbon catabolism in acetogens: analysis of carbon flow in *Acetobacterium woodii* and *Butyribacterium methylotrophicum* by fermentation and ^{13}C-nuclear magnetic reasonance measurement. J. Bacteriol. **155**:1208–1218.
8. **Kristjansson, J. K., P. Schonheit, and R. K. Thauer.** 1982. Different K_S values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. Arch. Microbiol. **131**: 278–282.
9. **Krzycki, J. A., R. W. Wolkin, and J. G. Zeikus.** 1982. Comparison of unitrophic and mixotrophic substrate metabolism by an acetate-adapted strain of *Methanosarcina barkeri*. J. Bacteriol. **149**:247–254.
10. **Lovley, D. R., D. R. Dwyer, and M. J. Klug.** 1982. Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. Appl. Environ. Microbiol. **43**:1373–1379.
11. **Lovley, D. R., and J. G. Ferry.** 1985. Production and consumption of H_2 during growth of *Methanosarcina* spp. on acetate. Appl. Environ. Microbiol. **49**:247–249.
12. **Lovley, D. R., and M. J. Klug.** 1982. Intermediary metabolism of organic matter in the sediments of an eutrophic lake. Appl. Environ. Microbiol. **43**:552–550.
13. **Lovley, D. R., and M. J. Klug.** 1983. Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. Appl. Environ. Microbiol. **45**:187–192.
14. **Lupton, F. S., and J. G. Zeikus.** 1984. Physiological basis for sulfate-dependent hydrogen competition between sulfidogens and methanogens. J. Bacteriol. **159**:843–849.
15. **Lynd, L., and J. G. Zeikus.** 1983. Metabolism of H_2-CO_2, methanol, and glucose by *Butyribacterium methylotrophicum*. J. Bacteriol. **153**:1415–1423.
16. **McInerney, M. J., M. P. Bryant, and N. Pfenning.** 1979. Anaerobic bacterium that degrades fatty acids in syntrophic association with methanogens. Arch. Microbiol. **122**:129–135.
17. **Moench, T. T., and J. G. Zeikus.** 1983. An improved preparation method for a titanium (III) media reductant. J. Microbiol. Methods **1**:199–202.
18. **Mountfort, D. O., and M. P. Bryant.** 1982. Isolation and characterization of an anaerobic syntrophic benzoate-degrading bacterium from sewage sludge. Arch. Microbiol. **133**:249–256.
19. **Nelson, D. R., and J. G. Zeikus.** 1974. Rapid method for the radioisotopic analysis of gaseous end products of anaerobic metabolism. Appl. Microbiol. **28**:258–261.
20. **O'Brien, J. M., R. H. Wolkin, T. T. Moench, J. B. Morgan, and J. G. Zeikus.** 1984. Association of hydrogen metabolism with unitrophic or mixotrophic growth of *Methanosarcina barkeri* on carbon monoxide. J. Bacteriol. **158**:373–375.
21. **Oremland, R. S., and B. F. Taylor.** 1978. Sulfate reduction and methanogenesis in marine sediment. Goechim. Cosmochim. Acta **42**:209–214.
22. **Pfenning, N., F. Widdel, and H. G. Truper.** 1981. The dissimilatory sulfate-reducing bacteria, p. 926–940. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. Springer-Verlag, Berlin.
23. **Postgate, J.** 1959. A diagnostic reaction of *Desulphovibrio desulphuricans*. Nature (London) **163**:481–482.
24. **Reddy, C. A., M. P. Bryant, and M. J. Wolin.** 1972. Characteristics of S organisms isolated from *Methanobacillus omelianskii*. J. Bacteriol. **109**:539–545.
25. **Robinson, J. A., and J. M. Tiedje.** 1984. Competition between sulfate-reducing and methanogenic bacteria for H_2 under resting and growing conditions. Arch. Microbiol. **137**:26–32.
26. **Scheifinger, C. C., B. Lindehan, and M. J. Wolin.** 1975. H_2 production by *Selenomonas ruminantium* in the absence and presence of methanogenic bacteria. Appl. Microbiol. **29**:480–483.
27. **Schink, B., and M. Steib.** 1983. Fermentative degradation of polyethylene glycol by a strictly anaerobic, gram-negative, non-sporeforming bacterium, *Pelobacter venetianus* sp. nov. Appl. Environ. Microbiol. **45**:1905–1913.
28. **Schonheit, P., J. K. Kristjansson, and R. K. Thauer.** 1982. Kinetic mechanism for the ability of sulfate reducers to outcompete methanogens for acetate. Arch. Microbiol. **132**: 285–288.
29. **Seiler, W., H. Gieil, and P. Roggendorft.** 1980. Detection of carbon monoxide and hydrogen by conversion of mercury oxide to mercury vapor. Atmos. Technol. **12**:40–45.
30. **Weimer, P. J., and J. G. Zeikus.** 1979. One carbon metabolism in methanogenic bacteria. Arch. Microbiol. **119**:49–57.
31. **Winfrey, M. R., D. R. Nelson, S. C. Klevickis, and J. G. Zeikus.** 1977. Association of hydrogen metabolism with methanogenesis in Lake Mendota sediments. Appl. Environ. Microbiol. **33**:312–318.
32. **Winfrey, M. R., and J. G. Zeikus.** 1979. Microbial methanogenesis and acetate metabolism in a meromictic lake. Appl. Environ. Microbiol. **37**:213–221.
33. **Winfrey, M. R., and J. G. Zeikus.** 1979. Anaerobic metabolism of immediate methane precursors in Lake Mendota. Appl. Environ. Microbiol. **87**:244–253.
34. **Winter, J. U., and R. S. Wolfe.** 1980. Methane formation from

fructose by syntrophic associations of *Acetobacterium woodii* and different strains of methanogens. Arch. Microbiol. **124**:73–79.
35. **Wolin, M. J.** 1974. Metabolic interactions among intestinal microorganisms. Am. J. Clin. Nutr. **27**:1320–1328.
36. **Zeikus, J. G.** 1977. Biology of methanogenic bacteria. Bacteriol. Rev. **41**:514–541.
37. **Zeikus, J. G.** 1983. Metabolic communication in nature, p. 423–462. *In* J. H. Slater, R. Whittenbury, and J. W. T. Wimpenny (ed.), Microbes in their natural environment. Cambridge University Press, Cambridge.
38. **Zhilina, T. N., and G. A. Zavarzin.** 1973. Trophic relationships between *Methanosarcina* and its associates. Mikrobiologiya **42**:266–273.