Terminal Reactions in the Anaerobic Digestion of Animal Waste†

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An anaerobic mesophilic digestor was operated using beef cattle waste (diluted to 5.75% volatile solids) as substrate; retention time was 10 days with daily batch feed. Volatile solids destruction was 36%. Daily gas production rate was 1.8 liters of gas (standard temperature and pressure) per liter of digestor contents (0.99 liters of CH_4 per liter of digestor contents). Acetate turnover was measured, and it was calculated that 68% of the CH_4 was derived from the methyl group of acetate. When the methanogenic substrates acetic acid or H_2/CO_2 were added to the digestor on a continuous basis, the microflora were able to adapt and convert them to terminal products while continuing to degrade animal waste to the same extent as without additions. The methanogenic substrates were added at a rate at least 1.5 times the microbial production rate which was measured in the absence of added substrates. Added acetate was converted directly to CH_4 by acetoclastic methanogens; H_2 addition greatly stimulated acetate production in the digestor. A method is described for the measurement of acetate turnover in batch-fed digestors.

It has been stated that methanogenic reactions are rate limiting in the complete anaerobic degradation of organic matter (7, 10, 16). Of the two predominant methanogenic reactions (4), the reduction of CO_2 with H_2 and the splitting of acetate, Kaspar and Würmann (10) reported the latter to be the rate-limiting reaction. This conclusion was based on their data that, in a well-digested sludge, H_2-oxidizing enzymes are less than 1% saturated whereas acetate-splitting enzymes are nearly half saturated. It is the purpose of this paper to examine the terminal reactions of anaerobic digestion and to determine whether they are rate limiting.

As a prerequisite to the study, it was necessary to develop a method for monitoring these reactions in digestors. The turnover rate (degradation rate divided by pool size, in units of reciprocal time) of acetate has been measured using the isotope dilution technique. As described by Jayasuriya and Hungate (9), the method requires constant production rate, degradation rate, and concentration of the measured intermediate (acetate) over a finite period of time (17). Measurements are made between batch feedings, with no liquid flow into or out of the system. Radioactively labeled intermediate is added, and, as the intermediate pool turns over, the produced cold intermediate dilutes the label. The loss of label from the extracellular

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pool of the intermediate is proportional to both the turnover of that pool and the amount of label in that pool (17):

$$dx/dt = -k_T x \qquad (1)$$

where x is the amount of radioactivity in the intermediate (disintegrations per minute per liter), t is time (hours), and k_T is the turnover rate (hour^{-1}) of the intermediate (17).

Reactions in anaerobic digestors can best be described using equations developed for continuous cultures. However, because of the nature of the substrates commonly used in anaerobic digestion, lab-scale digestors are almost universally operated on a daily batch-fed basis. In the interval between batch feedings, there is considerable variation in parameters such as gas production rate and concentrations and turnovers of intermediates (13, 14). During portions of this interval, there are periods of time when these parameters remain relatively constant, and turnover-rate measurements have been made during these periods and extrapolated to the entire 24-h interval (10, 11, 14, 18). Mackie and Bryant (13) infused labeled acetate into a digestor to measure acetate turnover continuously. However, this requires large amounts of radioactive label. An alternative approach is shortening the interval between batch feedings so that steady-state conditions are approached, and the interval over which measurements must be made is small. Then isotope dilution techniques could be used to

measure acetate turnover during the entire interval.

One problem which may prevent constant acetate concentration in digestors is that the concentration of acetate is normally higher in the influent substrate than in the digestor; there is, therefore, an instantaneous increase in digestor acetate concentration upon batch-feed addition. With a stably operating digestor, and with a short interfeed period, the concentration is assumed to drop linearly until the next feeding. Concentration could then be expressed by a linear equation:

$$p = a + bt \qquad (2)$$

where p is the pool size (millimolar concentration of acetate) and a and b are constants. With frequent small batch-feed additions, the drop in acetate concentration should be small. It is assumed that, under these conditions, the degradation rate is constant.

Since turnover rate (k_T) is the degradation rate (k, in units of millimolar per hour) divided by the pool size (p, or, from equation 2, $a + bt$) (18), one can substitute $k/(a + bt)$ for k_T in equation 1:

$$dx/dt = -kx/(a + bt) \qquad (3)$$

Rearrangement and integration gives:

$$ln(x_{t1}) - ln(x_{t0}) = -(k/b)ln(a + bt_1) + \\ (k/b)ln(a + bt_0) \qquad (4)$$

Equation 4 takes the form of a straight line when the two variables $ln(x_{t1})$ and $ln(a + bt_1)$ are plotted. Acetate degradation rate (k) can then be calculated from the slope of the line.

This method was used to measure acetate degradation in digestors which were fed animal waste and in digestors fed animal waste with ultimate methanogenic substrates added continuously to the fermentation. It was concluded that, in digestors where the concentrations of fermentation acids are low, methanogenic reactions do not limit the production of CH_4 from organic matter.

MATERIALS AND METHODS

Animal waste. Feces and urine without bedding and less than 24 h old were collected from young bulls fed the following ad libitum (percent dry weight): rolled barley (77.5), chopped wheat straw (20), calcium carbonate (1.0), calcium phosphate (0.4), urea (0.8), and sodium chloride (0.3).

The waste was diluted with tap water to about 12% volatile solids (VS), blended 2 min in a Waring blender, and stored frozen in polypropylene bottles. The waste was thawed rapidly, diluted to the desired VS concentration, and stored at 4°C for use within 1 day (21).

Digestor operation. A Multigen F-2000 fermentor (New Brunswick Scientific Co., New Brunswick, N.J.) served as a 1.5-liter anaerobic digestor. Agitation

was achieved with three sets of impellers at 230 rpm, and temperature was maintained at 40°C. Routinely, 150 ml of effluent was removed each day, and 150 ml of animal waste diluted to 5.75% VS was added to the digestor. This addition was not deoxygenated; however, care was taken to prevent gaseous air from entering the digestor during additions. Higher loading rates gave increased levels of volatile organic acids (VOA) in the effluent. Daily gas production was measured by displacement of acidic brine (200 g of NaCl and 5 g of citric acid per liter of tap water) (21). For some experiments an acetic acid solution was pumped into the digestor, and in that case the concentration of the animal waste added was increased and the volume of addition was decreased in a manner to maintain the same daily addition of animal waste and to maintain a 10-day hydraulic retention time. It was also necessary to add alkaline (up to 1 ml of 5 N NaOH) to maintain the pH at 7.1 to 7.2. This was necessary to neutralize higher levels of VOA and also to increase the bicarbonate concentration to balance the higher percentage of CO_2 in the produced gas. Acetic acid solution was pumped in using a model 341A syringe pump (Orion, Cambridge, Mass.). In other experiments a gas mixture (30% CO_2–70% H_2) was bubbled into the digestor (maximum rate, 3.4 ml/min). The gas mixture added was calculated to give a product gas (CH_4 and CO_2) with a gas composition similar to the gas produced in the digestor. Addition of methanogenic substrates (H_2 or acetic acid) made addition of nutrients necessary: increased gas production stripped away hydrogen sulfide from solution in the digestor and increased growth of methanogens required nutrients for growth. A nutrient solution (1 N NH_3–0.2 M KH_2PO_4–0.2 M K_2SO_4) was added to maintain ammonia and sulfide levels in the digestor. Phosphate was not measured, but it was assumed that phosphate demand for growth was about one-fifth of the ammonia demand for growth.

Analytical methods. VOAs were quantitated by gas chromatography using a Tracor 560 gas chromatograph (Tracor Instruments, Austin, Tex.). Samples were prepared by addition of 1 ml of 3 M phosphoric acid to 9 ml of effluent and centrifugation for 1 min at $12,800 \times g$ in an Eppendorf microcentrifuge (Brinkmann Instruments Inc., Westbury, N.Y.). Injection volume was 0.4 µl. The acids were separated on a glass column (2 mm inner diameter by 1.8 m) packed with 10% (wt/wt) SP-1000 (Supelco, Bellefonte, Pa.) and 1% (wt/wt) phosphoric acid on Anakrom 80/90 ABS. Operating temperatures were: inlet, 135°C; column, 120°C; detector, 135°C. Carrier gas (He) flow rate was 17 ml/min; detection was by flame ionization (12 ml of H_2 per min, 300 ml of air per min).

Radioactivity in individual VOAs was quantitated by gas chromatographic separation of acids (as described above except carrier gas flow rate was increased to 40 ml of He per min and injection volume was increased to 2 µl) followed by proportional counting (Packard Instrument Co., Inc., Rockville, Md.; model 894 gas proportional counter), converted to disintegrations per minute. Total radioactivity in the effluent (after acidification and centrifugation for VOA analysis) was measured by adding 10 µl to 10 ml of Aquasol scintillation cocktail, counted in a Beckman CPM-100 scintillation counter (83% counting efficiency), and converted to disintegrations per minute.

Gases were quantitated by gas chromatography,

using silica gel (18). VS, total solids, alkalinity, and pH were determined as described (21). Sulfide was determined by the methylene blue method (19), and ammonia was determined by membrane electrode (Orion, Cambridge, Mass.). Ether extract (1), cellulose (8), hemicellulose (8), lignin (8), Kjeldahl nitrogen (1), and crude protein (1) were determined by standard procedures. The above determinations were made at least in triplicate on single samples. When the results of these determinations were examined by least-squares analysis, weighting was used (weighting of each point was proportional to the reciprocal of its variance).

Gas production rates were measured using a valve assembly from a repipetter (Becton, Dickinson & Co., Rutherford, N.J.) (Fig. 1). A plastic syringe and needle were attached, and the side tube was connected to a manometer. The internal valve mechanism of the repipetter was removed so that gas flow was unrestricted. All gas flow from the digestor was shut off, and the needle was inserted through a septum to the gas space of the digestor. A second needle was briefly inserted through the septum to initialize the gas pressure to atmospheric. Afterwards, the syringe was continuously adjusted manually to maintain atmospheric pressure in the digestor (determined by monitoring the manometer). After a suitable period of time, the volume on the syringe and the time elapsed were recorded. After conversion to standard temperature and pressure, the gas production rate was calculated. This device can also be used to measure accumulated gas in sealed culture tubes or serum bottles.

RESULTS

Substrate composition. The animal waste used as digestor substrate was normally diluted to 5.75% VS. The composition of this suspension (in grams per liter) was (standard deviation in parentheses): TS, 64 (1.3); cellulose, 19 (0.4); hemicellulose, 15 (0.4); lignin, 5.2 (0.2); ether extract, 3.3 (0.02); Kjeldahl nitrogen, 1.68 (0.002); and crude protein, 10.5 (0.01). Other determinations of substrate composition are reported in Table 1.

Start-up of digestor. Fresh animal waste was diluted with tap water to 1.7% VS, and 1.5 liters was added to the digestor vessel. Calcium carbonate (70 g) was added, and the pH was then adjusted to 7.0 using 10 N NaOH. This suspension was incubated at 40°C. Each day the contents were sampled for pH and VOA determinations. When pH fell to 6.5, it was adjusted to 6.8 by addition of 10 N NaOH (cumulative addition was 7.4 ml). Total VOA levels reached a maximum of 79.5 mM (ca. 4,770 mg/liter as acetic acid) after 5 weeks of incubation, with acetic acid as the predominant acid (66% on a molar basis). Daily gas production began to increase, and there was a decrease in all VOAs except propionic acid; pH increased to 7.3, and was lowered to 7.2 by the addition of 1 ml of concentrated HCl. By 6 weeks, propionate levels dropped from a maximum of 15 mM to 8.4 mM, and daily feeding of the digestor was started at a
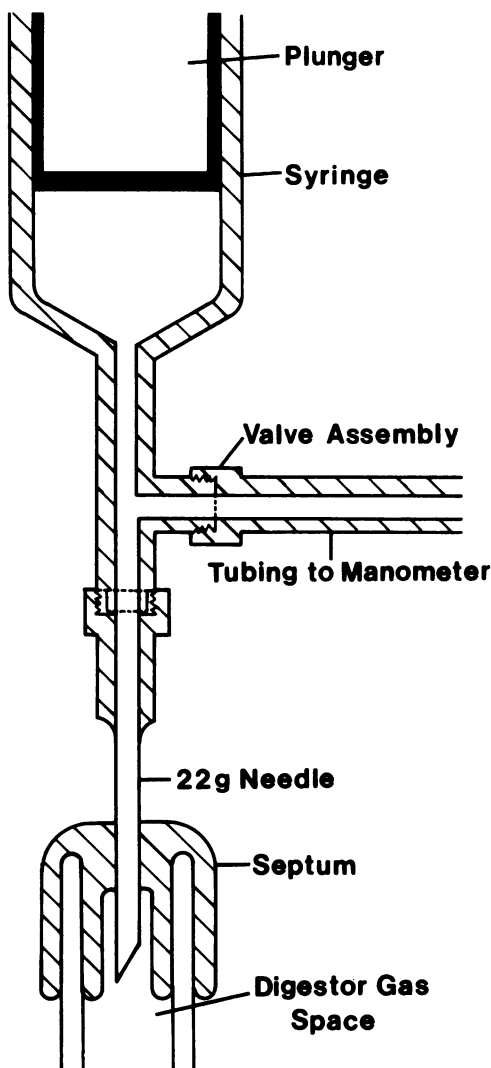

FIG. 1. Device for measuring gas production rates. A plastic syringe and a needle were attached to a repipetter valve assembly, with the internal valve mechanism removed to allow free gas flow. The syringe volume was manually increased, as gas was produced in the digestor, by monitoring internal pressure with the manometer and maintaining it at atmospheric pressure by the adjustments in the syringe volume.

Labels on figure: Plunger, Syringe, Valve Assembly, Tubing to Manometer, 22 g Needle, Septum, Digestor Gas Space

10-day hydraulic retention time and low loading rate (1.35 g of VS/liter per day loading rate, using influent substrate suspension of 1.35% VS). The influent substrate concentration was gradually increased to 5.75% VS (while maintaining the 10-day hydraulic retention time) over a 6-week period, with pH of effluent remaining within the range of 6.7 to 7.2 and total VOA levels of 4 to 25 mM. After stabilization, 250 ml of effluent was removed and 250 ml of digested

TABLE 1. Characterization of influent substrate suspension and digestor effluents of daily batch-fed digestors[a].

| Influent or effluent | pH[b] | VOA (mM) | | | Solids | | VS destruction[c] (%) | Alkalinity (mg of CaCO₃ per liter) | Ammonia[d] (mM) | Sulfide[e] (mM) |
		Acetate	Propionate	Longer (C4-C8)	% Total	%VS				
Influent substrate	6.88	46.0	6.7	7.0	6.7	5.75		1,550	32	ND[g]
	(0.03)[f]	(0.93)	(0.41)	(0.52)	(0.11)	(0.16)		(18)	(0.48)	
Effluent, no additions	7.15	0.8	0.5	Tr[h]	4.7	3.7	36	3,860	36	.08
	(0.03)	(0.08)	(0.12)		(0.14)	(0.18)		(31)	(0.52)	(0.01)
Effluent, acetic acid added	7.18	2.3	1.8	Tr	4.8	3.7	36	4,700	35	.08
	(0.02)	(0.22)	(0.16)		(0.07)	(0.15)		(74)	(0.73)	(0.01)
Effluent, hydrogen added	7.16	2.6	5.4	Tr	4.6	3.7	36	3,790	38	.09
	(0.02)	(0.18)	(0.32)		(0.15)	(0.18)		(52)	(1.02)	(0.01)

[a] Digestors with acetic acid or hydrogen-carbon dioxide addition had continuous addition of those methanogenic substrates for at least 30 days before characterization of effluent.
[b] Arithmetic average of three pH measurements (equivalent to negative log of geometric mean of [H⁺]).
[c] %VS destruction = 100 × %VS in effluent/%VS in influent substrate.
[d] Free ammonia plus ammonium.
[e] $S^{-2} + HS^- + H_2S_{aq}$.
[f] Standard deviations in parentheses.
[g] ND, Not determined.
[h] Tr. Trace (<0.05 mM).

domestic sewage sludge was added to ensure most-fit microflora. After restabilization, no effect of this inoculation was detected (as determined by daily monitoring of production and composition of gas and pH and VOA concentrations of effluent).

Characterization of the fermentation. The digestor was operated for at least 30 days (three complete turnovers) after stabilization (as determined by daily monitoring of production and composition of gas and of pH and VOA concentrations of effluent). Then effluent samples were collected for 5 consecutive days and individually analyzed for pH, alkalinity, VS, total solids, and concentrations of VOAs, ammonia, and sulfide (Table 1); gas production and composition were also measured (Table 2). Then gas production

rate was measured during the 24-h interval between feedings, and on the next day liquid samples were obtained over the interval for VOA analysis (Fig. 2A).

Measuring acetate turnover. After characterization of the fermentation, steady-state conditions were approached by increasing the frequency of feeding to three times per day for 3 days and then every 2 h for 8 h; the amount of feed was calculated to maintain the same hydraulic retention time and loading rate. Gas production rate became constant. VOA levels were measured during the last 2-h interval and found to vary linearly over the 2-h interfeed period (Fig. 3) as predicted and as assumed in equation 2:

$$Ac = 1.34 - 0.163t \qquad (5)$$
$$Pr = 0.76 - 0.098t \qquad (6)$$

TABLE 2. Effect of added methanogenic substrates on methane production from acetate or carbon dioxide, determined with daily batch feed.[a]

| Addition | mM/day added | Additional expected gas[b] | | | Total gas expected[b,c] | | | Measured gas production[b] | | |
		CH₄	CO₂	Total	CH₄	CO₂	Total	CH₄	CO₂	Total
None		0	0	0	[0.99][d]	[0.81][d]	[1.80][d]	0.99	0.81	1.80
								(0.04)[e]	(0.04)	(0.03)
Acetic acid	41	0.92	0.92	1.84	1.91	1.73	3.64	1.85	1.65	3.50
								(0.06)	(0.05)	(0.03)
Hydrogen/CO₂	100.5[f]	0.56	0.40	0.98	1.56	1.22	2.78[d]	1.48	1.20	2.68
								(0.05)	(0.05)	(0.03)

[a] Digestor with added acetic acid or hydrogen had continuous addition of those methanogenic substrates for at least 30 days before measurements.
[b] Measured in liters of gas per day per liters of digestor.
[c] Additional expected gas (stoichiometric from added methanogenic substrates) plus gas produced without added substrates.
[d] Measured.
[e] Standard deviations in parentheses.
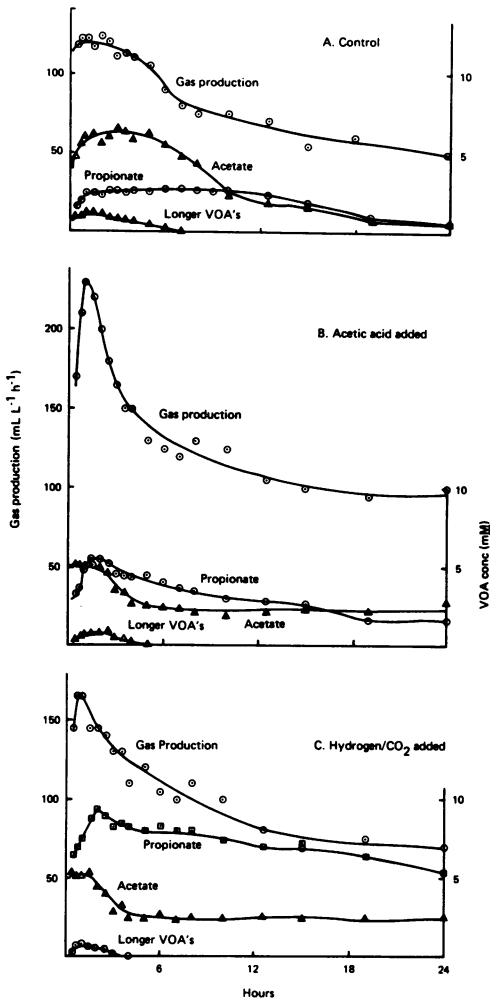[f] 100.5 mmol H_2 per liter of digestor per day.

FIG. 2. Gas production rates and VOA concentrations in daily batch-fed digestors receiving animal waste plus: no additional substrate (A); 41 mmol of acetic acid per liter of digestor per day (B); or 3.2 liters of 70% H_2, -30% CO_2 gas mixture per liter of digestor per day (C).

FIG. 3. Acetate and propionate concentrations during determination of acetate turnover (2-h batch feed) with no added methanogenic substrates.

where Ac and Pr are acetate and propionate concentrations (mM), respectively, and t is time in hours. Acetate and propionate concentrations were measured in this manner three times. The third time, acetate turnover was also measured using radioactive tracer. One ml of [2-^{14}C]acetic acid (0.2 mCi and 1 μmol) was added with the last feed addition. Samples were taken every 10 min for 2 h, and radioactivity in acetate was determined. Quantitation of ^{14}C by using the proportional counter showed no radioactivity in any peaks other than acetate in any sample. Determination of radioactivity in acetate by proportional counting and quantitation of total radioactivity in the supernatant gave results which
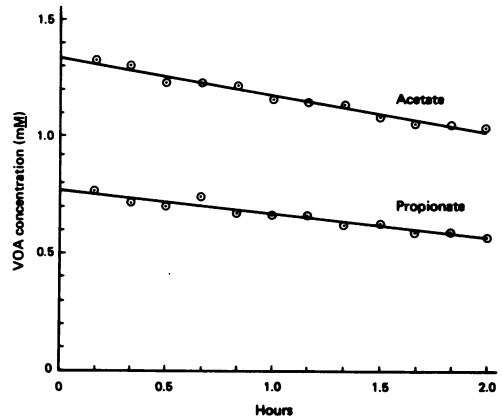
were not statistically different (95% confidence). The standard deviations for proportional counting quantitation (range 8 to 19%) were much higher than those for scintillation counting quantitation (range 3 to 7%), indicating that scintillation counting was more precise, and so these data were used in calculations of acetate turnover. $\ln(x_{t1})$ was plotted against $\ln(a + bt_1)$ (see equation 4), using concentrations of acetate derived from equation 5 at each time point. This gave a straight line with slope k/b. Acetate degradation rate (k) was calculated from the slope (Table 3). The linear decrease in the acetate pool size (Table 3) indicated that the acetate production rate was less than the acetate degradation rate and that the difference was equal to the rate of decrease in acetate concentration. Therefore, the production rate was calculated by subtracting the rate of decrease in pool size from the measured acetate degradation rate. Methane production from acetate, assumed to be 91% of the measured acetate degradation rate (13), was 68% of the total CH_4 production. This value is lower than that reported by other workers (13, 14, 18); however, their measurements were made on daily batch-fed digestors.

Addition of ultimate methanogenic substrates. Acetic acid was pumped into the digestor continuously while maintaining daily batch feeding of animal waste. The liquid volume of this addition was kept small (maximum 5 ml of addition per liter of digestor contents per day), so the liquid volume of the digestor did not significantly change during measurements. Acetic acid pumping was initially at a rate (3.5 mol/liter per day) that was 10% of the rate of microbial acetate production measured by labeled acetate turnover (Table 3), and gradually increased over a 4-week period. Even during these increases, VOA levels in the effluent remained low (less than 9

TABLE 3. Determination of acetate turnover while batch feeding the digestor every 2 h[a]

Determination	VOA pool size[b,c]				Acetate turnover[c]				Methane production[d]		
	Acetate		Propionate		Determined by proportional counting		Determined by scintillation counting		From acetate (mM/h)	From H$_2$[e] (mM/h)	Total (mM/h)
	A (mM)	B (mM/h)	A (mM)	B (mM/h)	Produced (mM/h)	Degraded (mM/h)	Produced (mM/h)	Degraded (mM/h)			
Control	1.34	−0.163	0.76	−0.098	1.34	1.51	1.40	1.57	1.41	0.65	2.06
	(0.05)	(0.005)	(0.04)	(0.030)	(0.14)	(0.14)	(0.07)	(0.07)	(0.06)	(0.07)	(0.05)
Acetic acid added	2.70	−0.160	1.44	−0.116	2.90[f]	3.06	2.91[f]	3.07	2.79	0.93	3.72
	(0.08)	(0.005)	(0.08)	(0.007)	(0.24)	(0.24)	(0.13)	(0.13)	(0.12)	(0.13)	(0.04)
Hydrogen added	2.82	−0.015	6.10	−0.023	1.95	1.96	1.96	1.98	1.80	1.18	2.98
	(0.07)	(0.006)	(0.15)	(0.030)	(0.15)	(0.15)	(0.10)	(0.10)	(0.09)	(0.10)	(0.03)

[a] Acetate turnover measured by dilution of [2-^{14}C]acetate over 2-h interfeed period.

[b] Pool size, varying over interfeed period, was determined by least-squares analysis; A and B are reported according to the formula: concentration = $A + B \times$ time (in hours).

[c] Weighted least-squares fit.

[d] Calculated from acetate degradation rate measured by scintillation counting (see text) and assuming 0.91 mol of methane produced per mol of acetate degraded (13).

[e] Measured as difference between total methane production and methane produced from acetate.

[f] Includes microbially produced plus amount pumped in.

mM total acids). To ensure sufficient concentrations of ammonia, sulfide, and phosphate for growth, ammonia and sulfide levels were periodically measured and continuous addition of nutrient solution containing ammonia, sulfate, and phosphate was adjusted to maintain ammonia and sulfide levels. Even at the maximum acetate addition rate used (41 mmol/liter per day), the gas production rate was at least 95% of the stoichiometrically expected rate (gas production rate without addition plus 1 mol each of CO$_2$ and CH$_4$ per mol of acetate added) (Table 2). After 30 days of stabilized operation, characterization (Table 1 and Fig. 2B) and measurement of acetate turnover (Table 3) were determined as with the control.

Acetic acid addition was stopped, and an H$_2$/CO$_2$ gas mixture (70:30) was bubbled into the bottom of the digestor, at first at 180 ml of gas mixture per liter of digestor contents per day (equivalent to 10% of the microbial H$_2$ production in the control digestor calculated stoichiometrically from the rate of CH$_4$ production from H$_2$ shown in Table 3). The rate of H$_2$/CO$_2$ addition was gradually increased, and, unlike the response to increases in acetic acid additions, digestor VOA levels increased (to as much as 40 mM) several times. H$_2$/CO$_2$ addition was then cut back, and the digestor recovered within 2 to 3 days. When H$_2$ addition rates of 100.5 mmol/liter per day were reached, the digestor operated stably for 30 days and measurements were made (Tables 1 and 2 and Fig. 2C). No H$_2$ was detected in the gas phase (<0.1%). As with acetic acid addition, the addition of H$_2$/CO$_2$ did not affect the degradation of organic matter in the animal waste, as determined by gas production and VS destruction (Tables 1 and 2).

DISCUSSION

Determination of turnover rates of intermediates such as acetate requires the use of radioactive label. Besides shortening the time over which measurements must be made, the data show that analytical techniques such as proportional counting may be unnecessary when these measurements are made over a short time-span. Essentially all of the label not in gases or suspended solids was in acetate, and so labeled acetate could be quantitated by acidification, centrifugation, and counting by scintillation. In other digestors where conversion of acetate to soluble nongaseous products such as longer VOAs might be more prominent, it would be necessary to first document that essentially all acetate conversions were directly to CH$_4$. Also, turnover rate determinations could normally be made on small portions of the digestor. However, in these experiments, methanogenic substrates were pumped into the digestor during determinations, and it was necessary to perform experiments using the entire digestor.

When acetic acid was pumped continuously into the digestor, there was an increase in acetate concentration in the effluent which was proportional to the increase in total acetate production (biological production plus amount pumped in), as expected (5). Propionate concentration also increased, perhaps due to product inhibition of propionate-degrading bacteria by the higher levels of acetic acid (3, 11) or due to increased propionate production by fermenta-

tive bacteria (4). When H_2/CO_2 was the added substrate, there were occasional instabilities in the fermentation immediately after increases in addition rate. The data therefore allow that abrupt changes in digestor influent substrates, which cause a rapid increase in H_2 production by H_2-producing microflora, could create instabilities causing digestor failure.

After stabilization, the VOA levels were higher with H_2/CO_2 addition than without, but these increases, as well as those which occurred with acetic acid addition, were small compared with the daily turnover of those intermediates. Digestors with added acetate or H_2/CO_2 converted at least 95% of added substrates stoichiometrically to CH_4, assuming gas production from the animal waste was the same as controls with no added substrates. Also, volatile solids destruction was similar in the presence and absence of added substrates, suggesting that fermentation of animal waste was not significantly affected by the added load on the methanogenic bacteria.

Acetate levels in the digestor with added H_2/CO_2 were higher, apparently due both to increased acetate production and inhibition of acetate dissimilation. Acetate turnover experiments (Table 3) indicated increased production of acetate. This increase could be due in part to production of acetate from H_2 and CO_2 by *Clostridia* (15). The acetate concentration when H_2/CO_2 was added was greater than when acetic acid was added, yet the acetate degradation rate was lower. This indicated inhibition of acetate degradation in the digestor with added H_2/CO_2. Inhibition by H_2 of methanogenesis by acetoclastic *Methanosarcinae* has been reported (2); however, the most numerous methanogen in my digestor (data not shown—determinations made as described by Mackie and Bryant [13]) was a rod morphologically similar (as determined by phase microscopy) to *Methanobacterium soehngenii*. These organisms have been shown to be insensitive to H_2 (20). It is therefore difficult to understand the reason for apparent inhibition of acetate dissimilation. More easily explained are increases in propionate levels in the effluent. Because the free-energy change of H_2 production from propionate is only marginally negative in ecosystems such as anaerobic digestors, propionate-degrading bacteria are very sensitive to H_2 levels (3, 4, 11). Increased propionate production by fermentative bacteria could also cause increases in propionate levels (4).

It is interesting that increases in H_2 addition caused temporary instabilities in the fermentation, whereas increases in acetate addition did not: acetate-splitting enzymes in other digestors were nearly half saturated, whereas H_2-oxidizing enzymes were less than 1% saturated (10). There may be limits in the activities of hydroge-

nases inherent in their nature; bacteria may not be able to construct enzymes with active sites of higher affinities. Their problem (the uptake of H_2 at very low concentrations) may have been solved by the maintenance of their hydrogenases mainly in the oxidized form, which would make the free-energy change for H_2 uptake more negative, allowing exergonic H_2 uptake at lower H_2 concentrations. In that case, measured enzyme saturation would be low even when the capacity of hydrogenases to take up H_2 at low concentrations was limiting the degradation of propionate.

In digestors where VOAs build up and cause digestor failure, it can be argued that the conversion of VOAs to gases limits anaerobic digestion. However, the data showed that the capacity for methanogenesis from ultimate methanogenic substrates is great under suitable conditions. When methanogenesis limits complete anaerobic digestion, inhibition of methanogenesis may occur by environmental factors such as ionic strength or by abrupt change in the nature or quantity of influent substrate. In the same manner, a buildup of acetic acid may be caused by increased H_2 concentration in the digestor, which may not only increase acetate production rate but also inhibit acetate degradation by acetoclastic methanogens. Also, all experiments in this study were carried out using a 10-day hydraulic retention time with daily batch-fed operation. The calculated microbial doubling time necessary for maintenance of populations in this digestor was 6.58 days. Although bacteria which degrade VOAs can grow somewhat faster than this (12), there is a limit to their generation times and consequently a limit to the volume of daily influent addition in any completely mixed digestor. This limitation can be attributed to the growth rates of obligate proton-reducing acetogens and methanogens (4, 13), and in that sense conversion of VOAs to gases becomes the rate-limiting step. In digestors where the concentrations of VOAs are low (such as in this study), the rate-limiting step is apparently the hydrolysis of polymers (6).

LITERATURE CITED

1. **Association of Official Agricultural Chemists.** 1965. Official methods of analysis, 10th ed. Association of Official Agricultural Chemists, Washington, D.C.

2. **Baresi, L., R. A. Mah, D. M. Ward, and I. R. Kaplan.** 1978. Methanogenesis from acetate: enrichment studies. Appl. Environ. Microbiol. **36:**186–197.
3. **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.
4. **Bryant M. P.** 1979. Microbial methane production—theoretical aspects. J. Anim. Sci. **48:**193–201.
5. **Chen, Y. R., and A. G. Hashimoto.** 1980. Substrate utilization kinetic model for biological treatment processes. Biotechnol. Bioeng. **22:**2081–2095.
6. **Eastman, J. A., and J. F. Ferguson.** 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. J. Water Pollut. Control Fed. **53:**352–366.
7. **Ghosh, S., J. R. Conrad, and D. L. Klass.** 1975. Anaerobic acidogenesis of wastewater sludge. J. Water Pollut. Control Fed. **47:**30–45.
8. **Goering, H. K., and P. J. van Soest.** 1970. Forage fiber analysis. Agricultural handbook no. 379. U.S. Department of Agriculture, Washington, D.C.
9. **Jayasuriya, G. C. N., and R. E. Hungate.** 1959. Lactate conversions in the bovine rumen. Arch. Biochem. Biophys. **82:**274–287.
10. **Kaspar, H. F., and K. Wührmann.** 1978. Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. Appl. Environ. Microbiol. **36:**1–7.
11. **Kaspar, H. F., and K. Wührmann.** 1978. Product inhibition in sludge digestion. Microbial Ecol. **4:**241–248.
12. **Lawrence, A. W., and P. L. McCarty.** 1969. Kinetics of

13. **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.
14. **Mountfort, D. O., and R. A. Asher.** 1978. Changes in proportions of acetate and carbon dioxide used as methane precursors during the anaerobic digestion of bovine waste. Appl. Environ. Microbiol. **35:**648–654.
15. **Ohwaki, K., and R. E. Hungate.** 1977. Hydrogen utilization by *Clostridia* in sewage sludge. Appl. Environ. Microbiol. **33:**1270–1274.
16. **Pretorius, W. A.** 1969. Anaerobic digestion. III. Kinetics of anaerobic fermentation. Water Res. **3:**545–558.
17. **Smith, P. H.** 1966. Microbial ecology of sludge methanogenesis. Dev. Ind. Microbiol. **7:**156–161.
18. **Smith, P. H., and R. A. Mah.** 1966. Kinetics of acetate metabolism during sludge digestion. Appl. Microbiol. **14:**368–371.
19. **Trüper, H. G., and H. G. Schlegel.** 1964. Sulfur metabolism in *Thiorhodacae.* I. Quantitative measurements on growing cells of *Chromatium okenii.* Antonie van Leeuwenhoek J. Microbiol. Serol. **30:**225–238.
20. **van den Berg, L., K. A. Lamb, W. D. Murray, and D. W. Armstrong.** 1980. Effects of sulfate, iron and hydrogen on the microbial conversion of acetic acid to methane. J. Appl. Bacteriol. **48:**437–447.
21. **Varel, V. H., H. R. Isaacson, and M. P. Bryant.** 1977. Thermophilic methane production from cattle waste. Appl. Environ. Microbiol. **33:**298–307.

methane fermentation in anaerobic treatment. J. Water Pollut. Control Fed. **41:**R1–R17.