APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1980, p. 217-222
0099-2240/80/08-0217/06$02.00/0
Vol. 40, No. 2

Effect of Temperature and Retention Time on Methane Production from Beef Cattle Waste

V. H. VAREL,* A. G. HASHIMOTO, AND Y. R. CHEN
Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933

The effect of temperature and retention time on the rate of methane production from waste of beef cattle fed a finishing diet was investigated by using continuously mixed 3-liter working volume anaerobic fermentors. The temperatures ranged from 30 to 65°C with 5°C increments between fermentors. The fermentors were fed once per day with 6% volatile solids (organic matter). Retention time for each temperature was varied from 18 to 2.5 days. After 3-volume turnovers, samples were obtained on 4 consecutive days. The highest methane production rate (liters/liter of fermentor per day) and methane yield at that rate (liters/gram of volatile solids) were 1.27 and 0.19 at 9 days and 30°C, 1.60 and 0.16 at 6 days and 35°C, 2.28 and 0.23 at 6 days and 40°C, 2.42 and 0.24 at 6 days and 45°C, 2.83 and 0.14 at 3 days and 50°C, 2.75 and 0.14 at 3 days and 55°C, 3.18 and 0.14 at 2.5 days and 60°C, and 1.69 and 0.17 at 6 days and 65°C. Volatile solids degradation at these retention times and temperatures was between 46 and 54%. The concentrations of volatile acids in the 30 to 55°C fermentors were generally below 2,000 mg/liter, with the exception of the 3-day retention time. The 60 and 65°C fermentors were usually above this level for all retention times. These studies indicate potential rates of methane production from the fermentation of untreated waste of beef cattle fed high-grain finishing diets. This information should serve as preliminary guidelines for various kinetic analyses and aid in economic evaluations of the potential feasibility of fermenting beef cattle waste to methane.

Buhr and Andrews (3) have reviewed the results of various studies on the effect of temperature and retention time (RT) on anaerobic fermentation. The advantages for thermophilic temperatures (50 to 65°C) include increased reaction rates in relation to the destruction of organic matter, and therefore less capital cost as a result of smaller fermentor size; increased efficiency with respect to the amount of organic matter destroyed; improved solid-liquid separation; and increased destruction of bacterial and viral pathogens. Disadvantages of a thermophilic system include higher energy requirements to heat influent substrate and maintain fermentor temperature; poor effluent quality as a result of large quantities of dissolved solids; and poor process stability.

Fermentation of livestock waste offers several advantages over other potential substrates. Adequate nutrients are available for the microbes to carry out the fermentation; in intensive livestock systems the substrate is easily collected; degradation of organic matter is usually between 30 and 65%; the fermentation provides a means of pollution control; and the fermentor residue has a potential value as a fertilizer or feedstuff (6).

Although considerable work has been done on methanogenesis from beef cattle waste (6, 11, 15, 16), sufficient data are not available to adequately evaluate the overall potential of this process. Specifically, not enough information is available as to the optimal temperature, solids loading rate, and solids RT at which the fermentation is conducted. Varel et al. (16) have previously found that 4.5 volumes of methane per volume of fermentor per day can be obtained in laboratory units. This rate of production has also been demonstrated in a 5.7-m^3 pilot-scale fermentor (6). This rate is four times higher than that for other conventional systems and was obtained by using thermophilic temperatures at either 55 or 60°C. Little is known about the maximum rates of gas production from animal wastes when lower temperatures are used.

The present studies were initiated using 4-liter laboratory fermentors to compare the efficiencies of methane production at mesophilic (30 to 45°C) and thermophilic (50 to 65°C) temperatures and at long and short RT, using waste of beef cattle fed a high-grain finishing diet. The results indicated that the effect of temperature on the rates of methane production was most noticeable at short RT, yet temperatures less then 60°C may be optimal for maximizing these rates.

MATERIALS AND METHODS

Fermentors. The fermentation vessels used in this study were 4-liter Pyrex aspirator bottles as previously described (16). Modifications from the previous study included enlarging the feed inlet port from 1.3 to 1.6 cm ID, and two 0.5-cm-thick Plexiglas baffles were glued with silicone sealant (Dow Corning Corp., Midland, Mich.) at 45° angles to the inside of each fermentor. The lower baffle, 1.5 by 4.0 cm, was positioned approximately 2 cm from the bottom of the fermentor, and the upper baffle was positioned to interface the 3-liter liquid volume level with that of the gas headspace. In operation, the objective of the baffles was to prevent a potential stratification effect that could be caused by the rotary shaker used for mixing.

The bags used to collect the gas were made of du Pont Tedlar material, fitted with a JACO polypropylene standard fitting (Pollution Measurement Corp., Chicago, Ill.). A 0.4-cm-ID polypropylene stopcock (A. H. Thomas Co., Philadelphia, Pa.) was connected to the fitting to regulate the flow of gas into or out of the bag.

Substrate. Approximately 150 kg of beef cattle waste (feces and urine without bedding) was collected in December 1977 from a 3-day accumulation on a partially covered concrete surface. The beef cattle weighed between 300 and 400 kg and were fed a finishing ration ad libitum consisting of 91% corn, 7% corn silage, and 2% soybean meal (dry matter basis). No antibiotics were fed. The waste was diluted with tap water in a slurry tank to approximately 15% volatile solids (VS, organic matter). The slurry was dispensed into screw-cap plastic bottles, each to provide the desired daily VS input for each fermentor. It was stored at −20°C until 1 day before use, when it was placed at 4°C. Just before daily feeding, hot tap water was added to bring the influent substrate to the desired volume and temperature. The fermentor shaker was in operation while the effluent was removed and stopped when substrate was added.

The substrate for a second experiment was collected from the same area in October 1978 and treated in the same manner. The diet fed to these animals consisted of 88% corn, 9% corn silage, and 3% soybean meal.

Experimental design. The experimental design shown in Table 1 was used to examine the effect of temperature and RT on methane production. Active microbial cultures were established in fermentors operated at 30 to 45°C by incubating beef waste at 1% VS plus a nitrogen and sulfur-free mineral solution as previously described for thermophilic fermentors (16). After 2 weeks of incubation, feeding was started. The 50 to 65°C fermentors were started by using effluent from a 55°C, 5.7-m^3 fermentor we have located on our research facilities. Feeding of the 50 to 60°C fermentors was started immediately. A 50-day acclimation period was permitted for the 65°C fermentor as previously described (16) before feeding was initiated. After cultures were established, the fermentors were fed once per day with waste containing 6% VS. Initially the fermentors were maintained at 18-day RT and a loading rate of 3.3 g of VS per liter per day for 3-volume turnovers, i.e., 54 days, after which four daily samplings were obtained and analyzed. After the samplings were completed, the RT and loading rate were adjusted to the next level over 4 days, and the equilibration time and sampling were repeated. This process was continued until the fermentors failed due to excess concentraton of waste or an RT of 2.5 days was reached.

TABLE 1. *Experimental design for study of methane production from beef feedlot waste at various temperatures and RT*

Fermenter no.	Temp (°C)	Loading rate (g of VS/liter per day) at given RT (days)[a]				
		18	12	9	6	3
1	30	3.3	5	6.7	10	20
2	35	3.3	↓	↓	↓	↓
3	40	3.3				
4	45	3.3	↓	↓	↓	↓
5	50	3.3				
6	55	3.3	↓	↓	↓	↓
7	60	3.3				
8	65	3.3				

[a] See text for the time period each fermentor was maintained at a particular RT.

In experiment 2, samples for analyses were not taken until 4-volume turnovers were completed, and the 18-day RT was not repeated.

Analytical procedures. Gas volume was determined by siphon displacement of a 20% NaCl and 0.5% citric acid solution as previously described (16). A manometer was connected to the rubber-stoppered solution jar. The solution displaced by the gas volume was weighed. Gas volume was corrected to standard pressure (760 mm of Hg), temperature (0°C), and water vapor content. Gas composition was analyzed with a Packard model 428 gas chromatograph with a stainless-steel Chromosorb 2 column, 182.9 by 0.64 cm OD, and a thermal conductivity detector. Helium served as the carrier gas for methane and carbon dioxide, whereas nitrogen was the carrier gas for the detection of hydrogen. Identification and percentage of methane, carbon dioxide, and hydrogen were based on a comparison of RT and peak areas of unknowns with those of standard amounts of the three gases.

Individual organic acids were determined by two procedures. The data which include nonvolatile and volatile acids were determined by the method of Salanitro and Muirhead (14) except that the column used was as described below, BF3-butanol was used as the butylating agent, and 0.4 ml of trifluoroacetic anhydride was used instead of 0.2 ml to react with the hydroxy acids and excess butanol in the reaction mixture. Waste containing 6% VS was initially centrifuged at 45,000 × *g* for 30 min before butyl esters were prepared. The gas chromatograph used was a Hewlett Packard model 5840A with a coiled glass column (182.9 by 0.2 cm ID) packed with 15% SP 1220/1% H_3PO_4 on 100/200-mesh Chromosorb W, acid washed (7). The injection port and detector temperatures were 200 and 250°C, respectively, and the initial column oven was set at 50°C followed by a 10°C/min temperature increase to 200°C. The total time for chromatographic separation and integration using a nitrogen carrier gas at a flow rate of 5 ml/min was 35 min for each sample.

A second procedure, in which only the volatile acids were analyzed (see Table 3), involved centrifugation (45,000 × g for 20 min), acidification to pH 2.0 using H_3PO_4, and direct injection of the supernatant on the same column as described above. The injection port, column, and detector temperatures were 200, 125, and 250°C, respectively. A nitrogen carrier gas was used at a flow rate of 40 ml/min.

Cellulose, hemicellulose, lignin, either extracts, sodium, potassium, and phosphorus were determined by previously published procedures (2). Total solids, VS, alkalinity (titration to pH 3.7), ammonia, and total volatile acids (salicic acid method) were determined by previously published methods (1). Kjeldahl nitrogen was determined by the method of Wael and Gehrke (17). Starch was determined by the method Wheeler et al. (18).

RESULTS

Substrate composition. Some constituents of the beef feedlot waste used in the study are listed in Table 2. The 6% organic acid fraction was comprised of acetate (58%), propionate and butyrate (16% each), lactate (9%), and a trace of succinate. Ammonia nitrogen accounted for 48% of the total nitrogen. The pH of the waste was 6.9, and the alkalinity concentration was 5,200 mg of $CaCO_3$ per liter.

Methane production. Figure 1 shows the liters of methane produced per gram of VS fed for the various temperatures and RT. Most of the values were between 0.20 and 0.30 liters per g of VS fed at 35 to 60°C and RT of 6 to 18 days. The fermentors produced less than 0.20 liters per g of VS fed when operated at 3-day RT, irrespective of temperature. Active fermentations could not be maintained at an RT of 6 days for 30°C, or at an RT of 3 days for 30, 35, 40, and 65°C. These fermentors presumably were inhibited because of high loading rates. Steady-state conditions at 2.5-day RT were achieved only for the 60°C fermentor.

TABLE 2. *Composition of dry matter of waste from beef cattle fed a high-grain finishing diet*[a]

Constituent	%[b]
VS	90.2 ± 0.20
Ether extract	3.4 ± 0.25
Cellulose	10.6 ± 0.27
Hemicellulose	20.2 ± 0.42
Lignin	3.5 ± 0.10
Starch	16.4 ± 0.51
Organic acids (as acetic)	6.0 ± 0.30
Kjeldahl N	4.8 ± 0.05
Ammonia N	2.3 ± 0.10
Phosphorus	1.1 ± 0.06
Potassium	1.8 ± 0.01
Sodium	0.3 ± 0.01

[a] See the text for methods of determination.

[b] Values are the mean and standard error of four determinations.

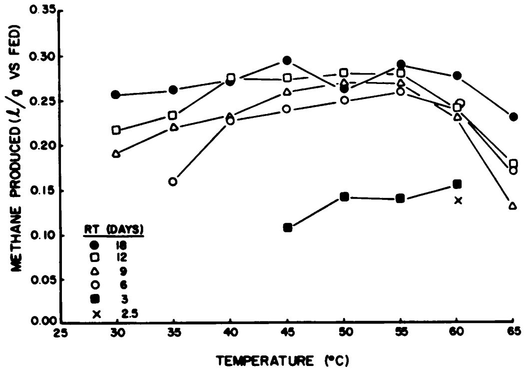

FIG. 1. *Methane production based on liters of methane per gram of VS fed as related to temperature at different RT.*

Figure 2 shows data on methane production at the various temperatures and RT based on liters of methane per liter of fermentor per day. At the 18-day RT, the rate of methane production was essentially the same at all temperatures, i.e., slightly less than 1 liter/liter per day. However, as the RT was decreased to 12, 9, and 6 days, a gradual increase in the amount of methane produced was observed when the temperature was increased from 30 through 55°C, followed by a decrease at 60 and 65°C. This effect was most dramatic at the 6-day RT. Thus, the effect of temperature on the rate of gas production is more apparent at short than at long RT. This trend is different from municipal refuse data, which showed peaks in gas production at 40 and 60°C (12).

Methane was found to be 46 to 55% of the total gas produced, with the balance being mainly carbon dioxide.

VS degradation. Figure 3 shows the relationship of temperature and RT to VS degradation. These data indicate that between 50 and 60% of the VS were degraded to methane and carbon dioxide under most temperatures and RT studied. In general, as the RT decreased and the loading rate increased, the VS degradation decreased.

Organic acid concentrations. Concentration of organic acids in a fermentor is a sensitive parameter used to determine fermentor stability. Figure 4 shows data on the levels of total volatile acids present in the fermentors stabilized at different temperatures and RT. Near the minimum and maximum temperatures of 30 and 65°C, a greater variability in the concentrations of acid with change in RT was observed, whereas

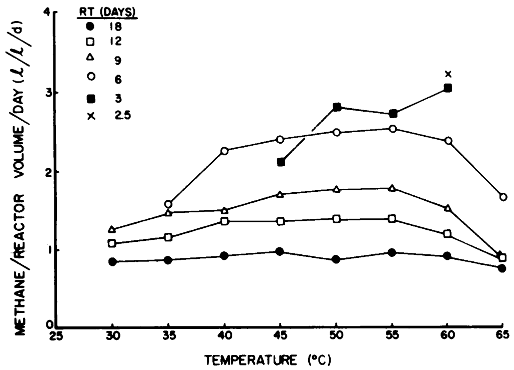

FIG. 2. *Rate of methane production based on liters of methane per liter of fermentor per day as related to temperature at different RT.*

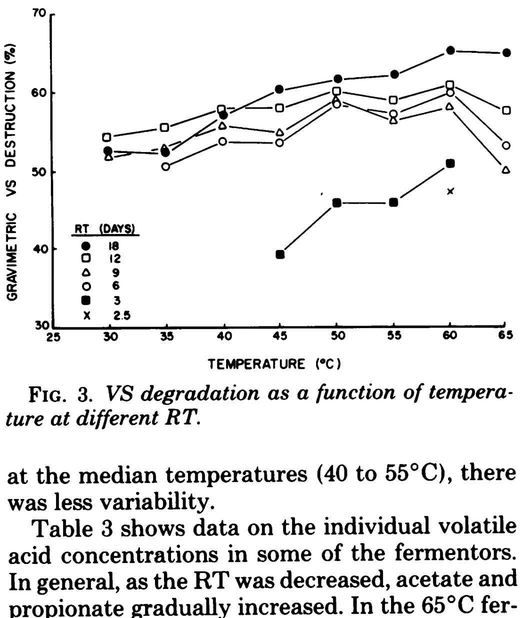

FIG. 3. *VS degradation as a function of temperature at different RT.*

at the median temperatures (40 to 55°C), there was less variability.

Table 3 shows data on the individual volatile acid concentrations in some of the fermentors. In general, as the RT was decreased, acetate and propionate gradually increased. In the 65°C fermentor, these acids plus isobutyric, butyric, isovaleric, and valeric acids were initially more prevalent than the 35, 45, and 55°C fermentors.

Experiment 2. In an effort to confirm the reproducibility of our results in experiment 1, a second experiment was conducted with a different lot of waste. This waste contained the following percentage composition (dry weight basis): VS, 88.2; ether extract, 7.4; cellulose, 11.1; hemicellulose, 13.4; lignin, 3.8; starch, 15.5; organic acids (as acetate), 8.6; Kjeldahl nitrogen, 4.4; ammonia nitrogen, 1.4; potassium, 1.1; phosphorus, 1.8; and sodium, 0.64. The alkalinity of the waste was 3,270 mg of $CaCO_3$ per liter, and pH was 5.5.

The results from experiments 2 (data not shown) showed significantly lower concentrations of acid in the 50, 55, 60, and 65°C fermentors at RT of 6, 9, and 12 days. Because of fermentor failures at the 3-day RT, data were obtainable from only the 60°C fermentor. The alkalinity concentrations were on the average 2,000 to 3,000 mg of $CaCO_3$ per liter lower, and the ammonia concentrations were 30 to 40 mM lower. The other parameters, including methane production rates, were similar to those for experiment 1.

DISCUSSION

These studies indicate that there is little difference in rates of methane production between 40 and 60°C at RT of 6 days or longer. The respective rates for these fermentors at a 6-day RT were 2.28, 2.42, 2.51, 2.57, and 2.41 liters/liter of fermentor per day, indicating that the higher temperatures are unjustified. Further studies should be conducted with large-scale fermentors verifying the effect of temperature and RT on methane production from beef waste. If one were interested in fine-tuning the rate of methane production as a function of temperature, VS concentrations of 7, 8, 9, and 10% should be investigated at RT of 4, 5, and 6 days. Hashimoto et al. (6) have concluded that for a given VS loading rate, higher methane production rates are possible at higher influent VS concentrations and longer RT. However, this is indicated to hold true only up to an influent VS concentration of about 8%.

A kinetic analysis, in which the optimum RT and maximum volumetric methane production rates were calculated based on results from our studies, indicates that a fermentor set at 60°C and an RT of 3.7 days will produce 90% more methane than one at 35°C and 5.9-day RT; 46% more than one at 40°C and 4.7-day RT; 28% more than one at 45°C and 4.1-day RT; 8% more than one at 50°C and 3.4-day RT; and 12% more

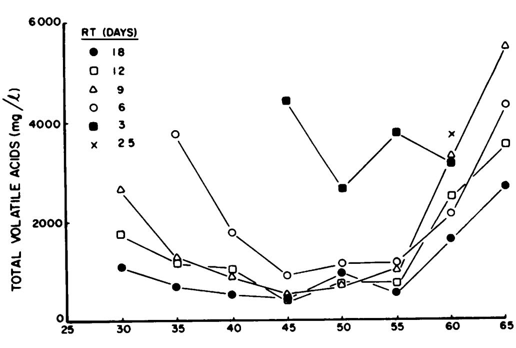

FIG. 4. *Total volatile acid concentrations as related to temperatures at different RT.*

TABLE 3. *Effect of temperature and RT on the concentration of individual volatile acids in some beef cattle waste fermentors*

Temp (°C)	RT (days)	Acid concn (mM)[a]						Total organic acids (mg of acetate/liter)[b]
		Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	
35	18	4.8	1.0	0	0	0	0.9	372
	12	12.5	2.9	0.2	0	0.4	1.1	949
	9	13.3	2.0	1.6	0	0.4	1.3	1,027
	6	16.9	36.4	5.5	0.9	3.4	5.1	3,357
	3[c]	90.9	32.3	6.9	24.1	6.2	12.7	9,055
45	18	3.1	0	0	0	0	1.5	242
	12	3.4	0	0	0	0	1.8	269
	9	3.2	0	0	0	0	1.7	252
	6	6.5	3.6	0	0	0.5	1.8	648
	3	16.0	28.1	6.5	36.1	7.1	4.1	4,476
55	18	2.4	0.9	0	0	0	2.0	263
	12	4.6	2.2	0	0	0	2.6	491
	9	5.7	5.8	0	0	0.2	3.6	762
	6	5.6	7.9	0	0	0.4	2.7	836
	3	19.9	17.9	4.5	34.4	3.3	3.9	3,937
65	18	9.1	13.3	3.3	2.0	4.7	2.9	1,720
	12	13.0	23.3	6.0	7.2	9.5	5.0	3,028
	9	51.4	23.2	6.8	10.0	10.2	5.0	5,453
	6	48.0	21.7	5.8	13.9	7.7	5.0	5,198
	3[c]	120.3	20.3	4.6	11.2	5.7	3.7	9,185
Influent[d]		20.9	4.3	0	6.0	0.7	3.6	1,863

[a] Values are the average of four determinations, two per sample on 2 consecutive steady-state days.
[b] Trace amounts of isocaproic and caproic acids were observed under some conditions and were included in these totals.
[c] Value obtained from samples just before complete fermentation failure as a result of overloading substrate.
[d] Average of three determinations.

than one at 65°C and 6.0-day RT (4). These data indicate that there is kinetic advantage in fermenting beef waste at thermophilic temperatures (>45°C) and short RT (<6 days). However, there is little advantage in fermenting waste at 60 rather than at 50°C. Increasing the fermentation temperature from 50 to 60°C, in fact, may result in a lower net energy production due to the higher heating requirements to maintain the fermentor temperature. The kinetic analysis showed that a net energy loss would also occur when operating a fermentor at long RT (>20 days) at thermophilic temperatures.

The concentrations of acid generally increased with an increase in loading rate. The concentrations of acid for the 60 and 65°C fermentors for all the RT studied, with the exception of the 60°C fermentor at 18-day RT, were above 2,000 mg/liter, which is sometimes considered to be inhibitory for mesophilic systems (8, 10). Our data (Fig. 4 and Table 3, 65°C fermentor) support other investigations (5, 9) which have shown that concentrations of volatile acids are higher in thermophilic fermentors than mesophilic ones. Based on the higher acid concentrations, Buhr and Andrews (3) concluded that a poorer-quality supernatant may be expected from thermophilic fermentations. The relative concentrations of propionate, and in some cases acetate, in the 60°C fermentor at all RT (data not shown) were higher by 13 to 15 mM than the concentrations in the 60°C fermentors used in a previous study (16) which were fed higher loading rates. The reason for these high concentrations are unknown, but may be a result of the differences in the percentage of VS (90 versus 72), lignin (3.5 versus 6.8), Kjeldahl nitrogen (4.7 versus 3.0), ammonia nitrogen (2.3 versus 0.55), and volatile acids (6.0 versus 1.2) of the two wastes. Although higher acid concentrations were found in this study than in the previous one, we do not feel that the fermentations were unstable. We have maintained fermentors for bacteriological studies for longer than 2 years with waste identical to that in experiment 2. These fermentors were fed 7% VS at a RT of 6 days and set at 55°C. The methane production rate and acid concentration have remained at approximately 2.91 liters/liter of fermentor per day and 1,835 mg of acetate per liter, respectively.

The reason for not obtaining steady-state data in experiment 2 at an RT of 3 days, with the exception of the 60°C fermentor, is unclear. Repeated efforts were made to stabilize the acid concentrations in the 45, 50, and 55°C, 3-day RT fermentors, with the additions of sodium hydroxide, decreasing the loading rate for extended periods, etc., but all efforts failed. Again, a possible explanation is the differences in the ammonia nitrogen, pH, alkalinity, and the concentration of volatile acids between the two wastes used and the fact that 3-day RT is close to the minimum RT. In experiment 2, the volatile acids and ammonia accounted for 8.6 and 1.4% of waste dry matter, respectively. The pH of the waste was 5.5, and the alkalinity concentration was 3,270 mg of $CaCO_3$ per liter. This compares with a volatile acid and ammonia concentration of 6.0 and 2.3%, respectively, pH of 6.9, and an alkalinity concentration of 5,200 mg of $CaCO_3$ per liter for the waste used in experiment 1. Thus, the waste used in experiment 2 is much higher in organic acids and has less ammonia nitrogen for buffering purposes, which is reflected in the lower alkalinity and pH values than for the waste used in experiment 1. This suggests that the composition of the waste plays an important role when fermentors are operated at high loading rates and short RT and requires further study.

Several studies (13, 16) have shown that when a fermentor is stressed, propionate is the first acid to increase. When further stressed, acetate also increases. Our data in Table 3 support this. In severely stressed fermentors, butyrate in particular accumulates to high levels; to a lesser degree, isobutyrate, isovalerate, and valerate also accumulate under mesophilic and thermophilic conditions. This indicates that similar metabolic pathways are used by the mesophilic and thermophilic microorganisms for anaerobic degradation of organic matter.

These studies indicate potential rates of methane production from the fermentation of untreated waste of beef cattle fed high-grain finishing diets. Similar rates and higher ones also have been demonstrated in a 5.7-m^3 fermentor, which indicates that these rates can be obtained on a larger scale. If field-size units are fed chemically similar materials and do not produce similar rates, one can assume it is not due to a lack of biological potential. These rates, along with the other parameters studied, should serve as preliminary guidelines for a more precise economic evaluation of fermenting beef cattle waste to methane.

ACKNOWLEDGMENTS

The aid of Bill Wheeler in determining starch content, the aid of Ron Mlejnek and Sandy Cummins in determining individual fatty acids, and the technical assistance of Sandy Fryda, Steve Robinson, Lynn Nieman, and Misi Eich are gratefully acknowledged.

LITERATURE CITED

1. **American Public Health Association.** 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association Inc., New York.
2. **Association of Official Analytical Chemists.** 1975. Official methods of analysis, 12th ed. Association of Official Analytical Chemists, Washington, D.C.
3. **Buhr, H. O., and J. F. Andrews.** 1977. The thermophilic anaerobic digestion process. Water Res. **11**:129–143.
4. **Chen, Y. R., V. H. Varel, and A. G. Hashimoto.** 1980. Effect of temperature on methane fermentation kinetics of beef cattle manure. Biotechnol. Bioeng. Symp. **10**: 69–81.
5. **Golueke, C. G.** 1958. Temperature effects on anaerobic digestion of raw sewage sludge. Sewage Ind. Wastes **30**: 1225–1232.
6. **Hashimoto, A. G., Y. R. Chen, and R. L. Prior.** 1979. Methane and protein production from animal feedlot wastes. J. Soil Water Conserv. **34**:16–19.
7. **Hauser, J. J., and R. J. Zabransky.** 1975. Modification of the gas-liquid chromatography procedure and evaluation of a new column packing material for the identification of anaerobic bacteria. J. Clin. Micribiol **2**:1–7.
8. **Kroeker, E. J., D. D. Schulte, A. B. Sparling, and H. M. Lapp.** 1979. Anaerobic treatment process stability. J. Water Pollut. Control Fed. **51**:718–727.
9. **Malina, J. F.** 1962. Thermal effects on completely mixed anaerobic digestion, p. 232–250. *In* Proceedings of the 16th Indiana Waste Conference. Purdue University Extension Series 109. Purdue University Press, Lafayette, Ind.
10. **McCarty, P. L., and R. E. McKinney.** 1961. Volatile acid toxicity in anaerobic digestion. J. Water Pollut. Control Fed. **33**:223–232.
11. **Miner, J. R., and R. J. Smith.** 1975. Livestock waste management with pollution control. North Central Region Research publication 222. Midwest Plan Service Handbook. Iowa State University, Ames.
12. **Pfeffer, J. T.** 1974. Temperature effects on anaerobic fermentation of domestic refuse. Biotechnol. Bioeng. **16**:771–787.
13. **Pohland, F. G., and D. E. Bloodgood.** 1963. Laboratory studies on mesophilic and thermophilic anaerobic sludge digestion. J. Water Pollut. Control Fed. **35**:11–42.
14. **Salanitro, J. P., and P. A. Muirhead.** 1975. Quantitative method for the gas chromatographic analysis of short-chain monocarboxylic and dicarboxylic acids in fermentation media. Appl. Microbiol. **29**:374–381.
15. **Smith, R. J., M. E. Hein, and T. H. Greiner.** 1979. Experimental methane production from animal excreta in pilot-scale and farm-size units. J. Anim. Sci. **48**:202–216.
16. **Varel, V. H., H. R. Isaacson, and M. P. Bryant.** 1977. Thermophilic methane production from cattle waste. Appl. Environ. Microbiol. **33**:298–307.
17. **Wael, L. L., and C. W. Gehrke.** 1975. An automated total protein nitrogen method. J. Assoc. Off. Anal. Chem. **48**:1221–1226.
18. **Wheeler, W. E., C. H. Noller, and C. E. Coppock.** 1975. Effect of forage to concentrate ratio in complete feeds and feed intake on digestion of starch by dairy cows. J. Dairy Sci. **58**:1902–1906.