The Effect of pH on Methane Production from Dairy Cattle Manure

Summer 1982

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THE EFFECT OF pH ON METHANE PRODUCTION FROM DAIRY CATTLE MANURE

BY

MARY GALZERANO STAFFORD
B.S., Stetson University, 1974

THESIS

Submitted in partial fulfillment of the requirements for the degree of master of Science in Microbiology in the Graduate Studies Program of the College of Arts and Sciences University of Central Florida Orlando, Florida

Summer Term
1982
The effects of pH upon methane production from anaerobic digestion of dairy cattle manure were investigated. One liter digesters were maintained by daily adjustment at the following pH levels: 7.6, 7.0, 6.0, 5.5, and 5.0. After 33 weeks of incubation the working volume of the digesters was increased to 3 liters. Digesters were incubated on a rotary shaker at 37°C. Digesters were loaded at the desired volatile solids concentrations, without an inoculum, and maintained from day one by daily additions and withdrawals to achieve a 3 day retention time. After 50 weeks of operation the manure from a second dairy was utilized as substrate.

Active digestion was achieved at all pH levels except pH 5.0. Biogas production was evident in 4 to 6 days after incubation. Biogas production was highest at pH 7.0 with manure from both dairies (3.047 ± 0.403 liters per liter of digester per day with Dairy II manure and 1.43 ± 0.09 liters per liter per day with Dairy I manure). Methane production was also highest at pH 7.0 (1.43 ± 0.292 liters per liter of digester per day with Dairy II manure and 0.611 ± 0.057 liters per liter per day with Dairy I manure although the highest percentages of methane in the biogas occurred at pH 7.6 (65.9 ± 5.2 from Dairy I manure and 50.4 ± 4.6 from Dairy II manure). Dairy II manure
produced significantly more biogas and methane at all pH levels. This increased production could not be attributed solely to differences in volatile solids concentrations of the two substrates.

Total volatile acid concentrations were highest at the highest pH levels and were higher with Dairy II manure as a substrate. Digesters at all pH levels had volatile acid concentrations above the 2000 mg/liter normally considered inhibitory for methane bacteria (2314-2890 mg/liter with Dairy I manure and 5.708-7.434 mg/liter with Dairy II manure).

The results reported here indicate that stable methane digestion of dairy cattle manure can be established and maintained at 37°C with a 3 day retention time. Digestion at pH levels as low as 5.5 continued for periods up to 24 months without a failure. High levels of volatile acids did not cause digester failure. Characteristics of the manure have significant effects on biogas and methane production.
ACKNOWLEDGEMENT

For his help and encouragement throughout this thesis endeavor I would like to thank Dr. R.J. Wodzinski. I would also like to thank the other members of my committee, Dr. R.N. Gennaro and Dr. D.H. Vickers, for their help in preparation of the thesis and their encouragement.

During the course of this study, the physical and spiritual help of my laboratory partners has been invaluable. I extend my deepest appreciation to Michael H. Scholla, Douglas A. Winkelmann, Sheril K. Charba, and Jesse W. Johnson. Sue Cox deserves a special thank you for all the feeding and grinding she did.

I would like to thank the Coordinating Council on the Restoration of the Kissimmee River Valley and Taylor Creek-Nubin Slough Basin for financing this research.

To my husband, Steven R. Stafford, goes the final thanks. I could not have done this without him. His help in feeding and grinding was above and beyond the call of duty and his encouragement throughout was essential.
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INTRODUCTION

In recent years there has been increased interest in the application of the anaerobic fermentation process in the treatment of a variety of wastes. These include domestic refuse, domestic sewage, livestock manure, agricultural wastes, and food processing wastes. Because of the variety of materials available as substrates, it is unlikely that any one set of optimum digestion conditions will be suitable. Significant differences have been reported in fermentation requirements for poultry, swine, and cattle wastes (9,13,15,18,34).

One of the considerations in defining optimum conditions must be the desired result. With domestic sewage a major objective is maximum destruction of organic material to reduce the quantity of effluent solids which must be disposed of, usually by landfill or land spreading (34,35). Recovery of methane gas is of secondary importance. Longer retention times generally provide greater solids destruction and higher percentages of methane in the gas. The objective in livestock waste treatment is to abate pollution at a cost that is not prohibitive to the farmer. Today's intensive livestock operations produce massive quantities of manure concentrated in feedlots. The value of the methane and the effluent solids becomes extremely important in reducing treatment facility installation and operation expenses. Emphasis is shifted to
stabilizing the solids rather than reducing them. The solids may then be sold as fertilizer or used as a feed additive. This may be accomplished by thermophilic digestion at retention times as low as 3 days. Under these conditions the rate of methane production is also increased. Experiments with beef cattle manure, 3 day retention time (RT) and thermophilic conditions, produced methane at the rate of 4.5 liters per day per liter of digester (39). This is the highest rate that has been reported.

Advantages of Anaerobic Digestion. Methane is a useful product whose sale or on-site use can offset the costs of system installation and operation (8,11,14,25,39,40,42). The effluent may also be economically important as a fertilizer or as a protein source (8,14,34,42). In an economic assessment of a hypothetical anaerobic digestion system for a 10,000 head beef cattle feedlot it was concluded that the system was economically feasible when the protein and methane values were both considered (14). Computer model studies by Wodzinski et al. (42) indicated that anaerobic digestion of dairy cattle waste was economically feasible if the solids are recovered and upgraded to a 6-6-6 fertilizer before sale. The methane may be sold or credit may be taken for its on-site use.

There is a reduction in the weight and volume of solids after anaerobic digestion (8,17,22,25,29,30,40). Anaerobic digestion provides little energy to the microorganisms. Most of the organic materials are converted into carbon dioxide and methane rather
than into production of new bacterial cells. This allows as much as 80 to 90% of the biodegradable organic portion of a waste to be stabilized by conversion to methane (25). Reductions in weight and volume of sewage sludge solids of 30% or more have been achieved (8). In a report on farm animal waste management, reductions in organic solids of 40 to 100% depending on the nature of the wastes have been reported (29).

Anaerobic digestion reduces offensive odors (14,29,30). This allows land spreading of the dried effluent in areas where direct spreading of manure would be offensive (29).

There is a reduction in the number of pathogenic organisms in the effluent, particularly under thermophilic digestion conditions (4,8,11,38,40).

The digested material has improved dewatering characteristics (8,20,22,29). One investigator used the capillary suction time (CST) analysis to determine the relative dewaterability of raw manure and the effluent of thermophilic digesters (20). CST reductions in the effluent of 25-50% were obtained. This material still had CST values considerably above the 40 to 50 seconds required for efficient sand bed dewatering.

Limitations of the Anaerobic Process. The major limitation of the process has been its perceived tendency to failure if one or more of the environmental factors changes suddenly (22,23,36). The rate at which the process can adjust to changing waste loads, temperatures, or other environmental conditions was thought to be
limited by the slow rate of growth of the methane bacteria (25). However, thermophilic methane bacteria have shorter generation times than mesophilic methane bacteria and relatively fast growth rates have been achieved at elevated temperatures. Stable fermentations at retention times of 2.5-3.0 days have been reported (38,39). According to Varel et al. (39) the fermentation is "highly stable to temperature changes between 55 and 60°C, to changes in RT between 15 and 3 days, and to increases in the amount of volatile solids (VS) in the feed from 2% to about 8 to 12% depending on the RT and lot of waste." Even at mesophilic temperatures, recent investigators have reported improved process stability (23). The importance of acclimation procedures was stressed in the stability of digesters to temporary hydraulic overloading, large daily fluctuations in influent organic solids, and temporary diurnal temperature fluctuations at RT's of 6 and 30 days.

The Anaerobic Digestion Process. The anaerobic digestion process may be considered to involve three general metabolic groups of bacteria (3) (Fig. 1). A complex group of fermentative bacteria catabolizes polysaccharides, proteins, and lipids to organic acids, alcohols, hydrogen, and carbon dioxide. A second group, the hydrogen-producing acetogenic bacteria, utilizes the organic acids and alcohols produced by the first group resulting in the production of acetate and hydrogen, and sometimes carbon dioxide. These two groups have often been collectively referred to as acid forming bacteria. The methanogenic bacteria then
Fig. 1. A scheme showing the relationships of the three general metabolic groups of bacteria or stages of fermentation involved in the methane fermentation (3).
utilize some of the products of the first two groups with the resultant production of methane and carbon dioxide. A complex interaction of these groups of organisms is necessary for the complete degradation of organic compounds to methane and carbon dioxide. The interaction of several factors will influence the types and numbers of each group of bacteria in an anaerobic fermentation. These include temperature, retention time, influent volatile solids concentrations, pH, and start-up conditions.

Anaerobic digestion studies have been performed at thermophilic (20,24,37,39) and mesophilic temperatures (10,14,20,21,24,34,37). It is generally considered that thermophilic systems are capable of generating high amounts of biogas at short RT (3 to 9 days) whereas mesophilic systems require longer RT to produce equivalent gas production rates (3,21,37,39). In a study on thermophilic methane production from beef cattle wastes the methane production rate was highest at 60°C with an RT of 3 days (39). Changes in temperature from 55 to 60°C produced little change but an increase to 65°C greatly lowered methane production. In another study there was little difference in rates of methane production between 40 and 60°C at RT of 6 days or longer (38). However, a kinetic analysis indicated that a digester at 60°C and an RT of 3.7 days will produce 90% more methane than one at 35°C with a 5.9 day RT. Results of a study of temperature effects on anaerobic fermentation of domestic refuse also indicate highest methane production rates at 60°C and 3 to 4 days RT (31).
Influent volatile solids are generally 6% or less for ruminant manures and 3% or less for non-ruminant manures (34,38). In one study, efficient fermentation at 8.2, 10.0, 11.6, and 11.6% VS for retention times of 3, 6, 9, and 12 days respectively was achieved (39). These VS feed concentrations were achieved by increases of 2% at 1 month intervals. Digestion was retarded at 14% VS.

The methanogenic bacteria have pH optima of 6.8-7.4 while the acid forming bacteria have optima of 5.0-6.0 (S. Ghosh and D.L. Klass, U.S. Patent #4022665). The process as a whole operates best near neutrality (2,3,20,22,26,41). Fermentations in the range of 6.6-7.6 are considered stable with the rate of methane production decreasing rapidly above or below these levels. Alkalinity and volatile acid concentrations influence pH level. In the pH range 6.0-7.5 volatile acids and ammonia are almost completely dissociated acting as a weak acid and base (6). The major system controlling pH is the carbon dioxide-bicarbonate system (6,26). Alkalinity formation and the dynamics of the buffering system are represented in Fig. 2. As volatile acids increase in the system they react with the bicarbonate alkalinity to form acid salts (volatile acid alkalinity) with the release of carbon dioxide (26,32). As the methane bacteria degrade the acid salts to produce methane, potential alkalinity will be released to the system to continue the cycle.
Fig. 2. Alkalinity formation. Modified from Pohland and Bloodgood (32).
When volatile acid concentrations are low, total alkalinity (TA) expressed as mg/liter CaCO$_3$ is approximately equivalent to bicarbonate alkalinity (BA) expressed as mg/liter CaCO$_3$. At increased volatile acid concentrations volatile acid alkalinity must also be considered:

$$\text{TA} = \text{BA} + (0.85)(0.833)\text{TVA}$$

where TVA = total volatile acid concentration, mg/liter as acetic acid. (After 26.) The factor 0.833 converts acid units from mg/liter acetic acid to mg/liter CaCO$_3$ and the factor 0.85 is included to account for the fact that only 85% of the volatile acid alkalinity is measured by titration of total alkalinity to pH 4 (26,32).

In a balanced digester, the volatile acids produced by the acid forming bacteria are utilized by the methanogenic bacteria as rapidly as they are formed (25). If the activity of the methanogenic bacteria is inhibited or if the acid forming bacteria are stimulated, an accumulation of volatile acids will occur. One group of investigators considers concentrations of volatile fatty acids above a critical limit (2000-3000 mg/liter) to be inhibitory to the methane bacteria at any pH level (5,16,27,33). The degree of inhibition is related to "salt toxicity" dependent on the type and concentration of cation in the volatile acid salt. Relatively high volatile acid concentrations can be tolerated provided they are neutralized with alkaline materials.
containing a cation of low toxicity (27). A second group of investigators believes that volatile fatty acids are toxic only indirectly by reducing the pH. An increase in volatile acids over and above the available cation component of the alkalinity could result in the production of free volatile acids and a destruction of the buffering potential of the system. The resultant low pH generally inhibits the methane bacteria further (32).

Start-up conditions are important in the establishment of successful fermentations. Digesters are usually started at low VS concentrations either with or without an inoculum from a working digester (17,18,35,38,39). The digester may be allowed to "acclimate itself" to the substrate before further additions (12,23,38,39) or loading may begin immediately at very low rates (17,18,35). Cassell and Sawyer (7) initiated digestion with undiluted sewage sludge. Digesters were filled with raw sewage and brought to temperature. Loading at the desired level was begun immediately and continued throughout the study. With a 20 day RT, normal digestion was achieved in 40-65 days. Maintenance of the pH between 6.8 and 7.2 was essential for these start-up procedures.

The purpose of this study was to investigate the effects of pH, feed ration and start-up conditions on methane production from dairy cattle waste.
MATERIALS AND METHODS

Digesters. In the initial phase of this study, one liter digesters with a working volume of 0.6 liters were outfitted as described by Varel et al. (39). After 33 weeks, the contents of the flasks, which were maintained at the experimental conditions described below, were transferred in a Coy anaerobic chamber to 4 liter digestion flasks of the same design with a working volume of 3 liters. The data reported here is the data obtained with the 3 liter digesters over a 33 week period. The digesters were maintained for an additional 10 months with essentially the same results.

Gas impermeable bags (60 cm x cm) (Pollution Measurement Corporation) equipped with a 200-AS valve, 7 cm from the bottom of the bag, were attached to the digesters. The digesters were incubated at 37°C on a New Brunswick Scientific Company G-10 rotary shaker at 110 rpm with a 5.1 cm diameter displacement.

Substrate. Dairy cow waste was obtained from two commercial dairies in Florida. Dairy I fed its cattle a ration of 86% Bermuda hay and 14% concentrate. The concentrate contained wheat middlings, soy bean meal, peanut meal, cane molasses, ground cornmeal, cotton seed hulls and brewer's grains. The exact percentage of the ingredients was not made available to us. Dairy waste (feces and urine) was collected from concrete feeding slabs.
Dairy II had its dry cows and replacement calves divided into two separate groups for feeding. One group was fed a diet of 17% dihydrolyzed poultry waste, 19% cotton seed hulls, 30% corn hominy, 12% grain mixture (soy bean and cotton seed meal), 8% molasses and 14% hay. The second group was fed a diet of 10% potato chips, 32% dihydrolyzed poultry waste, 30% cotton seed hulls, 14% corn hominy, 5% grain mixture (soy bean and cotton seed meal) and 8% molasses. Waste accumulated from the two groups for two to five days before the area was cleaned. The dairy waste (feces and urine) was collected from a feed area with a concrete floor and an aluminum roof.

The waste from both dairies was shoveled into 208 liter plastic-lined, steel barrels and transported to the laboratory at ambient temperature. The waste was stored at 4°C until total and volatile solids analyses were completed. The manure was diluted with tap water to the desired solids concentration. It was ground with a submersible grinder pump (Peabody Barnes, Model 203) in a 208 liter plastic-lined, steel barrel. The diluted ground manure was dispensed into 3.76 liter plastic jugs and stored at -20°C until used. The frozen manure was thawed overnight at 4°C and for 6 to 9 h at room temperature.

**Digester Start-Up and Maintenance.** The digesters were started by adding manure to the desired amount and gassing with nitrogen for 10 min. The digesters were sealed and incubated. Substrate was fed after 24 h and each 24 h thereafter for 24 mo at the
appropriate rates to achieve a 3 day RT.

Five digesters were maintained at the following pH levels: 7.6 ± 1.0, 7.0 ± 1.0, 6.0 ± 1.0, 5.5 ± 1.0, and 5.0 ± 1.0. The pH 7.6 digester represented the pH level of the unadjusted substrate. When the digesters were started, the pH levels were adjusted to the desired level with 5 N NaOH or 5 N HCl. After the effluent was removed each day, the pH was determined on a portion of the effluent. The effluent was titrated with 5N NaOH or 5 N HCl to the desired pH level of each digester. The pH of the fresh substrate was adjusted to the desired level and an appropriate amount of acid or base was added to the adjusted substrate to bring the pH level of the entire digester to the desired level. After addition of the substrate, the digester was vigorously shaken manually and allowed to shake on the rotary shaker for 15 min. The pH level was rechecked on a small aliquot from the digester. If further adjustment was necessary, it was made with either 5 N NaOH or 5 N HCl.

Analytical Procedures. When the gas bag to each digester contained 10 to 17 liters of biogas, it was removed and replaced with an evacuated bag. The gas volume was determined by fluid volume displacement (39). Samples of biogas for analysis were collected from the digesters using gas sample bulbs equipped with gas-tight stopcocks (Supelco, Inc.) or sealed 100 ml serum bottles. The gas sample container was evacuated and flushed three times with helium, then evacuated to 745 mm Hg. A double-draw needle
(Scientific Products) was first inserted into a port in the amber latex tubing leading into the gas bag, then into the sample collection container to obtain the sample.

Fifty microliter injection samples were collected from the sampling bulb with a Hamilton gas-tight syringe equipped with a side-port needle (Supelco, Inc.). The gaseous metabolic end products were determined with a Perkin-Elmer Sigma I gas chromatograph equipped with a thermal conductivity detector. A molecular sieve stainless steel column of 80/100 mesh Carbosieve (Supelco, Inc.), 2.3 m in length and 3.2 mm in diameter, was used to resolve methane, carbon dioxide, hydrogen, carbon monoxide and nitrogen. Optimum separation of gas components was achieved with the following temperature program: initial temperature of 60°C for 7 min; temperature increased to 110°C at a rate of 10°C per min and maintained for 2 min. Other instrument settings and parameters included: (1) an injection temperature of 75°C; a detector temperature of 150°C; (2) a carrier gas flow rate of 15 cc per min; (3) a thermal conductivity detector bridge current setting of 240 milliamps; and (4) a chart speed of 0.5 cm per min. Identification of gas components was based on the relative retention times compared to a commercially prepared standard. Concentration of gas components was determined by peak area integration after correlation of the peak areas with standards (28).

Total and volatile solids were determined on triplicate 50 g samples according to Standard Methods for the Examination of Water
and Wastewater (1).

Analysis of volatile fatty acids was performed by gas chromatography by the procedures in the *Virginia Polytechnic Institute Anaerobic Laboratory Manual* (19). The analysis of volatile fatty acids was done using a 7620A Hewlett Packard gas chromatograph equipped with a dual flame ionization detector. A 2 m stainless steel column (6.4 mm diameter) containing 15% SP1220/1% H₃PO₄ on 100/120 Chromosorb W AW (Supelco, Inc.) was used to resolve the fatty acids. Optimum separation of bacterial end products was achieved with the following temperature program: (1) initial column temperature 90°C for 2 min; (2) temperature increased to 120°C at a rate of 15°C per min and maintained for 2 min; (3) temperature increased to 150°C at a rate of 30°C per min and maintained for 2 min.; (4) temperature increased to 180°C at a rate of 30°C per min and maintained for 4 min. Other instrument settings and parameters were as follows: (1) nitrogen carrier gas with a flow rate of 40 cc per min; (2) a chart speed of 12.7 mm per min; (3) injection port and detector temperature of 250°C. Standards (Supelco, Inc.) were used to determine the relative retention times and concentrations. Concentrations of volatile acids in samples were determined by comparison of peak heights to standards and were expressed in mM.

Glucose was analyzed with an Auto Analyzer using method n-2b (Technicon Instrument Corporation) with pump II manifold #116-A151-01. The concentration of glucose was measured in
undiluted samples. The range of detection was 2.8 to 16.7 mM. Standard curves developed by this method had linear correlation coefficients of 0.97 ± 0.02.

Ammonia was analyzed with an Auto Analyzer using industrial method 18-69W (Technicon Instrument Corporation) with manifold #116-D001-01. This method was modified to include a dialyzer to remove particulate material from the manure since it interferes with the colorimetric analysis. In the modification employed, the sample was dialyzed prior to mixing with air and potassium sodium tartrate. The net effect of dialysis was to decrease the sensitivity by approximately 50%. The standard curve for this method gave correlation coefficients of 0.96 over a range of 0.714 to 5.36 mM. Since the ammonia concentrations in fresh manure and in digesters are higher than 5.36 mM, it was necessary to dilute the samples.

Methyl orange alkalinity was analyzed with an Auto Analyzer using industrial method 23-60W (Technicon Instrument Corporation) with manifold #116-D015-01. The method was modified to include dialysis of the sample prior to its combination with the methyl orange reagent and air. The net effect of dialysis is to reduce the sensitivity by approximately 50%. A correlation coefficient of 0.99 was obtained for the standard curve with standards ranging from 125-750 mg/liter CaCO₃. The fresh manure samples and digester samples were diluted in this range.
RESULTS

Preliminary Studies. Biogas production in the digesters was evident in 4 to 6 days after incubation. The pH of each digester fluctuated within narrow limits during the course of the fermentation (Tables 1,2). The pH of the digester adjusted daily to pH 7.6 varied the most, 7.4 to 7.2 with the manure from Dairy I and 7.1 to 6.7 with the manure from Dairy II. The digesters which were maintained at pH 7.0 and 7.6 tended to decrease in pH and those below pH 7.0 tended to increase in pH. When the pH was maintained below 7.0, the ammonia concentration increased (Tables 1,2). As would be expected, as the pH was lowered, the methyl orange alkalinity decreased (Tables 1,2). Methyl orange alkalinity was higher at all pH levels with manure from Dairy II. Essentially only methane and carbon dioxide were detected in the biogas. The percentage of carbon dioxide in the biogas increased as the pH was lowered (Tables 3,4). Glucose was not detected in any of the samples analyzed. The level of detection was 2.8 mM.

Effect of pH on biogas and methane production at 37°C with a three day retention time. More biogas and more methane were produced at pH 7.0 than at pH 7.6, 6.0, 5.5, 5.0 (Fig. 3). The highest yields of biogas were $3.047 \pm 0.403$ liters per liter of digester per day at pH 7.0 from Dairy II and $1.43 \pm 0.09$ liters
TABLE 1. Concentration of end products of methane fermentation of manure from Dairy I at various pH levels at 37°C with a 3 day retention time.

<table>
<thead>
<tr>
<th>pH of Reactor</th>
<th>Range of pH Before Daily Adjustment</th>
<th>Total Alkalinity mg/liter CaCO₃</th>
<th>Ammonia ((\text{NH}_3 + \text{NH}_4^-)) mM</th>
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<tbody>
<tr>
<td>7.6</td>
<td>7.2 - 7.4</td>
<td>4124 ± 176.4\textsuperscript{a}</td>
<td>30.8 ± 2.72\textsuperscript{a}</td>
</tr>
<tr>
<td>7.0</td>
<td>6.8 - 7.1</td>
<td>3409 ± 228\textsuperscript{a}</td>
<td>26.6 ± 2.49\textsuperscript{b}</td>
</tr>
<tr>
<td>6.0</td>
<td>6.0 - 6.3</td>
<td>1790 ± 138\textsuperscript{a}</td>
<td>42.4 ± 3.74\textsuperscript{a}</td>
</tr>
<tr>
<td>5.5</td>
<td>5.5 - 5.8</td>
<td>1429 ± 114\textsuperscript{c}</td>
<td>51.5 ± 4.58\textsuperscript{a}</td>
</tr>
<tr>
<td>5.0</td>
<td>4.8 - 5.8</td>
<td>1008 ± 82.0\textsuperscript{a}</td>
<td>51.2 ± 4.56\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The data represents the average of 17 weekly samples ± standard error.

\textsuperscript{b}The data represents the average of 16 weekly samples ± standard error.

\textsuperscript{c}The data represents the average of 15 weekly samples ± standard error.
TABLE 2. Concentration of end products of methane fermentation of manure from Dairy II at various pH levels at 37°C and a 3 day retention time.

<table>
<thead>
<tr>
<th>pH of Reactor</th>
<th>Range of pH Before Daily Adjustment</th>
<th>Total Alkalinity mg/liter CaCO₃</th>
<th>Ammonia (NH₃ + NH₄) mM</th>
</tr>
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<tbody>
<tr>
<td>7.6</td>
<td>6.7 - 7.1</td>
<td>6241 ± 1160</td>
<td>28.7 ± 1.88</td>
</tr>
<tr>
<td>7.0</td>
<td>6.6 - 6.8</td>
<td>5959 ± 297</td>
<td>28.9 ± 2.13</td>
</tr>
<tr>
<td>6.0</td>
<td>5.8 - 6.2</td>
<td>4152 ± 399</td>
<td>33.4 ± 2.28</td>
</tr>
<tr>
<td>5.5</td>
<td>5.5 - 6.0</td>
<td>3535 ± 344</td>
<td>37.4 ± 2.74</td>
</tr>
<tr>
<td>5.0</td>
<td>4.6 - 5.7</td>
<td>3457 ± 328</td>
<td>41.6 ± 3.40</td>
</tr>
</tbody>
</table>

| SUBSTRATE | 977 | 27.5 |

*a The data represents the average of 16 weekly samples ± standard error.

*b The data represents the average of 15 weekly samples ± standard error.

*c The data represents the average of 14 weekly samples ± standard error.
TABLE 3. Efficiency of methane fermentation of manure from Dairy I at various pH levels at 37°C and a 3 day retention time.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>TS&lt;sup&gt;c&lt;/sup&gt; W/V</th>
<th>VS&lt;sup&gt;c&lt;/sup&gt; W/V</th>
<th>% VS&lt;sup&gt;c&lt;/sup&gt; Destroyed</th>
<th>liters CH&lt;sub&gt;4&lt;/sub&gt;/g VS Fed&lt;sup&gt;a&lt;/sup&gt;</th>
<th>liters CH&lt;sub&gt;4&lt;/sub&gt;/g VS Destroyed</th>
<th>CH&lt;sub&gt;4&lt;/sub&gt; in Gas Phase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.6</td>
<td>5.69 ± 0.46</td>
<td>4.60 ± .11</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>6.25 ± 1.32</td>
<td>4.22 ± .20</td>
<td>8.3 ± .40</td>
<td>.038 ± .001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.46 ± .005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65.9 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>5.97 ± 0.72</td>
<td>4.31 ± .16</td>
<td>6.3 ± .23</td>
<td>.040 ± .0005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.632 ± .0007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.9 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>6.15 ± 0.98</td>
<td>4.31 ± .15</td>
<td>6.3 ± .23</td>
<td>.0233 ± .0005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.370 ± .0007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.4 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 5.5</td>
<td>5.69 ± 0.60</td>
<td>4.07 ± .15</td>
<td>11.5 ± .43</td>
<td>.0147 ± .0002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.127 ± .0002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.2 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>6.21 ± 0.77</td>
<td>4.85 ± .38</td>
<td>---</td>
<td>.00826 ± .000005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>---</td>
<td>26.5 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>The data represents the average of 17 weekly samples ± standard error.

<sup>b</sup>The data represents the average of 16 weekly samples ± standard error.

<sup>c</sup>The data represents the average of three determinations ± standard error.
TABLE 4. Efficiency of methane fermentation of manure from Dairy II at various pH levels at 37°C and a 3 day retention time.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>TS&lt;sup&gt;a&lt;/sup&gt; W/V</th>
<th>VS&lt;sup&gt;a&lt;/sup&gt; W/V</th>
<th>%VS Destroyed</th>
<th>liters CH&lt;sub&gt;4&lt;/sub&gt;/g VS Fed&lt;sub&gt;4&lt;/sub&gt;</th>
<th>liters CH&lt;sub&gt;4&lt;/sub&gt;/g VS Destroyed</th>
<th>CH&lt;sub&gt;4&lt;/sub&gt; in Gas Phase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.6</td>
<td>8.36 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.66 ± 0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>6.55 ± 0.38</td>
<td>4.90 ± 0.30</td>
<td>26.4 ± 1.6</td>
<td>0.135 ± 0.004</td>
<td>0.218 ± 0.006</td>
<td>50.4 ± 4.6</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>6.52 ± 0.33</td>
<td>4.96 ± 0.26</td>
<td>25.5 ± 1.3</td>
<td>0.064 ± 0.004</td>
<td>0.252 ± 0.015</td>
<td>46.6 ± 2.5</td>
</tr>
<tr>
<td>pH 5.5</td>
<td>6.99 ± 0.38</td>
<td>5.37 ± 0.36</td>
<td>19.4 ± 1.2</td>
<td>0.053 ± 0.004</td>
<td>0.274 ± 0.024</td>
<td>41.7 ± 3.0</td>
</tr>
<tr>
<td>pH 5.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.09 ± 0.33</td>
<td>5.60 ± 0.25</td>
<td>15.9 ± 0.7</td>
<td>0.036 ± 0.003</td>
<td>0.228 ± 0.020</td>
<td>39.2 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>7.28 ± 0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.66 ± 0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.0 ± 0.8</td>
<td>0.005 ± 0.0001</td>
<td>0.0336 ± 0.0007</td>
<td>20.3 ± 8.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>The data represents the average of 13 determinations ± standard error.

<sup>b</sup>The data represents the average of 11 determinations ± standard error.

<sup>c</sup>The data represents the average of 12 determinations ± standard error.

<sup>d</sup>Digester failed ten weeks after addition of Dairy II manure.
Fig. 3. Methane and biogas production from cattle manure from Dairy I and Dairy II at pH 5.0, 5.5, 6.0, 7.0, and 7.6.
per liter per day from Dairy I. The percentage of methane in the biogas was highest at the highest pH level and decreased as the pH was lowered to pH 5.0 (Table 3,4). Minimal amounts of biogas (0.484 ± 0.018 liters per liter of digester per day from Dairy I and 0.435 ± 0.119 liters per liter of digester per day from Dairy II) were produced at pH 5.0. The digester failed after 48 weeks.

Effect of feed ration on biogas and methane production at various pH levels. More biogas and more methane were produced from manure from Dairy II than from Dairy I (Fig. 3). This increased gas production was evident immediately after feeding with Dairy II manure was begun during week 51 (Fig. 4,5,6,7). At pH 7.6, 37% more biogas and 36% more methane were produced during week 51 than during week 50. For biogas and methane respectively, the increase in gas production was 45% and 38% for pH 7.0, 46% and 47% for pH 6.0, 38% and 47% for pH 5.5. In succeeding weeks methane and biogas production fluctuated well above the production achieved with Dairy I manure at all pH levels. The only exceptions were gas production for pH 6.0 during week 64 and for pH 5.5 during week 56. These discrepancies are attributed to leaks in the gas collection bags. The volatile solids concentration (W/V) was higher in Dairy II manure (6.66 ± 0.29) than in Dairy I manure (4.60 ± 0.11). If the volatile solids concentration was the only factor which determined the amount of biogas and methane produced, one would expect that 1.45 times as much (6.66/4.60) biogas and methane would be produced from Dairy II as from Dairy I manure. On this basis,
Fig. 4. Weekly gas production (methane and biogas) at pH 7.6 from week 33 through week 65. Symbols: ( ) biogas; ( ▲ ▲ ) methane.
Fig. 5. Weekly gas production (methane and biogas) at pH 7.0 from week 33 through week 65. Symbols; (---) biogas; (△△) methane.
Fig. 6. Weekly gas production (methane and biogas) at pH 6.0 from week 33 through week 65. Symbols: (---) biogas; (△△) methane.
Fig. 7. Weekly gas production (methane and biogas) at pH 5.5 from week 33 through week 65. Symbols: (---) biogas; (△—△) methane.
one would expect 1.29, 1.55, 1.20, 0.924, and 0.701 liters of biogas per liter of digester respectively for digesters at pH 7.6, 7.0, 6.0, 5.5, and 5.0. The yields of biogas were: 2.42, 3.05, 2.79, 2.06, and 0.435 at the respective pH levels. This data indicates that the diet fed the animals had an effect on methane production which cannot be attributed to volatile solids concentration alone.

**Effect of pH on organic acid concentrations.** Total organic acid concentrations were highest at the highest pH levels and decreased as the pH of the digesters was lowered (Tables 5,6). The exception to this trend was at pH 5.0 when manure from Dairy II was used and fatty acid concentrations of 8,796 mg/liter were recorded. This digester failed after 48 weeks. The concentration of fatty acids was higher when Dairy II manure was used. At pH 7.6, 61% more total fatty acids were produced from Dairy II manure than from Dairy I manure. Increases in fatty acid concentration of 64, 58.9, 59.5, and 71.8% were obtained at pH 7.0, pH 6.0, pH 5.5, and pH 5.0 respectively. Relatively high levels of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate were present in digesters at all pH levels with manure from both dairies (Tables 5,6) when compared with dairy waste digesters maintained at high levels of methane production at thermophilic temperatures (39). The levels of acetate (22 to 89 mM) and propionate (42 to 67 mM) are especially high.
<table>
<thead>
<tr>
<th>pH of Reactor</th>
<th>Acetic mM</th>
<th>Propionic mM</th>
<th>Isobutyric mM</th>
<th>Butyric mM</th>
<th>Isovaleric mM</th>
<th>Total Fatty Acids (Acetate) mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.6</td>
<td>27.4 ± 3.6e</td>
<td>17 ± 1.9</td>
<td>1.8 ± .29d</td>
<td>&lt;1</td>
<td>1.8 ± .28d</td>
<td>2890</td>
</tr>
<tr>
<td>7.0</td>
<td>22 ± 4.6c</td>
<td>16 ± 1.8c</td>
<td>1.6 ± .26c</td>
<td>&lt;1</td>
<td>1.7 ± .26c</td>
<td>2540</td>
</tr>
<tr>
<td>6.0</td>
<td>21 ± 3.2a</td>
<td>16 ± 2.1a</td>
<td>2.4 ± 0.3b</td>
<td>3.0 ± .51a</td>
<td>2.3 ± .27a</td>
<td>2689</td>
</tr>
<tr>
<td>5.5</td>
<td>15 ± 1.8b</td>
<td>15 ± 1.4a</td>
<td>2.3 ± .20a</td>
<td>2.9 ± .44a</td>
<td>2.5 ± .24a</td>
<td>2314</td>
</tr>
<tr>
<td>5.0</td>
<td>21 ± 3.5b</td>
<td>12 ± 1.5b</td>
<td>2.2 ± .15a</td>
<td>3.1 ± .27a</td>
<td>2.3 ± .14a</td>
<td>2481</td>
</tr>
</tbody>
</table>

a The data represents the average of 17 weekly samples ± standard error.
b The data represents the average of 16 weekly samples ± standard error.
c The data represents the average of 15 weekly samples ± standard error.
d The data represents the average of 12 weekly samples ± standard error.
e The data represents the average of 11 weekly samples ± standard error.

Valeric, isocaproic, and caproic acids were not detected with a sensitivity of 1 mM.
TABLE 6. Concentration of volatile fatty acids after methane fermentation of manure from Dairy II at various pH levels at 37°C and a 3 day retention time.

<table>
<thead>
<tr>
<th>pH of Reactor</th>
<th>Acetic mM</th>
<th>Propionic mM</th>
<th>Isobutyric mM</th>
<th>Butyric mM</th>
<th>Isovaleric mM</th>
<th>Valeric mM</th>
<th>Total Fatty Acids (Acetate) mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.6</td>
<td>44 ± 9.9 a</td>
<td>65 ± 7.8 a</td>
<td>3.8 ± 0.24 a</td>
<td>5.0 ± 1.2 a</td>
<td>3.9 ± 0.39 a</td>
<td>2.2 ± 0.33 a</td>
<td>7434</td>
</tr>
<tr>
<td>7.0</td>
<td>35 ± 5.3 b</td>
<td>67 ± 6.7 b</td>
<td>4.0 ± 0.03 a</td>
<td>4.5 ± 1.2 a</td>
<td>4.9 ± 0.42 a</td>
<td>2.2 ± 0.42 a</td>
<td>7056</td>
</tr>
<tr>
<td>6.0</td>
<td>22 ± 4.5 a</td>
<td>64 ± 6.6 a</td>
<td>3.7 ± 0.28 a</td>
<td>10 ± 1.2 a</td>
<td>3.9 ± 0.33 a</td>
<td>2.8 ± 0.36 a</td>
<td>6542</td>
</tr>
<tr>
<td>5.5</td>
<td>22 ± 6.4 c</td>
<td>54 ± 5.3 b</td>
<td>3.5 ± 0.23 b</td>
<td>8.8 ± 0.53 b</td>
<td>3.2 ± 0.28 b</td>
<td>3.4 ± 0.42 b</td>
<td>5708</td>
</tr>
<tr>
<td>5.0</td>
<td>89 ± 11 a</td>
<td>42 ± 45 a</td>
<td>2.5 ± 0.19 a</td>
<td>7.8 ± 0.84 a</td>
<td>2.4 ± 0.19 a</td>
<td>2.9 ± 0.33 a</td>
<td>8796</td>
</tr>
<tr>
<td>Substrate</td>
<td>53 ± 2.9 d</td>
<td>13 ± 1.3 d</td>
<td>1.56 ± 0.24 d</td>
<td>5.8 ± 0.63 d</td>
<td>1.6 ± 2.0 d</td>
<td>&lt;1 d</td>
<td>4551</td>
</tr>
</tbody>
</table>

a The data represents the average of 14 weekly samples ± standard error.

b The data represents the average of 13 weekly samples ± standard error.

c The data represents the average of 12 weekly samples ± standard error.

The data represents the average of 9 weekly samples ± standard error.

e Isocaproic and caproic acids were not detected with a sensitivity of 1 mM.
Efficiency of Fermentation. In general, the VS destruction based on gravimetric determinations decreased as the pH of the digesters was lowered from pH 7.6 to 5.0 (Tables 3,4). The trend was noted in all digesters except the pH 5.5 digester from Dairy I. This discrepancy is believed to be due to sampling error or analytical error. The liters of methane per g VS fed and liters of methane per g VS destroyed are relatively low when compared to other studies at higher temperatures and especially at the same temperature at longer retention times (37,39). The digester maintained at pH 7.6 had a comparable rate (0.135 ± 0.004 liters of methane per g VS fed) to that previously reported (0.15) at a 6 day retention time (37). The efficiency at the lower pH levels (7.0, 6.0, 5.5, and 5.0) is very low when compared to other reports (37,39) and to the pH 7.6 digester. This indicates that the digesters were stressed.

The percentage of VS destroyed (15.0 to 26.4) in the manure from Dairy II is relatively low when compared to previously published reports (38,39). The percentage of volatile solids destroyed (6.3 to 11.5) was even lower from Dairy I. The efficiency of the fermentation, when measured as liters of methane per g VS destroyed, was higher at most pH levels when manure was used from Dairy I than from Dairy II (Tables 3,4). This would be expected since the percentage VS fed was lower in the Dairy I manure than in the Dairy II manure.
DISCUSSION

These studies indicate that methane can be produced from dairy waste at 37°C with a 3 day retention time at pH levels from 7.6 to 5.0. The yield of methane at pH 5.0 (0.13 ± 0.003 liters of methane per liter of digester at Dairy I and 0.11 ± 0.002 at Dairy II) was minimal. The percentage of methane in the gas phase dropped from 26.5 ± 6.0 to 20.3 ± 8.2 as the fermentation progressed on the manure from Dairy II. This is an indication of imminent failure of the digester. Considering the low volumes of gas produced and the failure of the digester after 48 weeks, it is doubtful if multiplication of methanogens occurred at pH 5.0. It is likely that the methanogens in the daily influent accounted for the methane produced in this digester.

A previous report (38) in which digesters were charged with manure from steers fed a high grain finishing diet failed to maintain active fermentation with a RT of 3 days at 30, 35, 40, and 65°C. Two possibilities exist that might explain the discrepancy between the two studies. One is that the cattle were fed different diets and the manure differences did not permit the establishment of active fermentation. The substrate has been reported to affect the yields of methane in the fermentation (38). The other possibility is that the numbers and kinds of organisms
which can produce methane under these conditions were not present in the previous studies. It is possible that selection occurred that eliminated the microorganisms that can conduct an active fermentation at short retention times. In the previous study, the microbial fermentations were established by incubation for 2 weeks before substrate was fed. Once the substrate was fed, it was fed at a rate to maintain an 18 day RT. After steady state conditions were established, the RT was decreased in increments until the 3 day RT was reached. In contrast, the digesters in this study were charged with substrate and each day thereafter they were wasted and fed with the proper amount of substrate for the 3 day RT. Additionally, the pH was adjusted daily to the desired level. On a theoretical basis, the latter approach should select for the numbers and kinds of microorganisms that are best able to survive and propagate at these environmental conditions. There is a possibility that lowering the RT sequentially causes undue competition between an established population of microorganisms and the organisms required for a 3 day RT. Another reason for starting digesters by feeding and wasting daily from day one is to take advantage of the buffering capacity of the substrate. In addition to the results reported here, eight 3 liter digesters with dairy manure as substrate at 37°C and a 3 day RT, eighteen 50 liter digesters with dairy manure at 50°C, and thirty-seven 3 liter digesters with water hyacinths with a 6-12 day RT at 35°C have been started in our laboratories.
in the same manner.

The highest yield of biogas and methane production occurred in the digesters maintained at pH 7.0 at the 3 day RT. The stabilized pH level is near the pH level usually achieved with dairy cow waste once steady conditions are reached in the fermentation when the pH is not controlled. In a commercial digester, it is probably not necessary to control the pH level once the digester has achieved steady state. From the results of this study there would seem to be a distinct advantage to feed and waste the digester daily with compensatory pH control of the incoming substrate until steady state conditions are achieved.

The VA levels in this study were considerably higher than those normally associated with balanced digestion. Digesters at all pH levels using manure from Dairy I and Dairy II had VA concentrations above the 2000 mg/liter that many investigators consider inhibitory for methane bacteria (5,32,33). Only the digester at pH 5.0 failed to produce methane or biogas after 48 weeks. Its VA concentration (8796 mg/liter) was nearly double the VA concentration of the substrate (4551 mg/liter) from Dairy II. Digesters at all other pH levels produced significant quantities of methane and biogas at high VA levels (2314-2890 mg/liter from Dairy I manure and 5708-7434 mg/liter from Dairy II manure). These results indicate that methane bacteria can tolerate higher VA levels for longer periods of time than generally believed. Of special interest is the relationship between VA
levels and pH since these two parameters are considered to be indicators of digester stability. The highest VA levels occurred at the highest pH levels (Tables 5, 6). A digester is usually considered to be failing when pH decreases while VA concentrations increase.

The results reported here indicate that stable methane fermentations can be maintained for long periods of time at pH 7.6, 7.0, 6.0, and 5.5 at 3 day RT and 37°C. While stable, these fermentations do possess characteristics of stress including high VA concentrations, particularly acetic and propionic acids; low VS destruction; high percentages of CO₂ in the biogas. However, these stressed conditions did not lead to digester failure. Significant quantities of methane were produced at all pH levels including those levels generally considered to be characteristic of failed digesters.

Further work is necessary to determine the effects of cattle feed ration on subsequent methane production from the manure. It appears that minimum and maximum limits of tolerance for environmental conditions such as pH, VA concentration, VS loading, and RT are greatly affected by substrate composition and start-up conditions. To develop good predictive data for the rate of methanogenesis it will be necessary to study the consortia of microorganisms present under a wide variety of environmental conditions with different substrates and selection techniques.
LITERATURE CITED


