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# Anaerobic Fermentation of Beef Cattle Manure and Crop Residues

## Annual Report, 1980

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Prepared Under Subcontract No. DB-9-8372-1 for

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SERI TECHNICAL MONITOR:

DAN JANTZEN

# FOREWORD

This report describes the results of a research project to produce energy in the form of methane and a high-protein feed supplement from livestock manure. This work was jointly funded by the US Department of Agriculture, through the Science and Education Administration, and the US Department of Energy, through the Solar Energy Research Institute.

Mention of commercial or proprietary products in this report does not constitute recommendation or endorsement of these products by the US Departments of Agriculture and Energy or by the Solar Energy Research Institute.

Dan Jantzen, Sr. Project Manager
Solar Energy Research Institute

## SUMMARY

This report summarizes the research conducted at the Roman L. Hruska U.S. Meat Animal Research Center, during calander year 1980, on the feasibility of fermenting manure-crop residue mixtures to methane, and on factors affecting the rate and extent of methane production. Experiments were conducted to evaluate effects of temperature, pH, substrate concentration, and alkaline pretreatment on the rate and extent of hydrolysis of manure-straw mixtures. The rate of hydrolysis, as measured by the total organic acids (TOA) production in fermentors, was more rapid at 40°C compared to higher temperatures (50, 55 or 60°C), and very little TOA was produced at 60°C. This was even true when fermentors were inoculated with bacteria acclimated to 55°C. Inoculating fermentors with slurry from a thermophilic anaerobic fermentor decreased the time required to reach maximum TOA production from 3 days to 1 day for the 40°C fermentors, but had no effect on fermentors at 50, 55 or 60°C. Both pH adjustment and alkaline treatment (120°C, 80 g NaOH/kg VS straw) of the straw increased the extent of TOA production. However, alkaline treatment increased the extent of TOA production substantially more than pH adjustment (12% vs 56%).

The effect of mixing a highly carbonaceous substrate (molasses) and highly nitrogenous substrate (beef cattle manure) on methane ($CH_4$) production and effluent quality was evaluated. The manure and molasses were mixed so that they contributed varying percentages in the mixture, as follows: 100% manure (100:0); 75% manure and 25% molasses (75:25); and 50% manure and 50% molasses (50:50). Laboratory-scale, anaerobic fermentors (3-$dm^3$ working volume, continously mixed) were operated at 55°C and at 6, 9 and 18-day hydraulic retention times (HRT). At similar HRT and (VS) loading rates, fermentors receiving the 50:50 mixture consistently produced the highest volumetric $CH_4$ production rates ($m^3$ $CH_4$/$m^3$ fermentor·). The fermentor receiving only cattle manure produced the lowest rates. Kinetic evaluation showed that increased $CH_4$ production rates of molasses containing substrates were due only to higher ultimate $CH_4$ yields ($B_0$) of the manure-molasses mixtures, and not due to reduced inhibition nor increased microbial growth rate. $B_0$ were 0.325, 0.335, and 0.360 $m^3$ $CH_4$/ kg VS fed for the 100:0, 75:25 and 50:50 mixtures, respectively. The addition of molasses to manure also affected fermentor effluent characteristics. Of particular interest was a change in ammonia to total nitrogen ($NH_3$/TN) ratio. At a 6-day HRT, the $NH_3$/TN ratio decreased from 0.48 to 0.38 and to 0.23 for the 100:0, 75:25 and 50:50 mixtures, respectively. This shift from ammonia to organic nitrogen is desirable if fermentor effluent is used as a protein supplement in livestock feeds.

The pilot plant was modified into a two-stage fermentation system to accomodate manure-straw mixtures. Inputs to the system were 50% beef cattle manure and 50% wheat straw, based on VS content. The manure-straw mixture was mixed into a slurry and fermented in a hydrolysis tank for 1 day at 50 to 60°C. The slurry was then separated using a vibrating screen. Approximately 37% of the screened solids was returned to the hydrolysis tank. The screened liquid, which accounted for 35% of the VS in the manure and straw, was pumped to the anaerobic fermentor for conversion to $CH_4$. The fermentor, operated at 8-day HRT, 44 to 47°C, and influent concentration of 47.3 kg VS/$m^3$, produced 1.81 $m^3$ $CH_4$/$m^3$ fermentor·day.

The effects of temperature, ration constitutents, antibiotics and manure age on the ultimate methane yield ($B_0$ $m^3$ $CH_4$/kg volatile solids fed $VS_f$ ) were

investigaed using 4-$dm^3$, batch fermentors. The average $B_o$ for fermentors maintained at 30 to 60°C (at 5°C intervals) was 0.328 $m^3$ $CH_4$/kg $VS_f$. The $B_o$ at 65°C averaged 0.118 $m^3$ $CH_4$/kg $VS_f$, but this low yield was attributed to unstable fermentation rather than decreased substrate availability at that temperature. These results agreed well with $B_o$ values estimated from daily-fed fermentors. Chlortetracycline and monensin did not affect $B_o$; however, monensin did delay the start of active fermentation in batch fermentors. The average $B_o$ of manure from cattle fed 91.5, 40 and 7% corn silage were 0.173, 0.232 and 0.290 $m^3$ $CH_4$/kg $VS_f$, respectively. The average $B_o$ for 6 to 8 week old manure from a dirt feedlot was 0.210 $m^3$ $CH_4$/kg $VS_f$.

The effects of mixing duration and vacuum on methane production rates from anaerobically-fermented beef cattle wastes were determined. The results showed that continuously-mixed fermentors produced significantly ($P<0.05$) higher methane production rates than fermentors mixed two hour per day. However, the rates from the continuously-mixed fermentors were only 8 to 11% higher than the intermittently-mixed fermentors at 6- and 4-day HRT, respectively. There was no significant difference between the vacuum and conventional fermentors at 6-day HRT, but there was a significant difference at 4-day HRT. The $CH_4$ production rate of the vacuum fermentors was 5% higher than the conventional fermentors at 4-day HRT. The results of these experiments compared well with predicted $CH_4$ production rates. These results suggest that there is little potential for increasing the fermentation rates of livestock wastes by increased mixing or vacuum.

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# SECTION 1.0

## INTRODUCTION

Anaerobic fermentation research has been in progress since 1976 at the Roman L. Hruska U.S. Meat Animal Research Center. The overall objective of this project is to assess the technical and economic feasibility of recovering methane and high protein biomass through the anaerobic fermentation of beef cattle manure and crop residues. The major thrust of the project is to develop optimum fermentation systems that are compatible with livestock production enterprises. The project contributes to: establishment of design criteria and scale-up factors for efficient methane production; assesment of the economic feasibility of fermentation systems; and integration of this technology with the livstock production sector.

This report summarizes the research conducted during calander year 1980 on the feasibility of fermenting manure-crop residue mixtures to methane, and on factors affecting the rate and extent of methane production. This work is continuing through 1981.

## SECTION 2.0

## MICROBIAL HYDROLYSIS OF THERMOCHEMICALLY TREATED AND UNTREATED MANURE-STRAW MIXTURES

Andrew G. Hashimoto

### 2.1 INTRODUCTION

A two-stage fermentation system that converts manure-crop residue mixtures to methane ($CH_4$) is being evaluated at the Roman L. Hruska U.S. Meat Animal Research Center. A schematic diagram of the system is shown in Figure 2.1. The first stage is a completely-mixed, heated, well-insulated tank in which manure, crop residue and water are mixed to optimize cellulase and other hydrolytic activity. Optimum conditions for cellulase activity are reported to be temperatures between 55 to 60°C and pH between 5.8 to 7.2 under thermophilic conditions (Pye, 1978) and between 32 to 35°C and pH of 4 under mesophilic conditions (Ryu and Mandels, 1980). In order to prevent methanogenesis in the first stage, volatile solids (VS) loading rate is kept high (in excess of 30 kg VS/$m^3$·day) and the solids retention time is kept short (less than 4 days).

Effluent from the hydrolysis tank passes over a 10-mesh screen to remove coarse particles and allows the solubles and fines to pass to the second-stage fermentor. The coarse particles (hair, undigested feed, residue particles, etc.) are either recycled back to the hydrolysis tank, wasted (this could be used as roughage in ruminant rations) and/or thermochemically treated and then returned to the hydrolysis tank.

Potential advantages of this two-stage system are: easily hydrolyzable material is hydrolyzed in the first stage and fermented to $CH_4$ and $CO_2$ in the second stage; more resistant substrate is hydrolyzed for longer periods; only resistant substrate is exposed to alkaline treatment (reducing the amount residue to be treated and therefore the chemical and energy needed to treat the residue); the alkaline added to the system helps to maintain the pH in the first stage at optimum levels; neutralization of the treated material is not necessary; increased methane production rates may be achieved since the substrate to the second stage would presumably be more biodegradable; and problems associated with mixing and pumping the fermentor contents are minimized with the removal of the coarse material.

This study focuses on some design parameters needed to optimize the microbial hydrolysis stage. Four experiments were conducted to evaluate the effects of temperature, pH, substrate concentration, and alkaline pretreatment on the rate and extent of substrate hydrolysis.

### 2.2 METHODS

#### 2.2.1 Experiment Design

Table 2.1 shows the parameters evaluated in the four experiments. Each treatment was replicated twice. Experiment 1 compared effects of temperature (40°C vs 60°C) and pH (6 vs no pH control) on hydrolysis of a 130 kg VS/$m^3$, manure-straw mixture. Experiment 2 compared effects of substrate con-

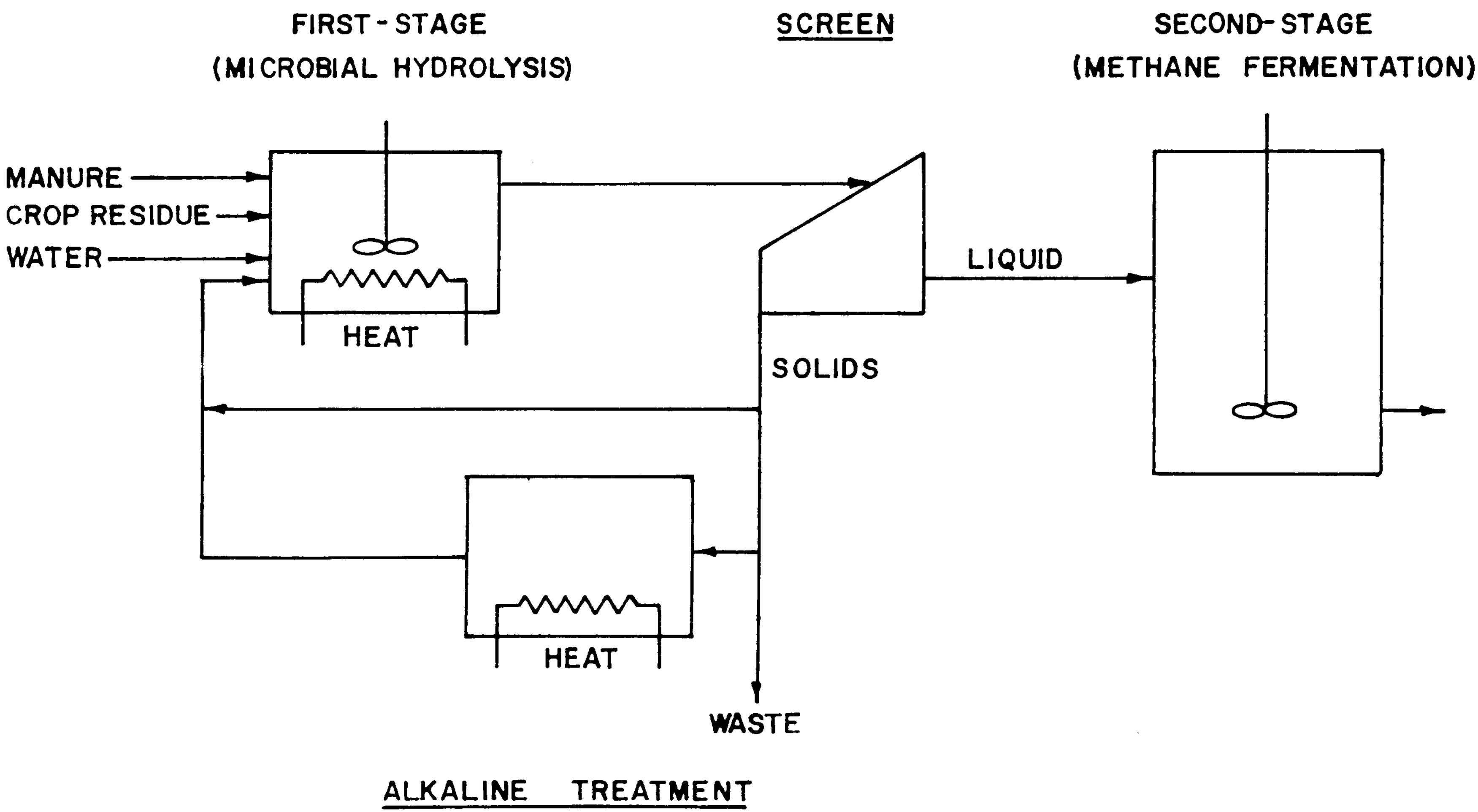


Figure 2.1. Schematic Diagram of a Two-Stage Fermentation System for Methane Production

TABLE 2.1. VARIABLES USED IN EXPERIMENTAL DESIGN

| Experiment | Treatment | Temperature °C | pH Control | VS Conc. kg/m$^3$ | Seeded | Straw Treated |
|---|---|---|---|---|---|---|
| 1 | 1A | 40 | Yes | 130 | No | No |
| | 1B | 40 | No | 130 | No | No |
| | 1C | 60 | Yes | 130 | No | No |
| | 1D | 60 | No | 130 | No | No |
| 2 | 2A | 50 | No | 130 | No | No |
| | 2B | 50 | No | 80 | No | No |
| | 2C | 50 | Yes | 130 | No | No |
| | 2D | 50 | Yes | 80 | No | No |
| 3 | 3A | 40 | No | 100 | Yes | No |
| | 3B | 50 | No | 100 | Yes | No |
| | 3C | 55 | No | 100 | Yes | No |
| | 3D | 60 | No | 100 | Yes | No |
| 4 | 4A | 40 | No | 100 | Yes | No |
| | 4B | 40 | No | 100 | Yes | Yes |
| | 4C | 55 | No | 100 | Yes | No |
| | 4D | 55 | No | 100 | Yes | Yes |

centration (80 vs 130 kg $VS/m^3$) and pH (5 vs no pH control) on rate and extent of hydrolysis. Experiment 3 compared effect of temperature (40, 50, 55 and 60°C) on rate and extent of hydrolysis of 100 kg $VS/m^3$, manure-straw mixtures seeded with anaerobic bacteria. Experiment 4 compared effects of temperature (40 vs 55°C) and alkaline treatment of straw on rate and extent of hydrolysis of seeded, 100 kg $VS/m^3$, manure-straw mixtures.

### 2.2.2 Fermentors

Fermentors used in Experiments 1 and 2 were 4-$dm^3$, pyrex, reaction kettles (Corning 6947) equipped with heating mantles and variable transformers to control temperature. Each fermentor was mixed by a 20 watt variable-speed mixer (220 revolutions per minute) and duel propellers (5.5-cm diameter, 3-blade propellers spaced 14 cm apart on the shaft).

Fermentors used in Experiments 3 and 4 were 5-$dm^3$, ceramic pots (crock pots) equipped with temperature controls and plastic covers. Mercury thermometers were inserted in the covers to monitor and adjust the temperature to the desired level.

### 2.2.3 Substrate

Manure used in this study was from beef cattle (weighing about 400 kg) fed a ration consisting of 85% corn, 13% corn silage and 2% soybean meal-mineral supplement (80.5% soybean meal, 11.5% limestone, 3% dicalcium phosphate, 0.8% vitamin A, D and E, 0.2% beef trace minerals, and 3.75% salt). The manure was less than 3 days old and scraped off concrete-floored pens. Straw used in this study was winter-wheat straw grown in Clay County, Nebraska. The straw was collected in the spring of 1979, stored in large (400 kg) round bales and passed through a hammer mill equipped with 1-cm diameter mesh screen. Alkaline treatment entailed placing a specified amount of straw in a 4-$dm^3$ flask, mixing NaOH (80 g NaOH/kg VS of straw) and diluting the mixture to 1 $dm^3$. The mixture was then placed in an autoclave and heated to 120°C for 1 hour.

Table 2.2 shows the composition of the manure, straw and alkaline-treated straw used in this study.

### 2.2.4 Experimental Procedures

At the start of each experiment, each fermentor (two fermentors per treatment) was filled with 3 $dm^3$ of water, and the fermentor temperature adjusted to the desired level. After 3 to 5 days for temperature equilibration, the fermentors were drained, and the specified amounts of manure, straw, alkaline-treated straw (ATS), hot water, and/or inoculum were added to the designated fermentor. The inoculum used in Experiments 3 and 4 was 0.5 $dm^3$ of slurry from a thermophilic (55°C) anaerobic fermentor (Hashimoto et al., 1978).

Fermentors in Experiments 1 and 2 were continuously mixed and pH was adjusted (using 4 N NaOH) 3 times each day during the first 2 days of operation, and once each day thereafter. The fermentor contents in Experiments 3 and 4 were mixed manually once each day or whenever samples were taken.

TABLE 2.2. COMPOSITION[a] OF MANURE, STRAW AND ALKALINE-TREATED STRAW

| Constituent | Manure | Straw | Alkaline Treated Straw |
|---|---|---|---|
| Total Solids | 15.5 | 92.6 | 17.0 |
| Volatile Solids | 84.3 | 88.7 | 66.2 |
| Carbon | 44.7 | 43.8 | 40.4 |
| Total Nitrogen | 3.9 | 1.7 | 1.3 |
| Chemical Oxygen Demand | 130.8 | 62.4 | 82.3 |
| Total Organic Acids | 7.6 | 1.3 | 2.3 |
| Cellulose | 9.4 | 41.2 | 34.6 |
| Hemicellulose | 13.4 | 22.6 | 3.5 |
| Lignin | 3.7 | 8.0 | 5.5 |
| Silica Ash | 2.2 | 2.6 | 2.0 |
| Phosphorous | 1.1 | 0.2 | 0.2 |
| Potassium | 1.9 | 2.8 | 2.8 |
| Sodium | 0.6 | 0.1 | 2.8 |

[a]Values expressed as percent of total solids except total solids. Total solids expressed as percent of wet weight.

### 2.2.5 Analytical Methods

Total solids (TS), volatile solids (VS), fixed solids (FS), ammonia (distillation method), chemical oxygen demand (COD), alkalinity (to pH 3.7), pH, and total organic acids (TOA, silicic acid method) were determined using the standard methods for wastewater analyses (APHA, 1975). Kjeldahl nitrogen was determined by the method described by Wael and Gehrke (1975). Samples for cellulose, hemicellulose, lignin, silica ash, phosphorous, potassium and sodium determinations were freeze dried, ground and analyzed using published procedures (AOAC, 1975). Carbon was determined on freeze dried, ground samples using a Perkin-Elmer, Model 240, Elemental Analyzer.

Samples for TOA and individual volatile fatty acids (VFA) were prepared by diluting 5 $cm^3$ of sample to 100 $cm^3$, adjusting the pH to 1.0 to 1.2 with concentrated $H_3PO_4$, and centrifuging at a relative centrifugal force of 12,062 for 30 minutes. Aliquots of the supernatant were used in the TOA analysis, or transferred into vials, sealed and frozen for future analysis.

The VFA, including acetic, propionic, butyric, i-butyric, valeric, i-valeric, and caproic acids, were measured using a Hewlett-Packard Model 5840A gas chromatograph with dual flame ionization detectors. Coiled glass columns (0.32 cm ID by 183 cm) packed with 15% SP-1220/1% $H_3PO_4$ on 100/120 mesh Chromosorb WAW (Supelco, Inc., Bellefonte, PA) were used for the fatty acid separation. Nitrogen carrier-gas flow was 0.67 $cm^3/s$ and injector, oven and detector temperatures were 200, 125 and 250°C, respectively.

Data were analyzed by least-squares procedures outlined by Harvey (1975). Main effects were: temperature, pH control and time for Experiment 1; VS concentration, pH control and time for Experiment 2; temperature and time for Experiment 3; and temperature, straw alkaline treatment and time for Experiment 4.

## 2.3 RESULTS

### 2.3.1 Experiment 1

Figure 2.2 shows the change in TOA (expressed as g TOA/g VS) with time. It shows that the rate and extent of TOA production is significantly ($P < 0.01$) faster at 40°C than at 60°C, and with pH control than without pH control ($P < 0.05$). After 4 days of fermentation, the 40°C fermentors (treatment 1B) produced 76% more TOA than the 60°C fermentors (treatment 1D), and the 40°C fermentors with pH control (treatment 1A) produced 12% more than the 40°C fermentors without pH control (treatment 1B).

This experiment was designed to control the pH at 6 for treatments 1A and 1C. Figure 2.3 shows that the manual addition of NaOH was not sufficient to maintain a constant pH of 6, especially during the period of maximum TOA production rates (days 1 to 4). The total amount of NaOH added to the fermentors was 58 g NaOH/kg VS for treatment 1A and 37 g NaOH/kg VS for treatment 1C.

Table 2.3 shows that, during the period of maximum TOA concentration (days 3 to 6), between 20 to 30 percent of the TOA was comprised of volatile fatty acids. This indicates that there are significant amounts of nonvolatile organic acids (e.g., lactate, succinate, etc.) or formate produced during the

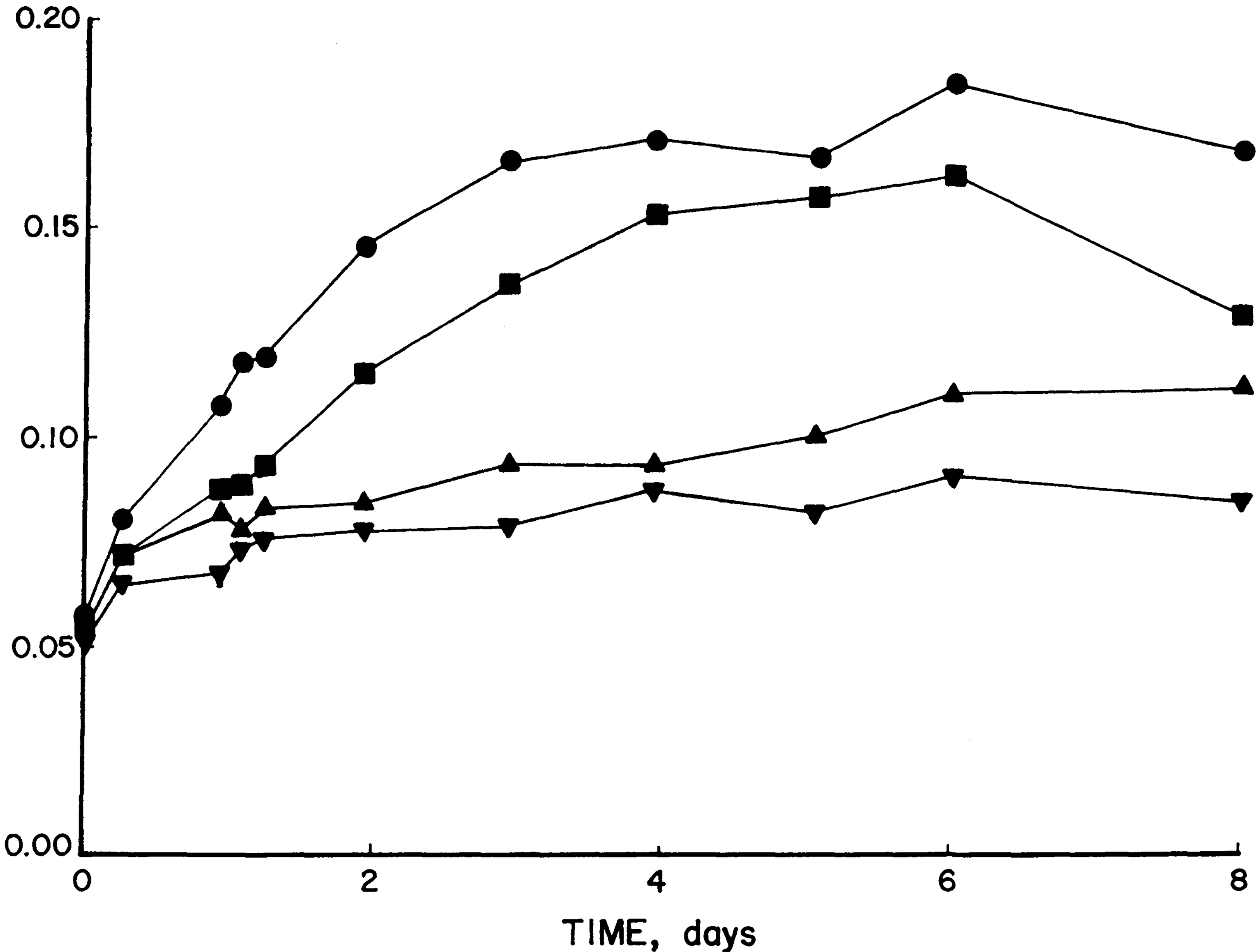


Figure 2.2. Total Organic Acids Profile for Experiment 1 (initial concentration = 130 kg VS/$m^3$; 40°C - pH control, ●; 40°C, ■; 60°C - pH control, ▲; 60°C, ▼).

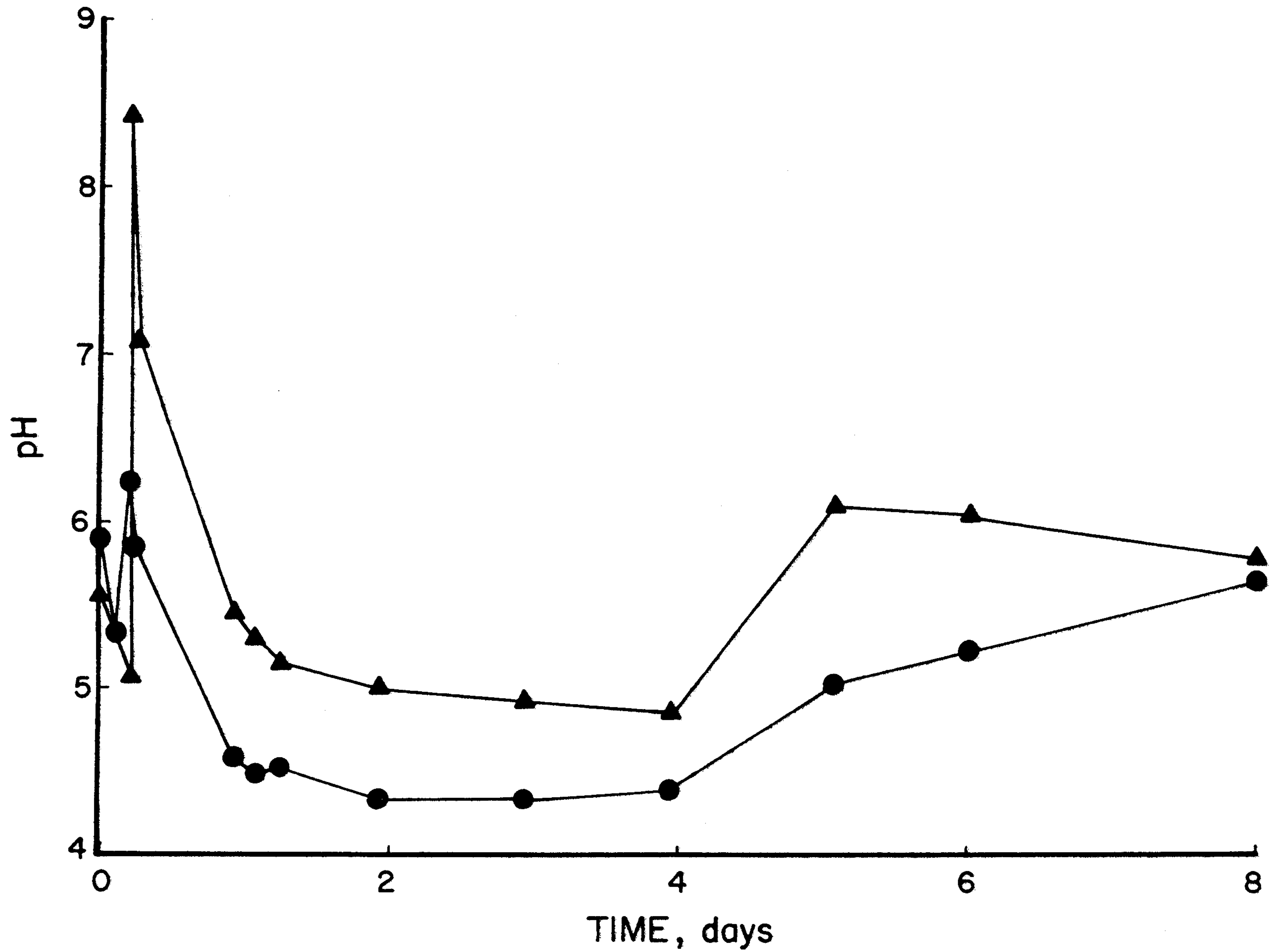


Figure 2.3. pH Profile for Experiment 1 (40°C - NaOH added, ●; 60°C - NaOH added, ▲)

TABLE 2.3. TOTAL ORGANIC ACIDS (TOA) AND TOTAL VOLATILE FATTY ACIDS (TVFA) PROFILES FOR EXPERIMENT 1

| Trt. | Time days | TOA $kg/m^3$ | TVFA/TOA % | Acid/TVFA, % Acetate | Propionate | Butyrate | Valerate | pH |
|---|---|---|---|---|---|---|---|---|
| 1A | 0 | 7.5 | 26.6 | 76.2 | 9.9 | 7.5 | 6.4 | 5.9 |
| | 0.11 | 7.8 | 29.4 | 77.8 | 9.0 | 7.1 | 6.0 | 5.3 |
| | 0.20 | 8.3 | 30.7 | 78.9 | 8.9 | 7.0 | 5.2 | 6.2 |
| | 0.25 | 10.4 | 27.1 | 79.4 | 8.8 | 6.8 | 5.0 | 5.8 |
| | 0.93 | 14.0 | 27.3 | 82.1 | 8.5 | 4.9 | 4.5 | 4.6 |
| | 1.07 | 15.3 | 22.4 | 82.2 | 8.7 | 4.9 | 4.3 | 4.5 |
| | 1.24 | 15.5 | 22.3 | 84.1 | 8.0 | 4.4 | 3.5 | 4.5 |
| | 1.93 | 18.9 | 22.4 | 82.3 | 9.3 | 4.6 | 3.8 | 4.3 |
| | 2.93 | 21.5 | 21.8 | 81.5 | 8.4 | 7.0 | 3.2 | 4.3 |
| | 3.94 | 22.2 | 22.7 | 80.6 | 7.6 | 9.1 | 2.7 | 4.4 |
| | 5.07 | 21.6 | 26.7 | 79.4 | 6.7 | 11.3 | 2.5 | 5.0 |
| | 6.01 | 24.0 | 28.6 | 77.0 | 5.4 | 15.3 | 2.4 | 5.2 |
| | 7.99 | 21.9 | -- | -- | -- | -- | -- | 5.6 |
| 1B | 0 | 7.1 | 26.6 | 78.5 | 8.3 | 7.5 | 5.7 | 4.3 |
| | 0.11 | 7.8 | 28.0 | 78.6 | 8.6 | 7.1 | 5.7 | 4.7 |
| | 0.20 | 8.2 | 30.3 | 79.3 | 8.9 | 6.9 | 4.9 | 4.6 |
| | 0.25 | 9.3 | 28.0 | 79.3 | 9.0 | 7.1 | 4.6 | -- |
| | 0.93 | 11.4 | 25.4 | 79.5 | 9.1 | 6.2 | 5.2 | -- |
| | 1.07 | 11.5 | 25.9 | 80.0 | 8.1 | 6.2 | 5.8 | -- |
| | 1.24 | 12.1 | 24.8 | 82.0 | 8.0 | 5.6 | 4.5 | -- |
| | 1.93 | 15.0 | 22.6 | 81.3 | 9.4 | 5.3 | 4.1 | -- |
| | 2.93 | 17.7 | 16.6 | 79.4 | 10.3 | 5.3 | 5.1 | -- |
| | 3.94 | 19.9 | 19.1 | 80.5 | 10.4 | 4.9 | 4.2 | -- |
| | 5.07 | 20.4 | 18.8 | 80.0 | 10.4 | 5.2 | 4.3 | -- |
| | 6.01 | 21.0 | 19.0 | 83.2 | 7.3 | 5.3 | 4.2 | -- |
| | 7.99 | 16.7 | -- | -- | -- | -- | -- | 4.2 |
| 1C | 0 | 6.9 | 27.8 | 78.8 | 7.1 | 7.0 | 7.2 | 5.6 |
| | 0.11 | 8.4 | 27.6 | 78.7 | 8.9 | 6.9 | 5.7 | 5.1 |
| | 0.20 | 8.8 | 25.2 | 80.9 | 7.5 | 6.4 | 5.3 | 8.4 |
| | 0.25 | 9.2 | 30.6 | 79.3 | 8.0 | 7.2 | 5.6 | 7.1 |
| | 0.93 | 10.6 | 34.8 | 75.2 | 6.2 | 13.8 | 4.8 | 5.4 |
| | 1.07 | 10.1 | 30.9 | 80.4 | 7.3 | 7.0 | 5.4 | 5.3 |
| | 1.24 | 10.8 | 27.8 | 81.9 | 7.0 | 6.7 | 4.5 | 5.2 |
| | 1.93 | 10.9 | 29.0 | 79.9 | 8.2 | 6.8 | 5.3 | 5.0 |
| | 2.93 | 12.1 | 27.8 | 81.7 | 7.2 | 6.7 | 4.6 | 4.9 |
| | 3.94 | 12.1 | 28.4 | 80.2 | 7.0 | 8.5 | 4.4 | 4.8 |
| | 5.07 | 13.0 | 26.9 | 75.6 | 5.6 | 15.3 | 3.6 | 6.1 |
| | 6.01 | 14.3 | 30.5 | 74.5 | 5.2 | 16.8 | 3.6 | 6.0 |
| | 7.99 | 14.4 | -- | -- | -- | -- | -- | 5.8 |
| 1D | 0 | 6.6 | 27.4 | 80.1 | 7.0 | 6.6 | 6.4 | 4.4 |
| | 0.11 | 7.6 | 28.4 | 79.1 | 8.2 | 6.8 | 5.8 | 4.6 |
| | 0.20 | 8.0 | 29.6 | 79.8 | 8.6 | 6.8 | 4.8 | 4.5 |
| | 0.25 | 8.5 | 28.6 | 78.6 | 8.7 | 7.3 | 5.3 | -- |
| | 0.93 | 8.8 | 29.3 | 79.7 | 8.2 | 6.7 | 5.4 | -- |
| | 1.07 | 9.5 | 28.0 | 79.6 | 7.9 | 7.3 | 5.2 | -- |
| | 1.24 | 9.8 | 28.6 | 78.9 | 7.8 | 7.3 | 6.0 | -- |
| | 1.93 | 10.1 | 26.1 | 79.3 | 7.8 | 7.3 | 5.6 | -- |
| | 2.93 | 10.2 | 27.6 | 79.3 | 8.2 | 7.4 | 5.1 | -- |
| | 3.94 | 11.3 | 26.2 | 79.6 | 7.9 | 7.2 | 5.2 | -- |
| | 5.07 | 10.7 | 28.0 | 80.6 | 7.5 | 6.6 | 5.3 | -- |
| | 6.01 | 11.7 | 28.2 | 79.7 | 7.1 | 7.2 | 5.9 | -- |
| | 7.99 | 11.0 | -- | -- | -- | -- | -- | 4.7 |

hydrolysis of manure and straw. Acetate accounts for 80 percent of the TVFA, and acetate and propionate account for nearly 90 percent of the TVFA.

### 2.3.2 Experiment 2

Figure 2.4 shows that, after 3 to 5 days of fermentation, there was no significant effect of substrate concentration on the extent of TOA production, and that pH control increased the extent of TOA production ($P < 0.01$). Treatments 2A and 2B showed no difference in extent of TOA production beyond 5 days of fermentation, but treatment 2C (130 kg VS/$m^3$) showed a continued increase in TOA production while treatment 2D leveled off after 5 days of fermentation. The different results of treatments 2C and 2D compared to 2A and 2B could be explained by the difficulties experienced in keeping treatment 2D mixed and thus achieving good temperature control. This inadequate mixing caused solids to accumulate on the fermentor walls and reduced heat transfer into the fermentor. As a result, the temperature of the fermentor in treatment 2D averaged about 47°C rather than 50°C. This inadequate mixing was the main reason why the ceramic pots, which facilitated complete manual mixing, were used as fermentors in subsequent experiments.

Table 2.4 shows results similar to Table 2.3. The TVFA comprised between 20 to 30% of the TOA, and about 80 percent of the TVFA was acetate. The total amount of NaOH added to the fermentors for pH control was 13 and 25 g NaOH/kg VS for treatments 2C and 2D, respectively.

### 2.3.3 Experiment 3

Figure 2.5 shows a significant ($P < 0.025$) effect of temperature on the rate and extent of TOA production. Treatment 3A (40°C) showed maximum TOA production after only 1 day of fermentation. Treatments 3B (50°C) and 3C (55°C) took between 3 to 6 days to achieve maximum TOA production, while treatment 3D (60°) showed only a slight increase in TOA production even after 7 days of fermentation. It should be noted that all of the fermentors in Experiment 3 were inoculated with slurry from a 55°C fermentor. Thus, it is surprising that treatment 3A produced TOA at a faster rate than treatment 3C.

Table 2.5 shows that the TVFA comprised between 25 to 30% of the TOA for treatments 3A, 3B and 3C. However, in treatment 3D, the TVFA comprised between 35 to 40% of the TOA. Acetate comprised about 80% of the TVFA for treatment 3A and slightly less than 80% for treatments 3B, 3C and 3D.

### 2.3.4 Experiment 4

Figure 2.6 again shows that significantly ($P < 0.01$) higher initial rates of TOA productions were achieved at 40°C than at 55°C even when the fermentors were inoculated with bacteria adapted to 55°C. Also, the alkaline-treated straw (ATS) significantly ($P < 0.01$) increased the extent of TOA production and allowed TOA production to continue even up to 14 days of fermentation. After 7 days of fermentation, treatment 4B produced 56% more TOA than treatment 4A, and treatment 4D produced 31% more TOA than treatment 4C. The continued TOA production of the ATS treatments (4B and 4D) compared to the untreated straw could be explained by the pH profiles (Figure 2.7). The pH for treatments 4A and 4C (without ATS) decreased to about 4, while treatments 4B and 4D (with ATS) decreased to about 4.5. Apparently, pH at or below 4

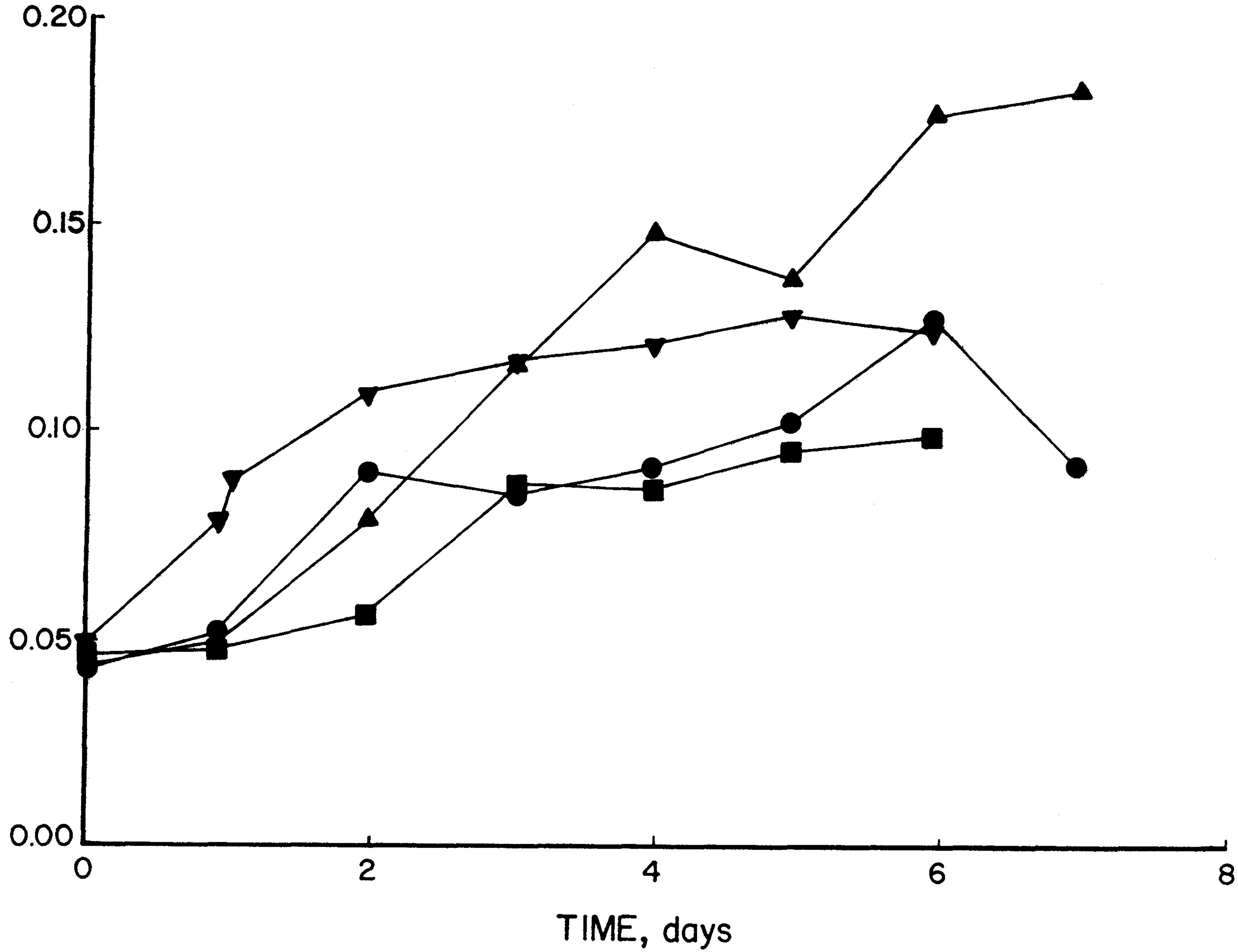


Figure 2.4. Total Organic Acids Profile for Experiment 2 (temperature = 50°C; 130 g VS/$m^3$, ●; 80 g VS/$m^3$ ■; 130 g VS/$m^3$ - pH control, ▲; 80 g VS/$m^3$ - pH control, ▼)

TABLE 2.4. TOTAL ORGANIC ACIDS (TOA) AND TOTAL VOLATILE FATTY ACIDS (TVFA) PROFILES FOR EXPERIMENT 2

| Trt. | Time days | TOA $kg/m^3$ | TVFA/TOA % | Acid/TVFA, % Acetate | Propionate | Butyrate | Valerate | pH |
|---|---|---|---|---|---|---|---|---|
| 2A | 0 | 5.5 | 33.5 | 79.4 | 6.1 | 6.5 | 8.0 | 5.0 |
| | 0.10 | 6.0 | 35.9 | 75.9 | 7.4 | 6.9 | 9.9 | 4.3 |
| | 0.17 | 6.0 | 35.7 | 76.6 | 7.6 | 6.8 | 9.1 | 4.6 |
| | 0.23 | 6.1 | 39.6 | 77.5 | 7.9 | 6.7 | 8.1 | 4.6 |
| | 0.92 | 6.7 | 20.2 | 81.9 | 6.4 | 8.2 | 3.7 | 4.6 |
| | 1.02 | 7.4 | 22.2 | 81.6 | 6.5 | 6.6 | 5.4 | 4.3 |
| | 1.15 | 7.1 | 26.5 | 81.3 | 6.7 | 7.0 | 5.1 | 3.9 |
| | 1.23 | 9.1 | 24.0 | 82.2 | 7.0 | 7.1 | 3.8 | 4.4 |
| | 1.96 | 11.7 | 21.4 | 83.6 | 5.8 | 7.0 | 3.7 | 3.8 |
| | 3.00 | 11.0 | 25.5 | 80.8 | 8.2 | 6.1 | 5.0 | 3.9 |
| | 3.96 | 11.9 | 24.3 | 78.9 | 9.2 | 6.0 | 6.0 | 3.9 |
| | 4.94 | 13.3 | 23.7 | 79.9 | 7.5 | 6.5 | 6.1 | 4.3 |
| | 5.92 | 16.6 | 20.3 | 75.7 | 9.9 | 7.0 | 7.5 | 4.2 |
| | 6.92 | 12.0 | 19.4 | 78.4 | 7.7 | 6.2 | 7.8 | 4.3 |
| 2B | 0 | 3.7 | 29.8 | 81.4 | 5.3 | 7.0 | 6.4 | 5.0 |
| | 0.10 | 3.8 | 37.0 | 76.4 | 6.8 | 6.7 | 10.3 | 4.6 |
| | 0.17 | 3.8 | 33.9 | 76.6 | 6.5 | 6.4 | 10.5 | 4.7 |
| | 0.23 | 3.6 | 24.1 | 82.2 | 5.8 | 8.2 | 3.9 | 4.6 |
| | 0.92 | 3.7 | 19.4 | 81.2 | 5.7 | 9.2 | 4.0 | 4.7 |
| | 1.02 | 3.8 | 21.8 | 79.4 | 6.0 | 8.8 | 6.0 | 4.7 |
| | 1.15 | 3.8 | 29.5 | 77.9 | 5.9 | 8.9 | 7.4 | 4.5 |
| | 1.23 | 3.8 | 27.6 | 79.0 | 6.2 | 10.3 | 4.6 | 4.6 |
| | 1.96 | 4.4 | 31.5 | 81.0 | 5.9 | 7.5 | 5.6 | 4.4 |
| | 3.00 | 7.0 | 28.2 | 83.8 | 6.8 | 4.4 | 5.1 | 3.7 |
| | 3.96 | 6.9 | 26.6 | 82.6 | 4.9 | 5.7 | 6.9 | 4.1 |
| | 4.94 | 7.6 | 24.9 | 84.4 | 4.6 | 4.5 | 6.6 | 4.1 |
| | 5.92 | 7.9 | 22.7 | 83.3 | 5.2 | 4.0 | 7.8 | 4.2 |
| | 6.92 | -- | -- | -- | -- | -- | -- | -- |
| 2C | 0 | 5.7 | 34.4 | 79.2 | 6.5 | 7.0 | 7.4 | 5.0 |
| | 0.10 | 5.8 | 36.0 | 77.1 | 6.6 | 6.5 | 10.0 | 4.8 |
| | 0.17 | 5.6 | 37.2 | 77.9 | 6.5 | 6.5 | 9.2 | 5.0 |
| | 0.23 | 6.5 | 20.9 | 81.6 | 5.6 | 8.8 | 4.1 | 5.1 |
| | 0.92 | 6.3 | 25.5 | 81.3 | 7.2 | 6.9 | 4.6 | 5.1 |
| | 1.02 | 7.8 | 21.0 | 82.6 | 5.9 | 6.6 | 5.0 | 4.8 |
| | 1.15 | 7.3 | 30.4 | 84.3 | 5.1 | 6.7 | 4.0 | 5.0 |
| | 1.23 | 7.7 | 29.9 | 83.4 | 5.6 | 8.0 | 3.1 | 4.6 |
| | 1.96 | 10.2 | 27.4 | 83.3 | 5.8 | 6.7 | 4.3 | 3.8 |
| | 3.00 | 15.0 | 24.2 | 83.0 | 7.1 | 5.7 | 4.4 | 4.4 |
| | 3.96 | 19.2 | 21.6 | 86.4 | 3.9 | 5.3 | 4.4 | 4.3 |
| | 4.94 | 17.8 | 25.3 | 81.7 | 10.7 | 4.2 | 3.4 | 4.0 |
| | 5.92 | 24.3 | 17.6 | 80.5 | 10.2 | 4.8 | 4.5 | 4.0 |
| | 6.92 | 23.7 | 15.5 | 81.5 | 10.6 | 4.2 | 3.9 | 4.6 |
| 2D | 0 | 3.9 | 33.2 | 76.4 | 6.3 | 7.1 | 10.2 | 5.0 |
| | 0.10 | 3.7 | 36.6 | 75.4 | 6.5 | 6.7 | 11.4 | 4.2 |
| | 0.17 | 3.7 | 36.2 | 77.5 | 6.2 | 6.5 | 9.9 | 4.9 |
| | 0.23 | 3.8 | 23.0 | 78.1 | 7.1 | 9.6 | 5.4 | 5.2 |
| | 0.92 | 6.3 | 19.7 | 83.7 | 5.8 | 5.8 | 4.8 | 3.6 |
| | 1.02 | 7.1 | 16.4 | 86.2 | 4.7 | 5.6 | 3.5 | 4.6 |
| | 1.15 | 7.3 | 22.6 | 87.4 | 4.5 | 5.4 | 2.8 | 4.6 |
| | 1.23 | 8.1 | 21.7 | 86.8 | 4.7 | 6.0 | 2.6 | 4.7 |
| | 1.96 | 8.7 | 22.3 | 87.3 | 4.7 | 5.4 | 2.6 | 4.6 |
| | 3.00 | 9.3 | 24.2 | 83.7 | 8.2 | 4.7 | 3.5 | 4.5 |
| | 3.96 | 9.6 | 23.7 | 85.4 | 4.4 | 4.5 | 5.7 | 5.0 |
| | 4.94 | 10.2 | 25.9 | 80.5 | 8.2 | 5.1 | 6.3 | 5.0 |
| | 5.92 | 10.0 | 23.0 | 84.6 | 5.5 | 4.6 | 5.3 | 5.0 |
| | 6.92 | -- | -- | -- | -- | -- | -- | -- |

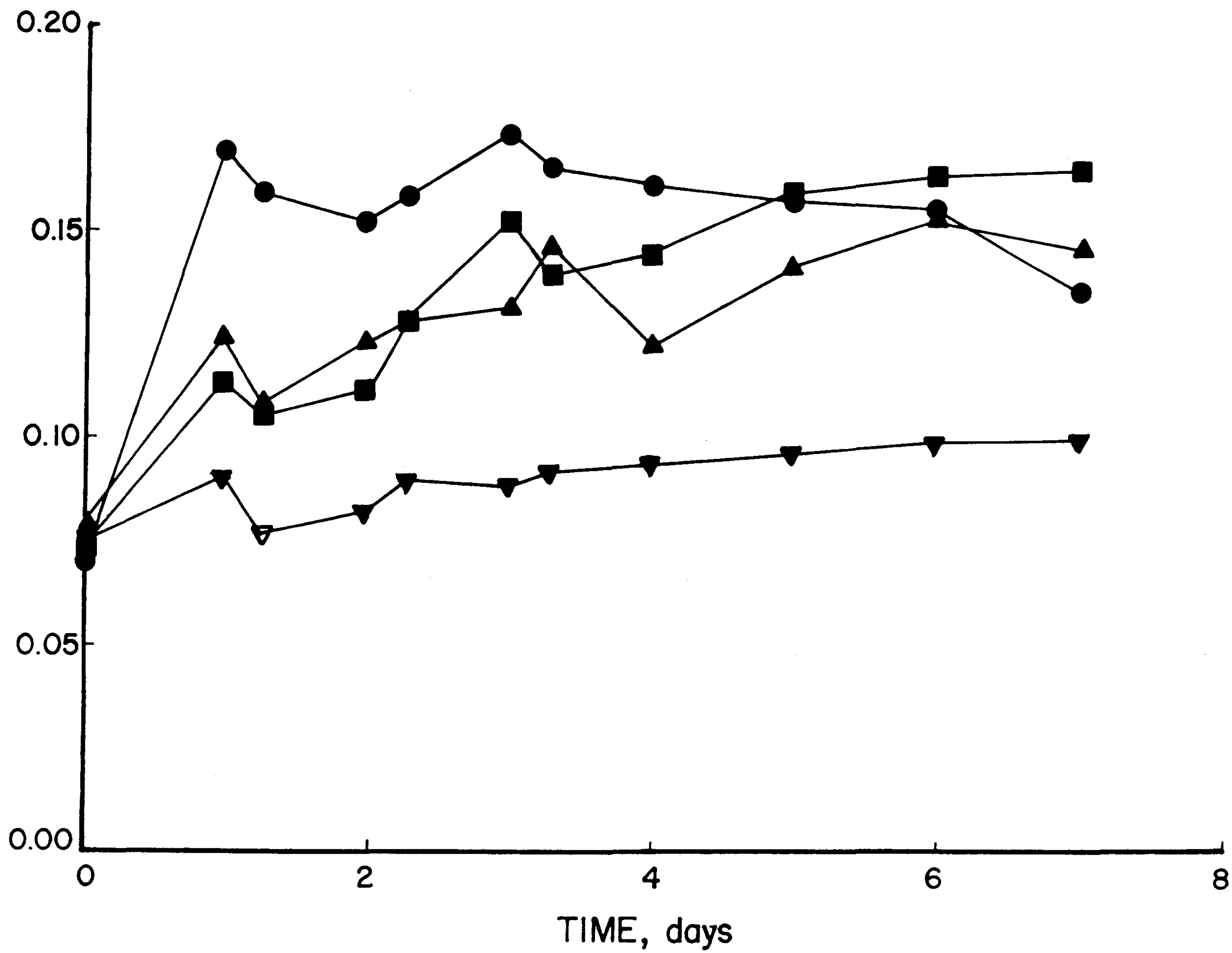


Figure 2.5. Total Organic Acids Profile for Experiment 3 (initial concentration = 100 g VS/$m^3$; all fermentors seeded; 40°C, ●; 50°C, ■; 55°C, ▲; 60°C, ▼)

TABLE 2.5. TOTAL ORGANIC ACIDS (TOA) AND TOTAL VOLATILE FATTY ACIDS (TVFA) PROFILES FOR EXPERIMENT 3

| Trt. | Time days | TOA $kg/m^3$ | TVFA/TOA % | Acid/TVFA, % | | | | pH |
|---|---|---|---|---|---|---|---|---|
| | | | | Acetate | Propionate | Butyrate | Valerate | |
| 3A | 0 | -- | -- | -- | -- | -- | -- | 5.4 |
| | 0.08 | 9.1 | 31.7 | 73.7 | 14.2 | 6.7 | 5.3 | 5.2 |
| | 0.25 | 13.8 | 27.2 | 77.3 | 13.0 | 6.0 | 3.8 | 4.7 |
| | 0.96 | 16.9 | 24.6 | 79.4 | 11.8 | 5.2 | 3.6 | 4.4 |
| | 1.25 | 15.9 | 25.5 | 78.5 | 13.4 | 5.1 | 3.0 | 4.3 |
| | 1.96 | 15.2 | 27.3 | 78.9 | 12.8 | 4.9 | 3.3 | 4.3 |
| | 2.25 | 15.8 | 28.0 | 81.2 | 11.3 | 4.8 | 2.9 | 4.3 |
| | 2.96 | 17.3 | 28.0 | 80.6 | 11.1 | 4.9 | 3.4 | 4.3 |
| | 3.25 | 16.5 | 27.9 | 82.0 | 10.5 | 4.8 | 2.7 | 4.3 |
| | 3.96 | 16.1 | 30.8 | 81.6 | 11.0 | 4.5 | 3.0 | 4.3 |
| | 4.96 | 15.7 | 30.5 | 79.0 | 12.2 | 5.1 | 3.9 | 4.4 |
| | 5.96 | 15.5 | 32.0 | 84.2 | 9.3 | 3.5 | 3.0 | 4.6 |
| | 6.96 | 13.5 | 38.1 | 82.7 | 9.8 | 4.7 | 2.8 | 4.7 |
| 3B | 0 | -- | -- | -- | -- | -- | -- | 5.3 |
| | 0.08 | 9.1 | 31.9 | 74.2 | 13.5 | 7.2 | 5.2 | 5.3 |
| | 0.25 | 11.6 | 29.1 | 74.9 | 13.7 | 7.1 | 4.2 | 5.0 |
| | 0.96 | 11.3 | 30.8 | 74.4 | 13.6 | 7.2 | 4.9 | 5.0 |
| | 1.25 | 10.5 | 33.5 | 72.7 | 16.1 | 7.7 | 3.6 | 5.0 |
| | 1.96 | 11.1 | 34.5 | 75.7 | 12.8 | 7.3 | 4.3 | 4.7 |
| | 2.25 | 11.9 | 31.1 | 75.4 | 13.8 | 7.4 | 3.4 | 4.6 |
| | 2.96 | 15.2 | 26.4 | 75.8 | 13.3 | 7.1 | 3.8 | 4.4 |
| | 3.25 | 13.9 | 25.0 | 80.4 | 10.2 | 5.6 | 3.7 | 4.4 |
| | 3.96 | 14.4 | 26.3 | 75.1 | 13.7 | 6.5 | 4.7 | 4.4 |
| | 4.96 | 15.9 | 25.8 | 79.5 | 12.4 | 4.6 | 3.5 | 4.2 |
| | 5.96 | 16.3 | 22.8 | 78.1 | 12.5 | 4.9 | 4.4 | 4.3 |
| | 6.96 | 16.4 | 20.5 | 79.0 | 11.2 | 5.9 | 3.9 | 4.2 |
| 3C | 0 | -- | -- | -- | -- | -- | -- | 5.4 |
| | 0.08 | 8.6 | 34.1 | 73.4 | 14.4 | 7.4 | 4.8 | 5.5 |
| | 0.25 | 8.8 | 37.5 | 73.3 | 14.2 | 7.9 | 4.7 | 5.5 |
| | 0.96 | 12.4 | 27.5 | 73.5 | 13.8 | 7.6 | 5.1 | 5.0 |
| | 1.25 | 10.8 | 31.7 | 73.0 | 12.2 | 10.4 | 4.4 | 4.8 |
| | 1.96 | 12.3 | 29.7 | 75.9 | 13.0 | 7.0 | 4.1 | 4.7 |
| | 2.25 | 12.8 | 30.0 | 74.8 | 13.7 | 7.1 | 4.4 | 4.6 |
| | 2.96 | 13.1 | 29.5 | 74.9 | 13.6 | 7.6 | 4.0 | 4.6 |
| | 3.25 | 14.6 | 25.9 | 75.7 | 13.5 | 7.2 | 3.7 | 4.6 |
| | 3.96 | 12.2 | 31.2 | 74.6 | 13.7 | 7.0 | 4.7 | 4.6 |
| | 4.96 | 14.1 | 24.9 | 76.8 | 13.7 | 6.1 | 3.5 | 4.8 |
| | 5.96 | 15.2 | 24.2 | 75.8 | 13.7 | 6.0 | 4.5 | 4.5 |
| | 6.96 | 14.5 | 22.5 | 77.7 | 11.7 | 6.7 | 4.0 | 4.5 |
| 3D | 0 | -- | -- | -- | -- | -- | -- | 5.4 |
| | 0.08 | 7.9 | 37.0 | 72.1 | 14.9 | 8.0 | 5.0 | 5.6 |
| | 0.25 | 9.1 | 34.8 | 71.7 | 13.9 | 8.7 | 5.7 | 5.5 |
| | 0.96 | 9.0 | 35.0 | 73.9 | 13.2 | 8.1 | 4.9 | 5.6 |
| | 1.25 | 7.7 | 42.2 | 73.7 | 13.0 | 8.6 | 4.8 | 5.5 |
| | 1.96 | 8.2 | 46.9 | 76.0 | 11.9 | 7.8 | 4.3 | 5.4 |
| | 2.25 | 8.9 | 45.7 | 76.0 | 11.9 | 7.8 | 4.3 | 5.3 |
| | 2.96 | 8.8 | 46.0 | 77.0 | 11.7 | 7.5 | 3.8 | 5.3 |
| | 3.25 | 9.1 | 43.4 | 78.1 | 11.2 | 6.8 | 4.0 | 5.2 |
| | 3.96 | 9.4 | 43.4 | 77.4 | 12.1 | 7.2 | 3.4 | 5.2 |
| | 4.96 | 9.6 | 41.4 | 79.2 | 11.9 | 5.7 | 3.2 | 5.4 |
| | 5.96 | 9.9 | 44.6 | 79.7 | 10.5 | 6.2 | 3.6 | 5.3 |
| | 6.96 | 9.9 | 42.4 | 78.9 | 11.1 | 6.5 | 3.5 | 5.2 |

TABLE 2.6. TOTAL ORGANIC ACIDS (TOA) AND TOTAL VOLATILE FATTY ACIDS (TVFA) PROFILES FOR EXPERIMENT 4

| Trt. | Time days | TOA $kg/m^3$ | TVFA/TOA % | Acid/TVFA, % | | | | pH |
|---|---|---|---|---|---|---|---|---|
| | | | | Acetate | Propionate | Butyrate | Valerate | |
| 4A | 0 | 6.6 | -- | -- | -- | -- | -- | 5.0 |
| | 1 | 13.5 | 20.0 | 83.6 | 9.4 | 6.1 | 1.0 | 3.9 |
| | 2 | 14.9 | 20.4 | 82.9 | 9.3 | 5.5 | 2.4 | 3.8 |
| | 3 | 14.1 | 20.3 | 83.5 | 8.8 | 4.8 | 2.9 | 3.7 |
| | 4 | 15.7 | 18.2 | 85.9 | 7.9 | 5.0 | 1.2 | 3.8 |
| | 7 | 14.9 | 19.5 | 83.8 | 7.7 | 4.4 | 4.1 | 3.9 |
| | 8 | 15.3 | 17.3 | 85.9 | 8.7 | 5.4 | 0 | 3.9 |
| | 9 | 15.5 | 12.0 | 81.3 | 9.4 | 6.0 | 3.4 | 4.1 |
| | 10 | 14.2 | 27.8 | 89.0 | 6.7 | 3.2 | 1.1 | 4.3 |
| | 11 | 14.5 | 30.4 | 89.4 | 6.2 | 3.0 | 1.3 | 4.2 |
| | 14 | 13.4 | 34.3 | 68.7 | 8.3 | 21.6 | 1.9 | 5.0 |
| 4B | 0 | 9.1 | 27.9 | 86.0 | 7.5 | 6.2 | 0.4 | 6.3 |
| | 1 | 17.8 | 27.8 | 89.2 | 5.8 | 4.0 | 1.0 | 4.5 |
| | 2 | 19.5 | 21.7 | 90.1 | 4.7 | 3.6 | 1.7 | 4.4 |
| | 3 | 19.2 | 25.1 | 90.2 | 5.5 | 3.1 | 1.4 | 4.3 |
| | 4 | 21.9 | 26.9 | 90.8 | 4.5 | 2.9 | 1.9 | 4.3 |
| | 7 | 23.3 | 29.4 | 91.4 | 4.5 | 2.7 | 1.5 | 4.2 |
| | 8 | 23.1 | 26.5 | 93.5 | 3.9 | 2.6 | 0.1 | 4.2 |
| | 9 | 24.7 | 18.4 | 89.3 | 5.8 | 3.1 | 1.9 | 4.3 |
| | 10 | 25.1 | 28.5 | 91.5 | 4.9 | 2.2 | 1.4 | 4.2 |
| | 11 | 25.0 | 30.3 | 90.7 | 5.5 | 2.4 | 1.5 | 4.1 |
| | 14 | 26.6 | 22.5 | 89.0 | 7.1 | 3.1 | 1.0 | 4.4 |
| 4C | 0 | 6.6 | 29.1 | 80.1 | 9.5 | 8.0 | 2.5 | 5.0 |
| | 1 | 7.5 | 31.4 | 82.2 | 8.7 | 8.1 | 1.1 | 4.7 |
| | 2 | 8.7 | 23.6 | 77.4 | 9.4 | 8.8 | 4.5 | 4.3 |
| | 3 | 12.6 | 16.0 | 80.4 | 9.9 | 6.9 | 2.9 | 3.9 |
| | 4 | 13.0 | 19.9 | 78.5 | 9.3 | 7.6 | 4.6 | 3.8 |
| | 7 | 14.1 | 19.6 | 82.0 | 9.7 | 7.3 | 1.0 | 3.7 |
| | 8 | 14.7 | 15.4 | 81.4 | 10.5 | 8.2 | 0 | 3.8 |
| | 9 | 15.0 | 15.7 | 81.4 | 10.7 | 6.3 | 1.7 | 3.8 |
| | 10 | 15.7 | 18.2 | 82.0 | 8.4 | 5.3 | 4.4 | 3.8 |
| | 11 | 15.5 | 19.9 | 81.7 | 9.1 | 5.2 | 4.1 | 3.8 |
| | 14 | 18.3 | 13.2 | 81.5 | 12.3 | 6.3 | 0 | 3.9 |
| 4D | 0 | 8.8 | 32.6 | 86.3 | 6.7 | 6.1 | 1.0 | 6.4 |
| | 1 | 12.1 | 31.6 | 85.8 | 6.4 | 6.3 | 1.5 | 5.7 |
| | 2 | 13.3 | 26.0 | 84.0 | 7.1 | 6.5 | 2.5 | 5.4 |
| | 3 | 16.6 | 23.1 | 87.1 | 6.5 | 5.2 | 1.4 | 4.7 |
| | 4 | 16.8 | 26.6 | 87.3 | 5.4 | 4.5 | 2.9 | 4.3 |
| | 7 | 18.5 | 23.1 | 88.7 | 6.1 | 5.1 | 0.2 | 4.5 |
| | 8 | 19.6 | 16.3 | 85.2 | 6.6 | 6.5 | 1.9 | 4.5 |
| | 9 | 20.5 | 19.9 | 90.6 | 5.5 | 3.5 | 0.4 | 4.6 |
| | 10 | 21.4 | 25.1 | 87.5 | 6.0 | 4.4 | 2.2 | 4.5 |
| | 11 | 21.6 | 22.1 | 86.6 | 7.1 | 4.9 | 1.4 | 4.5 |
| | 14 | 23.8 | 18.3 | 87.1 | 7.8 | 4.4 | 0.7 | 4.6 |

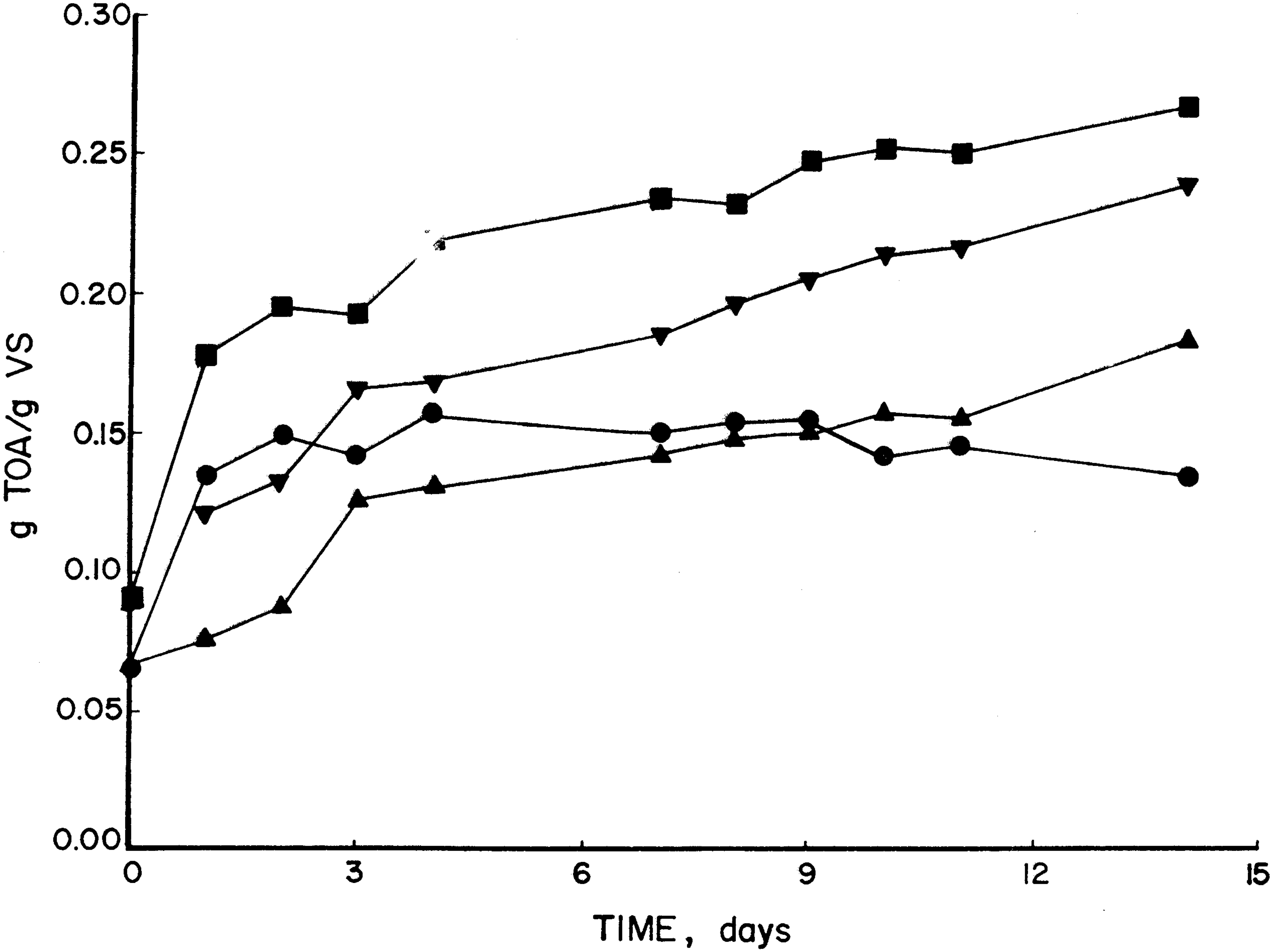


Figure 2.6. Total Organic Acids Profile for Experiment 4 (initial concentration = 100 g VS/$m^3$; all fermentors seeded; 40°C, ●; 40°C - ATS, ■; 55°C, ▲; 55°C - ATS, ▼)

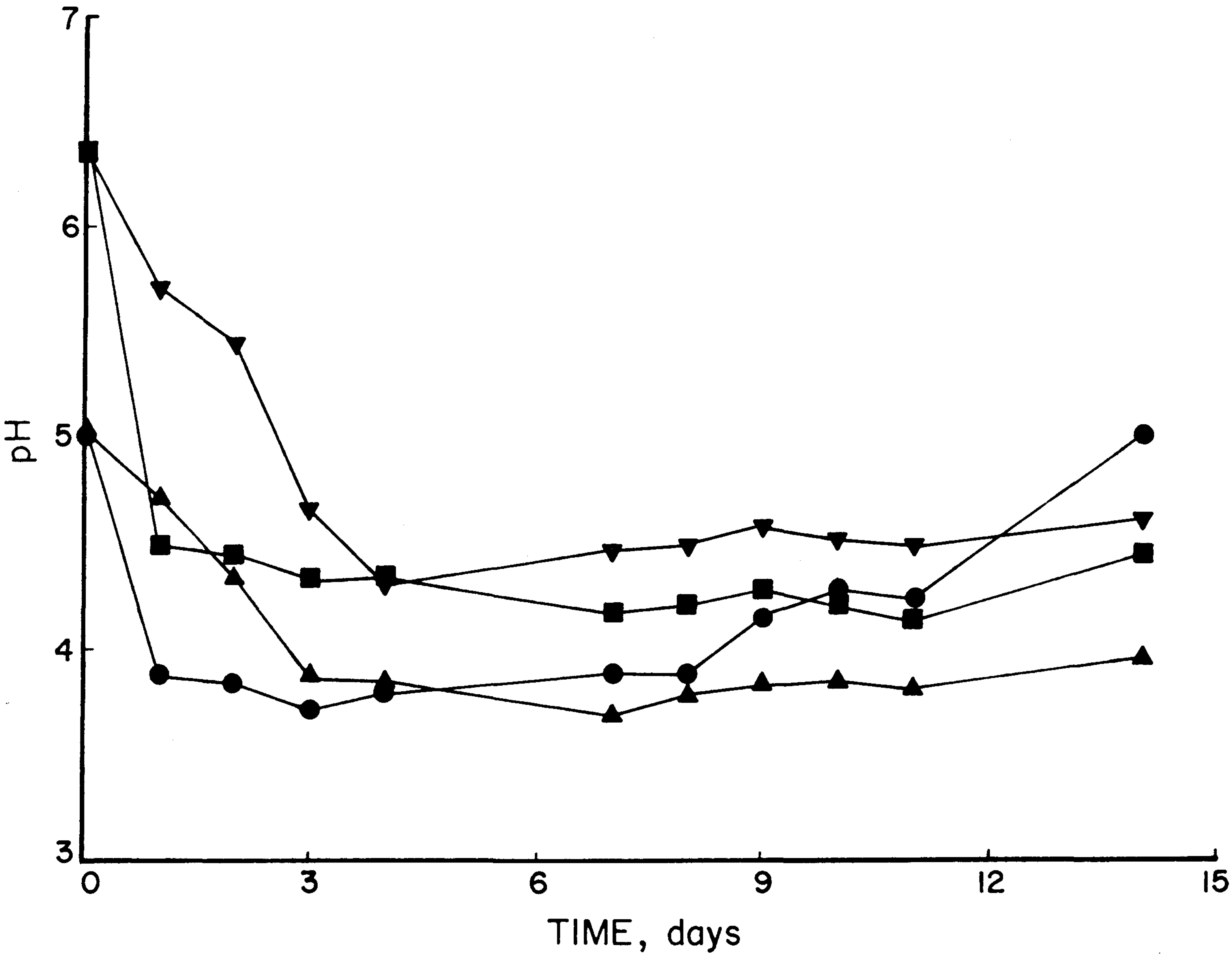


Figure 2.7. pH Profile for Experiment 4 (40°C, ●; 40°C - ATS, ■; 55°C, ▲; 55°C - ATS, ▼)

inhibit further TOA production while a pH of 4.5 allows some TOA to be produced.

## 2.4 DISCUSSION

These experiments show that the rate of TOA production was fastest at 40°C compared to higher temperatures, and that very little TOA was produced at 60°C. This was true even when the fermentors were inoculated with bacteria acclimated to 55°C. Temperatures below 40°C were not evaluated in this study, therefore, it is possible that even higher TOA production rates may be achieved at temperatures between 32 to 35°C as reported to be the optimum range for cellulase activity under mesophilic conditions (Ryu and Mandels, 1980). Inoculating fermentors with slurry from a thermophilic, anaerobic fermentor decreased the time required to reach maximum TOA production for the 40°C fermentors. Inoculated fermentors (40°) needed only 1 day to reach maximum TOA production (treatments 3A, 4A and 4B); whereas, the 40°C fermentors that were not inoculated (treatments 1A and 1B) required about 3 days to attain the maximum TOA production. However, fermentors operated at 50°C and higher needed about 3 days to achieve maximum TOA production, whether they were inoculated or not. Apparently, the hydrolytic bacteria/enzymes are sensitive to temperatures in the thermophilic range.

Both pH adjustment and alkaline treatment of the straw increased the extent of TOA production. However, alkaline treatment increased the extent of TOA production substantially more than pH adjustment. For example, at fermentor temperature of 40°C, pH adjustment increased the extent of TOA production by 12% (treatments 1A vs. 1B) and alkaline treatment increased the extent of TOA production by 56% (treatments 4A vs. 4B). The amount of alkaline needed to adjust the pH in treatment 1A was 58 g NaOH/kg VS while only 40 g NaOH/kg VS (since only the straw was treated) was used for treatment 4B. Since chemical cost is the most expensive component of alkaline treatment of crop residues, these results suggest that alkaline treatment of the straw is a more efficient and economical alternative than pH control to increase hydrolysis.

Substrate concentrations between 80 to 130 kg VS/$m^3$ had little effect on the rate and extent of TOA production. However, substrate concentration had a significant effect on physical properties of the mixtures. The 80 and 100 kg VS/$m^3$ mixtures were slurries while the 130 kg VS/$m^3$ mixture was semi-solid. Thus, different types of materials handling equipment will be needed depending on the substrate concentration.

These experiments were conducted to provide information to optimize the first-stage (microbial hydrolysis) fermentor of our pilot-scale, two-stage fermentation system shown in Figure 2.1. As a result of these findings, the microbiol hydrolysis fermentor is being operated at mesophilic temperatures (35 to 40°C) instead of at thermophilic temperatures, as originally conceived.

## 2.5 SUMMARY

Four experiments were conducted to evaluate effects of temperature, pH, substrate concentration, and alkaline pretreatment on the rate and extent of hydrolysis of manure-straw mixtures. The rate of hydrolysis, as measured by the total organic acids (TOA) production in fermentors, was more rapid at 40°C compared to higher temperatures (50, 55 or 60°C), and very little TOA was produced at 60°C. This was even true when fermentors were inoculated with bacteria acclimated to 55°C. Inoculating fermentors with slurry from a thermophilic anaerobic fermentor decreased the time required to reach maximum TOA production from 3 days to 1 day for the 40°C fermentors, but had no effect on fermentors at 50, 55 or 60°C. Both pH adjustment and alkaline treatment (120°C, 80 g NaOH/kg VS straw) of the straw increased the extent of TOA production. However, alkaline treatment increased the extent of TOA production substantially more than pH adjustment (12% vs 56%).

## SECTION 3.0

## METHANE PRODUCTION AND EFFLUENT QUALITY FROM FERMENTATION OF BEEF CATTLE MANURE AND MOLASSES

Andrew G. Hashimoto

### 3.1 INTRODUCTION

There has been recent interest in evaluating whether increased methane ($CH_4$) production rates can be achieved by mixing carbonaceous materials to livestock manures. The carbonaceous materials investigated have ranged from highly biodegradable substrate such as glucose and cellulose, to lignified crop residues like straw and corn stover.

Sievers and Brune (1978) used glucose and urea to adjust the carbon-nitrogen (C/N) ratio of swine manure between 2 to 25. Their fermentors were operated at 35°C, 15-day hydraulic retention time (HRT), and volatile solids (VS) loading rates of 1.12, 2.24 and 4.00 kg/$m^3$·day. They reported low $CH_4$ yields at low C/N ratios because of ammonia inhibition, and maximum $CH_4$ yields at C/N ratios between 16 to 19. They also reported decreasing $CH_4$ yields as the C/N ratio increased above 19, probably because of low nitrogen availability.

Fujita et al. (1980) compared the fermentation of 60 kg dried swine manure/$m^3$, to a mixture of 60 kg dried swine manure/$m^3$ and 20 kg dried corn stover/$m^3$ at thermophilic (55°C, 8-day HRT) and mesophilic (39°C, 16-day HRT) conditions. They stated that the $CH_4$ yield from the manure-stover mixture was substantially higher than from either the swine manure or corn stover alone. They attributed the higher $CH_4$ yield of the manure-stover mixture to the more advantageous C/N ratio.

Hills (1979) used glucose and reagent grade cellulose to increase the C/N ratio of screened dairy manure from 8 up to 52. He fermented these mixtures at 35°C, 25-day HRT, and loading rates of 1.0, 1.5 and 2.0 kg VS/$m^3$·day. He reported a linear increase in $CH_4$ yield as the C/N ratio increased; and that the highest $CH_4$ yield occurred at a C/N ratio of 25. He also reported a linear decrease in $CH_4$ yield as the C/N ratio increased above 25. Subsequently, Hills and Roberts (1981) fermented mixtures of dairy cattle manure combined with barley straw, rice straw or rice hulls. They reported maximum $CH_4$ yields at C/N ratios between 25 and 32.

Robbins et al. (1979) fermented 50, 60 and 70 kg dairy cattle manure/$m^3$; 50 kg manure/$m^3$ with 10, 20 and 30 kg cellulose/$m^3$; 50 kg manure/$m^3$ with 10, 20 and 30 kg delignified straw/$m^3$; and 50 kg manure/$m^3$ with 10 kg untreated straw/$m^3$. Fermentations were carried out at 37°C and 16-day HRT. They showed no difference in $CH_4$ yield from the 60 kg manure/$m^3$ and the 50 kg manure/$m^3$ + 10 kg untreated straw/$m^3$; however, they showed progressively higher $CH_4$ yields with increasing amounts of cellulose or delignified straw mixed with the manure.

Based upon the literature cited above, it is apparent that several mechanisms may be occuring when carbonaceous substrate is mixed with livestock manure. At low C/N ratio, carbon addition stimulates $CH_4$ yield by reducing ammonia inhibition. At high C/N ratios, carbon addition decreases $CH_4$ yield as nitrogen becomes a limiting nutrient. Also, the increase in $CH_4$ yield with increasing C/N ratio may be caused by mixing a carbonaceous substrate that is more biodegradable than the manure. The effects of these mechanisms on $CH_4$ yield can be quantitatively described by the following kinetic equation (Chen and Hashimoto, 1978):

$$B = \gamma_V \text{ HRT}/S_o = B_o \left(1 - K/(\text{HRT } \mu_m - 1 + K)\right) \tag{3.1}$$

where:

$B$ = $CH_4$ yield, $m^3$/kg VS·day

$B_o$ = ultimate $CH_4$ yield, $m^3$/kg VS·day as HRT approaches infinity

$\gamma_V$ = volumetric $CH_4$ production rate, $m^3$ $CH_4/m^3$ fermentor·day

HRT = hydraulic retention time, day

$\mu_m$ = maximum specific growth rate, $day^{-1}$

K = kinetic parameter, dimensionless.

We (Hashimoto, Chen and Varel, 1981) have reported that $\mu_m$ is primarily a function of temperature, and K reflects the degree of fermentation inhibition (i.e., K increases as the level of inhibition increases). Thus, if ammonia inhibition or nitrogen limitation inhibits the fermentation, then K would increase. Likewise, if $B_o$ is changed by mixing a carbon source with manure, then B would also change. This study was undertaken to determine which of the above mechanisms occur when molasses and beef cattle manure mixtures are fermented, and the effect these mixture have on the fermentor effluent quality.

## 3.2 MATERIALS AND METHODS

Beef cattle manure-molasses mixtures were prepared so that the manure and molasses contributed varying percentages of the VS in the mixture, as follows: 100% manure (100:0); 75% manure-25% molasses (75:25); and 50% manure-50% molasses (50:50). The manure was from steers (weighing 350 to 400 kg) fed a ration consisting of 85% corn, 13% corn silage and 2% soybean meal-mineral supplement (80.5% soybean meal, 11.5% limestone, 3% dicalcium phosphate, 0.8% vitamin A, D and E, 0.2% beef trace minerals, and 3.75% salt). The manure was less than 3 days old and scraped off concrete-floored pens.

The molasses was feed-grade molasses used in livestock feeds. The molasses had total solids (TS) concentration of 560 kg/$m^3$, VS concentration of 467 kg/$m^3$ and chemical oxygen demand (COD) of 985 kg/$m^3$.

Table 3.1 shows the analysis of the beef cattle manure and manure-molasses mixtures. There were no significant differences between the TS and VS concentrations of the three mixtures, however, there were noticeable trends in other constituents. As the molasses content increased, fixed solids (FS), COD, and potassium concentrations increased, while total nitrogen, ammonia, total volatile acids (TVA), cellulose, hemicellulose, lignin, silica ash and phosphorus concentrations decreased. The C/N ratios for the 100:0, 75:25, and 50:50 molasses-manure mixtures were 11, 14 and 19, respectively.

TABLE 3.1. Analyses of Beef Cattle Manure and Molasses Mixtures[a]

| Parameter | Manure-Molasses Content, % of VS | | |
|---|---|---|---|
| | 100-0 | 75-25 | 50-50 |
| Total Solids, $kg/m^3$ | 73.8 ± 2.4 | 75.9 ± 5.5 | 77.3 ± 3.8 |
| Volatile Solids, $kg/m^3$ | 67.6 ± 2.3 | 68.1 ± 4.9 | 68.8 ± 3.3 |
| Fixed Solids, $kg/m^3$ | 6.2 | 7.8 | 8.5 |
| COD, $kg/m^3$ | 68.1 ± 6.0 | 77.3 ± 5.4 | 84.0 ± 20.3 |
| Total Nitrogen, $kg/m^3$ | 2.91 ± 0.01 | 2.39 ± 0.14 | 1.80 ± 0.12 |
| Ammonia, $kg/m^3$ | 0.66 ± 0.10 | 0.51 ± 0.06 | 0.36 ± 0.02 |
| Volatile Acids, $kg/m^3$ | 6.84 ± 0.26 | 5.96 ± 0.39 | 5.00 ± 0.44 |
| Carbon, % TS | 44.7 | 45.2 | 43.8 |
| Cellulose, % TS | 11.6 | 8.4 | 5.7 |
| Hemicellulose, % TS | 22.3 | 11.2 | 8.4 |
| Lignin, % TS | 3.2 | 2.8 | 2.3 |
| Silica Ash, % TS | 2.6 | 1.6 | 1.0 |
| Phosphorus, % TS | 0.70 | 0.60 | 0.39 |
| Potassium, % TS | 1.19 | 2.21 | 2.61 |
| Carbon/nitrogen ratio | 11 | 14 | 19 |

[a]Data are means ± 1 standard deviation except when only 1 sample was analyzed, or when value was calculated by difference (FS)

The fermentors were 4-$dm^3$ Pyrex reaction kettles modified with an outlet fused to the bottom of each kettle (Figure 3.1). The feed-tube outlet was placed below the 3 $dm^3$ working-volume level to minimize the introduction of air during feeding. Mixing was accomplished by two, 5.5-cm diameter, 3-bladed propellers spaced 14 cm apart on the shaft. The mixer was a 20 watt variable speed motor operating at 220 revolutions per minute. The fermentor temperature (55°C±1°C) was maintained by a heating mantle controlled by a variable transformer. The fermentors were housed in a walk-in, constant-temperature chamber maintained at 25°C.

The biogas produced in the fermentor was collected in gas-impermiable bags and analyzed for gas volume and $CH_4$ concentrations. The gas volume was measured by a solution-displacement method, and the $CH_4$ content was measured by gas chromatography, as described previously (Hashimoto, Varel and Chen, 1981).

TS, VS, FS, ammonia (distillation method), COD, pH, alkalinity (to pH 3.7), and TVA were determined using standard methods for wastewater analysis (APHA, 1975). Kjeldahl nitrogen was determined by the method described by Wael and Gehrke (1975). Cellulose, hemicellulose, lignin, silica ash, phosphorous, potassium and sodium were determined (AOAC, 1975) on freeze-dried samples ground in a wiley mill. Carbon was determined on freezed-dried, ground samples using a Perkin-Elmers, Model 240, Elemental Analyzer.

The substrates were fed to the fermentors operated at 18, 9 and 6-day HRT's. Steady-state was assumed after 4 volume turnovers (i.e., 4 HRT). Steady-state $CH_4$ production and influent and effluent characteristics were analyzed for 5 consecutive days during steady-state. Data were analyzed by least-squares procedures outlined by Harvey (1975). Main effects were: manure-molasses mixtures, HRT, and day of sampling during steady state. Significance was tested at the $P < 0.01$ level.

## 3.3 RESULTS

Tables 3.2, 3.3 and 3.4 show the $CH_4$ production and effluent quality from the fermentors fed the 100:0, 75:25 and 50:50 manure-molasses mixtures, respectively. For a given mixture, $\gamma_v$ decreased significantly as HRT increased, and, at similar HRT and VS loading rates, $\gamma_v$ increased significantly as the molasses content of the mixture increased.

Ultimate $CH_4$ yields ($B_o$) were estimated by plotting B versus $HRT^{-1}$ as described by Chen and Hashimoto (1978). Using this procedure, the $B_o$ values were 0.325, 0.335 and 0.360 $m^3$ $CH_4$/kg VS fed for the 100:0, 75:25 and 50:50 manure-molasses mixtures, respectively. Thus, the addition of the molasses to the beef cattle manure increased $B_o$ of the mixture over only beef manure.

Tables 3.2, 3.3 and 3.4 also show the $CH_4$ yield (expressed as $m^3$ $CH_4$/kg VS fed, and $m^3$ $CH_4$/kg COD fed) from the three manure-molasses mixtures. The $CH_4$ yields from the 100:0 mixture at different HRT were identical when expressed on VS or COD basis, because the COD/VS ratio of the manure was 1.01 (Table 3.1). However, for the manure-molasses mixtures at the same HRT, the $m^3$ $CH_4$/kg VS fed increased as the proportion of molasses in the mixture increased, while the $m^3$ $CH_4$/kg COD fed remained relatively constant as the porportion of molasses in the mixture increased. These trends reflect the much higher COD/VS ratio of molasses (2.11) compared to manure (1.01) and help to explain the increase in $B_o$ as the molasses content increased. The high

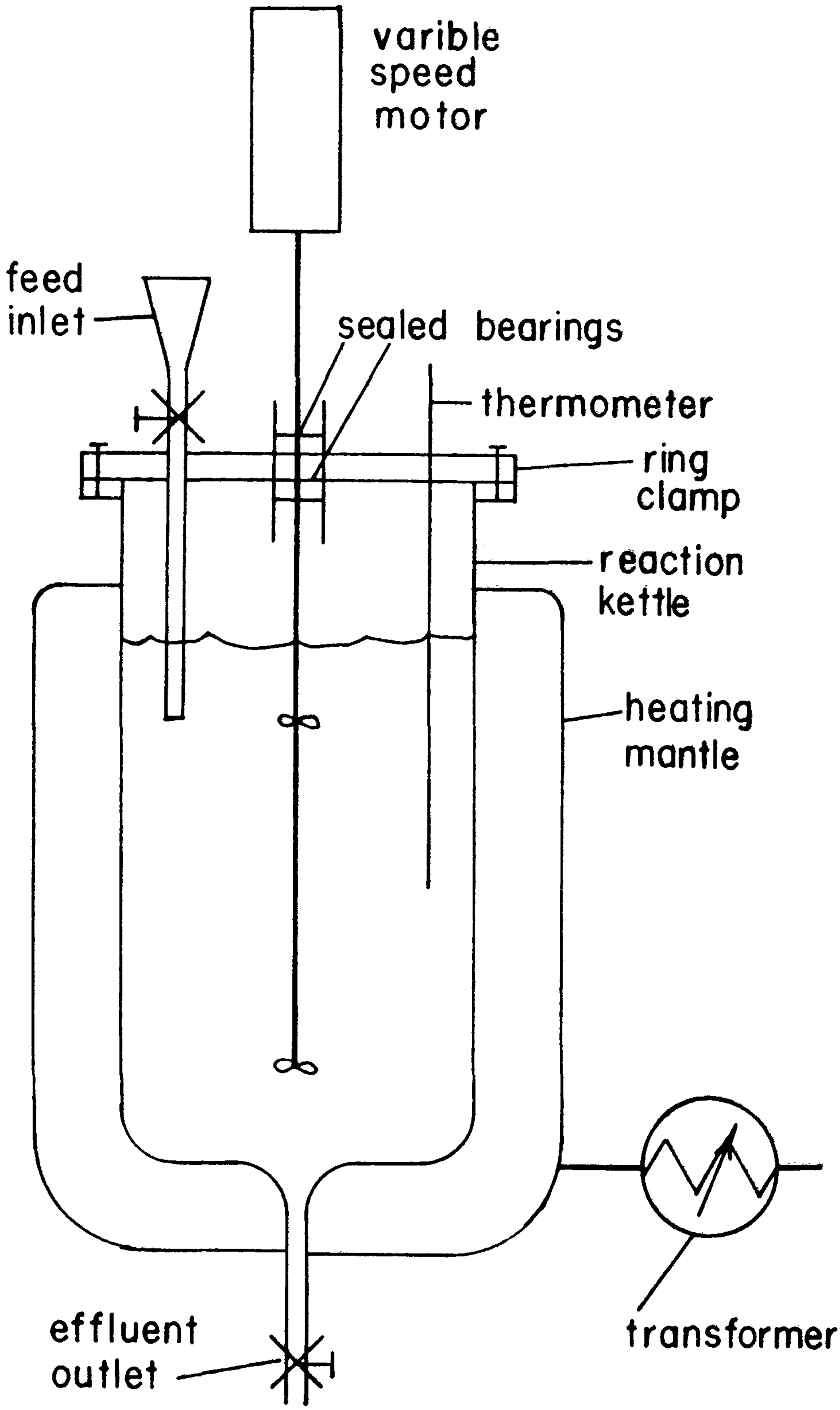


Figure 3.1. Laboratory Scale Anaerobic Fermentor

Table 3.2. Effluent Quality and Methane Production, From Continuously-Mixed, Thermophilic (55°C) Fermentors Receiving Beef Cattle Manure

| | Hydraulic Retention Time, days | | |
|---|---|---|---|
| Parameter | 18 | 9 | 6 |
| Total Solids, $kg/m^3$ | 26.2±1.1 | 27.2±0.4 | 29.1±0.4 |
| Volatile Solids, $kg/m^3$ | 21.4±1.0 | 21.6±0.3 | 23.4±0.4 |
| Fixed Solids, $kg/m^3$ | 4.8 | 5.6 | 5.7 |
| COD, $kg/m^3$ | 33.1±11.9 | 31.9±2.1 | 30.8±1.4 |
| Total Nitrogen, $kg/m^3$ | 2.75±0.09 | 2.57±0.04 | 2.57±0.03 |
| Ammonia, $kg/m^3$ | 1.40±0.02 | 1.28±0.04 | 1.24±0.05 |
| Organic Nitrogen, $kg/m^3$ | 1.35±0.09 | 1.29±0.06 | 1.34±0.07 |
| Volatile Acids, $kg/m^3$ | 0.59±0.32 | 0.35±0.02 | 0.63±0.07 |
| Alkalinity, $kg/m^3$ | 6.55±0.15 | 6.03±0.18 | 6.28±0.20 |
| Cellulose, % TS | 9.3 | 8.3 | 9.2 |
| Hemicellulose, % TS | 10.8 | 8.5 | 9.2 |
| Lignin, % TS | 7.0 | 7.6 | 7.4 |
| Silica Ash, % TS | 5.4 | 3.8 | 2.2 |
| Phosphorus, % TS | 1.77 | 1.86 | 1.65 |
| Potassium, % TS | 3.48 | 3.13 | 3.51 |
| Sodium, % TS | 0.65 | 0.86 | 1.00 |
| pH | 7.58±0.21 | 7.74±0.03 | 7.76±0.02 |
| Methane, % | 54.2±1.8 | 5.25±0.9 | 50.1±1.4 |
| Methane Production | | | |
| Rate, $m^3/m^3 \cdot day$ | 1.13±0.06 | 2.10±0.11 | 3.00±0.22 |
| Yield, $m^3$/kg VS fed | 0.30 | 0.28 | 0.27 |
| Yield, $m^3$/kg COD fed | 0.30 | 0.28 | 0.27 |

[a]Data are means ± 1 standard deviation except when only 1 sample was analyzed or when the values were calculated (FS, $CH_4$ Yield)

Table 3.3. Effluent Quality and Methane Production From Continuously-Mixed, Thermophilic (55°C) Fermentors Receiving 75% Beef Cattle Manure and 25% Molasses

| | Hydraulic Retention Time, days | | |
|---|---|---|---|
| Parameter | 18 | 9 | 6 |
| Total Solids, $kg/m^3$ | 30.2±0.1 | 29.5±0.5 | 29.3±0.5 |
| Volatile Solids, $kg/m^3$ | 23.8±0.3 | 22.6±0.4 | 22.4±0.4 |
| Fixed Solids, $kg/m^3$ | 6.4 | 6.9 | 6.9 |
| COD, $kg/m^3$ | 34.8±3.5 | 33.9±5.5 | 30.2±2.2 |
| Total Nitrogen, $kg/m^3$ | 2.36±0.10 | 2.17±0.03 | 2.41±0.26 |
| Ammonia, $kg/m^3$ | 1.13±0.02 | 0.84±0.03 | 0.85±0.06 |
| Organic Nitrogen, $kg/m^3$ | 1.23±0.08 | 1.33±0.05 | 1.56±0.29 |
| Volatile Acids, $kg/m^3$ | 3.83±0.44 | 0.35±0.06 | 0.47±0.05 |
| Alkalinity, $kg/m^3$ | 6.50±0.30 | 5.56±0.08 | 5.47±0.08 |
| Cellulose, % TS | 7.1 | 7.2 | 7.5 |
| Hemicellulose, % TS | 9.4 | 7.3 | 7.0 |
| Lignin, % TS | 6.6 | 6.6 | 6.1 |
| Silica Ash, % TS | 2.5 | 2.6 | 3.1 |
| Phosphorus, % TS | 1.18 | 1.50 | 1.40 |
| Potassium, % TS | 4.70 | 4.14 | 5.62 |
| Sodium, % TS | 0.50 | 0.59 | 0.84 |
| pH | 7.18±0.07 | 7.60±0.06 | 7.72±0.03 |
| Methane, % | 48.1±1.5 | 51.1±0.4 | 49.8±1.3 |
| Methane Production | | | |
| Rate, $m^3/m^3 \cdot day$ | 1.15±0.10 | 2.18±0.05 | 3.13±0.09 |
| Yield, $m^3/kg$ VS fed | 0.31 | 0.29 | 0.28 |
| Yield, $m^3/kg$ COD fed | 0.27 | 0.26 | 0.25 |

[a]Date are means ± 1 standard deviation except when only 1 sample was analyzed or when the values were calculated (FS, $CH_4$ Yield)

Table 3.4. Effluent Quality and Methane Production From Continuously-Mixed, Thermophilic (55°C) Fermentors Receiving 50% Beef Cattle Manure and 50% Molasses

| | Hydraulic Retention Time, days | | |
|---|---|---|---|
| Parameters | 18 | 9 | 6 |
| Total Solids, $kg/m^3$ | 27.7±0.5 | 29.2±0.9 | 30.9±3.0 |
| Volatile Solids, $kg/m^3$ | 20.2±0.7 | 21.0±0.8 | 22.1±2.2 |
| Fixed Solids, $kg/m^3$ | 7.5 | 8.2 | 8.8 |
| COD, $kg/m^3$ | 28.4±4.6 | 31.5±2.0 | 28.7±2.2 |
| Total Nitrogen, $kg/m^3$ | 1.83±0.06 | 1.70±0.02 | 1.68±0.07 |
| Ammonia, $kg/m^3$ | 0.65±0.01 | 0.49±0.04 | 0.38±0.02 |
| Organic Nitrogen, $kg/m^3$ | 1.18±0.07 | 1.21±0.04 | 1.29±0.07 |
| Volatile Acids, $kg/m^3$ | 1.91±0.08 | 0.37±0.02 | 0.43±0.03 |
| Alkalinity, $kg/m^3$ | 5.99±0.17 | 5.44±0.09 | 5.28±0.09 |
| Cellulose, % TS | 6.2 | 6.2 | 6.3 |
| Hemicellulose, % TS | 8.3 | 6.2 | 8.4 |
| Lignin, % TS | 4.4 | 5.4 | 4.3 |
| Silica Ash, % TS | 1.1 | 1.1 | 0.2 |
| Phosphorus, % TS | 1.02 | 1.18 | 1.02 |
| Potassium, % TS | 6.77 | 4.92 | 7.38 |
| Sodium, % TS | 0.52 | 0.54 | 0.76 |
| pH | 7.18±0.06 | 7.54±0.05 | 7.71±0.02 |
| Methane, % | 48.9±0.9 | 50.9±0.6 | 48.0±1.0 |
| Methane Production | | | |
| Rate, $m^3/m^3 \cdot day$ | 1.29±0.06 | 2.43±0.05 | 3.40±0.05 |
| Yield, $m^3$/kg VS fed | 0.34 | 0.32 | 0.30 |
| Yield, $m^3$/kg COD fed | 0.28 | 0.26 | 0.25 |

[a]Data are means ± 1 standard deviation except when only 1 sample was analyzed or when the values were calculated (FS, $CH_4$ Yield)

COD/VS ratio of molasses indicates it is a more biodegradable substrate than manure, thus $B_0$ increases when molasses is combined with manure.

Values for K were calculated using Equation (3.1) and the $B_0$ values listed above, and by assuming that $\mu_m$ was equal to 0.586 $day^{-1}$ at 55°C (Hashimoto, Chen and Varel, 1981). The mean (standard deviation) values for K were 0.64 (0.08), 0.71 (0.17), and 0.58 (0.05) for the 100:0, 75:25 and 50:50 manure-molasses mixtures, respectively. Analysis of variance showed no significant difference in K values between the three mixtures. The overall mean K value for the three mixtures was 0.64 with a mean standard deviation of 0.05. This is close to the K value of 0.6 reported for uninhibited fermentation of beef cattle manure (Hashimoto, Chen and Varel, 1981). These results show that increasing the C/N ratio from 11 to 19 did not alter the value of the kinetic parameter (K), and indicate that fermentation was not inhibited at these C/N ratios.

Table 3.5 compares the experimental values of $\gamma_V$ and the predicted $\gamma_V$. Equation (3.1) was used to predict $\gamma_V$ along with the values of $B_0$ listed above, and assuming $\mu_m$ to be 0.586 $day^{-1}$ at 55°C and K to be 0.6. The good prediction of $\gamma_V$ is illustrated by the mean ratio of predicted to experimental $\gamma_V$ being equal to 1.00 (standard deviation of 0.02), and by the plot of $\gamma_V$ versus HRT (Figure 3.2).

Tables 3.2, 3.3 and 3.4 also show the quality of the fermentor effluent. Of particular interest is the effect of molasses addition on the nitrogen constituents. Although molasses addition decreased the influent and effluent total nitrogen concentrations, there was no significant difference ($P < 0.01$) in the organic nitrogen concentration after fermenting the mixtures. The organic nitrogen concentrations averaged 1.32, 1.37 and 1.23 $kg/m^3$ for the 100:0, 75:25 and 50:50 manure-molasses mixtures, respectively. These trends in total nitrogen and organic nitrogen resulted in a decrease in the $NH_3$/TKN ratio as the molasses in the mixture increased. Figure 3.3 shows the decrease in the $NH_3$/TKN ratio as the proportion of molasses increased, and that the ratio increased as the HRT increased. At a 6-day HRT, the $NH_3$/TKN ratios were 0.48, 0.35 and 0.23 for the 100:0, 75:25 and 50:50 manure-molasses mixtures, respectively.

## 3.4 DISCUSSION

The results from this study agree with previous results showing an increase in $CH_4$ yield as the C/N ratio increases. However, this study shows that for C/N ratios between 11 to 19, the primary reason for the increase in $CH_4$ yield was the increase in $B_0$ of the substrate when molasses was mixed with cattle manure. This finding has practical implication because it shows that there is a range in C/N ratios where $CH_4$ production is not inhibited. Thus, the relative cost or abundance of substrates would determine the best C/N ratio rather than attempting to adjust the C/N ratio to a particular optimum ratio. This finding also implies that adding a carbonaceous substrate to manure may also decrease the $CH_4$ yield if the biodegradability of the carbonaceous substrate is lower than the manure. This is supported by the data of Hills and Robinson (1981). They showed significantly higher $CH_4$ yields when barley or rice straw was added to dairy-cattle manure compared to when rice hulls was combined with dairy-cattle manure. The higher lignin content (about 3-fold) of rice hulls compared to barley and rice straws was cited as the reason for the lower biodegradability of the rice hulls.

Table 3.5. Comparison Of Experimental And Predicted Volumetric Methane Production Rates

| Mixture % manure:molasses | $day^{-1}$ | $\gamma_V$, $m^3/m^3 \cdot day$ Experimental | Predicted[a] | $\gamma_V$ Ratio Pred/Exp |
|---|---|---|---|---|
| 100:0 | 18 | 1.13 | 1.15 | 1.02 |
| 100:0 | 9 | 2.10 | 2.14 | 1.02 |
| 100:0 | 6 | 3.00 | 2.95 | 0.98 |
| 75:25 | 18 | 1.15 | 1.19 | 1.03 |
| 75:25 | 9 | 2.18 | 2.22 | 1.02 |
| 75:25 | 6 | 3.13 | 3.07 | 0.98 |
| 50:50 | 18 | 1.29 | 1.29 | 1.00 |
| 50:50 | 9 | 2.43 | 2.41 | 0.99 |
| 50:50 | 6 | 3.40 | 3.33 | 0.98 |
| | | | | mean 1.00 ± 0.02 |

[a] Calculated by $\gamma_V = (B_o S_o/HRT)(1 - K/(\mu_m - 1 + K)$ where : $B_o$ = 0.325, 0.335, and 0.360 $m^3$ $CH_4$ $CH_4$/kg VS fed for 100:0, 75:25, and 50:50 (manure:molasses) respectively; $\mu_m$ = 0.586 $day^{-1}$; and K = 0.6

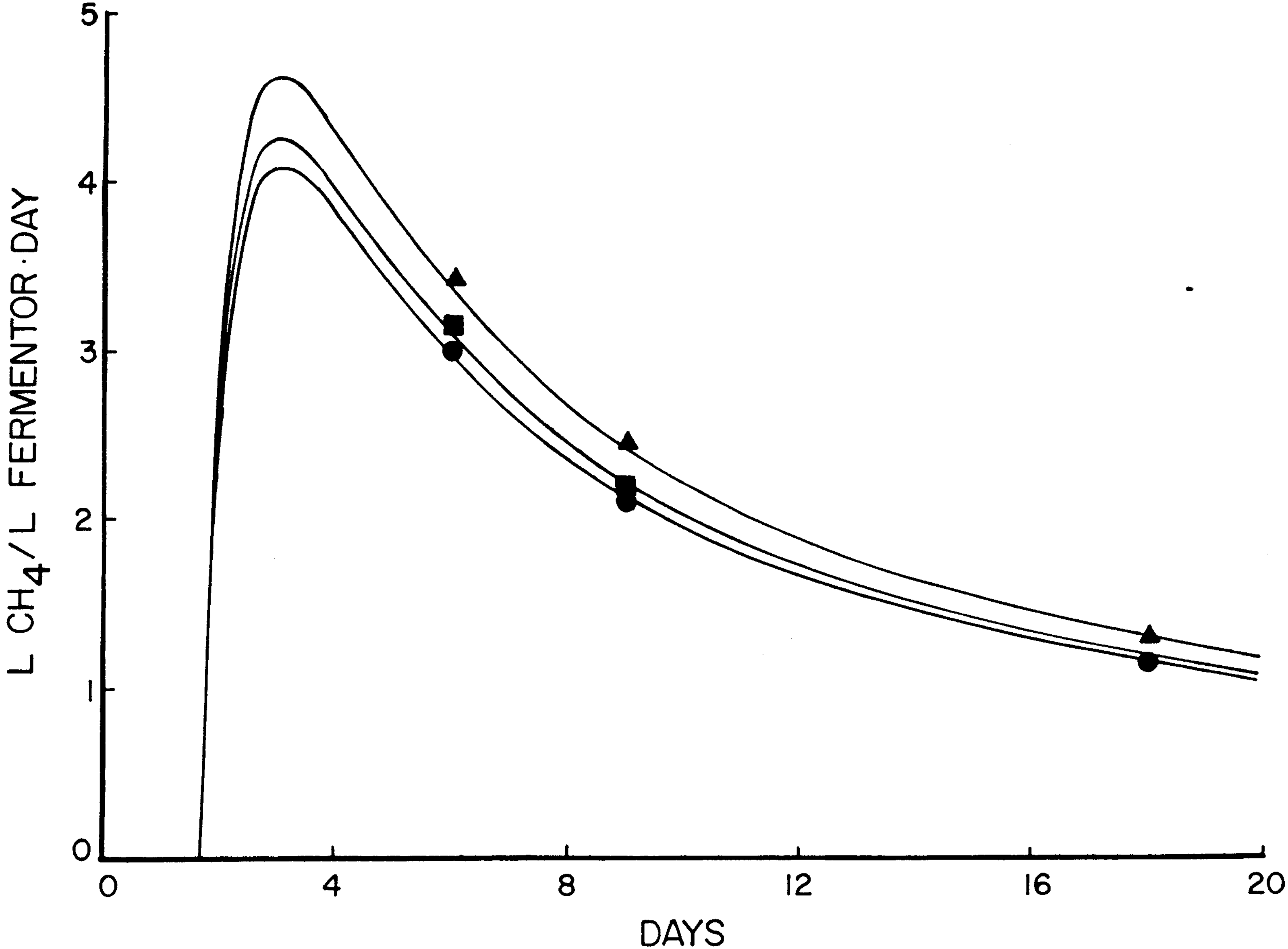


Figure 3.2. Relationship Between Specific Methane Production Rate and Hydraulic Retention Time (curve based on Eqa. 1; ●, 100% manure; ■, 75% manure:25% molasses; ▲, 50% manure:50% molasses)

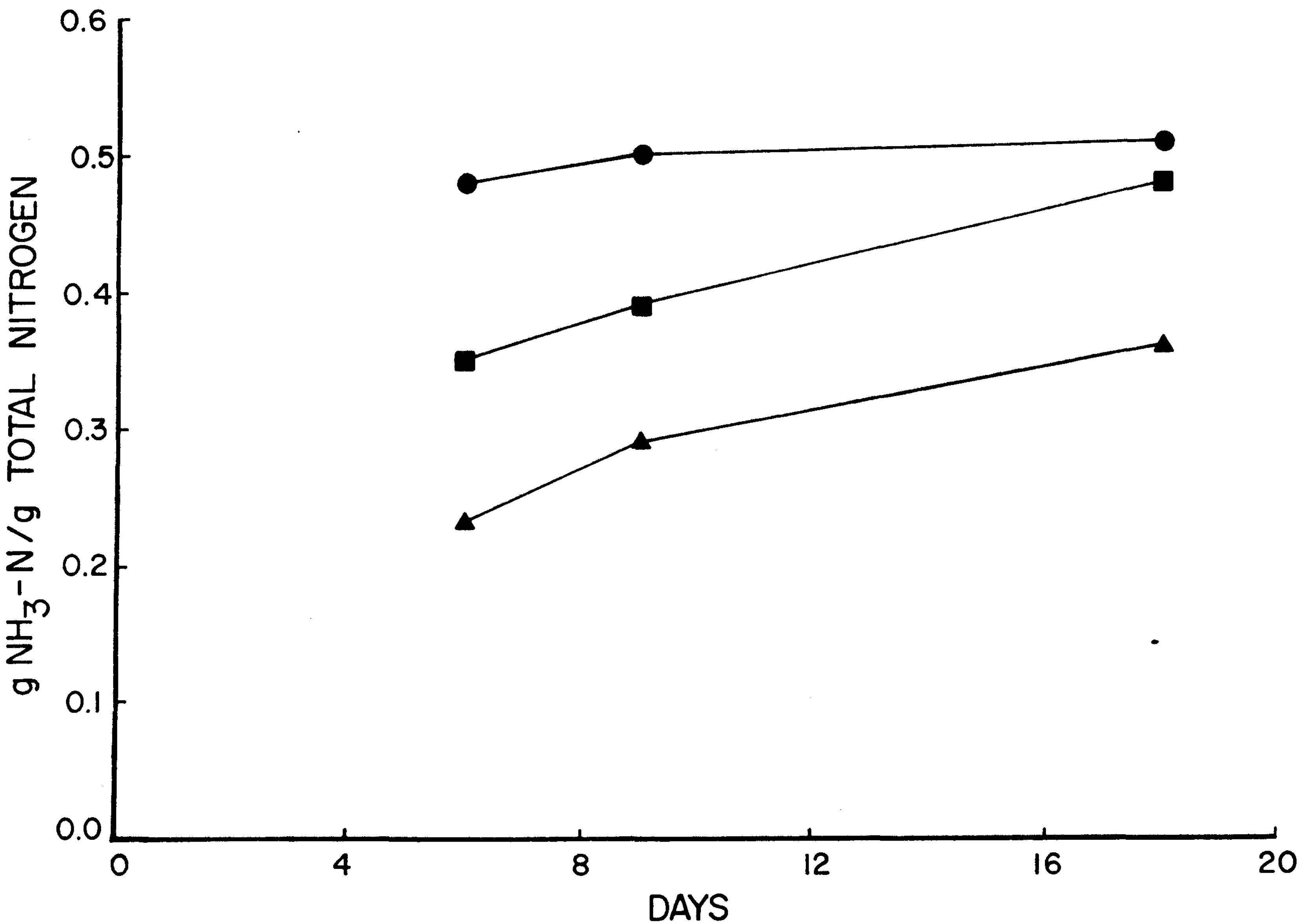


Figure 3.3. Effect of Hydraulic Retention Time on Ammonia to Total Nitrogen Ratio (●, 100% manure; ■, 75% manure:25% molasses; ▲, 50% manure:50% molasses)

In this study, the C/N ratio ranged from 11 to 19; therefore, the findings discussed above are only applicable over this range of C/N ratios. The literature does suggest that inhibition of $CH_4$ production occurs at very low and very high C/N ratios. Sievers and Brune (1978) showed extreme inhibition of $CH_4$ production at a C/N ratio of 1.7, and moderate inhibition at a C/N ratio of 5.5 using glucose as a carbon source. Hills (1979) showed stable fermentation at C/N ratio of 8 when glucose and cellulose were used. Thus, inhibition of $CH_4$ production probably begins at C/N ratios between 6 to 8.

At high C/N ratios, Hills (1979) showed a decline in $CH_4$ yield at C/N ratios above 25. Sievers and Brune (1978) showed that the $CH_4$ yield decreased above a C/N ratio of 20. It should be noted that highly biodegradable carbonaceous substrates (glucose and pure cellulose) were used to increase the C/N ratio when glucose or cellulose are mixed with manure.

From this discussion, it is apparent that uninhibited $CH_4$ production occurs over a range of C/N ratios. This study shows uninhibited fermentation between C/N ratios of 11 to 19. The literature suggests that C/N ratios between 8 to 25 would probably be satisfactory for systems fermenting mixtures of crop residues and manure.

An important aspect of the anaerobic fermentation research being conducted at the Roman L. Hruska U.S. Meat Animal Research Center is the evaluation of the fermentation residue as a protein supplement for livestock feeds (Prior et al., 1981). The potential credits to be realized from the use of the residue as a protein supplement has a significant impact on the economic feasibility of the anaerobically fermented livestock manures (Hashimoto et al., 1979).

This study showed that, by increasing the proportion of carbonaceous substrate to cattle manure, the total nitrogen concentration decreased, the ratio of ammonia to total nitrogen decreased, and organic nitrogen concentrate remained the same. Thus, for a given manure production rate, more crude protein (i.e., organic nitrogen x 6.25) would be produced if the manure is mixed with a carbonaceous substrate (e.g., crop residue) and fermented than if only the manure was fermented. Also, monogastric animals (e.g., swine, poultry, etc.), cannot use nonprotein nitrogen (e.g. ammonia), therefore, the ammonia in the residue must be removed before the residue can be fed to these animals. Thus, fermenting mixtures of manure and supplemental carbon (e.g., crop residues) has potential for increasing the amount and quality of the protein for use as a livestock feed supplement.

In summary, this study has shown that nitrogen-related inhibition (i.e., ammonia inhibition or nitrogen limitation) does not occur between C/N ratios of 11 to 19, and that the $CH_4$ yield is primarily determined by the relative biodegradabilities of the substrates within this range of C/N ratios. Also, the potential benefits of combining supplemental carbon with manures are increased total $CH_4$ production, because of the greater amount of substrate being fermented, and the increased quantity and quality of protein from the fermentation.

## 3.5 SUMMARY

The effect of mixing a highly carbonaceous substrate (molasses) and highly nitrogenous substrate (beef cattle manure) on methane ($CH_4$) production and effluent quality was evaluated. The manure and molasses were mixed so that they contributed varying VS percentages in the mixture, as follows: 100% manure (100:0); 75% manure and 25% molasses (75:25); and 50% manure and 50% molasses (50:50). Laboratory-scale, anaerobic fermentors (3-$dm^3$ working volume, continuously mixed) were operated at 55°C and at 6, 9 and 18-day hydraulic retention times (HRT). At similar HRT and VS loading rates, fermentors receiving the 50:50 mixture consistently produced the highest volumetric $CH_4$ production rates ($m^3$ $CH_4$/$m^3$ fermentor·day). The fermentor receiving only cattle manure produced the lowest rates, while the fermentor receiving the 75:25 mixture produced intermediate rates. Kinetic evaluation showed that increased $CH_4$ production rates of molasses containing substrates were due only to higher ultimate $CH_4$ yields ($B_o$) of the manure-molasses mixtures, and not due to reduce inhibition nor increase microbial growth rate. $B_o$ were 0.325, 0.335, and 0.360 $m^3$ $CH_4$/kg VS fed for the 100:0, 75:25 and 50:50 mixtures, respectively.

The addition of molasses to manure also affected fermentor effluent characteristics. Of particular interest was a change in ammonia to total nitrogen ($NH_3$/TN) ratio. At a 6-day HRT, the $NH_3$/TN ratio decreased from 0.48 to 0.38 and to 0.23 for the 100:0, 75:25 and 50:50 mixtures, respectively. This shift from ammonia to organic nitrogen is desirable if fermentor effluent is used as a protein supplement in livestock feeds.

# SECTION 4.0

# PILOT-SCALE FERMENTOR OPERATION

Andrew G. Hashimoto and Steven A. Robinson

## 4.1 INTRODUCTION

This section describes the extensive modifications of the pilot plant and operation of the two-stage fermentation system described in Section 2.1. The modifications were necessary to accomodate fermentation of manure-crop residue mixtures.

## 4.2 EQUIPMENT AND PROCEDURES

Figure 4.1 is a schematic diagram of the modified pilot-scale fermentation system. The pilot-scale facilities were orginally constructed under contract with Hamilton Standard Div. of United Technologies, Inc. and modified in 1980 to the present configuration.

Manure (1 to 10 days old) was gathered daily from steers housed on partially roofed, concrete-floored pens. The steers weighed from 340 to 570 kg. Their feed ration consisted of 85% yellow corn, 13% corn silage, 1.6% soybean meal, 0.2% limestone, 0.1% each of decalcuim phosphate and salt, and trace minerals and vitamins A, D and E.

The manure was transported to the pilot plant by a small front-end loader and dumped into the slurry tank. Water was added to form a slurry of 10 to 12% total solids (TS), and mixed with a 1-kW variable speed mixer.

Wheat straw, from hard-winter wheat grown in Clay Country, Nebraska, was baled in large round bales (approximately 400 kg) and stored in an open front barn. The wheat straw was ground in a tub grinder with a 1.9 cm screen. The straw was then re-ground in a rotary hammer-mill with 0.64 cm screen.

Based upon the volatile solids (VS) analysis of both the manure and ground wheat straw, the two were mixed in a 50:50 ratio (1 kg VS manure with 1 kg VS straw). This combination was mixed and water added to form a slurry of 10 to 12% TS. The slurry was then pumped by a diaphram pump to the hydrolysis tank. The hydrolysis tank was a 1-$m^3$, insulated, covered tank, equipped with a hot-water heat exchanger. The 1-$m^3$ fiber glass tank was insulated with 7.6 cm of polyurethane foam, and the insulation was protected with galvanized sheet metal. The heat exchanger consisted of 1.9 cm diameter by 27.7 m long coiled soft-copper tubing mounted on an aluminum pipe frame. Hot water in the heat exchanger was maintained between 77 to 70°C. The tank was equiped with a 3-phase, 2.2 kW variable-speed mixer with two 22.86 cm marine propellers.

After 24 hours in the hydrolysis tank at 50 to 60°C, the slurry was pumped, at a flow rate of 433 $cm^3/s$, to a 0.61-m diameter vibrating screen separator. The separator screen opening was 1.9 mm (10 mesh).

Solids removed from the slurry were weighed and approximately 180 kg, or 37%, of the screened solid were recycled to the slurry tank and mixed with fresh straw-manure slurry. The remaining solids were diposed of on land.

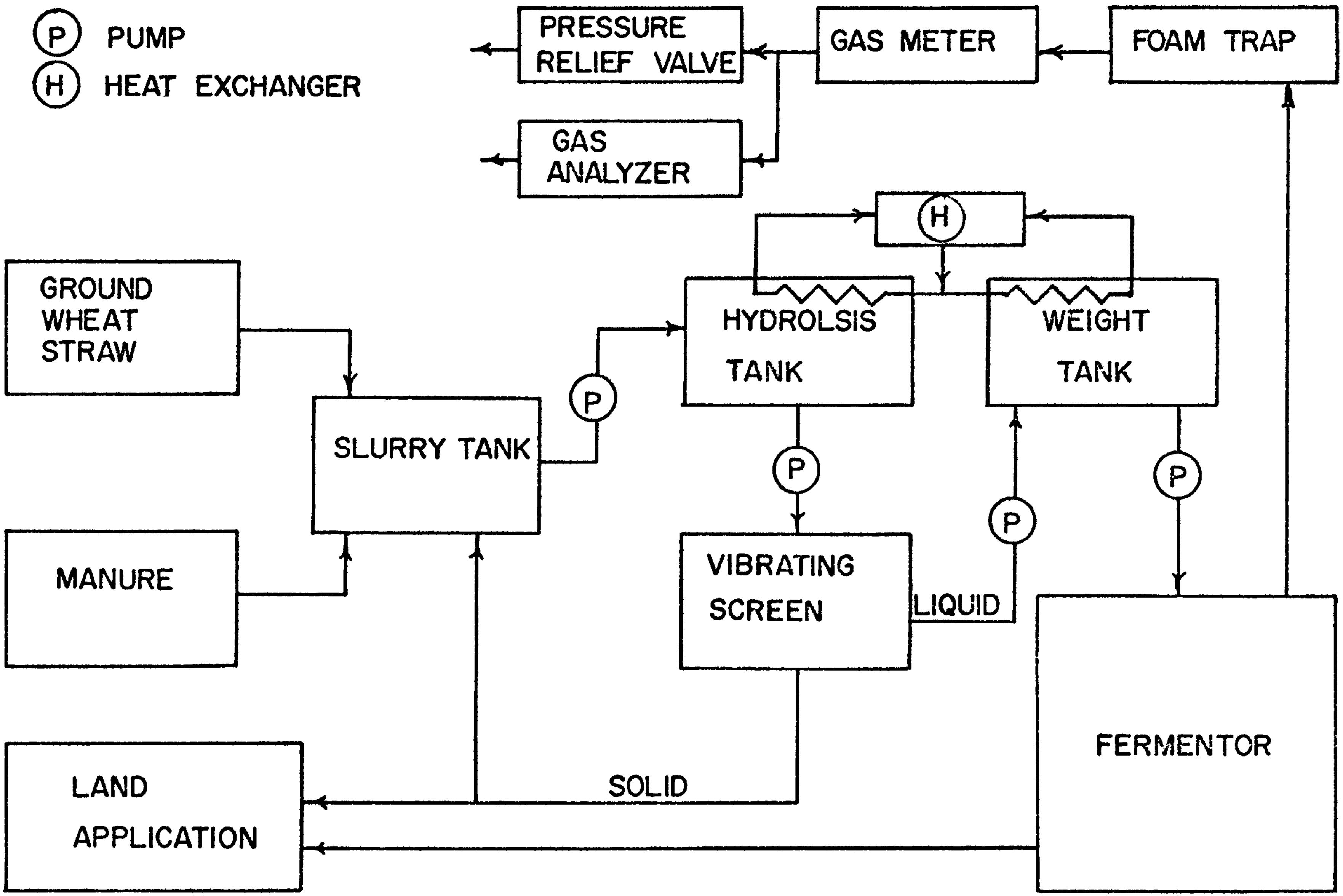


Figure 4.1. Schematic Diagram of Two-Stage Fermentation System

The screened liquid was pumped into another 1-$m^3$ tank (identical to the hydrolysis tank) mounted on a platform scale. This screened liquid then became the next days fermentor influent, and was heated to 65°C overnight then pumped into the fermentor the next day.

The fermentor contents were not mixed. The influent was pumped into the bottom of the fermenter at a flow rate of 250 $cm^3/s$. Withdrawl was accomplished through an overflow tube and the volume was kept at 4.9 $m^3$.

The gas produced during the fermentation passed through two condensate foam traps, a temperature compensated gas meter and a pressure relief valve. The condensate-foam traps consisted of cylindrical tanks, 0.53 m in diameter and 1.73 m high, with a siphon calibrated to discharge when the pressure exceeded 0.25 m of water columnm.

Samples of slurries, before and after screening, screened solids and fermentor effluent were routinely analyzed for various constituents. Total, volatile and fixed solids, ammonia (distillation method), chemical oxygen demand, alkalinity (to pH 3.7), pH and total volatile acids (silicic acid method) were determined by standard methods (APHA, 1975). Individual VFA's were determined by gas chromatography and Kjeldahl nitrogen was determined using technicon block digesters and Auto-Analyzer II as described by Wael and Gerke (1975). Gas volume was measured by an American AL-175 gas meter with temperature compensation to 15.6°C. Methane concentration was determined by a Beckman 864 Infra-Red gas analyzer.

A Macsym II (Analog Devices) process-controller microprocessor was used to control operating temperatures and calculate total gas and methane volumes. The microprocessor adjusted the gas volumes to 0°C, one atmophere and zero water vapor pressure.

## 4.3 SYSTEM OPERATION

### 4.3.1 Start-Up

Modification of the pilot-plant began in July, 1980. At that time the heat exchanger was dismantled and the fermentor temperature decreased from 55°C to 24°C in 21 days. The slurry was left in the fermentor until early September, while the rest of the plant was modified. Then the slurry was removed and stored in open tanks while the fermentor was modified. After one week, the slurry was pumped back to the fermentor. On September 19 the slurry was again removed from the fermentor and heated to 62° before being returned to the fermentor. The rest of the start-up procedure was as follows:

| | |
|---|---|
| Sept. 22-26 | Slurry was removed from fermentor and heated to 62°C, TVA = 3.3/kg $m^3$ |
| Sept. 29-Oct. 3 | Slurry was removed from fermentor and heated, by this time fermentor temperature had stabalized at 55°C |
| Oct. 6-9 | Fed fermentor at 15-day HRT and influent concentration 60 kg VS/$m^3$, TVA = 2 kg/$m^3$ |
| Oct. 10-13 | 12-day HRT, TVA = 1.25 kg/$m^3$, pH 7.9 |

| | |
|---|---|
| Oct. 14-20 | 10-day HRT, TVA = 1.5 kg/m$^3$, pH 7.9 temperature maintained at 52-55°C |
| Oct. 21 | 8-day HRT, TVA = 1.4 kg/m$^3$, pH 8.0 |
| Nov. 10 | TVA gradually increased to 3.5 kg/m$^3$ Hot water was fed instead of manure for two days at 8-day HRT. |

The 8-day HRT was continued and the TVA steadily decreased to about 0.3 kg/m$^3$. The influent was then changed from beef cattle manure to a mixture of beef cattle manure and ground wheat straw. Throughout the duration of the experiment the fermentor temperature fluctuated 3°C over 24 h. When the hot influent was introduced into the fermentor, the temperature increased to 47°C then cooled down to 44°C overnight. There was no apparent temperature stratification in the fermentor.

### 4.3.2 Steady-State Operation

The fermentor was operated at 44 to 47°C and 8-day HRT for 32 days before steady-state conditions were assumed. Table 4.1 shows the concentrations of various constituents in the system's flow streams during steady-state. Removing the coarse particles from the manure-straw mixture increased the COD/VS ratio from 0.89 to 1.36, for a 153% increase. This higher COD/VS ratio indicates a more biodegradable substrate for fermentation. Batch fermentations are in progress to determine whether the screened liquid is more biodegradable than the hydrolyzed manure-straw mixture.

The $CH_4$ production rate was 1.81 m$^3$ $CH_4$/m$^3$ fermentor·day and yields of 0.31 m$^3$ $CH_4$/kg VS fed (0.48 m$^3$ $CH_4$/kg VS used) and 0.22 m$^3$ $CH_4$/kg COD fed (0.36 m$^3$ $CH_4$/kg COD used). These rate and yields are comparable to those expected from beef cattle manure fermented at the same conditions (8-day HRT, 45.5°C).

Although fermentation of the screened liquid produced high $CH_4$ production rate and yield, there are several problems with this two-stage fermentation system. First, only about 35% of the VS in the raw manure and straw passed to the $CH_4$ fermentor. Thus the effective or overall $CH_4$ yield for the two-stage system was only 0.11 m$^3$ $CH_4$/kg VS fed. Secondly, the maximum VS concentration in the screened liquid (fermentor influent) was about 50 kg VS/m$^3$, while a more optimum concentration would be between 80 to 90 kg VS/m$^3$. Because of the limitations of the two-stage system noted above, the pilot-plant is being operated as a single-stage $CH_4$ fermentor receiving a 50:50 manure-straw mixture. This will determine whether $CH_4$ yields greater than 0.11 m$^3$ $CH_4$/kg VS fed can be achieved by a single-stage system.

## 4.4 SUMMARY

The pilot plant was modified into a two-stage fermentation system to accomodate manure-straw mixtures. Inputs to the system were 50% beef cattle manure and 50% wheat straw based on VS content. The manure-straw mixture was mixed into a slurry and fermented in a hydrolysis tank for 1 day at 50 to 60°C. The slurry was then separated using a vibrating screen. Approximately 37% of the screened solids was returned to the hydrolysis tank. The screened liquid, which accounted for 35% of the VS in the manure as straw, was pumped to the

TABLE 4.1. COMPOSITION OF PRODUCT STREAMS AT VARIOUS STAGES OF THE TWO-STAGE FERMENTATION SYSTEM

| Constituent[a] | Hydrolysis Tank | Screened Solids | Fermentor Influent | Fermentor Effluent |
|---|---|---|---|---|
| Total Solids | 108.3±9.5 | 175.1±10.5 | 60.8±8.9 | 25.8±5.3 |
| Volatile Solids | 88.3±6.0 | 152.0±10.5 | 47.3±7.2 | 16.8±3.6 |
| Fixed Solids | 20.0 | 23.1 | 13.5 | 9.0 |
| COD | 78.7±9.2 | 81.7±19.3 | 64.4±7.3 | 24.7±3.4 |
| Total Nitrogen | 2.63±0.32 | 2.71±0.35 | 2.27±0.31 | 2.21±0.18 |
| Ammonia - N | 0.63±0.08 | 0.58±0.10 | 0.62±0.10 | 1.07±0.03 |
| Volatile Acids | 6.09±2.43 | 6.45±1.32 | 9.26±1.11 | 0.28±0.16 |
| Alkalinity | -- | -- | 2.02±0.52 | 6.70±0.38 |
| pH | -- | -- | 4.19±0.13 | 7.62±0.52 |

[a]Expressed as $kg/m^3$ except for pH

anaerobic fermentor for conversion to $CH_4$. The fermentor, operated at 8-day HRT, 44 to 47°C, and influent concentration of 47.3 kg VS/$m^3$, produced 1.81 $m^3$ $CH_4$/$m^3$ fermentor·day and 0.31 $m^3$ $CH_4$/kg VS fed.

SECTION 5.0

# ULTIMATE METHANE YIELD FROM BEEF CATTLE MANURE: EFFECT OF TEMPERATURE, RATION CONSTITUENTS, ANTIBIOTICS AND MANURE AGE

A. G. Hashimoto, V. H. Varel and Y. R. Chen

## 5.1 INTRODUCTION

The rate of methane ($CH_4$) production has been reported to be a function of the ultimate $CH_4$ yield (i.e., substrate biodegradability), influent substrate (volatile solids, VS) concentration, retention time and the kinetic parameters K and $\mu_m$ (Chen and Hashimoto, 1978):

$$\gamma_V = (B_o S_o/\Theta)(1 - K/(\Theta \mu_m - 1 + K)) \quad (5.1)$$

where: $\gamma_V$ = volumetric $CH_4$ production rate, $m^3$ $CH_4/m^3$ fermentor·day

$B_o$ = ultimate $CH_4$ yield, $m^3$ $CH_4$/Kg $VS_f$ fed as $\Theta \rightarrow \infty$

$S_o$ = influent VS concentration, $kg/m^3$

$\Theta$ = hydraulic retention time, day

$\mu_m$ = maximum specific growth rate, $day^{-1}$

K = kinetic parameter, dimensionless.

We have previously reported that $\mu_m$ increases with temperature and that K is a kinetic parameter that increases as the fermentation becomes inhibited (Hashimoto, Chen and Varel, 1981).

This study focuses on some factors that affect $B_o$. Pfeffer (1974) published results indicating increased $CH_4$ yields and $CH_4$ production rates at 42° and 60°C. It was hypothesized that higher temperatures cause swelling of the lignin-cellulose complex and increases the availability of cellulose to microbial attack. This study investigates the hypothesis that higher temperature increases $B_o$.

We have assumed that the ration consumed by livestock affects the potential $CH_4$ yield from the manure of these livestock (Hashimoto et al., 1979). This study examines whether increasing silage content of beef cattle rations decreases $B_o$, whether antibiotics affect $B_o$, and whether manure from a dirt feedlot produces less $CH_4$ than freshly collected manure.

## 5.2 METHODS

### 5.2.1 Experiment Design

Two experiments were designed to examine the hypotheses presented above:

Experiment 1: Effect of temperature (30 to 65°C at 5°C increments) on $B_o$ of beef cattle manure.

Experiment 2: Effect of ration silage content (7, 40 and 91.5% corn silage), antibiotics (chlortetracycline and monensin), and manure age (6 to 8 week old manure from a dirt feedlot) on $B_0$ of beef cattle manure fermented at 55°C.

### 5.2.2 Fermentors

Figure 5.1 illustrates the experimental anaerobic fermentors used in both experiments. The fermentors were 4-$dm^3$ aspirator bottles with approximate working volumes of 3 $dm^3$. The fermentors were similar to those described by Varel et al. (1977) except that two 6 cm x 2 cm plexiglass baffles were glued to the side of the fermentor (one baffle near the liquid surface and one near the bottom) to aid in mixing, and commercially available, gas-collection bags (Tedlar bags, Pollution Measurement Corp., Chicago, IL) were used. The fermentors were placed on a rotary platform shaker, rotating at 140 revolutions per minute, and housed in a constant-temperature chamber. Fermentor temperatures in excess of 30°C were maintained by heating tapes wrapped around the aspirator bottles, and adjusted by variable transformers. The fermentors were maintained at the desired temperature with a variation of ± 1°C.

### 5.2.3 Substrate

Beef cattle manure (feces and urine) was the substrate used in these experiments. The manure for Experiment 1 was collected from steers (weighing over 400 kg) fed a high grain finishing ration consisting of 88% corn, 9% corn silage and 3% soybean meal-mineral supplement (80.5% soybean meal; 11.5% limestone; 3% dicalcium phosphate; 0.8% vitamin A, D and E; 0.2% beef trace minerals; and 3.75% salt). Manure less than three days old was scraped off concrete-floored pens and mixed with tap water to a total solids content of about 14%. The slurry was poured into 1-$dm^3$ polyethylene bottles and stored at -20°C until used. Before use, the bottles were placed in a refrigerator to thaw overnight, and the slurry was diluted with hot tap water to 10% total solids. The manure used in Experiment 1 came from the same batch of manure which was used by Varel et al. (1980) to investigate the effect of temperature on $CH_4$ production rate.

Table 5.1 shows the different rations fed to young steers (3 steers, weighing about 300 kg, per ration) from which manure was collected for Experiment 2. The steers were confined to indoor metabolism stalls on concrete floors, and the manure was collected daily until about 200 kg was accumulated. Rations A, B and C had increasing levels of corn, and rations D and E were identical to ration C except ration D contained chlortetracycline and ration E contained monensin. The manure in treatment F was from cattle (350 to 450 kg), fed a finishing ration containing monensin and confined to a dirt feedlot. The manure was collected from the feed bunk apron and was estimated to be about 6 to 8 weeks old.

### 5.2.4 Experimental Procedures

At the start of Experiments 1 and 2, each fermentor (two fermentors per treatment) was filled with 2 $dm^3$ of a mineral solution containing the following minerals (concentrations): $KH_2PO_4$ (900 $kg/m^3$); $NaCl_2$ (20 $kg/m^3$); $MgCl_2 \cdot 6H_2O$ (20 $kg/m^3$); $MnCl_2 \cdot 4H_2O$ (10 $kg/m^3$); and $CoCl_2 \cdot 6H_2O$ (10 $kg/m^3$). Each fermentor was adjusted to its designated temperature and monitored for two days to allow equilibration at that temperature. Inoculum (50

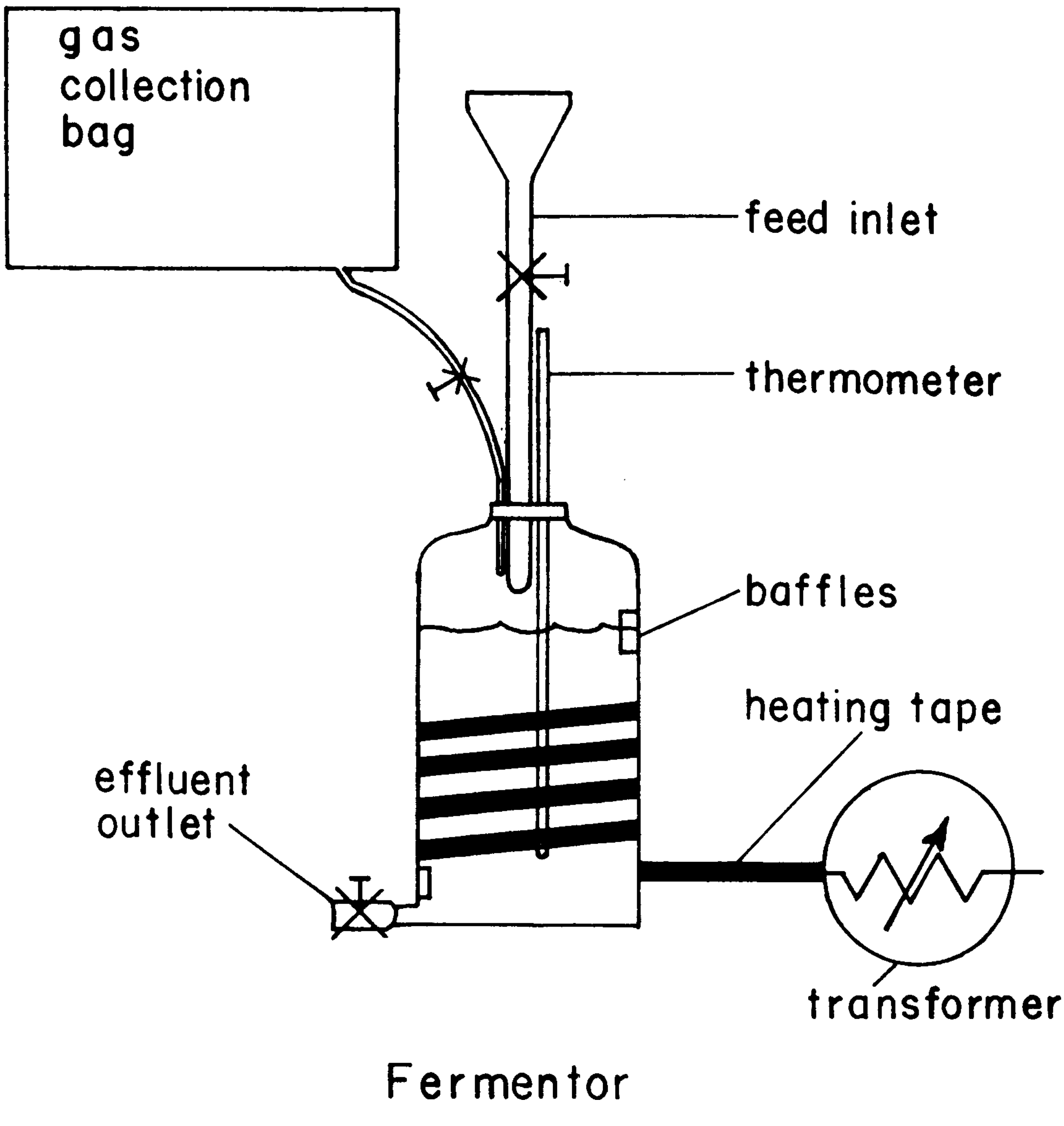


Figure 5.1. Anaerobic Fermentors Used in Experiment 1 and 2

TABLE 5.1. RATIONS USED FOR BIODEGRADABILITY AND INHIBITION EXPERIMENT (EXP. 2)

| | Ration, Dry Basis | | | | |
|---|---|---|---|---|---|
| Item | A | B | C | D | E |
| Corn Silage | 91.5 | 40.0 | 7.0 | 7.0 | 7.0 |
| Corn | 0 | 53.4 | 87.6 | 87.6 | 87.6 |
| Soybean Meal | 6.8 | 4.6 | 3.3 | 3.3 | 3.3 |
| Limestone | 0.7 | 1.2 | 1.6 | 1.6 | 1.6 |
| Dicalcium Phosphate | 0.5 | 0.3 | --- | --- | --- |
| Salt | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Trace Minerals[a] | +[e] | + | + | + | + |
| Vitamin ADE[b] | + | + | + | + | + |
| Chlortetracycline[c] | - | - | - | + | - |
| Monensin[d] | - | - | - | - | + |
| | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Calculated Composition | | | | | |
| TDN | 69.6 | 80.3 | 87.3 | 87.3 | 87.3 |
| Crude Protein, % | 11.0 | 11.0 | 11.1 | 11.1 | 11.1 |
| Calcium, % | 0.613 | 0.605 | 0.590 | 0.590 | 0.590 |
| Phosphorus, % | 0.327 | 0.357 | 0.345 | 0.345 | 0.345 |
| Dry Matter, % | 31.8 | 49.9 | 78.4 | 78.4 | 78.4 |

[a] 9.9 g Arizona-chelated trace minerals per Kg dry ration

[b] 29.3 g (ADE supplement of 8.8 x $10^6$ IU Vit. A/lb) per Kg of dry ration

[c] 10.8 chlortetracycline (110 g chlortetracycline per Kg carrier) per Kg of dry ration

[d] 22 g monensin (132 g monensin per Kg carrier) per Kg of dry ration

[e] + indicates addition of item, - indicates item not included in ration.

$cm^3$, 1.5 g VS) from fermentors operated at the same designated temperatures and fed beef manure daily, was then added to each fermentor. The slurry was then added over a 20-day period until a total of 600 g of slurry was added (60 g TS). The pH of each fermentor contents was measured periodically during this feeding period, and 4 N NaOH was added to maintain the pH above 6.5. The sample withdrawn for pH determination was returned to the fermentor. These batch fermentors were operated for 163 days in Experiment 1 and 184 days in Experiment 2. Gas volume and composition (% $CH_4$ and % $CO_2$) were measured periodically through the course of the experiments.

The volume of gas produced was measured using the apparatus shown in Figure 5.2. Before gas volume measurement, the top carboy (0.018 $m^3$) was filled with a solution containing 20% NaCl and 0.5% citric acid by pumping the solution from the bottom carboy. The electronic balance (Mettler Model PS 30) was then tared to zero and the gas collection bag attached to the apparatus. The stopcocks from the gas bag, manometer and bottom carboy were then opened to allow the solution to siphon from the top carboy and evacuate the gas bag. While the gas bag was being evacuated, 0.5-$cm^3$ gas samples were withdrawn with a syringe and analyzed for $CH_4$ and $CO_2$ contents. When the gas bag was completely evacuated (i.e., when solution displacement ceased), the weight of the solution displaced, the manometer reading and the gas temperature were recorded. The total gas volume was then calculated using the solution density (1028 $kg/m^3$) and corrected to standard pressure (760 mm Hg) and temperature (0°C) and zero water vapor. At the end of each experiment, the amount of $CH_4$ present in the fermentor head-space and the hose connecting the fermentor to the gas collection bag was measured and added to final $CH_4$ production.

### 5.2.5 Analytical Methods

The slurry fed to the fermentors and the fermentor contents at the end of the batch trials were analyzed for total solids (TS), volatile solids (VS), fixed solids (FS), ammonia (distillation method), chemical oxygen demand (COD), alkalinity (to pH 3.7), pH, and total volatile acids (TVA, silicic acid method) using standard methods for wastewater analyses (Am. Pub. Health Assoc., 1975). Total Kjeldahl nitrogen was determined by the method described by Wael and Gehrke (1975). Cellulose, hemicellulose, lignin, silica ash, phosphorus, potassium and sodium were determined by published procedures (AOAC, 1975).

Samples for TVA and individual volatile fatty acids were prepared by diluting 25 $cm^3$ of sample to 100 $cm^3$, adjusting the pH to 1.0 to 1.2 with concentrated $H_3PO_4$, and centrifuging at a relative centrifugal force of 12,062 for 30 minutes. Aliquots of the supernatant were used in the TVA analysis, or transferred into vials, sealed and frozen for future analysis.

The individual volatile fatty acids (VFA) including acetic, propionic, butyric, i-butyric, valeric, i-valeric, and caproic acids) were measured using a Hewlett-Packard Model 5840A gas chromatograph with dual flame ionization detectors. Coiled glass columns (0.32 cm ID by 183 cm) packed with 15% SP-1220/1% $H_3PO_4$ on 100/120 mesh Chromosorb WAW (Supelco, Inc., Bellefonte, PA) were used for the fatty acid separation. Nitrogen carrier-gas flow was 0.67 $cm^3/s$ and injector, oven and detector temperatures were 200, 125 and 250°C, respectively.

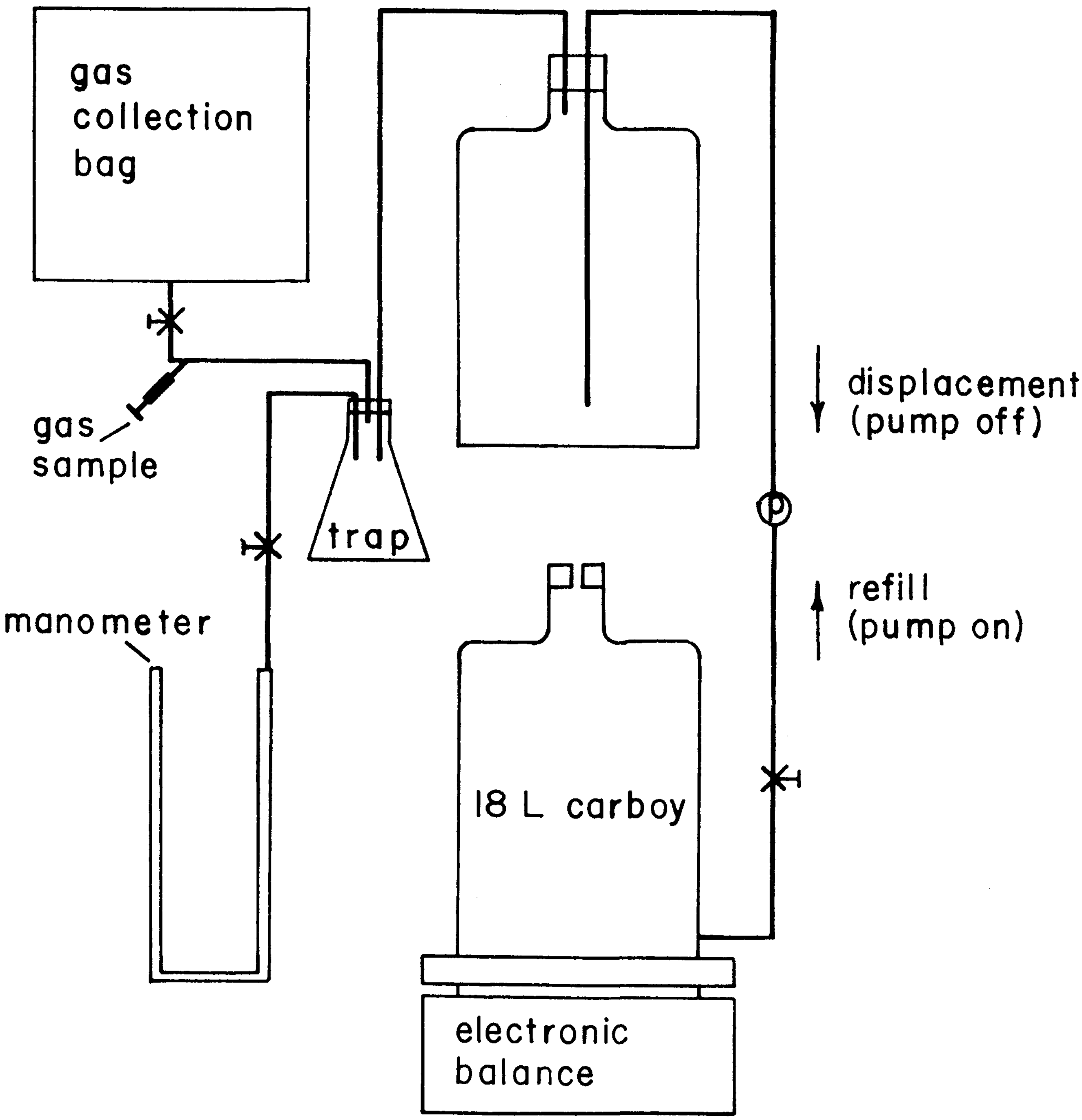


Figure 5.2. Apparatus for Measuring Gas Volume

$CH_4$ and $CO_2$ concentrations were measured using a Packard Model 428 gas chromatograph with dual thermal conductivity detectors. The stainless steel column (0.64 by 183 cm) was packed with 60/80 mesh Chromosorb 102. Injector, oven detector and filament temperatures were 100, 130, 70 and 350°C, respectively. The helium carrier gas flow was 0.67 $cm^3/s$.

## 5.3 RESULTS

### 5.3.1 Substrate

Table 5.2 shows the relative amounts of constituents in the manure fed to the batch fermentors in Experiments 1 and 2. The VS, COD, and nitrogen contents decreased and the cellulose, lignin and silica ash contents increased as the silage content in the ration increased. Although the 6 to 8 week old manure (treatment F) was from cattle fed a finishing ration similar to ration E, it contained much higher levels of lignin and silica ash than fresh manure. The manure used in Experiment 1 contained lower amounts of cellulose and hemicellulose, and higher amounts of nitrogen than manure from cattle fed similar finishing rations (rations C, D and E) in Experiment 2.

### 5.3.2 Effect of Temperature

Figure 5.3 shows the accumulated $CH_4$ production versus fermentation time of two representative fermentors operated at 35° and 45°C. Figure 5.3 shows that the $CH_4$ production rate for the 45°C fermentor is faster than the 35°C fermentor, but that there is no apparent difference in total $CH_4$ production at long fermentation time (i.e., there is little difference in $B_o$ between the 35° and 45°C fermentors).

Table 5.3 shows the values for $B_o$ ($m^3$ $CH_4$/kg VS fed $VS_f$ ) and $B_o'$ ($m^3$ $CH_4$/kg VS used $VS_u$ ). A least-significant-difference analysis showed no significant difference ($P < 0.05$) in $B_o$ for temperatures between 30° and 60°C (0.328 ± 0.022 $m^3$ $CH_4$/kg $VS_f$), but significantly lower $B_o$ at 65°C. $B_o'$ was fairly constant for all temperatures except fermentor 65-A. Table 5.4 shows the liquid volume, TS, VS and VFA concentrations at the end of the 163-day batch fermentation. The high VFA and VS of fermentor 65-B indicates acute stress while fermentor 65-A does not seem to be highly stressed.

### 5.3.3 Effect of Silage Content, Antibiotics and Manure Age

Table 5.5 shows that the values of $B_o$ and $B_o'$ obtained from fermenting manures from steers fed the rations shown in Table 5.1 and manure from the feeding apron of a dirt feedlot. The results show that $B_o$ decreases as the silage content of the ration increases (Figure 5.4). The $B_o$ of the manure from cattle fed ration A (91.5% silage) and ration B (40% silage) were 60% and 80%, respectively, of those fed ration C (7% silage).

Table 5.5 also shows the effect of chlortetracycline (D-1), monensin (E-1 and E-2) and dirt feedlot manure containing monensin (F-1 and F-2) on $B_o$. The results of fermentor D-2 was disregarded because of a leak in the gas collection bag. The $B_o$ from fermentor D-1 (0.292 $m^3$ $CH_4$/kg $VS_f$) was almost identical to the mean of fermentors C-1 and C-2 (0.290 $m^3$ $CH_4$/kg $VS_f$). As seen in Table 5.1, the only difference between rations C and D was the presence of chlortetracycline in ration D. Thus, the results show no adverse effect of chlortetracycline on $B_o$.

TABLE 5.2. COMPOSITION[a] OF MANURE FED TO BATCH FERMENTORS IN EXPERIMENTS 1 AND 2

| Constituent | Experiment 1 | Experiment 2 A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|
| Volatile Solids | 88.2 | 85.1 | 87.4 | 90.7 | 89.2 | 89.4 | 81.6 |
| Chemical Oxygen Demand | 114.0 | 80.8 | 86.3 | 102.2 | 95.9 | 91.0 | 81.6 |
| Cellulose | 9.4 | 24.7 | 16.8 | 13.4 | 14.2 | 13.7 | 19.0 |
| Hemicellulose | 13.4 | 21.1 | 20.2 | 20.9 | 20.7 | 23.2 | 17.8 |
| Lignin | 3.7 | 5.9 | 4.6 | 3.0 | 3.0 | 4.0 | 5.7 |
| Silica Ash | 2.2 | 4.8 | 3.2 | 0.7 | 1.2 | 0.7 | 9.3 |
| Total Volatile Acids | 6.0 | 3.6 | 3.1 | 3.6 | 3.3 | 3.7 | 5.3 |
| Kjeldahl N | 4.8 | 2.4 | 2.6 | 2.8 | 3.0 | 2.9 | 2.7 |
| Phosphorus | 1.1 | 0.9 | 1.0 | 0.8 | 0.8 | 0.8 | 0.7 |
| Potassium | 1.9 | 1.6 | 0.9 | 0.5 | 0.5 | 0.5 | 1.1 |
| Sodium | 0.6 | 0.6 | 0.4 | 0.3 | 0.2 | 0.4 | 0.2 |

[a]Values expressed as percent of total solids (manure slurry was 10% TS)

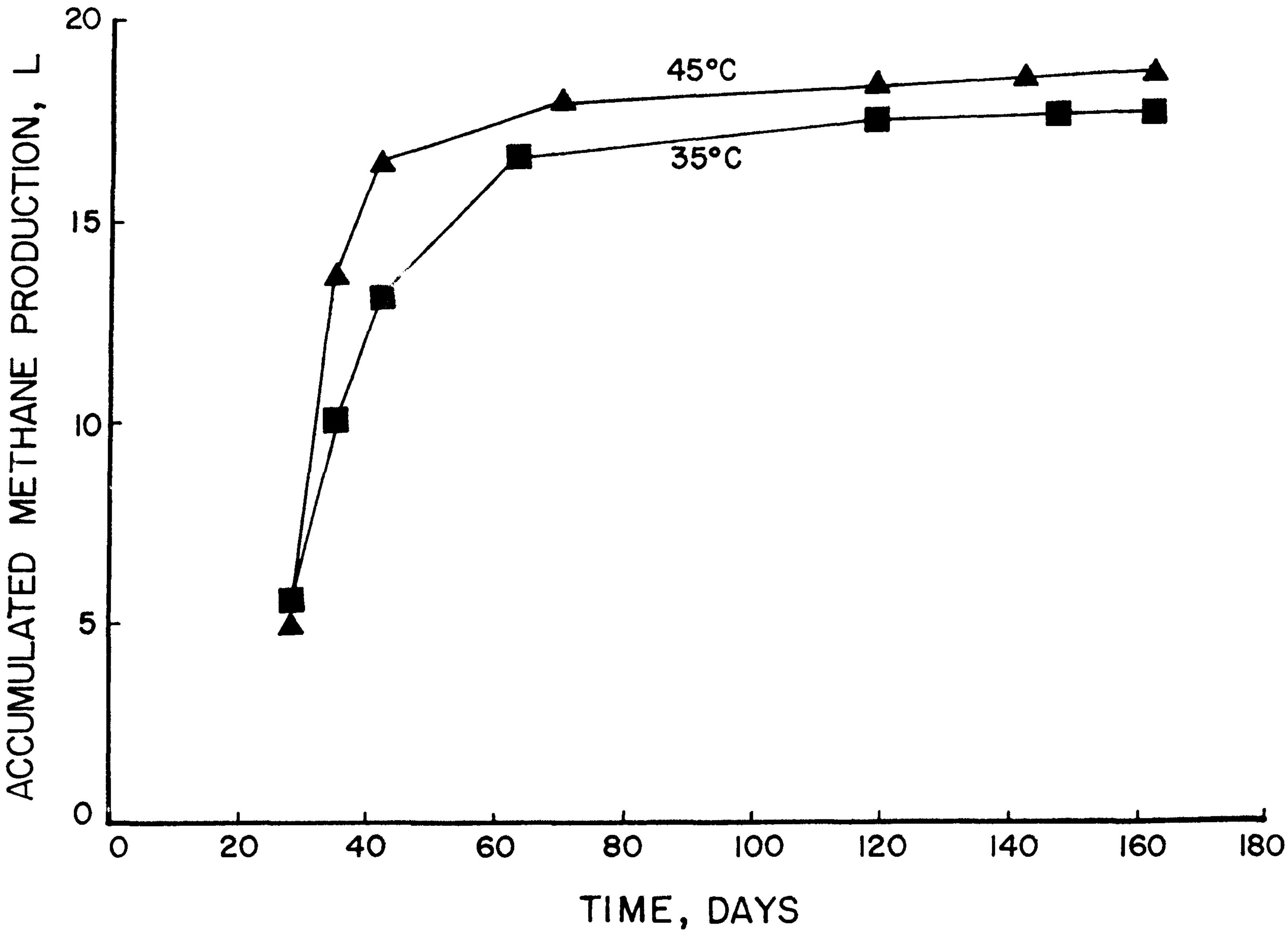


Figure 5.3. Accumulated Methane Production During Batch Fermentation

TABLE 5.3. EFFECT OF TEMPERATURE ON ULTIMATE METHANE YIELD OF BEEF CATTLE MANURE

| Temperature °C | $B_o$ $m^3$ $CH_4$/kg $VS_f$ | Mean $B_o$ | $B_o'$ $m^3$ $CH_4$/kg $VS_u$ |
|---|---|---|---|
| 30-A | 0.305 | 0.312[a] | 0.426 |
| 30-B | 0.318 | | 0.438 |
| 35-A | 0.336 | 0.338[a] | 0.449 |
| 35-B | 0.340 | | 0.463 |
| 40-A | 0.330 | 0.332[a] | 0.436 |
| 40-B | 0.334 | | 0.441 |
| 45-A | 0.354 | 0.353[a] | 0.499 |
| 45-B | 0.355 | | 0.489 |
| 50-A | 0.329 | 0.316[a] | 0.458 |
| 50-B | 0.304 | | 0.406 |
| 55-A | 0.316 | 0.332[a] | 0.439 |
| 55-B | 0.348 | | 0.454 |
| 60-A | 0.341 | 0.308[a] | 0.475 |
| 60-B | 0.276 | | 0.367 |
| 65-A | 0.098 | 0.118[b] | 0.131 |
| 65-B | 0.138 | | 0.437 |

[a]Means without a common superscript differ ($P < 0.05$)

TABLE 5.4. SLURRY CONSTITUENTS AT THE END OF BATCH FERMENTATION (EXPERIMENT 1)

| Temperature °C | Volume $dm^3$ | TS[a] $kg/m^3$ | VS[b] $kg/m^3$ | VFA[c], $kg/m^3$ | | | |
|---|---|---|---|---|---|---|---|
| | | | | $C_2$ | $C_3$ | $C_4$-$C_6$ | Total |
| 30-A | 2.83 | 9.1 | 5.5 | 26 | 0 | 0 | 26 |
| 30-B | 2.83 | 8.6 | 5.2 | 18 | 0 | 0 | 18 |
| 35-A | 2.86 | 8.5 | 4.8 | 25 | 0 | 0 | 25 |
| 35-B | 2.96 | 8.5 | 4.9 | 22 | 0 | 0 | 22 |
| 40-A | 2.86 | 8.4 | 4.6 | 17 | 0 | 0 | 17 |
| 40-B | 2.72 | 8.7 | 4.9 | 18 | 0 | 0 | 18 |
| 45-A | 2.86 | 9.0 | 5.5 | 21 | 0 | 0 | 21 |
| 45-B | 3.14 | 8.9 | 4.9 | 20 | 0 | 0 | 20 |
| 50-A | 2.81 | 9.0 | 5.5 | 16 | 0 | 0 | 16 |
| 50-B | 2.84 | 8.6 | 4.8 | 30 | 0 | 0 | 30 |
| 55-A | 2.83 | 8.9 | 5.4 | 23 | 0 | 0 | 23 |
| 55-B | 2.84 | 8.0 | 4.5 | 26 | 0 | 0 | 26 |
| 60-A | 3.04 | 9.0 | 5.1 | 41 | 0 | 0 | 41 |
| 60-B | 3.04 | 8.3 | 4.4 | 24 | 0 | 0 | 24 |
| 65-A | 3.20 | 9.5 | 4.3 | 206 | 20 | 43 | 269 |
| 65-B | 3.53 | 15.7 | 10.6 | 5266 | 355 | 308 | 6064 |

[a]Total Solids

[b]Volatile Solids

[c]Volatile Fatty Acids expressed as acetate ($C_2$ = acetic, $C_3$ = propionic, $C_4$-$C_6$ = sum of i-butyric, butyric, i-valeric, valeric, i-caproic and caproic)

TABLE 5.5. EFFECT OF RATION CONSTITUENTS ON ULTIMATE METHANE YIELD OF BEEF CATTLE MANURE FERMENTED AT 55°C

| Ration Code | $B_o$ $m^3$ $CH_4$/kg $VS_f$ | Mean $B_o$ | $B_o'$ $m^3$ $CH_4$/kg $VS_u$ |
|---|---|---|---|
| A-1 | 0.195 | 0.173[a] | 0.417 |
| A-2 | 0.151 | | 0.302 |
| B-1 | 0.220 | 0.232[b] | 0.366 |
| B-2 | 0.244 | | 0.426 |
| C-1 | 0.285 | 0.290[c] | 0.412 |
| C-2 | 0.296 | | 0.419 |
| D-1 | 0.294 | 0.294[c] | 0.416 |
| D-2 | --- | | --- |
| E-1 | 0.268 | 0.267[c] | 0.412 |
| E-2 | 0.266 | | 0.398 |
| F-1 | 0.210 | 0.210[a] | 0.413 |
| F-2 | 0.210 | | 0.398 |

[a,b,c]Means without a common superscript differ ($P < 0.05$)

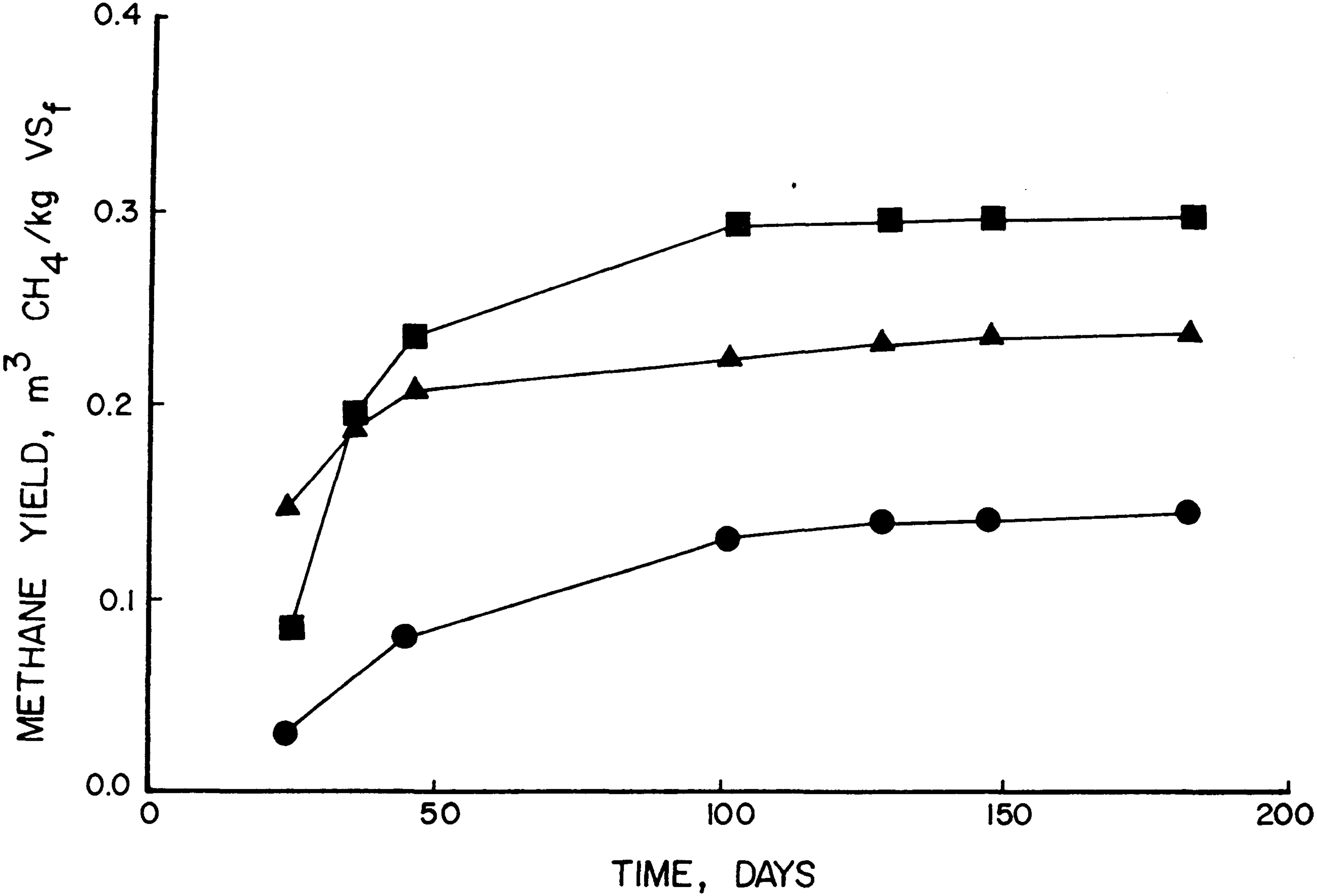


Figure 5.4. Effect of Silage Content on Methane Yield (●, 91.5% silage; ▲, 40% silage, ■, 7% silage)

The mean $B_o$ for fermentors E-1 and E-2 was 0.267 $m^3$ $CH_4$/kg $VS_f$, which is 92% of the mean $B_o$ of fermentors C-1 and C-2. The mean $B_o$ from fermentors F-1 and F-2 (0.210 $m^3$ $CH_4$/kg $VS_f$) was 72% of the mean $B_o$ from fermentor C-1 and C-2. A least-significant-difference analysis showed that there is no significant ($P < 0.05$) effect of chlortetracycline or monensin on $B_o$, but the $B_o$ for manure left on a dirt feedlot for 6 to 8 weeks is lower than the $B_o$ from freshly collected manure.

Although monensin has little effect on $B_o$, it delays the onset of $CH_4$ production. Figure 5.5 shows that nearly one-third of $B_o$ was achieved by fermentors C and D after 25 days of incubation, but only minimal amounts of $CH_4$ were produced in fermentors E and F after 40 days of fermentation. After 40 days of incubation, fermentor E began to produce $CH_4$ at a rate comparable to fermentor C, indicating little effect on kinetics after adaptation.

## 5.4 DISCUSSION

### 5.4.1 Substrate

The increase in cellulose, lignin and silica ash, and decrease in VS, COD, and nitrogen with increasing silage content (Table 5.2) are expected trends since silage contains more cellulose and lignin than corn. The high level of lignin and silica ash for the manure from the dirt feedlot (treatment F) reflect the effects of weathering, partial decomposition, and contamination of the manure with dirt and sand.

The differences in cellulose, hemicellulose and lignin between the manure used in Experiment 1 and manure from cattle fed similar finishing rations in Experiment 2 (rations C, D and E) may reflect the stage of growth of the cattle. As stated previously, the cattle in Experiment 1 were nearly ready for slaughter (weighing over 400 kg) while the cattle used in Experiment 2 were young cattle just started on a finishing diet. Since younger cattle have higher protein requirements and are more efficient in converting feed into meat, we would expect different manure composition from young and old cattle fed the same ration.

### 5.4.2 Effect of Temperature

This experiment showed no effect of temperature on increasing the $B_o$ of beef cattle manure for temperatures between 30 to 60°C. The reason for the low $B_o$ at 65°C was our inability to maintain stable fermentation at 65°C rather than a decrease in substrate availability. The high VFA and VS of fermentor 65-B are evidence to support the unstable fermentation. The discrepancy between the low $B_o$ and little indication of stress from fermentor 65-A could be explained by either a leak in the gas collection bag or by analyzing the wrong sample. It is unlikely that a leak occurred since $N_2$ and $O_2$ were not detected in the gas collection bag. Thus, it is likely that sample 65-A was mislabeled, but we could not confirm this since the samples were not kept for reanalysis. The average $B_o'$ was 0.445 ± 0.032 $m^3$ $CH_4$/kg $VS_u$ when the value for 65-A was not used. Omitting this value was justified because of the discrepancies noted above and because it was more than three standard deviations from the mean.

The results of this experiment also independently confirm our previous estimates of $B_o$ (using the same manure) based on steady-state data (Chen et al.,

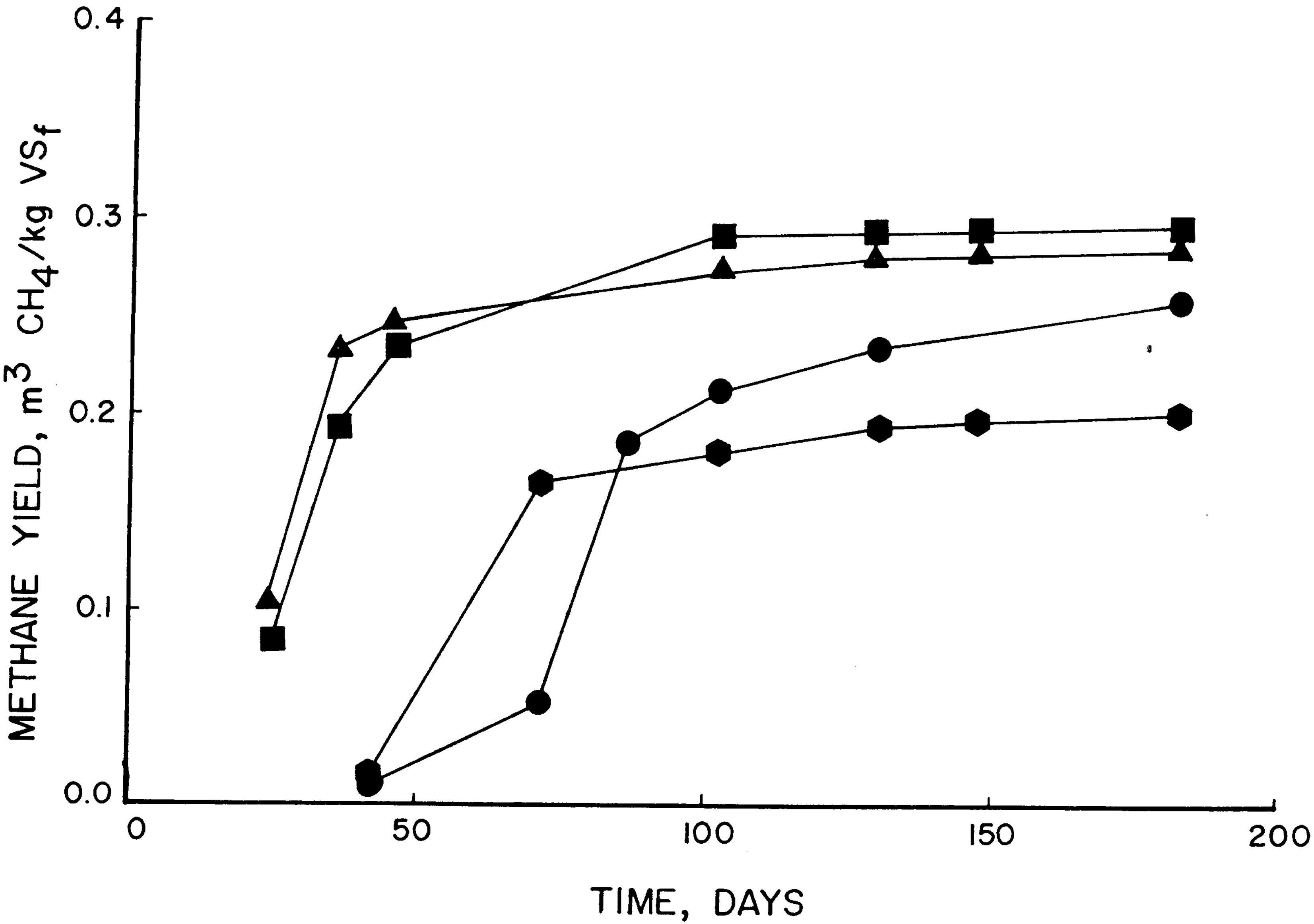


Figure 5.5. Effect of Antibiotics and Manure Age on Methane Yield ( ■, no antibiotics; ▲, chlortetracycline; ●, monensin; ⬢, monensin and dirt lot)

1980). Table 5.6 shows a comparison of the estimated $B_0$ using steady-state data and the $B_0$ obtained in this experiment. The mean $B_0$ was 0.32 ± 0.01 $m^3$ $CH_4$/kg $VS_f$ under steady-state conditions and the mean $B_0$ from this study was 0.328 ± 0.022 $m^3$ $CH_4$/kg $VS_f$ (neglecting the $B_0$ for 65°C as discussed earlier). The good agreement between the steady-state and batch results demonstrates that $B_0$ in Equation (5.1) can be obtained independently.

Thus, we can conclude from these results and other work we have previously published on beef cattle manure (Chen et al., 1980; Hashimoto, Chen and Varel, 1981) that temperature affects the rate at which $CH_4$ is produced but does not increase the amount of $CH_4$ that can be produced from a unit mass of substrate. We expect that this conslusion is also applicable to other livestock manures and other high cellulosic materials.

### 5.4.3 Effect of Silage Content and Manure Age

This study showed that the manure from cattle fed higher silage rations yield lower $B_0$ than manure from cattle fed high grain rations. Also, the age of the manure and the degree of contamination with inorganics (e.g., dirt) affects the yield. These results confirm our previous estimates of $B_0$ for various livestock species based primarily upon the grain content of their rations (Hashimoto et al., 1979).

### 5.4.4 Effect of Antibiotics

This study showed that antibiotics (chlortetracycline and monensin) do not affect $B_0$, but monensin does delay the onset of $CH_4$ production in batch fermentations. After adaptation to monensin, the fermentation proceeded at rates comparable to fermentors without monensin. Three possible mechanisms may explain the apparent adaptation of the microflora to monensin: a) mutant strains of bacteria develop resistance to the monensin; b) a shift in microbial populations caused by inhibition of some bacteria and an increase in others; and/or c) deactivation of the monensin during the 40-day lag period after which $CH_4$ production can proceed normally. Chen and Wolin (1979) have evidence to suggest that the first two mechanisms listed above explain the role of monensin in the rumen. The Rumensin Technical Manual (Anon., 1975) shows that one part per million of monensin in soil samples is deactivated in 14 days when incubated with animal feces, and 25 days when incubated without feces.

Results from feeding manure from cattle fed rations C, D and E to mesophilic (35°C) and thermophilic (55°C) fermentors operated at a hydraulic retention time of 9 days and influent concentration of 60 kg VS/$m^3$, showed almost immediate cessation of $CH_4$ production for those fed monensin and a 20% reduction in $CH_4$ production rate ($m^3$ $CH_4$/kg fermentor·day) for those fed chlortetracycline (Varel and Hashimoto, 1981). After 9 days of feeding the monensin, $CH_4$ production was not detectable, the pH dropped to 5.9 and the TVA exceeded 6.3 kg/$m^3$. $CH_4$ production did not resume even after 56 days of incubation. Microbial assays indicated no significant reduction in methanogenic or total viable counts due to monensin or chlortetracycline. These results suggest that monensin primarily affects the production of $CH_4$ precursors rather than the methanogenic bacteria.

This significant adverse effect of monensin on methanogenesis is of major concern because of the wide use of this product in beef production. Preliminary

TABLE 5.6. COMPARISON OF ULTIMATE METHANE YIELDS OBTAINED FROM STEADY-STATE DATA AND BATCH FERMENTATIONS

| Temperature °C | Steady-State $B_o$[a] $m^3$ $CH_4$/kg $VS_f$ | Batch $B_o$ $m^3$ $CH_4$/kg $VS_f$ |
|---|---|---|
| 30 | 0.32 | 0.312 |
| 35 | 0.31 | 0.338 |
| 40 | 0.33 | 0.332 |
| 45 | 0.33 | 0.353 |
| 50 | 0.32 | 0.316 |
| 55 | 0.31 | 0.332 |
| 60 | 0.32 | 0.308 |
| 65 | 0.33 | 0.118 |

[a]Chen et al. (1980)

results (Varel and Hashimoto, 1981) indicate that stable $CH_4$ production can be achieved at 25 days hydraulic retention time. The ability of anaerobic bacteria to adapt to monensin and produce $CH_4$ at hydraulic retention times less than 25 days must be studied.

### 5.4.5 Predicting $B_o$

It would be very conveient if $B_o$ could be predicted by analyzing the chemical composition of the substrate rather than performing the long-term batch fermentations described in this paper. Recently, Chandler and Jewell (1980) reported good correlation between the VS used during anaerobic fermentation ($\hat{B}_o = B_o/B_o' = VS_u/VS_f$) to the lignin content (%of lignin in VS) of the substrate. Figure 5.6 shows their data for swine, chicken, dairy cow and elephant manure and their line-of-best-fit. The average $\hat{B}_o$ in Experiment 1 was 0.736 ± 0.018 kg $VS_u/m^3$ $VS_f$ (excluding the data from fermentor 64-A), and is close to the reduction predicted by their regression equation. However, our results from Experiment 2, although showing good correlation between $\hat{B}_o$ and lignin content ($r^2 = 0.979$), did not agree with their equation. Thus, other factors besides lignin content affect $\hat{B}_o$ in anaerobic fermentation.

Even if a good predictive relationship between $\hat{B}_o$ and lignin content is obtained, values for $B_o'$ must also be obtained to estimate $B_o$. Chandler and Jewell (1980) reported values of $B_o'$ for dairy cow manure of 0.450, 0.465 and 0.495 $m^3$ $CH_4$/kg $VS_u$, and values of 0.560, 0.385 and 0.535 $m^3$ $CH_4$/kg $VS_u$ for swine, chicken and elephant manure, respectively. The mean $B_o'$ for Experiments 1 and 2 were 0.445 ± 0.032 (excluding the data from fermentor 65-A) and 0.398 ± 0.036 $m^3$ $CH_4$/kg $VS_u$, respectively. Thus, several factors affect the value of $B_o'$ and $\hat{B}_o$.

Figure 5.7 shows the effect of lignin content on $B_o$ for data from this study and from Chandler and Jewell (1980). There was good correlation ($r^2 = 0.935$) between lignin content and $B_o$ for the data in Experiment 2, but poor correlation for the rest of the data.

These results show that lignin content is not the only factor which affects $B_o$. Specie, stage-of-growth and other factors also affect $B_o$. More research on the factors affecting $B_o$, $B_o'$ and $\hat{B}_o$ is needed before accurate prediction of $B_o$ can be achieved. However, the relationships shown in Figures 5.6 and 5.7 may be useful in predicting changes in $B_o$ for a fairly well-defined source of manure. For example, seasonal changes in the lignin content of beef cattle manure used to feed the pilot-scale anaerobic fermentor at the Roman L. Hruska U.S. Meat Animal Research Center have been observed. Since the specie, stage-of-growth and ration are similar throughout the year, the seasonal changes in $B_o$ may be predicted by the changes in lignin content.

## 5.5 SUMMARY

The effects of temperature, ration constituents, antibiotics and manure age on the ultimate methane yield ($B_o$, $m^3$ $CH_4$/kg volatile solids fed ($VS_f$)) were investigated using 4-$dm^3$, batch fermentors. The average $B_o$ for fermentors maintained at 30 to 60°C (at 5°C intervals) was 0.328 $m^3$ $CH_4$/kg $VS_f$. The $B_o$ at 65°C averaged 0.118 $m^3$ $CH_4$/kg $VS_f$ but this low yield was attributed to unstable fermentation rather than decreased substrate availability at that temperature. These results agreed well with $B_o$ values estimated from daily-fed fermentors. Chlortetracycline and monensin did not affect $B_o$; however,

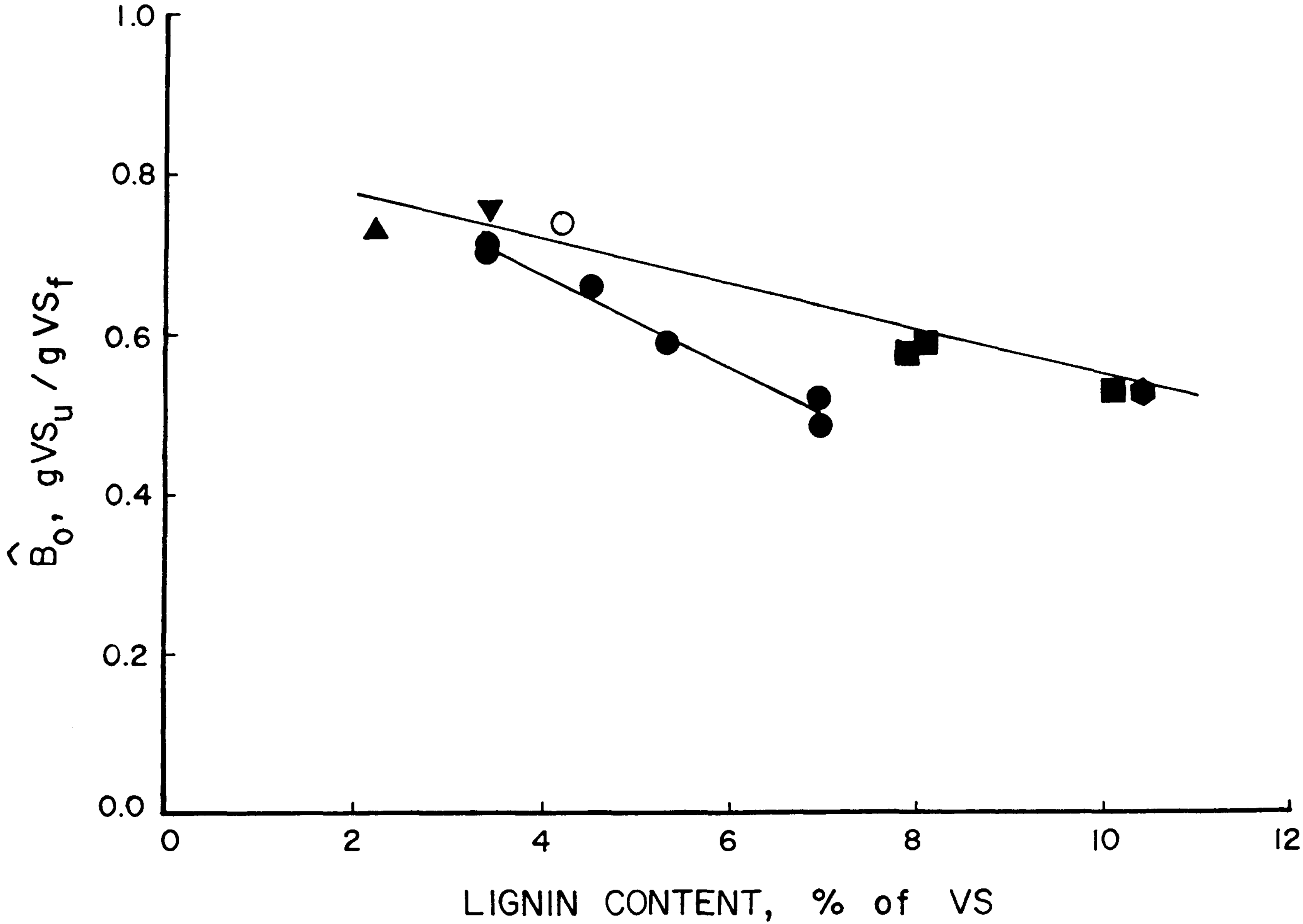


Figure 5.6. Effect of Lignin Content on $\hat{B}_o$ ( ○, beef (Exp. 1); ●, beef (Exp. 2); Data of Chandler and Jewell, 1980 (■, dairy; ▲, swine; ▼, chicken; ⬢, elephant))

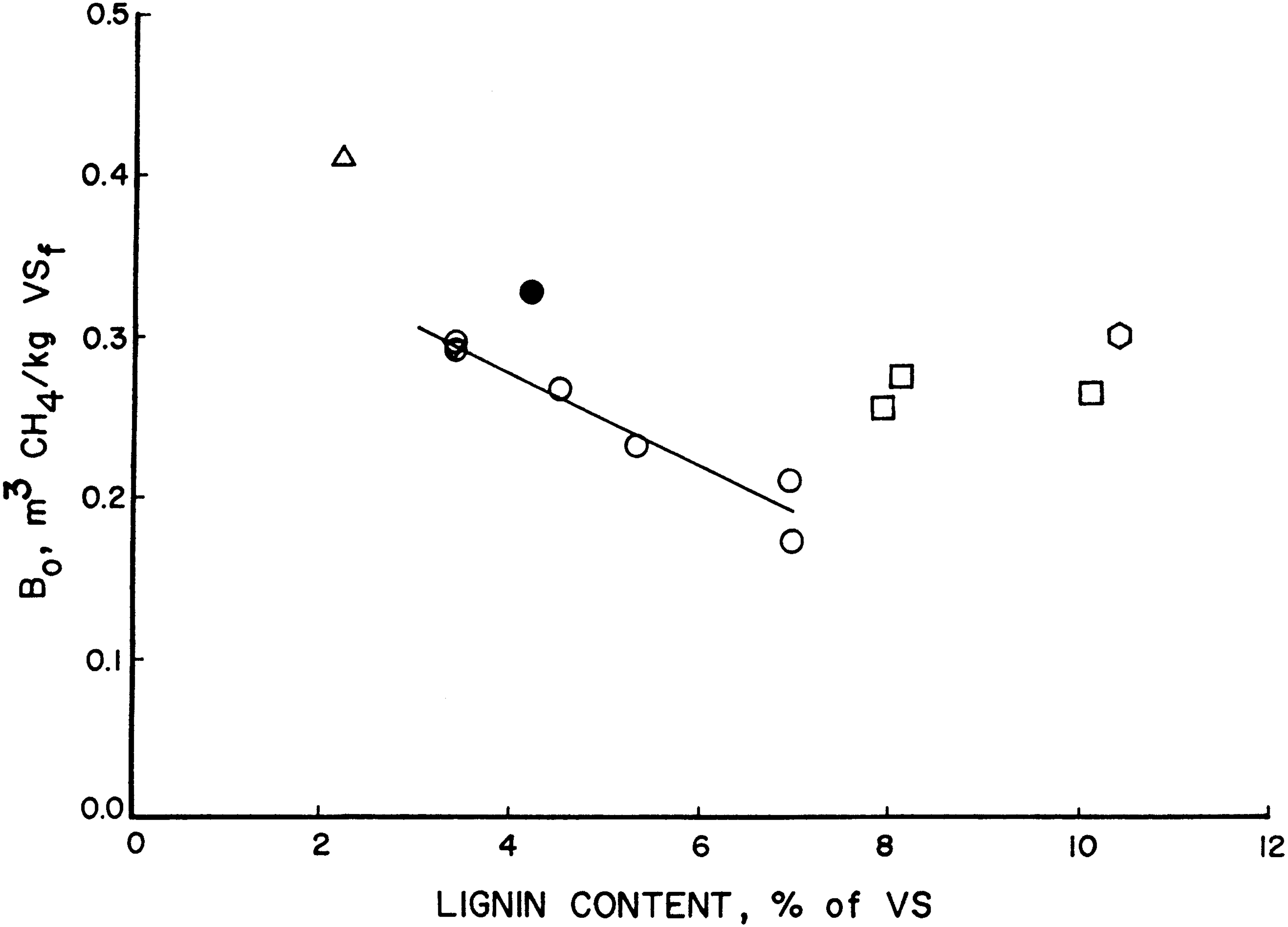


Figure 5.7. Effect of lignin content on $B_o$ (●, beef (Exp. 1); ○, beef (Exp. 2); Data of Chandler and Jewell, 1980 (□, dairy; △, swine; ▽, chicken; ⬡, elephant))

monensin did delay the start of active fermentation in batch fermentors. The average $B_o$ of manure from cattle fed 91.5, 40 and 7% corn silage were 0.173, 0.232 and 0.290 $m^3$ $CH_4$/kg $VS_f$, respectively. The average $B_o$ for 6 to 8 week old manure from a dirt feedlot was 0.210 $m^3$ $CH_4$/kg $VS_f$.

SECTION 6.0

# EFFECT OF MIXING DURATION AND VACUUM ON METHANE PRODUCTION RATE FROM BEEF CATTLE WASTE[a]

Andrew G. Hashimoto

## 6.1 INTRODUCTION

Finney and Evans (1975) hypothesized that the rate limiting step in the biological production of $CH_4$ is the phase transfer of products, and that the product gases ($CH_4$ and $CO_2$) inhibit methanogenic bacteria. They suggested that vigorous agitation, low pressure (vacuum), and elevated temperatures would increase the rate of phase transfer, and result in high $CH_4$ production rates.

However, Coppinger et al. (1979) reported no decrease in gas production from a full-scale dairy manure fermentor when mixing was discontinued. They reported that gas bubbling and thermal-convection currents provided sufficient mixing. Others (Miles, 1979; Smith et al., 1979) have recommended intermittent mixing for livestock waste fermentors operating under mesophilic conditions and loading rates of 6 kg volatile solids (VS)/$m^3$ fermentor·day or less. Under these conditions, $CH_4$ production rates of 1 $m^3$ $CH_4$/$m^3$ fermentor·day was expected. High-rate, thermophilic fermentations of beef cattle manure have produced over 4.5 $m^3$ $CH_4$/$m^3$ fermentor·day under continuously-mixed conditions (Varel et al., 1977; Hashimoto and Chen, 1979). This study was undertaken to determine whether continuous mixing was necessary to maintain high $CH_4$ production rates under thermophilic conditions, and whether vacuum fermentation would yield even higher $CH_4$ production rates.

## 6.2 METHODS

### 6.2.1 Experiment Design

Two sets of experiments were conducted to examine the hypotheses presented above:

Experiment 1: Effect of mixing duration on $CH_4$ production rate (temperature of 55°C, mixing speed of 220 revolutions per minute).

a. Four fermentors at 6-day HRT and mixed for either 1, 2, 3 or 24 h/day.

b. Four fermentors at 3-day HRT and mixed for either 1, 2, 3 or 24 h/day.

c. Duplicate fermentors at 6-day HRT and mixed for either 2 or 24 h/day.

d. Duplicate fermentors at 4-day HRT and mixed for either 2 or 24 h/day.

Experiment 2: Effect of vacuum (0.96 atm., -38 cm of water column) on $CH_4$ production rate (temperature of 55°C, continuous mixing).

a. Two vacuum fermentors and two conventional fermentors at 6-day HRT.

b. Two vacuum fermentors and two conventional fermentors at 4-day HRT.

### 6.2.2 Fermentors

Figure 6.1 illustrates the anaerobic fermentors used in Experiment 1. The fermentors were 4-$dm^3$ Pyrex reaction kettles (Corning 6947) modified with an outlet fused to the bottom of each kettle. The feed-tube outlet was placed below the 3-$dm^3$ working-volume level to minimize the introduction of air during feeding. Mixing was accomplished by two, 5.5-cm diameter, 3-bladed propellers spaced 14 cm apart on the shaft. The intermittently-mixed fermentors were mixed while samples were withdrawn and the fermentors were fed. The mixer was a 20 watt variable speed motor operating at 220 revolutions per minute. The fermentor temperature (55°C±1°C) was maintained by a heating mantle controlled by a variable transformer. The fermentors were housed in a walk-in, constant-temperature chamber maintained at 25°C.

Figure 6.2 illustrates the experimental anaerobic fermentors used in Experiment 2. The fermentors were 4-$dm^3$ aspirator bottles with approximate working volumes of 3-$dm^3$. The fermentors were similar to those described by Varel et al. (1977) except that two 6 cm x 2 cm plexiglas baffles were glued to the side of the fermentor (one baffle near the liquid surface and one near the bottom) to aid mixing. The fermentors were placed on a reciprocating platform shaker, reciprocating 140 times per minute, and housed in a constant-temperature chamber. Fermentor temperatures were maintained by heating tapes wrapped around the aspirator bottles, and adjusted by variable transformers. The fermentors were maintained at the desired temperature with a variation of 1°C.

The biogas produced by the fermentors in Experiment 1 and the conventional fermentors in Experiment 2 was collected in Tedlar bags (Pollution Control Corp., Chicago, IL). The vacuum fermentation gas collection system (Figure 6.3) consisted of two carboys with a 1.7 m difference in elevation. A transducer measured the vacuum on the fermentor head-space, and opened a solenoid valve when the transducer measured a pressure greater than 0.96 atm. The opened solenoid valve allowed the collection solution (20% NaCl and 0.5% citric acid) in the elevated carboy to flow to the lower carboy and maintain the designated vacuum. The biogas collected in the elevated carboy was displaced into gas collection bags by opening the 2-way stopcock and pumping the collection solution back to the elevated carboy.

### 6.2.3 Substrate

Beef cattle manure (feces and urine) was the substrate used in these experiments. The manure for Experiments 1a and 1b was collected from young steers (weighing about 300 kg) confined to indoor, metabolism stalls on concrete floors. The manure was collected daily and frozen until about 200 kg was accumulated. The ration for these steers consisted of 87.6% corn, 7% corn silage, 3.3% soybean meal, 1.6% limestone, 0.5% salt and trace mineral and vitamin A, D and E supplements.

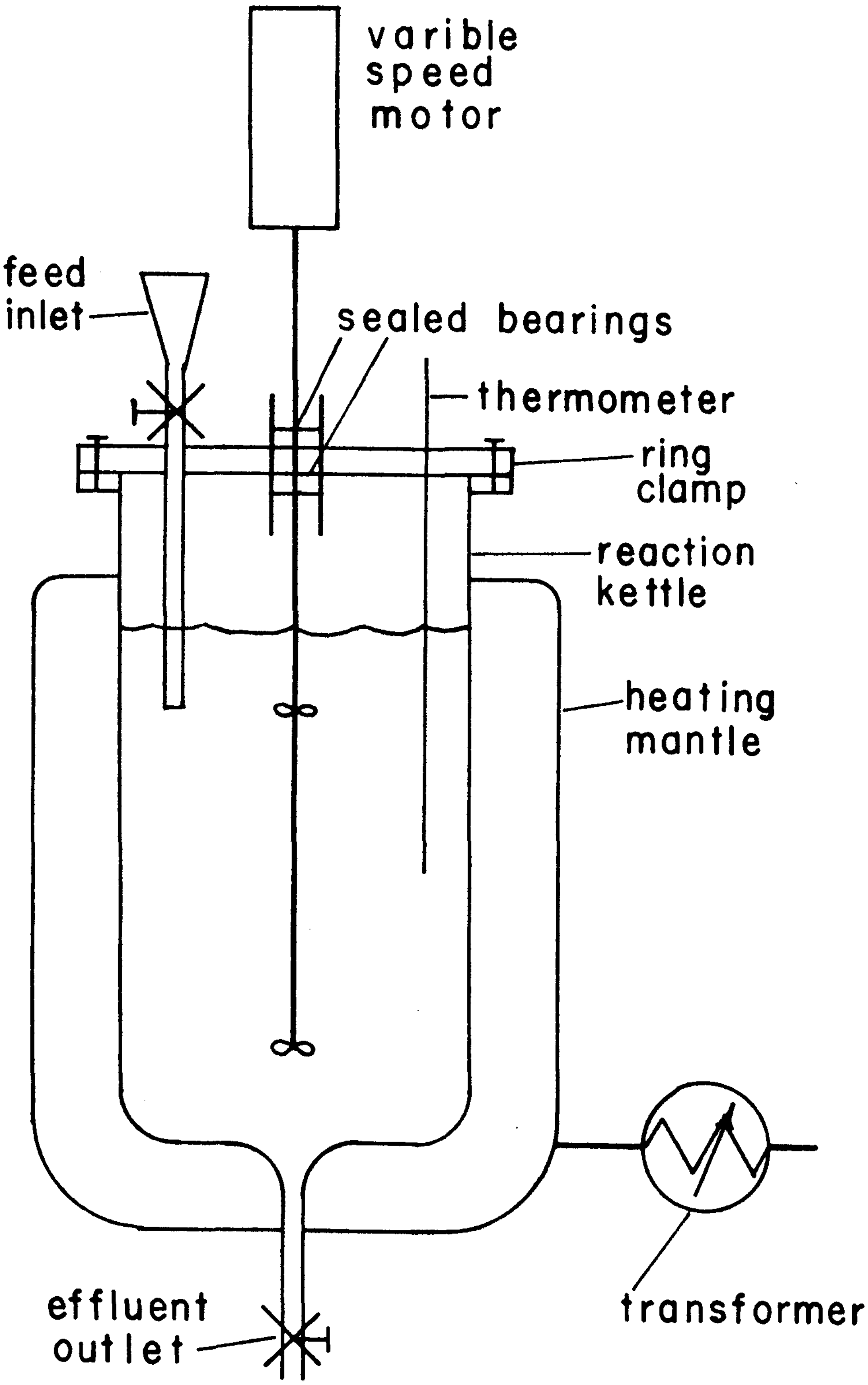


Figure 6.1. Anaerobic Fermentor Used in Mixing Experiments

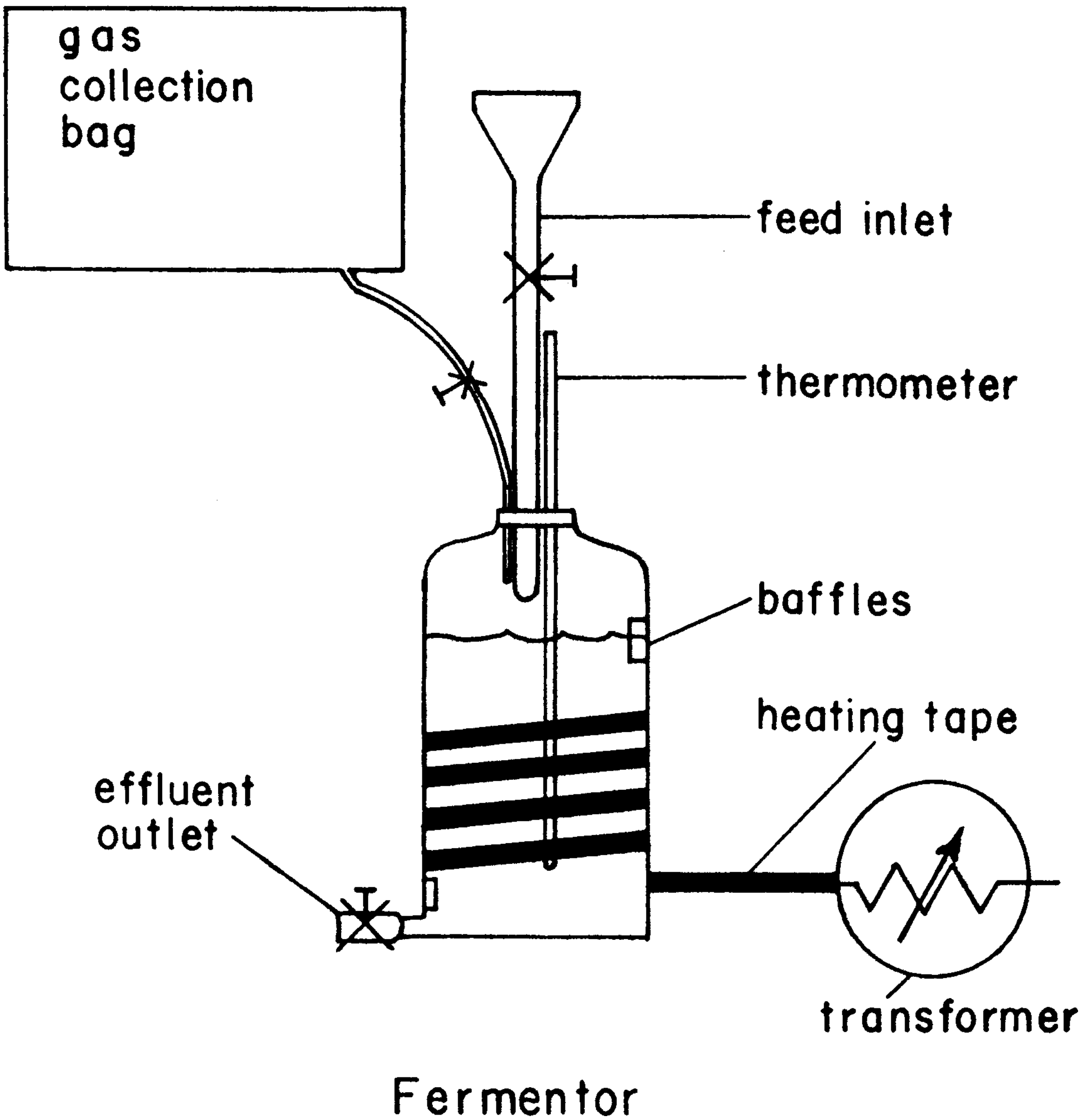


Figure 6.2. Anaerobic Fermentors Used in Experiment 2

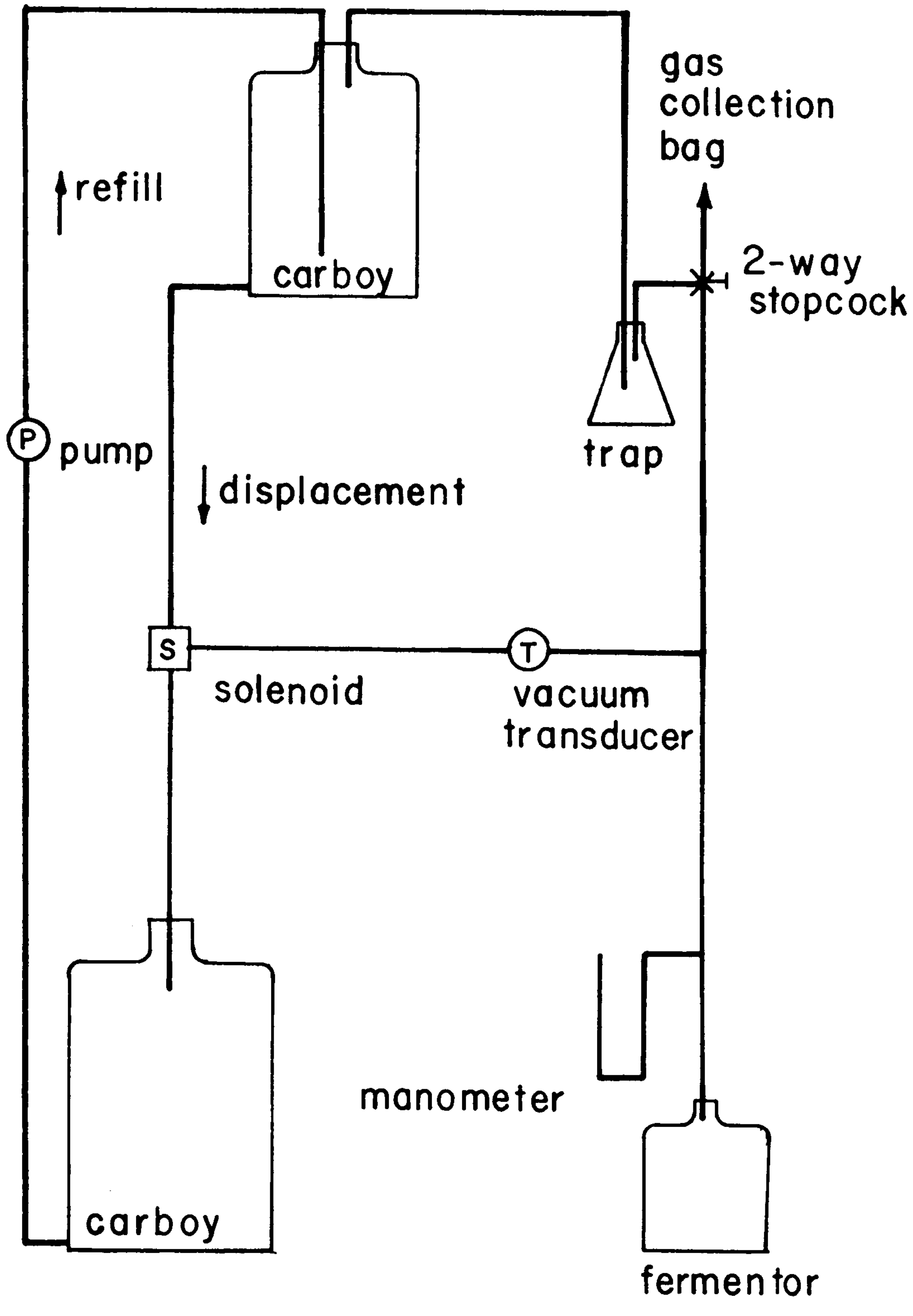


Figure 6.3. Schematic Diagram of Vacuum Fermentation Apparatus

The manure for Experiments 1c and 1d was collected from steers (weighing over 400 kg) fed a ration consisting of 85% corn, 13% corn silage and 2% soybean meal-mineral supplement (80.5% soybean meal, 11.5% limestone, 3% dicalcium phosphate, 0.8% vitamin A, D, and E, 0.2% beef trace minerals, and 3.75% salt). The manure was less than three days old and scraped off concrete-floored pens. The manure for Experiments 2a and 2b was from the same group of cattle as Experiment 1c and 1d, but was collected about one month later.

The manures for Experiments 1 and 2 were diluted with tap water to a total solids content of about 14%. The slurry was then poured into 1-$dm^3$ polyethylene bottles and stored at -20°C until used. Before use, the bottles were placed in a refrigerator to thaw overnight, and the slurry was diluted with hot tap water to 6 to 7% total solids.

### 6.2.4 Experimental Procedures

The fermentors in Experiment 1a were started by placing 3 $dm^3$ of slurry from our pilot-scale, thermophilic (55°C) fermentor into each fermentor. The temperature was adjusted to 55°C, and all four fermentors were mixed continously. After two days of acclimation, the mixers were connected to time clocks which allowed mixing periods of 1, 2, 3 or 24 h/day. The fermentors were operated at each retention time for 4 volume turnovers before steady-state gas production rates were measured and effluent quality were analyzed for 5 consecutive days. The HRT was then reduced to 3 days (Experiment 1b) and operated at this HRT for 4 volume turnovers before steady-state gas production rates were measured and effluent quality were analyzed.

The same start-up procedure used in Experiment 1a was used in starting Experiments 1c and 2a, except that duplicate fermentors were mixed at either 2 h/day or 24 h/day for Experiment 1c, and duplicate fermentors were either vacuum or conventional fermentors in Experiment 2a. After steady-state data at 6-day HRT were obtained, the HRT was reduced to 4 days (Experiments 1d and 2b).

After steady-state data at 4-day HRT were obtained for Experiment 2b, the 4 fermentors (2 vacuum and 2 conventional) were not fed and the $CH_4$ production was measured periodically until negligible gas was produced (102 to 126 days). The ultimate $CH_4$ yield was calculated by the following formula:

$$B_o = (\bar{V}_d \cdot \Theta + V_b)/S_o \cdot V_f \qquad (6.1)$$

where: $B_o$ = ultimate $CH_4$ yield, $m^3$ $CH_4$/kg VS fed ($VS_f$)

$\bar{V}_d$ = average steady-state $CH_4$ production rate, $m^3$ $CH_4$/day

$\Theta$ = hydraulic retention time, day

$V_b$ = total volume of $CH_4$ produced under batch conditions, $m^3$ $CH_4$

$S_o$ = influent VS concentration, kg VS/$m^3$

$V_f$ = liquid volume of fermentor, $m^3$

### 6.2.5 Analytical Methods

The slurry fed to the fermentors and the fermentor effluent during steady-state were analyzed for total solids (TS), volatile solids (VS), fixed solids (FS), ammonia (distillation method), chemical oxygen demand (COD), alkalinity (to pH 3.7), pH, and total volatile acids (TVA, silicic acid method) using the standard methods for wastewater analysis (APHA, 1975). Total Kjeldahl nitrogen was determined by the method described by Wael and Gehrke (1975).

Samples for TVA analysis were prepared by diluting 25 $cm^3$ of sample to 100 $cm^3$, adjusting the pH to 1.0 - 1.2 with 80% $H_3PO_4$, and centrifuging at a relative centrifugal force of 12,062 for 30 minutes. Aliquots of the supernatant were used in the TVA analysis.

The volume of gas produced was measured using the apparatus shown in Figure 5.2. Before gas-volume measurement, the top carboy (18 $dm^3$) was filled with a solution containing 20% NaCl and 0.5% citric acid by pumping the solution from the bottom carboy. The electronic balance (Mettler Model PS 30) was then tared to zero and the gas collection bag was attached to the apparatus. The stopcocks from the gas bag, manometer and bottom carboy were then opened to allow the solution to siphon from the top carboy and evacuate the gas bag. While the gas bags were being evacuated, 0.5 $cm^3$ gas samples were withdrawn with a syringe and analyzed for $CH_4$ and $CO_2$. When the gas bags were completely evacuated (i.e., solution displacement ceased), the weight of the solution displaced, the manometer reading and the gas temperature were recorded. The total gas volume was then calculated using the solution density (1028 $kg/m^3$) and corrected to standard pressure (1 atm.), and temperature (0°C), and zero water vapor.

$CH_4$ and $CO_2$ concentrations were measured using a Packard Model 428 gas chromatograph with dual thermal conductivity detectors. The stainless steel column (0.64 by 183 cm) was packed with 60/80 mesh Chromosorb 102. Injector, oven detector and filament temperatures were 100, 130, 70 and 350°C, respectively. The helium carrier gas flow was 0.67 $cm^3/s$.

## 6.3 RESULTS

### 6.3.1 Effect of Mixing

Table 6.1 summarizes the influent and effluent concentrations and $CH_4$ production of the fermentors operated at 6-day HRT, 55°C, loading rate of 9.6 kg $VS/m^3 \cdot day$ and mixed for 1, 2, 3 or 24 h/day. The concentration and percent reduction in TS, VS and COD were similar for the four fermentors. The influent and effluent FS concentrations were the same in three of the fermentors and only about 5% less in the other fermentor, indicating that the fermentor contents were being completely mixed. The low total volatile acids concentrations (less than 0.4 $kg/m^3$ as acetic acid), high alkalinity (over 5 $kg/m^3$ as $CaCO_3$) and stable pH (about 7.4) indicate that all of the fermentors were operating well and not stressed. The $CH_4$ production rates and yields show that the fermentors mixed for 1, 2 and 3 h/day produced slightly more $CH_4$ than the fermentor mixed 24 h/day.

The fermentors were then operated at the same temperature (55°C) and mixing periods (1, 2, 3 and 24 h/day) as the previous study, but at 3-day HRT and loading rate of 20.4 kg $VS/m^3$ fermentor·d. This high loading rate was imposed

TABLE 6.1. SUMMARY OF STEADY-STATE OPERATING PARAMETERS FOR THERMOPHILIC (55°C) FERMENTORS OPERATED AT SIX DAYS HYDRAULIC RETENTION TIME AND MIXED FOR VARIOUS PERIODS PER DAY[a]

| Parameter | Influent | 1h | 2h | 3h | 24h |
|---|---|---|---|---|---|
| Total Solids | | | | | |
| $kg/m^3$ | 63.9±1.5 | 34.7±0.9 | 33.5±0.09 | 34.6±1.3 | 34.9±0.8 |
| Change, % | --- | -45.7 | -47.5 | -45.9 | -45.4 |
| Volatile Solids | | | | | |
| $kg/m^3$ | 57.5±1.2 | 28.3±0.7 | 27.1±0.9 | 28.5±0.6 | 28.5±0.8 |
| Change, % | --- | -50.8 | -52.9 | -50.4 | -50.4 |
| Fixed Solids | | | | | |
| $kg/m^3$ | 6.4 | 6.4 | 6.4 | 6.1 | 6.4 |
| Change, % | --- | 0 | 0 | -4.7 | 0 |
| Volatile Acids | | | | | |
| $kg/m^3$ | 2.28±0.47 | 0.29±0.03 | 0.37±0.05 | 0.30±0.05 | 0.33±0.08 |
| pH | | | | | |
| Unit | --- | 7.38±0.07 | 7.37±0.08 | 7.46±0.06 | 7.45±0.07 |
| Methane | | | | | |
| % | --- | 49.7±3.3 | 49.5±2.9 | 49.2±3.4 | 49.4±3.0 |
| Methane Production | | | | | |
| $m^3/m^3 \cdot day$ | --- | 2.38±0.19 | 2.51±0.20 | 2.37±0.18 | 2.15±0.11 |
| $m^3/kg$ VS fed | --- | 0.25 | 0.26 | 0.25 | 0.22 |
| $m^3/kg$ VS used | --- | 0.49 | 0.50 | 0.49 | 0.45 |

[a]Mixed at 220 revolutions per minute

to determine whether continuous mixing is necessary when fermentors are heavily loaded or stressed. Two of the four fermentors failed in this experiment; therefore, meaningful conclusions could not be drawn.

Based on the previous results, a more detailed experiment was initiated to determine whether intermittent mixing was significantly different from continuous mixing. Two thermophilic (55°C) fermentors were mixed 2 h/day and two fermentors were mixed continuously. These fermentors were first operated at 6-day HRT then at 4-day HRT. Table 6.2 summarizes the mean steady-state results of this experiment. Analyses-of-variance showed no significant difference in effluent charracteristics between the 2 and 24 h/day fermentors operated at 6-day HRT; however, significant ($P<0.05$) differences were noted in fixed solids, total nitrogen, ammonia and pH between the fermentors at 4-day HRT. Table 6.2 also shows that the continuously-mixed fermentors produced $CH_4$ at significantly ($P<0.05$) higher rates than the fermentors mixed 2 h/day. The $CH_4$ production rates ($m^3$ $CH_4/m^3$ fermentor·day) of the continously-mixed fermentors were 8% (6-day HRT) and 11% (4-day HRT) higher than the fermentor mixed 2 h/day.

### 6.3.2 Effect of Vacuum

Table 6.3 summarizes the mean steady-state conditions of the vacuum (0.96 atm.) and conventional fermentors operated at 55°C and 6- and 4-day HRT. At 6-day HRT, there were significant ($P<0.05$) differences between the vacuum and conventional fermentors in TS, VS, COD, total nitrogen and methane concentration, but no significant difference in $CH_4$ production rate. At 4-day HRT, there were significant ($P<0.05$) differences between the vacuum and conventional fermentors in FS, COD, ammonia, alkalinity, pH, $CH_4$ production rate and $CH_4$ yield ($m^3$ $CH_4/kg$ $VS_u$). The $CH_4$ production rate for the vacuum fermentors was 5% higher than the conventional fermentors at 4-day HRT.

After steady-state at 4-day HRT was completed, the fermentors were not fed and the $CH_4$ production was measured. Using Equation 6.1 to estimate $B_0$, the mean $B_0$ was calculated to be $0.36 \pm 0.02$ $m^3$ $CH_4/kg$ $VS_f$.

## 6.4 DISCUSSION

### 6.4.1 Effect of Mixing

The preliminary mixing experiments (1a and 1b) indicated that intermittent mixing may produce more rapid $CH_4$ production rates than continuously mixed fermentors. However, the more controlled experiments (1c and 1d) showed that statistically higher $CH_4$ production rates were achieved when fermentors were mixed continuously. The increased $CH_4$ production rates for the continuously mixed fermentors were, however, only 8 to 11% higher than the intermittently mixed fermentors.

To investigate further the effect of intermittent mixing on anaerobic fermentation, our pilot-scale (5.7 $m^3$), thermophilic (50°C) fermentor (Hashimoto, Chen and Prior, 1979) was operated at 5-day HRT and mixed continuously for 5 volume turnovers (25 days, steady-state at days 20 to 25), then mixed 2 h/day for 25 days. The steady-state operating parameters of the fermentor (Table 6.4) indicates similar performance when mixed continuously and 2 h/day. Based upon the results presented here, it is difficult to justify the increased energy needed to continuously mix the fermentor when the

TABLE 6.2. SUMMARY OF STEADY-STATE OPERATING PARAMETERS FOR THERMOPHILIC (55°C) FERMENTORS OPERATED AT 4 AND 6 DAYS HYDRAULIC RETENTION TIMES AND MIXED[a] FOR 2 OR 24 HOURS PER DAY

| | | Hydraulic Retention Time[c] | | | |
|---|---|---|---|---|---|
| | | 6 day | | 4 day | |
| Parameter | Influent[b] | 2 h/day | 24 h/day | 2 h/day | 24 h/day |
| Total Solids, $kg/m^3$ | 65.0±2.3 | 34.2±0.6 | 33.8±0.4 | 34.8±0.8 | 35.6±0.8 |
| Volatile Solids, $kg/m^3$ | 56.1±2.2 | 25.8±0.7 | 25.9±1.0 | 26.8±1.2 | 27.0±0.6 |
| Fixed Solids, $kg/m^3$ | 8.9 | 8.4±0.1 | 7.9±0.6 | 8.0±0.3[f] | 8.6±0.2[g] |
| Chemical Oxygen Demand, $kg/m^3$ | 60.8±5.9 | 35.0±0.8 | 34.2±1.4 | 30.8±0.1 | 30.5±0.5 |
| Nitrogen | | | | | |
| Total, $kg/m^3$ | 2.75±0.12 | 2.80±0.02 | 2.80±0.02 | 2.68±0.04[f] | 2.77±0.01[g] |
| Ammonia, $kg/m^3$ | 0.99±0.13 | 1.54±0.03 | 1.56±0.01 | 1.38±0.01[f] | 1.28±0.03[g] |
| Total Volatile Acids, $kg/m^3$ | 6.48±1.00 | 0.77±0.09 | 0.70±0.08 | 1.60±0.34 | 1.14±0.04 |
| Alkalinity, $kg/m^3$ | 3.56±0.88 | 7.94±0.16 | 7.95±0.08 | 7.40±0.16 | 7.18±0.14 |
| pH | | | | | |
| Unit | 5.23±0.52 | 7.54±0.01 | 7.52±0.01 | 7.44±0.01[f] | 7.26±0.02[g] |
| Methane, % | --- | 56.1±0.5 | 56.2±0.2 | 55.6±0.2[f] | 56.4±0.4[g] |
| Methane Production | | | | | |
| $m^3/m^3 \cdot day$ | --- | 2.62±0[d] | 2.84±0.06[e] | 3.57±0.23[f] | 3.96±0.6[g] |
| $m^3$/kg VS fed | --- | 0.28±0[d] | 0.30±0.01[e] | 0.26±0.02[f] | 0.28±0.01[g] |
| $m^3$/kg VS used | --- | 0.52±0.01 | 0.56±0.04 | 0.49±0.01[f] | 0.54±0.02[g] |

[a] Mixed at 220 revolutions per minute

[b] Data presented as mean ± one standard deviation of 15 determinations

[c] Data presented as mean ± one mean standard deviation of 2 fermentors per treatment (5 obs./ferm.)

[d,e,f,g] Means bearing different superscripts in the same row are significantly different ($P < 0.05$)

TABLE 6.3. SUMMARY OF STEADY-STATE OPERATING PARAMETERS FOR VACUUM[a] AND CONVENTIONAL[b] THERMOPHILIC (55°C) FERMENTORS OPERATED AT 4 AND 6 DAYS HYDRAULIC RETENTION TIMES

| | | Hydraulic Retention Time[d] | | | |
|---|---|---|---|---|---|
| | | 6 day | | 4 day | |
| Parameter | Influent[c] | Vacuum | Conventional | Vacuum | Conventional |
| Total Solids, $kg/m^3$ | 76.6±4.6 | 31.8±1.2[e] | 34.0±0.1[f] | 37.4±0.8 | 36.4±0.8 |
| Volatile Solids, $kg/m^3$ | 68.4±4.0 | 25.2±1.0[e] | 27.2±0.2[f] | 29.8±0.6 | 29.5±1.0 |
| Fixed Solids, $kg/m^3$ | 8.2 | 6.6±0.1 | 6.8±0.2 | 7.6±0.2[g] | 6.9±0.2[h] |
| Chemical Oxygen Demand, $kg/m^3$ | 71.7±9.8 | 38.2±0.6[e] | 39.9±0[f] | 43.2±1.0[g] | 46.8±0.4[h] |
| Nitrogen | | | | | |
| Total, $kg/m^3$ | 3.01±0.20 | 2.84±0.01[e] | 2.95±0.02[f] | 2.84±0.01 | 2.82±0.04 |
| Ammonia, $kg/m^3$ | 0.73±0.09 | 1.48±0.04 | 1.42±0.04 | 1.38±0.03[g] | 1.30±0.02[h] |
| Total Volatile Acids, $kg/m^3$ | 7.61±0.56 | 1.48±0.20 | 1.25±0.01 | 2.04±0.02 | 2.02±0.01 |
| Alkalinity, $kg/m^3$ | 2.23±0.44 | 7.10±0.12 | 6.88±0.52 | 6.86±0.06[g] | 6.24±0.36[h] |
| pH | | | | | |
| Unit | 4.58±0.51 | 7.62±0.01 | 7.63±0.04 | 7.44±0.01[g] | 7.22±0.02[h] |
| Methane, % | --- | 52.2±0.02[e] | 54.6±0.6[f] | 53.8±1.0 | 53.9±0.6 |
| Methane Production | | | | | |
| $m^3/m^3 \cdot day$ | --- | 3.45±0.07 | 3.54±0.01 | 4.28±0.10[g] | 4.08±0.05[h] |
| $m^3$/kg VS fed | --- | 0.30±0.01 | 0.31±0.0 | 0.25±0.01 | 0.24±0 |
| $m^3$/kg VS used | --- | 0.48±0.02 | 0.52±0.01 | 0.44±0.01[g] | 0.42±0.01[h] |

[a]Vacuum of 0.96 atm.

[b]Atmospheric pressure

[c]Data presented as mean ± one standard deviation of 16 determinations

[d]Data presented as mean ± one mean standard deviation of 2 fermentors per treatment (5 obs./ferm.)

[e,f,g,h]Means bearing different superscripts in the same row are significantly different ($P < 0.05$)

TABLE 6.4. SUMMARY OF STEADY-STATE OPERATING PARAMETERS FOR THERMOPHILIC (55°C), PILOT SCALE FERMENTOR OPERATED AT SIX-DAY HYDRAULIC RETENTION TIME AND MIXED CONTINOUSLY AND TWO HOURS PER DAY[a]

| Parameter | Mixing Duration, h/day | |
|---|---|---|
| | 24 | 2 |
| Total Solids | | |
| Inf., $kg/m^3$ | 67.7±3.3 | 69.6±4.1 |
| Eff., $kg/m^3$ | 34.4±0.4 | 33.1±0.8 |
| Volatile Solids | | |
| Inf., $kg/m^3$ | 59.8±3.0 | 61.4±3.6 |
| Eff., $kg/m^3$ | 26.5±0.3 | 25.1±0.8 |
| Change, % | 55.7 | 59.1 |
| Fixed Solids | | |
| Inf., $kg/m^3$ | 7.9 | 8.2 |
| Eff., | 7.9 | 8.0 |
| COD | | |
| Inf., $kg/m^3$ | 68.9±3.5 | 70.2±6.9 |
| Eff., $kg/m^3$ | 34.0±4.3 | 34.8±5.1 |
| Total Nitrogen | | |
| Inf., $kg/m^3$ | 2.42±0.17 | 2.61±0.24 |
| Eff., $kg/m^3$ | 2.65±0.06 | 2.54±0.03 |
| Ammonia-N | | |
| Inf., $kg/m^3$ | 0.73±0.02 | 0.78±0.04 |
| Eff., $kg/m^3$ | 1.24±0.06 | 1.29±0.02 |
| Volatile Acids | | |
| Inf., $kg/m^3$ | 5.07±0.70 | 6.72±0.82 |
| Eff., $kg/m^3$ | 0.62±0.10 | 0.92±0.35 |
| Alkalinty | | |
| Inf., $kg/m^3$ | 3.33±0.15 | 3.10±0.26 |
| Eff., $kg/m^3$ | 6.57±0.22 | 6.79±0.27 |
| pH | | |
| Inf. | 5.45±0.37 | 4.80±0.04 |
| Eff. | 7.50±0.04 | 7.51±0.05 |
| Methane, % | 5.25±0.8 | 53.9±4.7 |
| Methane Production | | |
| $m^3/m^3 \cdot day$ | 2.59±0.06 | 2.60±0.19 |
| $m^3/kg$ VS fed | 0.26 | 0.25 |
| $m^3/kg$ VS used | 0.47 | 0.43 |

[a]Mixed at 160 revolutions per minute.

$CH_4$ production rate is increased, at the most, only about 10%. Thus, the recommendations for intermittent mixing of farm-scale fermentors is justified.

This study, however, only evaluated the effect of mixing on $CH_4$ production rate, and did not address the materials handling function that mixing also provides. If insufficient mixing causes solids deposition in the fermentor, the effective fermentor volume decreases. This decrease in effective volume affects important operational parameters such as HRT and loading rate. Thus, the minimum mixing requirement for fermentation systems may be based on the materials handling and fermentor design aspects rather than maximum $CH_4$ production rates. More research is needed in understanding the materials handling function of mixing systems in anaerobic fermentors.

### 6.4.2 Effect of Vacuum

This study showed that vacuum (0.96 atm.) did not increase the rate of (0.33 atm.) reported by Finney et al. (1977). However, the higher capital cost and operational problems associated with maintaining anaerobic conditions at high vacuum precludes the use of farm-scale, vacuum fermentation in the near future.

### 6.4.3 Comparison of Experimental to Predicted $CH_4$ Production Rates

Because the differences in $CH_4$ production rates between conventional, continuously-mixed fermentors and intermittently-mixed or vacuum fermentors were only 11% or less, these rates were compared to rates predicted by Equation 5.1. Values for $B_o$ were determined by long-term batch fermentations for Experiments 1a and 1b ($B_o$ = 0.29 $m^3$ $CH_4$/kg $VS_f$) and as described in this study for Experiments 1c, 1d, 2a and 2b. The values for $\mu_m$ (0.586 $day^{-1}$ at 55°C) and K were taken from the relations for $\mu_m$ vs temperature and K vs $S_o$ presented previously (Hashimoto, Chen and Varel, 1981).

Table 6.5 and Figure 6.5 show the experimental and predicted $\gamma_v$ for all the fermentations conducted in this study. The results show good correlation between the experimental and predicted $\gamma_v$, with a mean ratio of experimental to predicted $\gamma_v$ of 0.96 and a standard deviation of ± 0.06. These results show that the $\gamma_v$ obtained in these studies are comparable to those predicted for conventional fermentors.

A general conclusion from this study is that phase-transfer controlling mechanisms (i.e., mixing, vacuum) have minimal effect on the $CH_4$ production rate from fermentation of beef cattle waste, even for high-rate anaerobic fermentation systems. Thus, these results suggest that intermittently-mixed, conventional fermentors can produce high $CH_4$ production rates while minimizing energy and capital inputs. This study also shows that good prediction of $CH_4$ production rates can be achieved using a previously published kinetic model.

## 6.5 SUMMARY

The effects of mixing duration and vacuum on methane production rates from anaerobically-fermented beef cattle wastes were discussed. The results showed that continuously-mixed fermentors produced significantly ($P<0.05$) higher methane production rates than fermentors mixed two hours per day. However, the rates from the continuously-mixed fermentors were only 8 to 11% higher

TABLE 6.5. EXPERIMENTAL AND PREDICTED VOLUMETRIC METHANE PRODUCTION RATES[a]

| Fermentation Conditions | Mixing h/day | $B_o$ $m^3$ $CH_4$/kg $VS_f$ | $\Theta$ d | $S_o$ kg $VS_f/m^3$ | K | $\gamma_V$, $m^3$ $CH_4/m^3 \cdot$day Exp. | $\gamma_V$, $m^3$ $CH_4/m^3 \cdot$day Pred. | Ratio Pred./Exp. |
|---|---|---|---|---|---|---|---|---|
| Conventional | 1 | 0.29 | 6 | 57.5 | 0.60 | 2.38 | 2.24 | 0.94 |
| Conventional | 2 | 0.29 | 6 | 57.5 | 0.60 | 2.51 | 2.24 | 0.89 |
| Conventional | 3 | 0.29 | 6 | 57.5 | 0.60 | 2.37 | 2.24 | 0.95 |
| Conventional | 24 | 0.29 | 6 | 57.5 | 0.60 | 2.15 | 2.24 | 1.05 |
| Conventional | 2 | 0.36 | 6 | 56.1 | 0.60 | 2.62 | 2.72 | 1.04 |
| Conventional | 2 | 0.36 | 6 | 56.1 | 0.60 | 2.62 | 2.72 | 1.04 |
| Conventional | 24 | 0.36 | 6 | 56.1 | 0.60 | 2.78 | 2.72 | 0.98 |
| Conventional | 24 | 0.36 | 6 | 56.1 | 0.60 | 2.91 | 2.72 | 0.93 |
| Conventional | 2 | 0.36 | 4 | 56.1 | 0.60 | 3.80 | 3.49 | 0.92 |
| Conventional | 2 | 0.36 | 4 | 56.1 | 0.60 | 3.34 | 3.49 | 1.04 |
| Conventional | 24 | 0.36 | 4 | 56.1 | 0.60 | 3.90 | 3.49 | 0.89 |
| Conventional | 24 | 0.36 | 4 | 56.1 | 0.60 | 4.01 | 3.49 | 0.87 |
| Vacuum | 24 | 0.36 | 6 | 68.4 | 0.60 | 3.42 | 3.26 | 0.95 |
| Vacuum | 24 | 0.36 | 6 | 68.4 | 0.60 | 3.56 | 3.26 | 0.92 |
| Conventional | 24 | 0.36 | 6 | 68.4 | 0.65 | 3.55 | 3.26 | 0.92 |
| Conventional | 24 | 0.36 | 6 | 68.4 | 0.65 | 3.54 | 3.26 | 0.92 |
| Vacuum | 24 | 0.36 | 4 | 68.4 | 0.65 | 4.39 | 4.15 | 0.95 |
| Vacuum | 24 | 0.36 | 4 | 68.4 | 0.65 | 4.18 | 4.15 | 0.99 |
| Conventional | 24 | 0.36 | 4 | 68.4 | 0.65 | 4.13 | 4.15 | 1.00 |
| Conventional | 24 | 0.36 | 4 | 68.4 | 0.65 | 4.03 | 4.15 | 1.03 |

[a]Fermentation temperature = 55°C, $\mu_m$ = 0.586 $day^{-1}$

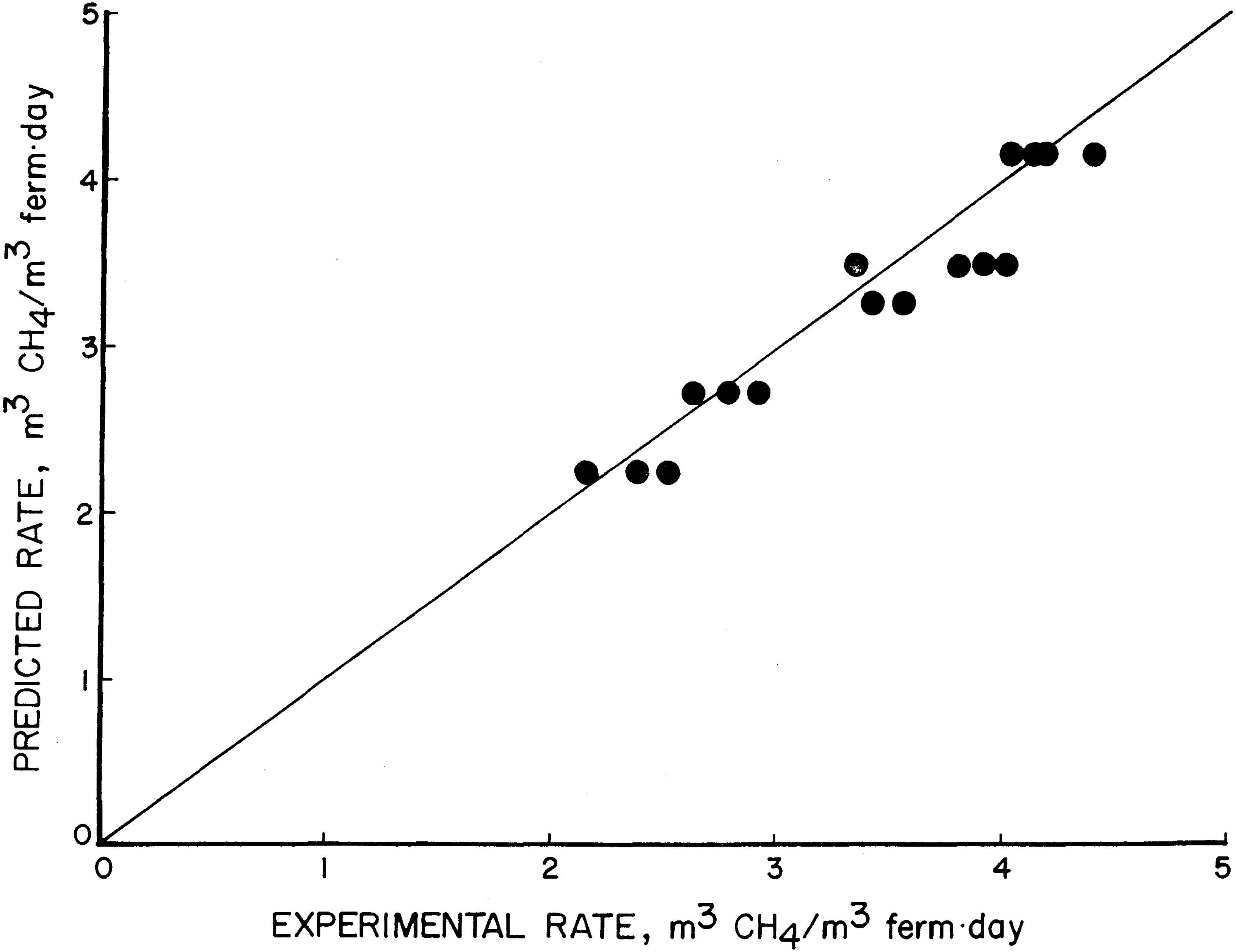


Figure 6.4. Comparison of Predicted and Experimental Methane Production Rates

than the intermittently-mixed fermentors at 6- and 4-day HRT, respectively. There was no significant difference between the vacuum and conventional fermentors at 6-day HRT, but there was a significant difference at 4-day HRT. The $CH_4$ production rate of the vacuum fermentors was 5% higher than the conventional fermentors at 4-day HRT. The results of these experiments compared well with predicted $CH_4$ production rates. These results suggest that there is little potential for increasing the fermentation rates of livestock wastes by increased mixing or vacuum.

## SECTION 7.0

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