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BIOLOGICAL CONVERSION OF BIOMASS TO METHANE
THE EFFECT OF REACTOR DESIGN ON KINETICS

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ABSTRACT

The advantage and need for mixing in the anaerobic fermentation of organic solids has been recognized. Because of the sequential nature of the microbial process, mixing has provided more stable systems that are less subject to operational problems. Recent reports in the literature have suggested that plug flow reactors are capable of providing higher rates of stabilization which result in greater methane production per unit volume of reactor. It has also been reported that separation of the acetogenic stage from the methanogenic stage increases gas yield.

An experimental program was conducted to evaluate the effect of reactor type on methane production. This study showed that if a balanced population of organisms can be maintained in the initial stage, multi-stage fermentation is more efficient than a complete mix system. However, when the system is stressed, failure in the multi-stage system is more rapid. If the objective is to maximize the conversion of solids to methane, a staged system will produce more methane per unit volume of reactor. If the objective is to maximize the methane production per unit volume of reactor, a single stage complete-mix reactor operating near the critical retention time is required.

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INTRODUCTION

Conventional digester technology has employed mixing as a prime requirement since the introduction of high rate digestion systems. Mixing has been found necessary to prevent stratification of the reactor contents. Without mixing a deep scum layer will form. This layer contains most of the unstable solids. The pH will be low and the methanogenic bacteria are inhibited by this low pH. The stable solids generally settle to the bottom of the reactor with an intermittent layer of supernatant resulting. Methane production occurs in the bottom layer and to some extent in the supernatant layer. The volatile acids generated in the scum layer must diffuse from this layer to the region where the methanogenic bacteria can survive.

In low rate systems, retention time is long. Therefore, this diffusion rate does not limit the stabilization of the sludge solids. However, in high rate systems, the diffusion rate is limiting and the continued buildup of undigested solids will cause digester failure. Mixing eliminates this stratification and provides for the various bacterial groups to be intimately mixed. This results in a greater overall rate of stabilization.

The literature in the past decade contains several articles that use mathematical models to show that plug flow reactors yield a higher rate of stabilization. The models are based on first order kinetics in which substrate level is the only rate limiting condition, or on the Monod model. Recent papers have reported that two stage digestion improves the yield. These studies attempt to separate the acetogenic and methanogenic phases.

THEORETICAL CONSIDERATIONS

1. Biological Mechanisms

Anaerobic treatment of complex organic fibers can be considered in its simplest form to be a three-stage process as shown in Figure 1. In the first stage, a group of anaerobic microorganisms, primarily cellulolytic bacteria, act upon the organic fibers. The reaction is an enzymatic hydrolysis of the polymers to the individual monomers. These monomers are soluble and become the substrate for the microorganisms in the second or acetogenic stage. In this stage, the soluble organic compounds are converted into short chain organic acids, primarily acetic acid. In addition, bacterial cells are formed as a result of the metabolism of these substrates.

The short chain organic acids become the substrate for a group of strictly anaerobic methanogenic bacteria. These bacteria ferment

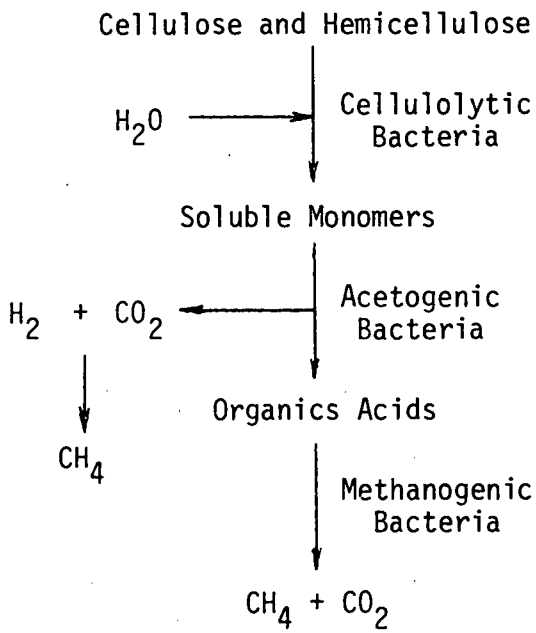

Figure 1. Anaerobic Fermentation of Organic Solids

acetic and propionic acids to methane and carbon dioxide. Methane can also be produced by bacteria that obtain energy for growth by reducing carbon dioxide utilizing hydrogen gas or formate produced by other species as the electron donor.

The methane formed in this last stage, being insoluble in water, is lost to the gas phase. It can be collected and used for its fuel value. The carbon dioxide evolved partially escapes to the gas phase. However, carbon dioxide is relatively soluble in water. It also reacts with any caustic (OH^-) in the system to produce bicarbonate (HCO_3^-) ion. Consenquently, carbon dioxide evolution is a function of several factors including pH, bicarbonate concentration, temperature and substrate composition.

It is in this third stage that stabilization occurs through the removal of oxygen demanding material inthe form of methane gas. Cell production is also minimal when compared to aerobic processes. This is a direct result of the high energy content of the products, in particular methane (McKinney and Conway, 1957). This is an advantage as the amount of solids requiring ultimate disposal is minimized by eliminating any substantial microbial protoplasm production in the process of stabilizing the organic material.

There are several different methanogenic bacteria responsible for the production of methane. McCarty (1964) suggests that the bacteria utilizing propionic and acetic acid are the most important. Since most of the methane is produced from the fermentation of these organic acids, this step is indicated as the rate limiting step in the overall process. However, Pfeffer (1968) found that in sewage sludge digestion at 35°C,

methane fermentation may be rate limiting at short retention times (10 to 15 days). At longer retention times, the rate limiting step is hydrolysis of the organic solids.

Chan and Pearson (1970) found that cellulose hydrolysis was the rate limiting step in the conversion of cellulose into methane. Pfeffer (1974) reported that cellulose hydrolysis was rate limiting when digesting municipal solid wastes. Pfeffer's studies were conducted at temperatures ranging from 35°C to 60°C. Since cellulose is a major component of all natural occurring organic fibers, it is probable that the breakdown of cellulose is the rate limiting step in the production of methane from biomass.

2. Kinetic Relationships

In most kinetic models developed for biological systems, the substrate level is assumed to be the rate limiting factor. In the simplest models, Equation 1 is used. The rate of substrate removal (dS/dt) is equal

$$dS/dt = -k_1 S \qquad (1)$$

to a constant (k_1) times the substrate level (S). Therefore, if this relationship is true, a plug flow reactor that has a high substrate level in the influent to the reactor will exhibit a high rate of substrate utilization. A mathematical model based upon this kinetic relationship will clearly show that the substrate stabilization per unit volume of reactor will be significantly greater in a plug flow reactor than in a completely mixed reactor.

The Monod model has been used more extensively in modelling of biological reactors. This relationship is shown in Equation 2 in which

$$-\frac{dS}{dt} = \frac{kSX}{K_S+S} \qquad (2)$$

X is the mass of microorganisms, k is the maximum rate of substrate utilization per unit weight of microorganisms and K_S is the half velocity coefficient, equal to the substrate concentration when $-dS/dt = (1/2)k$. This relationship shows that dS/dt is a function of both S and X.

In a plug flow reactor, S is equal to the influent substrate concentration at the reactor influent. When S is large, $K_S + S \rightarrow S$. Therefore, $-dS/dt \rightarrow kX$. This means that the substrate removal rate is a function of X. If no mixing occurs, i.e. there is no recycle of microbial mass from the reactor effluent, and the influent stream does not contain a large mass of microorganisms, the substrate removal rate is low because of the lack of microorganisms. Therefore, a true plug flow reactor is not the more efficient. Recycle of microbial mass is essential since the initial removal rates are more a function of X than S.

When methanogenesis is considered to be rate limiting, the time required to achieve a population of methanogenic bacteria is excessive since the doubling time for these bacteria is reported in days rather than hours. This is also complicated by inhibition that results from the environment associated with the multiple steps involved in the breakdown of the substrate. The cellulolytic and acetogenic bacteria grow more rapidly than the methanogenic bacteria. Consequently, the products of the acetogenic bacteria, organic acids, increase in the reactor. Unless adequately buffered, the pH is depressed to a level that is inhibitory to the methanogenic bacteria.

Therefore, it would appear that the plug flow reactor is not a viable alternative for methane fermentation. This was demonstrated by Andrews et al. (1964). The data in Table 1 were extracted from their study. This shows the data from a single stage reactor operating at 5.64 days retention time and from 4 series reactors operating at 1.41 days retention time each for a total of 5.64 days. The single stage reactor was stressed at this low retention time. The pH was 6.3 and the volatile acids was 2865 mg/l as acetic acid. However, even under these adverse operating conditions, the single stage reactor performed better as shown by these data.

Table 1. Comparison of Single- and Four-Stage Systems

Characteristic	Single Stage	Four Stages Combined
COD Removal, % Influent	39	32
$\frac{\text{mg } CH_4 \text{ carbon prod.}}{\text{l of reactor - day}}$	56	47
$\frac{\text{mg COD removed}}{\text{l of reactor - day}}$	783	578
$\frac{\text{mg } CH_4 \text{ carbon prod.}}{\text{mg V.S.S. in reactor - day}}$	0.111	0.069
$\frac{\text{mg COD removed}}{\text{mg V.S.S. in reactor - day}}$	1.56	0.85

3. Two-Stage Fermentation

Recognizing that the methane fermentation is a multiphase process, researchers have attempted to separate the acetogenic from the methanogenic stage. With this technique, it is possible to operate each stage under conditions that optimize the growth of the specific cultures. In biomass

fermentation, cellulose hydrolysis has been found to be the rate limiting step. Therefore, two-stage digestion should consider the optimum growth conditions for cellulolytic bacteria. Work by McBee (1948) reported growth of thermophilic cellulolytic bacteria to occur in a pH range of 6.4 to 7.4. Dubos (1928) found the cellulolytic bacteria of the soil had an optimum pH range of 6.5 to 8.5. He also reported that pH had little effect on the density of cellulolytic bacteria when nitrogen in the form of ammonium sulfate was added.

The following table, taken from Chan and Pearson (1970), illustrates the rates of cellulose hydrolysis obtained by various researchers.

Table 2. Summary of Hydrolysis Rate of Cellulose in Anaerobic Fermentation

Authority	System and Culture	Initial Cellulose Concentration (mg/ℓ)	Cellulose Material	pH	Hydrolysis Rate (mg/ℓ-day)
Maki	Batch, mixed 2 pure cultures from sewage, 38°C, mesophilic	2,000	Whatman's #1 filter paper	6.8	(1)* 260 (2)* 660
Heukelekian	Batch, pure culture from sewage, 25°C, mesophilic	3,120	Cellulose in sewage sludge	7.4	142
McBee	Batch, pure culture from (1)* soil and manure, 55°C, (2)* thermophilic	744 2,980	Adsorbent cotton	--- ---	(1)* 149 (2)* 429
Stranks	Batch, Mixed culture from rumen, 60°C, thermophilic	41,200	Whatman's #2 filter paper		11,400

*Indicate experiments with different strains

All studies show relatively low rates of hydrolysis for pure culture systems. However, Stranks (1956) reported extremely high rates of Hydrolysis when using a mixed culture of thermophilic microorganisms. This observation suggests that selection of operating conditions that limit the species of bacteria that will be present may have a negative effect on the hydrolysis rate.

Cellulose hydrolysis as well as acetogenesis is an enzymatic reaction. Extracellular cellulases are responsible for cellulose hydrolysis while both extracellular and intracellular enzymes are responsible for the production of organic acids from the cellulose hydrolysis products. The simplest enzyme reaction (Equation 3) can be described by the Michaelis-Menten relationship. The substrate (S) and enzyme (E) are in

$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} SE \xrightarrow{k_3} P + E \qquad (3)$$

equilibrium with substrate-enzyme complex. However, an irreversible reaction resulting in the product (P) and the enzyme is assumed. This reaction is the rate limiting step having a constant, k_3. The Michaelis-Menten expression shown in Equation 4 is developed from this equilibrium.

$$\frac{dS}{dt} = \frac{k_3 (S)(E_o)}{K_a + (S)} \qquad (4)$$

This equation is the same as the Monod expression except the initial enzyme concentration, (E_o), is substituted for microorganism mass, X.

Many enzyme reactions do not satisfy the restriction that the enzyme-substrate complex breaks down irreversibly but that it can also be formed from the product side as shown in Equation 5. As the concentration of product increases, most enzymatic reactions slow down. This is due to

$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} SE \underset{k_4}{\overset{k_3}{\rightleftarrows}} P + E \qquad (5)$$

the phenomenon of product inhibition. The rate equation shown in Equation 6 is developed from Equation 5. This equation shows that the substrate

$$-\frac{dS}{dt} = \frac{dP}{dt} = \frac{[K_1 k_3(S) - k_2 k_4(P)](E_o)}{[k_2 + k_3] + k_1(S) + k_4(P)} \qquad (6)$$

utilization rate is a function of not only the substrate and enzyme concentration but also the product concentration. Mahler and Cordes (1968) show that an enzymatic reaction slows down as equilibrium is approached, not only by virtue of the thermodynamic back reaction, but also because, as the product concentration increases, an increasing proportion of the enzyme is immobilized as an EP complex. This kinetic effect of product inhibition is thus an intrinsic property of any realistic, i.e., reversible, mechanism of enzyme catalysis.

Recognizing that product inhibition would reduce the kinetics of substrate utilization, Hammer and Bortchardt (1969) and Schaumburg and Kirsch (1966) attempted to separate the acetogenic phase from the methanogenic phase by semipermeable membranes. However, problems with the membranes made this technique unacceptable. Recent studies by Ghosh et al. (1975) attempt to achieve two stage digestion by operating the first stage at retention times too short to permit the growth of methane bacteria. Data on control single stage digestion are not presented so it is not clear what advantages were obtained with two stage digestion. Data presented on influent and effluent volatile solids show a very modest hydrolysis of solids in the acid digester. From the data presented, it would appear that the majority of the waste stabilization occurred in the methane fermentation stage.

EXPERIMENTAL PROCEDURE

1. Description of Experimental Fermentation System

The fermentation system shown in Figure 2 was designed to allow for evaluation of single stage complete-mix, two-stage and simulated plug-flow reactors simultaneously. Each of these reactors is made of 1/2 inch plexiglass and has a total operating liquid volume of approximately 43 liters. Mixing was provided with flat blade impellers fixed to a horizontal shaft. These impellers operate at a low speed, approximately 30 rpm. These reactors were operated on a semi-continuous feed schedule with appropriate volumes of substrate added either once or twice daily. In general, when retention times of less than 10 days were employed, substrate was added twice daily at approximately 12 hours intervals.

One reactor (C) contained four compartments as shown in Figure 3. Four mixed reactors in series provide a reasonable approximation to plug flow. The second reactor (B) operated as a two-stage reactor with the first compartment consisting of 33 percent of the total volume for Runs 1 and 2, and 50 percent of the total volume for Runs 3 and 4. In the third unit (A), all four compartments were combined into a single reactor. This served as the single-stage complete-mixing unit.

Flow from one compartment to the next was through a port cut in the dividing walls. When substrate is added to the first compartment, the difference in head caused the contents to flow into the next compartment. It was planned to have these ports open at all times. However it was recognized that intermixing of the contents of the compartments was possible. A tracer study was conducted to determine the degree of intermixing. This study showed that considerable intermixing between the

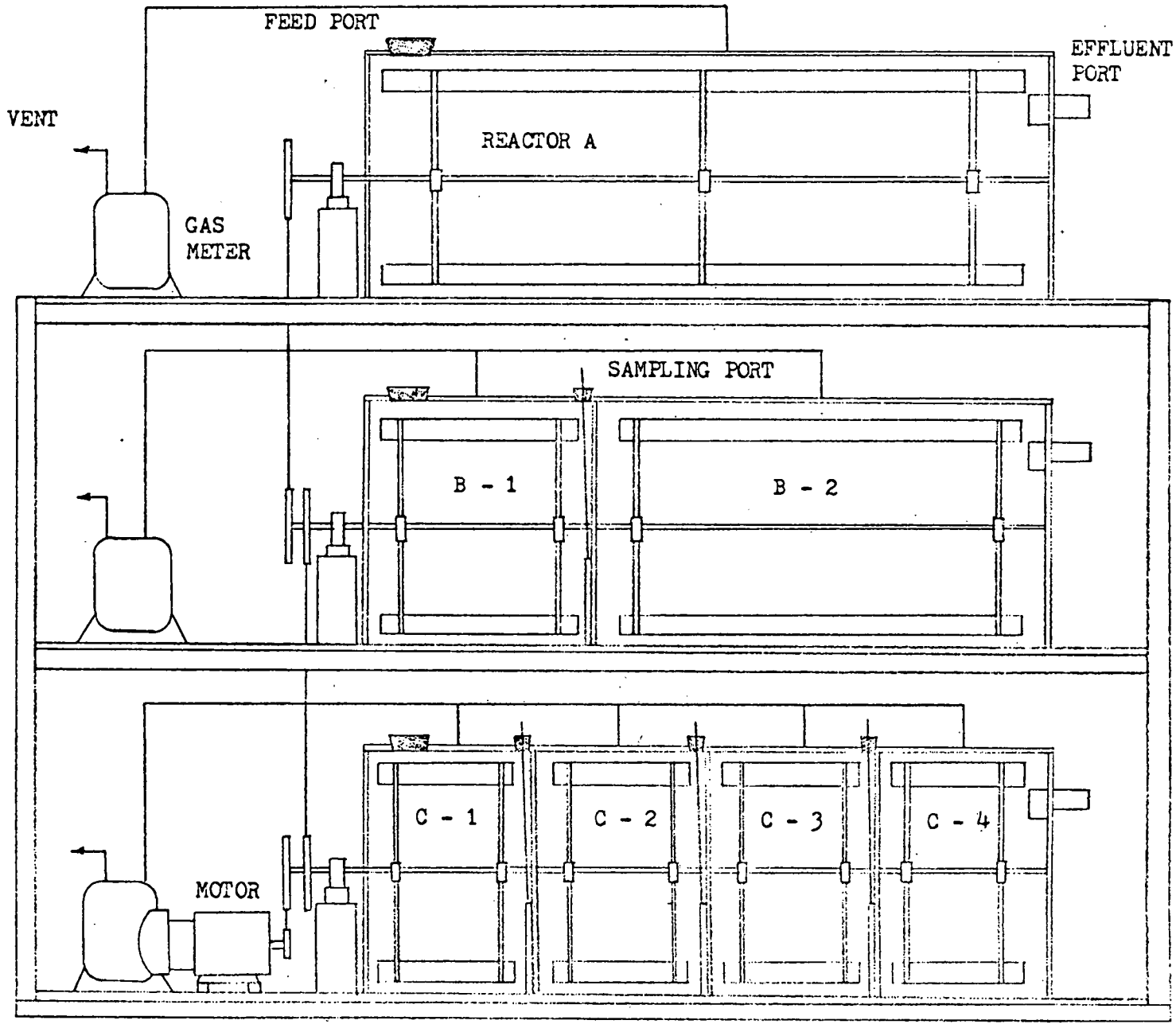

Figure 2. Schematic of Laboratory Fermentation System

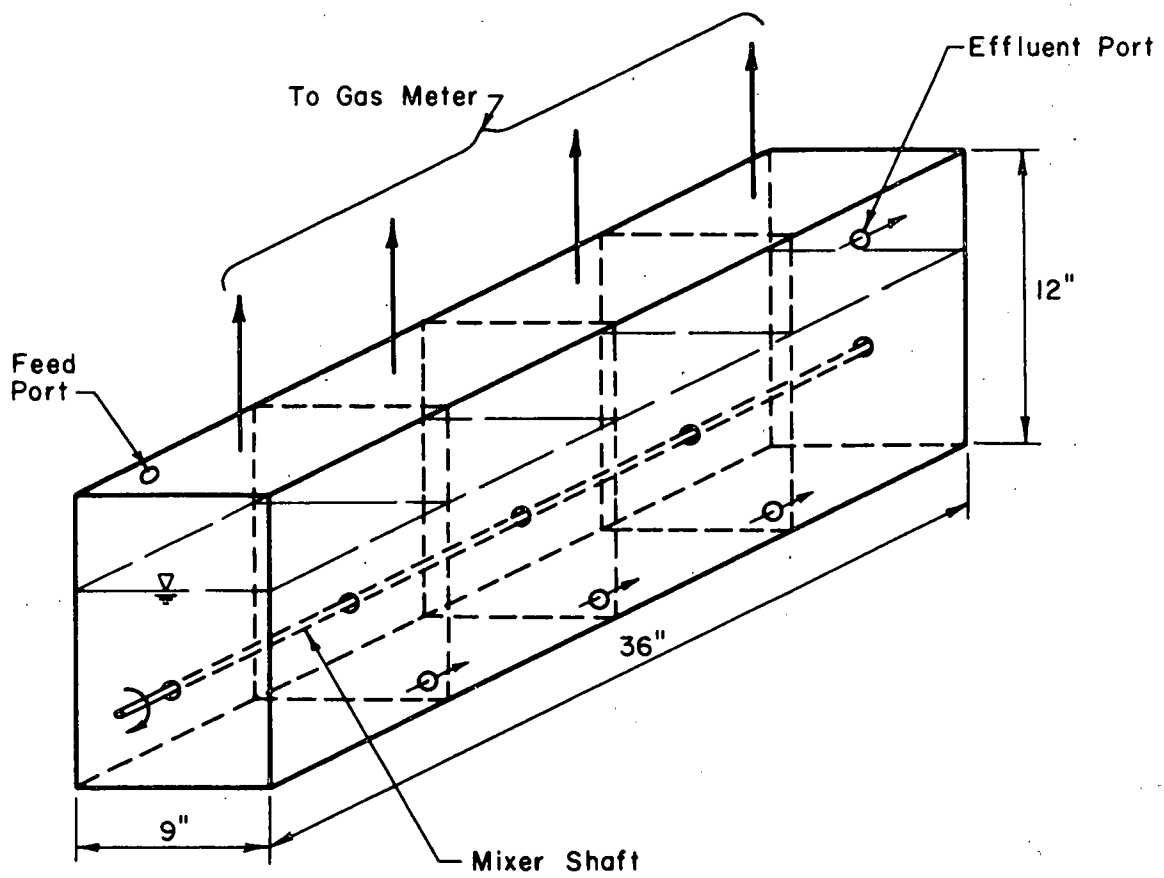

Figure 3. Schematic of Laboratory Fermentation Reactors

compartments in the two and four stage reactors did occur within a short period of time. Provisions were made to have sliding panels covering the ports at all times except during substrate addition.

Wet test gas meters were used to measure the total gas flow from each reactor. Provisions were made to allow for measuring the gas generated from each compartment if desired. The entire system was housed in a constant temperature room. This room provided thermophilic fermentation conditions, approximately 60°C, for the duration of the experimental program.

2. Fermentor Feed Stock

In order to maintain a uniform feed stock, shredded computer paper was selected as the carbon source. Large quantities of used computer paper are readily available at the University. This material is also more biodegradable than newsprint, and of more uniform quality. Before use, the paper is passed through a shredder with a one-inch screen. The composition of the feed slurry for Runs 1 through 4 is given in Table 3. In Run 1, the supplement was raw sewage sludge obtained from the Urbana-Champaign Sanitary District. During this run, it was observed that the systems were stressed. The addition of beef manure during the second run improved the systems operation. Therefore, for the remainder of Run 2, and for Runs 3 and 4 beef manure was substituted for the sewage sludge.

Additional nitrogen and phosphorous were provided to insure adequate nutrients. Also, calcium oxide and sodium hydroxide were added to provide the alkalinity required for pH control. The constituents in Tables 3 were added to tap water to provide the desired feed solids

concentration. The feed stock was prepared the previous day in one large container equipped with a rapid mix unit. This insured that each reactor received feed stock with identical characteristics.

Table 3. Composition of Feed Slurry - g/ℓ

Constituent	Run 1	Run 2	Run 3 and 4
Paper	22.3	44.6	38.55
NH_4Cl	0.55	1.11	1.0
K_2HPO_4	0.19	0.37	0.2
CaO	2.9	2.9	3.5
NaOH	1.0	1.0	0.5
Supplement (dry solids)	3.0	5.9	12.0

3. Process Evaluation

The objective of this study is to determine which flow pattern is the most efficient in producing methane from biomass. The primary parameter is the conversion efficiency, i.e., the portion of feed material converted to methane. A secondary parameter is the yield of methane per unit volume of reactor. This impacts on the cost of the system. Therefore, the primary emphasis was placed on these two parameters for the determination of the best reactor design.

Total gas production from each reactor and pH measurements from each compartment were determined daily. The composition of each off gas was determined periodically using a Fisher Gas Partitioner. Analysis of control variables, i.e., alkalinity and volatile acids, were conducted on an average of three times per week during normal operation and daily during

intensive sampling periods. A solids balance, including total and volatile total, as well as chemical oxygen demand (COD) analysis, were conducted only during the intense sampling periods. These data are given in the Appendix.

Standard Methods (1975) was the basis for most of the analytical techniques performed on the reactors. The samples for alkalinity and volatile acid determination were centrifuged at 10,000 rpm for 30 minutes prior to analysis. The column chromatography method was used for the volatile acid analysis. Total solids and COD measurements were also conducted according to Standard Methods. Samples for COD determination were diluted and mixed in an osterizer prior to analysis. Volatile solids were combusted at 600°C.

RESULTS AND DISCUSSION

1. Run No. 1

Substrate for this run was shredded computer paper plus a small quantity of sewage sludge added for microorganisms seed and micronutrients (see Table 3). Additional nitrogen and phosphorous are added to provide a balanced substrate. The quantity of base added to the substrate was to be sufficient to maintain a pH in the complete-mix reactor between 6.7 and 7.0. The reactors were initially seeded with effluent from a larger manure fermentation system located at the Department of Civil Engineering's Dynamic Testing Lab. The initial pH in all reactor stages was in the 7.2 to 7.5 range. The alkalinity was approximately 2700 to 2800 mg/l and the volatile acids approximately 300 mg/l as acetic acid. This run was initiated on January 15, 1977. The total retention time in all three reactors was 10 days with substrate being added once daily. The constant temperature room was set for thermophilic fermentation (60°C).

The amount of caustic added to the substrate was sufficient to maintain a pH in reactor A above 6.7 or greater under normal operating conditions. While the caustic was adequate to maintain the pH in Reactor A, the pH in all other reactor compartments eventually dropped to inhibitory levels. This data is shown in Figure 4. Reactor C failed in a short period due to these inhibitory levels. The pH in the first stage of Reactor B dropped to low levels, but it was only after about 30 days that the pH in the second stage began to drop. The associated volatile acids are shown in Figures 5 and 6.

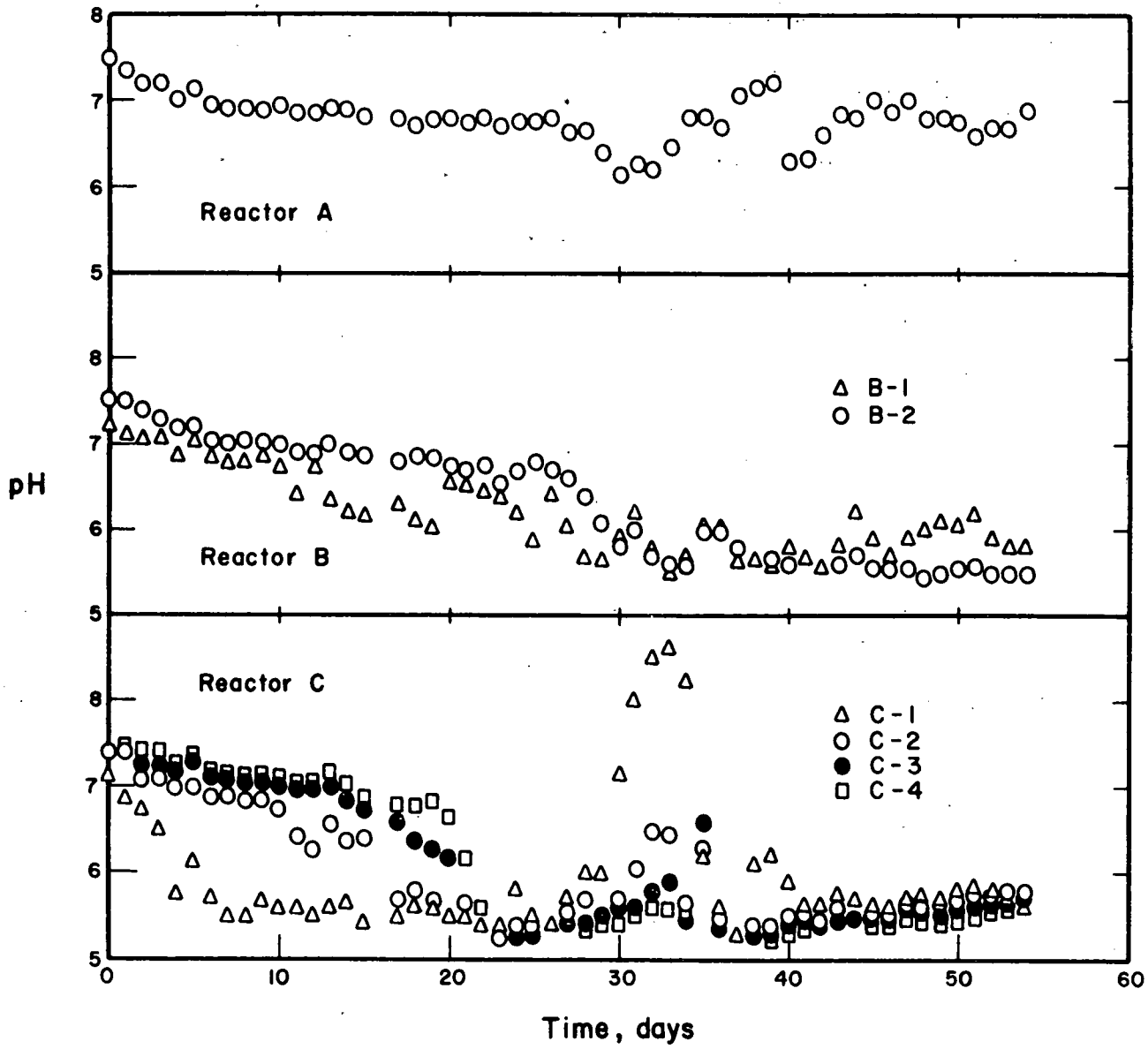

Figure 4. Variation in pH in Reactors A, B and C

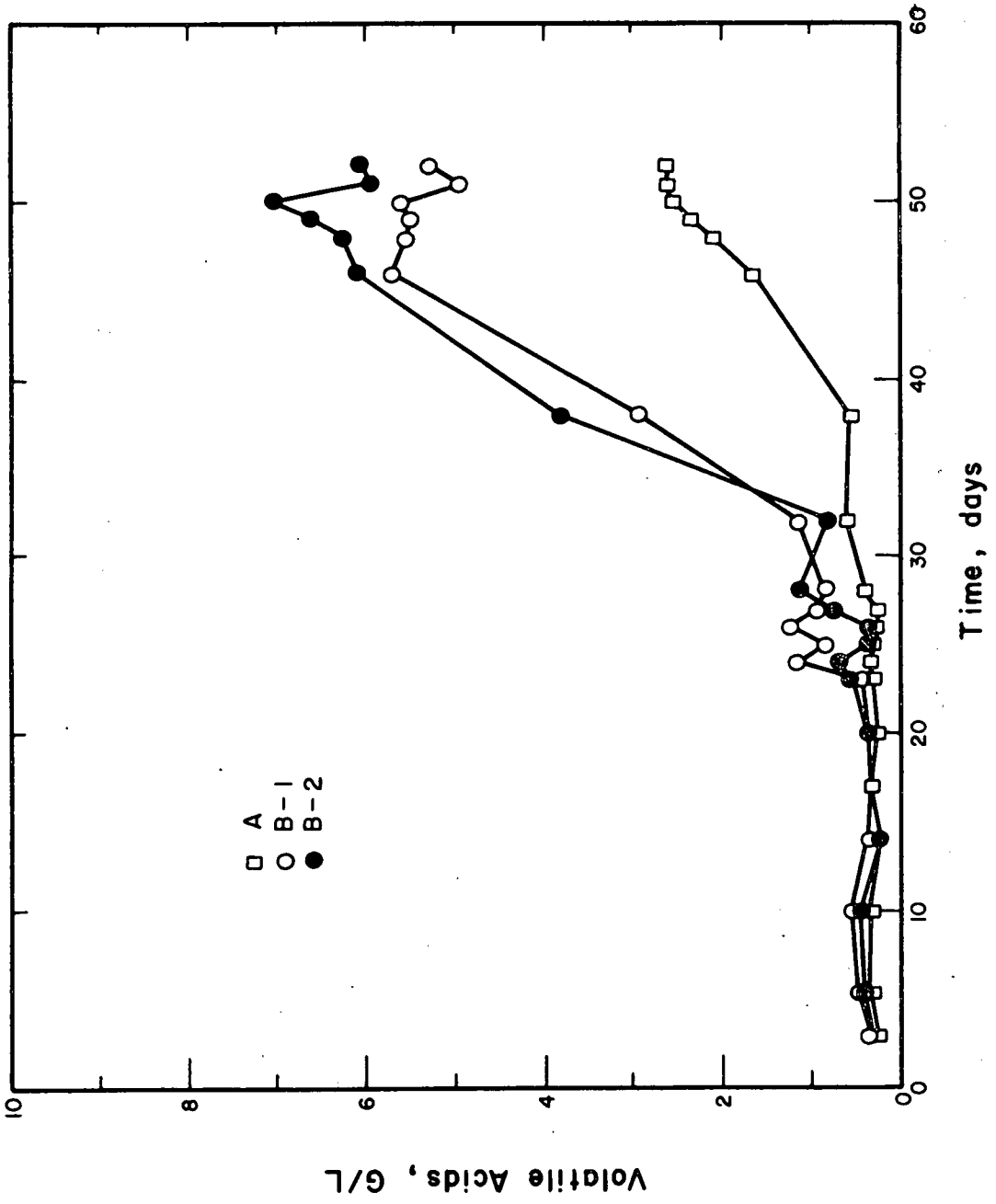

Figure 5. Variation in Volatile Acids for Reactors A and B

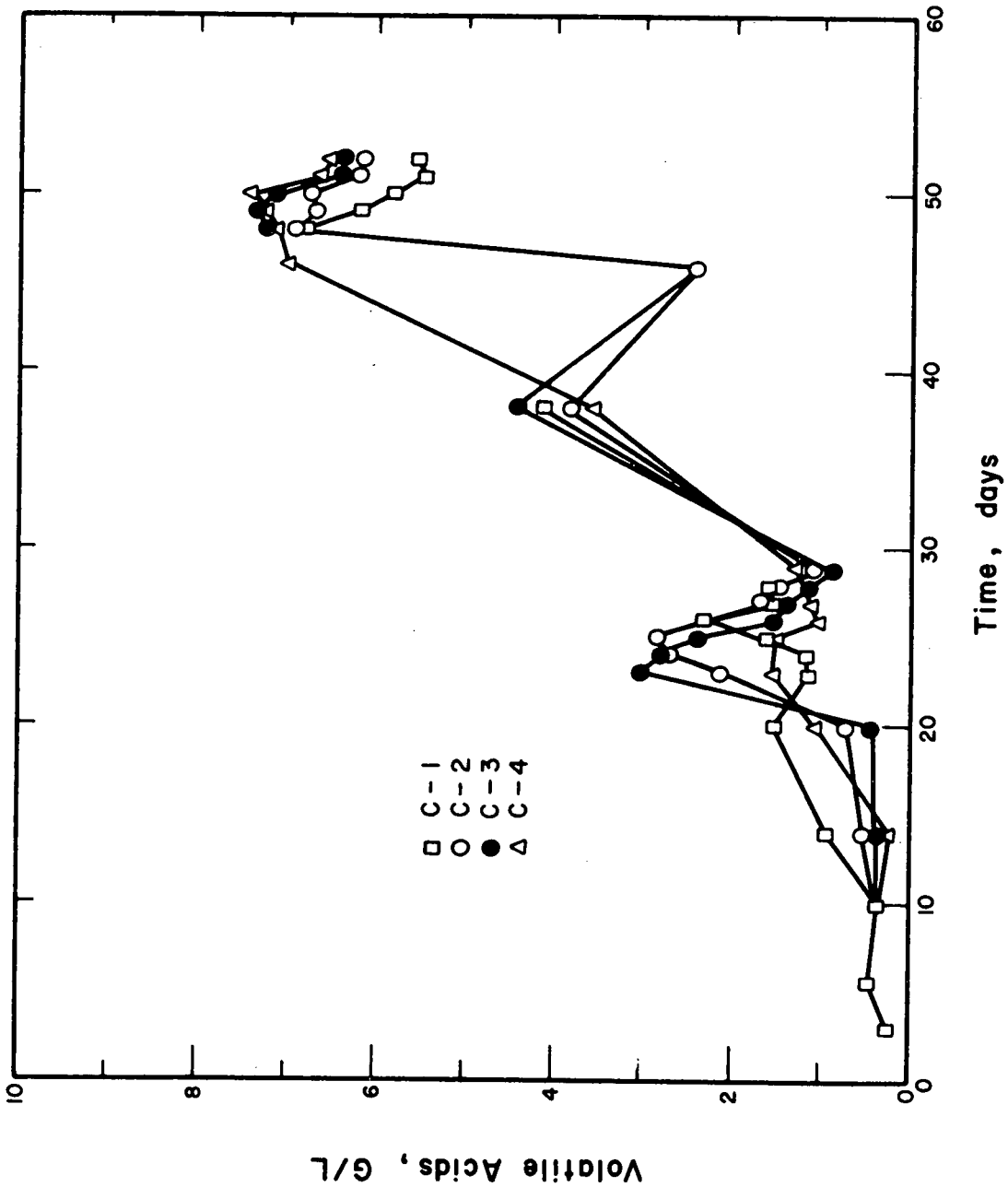

Figure 6. Variation in Volatile Acids for Reactor C

After about 30 days after start-up, the pH in Reactors A and B-2 began to decrease. Apparently some micronutrient was missing in the feed slurry. Additional caustic was required to maintain the pH in Reactor A. As a result of the increased caustic addition, the pH in Reactors B-1 and C-1 increased significantly.

The volatile acids in Reactor A increased slightly during this period. In Reactors B-1 and B-2, there was a significant increase in the volatile acids. In Reactor C, the volatile acids in the first stage started to increase after only about 10 days. A continued increase was observed in all stages during the balance of the run, reaching levels in excess of 6000 mg/l.

On about Day 30, a small quantity of sewage sludge was added to the feed slurry to supplement the trace nutrients. Reactor A responded favorably for about 15 days. The volatile acids in Reactor B-1 increased to greater than 6000 mg/l while B-2 increased to about 5500 mg/l. These data show that the two stage system functioned reasonably well when the system was not stressed. However, when stressed, the two stage system failed rapidly. The complete mix reactor was considerably more stable. Complete failure in Reactor C occurred within about 20 days.

The volatile solids and COD reduction are shown in Table 4. The feed slurry to the reactors contained 24.5 g/l of volatile solids and had a COD of 29.0 g/l. Table 5 shows the gas production for each of these reactors. These data clearly show that the complete mix reactor yielded a higher gas production than either of the other two reactor designs. Table 6 shows the measured methane production and the theoretical methane production based on COD reduction.

Table 4. COD and Volatile Solids Reduction

Reactor	θ Days	Vol. Solids - g/l		COD - g/l		K - Day^{-1}	
		S_o	S_e	S_o	S_e	Vol. Sol.	COD
A	10	24.5	6.3	29.0	7.6	0.289	0.282
B-1	3.3	24.5	12.8	29.0	22.1	0.277	0.095
B-2	6.7	12.8	8.3	22.1	21.7	0.081	0.081
C-1	2.5	24.5	16.6	29.0	26.9	0.190	0.031
C-2	2.5	16.6	14.1	26.9	27.0	0.071	-
C-3	2.5	14.1	12.4	27.0	26.9	0.055	0.001
C-4	2.5	12.4	12.1	26.9	25.8	0.010	0.017

Table 5. Gas Production

Reactor	m^3/m^3-Day	m^3/kg V.S. Fed	m^3/kg V.S. Destroyed
A	0.7	0.404 (6.48 SCF)	0.542
B	0.3	0.261 (4.20 SCF)	0.384
C	0.1	0.168 (2.70 SCF)	0.330

Table 6. Methane Production - lpd

Reactor	Measured	Theor. (COD Red)
A	30.1	32.2
B	13.3	11.2
C	6.3	5.0

The calculated rate constants from $S_e/S_o = 1/(1 + K\theta)$ for Reactor A as shown in Table 4, are comparable based either on volatile solids or COD. However, in Reactors B and C, their is no comparison between the constants. It is clear that the solids destruction resulted from the hydrolysis of the organic solids to volatile compounds that were lost when the solids analysis was performed. These low constants for COD reduction suggest severe inhibition of organic stabilization in both Reactors B and C.

Although these data show that the complete mix reactor operates more efficiently than the two stage or plug flow units, it appears that the microbial system was also stressed. Reactor A did not operate as efficiently as expected. At 60°C and a 10 day retention time, the volatile acids should have been lower, less than 500 mg/l. Also, other investigators have shown that efficient operation can be achieved with 5 day retention times at 60°C. Therefore, Reactor B should not have failed.

2. Run No. 2

This run was made with a modification of the substrate, see Table 3, and with a longer retention time. When the retention time was increased to 14.63 days, Reactors A and B began to recover slowly as evidenced by all parameters. This is shown by the first 30 days in Figures 7 through 10. On Day 35, the manure supplement was started. The operation of Reactors A and B further improved. Also, once the manure addition was started Reactor C improved significantly in all four stages. It appeared that the beef manure was supplying some micronutrient that had been lacking when sewage sludge was being used as the supplement. As shown in Figure 11, the volatile acids ranged from 2000 mg/l in the first stage to 200 mg/l in the last stage.

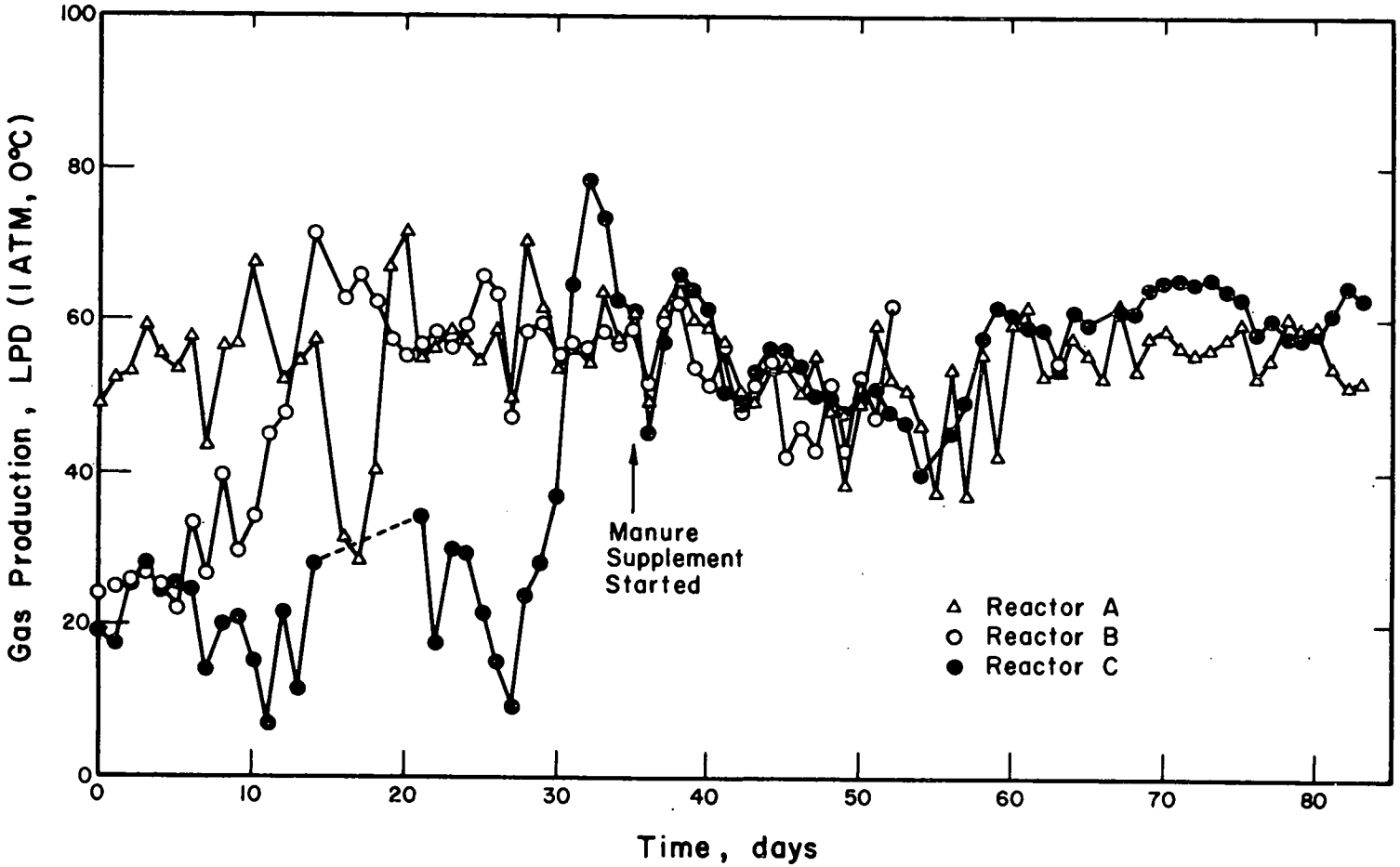

Figure 7. Gas Production from Reactors A, B and C

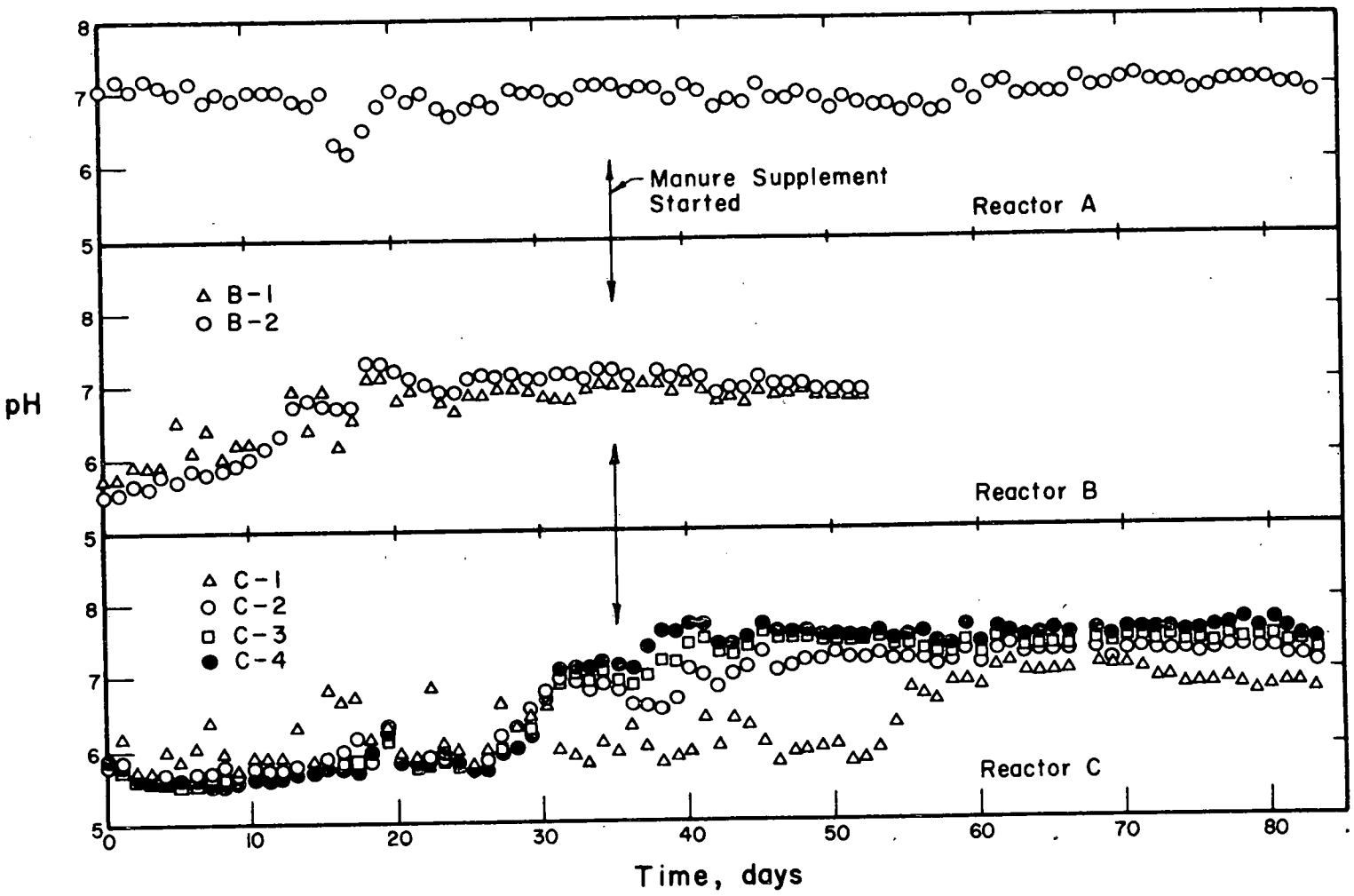

Figure 8. Variation in pH for Reactors A, B and C

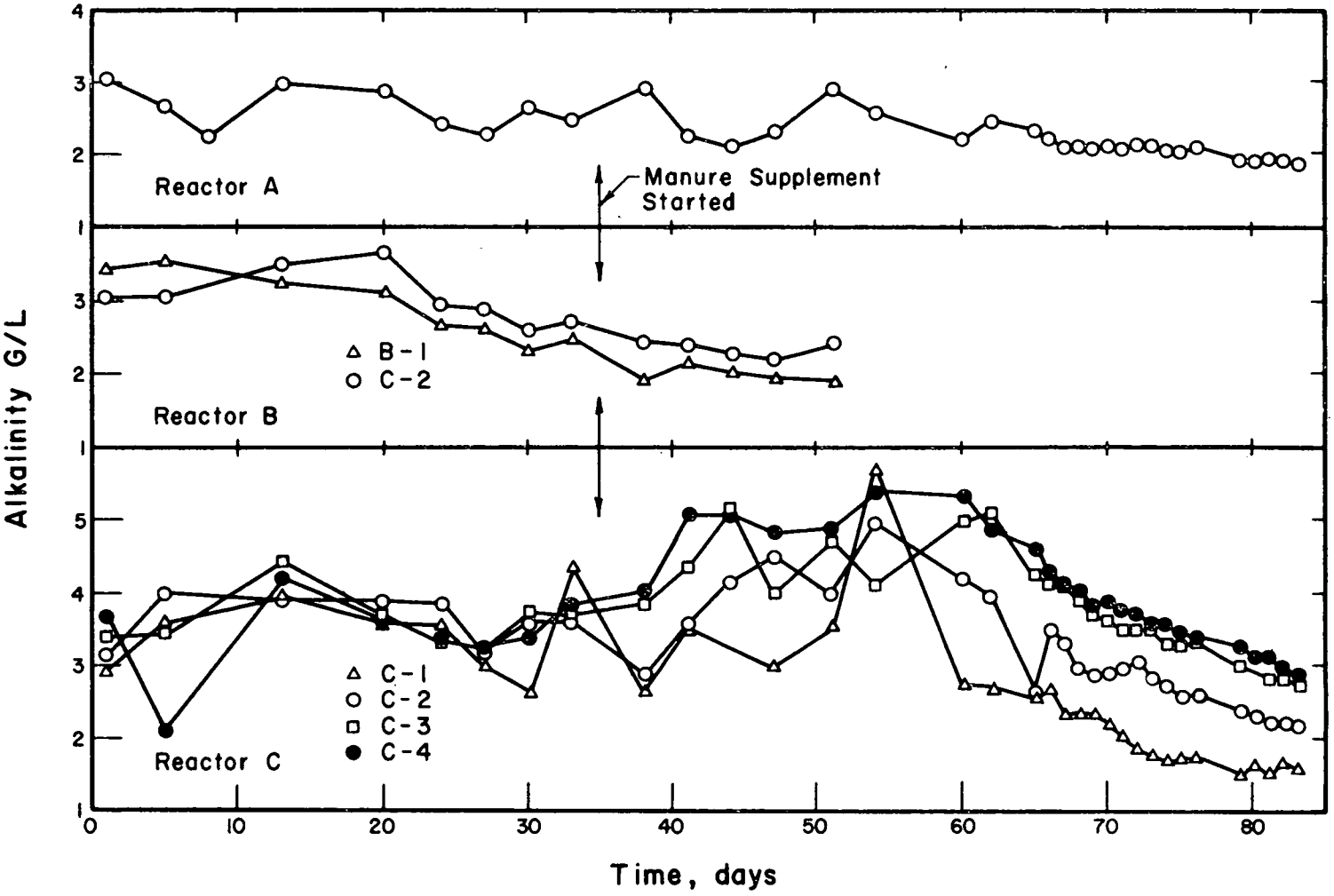

Figure 9. Variation in Alkalinity for Reactors A, B and C

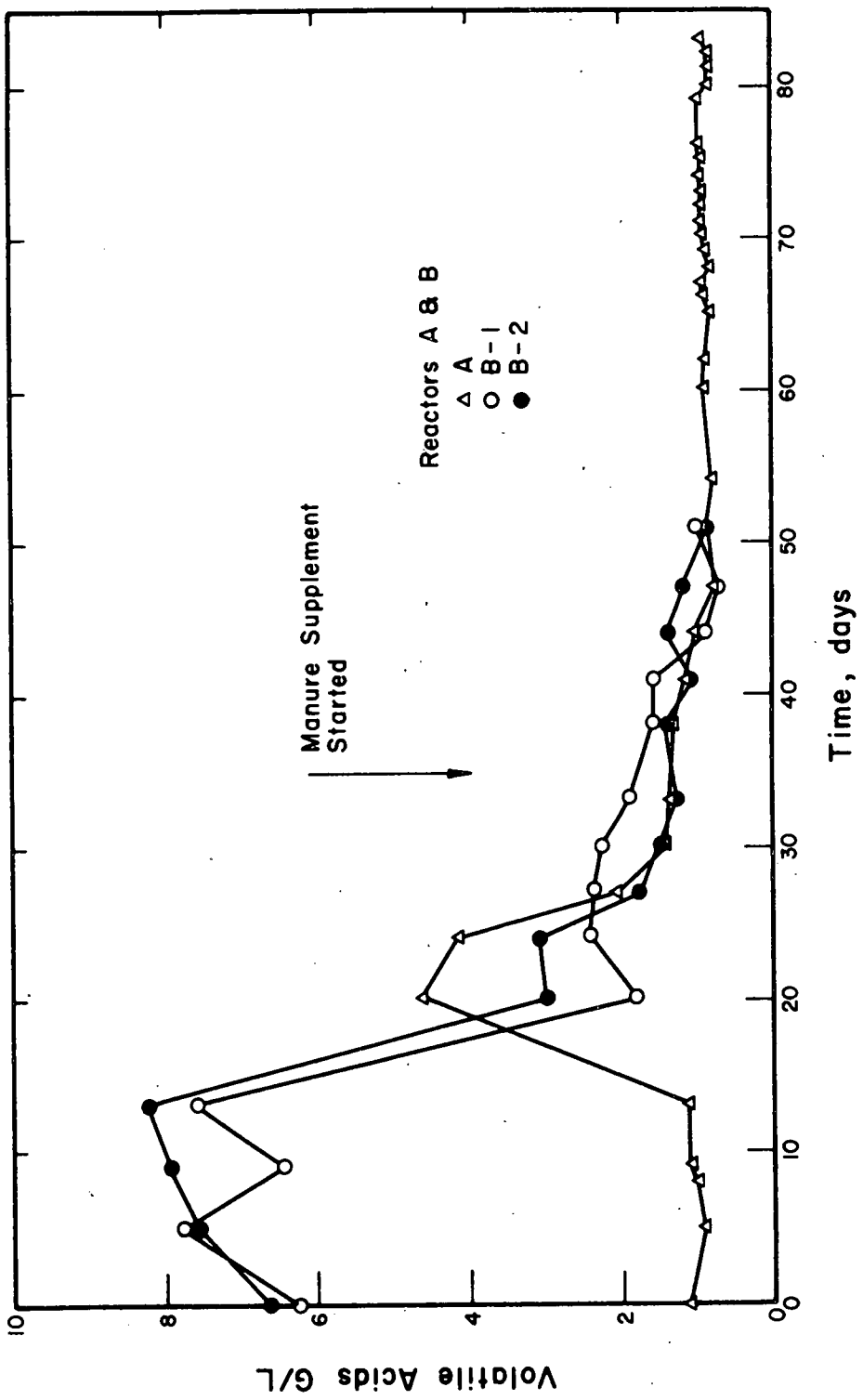

Figure 10. Variation in Volatile Acids for Reactors A and B

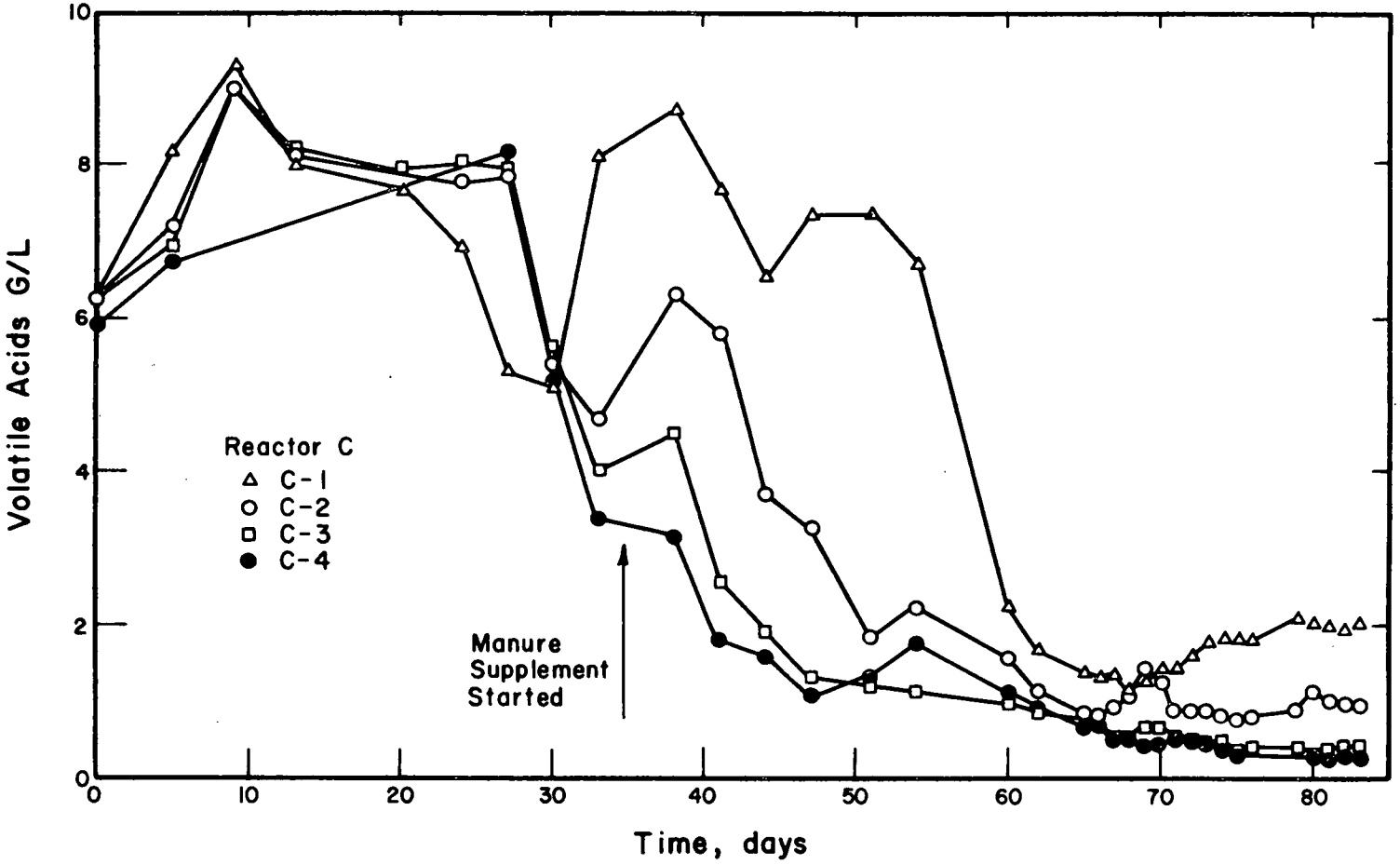

Figure 11. Variation in Volatile Acids for Reactor C

Initially both Reactors B and C required caustic in addition to that present in the feed slurry to prevent the pH from dropping to inhibitory levels. The volatile acids in Reactor B improved so that on Day 14, additional caustic was no longer required for pH control. On Day 33, the caustic addition to Reactor C was reduced to 10 g/day. By Day 54, it was no longer necessary to add any additional caustic to Reactor C.

Figure 7 shows the gas production from all three reactors. The gas from Reactor A was consistently in the range of 60 liters per day. The dip in gas production for Day 40 through 60 was a result of an inadvertent decrease in the feed solids concentration. Shortly after Day 50, the mixer shaft in Reactor B sheared in half due to a flaw in the material. Additional data could not be collected from this unit. During the period from Day 60 to 80, the gas production from Reactors A and C were reasonably constant, with C producing slightly more gas.

An intensive sampling period was initiated on Day 60. The average of 13 analysis for volatile solids concentration and COD is shown in Table 7. Using these data and the kinetic relationships $S_e/S_o = 1/(1 + K\theta)$, the rate constant K for each chamber was calculated. The total volatile solids destruction and COD reduction were higher in Reactor C than in Reactor A. In fact, the effluent from the second stage of Reactor C with a total retention time of 7.32 days was superior to the effluent from Reactor A with a 14.63 day retention time.

As shown in Table 8, the gas production in m^3/kg volatile solids fed and m^3/m^3 of reactor volume was greater in Reactor C than in Reactor A. As would be expected, the gas production per kg of volatile solids destroyed

Table 7. COD and Volatile Solids Reduction

Reactor	θ Days	Vol. Solids - g/l		COD - g/ℓ		K - Day^{-1}	
		S_o	S_e	S_o	S_e	Vol. Sol.	COD
A	14.63	41.5	10.4	51.0	15.2	0.197	0.162
C-1	3.66	41.5	19.5	51.0	25.2	0.309	0.278
C-2	3.66	19.3	10.4	25.2	14.3	0.234	0.210
C-3	3.66	10.5	9.2	14.3	12.4	0.039	0.042
C-4	3.66	9.2	9.0	12.4	12.2	0.003	0.004

Table 8. Gas Production

Reactor	m^3/m^3-Day	m^3/kg V.S. Fed	m^3/kg V.S. Destroyed
A	1.3	0.456 (7.32 SCF)	0.75
C	1.5	0.517 (8.30 SCF)	0.77

Table 9. Methane Production - 1 pd

Reactor	Measured	Theor. (COD Red.)
A	37.0	36.89
C	41.3	40.32

is constant. The methane production is given in Table 9. The measured methane production is in close agreement with the theoretical gas production as calculated from COD reduction. Also, based on COD reduction, 66.3 percent of the total methane from Reactor C was produced in the first stage. The methane produced in the second stage was 28.4 percent of the total.

The results of this run clearly show that if a balanced population of organisms can be maintained in the initial stage, multi-stage fermentation is more efficient than a complete mix system. Also, these results suggest that a retention time of 3.66 days in a complete mix system may be too short a time for efficient substrate utilization. The volatile acids in Reactor C-1 were at no time less than 1000 mg/l. Volatile acids of this magnitude are uncharacteristic of a balanced microbial system. Since volatile acids are an indication of solids hydrolysis, it would appear that the limitation was with the methanogenic bacteria.

3. Run No. 3.

For this run the systems were operating at a reduced retention time of 10 days. The feed slurry, as shown in Table 4, is of approximately the same feed solids concentration as Run 2, but with an increased amount of beef manure. In order to ease the shock of adding substrate to Reactor C, each reactor's feed volume was halved and added at 12 hour intervals. Also, in order to avoid temperature shock, the feed slurry was heated to 60°C prior to addition to the reactors.

In general, the systems responded well to the reduced retention time. The daily gas production, shown in Figure 12, was reasonably constant during the first 15 days. From then on, a steady decrease in the gas produced by Reactor A was observed. The pH and volatile acids (Figure 13 and 14) did not change significantly during this period. There was no ready explanation for this decrease in gas production. A check on the nitrogen and phosphorus level in each reactor proved interesting.

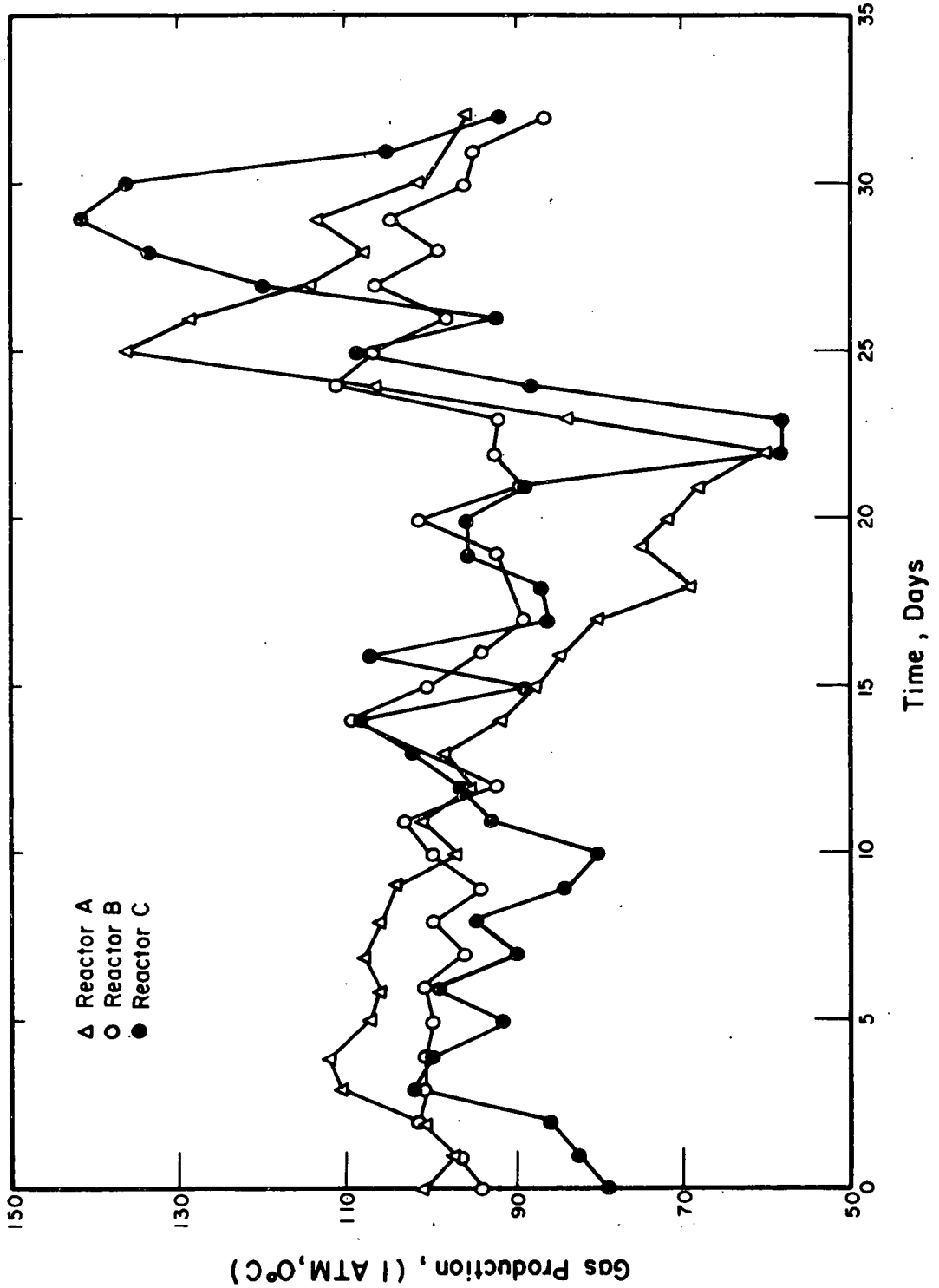

Figure 12. Gas Production from Reactors A, B and C

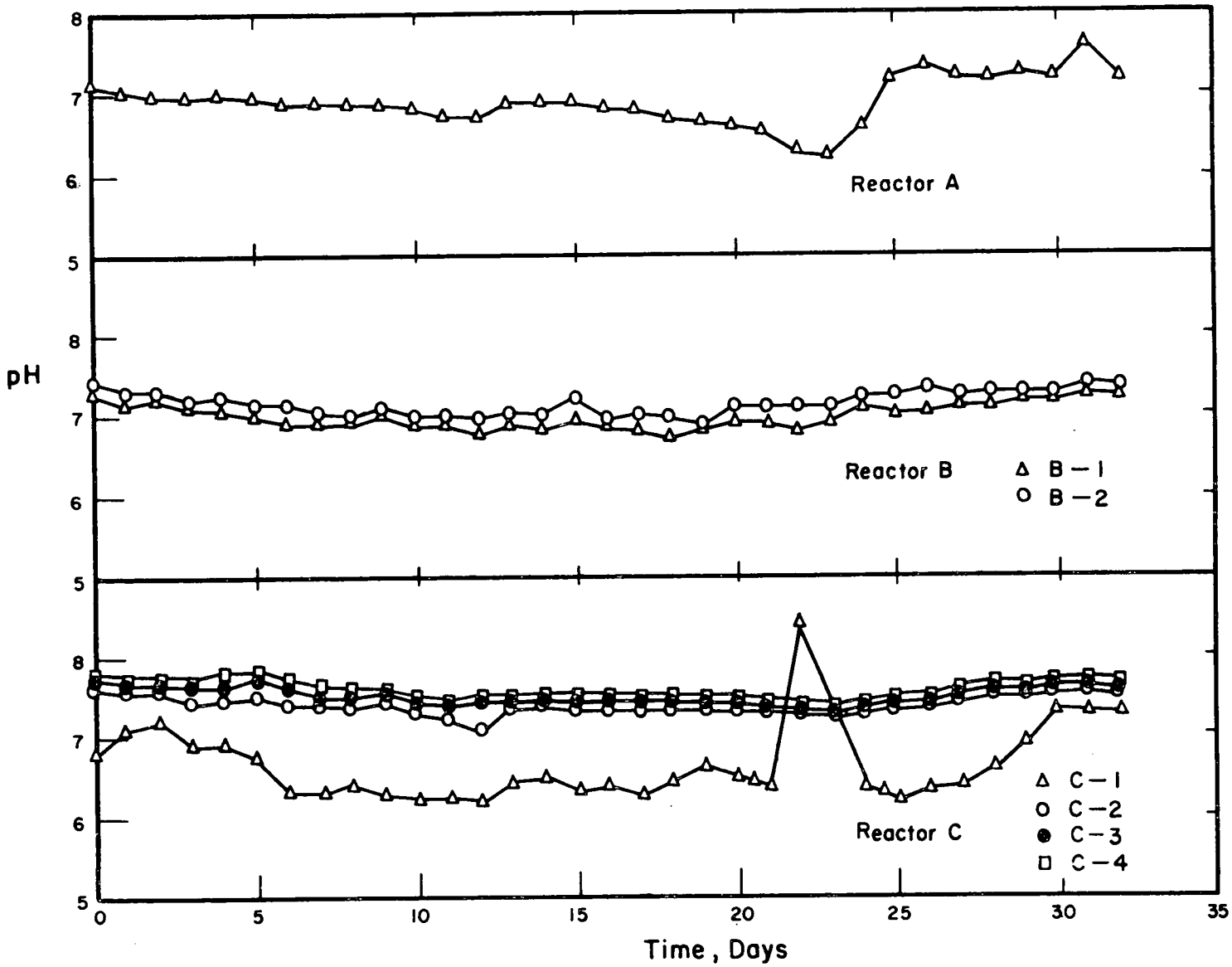

Figure 13. Variation in pH for Reactors A, B and C

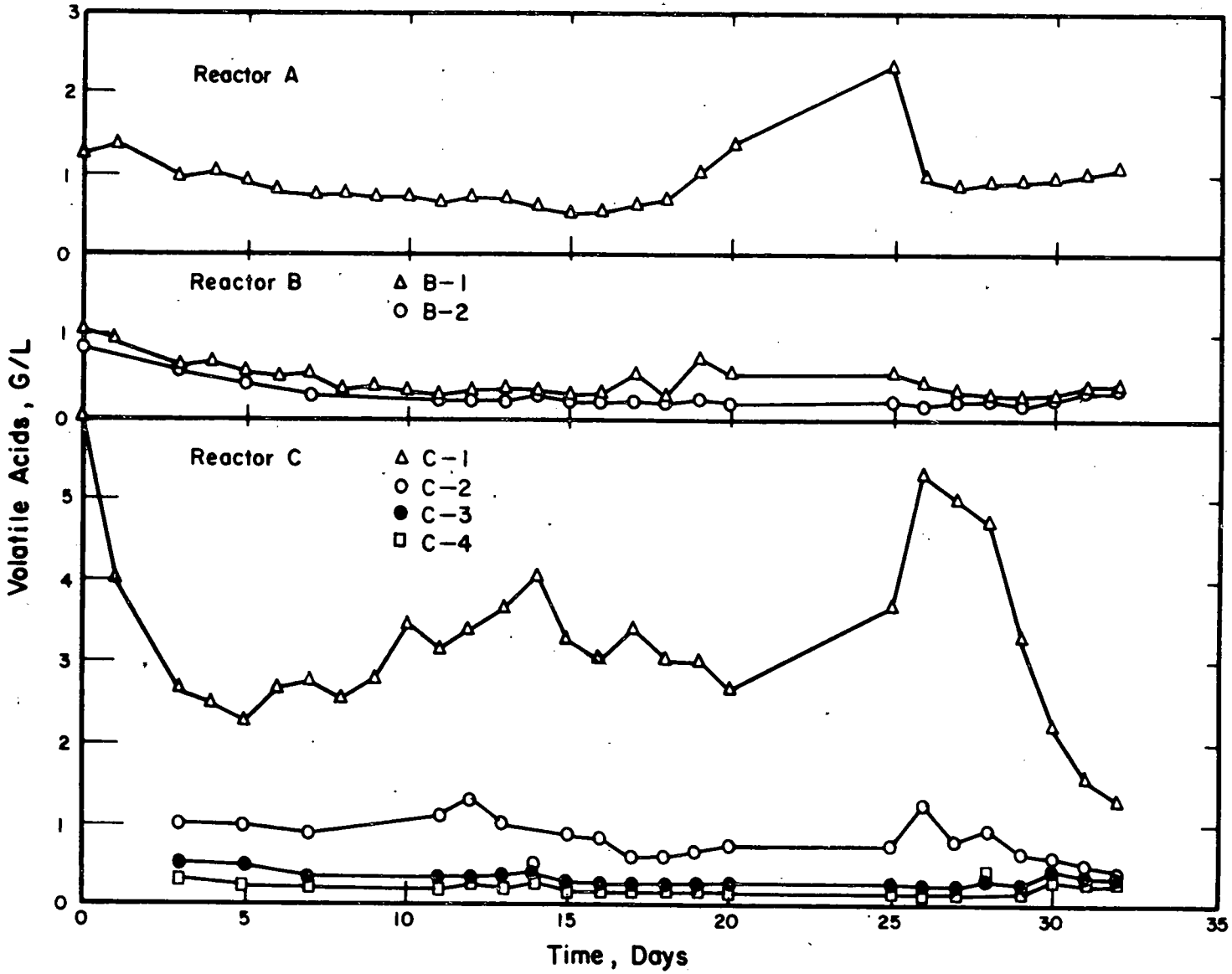

Figure 14. Variation in Volatile Acids for Reactors A, B and C

Phosphorus was found to be adequate in all reactors. However, the ammonia nitrogen in Reactor A was zero. In Reactors B and C, the ammonia nitrogen was less than 100 mg/l.

The cause of this low nitrogen was traced to the manner in which the feed was handled. The calcium oxide and sodium hydroxide added to the unbuffered feed slurry raised the pH to approximately 10.5. Storage of the feed in the hot room for 12 hours prior to adding to the reactors allowed a significant portion of the ammonia nitrogen to volatilize. This deficiency was not as apparent in Reactors B and C. Correction of this problem by adding the nitrogen and phosphorus directly into the reactors resulted in an immediate improvement in the gas production from Reactor A.

On Day 21, Reactor C received the entire feed volume in a single feeding. The pH increased to over 8.0 in the first chamber, resulting in an inhibition of the microorganisms. This caused a marked increase in the volatile acids. Resumption of the normal feed procedure with increased nitrogen substantially improved the operation of all three reactors. During the period of Day 25 to Day 33, the gas production increased significantly in all reactors. The volatile acids dropped to 1000 mg/l or lower in all reactors except C-1. Here the acids remained slightly above 1000 mg/l.

The alkalinity is shown in Figure 15. This parameter closely correlated with the volatile acid concentration as would be expected. Higher acids resulted in lower alkalinities.

The conversion efficiency of the reactors were evaluated on the basis of COD and volatile solids reduction. These data are shown in Table 10. Data collected during Day 15 to 25 were not included in these

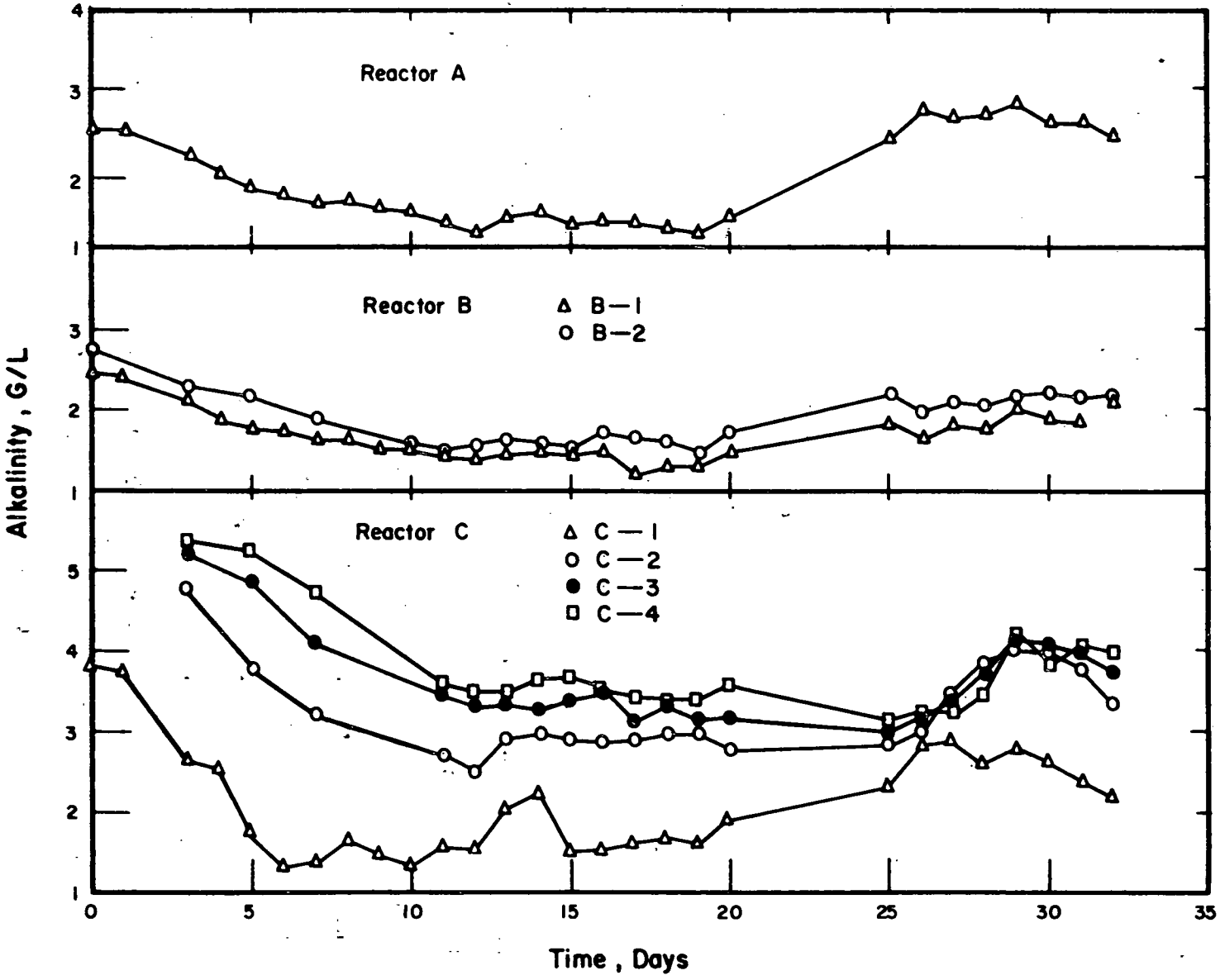

Figure 15. Variation in Alkalinity for Reactors A, B and C

Table 10. COD and Volatile Solids Reduction

Reactor	θ days	Vol. Solids - g/ℓ		COD g/ℓ		K - Day^{-1}	
		S_o	S_e	S_o	S_e	Vol. Sol.	COD
A	10	38.0	13.1	53.0	17.4	0.190	0.205
B-1	5	38.0	11.5	53.0	15.8	0.461	0.471
B-2	5	11.5	10.9	15.8	15.3	0.011	0.007
C-1	2.5	38.0	21.6	53.0	31.4	0.304	0.275
C-2	2.5	21.6	9.7	31.4	14.6	0.491	0.460
C-3	2.5	9.7	9.6	14.6	13.6	0.004	0.029
C-4	2.5	9.6	9.6	13.6	13.5	0	0.003

averages due to the nitrogen deficiency problem. A rate constant, K, was calculated using the COD and volatile solids data. Reactor C-2, followed by B-1, had the highest rate constants. In Run 2, where the total retention time was 14.63 days, Reactor C-1 has a higher rate constant than C-2. This may be due to the following. As suggested in Run 2, Reactor C-1 could not maintain a balanced microbial system with a retention time of 3.66 days. In this run, with the retention time being reduced to 2.5 days, the system was further stressed. This resulted in less efficient substrate utilization than experienced at 3.66 days. Therefore, a higher volatile solids and COD concentration were available for utilization in Reactor C-2.

The most interesting results are the COD and volatile solids level from Reactor A, B-1 and C-2. With a retention time of 2.5 days in each stage, the first two stages of Reactor C produced a lower effluent

COD and volatile solids than Reactor B-1, a complete mix system with a 5 day retention time. The effluents from both B-1 and C-2 were superior to Reactor A, a complete mix reactor with a 10 day retention time.

As shown in Table 11, the gas production in m^3/kg volatile solids fed and m^3/m^3 of reactor volume was slightly greater in Reactor C than in Reactor B. Reactor A, the complete mix system, had the lowest gas production of all three reactors. As expected, the gas production per kg volatile solids destroyed was relatively constant. The methane production is given in Table 12. The measured methane production, for Reactors A and B, is in close agreement with the theoretical gas production as calculated from the COD reduction. However, the measured gas production for Reactor C was about 8 percent low. It appears there was an error in the calibration of the wet test meter.

Table 11. Total Gas Production

Reactor	m^3/m^3-Day	m^3/kg V.S. Fed	m^3/kg V.S. Destroyed
A	2.18	0.576	0.89
B	2.29	0.602	0.85
C	2.31	0.609	0.82

Table 12. Methane Production - lpd

Reactor	Measured	Theor. (COD Red.)
A	52.3	54.2
B	55.4	56.3
C	55.4	60.0

The results of this run are compatible with those from Run 2. That is, if a balanced population of organisms can be maintained in the initial stage, multi-stage fermentation is more efficient than a complete-mix system. These data also indicate that a retention time of 5 days, in a complete-mix system, is sufficient for efficient substrate utilization. After Reactor B-1 had apparently recovered from the nitrogen deficiency, the volatile acids did not exceed 500 mg/l after Day 25. Volatile acids of this level are indicative of a balanced microbial system. Additional substrate utilization occurring in the second stage was minimal.

4. Run No. 4

This run was initiated with the intention of stressing Reactor C until complete failure occurred. This was accomplished by reducing the total retention time by intervals of 2 days and monitoring each systems response. The feed slurry composition for this run, as shown in Table 4, is identical to that used in Run 3. One day prior to Day 0, the feed schedule was changed from two daily feedings at 12 hour intervals, to one feeding per day while maintaining the same 10 day retention time. Unlike the inhibitory response encountered in Run 3, Reactor C was able to operate under these conditions. This may have been due to the following. First of all, it appeared that the microbial system in Reactor C-1 may have been stressed due to the nitrogen deficiency problem. Secondly, the mixing in Reactor C-1 appeared to be more adequate during this run. Poor material selection for the mixing equipment resulted in corrosion to the extent that 3 of 4 impeller blades had broken off in Reactor C-1. These blades may have been positioned in such a way in the reactor as to interfere with the mixing. The mixer was repaired prior to the start of Run 4.

During this period, it was evident that Reactor C's microbial system was stressed. On Day 1, after the hot room had reached a temperature of 61°C for only a short time, the daily gas production from Reactor C decreased approximately 33 percent (see Figure 16). The daily gas production shown in Figure 16 does not reflect the correction factors for the gas meters after they were recalibrated. Reactors A, B, and C are respectively 8.0, 14.4 and 4.0 percent too high. Reactor C recovered within 3 days, and on Day 4, the total retention time was reduced to 8 days. During this period, from Day 4 to 24, Reactor C was fed 1/2 the total feed volume at 12 hour intervals while Reactors A and B received the total feed volume in a single feeding. As before, the feed slurry was heated to 60°C prior to addition to the reactors.

In general, the systems responded well to the reduced retention time. After an initial adjustment period, the daily gas production remained reasonably constant. The pH, volatile acids and alkalinity (Figure 17, 18, and 19) did not change significantly during this period.

The COD and volatile solids reduction are shown in Table 13. These values are based on an average of 3 days of data. Therefore, too much emphasis should not be placed on their accuracy. However, they are very interesting. Based on both COD reduction and volatile solids destruction, Reactor C-1 had a higher rate constant than C-2. Each reactor operated at a retention time of 2 days. This is contridictory to results from Run 3 where Reactor C-2 had a higher rate constant than C-1 when operating at a retention time of 2.5 days per reactor.

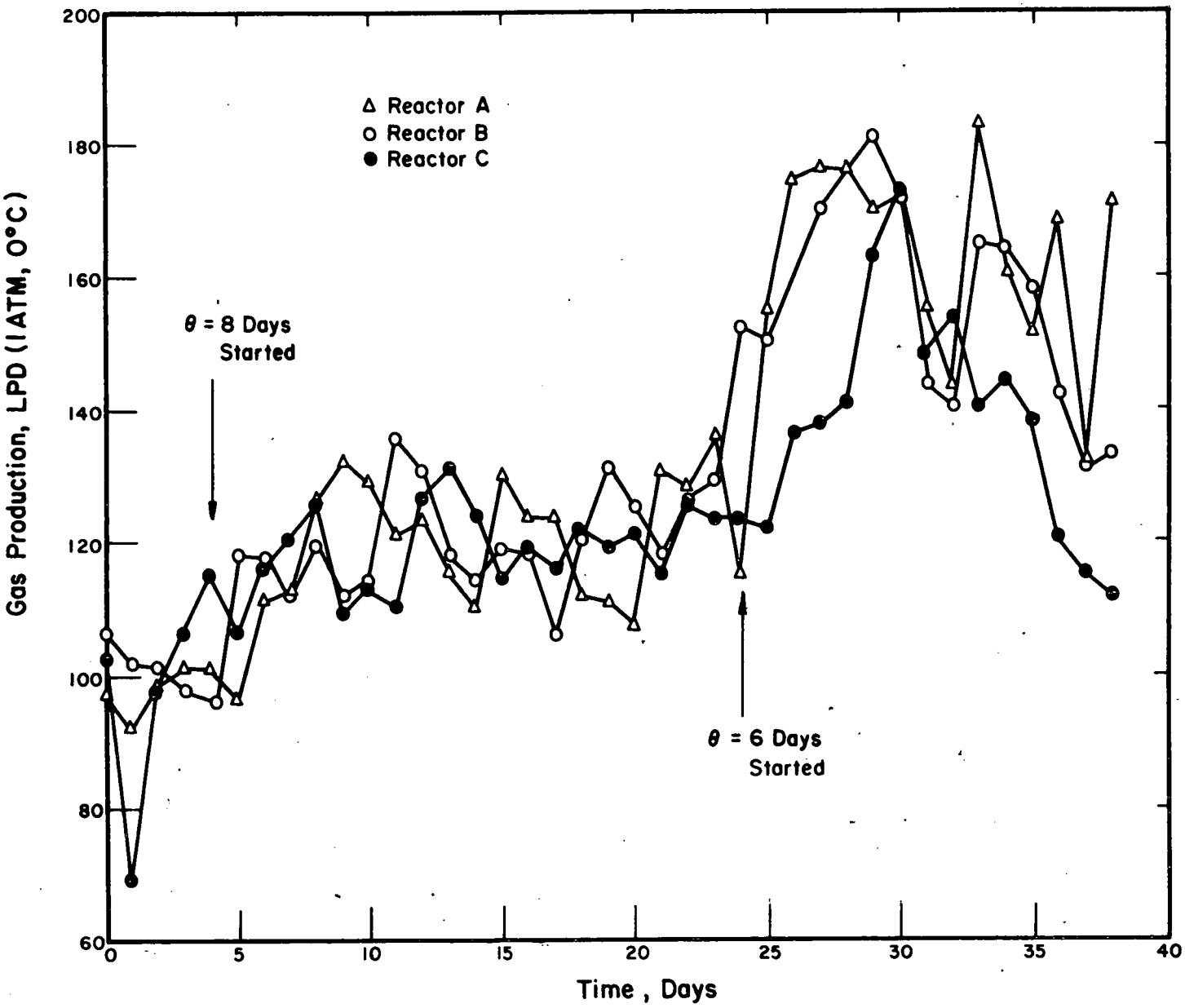

Figure 16. Gas Production from Reactors A, B and C

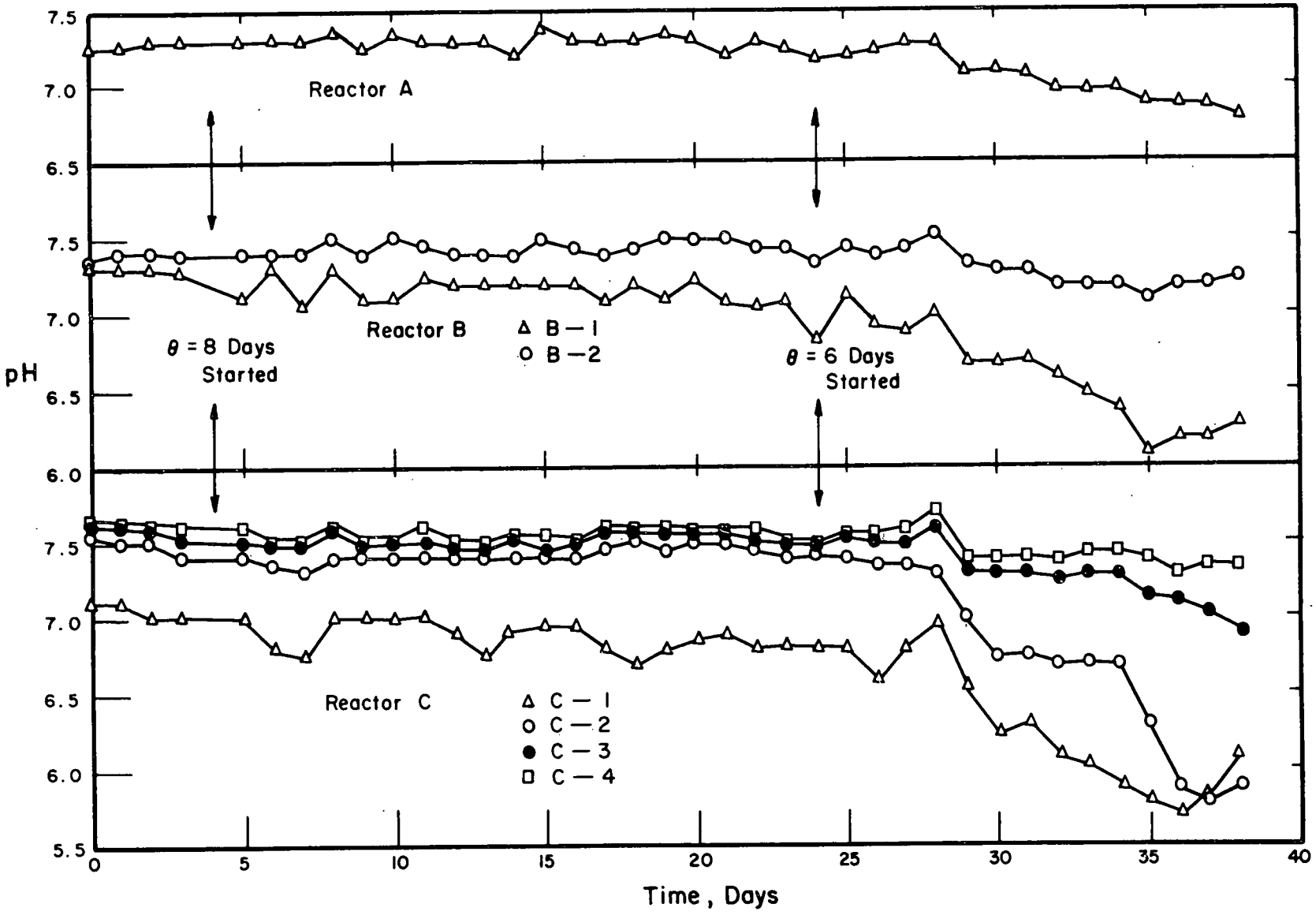

Figure 17. Variation in pH for Reactors A, B and C

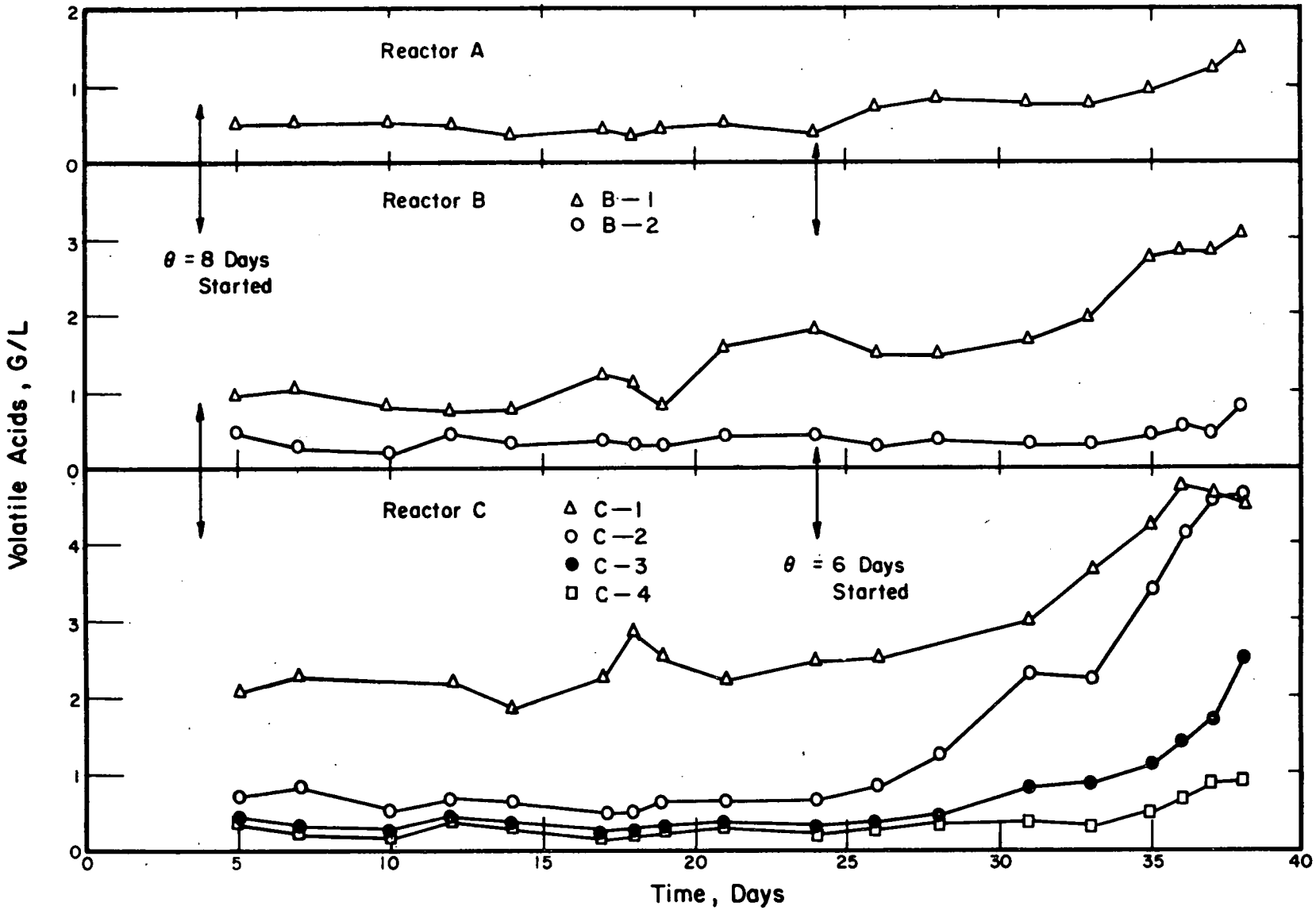

Figure 18. Variation in Volatile Acids for Reactors A, B and C

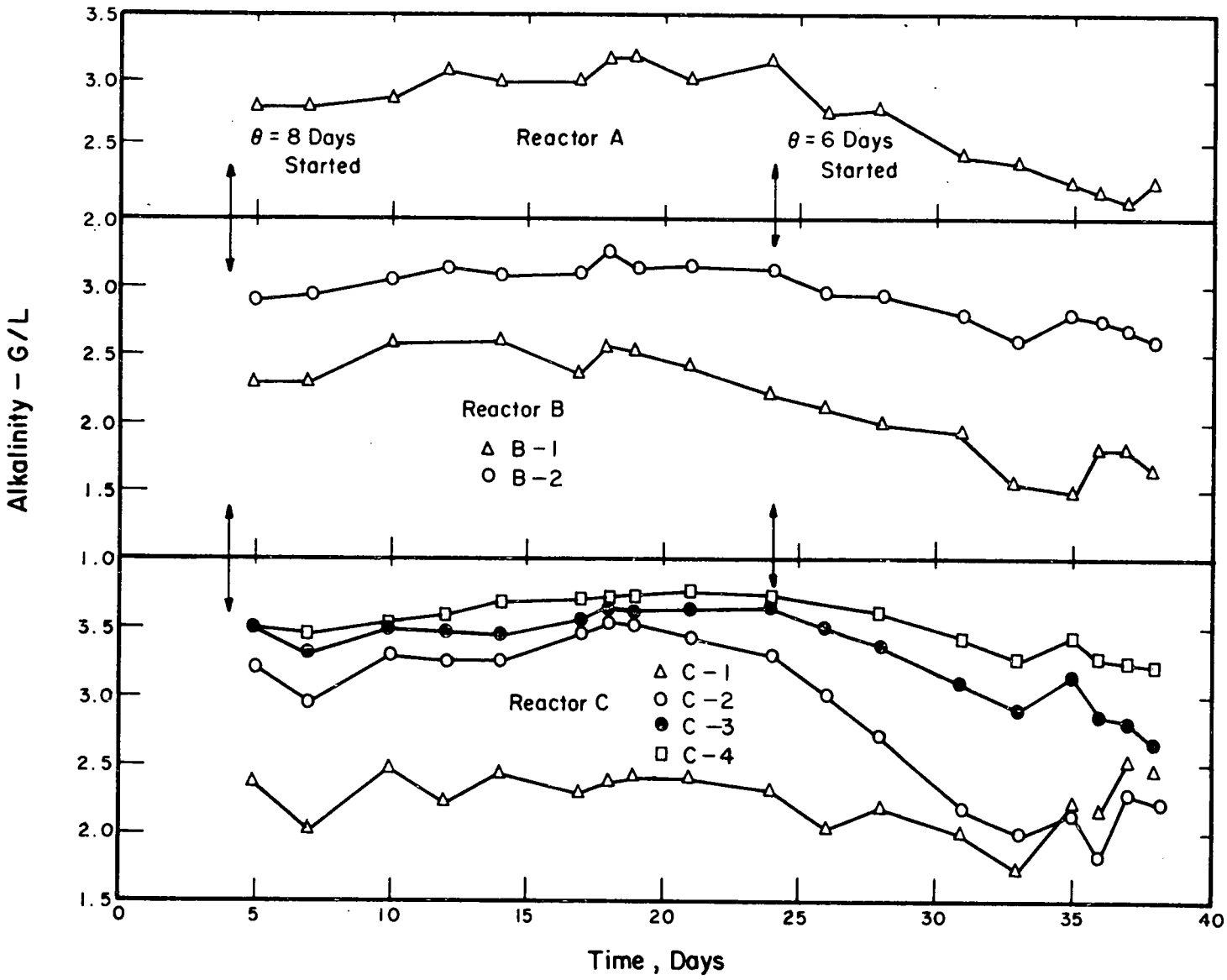

Figure 19. Variation in Alkalinity for Reactors A, B and C

A re-check of the laboratory data from Run 3 provided a ready explanation for this discrepancy. The majority of the data used to calculate the values in Table 8 were taken prior to our discovery of the nitrogen deficiency problem. Although it was not apparent that there was a nitrogen deficiency problem with Reactor C during Run 3, these data strongly indicate that was the case. Based on data from the last 4 days of Run 3 (see Table A-5 and A-6), the calculated rate constant for Reactors C-1 and C-2 are respectively, 0.509 and 0.212 day^{-1} for volatile solids reduction and 0.446 and 0.249 for COD reduction. It appears that Reactor C had sufficiently recovered from the nitrogen deficiency problem by this time. Reactor C-1 had a higher rate constant than C-2 as indicated by Run 4.

The data from Run 4 also show that with a retention time of 2 days in each stage, the first two stages of Reactor C produced a lower effluent COD and volatile solids than Reactor B-1, a complete-mix system with a 4 day retention time. The effluent from Reactor C-2 was superior to both Reactors B and A, each having a total retention time of 8 days. Based on both COD and volatile solids concentrations, Reactor A produced a slightly better effluent than Reactor B, a two-stage system with a 4-day retention time per reactor. However, the additional substrate utilization occurring in the second stage of Reactor B was minimal.

The gas production is given in Table 14. Based on the gas production per kg volatile solids destroyed, data for Reactors B and C appear to be low. These values are expected to be relatively constant. This may have been due to the following. Each gas meter was re-calibrated at the end of the run. Although these correction factors were applicable for data collected at the end of the run, they were inaccurate for correcting data during this period. As the off gas from each reactor

Table 13. COD and Volatile Solids Reduction[1]

Reactor	θ Days	Vol. Solids - g/l		COD - g/l		K - Day^{-1}	
		S_o	S_e	S_o	S_e	Vol. Sol.	COD
A	8	36.7	12.9	56.6	13.8	0.230	0.388
B-1	4	36.7	13.2	56.6	18.6	0.445	0.511
B-2	4	13.2	13.1	18.6	16.6	0.002	0.030
C-1	2	36.7	15.3	56.6	22.3	0.699	0.769
C-2	2	15.3	11.0	22.3	13.6	0.195	0.320
C-3	2	11.0	10.8	13.6	12.4	0.009	0.048
C-4	2	10.8	11.0	12.4	12.4	0.0	0.0

[1]Average of 3 days of data

Table 14. Total Gas Production

Reactor	m^3/m^3-Day	m^3/kg V.S. Fed	m^3/kg V.S. Dest.	Methane-lpd
A	2.61	0.533	0.846	59.8
B	2.31	0.490	0.753	59.2
C	2.62	0.555	0.780	59.6

passes through the gas meter, the liquid level in the meter increases due to the condensation of water vapor in the gas stream. An increase in the liquid level would result in a gas reading greater than that actually produced.

On Day 24, the total retention time in each reactor was reduced to 6 days. For the duration of the run, Reactors B and C were fed 1/2 the feed volume at 12 hour intervals while Reactor A received the total feed volume in a single feeding. As shown in Figure 16, there was an immediate improvement in the daily gas production from Reactors A and B. However, Reactor C required a longer period of time to adjust to this increased loading. By Day 30, the daily gas production from each reactor was approximately equal. The increase in gas production from Reactor B on Day 24 was the result of B receiving a 67 percent increase in the feed volume the previous day.

On Day 30, a blown fuse caused the temperature in the hot room to drop to 48°C for approximately 12 hours. Although it appeared that Reactor C was responding favorably to this reduced retention time, it was evident that C's microbial system was severely stressed. Figures 16 through 19, show that Reactor C was not able to recover from this temperature shock.

The pH and volatile acids data (Figure 17 and 18) show an interesting trend in Reactor C. From Day 31 to 36, the pH in Reactor C-1 decreased from 6.3 to 5.7. After Day 36, the pH began to increase. The pH in Reactor C-2 also decreased during this period and by the end of the run was less than the pH in Reactor C-1. During this same period, the volatile acids in Reactor C-1 increased from 2990 mg/l to 4730 mg/l. After

Day 36, the volatile acids began to decrease. The volatile acids in Reactor C-2 also increased during this period and by the end of the run were greater than the volatile acids in Reactor C-1.

It appears that the temperature shock had affected the microbial system in Reactor C by inactivating the methanogenic bacteria. As a result, the organic acids produced by the hydrolysis of solids were not utilized by the methanogenic bacteria and the volatile acids increased. The 1.5 day retention time per reactor was too short to allow these bacteria time to recover and the volatile acids continued to increase. Eventually, the volatile acids in Reactor C-1 were sufficiently high enough that its microbial system was "pickeled". As the feed schedule was continued, the volatile acids in Reactor C-1 began to decrease as they were washed out. Reactor C-1 was in effect becoming a holding chamber for the feed slurry. This sequence of events was observed progressing from one chamber to the next. Given sufficient time, the entire contents of Reactor C would have become "pickeled".

The alkalinity is shown in Figure 19. This parameter closely correlates with the volatile acids concentration. Higher acids resulted in lower alkalinities as expected.

Although the volatile acids had increased to slightly greater than 3000 mg/l in Reactor B-1, Reactor B did not fail from the temperature shock. With a retention time of 3 days per reactor, the methanogenic bacteria had sufficient time to recover. Reactor A, a complete-mix system with a retention time of 6 days, with the more stable of the three reactors. This had also been the case in previous runs whenever the systems were stressed. However, the volatile acids in Reactor A had

increased to approximately 1500 mg/l by the end of the run. Volatile acids of this magnitude are uncharacteristic of a balanced microbial system. A check in the nitrogen level in Reactor A showed a concentration of 850 mg/l as NH_3-N. Ammonia nitrogen concentrations of this level are reportedly non-toxic to microbial systems. It appears that some micronutrient may have been lacking in all reactors.

The COD and volatile solids reduction are shown in Table 15. These data are not averages and may reflect some experimental error. The stability of Reactor A is shown here by the consistency of both the COD and volatile solids data. Reactor B-1 shows a general decrease in the amount of COD and volatile solids reduced whereas, Reactor B-2 shows a general increase. However, the overall COD and volatile solids reduction was relatively constant. The results from Reactor C are interesting

Table 15. COD and Volatile Solids Reduction

Reactor	θ Days	% V.S. Reduction (% COD Reduction)			
		Day - 35	Day - 36	Day - 37	Day - 38
A	6	54.6 (61.0)	57.7 (63.4)	59.6 (60.3)	58.0 (61.9)
B-1	3	49.6 (41.8)	48.9 (53.2)	43.9 (45.4)	36.1 (37.2)
B-1	3	11.9 (24.9)	11.6 (14.9)	21.1 (20.7)	26.1 (29.3)
Total		61.5 (66.7)	60.5 (68.1)	65.0 (66.1)	62.2 (66.5)
C-1	1.5	3.8 (27.7)	2.9 (8.9)	7.1 (3.9)	0.0 (0.0)
C-2	1.5	46.3 (31.6)	39.4 (34.5)	32.1 (33.8)	29.9 (16.7)
C-3	1.5	12.1 (6.0)	17.1 (21.0)	21.1 (22.5)	28.7 (47.5)
C-4	1.5	1.4 (6.6)	1.9 (4.3)	2.6 (6.8)	4.3 (1.5)
Total		63.6 (71.9)	61.3 (68.7)	62.9 (67.0)	62.9 (65.7)

and tend to support the explanation for the failure. In Reactor C-1, the COD and volatile solids reduction decreased until virtually no biological activity was occurring, i.e., the COD and volatile soldis concentrations were equal to those of the feed slurry. This resulted in an increase of the organic loading to Reactor C-2. As the microbial system in Reactor C-2 became "pickeled", the organic loading to Reactor C-3 increased, and so on.

The gas production is given in Table 16. The gas production from Reactor A was relatively constant. The gas production per kg volatile solids destroyed for both Reactors B and C show a decreasing trend.

Table 16. Total Gas Production

Reactor		Day - 35	Day - 36	Day - 37	Day - 38
A	m^3/m^3-day	3.24	3.61	2.82	3.67
	m^3/kg V.S. Fed	0.459	0.512	0.401	0.522
	M^3/kg V.S. Dest.	0.837	0.883	0.672	0.902
	Methane-lpd	68.9	86.0	57.4	79.9
B	m^3/m^3-day	3.19	2.87	2.63	2.68
	m^3/kg V.S. Fed	0.452	0.407	0.373	0.380
	m^3/kg V.S. Dest.	0.736	0.675	0.572	0.615
	Methane-lpd	72.4	68.2	61.1	61.3
C	m^3/m^3-day	3.08	2.69	2.56	2.48
	m^3/kg V.S. Fed	0.437	0.381	0.363	0.353
	m^3/kg V.S. Dest.	0.690	0.623	0.575	0.566
	Methane-lpd	69.6	62.1	64.3	58.9

These values are expected to be constant. Although the first two chambers in Reactor C showed severe inhibition of organic stabilization, Reactor C-4 had not yet become too affected by the temperature shock. Therefore, the overall volatile solids destruction did not change significantly. In Reactor B, the volatile solids destruction resulted from the hydrolysis of the solids to volatile compounds that were lost when the solids analyses were performed.

The rate constants, K, for each reactor are given in Table 17. In Reactor A, the K value for volatile solids reduction and COD reduction are essentially equal. Reactor B shows a higher rate for volatile solids destruction in the first stage than for COD reduction. The K value for COD reduction was higher in the second stage of Reactor B. This same relationship is shown by Reactor C in the second and third stage. It would appear that the rate of COD reduction (methane fermentation) is inhibited by conditions not having a similar effect on the rate of solids hydrolysis.

It was only a matter of time before Reactor C failed completely. The rate constants for the first stage were essentially zero. In the second stage, the COD reduction constant was less than in the third stage. Based on the short period of operation, it would appear that failure is progressing through the reactor.

Table 17. COD and Volatile Solids Reduction[1]

Reactor	θ Days	Vol. Solids - g/l		COD - g/l		K - Day^{-1}	
		S_o	S_e	S_o	S_e	Vol. Sol.	COD
A	6.0	42.1	17.9	50.7	21.8	0.225	0.221
B-1	3.0	42.1	23.3	50.7	31.5	0.269	0.203
B-2	3.0	23.3	15.9	31.5	18.8	0.155	0.225
C-1	1.5	42.1	40.7	50.7	50.9	0.023	-
C-2	1.5	40.7	25.1	50.9	34.5	0.414	0.317
C-3	1.5	25.1	16.8	34.5	20.7	0.329	0.444
C-4	1.5	16.8	15.7	20.7	18.0	0.047	0.010

[1]Average of 4 days of data

5. Summary of Rate Constants

The rate constants calculated for the first stage complete-mix reactors are given in Table 18. The variation in these constants can be attributed to two primary factors. First, the stress applied to the biological populations as evidenced by high organic acids and low pH reduce the rate at which the solids are hydrolyzed and the COD stabilized. The calculated constants for these reactors have lower values.

The effect of retention time on the rate constant is very great. This would appear to result from the complex nature of the substrate. In the short retention times, the easily degraded organic material was rapidly stabilized. However, with the longer retention times, the microorganisms had to use more resistant substrates. This resulted in a decrease in the rate constant.

Table 18. Variation of K with Retention Time

θ Days	K - Day^{-1} Vol. Solids	COD
14.6	0.197	0.162
10.0	0.190	0.205
10.0	0.289	0.252
8.0	0.238	0.388
6.0	0.225	0.221
5.0	0.461	0.471
4.0	0.445	0.511
3.7	0.309	0.278
3.3*	0.277	0.095
3.0	0.269	0.203
2.5	0.509	0.446
2.5*	0.304	0.275
2.0	0.699	0.769
1.5	0.023	0

*Biological population stressed - High volatile acids

These data clearly show that this kinetic relationship is not valid since K is not constant. Because of the complex nature of the substrate, it is doubtful if any kinetic model can be adequately fitted to these data. It would appear that no single substrate component can be identified as the limiting substrate. At the very short retention times, hydrolysis of certain fiber constituents is rate limiting. At longer retention times, breakdown of the more complex fibers appear to be the rate limiting step. The lack of a defined limiting substrate significantly complicates the kinetic modeling.

SUMMARY

The results of this study clearly show that if a balanced population of organisms can be maintained in the initial stage, multi-stage fermentation is more efficient than a complete-mix system. However, if the system is stressed, failure of the multi-stage system is more rapid. When the first stage is not inhibited due to a short retention time, the additional waste stabilization is minimal. With a balanced microbial system in the first stage, it would appear that the optimum retention time in the first stage is between 2.0 and 3.0 days when operating at 60°C temperature.

Consequently, care must be exercised in the design of these units. If the objective is to maximize the conversion of solids to methane, a staged system will produce more methane per unit volume of reactor for a given quantity of substrate. If the objective is to maximize methane production per unit volume of reactor, a single stage reactor operating at near optimum retention time is required.

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APPENDIX

A. Total gas production per kg volatile solids destroyed. Assume the volatile solids to be 100 percent cellulose ($C_6H_{10}O_5$).

$$C_6H_{10}O_5 + HOH \rightarrow 3CO_2 + 3CH_4 \qquad (1)$$

$$\text{Total Gas} = \frac{6 \text{ moles}}{\text{mole cellulose}} \times \frac{1 \text{ mole cellulose}}{162 \text{ grams}} \times \frac{6.23 \times 10^{-2} \text{ m}^3}{\text{gram-mole}}$$

$$= 8.3 \times 10^{-2} \text{ m}^3/\text{gram}$$

$$= .83 \text{ m}^3/\text{kg volatile solids destroyed (standard conditions)}$$

B. Theoretical methane production based on COD reduction.

$$C_6H_{10}O_5 + 6O_2 \rightarrow 6CO_2 + 5HOH \qquad (2)$$

$$\text{COD of Cellulose} = \frac{6 \text{ moles } O_2}{\text{mole cellulose}}$$

$$\text{From Equations 1 and 2: } \frac{.5 \text{ moles } CH_4}{\text{mole } O_2} \times \frac{\text{lb.-mole } O_2}{32\text{-lbs.}} \times \frac{359 \text{ scf}}{\text{lb.-mole}}$$

$$= 5.61 \text{ scf Methane/lb. } O_2 \text{ destroyed}$$

Example: Reactor Volume = 50 liters

Retention Time = 10 days

Influent COD = 70 g/l

Effluent COD = 20 g/l

lbs COD destroyed per day:

$$\frac{(70\text{-}50)\text{g/l} \times 50 \text{ liters}}{10 \text{ days}} \times \frac{1\text{-lb.}}{454 \text{ grams}} = 0.55 \text{ lbs COD/day}$$

Theoretical Methane Production:

$$= \frac{0.55 \text{ lbs. COD}}{\text{day}} \times \frac{5.61 \text{ scf}}{\text{lb. } O_2} \times \frac{28.3 \text{ liters}}{\text{cf}}$$

$$= 87.3 \text{ liters/day}$$

Table A-1. Effluent Volatile Solids (g/l) - Run 1[1]

Date	Feed	A	B-1	B-2	C-1	C-2	C-3	C-4
2/8/77	26.3	5.6	8.7	6.4	15.7	11.2	9.6	9.2
2/9/77	23.3	5.4	9.0	6.1	14.8	11.0	10.4	10.3
2/10/77	20.7	5.5	10.0	6.1	12.5	11.8	11.5	-
2/11/77	23.8	5.8	10.7	5.6	14.4	11.9	10.8	11.1
2/12/77	23.6	5.6	11.8	5.2	15.8	12.8	10.9	11.0
2/13/77	28.2	6.7	11.3	6.7	16.2	13.4	10.7	11.3
3/3/77	24.5	6.9	16.1	11.2	18.4	15.5	14.0	13.5
3/4/77	22.8	6.6	16.6	10.5	18.0	15.9	14.2	13.5
3/5/77	24.3	6.6	16.8	10.5	18.0	15.3	13.8	13.2
3/6/77	24.4	7.2	11.7	11.2	19.9	17.8	15.4	14.5
3/7/77	24.2	7.9	18.1	11.6	19.0	18.7	15.6	13.1

[1]For Run 1, Day 0 corresponds to January 15, 1977

Table A-2. Effluent COD (g/l) - Run 1

Date	Feed	A	B-1	B-2	C-1	C-2	C-3	C-4
2/23/77	34.8	6.0	28.0	29.0	34.0	34.0	32.0	31.0
3/3/77	27.5	8.0	20.2	20.3	24.4	25.5	26.6	26.5
3/5/77	24.9	8.7	21.2	16.9	22.2	21.4	22.0	20.0

Table A-3. Effluent Volatile Solids (g/l) - Run 2[1]

Date	Feed	A	C-1	C-2	C-3	C-4
5/25/77	41.8	10.1	18.5	10.3	9.3	9.6
5/26/77	42.6	10.3	20.0	11.7	9.4	9.2
5/27/77	41.5	10.8	18.9	11.0	9.0	9.1
5/28/77	41.5	10.7	18.8	10.3	9.0	8.6
5/29/77	40.8	10.3	18.4	9.8	9.1	9.0
5/30/77	41.6	10.7	18.3	9.8	9.5	9.3
5/31/77	41.6	10.3	18.3	10.0	8.9	8.9
6/1/77	39.5	10.3	18.0	9.9	9.2	8.5
6/2/77	42.8	10.5	18.3	10.0	9.0	8.7
6/3/77	42.0	10.3	18.3	10.0	9.0	8.7
6/7/77	39.3	11.0	22.7	10.9	9.3	9.3
6/8/77	42.4	10.9	23.3	11.1	9.6	9.6

[1]For Run 2, Day 0 corresponds to March 19, 1977

Table A-4. Effluent COD (g/l) - Run 2

Date	Feed	A	C-1	C-2	C-3	C-4
5/23/77	53.0	15.0	23.3	13.2	13.0	11.9
5/25/77	51.0	14.3	-	15.1	12.1	-
5/26/77	45.3	13.4	24.5	16.1	12.1	13.0
5/27/77	51.9	15.7	-	13.3	12.6	12.5
5/28/77	58.2	15.3	26.8	14.5	12.7	-
5/29/77	53.4	15.8	24.3	14.0	-	12.9
5/30/77	49.4	15.1	25.9	14.5	12.8	12.6
5/31/77	50.8	15.5	26.0	14.2	12.2	12.8
6/1/77	51.4	15.7	25.2	14.5	-	12.0
6/2/77	58.6	14.9	22.4	15.0	-	-
6/3/77	46.0	13.7	23.5	12.5	-	11.1
6/7/77	47.5	14.8	32.5	13.9	11.7	11.4
6/8/77	51.0	15.4	29.3	14.1	12.3	11.2
6/9/77	46.5	15.9	25.9	14.9	11.8	12.9
6/10/77	51.1	16.8	27.9	14.6	12.8	12.3

Table A-5. Effluent Volatile Solids (g/l) - Run 3[1]

Date	Feed	A	B-1	B-2	C-1	C-2	C-3	C-4
9/6/77	41.3	12.4	10.2	10.6	26.4	9.7	9.0	8.4
9/7/77	37.1	13.7	12.9	10.8	26.9	10.1	8.8	9.1
9/8/77	39.6	16.1	12.3	10.6	25.2	9.0	9.2	9.0
9/9/77	39.0	13.6	11.0	10.7	24.5	8.7	9.2	9.0
9/10/77	38.0	14.6	11.3	11.3	-	9.8	10.0	10.0
9/11/77	38.9	14.0	11.4	10.8	24.0	9.7	9.5	9.8
9/12/77	36.7	14.9	12.4	10.8	25.3	7.8	9.9	10.0
9/23/77	39.3	12.1	10.9	11.5	21.4	12.3	10.2	10.1
9/24/77	34.7	10.9	10.2	11.0	16.5	9.9	9.7	9.5
9/25/77	34.6	11.3	10.6	11.5	13.0	9.6	10.0	9.9
9/26/77	36.0	11.0	10.6	8.7	12.6	9.8	10.1	11.2

[1]For Run 3, Day 0 corresponds to August 26, 1977

Table A-6. Effluent COD (g/l)- Run 3

Date	Feed	A	B-1	B-2	C-1	C-2	C-3	C-4
9/6/77	-	18.3	15.4	14.8	35.8	13.9	12.0	12.3
9/7/77	55.6	18.0	17.7	14.8	34.2	15.5	14.7	11.9
9/8/77	51.4	18.9	17.8	14.8	35.9	15.7	13.5	13.9
9/9/77	45.1	15.7	14.2	12.9	33.2	11.9	12.6	12.1
9/10/77	56.3	16.7	17.3	16.0	35.1	14.7	13.6	12.4
9/11/77	58.6	19.7	16.3	16.6	36.3	13.7	13.1	14.4
9/12/77	53.8	20.0	17.8	15.3	34.8	14.9	13.2	13.7
9/22/77	53.8	17.0	15.2	15.8	-	14.6	14.6	14.6
9/23/77	54.3	16.5	14.2	15.4	31.4	16.7	13.8	13.9
9/24/77	49.9	15.1	13.4	15.5	26.6	16.0	12.9	14.9
9/25/77	47.8	17.3	15.3	15.6	19.6	13.8	14.7	13.8
9/26/77	55.1	15.4	15.3	16.3	20.2	14.0	14.8	14.6

Table A-7. Effluent Volatile Solids (g/l) - Run 4[1]

Date	Feed	A	B-1	B-2	C-1	C-2	C-3	C-4
				θ_T = 8 Days				
10/17/77	35.3	11.8	12.2	12.3	14.1	9.6	9.8	10.1
10/18/77	37.1	13.3	13.2	13.4	16.5	10.8	10.8	10.7
10/21/77	37.8	13.7	14.1	13.5	-	12.5	11.8	12.3
				θ_T = 6 Days				
11/4/77	40.9	19.1	21.2	16.2	40.5	21.0	15.9	15.3
11/5/77	42.0	17.8	21.5	16.6	40.9	24.3	17.1	16.3
11/6/77	42.5	17.0	23.6	14.7	39.1	25.6	16.7	15.6
11/7/77	43.1	17.7	26.9	15.9	42.1	29.5	17.4	15.6

[1]For Run 4, Day 0 corresponds to September 20, 1977

Table A-8. Effluent COD (g/l) - Run 4

Date	Feed	A	B-1	B-2	C-1	C-2	C-3	C-4
				θ_T = 8 Days				
10/17/77	56.3	12.2	-	16.4	21.3	12.4	12.0	13.1
10/18/77	56.4	12.3	18.6	16.2	22.8	12.9	12.6	12.6
10/21/77	56.9	16.8	18.6	17.1	22.7	15.4	12.7	12.4
				θ_T = 6 Days				
11/4/77	41.2	22.1	33.0	18.9	21.0	23.1	19.7	15.9
11/5/77	65.0	20.8	26.5	18.1	51.6	32.1	20.2	17.7
11/6/77	48.5	22.5	31.0	19.2	54.5	35.4	22.6	18.8
11/7/77	48.3	21.6	35.6	19.0	56.7	47.2	20.3	19.5

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