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CONTROLLING FACTORS IN METHANE FERMENTATION

By
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and
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DEPARTMENT OF CIVIL ENGINEERING
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IN
METHANE FERMENTATION

By

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Organization of Report

In presenting this final report, all the various factors which were studied with regard to their effect on methane stimulation are discussed. In the first part of the report, a series of studies are summarized. Some of these factors indicated a low magnitude of stimulation of the methane fermentation. Other factors showed no effect. In the remaining body of the report, a series of separate studies is presented of the factors studied which gave strong, positive results.

Each of these studies is written up as a complete entity with separate figure numbers,table numbers, and bibliography.

Personnel

The Principal Investigator and Co-Principal Investigator for the entire period of the project has been R. E. Speece and R. S. Engelbrecht, respectively. Personnel employed on this project and their period of employment, were as follows:

Professional Personnel	Title	Period of Appointment	% of Time
C. V. RamaRao	Research Assistant	9-16-62 to 6-15-63	50%
C. V. RamaRao	Research Assistant	9-16-63 to 8-31-63	100%
C. V. RamaRao	Research Assistant	9-16-63 to 9-31-63	100%
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V. Kothandaraman	Research Assistant	9-16-63 to 6-15-64	50%
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Jan Kem	Research Assistant	9-16-63 to 6-15-64	50%
R. E. Speece	Ass't Prof. of San. Eng.	6-16-63 to 8-15-63	100%
R. E. Speece	Ass't Prof. of San. Eng.	6-16-64 to 8-15-64	100%
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I. Iijima	Research Assistant	9-1-64 to 6-15-65	50%
I. Iijima	Research Assistant	6-16-65 to 8-15-65	100%
I. Iijima	Research Assistant	9-16-65 to 2-1 -66	50%
R. E. Speece	Assoc. Professor	6-16-65 to 8-16-65	100%
R. E. Speece	Assoc. Professor	9-16-65 to 2-1 -66	25%

The contributions made by these persons in carrying out the objectives of this study are sincerely acknowledged.

Assay of Trace Metals, Trace Organics And Physical And Chemical Factors

Assay of Trace Metals and Trace Organics

The initial phases of the project were designed to evaluate the effects of a number of compounds which had produced stimulation in a previous study. These compounds were: iron, cobalt, thiamine, proline, glycine and benzimidazole. The addition of these separate compounds and combinations thereof allowed acetate utilization rates of 1000 mg/l/day. Whereas, the control, which received none of these compounds, operated at from 200 to 500 mg/l/day. However, even the maximum rate achieved was much lower than is commonly experienced in the anaerobic digestion of sewage sludge. Obviously, something was still lacking in the environment which inhibited the rates of methane production.

During this study period, ammonium bicarbonate was substituted for the sodium bicarbonate buffer system. This resulted in increasing the ammonia nitrogen content from 60 to 800 mg/l. However, no noticeable stimulation of methane production resulted.

Assay of Physical and Chemical Factors

Asbestos has been reported to stimulate methane production. In a study to evaluate this effect, it was found that a digester containing 30 grams per liter of asbestos resulted in doubling of the methane production over that of a control which had no asbestos. In an attempt to elucidate the effect of asbestos on methane production, asbestos was extracted under anaerobic conditions and the extract was assayed. The addition of the anaerobic asbestos extract resulted in no significant increase in methane production over the control digester.

References were found in the literature of bacteria which multiply more rapidly in the presence of material which increases the surface area, such as glass beads or an inert, finely divided precipitate. A slight, positive stimulation was found when powdered $CaCO_3$ was added to digesters, but the effect was only minor.

The effect of volatile acids concentration was evaluated and found to control the rate of methane production. The Michaelis-Menten Model has since been found by other investigators to reasonably predict acetate utilization rates.

The effect of detention time on acetate utilization rates was evaluated. At longer detention times, acetate utilization rates were proportionately greater due to the greater standing crop of organisms maintained. However, at increasing detention times, the unit activity of mg/l acetate utilized per gram of organisms per day decreases. There appears to be a relationship whereby the activity of the organisms decreases with mean cell age. The following results were obtained:

Acetate Utilization Rates

3.15 $\frac{\text{gm acetate/day}}{\text{gm cells}}$ at 6 day detention time.

1.00 $\frac{\text{gm acetate/day}}{\text{gm cells}}$ at 100 day detention time.

Net Synthesis Rate

5.3% synthesis of acetate at 6 day detention time.

1.0% synthesis of acetate at 100 day detention time.

The effect of mixing was strongly evident. Continuous mixing resulted in more than doubling of the acetate utilization rate as compared to a nonmixed control.

The surface charge of the methane producing organisms was altered by adding $AlCl_3$ and a cationic polyelectrolyte. There was no significant stimulation or repression in either case. However, it did indicate that the addition of a coagulant for removal and recycle of the bacterial mass in an anaerobic waste treatment process is feasible.

A chelating agent, EDTA, was added to a "washed-out" digester to assay its effects on methane production. There was no significant effect after several slug additions of 100 mg/l of the sodium salt of EDTA. This indicates that the

limited rates of methane production were not due to the inhibiting action of a toxic metal which was able to be chelated.

In summary, a number of factors were elucidated which stimulated the rate of methane fermentation. These were:

Iron alone

Iron and cobalt in combination

Asbestos

Calcium carbonate solids

Increased volatile acids concentration

Increased sludge retention time

Continuous mixing

Increased temperature to an upper limit of $45^{o}C$.

Fractionation and Assay of Digested Sludge Supernatant and Cattle Rumen Liquor

An extensive study of the nutritional requirements of a pure culture of methanogenic bacterium Methanobacterium ruminantium has been underway by Professor Marvin P. Bryant, Department of Dairy Science, University of Illinois, Urbana, Illinois. This organism grows in a liquid media containing H_2-CO_2 as an energy source. An unidentified growth factor found in rumen fluid and digested sewage sludge was required for growth. This growth factor was not found in yeast extract, cell-free extract of E. coli or many other crude materials commonly used to grow nutritionally exacting bacteria.

Therefore, a study was undertaken in the Sanitary Engineering Laboratory to fractionate cattle rumen fluid and digested sludge supernatant and assay its stimulation capacity in an enriched culture of acetate utilizing methanogenic bacteria. Very little energy is available to the microorganisms from anaerobic acetate utilization as compared to the very high amounts of energy available from hydrogen utilization. Consequently, much higher growth rates are possible to

organisms utilizing hydrogen as substrate. However, even though considerably lower growth rates would be anticipated with an acetate substrate, qualitative stimulation was considered to be sufficient to indicate the presence of growth factors.

The University of Illinois maintains several fistulated cattle from which the samples of rumen fluid were withdrawn. The digested sludge supernatant was taken from the Urbana-Champaign Sewage Treatment Plant. The procedures used to fractionate the rumen fluid were as follows and were used singularly or in combinations.

Centrifugation - 12,000 x g for 10 minutes.

Activated Carbon Adsorption - using chloroform extraction in Soxhlet apparatus with vacuum distillation for removal of chloroform.

Ion Exchange - Hydrogen cycle, hydroxyl cycle and mixed bed deionization.

Dialysis - Membrane used permitted dialysis of inorganic ions and organic coloring matters, but not proteins, lipids, carbohydrates or other macromolecules.

Solvent Extraction - Butanol and petroleum ether soluble fractions.

Ashing of Residue

The conclusions of this study were:

1. There was a limiting concentration for the addition of evaporated residue from digested sludge supernatant, beyond which the addition was inhibitory. This inhibition probably was related to salt toxicity.

2. The stimulatory factors in digested sludge supernatant were not eliminated by centrifuging at 12,000 x g for 10 minutes.

3. Solvent extraction of digested sludge supernatant with butanol indicated that the greater fraction responsible for stimulation was insoluble in butanol.

4. Salts responsible for reducing the activity of the methane organisms were dialysable and exist as organic chelates.

5. The ionic species of the dialysable inorganics in the digested sludge supernatant inhibited methane fermentation.

6. Anionic exchange of the centrifuged digested sludge supernatant at pH 7.0 did not alter the stimulation capacity.

7. Some component in the digested sludge supernatant with a carboxyl or phosphate fuctional group caused a slightly increased rate of methane fermentation.

8. Rumen liquor and the various fractions and combinations thereof, except the centrifuged rumen liquor solids, inhibited the rate of acetate utilization.

Dilution Studies

In the previous sections, the acetate utilization rates never approached the high rates reported by McCarty and Vath. The rates would rise to a certain value and reach a plateau. Some limitation prevented higher rates. This was verified in a study in which a 3-liter digester was fed acetate and buffered nutrient solution containing inorganic nutrients. Excess acetate substrate was always maintained in the system to keep from limiting methane production and the hydraulic and solids detention time was 15 days. At equilibrium, the completely mixed contents were equally divided between two digesters. One digester was maintained at a volume of 1.5 liters to serve as a control while the other digester was diluted back to the original volume of 3 liters with buffered nutrient solution. Operation of both digesters was continued on a 15 day detention time with excess acetate maintained at all times.

At the subsequent equilibrium, both digesters reached the same acetate utilization rate per unit volume of approximately 1000 mg/l/day. However, the total acetate utilization was consequently double in the digester, which had been diluted 100 percent with buffered nutrient solution. Therefore, this strongly indicates either the accumulation of toxic end products or a limiting nutrient concentration. Studies at Purdue in a dialysed system have also confirmed this observation. They noted gas production reached a maximum and decreased. However, after placing fresh media outside the dialysed system, gas production would reach a new maximum which was double the initial maximum. Thus, either a toxic end

product was lost from the system or a new supply of limiting nutrient was added when the old media was replaced by fresh media.

In an attempt to determine whether solubility of a required ion was limiting the rate of methane production, eight 10-liter digesters containing one liter of digested sludge were fed for 10 days with raw sludge. All digesters were then diluted to 10 liters volume with the following dilution waters:

1. Tap water
2. Buffered nutrient solution
3. Demineralized water
4. Stream water
5. Stream water - zeolite softened
6. Tap water - zeolite softened
7. Buffered nutrient solution - zeolite softened.
8. Centrifuged digester supernatant.

Zeolite softening treatment was used to remove any multivalent cations which may precipitate anions in the digester environment.

There was no significant difference in the acetate utilization rates of any of these digesters after dilution. This indicates that the presence or absence of multivalent cations in the dilution water had no effect on the system. However, this study is to be repeated in a "washed out" system which is more clearly defined as to the chemical constituents as opposed to this present preliminary study where raw sludge was initially fed.

THE EFFECT OF FREQUENT TEMPERATURE VARIATION ON METHANE PRODUCTION

THE EFFECT OF FREQUENT TEMPERATURE VARIATION ON METHANE PRODUCTION

By R. E. Speece, Associate Professor of Sanitary Engineering and
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Urbana, Illinois

Introduction

The methane-forming microorganisms are generally considered to be more sensitive to physical and chemical changes than the acid-forming microorganisms involved in the anaerobic digestion process. Also, because the methane-forming microorganisms utilize the volatile acid end-products produced by the acid forming microorganisms, failure of the methane-formers to utilize the volatile acids at approximately the same rate as they are produced, can result in a "stuck" digester. As a result, the fastidious nature of the methane-forming organisms combined with the critical position they occupy in the anaerobic digestion scheme, makes it very important that satisfactory environmental conditions be maintained for them in order to promote good digestion. However, in normal anaerobic digestion, the volatile acid concentration is low, indicating that the methane-forming organisms are capable of utilizing the volatile acids at least as fast as they are being formed. In other words, the acid formation is the rate-limiting step in normal digestion.

A number of studies have been made of the effect of temperature on the anaerobic digestion process (1), (2), (3). These studies have been made using a complex feed such as primary sludges. When using complex substrates, both the acid-formation and methane-formation rates are involved and the net overall effect being observed is actually controlled by whatever the rate-limiting step in the process happens to be. If the volatile acid concentration is low, then the acid forming step determines the overall rate which is observed.

In this study, acetate was fed as the sole substrate. Therefore, only the rate of activity of the methane-forming organisms was observed, since only these organisms can utilize acetate under anaerobic conditions.

The objectives of this study were to determine the effect on the methane-forming organisms in a mesophilic sludge under the following conditions:

1. A temperature change sustained for a number of hours as would occur if the temperature of the digester contents rose or fell over a 24 hour interval.

2. A temperature drop for a 15 minute interval as would occur when digested sludge is pre-mixed with the raw sludge before it is pumped to the digester.

3. A temperature drop for a 2 hour interval as would occur if a proposed "anaerobic" contact stabilization process were feasible..

Presently, cold, diluted wastes are uneconomical to treat by anaerobic digestion due to excessive heat requirements. However, if the wastes could be adsorbed on a bacterial or inert surface and thus concentrated, it may prove economical to heat this more concentrated form of the sludge and stabilize it by anaerobic digestion. Such a system is shown schematically in Figure 1.

Procedure

A 2 l Erlenmeyer flask was placed in a temperature controlled bath as shown in Figure 2. The digester was purged with nitrogen gas and 1.8 l of sludge from a well operating 35^{o} C digester was transferred to the flask. The digester contents were mixed by a magnetic stirrer placed underneath the bath. The temperature of the bath was controlled by a thermoregulator and it was kept in circulation by an air diffuser.

At the beginning of a run, the volatile acid concentration was determined. Sufficient acetate was then added to bring the volatile acid concentration to 700 to 1500 mg/l. The acetate was fed as the neutral salt in the form of calcium acetate. Acetate was continuously fed to the digester with an

electrolytic pump at approximately the same rate at which it was being utilized. Thus, feed concentration never limited the rate of gas production.

At the start of a temperature drop study, the temperature was maintained for about an hour at the level to which it was to be raised after the drop. Gas production was recorded at 10-minute intervals during this first hour, during the drop, and for a sufficient period after the drop to reach equilibrium. These readings were continued during the temperature drop.

Test runs were made to observe the effects of both temperature increases and decreases on the rate of gas production. Observations were made on gas production for the following temperature schedules:

Initial Temperature (^{o}C)	Drop Temperature (^{o}C)	Duration of Temperature Drop (Min)
35	10	15
35	10	120
35	20	15
35	20	120
50	10	120

It was desirable in the course of this study to confirm the effect of temperature on both the acid-formation rate and gas production rate. To accomplish this, an actively digesting sample of sludge was taken from the primary digester at the Champaign-Urbana Sewage Treatment Plant. This insured that both groups of microorganisms were functioning well. An increase in volatile acids concentration was simulated by adding sufficient calcium acetate to raise the volatile acids concentration in the sludge to 1800 mg/1. Then 10% by volume of primary sludge was added to insure adequate substrate for the acid-forming organisms. The sludge was then divided into 2 portions and placed in flasks which had been purged of oxygen by flushing with nitrogen and carbon dioxide gas.

The contents of the one flask were maintained at 35°C and the contents of the second flask were maintained at 45°C. Both flasks were continually mixed and once a day 1/20 of the flask contents was withdrawn and replaced by an equal amount of raw sludge. The raw sludge feed simulated normal digester operation and provided a food source for the acid forming microorganisms, and the volatile acids provided a food source for the methane-forming microorganisms. Thus, by comparing the volatile acids concentration and gas production in the 45°C digester, with that in the 35°C digester, the effect of a temperature increase on the relative rates of activity of the two groups of microorganisms (the acid - formers and the methane-formers) could be determined.

If the activity of both groups of microorganisms was dependent to the same degree on temperature, the volatile acids concentration in both the 35°C and 45°C digesters should be equal, because the increased volatile acid utilization would be matched by a corresponding increased volatile acid formation. However, if the volatile acids concentration was higher in the 45°C digester than in the 35°C digester, this would signify that the rate of production of volatile acids by the acid-formers increased to a greater degree with temperature than the rate of volatile acid utilization by the methane-formers. Finally, if the volatile acids concentration was lower in the 45°C digester than in the 35°C digester, this would indicate that the activity of the methane-formers increased at a greater rate with temperature than the acid-formers.

Results

The experimental results fall very much in line with those which would be predicted from theoretical considerations of the response of microorganisms to various temperature levels and temperature changes. Table 1 and Figure 3 give the relative gas production rates for a 35°C (mesophilic) acclimated sludge when the temperature is changed as indicated.

TABLE 1

GAS PRODUCTION RATES FOR EXTENDED INTERVALS OF TEMPERATURE

Temperature (°C)	Time Interval (Hrs)	Gas Production (ml/hr/l)	Relative Rate (%)
20	20.5	Nil	0
25	20.8	24	36
30	25.7	46	68
35		68*	100
40	5.9	90	132
45	54.0	117	171

*Calculated Value

Figure 4 indicated the relative gas production rates of a digester at 34°C which is then dropped to 27°C, raised back to 33°C and finally raised on up to 40°C.

Figures 5 through 8 shows the response of a mesophilic sludge held at approximately 35°C and then dropped to 10°C for both 15 minutes and 2 hours and also dropped to 20°C for the same two time intervals.

Figure 9 shows the corresponding effect of a temperature drop to 10°C for a 2 hour interval on a mesophilic sludge held at approximately 50°C before and after the temperature drops.

Figure 10 indicates the effect of an increase in temperature from 35°C to 45°C on the relative rates of activity of the acid-formers and the methane formers.

Figure 11 is the curve of rate of activity vs. temperature for acid-forming and methane-forming organisms as hypothesized from the results of this study.

Discussion of Results

Figures 3 through 9 reveal the effect of frequent temperature variations on just the methane production stage of anaerobic digestion, because acetate was fed as the sole substrate. Under anaerobic conditions, acetate can be used by only the methane-formers. The amount of volatile acids in the digester was continually maintained at sufficiently high concentrations which would not limit the rate of gas production. This is not the case in normal sewage sludge digestion which characteristically maintains a low and thus rate-limiting concentration of volatile acids.

The neutral calcium salt of acetic acid was fed so that the only gas which would evolve from a digester held at constant temperature would be that due to the activity of the methane-formers. No carbon dioxide evolution would be encountered when this neutral salt was fed to the digester. Also, the calcium would continually precipitate from solution as the acetate was consumed and there would be no cation toxicity problem. Finally, it was necessary to insure an unlimited food source for the methane-formers at all times and with the neutral calcium acetate being fed, there was no danger of an accidental drop in pH.

Feeding calcium acetate as the sole substrate results in a gas composition of 75% CH_4 and 25% CO_2. The CO_2 introduces an error into the gas production values with each temperature change, however, due to its high solubility in water and the great dependence of CO_2 solubility on temperature. Thus, following each temperature increase, there is an initial marked increase in gas production, after which gas production comes to an equilibrium at a somewhat lower rate. This spike in the gas production is accounted for by the decreased solubility of CO_2 at higher temperatures and therefore the release of CO_2 from solution. This correction was not made because the exact percentage of CO_2 in the digester atmosphere would have to be known both during the temperature drop when CO_2 would have been sucked

back into solution and after the raise in temperature when the CO_2 would be somewhat above the theoretical 25%. The CO_2 equilibrium of the digester contents and atmosphere was established soon after a temperature increase occurred because the volume at the top of the Erlenmeyer flask was small and was rapidly flushed out by the gas production.

Figure 3 shows the clear response of gas production from mesophilic sludge which had been incubated at 35°C and was then incubated for a number of hours at the temperatures shown. Figure 3 indicates gas production bears a linear response to temperature as opposed to the traditional logarithmic response. Below a temperature threshold of 20°C, gas production was nil. This observation is also borne out by later figures. Gas production could not be sustained at 50°C for more than a few hours before it commenced dropping off as noted in Figure 9 in which a mesophilic digester was raised to 50°C, then lowered to 10°C for 2 hours and finally raised back to 50°C.

From Figure 3, it is seen that a decrease in digester temperature from 37°C to 30°C reduces the gas production to approximately 50%. Golueke (1) found that the destruction of volatile matter decreased from 50% to 40% when the digestion temperature was decreased from the range of 35°C - 55°C down to 30°C. Thus, while volatile matter destruction at 30°C decreased to only 80% of that at 37°C, gas production would suffer much more severely by decreasing to 50% of the original. Golueke's results (1) were based on long term digester operation.

As shown in Figure 4, the response of the methane-formers to a temperature change is immediate. As the temperature was dropped over an hour's period of time from 34°C to 27°C, the gas production decreased to about 12% of that at 34°C. As the temperature was increased from 27°C back to 33°C, the gas production resumed at approximately 100% of that before the temperature drop occurred. A subsequent increase in temperature to 40°C was accompanied by gas production rates of about 140% of those at 34°C.

Figures 5 through 8 indicate that as long as the digester temperature is below 20°C, gas production is nil. However, as soon as the temperature is raised back to the normal range of anaerobic digestion, gas production resumes at a rate proportional to the temperature within the range 20°C to 45°C. There appears to be no carryover of the adverse effect on the methane-formers from exposure to temperatures of 10°C and 20°C for 15 minute periods. Gas production resumed at essentially the same rate as soon as the temperature was restored to the initial level.

Figure 6 showed a slight lag in recovery of gas production after maintaining a sludge at 10°C for two hours. It is noted that temperature recovery was also slow in this case due to the type of water bath used during this run. This equipment was modified in later experiments to allow more rapid temperature recovery. Figure 9 shows no gas production lag for a sludge held at 10°C for 2 hours and then raised to 50°C.

Thus, the methane-forming microorganisms appear to be very adaptable to frequent temperature changes with no adverse effects resulting from temperature drops. However, Golueke (1) reported in his studies on the anaerobic digestion of sewage sludge at various temperature levels that once a digester was well established, it became very sensitive to any abrupt temperature drop. He cited the case of a 35°C digester in which the temperature dropped to 25°C for about 16 to 18 hours, and resulted in a reduction in breakdown from 52.8% down to 44%. It is hypothesized that such a situation would develop when the methane-formers' rate of gas production is seriously retarded at the lower temperature. Thus, the volatile acids which are formed and accumulated are not converted to a gas and since the volatile acids are not volatile under the neutral pH conditions under which the solids are evaporated, they are measured as part and parcel of the volatile solids not destroyed.

Table II and Figure 10 revel a very significant comparison. It vividly shows the beneficial effect of increased temperature on a digester in which the methane-

formers are not inhibited by anything but temperature. That is to say, there are no adverse environmental or physiological conditions restricting the rate of activity of the methane-formers except temperature.

A digester with high volatile acids concentrations and continued raw sludge addition was inoculated by taking 3.6 liters of well digesting sludge from the primary digester at the Champaign-Urbana Sewage Treatment Plant and adding 0.4 of raw sludge. The volatile acids concentration in the primary digester at the time was approximately 300 mg/l as acetic acid. To this mixture of raw and digested sludge, sufficient calcium acetate was added to increase the volatile acids concentration to approximately 1800 mg/l. This 4 1 volume was thoroughly mixed and divided into 2-2 1 volumes and incubated at 35°C and 45°C respectively. Each day 100 ml of raw sludge was added to each digester to simulate a 20 day detention time.

TABLE II

COMPARISON OF VOLATILE ACIDS REDUCTION AT 35°C AND 45°C

Time	35°C			45°C		
	Vol. Acids Conc.	Comulative Gas Production		Vol. Acids Conc.	Cumulative Gas Production	
(Hrs.)	(mg/l)	(ml)		(mg/l)	(ml)	
0	1840	0	Gms Liquefied	1800	0	Gms Liquified
5.5	2040	240	(0.8)	1500	1000	(1.07)
7.5	1920	750	(1.41)	700	2840	(2.55)
8.5	1620	1250	(1.64)	340	4000	(3.75)
1.5	1440	1940	(2.43)	280	4610	(4.66)

Figure 10 shows that in less than 30 hours, the volatile acid concentration in the 45°C digester had dropped about 1500 mg/l, to the original level. However, in the 35°C digester the volatile acids concentration had dropped only to about 200 mg/l lower than it was initially.

It would be expected that the increase in temperature would not only increase the rate of activity of the methane-formers, but also the rate of activity of the acid-formers. This is borne out by calculations on the data from each 2 l digester. At 28.5 hours, 4000 ml of gas was produced by the 45°C digester and the volatile acids had decreased by 2.92 grams in the 2 l digester. Assuming 600 ml of gas is produced per gram of volatile acids destroyed, then 4000 ml of gas represents the destruction of 6.67 gms of volatile acids. Since the amount of volatile acids in the digester was only reduced by 2.92 grams, then 6.67 - 2.92 = 3.75 gm of volatile acids must have been contributed by the acid-formers during this time. By similar calculations, it is found that only 1.64 gms of volatile acids were contributed by the acid-formers in the 35°C digester after 28.5 hours.

It is concluded from Figure 10 that while an increase in temperature does increase the activity of both the methane-forming and acid-forming organisms, the methane-formers increase their activity at a considerably greater rate per °C. Thus, not only can the methane-formers consume the additional volatile acids contributed by the acid-formers at higher temperatures, but in addition they are able to reduce the pool of volatile acids which is initially present. This explains the rapid decrease in volatile acids in the 45°C digester as compared to the less rapid decrease in the 35°C digester.

Engineering Significance

Figure 11 is hypothesized as a characteristic plot of the temperature dependence of the acid-forming and methane-forming microorganisms in a favorable anaerobic digestion environment. Above a certain temperature, X, the methane-formers are capable of consuming volatile acids at a rate greater than the rate at which they are supplied by the acid-formers. Therefore, the volatile acids concentration remains low. Below this temperature, X, the rate of activity of the methane-formers

is lower than the acid-formers. The net result is that volatile acids start to accumulate in the digester.

Experience seems to indicate that the temperature, X, at which the rates are equal is approximately 30 to 35°C. There appears to be only a minor advantage to be gained by maintaining the temperature of a sewage sludge digester above this range, since the rate of liquefaction (acid-formation) is not appreciably increased according to Golueke (1). Above temperature, X, acid formation is the lower and thus the rate-limiting step in the two-step digestion process, and the increased potential capacity of the methane-formers cannot be exploited because they have only a limited food supply.

However, in the anaerobic digestion of a soluble industrial waste, the rate of acid formation may be appreciably greater than with a complex solid like sewage sludge. In such a case, advantage could be taken of the markedly greater rate of methane formation at the 40 to 45°C level as compared to the 35°C commonly used. This would result in a much smaller digester.

Pre-mixing of digested sludge with raw sludge before pumping the mixture into the digester appears to stop methane production if the temperature of the mixture would be lowered to less than 20°C. However, no retardation of gas production results as soon as the temperature is restored to normal inside the digester.

The flow diagram proposed in Figure 1 appears to be feasible with respect to the methane-forming organisms being able to adapt to the changing temperatures. The adsorptive concentration of the pollutant would be critical and require considerable study.

This study was supported by Grant WP-00394 from the Division of Water Supply and Pollution Control, United States Public Health Service.

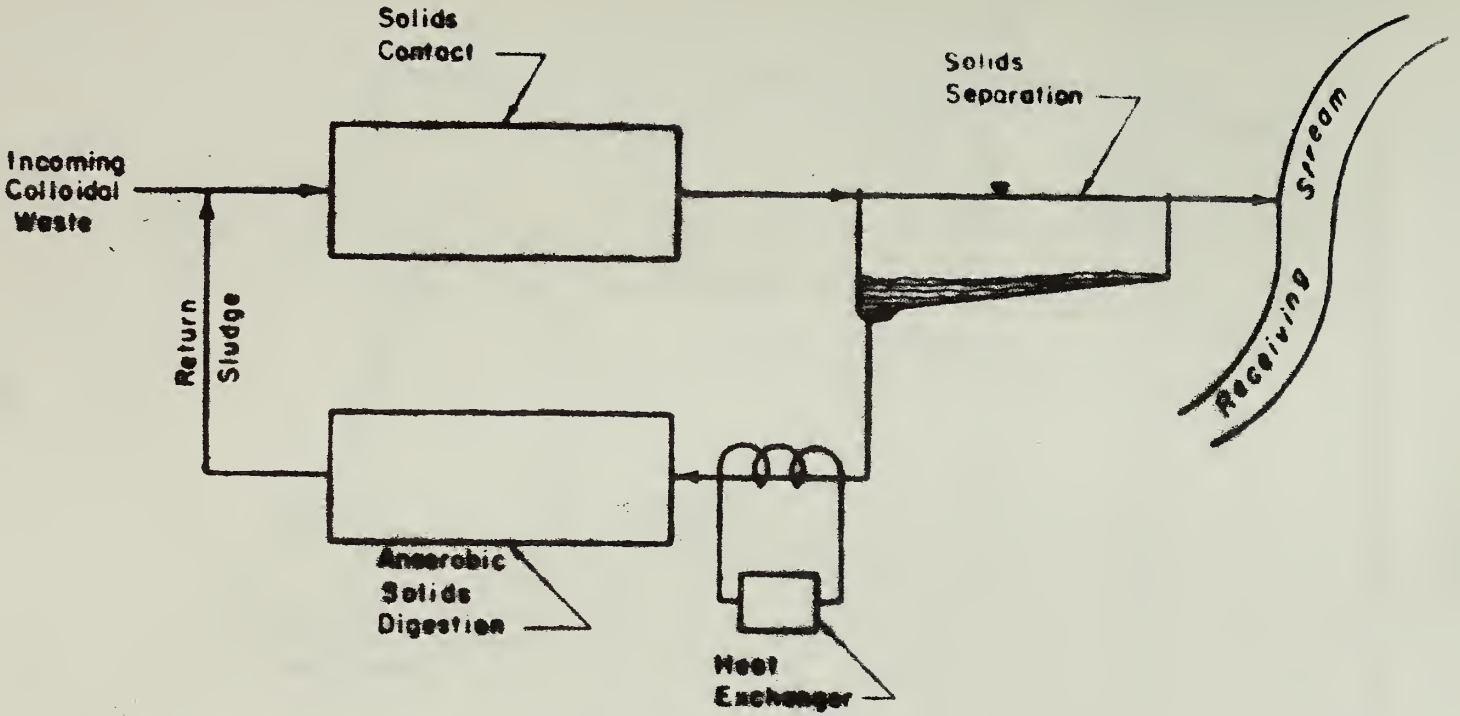

FIGURE 1. PROPOSED ANAEROBIC CONTACT STABILIZATION PROCESS.

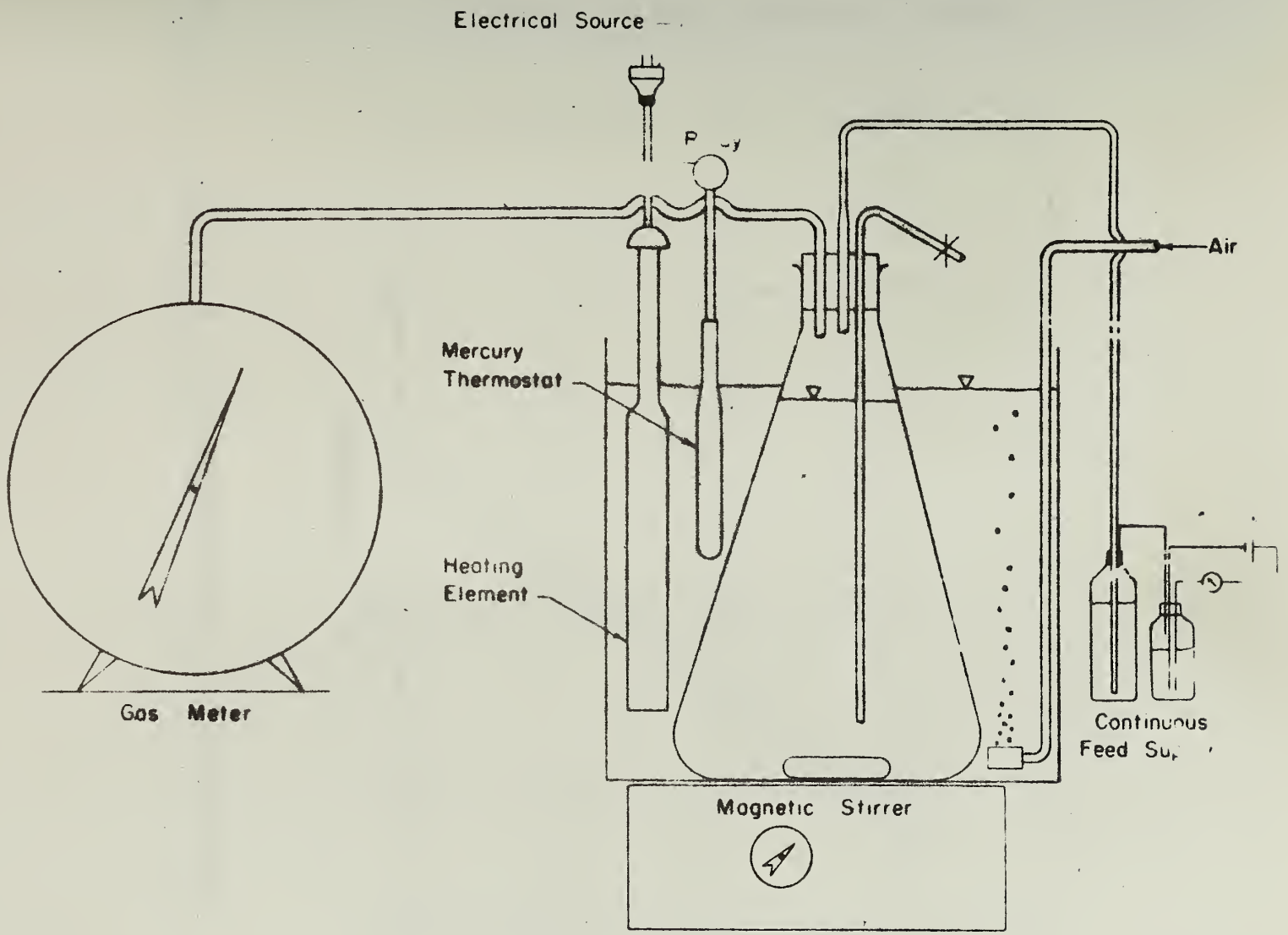

FIGURE 2 SCHEMATIC DIGESTION APPARATUS.

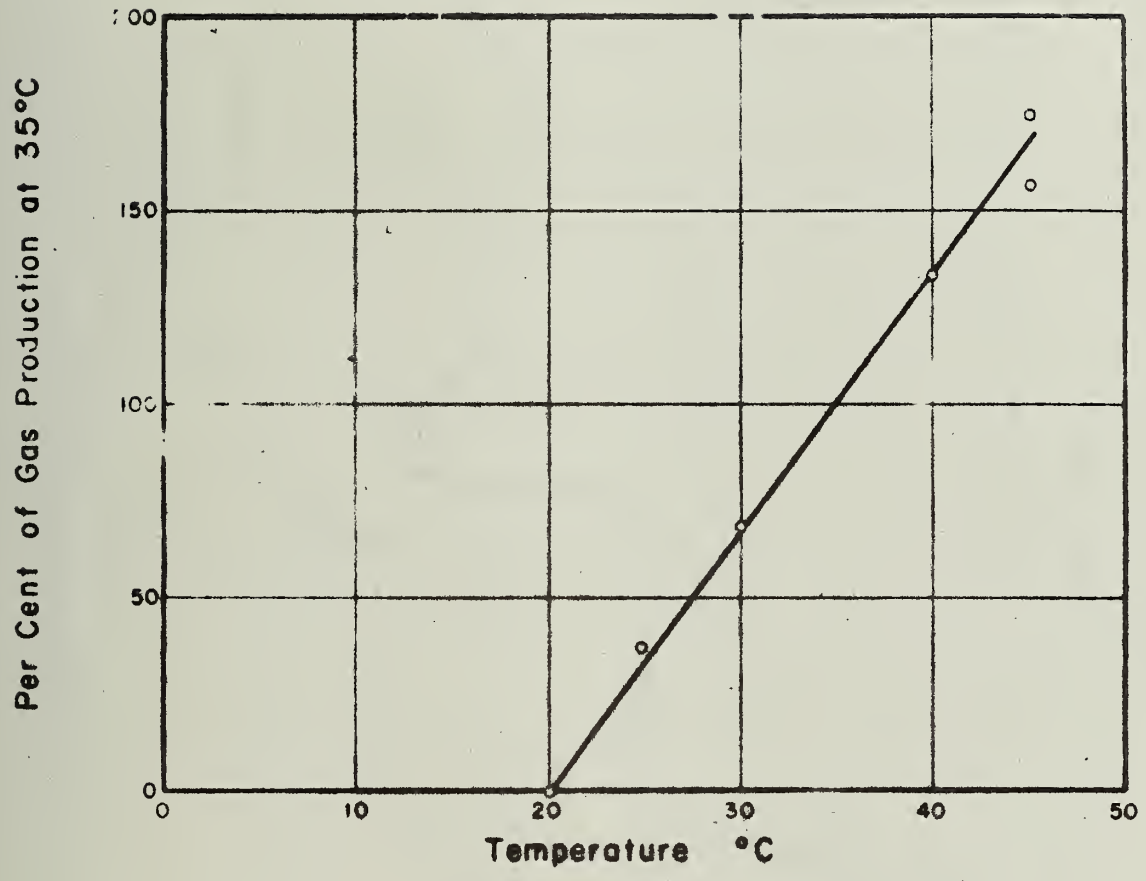

FIGURE 3. RELATIVE GAS PRODUCTION vs TEMPERATURE .

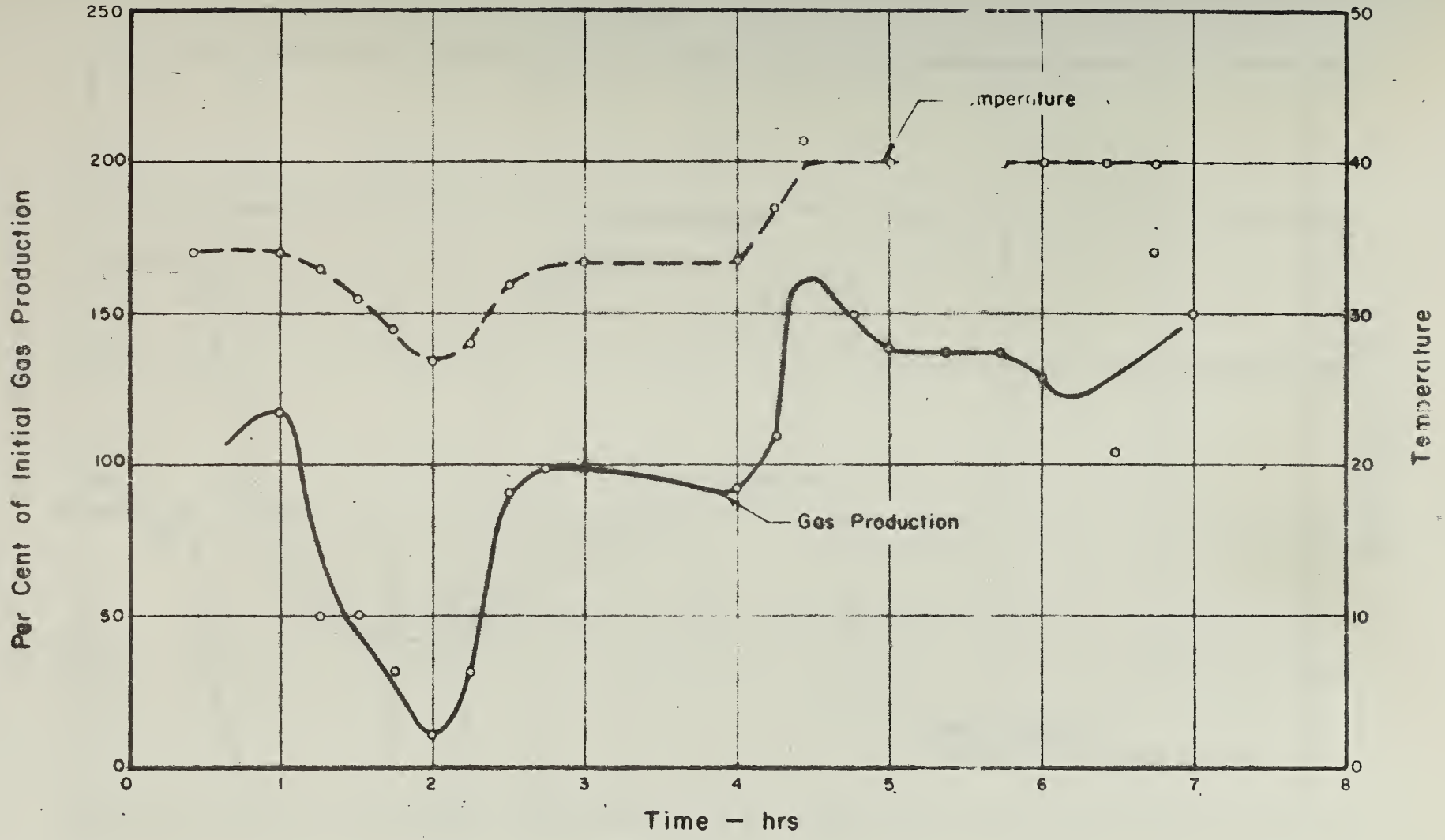

FIGURE 4. DEPENDENCE OF GAS PRODUC[illegible]ON TEMPERATURE.

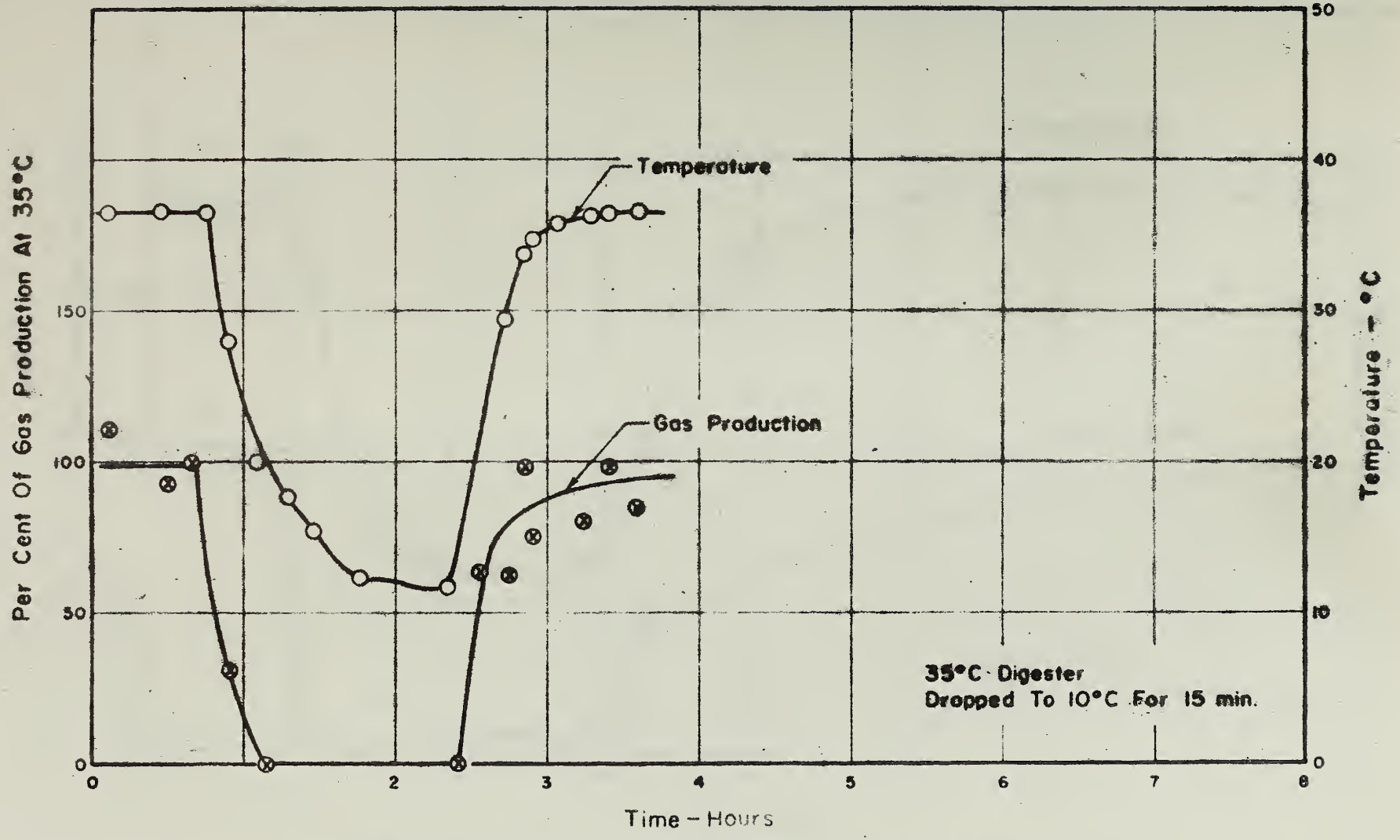

FIGURE 5. GAS PRODUCTION VS TEMPERATURE.

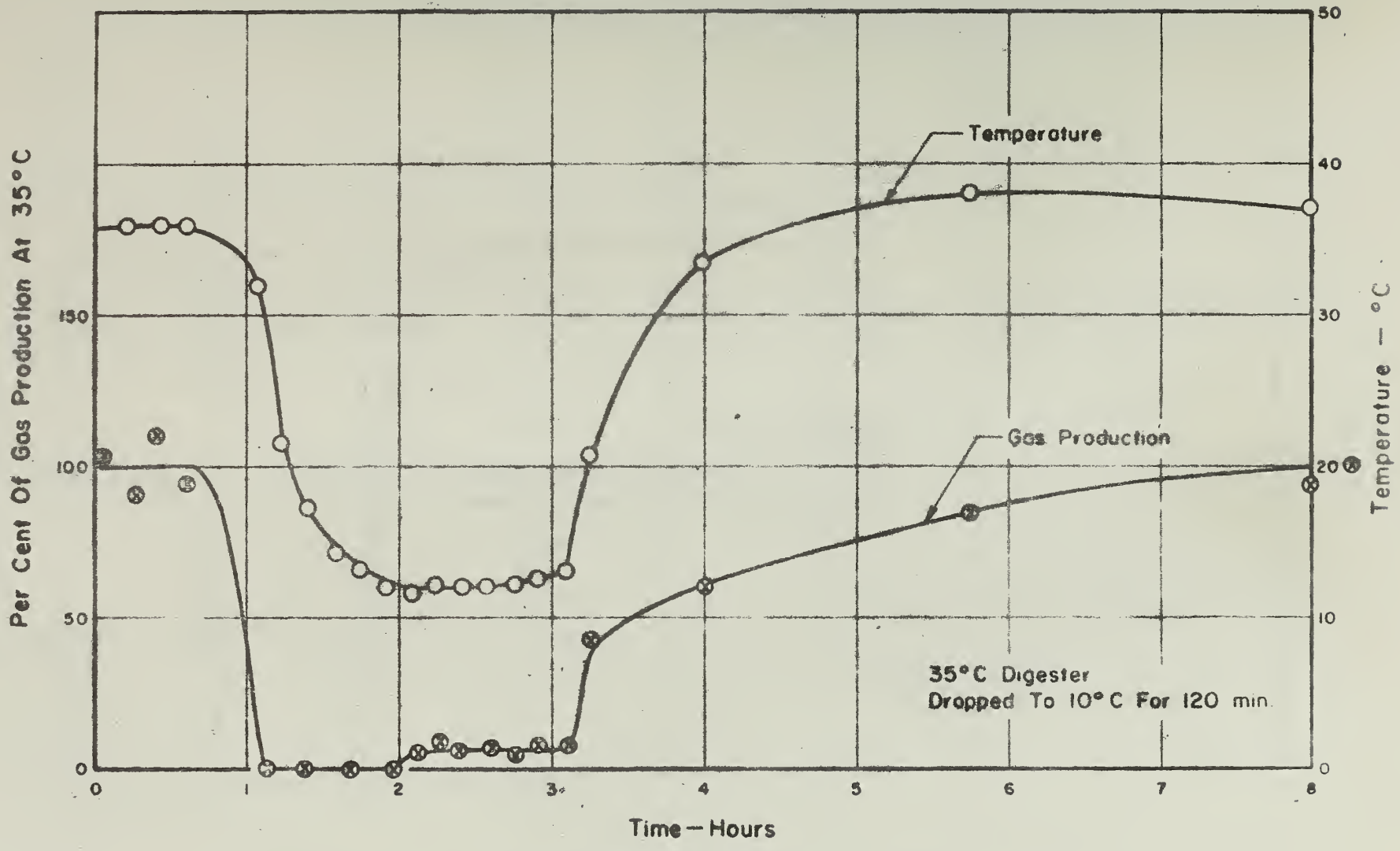

FIGURE 6. GAS PRODUCTION VS TEMPERATURE.

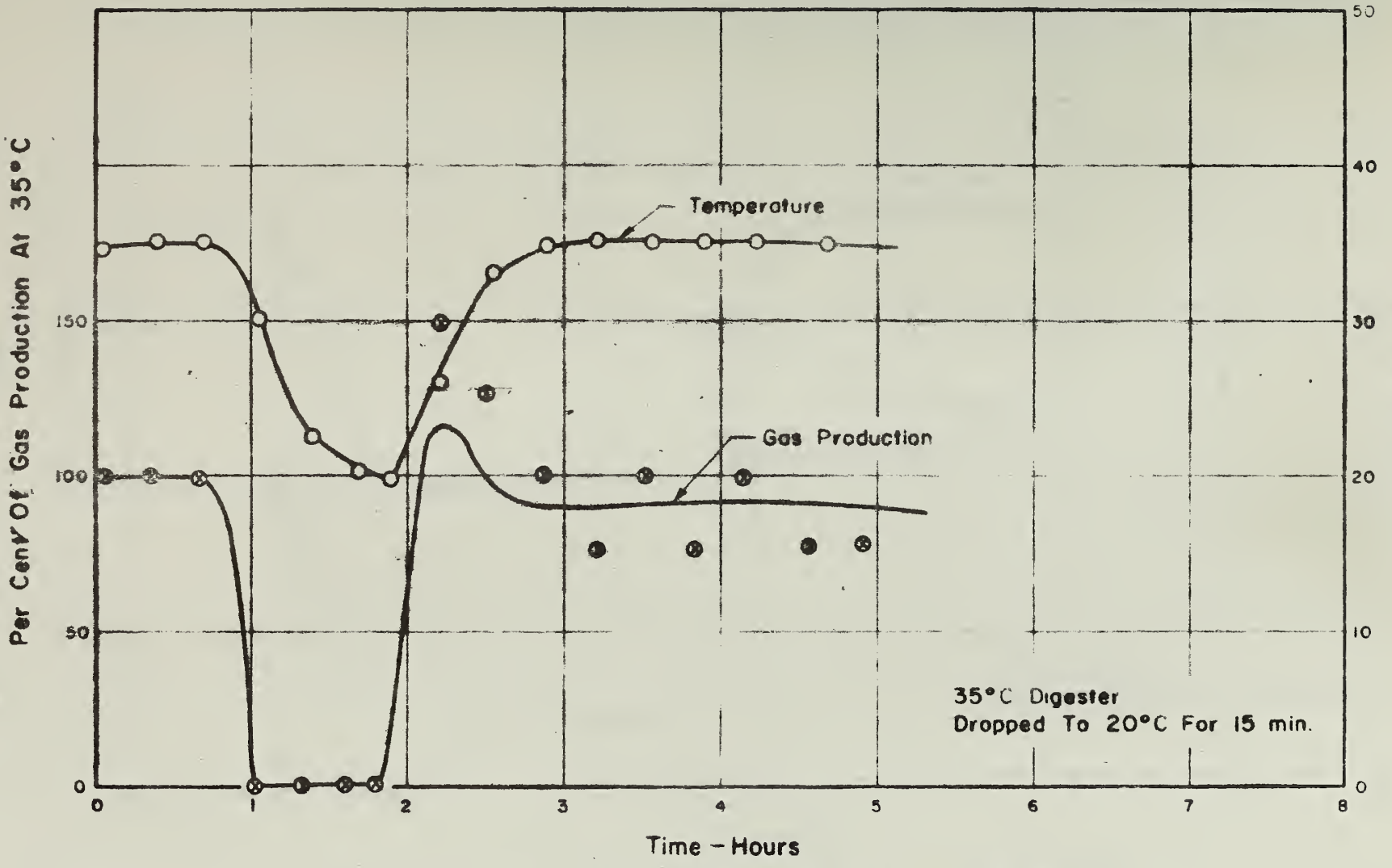

FIGURE 7. GAS PRODUCTION VS TEMPERATURE

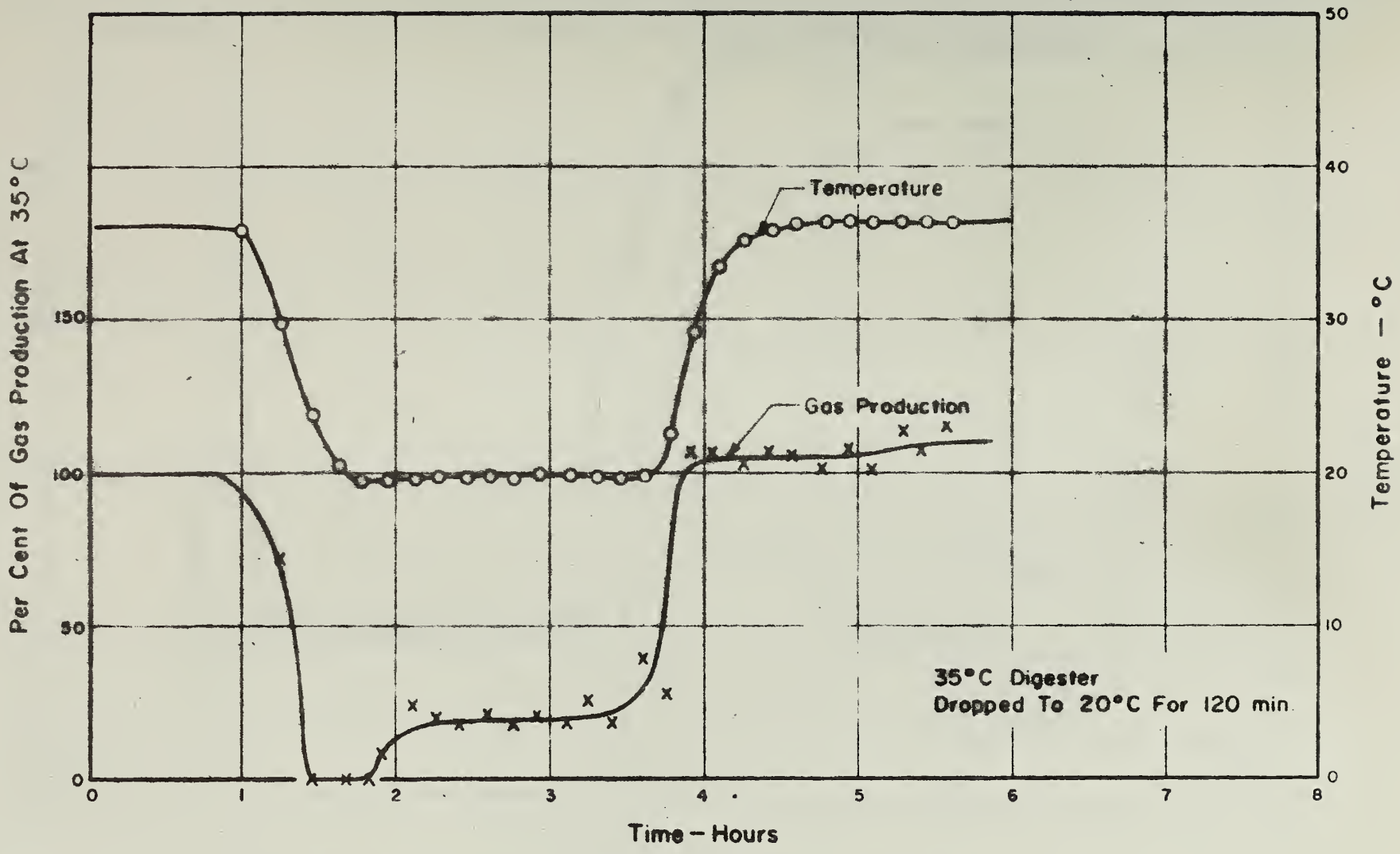

FIGURE 8. GAS PRODUCTION VS TEMPERATURE.

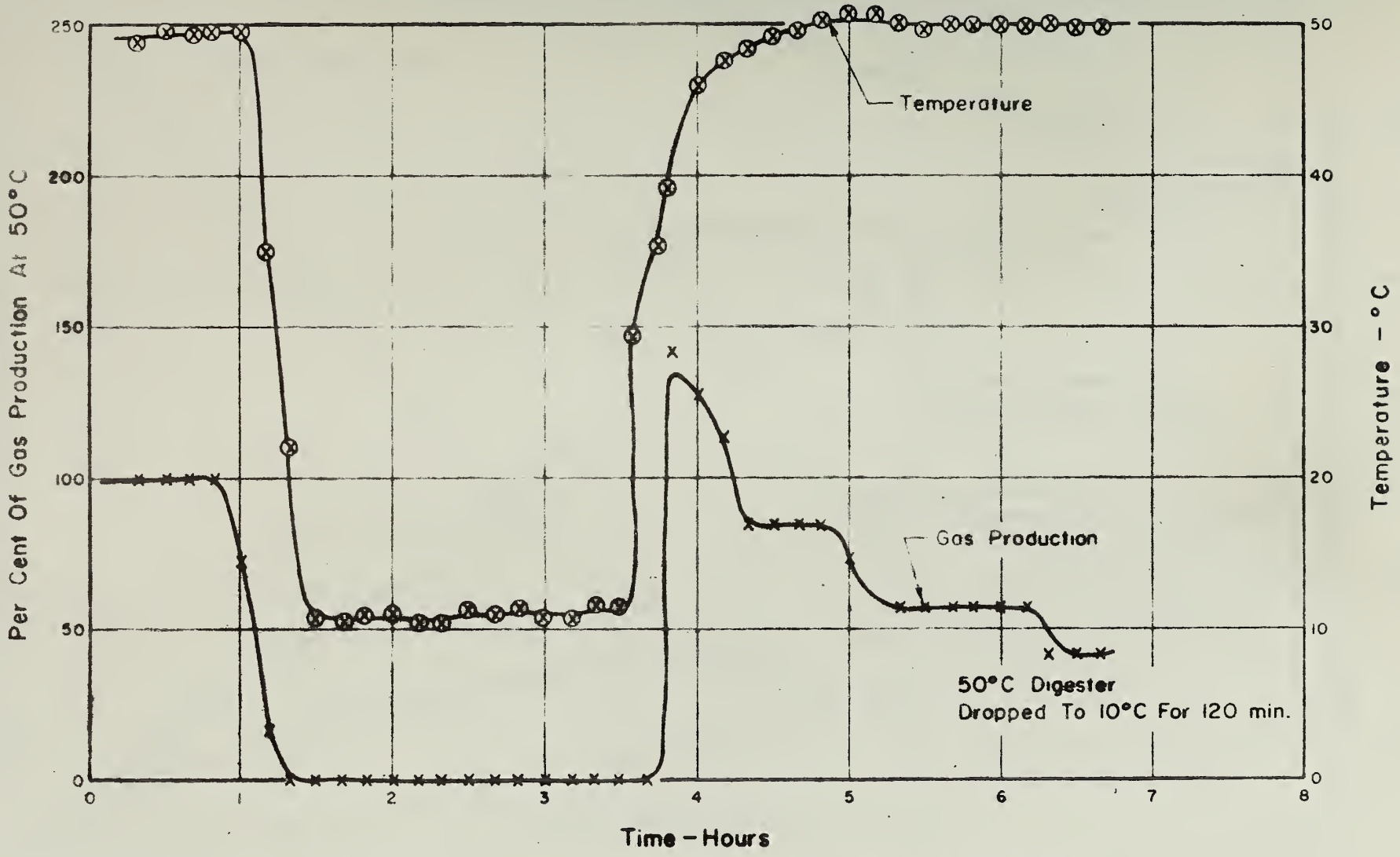

FIGURE 9. GAS PRODUCTION VS TEMPERATURE.

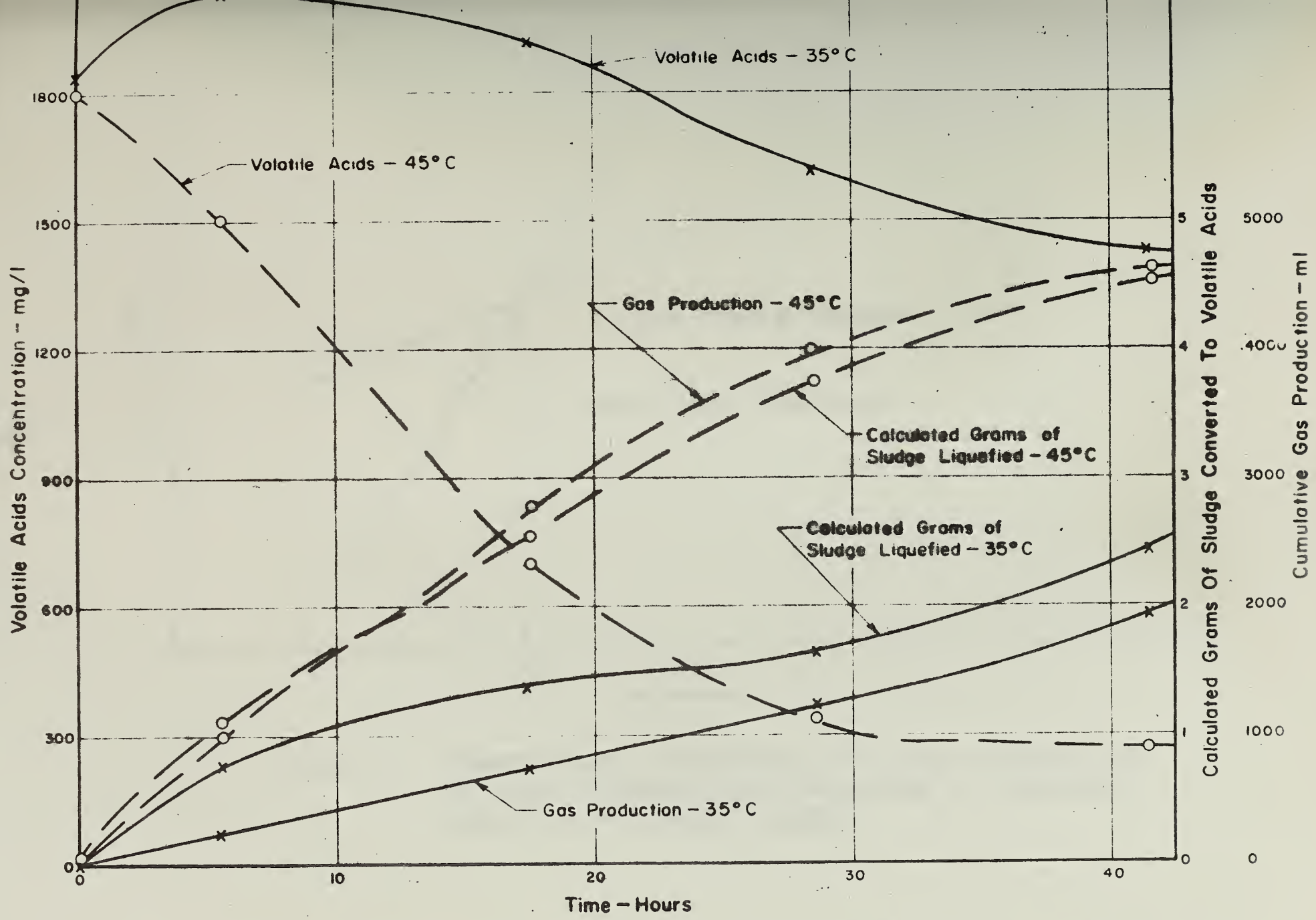

FIGURE 10. COMPARATIVE DIGESTER PERFORMANCE AT 35°C AND 45°C.

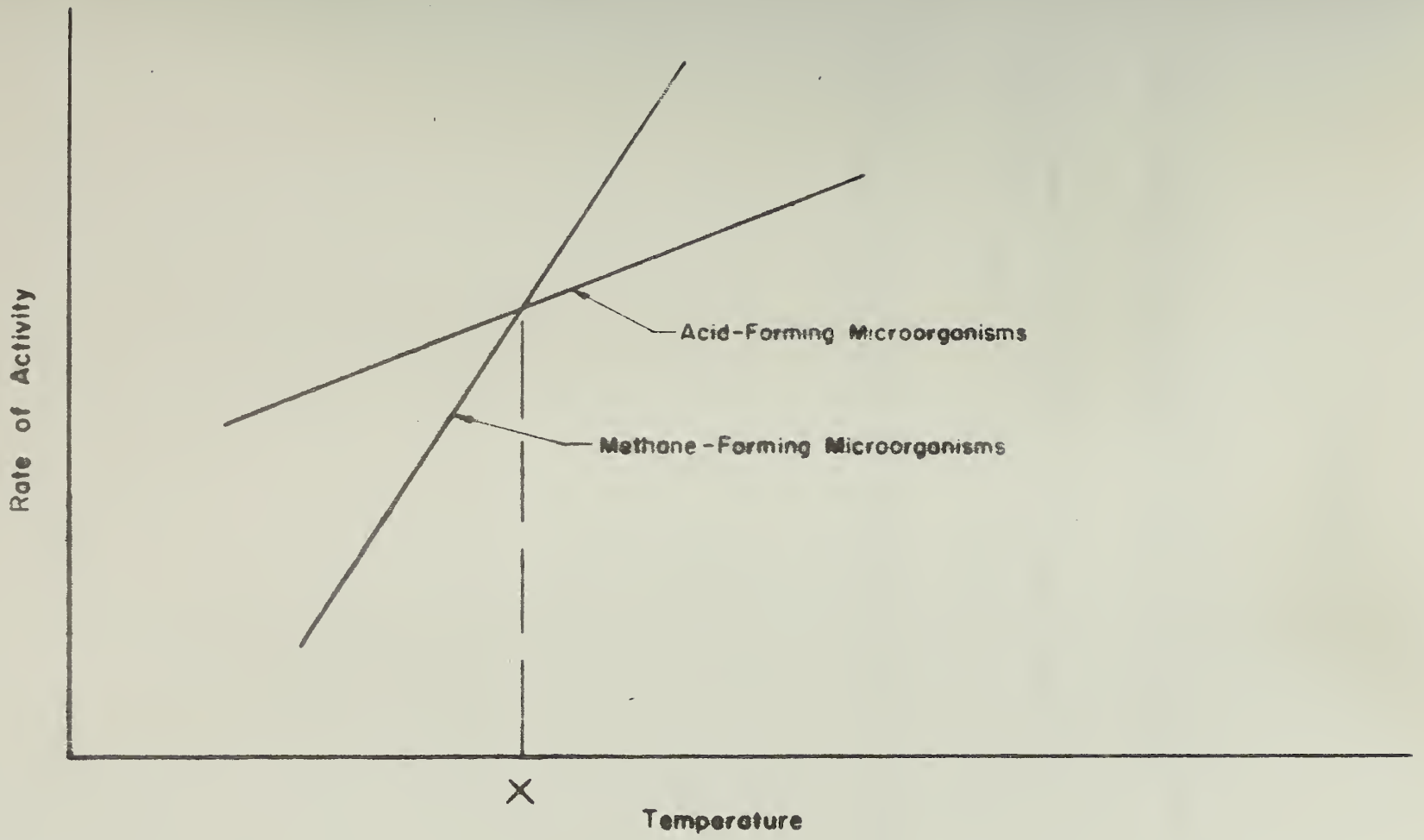

FIGURE 11. TEMPERATURE DEPENDENCE OF ACID-FORMING AND METHANE-FORMING MICROORGANISMS IN ANAEROBIC DIGESTION OF SEWAGE SLUDGE.

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THE EFFECT OF TEMPERATURE AND DETENTION TIME ON THE ACTIVITY OF METHANE FORMERS AND ACID FORMERS

THE EFFECT OF TEMPERATURE AND DETENTION TIME ON THE ACTIVITY OF METHANE FORMERS AND ACID FORMERS

Procedure:

The activity of methane formers and acid formers was studied at 25°C and 35°C. Two 750 ml digesters at the respective temperature were seeded with 675 ml of well digested sludge and fed with a mixed substrate of raw sewage sludge supplemented with acetic acid. Acetic acid was fed daily to make up the volatile acid concentration to 2000 mg/1. The detention time was varied to 10, 15, and 20 days, after the activity at each detention time reached equilibrium, by changing the volume of raw sludge feed each day.

The activity of the methane formers was calculated in terms of mg of COD/1/day from the volume of the methane produced by using the conversion factor of 400 ml of methane per gram of COD destroyed. The activity of the acid formers was also figured in terms of mg of COD/1/day by subtracting the COD of the acetic acid added supplementally to the respective digester from the activity of the methane formers.

Results:

The results are shown on Figure 1. The stable activity of methane and acid formers at each condition is listed in the following tabulation:

Period of Operation	Det. Time (Days)	Temp. (°C)	Methane Former Activity		Acid Former Activity	
			COD mg/1/day	#HAc/Ft^3/day	COD mg/1/day	#HAc/Ft^3/day
Days 117 - 130	10	25	1150	0.07	500	0.03
Days 0 - 54	10	35	3100	0.2	1700	0.10
Days 105 - 117	15	25	1600	0.1	850	0.055
Days 55 - 91	20	25	2250	0.14	850	0.055
Days 55 - 85	20	35	4800	0.30	1300	0.075

25°C Digester: Digestion failures resulted twice at a 10 day detention time on Day 20 and Day 48. Digestion was good at a 20 day detention time. After a

period of 50 days of operation on a 20 day detention time, the detention time was successfully reduced to 15 days at Day 105 and further reduced to 10 days at Day 117. The total period of operation was 75 days.

35°C Digester: This digester was started at a 10 day detention time. This detention time was then increased to 20 days on Day 55. Total operation time was 85 days.

Discussion

The equilibrium values of activity were plotted as bar graphs in Figure 1 for the temperatures and detention times studied. It appears that both acid formers and methane formers increase their activity with increasing temperature, but the acid formers increase their activity at a much lower rate than the rate at which methane formers increase their activity. Moreover, this trend is more distinct at longer detention times.

With respect to detention time, the methane formers increased their activity markedly with increasing detention time, while the activity of acid formers was not affected in such a definite way. The rate of acid formation was relatively independent of detention time. However, the quantity of substrate for the acid formers was decreased to achieve the longer detention times and the effect of detention time on the rate of acid formation was not clearly shown. This was evaluated more clearly in a subsequent study.

The activity of the methane formers is distinctly affected by the temperature and detention time. This dependence of activity on temperature most probably was due to that which characterizes ordinary enzymatic reactions, the rate of which increases with increasing temperature in the range lower than the optimum.

The effect of detention time on the methane production activity can be related to the generation time and equilibrium concentration of methane formers. The

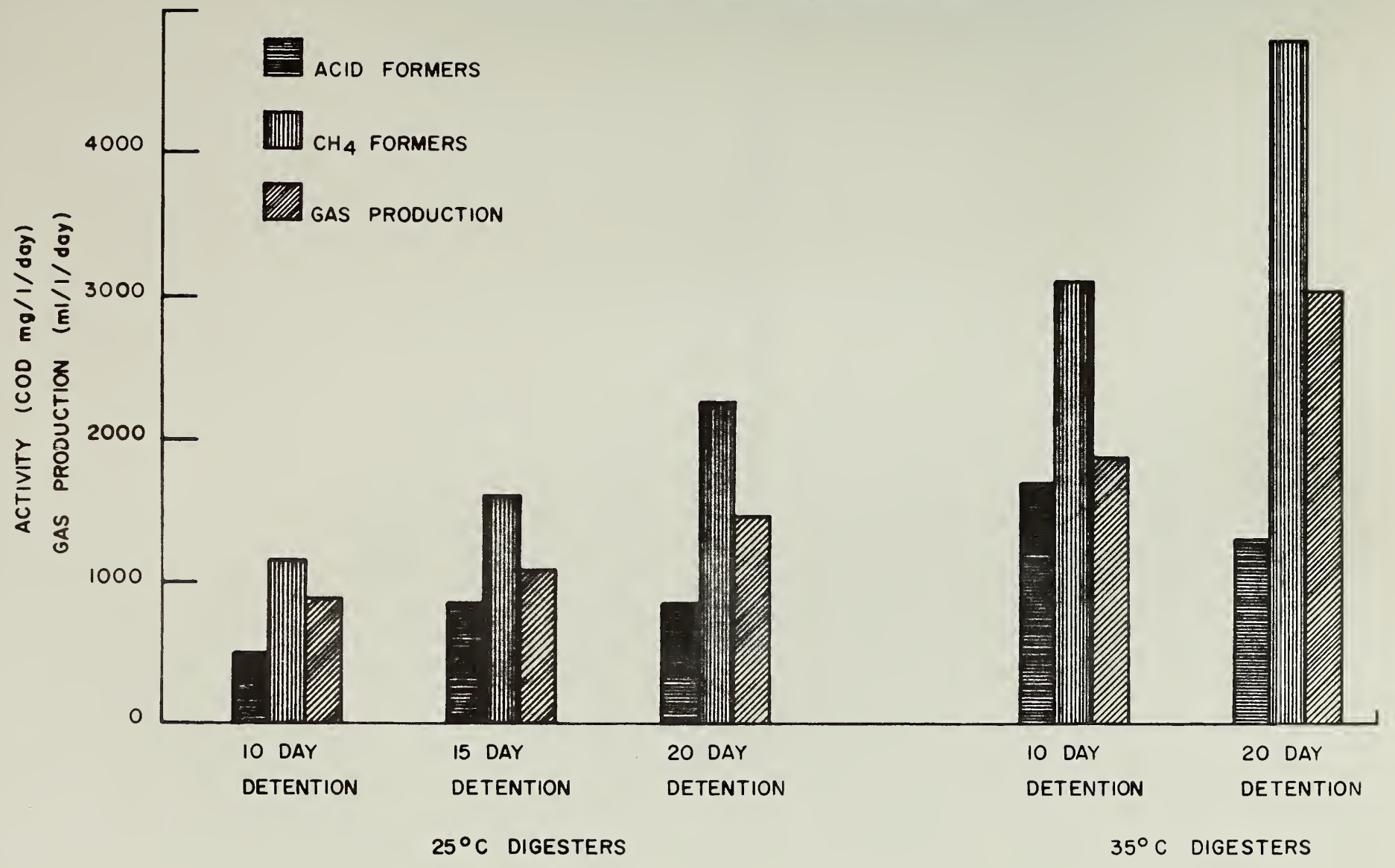
failures or the lower activities at the shorter detention times indicates that the majority or organisms responsible for methane production were washed out more rapidly than they reproduced, and their generation time was apparently longer than the average sludge detention time. At a given detention time, those species ppssessing shorter generation times than the hydraulic detention time will flourish. Therefore, at longer detention times, a greater number of methane formers will flourish in the digester and hence more methane production will result.

The effect of raw sludge loading was studied for a two week period at 25°C. During this period, the sludge loading was increased without changing the detention time of 20 days by feeding more concentrated raw sludge. Fifty ml of sludge (equivalent to a 15 day detention) was concentrated to thirty-five ml (equivalent to a 20 day detention.) The feed for thirty days was prepared from one sample of the raw sludge and frozen for storage. No distinct effect was observed on the activity of acid formers. A slight decrease of the activity of methane formers was observed.

It seems that a longer detention time is required to initiate digestion that is necessary after equilibrium is established. This fact was experienced in the operation of the 25°C digester. The period of time for which the digester must operate at extended detention times before equilibrium conditions are established is dependent on the change in temperature of the seed sludge from that to which it was acclimated. A stable activity was finally established in the 25°C digester at 10 days detention time after a period of operation on a 20 day detention time.

FIGURE 1

ACTIVITY AND GAS PRODUCTION



THE EFFECTS OF CONSTANT DETENTION TIME AND VARIED LOADING RATES ON ANAEROBIC DIGESTION

THE EFFECTS OF CONSTANT DETENTION TIME AND VARIED LOADING RATES ON ANAEROBIC DIGESTION

Introduction

In previous studies it was found that the activity of the methane formers was considerably enhanced by feeding a mixed substrate of raw sludge and acetic acid as compared with a digester fed with acetic acid alone, or with raw sludge alone at the same detention time. Furthermore, it was noticed that there was a significant interdependency between the activity of acid formers and methane formers in many cases. However, from the many cases of failure in sour digesters, it was apparent that acid formers can normally work effectively irrespective of the activity of methane formers. Therefore, this relation is a dependency of the methane formers on the acid formers.

Therefore, the objective of this study was to establish the relationship between type of substrate, loading rate and methane production when volatile acids were not limiting the rate of methane production. Raw sludge, activated sludge, Metrecal and glucose served as the various substrates. Anaerobic digestion is commonly considered to be accomplished by two groups of microorganisms - acid formers and methane formers. All of the substrates used required the activity of the acid formers, because of their complex nature. To insure that excess volatile acids were present continually so that substrate limitation did not control methane production, sufficient acetate was added daily to increase the volatile acids concentration to at least 2000 mg/1.

The loading rates used were 0.005, 0.05, 0.10 and 0.20 # volatile matter/ft^3/day for raw sludge and activated sludge. For Metrecal and glucose, 0.005, 0.05, 0.10 and 0.20 # COD/ft^3/day loading rates were used.

Activated sludge and raw sewage sludge were chosen to determine whether the effect of stimulation was in the degradation of a biologically synthesized substance. Metrecal and glucose were chosen to investigate the effect of the degradation of less complex organic substances on the methane fermentation. The latter was chosen also to observe the effect of the degradation of a pure organic substance on the rate of methane fermentation.

Literature Review

Although many studies have been conducted on the fermentation of volatile acids, few have attempted to show the correlation between the rate of volatile acid utilization and that of volatile acid production.

McCarty and Vath (9) have shown that the acetic acid utilization rate was remarkably increased by the continual addition of the dried solids of digester supernatant liquor. The rate of stimulation appeared to be proportional to the weight of solids added. The methane fermentation rate decreased gradually after the addition of the solids was stopped. They further noted stimulation was lost when the solids were ashed at 600^{o}C, even though the stimulative effect was not lost by sterilizing the solids under the condition of 120^{o}C for five minutes.

Andrews, Cole and Pearson (2) investigated the feasibility of multi-stage digestion with a soluble synthetic substrate. Although their emphasis was on the kinetic aspects, their results showed that the acetic acid utilization rate was highest in the stage where the acid production rate was highest. About half of the total gas produced was from the first stage digester where the acid production rate was highest, while the concentration and distribution of volatile acids were almost constant throughout the subsequent stages of fermentation.

Todd (12) reported that in the presence of butyric acid, propionic acid was decomposed more rapidly than when only propionic acid was present. Furthermore, he traced the fate of some individual intermediate acids when a complex pure organic substance was degraded . He showed that the rate of degradation of acetic acid in the glucose or xylose digester was definitely higher than that in the butyric acid digester. It also appeared that acetic acid was more rapidly degraded when it is accompanied with propionic acid than with butyric acid.

These observations might be explained by the finding reported by Speece and McCarty (11) that the acid formers exhibited exceptionally high net biological synthesis from carbohydrates. Jeris and McCarty (7) showed that the carbohydrate acclimated sludge utilized acetic acid effectively. Acetic acid cannot be degraded by the acid forming microorganisms, but the high population or high activity of the acid formers which degrade carbohydrates to lower fatty acids apparently benefits the methane forming organisms utilizing acetic acid.

Also, it should be noted that acetic acid acclimated sludge could not utilize carbohydrates (7). The predominant species of methane forming organisms could possibly be different in digesters receiving carbohydrates as compared to acetate digesters.

Procedure

Four 750 ml digesters for each respective substrate, were operated at 35°C for 27 - 29 days. Volatile matter or COD loading rates of 0.005, 0.05, 0.1 and 0.2 pound per cubic feet of digester per day were fed to the respective digesters. The detention time was 15 days for all digesters and accordingly 50 ml of the well-mixed digester contents was withdrawn and the feed was made up to the same volume and fed once a day. At the same time, acetic acid was fed in sufficient amount to maintain the volatile acid concentration in the range of 2000 - 2500 ppm at the beginning of every 24-hour period.

The total volatile acids content and the gas production were determined every day. The percentage of CO_2 in the digester gas was analyzed every 3 to 5 days and the remainder was assumed to be methane. The volume of methane was then used to calculate the activity of the methane formers. The activity of the acid formers was determined by subtracting the COD of the supplemental acetate added to the digester from the activity of the methane formers. The pH and alkalinity were also measured periodically. In order to maintain the pH above 6.8 a measured amount of ammonium bicarbonate was added as needed.

The necessary amounts of raw and activited sludge were taken at one time from the Urbana-Champaign Sanitary District Treatment Plant. The volatile content of the sludge was determined after concentration and homogenization. These slurries were again diluted the proper amount with water so that 40 ml of each diluted slurry contained the calculated volatile matter for each of the prescribed loadings. Then they were divided into 50 ml plastic centrifuge tubes and frozen for storage. Each feeding was made up to 50 ml with a solution containing inorganic nutrients before adding to each digester. Seventeen digesters were used in all, including one control digester. Yeast extract was fed to the glucose digesters and the acetate control digester at the rate of 10 mg/l in the feed.

All digesters were started in the same fashion with 700 ml of well digested sludge as the seed. Then 1000 mg/l of volatile acids, as acetic acid, was fed in the form of calcium acetate. Finally 50 ml of each respective feed was added to each digester. The ratio of COD to volatile matter was 1.57 for Metrecal and 1.06 for glucose. The composition and concentration of inorganic nutrients added in the feed to all digesters is shown in Table I.

TABLE I

Composition of Inorganic Nutrients A and B

Nutrient A

NH_4HCO_3	1.32 g/l - feed
$KHCO_3$	1.00 g/l - feed
$NaHCO_3$	1.67 g/l - feed
$(NH_4)_2HPO_4$	50.00 mg/l- feed
$NH_4Mo_7O_{24}$	30.00 mg/l- feed

Nutrient B

$CaCl_2$	1.47 g/l - feed
$MgCl_2 6H_2O$	1.02 g/l - feed
$MnCl_2 4H_2O$	9.00 mg/l- feed
$CoCl_2$	0.25 mg/l-feed as cobalt
$AlCl_3$	10.00 mg/l-feed

Results

These digesters were operated for approximately thirty days. The average rates of activity are plotted in Figures 1 and 2 as bar graphs. The activities of the acid formers and methane formers are plotted along with gas production.

Raw Sludge

With raw sludge as the substrate, the methane formers utilized approximately 1000 mg/l/day of acetate, at all loading rates, over and above the volatile acids produced by the acid formers. This indicates that the methane formers operate below their potential capacity in normal digestion because they only receive volatile acids at the rate at which they are produced by the acid formers. This observation is also borne out by operating experience in that the volatile acid concentration is consistently low, indicating that acid production is the rate limiting step.

With raw sludge, the activity of the methane formers was approximately 1300 mg of COD/l/day at the lowest loading rate and gradually increased to approximately 3600 mg of COD/l/day at the highest loading rate. Whereas, the rate of activity of the acid formers varied from approximately 100 mg of COD/l/day at the lowest loading to approximately 2600 mg of COD/l/day at the highest loading.

Activated Sludge

There was also a considerable difference between the rates of activity of the acid formers and methane formers with activated sludge as the substrate. The additional methane former activity amounted to 1200 to 1600 mg of COD/l/day in excess of the activity of the acid formers. Activated sludge is not as easy to degrade as raw sludge and consequently, the rates of activity of the acid formers varied from approximately 300 mg of COD/l/day at the lowest loading to approximately 1400 mg of COD/l/day at the highest loading. Both the magnitude of activity of the acid formers and the variation in magnitude from the lowest loading to the highest loading were considerably less with activated sludge than with raw sludge.

Metrecal

At the three lowest loading rates used with Metrecal, the rate of activity of the methane formers exceeded that of the acid formers by approximately 1800 mg of COD/1/day. However, at the highest loading rate of 0.2#COD/1/day, unsatisfactory digestion developed and the methane formers could not utilize the volatile acids at a rate equal to that at which they were produced. Metrecal was readily degradable and 80%, 85%, and 97% of the applied COD was transformed by the acid formers at loading rates of 0.20, 0.10 and 0.05#COD/of/day respectively.

Glucose

The rate of activity of the methane formers at the lowest loading rate was approximately 600 mg of COD/1/day. This is only about half the rate achieved with raw sludge, activated sludge and Metrecal at the same loading rate. However, at the two intermediate loading rates, the methane formers operated at a higher rate than the other three substrates. With glucose, as with Metrecal, unsatisfactory digestion developed at the highest loading rate and the results are inconclusive.

Discussion

The synergistic effect of acid formers upon the methane formers was demonstrated to be independent of the type of substrate used by the acid formers. Trace materials might be expected from the raw sludge, activated sludge and Metrecal substrates. However, the fact that glucose exhibited the same effect demonstrates that trace materials are not necessary, since none are produced from glucose during its degradation. Therefore, the synergistic effect appears to be produced as a result of the metabolic activity of the acid formers.

Several possibilities exist which may explain the synergism. The kinetic theory of enzymatic reactions indicates that the reciprocal of the substrate utilization rate per unit weight of organisms is proportional to the reciprocal

of the substrate concentration (1), (2). Since the volatile acid concentration in all digesters was consistently at the same high level, the higher methane production rates at the higher loading rates cannot be explained on this basis. Also Golueke (5) indicated that the activity of gas production was not affected by the high acid content in his study of raw sludge digestion.

Agardy, et al (1) and Lawrence and McCarty (8) fitted substrate removal rates in terms of COD to the Michaelis-Menten model. Lawrence and McCarty (8) evaluated the constants for several substrates and at several different temperatures. The Michaelis-Menten equation predicts that the substrate removal rate is a function of the substrate concentration and the number of organisms present. However, at high concentrations, the substrate removal rate becomes essentially constant. The smaller the value of the constant, K_m, the concentration at which the removal rate is half the maximum, the less dependent substrate removal rate is upon substrate concentration at higher concentrations. At 35°C with acetate substrate, Lawrence and McCarty (8) found K_m equal to 220 mg/l. Since this value is comparatively low and the fact that the volatile acid concentrations at all loading rates were comparable, the difference in methane production rates does not appear to be due to substrate concentration.

Buswell (4) and Todd (11) reported that an anaerobic digester receiving a high concentration of glucose and having a volatile acids concentration of 7000 mg/l evolved gas with a high hydrogen content and no methane. Smith (10) reported that hydrogen gas accounted for 23% of the methane produced from raw sludge and acetate accounted for 73% of the methane. However, three times as much energy was derived from the hydrogen even though it accounted for only one fourth of the total methane production. This provides a possible explanation for the synergism

demonstrated in this study. The hydrogen produced during the acid production phase of anaerobic digestion may have been utilized by the methane producing organisms and have substantially increased their synthesis rate. Thus, a greater quanity of methane producing organisms would be present in the system and consequently, increased methane producing organisms can utilize both hydrogen gas and acetate as their energy source. It is also assumed that the fermentation in these digesters was normal and the activity of the methane formers mainly represents the consumption rate of acetic acid, with minor amounts of propionic acid, as indicated by many researchers (3), (6), (7).

The presence of an inert surface was reported by Buswell and Hatfield (4) to be beneficial to methane production. This explanation of the stimulation observed in this study does not hold, since glucose was a soluble substrate and yet good methane production rates were observed. The only surface present in the glucose digester would be due to the synthesis of microorganisms because of the soluble nature of the substrate.

Another possible explanation of this synergism may be the presence of refractory organic materials excreted during metabolism. These humic-like materials characteristically exhibit good chelation characteristics. In the highly alkaline environment of the digester, many of the common trace metals precipitate out of solution and thus are unavailable as biochemical catalysts. Fe^{++}, Mn^{++}, Zn^{++} and Cu^{++} precipitate from alkaline solutions at or near neutral pH. The presence of chelating agents holds such trace metals in solution. The trace metals may either be made available to the microorganisms or prevented from exerting a toxic effect by the presence of chelators. Thus, the end-products of metabolism of the acid formers may provide the necessary chelation which results in stimulation of the methane formers. In the culturing of algae, iron is commonly added in a chelated

form so that it will be available as a biochemical catalyst. A study is presently underway in which chelated trace metals are being added directly to digesters for assay of their methane production stimulation potential.

Summary

The methane production capacity exceeded the acid production capacity in all but two of the sixteen anaerobic digesters receiving four different substrates and with four different loading rates for each substrate. The methane forming organisms were able to utilize an additional 1000 to 1500 mg of COD/l/day of acetate in excess of the end products produced by the acid forming microorganisms. The total methane production was dependent on the loading rate and to a lesser extent on the type of substrate. Hydrogen production and/or production of metabolic end-products which serve as chelators of trace metals is considered to play an important role in the stimulation observed in this study.

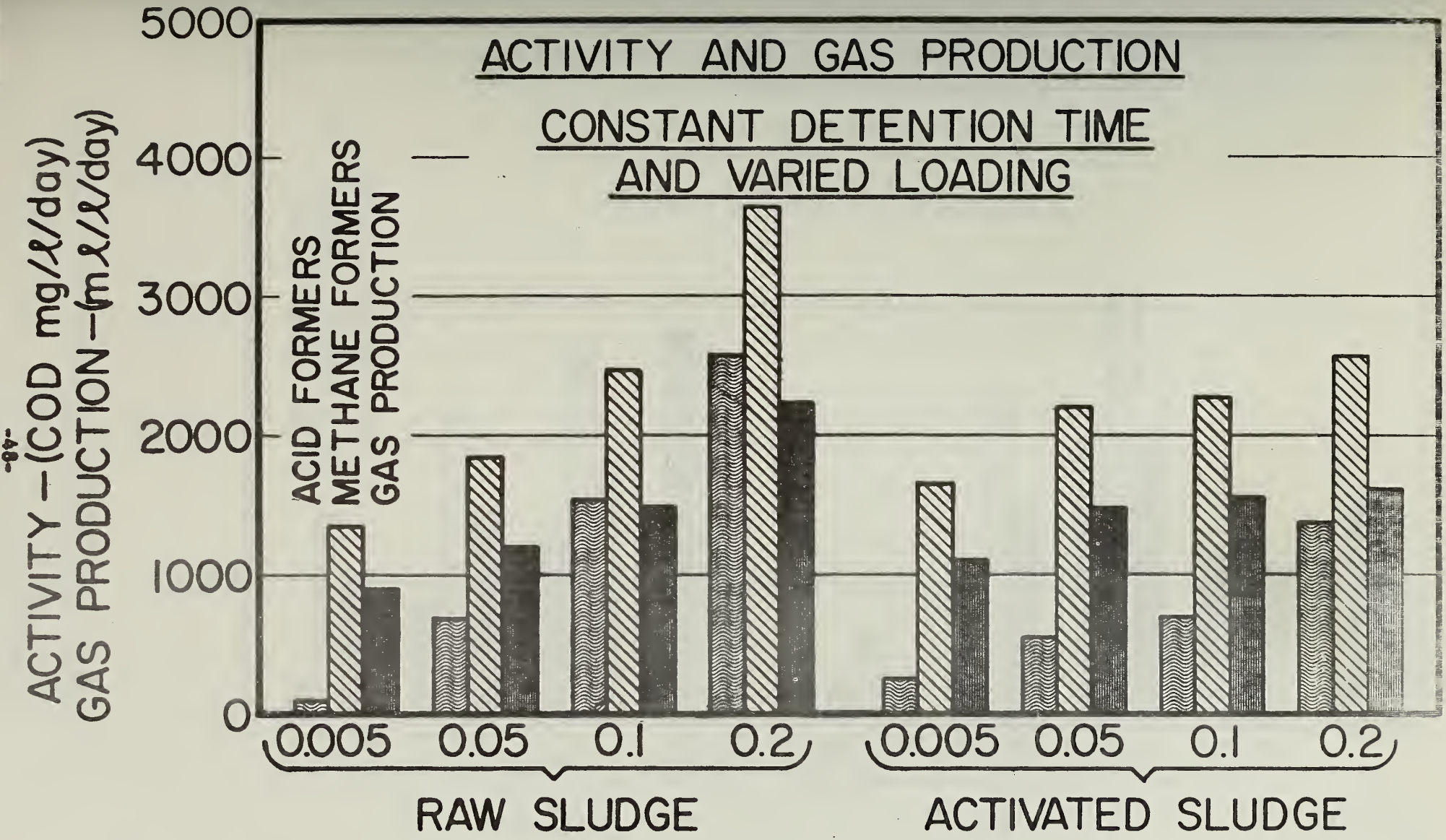

ACTIVITY AND GAS PRODUCTION
CONSTANT DETENTION TIME
AND VARIED LOADING
ACTIVITY – (COD mg/ℓ/day)
GAS PRODUCTION – (mℓ/ℓ/day)
ACID FORMERS
METHANE FORMERS
GAS PRODUCTION
5000
4000
3000
2000
1000
0
0.005
0.05
0.1
0.2
0.005
0.05
0.1
0.2
RAW SLUDGE
ACTIVATED SLUDGE

FIG. 1

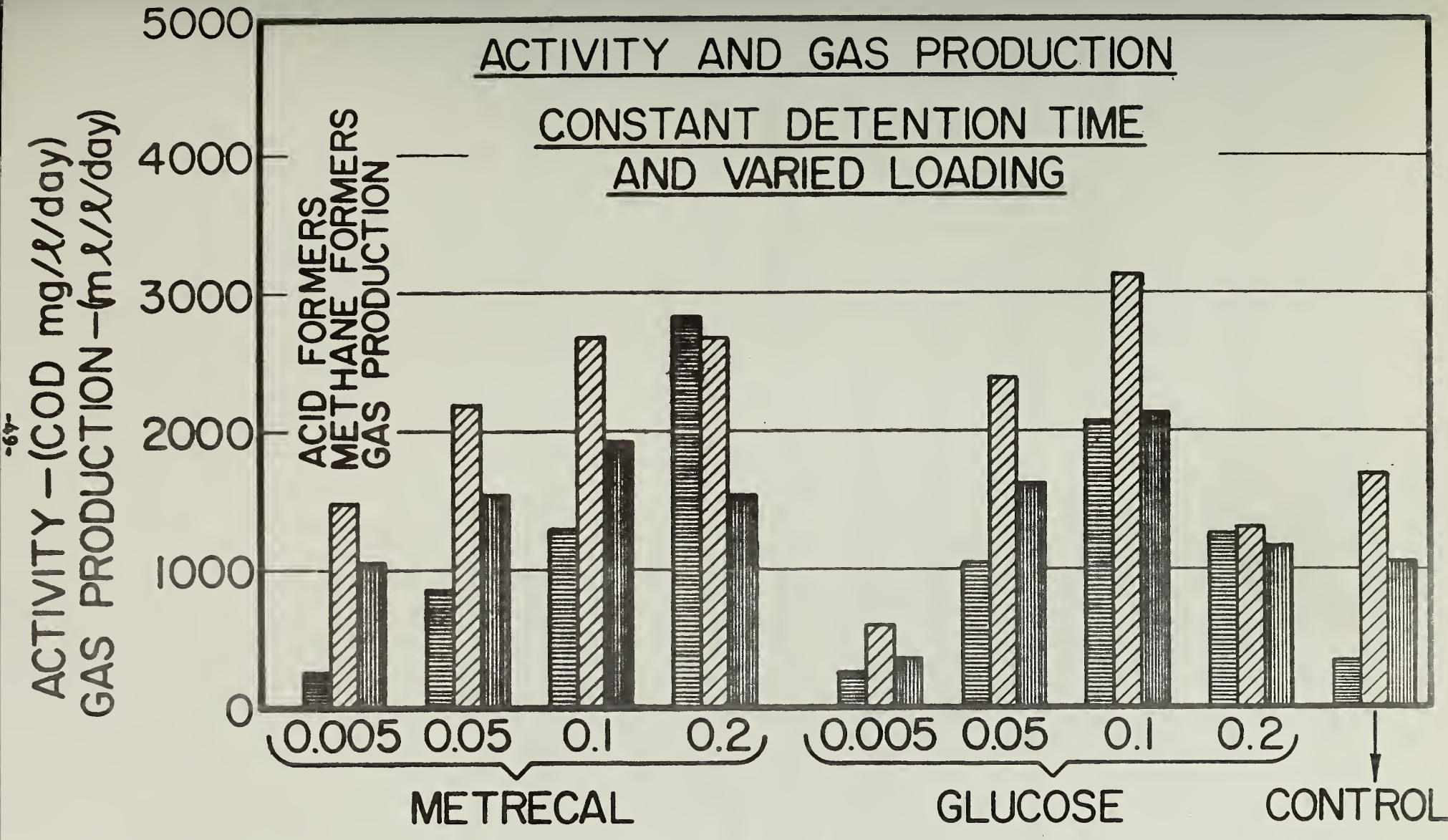

ACTIVITY AND GAS PRODUCTION
CONSTANT DETENTION TIME
AND VARIED LOADING
ACTIVITY – (COD mg/ℓ/day)
GAS PRODUCTION – (m ℓ/ℓ/day)
5000
4000
3000
2000
1000
0
ACID FORMERS
METHANE FORMERS
GAS PRODUCTION
0.005
0.05
0.1
0.2
0.005
0.05
0.1
0.2
METRECAL
GLUCOSE
CONTROL

FIG. 2

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APPENDIX

TABLE 1

Average Activity and Average HAc Feed Rate

Constant Detention and Varied Looding

NO	Days of Average	Substrate and Loading	Acid Formation (COD mg/1/day)	Methane Formation (COD mg/1/day)	Gas Reduction (ml/1/day)	Acetic Acid Fed (mg/1/day)	Residual Vol. Acid (mg/l)
		R					
1	19-27	0.005	96	1340	895	1280	701
2	"	0.05	675	1830	1200	1200	778
3	"	0.1	1530	2470	1490	930	1059
4	"	0.2	2590	3640	2240	1050	951
		A					
5	"	0.005	254	1660	1115	1410	592
6	20-27	0.05	560	2200	1480	1700	595
7	"	0.1	717	2280	1570	1490	755
8	"	0.2	1380	2580	1620	1130	810
		M					
9	"	0.005	267	1490	1060	1180	708
10	"	0.05	855	2207	1560	1320	651
11	18-26	0.1	1307	2680	1930	1370	628
12	18-22	0.2	2840	2680	1540	0	1824
		G					
13	18-26	0.005	267	615	358	413	1533
14	"	0.05	1050	2380	1620	1320	682
15	"	0.1	2060	3130	2140	1120	863
16	21-27	0.2	1270	1310	1190	0	2288
		Set					
17	20-26	Sew	127	815	465	760	1280
18	20-27	Control	338	1698	1055	1510	830

* R: raw sludge M: Metrecal
A: activated sludge G: Glucose

TABLE 2

Volatile Matter Destruction and Gas Production Efficiency

Constant Detention and Varied Loading

NO	Substrate and Loading #/cf/dy)	Complex Org. Dest. (mg/1/day)	Tot. Vol. Matter Dest. (mg/1/day)	Gas Prod. Per Tot.Vm. Dest. (cf/lb/Vm)	Methane Per Tot. Vm. Dest. (cf/lb/Vm)	Gas Prod.Per Tot. Vm. fed (cf/lb/Vm)
	R					
1	0.005	27	1430	12.2	6.6	11.7
2	0.05	1560	2740	7.1	4.3	9.7
3	0.1	1230	2240	10.6	7.0	8.9
4	0.2	1800	2790	12.5	8.1	8.0
	A					
5	0.005	101	1620	12.5	7.4	12.7
6	0.05	298	1960	11.7	7.0	9.5
7	0.1	553	2130	12.2	7.0	8.5
8	0.2	1075	2180	11.9	7.6	6.4
	M					
9	0.005	-----	967	17.0	9.9	13.0
0	0.05	322	1650	15.2	8.7	13.1
1	0.1	770	2160	14.0	7.8	12.1
2	0.2	-----	1720	14.9	10.4	11.6
	G					
3	0.005	-----	206	27.8	21.0	11.8
4	0.05	560	1810	14.2	8.3	12.5
5	0.1	1190	2150	16.2	9.4	13.7
6	0.2	1740	1610	13.1	6.4	10.5
	Set					
7	Sew.	-----	443	14.2	9.3	9.5
8	Control	-----	1300	12.5	8.3	10.7

DESTRUCTION OF THE PROTEIN AND LIPID COMPONENTS OF RAW SLUDGE DURING ANAEROBIC DIGESTION

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Introduction

The objective of this study was to observe the degradation of the protein and lipid fraction of raw sludge in anaerobic digestion. This was observed under natural conditions prevailing when only raw sludge was added as well as under a condition in which supplemental additions of acetate were made to increase the volatile acid concentration to 2000 mg/l daily.

These observations were made while varying the detention time and temperature. The studies were made under constant as well as varied loading conditions. Constant loading rates were achieved by adding the same quantity of sludge each day to digesters operating at the various detention times and adding sufficient dilution water to achieve the desired detention time. This had the obvious advantage of being able to elucidate the effect of detention time alone without having to consider the effect of varied loading rate which results when detention time is controlled by feeding varied quantities of sludge.

The total solids, volatile solids, protein and lipid components of the raw sludge feed were determined. The degradation of these components during anaerobic digestion was ascertained by analyzing the effluent samples and comparing with the feed. An attempt was then made to fit the results to a model developed by Herbert, Elsworth, and Telling (1).

Procedure

Laboratory digesters containing 750 ml of actively digesting sludge were used in this study. Raw sludge from the Urbana-Champaign Sewage Treatment Plant was used as the feed. In some instances it was concentrated by allowing natural flotation to occur overnight and draining off the solids-free water.

Digesters were set up according to the following schedule for the first phase:

	35°C		25°C	
	Constant Loading	Varied Loading	Constant Loading	Varied Loading
Loading Rate (#volatiles/ft^3/day	0.15	0.09-0.35	0.15	0.038-0.23
Detention Time (Days)	6-25	6-25	6-25	10-60

During the second phase of the study, the volatile acid concentration was supplemented by adding acetate in order to increase the concentration to 2000 mg/l at the start of each day. The detention times studies were from 10-30 days.

Discussion of Results

The first phase of this study was conducted for 51 days with those digesters which operated well and for 81 days with those digesters which proved difficult to operate at the shorter detention times. The former, stable digesters were used for the second phase of this study. During this second phase, the raw sludge was supplemented with acetate to continually maintain a high volatile acids concentration.

The operation of these digesters is indicated in Figure 1, 2, 3, and 4. The three-day average gas production of the respective digesters is plotted vs days of operation. In order to achieve satisfactory digestion in some of the shorter detention time digesters, it was necessary to lengthen the detention time for the periods indicated in the figures.

Difficulty was encountered in achieving satisfactory operation of the following digesters:

Temp. (C°)	Type of Loading	Loading Rate (#/ft^3/day)	Detention Time (Days)
35	constant	.15	6
35	constant	.15	7.5
35	varied	.35	6-6.25
25	constant	.15	7.5
25	varied	.23	10

The highest methane production was obtained from the following digesters:

Temp. (C°)	Type of Loading	Loading Rate (#/ft^3/day)	Detention Time (Days)	CH_4 Production (Vol./vol./day)
35°C	Constant	.15	15	1.31
35°C	Varied	.30	7.5	2.18
25°C	Constant	.15	15	1.17
25°C	Varied	.20-.15	11.5-5	1.17

Figure 5 shows the total gas production and gas production per pound of volatile matter added under varied loading conditions. It is to be expected that the total gas production decreased with increasing detention time in the varied loading rate study, since less volatile matter is added to achieve the longer detention time. However, at detention times of 15 days or more, total gas production was essentially the same at both 25°C and 35°C. The same was true for the amount of gas produced per pound of volatile matter added at these temperatures.

Figure 6 reinforces the above observations, since a constant loading rate was used at all detention times. Figure 6 also indicates that gas production is relatively independent of detention time and temperature within the limits of this study. These appears to be a slightly greater total gas production at 15 days detention times as compared to shorter or longer detention times. The somewhat reduced gas production at shorter detention times is probably due to incomplete degradation of the raw sludge. At the longer detention

times, endogenous metabolism may consume a greater amount of intermediate degradation products, leaving less available for gas production.

Figures 7 and 8 reveal the fact that when the protein and lipid concentration was plotted versus detention time, each had a minimum value in the effluent sludge at a short detention time followed by an increase. It has been anticipated that there would be a consistent decrease. In the series of varied loading rate studies, the 35°C digesters showed a minimum value of organics in the effluent at 7.5 days detention time and an increased value at 10 days detention time. With the 25°C digesters, comparable values were noted at 15 days detention time and 20 to 25 days detention time respectively. In the latter studies, the reduction of the volatile matter was determined only with the digesters of detention times longer than ten days. Therefore, the volatile matter in the effluent vs detention time curve showed a consistent decrease with increasing detention time.

The first trend has some similarity to the steady-state relationship between detention time and bacterial concentration developed theoretically and examined experimentally by Herbert, Elsworth and Telling,[1] although their data was collected with a pure culture.

Their theory is based on the material balance in a completely-mixed type of continuous culture vessel:

$$\text{Change in bacterial concentration} = \text{growth} - \text{out put}$$

$$\frac{dx}{dt} = u\,x - Dx \qquad (1)$$

$$\text{Change in substrate concentration} = \text{input} - \text{output} - \text{consumption}$$

$$= \text{input} - \text{output} - \frac{\text{growth}}{\text{yield constant}}$$

$$\frac{dS}{dt} = DS_R - DS - \frac{u\,x}{Y} \qquad (2)$$

x = bacterial concentration in vessel

S = substrate concentration in vessel

S_R = feeding substrate concentration

D = dilution rate ($\frac{\text{inflow rate}}{\text{volume of vessel}}$, number of complete volume changes or reciprocal of detention time)

u = specific growth rate

y = yield constant = $(\frac{\text{weight of bacteria formed}}{\text{weight of substrate used}})$

By using the Michaelis-Mentern equation:

$$u = \frac{u_m S}{K_S + S}$$

u_m = Maximum growth rate

K_S = substrate concentration when growth rate is half of maximum

they derived:

$$\frac{dx}{dt} = x\left[u_m \left(\frac{S}{K_S + S}\right) - D\right] \qquad (3)$$

$$\frac{dS}{dt} = D(S_R - S) - \frac{u_m X}{Y}\left(\frac{S}{K_S + S}\right) \qquad (4)$$

At steady state:

$$\frac{dx}{dt} = \frac{dS}{dt} = 0$$

$$\bar{S} = K_S \left(\frac{D}{u_m - D}\right) \qquad (5)$$

$$\bar{x} = Y(S_R - \bar{S}) = Y\left[S_R - K_S \left(\frac{D}{u_m - D}\right)\right] \qquad (6)$$

The plot of this relation is shown in Figure 15. In these results it could be assumed that the protein concentration as well as lipid and volatile matter concentration shown in Figure 7 (but not in Figure 8) is proportional to the total concentration of undegraded substrate and the cell mass in the effluent, that is, in the vessel. The summation of $\bar{S}$ and $\bar{x}$ of Figure 15 will yield the curve shown

in Figure 16. This plot of the summation of $\bar{x} + \bar{S}$ resembles the mirror image of the curves in Figure 7, since the inverse of detention time is plotted. However the difference lies in the fact that the summation of $\bar{x}$ and $\bar{S}$ in Figure 16 does not have the minima and maxima shown in Figure 7. This latter difference might be explained by considering endogenous respiration.

The derivation of equation (5) and (6) has neglected the endogenous consumption of cell masses. Although Speece and McCarty[2] have indicated that the endogenous consumption rate in anaerobic digestion in terms of specific maintenance "a" is 0.005 (day^{-1}) or 0.0002 (hr^{-1}) under starved condition, several researchers have reported that the "a" value varies depending on the stage of the growth as well as on the temperature. Marr, Nilson and Clark[3] have reported that the value of "a" with E. coli is 0.028 (hr^{-1}) under glucose limited growth and is almost zero at unrestricted growth.[7] Therefore, it could be reasonable to assume that the endogenous consumption of the material in the vicinity of the washing-out detention time is almost zero and at the detention time where the substrate-limited growth governs, "a" is 0.02 to 0.03 (hr^{-1}) for an aerobic system with glucose substrate. As for the effect of temperature on "a", Marr et.al.[3] reported the following with E. coli under aerobic conditions:

at 15°C $\quad a = 0.005\ hr^{-1}$

at 28°C $\quad a = 0.028\ hr^{-1}$

Then if we take endogenous respiration into account, equations (1) through (6) could be replaced by equations (7) through (10):

$$\frac{dx}{dt} = u\,x - Dx - ax \tag{7}$$

$$\frac{dS}{dt} = DS_R - DS - \frac{u\,x}{Y} - ax \tag{8}$$

By the same procedure:

$$\bar{S} = \frac{K_S(a + D)}{u_m - (a + D)} \tag{9}$$

$$\bar{x} = \frac{Y\left[S_R - K_S \frac{a + D}{u_m - (a + D)}\right]}{1 + \frac{a(1-Y)}{D}} \qquad (10)$$

The magnitude of the endogenous respiration rate may vary from nil at unrestricted growth to as high as 10% of the specific growth rate under starvation conditions.

In order to check the adaptability of the protein concentration to the Herbert type curve the protein curve of Figure 7 was replotted in Figure 17 using dilution rate on the abscissa. The hypothesized lines of bacterial concentration and degradable substrate concentration were also added. Since some of the protein is undegradable, there will never be complete degradation. However, the zero scale could be shifted upward to coincide with the level of undegraded protein at infinite detention times and then the Herbert-type curve would more nearly describe the results of this study. The hump in the curve cannot be explained.

The protein and lipid curves must be examined in conjunction with the gas production curves. By comparing Figure 7 with Figure 5, it can be noticed that the gas production rate at 35°C is highest at the detention time where the protein and lipid curves show a minimum. The protein and lipid curves are representative of bacterial population as well as raw sludge substrate concentration. The protein, lipid, volatile matter and total solids analyses would measure the quantity of these materials in the raw sludge substrate as well as that found in the bacteria which are synthesized during degradation of the raw sludge. The great majority of protein, lipid, volatile matter and total solids would be attributed to the raw sludge, while only a minor fraction would be contributed by the bacteria which are synthesized during the degradation process. In anaerobic digestion, the fraction of synthesis is quite low. In the above discussion the case of constant loading was omitted. This is because the experiment of constant loading seems to be a study of quite a different scheme of investigation from the view point of the steady-state relation such as the Herbert-type curves shown in Figure 15. With the varied loading rate study, application of Herbert's curves

was not as successful, but with the results from the constant loading study, it provided a good basis of explanation.

In the case of constant loading rates, the concentration of the substrate decreases proportionately to the reciprocal of the detention time (proportionately to the dilution rate "D"). Therefore, if the experimental condition of this scheme is expressed in terms of the substrate concentration "S_R" and the detention time (or dilution rate "D"), each detention time at a constant loading refers to a point on a different curve in Figure 15, since S_R is different in each digester. The protein concentration was treated like a bacterial concentration and replotted on Figure 18 using Table 4 in the Appendix. The broken lines are hypothetical qualitative Herbert-type curves. Each plot is equal to the sum of the substrate concentration and the bacterial concentration in terms of protein. For example, the digester of 7.5 days detention was fed with a substrate having a concentration $S_R = \frac{7.5}{15} C_o = 1/2\ C_o$. Thus the plotted value should be equal to the sum of the substrate concentration and the bacterial concentration at the 7.5 day detention period.

Herbert et.al.[1] have defined the output as "(D) $(\bar{x})$" which means the production of cells per unit volume of the vessel per unit time $(\frac{1}{hr}) \times (\frac{gm}{l}) =$ gm/l/hr). They examined the theoretical equation expressing the relation between the output and detention time experimentally and gave such an output curve as shown in Figure 16.

If we assume that the gas production is proportional to this "output", the gas production vs detention time at constant loading could be expressed as a tieline connecting the corresponding point of each Herbert-type curve as shown in Figure 19 and 20. By changing the dilution rate scale to a detention time at the larger values of "D", the scale will be contracted and at the smaller values of "D' the scale will be expanded as can be seen in the scale of Figure 17. It can be seen from Figures 5 and 6 that the gas production curve at constant loading is much flatter than that at the varied loading.

An interesting comparison is shown in Figure 21. The gas production rates at equilibrium operation were plotted as Curve I for the study of varied loading rate and varied detention time. After this study was completed, the digesters continued to receive raw sludge at the same rate and at the same detention time. However, acetic acid (HAc) was added daily in sufficient amounts so that the volatile acid concentration was maintained in excess of 1000 mg/l. Thus, the rate of gas production was not limited by low volatile acid concentrations as is normally the case when only raw sludge is added. The gas production values under conditions of excess volatile acids are plotted as Curve II in Figure 21.

Curve I describes the rate of activity of the acid-forming stage of digestion at the respective detention times, because the rate-limiting step in the over all transformation was acid formation under these conditions. However, Curve II can be said to more nearly describe the rate of activity of the methane-forming stage of digestion, because the substrate for the methane-forming microorganisms was not limited under the conditions in which this data was obtained.

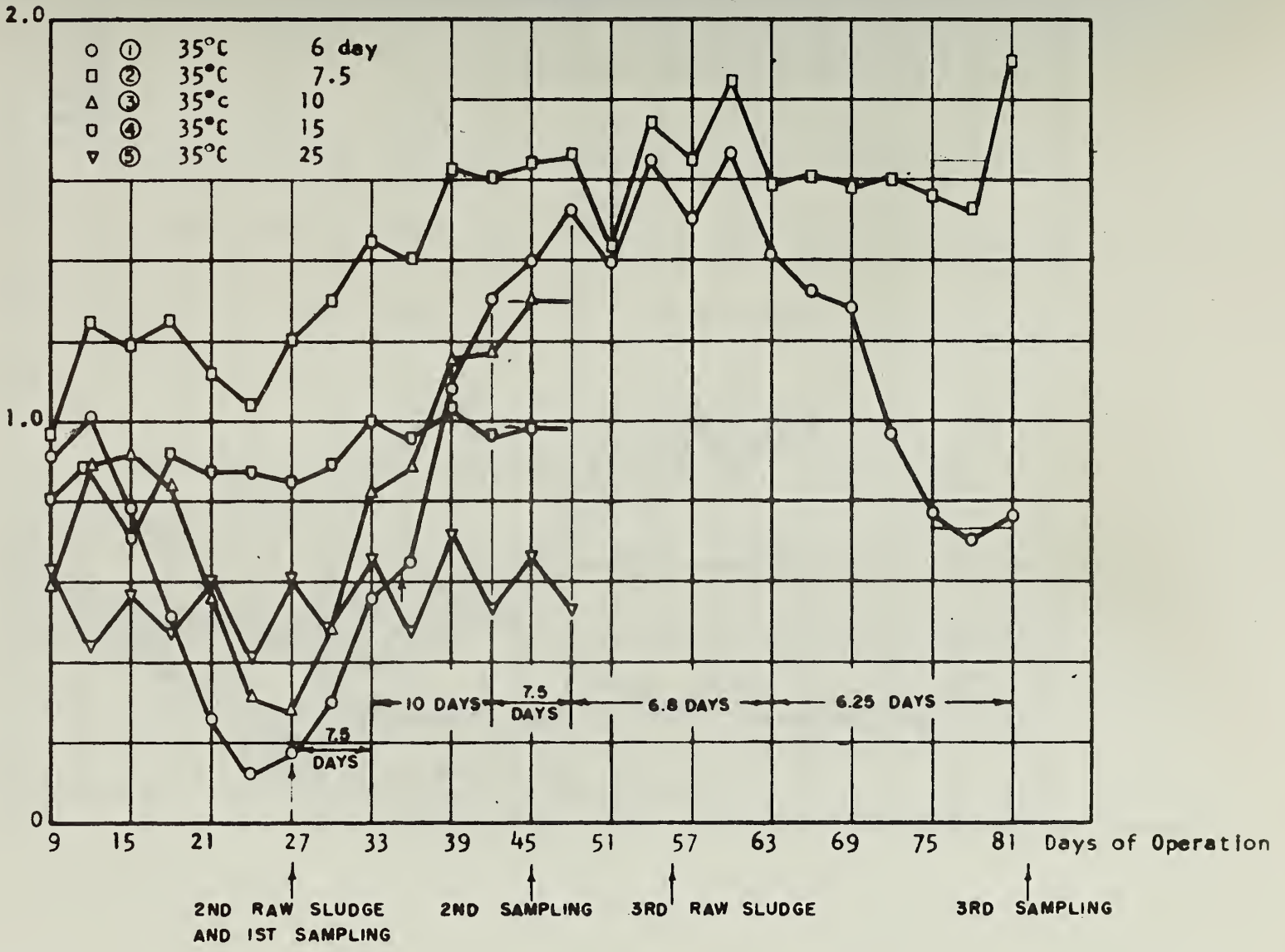
Summary

1. A reaction kinetic scheme developed by Herbert, et.al. for continuous pure culture in the steady state may be applicable with some modifications to domestic wastewater sludge digestion.
2. There is a narrow range of short detention times at which the effect of protein and lipid reduction is nearly equal to that reduction which occurred at much longer detention times.
3. The optimum sludge detention time for methane formers again came out to be 15 days when the volatile acid concentration was kept over 500 mg/l by feeding acetic acid with sludge. However, it was 7.5 days when only raw sludge was fed and at this detention time the concentration of proteins and lipids was at a minimum.

4. The washing-out time appears to become longer by 3 to 4 days when the temperature is lowered from 35°C to 25°C.

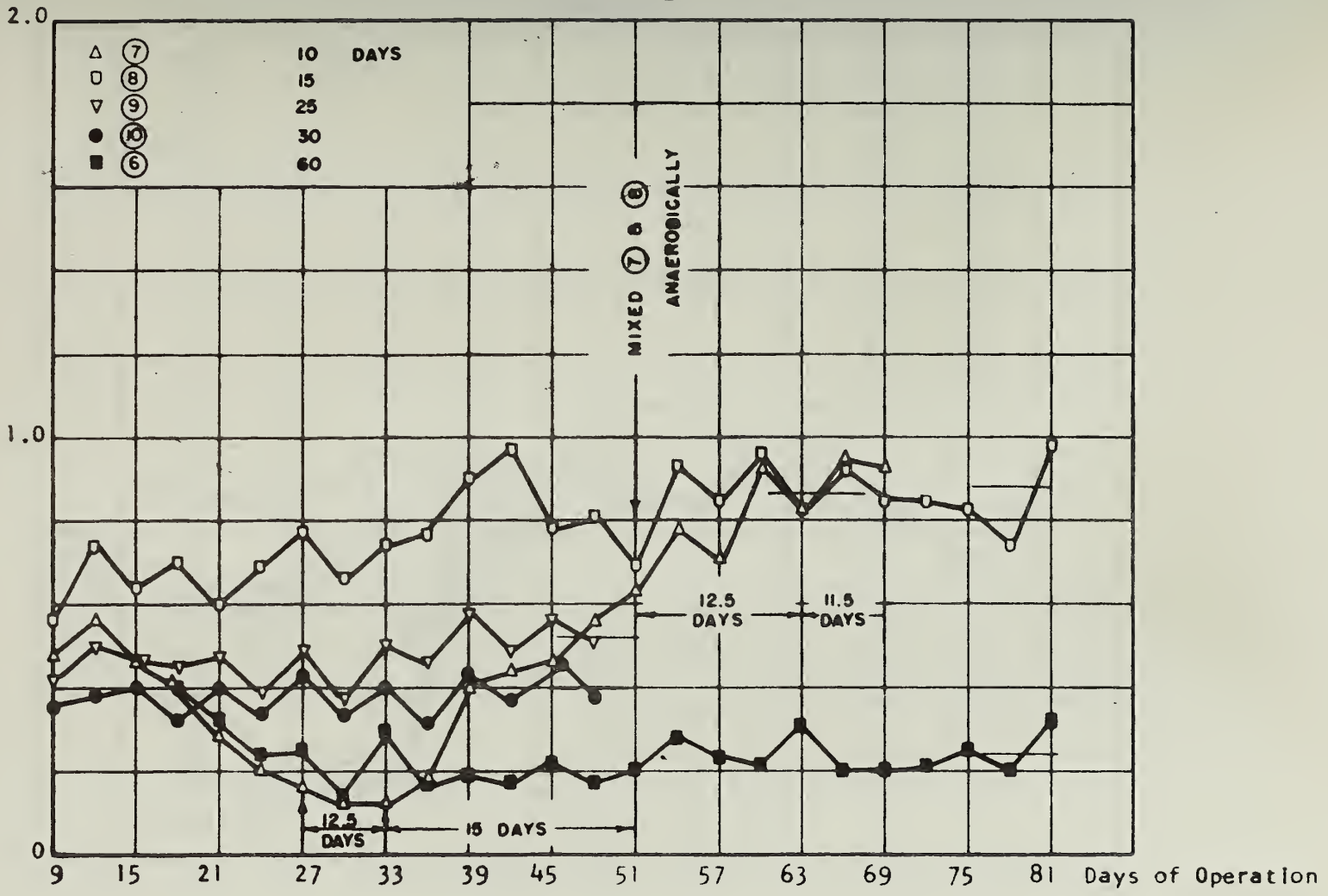
GAS PRODUCTION WITH VARIED LOADING AND VARIED DETENTION AT 35° C

FIGURE 1



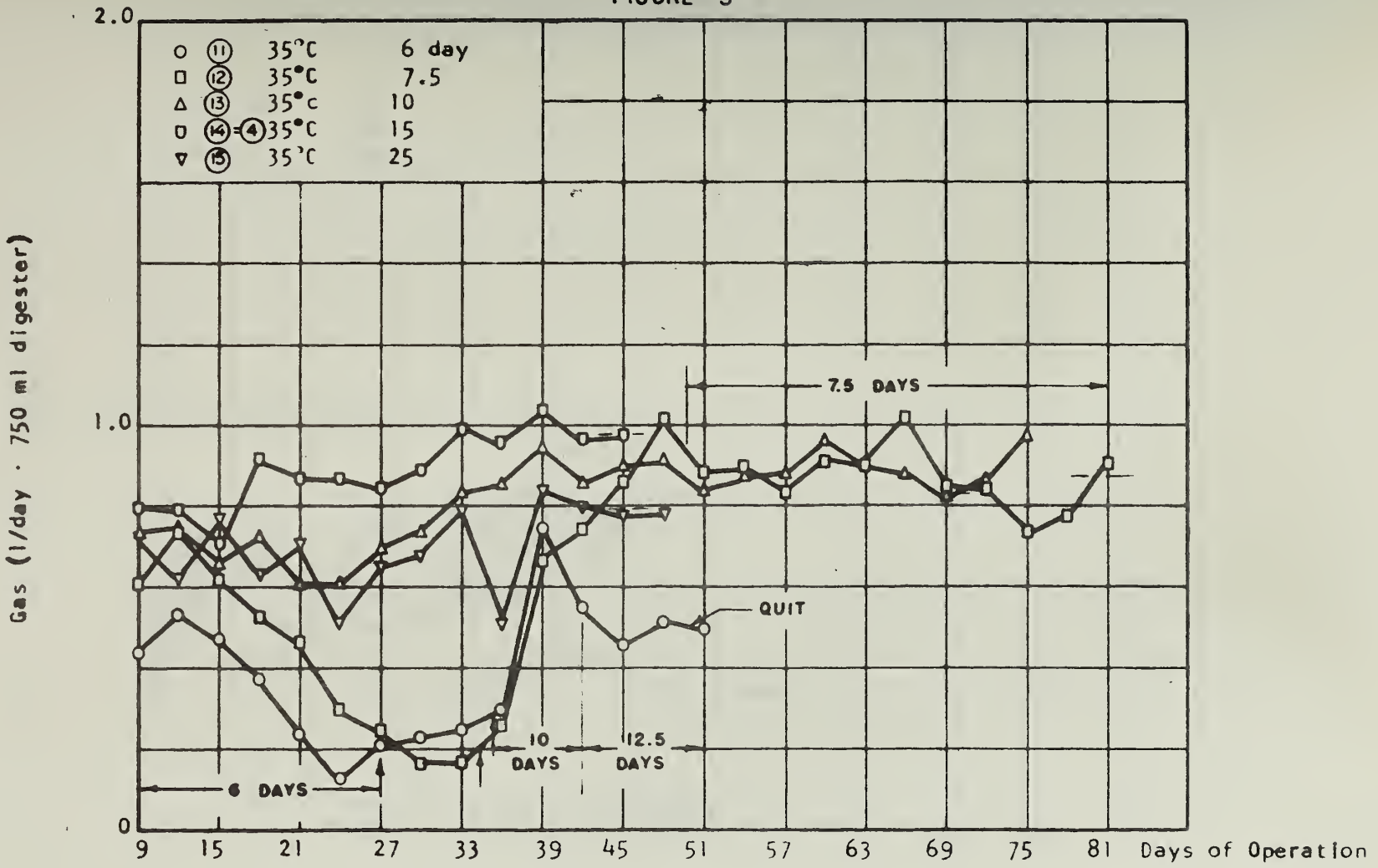
GAS PRODUCTION WITH VARIED LOADING AND VARIED DETENTION AT 25° C

FIGURE 2



GAS PRODUCTION WITH CONSTANT LOADING AND VARIED DETENTION AT 35° C

FIGURE 3



GAS PRODUCTION WITH CONSTANT LOADING AND VARIED DETENTION - TIME 25° C

FIGURE 14

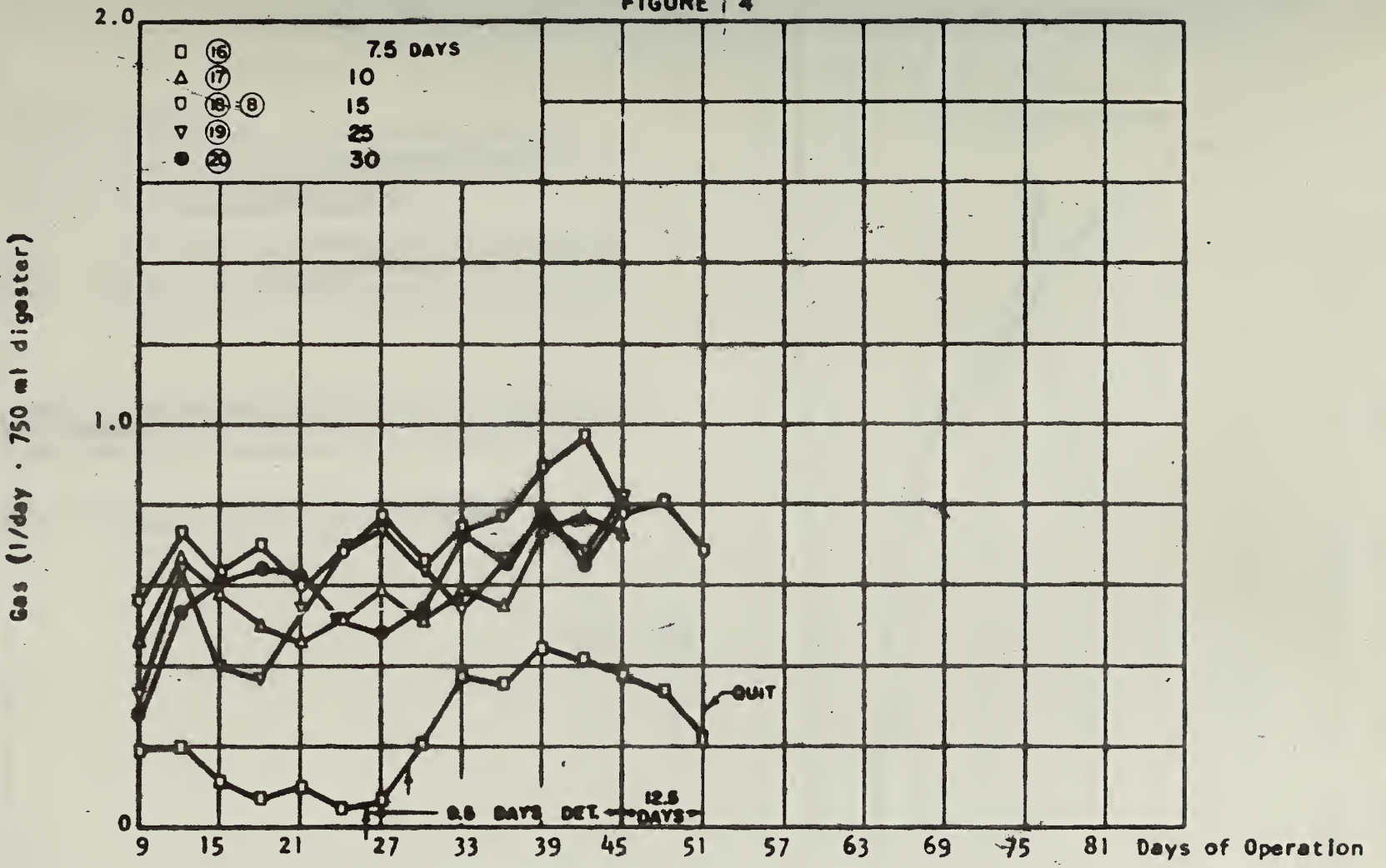


FIGURE 5

EFFECT OF VARIED LOADING RATE AND VARIED DETENTION TIME ON DIGESTION OF RAW SLUDGE

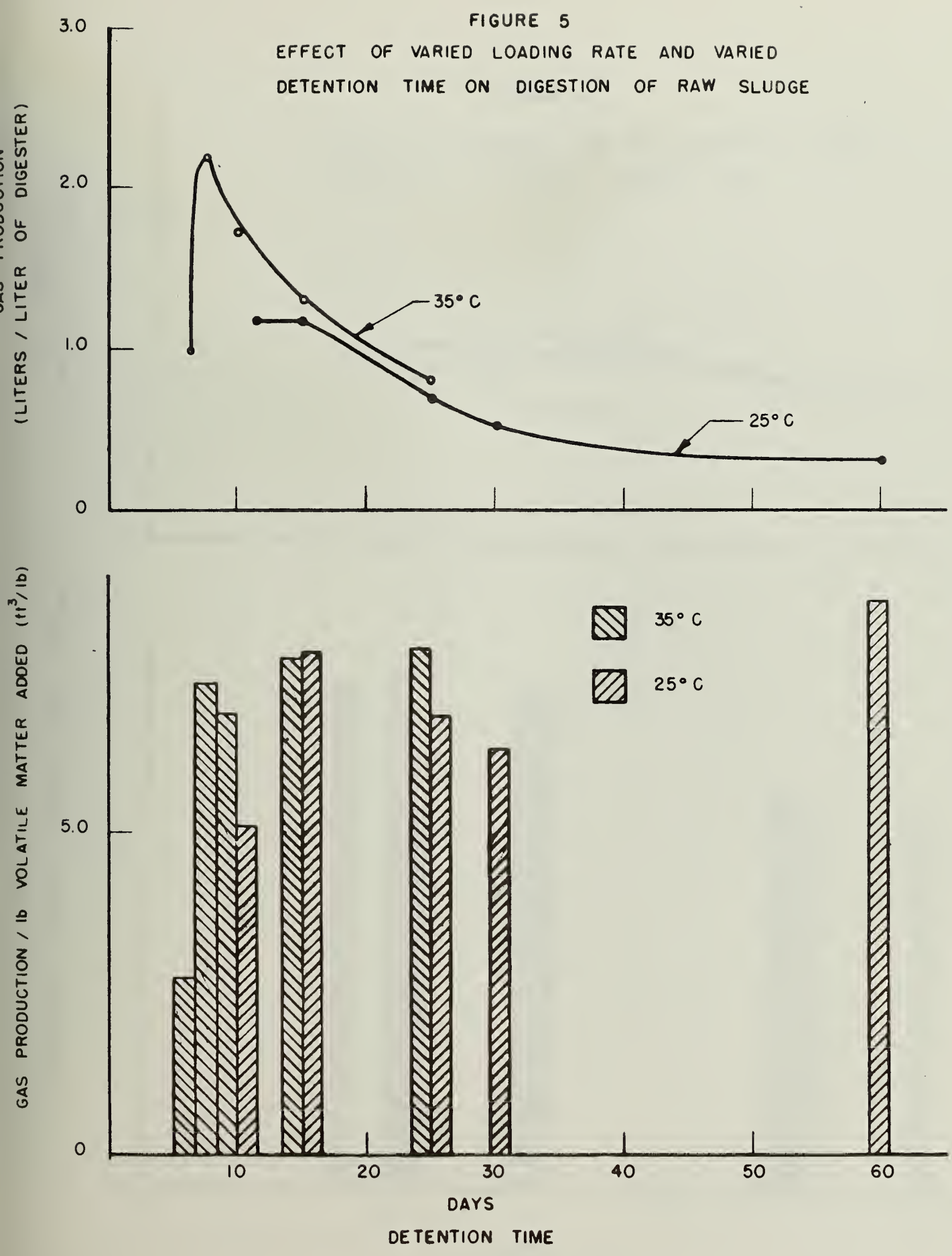


FIGURE 6

EFFECT OF DETENTION TIME ON DIGESTION OF RAW SLUDGE WITH CONSTANT LOADING RATE

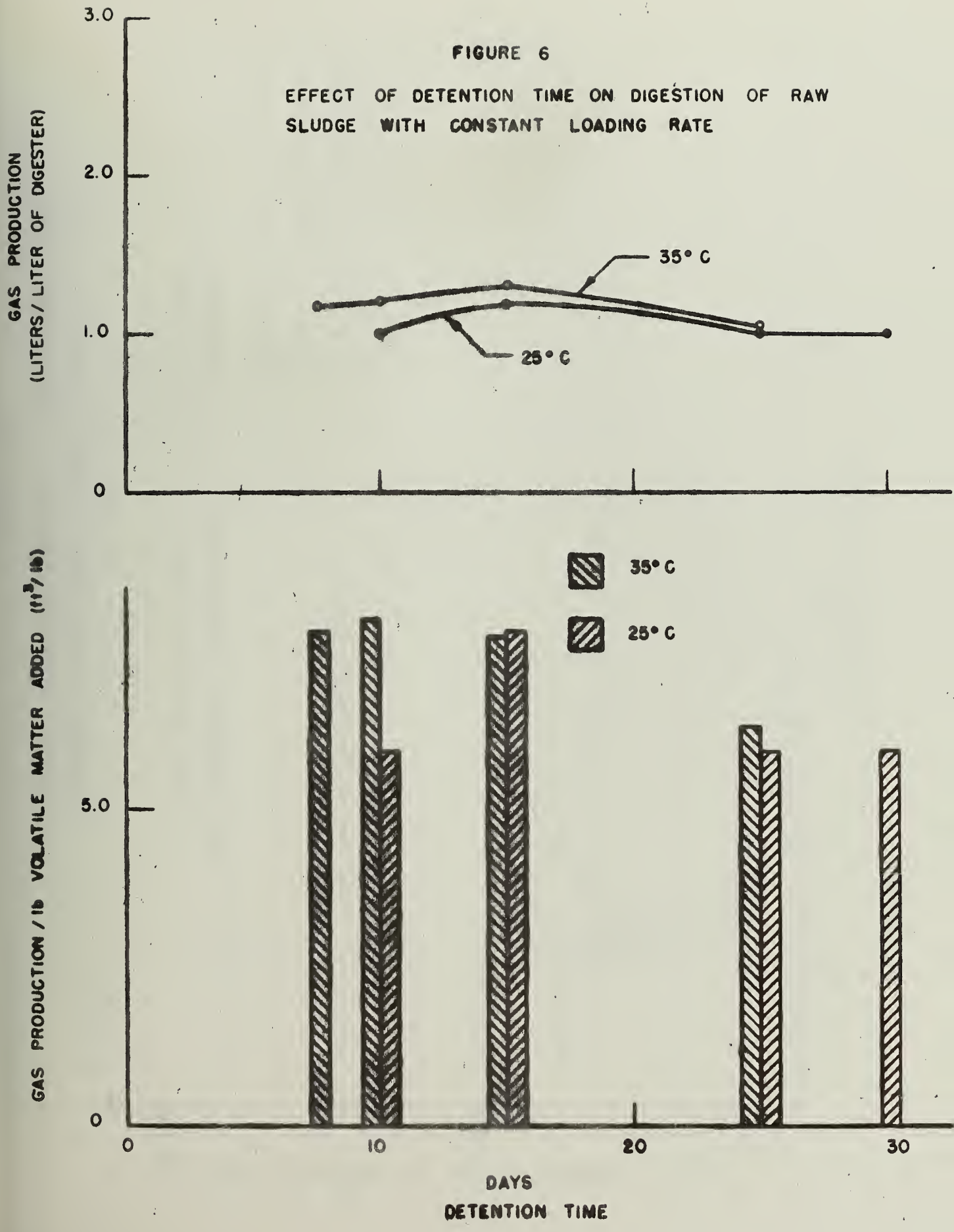
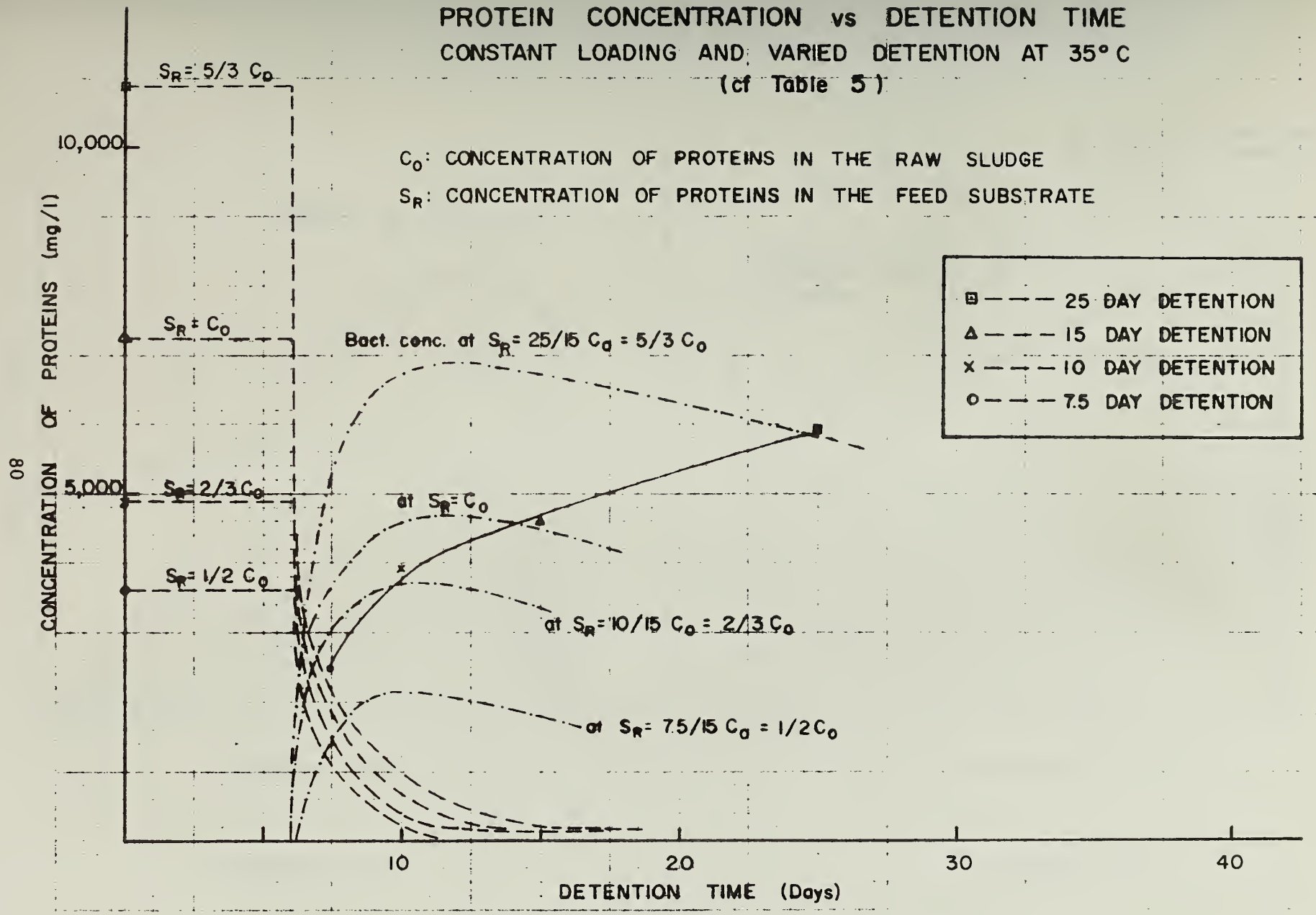


FIGURE 18

PROTEIN CONCENTRATION vs DETENTION TIME

CONSTANT LOADING AND VARIED DETENTION AT 35° C

(cf Table 5)



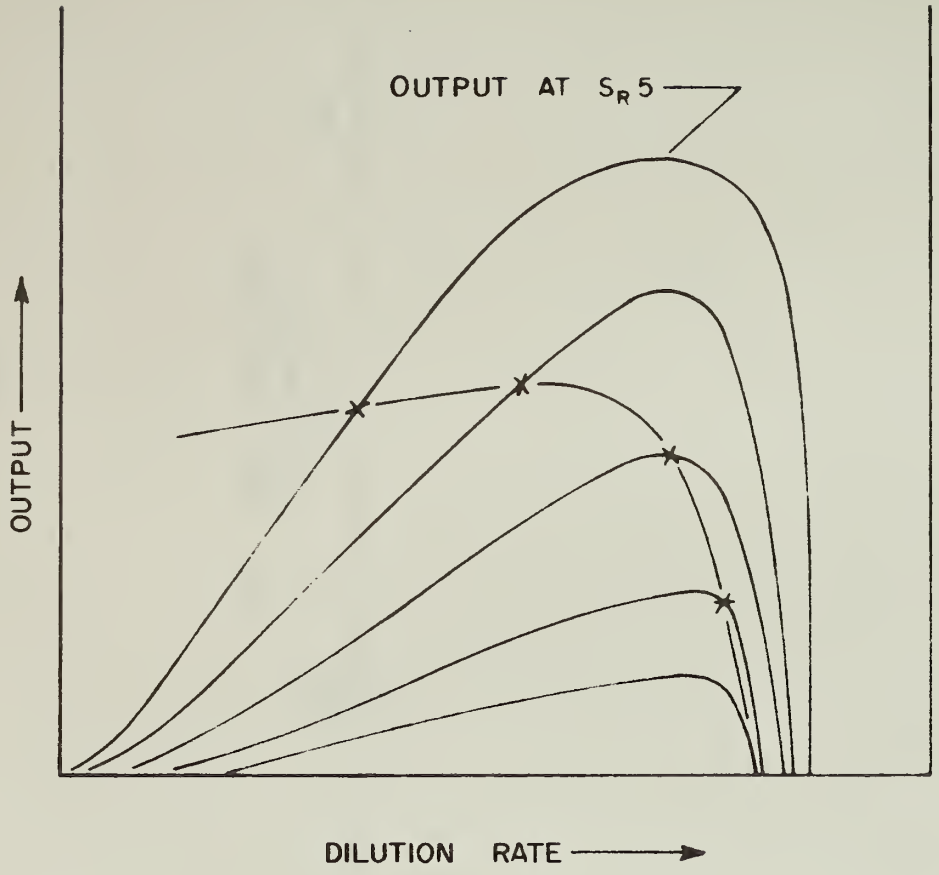


FIGURE 19

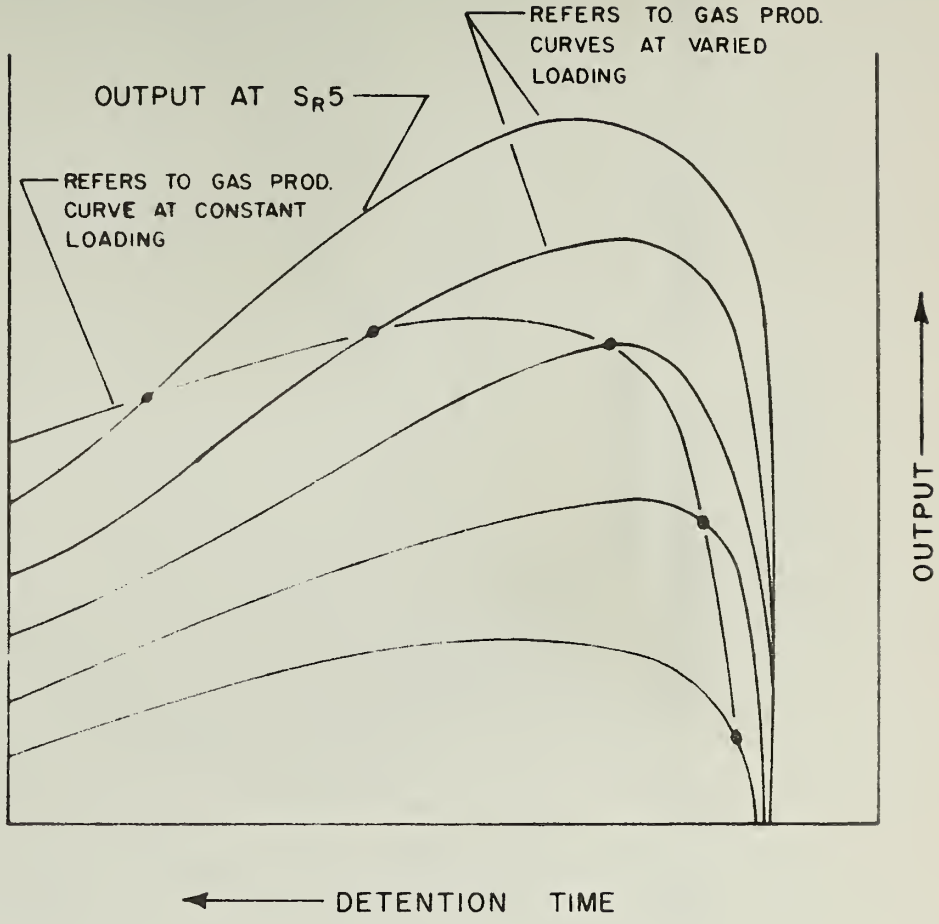


FIGURE 20

FIGURE 21

EFFECT OF ACETATE SUPPLEMENTATION ON GAS PRODUCTION

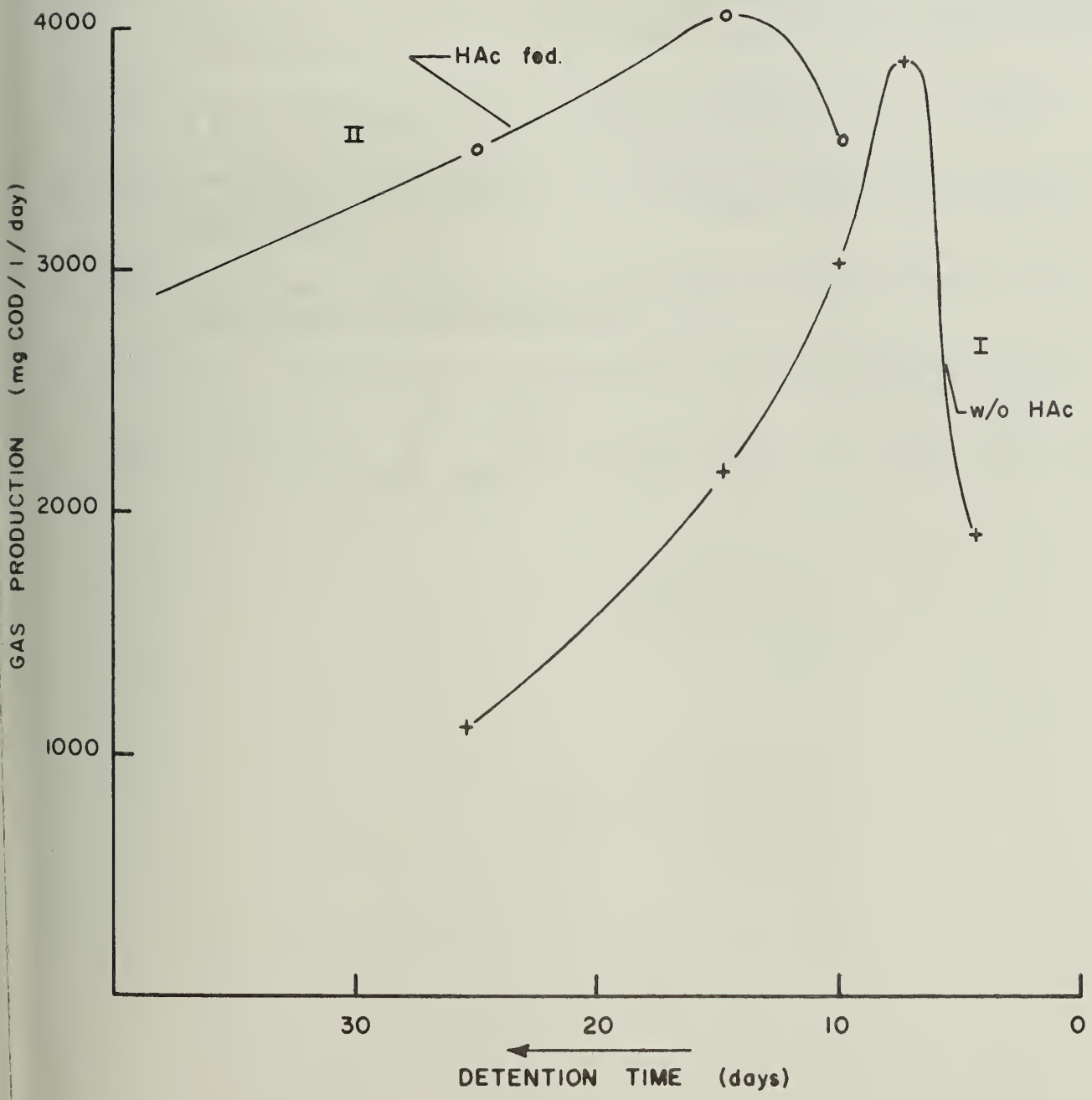


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FIGURE 14

ACTIVITY AND GAS PRODUCTION AT 25° C RAW SLUDGE AND HAc FEED

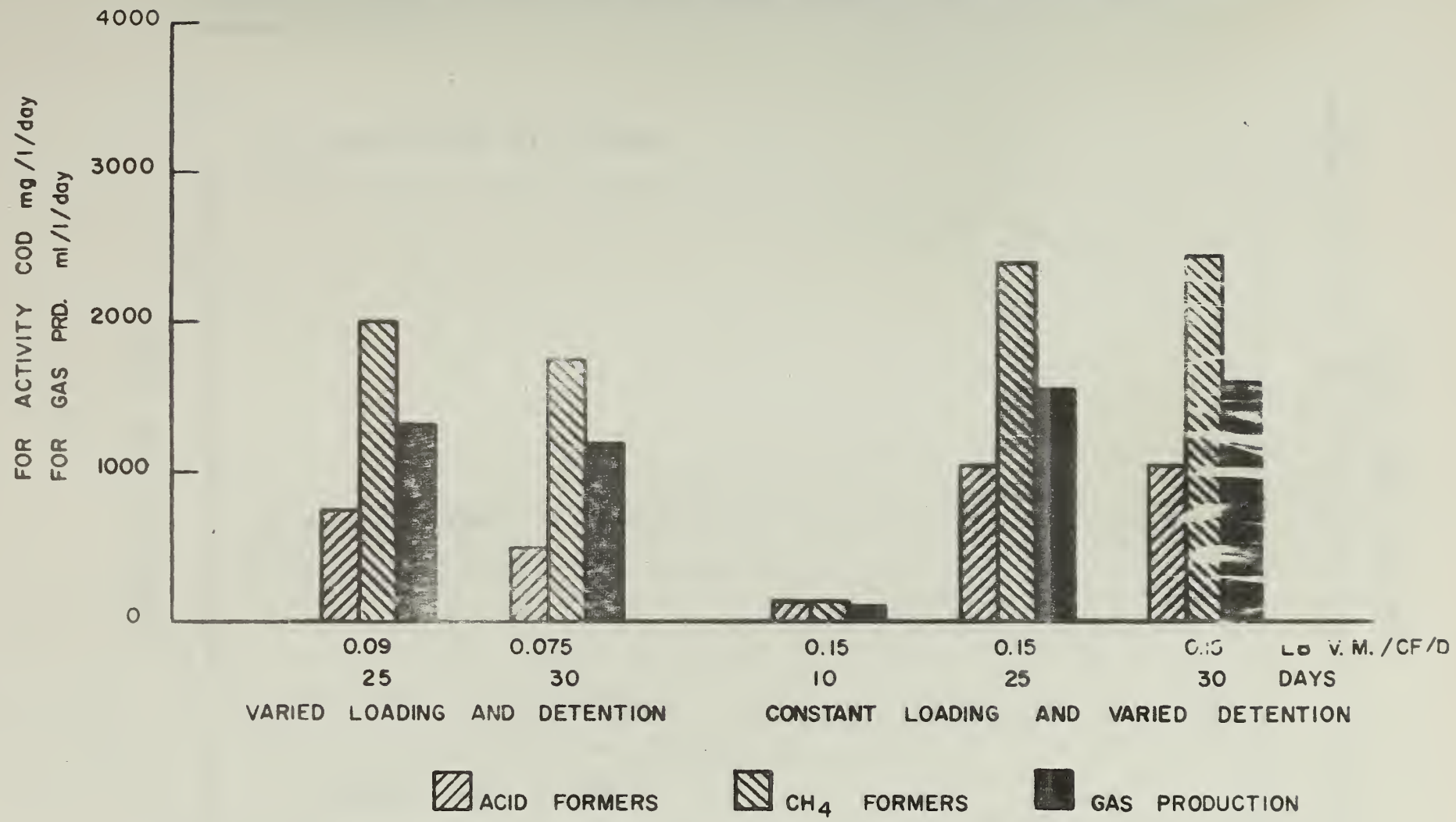


FIGURE 15

EFFECT OF VARYING THE CONCENTRATION OF SUBSTRATE IN THE INFLOWING MEDIUM (S_R) ON THE STEADY-STATE RELATIONSHIP (after Herbert & et. al.)

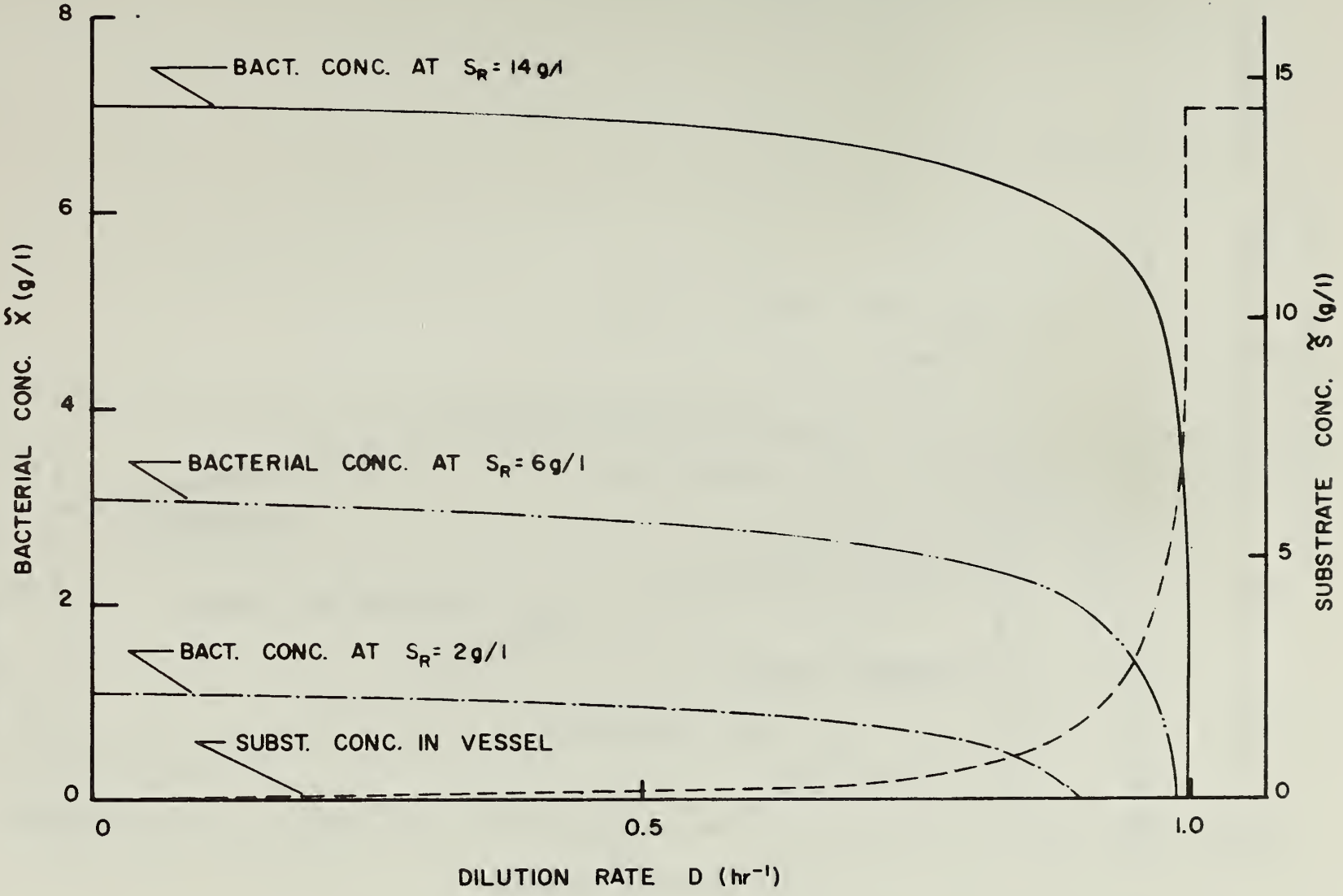


FIGURE 16

THE VARIATION OF THE BACTERIAL AND SUBSTRATE CONCENTRATION WITH DETENTION TIME

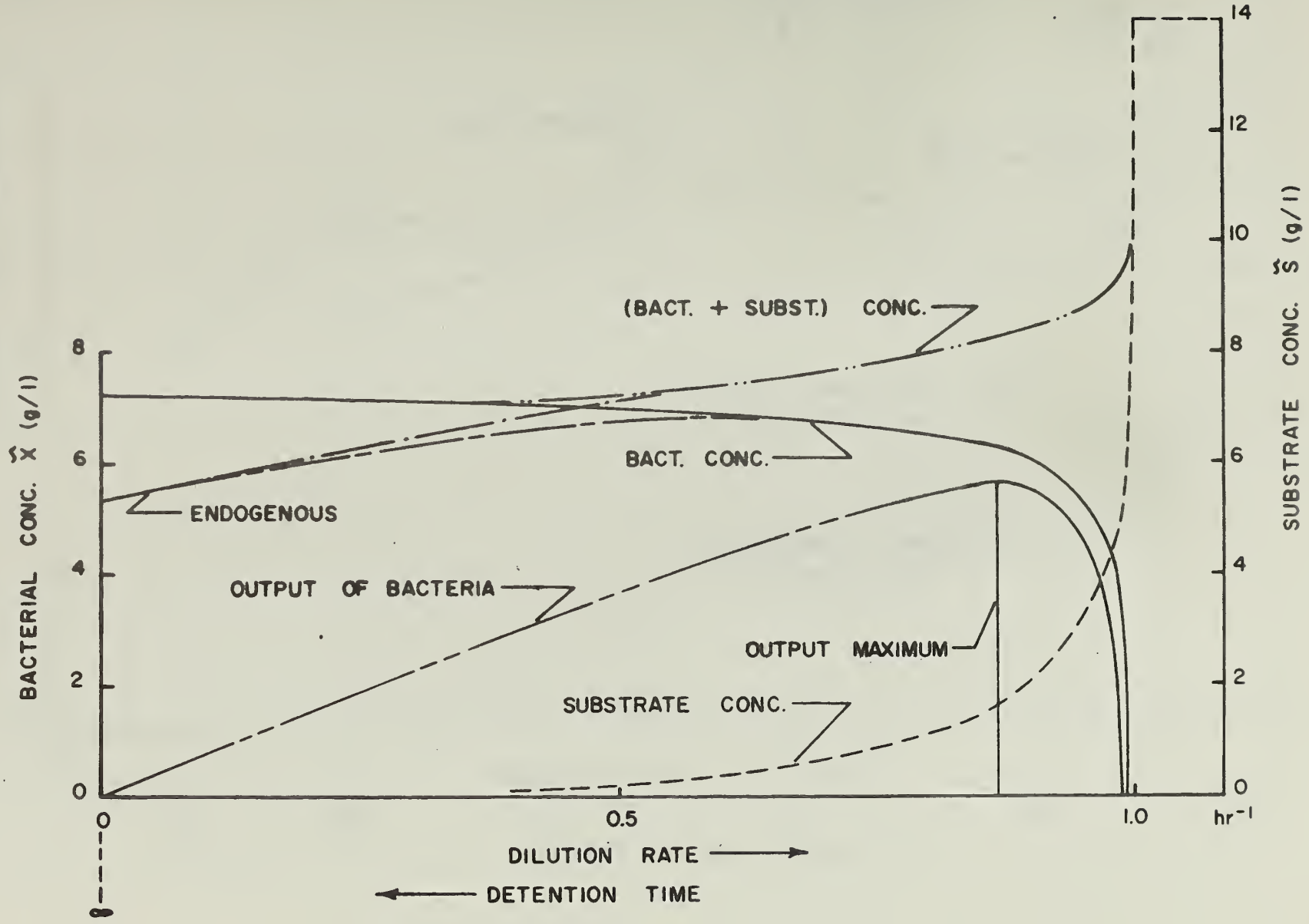


FIGURE 17

PROTEIN CONCENTRATION IN THE EFFLUENT SLUDGE vs DILUTION RATE (VARIED LOADING)

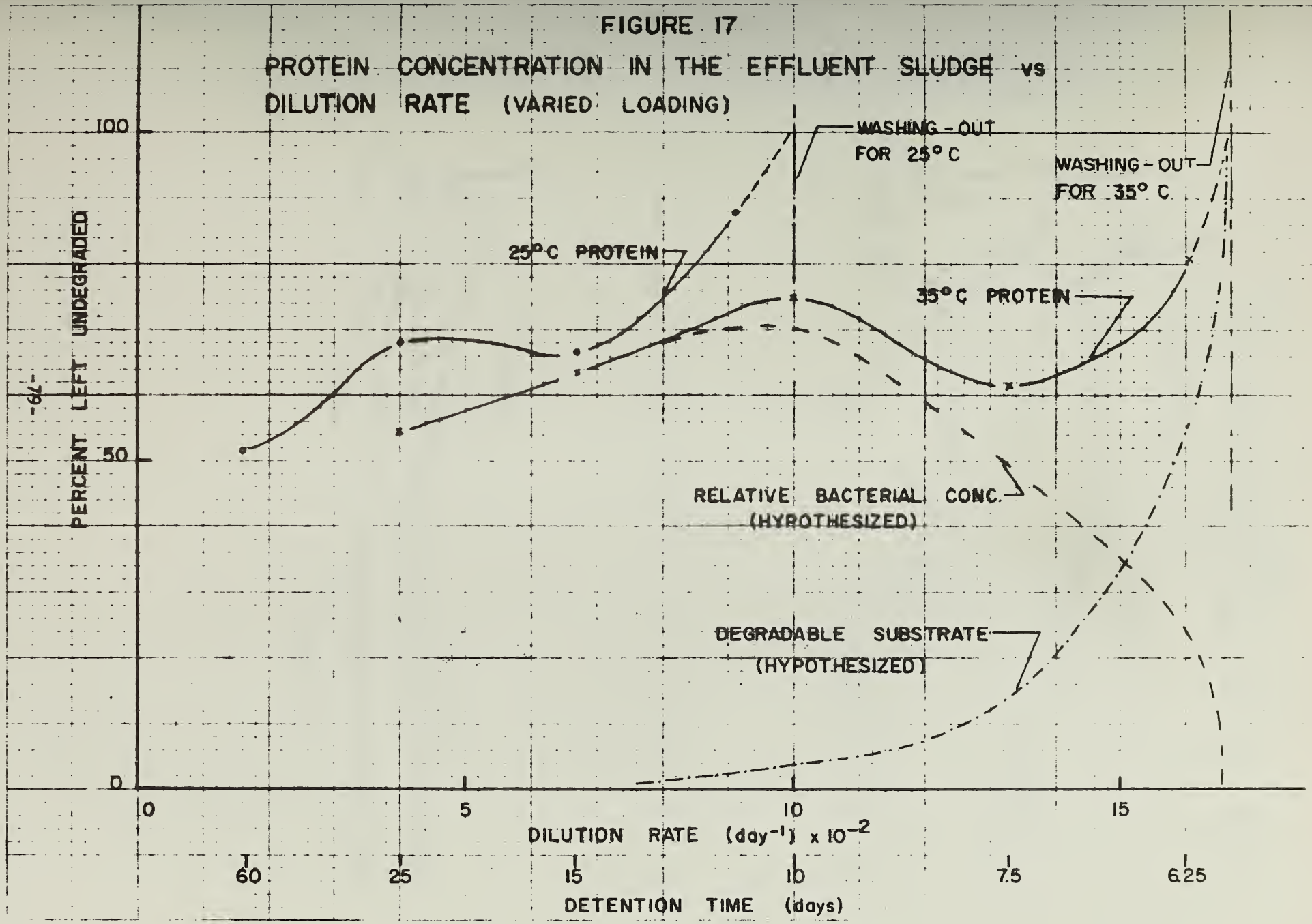
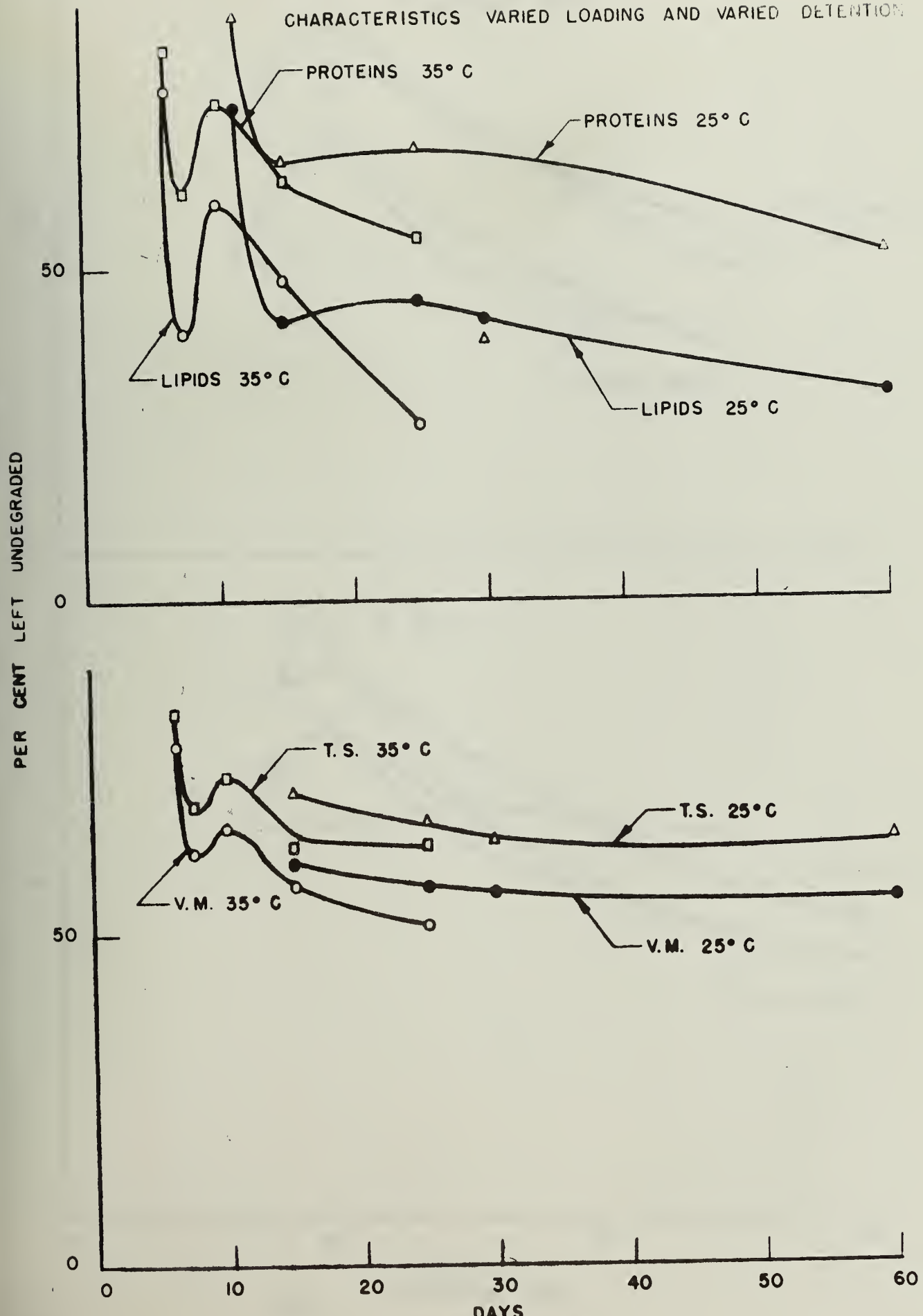


FIGURE 7

EFFECTS OF SOLIDS DETENTION TIME ON EFFLUENT CHARACTERISTICS VARIED LOADING AND VARIED DETENTION



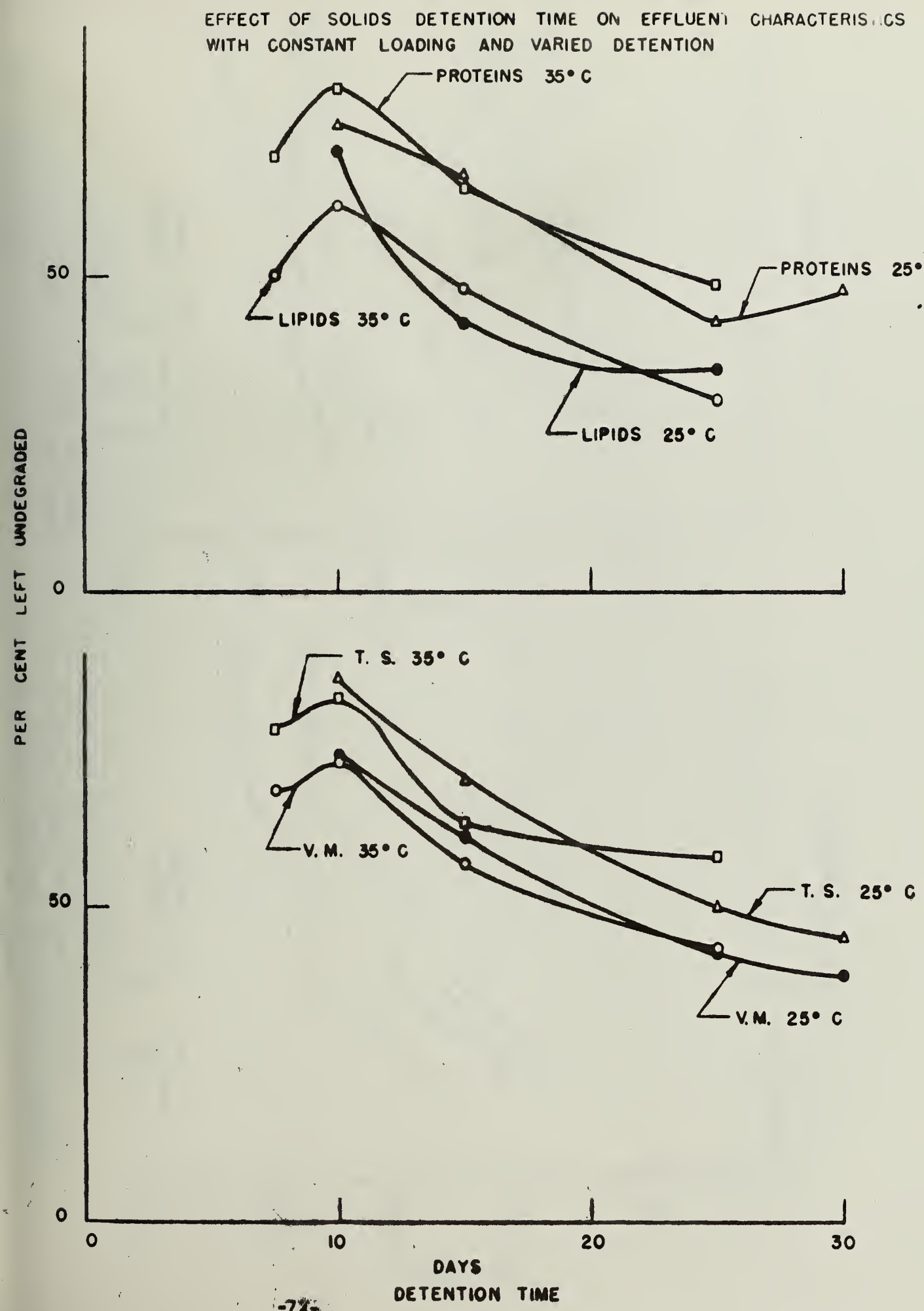

EFFECT OF SOLIDS DETENTION TIME ON EFFLUENT CHARACTERISTICS
WITH CONSTANT LOADING AND VARIED DETENTION
PROTEINS 35° C
PROTEINS 25° C
LIPIDS 35° C
LIPIDS 25° C
T. S. 35° C
T. S. 25° C
V. M. 35° C
V. M. 25° C
PER CENT LEFT UNDEGRADED
50
0
50
0
0
10
20
30
DAYS
DETENTION TIME

FIGURE 9

ACTIVITY IN COD WITH CONSTANT LOADING OF RAW SLUDGE AND WITH HAc FEED AT 35° C

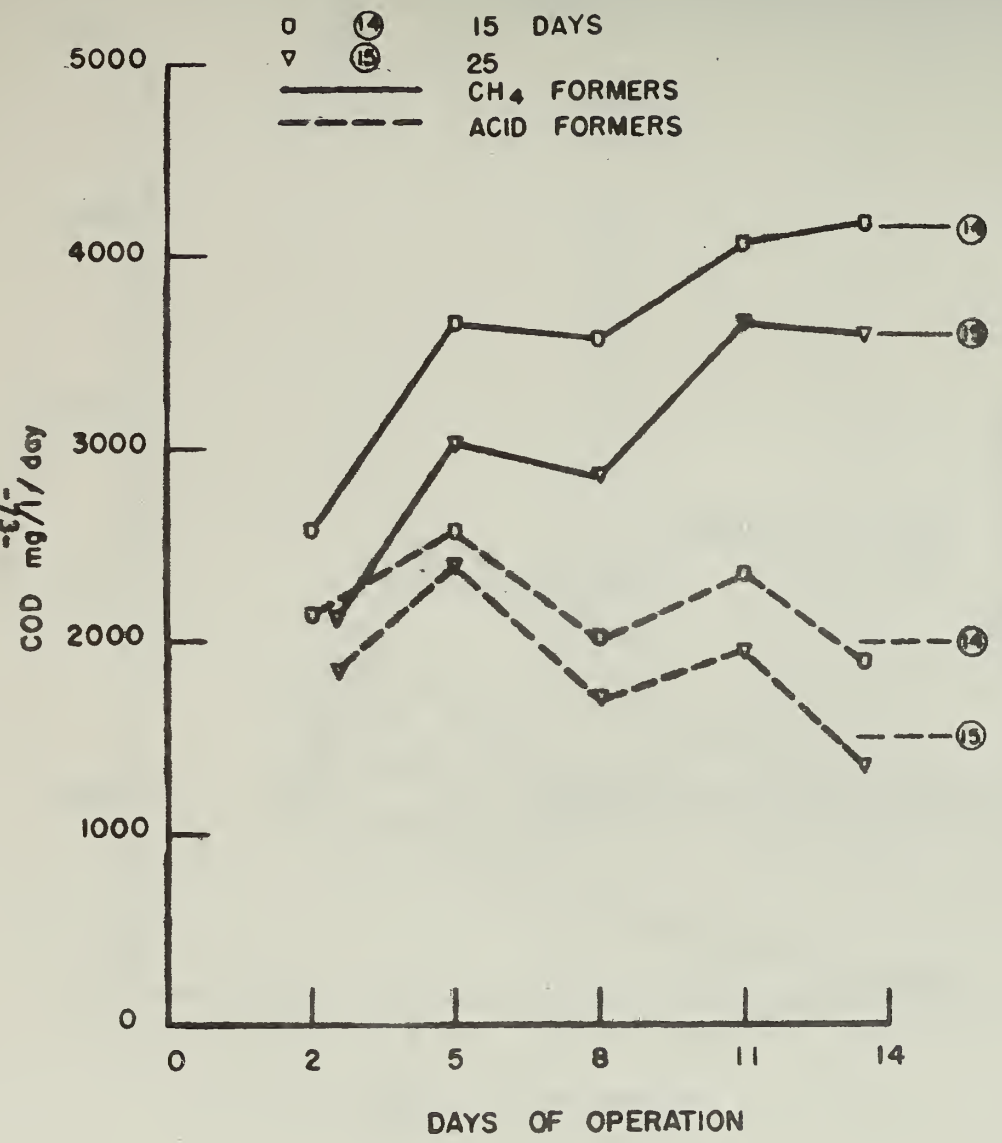


FIGURE 10

ACTIVITY IN COD WITH VARIED LOADING AND VARIED DETENTION OF RAW SLUDGE WITH HAc FEED AT 35° C

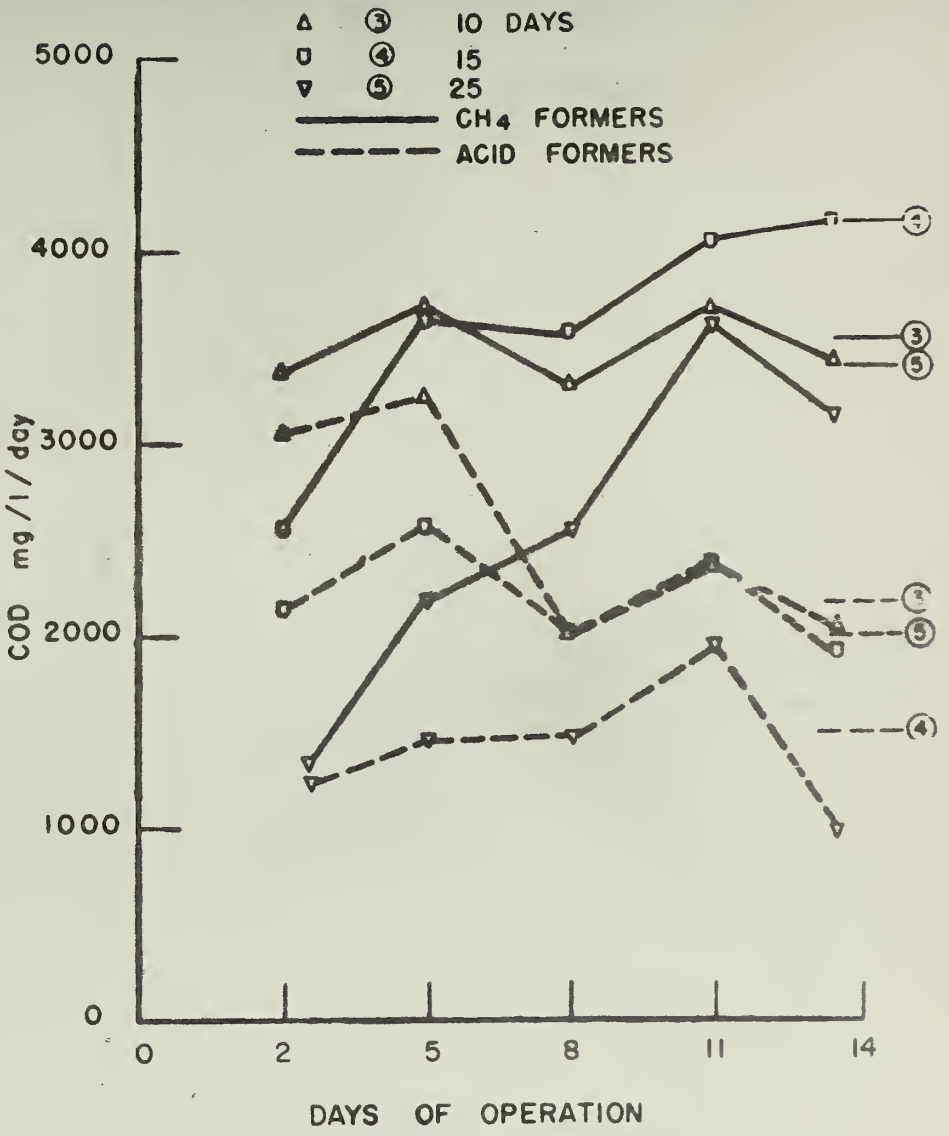


FIGURE 11

ACTIVITY IN COD WITH CONSTANT LOADING OF RAW SLUDGE AND WITH HAc FEED AT 25° C

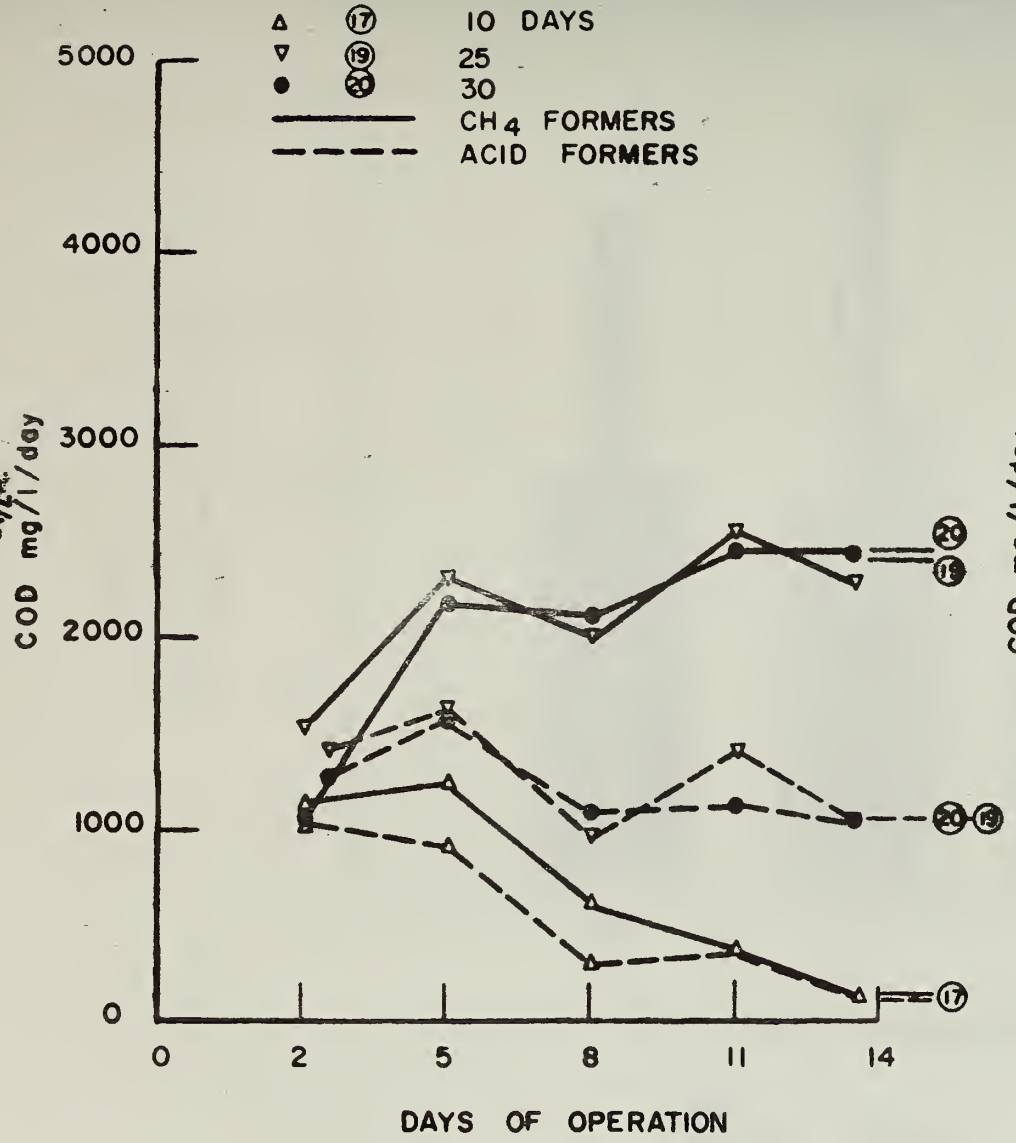


FIGURE 12

ACTIVITY IN COD WITH VARIED LOADING AND VARIED DETENTION OF RAW SLUDGE WITH HAc FEED AT 25° C

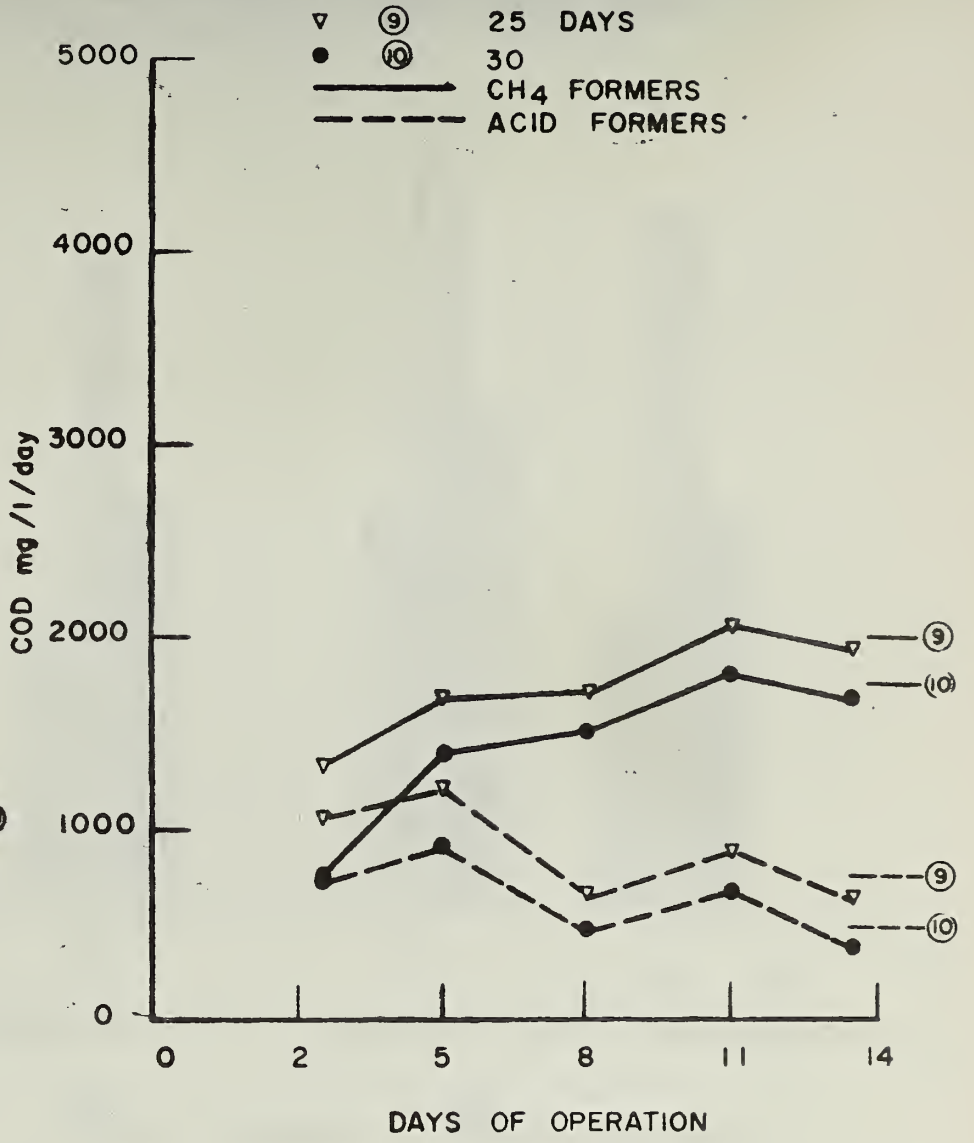
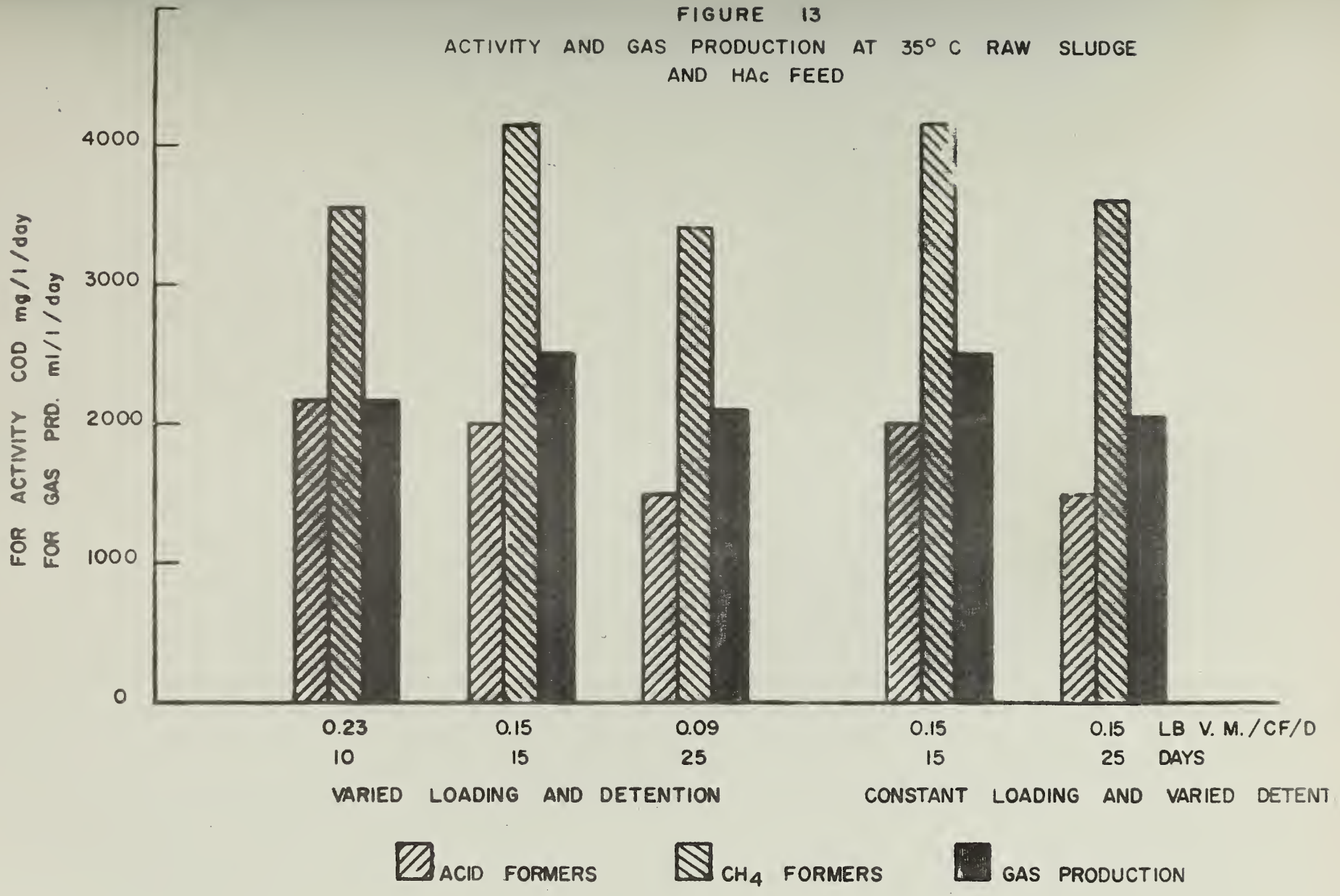


FIGURE 13

ACTIVITY AND GAS PRODUCTION AT 35° C RAW SLUDGE AND HAc FEED



APPENDIX

Analytical Procedures

The Folin reaction was applied to the analysis of proteins in sewage sludge by procedure reported by Orme-Johnson and Woods[4]. A modification was made in replacing centrifugation with filtration using glass fiber filterpaper 30 minutes after the addition of Folin reagent. The precision ($\frac{\text{standard deviation}}{\text{average}}$) was 10-20%.

The lipid analyses was carried out according to the method reported by Loehr and Rohlich[5], and Loehr and Higgins.[6] The reproducibility was such that the lipids of a thickened raw sludge came out to be .99% and .98% on two successive analyses.

Sample Preparation

The total solids of fresh, raw sludge taken from Urbana-Champaign Sanitary District Plant was usually 3.0 to 4.0%. By standing overnight, a thickened layer of sludge floated. By siphoning off the supernatant, a condensed sludge of 5 - 6% total solids was obtained. All of the raw sludge in this experiment was thickened by this method.

TABLE 1

Properties of Raw Sludge

Items	1st Raw Sludge Sept. 25, 1965	2nd Raw Sludge Oct. 23, 1965	3rd Raw Sludge Nov. 25, 1965
Total Solids %	5.10	5.63	4.94
Volatile Solids %	3.25	4.07	3.59
Proteins (mg/l)	7400	7240	7450
Lipids (mg/l)	9784	8486	10,100

TABLE 2

Properties of Effluent and Gas Production with Varied Loading and Varied Detention Time at 35°C

Items	Period of Exper.	Raw Sludge	No. Ldg. Det.	1 0.35 6.25	2 0.30 7.5	3 0.23 10	4 0.15 15	5 0.09 25
teins g/l)	1 2 3	7400 7240 7450		3810(53) -------- 6195(83)	4100(56) 6180(86) 4560(61)	3060(41) 5460(75) --------	3780(51) 4590(63) --------	4050(55) 3939(54) --------
ids /l)	1 2 3	9784 8486 10,100		5806(60) 5942(70) 7734(77)	4544(47) 4920(58) 4030(40)	4406(45) 5972(70) --------	2900(30) 4080(48) --------	2616(27) 2176(26) --------
S. /l)	1 2 3	5.10 5.63 4.94		4.16(82) -------- 4.08(83)	3.93(77) 4.55(81) 3.38(69)	4.44(87) 4.17(74) --------	3.89(76) 3.53(63) --------	3.89(76) 3.52(63) --------
S.)	1 2 3	3.25 4.07 3.59		2.46(76) -------- 2.81(78)	2.13(66) 2.80(69) 2.22(62)	2.48(76) 2.67(66) --------	2.77(85) 2.31(57) --------	1.79(55) 2.08(51) --------
l-d)	1 2 3			.99	2.18	1.73	1.31	.80
/ 3/LB)	1 2 3			2.75	7.30	6.82	7.70	7.85

le 2 through 4: Figures in parenthesis are percentages left undegraded.
le 2 through 4: Figures underlined were used for graphs plotted.

TABLE 3

Properties of Effluent and Gas Production Varied Loading and Varied Detention Time at 25°C

Items	Period of Exper.	Raw Sludge	No. Ldg. Det.	7 .23-.20 10-11.5	8 0.15 15	9 0.09 25	10 0.075 30	6 0.038 60
roteins (mg/l)	1	7400		4230(57)	4440(60)	3270(44)	2850(39)	3270(44)
	2	7240		6360(88)	5160(71)	4890(68)	--------	3120(43)
	3	7450		--------	4530(61)	--------	--------	4530(61)
ipids (mg/l)	1	9784		5930(61)	3760(38)	2480(25)	2500(26)	1620(16)
	2	8486		6284(74)	4270(50)	3790(45)	3548(42)	2632(31)
	3	10100		--------	3462(34)	--------	--------	3010(30)
. S. (%)	1	5.10		3.82(75)	3.38(66)	2.60(51)	2.46(48)	1.87(37)
	2	5.63		--------	4.00(71)	3.76(67)	3.62(64)	3.63(65)
	3	4.94		--------	3.40(69)	--------	--------	3.07(62)
. S. (%)	1	3.25		2.57(78)	1.70(52)	1.62(50)	1.58(49)	1.19(37)
	2	4.07		--------	2.45(60)	2.32(57)	2.28(56)	2.29(56)
	3	3.59		--------	2.20(61)	--------	--------	1.04(54)
as l/l-d)	1							
	2					.69	.54	
	3			1.15	1.17			.32
as/ ft^3/LB)	1							
	2					6.8	6.3	
	3			5.1	7.8			8.6

TABLE 4

Properties of Effluent and Gas Production with Constant Loading and Varied Detention Time at 35°C (0.15 #Volatile Solids/ft^3/day)

Items	Period of Exper.	Raw Sludge	No. Det.	11 6	12 7.5	13 10	14 15	15 25
Proteins (mg. withdrawn/day)	1 2 3	370 362 373		210(57) ------- -------	192(52) ------- 258(69)	155(42) 280(77) 313(84)	189(51) 230(64) -------	81(22) 178(49) -------
Lipids (mg. withdrawn/day)	1 2 3	490 425 505		229(47) ------- -------	182(37) ------- 251(50)	153(31) 215(51) 357(71)	145(30) 204(48) -------	94(19) 128(30) -------
T. S. (gm withdrawn/day)	1 2 3	2.55 2.82 2.47		202(79) ------- -------	1.87(73) -------- 1.92(78)	1.74(68) 2.55(80) 2.12(86)	1.95(76) 1.77(63) --------	1.38(54) 1.62(58) --------
V. S. (gm withdrawn/day)	1 2 3	1.63 2.04 1.80		1.13(70) -------- --------	1.01(62) -------- 1.22(68)	0.92(56) 1.41(69) 1.39(77)	1.39(85) 1.16(57) --------	.68(42) .882(43) -------
Gas (l/l-d)	1 2 3				1.17	1.2	1.31	1.07
Gas/ (ft^3/LB)	1 2 3				7.8	8.0	7.7	6.27

TABLE 5

Properties of Effluent with Constant Loading and Varied Detention Time at 35°C (0.15 #/ft^3/day)

Items	Period of Exper.	No.	11		12		13		14		15	
		Det.	6.0		7.5		10		15		25	
			Raw Feed	Effluent	Raw Feed	Effluent	Raw Feed	Effluent	Raw Feed	Effluent	Raw Feed	Effluent
Proteins conc (mg/l)	1		2960	1680	3700	1920	4930	2070	7400	3780	12,000	2700
	2						4840	3720	7240	4590	11,800	5940
	3				3620	2580	4970	4170				
Lipids conc (mg/l)	1		3920	1830	4892	1820	6520	2040	9784	2900	16,300	3120
	2						5650	2872	8486	4080	14,200	4266
	3				5050	2514	6740	4766				
Total Solids (%)	1		2.04	1.62	2.55	1.87	3.40	2.32	5.10	3.89	8.50	4.58
	2						3.76	3.00	5.63	3.53	9.38	5.40
	3				2.81	1.92	3.29	2.83				
Volatile Solids (%)	1		1.30	0.90	1.63	1.01	2.16	1.22	3.25	2.77	5.40	2.27
	2						2.72	1.88	4.07	2.31	6.80	2.94
	3				2.04	1.22	2.40	1.85				

The figures underlined were used for Fig. 17, since they can be assumed to be the values for steady state.

TABLE 6

Properties of Effluent and Gas Production with Constant Loading and Varied Detention Time at 25°C (0.15# Volatile Solids/ft^3/day)

Items	Period of Exper.	Raw Sludge	No. Det.	16 7.5	17 10	18 15	19 25	20 30
oteins ng.with- rawn/day)	1 2 3	370 362 373		350(95) ------- -------	272(74) 268(74) -------	222(60) 258(71) 226(61)	220(60) 156(43) -------	248(67 174(48 -------
pids ng.with- rawn/day)	1 2 3	490 425 505		290(59) ------- -------	220(45) 296(70) -------	188(38) 214(50) 173(34)	138(28) 148(35) -------	95(20) 357(?) ------
S. gm with- rawn/day)	1 2 3	2.55 2.82 2.47		1.54(60) -------- --------	1.34(53) 2.44(86) --------	1.69(66) 2.00(71) 1.70(69)	1.24(49) 1.42(50) --------	0.94(37) 1.28(45) -------
S. gm with- rawn/day)	1 2 3	1.63 2.04 1.80		1.02(63) -------- --------	.75(46) 1.50(74) --------	0.85(52) 1.23(60) 1.10(61)	0.76(47) 0.85(42) --------	0.58(36 0.79(39) -------
s /l-d)	1 2 3				1.0	1.17	1.0	1.0
s/ ft^3/LB)	1 2 3				5.9	7.8	5.9	5.9

TABLE 7

Properties of Effluent with Constant Loading and Varied Detention Time at 25°C (0.15# Volatile Solids/ft^3/day)

Items	Period of Exper.	No. Det.	16 7.5		17 10		18 15		19 25		20 30	
			Raw Feed	Effluent	Raw Feed	Effluent	Raw Feed	Effluent	Raw Feed	Effluent	Raw Feed	Effluent
Proteins conc (mg/1)	1		3700	3500	4930	3630	7400	4440	12,000	7300	14,500	9900
	2				4840	3570	7240	5160	11,800	5190	14,146	6960
	3						7450	4530				
Lipids conc (mg/1)	1		4892	2896	6520	2940	9784	3760	16,300	4606	19,568	3810
	2				5650	3950	8486	4270	14,200	4920	16,972	14260
	3						10,100	3462				
Total Solids (%)	1		2.55	1.53	3.40	1.78	5.10	3.38	8.50	4.13	10.20	3.77
	2				3.76	3.26	5.63	4.00	9.38	4.71	11.26	5.10
	3						4.94	3.40				
Volatile Solids (%)	1		1.63	1.02	2.72	1.99	3.25	1.70	5.40	2.52	6.50	2.30
	2						4.07	2.45	6.80	2.82	8.14	3.15
	3						3.59	2.20				