METHANE FERMENTATION OF ORGANIC ACIDS

Thesis for the Degree of M. Sc.

MICHIGAN STATE UNIVERSITY

B. Naga Raju

1956

This is to certify that the

thesis entitled

Methane Fermentation of Organic Acids

presented by

B. Naga Raju

has been accepted towards fulfillment
of the requirements for

M.S. degree in Civil Engineering

Frank P. Theroux
Major professor

Date 11-5-56

O-169

METHANE FERMENTATION OF ORGANIC ACIDS

by

B. Naga Raju

AN ABSTRACT

Submitted to the College of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Civil and Sanitary Engineering

November, 1956

Approved ________________________

B. NAGA RAJU ABSTRACT

Volatile acids are one of the intermediates in sludge digestion. Buswell and other workers in this field have shown 2000 to 3000 p.p.m. of volatile acids to be the critical limit in methane fermentation. Kaplovsky in his experiments on the digestion of sewage sludge had found that only three acids, acetic, propionic, and butyric were found in measurable amounts. Hence these acids were selected for this study on maximum fermentation rates, rates of gas production and their relationship to volatile acids.

When pure acids were used as a feed, the gas production showed marked decrease at a volatile acids concentration of about 3000 p.p.m. in all three cases. This was, therefore, considered to be the critical volatile acids concentration. At the same acid level, the pH dropped below 7.0 into the acid range. Therefore, in the second series of experiments, ammonium and potassium salts were used to eliminate the effect of pH changes. The critical acid concentration for ammonium and potassium salts, except for potassium butyrate were the same as for pure acids. From this it was concluded that the decrease in gas rate caused not by the changes in pH but by the acutal accumulation of volatile acids above the critical limit. In the case of potassium butyrate the gas rate showed a marked increase at an acids level beyond the previously established maximum concentration. It was assumed that in this case potassium ion had a beneficial effect on methane fermentation.

Gas rates, volatile acids concentration and pH were plotted to demonstrate the relationship between the acids concentration and the rate of gas production.

The average maximum gas rates for an active digester volume of one liter were about 200, 797, and 1680 ml. per day for propionic, acetic, and butyric acids and the corresponding gas ratio was 1 : 4 : 8. The average maximum rate of acid fermentation was 0.2, 0.983, and 1.51 grams for propionic, acetic and butyric acid which could be expressed by the ratio of 1 : 5 : 8. A given volume of digester could ferment five times more acetic and eight times more butyric per day than propionic acid. An explanation for the different rates of acid fermentation could not be offered because the mechanism of methane fermentation is not yet fully understood at this time.

METHANE FERMENTATION OF ORGANIC ACIDS

by

B. Naga Raju

A THESIS

Submitted to the College of Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Civil and Sanitary Engineering

1956

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Professor Robert F. McCauley and to Dr. Karl L. Schulze for their valuable guidance and assistance in connection with this thesis.

Appreciation is also extended to Professor Frank R. Theroux for proof reading of the final draft of this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SECTION I

INTRODUCTION

Methane as a combustible gas has been observed in nature since early times. Thaysen and Bunker [24] stated that the marsh gas which escapes from the ground in some localities, has been used as a combustible gas for domestic purposes for many years. However, the commercial production of gaseous fuels by the microbiological decay of vegetable debris have not yet been successful.

It was not until 1897 that a waste disposal tank serving a leper colony in Bombay (India) [7] was equipped with gas collectors and gas was used to drive a gas engine. In the United States, in 1915 Hommon [24,7] equipped a waste treatment tank with gas collectors and used the gas. In 1920 John Watson of Birmingham, England [24,7] reported a study of methane production from sludge digestion and called attention to the fact that a considerable amount of methane could be produced in this way.

In 1925, Imhoff in Germany [7] had equipped a sludge digestion tank with gas collectors. The gas was found satisfactory for municipal use and was sold to the city. Since then, gas has been produced from municipal waste and the present practice in almost all sewage treatment plants, is to use the gas for heating and power generation.

Steel [21] has stated that inthe digestion of sewage sludge, anaerobic and facultative anaerobic organisms of various types are responsible for the biochemical changes. These changes were classified [21] into three stages as follows:

The first or the acid stage starts immediately. More easily fermentable compounds such as carbohydrates are attacked producing carbon dioxide and organic acids. The pH will be on the acid side.

In the second stage of acid regression, organic acids and nitrogeneous compounds are attacked with the production of carbon dioxide, hydrogen, nitrogen, and hydrogen sulfide.

In the last stage or period of intensive digestion, the accumulated volatile acids decrease. The pH moves into an alkaline range and large volumes of gas with a high percentage of methane are produced.

Kaplovsky [13] found that only three volatile acids occurred in measurable quantities, namely acetic, propionic, and butyric. Hence these three acids were selected for this study.

(A) Purpose of Study

The purpose of the study was two-fold. First it was intended to investigate the maximum rate of gas production per day using acetic, propionic, and butyric acids as feed. This is of particular interest since there is not much information available on this point. Secondly, it was intended to study the relationship between the rate of gas production and volatile acids concentration. Buswell and co-workers stated that above a critical limit of 2000 to 3000 p.p.m. volatile acids, methane fermentation is markedly impaired. Since not all the workers in the field agree with Buswell's point of view, it seemed important to provide more information in this problem.

SECTION II

REVIEW OF LITERATURE

Buswell and Hatfield [3] found that the organic acids formed in the digestion of sewage sludge and industrial wastes, consist of formic, acetic, propionic, butyric, valeric, and caproic acids. They stated that when the acids concentration is kept below 2000 to 3000 p.p.m., methane fermentation proceeds with ease, provided methane bacteria are present and the buffering capacity of the medium is sufficient to maintain the pH above 6.5. Buswell and Hatfield also stated that the accumulation of organic acids and their salts to the extent of 2000 to 6000 p.p.m. as acetic acid overtaxes the buffering capacity of the medium. These workers reported that methane producing organisms work best in the absence of high concentrations of organic acid salts and stated that alkalies should not be added if one is endeavoring to produce methane as the main end product. They also found that when butyric acid was used as a substrate, acetic acid was an intermediate. Buswell and Nave [4] showed that when a mixture of butyric and acetic acids were fed to ripe sludge, butyric acid disappeared more rapidly than acetic acid. Propionic acid had acetic and formic acids as intermediates.

Active bacterial flora is an important factor in proper fermentation. Kaplovsky [13] stated that acids accumulation is a secondary factor and that without the active bacterial flora trouble will be experienced even at a low acid level.

Heukelekian [12] found that there exists an intimate relationship between gas production and volatile acids concentration. He observed that when volatile acids concentration was high, the rate of gas production decreased; on the other hand with decreasing volatile acids concentration, the rate of gas production increased rapidly.

(A) Gas Rate

The rate of fermentation varies with the substrate fed as shown in Table 1. Feeding must be such as to recover the substrate as gas before more of that material is added.

Very little data on the rates of fermentation of lower fatty acids at mesophilic temperatures are available. Buswell and Hatfield [3] have shown that lower fatty acids can be quantitatively fermented to methane and carbon dioxide. Under favorable conditions it is possible to recover 95 to 100 per cent gas from the substrate fed. Using a thermophilic range they found that 2.54 grams of acetic acid per day could be fermented with an active digester volume of 3500 ml with a rate of gas production of 1900 ml per day. Table 1 gives a comparison of the rates of fermentation of acetic and other acids used in their experiments.

TABLE 1. Rates of fermentation of organic acids with an active digester volume of 3500 ml.

Acid Added	Temperature in Centigrade	Average Volume of gas per day in ml.	Acid Decomposed per Day in Grams
Acetic	55	1900	2.54
Succinic	35	1320	1.72
Succinic	35	1340	1.76
Malic	55	1720	2.65
Malic	55	2490	3.85
Tartaric	35	2000	3.24
Tartaric	35	1900	3.18

(B) Use of Alkalies in Fermentation

One of the earlier practices [22] for adjusting pH in sludge digestion was to add alkalies such as lime and sodium hydroxide.

Schlenz [18] was of the opinion that conditions for proper digestion was not obtained by mere adjusting of the pH. Schlenz also stated that liming to correct a low pH appeared to do more damage than good and that "liming in any form or amount has no place in a digester."

Buswell and Hatfield [3] reported that the addition of lime as a means of controlling the pH was not the proper approach to solve acid conditions since this accelerated the production and accumulation of organic acids by neutralizing the free acids. Buswell [5] stated that one of the ways of limiting the accumulation of acids in the fermentation vessel was to limit the rate of addition of the substrate. If the acid concentration had developed too much, the only remedy suggested was dilution.

Kaplovsky [13] has shown that lime does not alter the manner of decomposition but merely increases the rate of hydrolysis. He was of the opinion that the addition of lime was of considerable aid in controlling digestion if administered at the proper acids level. Instead of adding lime at an acids concentration of 2000 to 6000 p.p.m., it was recommended that liming begin at a volatile acids level of approximately 100 p.p.m.

(C) Temperature

The usual practice is to maintain the digester temperature at 29 - 35°C. Proff [14] stated that the optimum temperature for the fermentation of methane was 40°C. But, Omelianski and Gronewage [16] believed that 30 - 35°C was the most favorable temperature. Buswell and Hatfield [3] showed that the best results were obtained at 33 - 35°C. According to a table given by Fair and Geyer [10] digestion of sludge at 27°C averaged thirty days while for a temperature of 43°C twenty-six days was required.

(D) Water Required in the Fermentation of Lower Fatty Acids

The anerobic breakdown of lower fatty acids requires water [15]. The number of moles of water needed to balance the equations expressing the observed gas to acids ratio is found to increase regularly with the length of carbon chain. The moles of water required per mole of acid is equal to (n-2)/2, where n is the number of carbon atoms in the acid molecule.

SECTION III

THEORETICAL CONSIDERATIONS

(A) Characteristics of Methane Fermentation

(1) Mixed Culture.--Methane fermentation differs in many respects from other industrial fermentations. The most important difference is that it does not require a pure culture of organisms [6] in order to obtain uniform results nor is it necessary to maintain pure cultures for inoculation. This quality of fermentation with mixed cultures may be termed the first characteristic of this type of digestion.

The bacteria which are capable of producing methane are found universally in nature and are present in great numbers in decaying organic matter. Under proper conditions these bacteria can be cultivated to a high degree of activity which continues indefinitely. Several workers in this field have made attempts to isolate pure methane producing cultures. Two different rod shaped forms and two different spherical or coccus forms have proved capable of producing methane. The coccus forms were distinctly different in size. One was usually four to five micron in diameter; the other organisms were usually less than one micron in size. These bacteria in addition to producing methane, also possessed the following common characteristics: they were non-motile, non-spore forming and gram negative [5].

(2) Independence of Substrate.--A second characteristic of these fermentations is that practically any kind of organic matter may be used as a substrate. Nearly one hundred different pure substances and some thirty to forty natural plant and animal products such as corn stalks, milk whey etc. have been used successfully as fermentation material.

(3) Continuous Process.--It is possible to carry on methane fermentation indefinitely by proper addition of the substrate, while the products, methane and carbondioxide, are given off at a steady rate and the inert residue is removed continuously. This quality may be termed the third characteristic of methane fermentation.

(4) Quantitative Yields.--The fourth characteristic, the quantitative yield of two simple products, methane and carbon dioxide regardless of the substrate, is unique. In alcoholic fermentations, starch is practically quantitatively converted to alcohol and carbon dioxide but fats, protein and fibre are not attacked at all. Methane fermentation converts the entire grain with the possible exception of a small amount of fibre to carbon dioxide and methane. The reaction is of the oxidation-reduction type involving water and has been represented by Buswell and Hatfield [3] by the empirical equation: $C_nH_aO_b + (n-a/4 - b/4)\ H_2O = (n/2 - a/8 + b/4)\ CO_2 + (n/2 + a/8 - b/4)\ CH_4$. With a little care it is possible to get 95 to 100 per cent yield as calculated from the above equation.

(5) Wide Temperature Range.--The fifth characteristic of methane fermentation is the wide range of temperature in which it can be carried on. The rate of fermentation increases with an increase of temperature from 0°C to 55°C or a little higher. Maximum rates of fermentation have been reported at 26°C to 37°C and at 50° to 55°C [6]. Oliver [9] stated that uniformity of temperature is very important in that sudden small changes will result in a decrease of bacterial activity.

(6) pH Range.--Buswell and Mueller [6] have reported that methane fermentation proceeds continuously at a pH range of 6.5 to 8.0. It is normally conceded that the optimum pH of the digester tank contents is between 6.8 and 7.2 [18,21].

(7) Stirring or Mixing.--Stirring is of primary importance in methane fermentation. Heukelekian [11] observed that proper agitation or mixing in the digester caused a twenty-six per cent increase in the gas production. He also observed that agitation of digestion mixtures resulted in an acceleration of gas production. Oliver [8,9] reported that thorough mixing brings the added material into contact with the organisms, increasing bacterial use of the substrate and releasing the entrapped gases. Mixing also increases the uniformity of temperature by eliminating the stagnant pockets of material.

(B) Mechanism of Methane Fermentation

According to Buswell and Mueller [6] three possible mechanisms for the fermentation of methane have been suggested. Previous workers have reported that acetic acid was always found as an intermediate during methane fermentation and regarded this acid as an important step in the process.

Sohngen [19] showed that a preliminary decomposition of acetic acid to hydrogen and carbon dioxide occurred with the subsequent reduction of carbon dioxide to methane:

$$CH_3COOH + 2H_2O = 2\ CO_2 + 4H_2$$

$$4H_2 + CO_2 = CH_4 + 2H_2O$$

This mechanism was based on the similarity of hydrogen and methane fermentations. But Buswell and Hatfield [3] considered the absence of hydrogen in their experiements as evidence against this mechanism.

Van Niel's theory supported by Barker [1,2], stated that methane fermentation of acetic acid was a dehydrogenation of the acid accompanied by a reduction of carbon dioxide. Buswell and Mueller [6] have reported that this mechanism avoided the weakness of the first mechanism by implying a direct reduction of the carbon dioxide without the intermediate stage of free hydrogen.

The third mechanism was suggested by Buswell and Nave [4] who reported that methane originated primarily by a decarboxylation of acetic acid. This mechanism appeared to be the simplest and most direct. It could be expressed as follows:

$$CH_3COOH = CH_4 + CO_2$$

(C) Computation of Theoretical Amounts of Gas

According to Buswell and Hatfield [3] the yield of gas can be calculated by the following oxidation-reduction reaction involving water.

$$C_nH_aO_b + (n-a/4-b/2)H_2O = (n/2-a/8+b/4)\ CO_2 + (n/2+a/8-b/4)CH_4$$

where

n is number of carbon atoms

a is number of hydrogen atoms

b is number of oxygen atoms.

For acetic acid

$$(CH_3COOH) - (CO_2) + (CH_4)$$

60 grams = 2(22.4) liters of gas at 0°C and at 760 mm mercury pressure.

1 gram = 750 ml of gas at 0°C and at 760 mm mercury pressure.

For propionic acid

$$(CH_3.CH_2.COOH) + 1/2\ (H_2O) = 1\ 1/4(CO_2) + 1\ 3/4(CH_4)$$

74 grams of acid = 3(22.4) liters of gas at 0°C and at 760 mm. mercury pressure.

1 gram of acid = 906 ml of gas at 0°C and at 760 mm mercury pressure.

For butyric acid

$$(CH_3.CH_2.CH_2COOH + (H_2O) = 1\ 1/2\ (CO_2) + 2\ 1/2\ (CH_4)$$

88.06 grams of acid = 4 (22.4) liters of gas at 0°C and at 760 mm. mercury pressure.

1 gram of acid = 1020 ml. of gas at 0°C and at 760 mm. mercury pressure.

Since in the laboratory the gas measurements were corrected for 25° C and 760 mm. mercury pressure, the corresponding gas amounts are given here:

1 gram of acetic acid is equal to 811 ml. gas.

1 gram of propionic acid is equal to 989 ml. of gas.

1 gram of butyric acid is equal to 1113 ml. of gas.

SECTION IV

EXPERIMENTAL MATERIAL AND APPARATUS

Sludge from the East Lansing sewage treatment plant digester was used as a starting material. The sludge was in a working condition nearing complete digestion as shown in Figures 5 and 12, and Tables 9 and 16. The sludge was brought in a glass bottle about twenty liters in capacity and stored in the laboratory for a few days to allow the solid particles of sludge to settle and the supernatent to be decanted. Table 2 shows the analysis of the sludge used in both sets of experiments.

TABLE 2. Composition of sludge used in the experiments

Sludge	Experiment 1	Experiment 2
Total solids	7.3%	6.2%
Volatile matter	43.8%	34.0%
Ash	56.2%	57.0%
Volatile acids	420 ppm.	610 ppm.
pH	7.4	7.1

Acetic, propionic and butyric acids were of about five normal concentration. The acids were used either in pure form or as ammonium or potassium compounds. Method of preparation for the acid salts was as follows:

To a known volume of acid, ammonium hydroxide or potassium hydroxide was added until a pH of 6.0 was reached. This was done in order to keep the alkali concentration low and to minimize the changes in pH.

(A) Description of Equipment

Fermentation was carried on in a fermentation vessel shown in Figure 1. The laboratory fermentation units were constructed of lucite plastic and each one had a volume of about four liters. The top of the digester was fastened to the bottom by means of bolts with ring nuts. A rubber gasket was placed between the cover and body of the vessel to insure gas tightness.

The material in each of the unit was kept in constant agitation by a stirring mechanism which consisted of a vertical shaft in the center of the units with three horizontal brass rods attached. The shaft rotated at four revolutions per minute. The stirring rod passed through a half-inch long Tygon gas tight bearing set in the plastic cover of the unit.

Power for the stirring mechanism was provided by a motor and gear reduction box which, in turn, was connected to a long horizontal steel shaft. By a suitable bevel gearing arrangement, the vertical shafts of the units were made to revolve.

The units were placed in a water bath maintained at 32°C by means of heating units and Fenwall thermostats. The temperature ineach bath was kept uniform by air stirrers. A thin film of oil on top of the water bath prevented evaporation.

The fermentation unit had two openings as shown in Figure 1. One end of the 2.5 liter gas collecting cylinder D was connected to one opening in the digestion unit. The other end was connected to a leveling bulb E. Gas was collected in the gas cylinder over a saturated solution of sodium chloride.

The second opening in the top of the digester units was drilled to fit a No. 8 rubber stopper. This stopper had two holes, one being a small gas tight opening through which the spout of the burette B was passed for the addition of the required amount of acid. The second opening in the No. 8 stopper was closed with a '00' size stopper which could be withdrawn to permit sampling by means of a glass tube.

The volume of gas collected in the graduated cylinder varied with temperature and barometric pressure. All readings were corrected to a temperature of 25°C and 760 mm. mercury pressure by means of a special slide rule as described by Snell [17].

(B) Other Equipment

Volatile acids concentration was determined according to Standard Methods [20]. A Beckman pH meter was used for the determination of pH; a hot air oven maintained at 105°C and a muffle furnace which could be operated at 600°C were used for the determination of total solids, volatile matter and ash.

Figure 1. Fermentation Apparatus

A	Sludge in the unit
B	Burette through which acid was added
C	Tygon tube bearing
D	Gas collecting cylinder
E	Levelling flask
F	Thermostat
G	Heating Unit
H	Air stirrer
I	Gear mechanism
J	Water bath
K	Thermometer
L	Vertical shaft

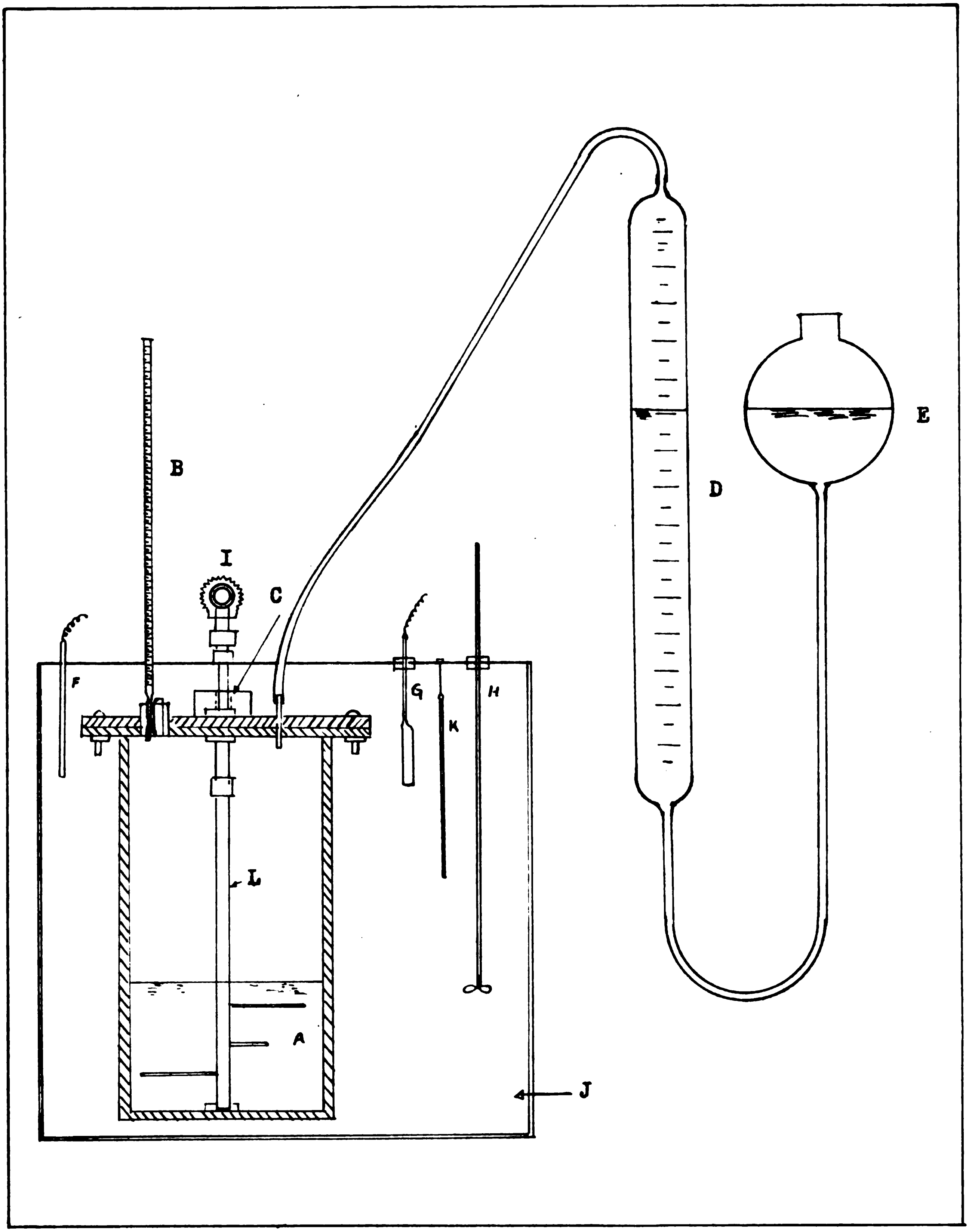

FIGURE 1. FERMENTATION APPARATUS

SECTION V

EXPERIMENTAL PROCEDURE AND RESULTS

The first set of experiments was preliminary in nature, the object being to study the maximum gas rates and volatile acids concentration in the fermentation vessel.

The purpose of the second set of experiments was to check the critical acid concentration limits and the gas rates established in the first set and to study the behavior of fermentation when using ammonium and potassium compounds as substrate.

Theoretical computations of gas production are shown on pages 13 and 14. These data were used as a guide for feeding the amount of acid that could be fermented quantitatively each day. The rates of fermentation of these acids were found to be different from one another and feeding was adjusted to these different rates so that almost all of the material fed was recovered as gas before more was added. This procedure resulted in a high yield of gas.

(A) First Set of Experiments with Acetic, Propionic and Butyric Acids

Before starting the experiments the units were checked for gas tightness by using air under pressure. Four units were started, one for each of the three acids and the last as a control unit.

One liter of active sludge was added to each of the units to support the methane producing bacteria and the units were flushed with nitrogen in order to remove oxygen.

The acids used for the fermentation were 9.65 N acetic, 4.32 N propionic and 4.62 N butyric. One milliliter acid solution contained; acetic 0.579 grams, propionic 0.32 grams, and butyric 0.407 grams.

(1) Acetic Acid. [Figure 2 and Table 6]--The acetic acid unit received one milliliter solution equal to 0.579 grams of acetic acid per day starting from the third day to the ninth day. The rate of gas production recorded on the fourth day was 534 ml. somewhat above the theroetical amount expected of 470 ml. per day. Accordingly the volatile acids concentration in the unit remained at a low level, 313 p.p.m. at a pH of 7.3 on the eighth day. The gas rate on the ninth day was 520 ml. per day, therefore, the entire amount of acid was being daily converted into gas.

On the tenth, eleventh, twelfth, fourteenth, sixteenth, seventeenth, and twentieth days, 1.158 grams of acid was added and which was equal to the theoretical amount of 940 ml. of gas per day. In this case not all the acid added was fermented to gas. A maximum gas rate of 760 ml. per day was recorded on the twelfth and the thirteenth day. The gas rate commenced to drop on the twelfth, thirteenth, and fifteenth days and showed 200 ml. of gas to be missing daily when compared with the theoretical rate. As expected, the volatile

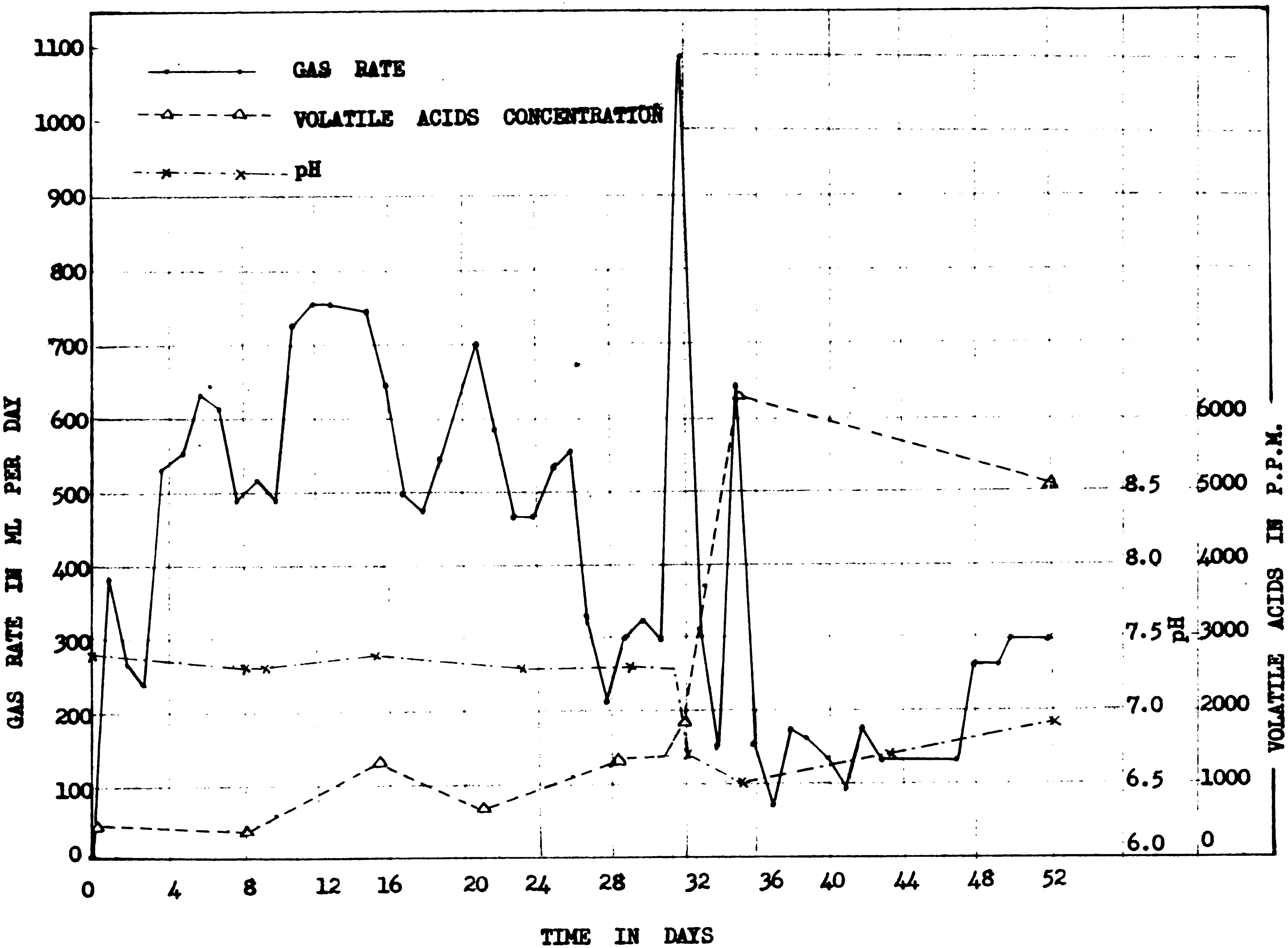

FIGURE- 2. ACETIC ACID FERMENTATION SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH.

acids concentration on the fifteenth day increased to 1289 p.p.m.Gas production rate decreased to 650 ml. on the sixteenth day, indicating a maximum gas production rate for acetic acid of 760 ml. per day per liter of active digester volume.

By the twenty-second day the gas rate had decreased to 590 ml. because of acids accumulation. In order to keep the acids concentration low, the feed rate was reduced to 0.579 grams of acetic acid on the twenty-second and twenty-third day. On the twenty-fourth day and on the twenty-sixth day 0.868 grams of acid was added and the gas rate observed on the twenty-sixth day was 560 ml. per day indicating volatile acids were still increasing.

In order to increase the volatile acids concentration to a still higher level, 3.474 grams of acid equal to 2820 ml. of gas, were added on the thirty-first day and the gas rate observed on the thirty-second day was 1100 ml. of gas. A sudden drop to 300 ml. and 150 ml. of gas on the respective following days was then recorded. Due to the marked lag of gas production as related to the theoretical amount expected, a steep rise in volatile acids concentration could be anticipated.

On the thirty-fourth day a further heavy load of acetic acid (3.474 grams) was added. After a short increase in the gas rate to 650 ml. per day, the rate of gas production dropped to 160 ml. and 70 ml. on the following two days. On the thirty-fifth day volatile acids concentration was 6320 p.p.m. and pH dropped to 6.5. For the following thirteen days the gas rate stayed low (between 70 to 180 ml. per day). A gradual recovery was then noted on the forty-ninth day.

With volatile acids concentration below 3000 p.p.m., the pH varied between 7.3 and 7.4. When the acids level reached 6000 p.p.m. the buffering capacity was overtaxed, the pH dropped to 6.5 and the gas rate decreased substantially.

The maximum rate of gas production observed in the experiment was 760 ml. per liter of active digester volume per day and the fermentation rate was about one gram of acetic acid per day.

(2) Propionic Acid. [Figure 3 and Table 7]--On the third day of the experiment, daily addition of one ml. of 4.32 N propionic acid solution, equal to 0.32 grams of acid was commenced. Theoretically 316 ml. of gas was expected from this amount of acids but actually about 245 ml. of gas per day was the maximum production rate observed on the ninth day. As a result, volatile acids were accumulating in the unit. Analysis showed 1584 p.p.m. on the eighth day, 2120 p.p.m. on the fifteenth day, and 3050 p.p.m. on the twenty-second day. In order to reduce volatile acids concentration the rate of acid addition was lowered to one-half on the twenty-fifty day and continued at that rate up to the thirty-third day. On the twenty-seventh day volatile acids concentration was 2850 p.p.m. and the gas rate was 130 ml. per day. The

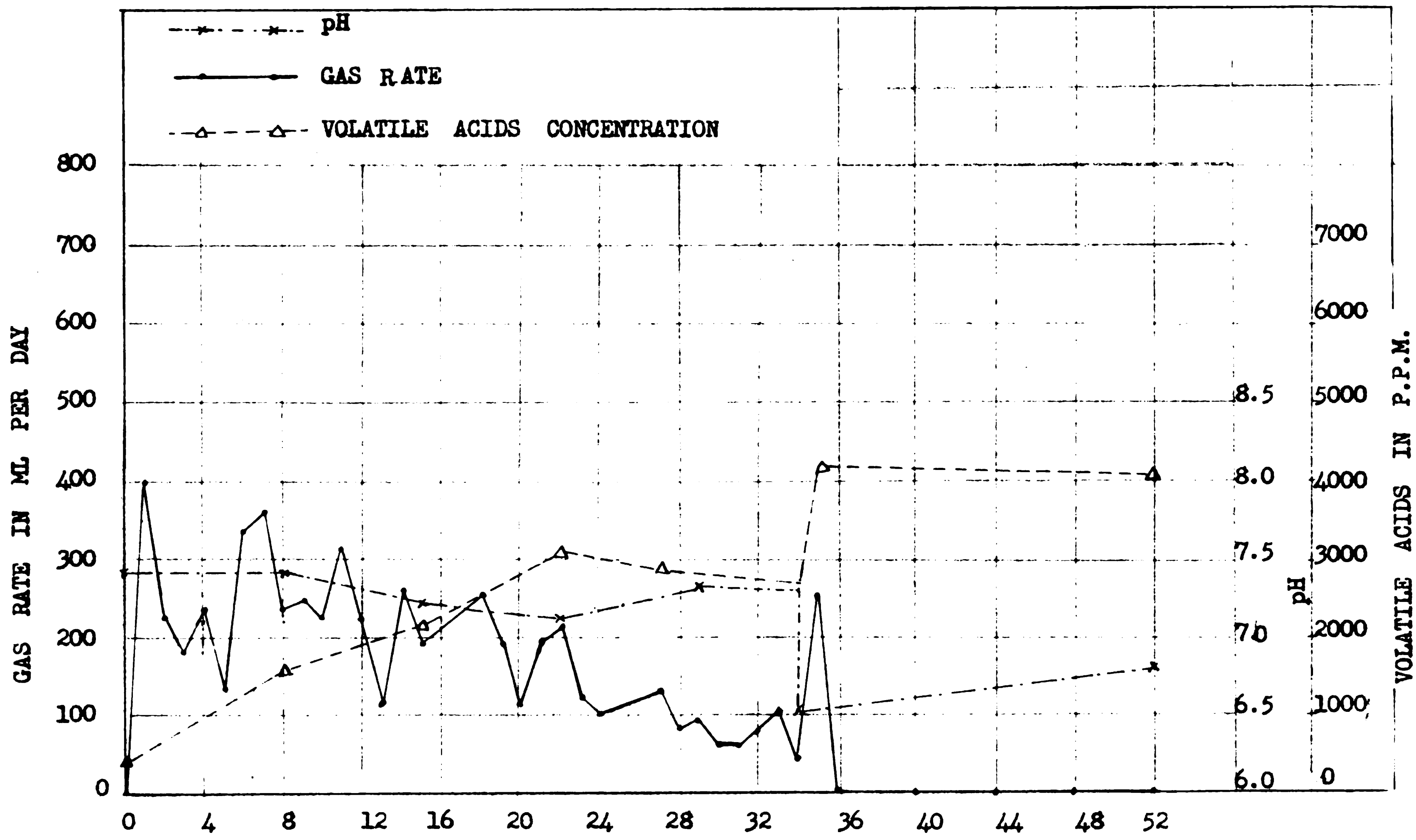

FIGURE- 3. PROPIONIC ACID FERMENTATION SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH.

acids concentration of 2850 p.p.m. seemed to be the critical limit as the rate of gas production was not improved at that level.

An attempt to study the behavior of fermentation at still higher acids concentration was then made. Ten times the original amount of acid (3.2 grams) was added on the thirty-fourth day. On the following day the gas rate observed was 250 ml and thereafter fermentation stopped altogether. Volatile acids concentration in the unit on the thirty-fifth day was 4120 p.p.m. and pH was 6.5.

Apparently 4000 p.p.m. of acids concentration combined with a drop in pH was enough to stop the methane production completely. From the data in Table 7, it can be seen that at volatile acids concentration above 3000 p.p.m. the buffering capacity of sludge was overtaxed and the pH dropped below 7.0. Table 7 also shows that a maximum rate of gas production of about 200 ml. per liter of active digester volume per day and a fermentation rate of 0.2 grams of acid per day could be assumed.

(3) Butyric Acid. [Figure 4 and Table 8]--For the third unit 4.62 N butyric acid was added. Beginning on the third day of the experiment, one milliliter of acid solution equal to 0.407 grams of acid per day was added to the unit and continued at that rate up to the ninth day. The theoretical amount of gas was 453 ml. at that rate of acid feed. The actual rate of gas production was 512 ml and 517 ml. on

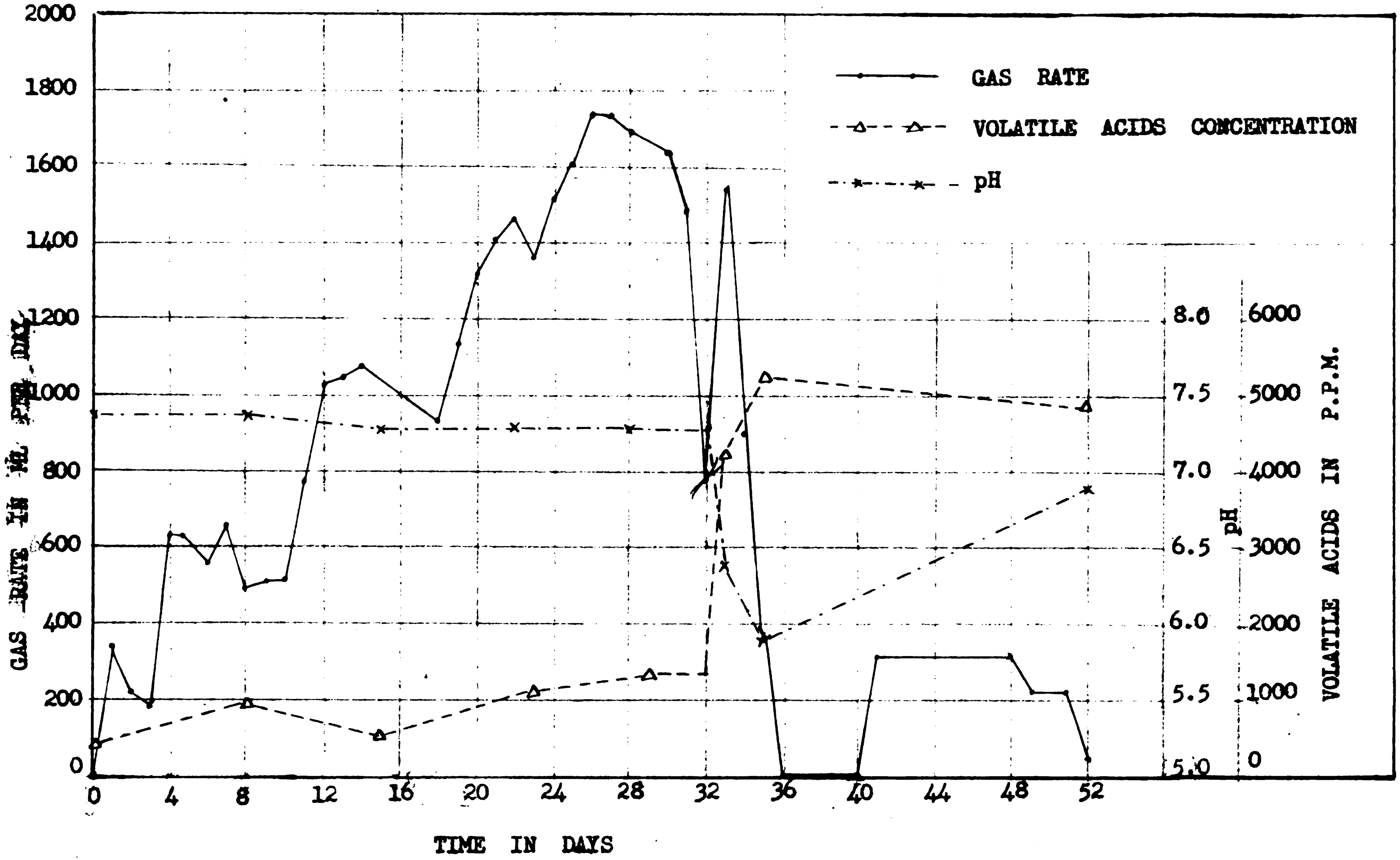

FIGURE- 4. BUTYRIC ACID FERMENTATION SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH.

the ninth and the tenthdays respectively. The additional gas was due to the digestion of sludge.

On the tenth day the amount of acid added was doubled to 0.814 grams of acid per day. This feed rate was continued up to the seventeenth day. Volatile acids concentration remained low and on the fifteenth day was 550 p.p.m. with a pH of 7.3. Gas rate observed was 1030 ml., 1050 ml., and 1085 ml. on the twelfth, thirteenth, and fourteenth days respectively, while the theoretically expected amount of gas was 910 ml.

Beginning on the eighteenth day, the feed rate was trippled to 1.221 grams of acid per day. The theoretically expected 1400 ml. of gas per day were formed for each of six days. Next, 1.628 grams of acid was added for seven days beginning on the twenty-fourth day. The maximum rate of gas production was about 1730 ml., slightly less than the maximum expected amount of 1810 ml. Consequently volatile acids concentration increased to 1320 p.p.m. on the twenty-ninth day and the rate of gas formation decreased to 1490 ml. per day on the thirtieth day of the experiment.

A 3.256 gram portion (eight times the amount of acid added at the beginning) was added on the thirty-second day in an attempt to study the behavior of fermentation at higher acids concentration levels. On the thirty-third day the pH dropped to 6.4 at an acid concentration of 4250 p.p.m. On the thirty-fourth day a further portion of 3.256 grams of acid was added and the gas dropped to 320 ml. per day on the thirty-fifth day, while the volatile acids concentration was 5770 p.p.m. and pH had dropped to 5.9 as shown in Figure 4 and Table 8.

Fermentation then stopped and no gas formation was observed until the forty-first day, when gradual recovery began. Gas rate from the forty-second day to the fifty-second day was about 300 ml. per day.

About 4000 p.p.m. of acids concentration in the unit seemed to be the critical limit. When the acids level in the units was below 3000 p.p.m. pH was almost stable at 7.3 and 7.4. But when acids level reached beyond 4000 p.p.m., pH commenced to drop rapidly. The maximum rate of gas production was about 1735 ml. per day which meant that approximately 1.6 grams of butyric acid could be fermented per day.

The importance of stirring mechanism of the fermentation vessel cannot be over-looked. A few occasional stoppages of stirring mechanism due to mechanical trouble showed a considerable reduction in gas formation. In one instance(shown in Table 8) gas rate dropped from 1690 ml. to 1360 ml. per day because of failure of the stirring mechanism.

(4) Control Unit. [Figure 5 and Table 9]--The last unit was started under identical conditions and served as a control unit. The gas rate on the first day was 400 ml. and gradually decreased to 10 ml. per day on the ninteenth day and to zero on the thirty-first day. The amount of gas produced was very small compared to that produced by the acid

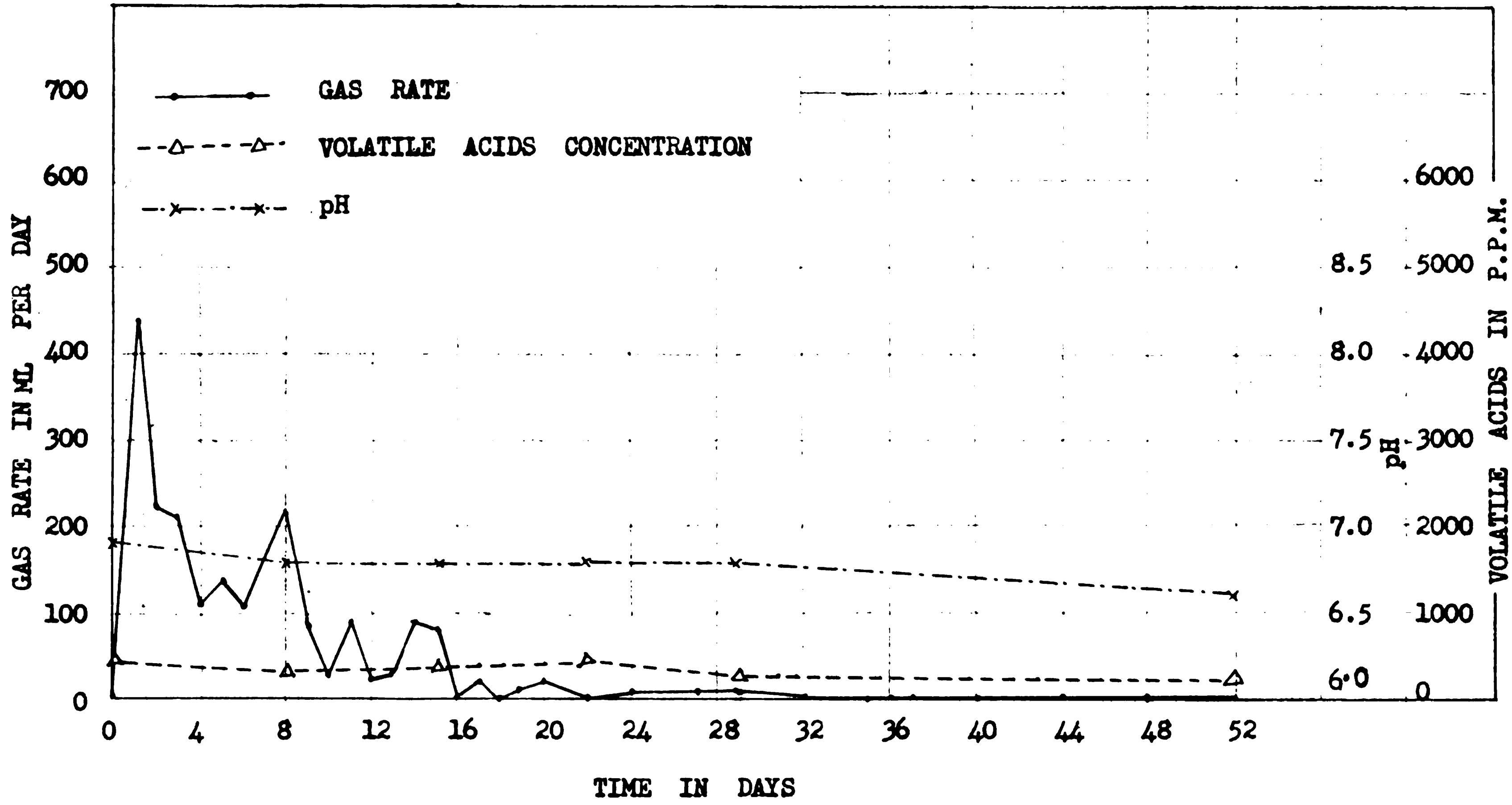

FIGURE- 5. CONTROL UNIT SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH.

fermentation. Therefore, no correction was made for gas produced by the sludge.

Volatile acids concentration in the unit gradually decreased from an initial acids concentration of 420 p.p.m. to 260 p.p.m. on the fifty-second day. Variation in pH was between 7.4 to 7.1.

(B) Second Set of Experiments with Acetic Acid, Propionic Acid, and Butyric Acid

The object of this experiment was to check the results of the first work and to use ammonium and potassium compounds for buffering the pH.

The experiment was started with sludge in a condition similar to that used in the first set of experiments (shown in Table 2). Two parallel units were used for each of the three acids. The acids used were 4.72 N acetic, 4.7 N propionic, and 4.75 N butyric. One milliliter of these solutions contained 0.283 grams, 0.348 grams, and 0.418 grams of acid respectively.

(1) Experiment with acetic acid using ammonium and potassium salts. [Figures 6 and 7, and Tables 10 and 11]--Fermentation was started by adding 0.929 grams of acetic acid to each of the two Units A and B, a rate slightly less than the critical value of one gram of acid per day which was obtained in the first set of experiments. This feed rate was continued up to the twentieth day and the maximum gas rates observed were 780 ml., 760 ml., 800 ml., and 800 ml. per day

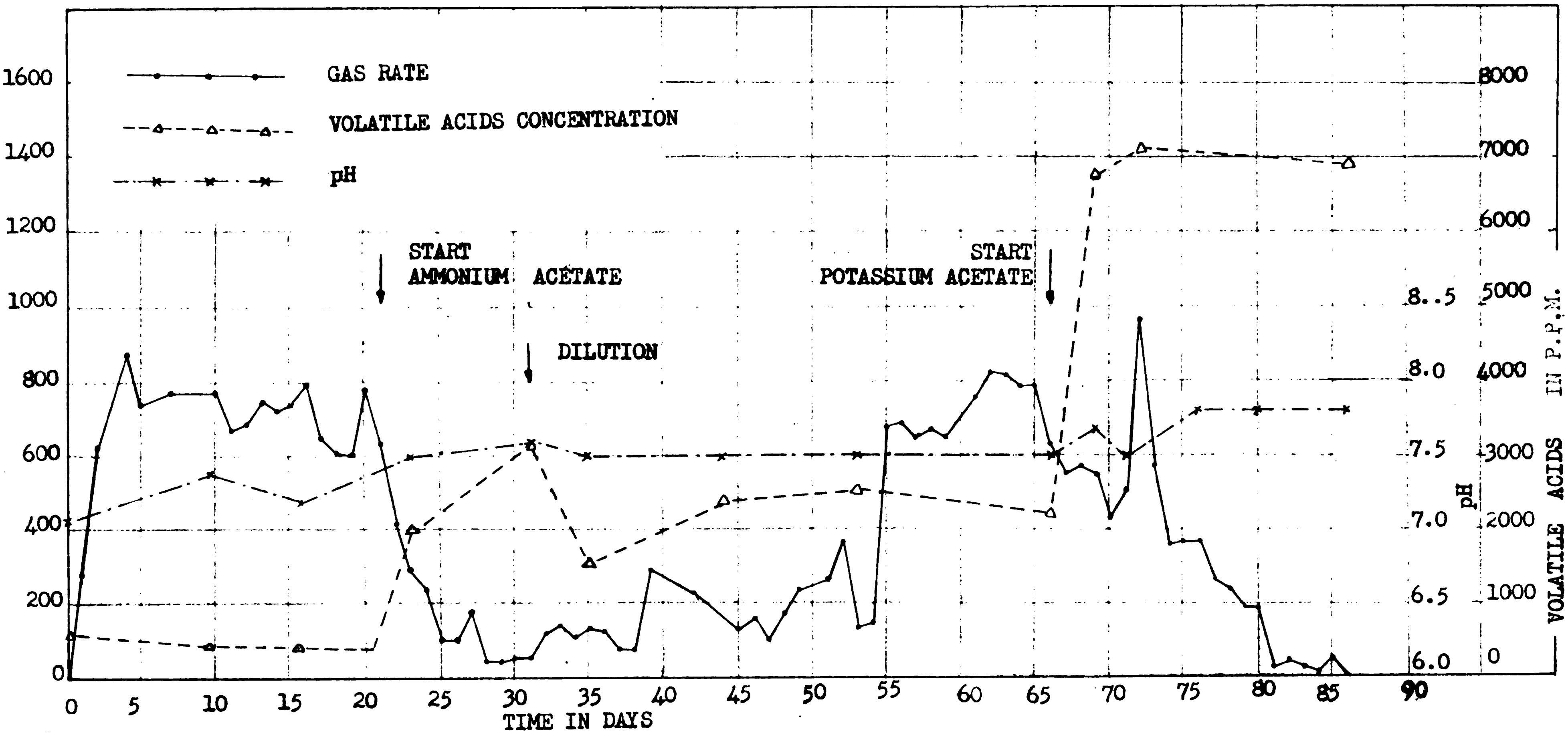

FIGURE- 6. ACETIC ACID FERMENTATION SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH (UNIT A)

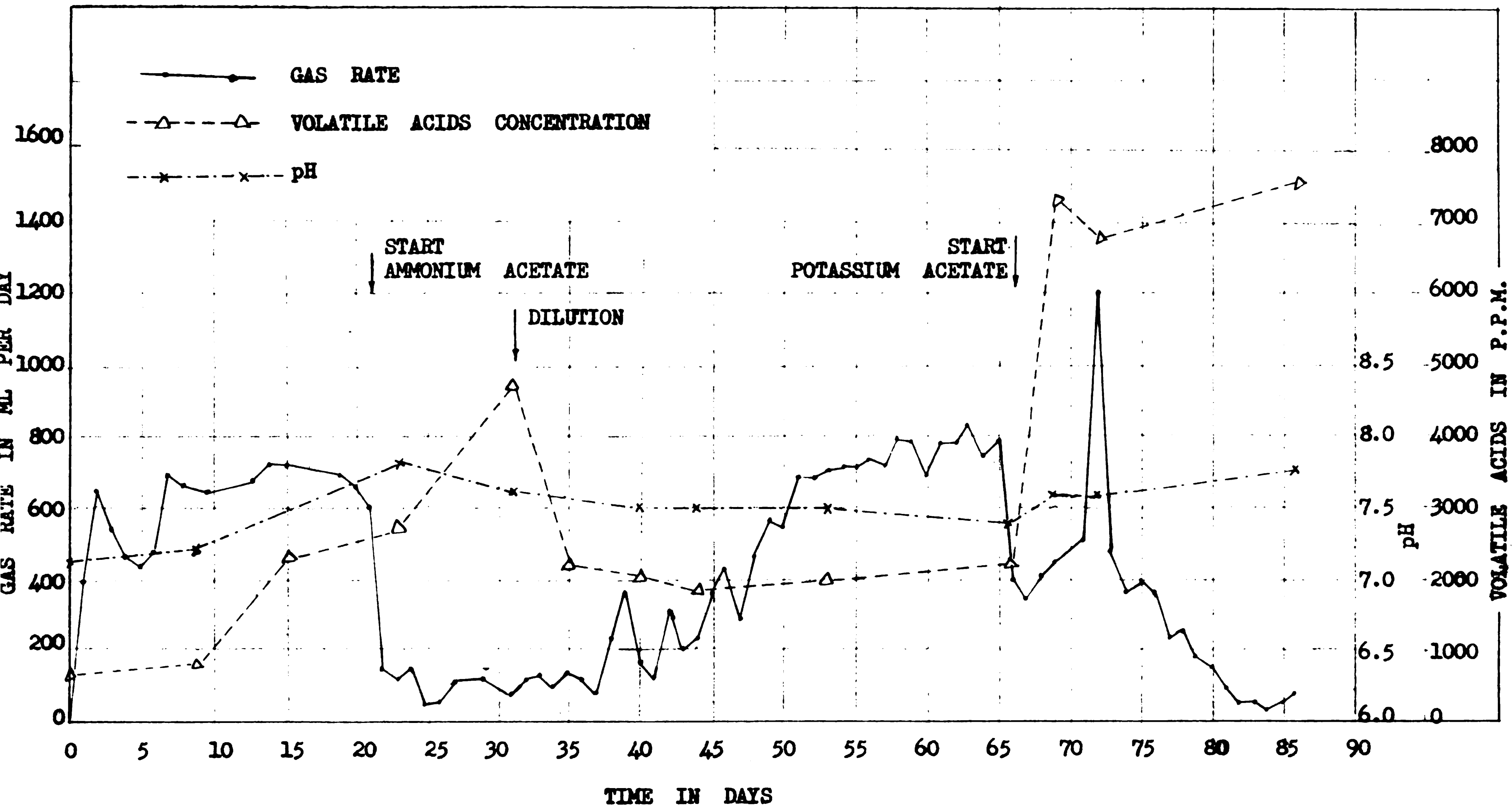

FIGURE- 7. ACETIC ACID FERMENTATION SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH (UNIT B) on the tenth, thirteenth, sixteenth, and twentieth days in Unit A, and 720 ml. and 770 ml. on the fourteenth and nineteenth day in Unit B. This compared favorably with the maximum gas rate of 760 ml. per day reported in the first set of experiments. Since the theoretical amount of gas expected (760 ml.) was produced, volatile acides did not increase.

On the twenty-first day, a portion of ammonium acetate which contained 1.701 grams of acid and 0.498 grams of ammonia was added. The amount was almost double the quantity of acid added before. The rate of gas production dropped to 420 ml. and 150 ml. in Units A and B respectively on the twenty-second day. Equal amounts of ammonium acetate were added on the twenty-third, twenty-fourth, and twenty-sixth days and the gas rate dropped to 190 ml. and 110 ml. in Units A and B respectively. On the thirty-first day, volatile acids concentration in Unit A rose to 3180 p.p.m. and to 4470 p.p.m. in Unit B. Since there was no drop in pH due to buffering effect of the ammonium ion, the decrease in the gas production in this case was apparently caused by the actual concentration of acid or acid salts.

The dilution method [5] was used to bring the units back to good operating condition. One liter of sludge (in an active condition) from the East Lansing Sewage treatment plant was added to each of the two units. This resulted in a reduction of the volatile acids concentration from 3180 to 1540 p.p.m. in Unit A, and from 4470 to 2020 p.p.m. in Unit B. The pH variation in the units was slight, from 7.6 to 7.5.

The gas rate gradually improved increasing to 150 ml. on the thirty-third day in Unit A and 125 ml. in Unit B on the same day.

On the thirty-eighth day, 1.490 grams of pure acid were added but the gas rate remained low at 300 ml. per day in Unit A and 365 ml. in Unit B. Further additions of pure acid to both units did not increase gas production; probably because the stirring mechanism in the units was not designed for two liters of sludge. Hence one liter of sludge from each of the two units was withdrawn on the fiftieth day.

Acid fermentation was then continued by adding 0.894 grams of pure acid per day from the fifty-first day to the sixtieth day. Maximum rate of gas production then increased to 800 ml. in Unit B on the forty-eighth day showing that the units were in good operating condition. Again, slightly more than one gram of acid (1.198 grams) was added on the sixty-first day to sixty-fourth day to establish the maximum rate of gas production which was 830 ml. in Unit A and 800 ml. in Unit B. This gas rate was again in good agreement with the 760 ml. per day established in the first set of experiments.

Potassium acetate containing 1.894 grams of acetic acid and 1.212 grams of potassium was added from the sixty-sixth day to the sixty-ninth day to both units. This acid feed rate was about twice the level established as maximum in the first set of experiments. As a result of this overloading, the volatile acids concentration rose to 6800 p.p.m. per day in

Unit A and 7400p.p.m. in Unit B on the sixty-ninth day, while the gas rate gradually dropped to 550 ml. per day. The pH level remained between 7.7 and 7.6.

In order to minimize the accumulation of potassium a portion of pure acid containing 1.788 grams was added to each of the two units on the seventy-first day. Two further doses of potassium acetate were added, one on the seventy-second day and the other on the eightieth day. Volatile acids concentration reached 6950 p.p.m. in Unit A and 7600 p.p.m. in Unit B with pH 7.8. With the exception of one day, the gas rate showed a continuous decrease in both units, reaching zero in Unit A and 90 ml. in Unit B on the eighty-sixth day. The experiment was therefore terminated.

It was noted that 3000 to 4000 p.p.m. constituted a critical acids concentration level when pure acid or ammonium acetate was used. In the case of potassium acetate the decrease in gas rate started at a much higher level of about 7000 p.p.m. volatile acids concentration. Since the pH factor had been eliminated in the case of both ammonium and potassium acetate, it may be concluded that the potassium ion had a beneficial effect on methane fermentation. Such conclusion is substantiated by the fact that the curves in Figure 6 and 7 show a close agreement throughout the experiment.

(2) Experiment with propionic acid using ammonium and potassium salts. [Figures 8 and 9, and Tables 12 and 13.]--Fermentation was started by adding 0.174 grams of acid to each of the two Units C and D, a rate slightly less than the critical value of 0.2 grams of acid per day which was obtained in the first set of experiments. This amount was increased to 0.209 grams per day and continued up to the twentieth day. The theoretical rate of gas production at this rate of acid feed was 207 ml. and the actual gas rates in both the units were fairly constant at about 200 ml. per day. This confirmed the rate obtained in the first set of experiments. Since the expected amount of gas was not quite realized, volatile acids concentration increased to 1690 p.p.m. and 2043 p.p.m. in Units C. and D. respectively on the fourteenth day.

Ammonium propionate solution containing 0.342 grams of acetic acid and 0.078 grams of ammonia was added from the twenty-first day to the twenty-fourth day and on the twenty-sixth day to Units C. and D. The gas rate in Unit C was 130 ml., 150 ml., and 130 ml. per day on the twenty-second, twenty-third, and twenty-fourth day and the corresponding values for Unit D were 70 ml., 60 ml., and 40 ml. per day. The expected amount of gas 338 ml. per day was not produced, therefore volatile acids concentration reached 2860 p.p.m. in Unit C and 2640 p.p.m. in Unit D on the twenty-third day. pH was between 7.6 and 7.7 in the units.

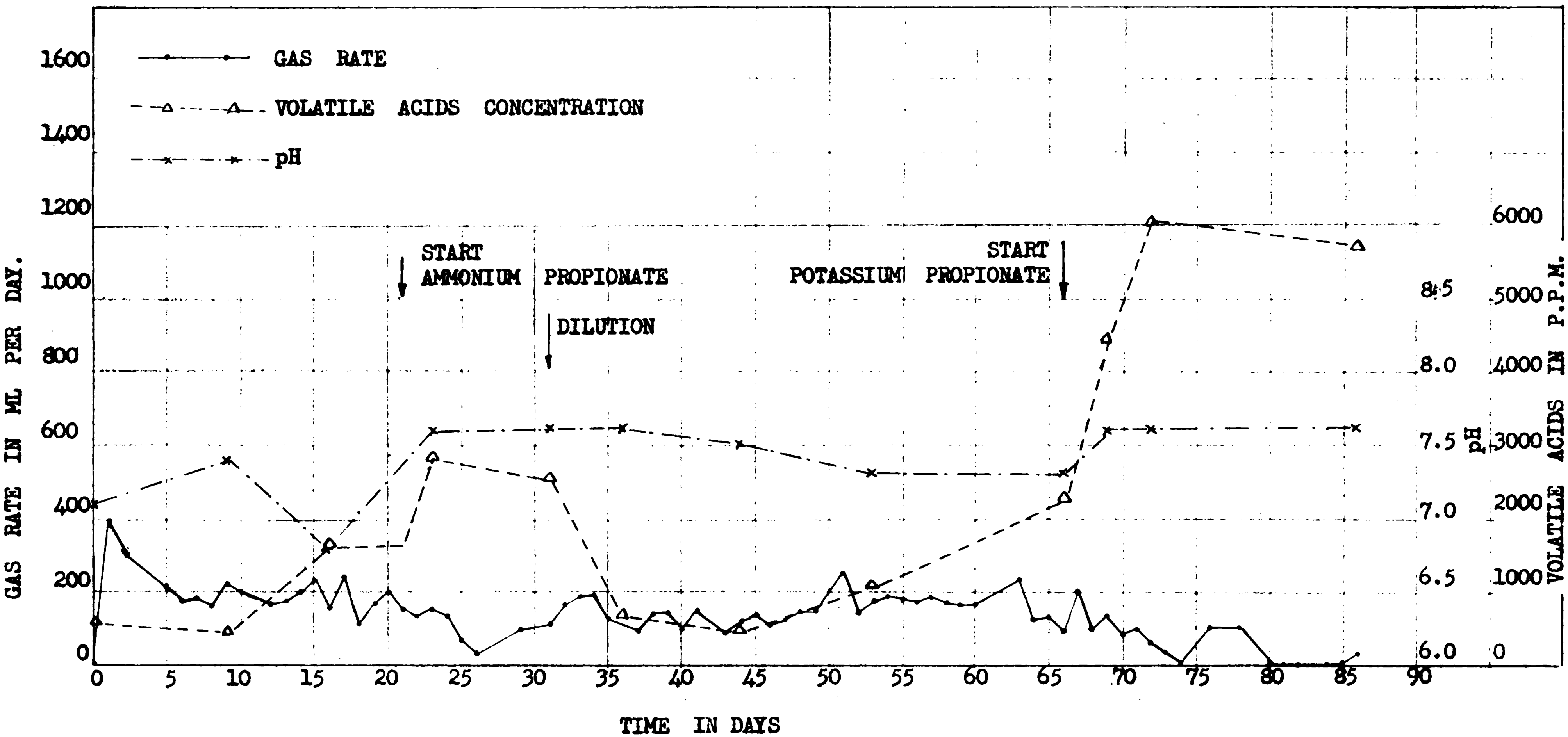

FIGURE- 8. PROPIONIC ACID FERMENTATION SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH (UNIT C)

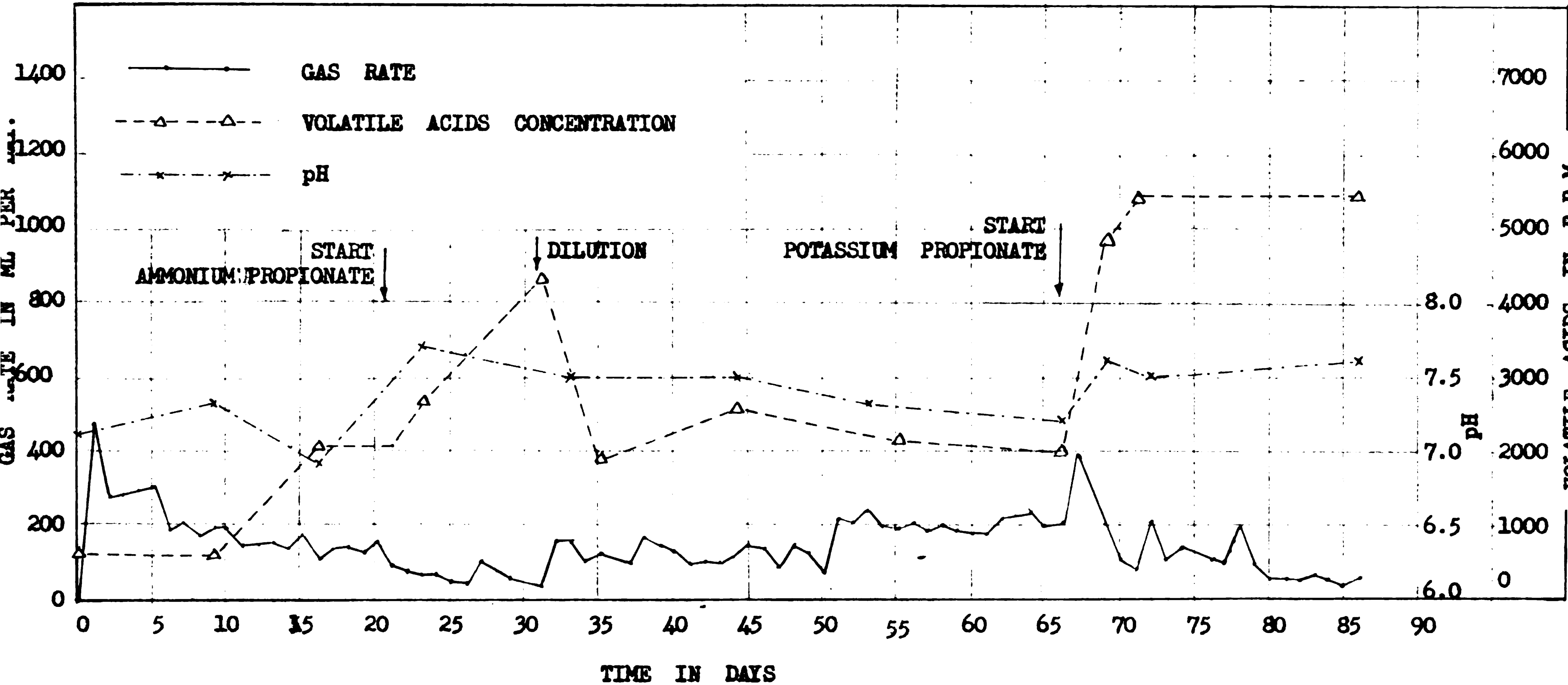

FIGURE- 9. PROPIONIC ACID FERMENTATION SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH. (UNIT D)

The gas rate was low when ammonium propionate was being added. On the thirty-first day the volatile acids concentration was 2520 p.p.m. in Unit C and 4350 p.p.m. in Unit D. The low rate of gas production was due to the combined effect of acids concentration and the depressing effect of ammonium ion on gas production.

As in the case of acetic acid experiment, one liter of ripe sludge from the East Lansing sewage treatment plant was added for dilution purposes on the thirty-first day. Feeding was discontinued for six days. The rate of gas production increased from 105 ml. to 165 ml. on the following days. Volatile acids on the thirty-sixth day was 670 p.p.m. in Unit C and 1900 p.p.m. in Unit D and the corresponding pH was 7.6 and 7.5. A maximum rate of gas production was not reached, the level being about 185 ml. and 155 ml. per day in Units C and D respectively.

From the thirty-seventh day to the forty-ninth day 0.348 grams of pure acid were fed daily to both units, with the exception of three days. The maximum rate of gas production recorded was about 140 ml. per day in both units. As already mentioned this was probably due to the stirring mechanism being inadequate for two liters of sludge. So on the fiftieth day, one liter of sludge was removed from the units. From the fifty-first to the sixtieth day 0.174 grams of pure acid were added daily. Maximum gas rate recorded was 200 ml. per day, almost identical to that in the first experiment. This showed that the unit was back in good operation.

On the sixty-first day and on the sixty-second day, double the previous amount of acid (0.348 grams) was added but the rate of gas production did not go beyond 230 ml.per day which was equal to 0.232 grams of acid. Therefore, the feed rate was reduced to 0.174 grams on the next two days.

Potassium propionate containing 0.776 grams of propionic acid and 0.4 grams of potassium was added from the sixty-sixth day to the sixty-ninth day. This portion represented 3.4 times the previously established maximum loading.

Accordingly, the volatile acids concentration rose to 4430 and 4900 p.p.m. in Units C and D. on the sity-ninth day, and 6100 and 5400 p.p.m. on the seventy-second day. pH in both units stayed in the alkaline range between 7.5 and 7.6. Nevertheless the gas rate dropped to 90 ml. and 100 ml. on the seventieth day, showing that the critical volatile acids concentration has been reached. During the following eighteen days the gas rate gradually decreased almost to zero and the experiment was discontinued on the eighty-sixth day.

<u>(3) Experiments with butyric acid using ammonium and potassium salts</u>. [Figures 10 and 11, and Tables 14 and 15]-- Two Units E and F were started by adding 1.254 grams of butyric acid per day to each of the units from the first day to the twentieth day. This feed rate was slightly less than the maximum of 1.6 grams per day found in the first experiment. The theoretical amount of gas expected was 1396 ml. per day and the average gas rate was 1319 ml. for the Unit E and 802 ml.

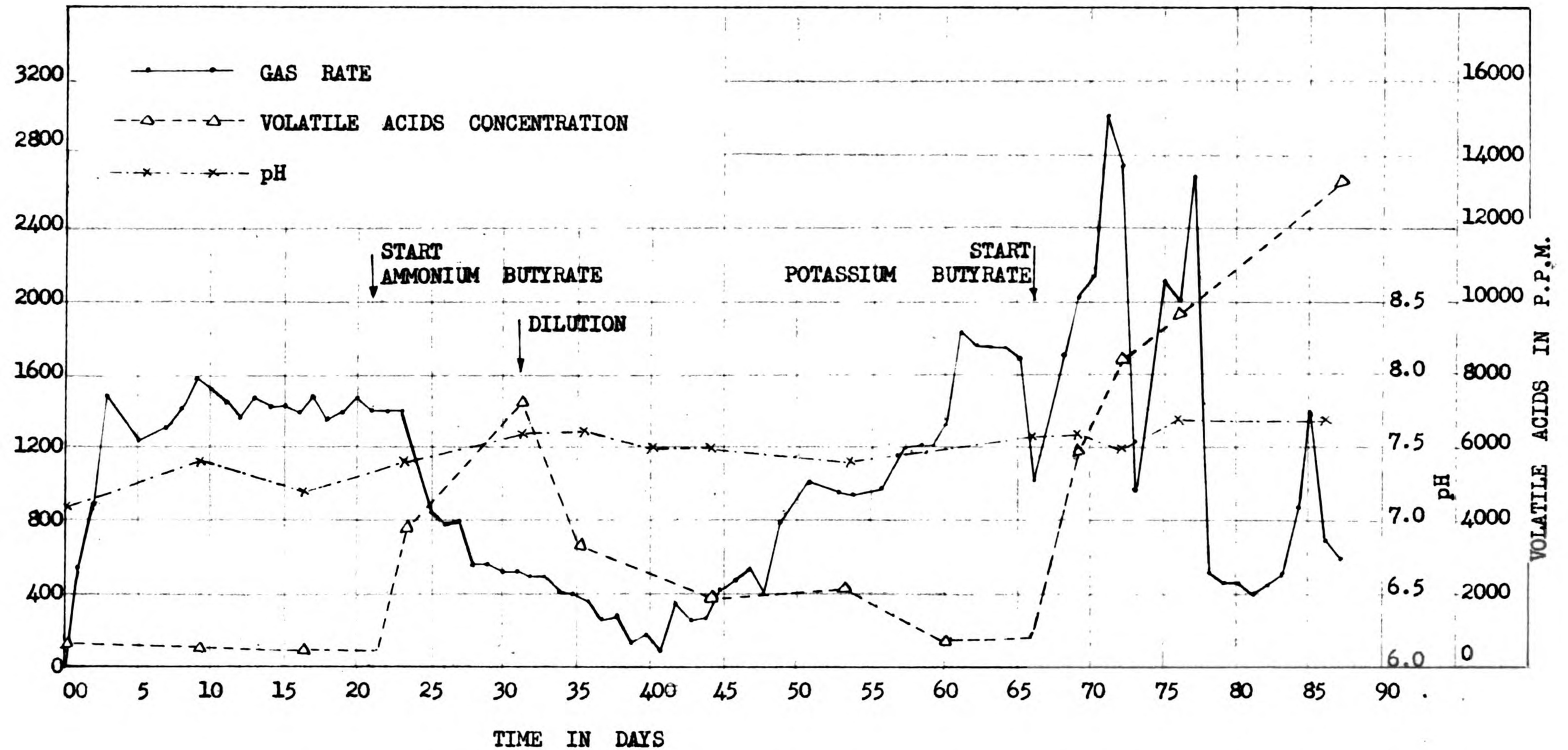

FIGURE- 10. BUTYRIC ACID FERMENTATION SHOWING GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH. (UNIT E)

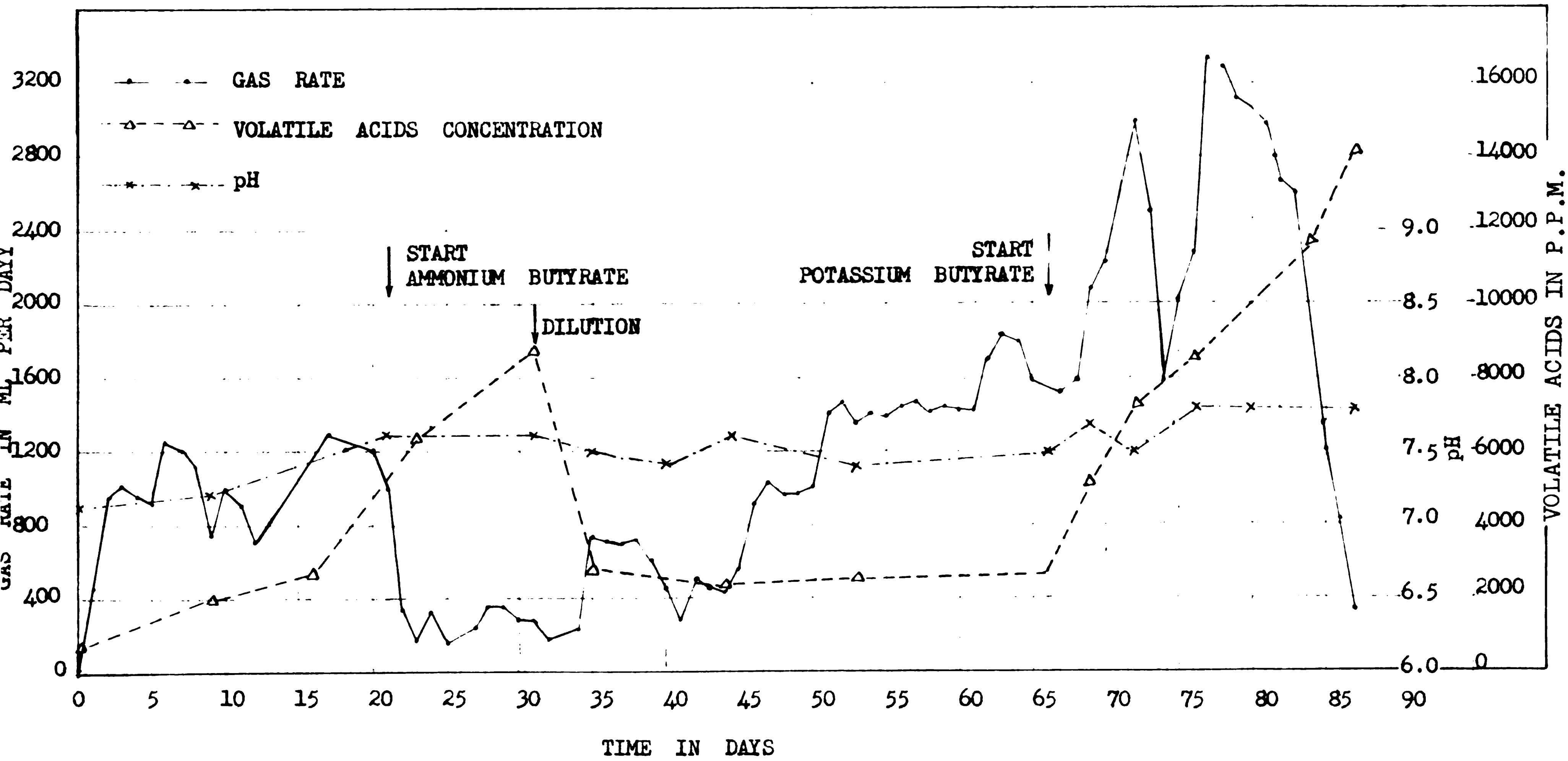

FIGURE- 11. BUTYRIC ACID FERMENTATION SHOWING GAS RATES, VOLTILE ACIDS CONCENTRATION AND pH. (UNIT F) for Unit F. The volatile acids concentration in Unit E was 470 p.p.m. and 398 p.p.m. on the ninth and sixteenth days which confirmed that all the acid added was converted to gas. Since Unit F did not work well, the respective volatile acids concentration were 1962 and 2300 p.p.m.

Beginning on the twenty-first day, ammonium butyrate which contained 2.512 grams of butyric acid and 0.504 grams of ammonia was added daily. This level of acid feed represented twice the level used during the previous twenty day period and was continued for six days in both units. Within two days volatile acids concentration rose to 3830 p.p.m. in Unit E and 6150 p.p.m. in Unit F, and after ten days the concentration was 7250 p.p.m. in Unit E and 8350 p.p.m. in Unit F. At the same time the gas rate dropped to 580 ml. in Unit E and to 200 ml. in Unit F. The pH remained at 7.6 in both units even though the acids concentration was high. The drop in gas rate occurred at a volatile acids concentration of 4000 to 5000 p.p.m. in both units. This was the same range found in the first set of experiments, the difference being that this time no lowering of pH took place. The decrease in gas rate was, therefore, not related to the change in pH but to the critical volatile acids level.

As in the other cases, one liter of ripe sludge in an active condition from the East Lansing sewage treatment plant was added for dilution purposes on the thirty-first day. Volatile acids concentration was reduced to 3320 p.p.m. in Unit E and to 2770 p.p.m. in Unit F on the thirty-fifth day.

The units were allowed to recover without adding any further amount of acid.

On the forty-first day 1.248 grams of pure acid was added to each of the two units and the gas rate increased to 825 ml. in Unit E and to 975 ml. in Unit F on the forty-ninth day. The volatile acids concentration in both units was about 2000 p.p.m. on the forty-fourth day. Fermentation never reached a high rate though the same amount of acid was added up to the forty-ninth day. Again it was suspected that the stirring mechanism was not sufficient for two liters of sludge and, therefore, one liter of sludge was withdrawn on the fiftieth day. This improved the fermentation rate considerably. Up to the fifty-ninth day 1.248 grams of pure acid were added daily in Unit F. In Unit E feeding was interrupted for three days because gas production lagged somewhat behind expectation. From the sixieth day to the sixty-fourth day the feed rate was increased to 1.664 grams for both units, which was approximately the maximum dose established in the first set of experiments. This feed rate corresponded to a theoretical gas production of 1852 ml. per day. The actual average gas rate was 1736 ml. for Unit E and 1692 ml. for Unit F. These gas rates proved the complete restoration of the activity in the units. Therefore, the potassium butyrate experiment was started on the sixty-fifth day in Unit E and on the sixty-sixth day in Unit F.

The daily portion of potassium butyrate contained 1.4 grams of potassium and 3.248 grams of acid, representing twice the previously demonstrated maximum loading. This feed rate was continued until the seventy-fifth day. To avoid an excessive accumulation of potassium, pure acid (6.24 grams, about four times the maximum) was fed to Unit E on the seventy-sixth day only. Unit F received 4.16 grams of pure acid on the seventy-sixth and seventy-seventh day. One further feeding of potassium butyrate (5.969 grams acid) was made to Unit E on the eighty-fourth day and the same feeding was made to Unit F on the seventy-eighth and eighty-fourth days.

This excessive loading caused volatile acids to rise to 9750 p.p.m. in Unit E and 8550 p.p.m. in Unit F on the seventy-sixth day. By the eighty-seventh day this concentration had reached 13300 p.p.m. in Unit E and 14200 p.p.m. in Unit F. The pH remained between 7.5 and 7.8. Contrary to the previous experiment, the gas rate did not decrease as soon as the excessive feeding had begun. Gas production increased instead and showed two distinctive peaks at 3020 ml. and 2690 ml. per day in Unit E and 3000 ml.and 3340 ml. per day in Unit F. These peaks occurred while the volatile acids concentration ranged from 6000 to 10,000 p.p.m. From the seventy-seventh day the gas rate decreased sharply in both units and could not be restored even by an additional feeding. On the eighty-seventh day Unit E produced 600 ml. and Unit F 350 ml. of gas and the experiment was terminated.

From the two curves in Figure 10 and 11, it can be stated that 1750 ml. to 1800 ml. per day was the maximum gas rate and that 1.6 grams of acid could be fermented per day. This was in close agreement with the 1735 ml. obtained in the first experiment. As far as the critical acids concentration was concerned, the gas production started to decrease at the following levels: 3000 to 4000 p.p.m. for pure acid feeding, 4000 to 5000 p.p.m. for the amonium compound, and 6000 to 10,000 p.p.m. for the potassium compound. In the case of the acid compounds, the pH could not be made responsible for this effect. It appears, therefore, that an actual critical acids concentration level existed beyond which the gas production was decidedly depressed. The high degree of acid tolerance together with the temporary peak in gas production in the case of the potassium compound demonstrated again a beneficial effect of the potassium ion, similar to the previous experiments with the acetate compound.

(4) The Control Unit. [Figure 12 and Table 16]-- The last unit in the second set of experiments was a control unit. The gas rate on the first day was 360 ml. per day and decreased gradually to 10 ml. on the twelfth day. The gas formation stopped on the twenty-first day. The amount of gas produced by the sludge was low and almost all of the gas was produced during the first twenty days. During this period the average rate of gas production was 60 ml. per day. As this rate was low when compared with the gas rates of the organic acids used, it has not been taken into account in the fermentation experiments.

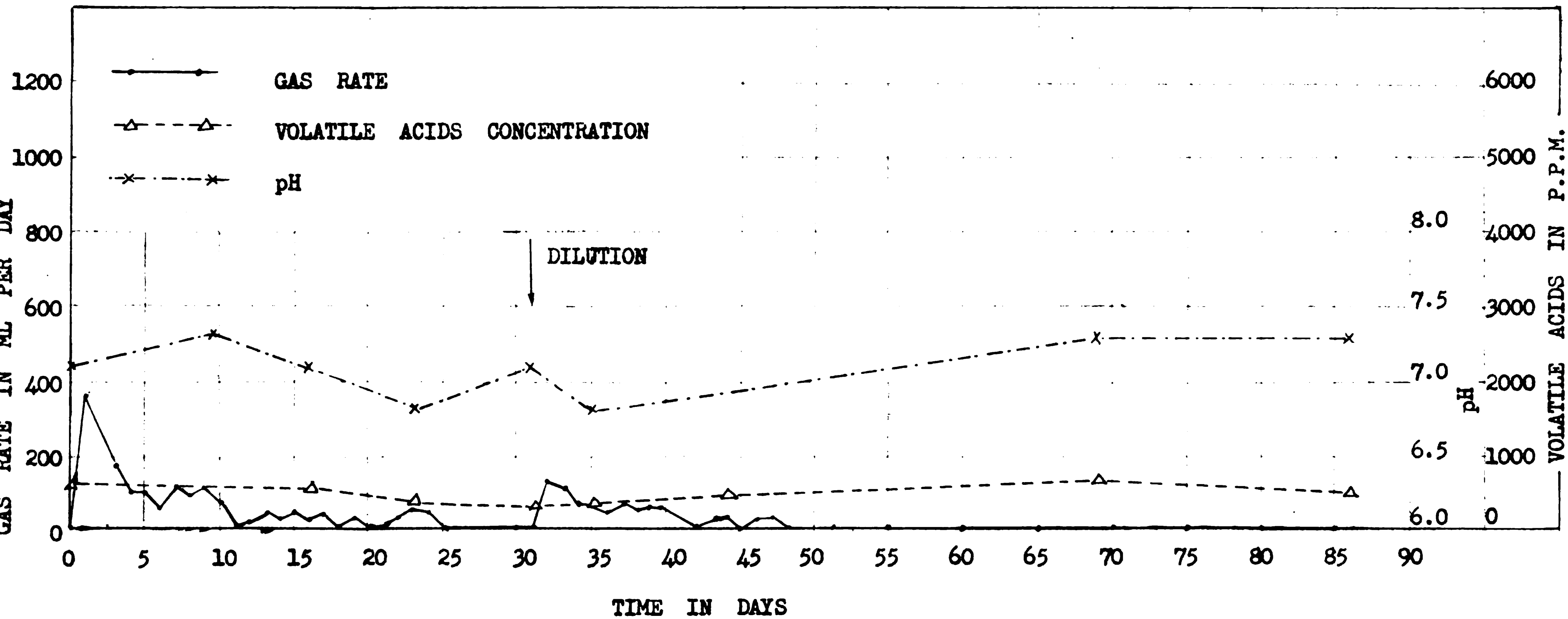

FIGURE- 12. GAS RATES, VOLATILE ACIDS CONCENTRATION AND pH IN THE CONTROL UNIT.

When another liter of sludge was added for dilution purposes as in other experiments, the gas rate rose to 135 ml.per day on the thirty-second day and gradually decreased to 10 ml. per day on the forty-seventh day. Volatile acids concentration remained low through the experiment being 610 p.p.m. on the first day, 342 p.p.m. on the twenty-third day, and 321 p.p.m. on the thirty-fifth day. The pH remained between 7.1 and 7.3.

SECTION VI

DISCUSSION

According to Buswell and co-workers [6] the volatile acids concentration in a digester should not be allowed to increase beyond 2000 to 3000 p.p.m. in order to prevent a drop in pH and a marked decrease in gas production.

In the first set of experiments, using pure acetic, propionic, and butyric acids as feed, it was found that this is true in all the cases (Figures 2, 3, and 4). The gas rate started to decrease whenever the volatile acids concentration level increased beyond 3000 p.p.m. Since this decrease in gas production could be due to the drop in pH as well as to the acids concentration, ammonium and potassium compounds were used in the second set of experiments. Thus it was possible to study the effects of increasing volatile acids concentration without moving the pH into the acid range. The results given in Figures 6, 7, 8, 9, 10, and 11 show that independently of the pH the gas rates also decreased when the volatile acids concentration increased beyond certain critical limits.

With the ammonium compounds, the gas rates started to drop at the following volatile acids concentration:

2000 to 2500 p.p.m.	(acetic)
2000 to 2800 p.p.m.	(propionic)
4000 to 5000 p.p.m.	(butyric)

With the exception of butyrate these are the same levels found with pure acids. Thus it can be concluded that the volatile acids concentration as such, independent of pH, constitutes a limiting factor in methane fermentation.

For the potassium compounds, the corresponding critical levels could not readily be established from the curves, due to the erratic behavior of the gas production rates and the steep rise in volatile acids concentration. With potassium propionate the gas rate decreased as soon as the acids concentration went beyong 3000 p.p.m. The same was true for potassium acetate, but there occurred a sharp peak in gas production at approximately 7000 p.p.m. In the case of potassium butyrate the gas rate did not decrease but showed a considerable increase beyond the previously established maximum up to a volatile acids concentration of 8000 to 10,000 p.p.m. These excessive gas rates cannot be explained under our present knowledge. It seems possible that the potassium ion has a beneficial effect on methane fermentation.

In several instances a complete stop in gas production was experienced when the acids concentration level was increased beyond the critical limit. In the first set of experiments with pure acid feeding, the gas rate decreased to zero at 4000 p.p.m. volatile acids concentration for propionic acid and at 5000 p.p.m. for butyric acid. With acetic acid 6000 p.p.m. were not quite sufficient to stop gas production completely. When compounds were used for feeding, higher acid concentrations were necessary to halt methane production. With potassium propionate gas production ceased at 6000 p.p.m. and with potassium acetate at 7000 p.p.m., the limit for potassium butyrate appeared to be beyond 14,000 p.p.m.

For the purpose of comparing the rates of gas production in the two sets of experiments, the mean average and the maximum rates of gas production were computed when using pure acids as shown in Tables 3, 4, and 5. The average maximum rate of gas production for acetic acid was 797 ml. per day equal to 0.983 grams of acid.

TABLE 3. Acetic acid fermentation showing the mean average and the maximum gas rates

Unit No.	Table No.	Days From and To	Number of Days	Acid Added in Grams	Theoretical Gas Rate	Mean Average
-	6	3 to 30	28	18.257	536	515
-	6	10 to 17	8	9.264	939	650
A	10	1 to 20	20	15.799	642	625
A	10	50 to 65	16	11.529	585	540
A	10	61 to 64	4	4.792	971	800*
B	11	1 to 20	20	11.88	483	445
B	11	50 to 65	16	14.626	740	749
B	11	61 to 64	4	4.792	971	793*

*Maximum figures taken for average.

With propionic acid, the average maximum rate of gas production was about 200 ml. per day, equal to 0.2 grams of acid.

TABLE 4. Propionic acid fermentation showing the mean average and the maximum gas rates

Unit No.	Table No.	Days From and To	Number of Days	Acid Added in Grams	Theoretical Gas Rate	Mean Average
-	7	10 to 13	4	0.96	240	213*
C	12	1 to 20	20	3.796	199	208*
C	12	50 to 65	16	2.198	176	158
C	12	10 to 16	7	1.463	206	186
D	13	1 to 20	20	3.656	182	191*
D	13	50 to 65	16	3.136	193	189
D	13	5 to 10	6	1.289	212	175

*Maximum figures taken for average.

In the case of butyric acid, the average maximum rate of gas production was 1680 ml. per day, equal to 1.51 grams of acid.

TABLE 5. Butyric acid fermentation showing the mean average and the maximum gas rates

Unit No.	Table No.	Days from and To	Number of Days	Acid Added in Grams	Theoretical Gas Rate	Mean Average
-	8	3 to 31	29	27.676	1064	995
-	8	24 to 30	7	11.396	1812	1613*
E	14	1 to 20	20	24.871	1382	1319
E	14	50 to 64	15	17.056	1265	1258
E	14	60 to 64	5	8,320	1852	1736*
F	15	1 to 20	20	17.974	1020	802
F	15	50 to 65	16	20.384	1420	1470
F	15	61 to 64	4	6.656	1852	1692*

*Maximum figures taken for average.

If these maximum gas rates are compared they show a ratio of 200 : 797 : 1680 which is very close to 1 : 4 : 8 as propionic : acetic : butyric. The ratio of fermentation of the acids per one liter of active digester capacity is about 0.2 grams : 0.983 grams : 1.51 grams or close to 1 : 5 : 8 as propionic : acetic ; butyric acid. Thus it appears that propionic acid is converted at the slowest rate. A given volume of digester is able to ferment five times more acetic and eight times more butyric than propionic acid per day. The maximum rate of fermentation for acetic acid was equal to 0.983 grams per liter active digester volume per day. The figure given by Buswell and coworkers [3] for this acid was 2.54 grams but they used 3.5 liters active digester volume. If this is taken into account, a value of 0.736 grams of acetic acid per liter of active digester volume per day results. This is in close agreement with the figures given above. Buswell and coworkers [3] also noted that butyric acid was more readily fermented than acetic acid. When a mixture of acetic and butyric acid was fed to their units, the butyric acid disappeared more rapidly than the acetic acid.

An explanation of the marked difference in fermentation rates as expressed by the ratio of 1 : 5 : 8 for propionic : acetic : butyric acid cannot be given here. Our knowledge of the enzymatic reactions involved in the fermentation of methane is now very limited. From what is known in general biochemistry the conversion of propionic acid, a three-carbon molecule, is more complicated than that of two-carbon acetic or four-carbon butyric acid.

SECTION VII

CONCLUSION

The following conclusions resulted from the work described in the thesis.

1. The maximum rates of fermentation were 0.2 grams, 0.983 grams and 1.51 grams per liter of active digester volume per day in the case of propionic, acetic, and butyric acids respectively. The corresponding average maximum rates of gas production at these rates of acid feeding were 200, 797, and 1680 ml. per day. When the acid feed was increased beyond the amount stated above, volatile acids concentration increased in the fermentation vessel and at an acid concentration of about 3000 p.p.m. the gas rate started to decrease. This was true for all three acids used. At the same volatile acids level the pH showed a sudden drop below 7, indicating that the buffering capacity of the sludge was overtaxed.

2. When ammonium compounds were used, no drop in pH occurred and the critical volatile acids concentrations were practically the same as for pure acids with the exception of ammonium butyrate which was markedly higher. Independently of the pH, the volatile acids, as such, depressed gas production at an acids concentration of about 3000 p.p.m.

The potassium compounds produced the same results as far as potassium propionate was concerned. With potassium acetate and especially with potassium butyrate excessive gas production rates were experienced temporarily at volatile acids concentration levels above 3000 p.p.m. It was assumed that the potassium ion had a beneficial effect on methane fermentation.

3. The rates of fermentation measured as grams of acid fermented per liter of active digester volume per day were in the ratio of 1 : 5 : 8 and the maximum rates of gas production per day were in the ratio of 1: 4 : 8 as propionic : acetic : butyric acid.

BIBLIOGRAPHY

1. Barker, H. A. Archiv Mikrobiology 7, 404, (1936).

2. Barker,H. A. Biological fermentation of methane, Industrial Engineering Chemistry, 48, 1438, September 1956.

3. Buswell, A. M., and Hatfield. Illinois State Water Survey Bulletin No. 32 (1936).

4. Buswell, A. M., and Nave, S. L. Illinois State Water Survey Bulletin No. 30 (1930).

5. Buswell, A. M.. Microbiology and Theory of Anaerobic Digestion, Sewage Works Journal, 19, 28, (1947).

6. Buswell, A. M., and Mueller, H. F. Mechanism of Methane Fermentation, Industrial Engineering Chemistry, 44, 550, March 1952.

7. Buswell, A. M. Production of fuel gas by Anaerobic Fermentations, Industrial Engineering Chemistry, 22, 1168, (1930).

8. Gorden,E. Oliver. Anaerobic Digestion of Pea-Blancher Waste, Thesis, The State College of Washington (1954).

9. Gorden, E. Oliver and Dustan, Gilbert H. Anaerobic Digestion of Pea-Blancher Waste, Sewage and Industrial Wastes, 27, 1171, (1955).

10. Fair, Gorden M. and Geyer, John C. Water Supply and Waste Disposal, John Willy and Sons, Inc., (1954).

11. Heukelekian H. Thermophilic digestion of daily charges of fresh solids and activated sludge, Sewage Works Journal, 3, 3, (1931).

12. Heukelekian, H. Volatile acids in Digesting Sewage Sludge, Industrial Engineering Chemistry, 20, 752, (1928).

13. Kaplovsky, Joel. Volatile acids production during the digestion of Seeded, Unseeded and Limed fresh solids, Sewage and Industrial Wastes, 23, 713, (1951).

14. Nave, S. L., and Buswell, A. M. Alkaline Digestion of Sewage Grease, Industrial Engineering Chemistry, 20, 1368, (1928).

15. Nave, S. L., and Buswell, A. M. Anaerobic Oxidation of Fatty Acids, Journal American Chemical Society, 52, 3308, (1930).

16. Proff, Omelianski, and Groenewage. Illinois State Water Survey Bulletin No. 32, (1936).

17. Snell, J. R. Correction of errors during the measurement and analysis of the gas, Sewage Works Journal, 14, 1304, (1942).

18. Schlenze, H. E. Important Consideration in Sludge Digestion, Practical Aspects, Sewage Works Journal, 19, 19, (1947).

19. Sohngen, N. L. Recveil des travanx Chimiques, 29, 238, (1910).

20. Standard Methods for the Examination of Water and Sewage, tenth Edition (1955).

21. Steel. Water Supply and Sewage. McGraw Hill Book Company (1953).

22. Stuart, E. Coburn. Practical Application of Hydrogen ion Control in the Digestion of Sewage Sludge, Industrial Engineering Chemistry, 19, 235, (1927).

23. Thyer, A. L. Bacterial Gnesis of Hydro carbons from Fatty Acids, Bulletin American Association of Petroleum Geology, 15, 441, (1931).

24. Thysen, A. C. and Bunker, H. J. Microbiology of Cellulose, Hemicellulose, Peptin and Gums, Oxford University Press, London (1927).

APPENDICES

Table 6. Gas rates, Volatile Acids concentration and pH in Acetic Acid fermentation

Days	Temp. of Unit	Gas Rate Per Day	Acid Added in Grams	Volatile acids in p.p.m.	pH	Remarks
0	32°C	0	-	420	7.4	
1		386	-			
2		276	-			
3		240	0.579			
4		534	0.579			
5		557	0.579			
6		636	0.579			
7		620	0.579			
8		490	0.579	313	7.3	
9		520	0.579			
10		490	1.158			
11		730	1.158			
12		760	1.158			
13		760	-			
14		560	1.158			
15		750	1.158	1289	7.3	
16		650	1.158			
17		500	1.158			
18		480	0.579			
19		550	-			
20		50*	1.158			
21		710	0.579			
22		590	0.579	606	7.3	
23		470	0.579			
24		470	0.868			
25		540	0.868			
26		560	-			
27		355	-			
28		215	0.579			
29		305	0.579	1297	7.3	
30		330	-			
31		300	3.474			
32		1100	-	1770	6.7	
33		300	-			
34		150	3.474			
35		650	-	6320	6.5	
36		160	-			
37		70	-			
38		180	-			
39		168	-			
40		137	-			
41		100	-			
42		185	-			
43		185	-			

*Leak developed

Table 6--Continued

Days	Temp. of Unit	Gas Rate Per Day	Acid Added in Grams	Volatile Acids in p.p.m.	pH	Remarks
44	-	185	-	-	-	-
45		185	-			
46		185	-			
47		137	-			
48		137	-			
49	32°C	270	-			
50		207	-			
51		207	-			
52		207	-	5020	6.8	Experiment Closed

Table 7. Gas rates, Volatile Acids concentration and pH in Propionic Acid fermentation

Days	Temp. of Unit	Gas Rate Per Day in ml.	Acid Added in grams	Volatile Acids in p.p.m.	pH	Remarks
0	32°C	0	-	420	7.4	
1		397	-			
2		222				
3		180	0.32			
4		235	-			
5		135	0.32			
6		334	0.32			
7		361	0.32			
8		236	0.32	1584	7.4	
9		245	-			
10		212	0.32			
11		310	0.32			
12		220	-			
13		110	0.32			
14		260	0.32			
15		190	-	2120	7.2	
16		70*	-			
17		77*	0.32			
18		251	0.32			
19		190	-			
20		110	-			
21		190	0.32			
22		210	0.32	3050	7.1	
23		120	-			
24		100	-			
25		10*	0.16			
26		80*	-			
27		130	0.16	2850	7.3	
28		80	0.16			
29		90	0.16			
30		60	-			
31		60	0.16			
32		80	0.16			
33		100	0.16			
34		40	3.20			
35		250	-	4120	6.5	
36		0	-			
37		0	-			
38		0	-			
39		0	-			
40		0	-			
41		0	-			
42		0	-			

*Leak developed

Table 7--Continued

Days	Temp. of Unit	Gas Rate Per Day in ml.	Acid Added in grams	Volatile Acids in p.p.m.	pH	Remarks
43		0	-			
44		0	-			
45		0	-			
46		0	-			
47		0	-			
48		0	-			
49		0	-			
50		0	-			
51		0	-			
52		0	-	4020	6.8	Experiment Closed

Table 8. Gas rate, Volatile Acids concentration and pH in Butyric Acid fermentation

Days	Temp. of Unit	Rate of Gas per day in mol.	Acid Added in grams	Volatile Acids in p.p.m.	pH	Remarks
0	32°C	0	-	420	7.4	
1		350	-			
2		227	-			
3		190	0.407			
4		640	0.407			
5		634	0.407			
6		564	0.407			
7		667	0.407			
8		490	0.407	970	7.4	
9		512	0.407			
10		517	0.814			
11		770	0.814			
12		1030	0.814			
13		1050	0.814			
14		1085	0.814			
15		400*	-	550	7.3	
16		10*	-			
17		29*	0.814			
18		937	1.221			
19		1140	1.221			
20		1320	1.221			
21		1410	1.221			
22		1470	1.221	1138	7.3	
23		1360	1.221			
24		1520	1.628			
25		1610	1.628			
26		1735	1.628			
27		1730	1.628			
28		1690	1.628			
29		1360**	1.628	1320	7.3	
30		1640	1.628			
31		1490	1.221			
32		780	3.256			
33		1540	-	4250	6.4	
34		900	3.256			
35		320	-	5770	5.9	
36		0	-			
37		0	-			
38		0	-			
39		0	-			
40		0	-			
41		0	-			
42		322	-			
43		322	-			

*Leak developed **Stirring stopped

Table 8--Continued

Days	Temp. of Unit	Rate of Gas per Day in ml.	Acid Added in grams	Volatile Acids in p.p.m.	pH	Remarks
44		322	-			
45		322	-			
46		322	-			
47		322	-			
48		322	-			
49		230	-			
50		230	-			
51		230	-			
52		53	-	3940	6.8	Experiment Closed

Table 9. Gas rate, Volatile Acids concentration and pH in the control unit

Days	Temp. of Unit	Rate of Gas per Day in ml.	Volatile Acids in p.p.m.	pH	Remarks
0	32°C	-	420	7.4	
1		438			
2		222			
3		210			
4		107			
5		134			
6		104			
7		164			
8		218	300	7.3	
9		85			
10		28			
11		90			
12		20			
13		30			
14		90			
15		80	314	7.4	
16		0			
17		20			
19		10			
20		20			
21		5			
22		10			
23		10			
24		10			
25		10			
26		10			
27		10			
28		10			
29		10	266	7.4	
30		10			
31		10			
32		10			
33		10			
34		10			
35		0			
36		0			
37		0			
38		0			
39		0			
40		0			
41		0			
42		0			
43		0			

Table 9--Continued

Days	Temp. of Unit	Rate of Gas Per Day in ml.	Volatile Acids in p.p.m.	pH	Remarks
44		0			
45		0			
46		0			
47		0			
48		0			
49		0			
50		0			
51		0			
52		0	260	7.1	Experiment Closed

Table 10. Acetic Acid fermentation showing gas rate, Volatile Acids concentration and pH (Unit A)

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
0	32°C	0	-	-	-	601	7.1	
1		330	1.132					
2		630	0.566					
3		560	0.849					
4		880	0.849					
5		750	0.566					
6		490	0.929					
7		780	0.849					
8		50*	-					
9		110*	0.929			390	7.1	
10		780	0.849					
11		680	0.849					
12		700	-					
13		760	0.929					
14		730	0.929					
15		750	0.929					
16		800	0.929			392	7.2	
17		650	0.929					
18		620	0.929					
19		608	0.929					
20		800	0.929					
21		640	1.701	0.498				
22		420	-	-				
23		300	1.701	0.498		2720	7.5	
24		250	1.701	0.498				
25		110	-	-				
26		110	1.701	0.498				

*Leak developed

Table 10--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Amonia	Potassium			
27		190	-					
28		55	-					
29		55						
30		60						
31		60						
32		125				3180	7.6	One liter of sludge added
33		150						
34		115						
35		140						
36		133				1540	7.5	
37		85						
38		80	1.490	-				
39		300	-					
40		70*	-					
41		35**	1.198					
42		235	-					
43		40**	-					
44		70**	0.596			2440	7.5	
45		135	0.596					
46		165	-					
47		105	-					
48		175	0.894					
49		245	-					
50	32 C	68*	0.497					One liter of sludge removed

**Stirring stopped

Table 10--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
51		266	0.894					
52		380	-					
53		140				2560	7.5	
54		150	0.894					
55		680	0.894					
56		690	0.894					
57		650	0.894					
58		670	0.894					
59		650	-					
60		329	0.894					
61		760	1.198					
62		830	1.198					
63		820	1.198					
64		790	1.198					
65		790	-					
66		630	1.894	-	1.212	2220	7.5	
67		550	1.894		1.212			
68		570	1.894		1.212			
69		550	1.894		1.212	6800	7.7	
70		430	-	-	-			
71		500	1.788	-	-			
72		970	1.894	-	1.212	7200	7.5	
73		570	-	-	-			
74		360						
75		370						
76		370					7.8	
77		260						
78		240						
79		190						

Table 10--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
80		190	3.160	-	2.02		7.8	
81		30						
82		40						
83		30						
84		10						
85		60						
86		0				6950	7.8	Experiment Closed

Table 11. Acetic Acid fermentation showing gas rate, Volatile Acids concentration and pH (Unit B)

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
0	32 °C	0	-	-		610	7.1	
1		380	1.132					
2		660	0.566					
3		530	0.566					
4		460	0.566					
5		430	0.566					
6		470	0.929					
7		690	0.929					
8		660	-					
9		0*	0.929			728	7.3	
10		640	-					
11		120*	0.566					
12		330*	0.929					
13		670	0.929					
14		720	0.566					
15		140*	0.000					
16		160*	0.283			2320		
17		220*	0.566					
18		180*	0.929					
19		770	0.929					
20		650	-					
21		590	1.701	0.498				
22		150	-	-				
23		120	1.701	0.498		2700	7.8	
24		150	1.701	0.498				
25		50	-	-				

*Leak developed

Table 11--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams: Acid	Substances Added in Grams: Ammonia	Substances Added in Grams: Potassium	Volatile Acids in p.p.m.	pH	Remarks
26		50	1.701	0.498				
27		110	-	-				
28		110						
29		110						
30		65						
31		65	-	-		4470	7.6	One liter of sludge added
32		110						
33		125						
34		100						
35		130						
36		120						
37		75						
38		115	1.490					
39		365						
40		165				2020	7.5	
41		120	1.198					
42		318						
43		200						
44		230	0.894			1890	7.5	
45		360	1.490					
46		440						
47		280	1.198					
48		465	1.788					
49		575	1.490					
50	32°C	550	0.894					One liter of sludge removed

Table 11--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams: Acid	Ammonia	Potassium	Volatile Acids in p.p.m.	pH	Remarks
51		700	0.894					
52		690	0.894					
53		710	0.894			2010	7.5	
54		720	0.894					
55		720	0.894					
56		740	0.894					
57		730	0.894					
58		800	0.894					
59		790	0.894					
60		690	0.894					
61		780	1.198					
62		790	1.198					
63		850	1.198					
64		750	1.198					
65		800	-					
66		390	1.894		1.212	2300	7.4	
67		348	1.894		1.212			
68		410	1.894		1.212			
69		450	1.894		1.212	7400	7.6	
70		480	-	-	-			
71		510	1.788					
72		1210	1.894		1.212	6800	7.6	
73		480	-	-				
74		370						
75		390						
76		369						
77		240						
78		250						
79		190						

Table 11--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
80		150	3.16		2.02			
81		100						
82		70						
83		60						
84		50						
85		60						
86		90				7600	7.8	Experiment Closed

Table 12. Propionic Acid fermentation showing gas rate, Volatile Acids concentration and pH (Unit C)

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
0	32°C	-	-			610	7.1	
1		390	0.174					
2		300	0.174					
3		270	0.174					
4		240	0.174					
5		210	0.209					
6		170	0.209					
7		180	0.209					
8		160	0.209					
9		220	0.244			642	7.4	
10		200	0.209					
11		190	0.209					
12		160	0.209					
13		180	0.209					
14		200	0.209					
15		230	0.209					
16		150	0.209			1690	6.8	
17		240	0.174					
18		110	0.174					
19		168	0.209					
20		200	-					
21		150	0.342	0.078				
22		130	0.342	0.078				
23		150	0.342	0.078		2860	7.6	
24		130	0.342	0.078				
25		70	-	-				
26		30	0.342	0.078				
27		170						

Table 12--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
28		100						
29		100						
30		105						
31		105				2520	7.6	One liter sludge added
32		165						
33		185						
34		195						
35		125						
36		115				670	7.6	
37		90	0.348					
38		140	0.348					
39		140	-					
40		100	0.348					
41		150	0.348					
42		105	-					
43		85	0.348					
44		110	0.348			470	7.5	
45		125	0.348					
46		115	-					
47		120	0.348					
48		140	0.348					
49		140	-					
50		60*	0.209					One liter of sludge removed
51		250	0.174					

* Leak developed

Table 12--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
52		140	-					
53		170	0.174			1010	7.3	
54		180	0.174					
55		170	0.174					
56		170	0.174					
57		180	0.174					
58		170	0.174					
59		160	0.174					
60		160	0.174					
61		40*	0.348					
62		200	0.348					
63		230	0.174					
64		120	0.174					
65		130	-					
66		90	0.776	-	0.4	2240	7.3	
67		190	0.776	-	0.4			
68		90	0.776	-	0.4			
69		130	0.776	-	0.4	4430	7.6	
70		80	-					
71		100	0.348	-				
72		50	0.194	-	0.1	6100	7.6	
73		30						
74		0						
75		60						
76		100						
77		100						
78		110	0.194	-	0.1			
79		50						
80		0						

Table 12--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
81		0						
82		0						
83		0						
84		0						
85		0						
86		30				5700	7.6	Experiment Closed

Table 13. Propionic Acid fermentation showing gas rate, Volatile Acids concentration and pH (Unit D)

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
0	32°C	-	-			610	7.1	
1		470	0.174					
2		270	0.174					
3		280	0.174					
4		290	0.174					
5		300	0.209					
6		183	0.209					
7		200	0.209					
8		170	0.209					
9		190	0.244			567	7.3	
10		180	0.209					
11		130	0.174					
12		140	0.174					
13		150	0.209					
14		130	0.174					
15		170	0.209					
16		100	0.209			2043	6.9	
17		130	0.174					
18		130	0.174					
19		105	0.174					
20		148	-					
21		80	0.342	0.078				
22		70	0.342	0.078				
23		60	0.342	0.078		2640	7.7	
24		60	0.342	0.078				
25		40	-	-				
26		40	0.342	0.078				

Table 13--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
27		120						
28		60						
29		60						
30		25						
31		25				4350		One liter of sludge added
32		150						
33		155						
34		100						
35		115				1900	7.5	
36		40						
37		95	0.348					
38		165	0.348					
39		135	-					
40		120	0.348					
41		90	0.348					
42		94	-					
43		90	0.348					
44		105	0.348			2520	7.5	
45		135	0.348					
46		130	-					
47		80	0.348					
48		135	0.348					
49		120	-					
50		65	0.348					One liter of sludge removed

Table 13--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
51		210	0.174					
52		200	0.174					
53		230	0.174			2100	7.3	
54		190	0.174					
55		180	0.174					
56		210	0.174					
57		170	0.174					
58		190	0.174					
59		180	0.174					
60		170	0.174					
61		170	0.348					
62		220	0.348					
63		220	0.174					
64		230	0.178					
65		190	-					
66		200	0.776	-	0.4	1990	7.2	
67		380	0.776	-	0.4			
68		290	0.776	-	0.4			
69		190	-	-	-	4900	7.6	
70		100	-	-	-			
71		60	0.348					
72		200	0.194		0.1	5400	7.5	
73		100	-					
74		130						
75		120						
76		100						
77		100						
78		190	0.696					
79		80						
80		50						

Table 13--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
81		60						
82		50						
83		70						
84		50						
85		30						
86		50				5040	7.6	Experiment Closed

Table 14. Butyric Acid fermentation showing gas rate, Volatile Acids concentration and pH (Unit E)

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
0	32°C	-	-			610	7.1	
1		567	1.254					
2		900	1.045					
3		1490	0.836					
4		930	1.254					
5		1260	0.836					
6		865	1.254					
7		1320	1.254					
8		1420	1.463					
9		1600	1.463			470	7.4	
10		1540	1.254					
11		1470	1.254					
12		1390	1.254					
13		1490	1.254					
14		1450	1.254					
15		1450	1.463					
16		1420	1.463			398	7.2	
17		1510	1.254					
18		1380	1.254					
19		1424	1.254					
20		1508	1.254					
21		1420	2.512	0.504				
22		1420	2.512	0.504				
23		1430	2.512	0.504		3830	7.4	
24		1160	2.512	0.504				
25		870	2.512	0.504				
26		800	2.512	0.504				
27		780						

Table 14--Continued

Days	Temp. of Unit	Gas Rate Per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
28		585						
29		585						
30		540						
31		540				7250	7.6	One liter of sludge added
32		520						
33		510						
34		435						
35		420						
36		375				3320	7.6	
37		285						
38		290						
39		165						
40		200						
41		115	1.248					
42		385	-					
43		280	-					
44		285	1.248			1980	7.5	
45		455	1.248					
46		500	1.248					
47		570	-					
48		430	1.248					
49		825	1.248					
50		550	1.248					One liter of sludge removed
51		1050	-					
52		470	1.248					
53		990	-			2200	7.4	

Table 14--Continued

Days	Temp. of Unit	Gas Rate Per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
54		970	-					
55		700	1.248					
56		1220	1.248					
57		1400	1.248					
58		1410	1.248					
59		1420	1.248					
60		1550	1.664					
61		1840	1.664					
62		1770	1.664					
63		1760	1.664					
64		1760	1.664					
65		1700	3.248		1.4			
66		1010	3.248	-	1.4	780	7.6	
67		1350	3.248	-	1.4			
68		1710	3.248	-	1.4			
69		2030	3.248	-	1.4	5920	7.6	
70		2130	3.248	-	1.4			
71		3020	3.248	-	1.4			
72		2750	3.248	-	1.4	8500	7.5	
73		980	3.248	-	1.4			
74		1640	-	-	-			
75		2110	4.782	-	2.1			
76		2020	6.240	-	-	9750	7.7	
77		2690	-					
78		530						
79		460						
80		450						
81		400						
82		450						

Table 14--Continued

Days	Temp. of Unit	Gas Rate Per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
83		520						
84		870	5.960	2.1				
85		1380						
86		700						
87		600				13300	7.7	Experiment Closed

Table 15. Butyric Acid fermentation showing gas rate, Volatile Acids concentration and pH (Unit F)

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
0	32°C	-	-			610	7.1	
1		415	1.254					
2		950	0.836					
3		1000	0.836					
4		940	0.836					
5		910	1.254					
6		1240	1.254					
7		1200	1.254					
8		1090	-					
9		720	1.254			1962	7.2	
10		990	1.254					
11		890	0.418					
12		690	1.254					
13		800	0.836					
14		600	1.254					
15		430*	-					
16		0*	1.254			2300		
17		1280	0.836					
18		670	-					
19		0*	1.254					
20		1200	0.836					
21		980	2.512	0.504				
22		310	-	-				
23		160	2.512	0.504		6150	7.6	
24		310	2.512	0.504				
25		130	-	-				

*Leak developed.

Table 15--Continued

Days	Temp. of Unit .	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
26		180	2.512	0.504				
27		230						
28		345						
29		345						
30		275						
31		275						
32		160				8350	7.6	One liter of sludge added
33		0*						
34		220						
35		730				2770	7.5	
36		695						
37		680						
38		710						
39		605						
40		460						
41		265	1.248					
42		495	-					
43		460	-					
44		430	1.248			2320	7.6	
45		565	1.248					
46		915	1.248					
47		1040	1.248					
48		970	1.248					
49		975	1.248					
50		1005	1.248					One liter of sludge removed

Table 15--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
51		1400	1.248					
52		1460	1.248					
53		1350	1.248			2450	7.4	
54		1400	1.248					
55		1390	1.248					
56		1440	1.248					
57		1460	1.248					
58		1410	1.248					
59		1440	1.248					
60		1420	1.248					
61		1420	1.664					
62		1710	1.664					
63		1840	1.664					
64		1800	1.664					
65		1590	-					
66		620	3.248	-	1.40	420	7.5	
67		1520	3.248	-	1.40			
68		1590	3.248	-	1.40			
69		2080	3.248	-	1.40	5150	7.7	
70		2220	3.248	-	1.40			
71		2640	3.228	-	1.40			
72		3000	3.248	-	1.40	7250	7.5	
73		2490	3.248	-	1.40			
74		1610	-					
75		2050	4.872	-	2.10			
76		2280	4.160	-	-	8550	7.8	
77		3340	4.160					
78		3300	5.960	-	2.10			
79		3130						

Table 15--Continued

Days	Temp. of Unit	Gas Rate per Day in ml.	Substances Added in Grams			Volatile Acids in p.p.m.	pH	Remarks
			Acid	Ammonia	Potassium			
80		3070					7.8	
81		2990						
82		2660						
83		2600						
84		2000	5.960	-	2.10	11700		
85		1230						
86		480						
87		350				14200	7.8	Experiment closed

Table 16. Gas rate, Volatile Acids concentration and pH in the control unit

Days	Temp. of Unit	Gas Rate Per Day in ml.	Violate Acids in p.p.m.	pH	Remarks
0	32°C	-	610	7.1	
1		360			
2		0*			
3		175			
4		100			
5		100			
6		58			
7		120			
8		70			
9		110	580	7.3	
10		60			
11		0			
12		10			
13		50			
14		30			
15		50			
16		30	543	7.1	
17		40			
18		0			
19		15			
20		0			
21		0			
22		30			
23		50	342	6.8	
24		40			
25		0			
26		0			
27		0			
28		0			
29		0			
30		0			
31		0	316	7.1	One liter of sludge added
32		135			
33		115			
34		75			
35		75	321	6.8	
36		45			
37		65			
38		55			
39		60			
40		60			

*Leak developed

Table 16--Continued

Days	Temp. of Unit	Gas Rate Per Day in mi.	Violate Acids in p.p.m.	pH	Remarks
41		25			
42		15			
43		35			
44		35	480		
45		0			
46		25			
47		30			
48		5			
49		5			
50	32°C	10			One liter of sludge removed
51		10			
52		20			
53		10			
54		10			
55		10			
56		0			
57		0			
58		0			
59		0			
60		0			
61		0			
62		0			
63		0			
64		0			
65		0			
66		0			
67		0			
68		0			
69		0	670	7.3	
70		0			
71		0			
72		0			
73		0			
74		0			
75		0			
76		0			
77		0			
78		0			
79		0			
80		0			
81		0			
82		0			
83		0			

Table 16--Continued

Days	Temp. of Unit	Gas Rate Per Day in ml.	Violate Acids in p.p.m.	pH	Remarks
84		0			
85		0			
86		0			
87		0	520	7.3	Experiment Closed

Date Due
