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TITLE FACTORS AFFECTING THE RATIOS O

GASES PRODUCED DURING THE

DIGESTION OF SLUDGE

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FACTORS AFFECTING THE RATIOS OF GASES PRODUCED DURING THE DIGESTION OF SLUDGE

by

William C. Alegnani

A DISSERTATION

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

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To my wife

PEGGY

who has inspired me in moments
of depression, this thesis is
affectionately dedicated

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INTRODUCTION

The utilization of sewage gas has proved to be an economical asset to many city sewage treatment plants. The gas is used for heating the plants in cold weather, for maintaining the temperature of the anaerobic digesters, and for the operation of gas engines which, in turn, run dynamos for the production of electricity. Hurley (24), in Great Britian, reported that, "during the war quite a lot of compressed sludge gas was used as motor fuel for cars and publicly owned vehicles. Some of it was used for making a particularly devastating type of fire bomb". This is probably the only recorded instance of sewage being used as a weapon of war. Wirts (45), in regard to commercial utilizati of waste digester gas, reported that "the cost of meeting the conditioning and purification requirements for augmenting the gas utility supply with waste digester gas would be of no gain under present economic conditions".

Although the production of sewage gas is not the primary function of anaerobic digestion, its economical value and usefulness cannot be overlooked. Thus, the factors which influence the production of sewage gas are becoming more and more important. In addition to this, the ratio of non-combustible to combustible components serves as an index of the progress or state of the digesting material. A noticeable shift in the ratios will often indicate undesirable conditions and improper digestion.

Purpose of study:

The purpose of this study was to determine the effect of several factors on the digestion of sludge. Particular attention was paid to the total gas production, ratios of carbon dioxide to methane produced, and the bacterial flora with respect to the coliform and gram positive micrococcus groups. Special attention was given to the synthetic detergents (cationics, anionics, and non-ionics). An attempt was made to correlate the relationship between gas production and specific groups of microorganisms. A study on the decomposition of some organic compounds and their effect on the microflora of digesting sludge was also carried out.

REVIEW OF LITERATURE

The primary function of anaerobic digestion is to convert the solid material obtained from the primary settling tanks into an innocuous, easily dried residue. The conversion of this material is dependent upon the presence and growth of numerous microorganisms. These microorganisms utilize the organic matter which is present, for growth, reproduction and energy. The gases, which are produced, result from the various stages of microbial metabolism. Gases are produced from the breakdown of carbohydrates, fats, and proteins. Different gases may be liberated from the utilizati of these various compounds. Some of the gases reported in the literature are: carbon dioxide, methane, hydrogen, oxygen hydrogen sulfide, nitrogen, nitrous oxide, nitric oxide, ammonia, and phosphine. Carbon dioxide and methane are the two gases found most abundantly in sewage gas.

Composition of sewage gas:

In a study on the composition of sludge digestion gas taken from several different cities, Haseltine (15) reported that the principal ingredients vary somewhat as follows: methane - 60 to 80 percent, carbon dioxide - 10 to 30 percent, and nitrogen - 1 to 15 percent. The gas usually contained detectable amounts and often measurable amounts of hydrogen sulfide. It may also contain small amounts of hydrogen or oxygen. Carbon monoxide and illuminants are seldom present. He also concluded that the daily variations in the composition of the gas are as great as are those in the quantity of gas. Roberts (30) also has presented data on the variation in sewage gas from digesters.

The presence of phosphine has been reported in sludge gas. It was claimed that it occurred during the bacterial reduction of organic and inorganic phosphorus compounds. Rudolfs and Stahl (34), however, carried out a series of experiments in which various organic and inorganic phosphorus compounds were added to digesting sludge. All of the compounds studied gave qualitative tests at various stages during the digestion, but none were confirmed by quantitative tests when the gas was oxidized with chlorine and the phosphorus determined as phosphomolybdate. They concluded that if phosphine was present it was in a concentration less than the quantitative test could detect.

Eliassen, Heller, and Kisch (12) presented a comprehensive review of the literature on the mechanisms of hydrogen sulfide production. According to the authors, the mechanisms of hydrogen sulfide production could be divided into two classes and are categorized by the source of sulfur for the biochemical reactions. The most prevalent reaction was the production of hydrogen sulfide by the reduction of sulfates. Hydrogen sulfide may also be produced by the decomposition of organic matter containing sulfur, such as proteins and their derivatives. Both Hotchkiss (23) and Heukelekian (16) reported on the numbers of sulfatesplitting organisms in sewage.

Snell (36) showed that organic nitrogen was not lost as a gas in anaerobic digestion, but that about 75 to 90 percent was broken down into ammonia nitrogen. He also reported that no nitrogen, nitrous oxide, or nitric oxide could be evolved unless nitrates or nitrites were present and then only in a quantity equivalent to the weight of the nitrates or nitrites present. Snell (35) also showed that the presence of nitrogen gas reported in sludge digestion was false and is accounted for by infiltration of disolved nitrogen gas, by leaks, and by errors in gas analysis. He also pointed out that since nitrates and nitrites constitute only a small percentage of the total nitrogen present in activated sludge and are entirely negligible in sludge produced by plain sedimentation of sewage, the above mentioned gases need not be considered important.

One of the first important pieces of work covering the existence of methane, the biological processes which produce methane, and the utilization or disappearance of methane by biological means was carried out by Sohngen (37). Since then numerous investigations on the mechanisms of methane fermentation have been made (1, 2, 3, 4, 7, 8, 9, 26, 37, 38, 39, 40, 41, 42). Walker (44) presented an excellent discussion on the principles of methane production in sludge digestion. He stated that in the establishing of normal digestion the raw sewage passes through three stages as follows: 1) the acid production or accumulating stage, 2) the slow acid digestion and regression stage, and 3) the alkaline or methane digestion stage. This third stage may be called the normal digestion stage. During this third, or methane stage of digestion, the methane bacteria more or less take control of the process and this stage continues until nearly all of the organic matter is broken down. This desirable third stage can be made to continue indefinitely in normal digesters providing certain conditions are maintained.

Heukelekian and Heinemann (18, 19, and 20) have also contributed to the study of methane bacteria in sludge. Their work was primarily concerned with the enumeration of methane-producing bacteria in digesting solids, the effect of adding enriched cultures of methane-producing bacteria in supernatant liquors.

Synthetic detergents in sewage:

For an extensive review of the literature concerning the synthetic detergents, the work by Glassman (13) should be consulted. Since the advent of synthetic detergents, the use of soaps in hard water regions has markedly decreased. Consequently, synthetic detergents have been held responsible for many interferences occurring in sewage treatment plants. A symposium on new problems in sewage treatment with special reference to synthetic detergents was presented in the Sewage Works Journal (14, 17, 21, 22, and 28). This was presented in five parts and showed some of the difficulties encountered from the use of these detergents.

In the treatment of sewage wastes, soaps have not presented a problem because they are readily precipitated by the mineral constituents (calcium, magnesium, and iron) normally found in the water. In this form they are utilized for food (and gas production) by the microorganisms present in the digesting tanks. The synthetic detergents, however, are not affected by these mineral salts. As was stated by Goldthrope and Nixon (14), "the surface active agents do not form permanent chemical combinations, but merely a loosely held physical association. The surface active agents have also an inhibitory influence on certain bacteria. They lower the surface tension and may affect the transfer of oxygen in aerobic processes. They affect the interfacial tension and will bring more substances to the interface of the bacterial cell and water."

Sperry (43) mentioned that the process of sludge digestion was not affected by synthetic detergents, but plant operating data seemed to indicate that the volume of gas to be expected was diminished.

Rudolfs (33) showed the principal effects of the syntheti detergents to be : 1) decreased efficiency of settling tanks; 2) foaming, floc agglomeration, and carry over in activated sludge units; 3) reduction in gas production. He also noted that with 100 ppm of Nacconol N.R., the peak of daily gas production was reached two days earlier than without the detergent.

Keefer (25) investigated the content of synthetic detergents in raw sewage and found that the removal of detergent by primary settling was about 14 percent. He concluded that the concentration of detergent was so small that it would have no deterimental effect on the treatment process.

Eliassen (11) presented an excellent article on the manner in which synthetic detergents reduce the efficiency of sewage sedimentation.

Degens and Van der Zee (10) showed that methane production would not be affected provided the concentration in the raw sludge of the synthetic detergent investigated was below 500 ppm of active material.

<u>Influence of organic matter</u>:

An extensive literature has appeared regarding this particular subject. This review will be limited to only a few articles pertaining to the understanding of this thesis.

Buswell and Boruff (6) showed that the methane content, as well as the total gas produced in the anaerobic decomposition of organic matter, depends on the ratio of C : H : O : N in the material being decomposed.

Larson, Boruff, and Buswell (27) did a carbon balance study of sludge digestion. Their data indicated that the greater portion of the gas formed during sludge digestion was due to the decomposition of fats and soaps. The fatty acids and soaps, due to their low oxygen content, furnished larger volumes and weights of gas per unit weight of material digested. They cited as an example the fermentation of glycerol stearate:

$$4\ (C_{17}H_{35}COO)C_3H_5 + 106\ H_2O \text{ --- } 65\ CO_2 + 163\ CH_4$$

Each gram of this fat upon digestion furnished 1433 cc of gas which weighed 1.54 grams and contained 72 percent methane. The other constituents found in sludge furnished lower volumes and less weight of gas per unit weight digested.

Rudolfs (32) studied the effects of different solvents on sludge digestion. He showed that different solvents had various effects on volatile matter and gas production, and that the limits of tolerance were not the same.

Rudolfs (31) showed that large quantities of grease added to digesting mixtures may result in a rapid accumulation of fatty acids (lower pH values). Acidic conditions in turn retard the methane producing organisms, but do not affect CO_2 production. He also showed that soaps decompose at a rapid rate and are an excellent source of gas. Small quantities of mineral oils (gasoline, crank case oil) did not materially affect the digestion process or the gas production of seeded solids.

Buswell (5) classified the stablization processes involved in sewage treatment into two simple types of reactions

1) Aerobic:

$$CHX + O_2 \text{ --- } CO_2 + H_2O + X$$

2) Anaerobic:

$$CHX + H_2O \text{ --- } CO_2 + CH_4 + X$$

C represented carbon, H - hydrogen, and X - small amounts of other elements which might be present. Thus, CHX represented organic matter.

MATERIALS AND METHODS

Sludges:

Raw sludge or sewage, was obtained from the primary settling tank of the East Lansing Sewage Disposal Plant. The material was collected in two liter erlenmeyer flasks.

Digested sludge was collected from the same plant. This material came from the anaerobic digester. The sludge was collected in a 5 gallon milk can and brought to the laboratory for immediate use. The sludges investigated were collected between 9:00 and 10:00 A.M. on the days when each experiment was carried out.

Synthetic detergents:

Three synthetic detergents were used in this study.

1) Anionic - Nacconol N.R.S.F. - alkyl aryl sulfonate - National Aniline Co. It is used principall as a detergent and wetting agent. Many of the common household detergents such as Tide, Dreft, Vel, Breeze, etc. belong to this group.

2) Cationic - BTC - alkyl dimethyl benzyl ammonium chloride - Onyx Oil & Chem. Co. It is used primarily as a germicidal agent for sanitization purposes.

3) Non-ionic - Triton X-100 - alkylated aryl poly ether alcohol - Rohm & Haas Co. It is used as a wetting, detergent, and emulsifying agent. It has applications in insecticides and agricultural sprays, industrial cleaners, paper processing, textiles, and other products.

Culture media:

Lactose broth, as recommended by Standard Methods of Water Analysis, was used for the determination of the coliform group.

Dextrose azide broth was used for the enumeration of the gram positive micrococci.

Bacterial counts:

Both coliform and gram positive micrococci counts were determined by employing the three tube dilution technique. The counts throughout this experiment are expressed in terms of the "Most Probable Number" (MPN) of organisms per 100 ml of sample. All tubes were incubated at 35 C. Final counts were made at the end of 48 hours.

Fermentation systems:

The apparatus shown in Figure I was constructed in order to carry out the various fermentations. One liter erlenmeyer flasks were used in the experiments. The total inoculum never exceeded 500 ml. When 500 ml flasks were used, allowing a head-space of approximately 100 ml, considerable difficulty was encountered due to frothing and subsequent eruption of the digesting material. The 1 liter flasks allowed for a head-space of approximately 600 ml and eliminated these difficulties.

After inoculation, the flasks were connected to the gas collecting columns (B) and flushed with nitrogen. Tank nitrogen was used and was purified by passing it through alkaline pyrogallol and concentrated sulfuric acid. Nitrogen entered through the sidearm of stopcock (W), passed through the fermentation flask into the sidearm of stopcock (X), and was expelled into the surrounding atmosphere through stopcock (Y). The displacing liquid* in the gas collecting column was level with stopcock (Y). Approximately 10 volumes of nitrogen was allowed to flow through the system in order to eliminate or displace the oxygen present, and to establish anaerobic conditions. The valve on the nitrogen tank was closed, and

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* An aqueous solution containing 20 percent sodium sulfate by weight and 5 percent sulfuric acid by volume was used. A few drops of methyl orange were added to facilitate reading and to indicate any change from an acid to an alkaline condition. The indicator changes from a pink to an orange at pH 5.

Figure 1. Fermentation apparatus used for the collection of gas during the digestion of sludge.

A - One liter erlenmeyer flask

B - Gas collecting column (300 ml capacity)

C - Displacing bottle (500 ml capacity)

D - Calcium chloride tube containing - Magnesium perchlorate and Ascarite.

W - Two-way stopcock

X and Y - Three-way stopcocks


W
X
Y
A
III
B
C
D

stopcock (W) was closed. Stopcock (Y) was adjusted so that the gases formed during the fermentation process would pass directly into the collecting column. The gas drawn over immediately after the collecting column was opened to the fermentation flask, was expelled through stopcock (Y), and the stopcock readjusted. The entire system was then placed in a 35 C. incubator for the desired intervals.

Samples of gas were taken at various intervals by closing stopcocks (X) and (Y) and disconnecting the fermentation flask from the collecting column. The gas was transferred to the gas analyser as described in the next section. The collecting column was then reattached to the fermentation system. The gas remaining in the collecting column was expelled through stopcock (X). In this manner, atmospheric gases were prevented from entering the fermentation system during sampling periods.

Method of gas analysis:

The mixture of gases obtained in the following experiments was analysed in the Burrell Gas Analyser shown in Figure II. The procedure involved was as follows:

Carbon dioxide was determined by absorption in potassium hydroxide*.

Oxygen was determined by absorption in alkaline pyrogallol**.

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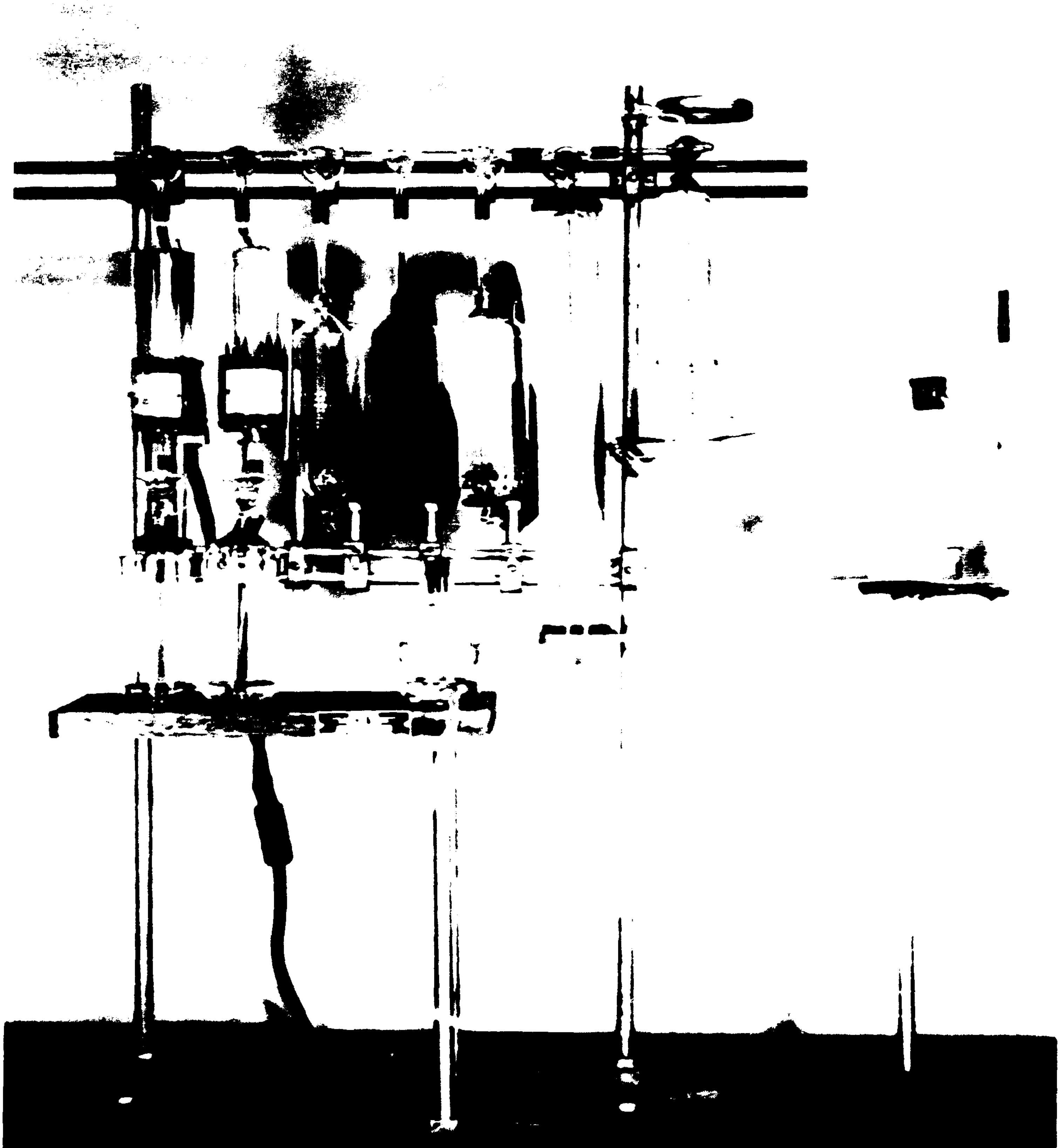
* 50 % KOH - 1 ml absorbs 40 ml of carbon dioxide

** 5 gms pyrogallic acid disolved in 100 ml of 50 % KOH - 1 ml absorbs 2 ml of oxygen

Figure II. Gas analysis apparatus. Burrell Build-up Model # 39-241.

Picture illustrates method used to transfer gas from the collecting column to the burette of the gas analyser.



Hydrogen was determined by oxidation over copper oxide[*].
Methane was determined by oxidation in the presence of catalyst[**].
Nitrogen was determined as the residual gas remaining after the removal of all of the above gases.

Prior to each analysis the entire apparatus was flushed with nitrogen to remove atmospheric gases. The collecting column containing the gas to be analysed was attached to the gas burette. The stopcocks were adjusted so that nitrogen passed through the manifold system of the gas analyser and the sidearm of the collecting column into the surrounding atmosphere. After flushing, the stopcocks were closed to the atmosphere and the entire system brought to atmospheric pressure. The gas in the collecting column was then transferred to the gas burette. One hundred ml samples were used for each analysis. In each instance the gas in the burette was brought to atmospheric pressure by allowing the displacing liquid in the leveling bottle and the gas burette to come to the same level. Manifold stopcocks were adjusted to allow the gas to pass into the first absorption tube containing KOH. The gas was then drawn back into the gas burette. Subsequent passages were made until a constant reading was obtained in the gas burette. Readings were made by bringing

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* CuO tube heated to 300 C. Gas was allowed to pass through at a rate of 10 ml per minute. Reaction envolved:

$$H_2 + CuO \text{ --- } H_2O + Cu$$

** Catalyst tube heated to 500 C. The gas was allowed to pass through at a rate of 30 ml per minute. Reaction envolved:

$$CH_4 + 2\,O_2 \text{ --- } CO_2 + 2\,H_2O$$

all of the gas back into the gas burette and closing the stopcocks. The liquid in the gas burette and the leveling bottle was adjusted to the same level and the final reading recorded. The gas was next passed into the second absorption tube containing alkaline pyrogallol, and the above procedure repeated. The remaining gas was passed through the heated copper oxide tube for the determination of hydrogen.

For the determination of methane a measured quantity of the remaining gas was stored in the KOH tube (usually 30 or 50 ml depending on the sample), and the remainder was expelled into the surrounding atmosphere. Since oxygen is necessary for the oxidation of methane, a given quantity was added to the gas burette to bring the combined volume of the stored gas and the added gas to a total of 100 ml. After mixing of the stored gas and oxygen, it was passed through the heated catalyst tube. The contraction due to oxidation was measured after each passage until a constant reading was obtained on the burette. The carbon dioxide produced from the oxidation of methane was absorbed into KOH and the final volume recorded. The percent methane was determined by the following formula:

Contraction due to oxidation ------ (r)

Carbon dioxide absorbed ----------- (s)

$$\text{Percent methane} \text{ --- } \frac{(r) + (s)}{3}$$

The total amount of methane present in the original 100 ml sample was determined by a simple proportion.

Nitrogen was determined by subtracting the total of the above determined gases from 100.

A record was kept on each reagent so that it was replaced before it lost its absorbing qualities.

The displacing liquid used in the gas burette and gas reservoirs was the same as that described for the gas collectin columns in the fermentation system.

RESULTS AND DISCUSSION

<u>Raw and digested sludge</u>:

A preliminary experiment was carried out to determine what the changes in carbon dioxide to methane ratios would be when increasing amounts of raw sludge were added to digested sludge. A mixture of raw and digested sludge was desired, which would, in a short period of time, yield a gas containing more methane than carbon dioxide. Four fermentation systems were set up and inoculated as follows:

Flask I - 500 ml of digested sludge
Flask II - 450 ml of digested sludge + 50 ml of raw sludge
Flask III - 400 ml of digested sludge + 100 ml of raw sludge
Flask IV - 300 ml of digested sludge + 200 ml of raw sludge

From the results presented in Table 1, it was noted that a marked increase in the total gas production occurred when increasing amounts of raw sludge were incorporated in the flasks. All the flasks which were seeded with raw sludge showed a favored methane production after 24 hours of incubation. The ratios of carbon dioxide to methane obtained, showed only slight variations. In order to confirm these results, six flasks were again seeded as follows:

Flask I, II, III, and IV contained the same quantities of raw and digested sludge as in the above experiment
Flask V - 200 ml of digested sludge + 300 ml of raw sludge
Flask VI - 500 ml of raw sludge

The results presented in Table 2, supported the evidence obtained in the first experiment. It was noted, however,

Table 1. The relationship between gas production and carbon dioxide - methane ratios when various amounts of raw sludge are added to digested sludge.

Flask *	I			II			III			IV		
Time In Hrs.	5	28	48	6	29	46	7	27	47	9	30	49
TGP **	95	240	340	130	380	480	225	545	735	290	690	1040
% CO_2	-	10.8	19.8	2.2	13.4	18.5	5.0	17.6	22.0	7.4	24.6	27.8
% O_2	-	2.0	0	2.4	1.0	0	6.4	0	0	1.6	0	0
% H_2	-	0.4	0.4	0	0	0	0	0	0	9.0	0	0.2
% CH_4	-	5.7	24.4	1.5	21.7	37.1	7.6	32.9	35.4	8.5	33.6	27.3
% N_2	-	81.1	44.6	93.9	63.9	44.4	81.0	49.5	42.6	73.5	41.8	44.7
$\frac{CO_2}{CH_4}$	-	$\frac{1.89}{1}$	$\frac{1}{1.23}$	$\frac{1.46}{1}$	$\frac{1}{1.62}$	$\frac{1}{2.00}$	$\frac{1}{1.52}$	$\frac{1}{1.86}$	$\frac{1}{1.60}$	$\frac{1}{1.15}$	$\frac{1}{1.36}$	$\frac{1.02}{1}$

* Flask I - 500 ml of digested sludge
II - 450 ml of digested sludge + 50 ml of raw sludge
III - 400 ml of digested sludge + 100 ml of raw sludge
IV - 300 ml of digested sludge + 200 ml of raw sludge

** TGP - Total ml of gas produced

Table 2. Carbon dioxide - methane ratios obtained when raw sludge was added to digested sludge.

Flask *	I		II		III		IV		V		VI	
Time In Hrs.	24	48	25	49	26	50	28	52	29	53	30	54
TGP **	135	235	590	870	650	1110	610	1180	530	950	250	460
% CO_2	4.6	11.0	20.2	23.8	21.0	25.4	26.4	34.8	28.2	43.6	17.8	35.0
% O_2	1.6	0.8	0	0	0	0	0	0	0	0	0.4	0.2
% H_2	0	0	0	0	0	0	0	0	0	0	0	0
% CH_4	0.9	11.2	35.9	49.3	36.6	52.5	28.2	48.7	15.6	32.2	1.3	6.2
% N_2	92.9	77.0	43.9	26.9	42.4	22.1	45.2	16.5	56.2	24.2	80.5	41.4
$\frac{CO_2}{CH_4}$	$\frac{5.11}{1}$	$\frac{1}{1.01}$	$\frac{1}{1.77}$	$\frac{1}{2.07}$	$\frac{1}{1.74}$	$\frac{1}{2.06}$	$\frac{1}{1.06}$	$\frac{1}{1.39}$	$\frac{1.81}{1}$	$\frac{1.35}{1}$	$\frac{13.7}{1}$	$\frac{5.64}{1}$

* Flask I - 500 ml of digested sludge
II - 450 ml of digested sludge + 50 ml of raw sludge
III - 400 ml of digested sludge + 100 ml of raw sludge
IV - 300 ml of digested sludge + 200 ml of raw sludge
V - 200 ml of digested sludge + 300 ml of raw sludge
VI - 500 ml of raw sludge

** TGP - Total ml of gas produced

that when the quantity of raw sludge exceeded the amount of digested sludge (Flask V), both the total gas production and the ratio of gases were affected. Total gas production decreased and carbon dioxide production increased. In Flask VI (raw sludge) most of the gas produced was carbon dioxide, only a small amount of methane was produced. The differences noted in the ratios of the digested sludge (Flask I of both experiments) can be readily explained. The character of the digested sludge on any one day, depends upon the amount of raw sludge added on the preceding day. Since the amount and composition of the raw sludge varies from day to day, it can be expected that the digested sludge will be in a different state of decomposition each day. This accounts for both the differences in the total gas production and the ratios of gases obtained in these experiments from digested sludge alone. For this reason two controls were set up with most of the experiments. The first control contained only digested sludge. The second control contained digested sludge plus raw sludge. These controls indicated the condition of the digested sludge and the raw sludge at the time the experiment was carried out.

The presence of oxygen in the samples was due to insuffici flushing of the system with nitrogen. The decrease observed in the percentage of oxygen and nitrogen in the samples is readily explained. Since neither oxygen nor nitrogen are produced during the anaerobic digestion of sludge, the volume of these two gases originally present will not change. Howeve as the fermentation gases are produced, diffusion of the gases occurs. When samples of the gas are taken for analysis, the concentration of nitrogen and oxygen is reduced. This accounts for the reduction in the percentage of these two gases in subsequent samples.

It was concluded from this experiment that the amount of raw sludge to be added to the digested sludge in future experiments would be that found in Flask II (50 ml of raw sludge). This quantity of raw sludge gave a rapid methane production and also presented a more favorable and a more desirable working mixture than the other mixtures tested.

<u>Cationic detergents and sludge digestion</u>:

At the same time the first experiment was set up, two given concentrations of a synthetic detergent (BTC) were introduced into two fermentation flasks containing raw and digested sludge. This was done in order to obtain some idea of the immediate effect of this compound on the digestion processes. Flask I and II in Table 1, served as controls for this experiment. The flasks containing the synthetic detergent were seeded as follows:

Flask V - 400 ml of digested sludge + 50 ml of raw sludge
+ 50 ml of BTC (final conc. 50 ppm*)

Flask VI - 400 ml of digested sludge + 50 ml of raw sludge
+ 50 ml of BTC (final conc. 100 ppm)

The results presented in Table 3, indicated that concentrations of 50 and 100 ppm of BTC had no detrimental

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* ppm - parts per million

Table 3. The effect of a synthetic detergent (BTC) on the digestion of sludge.

Flasks*	V			VI		
Time In Hours	10	32	50	11	33	51
TGP**	190	400	510	170	370	490
% CO_2	4.6	13.4	17.6	5.0	13.4	17.0
% O_2	2.0	0.4	0	2.0	0	0
% H_2	0	0	0	0	0.4	0.2
% CH_4	6.9	20.5	35.4	6.1	25.3	53.6
% N_2	86.5	65.7	47.0	86.9	60.9	29.2
$\frac{CO_2}{CH_4}$	$\frac{1}{1.50}$	$\frac{1}{1.53}$	$\frac{1}{2.01}$	$\frac{1}{1.22}$	$\frac{1}{1.89}$	$\frac{1}{3.15}$

* Flasks V - 400 ml of digested sludge ≠ 50 ml of raw sludge ≠ 50 ml of BTC (final conc. 50 ppm)
VI - 400 ml of digested sludge ≠ 50 ml of raw sludge ≠ 50 ml of BTC (final conc. 100 ppm)

** TGP - Total ml of gas produced

effect on the digestion processes. The presence of 100 ppm of the synthetic detergent appeared to stimulate methane production. Rudolfs, Manganelli, and Gellman (33) also observed that 100 ppm of Nacconol N.R. hastened the digestion processes. The phenomenon, however, was not explained. It was thought that the microorganisms present in the sludge might possess the ability to utilize the detergent as a source of food material with the liberation of methane. Also, since detergents were known to lower the interfacial tension, thereby bringing more substances to the surface of the bacterial cells, that this might be the cause for stimulated methane production. Another factor which was considered, was the composition of the raw sludge. Since 50 ml of raw sludge was added, there was no way of governing the amount of solid material in the aliquot. Thus, increased methane production may have been due to a larger amount of organic material (fats, proteins, etc.) in the sample.

Direct effect of a cationic and an anionic detergent:

In order to determine which of these factors might be responsible for the increased methane production, the following experiment was carried out. Five fermentation flasks were seeded as follows:

Flask I - 500 ml of digested sludge

Flask II - 450 ml of digested sludge + 50 ml of BTC (final conc. 100 ppm)

Flask III - 450 ml of digested sludge + 50 ml of BTC (final conc. 500 ppm)

Flask IV - 450 ml of digested sludge ≠ 50 ml of Nacconol (final conc. 100 ppm)

Flask V - 450 ml of digested sludge ≠ 50 ml of Nacconol (final conc. 500 ppm)

The results presented in Table 4, showed that in all the flasks containing synthetic detergents, normal digestion was impaired with respect to total gas production. The production of methane was again stimulated in the flasks containing 100 ppm of both BTC and Nacconol. A concentration of 500 ppm of BTC had a more inhibitory effect on both the total gas produced and methane production than the same concentration of Nacconol.

It was concluded that raw sludge was not responsible for the increased methane in the gas sample, as raw sludge was not used in this experiment. Since the total volume of gas was less in all the flasks seeded with detergents, it was also concluded that the microorganisms in digested sludge were not able to utilize the detergents for methane production. The stimulated production of methane, therefore, appeared to be due to the lowering of the interfacial tension between the bacterial cells and water by the detergents. Thus, 100 ppm of the detergent caused more food material to come in contact with the methane producing organisms.

<u>Gas ratios at different intervals of time</u>:

The next experiment was set up to determine if the ratios of carbon dioxide to methane would vary at different

Table 4. The ratios of carbon dioxide to methane produced when various concentrations of synthetic detergents are added to digested sludge and incubated at 35 C.

Flasks*	I	II	III	IV	V
Time In Hrs.	48	49	50	52	53
TGP**	380	240	100	270	260
% CO_2	10.0	9.6	9.0	9.4	12.2
% O_2	1.6	0.6	0.4	1.2	0.6
% H_2	0	0	0	0	0
% CH_4	12.4	13.8	2.1	13.5	11.8
% N_2	76.0	76.0	88.5	75.9	75.4
$\frac{CO_2}{CH_4}$	$\frac{1}{1.24}$	$\frac{1}{1.45}$	$\frac{4.28}{1}$	$\frac{1}{1.43}$	$\frac{1.03}{1}$

* Flasks I - 500 ml of digested sludge
II - 450 ml of digested sludge + 50 ml of BTC (final conc. 100 ppm)
III - 450 ml of digested sludge + 50 ml of BTC (final conc. 500 ppm)
IV - 450 ml of digested sludge + 50 ml of Nacconol (final conc. 100 ppm)
V - 450 ml of digested sludge + 50 ml of Nacconol (final conc. 500 ppm)

** TGP - Total ml of gas produced

intervals of time. An interval was desired which would give an accurate picture of the effect of the synthetic detergents on the digestion processes. The effect of the detergent on the total volume of gas was also determined. Various concentrations of the synthetic detergent (BTC) were tested. Samples of gas were analysed after 5, 48, 96, and 168 hours. A series of six fermentation flasks were seeded as follows:

Flask I - 500 ml of digested sludge

Flask II - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of water

Flask III - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 50 ppm)

Flask IV - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 100 ppm)

Flask V - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 200 ppm)

Flask VI - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 500 ppm)

The results presented in Table 5 (a, b, c, d, e, and f) showed that concentrations of 50 and 100 ppm of BTC stimulated both the total gas production and the production of methane. Although the fermentations were carried out over a long period of time, no significant changes were observed in the gas ratios after 48 hours of incubation. The total volume of gas produced during the 168 hour period was recorded in Table 6, and presented in the form of a graph in Figure III. From this graph it can readily be seen

Table 5. A comparative study of gas ratios and total gas production over various periods of time when different concentrations of synthetic detergent are present.

a) 500 ml of digested sludge

Time In Hrs.	8	48	98	168
TGP*	120	300	440	550
% CO_2	6.4	14.6	17.6	19.4
% O_2	0	0.4	0	0
% H_2	0	0	0	0
% CH_4	1.0	13.6	27.4	36.6
% N_2	92.6	71.4	55.0	43.9
$\frac{CO_2}{CH_4}$	$\frac{6.4}{1}$	$\frac{1.07}{1}$	$\frac{1}{1.55}$	$\frac{1}{1.89}$

* TGP - Total ml of gas produced

b) 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of water

Time In Hrs.	6	49	97	169
TGP	240	1110	1420	1655
% CO_2	7.8	22.6	24.4	25.4
% O_2	0.6	0	0	0
% H_2	0	0	0	0
% CH_4	6.6	35.0	50.4	48.0
% N_2	85.0	42.4	25.2	26.6
$\frac{CO_2}{CH_4}$	$\frac{1.18}{1}$	$\frac{1}{1.54}$	$\frac{1}{2.05}$	$\frac{1}{1.89}$

c) 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 50 ppm)

Time In Hrs.	5	50	96	170
TGP	190	1180	1500	1750
% CO_2	6.0	27.0	27.8	28.4
% O_2	1.6	0	0	0
% H_2	0	0	0	0
% CH_4	5.5	56.9	60.9	63.0
% N_2	86.9	16.1	11.3	8.6
$\frac{CO_2}{CH_4}$	$\frac{1.09}{1}$	$\frac{1}{2.10}$	$\frac{1}{2.19}$	$\frac{1}{2.21}$

d) 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 100 ppm)

Time In Hrs.	9	51	100	172
TGP	280	1190	1580	1830
% CO_2	8.2	26.8	26.4	26.4
% O_2	1.8	0.2	0	0
% H_2	0	0	0	0
% CH_4	8.0	52.8	60.4	60.8
% N_2	82.0	20.2	13.2	12.8
$\frac{CO_2}{CH_4}$	$\frac{1.02}{1}$	$\frac{1}{1.89}$	$\frac{1}{2.25}$	$\frac{1}{2.30}$

e) 400 ml of digested sludge ≁ 50 ml of raw sludge ≁ 50 ml of BTC (final conc. 200 ppm)

Time In Hrs.	10	52	101	173
TGP	190	680	1060	1400
% CO_2	7.8	25.6	29.0	26.0
% O_2	1.4	0	0	0
% H_2	0.4	0	0	0
% CH_4	5.4	34.0	45.2	44.4
% N_2	85.0	40.4	25.8	29.6
$\frac{CO_2}{CH_4}$	$\frac{1.44}{1}$	$\frac{1}{1.32}$	$\frac{1}{1.56}$	$\frac{1}{1.70}$

f) 400 ml of digested sludge ≁ 50 ml of raw sludge ≁ 50 ml of BTC (final conc. 500 ppm)

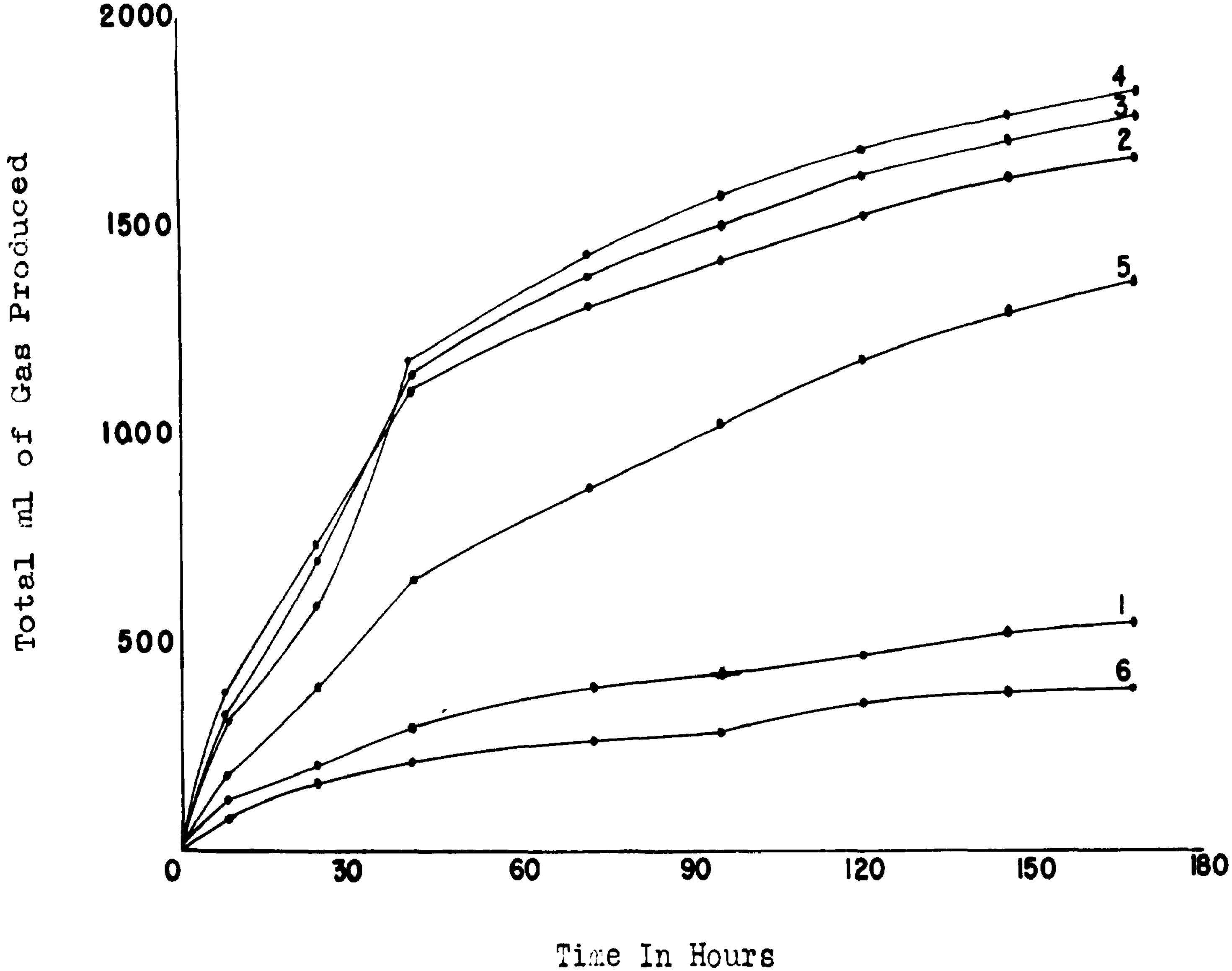
Time In Hrs.	11	53	102	174
TGP	110	220	320	390
% CO_2	5.6	17.2	24.0	-
% O_2	1.6	0.2	0	-
% H_2	0.2	0	0	-
% CH_4	1.2	6.0	9.4	-
% N_2	91.4	76.6	66.6	-
$\frac{CO_2}{CH_4}$	$\frac{4.66}{1}$	$\frac{2.86}{1}$	$\frac{2.55}{1}$	-

Table 6. The effect of different concentrations of synthetic detergent (BTC) on the total volume of gas produced by digesting sludge.

Time In Hours	Flasks *					
	I	II	III	IV	V	VI
8	120	350	320	270	180	95
24	205	720	690	580	380	160
48	300	1100	1140	1170	650	220
74	400	1310	1380	1440	880	270
96	430	1410	1500	1570	1020	295
120	495	1530	1620	1690	1180	375
148	520	1600	1700	1750	1290	390
168	550	1655	1745	1820	1370	395

* Flasks I - 500 ml of digested sludge
II - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of water
III - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 50 ppm)
IV - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 100 ppm)
V - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 200 ppm)
VI - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 500 ppm)

Figure III. The effect of various concentrations of BTC on the total volume of gas produced by digesting sludge.



1 - 500 ml of digested sludge
2 - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of water
3 - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (50 ppm)
4 - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (100 ppm)
5 - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (200 ppm)
6 - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (500 ppm)

that 50 and 100 ppm of BTC had a slightly stimulated effect on gas production. It was also observed that with 200 and 500 ppm of BTC the total gas production was retarded.

It was concluded that a 48 hour incubation period would be sufficient to show the type of result sought after in these experiments. That is, it would not be necessary to analyse samples of the gas at different intervals of time in order to detect the effect of the synthetic detergents on the digestion processes.

Non-ionic detergents and sludge digestion:

A study of the effect of a non-ionic synthetic detergent (Triton X-100) on the total gas production and the ratio of carbon dioxide to methane was undertaken. Six fermentation systems were seeded as follows:

Flask I - 500 ml of digested sludge

Flask II - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of water

Flask III - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of Triton X-100 (final conc. 100 ppm)

Flask IV - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of Triton X-100 (final conc. 500 ppm)

Flask V - 450 ml of digested sludge + 50 ml of Triton X-100 (final conc. 100 ppm)

Flask VI - 450 ml of digested sludge + 50 ml of Triton X-100 (final conc. 500 ppm)

The results presented in Table 7, showed that a

Table 7. The effect of a non-ionic synthetic detergent (Triton X-100) on the ratio of gases when added to digesting sludge.

Flasks*	I	II	III	IV	V	VI
Time In Hrs.	48	49	50	52	53	54
TGP **	200	540	570	220	210	250
% CO_2	8.2	15.4	16.8	11.8	9.4	10.2
% O_2	1.8	0	0	0.4	1.4	1.6
% H_2	0	0	0	0	0	0
% CH_4	3.6	30.2	34.7	8.6	10.6	11.7
% N_2	86.4	54.4	48.5	79.2	78.6	76.5
$\frac{CO_2}{CH_4}$	$\frac{2.28}{1}$	$\frac{1}{1.96}$	$\frac{1}{2.06}$	$\frac{1.38}{1}$	$\frac{1}{1.12}$	$\frac{1}{1.14}$

* Flasks I - 500 ml of digested sludge
II - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of water
III - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of Triton X-100 (final conc. 100 ppm)
IV - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of Triton X-100 (final conc. 500 ppm)
V - 450 ml of digested sludge + 50 ml of Triton X-100 (final conc. 100 ppm)
VI - 450 ml of digested sludge + 50 ml of Triton X-100 (final conc. 500 ppm)

** TGP - Total ml of gas produced

a concentration of 100 ppm of the detergent (Flask III) stimulated methane production and total gas production. Five hundred ppm of the detergent reduced both methane production and total gas production. However, when the detergent in a concentration of 100 and 500 ppm was added to digested sludge (Flask V and VI), methane production and total gas production were slightly stimulated.

It was concluded that at a concentration of 100 ppm of Triton X-100, the non-ionic detergent would have no effect on the digestion processes. A concentration of 500 ppm was tolerated, however, the process was retarded.

<u>Effect of detergents on microflora of sludge</u>:

The coliform and the gram positive micrococcus groups of microorganisms comprise a large portion of the bacterial population of digested sludge. The following experiment was set up to determine if these two groups of organisms were affected by the presence of the detergents, and if the total gas production and methane production were associated with these two groups. Six fermentation flasks were seeded as follows:

Flask I - 500 ml of digested sludge

Flask II - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of water

Flask III - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 100 ppm)

Flask IV - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of BTC (final conc. 500 ppm)

Flask V - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of Nacconol (final conc. 100 ppm)

Flask VI - 400 ml of digested sludge + 50 ml of raw sludge + 50 ml of Nacconol (final conc. 500 ppm)

Bacterial counts were made after seeding the flasks. The results of the experiment are presented in Table 8.

No significant change in the microflora with respect to the above two groups was noted. Although the ratio of the gases was disturbed in Flask IV and VI, no germicidal activity was observed. It was again observed, that the cationic detergent (BTC) had a more inhibitory effect on the total gas production and the ratio of gases than the anionic detergent (Nacconol).

It was concluded that the decreased gas production and the altered gas ratios obtained in Flasks IV and VI were not due to the germicidal action of the detergents on the above mentioned groups of microorganisms. Although the concentrations of the detergents were not sufficient to kill off the microorganisms, the possibility that the metabolic activities were retarded by the higher concentrations should not be excluded.

Table 8. The effect of different concentrations of synthetic detergents on the total gas production, the ratios of carbon dioxide to methane, and the coliform and gram positive micrococcus organisms when added to digesting sludge.

Flasks*	I	II	III	IV	V	VI
Time In Hrs.	48	49	50	52	53	54
TGP**	320	950	850	150	960	535
% CO_2	10.8	24.2	21.6	10.8	21.4	17.2
% O_2	1.0	0	0	0.8	0	0
% H_2	0	0	0	0	0	0
% CH_4	11.5	51.5	43.4	3.2	47.9	28.6
% N_2	76.2	24.3	35.0	85.2	30.7	54.2
$\frac{CO_2}{CH_4}$	$\frac{1}{1.06}$	$\frac{1}{2.12}$	$\frac{1}{2.01}$	$\frac{3.37}{1}$	$\frac{1}{2.23}$	$\frac{1}{1.66}$
Coliform# Before	4.3 M	43 M	25 M	4.3 M	25 M	43 M
Coliform After	4.3 M	2.4 M	15 M	9.2 M	9.1 M	250 M
Cocci Before	730 T	2.4 M	2.4 M	9.2 M	4.3 M	2.4 M
Cocci After	250 T	430 T	1.5 M	2.5 M	430 T	430 T

* Flasks I - 500 ml of digested sludge
II - 400 ml of digested sludge ≁ 50 ml of raw sludge ≁ 50 ml of water
III - 400 ml of digested sludge ≁ 50 ml of raw sludge ≁ 50 ml of BTC (final conc. 100 ppm)
IV - 400 ml of digested sludge ≁ 50 ml of raw sludge ≁ 50 ml of BTC (final conc. 500 ppm)
V - 400 ml of digested sludge ≁ 50 ml of raw sludge ≁ 50 ml of Nacconol (final conc. 100 ppm)
VI - 400 ml of digested sludge ≁ 50 ml of raw sludge ≁ 50 ml of Nacconol (final conc. 500 ppm)

** TGP - Total ml of gas produced

\# MPN of organisms per 100 ml of sample (M-million)(T-thousand)

<u>Fatty acids and sludge digestion</u>:

The effect of various organic compounds on the digestion of sludge has been reported in the literature by several investigators. The purpose of the next experiment was to observe the effects of a few organic compounds on the ratio of gases and the bacterial population with respect to the coliform and gram positive micrococcus groups. Six fermentation flasks were seeded as follows:

Flask I - 500 ml of digested sludge

Flask II - 500 ml of digested sludge ≁ 1 gm of palmitic acid

Flask III - 500 ml of digested sludge ≁ 5 gm of palmitic acid

Flask IV - 500 ml of digested sludge ≁ 1 gm of stearic acid

Flask V - 500 ml of digested sludge ≁ 5 gm of stearic acid

Flask VI - 500 ml of digested sludge ≁ 5 gm of oleic acid

Bacterial counts of the sludge were made at the time of seeding. The results of this experiment are presented in Table 9.

No significant change was observed in the coliform or gram positive micrococcus counts. More gas was produced from the 5 gm samples than the 1 gm samples. Increased methan production was observed in all flasks containing fatty acids. The greatest amount of methane was produced in those flasks containing the 5 gm samples. There was a drop in the pH values, but not enough to influence gas production. It was concluded that the presence of fatty acids in the above stated concentrations would have no detrimental effect on

Table 9. The effect of different amounts of fatty acids on the total gas production, the ratios of carbon dioxide to methane, and the bacterial counts with respect to the coliform and gram positive micrococcus groups when added to digesting sludge.

Flasks*	I	II	III	IV	V	VI
Time In Hrs.	48	49	50	52	53	54
TGP**	240	610	860	550	930	980
% CO_2	7.2	18.6	32.0	17.2	33.4	29.0
% O_2	1.0	0	0	0.2	0	0
% H_2	0	0	0	0	0	0
% CH_4	1.5	30.1	33.8	22.0	44.0	46.2
% N_2	90.3	51.3	34.2	60.6	22.6	24.8
$\frac{CO_2}{CH_4}$	$\frac{4.80}{1}$	$\frac{1}{1.62}$	$\frac{1}{1.06}$	$\frac{1}{1.27}$	$\frac{1}{1.31}$	$\frac{1}{1.59}$
Coliform# After	4.3 M	430 T	2.5 M	920 T	43 M	920 T
Cocci After	920 T	430 T	250 T	920 T	250 T	920 T
pH##	7.3	6.6	6.3	7.2	6.8	6.0

* Flasks I - 500 ml of digested sludge
II - 500 ml of digested sludge + 1 gm palmitic acid
III - 500 ml of digested sludge + 5 gm palmitic acid
IV - 500 ml of digested sludge + 1 gm stearic acid
V - 500 ml of digested sludge + 5 gm stearic acid
VI - 500 ml of digested sludge + 5 gm oleic acid

** TGP - Total ml of gas produced

\# Coliform count before fermentation - 9.2 M, micrococci count - 4.3 M. (M - million) (T - thousand)

\## pH - prior to the addition of the various compounds the pH of the digested sludge was 7.3

gasification, and that the fatty acids studied could be used as a source of food material with the subsequent liberation of methane.

<u>Tolerance of fatty acids</u>:

In order to determine the tolerance of the fatty acids on sludge digestion, the next experiment was carried out. For comparative purposes, sodium formate and dextrose were also included in this experiment. Six fermentation flasks were seeded as follows:

Flask I - 500 ml of digested sludge
Flask II - 500 ml of digested sludge + 10 gm sodium formate
Flask III - 500 ml of digested sludge + 10 gm dextrose
Flask IV - 500 ml of digested sludge + 10 gm oleic acid
Flask V - 500 ml of digested sludge + 10 gm palmitic acid
Flask VI - 500 ml of digested sludge + 10 gm stearic acid

The results presented in Table 10, showed no significant change in the coliform count of all six flasks. A marked difference was observed in the gram positive micrococcus count. Flasks II and III, showed an increased micrococcus count, whereas, Flasks IV, V, and VI all showed a decreased count. The pH values observed in the various flasks indicated that this was not the influencing factor. The responsible factors were, no doubt, the compounds which were introduced into the flasks. High concentrations of fatty acids are known to be toxic to certain groups of bacteria.

With the exception of dextrose, no interference in

Table 10. The effect of various organic compounds on the total gas production, the ratios of carbon dioxide to methane, and the bacterial counts with respect to the coliform and gram positive micrococc groups when added to digesting sludge.

Flasks*	I	II	III	IV	V	VI
Time In Hrs.	49	50	48	53	72	51
TGP**	240	410	890	1220	900	520
% CO_2	9.6	6.6	69.6	36.2	40.2	42.8
% O_2	1.0	0.4	0	0	0	0
% H_2	0	16.4	24.0	0	0.4	0.4
% CH_4	8.5	15.8	2.7	37.6	25.5	10.2
% N_2	80.9	60.8	3.7	26.2	33.9	46.6
$\frac{CO_2}{CH_4}$	$\frac{1.13}{1}$	$\frac{1}{2.40}$	$\frac{25.8}{1}$	$\frac{1}{1.03}$	$\frac{1.57}{1}$	$\frac{4.19}{1}$
Coliform# After	4.3 M	2.9 M	4.3 M	9.2 M	2.4 M	43 M
Cocci After	240 T	25 M	92 M	<30 T	<30 T	30 T
pH##	7.3	8.2	4.8	6.6	6.7	6.7

* Flasks I - 500 ml of digested sludge
II - 500 ml of digested sludge ≠ 10 gm NaOOCH
III - 500 ml of digested sludge ≠ 10 gm dextrose
IV - 500 ml of digested sludge ≠ 10 gm oleic acid
V - 500 ml of digested sludge ≠ 10 gm palmitic aci
VI - 500 ml of digested sludge ≠ 10 gm stearic acid

** TGP - Total ml of gas produced

\# Coliform count before fermentation - 4.3 M, micrococci count - 730 T. (M - million) (T - thousand)

\## pH - prior to the addition of the compounds the pH of the digested sludge was 7.3

methane production was observed. Because of the low acidity produced in this case (pH 4.8), the activities of the methane producing bacteria were probably inhibited. As mentioned in the review of the literature, Rudolfs (31) stated that acid conditions retard methane production, but do not affect carbon dioxide production. Stadtman and Barker (41) also indicated that the pH range for growth of the methane producing organism, Methanococcus vanniellii, was between 7.4 and 9.2.

It was noted that large quantities of hydrogen were obtained from the fermentation of sodium formate and dextrose, and practically none from the fatty acids.

The data also showed a marked difference in the amount of methane obtained from the decomposition of the three fatty acids used. Almost four times more methane was obtained from the decomposition of oleic acid than stearic acid. This difference could not be explained and prompted the next series of experiments.

With the exception of sodium formate and dextrose, four flasks were seeded exactly as the ones in the above experiment. The data from the fermentations are presented in Table 11.

Here, as in the previous experiment, the coliform count showed no significant change. The micrococcus count, however, dropped as before. There was a marked decrease in the amount of methane produced from oleic acid as compared to that obtained in the previous experiment. The

Table 11. The effect of fatty acids on the total gas production, the ratios of carbon dioxide to methane, and the bacterial counts with respect to the coliform and gram positive micrococcus groups when added to digesting sludge.

Flasks*	I	II	III	IV
Time In Hrs.	48	49	50	51
TGP**	170	270	465	520
% CO_2	6.4	21.8	28.0	31.0
% O_2	1.6	0.6	0.8	0.4
% H_2	0	0	0	0
% CH_4	1.3	1.6	14.0	10.8
% N_2	90.7	76.0	57.2	57.8
$\frac{CO_2}{CH_4}$	$\frac{4.92}{1}$	$\frac{13.5}{1}$	$\frac{2.00}{1}$	$\frac{2.87}{1}$
Coliform# Before	4.3 M	2.5 M	2.5 M	4.3 M
Coliform After	2.5 M	2.5 M	4.3 M	4.3 M
Cocci Before	920 T	920 T	430 T	430 T
Cocci After	920 T	43 T	11 T	92 T
pH##	7.2	5.3	5.5	6.8

* Flasks I - 500 ml of digested sludge
II - 500 ml of digested sludge + 10 gm oleic acid
III - 500 ml of digested sludge + 10 gm palmitic aci
IV - 500 ml of digested sludge + 10 gm stearic acid

** TGP - Total ml of gas produced

\# MPN of organisms per 100 ml of sample (M-million)(T-thousan

\## pH - prior to the addition of the compounds the pH of the digested sludge was 7.3

flask containing palmitic acid also showed a decrease in methane production. The total gas production was also affected in both of these flasks (II and III). Flask II produced 270 ml of gas and Flask III produced 465 ml, as compared to 1220 ml and 900 ml respectively in the previous experiment. The differences may have been due to several factors. 1) The observed pH values in Flask II and III respectively, were 5.3 and 5.5. This drop in pH may have been sufficient to stop or retard the gasification processes. 2) Since a greater drop in pH was observed in the last experiment, there may have been a difference in the buffering capacities of the sludge. In the last case, the buffering capacity of the sludge was not sufficient to maintain a high pH level upon the addition of the fatty acid. 3) A comparison of the gas ratios obtained in the controls of both experiments, revealed that the digested sludges were in different stages of decomposition. The ratio of carbon dioxide to methane in the previous experiment was 1.13/1, whereas in the control of the last experiment it was 4.92/1. In the latter case, conditions were, no doubt, unfavorable for methane production. There may have been less methane producing bacteria present and the addition of fatty acid in large quantities suppressed the metabolic activities of those present.

It was concluded from these experiments, that when testing the effect of various organic materials on the digestion processes, the condition of the digested sludge must be taken into consideration. As shown in the above experiment results may differ from day to day. Small amounts of the fatty acids produced no detrimental effects in the digesting sludge. Large amounts of the fatty acids affected both the total gas production and methane production. The gram positiv micrococci were also destroyed by large concentrations of the fatty acids.

Contact time and gas ratios:

The next experiment was carried out to determine if the gas ratios would differ if the gas was allowed to remain in contact with the digesting liquor for longer periods of time. Two individual experiments were set up. The flasks were seeded as follows:

Flask I - 500 ml of digested sludge
Flask II - 500 ml of digested sludge + 10 gm sodium formate
Flask III - 500 ml of digested sludge + 10 gm sodium formate
Flask IV - 500 ml of digested sludge + 10 gm oleic acid
Flask V - 500 ml of digested sludge + 10 gm oleic acid

Flasks I, II, and IV were set up as shown in Figure I. The remaining two flasks (III, and V) were connected to a collecting column having a two liter capacity. Samples of gas were analysed from flasks (I, II, and IV) at 24 and 96 hours. At the time of sampling, all the gas in the collecting column was expelled. The gas in Flasks (III and V) was analysed at 96 and 144 hours. Only the amount of gas necessary for an analysis was removed from the collecting columns. The remainder of the gas was allowed to remain in contact with the digesting liquor. The results are presented in Table 12 (a and b).

In the case of sodium formate (12 a), it was noted that the total gas production in Flasks II and III varied only slightly. Most of the gas was formed during the first 48 hours. A loss of carbon dioxide was observed after 96 and 144 hours of incubation. It appeared that methane was formed at the expense of carbon dioxide. This is in agreement with the carbon dioxide reduction theory, Barker (1). According to Stephenson and Stickland (42) formic acid was decomposed to hydrogen and carbon dioxide. The hydrogen was activated by the enzyme hydrogenase and the carbon dioxide was, at the same time, reduced bringing about the reaction:

$$HCOOH \text{ --- } H_2 + CO_2$$

$$4\ H_2 + CO_2 \text{ --- } CH_4 + 2\ H_2O$$

This would account for the decrease in carbon dioxide content and the increase in methane production.

In the case of oleic acid (12 b), an increase in methane production was also observed in the flask which was allowed to incubate for the longer period. No hydrogen was observed in these fermentations. Neave and Buswell (29) stated that the higher fatty acids were decomposed to carbon dioxide and methane, water acting as the oxidizing - reducing agent. Using a highly purified culture of <u>Methanobacterium suboxydans</u> Stadtman and Barker (39) showed through tracer experiments that methane was formed from carbon dioxide in the butyrate, valerate, and caproate fermentations. However, the exact

Table 12. The differences in carbon dioxide - methane ratios when the gases are left in contact with the digesting liquor over long periods of time.

a) sodium formate

Flasks*	I		II	III	
Time In Hrs.	48	96	49	96	144
TGP**	160	260	335	300	410
% CO_2	6.0	12.6	6.2	2.8	2.6
% O_2	1.8	0.4	0.6	1.4	1.0
% H_2	0	0	8.8	12.2	15.6
% CH_4	1.7	14.4	15.4	16.9	23.5
% N_2	90.5	72.6	69.0	66.7	42.7
$\frac{CO_2}{CH_4}$	$\frac{3.53}{1}$	$\frac{1}{1.14}$	$\frac{1}{2.48}$	$\frac{1}{6.04}$	$\frac{1}{9.03}$
pH#		7.2	8.5		8.3

* Flasks I - 500 ml of digested sludge

II - 500 ml of digested sludge + 10 gm sodium formate (after gas was removed for analysis and the system reattached, not enough gas was evolved for an additional analysis)

III - 500 ml of digested sludge + 10 gm sodium formate. Only 100 ml of the gas was removed for the analysis. The remaining gas was left in contact with the digesting liquor.

** TGP - Total ml of gas produced

\# pH - prior to the addition of the compounds, the pH of the digested sludge was 7.3

b) oleic acid

Flasks*	VI		V	
Time in Hrs.	50	97	98	146
TGP**	275	520	550	2800
% CO_2	24.2	39.6	28.6	29.0
% O_2	0.4	0	0.6	0.2
% H_2	0	0	0	0
% CH_4	2.3	7.1	32.8	45.8
% N_2	73.1	53.3	48.0	25.0
$\frac{CO_2}{CH_4}$	$\frac{10.5}{1}$	$\frac{5.57}{1}$	$\frac{1}{1.14}$	$\frac{1}{1.58}$
pH#		6.5		6.6

* Flasks VI - 500 ml of digested sludge + 10 gm oleic acid. All of the gas in the collecting column was expelled at the time of analysis.

V - 500 ml of digested sludge + 10 gm oleic acid. Only 100 ml of the gas was removed for analysis. The remaining gas was left in contact with the digesting liquor.

** TGP - Total ml of gas produced

\# pH - prior to the addition of the compounds, the pH of the digested sludge was 7.3

mechanism for methane fermentation of the higher fatty acids is not known.

The carbon dioxide content in Flask VI was greater than in Flask V after 98 hours of incubation. The methane content was just the opposite, although the total gas volume in the two flasks had not changed significantly. It appeared that the undisturbed material in Flask V, had adapted itself more rapidly to a methane producing state.

It was observed that if the carbon dioxide to methane ratios in the 48 hour Flask I (Table 12 a) and the 50 hour Flask VI (Table 12 B), were compared to the carbon dioxide-methane ratios in Flask I and II (Table 11), similar results were obtained. Likewise, it was noted that if the carbon dioxide - methane ratios in the 96 hour Flask I (Table 12 a) and the 98 hour Flask V (Table 12 b), were compared to the carbon dioxide - methane ratios in Flasks I and IV (Table 10), similar results were again obtained. This showed that the condition of the digested sludge was responsible for the differences in carbon dioxide - methane ratios obtained in the previous experiments. The large amount of gas produced in Flask V (Table 12 b) indicated that even large concentratio of fatty acids will be decomposed, but longer digestion periods are necessary.

It was concluded that gases left in contact with the digesting liquor will show carbon dioxide - methane ratios which are different from the gases pulled off after short incubation periods. This difference is probably due to the reduction of carbon dioxide to methane as stated previously.

SUMMARY AND CONCLUSIONS

The addition of large quantities of raw sludge to digested sludge decreased the total gas production and the production of methane.

The presence of 100 ppm of cationic, anionic, and non-ionic detergents in digesting sludge, showed no harmfull effects on the digestion processes.

Total gas production and methane production were stimulated by the presence of 100 ppm of the synthetic detergents in the digesting material.

Concentrations of 500 ppm of the detergents retarded the digestion processes.

The presence of 100 and 500 ppm of a cationic and an anionic detergent in digesting sludge had no germicidal effect on the coliform or gram positive micrococcus groups of organisms.

Small amounts of fatty acids were readily decomposed to carbon dioxide and methane, and had no effect on the coliform or gram positive micrococcus groups of organisms.

Large amounts of fatty acids were decomposed, but retarded gasification processes. A germicidal effect was observed against the gram positive micrococci.

Gases left in contact with the digesting liquor were shown to contain more methane than those which were drawn off and analysed at earlier intervals.

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