UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SUBJECT: The Mechanism Whereby Methane Gas is Generated in an Anaerobic Digestion of Organic Material

DATE: August 16, 1974

FROM: Edmond P. Lomasney
Region IV, Research & Development Program

TO: Regional Personnel

SUMMARY

We take it for granted that most everything worth knowing about anaerobic digestion of organics has appeared in the literature and that the process is well understood. This is not so--in fact, a good deal of the published literature regarding the behavior of this process may be misleading, especially as concerns the mechanism for generation of methane.

In January of this year, the attached paper pertaining to this subject was presented at an agricultural seminar in Atlanta. At that time there unfortunately were no printed copies available, though many requests were made for copies. Recently, we have obtained copies and as a consequence I am forwarding the attached for your edification.

ACTION

None--the paper is only intended for educational purposes; relating to a biological process for the generation of energy.

BACKGROUND

Our program has had research projects which utilized anaerobic digestion procedure for organics and have yielded strange or unusual results. Some of the reasons for this behavior are indirectly related to the subject matter of the attachment. Though in many respects the generation of methane by this process was considered a known fact, there were times when a specific anaerobic process performed in a relatively inefficient manner for no apparent reason; methane production ceased with the system going sour. With the information generated by Dr. Paul H. Smith at the University of Florida, Gainesville, we were able to have a more thorough understanding of the anaerobic system for generation of methane, and consequently avoid the pitfalls experienced in the past.

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SOUTHEASTERN POULTRY AND EGG ASSOCIATION SEMINAR

Atlanta, Georgia
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TECHNOLOGY OF METHANE GAS PRODUCTION RELATED TO ANAEROBIC DIGESTION OF ORGANIC MATERIAL

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Presented through the courtesy of:

Dr. Paul H. Smith, Project Director, University of Florida, Gainesville, Florida

Cecil Chambers, Project Officer, National Environmental Research Center, Environmental Protection Agency, Cincinnati, Ohio

For Figures, Charts, Graphs, Tables and Pictures

see Appendix

Anaerobic Digestion of Organic Material

This presentation is a summary of work conducted under an EPA Grant on the breakdown of organic material under anaerobic conditions. The work deals with the conversion of complex organic matter such as the material found in domestic and agricultural wastes to methane and carbon dioxide. The conversion is attractive for waste disposal because the products are innocuous, the conversion rates rapid, and the process is economical. The process requires little energy input and produces a product, methane, which may be utilizable as a secondary energy source. The anaerobic digestion processes, however, are made unattractive by the current limited ability for control and regulation. It is probable that our limited abilities to control and regulate the process is based on our limited knowledge of the critical biological steps involved. I shall deal largely with this subject.

The first figure, one (1), illustrates the generally accepted concept of the steps involved in the conversion of insoluble organic material to methane and carbon dioxide. In this scheme there is a conversion of insoluble organic material to soluble organic material, catalyzed by a wide variety of extra-cellular enzymes. The existence of this step has been convincingly demonstrated by a large amount of experimental evidence. Organisms and enzymes capable of carrying out this step have been isolated and studied over a long period of time.

Similarly step two has been definitely established. In this step soluble organics are converted to volatile acids, plus hydrogen and carbon dioxide. Step three however has never been experimentally verified. Volatile acids, primarily acetate, propionate, and butyrate, have been isolated, but repeated efforts in many laboratories to culture organisms capable of metabolizing these acids have all failed. The reason these efforts have failed is that the supposition of the scheme is not correct. Contrary to the general view, the digestion of complex organic substances consists of four stages, as shown in the second figure, two (2). Digestion consists of: hydrolysis of complex organic molecules, acid production, hydrogenogenesis from acids, and methane formation. The second scheme differs from the first scheme in that some fatty acids are converted to hydrogen and carbon dioxide prior to the formation of methane, by a microflora that does not produce methane. What evidence, you may ask, exists in support of the second scheme. I shall now discuss the shaded part of the four stage scheme since this is the portion which differs from the three stage scheme. If the new scheme is correct, it should be possible to quantitatively demonstrate volatile acids including acetate, as intermediates in the process using isotope dilution techniques. With this technique a rate constant is calculated from a linear change in specific activity of added radioactive label. This constant multiplied by the fatty acid pool size gives the rate

of formation of the acid which is then used to calculate the percentage contribution of the specific acid to total methanogenesis. Such rate constants were experimentally determined as shown in the third figure, three (3). This graph shows a linear change in a plot of the log of counts per minutes in acetate against time in a fermentation producing methane at a rate of 0.033 micromoles per milliliter per minute. From the data on this graph a rate constant of 0.0052 per minute is obtained. The acetic acid pool size in the samples was 4.7 micromoles/milli iter. The product of the rate constant times the pool size gives a rate of formation of acetic acid of 0.024 micromoles per milliliter per minute. Assuming that 1 mole of methane is formed from 1 mole of acetic acid, the acetic acid would account for approximately 73% of the methane produced during the fermentation. In the fourth figure, four (4), similar experiments with propionic acid, n-butyric acid and isobutyric acid show that 95% $\pm$ 5% of the methane formed during digestion of domestic waste, had these volatile acids as intermediates. The results show a minimal contribution of the fatty acids to methane of approximately 90% leaving 10% for hydrogen and other oxidizable substrates. In the fifth figure, five (5), the experiments also show that acetate was formed 40% from butyrate and 23% from propionate.

These results are in agreement with both schemes. Sludge would rapidly metabolize the fatty acids, and hydrogen and carbon dioxide

were also rapidly converted to methane. Efforts to isolate fatty acid and hydrogen utilizing methanogenic bacteria resulted in the isolation of a large number of methanogenic bacteria from the sludge. In the sixth figure, six (6), the picture shows some bacterial species isolated from high dilutions of sludge. All of these isolates were present in digesting sludge in numbers exceeding one million per milliliter. The large sarcina converts acetate to methane and carbon dioxide. The others all form methane from hydrogen and carbon dioxide but will not produce methane from fatty acids. Figure seven (7) shows bacteria representing other isolates from sludge. They all form methane from hydrogen and carbon dioxide but do not metabolize fatty acids. Figure eight (8) shows a spirillum, isolated from a high dilution of a propionate enrichment, which is methanogenic and metabolizes hydrogen and carbon dioxide but not fatty acids. Figure nine (9) represents a colony of the spirillum and is included to show its beauty.

The large number of hydrogen oxidizing methanogenic bacteria in digesting sludge suggested that the capacity of digesting sludge for hydrogen metabolism should be great. Capacity of digesting sludge for hydrogen metabolism was determined by incubating digesting sludge under a gas phase of 70% hydrogen and 30% carbon dioxide. Hydrogen uptake and methane formation were determined quantitatively. Methane formation was also quantitatively determined for digesting sludge

incubated under a gas phase of 70% nitrogen and 30% carbon dioxide. The results are shown in figure ten (10). Taking average values, 447 micromoles of hydrogen were utilized in the hydrogen flasks and 210 micromoles of methane was formed. Assuming 1 micromole of methane from 4 micromoles of hydrogen, 112 micromoles of methane would have been produced from added hydrogen and carbon dioxide, leaving 98 micromoles of methane formed from the sludge. However, 134 micromoles of methane were produced from sludge in the nitrogen flasks. The difference between the 134 micromoles of methane produced from sludge in the absence of added hydrogen and the 98 micromoles calculated from sludge in the presence of hydrogen indicates that either hydrogen inhibited methane production from other sludge precursors, or hydrogen and carbon dioxide were converted to molecules other than methane. The rate of methane formation was determined for sludge samples which had been equilibrated for two hours with a gas phase of 70% hydrogen and 30% carbon dioxide. This rate was compared to the rate of methanogenesis from sludge which had been equilibrated for two hours with a gas phase of 70% nitrogen and 30% carbon dioxide as shown on the next figure, eleven (11). The rates were calculated from the methane evolved during the seventy-five minutes immediately following the equilibration period. The lowered rate of methane evolution following exposure to hydrogen gas shows that an inhibition of methanogenesis had occurred. Calculations from

average values of gas utilization and production given in next to last table show an inhibition of 30% during exposure to a 70% hydrogen atmosphere.

The data suggests that hydrogen inhibits the methane formation from some substrates other than hydrogen. In figure twelve (12), the 30% inhibition corresponds to the total methane expected from propionate. This graph shows that turnover of propionic acid metabolism was strongly inhibited by hydrogen since in the presence of molecular hydrogen labeled propionic acid was not diluted, suggesting that an important ecological role of the hydrogen oxidizing methanogenic bacteria in dissimulation processes is the maintenance of a hydrogen concentration low enough to prevent the inhibition of propionic acid metabolism, and the concurrent cessation of the fermentation.

Efforts were made to isolate methanogenic bacteria from propionic acid enrichments. In every case the enrichments were found to contain large numbers of hydrogen oxidizing methanogenic bacteria. These organisms could not metabolize propionate, suggesting that propionate metabolism resulted in the formation of free molecular hydrogen which served as the substrate for the growth of the hydrogen oxidizing methanogens which were isolated.

Propionate acid enrichments were then established under an atmosphere of 100% carbon dioxide. The enrichments were then fed propionate and vigorously gassed with 100% carbon dioxide.

A carbon dioxide absorbing train was placed at the outlet of the fermentation vessel. The carbon dioxide was absorbed in potassium hydroxide and the remaining gas analyzed. The concept here is that under the experimental conditions created where hydrogen was formed from propionate, most of it would be removed from the fermentation liquid before it could be converted to methane and would then be present in the collected gas. This would be consistent with the new scheme. If propionate were converted directly to methane (as proposed in the old scheme) then there would be no hydrogen in the collected gas.

The results are shown in figure thirteen (13). A large amount of hydrogen was obtained. This definitely demonstrates a formation of molecular hydrogen from propionate. Similar experiments are in progress with butyrate. Butyrate enrichments yield hydrogen utilizing methanogenic bacteria which lack the capacity to metabolize butyrate.

The data demonstrates a four step process in methane formation from organic matter.

Every bit of this constitutes basic considerations in terms of the methane production via biological processes. Much of the past information relating to the methanogenic bacterial process has been misleading. The correct interpretation of this basic phenomena must be understood before we can tap this source of gaseous energy. The information and data disclosed in this presentation is the result of long-term work by Dr. Paul H. Smith at the University of Florida,

Gainesville, under funding provisions by the Environmental Protection Agency, Research and Development Program. At present, Dr. Smith continues to investigate this subject, and further disclosures on the mechanism of biological methane production will undoubtedly be forthcoming.

Appendix

FIGURE 1

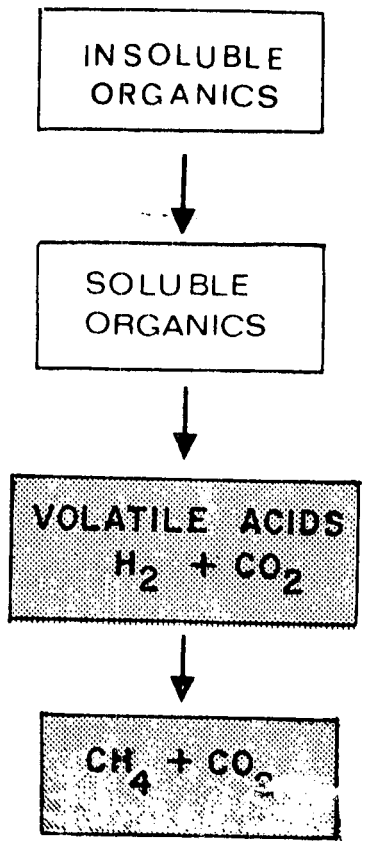
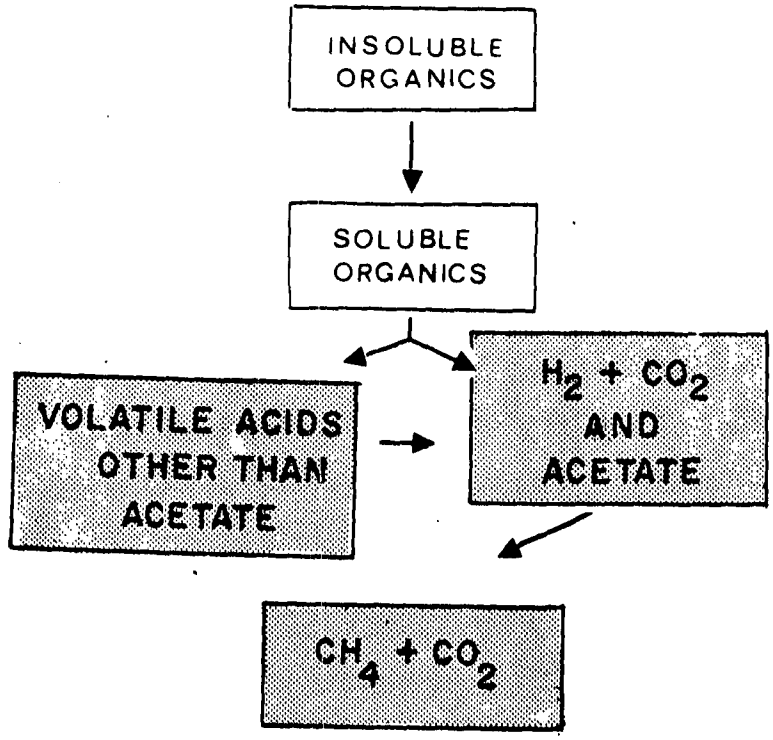

INSOLUBLE ORGANICS
SOLUBLE ORGANICS
VOLATILE ACIDS
$H_2 + CO_2$
$CH_4 + CO_2$

FIGURE 2


INSOLUBLE ORGANICS
SOLUBLE ORGANICS
VOLATILE ACIDS OTHER THAN ACETATE
$H_2 + CO_2$ AND ACETATE
$CH_4 + CO_2$

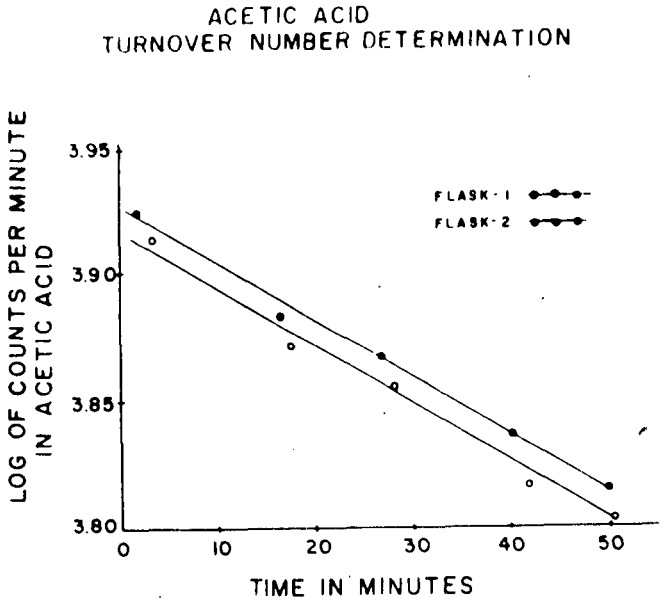


FIGURE 3

PRECURSORS OF METHANE IN DOMESTIC SLUDGE

	% of Total Methane
Acetic Acid	73
Propionic Acid	13
n-Butyric Acid	9
Isobutyric Acid	1.5
Formic Acid	?
TOTAL	95.5 ± 5

FIGURE 4

PRECURSORS OF ACETIC ACID IN DOMESTIC SLUDGE

Precursor	% of total acetic acid
Butyric acid	40
Propionic acid	23
Others	37

FIGURE 5

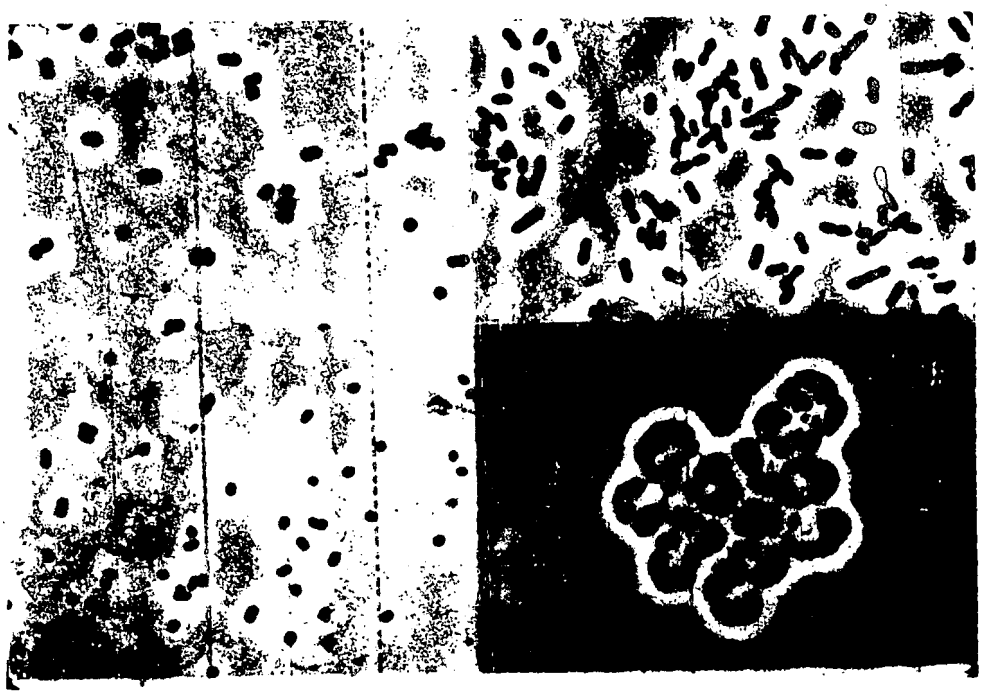


FIGURE 6

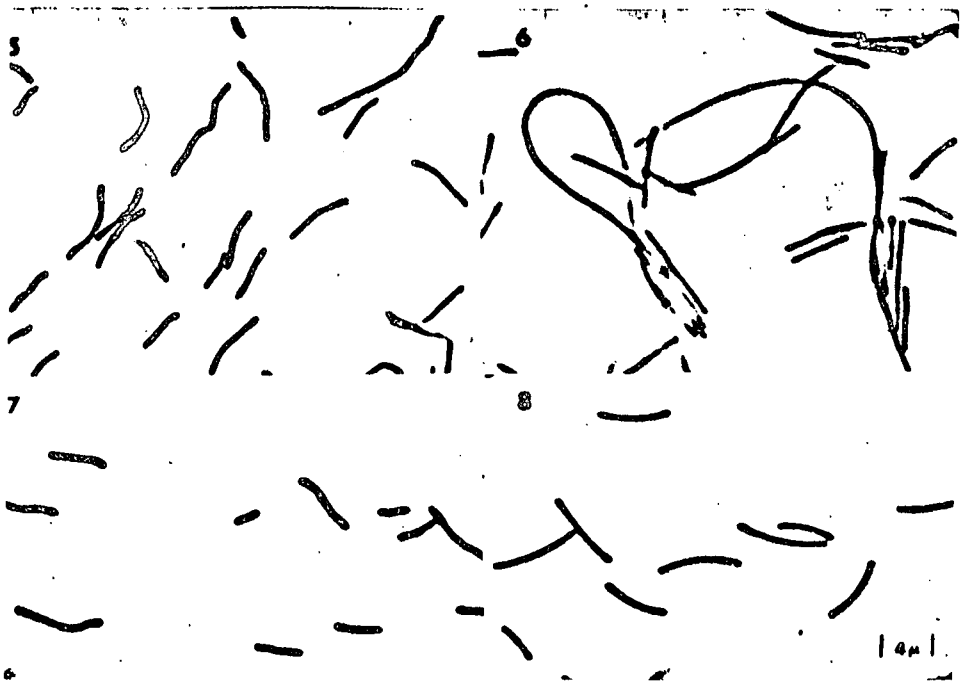


FIGURE 7

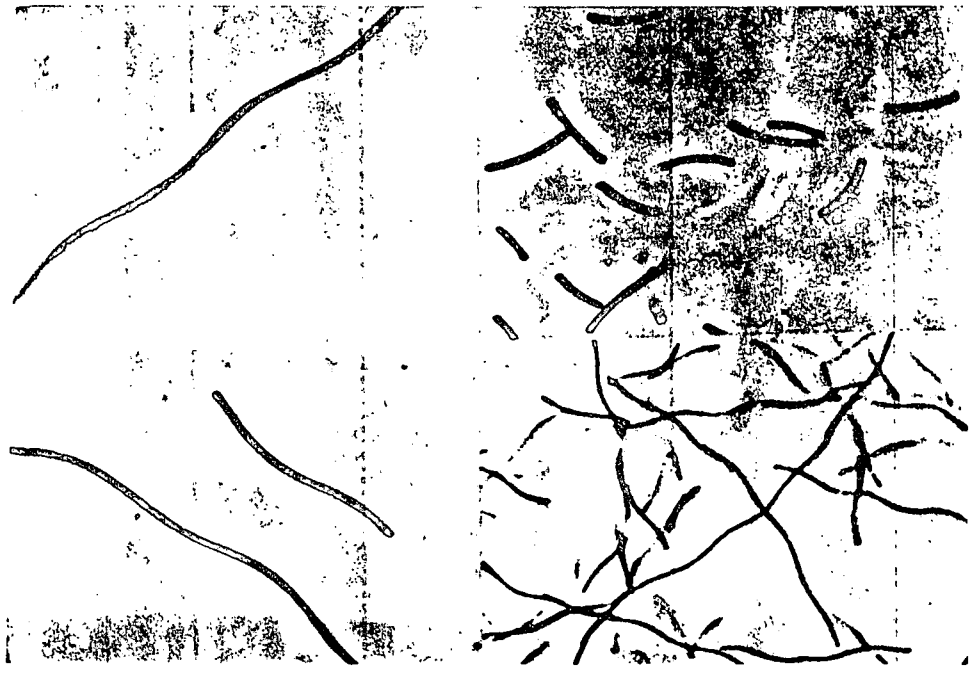


FIGURE 8

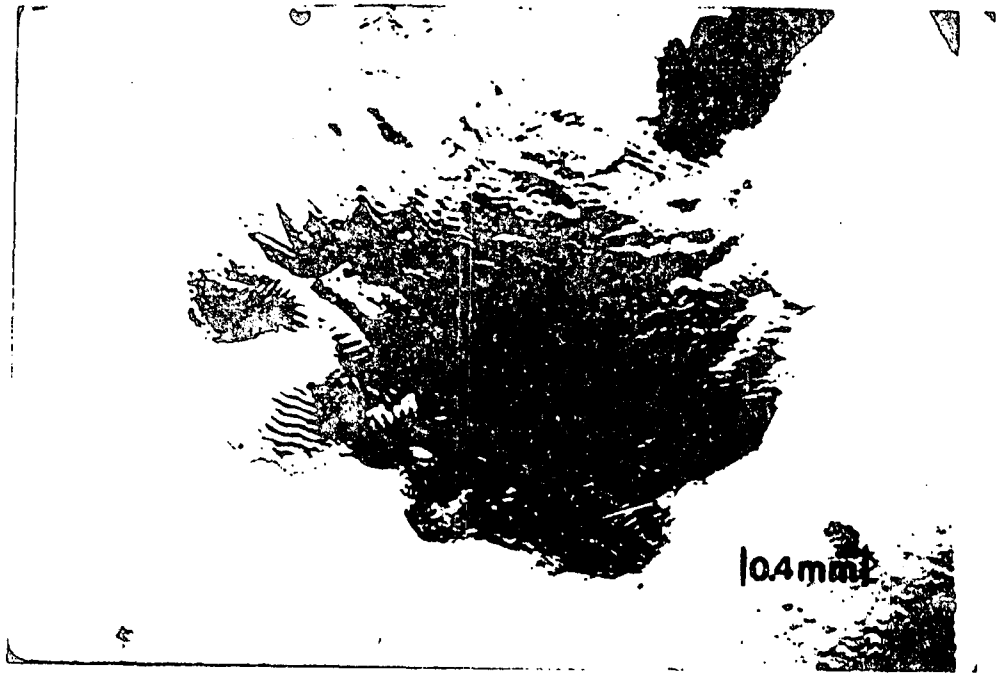


FIGURE 9

HYDROGEN UTILIZATION BY DOMESTIC SLUDGE

Gas Phase	CH_4 Formed in µmole	Initial H_2 in µmole	Final H_2 in µmole
70% H_2, 30% CO_2	207	1650	1220
" "	214	1695	1230
70% N_2, 30% CO_2	132	0	3
" "	136	0	3

FIGURE 10

EFFECT OF HYDROGEN GAS ON SLUDGE METHANOGENESIS

Treatment Prior to Inoculation	CH_4 Formed in μmole	Rate of CH_4 Formation in μmole/ml min^{-1}
Exposed to H_2 gas*	62	0.017
" " "	66	0.018
Exposed to N_2 gas+	75	0.020
" " "	78	0.021

* Equilibrated with 70% H_2 and 30% CO_2 for two hours.

\+ Equilibrated with 70% N_2 and 30% CO_2 for two hours.

FIGURE 11

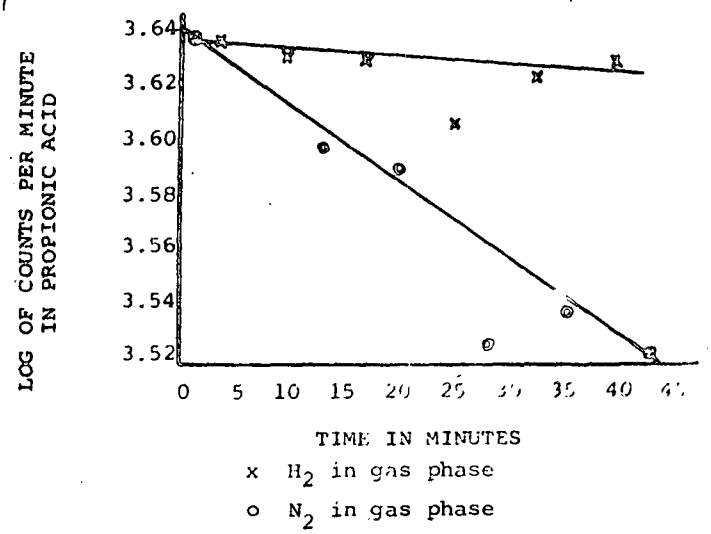


FIGURE 12

TIME IN MINUTES	PERCENT HYDROGEN	PERCENT METHANE
1 — 10	0.9	99
10 — 20	40	60
20 — 30	43	57
30 — 40	43	57
40 — 50	43	57
50 — 60	43	57

FIGURE 13