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STATE OF ILLINOIS
DEPARTMENT OF REGISTRATION AND EDUCATION

DIVISION OF THE
STATE WATER SURVEY

A. M. BUSWELL, Chief
W. D. HATFIELD, Editor

BULLETIN NO. 32

ANAEROBIC FERMENTATIONS



[Printed by authority of the State of Illinois.]
URBANA, ILLINOIS

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ORGANIZATION

STATE OF ILLINOIS
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DEPARTMENT OF REGISTRATION AND EDUCATION
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PREFACE.

This bulletin presents the results of some ten years' study of the process of anaerobic fermentation as it occurs in the degradation of organic matter in nature. Though much of the work reported here has been previously published in technical journals, a considerable amount of unpublished material is included, especially on the fermentation of natural substances. All the material has been condensed and rewritten so as to present a coherent discussion of this very interesting subject. Three previous publications have been issued by this laboratory in this field, namely: "Chemical Studies on Sludge Digestion," Circular No. 8; "Studies on Two-Stage Sludge Digestion," Bulletin No. 29; and "Laboratory Studies of Sludge Digestion," Bulletin No. 30. In the earlier work the emphasis was on the anaerobic phase of waste treatment while the studies reported here stress the anaerobic production of methane. The mechanism of the fermentation of fats to CH_4 and CO_2 reported by Neave has been extended to carbohydrates and proteins in this report.

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I

SUMMARY.

The formation of methane in nature has interested bacteriologists and chemists for more than sixty years. The studies in this field, commencing with a search for an explanation of the "Will O' the Wisp" have progressed to a point where methane enough for fuel for power plants of several thousand horsepower is produced by anaerobic fermentation.

The literature in this field was summarized some years ago by McBeth and Scales⁽¹⁷⁹⁾, and by Stephenson⁽³²⁴⁾. Briefly, it was known that moist organic matter when allowed to decompose under restricted oxygen conditions yielded H_2 , CO_2 , CH_4 , and a variety of organic acids in greater or less degree. Little or no quantitative data on the yield of the various products were available and little had been done with pure compounds although Sohngen⁽³¹⁴⁾ had shown that lower fatty acids with an even number of carbon atoms could be decomposed by mixed cultures giving CH_4 and CO_2 . Whether H_2 and fatty acids were necessary intermediates in the process of methane formation was not known. The information on the susceptibility of various natural products to methane fermentation was conflicting. Cellulose was known to ferment to some extent^(249, 224) but "ligno-cellulose" so called was said to be resistant⁽⁸⁶⁾. One author held that grease would decompose anaerobically⁽²³¹⁾ while another held that it would not ferment to any substantial extent⁽³⁵⁷⁾. The addition of lime to favor methane production was preferred by one group of workers and opposed by another.

Of the earlier systematic studies of this fermentation those of Sohngen were the most extensive⁽³¹⁴⁾. More recent studies are those of Fowler and Joshi⁽⁸⁶⁾, Sen, Pal, and Ghosh⁽³⁰¹⁾; and Fischer, Lieske, and Winzer⁽⁷⁷⁾.

CHARACTERISTICS OF METHANE FERMENTATION.

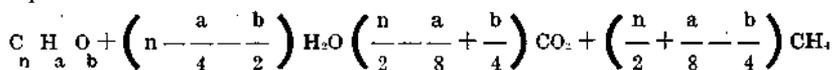
Mixed Culture. The anaerobic fermentations as carried out for the production of methane differ in many respects from other types of fermentations. The most important difference is perhaps the fact that it is not necessary to use a pure culture of organisms nor is it necessary to maintain "purified" cultures for inoculation or reinoculation. The bacteria which are capable of producing methane are found almost universally in nature although in preponderating numbers in mud and decaying matter. Under proper conditions these bacteria can be cultivated to a high degree of activity within a few days. The culture can then be maintained at this high degree of activity indefinitely, providing a few simple rules concerning chemical and physical environment are followed.

In fact, microscopic and subculture studies of this fermentation reveal such a mixed and variable flora that it appears at present to be environment rather than flora which determines the result obtained.

Continuous Process. This situation makes possible the continuous operation of the fermentation, a procedure which is at least very unusual in fermentology. It is possible to carry on this process in apparatus arranged to allow the substrate to enter continuously at one point and the exhausted or inert residue to be discharged continuously at another, while the products, methane and carbon dioxide, are given off at a steady rate. - There is apparently no limit to the size of apparatus which can be used. Large tanks yielding several hundred thousand cubic feet of gas a day operate as smoothly as laboratory size flasks.

Independent of Substrate. A third characteristic of these fermentations is that practically any sort or kind of organic matter may be used as a substrate. Nearly a hundred different pure substances^(5, 332, 335) and some thirty or forty natural plant and animal products^(22, 23, 43) (corn-stalks, milk whey, etc.) have been used successfully as fermentation material. There is apparently no decomposition of mineral oils, and lignin, when isolated, is attacked not at all or with difficulty^(25, 174, 218).

Quantitative Yields. Fourthly, the nearly quantitative yields of the two simple products carbon dioxide and methane are somewhat unique. It is true that in the various commercial fermentations of grains the starch is practically quantitatively recovered in the products but the fats, proteins and fiber are not attacked at all. The methane fermentation converts the entire grain, with the possible exception of a small amount of fiber, to CO₂ and CH₄ within 24 to 72. hours. The reaction is an oxidation-reduction involving water. If the composition of the substrate is known, the yield of gas can be calculated from the following equation⁽³³²⁾.



With a little care, it is possible to get 95-100 per cent agreement with this equation. The uniformity of the end products, CO₂ and CH₄, was at first puzzling. A study of the energy⁽³³²⁾ of the reaction of various compounds with water showed the maximum free energy when the final products were CO₂ and CH₄. In our experience, neither carbon monoxide nor higher hydrocarbons have been observed among the products.

Wide Temperature Range. A fifth characteristic is the absence of any narrow optimum temperature range. The rate of fermentation increases with increase in temperature from 0° C. or perhaps below to 55° C. or a little higher. Maxima have been reported at 26°, 37°, and about 50° -55°⁽⁷⁴⁾. These maxima are not very pronounced and have not been noticed by some observers.

Although this fermentation can be carried on over a wide range of temperature it may be upset by sudden temperature changes. A drop of five degrees from that at which the culture has been working will frequently arrest the production of gas without materially affecting the

production of acids. This may result in a dangerous accumulation of acids.

CONTROL OF METHANE FERMENTATION.

The high yields, uniformity of products, and successful inoculation and cultivation are dependent on four conditions.

Large Surface. First, the organisms seem to require a certain amount of surface for their propagation. When material of a fibrous or granular nature is fermented, this requirement is satisfied by grinding or shredding the substrate, but when dissolved substrates are employed, some sort of inert surface must be supplied. We have used shredded washed asbestos for this purpose⁽³⁰⁾ employing 25 grams per liter. Fischer, Lieske, and Winzer⁽⁷⁷⁾ have found ferric hydroxide or ferrous sulfide useful for the same purpose. With this technique a large number of soluble substances have been used as substrates and over considerable periods of time. Successful inoculation can be made by transferring single fibers of asbestos⁽³⁰⁾ from an active culture to a flask or test tube containing sterile asbestos and culture media.

Volatile Acids. The second condition is that the volatile acids⁽⁴⁵⁾, which are intermediates in the decomposition of higher compounds, must not exceed a predetermined value usually from 2,000 to 3,000 p.p.m. (calculated as acetic).

If the volatile acid value is allowed to rise much above 2,000 p.p.m. (as acetic) gas formation drops off, the acids increase rapidly, and usually within 24 to 48 hours all fermentation has ceased.

This factor is followed by making daily determinations of the volatile acids by the method of Duclaux⁽⁶⁹⁾ after acidification of the sample with phosphoric or sulphuric acid. This procedure gives a value which includes both the free acids and their salts and is independent of the pH value. It is somewhat surprising to encounter such a situation, especially since we have come to think of pH as an almost all important factor in fermentation control. Since it is the total of acid plus its salt which controls the fermentation, the addition of alkali is of little use. In fact, the production of acid is stimulated in some cases by the addition of lime.

There is only one way to limit the accumulation of volatile acids. That is to limit the rate at which the substrate is added to the fermentation vessel, so that the acids will be fermented to CH_4 and CO_2 as rapidly as they are formed from the raw substrate. Many of the early failures are due to the "batch" type of fermentation in which too much substrate was present.

If a culture has developed too much acid the only remedy is dilution. This has been adapted to a series or stage digestion for certain industrial wastes⁽³⁷⁾.

Scum. A third condition which must be avoided is the formation of any considerable amount of scum. This is sometimes encountered when fermenting greasy material⁽²⁰⁹⁾. The objection to scum is that it constitutes a zone of high substrate concentration in which a high concentration of acids is likely to develop. In laboratory fermentations, this can be avoided by mechanically breaking up the scum. In plant scale

fermentations, the power required for a scum breaker is too great. Moistening the scum with liquid pumped from beneath it is a very effective and inexpensive remedy⁽³⁵⁾.

Fibrous Material. A fourth difficulty is encountered with fibrous material such as paper, shredded cornstalks, etc. These substances form a tough mat at the top of the fermentation vessel⁽²⁷⁷⁾. The objection to this mat is, as above, that it favors the accumulation of large amounts of acid. A mat cannot be broken up mechanically nor by the circulation of liquor with any success. It is necessary to provide the fermentation vessel with suitable connections so that it can be operated alternately in an upright and an inverted position. In this manner, the mat is broken up after each inversion by the fermentative action itself. A fermenter containing a slowly rotating drum has been developed for large scale operation⁽³⁹⁾.

LOADINGS.

We wish to point out that the above difficulties are not met with in the ordinary plants for the reduction of municipal wastes except at times of starting new plants or of inexcusably bad supervision. The digestion or fermentation tanks are so large in proportion to the load that volatile acids seldom accumulate and any scum which may form may be allowed to accumulate for a year or more without seriously interfering with the operation of the tank. It is common practice to design municipal plants with 50 cu. ft. of digester capacity for each pound (3 m³ per kilo) of organic matter which is to be fermented per day.

The writer uses loadings of from 0.2 lb. per cubic foot (3 kilo per cu. meter) per day up to 1 lb. per cu. ft. (16 kilo per cu. meter) per day quite successfully and continuously. It is necessary, however, to observe the rules laid down above.

GAS YIELDS.

The actual gas yields⁽⁴⁰⁾ range from 12 cu. ft. per pound decomposed (700 c.c. per gram) for protein to about 20 cu.ft. per pound decomposed for fats (1250 cc. per gram). From the loadings given above, it is seen that the ratio of the volume of gas yield to the volume of fermenter varies from 1:1 to 4:1 in most cases. In laboratory experiments as much as 10 liters of gas have been obtained per day. per liter of fermenter volume.

COMMERCIAL DEVELOPMENT.

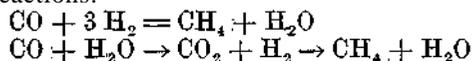
The commercial application of this fermentation dates well back into the last century when it was employed merely to stabilize and humify organic wastes (i. e. in septic tanks).

It was not until 1897 that a waste-disposal tank serving a leper colony in Matunga, Bombay, was equipped with gas collectors and the gas used to drive gas engines⁽⁸⁵⁾. At about the same time, the waste-disposal tanks at Exeter, England, were partially equipped with gas collectors and the gas was used for heating and lighting at the disposal works. In 1911, a company was formed in Australia for the purpose of

producing and using fuel gases which resulted from the biological decomposition of municipal wastes. In this country in 1915, Hommon equipped some waste-treatment tanks with gas collectors and used the gas⁽¹²⁸⁾. In 1920, John Watson, of Birmingham, England, reported a study of methane production from sludge digestion and called attention to the fact that a considerable amount of methane can be produced in this way⁽³⁸¹⁾. Following his suggestion, the new disposal plant which was put into operation in 1927 by his successor, Mr. Whitehead, is equipped with gas engines that are being operated on the gases produced from sludge digestion. This use of the gas cuts down very materially the operating cost of the disposal works. In the meantime (1925) Imhoff in Germany had equipped the sludge-reduction tank in Essen with gas collectors and connected them to the city mains. The gas is found satisfactory for general municipal use and is sold to the city. In the same year, Buswell and Strickhouser⁽⁴⁶⁾ observed that the sludge-reduction tanks at Decatur, Illinois, were producing about 200,000 cubic feet of gas a day. This large yield is due to a considerable amount of wastes from a starch works which are discharged into the city drainage system. The average yield at Decatur is about 125,000 cubic feet of gas per day. At present the use of gas for power or heat at municipal disposal plants is almost universal in America.

When an industrial waste is very concentrated (1% to 6% solids) as in the case of distillery, dairy, and packing-house wastes, methane fermentation offers an attractive method of treatment^(21, 24, 36, 43). The operating cost is small although the original investment is rather large. Altogether it appears that gas can be produced from these wastes at from 5 cents to 10 cents per thousand cubic feet.

An interesting application of this fermentation is that proposed by Fischer, Lieske, and Winzer⁽⁷⁹⁾, namely, the detoxication of coal gas by means of the reactions:



Sohngen⁽³¹⁴⁾ found that hydrogen was formed in this reaction and suggested that it was intermediate in the formation of methane. He showed that in the presence of the methane-forming culture the reaction $3\text{H}_2 + \text{CO}_2 = \text{CH}_4 + \text{H}_2\text{O}$ took place quite readily. The writer has confirmed this observation of Sohngen⁽³³¹⁾. The volume efficiencies have not been developed to date but this phase of the process holds interesting possibilities.

HYDROGEN FORMATION.

Since the topic assigned includes "other combustible gases" a brief statement covering this phase of the subject must be included.

So far as we are aware hydrogen is the only other combustible gas formed by fermentation. Occasional reports of ethane are to be found in the literature and carbon monoxide has been mentioned. The amounts have been so small that when the limits of accuracy in even the most careful gas analyses are considered it is very doubtful whether the data

have justified the conclusions in regard to the presence of the last two gases.

Hydrogen is not formed in detectable amounts during the methane fermentation of the lower fatty acids although it may well be an intermediate. When carbohydrates are used as substrates the hydrogen may be as high as 30% by volume⁽³³⁵⁾ although with proper control the hydrogen can be held to about 1%.

There appear to be only two types of fermentations yielding hydrogen which have been subjected to quantitative investigation. One is the fermentation of sugars by the coli group of organisms and the other the "butanol-acetone" fermentation.

Extensive quantitative data on the coli fermentation are given by Eogers, Clark and Adams⁽²⁷²⁾. Their work indicated that in the main from 30% to 50% of the carbohydrate was decomposed and probably not more than a few per cent occurred as gas. Three types of fermentation reactions were encountered. The one giving the highest yield of gas produced CO_2 and H_2 in the ratio of about 2:1. The second type produced about half as much gas with a CO_2 : H_2 ratio of 1:1. The third type gave CO_2 only and showed relatively little gas yield. The second type has been investigated by Scheffer⁽¹⁵⁰⁾ who found about 10% yield of gas of a CO_2 : H_2 ratio of 1:1.

A discussion of the butanol fermentation is beyond the scope of the present paper. As commercially carried out there is a yield of one and one-half pounds of CO_2 and H_2 for every pound of mixed solvents⁽¹⁰⁰⁾ produced. These gases are utilized to produce methanol by the catalytic process.

II

REVIEW OF THE LITERATURE.

A. GENERAL.

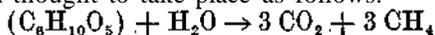
The biological decomposition of cellulose and cellulosic materials has been studied for over 70 years. In spite of this fact there is still much that is unknown about the mechanism and the agents that bring about the different types of decomposition. This review will cover only those articles dealing with the bacteriological degradation of these substances. Special attention will be given the facultative and anaerobic work.

References on the mechanism of the bacteriological degradation of cellulose and cellulosic materials and especially those dealing with the anaerobic fermentation of these materials, are scarce and far from enlightening. As little original work along this line is offered in this bulletin, this phase of the problem will be referred to only incidentally. Additional information may be found in the periodic literature, in texts to be referred to later and in M. Stephenson's book "Bacterial Metabolism"⁽³²⁴⁾.

For a review of the literature which covers not only the bacterial degradation of cellulose and cellulosic materials but also those decompositions attributed to other forms of biologic life, the reader is referred to Thaysen's and Bunker's book "The Microbiology of Cellulose, Hemicelluloses, Pectin and Gums"⁽³⁴⁷⁾, and to Thaysen and Galloway's "The Microbiology of Starch and Sugars"⁽³⁵³⁾.

From the references cited below one may conclude that:

1. There are both mesophiles and thermophiles that are known to decompose cellulose and cellulosic materials.
2. There are a large number of mixed and pure cultures of aerobes that have been found capable of decomposing cellulose and cellulosic materials to numerous end products, the main ones being organic acids, hydrogen, carbon dioxide, and water.
3. There have been a large number of cases cited where mixed flora have been able to anaerobically decompose cellulose and cellulosic materials to organic acids, humus and carbon dioxide and hydrogen or carbon dioxide and methane.
4. The gasification of cellulose to give a 1:1 ratio of CO₂ and CH₄ has been thought to take place as follows:



Many think that the gaseous end products, CO₂ and H₂, are formed first. If the proper bacteria (enzymes) are present, part of the CO₂ is thought to react with all the H₂ to give as the

final end products CO_2 and CH_4 . Low rates of gasification as well as low gas yields per gram of material added accompanied with moderately high organic acid yields characterize all studies prior to those of Boruff and Buswell. There is some evidence that cellobiose and glucose as well as numerous fatty acids are formed in the anaerobic degradation of cellulose.

5. The few pure cultures of anaerobic cellulose decomposers reported either give no gas or only carbon dioxide and hydrogen. Omelianski reported a methane producer but the purity of his cultures have been doubted by certain modern investigators.
6. Many have noted the quantities of combustible gases, mainly mixtures of carbon dioxide and methane, that are formed in the anaerobic degradation of crude plant tissue by impure crude cultures of anaerobes but it remained for Fowler and his co-workers to try to develop this to a state where it would be possible to use these materials for the development of power and fuel gas. He failed to recover attractive volumes of gas nor did he develop a continuous process.
7. The hexosans, pentosans, hexoses, pentoses, waxes, resins, fats, pectin, and nitrogenous complexes found in cellulosic materials (crude plant tissues) are fermented both aerobically and anaerobically to form organic acids, alcohols and gaseous end products. Methane is formed by crude anaerobic cultures.
8. There is a question as to whether lignin may be fermented to gaseous end products by aerobes.

B. DETAILED HISTORICAL SUMMARY.

Cellulose

Even while the theory of spontaneous generation was still held in high regard in some quarters, records were made of observations on the decomposition of plant materials. Volta (1776) showed that moist soil heavily laden with organic matter almost always contained a combustible gas. Later Bunsen and Hoppe-Seyler confirmed this observation and noted further that it was particularly energetic during the warm summer months. Omelianski⁽²²⁷⁾ reported that so much gas (CH_4 and CO_2) was liberated from the delta of the Mississippi River that at one time plans were made to use it for lighting purposes.

In 1850 Mitscherlich⁽¹⁹⁴⁾ observed a rod shaped vibrio which was capable of removing the structural walls from around the starch grains in a potato. After this observation he suggested that microorganisms might be the cause of the disappearance of cellulose in nature. In 1856 Eiseit⁽²⁶⁷⁾ found methane being liberated from decomposing manure piles and with this as a basis he proposed studying these gases hoping that such a study would throw light on the reactions by which nature was disposing of its dead organic matter.

Sellers⁽²⁹⁹⁾ obtained in 1863-4, two U. S. Patents for the removal of non-fibrous matter from plant materials. The first was a steaming process but the second was based on the aerobic fermentation of these constituents. Shortly after this (1873) Eoutledge⁽²⁷⁴⁾ obtained a

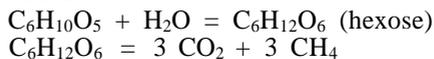
U. S. Patent on a retting process which depended on the natural fermentation of the non-fibrous materials following lime treatment. The tanks were covered and no air was introduced. No gas collection or data are reported. Pennington⁽²³⁷⁾ (1893) patented a synthetic salt mixture and the use of a certain "microbe mixture" to aid in the retting process. No gas data are given.

Trecul writing in 1865⁽³⁵⁹⁾ described three cellulose-decomposing bodies which he isolated from macerated plant tissues. He gave them the name, *Amylobacter*, because they were stained blue with iodine. He, however, was still of the old school, because he regarded these bodies as having arisen in the liquids through spontaneous generation. This same year Nylander⁽²¹⁹⁾ showed that these bodies were motile anaerobes. Van Tieghem (1877)⁽³⁶⁵⁾ reported Trecul's bodies to be anaerobic, motile bacilli capable of dissolving cellulose and changing it to glucose, as well as to CO₂, H₂ and certain organic acids. He also reported (1879)⁽³⁶⁶⁾ that the various forms noted by Trecul were merely stages in the life cycle of the organism. He saw fit to state that the organism was identical with Pasteur's *Vibrio butyrique* as well as Mitscherlich's vibrio.

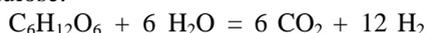
In 1875 Popoff⁽²⁴⁹⁾ published one of the early classics in the microbiology of cellulose. He investigated the causes of the natural evolution of methane in stagnant ponds, as well as the anaerobic decomposition of pure filter paper, potatoes, hay, gum arabic, glucose, certain fatty acid salts and numerous other products. Popoff found 40 degrees C. to be the optimum temperature for gas production. His gas showed, after a two-month period, a 1:1 ratio of CO₂:CH₄ which ratio he claimed to be that characteristic of the anaerobic fermentation of cellulose.

In 1880 Prazmowski⁽²⁵⁰⁾ announced the finding of two anaerobes, *Clostridium polymyxa* and *Vibrio rugula*, that were capable of fermenting cellulose to CO₂ and H₂.

The papers of Hoppe-Seyler⁽¹³⁰⁻¹³³⁾ are classics. He placed 25.773 grams of pure filter paper in a bottle, inoculated it with a small quantity of slime and filled it almost full of water. The bottle was closed and the gas evolved was collected over mercury. In all, 95 gas analyses were run. After a period of four years he found there had been formed from the 15 grams of filter paper that had disappeared, 3.281 cc. of CO₂ and 2,571 cc. of CH₄. From these data he concluded that the following two reactions must represent the mechanism of the methane type of decomposition:



He was unable to establish whether humus was formed. He found that ferric iron, manganese and sulfates inhibited the formation of methane. Gypsum did not interfere. He reported that he noted Van Tieghem's *Bacillus amylobacter* in his cultures. He also studied the decomposition of calcium acetate and xylan^(132, 133) and secured methane from both. The following reaction was proposed as representing the H₂ type of fermentation of cellulose:



Tappeiner contributed many articles on the decomposition of cellulose (cellulose, hemicellulose and pectin). In 1882-3^(333, 334) he associated microorganisms with the intestinal decomposition of cellulose in ruminants and questioned the idea of an intestinal cellulose. Haubener and Sussdorf (1859)⁽¹¹⁸⁾ had previously called attention to the fact that only about fifty per cent of the cellulose fed to ruminants could be recovered. Tappeiner also stated that there were two types of ferments, namely, the H_2 - CO_2 type which was associated with a slightly alkaline reaction, and a CH_4 - CO_2 type which was associated with a slightly acid medium. He regarded the methane type as the principal one found in the paunches and intestines of animals. He obtained high yields of organic acids. In a small experiment in which he used filter paper as the substrate, he recovered approximately 60 per cent of the carbon that was fermented as acids, and only 10 per cent by weight as methane, and 23 per cent as CO_2 .

Van Senus (1890)⁽³⁶⁴⁾ was the first to attribute the decomposition of cellulose and cellulosic materials to the joint activity of organisms. He was also of the opinion that the primary end-products were H_2 - CO_2 , and acetic acid, and that the CH_4 came from the interaction of the H_2 and the acetic acid to give acetaldehyde, then alcohol, and finally CH_4 . Van Senus' ideas concerning the associative action of organisms were strengthened in 1899 by Macfayden, Allen and Blaxwell's⁽¹⁸²⁾ announcement that they considered their thermophilic cellulose decompositions to be symbiotic in nature. The role of symbiosis in the gas metabolism of bacteria has been extensively studied by Castellani and others⁽⁵¹⁾.

Passing over a number of minor studies of this period one's attention is called to the classical studies of Omelianski⁽²²¹⁻²²⁸⁾. For over ten years (1894-1895) he published the results of his studies on cellulose and closely related subjects. These included the isolation in pure culture of two anaerobes, one *Bacillus methanizenes*, a CO_2 - CH_4 producer, and the other *Bacillus fossicularum* (*B. fermentationis cellulosa*), a CO_2 - H_2 producer. The purity of these cultures has been questioned by McBeth and Scales⁽¹⁷⁹⁾ and Clausen⁽⁵³⁾ but upheld by Thaysen⁽³⁴⁷⁾. Both organisms were grown at 35 degrees C. and were found to be morphologically very similar. The CH_4 producing organisms could be killed by heating at 75 degrees C. for 15 minutes. The H_2 producing organism could withstand this heat treatment. Omelianski took advantage of this difference in heat tolerance and used it to clear his mixed cultures of the methane producing form. In the CO_2 - H_2 type of fermentation he recovered⁽²²³⁾ about 65 per cent of the weight of the cellulose added as acids while only 29 per cent and 4 per cent were recovered as CO_2 and H_2 , respectively. The organic acids were found to consist of a 1.7 to 1 mixture of acetic and butyric with traces of valeric. In the CO_2 - CH_4 type of fermentation Omelianski⁽²²⁴⁾ got about 50 per cent by weight of the cellulose added converted to volatile organic acids, while 7 per cent and 42 per cent were converted to CH_4 and CO_2 , respectively. The volatile acids were found to be composed of 9 parts of acetic to every part of butyric. No non-volatile acids were reported.

In 1903, Maze⁽¹⁸⁷⁾ reported a pseudo-sarcina which was capable of decomposing dead leaves to give a gas containing 65 to 66 per cent CH₄. No data are given to show the source of the gas. Upon heating his impure cultures at 60 degrees C. for eight minutes, the sarcina was killed. The other forms left following this treatment were capable of producing acids (acetic and butyric) as well as CO₂ and H₂. When the sarcina form was present the acids did not accumulate and CH₄ was formed instead of H₂. Impure cultures of the sarcina were not capable of producing gaseous end-products. These anaerobic studies also show the importance of symbiosis in the natural decay of organic matter. Maze reported further on these organisms in a paper published in 1915⁽¹⁸⁸⁾.

Van Iterson (1904)⁽³⁶³⁾ seems to have been the first to report laboratory studies on the aerobic decomposition of cellulose by bacteria. He found that his mixed cultures gave CO₂ as the main end-product.

Sohngen^(314, 315) not only verified Omelianski's methane and hydrogen types of fermentation of cellulose, but also did important work on the methane decomposition of fatty acids. He also favored Omelianski's idea that the reaction, $4 \text{H}_2 + \text{CO}_2 = \text{CH}_4 + 2 \text{H}_2\text{O}$, might be the key reaction that determines whether hydrogen or methane is to be recovered. He was able to quantitatively convert a mixture of CO₂ and H₂ to CH₄ by bubbling it through a formate decomposing medium.

The classical studies of Pringsheim⁽²⁵¹⁻²⁶²⁾ have contributed much to the knowledge of cellulose degradation. In 1910 he called attention to the symbiotic relation existing between the cellulose decomposers and the nitrogen fixing organisms in the soil^(255-257, 260). In some cases about 45 per cent of the destroyed cellulose was recovered as acetic acid, small amounts of formic acid, and large amounts of CO₂ and H₂. He was also successful, in isolating cellobiose and dextrose as bacteriologically split products of cellulose. As to their importance in nature, Pringsheim⁽²⁵³⁾ classified the cellulose decomposing organisms in the following order: (1) mold fungi, (2) aerobes, (3) denitrifiers, (4) methane producers, (5) hydrogen producers, and (6) thermophiles. He was also the first to report investigations on the anaerobic decomposition of lignin. Frau Lichtenstein⁽¹⁷⁵⁾ had already reported two aerobes that could modify it. Pringsheim and Puchs⁽²⁶²⁾ found, in 1923, that (anaerobically at 37 degrees C.) the ammonia salt of lignin was altered, but no gaseous end-products were obtained.

Kroulik described, in 1912⁽¹⁵⁶⁾, two new cellulose-decomposing organisms, one an aerobic thermophile and the other a facultative anaerobic thermophile. The aerobe decomposed 70 per cent of the cellulose added to carbon dioxide and acetic and butyric acids and was much more active than the anaerobe which gave as end-products, carbon dioxide and hydrogen, and the same acids as the aerobe only in larger amounts.

Kellerman, McBeth and Scales (1912-13)^(145, 179) report that the methods of Omelianski and Van Iterson are unsuccessful in producing pure cultures. They have reported two contaminating cellulose destroyers in Omelianski's hydrogen cultures and likewise five such organisms in his methane cultures. They also found that Omelianski's hydrogen cultures could be grown aerobically. They isolated seventeen new aerobes, one a thermophile, and seventy-five species of molds that were

able to decompose cellulose. They report that none of the bacteria isolated were able to produce gas in pure cultures. Some species⁽¹⁷⁹⁾ formed formic and acetic acids while others gave only traces of fatty acids. None gave tests for alcohol, aldehydes, ketones or carbohydrates capable of reducing Fehling's solution. In 1916 McBeth⁽¹⁷⁸⁾ announced the isolation of more new aerobic cellulose-decomposing bacteria. He found that nitrogen in the form of peptone, casein, $(\text{NH}_4)_2\text{SO}_4$ or KNO_3 could be utilized by the aerobes studied. The work of Löhnis and Lochhead (1913 and 1923)^(176, 177) was much of the same type as that of the authors just mentioned. They found beef extract (0.1 per cent) to be the most suitable source of nitrogen. They also favor the idea that the decomposition of cellulose in nature is a symbiotic process. Horovitz^(133a) reported in 1915, that he had isolated three new aerobes that would decompose cellulose at 37 degrees C.

Oechsner (1916)⁽²²⁰⁾ like Omelianski, got a large per cent (50.2) of his cellulose converted to organic acids. The remainder of his material fermented to carbon dioxide and methane. The acids formed were found to be composed of acetic and butyric with traces of propionic.

Schmity (1919)⁽²⁹²⁾ has studied the relation of bacteria and fungi to cellulose fermentations in nature. He says: "Cellulose-destroying bacteria take no important part in the decay of wood under natural conditions, but the rate of decay by fungi may be increased materially by the presence of ordinary saprophytic bacteria."

Fowler and Joshi⁽⁸⁶⁾ in 1920, made the first attempt toward developing a process for the production of power and fuel based on the idea that anaerobic bacteria give methane as a fermentation product of cellulose and cellulosic materials. In their studies they used filter paper as representative of "normal celluloses," newspaper as representing "lignocelluloses" and banana skins, banana stems and mahua waste as representing the "hemicelluloses." They inoculated these materials and others with sewage sludge and collected the gases evolved in the composition. Their gas analyses show the effect of the large quantity of organic (sewage) matter present in the inoculum, but even in the presence of this material, they obtained low gas yields. The gases from their filter paper-inoculum bottles contained 81 per cent CH_4 and 14.5 per cent H_2 . The rest was CO_2 . They state that they were able to obtain a daily volume of combustible gas equal to 80 per cent of the volume of the space occupied by the fermenting material, but they fail to state what the material was or for how long a time they were able to get this yield. They observed a greater number of rod-like bacteria in the sludge than in the supernatant liquor. They also noted that they could get more rapid fermentation when they replaced part of the mother liquor each day with distilled water. On the basis of their low gas yields, they concluded that commercially the process had no possibilities. In speaking of the digestion of plant residues in the crude state they say: "These was no fermentation at all, showing that cellulose is not attacked when it is in, combination with substances like pectin, lignin, etc., which are always present in the raw vegetable tissues." Their gas recovery data are summarized in Table I. Dubdin⁽⁶⁶⁾ also reports that "woody fiber, such as paper pulp and debris from street

paving, is especially refractory . . . " Sen, Pal, and Ghosh⁽³⁰¹⁾, also of India, have recently (1929) tried to ferment anaerobically a water hyacinth. Their digestion mixture went acid (pH 3.8) in seven days, during which time, however, they had recovered 1800 cc. of gas which contained about 22 per cent carbon dioxide and 52 per cent methane. The experiment was carried out on a 100 gram sample of the green hyacinth. These data also show low gas yields. It is quite probable that the air (oxygen) which was present in the gas above the digestion mixture was what brought about the acid condition and stopped the anaerobic digestion.

Esselen⁽⁷²⁾ has made some calculations, seemingly based on Fowler's work, as to the size of a "pit" necessary to furnish gas for a home. He, however, gives Fowler's data every benefit of the doubt and fails to realize that Fowler's data are not based on a continuous feeding and gas evolution basis. No such experiments were conducted by Fowler.

TABLE I.
COMPARATIVE GAS YIELD DATA, (CO₂ + CH₄), BATCH EXPERIMENTS".

Material fed.	Fowler and Joshi. ¹		Omelianski. ²		Schloesing. ³		Buswell and Boruff. ⁴	
	Time days.	Yield, cc. per gm. fed.	Time days.	Yield, cc. per gm. fed.	Time days.	Yield, cc. per gm. fed.	Time days.	Yield, cc. per gm. fed.
Filter paper.....	30	15	135	267	-----	-----	30	676
Newspaper.....	30	25	-----	-----	-----	-----	30	300
Banana skins and stems.....	30	41	-----	-----	-----	-----	30	360
Flax straw.....	-----	-----	180	23 to 31% loss in weight. No gas data.	-----	-----	20	300
Flax shives.....	-----	-----	-----	-----	-----	-----	20	210
Wheat straw.....	-----	-----	-----	-----	-----	-----	20	245
Cornstalks.....	-----	-----	-----	-----	-----	-----	20	275-375
Stable manure.....	-----	-----	-----	-----	60	294	30	*300-500

¹ Used large quantities of sludge as inoculum J. Indian Inst. Sci. 3, 39 (1920).

² Omelianski, Centr. f. Bakt., Abt. II, 8, 353 (1902) and 12, 33 (1904).

³ Schloesing, Comptes rend. de l'Academie de Sci., Paris, 109, 835 (1889).

⁴ Using small amounts of inoculum. Gas data corrected for gas from inoculum.

* Cornstalks used as bedding.

** Summary gives the best data reported in literature. Data of all are taken from batch experiments.

In 1920, Groenewege^(108, 111) studied the aerobic decomposition (37 degrees C.) of cellulose and called attention to the fact that the decomposition products of the cellulose-dissolving organisms were utilized by the denitrifiers. On the basis of this, he proposed a symbiotic classification of the cellulose-dissolving organisms. In later studies^(109, 110) he found that he got a slight accumulation of cellobiose under faintly alkaline conditions. A spore-forming flora was found to hydrolyze this cellobiose to formic, acetic, and a higher fatty acid, probably valeric. A second flora, partly spore formers, oxidized the acids to CO₂ and H₂O. Both nitrifiers and denitrifiers were present. Another paper⁽¹¹²⁾ reports studies on the anaerobic and aerobic decomposition (28 degrees C.) of certain alcohols, fatty acid salts of cellulose and other materials. The predominating organism in his anaerobic mixed cultures was a small capsulated micrococcus.

Krogh and Schmidt (1920-21)^(154, 155) have studied the fermentation of cellulose in the paunch of the ox. They have proposed the following reaction which they base on the CO_2/CH_4 ratios they obtained:



They found no production or absorption of gaseous nitrogen.

Schellenberg (1920)⁽²⁹⁰⁾ studied the decomposition of wood under natural conditions and came to the conclusion that like the decomposition of most plant materials, the fungi play the most important role during the earlier process while the bacteria and the fungi share in the decomposition during the latter stages.

Issantchenko (1921)⁽¹³⁹⁾ isolated some aerobes from the slime of Lake Saki (Crimea) and found that they decomposed filter paper rather rapidly to a substance that reduced Pehling's solution.

Thaysen and associates have contributed a great deal of information to this subject. Thaysen and Bunker (1927)⁽³⁴⁷⁾ have written a 363 page book on "The Microbiology of Cellulose, Hemicellulose, Pectin, and Gums" and recently Thaysen and Galloway (1930)⁽³⁵³⁾ have published a similar book on "The Microbiology of Starch and Sugars." These books are probably the most complete treatises published in this field. In this connection Smyth and Obold⁽³¹¹⁾ have recently (1930) published a book—"Industrial Microbiology" which also contains short chapters on the microbiology of cellulose and cellulosic materials. Thaysen and others^(341, 344, 346, 350, 351) have noted the decomposition of cotton and fabrics under aerobic and anaerobic conditions. They conclude that such decompositions are greatly accelerated by a moisture content of over nine per cent and by warm temperatures. They found cellulose acetate and nitro silks not readily attacked. Viscose silk, however, was found to be less resistant and cuprammonium silk very susceptible to microbiological action. Thaysen and Bunker⁽³⁴⁵⁾ also noted, in 1924, the different rates of deterioration of cottons from different sources. They have been unable to establish the reason for this difference. Thaysen^(348, 349) has studied the butyl alcohol-acetone fermentation of Jerusalem artichokes and the production of power alcohol from vegetable wastes. Both of these fermentations, however, are carried out following hydrolysis by the use of 0.2 per cent H_2SO_4 .

Carpentier (1922)⁽⁵⁰⁾ has shown that the presence of nitrogen, notably in the form of ammonia, facilitates the disposal of cellulose in soils. These data have been substantiated by Barthel and Bengtsson^(12, 14). These latter authors reported in 1926 that the rate of decomposition was proportional to the nitrogen content. They used this in explaining the reason why straw decomposes more rapidly than filter paper.

Gescher (1922)⁽¹⁰³⁾ studied the aerobic decomposition of cellulose and pectin. Cellulose- and pectin-destroying organisms were found present and were isolated from garden soil. The strains were found to be highly selective as to their source of carbon. The cellulose-decomposing forms were favored by high temperatures (30-60 degrees C), by alkalinity and by aeration. Hence in order to avoid injury to the cellulose fibers in the retting process, these conditions should be avoided.

Khouvine^(148, 149) announced in 1922 that she had isolated a very

small cellulose-fermenting anaerobe. *B. cellulosa dissolvens*, n. sp. Many different morphological forms were noted in pure cultures of the organism. It would not grow on ordinary laboratory media but grew well at 33 degrees C. in the presence of cellulose and sterilized fecal extract. It fermented cellulose to CO₂, H₂, acetic and butyric acids and ethyl alcohol and left a yellow pigment which corresponded to about 60 per cent of the cellulose digested. The bacillus would destroy one gram of cellulose in six days alone, or five times this quantity when associated with other bacteria.

In 1923 the report⁽²⁰⁷⁾ of a group discussion held on the action of bacteria on cellulosic materials was published. Sir Frederic Nathan acted as chairman. It is interesting to note that he saw possibilities in a microbiological process based on the production of carbon dioxide and methane from cotton wastes, etc., but he called attention to the fact that such a process, so far at least, had not been found commercially possible. Lynn and Langwell reviewed their fermentation process for the manufacture of organic acids and outlined a probable chemical mechanism. Thaysen, Page, Harland, Cross, Hutchinson, Ormandy, and Lessing who also contributed to the discussion most of the numerous Langwell patents⁽¹⁵⁷⁻¹⁶²⁾ call for the fermentation of cellulosic materials, with aeration at a temperature of about 60 degrees C., and with the addition of certain inorganic salts and enzymes, the latter being derived from decomposing stable manure⁽¹⁶³⁾. The organisms decompose cellulose and cellulosic materials of many types. By varying the conditions the following compounds are formed within the limits stated:

	PerCent
Acetic acid.....	20 to 80
Butyric acid.....	0 to 30
Lactic.....	0 to 60
Ethyl alcohol.....	0 to 30
Methane.....	0 to 8
H ₂	0 to 1
CO ₂	28 to 50

Here, as in previous studies, the major portion of the materials fermented are recovered as organic acids. Some of the patents also cover the production of butanol, acetone, and related compounds⁽³⁷⁶⁾, as well as methods of recovering the organic materials produced and of the ammonia present⁽¹⁶¹⁾. Legg and Christenson⁽¹⁶⁹⁾ have also studied the production of organic acids from cellulosic materials. May and Herrick⁽¹⁸⁶⁾ have recently (1932) reviewed the literature on the biological production of acids from various carbohydrates.

Neuberg and Cohn (1923)^(213, 214) have classified the cellulose-decomposing organisms into seven groups. One group is composed of the filamentous fungi while the other six are bacteria. Since they secured positive tests for aldehyde in some of their anaerobic fermentations, they proposed the following scheme:

cellulose anhydrobirose cellobiose glucose acetaldehyde

Gray and Chambers (1924)⁽¹⁰⁷⁾ have reported the isolation of a new aerobe, *Microspora* or *Vibrio agarliquefaciens*, which not only dissolves cellulose but also attacks agar. Xylose, lignin and other substances stimulate its growth.

Heukelekian and Eudolfs^(122-5, 278, 279) have studied the distribution of cellulose and cellulosic materials in sewage. They have found that the presence of these materials not only tends to retard the rate of digestion but also leads to higher organic acid concentrations and lower pH values. These materials also increase the CO₂ content of the gas. Eudolfs and Heisig⁽²⁷⁷⁾ have reported the Milwaukee experiment on the digestion and gasification of sewage screenings. The accumulation of the fermenting cellulosic materials at the top of the tank in the form of a thick heavy mat proved to be too great a handicap so the idea was abandoned. From 5.1 to 5.7 cu. ft. of gas were collected per pound of screening fed. Much of this gas undoubtedly came from the sewage particles trapped in the cellulosic material. By the use of the special digester to be described later, Buswell and Boruff⁽³⁹⁾ have been able to overcome the operating difficulty described by Eudolfs and Heisig.

Mullin (1924)⁽²⁰¹⁾ has reported the various molds and bacteria that attack cellulose and bring about injury to fibers and fabrics. He has noted that warmth, moisture, stagnant air, starches and gums all favor disintegration.

Sack⁽²⁸²⁾ reported in 1924 three new bacilli and one coccus that were capable of decomposing cellulose under aerobic conditions. All gave nitrites and then ammonia from KNO₃. He calls attention to the fact that Bergey's manual gives 31 cellulose-attacking bacteria in the *cellulomonas* group.

Sanborn has reported the effect of pH on the cellulose-decomposing power of organisms⁽²⁸³⁾ and has also devised special media⁽²⁸⁵⁾ for the isolation of cellulose-decomposing bacteria. He published in 1926 and 1927 papers^(284, 286) on the effect of certain essential food substances of the soil on the activity of these organisms. He found that certain inorganic ions, as well as lignin, xylose, alcohol, nucleic acids, and certain products of bacterial metabolism, aided decomposition. Sanborn⁽²⁸⁷⁾ outlined in 1928 the important or controlling factors involved in the degradation of cellulose. In summary they are as follows:

1. Form of cellulose being attacked.
2. Nitrogenous constituents of the medium.
3. Essential food products or stimulating factors present.
4. Associative action of microorganism.

In 1929 he announced additional studies bearing on the importance of symbiosis in aerobic cellulose decompositions.

Heukelekian and Waksman⁽¹²⁷⁾ have isolated in pure culture an aerobic *Trichoderma koningi*, that is capable of fermenting cellulose to CO₂ and H₂O. Waksman and Skinner⁽³⁷³⁾ and Waksman and Carey⁽³⁷¹⁾ have published good summaries of their cellulose studies. The first authors have called attention to the fact that anaerobes act independently of nitrates and that they require less oxygen, less energy, and hence less nitrogen than do the aerobic cellulose-digesters. They have noted that under aerobic conditions fungi, bacteria, and to some extent the actinomycetes, play the important roles in the decomposition of cellulosic materials⁽³⁶⁹⁾. Under anaerobic conditions the spore-forming bacteria are largely responsible for the process. Waksman has published a very complete series of articles in volume 22 of *Soil Science* on the

origin and nature of soil organic matter or soil humus. For anyone interested in this subject the original papers should be consulted. He has also written a chapter in Jordon and Falk's, "The Newer Knowledge of Bacteriology and Immunology"⁽¹⁴²⁾. This chapter deals with the nature, distribution and functions of soil microorganisms. Waksman, Tenney, and Diehm⁽³⁷⁹⁾ reported in 1929 studies on the chemical and microbiological principles underlying the transformation of organic matter in the preparation of artificial manures. In the presence of sufficient nitrogen, the water soluble portion, the pentosans, and cellulose were found to undergo rapid decomposition. There was, however, an accumulation of lignin, protein and ash. They state that the microbial protoplasm formed by bacteria and fungi may be equivalent to one-fifth or more of the actual organic matter decomposed. They found that the decomposition of cellulosic materials could be controlled by regulating the temperature, moisture content, and by the addition of the required amounts of N, P₂O₅, K₂O and CaCO₃. Mundy⁽²⁰²⁾ has reported studies of much the same character. Waksman and Stevens⁽³⁷⁷⁾ recently published a study of the processes involved in the decomposition of wood reporting the action of bacteria and fungi under both aerobic and anaerobic conditions. They confirmed earlier work and in addition called attention to the fact that part of the water and alkali soluble fractions of fossilized wood are made up largely of fungi mycelium. Recently Waksman and Purvis⁽³⁷²⁾ have announced studies which show that facultative anaerobes which are capable of growing in fairly high acidities play an important part in the transformation of peat. They are firmly convinced that lignin is not fermented anaerobically but believe that certain aerobic organisms are capable of altering it. This seemingly universal idea is further supported by the data of Tenney and Waksman (1930)⁽³³⁶⁾ and Schrader⁽²⁹⁶⁾. The decomposition of wood has been found to be of two types, (1) destruction, that is, that type wherein the cellulose is decomposed but the lignin accumulates, and (2) corrosion, that is, that type in which the cellulose is fermented and the lignin altered. In summary, the decomposition was found to depend on (a) the organisms active in the process; (b) the environmental conditions; and (c) the composition of the plant. Further studies on the composition and formation of peat⁽³⁶⁸⁾, as well as studies on the decomposition of certain cellulosic materials⁽³⁷⁶⁾ and of vegetable proteins⁽³²⁴⁾ have recently been published.

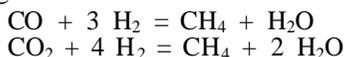
Grosman (1925)⁽¹¹³⁾ found organisms in the intestines of man that were capable of disintegrating cellulose to soluble end-products. This action was found to take place more actively under anaerobic than under aerobic conditions. Berkefeld filtrates of emulsions of the bacteria would not digest cellulose. Strauss⁽³²⁸⁾ has reported that the normal man can digest 50 per cent of the cellulose he ingests. Yonge⁽³⁹⁴⁾ and Brahm⁽²⁹⁾ have also called attention to the important role played by anaerobes in the digestion of cellulose in the intestinal tract. Armandi⁽⁶⁾ has studied the cellulose-decomposing organisms found in the rumen of the ox. Several species have been isolated.

Coolhaas (1926)^(56, 58) has studied the thermophilic degradation of cellulose as well as certain carbohydrates and fatty acid salts⁽⁵⁷⁾.

Most of his work has been done with anaerobes. Ardern⁽⁵⁾ reports he is able to digest paper anaerobically and at thermophilic temperatures with the production in ten days, of 700 cc. of gas per gram of organic matter added. He has been able to confirm many of Omelianski's results. He, like Omelianski, Sohngen, Groenewege, Bach, Sieck, Imhoff and others, favors the following exothermic reaction as a possible explanation for the source of CH₄ and as showing a possible relationship between the hydrogen and methane types of fermentation:



The fourth transfer of his thermophilic methane-producing organisms gave only CO₂ and H₂ from cellulose. Fischer and others^(77, 78) have recently been able to biologically reduce CO and CO₂ to CH₄. They propose the following reactions:



Using 100 grams of cabbage leaves inoculated with fecal matter and river slime, Coolhaas⁽⁶⁷⁾ tested the relative rates of methane production at 26°, 37° and 60° C. Little gas was given off at 37° C. During a period of 20 days the bottle held at 60° C. gave 5.3 liters of CH₄, while that held at 26° gave only 2.8 liters. Partially purified cultures gave CO₂ and H₂ but no CH₄. Pure cultures of his thermophiles gave no gas. Coolhaas proposed, as an explanation of this fact, the idea that the metabolism of cellulose by certain bacteria produced substances which served as sources of food for the gas-forming organisms which were unable to metabolize cellulose directly.

Speakman (1926)⁽³¹⁷⁾ has studied the fermentation of cellulose, but more especially the derivatives of cellulosic materials, namely, glucose, xylose and galactose. He also studied the acetic fermentation⁽³¹⁸⁾ of sulfite liquors.

Winogradsky has published a review^(391, 392) of the aerobic and anaerobic decomposition of cellulose in the soil.

Out of 39 soil samples tested, Bradley and Eettger report (1927)⁽²⁸⁾ that 36 gave positive tests for cellulose-decomposing bacteria. In some quantitative studies on the destruction of cellulose by the different aerobic strains they had isolated, they found that from 3 to 14 per cent of the filter paper added was removed in 15 days. All strains that were tested reduced nitrates. The presence of the enzyme cellulase was shown by the auxanographic method on cellulose casein-digest agar. Snieszko (1929)⁽³¹³⁾ and Tetrault (1930)⁽³³⁸⁾ have also shown the presence of cellulase.

Werner⁽³⁸⁶⁾ has isolated in pure culture an anaerobe *B. cellulosumfermentans*. It grows only in the presence of cellulose. Gas formation was observed but the fermentation products in the pure culture studies were not investigated. No CH₄ was formed by the mixed cultures studied.

Eege (1927)⁽²⁶⁵⁾ like other earlier workers, found fungi to be more important than bacteria in the early stages of the decomposition of cellulosic materials in nature. Bacteria were found to take the more important part in the latter stages of the decomposition. Eege states that

when the ratio of the energy factor (pentosans) to the inhibiting factor (lignin) is greater than one, then the material is easily attacked, but if less than 0.5, the material is resistant to attacks by microorganisms.

Dubos (1929)⁽⁶⁸⁾ has studied and isolated some new aerobic, cellulose-decomposing bacteria. He also worked with an anaerobic, cellulose-decomposing bacterium that could utilize peptone as a source of nitrogen. A pure culture of the organism produced no gas from cellulose. Dubos⁽⁶⁷⁾ explained the lag noted in the development of anaerobic cultures as due to (1) lack of sufficient inoculum, and (2) lack of the proper medium for anaerobiosis. Simola⁽³⁰⁴⁻⁶⁾ studying the aerobic fermentation of cellulose, gave special attention to *Cellulobacillus myxogenes* and *Cellulobacillus mucosus*. They form cellulase and cellobiase and produce acids and CO₂ from cellulose. For each gram of cellulose or glucose metabolized, 30 milligrams of inorganic nitrogen are converted to an organic form.

Tuorila has shown (1928)⁽³⁶¹⁾ that although certain nitrogen-fixing bacteria cannot utilize cellulose directly, they can do so if associated with other organisms which decompose cellulose to soluble products. Skinner^(308,309) has also studied these and other aerobic cellulose fermenters.

Rubenchik⁽²⁷⁵⁾ has isolated some aerobic halo-tolerant cellulose-destroying organisms from the Odessa salt marsh.

Woodman and Stewart⁽³⁹³⁾ working with an aerobic thermophile have been able, by the use of a collodion sack, to isolate glucose as a microbiological split product of cellulose and of oat fibers.

The anaerobic fermentation of cellulose and cellulosic materials and their relation to sewage disposal was first investigated by the State Water Survey in 1923. No conclusive data were obtained. This problem was again attacked in 1928. The present writers (Boruff and Buswell)^(22, 23) have reported data on the anaerobic fermentation of cellulose in which they got almost 90 per cent of the cellulose converted to CO₂ and CH₄ (See Table XXVIII). These and other studies on the methane fermentation of cellulose and cellulosic materials, are given in detail elsewhere in this bulletin. The authors have also published in *Cellulose*, two general articles on the "Decomposition of Cellulose and Cellulosic Materials by Bacteria."⁽³⁸⁾

Cowles and Eettger⁽⁶⁰⁾ have reported (1931) the isolation of a spore-forming, cellulose-fermenting anaerobe, *Clostridium cellulosolvens* (*n. sp.*) which attacks from among a number of carbohydrates studied, only cellulose, dextrin, arabinose, and xylose. Glucose is not utilized, a fact in contradiction of the so-called theory of carbohydrate gradients. When associated with certain other organisms, as for example *B. aerogenes*, it decomposes cellulose more rapidly. It converts cellulose to organic acids and a gas composed of 3 parts of H₂ and 1 part of CO₂.

Tetrault (1930)^(337, 338) has reported studies on the cultivation of pure and mixed cultures of thermophilic cellulose-digesting bacteria. He has worked with both aerobes and anaerobes and has shown that some produce cellulase and a reducing sugar. Snieszko⁽³¹³⁾ has also demonstrated the production of cellulase by certain aerobic bacteria.

Eobinson⁽²⁷¹⁾ (1931) has reported the necessity of and the methods used in the preservation of cotton fishing nets.

Snieszko⁽³¹²⁾ has reported (1931) the isolation of a thermophilic cellulose fermenter.

Straw

Hebert (1892)⁽¹²⁰⁾ studied the mesophilic and thermophilic decomposition of straw under both aerobic and anaerobic conditions. During a three-month period he got a loss of material amounting to almost 50 per cent of the weight of the straw added. The loss occurred principally in cellulose, vasculose and straw gum. Potassium or ammonium carbonate was added to the straw. His work is of further interest because he reports certain anaerobic experiments in which the fermentation at first was of the CO₂-H₂ type, but which later produced CO₂ and CH₄. No explanation was offered. Krabbe (1890)⁽¹⁵²⁾ noted that bacteria decomposed the middle lamella of plants and that the end-products were CO₂, CH₄, H₂, butyric and acetic acids, aldehydes, and alcohols.

Eichards (1917)⁽²⁶⁸⁾ studied the aerobic decomposition of cellulosic materials and in conjunction with Hutchinson and Clayton^(137, 138) devised the first method for the rapid production of artificial manure. Their patents^(269, 270) describe how straw and similar cellulosic materials should be piled and treated with soluble sources of nitrogen (synthetic or wastes from industrial plants) in order that a manure may be formed containing appreciable quantities of organic nitrogen. From their material they isolated an aerobe, *Spirochaeta cytophage*, which was found to have an interesting life cycle involving many different morphological forms. This aerobe, or the natural flora found on straw, with the aid of nitrogen salts of almost any form, was found capable of changing a straw pile to a black humus in as short a time as three weeks.. They report the end-products as follows: (1) a yellow pigment, fat-like in nature, (2) fatty acids, apparently including butyric, and (3) some mucilaginous materials, of pectin-like properties which would not reduce Fehling's solution and (4) humus.

Murray (1921)⁽²⁰³⁾ has called attention to some data that show that when straw is added to soil it stimulates the reproduction of bacteria which use the carbon of the straw and the available nitrogen of the soil to form organic nitrogen, thus temporarily robbing the soil and hence the growing plants of the available nitrogen.

Starkey⁽³²⁰⁾ noted, in 1924, that 20 per cent of the rye straw introduced into fertile soils was decomposed in ten days. In less fertile soils the decomposition was much slower. The relative rate of CO₂ evolved from carbon-containing material placed in different soils was also studied⁽³²¹⁾.

In 1925, Collison and Cohn⁽⁵⁵⁾ noted the effect of straw on plant growth and explained the retardation noted as due to some toxic chemical agent found in straw and to the competition noted between the growing plants and the microorganisms for the available nitrogen present.

Falck (1928)⁽⁷⁵⁾ has classified the bacteria taking part in the composting of straw into two divisions, namely, (1) those leading to the destruction (the thermophiles), and (2) those leading to corrosion, namely, the nitrate- and humus-forming bacteria. Data are given which

show the decomposition of straw over a period of 30 months. A general decrease is noted in the pentosans and in cellulose with a corresponding accumulation of lignin and an increase in the alkali-soluble fraction. Norman⁽²¹⁷⁾ has recently (1929) published similar studies as also has Page⁽²³²⁾ and co-workers (1930). Page has given special attention to the carbon-nitrogen cycles in soils and to the formation of humus.

Mabee (1929)⁽¹⁸¹⁾ has been granted a patent on an apparatus and a fermentation method for pretreating hay, straw, clover and other roughage in order that it may be changed into a better grade of stock food.

Lignin

Wehner (1925)⁽³⁸³⁾ has also tried to cultivate bacteria on lignin but so far his attempts have been fruitless. Pringsheim⁽²⁶¹⁾ was able to anaerobically ferment sulfite cellulose waste liquors containing lignin compounds, pentosans, sulfite, etc., providing they were diluted 20 times and suitably inoculated. The gas contained 57 per cent CO₂, 40 per cent H₂ and 3 per cent CH₄.

Burkey⁽³³⁾ has published a report on the aerobic and anaerobic fermentation of pectin. No chemical data are presented. Pitman and Cruess (1929)⁽²⁴⁸⁾ have published a similar paper which considers molds and yeasts, as well as bacteria. Burkey, in a letter to the writers⁽³⁴⁾ concerning the fermentation of lignin, said "I have made some observations on the fermentation of pure lignin as well as that combined in plant residues. I have never obtained a definite production of gas with either pure or mixed cultures."

Egozinski and Starzlioska⁽²⁷³⁾ have shown that lignin is not digested by ruminants. Csonka, Phillips and Breese-Jones⁽⁶¹⁾, however, have noted an increase in the hippuric acid content of the urine from cows or dogs that have been fed lignin.

Makrinov⁽¹⁸³⁾ has studied the action of two pectin-destroying organisms *Granulobacter pectinovarium* and *Pectinobacter amzlophilum*. They produce acids, CO₂ and H₂. He got greater tissue destruction under aerobic than anaerobic conditions.

Organic Acids

Peterson and Fred and their associates have contributed much to our knowledge of the subject under consideration. They have studied the production of acetic and lactic acids^(87-90, 239, 240) from pentosans and pentoses and have secured a 90 per cent conversion of the latter substrate into these acids. The reaction, as carried out by either aerobes or facultative anaerobes at 30 degrees C, was found to take place in accordance with the following exothermic reaction:



They have also studied the biological production of propionic acid from carbohydrates^(84, 243). In 1921 and 1923 they found that during the first 66 days^(241, 242) there was a loss of from 15 to 20 per cent in the pentosan content of corn fodder silage. Fred, Peterson, and Viljoen⁽⁹⁵⁾ reported in 1924 that by the use of an aerobic thermophile they were able, in five to six days, to convert 60 to 80 per cent of a sample of cellulose pulp to gas, acetic acid (56.5 per cent) and ethyl alcohol (11.0 per cent). Us-

ing corncobs they recovered after 16 days, 26.7 per cent of the weight of the cobs as acetic, and 0.11 per cent as ethyl alcohol. In 1926⁽³⁶⁷⁾ they isolated the organism and reported the end-products as acetic acid, 55 per cent; butyric acid, trace; ethyl alcohol, 5 to 25 per cent; and CO₂ and H₂. Stiles, Peterson and Fred (1929⁽³²⁵⁾) reporting on the activity of *Clostridium acetobutylicum* on maize, state that appreciable quantities of formic acid are produced, as well as smaller amounts of acetic, butyric and an alpha hydroxy acid of some kind found in the non-volatile fraction. Fred, Peterson, et al. have also studied the production of solvents and organic acids by the hydrolysis (dilute H₂SO₄) and fermentation⁽⁹¹⁻⁹⁴⁾ of mill sawdust, oat and peanut hulls, corn-cobs, and wood. Scott, Fred and Peterson⁽²⁹⁸⁾ have been able to obtain 45 to 65 per cent yield of acetic acid by the thermophilic fermentation of cellulose. Both aerobic and anaerobic methods have been studied. Purified cultures gave higher yields than mixed cultures. They also obtained positive tests for glucose. No CH₄ was reported. Werkman and Carter⁽³⁸⁴⁾ and Peterson (C. J.)⁽²³⁸⁾ have also developed a thermophilic process for the production of acetic acid. As much as 20 to 50 per cent by weight of the corn-cobs or stalks added may be recovered as acetic acid in 5 to 8 days. Werkman and Stretar⁽³⁸⁵⁾ have also recovered low yields of acids by the anaerobic fermentation of sugar beet pulp. Peterson et al.⁽²⁴⁴⁾ have noted the production of reducing sugars from starch as well as cellulose. The energy relationships involved in fermentations by heterotrophic bacteria have been thoroughly discussed by Wilson and Peterson⁽³⁹⁰⁾.

Composting

In 1884 Gayon⁽¹⁰²⁾ studied the fermentations taking place in a manure pile. He came to the conclusion that there were two types, one which proceeded in the presence of air and with an excess of it gave high temperatures and only CO₂, and the other which took place in the absence of air and produced CH₄. In the latter case the temperature remained constant. He found that 1 cubic foot of manure when moistened and kept at 35 degrees C. gave 100 liters of gas in 24 hours. This gas contained considerable methane. Gayon attributed the decomposition to an extremely small anaerobe. Deherain (1884)⁽⁶³⁾ noted that at the bottom of a manure pile the gases formed were CO₂ and CH₄ while nearer the top the gases were mainly CO₂. He also reported an H₂-CO₂ fermentation. Numerous extremely fine rods were reported present in the ferments. Further work on the decomposition of manure was reported by Schloessing in 1889⁽²⁹¹⁾. He reported that CO₂ was the only gas liberated aerobically and that the ferment was very active at 72.5 degrees C. The optimum temperature under anaerobic conditions was found to be 52 degrees C. From 124.4 grams of manure (29.8 gms. dry weight), which was held in an atmosphere of CO₂ and at a temperature of 52 degrees C. for two months, he recovered 4,217.5 cc. of CO₂ and 4,577.4 cc. of CH₄. This is an average of 4.9 cc. of gas per gram (dry weight) per day. The most active gas production noted for any one day was 6.0 cc. per gram. He found practically no loss in nitrogen. From his analytical data he concluded that water took part in the decomposition.

König (1900)⁽¹⁵¹⁾ found that well rotted manures still contained fully 50 per cent of their original pentosan content. Gran (1902)⁽¹⁰⁶⁾ proposed the idea that the hemicelluloses present were hydrolyzed to soluble end-products by the enzymes of bacteria. His ideas were strengthened by the work of Sawamura (1902)⁽²⁸⁹⁾ who reported the microbiological hydrolysis of mannans, galactans and arabans.

Sjollema and others⁽³⁰⁷⁾ pointed out in 1908 that the pentosans found in manure were more readily decomposed under anaerobic conditions at 35 degrees C. than they were under aerobic conditions at room temperatures. These data have been verified by Shorey and Lathrop⁽³⁰³⁾ who also found that pentosans and pentoses were widely distributed in arable soils while hexosans were not found in measurable quantities. On this basis they concluded that the former was less readily attacked than the latter.

In 1911 Choukevitch⁽⁵²⁾ found a small cellulose decomposing anaerobe, *Bacillus gasogenus*, in the large intestine of a horse. In pure culture this organism would not ferment cellulose to gaseous end-products. Distaso⁽⁶⁴⁾ has isolated a facultative anaerobe, *Bacillus cellulosaes desagregans*, from the intestines of fowls. This new bacillus also failed to give gaseous end-products from cellulose. In the same year Miede⁽¹⁹³⁾ called attention to the fact that, although the fungi were the more important class of organisms decomposing vegetable materials aerobically, the bacteria were the most important in the anaerobic degradation processes. This idea was also sponsored by Mutterlein in 1913⁽²⁰⁴⁾. Miede studied manures and the spontaneous heating of hay stacks. In the latter he noted a 40 per cent decrease in the pentosans and a total nitrogen decrease of 50 per cent. The protein nitrogen content, however, was found to remain almost constant. He realized the role played by the plant enzymes but claimed that these alone could not account for the high degree of decomposition noted under natural conditions. (In this connection Browne⁽³⁷⁾ has recently (1929) published a bulletin—"The Spontaneous Combustion of Hay." He discusses the role played by the thermophilic bacteria, the plant enzymes and the chemical constitution of the hay itself). And in 1911 Merkes⁽¹⁹⁰⁾ isolated an aerobe, *Micrococcus cytophagus*, which was decomposing the cellulose in the hydrophyte *Elodea*.

In 1922 Henneberg⁽¹²¹⁾ attempted to study the microflora of a rotting compost heap by direct examination. The predominant flora was found to be bacteria and not fungi.

Bottini⁽²⁷⁾ reported in 1925 his studies on the maturation of horse manure. He noted that cellulose was resistant at first but after four months only 40 per cent was left and after eight months only 26 per cent remained. The fatty substances decreased noticeably during the first two months and even more rapidly in the succeeding two months. The pentosans and methyl pentosans gradually decomposed from the first. After eight months 16 per cent of the pentosans remained, while 40 per cent of the methyl pentosans were left unattacked. There was practically no change in the protein or mineral content of the material.

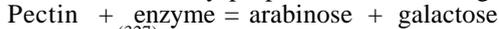
Itano^(140, 141) reported in 1926 some studies on an aerobic thermophilic composting process by which he was able, in three weeks, to re-

duce vegetable and plant residues to a humus which on an average contained 2 per cent of N, 1.44 per cent of K and 0.85 per cent of phosphates. Although the process is started at normal temperatures the contents in the "zymotic tank" is reported to reach a temperature of 70 degrees C. in five days. Halversen and Torgerson (1927) have reported similar studies.

Goeters (1929)⁽¹⁰⁵⁾ has studied the relative numbers of aerobic and anaerobic bacteria found in manures during the different stages of decomposition. He has also found processed manures to be more valuable as fertilizers than natural farm manures.

Betting

Beijerink and Van Delden⁽¹⁸⁾ studied the anaerobic retting bacteria of Trecul and Van Tieghem and came to the conclusion that there was a pectosinase formed by the bacteria which dissolved the middle lamellae of the cells of the flax stem. They proposed the following:



Stormer reported in 1904⁽³²⁷⁾ that he had isolated a retting anaerobe, *Plectridium pectinovarum*, which gave as gaseous end-products, CO₂ and H₂ and as organic acids appreciable quantities of acetic and butyric with traces of lactic and valeric. Carbone (1921)⁽⁴⁹⁾ working along this same line proposed a special type of retting process in which an anaerobe that he had isolated, *B. felsineus*, was the active agent. He was unable to cultivate his organism in laboratory media and hence could not secure a pure culture. Behrens⁽¹⁷⁾ also carried on extensive studies with anaerobic retting bacteria.

Toles⁽³⁵⁸⁾ secured, in 1915, a patent on a novel method of retting flax. He heated baled flax with retting liquors under pressure. Lathrop⁽¹⁶⁵⁾ has patented a method for limiting the decomposition of plant fibers especially bagasse. The heating of the freshly baled crude plant tissue pasteurizes it sufficiently so that, if it is kept dry, the valuable part of the fiber will not be attacked. A second patent⁽¹⁶⁶⁾ elaborates on the above method and provides for a secondary fermentation by microorganisms of the "mushroom type."

Kayser and Delaval (1920)⁽¹⁴⁴⁾ studied an anaerobic retting process and found that formic, acetic, butyric, succinic and lactic acids were produced, as well as ethyl alcohol, acetone sugars, soluble nitrogen compounds, and CO₂ and H₂. They, like Fowler and Joshi⁽⁸⁶⁾, found it advantageous to remove the fermentation liquors periodically during the process in order that the above formed materials might be washed out., Reilly's investigations (1920)⁽²⁶⁶⁾ support the presence of the substance mentioned by Kayser and Delaval.

Euschmann (1922)⁽²⁸¹⁾ has proposed an anaerobic, cold water, fill and draw method for the retting of flax (Ochmann process). He claims that-only 6.5 per cent of the fiber is lost in the process. Sweeney⁽³³⁰⁾ has patented (1927) a process for the retting of cornstalks prior to their use in the manufacture of pulp or wall board. He submerges the stalks in water and the natural flora present on the stalks carries out the fermentation. No gas data are given. Toles⁽³⁵⁸⁾ has also patented a retting process which involves fermenting baled flax under pressure (8-12 lbs.) in an anaerobic tank at 95° to 100° F. for 2½ to 3 days.

Peat

White and Thiessen (1913)⁽³⁸⁷⁾ have studied the processes involved in the formation of peat and coal. They have emphasized the important role played by fungi, and to some extent the bacteria, in the early stages of the process as well as the major part played by the bacteria in the later phases of the decomposition. A recent publication by Thiessen and Johnson (1929)⁽³⁵⁶⁾ follows the decrease in cellulose and lignin, with the corresponding increase in humus, as noted in the samples of peat taken at increasing depths. Studies have also been made by the use of the Spierer lens⁽³⁵⁴⁾. Humic acids have been further studied by Thiessen and Engelder⁽³⁵⁵⁾.

Fisher and Schrader (1920-24)⁽⁸⁰⁻⁸³⁾ have proposed the idea that peat and coal are formed microbiologically from lignin and that cellulose is almost quantitatively removed as gaseous end-products. This theory with some slight modification is now accepted by most workers⁽³⁴⁷⁾.

Thaysen, Bakes and Bunker⁽³⁴³⁾ found that the fungi count in peat-like soils decreases rapidly with increasing depth. This limits peat production to bacteria and actinomycetes. Three year old silage was found to be low in pentosans (17.54 per cent) and cellulose (27.36 per cent) and to contain 26 per cent of humus. They studied the decomposition of cellulosic materials in nature^(340, 342) and divided the humus substances formed⁽³⁴³⁾ into two types based on the solubility of their chlorine derivatives in ether. Thaysen also studied in 1926⁽³³⁹⁾ the action of bacterium of the amylobacter group on starch. He reported the following recoveries: acetone, 10.77 per cent; butyl alcohol 25.07 per cent; CO₂, 62.61 per cent; H₂, 1.6 per cent; and organic acids, 1.8 per cent. He estimates that 1,000 cubic feet of H₂ can be produced by this process for \$0.25. Thaysen and Bakes⁽³⁴²⁾ have studied the aerobic humification of vegetable tissues. They conclude that an appreciable portion of humus of carbohydrate origin is formed along with that of lignin origin. Their data tend to show that the greater portion of the carbohydrate humus comes from the pentosans. In the study of coal no definite proof was found of the presence of humus of carbohydrate origin.

Tropsch⁽³⁶⁰⁾ has reviewed the literature on the decomposition of cellulosic materials and their relation to the formation of coal. Lignin is thought to be the main mother substance of coal.

Grosskopf (1926)⁽¹¹⁴⁾ has studied the formation of brown coals from coniferous plant residues, and has given special attention to the humic acids and humus. The relative amounts of certain constituents as he found them in the original plant materials, and in the resulting coal may be summarized as follows:

	Plant Per cent	Coal Per cent
Pentosans	10.0	0.0
Cellulose	26.3	0.1
Lignin	37.6	3.2
Humic matter	0.0	98.3

On the basis of these and other data showing the rapid and almost complete destruction of cellulose by bacteria and fungi, he has concluded

that the humic matter comes from the lignin. Similar conclusions have also been reported by Marshall and Page⁽¹⁸⁵⁾.

Melin, Norrbin, and Oden⁽¹⁸⁹⁾ reported in 1926 an attempt to secure methane by the anaerobic fermentation of peat. Only a small amount of gas was formed and it came from the fermentation of the hydrocelluloses, mucilage substances, cellulose, and to some extent from the pentosans present. No indication of attack on the lignin or the humic acids was noted.

Waste

Neave and Buswell (1928)⁽²¹⁰⁾ and Hatfield (1930)⁽¹¹⁶⁾ have studied the mesophilic anaerobic digestion of distillery slop waste which contains appreciable quantities of fibrous material and have found that it decomposes upon dilution with sewage, to give CO₂ and CH₄. Boruff and Buswell⁽²⁴⁾ have more recently (1931) developed a method for the thermophilic and anaerobic fermentation of undiluted hot distillery waste. They recover large volumes of CO₂ and CH₄.

A recent Dutch patent⁽²⁰⁵⁾ (1930) describes an anaerobic fermentation method for the preparation of a gas mixture composed of 12 to 30 per cent CO₂, a few per cent of H₂ and the rest CH₄. Dilute molasses, in the presence of 5 to 10 per cent sugar cane stalks, is passed through compartments of a closed septic tank. The mesophilic methane-producing bacteria are obtained by treating sewage sludge or manure with a weak alkali like calcium acetate or butyrate or by suitable culture fluids. The optimum concentration of molasses has been found to be about 4 per cent and the optimum temperature 32 degrees C. The fermentation takes place in 3 to 7 days.

Miscellaneous

Schottelius⁽²⁹³⁻⁵⁾ has shown that microorganisms play an important part in the digestive mechanism of chicks. He attempted to raise sterile chicks on sterile vegetable tissues. His results were such that he concluded microorganisms were absolutely necessary.

Stocks (1904)⁽³²⁶⁾ strengthened Omelianski's work on the decomposition of gums. He noted that several types of organisms were seemingly involved in the breakdown.

Esten and Mason (1912)⁽⁷³⁾ have studied the formation of silage. They attribute the action largely to bacteria and yeasts. They noted that the sugars were fermented and recognized alcohol, and acetic and lactic acids as end-products. Hunter and Bushnell's work (1921)^(134, 135) lead to similar conclusions. They found large numbers of organisms of the *bulgaricus* and colon groups.

Smith (1923)⁽³¹⁰⁾ has shown that there are thermophiles that can decompose gums. Additional information on the aerobic and anaerobic decomposition of gums has been summarized by Thaysen and Bunker⁽³⁴⁷⁾.

Mizuno⁽¹⁹⁵⁾ received, in 1924, a French patent on an anaerobic fermentation tank to be utilized for the production of combustible gas. He filled this tank with inverted V-shaped troughs. These kept the fermenting materials submerged and hence he had no trouble with the formation of a heavy mat on the surface of the liquor. He started his

fermentation with 60 per cent rice or wheat bran, 10 per cent sewage sludge, and 30 per cent water. He was unable to use the gas for three weeks, after which time the methane content reached as high as 30 per cent. No gas yield data are given. Bran would seem an expensive material from which to produce a gas containing at most only 30 per cent of methane. Since bran contains mainly starch with very little cellulose and only small amounts of less easily digestible crude fiber, this author probably did not observe the accumulation of undigested residue and made no provision for its removal. Such would be necessary if crude fibrous materials were to be fermented.

Eedish and Rettger⁽²⁶⁴⁾ reported in 1924, biochemical studies on pure cultures of twelve representative anaerobes. No CH₄ production was reported. Anderson⁽³⁾ has studied the gas metabolism of a number of pure cultures of anaerobes. He recovered CO₂ and H₂ from carbohydrates but reports no CH₄ formation. Aibel and Salabartan⁽⁹⁾ and Nord⁽²¹⁶⁾ carried out similar studies with *B. coli* (anaerobic). No CH₄ formation was reported.

Waksman⁽³⁷⁵⁾ has investigated the role played by bacteria and other organisms in the process of humifying plant materials. He stated in 1924 that for every milligram of available nitrogen, 40 to 50 milligrams of cellulose could be decomposed. Anderson⁽⁴⁾ has found the optimum ratio of cellulose to nitrogen to be 35:1.

Beckman (1930)⁽¹⁶⁾ has worked out a process whereby he utilized the protein and carbohydrate splitting properties of *Bacillus delbrueckia*, a thermophilic anaerobe, to liberate the natural oils from certain nuts. A better grade of oil is obtained at less expense than by the older pressure process.

III. FERMENTATION OF PURE COMPOUNDS.

THE METHANE FERMENTATION OF CARBOHYDRATES*.

BATCH EXPERIMENTS.

After the studies of sludge digestion by Buswell and Neave⁽⁴⁵⁾ it became apparent that a clearer understanding of such digestion could best be acquired by observing the decomposition of pure organic compounds. In other words, a knowledge of the digestion as a whole must be approached through a study of its individual parts. It was with this purpose in mind that an extensive investigation of the fermentation of a variety of compounds was undertaken.

"Batch" experiments were first carried out for the purpose of ascertaining whether any correlation existed between the structure of the substrate and the products produced. Would each carbohydrate, for example, break up in its own characteristic manner, evolving some gas and leaving behind some inert, tell-tale parts? In some cases these experiments were not successful. The reason for failure was obvious after the introduction of the volatile acid test. This test showed that one large feeding, such as was used in the "batch" experiments, often gave rise to a volatile acid concentration high enough to prohibit further fermentation by the culture.

The difficulty was obviated by the initiation of controlled feeding. By this method a check was maintained on the volatile acid concentration by feeding the substrate intermittently in small portions.

A further refinement in technique resulted from the discovery of Breden and Buswell⁽³⁰⁾ that asbestos could serve as well as sewage sludge as a nidus or resting place for the bacteria. Since sludge introduced a considerable amount of undesirable organic matter into a culture, its elimination made possible the excellent balances obtained with pure compounds.

In the early experiments the quantity of substrate needed to produce a 1 per cent solution was added. The inoculum was digested sludge from an anaerobic digestion of domestic wastes and contained 4-5 per cent solids. Seven to eight hundred ml. of this inoculum (plus substrate) was placed in a 1-liter, wide-mouthed, brown bottle. This was closed by a one-hole, rubber stopper, (containing a 7 m.m. glass delivery tube) and sealed with wax. The delivery tube was connected to a gas collecting bottle filled with saturated salt solution. Brine displaced by

* The work under this topic was done by Dr. G. E. Symons and the results were taken from his Ph.D. Thesis, U. of Ill., 1932 and the publication: Symons with Buswell, J. Am. Chem. Soc., 55, 2028 (1933).

gas was collected in a movable reservoir in such a manner that a slight positive pressure was maintained on the gas. Two controls (inoculum to which no substrate had been added) were set up with each series of experiments. All gases produced were measured and analyzed. The fermentations were allowed to proceed from 1 to 2 months or longer.

The large amount of inoculating material used was to insure the most favorable conditions for the digestion, i. e., sufficient bacterial inoculation, proper buffer capacity, etc. Even under these conditions, in some instances, acids were produced in quantities which stopped fermentation within a few days.

This fact led to the adoption of a daily feeding type of experiment in which a feeding tube was added to the digestion bottle and the substrate added in small quantities daily or whenever gasification subsided. Under these conditions the most favorable results could be expected.

Since in these experiments it was impossible to strike a carbon balance, the per cent yield could not be calculated in the same manner as it was in the balanced experiments conducted later. It was calculated as follows:

$$\text{Per cent yield} = \frac{\text{weight of gas produced per gram fed}}{\text{theoretical weight of gas per gram fed}}$$

This figure is comparable to the per cent recovery of carbon as gas in the carbon balances. Results appear in Table II.

From Table III it can be observed that amyl alcohol (commercial mixture of iso-and active forms) did not ferment when fed in concentrations of 1 per cent. Ethyl ether, too, gave less gas than the control. Petroleum ether was apparently unattacked for practically no more gas was obtained than from the control. This may have been due to lack of solubility. Salicin fermented vigorously the first 2 days and then ceased. It is quite possible that the glucose part of the molecule was attacked and removed first, setting free salicylic acid which stopped the fermentation.

Acetone was not attacked for more than 30 days but then vigorous gasification set in lasting about a week. Trimethylene glycol was at-

TABLE II.
QUALITATIVE EXPERIMENT—33 DAYS.

Substrate.	Grams fed.	Gas volume (CO ₂ +CH ₄).	Gas composition ratio CO ₂ : CH ₄ .		Per cent yield.
			Theory.	Actual.	
Dextrin.....	8.4	6,395	1 : 1	1.04 : 1.00	93.5
Ethyl alcohol.....	6.9	5,615	1 : 3	1 : 4	76.8
Galactose (d).....	9.0	5,715	1 : 1	1.2 : 1.0	88.0
Lactose.....	7.7	5,220	1 : 1	0.97 : 1.00	85.6
Levulose.....	7.4	3,228	1 : 1	1.39 : 1.00	64.5
Maltose.....	7.8	4,960	1 : 1	1.1 : 1.0	83.0
Mannitol (d).....	8.2	5,676	11 : 13	11.6 : 13.0	95.0
Raffinose.....	6.7	4,573	1 : 1	0.94 : 1.00	81.2
Xylose (d).....	7.0	3,649	1 : 1	1.01 : 1.00	70.0

TABLE III.
 QUALITATIVE EXPERIMENT—110 DAYS.

Substrate.	Grams fed.	Gas volume (CO ₂ +CH ₄).	Gas composition ratio CO ₂ : CH ₄ .		Per cent yield.
			Theory.	Actual.	
Acetone.....	7.0	7,260	1:2	0.975:2	89.5
Amyl alcohol (mixt.).....	7.0	-2,500	1:3		
Arabinose.....	7.0	5,720	1:1	0.77:1	105.0
Dulcitol.....	7.0	5,240	11:13	11:13	97.0
Ethyl ether.....	7.0	-2,000	1:3		
Glycerol.....	7.0	4,655	5:7	4.79:7	88.7
Inulin.....	7.0	5,820	1:1	0.87:1.0	97.3
Levulose.....	7.0	4,440	1:1	0.83:1	81.5
Petroleum ether.....	7.0	10			
Rhamnose.....	7.0	3,980	11:13	9.7:13	58.7
Saccharose.....	7.0	5,515	1:1	0.95:1	100.0
Salicin.....	7.0	-2,000	6:7		
Starch.....	7.0	5,460	1:1	0.82:1	90.2
Trimethylene glycol.....	7.0	3,080	1:2	0.77:2	48.8

tacked very slowly. In 9 months less than half had been gasified. This confirms Buswell and Neave's observations⁽⁴⁵⁾.

Table IV shows that the mixture of amyl alcohols will ferment if fed in small concentrations. The total amount of ethers fed was added at the beginning of the experiment. Duplicate control bottles gave amounts of gas varying by 200 cc. Any amount of gas differing from the control by less than that volume was taken as an indication that the substance was not fermenting.

Table V presents data obtained from a feeding type of qualitative experiment. Acetaldehyde feedings were increased gradually from 0.1 g. per day to 1.0 g. per day. The other substrates were added in 1 g. quantities the first day; this concentration (0.012 per cent) should not have been bacteriostatic. The ethers again appeared not to be attacked. Heptadecyl amine was fed as a water suspension of the hydrochloride.

 TABLE IV.
 QUALITATIVE EXPERIMENT—140 DAYS.

Substance.	Grams fed.	Gas volume (CO ₂ +CH ₄).	Gas composition ratio CO ₂ : CH ₄ .		Per cent yield.
			Theory.	Actual.	
Amyl alcohol (mixt.).....	4.8	5,340	1:3	0.95:3	87.0
Butyl ether.....	9.1	60	1:3		
Ethyl ether.....	7.2	140	1:3		
Isopropyl ether (commercial)	7.2	-400	1:3		

Formaldehyde when added in concentrations of 0.5 per cent did not ferment. In this experiment 0.1 g. was added at a time. This was added twice as a formalin solution (38.7 per cent). When it did not gasify, an attempt was made to depolymerize it by distilling and collecting as a dilute solution. This dilute solution was fed at the rate of 0.1

TABLE V.
 QUALITATIVE EXPERIMENT.

Substrate.	Grams fed.	Gas volume (CO ₂ +CH ₄).	Gas composition ratio CO ₂ : CH ₄ .		Per cent yield.
			Theory.	Actual.	
Acetaldehyde.....	11.4	11,620	3 : 5	2.72 : 5	98.0
n-Butyl ether.....	1.0	-323	1 : 3	-----	-----
Formaldehyde.....	1.0	-80	1 : 1	-----	-----
Heptadecylamine.....	1.0	-115	1 : 3	-----	-----
Isopropyl ether.....	1.0	-240	1 : 3	-----	-----

The following compounds were fed to the same inoculum at different periods. Each period was from 5-10 days. Substances listed in chronological order.

Formic acid.....	5.0	1,530	3 : 1	2.1 : 1	98.0
Oxalic acid.....	8.0	2,800	7 : 1	5 : 1	85.0
Acetic acid.....	5.0	3,500	1 : 1	1 : 1	94.3
*Acetaldehyde.....	4.8	4,970	3 : 5	2.8 : 5	87.0
**Tartaric acid.....	8.0	4,320	11 : 5	9 : 5	89.9
Succinic acid.....	5.0	2,640	9 : 7	9.4 : 7	85.0

* Depolymerized.

** Optically active form.

g. every third day for 3 weeks. The extent to which depolymerization was brought about is not known and this may account for the lack of gasification.

That polymerization is important is evident from an experiment in which 4.0 g. of paraldehyde was fed during the course of 8 days to 2 liters of culture solution. There was no gasification. The culture remained active however for when dextrose was fed, gas was immediately produced. Later the paraldehyde was distilled with a trace of sulphuric acid present and collected in water. This dilute solution containing 375 mg. per ml., when fed to active cultures, was fermented as indicated by the data in Table V.

It is not apparent why ethers are not attacked but it may be due to the ether linkage or to their non-ionizability. Coupin⁽⁵⁹⁾ reported that ethyl ether was not a suitable source of carbon for a mold, *Penicillium glaucum*.

SHIFTING GAS RATIOS.

In the "batch" experiments it was noted that the carbon dioxide: methane ratio changed as the fermentation of a substrate proceeded. The percentages of these two gases occurring at different periods of the fermentation are given in Tables 39 and 40 in Symons' thesis. It is noted that the carbon dioxide percentage is always higher at the beginning of the fermentation than the over-all theoretical ratio of the two gases.

Hoppe-Seyler⁽¹³¹⁾ observed this phenomena of shifting gas ratios and Omelianski⁽²²⁴⁾ attributed it to the oxidation of the methane by free oxygen. This explanation will not suffice in the present experiments because the amount of oxygen present at the beginning of the experi-

ments was too small to cause any such large oxidation of methane as would have been necessary to produce the noted amount of excess carbon dioxide. Also the air was soon swept out of the culture flask by the active gasification of the substrate. Finally, had the methane been oxidized, the final over-all gas ratio could not have approached the theoretical gas ratio as it did.

It is possible that a combination of two explanations may apply to account for the shifting ratios. First, acids formed early react with the bicarbonate present to liberate a large amount of carbon dioxide and second, the part of the molecule decomposed during the acid formation has a higher carbon dioxide content.

Tarvin studied in detail the change in composition of the gas evolved during the fermentation of dextrose. By this method he hoped to interpret the path of decomposition taken by the sugar. The data on this work are presented in Part IV.

QUANTITATIVE EXPERIMENTS.

Inverted bottles or large filter flasks were used for these experiments. The set-up shown in Figure I was connected to a gas collector and gasometer. Inoculation for these tanks was first, liquor from anaerobic digestion tanks; later, liquor from one experiment was used as the inoculum for another.

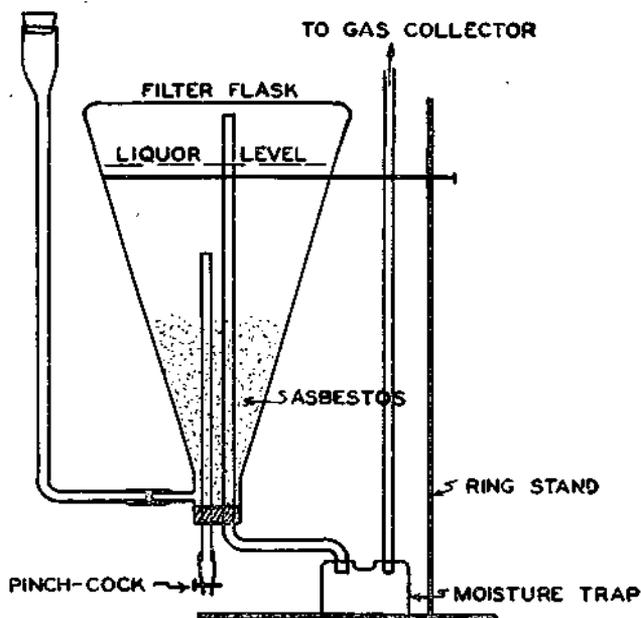
The amount of substrate fed was regulated so that the weight of gas produced between feedings was equivalent to not less than 85 per cent of the theoretical yield. At the same time that the substrate was fed, inorganic nitrogen in the form of ammonium hydroxide was added at the rate of 6 mg. nitrogen per gram of substrate. The concentration of ammonia nitrogen in the liquor was maintained at 400-600 mg. per liter.

Sludge containing high amounts of organic matter introduced a large possible error into the carbon balances. To obviate this difficulty and still provide a resting place for the bacteria, finely chopped asbestos⁽³⁰⁾ (previously ignited to remove volatile matter) was added to the flasks in amounts of 25 g. per liter of solution. To each liter of inoculating solution 100 mg. of each of the following salts was added: K_2HPO_4 , $Mg_3(PO_4)_2$, $(NH_4)_2SO_4$.

Analyses were made on all inoculating media. The following determinations were made: residue, volatile matter, total CO_2 , ammonia nitrogen, organic (Kjeldahl) nitrogen, volatile acids (reported as acetic acid), lactic acid, chlorides and alkalinity. At the end of an experiment the same determinations were made on the culture medium. Analysis of the liquor was also made for unfermented substrate and qualitative tests were made for ethyl alcohol and formic acid. The asbestos was analyzed to determine total increase in dry weight, total increase in volatile matter, and gain in total organic nitrogen. Gas was analyzed every few days. From these data it was possible to strike a balance and account for the amount of substrate fed that was not converted to CO_2 and CH_4 , and the amount of CO_2 that was dissolved in the solution, as well as the gas formed. Table VI includes a summary of all of the

data on experiments in which a complete carbon balance, was determined.

Popoff⁽²⁴⁹⁾ stated that the optimum temperature for the methane fermentation was 40° C. but Omelianski⁽²²³⁾ and Groenewege⁽¹¹²⁾ found 30-35° C. the most favorable, as did Hatfield, Symons and Mills⁽¹¹⁷⁾. In the present investigation it was found that the rate of gasification increased rapidly with temperatures above 20° C. and it appeared that at 33-35° C. the best results were obtained when working at mesophilic temperatures. Coolhaas showed that the fermentation also proceeds at thermophilic temperatures⁽⁵⁷⁾. One series of experiments was carried out at 58-60° C.



APPARATUS FOR BALANCED FEEDING EXPERIMENTS

FIG. I

The question of specificity of the organisms was next investigated. It was desirable to know whether a flora built up from the fermentation of one substance would suffice for the fermentation of another or whether a different flora would be necessary. In this experiment different substances were fed to the same flask without analyzing the flask contents between different experiments except for total CO_2 . Fifteen to twenty-five grams of substrate was fed over a period of several days. After the

TABLE VI.

SUMMARY OF DATA ON EXPERIMENTS IN WHICH A COMPLETE CARBON BALANCE WAS DETERMINED.

A. Conditions of Experiment and Substrate.

Substrate.	Grams fed.	Temperature °C.	Duration (days).	Volume of culture flask (liters).	Asbestos (grams).	Inoculum (grams).		Carbon in substrate fed (grams).
						Source.	Ash free.	
Cellulose.....	10.0	35.0	35	2.0	50	L.....	2.60	4.25
Dextrose.....	1004.0	29.5	136	7.3	S.....	136.80	402.0
Dextrose.....	652.0	33.1	155	7.3	135	L-A ₂	67.40	272.5
Ethylene glycol.....	38.7	35.0	79	2.0	50	L-A ₄	1.96	14.98
d-Galactose.....	9.9	35.0	35	2.0	50	L.....	2.64	44.05
Lactose.....	253.6	34.7	74	7.3	176	L-B ₂	24.50	103.90
Levulose.....	29.5	35.0	75	2.0	50	L-A ₄	1.96	11.77
Maltose.....	10.0	35.0	33	2.0	50	L.....	2.64	14.33
d-Mannitol.....	79.6	35.2	45	7.3	185	L-A ₄	3.90	31.40
Raffinose.....	21.0	35.0	100	2.0	50	L.....	2.30	9.00
Sucrose.....	70.8	35.0	105	2.0	50	L-A ₄	6.4	29.30
Xylose.....	474.0	31.1	46	7.3	S.....	257.00	189.30
Xylose.....	242.5	30.7	70	7.3	185	L-A ₃	14.00	97.09

B. Products.

Substrate.	Carbon recovered as—				Carbon accounted for as protoplasm.		Total carbon accounted for.		Moles CO ₂ / moles CH ₄ .	
	CO ₂ grams.	CH ₄ grams.	Total gas.		Grams.	Per cent of total fed.	Grams.	Per cent of total fed.	Actual.	Theoretical.
			Grams.	Per cent of total fed.						
Cellulose.....	1.60	1.6	3.2	75.3	1.01	23.8	4.21	99.1	1/1	1/1
Dextrose.....	174.43	175.05	349.48	37.0	48.50	12.1	368.75	99.3	0.996/1	1/1
Dextrose.....	123.10	124.8	247.9	91.0	19.30	6.9	267.20	97.9	0.986/1	1/1
Ethylene glycol.....	4.33	8.08	12.59	36.1	1.75	11.7	14.54	97.3	2.994/5	3/5
d-Galactose.....	1.69	1.66	3.35	32.6	0.80	19.7	4.16	102.3	1.018/1	1/1
Lactose.....	50.50	49.7	100.2	92.1	7.40	6.8	107.60	98.9	1.028/1	1/1
Levulose.....	4.76	5.03	9.79	53.0	1.35	11.5	11.44	94.5	0.944/1	1/1
Maltose.....	1.79	1.78	3.57	82.4	0.79	18.2	4.36	100.6	1.005/1	1/1
d-Mannitol.....	13.00	15.20	28.20	89.5	4.10	13.0	32.30	102.5	11.08/13	11/13
Raffinose.....	3.91	4.00	7.91	87.9	1.00	11.1	8.91	99.0	0.978/1	1/1
Sucrose.....	12.20	13.10	25.3	85.0	3.9	13.1	29.2	98.1	0.953/1	1/1
Xylose.....	86.42	92.07	178.49	94.3	15.30	8.0	193.80	102.3	0.938/1	1/1
Xylose.....	46.11	45.98	92.09	94.7	4.95	5.1	97.04	99.8	1.006/1	1/1

^a Gain in organic nitrogen x 5. ^b Liquor from anaerobic digestion of domestic wastes. ^c Sludge from anaerobic digestion of domestic wastes. ^d 0.75 grams carbon recovered as lactic acid. ^e Liquor from Experiment A2. ^f Includes the carbon in 0.28 grams of acetic acid decomposed from inoculum. ^g Anhydrous. ^h Includes the carbon in 0.12 grams of acetic acid and 0.24 grams of lactic acid decomposed from inoculum.

last feeding the culture was allowed to stand until gas production had practically ceased, then another substrate was similarly fed. The data are shown in Table VII. The organisms are not specific because when new substances were added they began fermenting at once.

THE ROLE OF WATER IN THE REACTION.

Hoppe-Seyler⁽¹³¹⁾, Sohngen⁽³¹⁴⁾, Coolhaas⁽⁵⁷⁾, et al., noted that water is involved in the fermentation. Buswell and Boruff⁽²²⁾ reported

data which indicated that the weight of gas recovered from an anaerobic fermentation of mixed organic matter is greater than the weight of material decomposed, by the amount of water which has entered the reaction. Larson, Boruff and Buswell⁽¹⁶⁴⁾ made a carbon study of sludge digestion and concluded that the average weight of gas collectable per pound of volatile matter digested is about 1.2 pounds.

TABLE VII.
SUMMARY OF DATA ON EXPERIMENTS IN WHICH THE GAS YIELD ONLY
WAS DETERMINED.

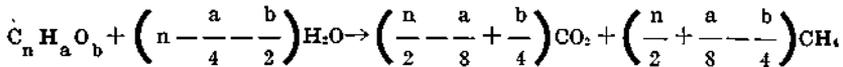
A. Experiments at Mesophilic Temperature (33-35°).

Substrate.	Grams fed.	Recovery of carbon as gas (per cent).	Moles CO ₂ / moles CH ₄ .	
			Actual.	Theoretical.
Acetic acid.....	15.0	91.5	0.994/1	1/1
n-Amyl alcohol.....	25.2	88.4	0.965/3	1/3
Amyl alcohol (act.).....	15.5	67.9	1/3	1/3
n-Butyl alcohol.....	27.6	95.4	0.996/3	1/3
Dextrin.....	10.0	87.0	0.946/1	1/1
Ethyl acetate.....	12.8	91.6	3.13/5	3/5
Ethyl alcohol.....	15.0	95.7	1.042/3	1/3
Ethyl alcohol.....	30.5	88.5	0.994/3	1/3
Formic acid.....	9.0	82.6	2.45/1	3/1
Glycerol.....	10.0	97.5	4.95/7	5/7
Inulin.....	10.75	100.0	0.993/1	1/1
Lactic acid.....	9.0	100.0	0.948/1	1/1
Methyl alcohol.....	14.0	98.6	0.989/3	1/3
Methyl alcohol.....	50.9	85.6	1.001/3	1/3
Oxalic acid.....	14.95	90.0	6.89/1	7/1
Propyl alcohol.....	25.0	75.3	0.997/3	1/3
Pyruvic acid.....	15.0	96.7	6.55/5	7/5
Starch.....	18.8	94.5	0.937/1	1/1
Succinic acid.....	5.0	90.0	8.64/7	9/7
Xylose.....	15.0	86.1	0.932/1	1/1

B. Experiments at Thermophilic Temperature (58-60°).

Acetaldehyde.....	31.0	95.4	3/5	3/5
Dextrose.....	24.5	98.1	0.996/1	1/1
Isoamyl alcohol.....	25.4	85.9	1/3	1/3
Lactose.....	23.7	88.2	0.966/1	1/1
Starch.....	25.2	80.1	0.965/1	1/1
Sucrose.....	25.0	81.2	0.995/1	1/1
Xylose.....	22.5	96.4	0.978/1	1/1

In the present study the weight of water entering into the overall catabolic phase of the fermentation was determined to show the agreement of the data obtained with the theoretical results predicted by the general equation (R):

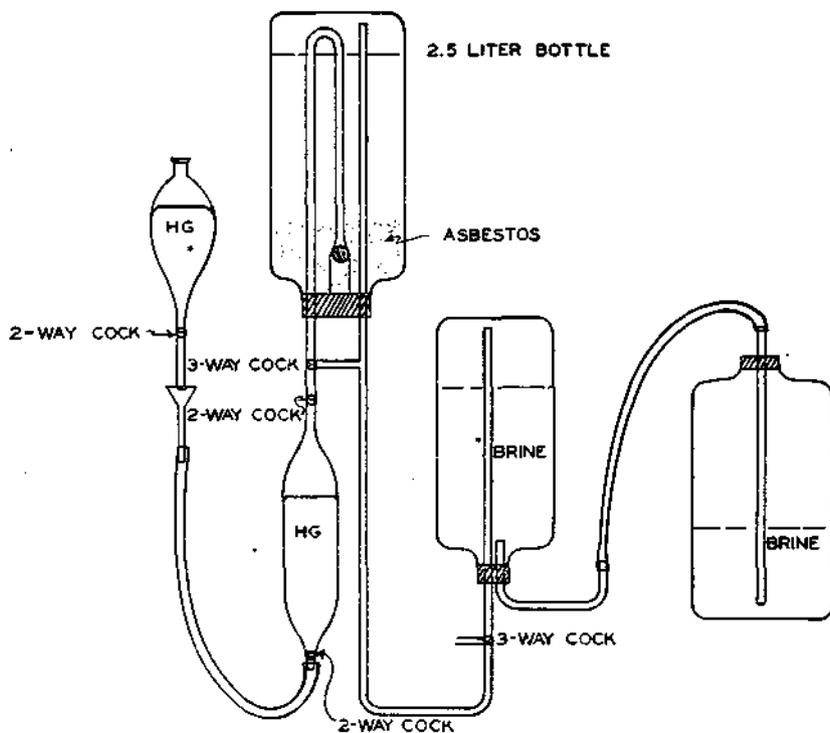


It was apparent from the carbon balances that the amount of substrate not converted into cell substance was converted into gas. Therefore, the percentage of substrate available for the gasification reaction was taken as the same as the amount of carbon recovered as

gas. Using this value the weight of the water that should theoretically enter the reaction was calculated. The weight of the water entering the reaction according to the experimental data was determined by comparing the weight of the gas produced (based on the methane volume) with the weight of substrate entering the reaction. See Table VIII.

THE REACTION OF CARBON DIOXIDE AND HYDROGEN.

One other type of feeding experiment utilized the apparatus shown in Figure 2. Seeded asbestos and culture liquor were added to the bottle



APPARATUS FOR STUDYING THE BACTERIAL
REDUCTION OF CARBON DIOXIDE

FIG. 2

and thereafter a mixture of CO_2 and H_2 was bubbled through the fermentation mixture. The apparatus was so arranged that the gases could be removed, analyzed, and replaced by more CO_2 and H_2 . Results appear in Table IX. From data collected in this experiment it was possible to verify the work of Sö hngen⁽³¹⁴⁾ and Fischer, Lieske, and Winzer⁽⁷⁷⁾ on the reaction:

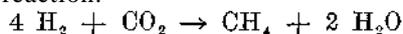


TABLE VIII.
 WEIGHT OF WATER ENTERING THE OVERALL REACTION.

Substrate.	Grams fed.	Per cent yield.	Grams substrate catabolized.	Grams of gas produced.	Grams of water added in reaction.	
					Theoretical.	Actual.
Dextrose.....	1,004.0	87.0	872.0	878.00	0.0	+6.0
Xylose.....	474.0	94.3	448.0	462.00	0.0	+14.0
Dextrose.....	682.0	91.0	621.0	627.00	0.0	+6.0
Xylose.....	242.5	94.7	229.5	230.50	0.0	+1.0
Mannitol.....	79.8	89.5	71.3	67.80	-3.7	-3.5
Lactose.....	258.6	92.1	238.1	248.00	+12.4	+9.9
Sucrose.....	70.8	85.0	60.2	65.90	+3.3	+5.7
Cellobiose.....	10.0	75.3	7.53	8.04	+0.31	+0.5
Levulose.....	29.5	83.0	24.5	25.20	0.0	+0.7
Galactose.....	9.9	82.6	8.18	8.34	0.0	+0.16
Ethylene glycol.....	38.7	86.1	33.3	28.55	-4.8	-4.75
Maltose.....	10.0	82.4	8.24	8.80	+0.44	+0.56
Raffinose.....	21.0	87.9	18.48	20.00	+1.24	+1.52
Methyl alcohol.....	50.9	85.6	43.6	31.30	-12.35	-12.30
Ethyl alcohol.....	30.5	88.5	27.0	28.20	0.0	+1.2
Propyl alcohol (n).....	25.0	75.3	18.85	21.70	+2.8	+2.9
Butyl alcohol (n).....	27.6	95.4	26.3	32.60	+6.3	+6.3
Amyl alcohol (n).....	25.2	88.4	22.3	29.20	+6.8	+6.9
Amyl alcohol (act.).....	15.5	67.9	10.5	13.80	+3.3	+3.3
Starch.....	18.8	94.5	17.78	19.85	+2.0	+2.1
Inulin.....	10.75	100.0	10.75	12.00	+1.2	+1.25
Dextrin.....	10.0	87.0	8.70	9.98	+0.95	+1.23
Dextrose.....	24.5	98.1	24.0	24.1	0.0	+0.1
Xylose.....	22.5	96.4	21.7	21.9	0.0	+0.2
Sucrose.....	25.0	81.2	20.25	21.4	+1.10	+1.15
Lactose.....	23.7	88.2	20.9	22.2	+1.10	+1.3
Starch.....	25.2	80.1	20.2	22.8	+2.2	+2.6
Isoamyl alcohol.....	25.4	85.9	21.8	28.6	+6.7	+6.8
Acetaldehyde.....	21.0	95.4	20.0	24.2	+4.15	+4.20
Pyruvic acid.....	15.0	96.7	14.5	16.3	+1.45	+1.8
Formic acid.....	9.0	82.6	7.44	5.86	-1.47	-1.58
Ethyl acetate.....	12.8	91.6	11.7	14.08	+2.42	+2.38
Oxalic acid.....	14.96	90.0	13.45	12.10	-1.35	-1.35
Glycerol.....	10.0	97.5	9.75	8.82	-0.95	-0.93
Succinic acid.....	5.0	90.0	4.50	4.82	+0.35	+0.32

 TABLE IX.
 FORMATION OF CH₄ FROM H₂ AND CO₂.

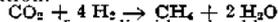
Date.	Additions.				Removals.			
	CO ₂ .	H ₂ .	CH ₄ .	N ₂ .	CO ₂ .	H ₂ .	CH ₄ .	N ₂ .
10-31-31 set up.....								
11-10-31 contents.....	137		171	225				
11-10-31.....	585	2,630		40				
11-30-31.....	660	2,340		40	130	112	454	80
12-16-31.....	570	2,650		45	82	170	596	80
12-28-31.....	529	2,690		40	40	215	563	77
12-29-31 (loss).....					78	394	42	31
1-12-32.....	525	2,990		30	28	31	441	56
1-29-32.....	110	1,100		20	42	125	643	70
2-6-32.....		1,885		35	19	73	168	16
2-17-32 left.....					32	880	695	60
From solution.....	800							
Total.....	3,866	15,685	171	470	451	2,000	3,661	550

Carbon dioxide disappearing = 3,866 - 451 = 3,415 = 1,841 g. carbon

Hydrogen disappearing = 15,685 - 2,000 = 13,685 = 1,230 g. hydrogen

Methane formed = 3,661 - 171 = 3,490 = 2,502 g. methane

According to the equation:



12 grams of carbon as carbon dioxide should react with 8.0624 grams of hydrogen to give 16.0312 grams of methane. The above data show 12,000 grams of carbon reacting with 8,019 grams of hydrogen to give 16,305 grams of methane.

THE EFFECT OF HIGH PRESSURE.

No evidence has yet been submitted to show that any hydrocarbon other than methane is formed by the fermentation. It was with the hope of producing some higher hydrocarbons that a high pressure experiment was attempted. Inoculated asbestos, liquor and dextrose were incubated at 25-30° C. for 2 months at 10,000 lbs. pressure per square inch. A steel bomb was used as the reaction chamber. Gas production was apparently inhibited by the pressure and the reaction stopped before the intermediate steps were completed. Prior to being subjected to this pressure the culture was fed the same amount of dextrose (6 grams) and this was all gasified within a period of 3 weeks. However, when the culture and the same amount of dextrose were incubated under pressure the amount of gasification was negligible, indicating that this phase of the reaction was inhibited by the pressure. The actual cessation of bacterial action was probably due to the inhibiting concentrations of acids present (pH 4.0).

From Table X it can be seen that the lactic acid produced was a far larger amount than any of the other products. Since the method employed to determine lactic acid would probably include pyruvic as well, it may be that some of the acid reported here as lactic was pyruvic

TABLE x.

Substrate: Dextrose. Amount fed: 6.0 grams.
 Temperature: 25-30° C. Pressure: 10,000 lbs. per sq. inch.
 Duration: 60 days. Volume of culture flask: 600 ml.
 Inoculum: Seeded asbestos and liquor from a previous fermentation.
 Asbestos: 15 grams (seeded).

Data.	ml.	Grams.
Volume of gas produced (N ₂ free).....	15.4	
CH ₄	91.0%	14.0
H ₂	1.3%	0.18
CO ₂	7.8%	1.2
Dissolved CO ₂	20.0	
Weight of gas produced.....		0.05
Analysis of liquor:		
pH = 6.0.		
Acetic acid.....	880 mg./liter	0.53
Propionic acid.....	1,865 mg./liter	1.00
Lactic acid.....	3,000 mg./liter	1.501
Succinic acid.....	60 mg./liter	0.036
Dextrose.....	378 mg./liter	0.53
Protoplasm (estimated).....	10%	0.6
Total recovery.....		4.647

Per cent recovery = 77.3.

acid. Acetic and propionic acids were determined by the/ partition method of Osborn and Werkman⁽²³⁰⁾. The succinic acid determined was doubtful and the amount of dextrose remaining unchanged in the liquor was probably not exact. However, the large amounts of acids recovered strongly favors the suggestion that propionic, lactic, and acetic acids are intermediates.

THE BACTERIAL FLORA.

Stained slides of the bacterial flora were made on all experiments by Dr. C. R. Breden. Since pure cultures of the methane formers have not been isolated (Omelianskf's cultures⁽²²³⁾ were shown to be impure by McBeth and Scales⁽¹⁷⁹⁾), inoculum was obtained that was known to contain the methane organisms as well as a large number of other varieties. These indicated that the heterogeneity of the culture changed but little during the course of an experiment where but one substance was fed. Any apparent diminution of one form seemed to have little significance because with a change of food material it might reappear. Micrococci and short thin rods were found in all cultures. In the mechanism study where only carbon dioxide and hydrogen were fed these forms seemed to be the predominant ones, though a transfer of the culture to an enrichment medium brought forth apparently as many forms as were originally present in the culture.

NITROGEN RATIOS AND REQUIREMENT.

Asbestos to which organisms were attached was dried and the organic nitrogen and organic carbon were determined on one-gram samples. This was done in duplicate in samples of culture • asbestos from two different experiments. In Table XI are presented the data calculated on the basis of one gram of bacterial protoplasm. From these data it is apparent that the percentage of each of these constituents varied somewhat from different samples but that the ratio was very nearly the same in both cases. It is quite likely that this ratio would vary more in some cases than is shown by these data, but in view of the information available⁽³²⁾ this figure is very near the average.

When an experiment was completed and the final analysis made of the flask contents, both the volatile matter attached to the asbestos and the organic nitrogen were determined. From these data it was possible to determine the percentage of nitrogen in the cell substance, i. e., the nitrogen-volatile matter ratio. These data are shown in Table XII and the average shows that the nitrogen content of the cells was 9.5

TABLE XI.
NITROGEN-CARBON RATIO IN BACTERIAL PROTOPLASM.

Experiment.	Trial.	Milligrams per gram of cell substance.		Ratio—N : C.
		Organic nitrogen.	Organic carbon.	
I.....	1.....	113	550	1 : 3.17
	2.....	105	580	
	Average.....	109	565	
II.....	1.....	85	415	1 : 4.86
	2.....	80	385	
	Average.....	82.5	400	
Total average.....				1 : 5.03

TABLE XII.
ORGANIC NITROGEN-VOLATILE MATTER RATIO IN BACTERIAL
PROTOPLASM.

Substrate	Org. Nit. :Vol. Mat. Ratio
Dextrose	1:10.3
Xylose	1:10.0
Dextrose	1:12.0
Xylose	1:10.1
Mannitol	1:8.0
Lactose	1:10.0
Sucrose	1:9.0
Cellobiose	1:12.1
Levulose	1:11.5
Galactose	1:10.5
Ethylene glycol	1:11.5
Maltose	1:11.5
Raffinose	1:10.2
Average	1:10.5

per cent. This figure agrees very well with the average of the data listed by Buchanan and Pulmer⁽³²⁾. That it is in error due to the fact that inorganic salts were not considered is no doubt true, but it furnished an estimate to use in certain calculations.

By determining the gain in the organic nitrogen of the culture it was possible to calculate the amount of nitrogen necessary for metabolic needs. This calculation is expressed in milligrams of inorganic nitrogen required per gram of substrate fed. These data, Table XIII, show that where the inoculation is small the amount of material converted into protoplasm is large. Thus it appears that the first thing a culture does is to build itself up in preference to carrying on the gas reaction. The average of these data show that 9.8 mg. of nitrogen is required per gram fed but a plot of the data shows that when amounts of 100 grams or more are fed the nitrogen requirement of the culture is about 6 mg. per gram of substrate fed. These data were checked by determining the amount of ammonia nitrogen before and after fermentation as well as that added.

TABLE XIII.
NITROGEN REQUIREMENT.

Substrate.	Grams fed.	Mg. nitrogen required per gram substrate fed.
Dextrose.....	1,004.0	8.6
Xylose.....	474.0	6.5
Dextrose.....	682.0	5.7
Xylose.....	242.5	4.1
Mannitol.....	79.6	10.3
Lactose.....	258.6	5.7
Sucrose.....	70.8	6.8
Cellobiose.....	10.0	20.2
Levulose.....	28.5	9.1
Galactose.....	9.9	16.0
Ethylene glycol.....	38.7	9.1
Maltose.....	10.0	15.8
Raffinose.....	21.0	9.4

THE METHANE FERMENTATION OF ORGANIC ACIDS*

QUANTITATIVE STUDIES.

Continuous fermentations were carried on in inverted 4-liter flasks. The inoculum consisted of either overflow liquor from a sewage digestion tank or liquor from a previous fermentation. At first 5-10 g. of sludge per liter of liquor was deemed necessary for satisfactory decomposition of the substrate. These large quantities were used in the first fermentation of acetic acid and in the fermentation of n-butyric acid. It was evident, however, that the presence of such a large amount of digestible material with the decomposing pure substance might cause erroneous results in the data obtained from the overall decomposition of the pure substance. Later it was found possible to omit all sludge and to use only shredded asbestos⁽³⁰⁾ and liquor filtered through an asbestos mat and diluted with an equal volume of tap water.

The manner of feeding varied with the particular acid fed. In some runs the liquor was analyzed and the sodium salt of the desired acid fed at once; in other runs, active fermentations were first obtained by feeding sodium acetate and acetic acid. Where acetate was fed at the beginning, the fermentation was allowed to subside and the liquor was analyzed before feeding the desired substrate. The sodium salt of the acid was fed until sufficient buffer capacity (due to NaHCO_3) had developed to maintain a pH of 6.6-6.8, then a dilute solution of the free acid was fed. The free acid could not be fed in every case, however, due to low solubility. In the case of valeric and stearic acids a mixture of the sodium salt and the free acid was fed. In the case of aromatic acids it was necessary to feed the sodium salts entirely.

The sodium salts were prepared by titration of the free acids with NaOH, using ethyl alcohol to dissolve the acid where necessary. The alcohol was evaporated on the water bath, the residue washed with ether, dried, weighed, made up quantitatively with distilled water, and fed with a pipette.

The rate of fermentation varied with the substances fed and feeding was at such a rate that most of the material fed was recovered as gas before more of that material was fed. This method resulted in a high yield of gas since insufficient food was present to allow high conversion into protoplasm.

All gas evolved during the fermentations was collected and analyzed at intervals. The inoculum liquor was analyzed before the fermentation and again some days after the last feeding was made, i. e., when the fermentation was apparently complete. Total residue, volatile matter, total carbon dioxide, ammonia nitrogen, organic nitrogen, volatile acids, lactic acid, chlorides, and alkalinity were determined. The asbestos was analyzed before and after fermentation to determine increase in dry weight, increase in volatile matter and gain in organic nitrogen. From the data obtained carbon balances were made which include substrate carbon gasified, recovered as acids, and converted into protoplasm. These are presented in Table XIV.

* The work under this topic was done by Dr. D. Tarvin and the results were taken from his Ph.D. Thesis, U. of Ill., 1933 and the publication: Tarvin and Buswell, J. Am. Chem. Soc., 56, 1951 (1934).

TABLE XIV.

SUMMARY OF FERMENTATIONS OF ACIDS.

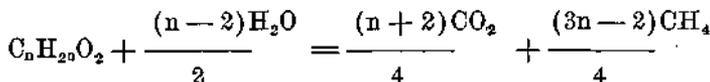
Numbers 3, 7, S, 17 at 55°; Number 9 at 25-30°; all others at 32-34°.

Acid.	Grams fed.	Inoculum volatile matter, grams.	Duration, days.	Grams carbon in substrate fed.	Grams carbon accounted for as—		Acids.	Proto-plasm.	Total carbon accounted for, per cent.	Ratio CO ₂ : CH ₄ .	
					CO ₂ .	CH ₄ .				Actual.	Theoretical.
Formic.....	125.5	3.55	118	32.70	23.70	8.10	-----	0.45	98.6	2.92 : 1.00	3.09 : 1.00
Acetic I.....	186.0	18.00	118	74.40	36.90	39.30	2.90	-----	103.1	0.94 : 1.00	1.00 : 1.00
Acetic II.....	118.7	5.91	75	47.48	23.10	23.60	+2.50	.50	99.5	.93 : 1.00	1.00 : 1.00
Propionic.....	72.0	3.19	128	35.00	14.40	21.00	0.30	.60	100.0	.69 : 1.00	0.71 : 1.00
Butyric.....	168.0	10.66	125	92.10	31.15	53.05	9.85	1.40	101.4	.59 : 1.00	.60 : 1.00
Isobutyric.....	84.8	2.58	122	46.65	16.41	28.25	0.26	1.15	98.7	.58 : 1.00	.60 : 1.00
Valeric.....	53.3	2.56	100	31.60	9.10	17.90	1.54	1.79	96.0	.51 : 1.00	.54 : 1.00
Stearic.....	12.4	3.40	180	9.45	2.47	7.03	-----	0.25	103.0	.35 : 1.00	.38 : 1.00
Benzoic I.....	45.5	5.59	160	31.20	13.58	16.20	0.34	.96	98.6	.84 : 1.00	.87 : 1.00
Benzoic II.....	45.9	3.94	130	31.50	13.82	16.52	-----	.98	99.3	.84 : 1.00	.87 : 1.00
Phenylacetic.....	31.3	2.61	144	22.50	8.16	11.33	-----	.64	91.5	.72 : 1.00	.78 : 1.00
Hydrocinnamic.....	34.9	2.58	135	25.04	9.07	13.70	-----	1.40	96.4	.66 : 1.00	.71 : 1.00
Cinnamic.....	49.0	3.46	120	35.75	15.30	21.15	-----	0.42	103.0	.72 : 1.00	.86 : 1.00
Lactic.....	85.7	2.61	125	34.30	18.30	16.50	0.20	1.35	100.00	.99 : 1.00	1.00 : 1.00
Oxalic.....	90.6	2.55	145	24.15	19.53	3.20	-----	0.32	95.5	6.10 : 1.00	7.00 : 1.00
Succinic.....	112.4	2.93	130	45.85	25.00	18.95	-----	.90	100.0	9.20 : 7.00	9.00 : 7.00
Malic.....	126.9	3.72	125	45.40	27.10	15.65	-----	.94	96.5	10.40 : 6.00	10.00 : 6.00
Tartaric.....	128.5	3.87	132	41.10	27.40	12.72	-----	1.60	97.6	11.75 : 5.00	11.00 : 5.00
Alanine.....	19.9	1.15	300	7.53	3.02	3.05	.005	0.275	83.2	0.99 : 1.00	1.00 : 1.00
Tyrosine.....	15.0	1.15	300	8.95	2.45	3.01	b.38	.42	70.0	.81 : 1.00	0.89 : 1.00

^a Inoculum carbon gasified.^b Phenol.

The amounts of water involved in the decomposition of the acids studied are given in Table XV. These amounts were calculated from a consideration of the grams of material gasified with the actual and theoretical CO_2 and CH_4 produced.

The work on fatty acids yielded results which check those of Neave and Buswell for the decomposition according to the formula given by them⁽²¹¹⁾.



No difficulty was observed in obtaining the fermentation of propionic and valeric acids in contrast to the findings of Sö hngen⁽³¹⁴⁾. Stearic acid decomposed much more slowly than other fatty acids.

FERMENTABILITY OF AROMATIC COMPOUNDS.

The aerobic decomposition of certain aromatic compounds, including benzene, has been reported. Whether such compounds could be decomposed anaerobically and what effect substituent groups might have upon decomposition of the ring were matters of considerable specula-

TABLE XV.
ADDITION OF WATER IN ACID FERMENTATIONS.

Acid.	Grams fed.	Per cent gasified.	Grams gasified.	Grams CO_2 and CH_4 actual.	Grams CO_2 and CH_4 theoretical.	Water added.	
						Actual.	Theoretical.
Formic.....	125.5	97.2	122.00	97.44	98.04	-24.56	-25.96
Acetic I.....	186.0	102.4	188.00	187.53	186.00	+1.53	0.00
Acetic II.....	118.7	98.5	116.80	119.96	116.80	+3.16	0.00
Propionic.....	72.0	100.0	72.00	79.79	80.75	+7.79	+8.75
Butyric.....	168.0	91.2	153.20	184.82	184.00	+31.62	+31.70
Isobutyric.....	84.8	95.7	81.10	97.81	97.60	+16.71	+16.50
Valeric.....	58.3	85.5	45.50	57.28	57.60	+11.78	+12.10
Stearic.....	12.4	101.0	12.40	18.52	18.70	+6.12	+6.30
Benzoic I.....	45.5	95.5	43.50	71.25	72.50	+27.75	+29.00
Benzoic II.....	45.9	96.2	44.10	72.75	73.50	+28.65	+29.40
Phenylacetic.....	31.3	88.6	27.75	45.80	46.05	+18.05	+18.30
Hydrocinnamic.....	34.9	91.0	31.75	51.50	52.65	+19.75	+20.90
Cinnamic.....	49.0	100.2	49.00	84.40	84.60	+35.40	+35.80
Lactic.....	85.7	95.6	82.00	79.84	82.00	-2.36	0.00
Oxalic.....	90.6	94.3	85.50	78.63	76.85	-6.87	-8.55
Succinic.....	112.4	98.1	110.80	116.85	118.85	+6.55	+8.55
Malic.....	126.9	93.4	118.20	120.28	118.20	+2.28	0.00
Tartaric.....	128.5	95.1	122.10	117.26	115.00	-4.84	-7.10
Alanine.....	19.9	80.7	16.05	15.17	16.22	-0.98	+0.05
Tyrosine.....	15.0	81.0	9.15	13.06	13.30	+3.91	+4.15

tion. In order to obtain relevant data, two series of bottle experiments were set up, using 1-liter brown bottles. The bottles were closed with rubber stoppers which were equipped with feeding and gas delivery tubes covered with sealing wax and connected to closed systems containing saturated, acidified, NaCl solution. Rubber connections were cut to a minimum because of permeability to nitrogen. Asbestos was placed in each bottle. Analyzed, overflow liquor from a sewage digestion tank was used for inoculum in the first series. The desired sub-

stance was then fed in small quantities. In the second series liquor and asbestos from a previously active dextrose fermentation were used and 1 g. of sodium acetate was fed at the beginning. Following the decrease in activity at completion of decomposition of the acetate, the liquor was analyzed and the desired substance fed in small quantities. Where any gas evolution was exhibited upon standing a few weeks, more of the substrate was fed. All gas evolved was collected and analyzed. After several months the apparatus was dismantled and the liquor analyzed. Results appear in Table XVI.

o-Phthalic acid, salicylic acid, and phenol can apparently be decomposed. Other substances gave either negative or doubtful results. Benzyl alcohol was believed to be oxidized to benzoic acid but positive identification could not be made. No decomposition of benzene, toluene, benzaldehyde, bromobenzene, or aniline was noted.

Previous quantitative experiments showed that benzoic, phenyl-acetic, hydrocinnamic and cinnamic acids could be decomposed rather

TABLE XVI.
FERMENTABILITY OF AROMATIC COMPOUNDS.

Series A.

	Inoculum V. M. grams.	Substrate fed grams.	Gas produced (246 days).			Increase grams gas.	Per cent substrate gasified.
			CO ₂ grams.	CH ₄ grams.	Total grams.		
Control.....	1.45		0.091	0.085	0.176		
Benzene.....	1.45	0.85	0.126	0.138	0.264	0.108	12.3
Phenol.....	1.45	1.00	0.188	0.230	0.418	0.242	24.2

Series B.

	Inoculum V. M. grams.	Substrate fed grams.	Gas produced (148 days).			Increase V. M. grams.	Per cent substrate gasified.
			CO ₂ grams.	CH ₄ grams.	Total grams.		
Benzene.....	1.86	0.83					
Bromobenzene.....	1.86	2.99					
Aniline.....	1.86	1.03					
Toluene.....	1.86	0.86					
Benzyl alcohol.....	1.86	3.15	0.490	0.001	0.491	2.95	15.5
Benzaldehyde.....	1.86	4.23					
Salicylic acid.....	1.86	4.90	1.420	0.530	1.950	2.40	39.8
o-Phthalic acid.....	2.00	4.70	3.770	0.780	4.550	0.33	96.9

completely. The decomposition of phenyl-acetic acid was more difficult to start than the others. A long lag phase appeared with benzoic acid also. No aromatic derivatives were isolated from the fermentation of benzoic, phenyl-acetic, or hydrocinnamic acids. Fatty acids appeared as intermediates. Tyrosine gave rise to phenol and this phenol was apparently decomposed.

FERMENTATION RATES.

There is very little data available concerning the rates of decomposition of various pure substances. Thermophilic decomposition seems to be somewhat more rapid than mesophilic. In order to investigate the rate of decomposition, 1-normal acetic acid was fed continuously to a culture. As much was fed as could be metabolized without causing the tank to go sour.

The decomposition of certain cannery wastes suggested an investigation of the decomposition rates of succinic, malic, and tartaric acids. Following quantitative fermentations of these acids as much acid was fed intermittently as could be metabolized.

A comparison of the rates of decomposition of acetic, succinic, malic, and tartaric acids is given in Table XVII. Whether or not the rates of decomposition of these acids are a function of the amount of oxygen present in the molecule is not known, but it is evident that larger quantities of tartaric and malic can be decomposed per day than of succinic. Propionic acid was apparently produced from each of the acids fed which indicates a reduction of hydroxyl groups and a decarboxylation of one end of the molecule in each case.

TABLE XVII.

RATES OF FERMENTATION.

TANK VOLUME: 3,500 c. c. in all cases.

Acid fed.	Asbestos (grams).	Temperature.	Days.	Average volume gas per day (c. c.).	Acid decomposed per day (grams).	Maximum propionic (p. p. m.).
*Acetic.....	20	55°	32	1,900	2.54
Succinic.....	40	35°	28	1,320	1.72	2,350
	40	35°	17	1,340	1.76	(not determined)
Malic.....	46	55°	24	1,720	2.65	2,250
	46	55°	21	2,490	3.85	(not determined)
Tartaric.....	40	35°	20	2,000	3.24	1,400
	40	35°	21	1,900	3.18	(not determined)

* The acetic acid was fed continuously; the other acids were fed intermittently.

INTERMEDIATES.

Attempts were made to isolate and identify intermediates suspected of playing important roles in the degradation of the various substrates fed.

Where continuous fermentations were carried on in 4-liter flasks, liquor was withdrawn at intervals, examined and the accumulated residues saved for identification purposes. Because of the limited quantity of liquor which could be withdrawn at one time from a 4-liter flask without affecting the fermentation, only small amounts of material were obtainable for examination. In order to obtain more workable quantities of material, a 20-liter bottle was used for production of fatty acids from dextrose. The concentration of acids in the bottle was built up by over-feeding of dextrose, then 10 liters of liquor was

withdrawn and used for identification of intermediates. For identification of volatile acids produced during fermentation, sodium salts were concentrated and concentration followed by Duclaux distillation constant determinations, application of color tests and a specific test for formic acid. In the case of acids produced from dextrose, the boiling point, specific gravity, refractive index and neutral equivalent were determined and derivatives prepared according to the method of Drake and Bronitsky, using p-phenylphenacyl bromide⁽⁶⁵⁾. Lactic acid was determined where it occurred^(33b). Phenol was identified in the tyrosine fermentation by means of its tribromo derivative and was quantitatively determined at intervals by Baylis' modification of the Gibbs method⁽¹⁵⁾. A summary of intermediates found in the various fermentations and manner of identification of each is given in Table XVIII.

Alanine and tyrosine were deaminated with increases in the ammonia nitrogen content of the liquor of 2,120 mg. and 640 mg. respectively. Of 450 mg. of phenol present after feeding tyrosine, 150 mg. or 33 % was decomposed in 16 days.

The bacterial reduction of lactic to propionic acid and subsequent disappearance of the latter was observed. Tests made after feeding pyruvic acid showed lactic and propionic acids to be absent from the fermentation liquor. After a few hours acetic and formic acids were found, indicating the true hydrolysis of an alpha-keto acid. Formic and oxalic acids were found to be decarboxylated, a portion of the resulting carbon dioxide and all of the hydrogen being converted quantitatively to methane and water.

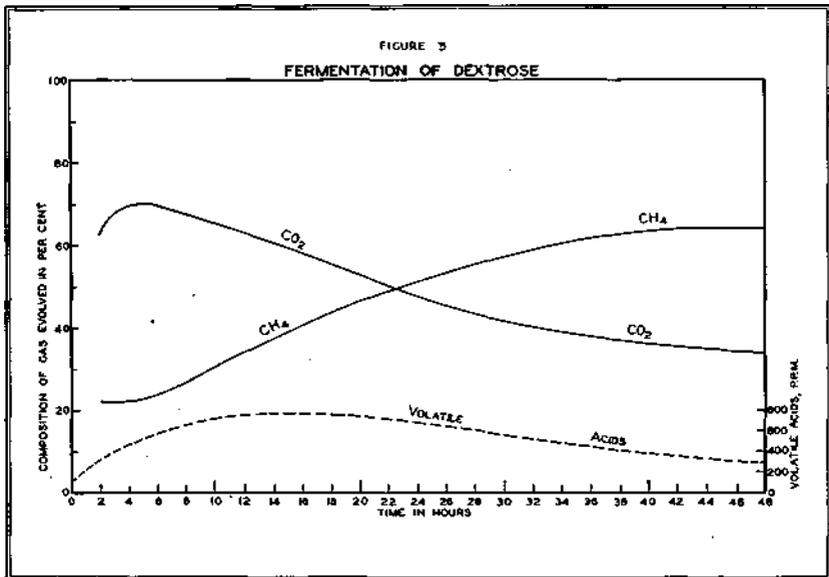
TABLE XVIII.
INTERMEDIATE STUDIES.

Substrate.	Substances indicated.	Manner of identification.				
		Gas ratio.	Duclaux constants.	Color test.	Specific test.	Derivative.
Propionic acid.....	Acetic acid.....			+		
	Formic acid.....			+	+	
Isobutyric acid.....	Lower fatty acids.....		+			
Benzoic acid.....	Formic acid.....		+		+	
Phenylacetic acid.....	Acetic acid.....		+			
Formic acid.....			+			
Hydrocinnamic acid.....	Acetic acid.....		+			
	Formic acid.....		+			
Lactic acid.....	Propionic acid.....	+		+		
Succinic acid.....	Propionic acid.....		+	+		
Malic acid.....	Propionic acid.....		+	+		
Tartaric acid.....	Propionic acid.....		+	+		
Pyruvic acid.....	Acetic acid.....		+	+		
	Formic acid.....			+	+	
	Acetaldehyde.....					+
Alanine.....	Ammonia.....	Distillation and titration				
Tyrosine.....	Ammonia.....	Distillation and titration				
	Phenol.....			+	+	+
Dextrose.....	Butyric acid.....	+	+	+		
	Propionic acid.....		+	+		+
	Acetic acid.....		+	+		+
	Formic acid.....				+	
	Lactic acid.....				+	
Ethyl alcohol.....	Propionic acid.....		+			
	Acetic acid.....		+			
	Acetaldehyde.....				+	
Milk waste.....	Propionic acid.....		+			
	Acetic acid.....		+			
	Formic acid.....				+	
	Ester.....					

VARIANCE IN GAS COMPOSITION.

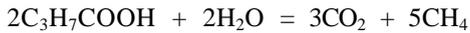
If the nature of a fermenting substrate changes, the change in composition of gas evolved during the fermentation should reflect the nature of that change. For example, the over-all ratio of carbon dioxide to methane obtained in the fermentation of formic acid is 3:1, for acetic it is 1:1 and for butyric it is 3:5. It is apparent that if dextrose is converted to a fatty acid, the nature of that acid should be revealed in the composition of gas evolved during its subsequent decomposition. Accordingly, attempts were made to determine the mechanism of carbohydrate fermentation by investigation of change of composition of the gas produced during fermentation. For this purpose, the 20-liter fermentation bottle was used and 50 g. of substrate fed at one time. Gas evolved was analyzed every two hours until fermentation subsided. Analyses of the liquor were also made at intervals; these included dissolved carbon dioxide, volatile acids, and pH.

The results obtained with dextrose are shown in Figure 3. A nearly constant 3:1 ratio of carbon dioxide to methane was observed during



the first six hours of fermentation. Following this, there was a steady downward drift of the carbon dioxide curve and a corresponding upward drift of the methane curve. At thirty-six hours the curves again approached the horizontal and an approximate 3:5 ratio of carbon dioxide to methane continued during the last twelve hours. These ratios would

appear to indicate production and decomposition of butyric acid in accordance with



Butyric acid had been previously identified in dextrose fermentation, together with propionic and acetic (Table XVIII), but no evidence was obtained as to which was produced first.

The above postulated reactions do not agree completely with the facts observed. A comparison on the weight basis, from data obtained, shows that during the first six hours of fermentation 9.70 g. of butyric acid was produced, together with 13.90 g. of carbon dioxide and 1.41 g. of methane, whereas the theoretical would be 7.30 g. of carbon dioxide and 0.88 g. of methane. Therefore, it appears that some other intermediate such as a non-volatile substance must be produced, either in conjunction with or separate from butyric acid. The exact substance cannot be stated at present, but the observed 3 : 1 ratio of carbon dioxide to methane at the beginning of fermentation theoretically may be correlated with production of either succinic or levulinic acid, as well as butyric.

The other carbohydrates studied, i. e., levulose, sucrose and starch, produced methane and, carbon dioxide curves similar to the one for dextrose. High carbon dioxide and low methane production at the beginning of fermentation were reversed at the end. Volatile acids were produced as intermediates but calculations of these as butyric acid do not correlate directly with the weights of carbon dioxide and methane produced at the beginning of fermentation. Starch showed a twelve hour lag phase before beginning of fermentation which may possibly indicate hydrolysis during that time.

As stated above, the methane and carbon dioxide curves for the mono-, di- and polysaccharides are quite similar. However, the curves for hydrogen are somewhat different and the average amounts of hydrogen produced from/ 50 g. each of the various carbohydrates may be significant. These are indicated in Table XIX.

TABLE XIX.

	Total fermentation time, hours.	Total hydrogen average volume, cc.	Maximum hydrogen in evolved gas for any 2 hour period, per cent.
Dextrose.....	48	933	11.0
Levulose.....	72	925	15.6
Sucrose.....	60	3,893	30.7
Starch.....	120	2,030	22.0

A survey of the above data indicates that the mono-saccharides produce less hydrogen than the disaccharide and polysaccharide. In contrast to the carbohydrates, no hydrogen was produced from any of

the lower fatty acids in the series formic to valeric, with the exception of a little from formic. Further evidence that the nature of the compound fermented rather than the culture at hand is the dominating-factor in hydrogen production is shown in the following experiment: 50 g. of sucrose was fed to an active culture and allowed to ferment until gasification ceased. Acetic acid-sodium acetate mixture was then fed under the same conditions, allowed to ferment, and finally sucrose was fed a second time. The amounts of hydrogen produced from sucrose, acetate and sucrose were 4,000, 120, and 3,587 cc, respectively.

ACID INTERMEDIATES IN THE METHANE FERMENTATION*

ACIDS FROM THE FERMENTATION OF DEXTROSE.

When dextrose is fermented there is a rapid evolution of gas in the beginning which later subsides to a slower, more steady evolution as the reaction proceeds. It is during this initial, rapid period that the acids are formed. The later evolution of gas results from the decomposition of the acids first formed.

When dextrose was fed in large amounts high acid concentrations were built up. Analyses employing the methods of Osburn and Werkman⁽²³⁰⁾ on liquor containing 6,535 p.p.m. volatile acid showed 28.0 per cent acetic and 72.0 per cent butyric acid.

A large portion of the acid liquor could be removed, the remainder diluted to original volume with water, and acids again built up by feeding dextrose. This procedure could be accomplished repeatedly with no apparent detrimental effect on the bacteria producing the acids. This was not a normal fermentation because the acids formed did not continue to ferment to gases. The reaction stopped at the acid-intermediate stage.

The gas evolved from this high acid liquor was of a different composition from that of a normal methane fermentation. There was no methane present at all and the hydrogen, which is usually present in quite small amounts, was now one of the two most important constituents. An analysis of a sample of the gas taken after the volatile acid concentration had reached 7,000 p.p.m. showed an approximate $\text{CO}_2:\text{H}_2$ ratio of 1:1.

The variance of the acid intermediates during the progress of a normal fermentation of dextrose was next studied. This made it necessary to draw off samples of the culture liquor for analysis at intervals during the course of the reaction. For this purpose a 12-gallon culture bottle was used. The culture was maintained at 53° C. because the fermentation proceeded more rapidly at this thermophilic temperature. The Duclaux method of analysis was used. Numerical results appear in Table XX. Figure 4 is a graphical representation. A second fermentation gave similar results.

* The work under this topic was done by Dr. H. R. Todd and the results were taken from his Ph.D. Thesis, U. of Ill., 1936.

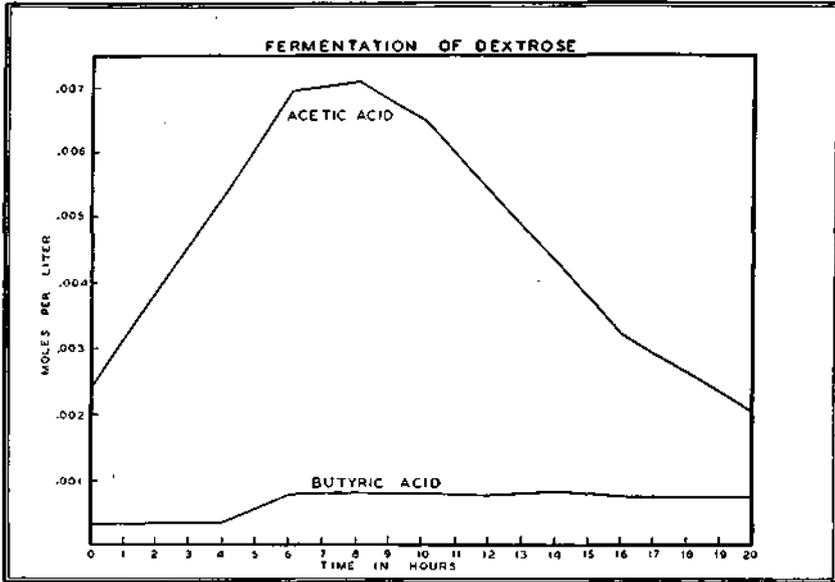


FIGURE 4

TABLE XX.

SUBSTRATE: Dextrose.
 AMOUNT FED: 42.2 g.
 TEMPERATURE: 53° C.
 VOLUME OF LIQUOR: 42.2 liters.

Time (hours).	Acetic acid (p. p. m.).	Butyric acid (p. p. m.).	Acetic acid (per cent).	Butyric acid (per cent).	Acetic acid (moles/liter).	Butyric acid (moles/liter).
Present before feeding..	146	30	83.0	17.0	.00243	.00034
After feeding:						
4.....	322	33	90.7	9.3	.00537	.00037
6.....	416	68	86.0	14.0	.00693	.00077
8.....	423	71	85.6	14.4	.00705	.00081
10.....	389	70	84.8	15.2	.00648	.00080
12.....	325	69	82.5	17.5	.00542	.00078
14.....	256	72	78.0	22.0	.00427	.00082
16.....	193	64	75.1	24.9	.00322	.00073
20.....	125	64	66.1	33.9	.00208	.00073

ACIDS FROM VARIOUS SUBSTRATES.

A. *Lactic Acid*. This substrate was fed to a culture which had been used to ferment sodium acetate. There was 397 p.p.m. acetic acid remaining from the previous fermentation and an amount of lactic acid was fed such as would produce a concentration of 727 p.p.m. Results shown below indicate a transformation to propionic acid as an intermediate. The acetic acid was decomposed more rapidly than the propionic acid so that after a time only propionic acid was found in the liquor. This being the case propionic acid may pass through an acetic

Time.	Acetic acid.	Propionic acid.
0 hours	397 p. p. m.	
24	357	600 p. p. m.
48	180	536
64	57	522
88		414

acid stage without any accumulation of the latter to indicate its formation.

B. *Butyric Acid.* This acid decomposed rather rapidly into acetic acid and gas as shown in Table XXI and Figure 5. It decomposed more rapidly than the acetic acid as evidenced by the steady accumulation of the latter. The acetic acid concentration did not decrease until almost all of the butyric acid has decomposed. The fact that after the fermentation had been going for 76 hours there were more molecules of acetic acid than there were of butyric acid in the beginning indicates that one molecule of butyric acid must form more than one molecule of acetic acid. In other words, one molecule of butyric acid does not merely break down to one molecule of acetic acid and evolve the remainder of the molecule as gas. It is quite possible, however, that hydrogen and carbon dioxide are formed and that they react immediately while still in the liquor to form acetic acid.

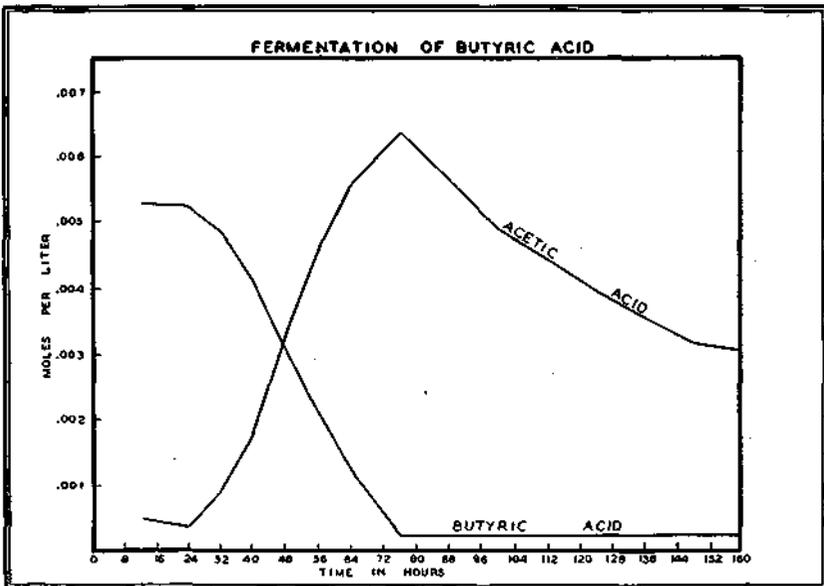


FIGURE 5

C. *Propionic Acid.* This acid fermented without the accumulation of any other acid. This is what would be expected from the results secured on the fermentation of lactic acid. Acetic acid may have been formed as an intermediate but decomposed more rapidly than the propionic acid so that it was not found in the liquor.

TABLE XXI.
 SUBSTRATE: Butyric acid.
 AMOUNT FED: 15 g.
 TEMPERATURE: 53° C.
 VOLUME OF LIQUOR: 45 liters.

Time (hours).	Acetic acid (p. p. m.).	Butyric acid (p. p. m.).	Acetic acid (per cent.).	Butyric acid (per cent.).	Acetic acid (moles/liter).	Butyric acid (moles/liter).
Present before feeding..	43	20	68.2	31.8	.00072	.00023
After feeding:						
12.....	28	465	5.7	94.3	.00047	.00528
24.....	21	463	4.3	95.7	.00035	.00526
32.....	53	430	11.0	89.0	.00058	.00459
40.....	105	365	22.3	77.7	.00175	.00415
48.....	195	268	42.1	57.9	.00325	.00305
56.....	273	186	59.5	40.5	.00455	.00211
64.....	334	110	75.2	24.8	.00557	.00125
76.....	331	20	95.0	5.0	.00635	.00023
100.....	294	21	93.3	6.7	.00490	.00024
124.....	235	33	91.2	8.8	.00397	.00026
148.....	191	22	89.7	10.3	.00318	.00025
313.....	75	21	78.1	21.9	.00125	.00024

D. *Propionic and Butyric Acids.* The propionic acid disappeared rather rapidly when these two acids were fermented together as indicated in Table XXII and Figure 6. As was noted before, the decomposition of butyric acid produced an accumulation of acetic acid. Although the Duclaux method cannot be very accurate when used for estimating a mixture of three acids, still the time of disappearance of one of the acids can be rather definitely established. To be more specific, although the amounts of propionic acid as given are somewhat questionable, there is little doubt but that most of this acid had disappeared at the end of 56 hours.

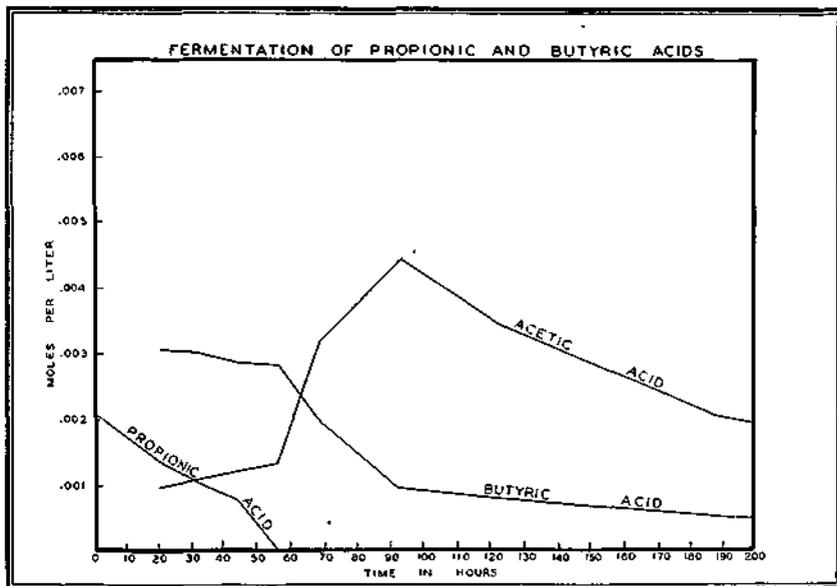


FIGURE 6

TABLE XXII.

SUBSTRATE: Propionic and butyric acids.
 AMOUNT FED: 3.25 g. Propionic acid.
 6.75 g. butyric acid.
 TEMPERATURE: 53° C.
 VOLUME OF LIQUOR: 45 liters.

Time (hours).	(p. p. m.).			(per cent.).			(moles/liter).		
	HOAc	HOPr	HOBu	HOAc	HOPr	HOBu	HOAc	HOPr	HOBu
Present before feeding		153			100.0			.00207	
After feeding:									
20	59	101	270	13.7	23.5	62.8	.00098	.00136	.00307
32	65	77	266	15.9	18.9	65.2	.00108	.00104	.00302
44	73	58	254	18.9	15.1	66.0	.00122	.00078	.00289
56	80		250			75.8	.00133		.00284
68	192		174	52.5		47.5	.00320		.00198
92	265		83	76.2		23.8	.00442		.00094
122	207		69	75.0		25.0	.00345		.00078
158	124		44	73.8		26.2	.00207		.00050
260	88		30	74.6		25.4	.00147		.00034

E. *Formic Acid*. This substrate decomposed without any large accumulation of higher acids. Data indicated some accumulation of butyric acid but this cannot be regarded a certainty due to the small difference in values.

P. *Xylose*. Since the acids resulting from dextrose had been examined, it was desirable to know what acids would result from a 5-carbon sugar. Results on the fermentation of xylose are shown in Table XXIII and Figure 7. Here, as in the fermentation of dextrose, there was a small production of butyric acid but acetic was the principal acid intermediate.

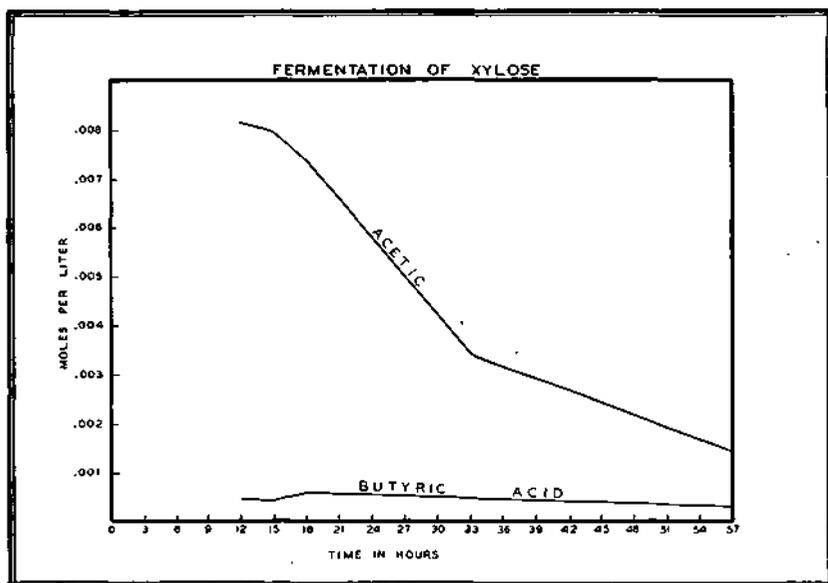


FIGURE 7

TABLE XXIII.

SUBSTRATE: Xylose ($C_5H_{10}O_5$).
 AMOUNT FED: 60 g.
 TEMPERATURE: 53° C.
 VOLUME OF LIQUOR: 45 liters.

Time (hours).	Acetic acid (p. p. m.).	Butyric acid (p. p. m.).	Acetic acid (per cent).	Butyric acid (per cent).	Acetic acid (moles/liter).	Butyric acid (moles/liter).
After feeding:						
12.....	459	36	93.1	6.9	.00815	.00041
15.....	478	34	93.4	6.6	.00797	.00039
18.....	445	48	90.3	9.7	.00742	.00055
33.....	205	39	84.0	16.0	.00342	.00044
57.....	85	24	78.0	22.0	.00142	.00027

G. *Methyl Alcohol*. The accumulation of an acid intermediate higher than formic acid from this substrate would definitely indicate a carbon to carbon synthesis. Results were disappointing, however, for no noticeable accumulation of acid resulted when 45 g. of the alcohol was fermented over a period of three days. The alcohol decomposed rapidly as indicated by the gas evolved.

H. *Ethyl Alcohol*. Data are given in Table XXIV. The alcohol fermented for several days before there was very much increase in acidity. After this time, however, the acidity rose rapidly. The acidity was principally due to an accumulation of acetic acid. A very small accumulation of butyric acid appeared. The acids began to fall after the last determination shown in the data.

TABLE XXIV.

SUBSTRATE: Ethyl alcohol.
 AMOUNT FED: 45 g.
 TEMPERATURE: 53° C.
 VOLUME OF LIQUOR: 45 liters.

Time (hours).	Acetic acid (p. p. m.).	Butyric acid (p. p. m.).	Acetic acid (per cent).	Butyric acid (per cent).	Acetic acid (moles/liter).	Butyric acid (moles/liter).
After feeding:						
25.....	62	8	88.6	11.4	.00103	.00009
37.....	65	16	80.2	19.8	.00108	.00018
49.....	77	22	77.8	22.2	.00128	.00025
97.....	288	33	89.7	10.3	.00480	.00038
121.....	337	42	88.9	11.1	.00562	.00048
125.....	375	28	93.1	6.9	.00625	.00032

THERMODYNAMIC CONSIDERATIONS*.

Acquisition of energy being the motivating force in bacterial degradation of organic matter some treatment of the energetics concerned in these reactions is of interest at this point. It has been shown that the use of heats of formation and heat of combustion do not give true information concerning the energy of a reaction and that the proper cri-

* Taken from Symons with Buswell, J. Am. Chem. Soc., 55, 2028 (1933).

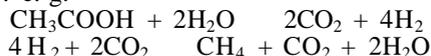
terion is the free energy decrease⁽³¹⁴⁾. It has further been shown that the use of free energies at standard state is not the true criterion because of the change in free energy with concentration and temperature⁽¹⁵⁾. Various other factors (not easily determinable) also enter the consideration and make the calculation of the true free energy decrease practically impossible. However, the free energy decrease in the reaction for all of those compounds for which the free energy of formation is known has been calculated at standard state. In all cases, the reaction is indicated to give off energy (i. e. P is negative.)

If water is the only source of oxygen besides that in the compound undergoing fermentation, the greatest decrease in free energy occurs when carbon dioxide and methane are the end products. In considering that the reaction is a stepwise breakdown as suggested above, it appears that most of the intermediate steps (for which free energy data are available) take place with a decrease in free energy. The step involving pyruvic acid appears to require energy but this reaction may proceed with a decrease in free energy at the concentrations actually existing in the bacterial system⁽³⁹⁰⁾. It has also been suggested⁽⁹⁾ that bacteria may do work on an intermediate reaction if they can obtain energy from the over-all reaction.

The amount of the energy available to the organism appears to be of the same order of magnitude as that previously reported⁽³⁹⁰⁾. In the present investigation only a small amount of the substrate was converted into bacterial cell protoplasm. The fermentation of the remainder was more than sufficient for the energy requirements of the bacteria as calculated by Wilson and Peterson⁽³⁹⁰⁾. Boruff and Buswell⁽²²⁾ calculated from heats of combustion that 6.7% of the total energy available from the complete oxidation of cellulose is lost to the bacteria by its fermentation to carbon dioxide and methane. A similar calculation using the free energy at standard state has been made for the substances in this investigation. The loss varied widely. For carbohydrates it averaged about 12%.

MECHANISM OF THE FERMENTATION.

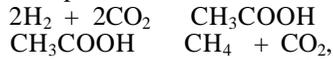
Sohngen intimated that these fermentations proceeded through oxidation-reduction to form hydrogen, carbon dioxide and acetic acid, and showed that the hydrogen reacted with carbon dioxide to form methane and also assumed that the acetic acid was decarboxylated to form methane and carbon dioxide. We have verified Sohngen's experimental results and have felt that his hypothesis was correct. Recently Barker contended that even in the case of the methane fermentation of acetic acid, hydrogen and carbon dioxide formed first and then reacted to form methane: e. g.



Beside the cumbersome character of this mechanism there are several experimental facts which oppose it. In the first place we have never found the slightest trace of hydrogen in the gases formed by the fermentation of acetic acid. In the case of the carbohydrates however

a small amount of hydrogen is always formed. If hydrogen were formed according to the above equations, it should be found in the gaseous products of the fermentation. Secondly, Fischer, Lieske and Winzer⁽⁷⁷⁾ have reported the formation of acetic acid when hydrogen and carbon dioxide are bubbled through the methane inoculum.

This would suggest the steps,



which is exactly opposite to those proposed by Barker^(10a).

IV.

CELLULOSE AND CELLULOSIC MATERIALS. GENERAL METHOD OF ATTACK.

The preliminary studies on the anaerobic decomposition of cellulose and cellulosic materials were carried out by placing a definite weight of the material to be tested in a dark glass, wide-mouthed bottle which was then completely filled with the inoculating liquor or other suitable medium. By the use of a one-hole, tight-fitting, rubber stopper, connection was made by the use of glass tubing (6 mm.) (no rubber connections) to a second and larger bottle which was filled with a slightly acid, saturated brine solution and served as the gas collector. (See Figure 8). The generation of gas in the digestion bottle displaced brine in the second causing the latter to flow into the third bottle which served as a reservoir. By altering the liquid levels gas could be withdrawn at will. The digestion and gas-collector bottles were tightly sealed by pushing a tight-fitting rubber stopper well into the mouth of the bottle and then filling the space left above the stopper ($\frac{1}{4}$ to $\frac{1}{2}$ inch) with hot sealing wax. This was in turn, when cool, covered with paraffin. In some of the later studies, bottles were fitted with mercury-sealed stirrers and still others with flexible rubber connections so that the materials could be thoroughly mixed and agitated at will. These experiments lead to the designing of a special type of digester to overcome the mechanical difficulties experienced by the present writers and earlier workers when fermenting fibrous materials.

Earlier investigators had used river slime, fecal extract, manure, and other substances as the source of their anaerobic organisms. As the overflow liquors and sludge from an anaerobic sewage disposal tank are known to contain a high count of anaerobes as well as suitable sources of nitrogen, and as these liquors were easy to obtain, these were used as the inoculum in the investigations herein reported. In most of the later studies the amount of organic matter introduced in the inoculum was materially decreased (total to about 1.0 gram per liter) by using settled and diluted overflow liquors or asbestos fibers from a bottle or tank that had previously been started with sludge and some asbestos, and either cellulose, cellulosic material, or whatever was to be investigated. The asbestos fibers from such a bottle contain a sufficient number of organisms for inoculation and serve as a suitable inert contact material for starting other bottles or tanks.

Analyses were made of all inoculum solutions used. A bottle containing only the inoculum solution was set up as a control on the bot-



FIGURE 8.

Arrangement of Apparatus Used in Preliminary Bottle Investigations.

tles containing the inoculum plus the substances under consideration. A few synthetic media were tried but they gave at best only very slow rates of gas evolution.

After a certain length of time had elapsed, such time being determined by the amount of gas being liberated, the bottles or tanks were opened, the contents analyzed and the loss in organic matter or in the weight of the cellulose or cellulosic material decomposed, compared with the volume and weight of gas evolved.

Most of the later and more conclusive experiments were carried out by the use of a special "tip-top" bottle set-up, to be described later, or by the use of small digestion tanks (Figure 2) of from 7 to 27 liters capacity built in a manner to resemble standard anaerobic digestion tanks now in use. Three pilot unit tanks of special design and of 1,287, 1,376, and 92 gallons capacity, respectively, were used in plant scale experiments. By the use of these tanks, academic as well as commercial size experiments could be made for the tanks could be fed large quantities of material at will; the gas could be drawn, measured, analyzed, and burned; the residue formed in the process could be drawn periodically and studied; and the general mechanics of operation observed, all without interrupting the activity of the tank. Strict

chemical control was maintained at all times. Special mechanical investigations were also made.

All sanitary chemical analyses were made as outlined in "Standard Methods for the Examination of Water and Sewage," sixth edition, 1925⁽³¹⁹⁾. The gases drawn were measured in a gas burette and analyzed by the use of a modified Orsat apparatus (Illinois Gas Apparatus)⁽²³³⁾. Volatile organic acids were determined by a modified Duclaux method and calculated as acetic. Lactic acid was determined as described in the appendix. Dissolved and total CO₂ were determined by the use of a new and specially designed method which has also been described in the appendix.

Throughout all the experiments, special care has been used in keeping out air (oxygen), thus maintaining as nearly as possible strict anaerobic conditions. Particular care has also been used in keeping all connections gas tight and in collecting the gases liberated during the fermentations. Unless otherwise stated all fermentations were conducted at room temperatures, namely, 25° -30° C.

The fermentation of cellulose will be discussed first. This will be followed by a presentation of laboratory and pilot unit data collected on the anaerobic fermentation of cornstalks, straw, and other cellulosic materials. This will be followed by a discussion of the factors influencing these fermentations.

THE ANAEROBIC FERMENTATION OF CELLULOSE AND STARCH

The first studies carried on were small scale batch experiments in which 10 grams or so of cellulose were added at the start, none being added during the run. It seems advisable, however, to first discuss a few continuous feeding experiments, and follow these with additional data gathered from the other experiments.

A. FEEDING EXPERIMENTS

Prior to 1929, there was no report in the literature of a carefully controlled anaerobic investigation in which an appreciable amount of cellulose was decomposed. Most of the investigators used from 1 to 10 grams and a very few used as much as 25 grams. It was, therefore, thought advisable to conduct some investigations in which large quantities of cellulose were fed, not necessarily all at once, but rather at certain intervals. Such continuous feeding experiments would not only give interesting chemical and bacteriological data but would also involve mechanical manipulations.

Filter paper, Tank A. In order to carry out a continuous feeding investigation, a 27.5 liter galvanized tank (Tank A) was made and equipped (Figure 9) with the necessary feeding tube, a tap at the bottom for withdrawing materials, a gas-collecting dome and connections so that liquor could be drawn from the middle of the tank, circulated through a small air pump that was remodeled to handle liquor, and then discharged back into the tank close to the gas collecting dome.

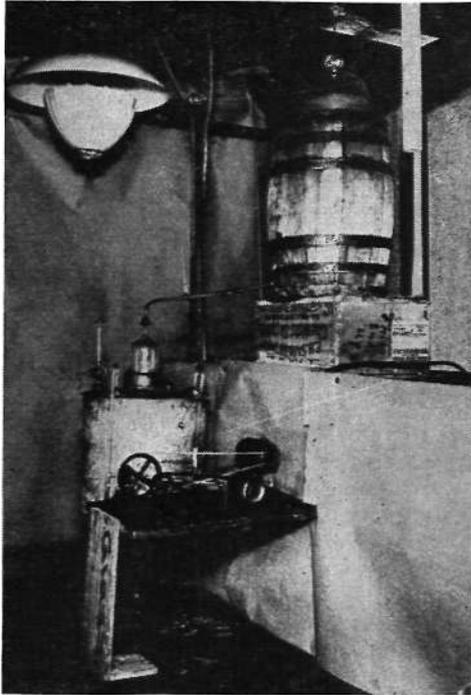


FIGURE 9.

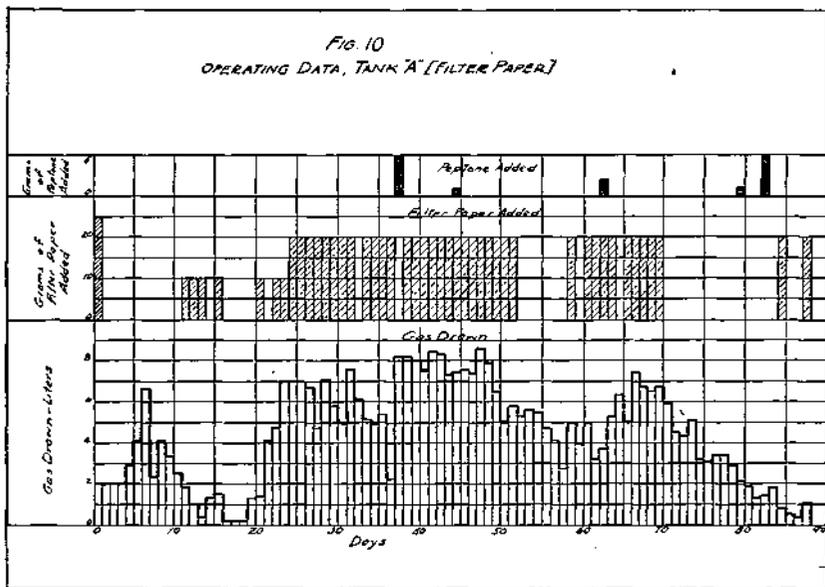
Apparatus Used in Continuous Feeding Investigation on Anaerobic Fermentation of Cellulose, Tank A.

Previous small scale bottle experiments had shown the necessity of keeping the mat of fermenting cellulose that tends to collect at the top of the receiver thoroughly wetted, and if possible, broken up, so as to release the entrapped gas bubbles and thus permit the material to settle into the mother liquor. The gas-collecting dome was connected to a suitable gasometer where the gases were collected and measured. (Figure 2.)

Figure 10 graphically shows the volume of gas drawn daily, as well as the periodic additions of filter paper and the occasional additions of peptone which at this time was thought to be the best source of nitrogen for the organisms. Ammonia nitrogen has since been found to be better.

The fermentation was started by adding an inoculum composed of three parts of overflow liquor from an anaerobic sewage disposal tank and one part of a thin mixture that was actively decomposing cellulose. This inoculum contained 274 p.p.m. of ammonia nitrogen and 497 p.p.m. of total nitrogen. Twenty-five grams of pure cellulose (Whatman Quantitative filter paper No. 40) were also added to the tank. Subsequent additions of filter paper were made in three different ways, namely, in a dry form, as an unsterilized suspension (filter paper in distilled water or liquor from the tank), and as a sterilized suspension. The addition of the filter paper suspensions involved the withdrawal of liquor from

the tank. These liquors were all collected, composited, iced, and at certain intervals, analyzed. A summary of the analyses of the inoculum solution, the overflow composites and the mother mixture left at the end of the run are recorded in Table XXV. The data in parenthesis are in terms of parts per million.



From these data one notes that in all 855 grams (dry weight) of filter paper were added and that from this (Table XXVI) 380.5 liters of gas were collected. This gas volume, when corrected for the increase in total CO_2 content of the tank at the end over what it was at the start, brings the total gas volume to 411.4 liters. The weight of the gas, as drawn and as calculated from its analysis from day to day, was 482.93 grams, which, when corrected for the retained CO_2 , is increased to 543.43 grams. As noted in Table XXVII, these gas weights amount to 56.5 per cent and 63.5 per cent, respectively, of the filter paper added. These gas yields are not of the magnitude noted in certain other experiments which will be discussed later, where 100 per cent of the cellulose added was converted to gas, but they are of a much greater magnitude than any other experiments previously reported in the literature. The writers felt that more cellulose was being added than the tank could metabolize, but the heavy feedings were continued merely to see if the tank would finally "take hold" and ferment the accumulated paper. Toward the end of the run the total solids became quite high (20.9 gm. per l.) and the cellulose very gelatinous. This policy of feeding undoubtedly led to lower yields than would otherwise have been obtained. The small pump, attached to keep the tank stirred, was unable to function in the liquors containing so much filter paper. It was, therefore, necessary to open the tank to the

TABLE XXV.
FERMENTATION OF CELLULOSE, OPERATING DATA.

Substances Added to and Removed from Tank A^a.

Substances Added.

	Volume or weight.	pH.	Total solids, grams.	Organic solids, ^b grams.	Volatile Acids as AcOH, grams.	Ammonia N, p.p.m.	Total N, p.p.m.	Total CO ₂ , grams.
Inoculum solution.....	27.5 liters.	7.4	273.9 (9,960)	149.2	6.05 (220)	(274)	(497)	44.0 (1,600)
Filter paper.....	855.0 grams		855.0	855.0				
Peptone.....	14.0 grams		13.1	12.3				
Inorganic salts.....	22.0 grams		22.0					
Total.....			1,164.0	1,016.5				44.0

Substances Removed in Liquor.

Time:	Volume	pH.	Total solids, grams.	Organic solids, grams.	Volatile Acids as AcOH, grams.	Ammonia N, p.p.m.	Total N, p.p.m.	Total CO ₂ , grams.
1st—23rd..	3.50 liters	6.6	9.56 (2,729)	5.83	1.35 (374)	(294)	(330)	
24th—45th..	5.85 liters	6.5	14.62 (2,500)	6.05	1.43 (244)	(159)	(180)	
46th—62nd..	7.80 liters	6.5	17.63 (2,320)	7.54	3.50 (460)	(76)	(94)	
63rd—67th..	3.60 liters	6.0	8.75 (2,430)	5.25	3.06 (850)	(62)	(75)	
68th—80th..	5.95 liters	5.8	18.10 (3,040)	12.65	7.50 (1,260)	(32)	(90)	
90th (end)...	27.5 liters	7.0	575.0 (20,900)	483.0	48.10 (1,750)			60.5 (2,200)
Total.....			674.66	520.32	64.94			

^a Data in parenthesis are in p. p. m.

^b Volatile matter 700-900° C.

TABLE XXVI.
FERMENTATION OF CELLULOSE, GAS DATA.

Tank A (Filter Paper) As Drawn.

	Composition, per cent.	Volume, liters.	Weight, grams.
CO ₂	47.5	180.48	353.74
CH ₄	47.5	180.71	128.21
H ₂	2.9	10.95	0.98
N ₂	2.1	8.36	*
Total.....		380.50	482.93

Corrected for Increase in CO₂ (Dissolved, etc.).

Gain in total CO ₂		30.9	60.5
Grand total.....		411.4	543.43

^a Weight of N₂ (from air) not added in.

TABLE XXVII.
FERMENTATION OF CELLULOSE, SUMMARY, TANK A.

	Grams.	Per cent in terms of filter paper added.	Per cent in terms of organic matter digested.
Filter paper added.....	555.0	-----	-----
Cellulose digested.....	496.2	58.0	-----
Organic acids formed.....	58.9	6.9	11.9
Gas, drawn.....	482.9	56.5	97.4
Gas, total.....	543.4	63.5	109.5

^a One test showed: 6 parts acetic, 4 parts butyric, trace of some higher fatty acid.

air and stir the contents by hand in order to break up the thick cellulose scum (2 to 3 inches) that collected at the top of the tank. This procedure introduced air and undoubtedly was one of the main factors which brought about the accumulation of organic acids and the high content of CO₂, noted in the gas toward the end of the experiment (55 per cent). The addition of peptone, as a source of nitrogen, and the accumulation of undigested cellulose also probably contributed to the sour condition. Due to dilution, the ammonia content during the run decreased from 274 p.p.m. to only 32 p.p.m. and the total nitrogen content to only 90 p.p.m. (Table XXV). Subsequent work has shown these concentrations to be too low for normal fermentation. The pH during the entire experiment (5.7 to 6.8) was lower than that noted in later studies (Eun 40-3) which gave higher yields of gas from cellulose. As noted toward the end of the experiment, regulation of the pH by the addition of alkalies seemed to aggravate rather than aid the sour condition. The volatile acids during the latter part of the experiment were found to be composed of 6 parts of acetic and 4 parts of butyric. Lactic was present to the extent of about 0.9 p.p.m. Tests for formic acid showed the presence of not over a trace. Qualitative tests for succinic, malic, tartaric, citric, oxalic, propionic, and valeric were all negative.

Filter paper, Run 40-3. Before discussing the significance of certain data collected during the Tank A experiment, the writers wish to introduce the results of two other experiments, one on the anaerobic fermentation of cellulose and one on starch. These feeding experiments were carried out in two-liter bottles equipped with a tube for withdrawing the gas, and one which ran to the bottom of the bottle through which filter paper or starch could be added. These tubes were sealed in with sealing wax. The gas outlet tube was connected to a bottle gasometer (Figure 8) by a short piece of rubber tubing, thus making it possible to thoroughly shake and mix the contents at will. These two bottles and a control were then filled with overflow liquor (1,900 cc.) and 100 cc. of sludge from an active anaerobic sewage digestion tank. Three grams of asbestos were added to the starch bottle. The control gave 1,700 cc. of gas in 35 days. To the other two bottles were added cellulose and starch, respectively. From time to time, further additions were made.

For a time the filter paper added to the one bottle (Run 40-3) was torn in half-inch squares and then shredded by suspending it in water which was violently stirred by a motor-driven piece of broken glass tubing. In five minutes the sharp edges of the tubing had cut the paper into fine shreds which were then filtered off on a Buchner funnel and fed to the bottle. The rate of feeding of this shredded cellulose was practically the same as that when the half inch squares were fed, but the average daily gas production was a little greater (1.0 to 2.3 liters as compared with 1.6 liters per day). These gas production and recovery data are summarized in Table XXVIII. These data are all corrected for the small amount of gas given by the inoculum, which incidently was insignificant as compared with the total amount of gas formed. No liquors were added or drawn during the run except that small volume drawn for analytical purposes. No additional source of nitrogen other than that in the inoculum solution was added during the investigation.

From these data it is evident that at least 2.5 grams of cellulose can be fed to a 2-liter fermentation vessel per day. From this 1.6 to 2.3 liters of gas can be recovered daily. The gas analyses, when corrected for

TABLE XXVIII.
CAPACITY DATA, CONTINUOUS FERMENTATION OF CELLULOSE (FILTER PAPER)
DAILY MIXING.

Run 40-3.

					Total for period.	Extra period.	Recovery summary.
Volume of fermentation bottle ^f , liters....	2	2	2	2	2	2	2
Duration of test, days.....	20	16	20	30	86	11	97
Cellulose fed, grams.....	30	40	50	65	135	0	135
Cut and shredded, grams.....	30	40	0	65	135	0	
Cut only, grams.....	0	0	50	0	50	0	
Average per day, grams.....	1.5	2.5	2.5	2.2	2.2	0	
Gas: ^a							
Total volume, S.T.P., liters.....	20.5	36.7	33.0	47.7	137.9	3.4	152.3
Average per day, liters.....	1.0	2.3	1.6	1.6	1.6	0.8	
Maximum, liters.....	2.9	3.5	3.5	2.7	3.5	1.3	
Total weight, grams ^b	26.6	48.0	42.3	59.2	176.1	10.2	*188.2
Per cent gasification.....	89	120	85	91	95		*107
Representative data:							
pH.....	7.4			6.7		6.5	
Volatile acids, p.p.m.....	300			608		2,600	
Ammonia nitrogen, p.p.m.....	d400			63		0	
Total nitrogen, p.p.m.....	d1,000					903	
Total solids in bottle, per cent.....		3 ^c	3 ^c	3 ^c		2.6	
Gas analysis, per cent:							
CO ₂	48.0	49.0	46.0	43.3	46.3	42.0	48.1
CH ₄	49.2	48.0	52.5	54.0	51.3	56.6	49.6
H ₂8	.5	.3	.5	.5	.4	.5
N ₂	2.0	2.5	1.2	2.2	1.9	1.0	1.8

^a Corrected for control 1.5 liters.

^b Weight of N₂ not included.

^c Corrected for dissolved CO₂.

^d Estimated.

^e Not corrected for residual cellulose; about 5.0 grams.

^f Tip-top apparatus. See Figure 12.

the CO₂ retained in the liquor, show a 1:1 ratio of CO₂:CH₄. The weight of the gas recovered was 107 per cent of the weight of cellulose fed. About 5 grams of undigested cellulose was left in the bottle when the experiment ended.

Starch. The data collected during the continuous feeding experiment on the anaerobic fermentation of starch (Eun 38-3) are given in Table XXIX. No liquors were added or withdrawn during the run except for the small amounts drawn for analytical purposes. No additional nitrogen was added. As much as 14 grams of starch were fed per day for 37 days. The average for the 74-day experimental period was 0.93 grams per day. An average of 1.02 liters of gas per day was collected during the rapid feeding period. The average for the entire experiment was 0.77 liters or 0.39 liters per day per liter of tank volume. The authors feel that the maximum rate at which pure starch can be fed, without the accumulation of acids, must be but slightly over 0.7 grams per day per liter of tank capacity.

As noted in Table XXIX, when corrected for dissolved CO₂, the gas formed from the starch had a CO₂:CH₄ ratio of 1:1. The weight of gas recovered, 75.16 grams equals 110 per cent of the weight of starch added.

An earlier experiment on the fermentation of starch was evidently fed too much at the start (10 gms. per liter) for it developed a combination CO₂-CH₄ and CO₂-H₂ fermentation and turned sour (pH of 4.8, volatile acids 6,300 p.p.m.)

TABLE XXIX.
CONTINUOUS FERMENTATION OF STARCH^a.
Run 38-3.

				Summary.
Volume of bottle, liters.....	2	2	2	2
Time, days.....	20	37	17	74
Starch fed, grams.....	6.5	52	10	68.5
Average starch fed per day, grams.....	0.3	1.4	0.6	0.9
Gas produced, S.T.P.:				
Total volume, liters ^c	5.15	37.60	9.46	56.71
Average volume per day, liters.....	0.26	1.02	0.56	0.77
Analysis, per cent:				
CO ₂	41.2	46.2	41.5	49.2
CH ₄	58.0	52.7	57.1	49.7
H ₂4	.5	.4	.4
N ₂4	.6	1.0	.7
Total weight, grams ^b	6.28	48.50	11.53	75.16
Per cent gasification.....	96.7	93.3	115.3	110.0
Representative data:				
Volatile acids, p.p.m.....	50.0	140.0	270.0	
pH.....	7.4	7.4	7.0	
Ammonia nitrogen, p.p.m.....			49.0	
Total nitrogen, p.p.m.....			46.9	

^a All data corrected for inoculum solids.

^b Does not include N₂.

^c Corrected for increase in dissolved CO₂.

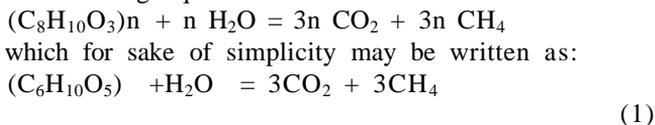
^d Theoretical is 111.

Cracked Corn. In a continuous feeding experiment on the fermentation of cracked corn, which is about 88 per cent starch, the authors were able to feed an average of 1.89 grams per day per liter of tank volume for an experimental period of 77 days (2.46 grams per day to a 1.3 liter bottle). A total of 101.2 liters of gas (128.7 grams), not corrected for dissolved CO₂, were recovered from the 189.0 grams of corn added. The gas, as drawn, averaged 53.4 per cent CH₄.

Gas Recoveries. These data, Tank A and Bun 40-3 on cellulose, and Bun 38-3 on starch, as well as certain earlier preliminary studies reported by the writers, show that the cellulose and starch which are decomposed in anaerobic fermentations are quantitatively converted into gas and that the ratio of CO₂ to CH₄, in those normal experiments where the amount of inoculum solids is low, is always 1:1.

The solubility of CO₂ in the mother liquor and the CO₂ equilibria set up in this liquor tend to give low CO₂ recoveries. Analysis of this liquor for dissolved CO₂ and increase in bicarbonate, etc., as described in the appendix, aids, but still at times only approximate CO₂ recoveries are noted. Most, if not all, of the small amount of unaccounted for material can be accounted for in the formation of bacteriological protoplasm.⁽³²⁾ All procedures used in feeding, withdrawal of samples for analysis or recirculation and the like, lead to a greater loss in CO₂ than in CH₄. High CO₂ recoveries, as in Tank A, are due to aeration, accumulation of acids and other causes to be discussed later. The gas ratios (CO₂ to CH₄) in the blanks (inocula) may become as great as 1 to 8. This 1:1 gas ratio for the anaerobic fermentation of cellulose has been noted by earlier workers⁽⁸⁶⁾. Most of these investigators, however, got only low gas yields and an appreciable accumulation of organic acids in spite of the fact that their digestion periods were usually very long.

On the basis of this 1:1 ratio of CO₂:CH₄ as noted for cellulose and starch one may draw that the over-all reaction must take place in accordance with the following equation:



$$162 + 18 = 180; 180/162 = 111 \text{ per cent}$$

One notes how nearly quantitatively the agreement of the analytical data is with this equation. A variety of side reactions might have been anticipated. Such agreement, however, is not surprising when one considers that, of all the possible biological reactions between cellulose and water or starch and water in the absence of air and practically all other reagents, the anaerobic fermentation to CO₂ and CH₄ liberates the greatest possible quantity of energy. The exact amount of this *energy* loss will be considered later.

Returning to the laboratory investigation, there was noted in Tank A an over-all decrease in organic matter or cellulose* amounting to 496.2 grams or 58.0 per cent of the weight of the filter paper added. This organic matter (cellulose) gave 543.4 grams of gas and 58.9 grams of acids or a total of 602.3 grams of end-products. These end-products account for 121 per cent of the cellulose digested (496.2 grams).

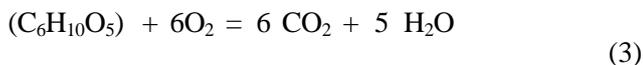
* The decrease in organic matter in the inoculum solids with the corresponding production of gas has been disregarded, since a control bottle containing a 1 liter sample of the inoculum liquor gave only 0.4 liter of gas. The total volume of 27.5 liters would, therefore, have given 11 liters, or only two per cent of the total amount evolved during the experiment. The residue left at the end of the experiment, other than the inoculum solids, has been considered as unmodified cellulose, since, physically as well as chemically, it answered the requirements for this substance.

From equations 1 and 2 it will be noted that cellulose or starch in decomposing to CO_2 and CH_4 or to acetic acid, should give 111 per cent of its weight as end-products. Its reaction with water accounts for this increase.



$$162 + 18 = 180; 180/162 = 111 \text{ per cent}$$

Since the CO_2 content of the gas collected during the Tank A investigation exceeds the CH_4 content by 30.7 liters* (60.2 grams) it is apparent that some cellulose decomposed in accordance with equation 3.



$$162 + 192 = 264 + 90; 354/162 = 218 \text{ per cent}$$

Attention has already been called to the fact that frequently during the latter part of the run the tank had to be opened to the air in order that the sum of cellulose might be broken up. This introduced air (oxygen), which in turn brought about higher acid concentrations, and higher percentages of CO_2 in the gas (45 per cent under normal conditions as compared with 55 per cent under conditions following the introduction of small amounts of air). The extent to which equation 3 took part in the decomposition can be estimated by noting the amount of CO_2 formed in excess of the CH_4 . Subtracting this amount from the products and an equivalent amount from the cellulose, as calculated* from equation 3, it is noted that 483.2 grams of gas plus 58.9 grams of acids or a total of 542.1 grams of end-products were formed in the decomposition of 459.0 grams of cellulose. The actual recovery then becomes 118 per cent as compared with a theoretical of 111 per cent. This error is greater than noted in the other controlled investigations (Eun 40-3, 38-3) but still it is not unreasonable when one considers the difficulty in handling biological balances.

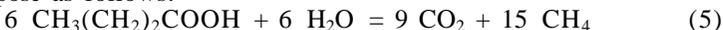
Referring to the data given in Tables XXVII and XXIX on the fermentation of cellulose (Bun 40-3) and starch (Eun 38-1), respectively, it is noted that from the 185 grams of cellulose fed there was recovered 198.2 grams of gas or 107 per cent of the weight of cellulose added. Likewise, from the 68.5 grams of starch added, there was recovered 75.16 grams of gas or 110 per cent of weight of starch added. As biological reactions, these gas recoveries check exceptionally well with the theoretical reaction, according to which, if complete fermentation took place, 111 per cent of the weight of the cellulose or starch added should be recovered as gas. These recoveries along with the facts that (1) equation 1 is the only balanced equation that can be written for the quantitative anaerobic fermentation of cellulose and starch to give CO_2 and CH_4 and (2) that this reaction gives the greatest possible change in free energy, leave no doubt as to the correctness of this equation.

* 60.2 grams (30.7 liters) of excess CO_2
 $162/x = 264/60.2 \quad x = 37.2$ grams of cellulose
 $496.2 - 37.2 = 459.0$ grams of cellulose
 $543.2 - 60.2 = 483.2$ grams of gas (1:1 mixture of CO_2 : CH_4)

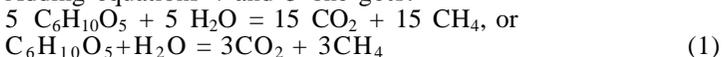
The accumulation of mono-basic acids higher than acetic, like aerobic oxidation, also increases the CO₂ content of the gas. One test, at a time when the cellulose digesting tank (A) was sour and when the CO₂ content of the gas was 55 per cent, showed the presence of 4 parts of butyric and a trace of a higher acid with every 6 parts of acetic. The production of butyric acid and CO₂ anaerobically may be represented as follows:



If normal fermentation is again established the butyric acid will decompose as follows:



Adding equations 4 and 5 one gets:



which is the same as equation 1. In other words, the accumulation of certain anaerobic fermentation products will alter the gas ratios in proportion to their concentration and degree of oxidation or reduction as compared with the mother substance. If these products are finally fermented anaerobically to CO₂ and CH₄ one obtains the same over-all 1:1 gas ratio characteristic of cellulose and starch.

B. SMALL SCALE BATCH INVESTIGATIONS.

It might be well at this point to briefly summarize some of the small scale (bottle) experiments on the fermentation of cellulose (high grade filter paper and cotton) and other closely related materials. As outlined earlier these investigations were carried out in dark glass bottles which were tightly sealed following the introduction of the substrate and inoculum solution. The gases formed during the digestion were collected, as shown in Figure 8.

Cellulose, Toilet Paper, Wood Pulp, and Kotex. In one experiment 9.56 grams (dry weight) of filter paper was added to one bottle and 8.923 grams of toilet paper to another. The digestion bottles were then completely filled with overflow liquor from a sewage disposal plant. The gases formed during the anaerobic decomposition of the cellulose were collected, measured, analyzed, and calculated to standard conditions. A dissolved CO₂ determination was made on the mother liquor at the end of the experiment and the corresponding correction made in the final data. These and additional data have been summarized in Tables XXX, XXXI, and XXXII.

On the basis of frequent analyses, the 7,312 cc. of gas collected from the digested cellulose had a 1.0 to 0.94 ratio of CO₂:CH₄. The CO₂, H₂, and CH₄ found in the gas weighed 9,307 grams, or 98 per cent of the weight of the cellulose added (9.56 gm.). From Table XXXII it will be noted that 34 per cent and 56 per cent of the total volume of gas was given off during the first 10 and the first 15 days, respectively. In other words, the major part of the fermentation took place within the first 15 to 20 days.

The CO₂:CH₄ ratio in the gas collected from the toilet paper was 1.0 to 0.994. The gas production in this experiment was inhibited due

TABLE XXX.
DIGESTION OF FILTER AND TOILET PAPER, CHEMICAL DATA.

	Volume cc.	pH.	Free CO ₂ p.p.m.	Total solids p.p.m.	Loss on ignition, per cent.	Vola- tile acids as acetic p.p.m.	Total vola- tile matter gm.	Cl p.p.m.	N as NH ₄ p.p.m.	Total N p.p.m.	Alka- linity M. O.
Overflow liquor used as the in- oculum.....	2,660	7.2	328	1,730	55.3	304	3,390	85	266	336	2,080
Filter paper solu- ble after diges- tion.....	2,660	6.7	1,035	1,614	51.6	93	2,466	80	255	308	2,080
Toilet paper solu- ble after diges- tion.....	2,660	6.9	650	3,018	67.5	43	5,544	80	260	337	2,060

TABLE XXXI.
FILTER AND TOILET PAPER DIGESTION, BALANCE SHEET.

Digestion composition.	Volatile matter.			Gas collected, cc.	CO ₂ , CH ₄ and H ₂ grams.	CO ₂ : CH ₄ : H ₂ .
	At start grams.	At close grams.	Gasified grams.			
Inoculum + filter paper.....	*12.950	2.466	10.484	7,724	9.307	15 : 15 : 1
Inoculum ^b + toilet paper.....	*12.313	5.644	6.769	5,560	6.723	12 : 12 : 1

^a Of this, 3,390 grams was inoculum and 9,560 grams paper.

^b Hg from gas seal sucked into digestion bottle. Digestion not complete.

^c Of this, 3,390 grams was inoculum and 8,923 grams paper.

TABLE XXXII.
GAS RECOVERIES FROM FILTER AND TOILET PAPERS.

Number of days.	Cellulose (Filter paper).		Toilet paper.	
	Total volume S. T. P. cc.	Per cent of total volume.	Total volume S. T. P. cc.	Per cent of total volume.
5.....	765	11	1,776	32
10.....	2,522	34	2,898	52
15.....	4,088	56	3,756	68
20.....	5,012	69
25.....	5,590	77	4,100	74
35.....	5,985	82	4,475	80
46.....	6,362	87
69.....	6,732	92
72.....	6,777	93	5,125	92
72.....	*7,312	100	*5,560	100

^a Corrected for increase in CO₂ of mother liquor but not for gases formed from inoculum solids. Latter was low.

to loss of mercury into the bottle but, in spite of this, 76 per cent of the weight of paper added was recovered as gas. Most of the gas was formed prior to the time the mercury was accidentally introduced, namely, 52 per cent of the total in 10 days and 68 per cent in 15 days. These data characterize numerous of the other early, small scale investigations made by the authors on the decomposition of filter paper, cotton, toilet paper, and the like. The results of the first preliminary experiment are summarized in Table XXXIII. Here again the 1:1 ratio of CO_2 : CH_4 as well as the excess in weight of gas over that of volatile matter digested, is noted.

C. POSSIBLE SOURCES OF ERRORS IN QUANTITATIVE BALANCES.

The difficulties involved in establishing quantitative balances in bio- and zymochemical studies are self-evident to those who have studied in these fields. Some of the more specific difficulties and certain possible errors involved in the studies herein reported have already been referred to. These difficulties may be summarized as follows:

(1) One source of error is involved in the fact that equation 1 does not take into consideration the small amount of H_2 (0.1 to 3.0 per cent by volume) that is formed in these fermentations.

TABLE XXXIII.
PRELIMINARY CELLULOSE DIGESTION BALANCES.

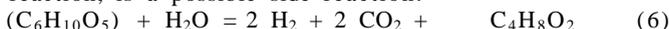
Digestion composition.	Volatile matter.			Gas as drawn.			Gas data, corrected.		
	At start, grams.	At close, grams.	Gasified, grams.	CO_2 cc.	CH_4 cc.	H_2 cc.	Gas less N_2^a , grams.	Ratio L.V.M.: G. ^b	Ratio CO_2 : CH_4^c
Filter paper (inoculum + 9.56 grams, dry basis).....	15.44	6.51	8.93	2,864	3,953	223	10.85	1 : 1.21	1 : 0.95
Cotton (inoculum + 10.429 grams, dry basis).....	16.31	7.00	9.31	2,961	4,440	320	10.39	1 : 1.12	1 : 1.06
Toilet paper (inoculum + 9.3 grams, dry basis).....	14.80	9.05	5.75	1,786	2,355	233	6.93	1 : 1.20	1 : 0.91
Wood pulp (inoculum + 10.12 grams, dry basis).....	15.85	12.98	2.87	733	2,063	99	4.32	1 : 1.50	1 : 1.12
Kotex (inoculum + 13.777 grams, dry basis).....	19.56	6.56	13.00	4,756	5,710	26	14.82	1 : 1.14	1 : 0.95
Inoculum (200 cc. digested sludge + 2,460 cc. settled sewage).....	7.03	5.80	1.23	114	944	38	1.49	1 : 1.21

^a Corrected for dissolved CO_2 .

^b Ratio of loss in volatile matter to weight of gas recovered (corrected for dissolved CO_2).

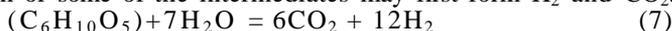
^c Corrected for dissolved CO_2 and inoculum gas.

This small amount of H_2 is probably due to one or a series of the many side reactions that usually accompany any biological change. It may be one of the products formed through oxidation by dehydrogenation. The anaerobic fermentation of carbohydrates (starches and di- and mono-saccharides) to give H_2 , CO_2 , and butyric acid, in accordance with the following reaction, is a possible side reaction:



Small to moderate amounts of butyric acid have been found in all cellulose digestions examined for this substance.

As stated before, it is also possible and very probable that cellulose and starch or some of the intermediates may first form H_2 and CO_2 .



All of the H_2 may then unite with half of the CO_2 to give CH_4 .



Each of these separate reactions is well known. The present writers have studied the latter one. It will be discussed later under the topic—"Effect of Hydrogen." If the CH_4 produced in the biological degradation of carbohydrates is produced through H_2 and CO_2 , the lack of a quantitative reaction could account for the small amount of H_2 noted in the gases drawn.

The trace of H_2 may come from the metabolic reactions of the bacteria themselves or from the degradation of their protoplasm following death. Neave and Buswell⁽²¹¹⁾ in connection with their studies on the fermentation of fatty acids, have called attention to three other possible sources of the H_2 , namely, (1) $CH_3COOH + 2 H_2O = 2 CO_2 + 4 H_2$ (2) $RCH_2COOH + NH_3 = RCH(NH_2)COOH + H_2$ (3) Production of higher homologues (e.g. succinic from acetic).

(2) No correction has been made for the traces of non-volatile acids formed during the fermentation. This correction, on the basis of tests made for these compounds, would be very small and hence is of little significance.

(3) The volatile acids were all calculated as acetic although butyric is known to be present in lesser amounts. Dr. S. L. Neave (formerly of State Water Survey Laboratory) has shown that the errors involved in this practice are, for all practical purposes, compensating.

(4) There may be unknown reactions which may tend to give higher gas or acid yields than that shown by equations 1 and 2. This however, seems improbable.

(5) There are always determinate and indeterminate errors that play an important part in such biological balances as the ones herein reported. One of the greatest errors is that involved in sampling.

(6) The fact that a variable but small amount of the substrate goes into the building of bacterial protoplasm introduces a corresponding error in quantitative balances.

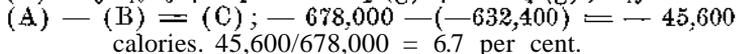
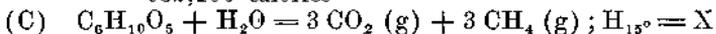
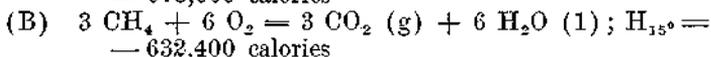
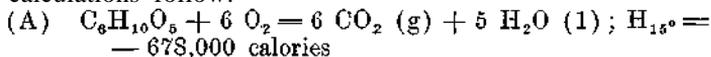
D. HUMUS FORMATION FROM CELLULOSE.

On the basis that practically theoretical weights of the gaseous end-products (CO_2 and CH_4) are recovered, one may draw the conclusion that there is no humus formed. If it were formed there would be a greater loss in weight of cellulose than could be accounted for by the acids and gas produced. Furthermore, the formation of humus, which contains 50 per cent or greater of carbon, would require some type of a reaction involving the removal of H and O from the original cellulose in order to increase its carbon content from 44.5 per cent to 50 per cent or greater. This would alter the gas ratio. This proof, along with the fact that the mixture left at the end of these digestions was of a light gray color and not black as it would have been if much humus had been formed, leads to the conclusion that humus is not formed during the

anaerobic decomposition of cellulose. The accumulation of *dead bacterial protoplasm* in a tank that was fed cellulose for a long period of time might lead to the production of a residue which some, at least, might call humus.

E. ENERGY CALCULATIONS.

It is interesting to note the energy relationships involved in Equation 1, or in other words, the energy consumed by the bacteria in bringing about the fermentation of cellulose to equal volumes of CO_2 and CH_4 . As data on the free energy of cellulose are not available, heat data, taken from the International Critical Tables, Vol. V, page 163, were used. The calculations follow:



From the above, one concludes that 45.6 kilogram calories or only 6.7 per cent of the total heat value of the cellulose (heat of oxidation or combustion) is consumed in the biological hydrolysis of cellulose to form equal volumes of CO_2 and CH_4 . This loss appears small in comparison with the advantages of a gaseous fuel. Although this loss is small, additional calculations show that of all the other possible quantitative reactions involving the anaerobic decomposition of cellulose in the absence of all other reactants except water, the above represents the greatest possible loss of energy. Such thermodynamic considerations account for the fact that, given the proper biological conditions, one recovers, in time, quantitative yields of CO_2 and CH_4 .

The above value checks very well with the heat of reaction as calculated on the basis of heats of formation. Although the actual molecular heat of formation of cellulose is not known it can be roughly calculated by the following formula (Int. Crit. Tables V, 162 (1929)):

$$H = -Q + 94.38a + 34.19b + Oc$$

where

H = heat of formation of cellulose

Q = heat of combustion

a = number of C atoms

b = number of H atoms

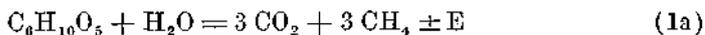
c = number of O atoms

Inserting the correct values in the above equation one obtains:

$$H = -678.0 + (94.38 \times 6) + (34.19 \times 10)$$

$$H = 230.2 \text{ kg. cal. per unit of } \text{C}_6\text{H}_{10}\text{O}_5$$

Using this value for H (230.2) and the standard values (Int. Crit. Tables, V) for the other members of the equation, one obtains the following:



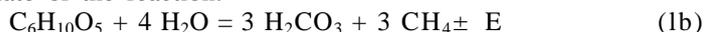
$$230.2 + 68.38 = (3 \times 94.4) + (3 \times 19.1) \pm E$$

$$298.6 = 340.5 - E$$

$$E = 41.9 \text{ kg. cal. per } (\text{C}_6\text{H}_{10}\text{O}_5) \text{ unit.}$$

Therefore, in accordance with the above calculations, equation 1a is exothermic to the extent of 41.9 kg. cal. per unit of $\text{C}_6\text{H}_{10}\text{O}_5$ fermented.

On the basis that the bacteria undoubtedly excrete H_2CO_3 rather than CO_2 , the following reaction probably represents more nearly the true state of the reaction.



Making the same type of calculations as above, one obtains the following:

$$230.2 + (4 \times 68.38) = (3 \times 167.5) + (3 \times 19.1) \pm E$$

$$503.8 = 559.8 - E$$

$$E = 56.0 \text{ kg. cal. per } \text{C}_6\text{H}_{10}\text{O}_5 \text{ fermented}$$

On this basis, equation 1b is exothermic, to the extent of 56.0 kg. cal. per unit of $\text{C}_6\text{H}_{10}\text{O}_5$ fermented.

P. SIGNIFICANCE OF THE GAS YIELD DATA.

The present writers do not claim priority in the idea of bacteriologically producing methane from cellulose. They do, however, claim to be the first to produce conditions such that quantitative yields of CO_2 and CH_4 are recovered from the fermentation of this and closely related substances. As was noted in the historical chapter, the early workers got low gas yields and high acid recoveries. Usually the acids recovered amounted to as much as 50 to 60 per cent of the cellulose added. These gas yields did not attract further study. In the best investigations herein reported, small amounts of acids and almost quantitative yields of gas were recovered as end-products, namely, 107 to 110 per cent as compared with 111 per cent for the theoretical yield of CO_2 and CH_4 from cellulose. Some of the factors contributing to this success have already been referred to. Others will be discussed in a later chapter on "Factors Influencing the Fermentation of Cellulose and Fibrous Materials."

High yields of a gas containing 50 to 60 per cent CH_4 (B. t. u. of 500 to 600) gave promise for the development of a commercial method for the preparation of this important power and fuel gas based on the direct anaerobic fermentation of waste cellulose and excess, or inefficiently utilized crude fiber. Although Fowler⁽⁸⁶⁾, Dubdin⁽⁶⁶⁾, and others had found crude plant fibers to be very resistant to anaerobic fermentation, the authors felt it worth while to see if they could take advantage of the information they had gathered on the fermentation of pure cellulose, and use it to an advantage to obtain greater gas yields from crude plant materials.

Living in the heart of the Corn Belt, one of the first materials to be selected for consideration was cornstalks. The early bottle (batch) experiments gave promising results which, in turn, led to studies similar in type but more extensive than the ones made on pure cellulose. The results are summarized in the following chapter.

THE ANAEROBIC FERMENTATION OF CRUDE PLANT MATERIALS.

As in the preliminary studies on the anaerobic fermentation of cellulose, the first experiments on the fermentation of cornstalks and other inefficiently utilized plant materials were carried out in small bottle set-ups. The results of some of these small scale studies have already been published²³. They bear out the fact that the following volumes of gas may be generated from cornstalks in the times recorded. (See Table XXXIV.)

TABLE XXXIV.
GAS PRODUCTION FROM CORNSTALKS.

Time of fermentation (days)	Volume of gas (normal T. and P.).	
	Liters per 10 grams of cornstalks.	Thousands of cubic feet per ton of cornstalks.
10.....	1.5-2.0	4.8- 6.5
20.....	2.5-3.5	8. -11.
30.....	3.0-4.5	10. -15.
50.....	4.0-6.0	13. -20.

Attention is called to the fact that these data are based on small scale experiments in which the material was all added to the bottle at once. These digestions were not kept under close chemical control, nor were the contents stirred at any time during the investigation. As stated before, these small scale, bottle experiments lack many of the factors that would be present if the digestion were conducted on a larger scale. These factors, such as intermittent feeding and withdrawing of residue, stirring, etc., as has been shown for cellulose, lead to higher yields per unit of time. The above data, therefore, represent minimum, or average, and not maximum yields. As these data showed promise of a method of recovering appreciable volumes of methane from such materials, the work was continued.

A. LABORATORY FEEDING EXPERIMENTS.

1. *Cornstalks. Tank C.* In order to test out the continuous production of methane in the laboratory, a 25-liter bottle was arranged with tubes so that cornstalks could be added and withdrawn at will without opening the tank to the air. A mercury-sealed, mechanical stirrer served as a means of keeping the bottle contents mixed. This method of keeping the surface mat of cornstalks broken up was only partially successful. Better mixing could undoubtedly have been obtained if the bottle had been set up so that it could have been inverted from time to time (Figure 12). To this bottle were added 50 grams of dry cornstalks and 22 liters of overflow liquor from a sewage-disposal plant. This liquor served as an inoculum as well as a suitable source of nitrogen for the bacteria. It contained 223 p.p.m. of ammonia nitrogen and 44 p.p.m. of organic

nitrogen. At the end of the experiment the liquor contained 158 p.p.m. of ammonia nitrogen and 45 p.p.m. of organic nitrogen. During the 90-day period of the experiment, cornstalks were added from time to time and samples of the mother liquor were withdrawn and the volume made up by adding raw sewage or water. Samples of the cornstalks that settled to the bottom of the tank during the digestion were also withdrawn and composited with those remaining at the end of the experiment. An analysis was made of everything that was put into and taken out of the tank. Table XXXV gives a summary of these data. Figure 11 shows graphically the amounts, as well as the days, on which cornstalks were added as well as the daily volumes of gas generated.

At the end of the experiment the active cornstalks remaining in the tank were separated from the mother liquor by means of a screen filter. These stalks were then washed with distilled water, dried, composited with those drawn during the run, ground, and analyzed (Table XXXVII.) The mother liquor and the washings were also analyzed and the data recorded in Table XXXV under the heading "Sludge and Soluble Solids." Additional data as to the nature of these solids are given in Tables XXXVIII and XXIX. A portion of the total solids' was extracted with cold water and the water-insoluble sludge analyzed separately (Table XXXIX). The ultimate analysis of this material throws little light upon its chemical nature. It possessed humus-like characteristics and probably would be called such by most investigators^(250, 366).

During the experiment 1,535 grams of dry cornstalks were fed and from this 370.02 liters of gas were collected. This volume of gas, when corrected for the 4.56 liters of carbon dioxide found to be dissolved in the mother liquor at the end of the run, totals 374.58 liters. The gas data may be summarized as follows:

	Liters	Per cent by volume	Grams
CO ₂	178.81	47.7	350.5
H ₂	6.16	1.7	0.6
CH ₄	159.30	42.5	113.9
N ₂	30.31	8.1	37.9
Total	374.58		502.9

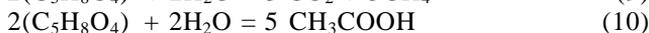
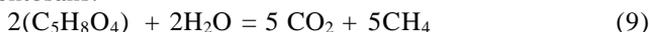
No correction has been made for the gases coming from the digestion of the inoculum or sewage solids added, since the organic matter in these solutions (35.7 grams) amounted to only 2.5 per cent of that added as cornstalks (1,412.2 grams). It will be noted that the volume ratio of CO₂:CH₄ is 1.0 to 0.89. This corresponds well with the theoretical (1:1) for cellulose. The simplest reactions that can be written to illustrate the gasification of cellulose and the pentosans, as well as the formation of acetic acid from these materials, are as follows:

Cellulose:



$$162 + 18 = 180$$

Pentosans:



$$264 + 36 = 300$$

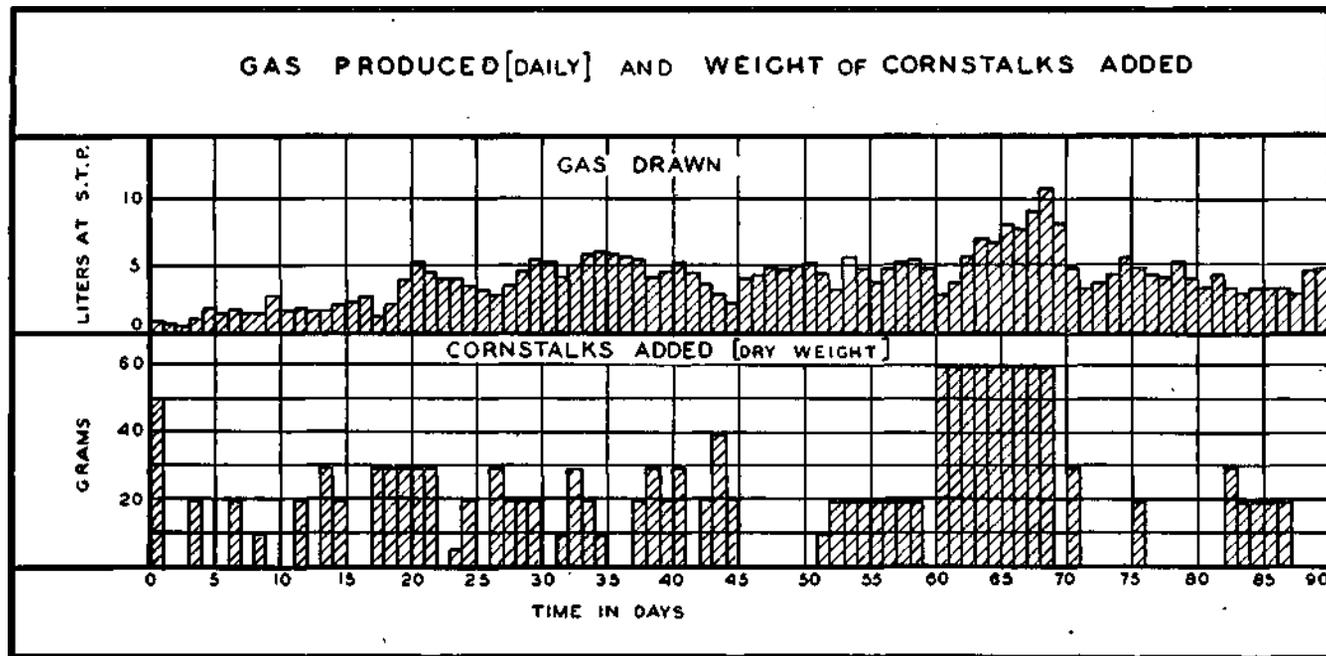


FIGURE II

As noted previously cellulose should give 111 per cent of its weight either as acetic acid or as carbon dioxide and methane. Similarly, the pentosans should give 114 per cent. Buswell and Neave, of this laboratory, have noted that the weight of gas generated from the decomposition of fats may amount to 150 per cent of the weight of the material decomposed. Other materials that give more than a gram of gas per gram of volatile matter decomposed have been reported on by Buswell and Boruff⁽⁴⁰⁾. The 485.0 grams of carbon dioxide, hydrogen, and methane plus the 86.3 grams of organic acids collected in this experiment represent a decrease of 466.4 grams (Table XV) in the organic matter. In other words, the gas and acids represent 118 per cent of the weight of the organic matter decomposed. The difference between the theoretical yields for cellulose and the pentosans and the actual yield as found may be due in part to the decomposition of the lignin, resins, and waxes found in the original cornstalks. No equation has been suggested for the decomposition of lignin. From its empirical formula, $C_{40}H_{46}O_{16}$ ⁽²⁴⁵⁾, it would be expected that it would give a higher yield of gas per gram of material decomposed than would cellulose or the pentosans. Furthermore, the fact that the carbon dioxide content of the gas exceeds the methane indicates a certain amount of oxidation. As noted before this would give an appreciably higher weight of gas than indicated by the above equations (1, 8, 9, and 10).

The apparent ash digestion of 34.1 grams (Table XXXV) is due in part to the digestion of sulfates to sulfides and hydrogen sul-

TABLE XXXV.
SUMMARY OF GENERAL DATA, TANK C.
Materials Added.

	Quantity.	Total solids, grams.	Organic solids, grams.	Volatile acids as acetic, grams.	Ash, grams.
Inoculum solution.....	22 liters...	41.844	22.596	5.368	19.248
Buffer salts.....	5.1 grams...	5.1	5.1
Sewage.....	17 liters...	16.490	6.002	1.502	10.488
Cornstalks (dry).....	1,535 grams...	1,535	1,412.2	122.8
Total.....	1,598.4	1,440.8	7.170	157.6

Substances Removed.

Overflow liquors.....	26.7 liters...	150.3	103.4	57.0	46.9
Sludge and soluble solids	115.4	50.3	36.5	34.6
Cornstalks:					
Top (active).....	798.9 grams...	798.9	758.6	40.3
In sludge (inert).....	33.3 grams...	33.3	31.6	1.7
Total.....	832.2 (cornstalks)	1,097.9	974.4	93.5	123.5

Digested.

	702.8 (cornstalks)	500.5	466.4	36.33 (formed)	34.1
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fide^(47, 70, 236). As the sulfur content of cornstalks is only about 0.1 per cent⁽¹¹⁹⁾, and that of overflow liquor and sewage about 40 parts per million (as S), the decomposition of sulfates cannot account for all the apparent loss in ash. The remainder is undoubtedly due to errors in sampling and in the ash determination. It is commonly recognized that these errors are quite appreciable in such mixtures as herein described, and especially is this the case when large calculation factors are used⁽⁴⁷⁾.

Table XXXVI gives a distribution of the products formed in the digestion. The 465 grams of gas represent the gasification of 30.3 per cent of the cornstalks added. Table XXXV shows that there was an increase in the volatile acid content of the tank liquors amounting to 86.3 grams. This represents 5.6 per cent of the weight of the stalks added. This amount could be kept to a much lower figure by proper regulation of digestion conditions. As the total ash content of the original stalks was 122.8 and that of the residual cornstalks only 42.0 grams, there must have been 80.8 grams of mineral matter leached from the stalks. This decrease in ash amounts to 5.3 per cent of the total weight of stalks added. The total organic solids found in the overflow liquors, and in the sludge and soluble matter at the end of the run (184.2), less that added at the beginning of the run (28.6), represents the "sludge" or "humus" formed during the digestion of the cornstalks. This amounts to 155.6 grams. Of this amount 83.3 grams were recovered at the end of the experiment as water-insoluble sludge.

TABLE XXXVI.
DISTRIBUTION OF PRODUCTS FORMED IN THE DIGESTION OF CORNSTALKS.

	Grams.	Per cent of stalks added.
Cornstalks added.....	1,535.0	
Cornstalks recovered.....	832.2	54.3
Cornstalks digested.....	702.8	45.7
Recovered as:		
Soluble and suspended solids:		
Ash.....	80.8	5.3
Organic matter.....	155.6	10.0
Acids, volatile organic.....	88.3	5.6
Gas.....	465.0	30.3
Total.....	787.7	51.2

Table XXXVII gives an analysis of the cornstalks that were used in the experiment as well as an analysis of a composite of the stalks drawn from the tank during the digestion and those left at the end of the experiment. From this table it is apparent that the water-soluble constituents are almost entirely removed. Although there is not a very pronounced difference in the analysis of the water-insoluble matter of the cornstalks before and after being exposed to anaerobic digestion, it is noted that there is a greater decrease in the cellulose content than in any other constituent. The sugars are, of course, an exception to this statement. They are quantitatively removed. The analytical data pre-

TABLE XXXVII.

ANALYSIS OF CORNSTALKS BEFORE AND FOLLOWING ANAEROBIC FERMENTATION^a

Component.	Original cornstalks.		Following fermentation.		
	Compo- sition.	Weight added.	Compo- sition.	Weight recovered.	Removed.
	Per cent ^b .	Grams.	Per cent ^b .	Grams.	Per cent.
Total.....		1,535.0		832.2	46
Ash.....	8.0	123.0	5.05	42.0	
Cold-water soluble 1 hour.....	12.2	187.0	1.4	11.6	594
Ether soluble.....	1.8	27.6	2.4	20.0	28
Alcohol-benzene soluble.....	8.5	131.0	5.8	48.3	63
Pentosans, total.....	22.1	339.0	25.9	215.0	37
Lignin, total.....	27.4	421.0	29.6	246.0	42
Cellulose, C. and B., pure.....	32.1	493.0	29.6	246.0	50
Ammonia nitrogen.....	0.066	1.01	0.0366	0.31	69
Total nitrogen.....	0.729	11.20	0.917	7.64	32
Alcohol-water soluble sugars ^c :					
Reducing, as dextrose ^d	1.09	16.7	0.00	0.00	100
After hydrolysis, as dextrose ^e	1.36	20.9	0.00	0.00	100
Xylose ^f	0.28	4.3	0.00	0.00	100

^a Except where otherwise stated, analysis was made as per Bray, Paper Trade J., 87, 59 (1928).^b Dry weight.^c Assocn. Official Agr. Chem., 1925, p. 118.^d Munson and Walker method.^e Twenty-five cc. sample hydrolyzed by 2.5 cc. coned. HCl at 70° C. for 7 minutes.^f Six-carbon sugars removed from hydrolyzed sample by use of *Saccharomyces cerevesiae*.^g Loss in ash recovered in part in mother liquor.

sented in this paper bear out the fact that cellulose is more readily attacked anaerobically than is lignin. Only a trace of cellulose was found in the sludge or slime at the bottom of the vessel, but the lignin and pentosan contents were found to be 34.4 and 11.1 per cent, respectively. Making these corrections to the data given in Table XXXVII, there was noted an over-all removal of 35 per cent of the lignin, 34 per cent of the pentosans, and 50 per cent of the total cellulose added.

TABLE XXXVIII.

EXTRACTION ANALYSIS OF WATER SOLUBLE AND WATER-INSOLUBLE SLUDGE.
Tank C.

	Per cent of original weight.
Hot-water soluble.....	31.0
Ether soluble, after 1.....	1.7
Alcohol-benzene soluble, after 2.....	2.9
Pyridine soluble, after 3.....	1.2
NaOH (3%) soluble, after 4.....	34.0

2. *Cornstalks in Tip-Top Apparatus.* An investigation of the advantages of mixing the contents of the digestion bottle and releasing the entrapped gases from the floating cornstalk mat, which soon collects at the top of any container in which this type of material is being fermented, was made by using the "tip-top" apparatus shown in Figure 12.

TABLE XXXIX.
ANALYSIS OF WATER-INSOLUBLE SLUDGE, TANK C. (TOTAL WEIGHT, 83.3 GRAMS).

	Per cent*.
Ash.....	28.11
Lignin (ash-free).....	34.4
Pentosans.....	11.1
Cellulose.....	trace
Undetermined (by difference).....	31.4
ULTIMATE ANALYSIS.	
Total nitrogen.....	2.76
Carbon.....	30.21
Hydrogen.....	5.20
Sulfur (total).....	0.56
Ash.....	23.11
Oxygen (by difference).....	38.16

* Dry basis.

Inversion of the bottle always released the entrapped gases and thoroughly mixed the bottle contents. The bottle had a capacity of 1.7 liters and was arranged with tubes so that cornstalks could be added and gas drawn at will. In the upright position the gas was drawn from the top. After 12 hours or so this gas outlet was closed, the bottle was inverted, and a similar gas outlet in the bottom, which was now upright, was opened. Due to the smallness of the apparatus it was impossible to keep the digested material separated from the fresh. The residue which was drawn by releasing the stopper of the bottle while in an inverted position always contained some material that was only slightly digested. To partially overcome this difficulty the bottle was not fed daily but was fed at 10 to 20 day intervals. The residue was always drawn just prior to feeding. At times the liquor volume lost in withdrawing residue was replenished by adding water; at other times settled raw sewage was added. Figure 13 gives a graph of the data collected during one of these

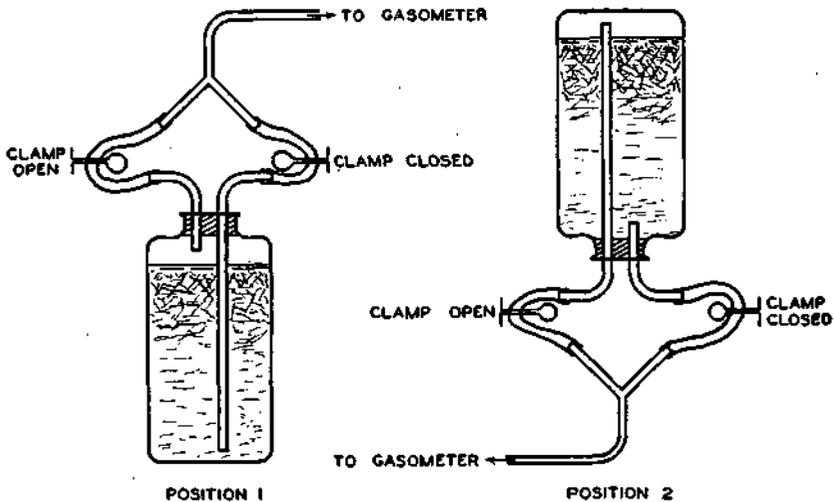


FIGURE 12.

Tip-Top Digester for Laboratory Tests on Fermentation of Fibrous Materials.

investigations. It shows the daily volume of gas drawn as well as the amounts and times at which fresh cornstalks were added and residue withdrawn.

In starting such an investigation as this it has repeatedly been found advisable not to feed too heavily at the start. After the fermentation has got started and the proper flora has become habituated to the substrate and the environment, then the bottle or tank may be fed at a greater rate. The first "tip-top" digestion tried with cornstalks turned sour (pH 5.6, volatile acids 4,080 p.p.m.) not because it was fed too rapidly at the start but because the nitrogen content of the inoculum was too low, namely, only 13 p.p.m. of ammonia nitrogen and 88 p.p.m. of total nitrogen. Similar experiments in which over 100 p.p.m. of ammonia nitrogen was present at the start did not go sour but produced normal gasification of the cornstalks added. The data plotted in Figure 13 may serve to illustrate. They show characteristic yields and the effect on the gas production of the introduction of fresh materials. For a smoother rate of gas production, a smaller amount of material would be fed at more frequent intervals. It is interesting to note that once the digestion is well under way, large amounts may be fed with seemingly no effect on the digestion except that larger volumes of gas are formed each day. The introduction of 60 grams of cornstalks on the 36th day (See Figure 13), when once the stalks became soaked, occupied over three-fourths the volume of the digestion bottle. Thus, it is noted that commercially the operator could regulate the feedings in accordance with the expected gas needs. From the 23rd to the 73rd day, 43.02 liters of gas were formed. This amounts to an average daily gas production of 0.86 liters or 50.6 per cent of the digestion tank volume per day. This is the highest *average* yield per day of gas that has been recovered from cornstalks in laboratory digesters. From the 20th to the 75th day there were added 180 grams of chopped cornstalks. During this period a total of 45.23 liters of gas were recovered. This amounts to 251 cc. per gram or 8,040 cu. ft. per ton of stalks. The authors feel that the main reason why these gas rates were obtained and why the fermentation withstood the introduction of such large volumes of cornstalks at one time, was that the fibrous mat was kept broken up and the entire contents thoroughly mixed. This prohibits the accumulation of acid zones and keeps the fibrous materials wetted at all times. In a comparative batch experiment on the fermentation of 10 grams of cornstalks there was formed in 10 days 1,500 cc. of gas from a stationary bottle as compared with 2,400 cc. from a same sized bottle which was equipped so it could be inverted from time to time. (Figure 12.)

3. *Cornstalks in Packed Tanks.* The present writers have investigated the results of packing small tanks with cornstalks and then adding a suitable inoculum solution. Seven-liter digesters of the type shown in Figure 8 were filled with chopped cornstalks (625 grams) and overflow liquor (6,700 cc.) from a sewage disposal tank. A coarse wire screen was fitted inside and at the top of the tank to keep the cornstalks from pushing up into the gas line. A 1-liter surge bottle was attached to the side to take care of expansion and contraction in liquor volume due to generation and release of gas bubbles from the fermenting mass.

In two days only 4.67 liters of gas had been generated and the pH had dropped to 5.6. The gas contained 58.2 per cent CO_2 , 10 per cent H_2 , and only 13.3 per cent CH_4 . Two liters of the sour and foul smelling liquor was replaced with overflow liquor from a sewage digestion tank. No change was noted. The addition of ammonia and of ammonium sulfide had no effect. In the meantime gasification had ceased. The pH remained between 6.3 and 6.6. Other tanks filled with as much as 380 grams of cornstalks per 7 liters of tank volume acted similarly.

On the basis of these experiments one concludes that the charging of tanks with cornstalks and overflow liquor from a sewage tank does not lead to normal fermentation and gasification. As the inoculum used has been found satisfactory (sufficient ammonia also present) in other experiments in which cornstalks were fed at slow rates, at the first at least, the difference in behavior may be due to the interference in biological action induced by the presence of such large quantities of fresh substrate. As noted in previous experiments appreciable quantities of cornstalks can be fed at one time provided there is already present in the digester considerable cornstalks or other materials already undergoing normal fermentation and gasification. The sour liquors from packed tanks could be slowly circulated through cornstalk tanks that were undergoing the desired fermentation, thereby gasifying the acids to CO_2 and CH_4 but such a practice would require greater tank capacity than that required for slow continuous feeding.

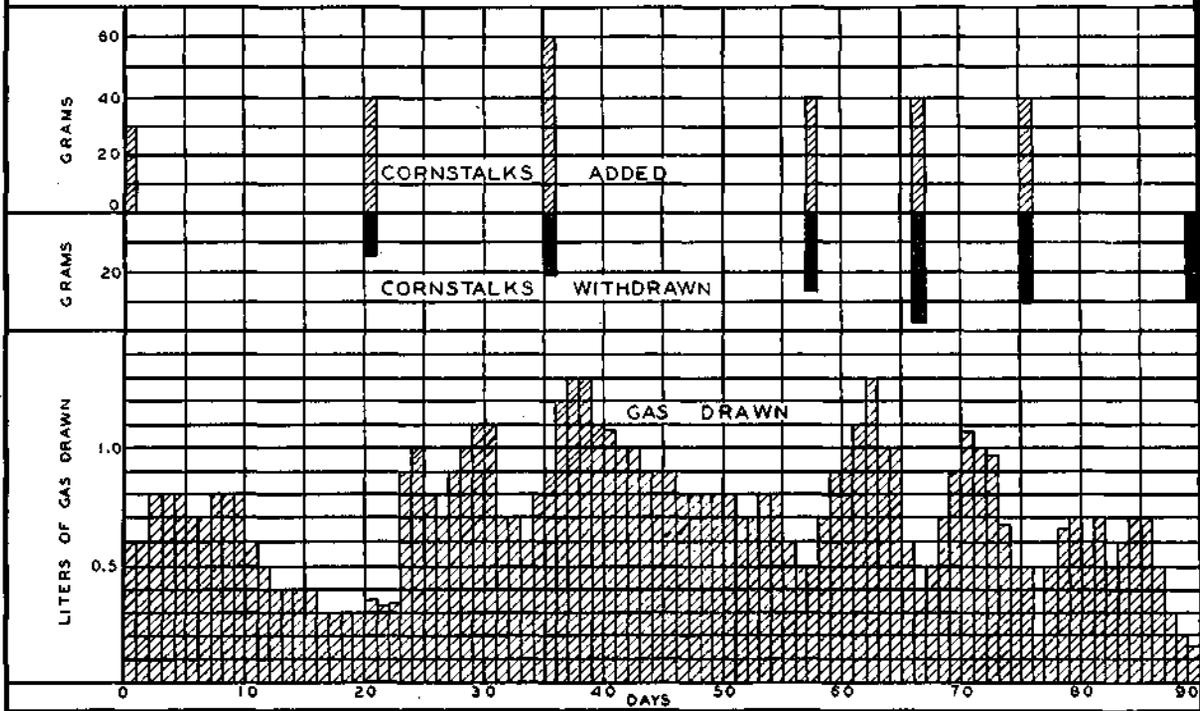
TABLE XL.
FERMENTATION OF GREEN CORNSTALKS.

	Green cornstalks
Digestion volume, liters.....	2.0
Duration of run, days.....	46.0
Total dry weight fed, grams.....	90.0
Total gas recovered, S. T. P:	
Volume, liters.....	27.4
Weight, grams.....	32.2
Analysis, per cent:	
CO_2	58.2
H_2	10.0
CH_4	13.3
N_2	1.2

^a During first 19 days fed at rate of 2.1 grams per day per liter of digestion capacity (0.13 lb./cu. ft.).

4. *Fermentation of Green Cornstalks.* The rate of digestion of green cornstalks (stalk and corn mature but still very green) was investigated by setting up a 2-liter "tip-top" digestion bottle (Figure 12) to which were added overflow liquors from a sewage tank and the shredded stalks and pulp respectively. Fresh materials were added periodically and the generated gas drawn and analyzed. The data are summarized in Table XL. During the first 19 days the green cornstalks were fed at the rate of 2.1 grams per day per liter of tank capacity. (.13 lb. per cu. ft.) Gas was collected at the rate of 376 cc. per day per liter of bottle capacity. During the rest of the run only 10 grams

FIGURE 13
 OPERATING DATA T-T APPARATUS
 [RUN XXIII-3] [VOLUME OF APPARATUS, 1.7 LITERS]



of stalks were added. No residue was withdrawn during the experiment. On the 46th day the residue occupied about one-third of the bottle. The average rate of feeding for the entire experiment (46 days) was 0.98 grams per day per liter of bottle capacity. The average rate of gas production was 298 cc. per day per liter of bottle capacity. The pH during the experiment varied from 7.2 to 7.7 and the volatile acids from 178 to 470 p.p.m. as acetic. On the basis of these data it is apparent that green cornstalks can be fermented at rates undoubtedly somewhat above the maximum rate noted in this experiment.

B. SMALL SCALE (BATCH) EXPERIMENTS.

1. *Gas Production from Water Soluble as Compared with Water Insoluble Constituents of Cornstalks.* In order to obtain more information as to what constituents found in cornstalks were furnishing the gas the writers extracted 10 grams of chopped cornstalks with cold water for 1 hour and then noted the gas produced from the water soluble and water insoluble portions. The water removed 0.69 grams of material which contained 0.036 grams of sugars. This material was added to one digestion bottle while the water insoluble portion was fermented in another 1-liter bottle. In 67 days the water soluble portion had produced 498 cc. or 0.63 grams of gas. The water insoluble portion gave 3,745 cc. or 4.89 grams of gas. All data are corrected for the small amount of gas generated from the inoculum. In other words, the water soluble constituents furnished only 11.8 per cent of the total gas while the water insoluble constituents furnished 88.2 per cent. The data are summarized in Table XLI.

2. *Plant Materials other than Cornstalks.* Although the writers have seen fit to direct most of their attention to the anaerobic fermentation of cornstalks, they have also investigated in a small way the possibilities of obtaining gas from other plant residues. All of the tests, except some pilot unit studies on straw, manures, and sewage screenings which will be presented later, were made by setting up batch experiments on the plant or fibrous materials in question. These experiments were all made before the importance of agitation was fully realized, hence the data do not represent optimum conditions or maximum gas recover-

TABLE XLI.
FERMENTATION OF WATER SOLUBLE AND WATER INSOLUBLE CORNSTALK
CONSTITUENTS.

Gas formed.	Water insoluble portion, (9.31 grams).		Water soluble portion, (0.69 grams).		Total, 10 gram sample.	
	cc.	Grams.	cc.	Grams.	cc.	Grams.
CO ₂	1,760	3.47	218	0.43	1,978
CH ₄	1,985	1.42	280	0.20	2,265
Total (67 days).....	3,745	4.89	498	0.63	4,243	5.52

NOTE.—All gas data corrected for gas from inoculum solids, gain in dissolved CO₂ and to S. T. P.

TABLE XLII.
ANAEROBIC FERMENTATION OF WASTE PLANT MATERIALS.
Batch Experiments, Temperature 22 to 28° C.

Plant material name.	Added dry weight, grams.	Di-gestion time, days.	Gas data, S. T. P. (corrected for inoculum).		
			Volume, cc.	Weight, grams.	Ratio, CO ₂ :CH ₄ .
Cornstalks.....	10	67	4,243	5.52	1:3.14
Peanut hulls.....	10	62	982	1.20	1:1.61
Broomcorn stalks.....	10	62	3,852	4.50	1:1.37
Corn cobs.....	10	62	4,254	5.13	1:1.25
Wheat straw.....	10	62	3,422	3.85	1:1.38
Alfalfa hay.....	10	62	3,266	3.78	1:1.35
Cat tails.....	10	62	1,297	1.25	1:1.55
Pithy weeds.....	10	62	208	.30	1:0.38
Non-pithy weeds.....	10	62	1,220	1.46	1:1.44
Newspaper.....	10	62	2,724	3.25	1:1.27
Banana stems.....	10	62	3,739	4.30	1:1.63
Mixed leaves.....	10	62	2,939	3.40	1:1.46
Excelsior.....	10	62	2,782	3.39	1:1.19
Thrashing machine chaff.....	10	62	6,151	6.83	1:1.6
Excelsior (autoclaved).....	10	62	2,526	2.95	1:1.17
Bagasse.....	10	62	3,260	3.68	1:1.08
Pine tree needles.....	10	63	4375	40.41	41:2.18
Pine tree needles (alkali treated).....	10	63	41,078	41.35	41:1.31
Extracted Jerusalem artichokes.....	10	63	45,330	45.55	41:2.8
Soft coal.....	10	131	41,131	41.76	41:3.7
Meizewood shavings.....	10	62	41,948	42.36	41:1.24
Flax shives.....	10	62	42,465	42.94	41:1.33
Flax straw.....	10	62	43,590	44.48	41:1.44
Rice hulls.....	10	32	475	.52	1:1.62
Tannin chips.....	10	32	575	.65	1:1.65

^a Not corrected for dissolved CO₂.

TABLE XLIII.
FERMENTATION OF PLANT RESIDUES.^a

Substances added.	Per cent (by weight) fermented to CO ₂ , H ₂ and CH ₄ .	Duration of experiment (days).
1. Filter paper.....	77-98	50-70
2. Cotton, pure.....	78	70
3. Kotex.....	91	76
4. Toilet paper.....	50-65	40-50
5. Cornstalks.....	35-50	50
	50-35	140
6. Bagasse, fiber.....	50	100
7. Thrashing machine chaff.....	68	50
8. Citrus pulp waste.....	50	60
9. Corn cobs.....	80-90	150
10. Wheat straw.....	51	50
11. Broom cornstalks.....	49	50
12. Banana stems.....	45	50
13. Alfalfa hay.....	43	50
14. Leaves, mixed.....	38	50
15. Newspaper pulp.....	34	50
16. Excelsior.....	34	50
17. Wood flour.....	30-34	50
18. Weeds, swamp.....	20	76
19. Cat tail stalks.....	15	50
20. Peanut hulls.....	13	50
21. Soybean vines ^b	12	50
22. Soybean vines ^b	39	15
23. Sunflower stalks ^b	47	50
24. Sunflower stalks ^b	29	15
25. Sunflower stalks ^b	61	60
26. Sewage screenings.....	31	40

^a Data on filter paper etc. included. Gives basis for comparison. All the above data, except the first five, are based on one investigation. All are bottle experiments.

^b By courtesy of Buswell and Symons.

ies. For comparative reasons, however, they will be presented. The various materials listed in Table XLII were added to one-liter bottles which were then filled with the inoculum solution.* The gas was collected over a saturated brine solution. The gas volumes and weights recorded have been corrected for the volume and weight of gas produced in the control (inoculum) bottle. The reader should find the table self-explanatory. From these data it is noted that the more porous or the thinner the cell walls of the material being fermented, the greater is the quantity of gas recovered.

3. *Long Time Digestion of old Cornstalks; Pre-treatment.* The following series of 1-liter (batch) experiments were set up in order to determine the effect of a long period of digestion on the different constituents found in cornstalks. The cornstalks used in this experiment were not collected in season but were old stalks found on the ground in July and August. Only those stalks that were in good physical condition were used. All bottles except the duplicate controls contained 15 grams of dried, shredded cornstalks.

Bottle No.	Bottle contents, and pre-treatment.
1	Control, 750 cc. inoculum solution and 200 cc. distilled water.
2 and 3	15 gm. cornstalks + 200 cc. distilled water + 750 cc. inoculum.
4 and 5	15 gm. cornstalks + 1 hour boil in 200 cc. of distilled water. Cooled, 750 cc. inoculum added.
6 and 7	15 gm. cornstalks + 2-day soak in 200 cc. of distilled water. Then 750 cc. inoculum added.
8 and 9	15 gm. cornstalks + 2-day soak in saturated lime water. Neutralized with H_3PO_4 , then 750 cc. inoculum added.

The inoculum was composed of 6 volumes of settled overflow liquor from a sewage digestion tank, 2 volumes of liquor from a tank actively digesting cornstalks and 2 liters of liquor from a tank actively digesting cellulose. It contained 1.4 grams of organic matter per 750 cc.

The analysis of the solutions at the start and the contents of the bottles after digestion are given in Table XLIV.

From the data given in Table XLV it is apparent that from 7.21 to 7.92 grams or 48 to 53 per cent of the original 15.08 grams of organic matter was converted to gas in 136 days. The weight of the gas, as noted in previous experiments, was always greater than the weight of organic matter decomposed. Those digestions that were permitted to run for 600 days (bottles 2, 3, 8, and 9) gave additional volumes and weights of gas. These 4 digestions produced an average of 9,167 cc. or 10.16 grams of gas. This weight of gas represents 67 per cent of the weight of organic matter present at the start (15.08 gms.)

The loss in total nitrogen noted in bottle No. 5 during the 136-day digestion was only 10 mg. The weight of nitrogen found in the gas was 200 mg. (160 cc). The nitrogen content of bottle No. 7

* 6.3 gm. total solids per liter, 55% volatile, ammonia nitrogen 144 p.p.m., total nitrogen 373 p.p.m.

apparently increased 11 nig. The gas from bottle No. 7 also contained 200 mg. (161 cc.) of nitrogen. The total nitrogen changes in these bottles are too small to be of significance. The nitrogen noted in the gases drawn can be accounted for by taking into account the solubility of gaseous nitrogen from the air in the original solution added, the salt water used in the gas burettes employed in drawing the gas, and in the possible error involved in determining CO₂, O₂, H₂, and CH₄ and calling the remainder N₂.

TABLE XLIV.
LONG TIME DIGESTION OF CORNSTALKS, ANALYTICAL DATA.
At the Start.

	pH.	NH ₂ -H mg.	Total N mg.	Total solids gms.	Organic solids gms.	Volatile acids (AcOH) gms.	Ash gms.	Total CO ₂ gms.
Inoculum solution.....	7.1	142	240	2.24	1.40	0.208	0.84	0.965
Cornstalks added.....		trace	57	15.00	14.40	-----	0.60	-----
Total at start.....		142+	297	17.24	15.80	0.208	1.44	0.965

RECOVERED AFTER 136 DAYS (SERIES A).
Bottle 5.

Mother solution.....	6.0	132	168	2.31	1.13	0.084	1.18	1.345
Cornstalk wash liquor.....		13	65	0.91	0.73	-----	0.18	-----
Cornstalks.....		3	54	6.26	6.15	-----	0.10	-----
Total.....		148	287	9.47	8.01	0.084	1.46	1.345

Bottle 7.

Mother solution.....	6.1	142	212	2.57	1.29	0.075	1.28	1.821
Cornstalk wash liquor.....		15	40	0.74	0.73	-----	0.01	-----
Cornstalks.....		0	56	6.52	6.40	-----	0.12	-----
Total.....		157	308	9.83	8.42	0.075	1.41	1.821

Bottle 4 (Duplicate of 5).

Mother solution.....	6.1	-----	-----	2.22	1.20	0.145	1.02	-----
Cornstalk wash liquor.....		-----	-----	1.08	0.87	-----	.21	-----
Cornstalks.....		-----	-----	6.69	6.58	-----	.11	-----
Total.....		-----	-----	9.99	8.65	-----	1.34	-----

Bottle 6 (Duplicate of 7).

Mother solution.....	6.2	-----	-----	2.28	1.05	0.074	1.20	-----
Cornstalk wash liquor.....		-----	-----	0.78	0.67	-----	.11	-----
Cornstalks.....		-----	-----	6.65	6.53	-----	.12	-----
Total.....		-----	-----	9.71	8.28	-----	1.43	-----

RECOVERED AFTER 600 DAYS (SERIES B).
Composite of Bottles 2, 3, 8 and 9.

Cornstalks plus filterable residue.....	-----	-----	-----	21.4	18.7	-----	2.7	-----
Mother solution.....	-----	-----	-----	9.1	5.0	-----	4.1	-----
Total.....	-----	-----	-----	30.5	23.7	-----	6.8	-----

TABLE XLV.
LONG TIME DIGESTION OF CORNSTALKS, GAS DATA.

Bottle No.	Time of digestion—days.	Total loss in organic matter grams.	Gas data. ^a		Ratio CO ₂ /CH ₄ corrected for control gas.	Weight of gas loss in organic matter.
			Total weight of CO ₂ + CH ₄ grams.	Total volume of gas drawn cc.		
2.....	600	***	10.48	8,830	1/1.16	***
2.....	600	***	11.23	9,490	1/1.17	***
4.....	136	7.21	8.06	6,874	1/1.22	1.09
5.....	136	7.92	8.21	6,804	1/1.22	1.01
6.....	136	7.85	8.43	6,688	1/1.02	1.07
7.....	136	7.52	8.70	6,807	1/1.02	1.13
8.....	600	***	10.99	9,200	1/1.14	***
9.....	600	***	10.92	9,150	1/1.14	***
10 ^b	600	-----	.66	731	1/3.0	-----

^a Corrected to S. T. P. Also for dissolved CO₂.

^b Inoculum.

*** Composite data, bottles 2, 3, 8 and 9.

Loss in organic matter = 39.5 grams.

Weight per gallon of gas / loss in organic matter = 43.6 / 39.5 or 1.10.

As shown in Figure 14 the water-soaked and lime-soaked comstalks digested at practically the same rate as the untreated samples. The close checks noted in the duplicates are also encouraging. The

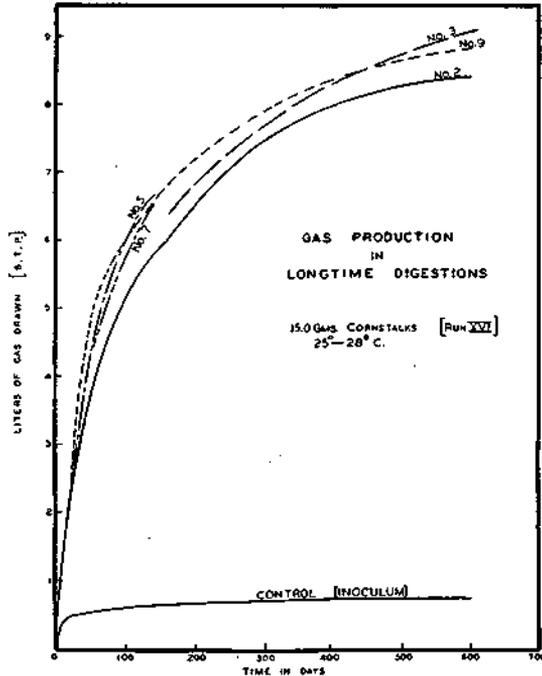


FIGURE 14

more rapid stage of digestion usually noted when feeding fresh cornstalks was not as marked in this experiment in which old cornstalks were used. The more readily utilizable food constituents were undoubtedly leached out of these old stalks.

Table XLI gives the analysis of the cornstalks as fed to these bottles as compared with a composite sample of those removed from bottles 4, 5, 6, and 7 at the end of the 136-day digestion period and a composite of those removed from bottles 2, 3, 8, and 9 at the end of the 600-day fermentation.

TABLE XLVI.
ANALYSIS OF MATERIALS ADDED TO SERIES A^a AND SERIES B^a DIGESTIONS.
Run 16.

	Cornstalks added.		Inoculum added.		Total added grams.
	Per cent composition.	Weight grams.	Per cent composition.	Weight grams.	
Total solids.....		60.0		8.06	69.0
Total volatile matter.....		57.6	82.5	5.60	63.2
Ash.....	4.0	2.40	37.5	3.36	5.8
Water soluble.....	6.09	3.65	^b 100.	8.90	12.6
Ether soluble.....	2.32	1.39			1.4
Ethyl alcohol-benzene soluble.....	6.54	3.92			3.9
Pentosans, total.....	21.1	12.66	nil.		12.7
Cellulose C. & B., pure.....	37.2	22.32	nil.		22.3
Lignin.....	25.1	15.06	nil.		15.1
Protein.....	2.38	1.43	27.4	2.45	3.9
Total.....	104.63				

^a Series A = bottles 4, 5, 6 and 7. Series B = bottles 2, 3, 8 and 9.

^b Practically all water soluble.

TABLE XLVII.
ANALYSIS OF MATERIALS REMOVED FROM SERIES A BOTTLES AFTER DIGESTING
136 DAYS, 20-30° C.

	Cornstalk residues.		Mother liquor drawn.	Total recovered grams.	Total fermented grams.	Per cent.
	Per cent composition.	Weight grams.				
Total solids.....		26.11	12.89	39.0	30.0	44
Total volatile matter.....	98.3	25.67	7.70	33.4	29.8	47
Ash.....	1.7	.44	5.19	5.6	0.2	
Water soluble.....	2.82	.74				
Ether soluble.....	.68	.17				
Ethyl-alcohol-benzene solution.....	6.65	1.74				
Pentosans, total.....	22.7	5.93	nil.	5.9	6.8	54
Cellulose, C. & B., pure.....	36.8	9.61	nil.	9.6	12.7	57
Lignin.....	33.5	8.75	nil.	8.8	6.3	42
Protein.....	4.68	1.22				

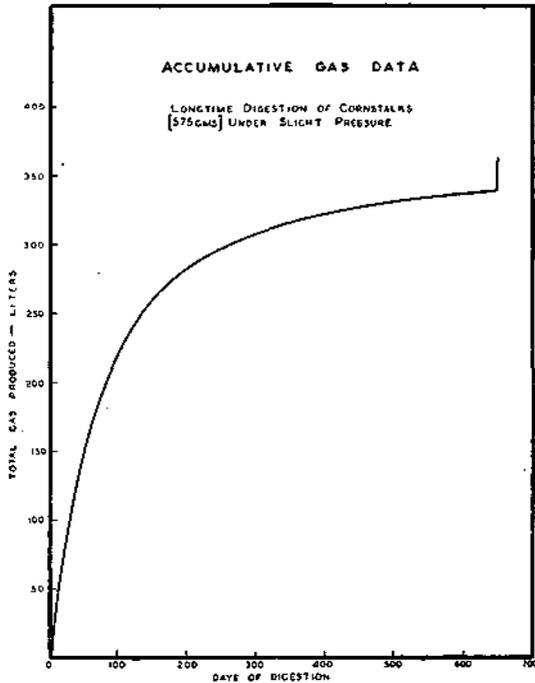
TABLE XLVIII.

ANALYSIS OF MATERIALS REMOVED FROM SERIES B^a BOTTLES AFTER 600 DAY DIGESTION, 20-30° C.

	Residues recoverable (filterable).		Filtered mother liquor grams.	Total recovered grams.	Total fermented.	
	Per cent composition.	Weight grams.			Grams.	Per cent.
Total solids.....		21.4	9.1	30.5	38.5	56
Total volatile matter.....	57.6	18.7	5.0	23.7	39.5	63
Ash.....	12.4	2.7	4.1	6.8	61.0	
Water soluble.....	8.5	1.8	9.1	10.9	1.7	14
Ether soluble.....	0.7	0.2				
EtOH soluble.....	0.2	0.0				
Pentosans, total.....	17.3	3.7	nil.	3.7	9.0	71
Cellulose, C. & B.....	15.7	3.4	nil.	3.4	18.9	85
Lignin.....	33.2	7.1	nil.	7.1	8.0	54
Protein.....	12.5	2.7				

^a Bottles 2, 3, 8 and 9.^b Accumulation of ash (due to lime water and H₃PO₄ added to bottles 8 and 9).

4. *The Fermentation of Cornstalks Under Slight Pressure; Chemical Studies.* The effect of pressures up to 35 pounds per square inch on the digestion of cornstalks was found to be of little if any consequence. In certain cases it might be advisable to conduct this fermentation in this manner thereby eliminating the necessity of a gasometer. This particular experiment was conducted by charging a 16 liter (4.23 gallon) pressure cooker with a mixture of 1 part of actively fermenting cornstalks and 8 parts of fresh cornstalks, the voids being filled by adding 12.5 liters of settled liquor from a tank actively fermenting cornstalks. A total of 575 grams (dry weight) of cornstalks was added. A composite of the cornstalks added as well as a sample of the mother solution, was analyzed. The mother solution contained only 1.2 grams of organic matter per liter or a total of 15.0 grams. The gas generated as a result of the fermentation of this mixture was collected in the small space between the digesting materials and the tight fitting cover. When a pressure of 30 to 35 pounds had built up the gas was withdrawn to a suitable gasometer, mixed, measured and analyzed. As might be expected the first gas drawn (pressure at 35 lbs.) was found to contain a little more methane and less CO₂ than the very last portion drawn at this same time (pressure of 1 lb.). During one investigation the first liter drawn contained 53.4 per cent methane as compared with 48.1 per cent as noted in the 17th liter drawn. The accumulative gas data are plotted in Figure 15. There is a remote possibility that gas was lost around the cover of the pressure cooker because during the latter part of this experiment, which was run for a total of 644 days without being opened, the rate of gas production, as in all such fermentations carried on for such a long period of time, was very slow, yet the pressure within the digester gradually increased. The 13 liters of gas drawn at the close of this experiment (October 24, 1932) had been collecting for 193 days.



At the end of the experiment the gas cock on the digester was connected to a small gasometer. The weights of the gasometer were regulated so as to produce a slight vacuum (2 inches of water) on the system in order to draw as much of the free and dissolved gases out of the digester as practical. After about four hours the gas collected was measured and analyzed. The digester was then opened, the cornstalks and liquor separated and a complete analysis of each made (Table XLIX).

From the data given in Tables XLIX and L it is apparent that the 363 liters of gas generated and which weighed 462 grams, was produced by the gasification of 416.1 grams of volatile matter. Here again one notes that the weight of gas exceeds the weight of volatile matter digested by 11 per cent. This is due, as referred to before, to the weight of the water entering into the decomposition of the various constituents found in the stalks. A total of 68 per cent of the weight of the total solids added (75 per cent of volatile matter) was gasified during the 644-day period. The gas drawn (not corrected for CO_2 retained in the digester) was of the following average composition:

$$\begin{aligned} \text{CO}_2 &= 42.3\% \\ \text{H}_2 &= 0.7 \\ \text{CH}_4 &= 53.7 \\ \text{N}_2 &= 3.3 \end{aligned}$$

The analysis of the cornstalks added to and removed from the digester, as shown in Table XLIX indicates that the main portion of the gas comes from the digestion of the hemicelluloses, the cellulose and lignins. During the 644 days of digestion 88 per cent of the total weight of celluloses added was gasified as was also 93 per cent of the cellulose and 52 per cent of the lignin.

Prom the above experiment one concludes that small pressures have little if any effect on the rate, type, or degree of gasification of cornstalks. It was also noted that the gases recovered at various times throughout the 644-day digestion period were for all practical purposes

TABLE XLIX.
ANALYSIS OF CORNSTALKS ADDED TO AND RECOVERED FROM PRESSURE
EXPERIMENT.^b
Run 34.

	Cornstalks added.		Inoculum liquor 12.5 liters.	Residue recovered after 644 days.		Solids in mother liquor 12.5 liters.		Materials digested.	
	Per cent composition.	Total grams A.	Total grams B.	Per cent composition.	Total grams C.	Per cent composition.	Total grams D.	A+B+C+D grams.	Per cent.
Total.....		575.0	32.8		144.0		51.6	412.2	68
Volatile matter.....	93.6	538.2	15.0	79.4	114.4	44.0	22.7	416.1	75
Hot water soluble ^a	12.8	73.5	10.0	9.7	14.0	12.7	6.6	52.9	72
Ether soluble.....	2.5	14.4	trace	2.4	3.5	trace		10.9	76
Ethylalcohol-benzene (1:2) soluble	0.3	1.7	trace	1.6	2.3	0.6	0.3	+0.9	
Hemicelluloses.....	29.1	167.2	nil.	13.6	19.6	trace		147.6	38
Cellulose.....	31.3	180.0	nil.	7.7	11.1	2.1	1.1	167.8	93
Lignin ^a	19.5	112.0	nil.	33.0	47.5	11.6	6.0	58.5	52
Protein ^d	4.4	25.3	7.8	12.5	18.0	18.2	9.4	5.7	18
Ash.....	6.4	36.8	17.8	20.6	29.6	56.0	28.9	+3.9	
Accounted for by analysis.....	106.3			101.1		101.2			

^a Corrected for ash content. 61% of total 32.7% water soluble.

^b Analysis in duplicate made as per Wakeman and Stevens, Ind. Eng. Chem. Anal. Ed. 2, 167 (1930).

^c Lignin also determined as per Ritter, Seborg and Mitchell, Ind. Eng. Chem. Anal. Ed. 4, 202 (1932).

^d (Total nitrogen-Ammonia nitrogen) x 6.25 = protein.

^e Not corrected for ash.

TABLE L.
GAS DATA, DIGESTION OF CORNSTALKS PRESSURE EXPERIMENT.
Run 34.

25°-30° C; 0 to 35 pounds pressure; 644 days.

	Total gas volume liters.	CO ₂ liters.	CH ₄ liters.	CH ₄ /CO ₂ .	Weight of CO ₂ +CH ₄ grams.
Gas as drawn, S. T. P.....	338	143	182	1.27	413
Gas corrected for increase in dissolved CO ₂	363	168	182	1.08	462

Average Gas Analysis (As Drawn):

CO₂ = 42.3%

H₂ = .7%

CH₄ = 53.7%

N₂ = 3.3%

Weight of gas collected / volatile matter digested = 462 / 416 = 1.11.

of the same composition. This along with the analyses of the residual materials noted in this and previous experiments shows that there is no one of the three major constituents which is quantitatively removed first. The fermentation removes all of the three major constituents showing only a slight preference for cellulose. The rate of gasification noted during the first days was materially greater than noted during the later part of the study.

C. FERMENTATION OF LIGNIN.

While studying the anaerobic fermentation of fibrous materials, it was noted that the pith in materials such as cornstalks was quickly and very completely fermented under anaerobic conditions to carbon dioxide and methane⁽²²⁾. From data obtained in numerous studies, it was apparent that the lignin fraction in the pith and in the general body of the cornstalks was furnishing part of the gas recovered⁽²³⁾. The large amount of organic matter used to start these earlier fermentations, however, made it difficult to definitely establish this fact. Since the gasification of lignin had not been previously observed^(370, 377, 378), the work was repeated under conditions designed to give quantitative data on lignin.

STUDIES ON ISOLATED LIGNIN.

The anaerobic fermentation of lignin isolated from cornstalks by four different methods, namely Kalb, Friedrich, and the two different Phillips procedures, has been investigated. Lignin prepared by the Kalb procedure⁽⁹⁷⁾ when inoculated with organic matter from cultures of carbon dioxide-methane producing bacteria gave only small volume's of gas over that from the control (5 g. of lignin gave 133 cc. of methane and 43 cc. of carbon dioxide in one experiment and 368 cc. of methane and no carbon dioxide in another; both experiments were incubated at 25-30° for thirty-three days). Friedrich⁽⁹⁷⁾ lignin also gave small volumes of gas, namely, 140 cc. of methane and 6 cc. of carbon dioxide from 2.0 g. of lignin in thirty-three days. In these three studies no correction was made for the carbon dioxide retained in the mother liquor. This undoubtedly averaged about 1,000 cc. Lignin prepared by hydrochloric acid⁽²⁴⁷⁾ and by sodium hydroxide⁽²⁴⁶⁾ treatment, in 235 days at 25-30°, gave somewhat more gas, but still the carbon recovered in the gas (corrected for dissolved carbon dioxide), namely, 1.31 g. and 1.51 g., respectively, represented only 43 and 25%, respectively of the weight of carbon added as lignin, assuming the formula for the lignin to be $C_{40}H_{46}O_{16}$ ⁽³³²⁾. The methane to carbon dioxide ratios obtained, namely, 1 to 2.2 and 1 to 2.1, indicate too great a recovery of carbon dioxide to represent complete and normal fermentation of a material having an approximate formula of $C_{40}H_{46}O_{16}$ ⁽²⁴⁵⁾.

Asbestos fiber inoculum⁽³⁰⁾ was placed in three two-liter anaerobic fermentation tanks held at 25 to 30° and fed glucose. Normal fermentation was soon established as evidenced by the one to one carbon dioxide to methane ratio characteristic of glucose⁽³³²⁾. To the fermentations were then added 5 g. of lignin isolated from corncobs and fur-

nished through the courtesy of Max Phillips of the U. S. Department of Agriculture. Gasification stopped at once. Further additions of glucose failed to revive the fermentation.

From the above data it is evident that isolated lignin is incompletely fermented and is undoubtedly somewhat bacteriostatic to the flora responsible for the production of carbon dioxide and methane. Waksman⁽³⁷⁰⁾ and Schrader⁽²⁹⁷⁾ have each reported they were unable to get soil organisms to attack isolated lignin. The present writers, however, are reluctant to state that isolated lignin will not ferment. Many times in the past other materials have acted in a similar manner until the proper inoculating, feeding and incubating conditions were discovered. The high carbon dioxide content noted in the lignin gas is characteristic of abnormal or incomplete fermentation⁽³³²⁾.

Even though isolated lignin cannot be fermented by a natural anaerobic flora, this is no definite proof that lignin in its natural state will not ferment, since it is general knowledge that the methods commonly used for the isolation of lignin bring about certain modifications in its general constitution⁽³⁰²⁾.

FERMENTATION OF LIGNIN IN ITS NATURAL STATE.

A series of eight one-liter anaerobic fermentation cultures were set up and to each was added 15.0 g. dry weight of chopped cornstalks. Each bottle also contained 2.24 g. of inoculum solids (1.40 g. volatile on ignition). The cornstalks and inoculum liquor were analyzed separately for cellulose, pentosans, lignin, etc. The inoculum liquor was practically 100% water soluble and contained no pentosans, cellulose or lignin. Four of these fermentations were permitted to run for 136 days. As the volume of gas developed in each was practically the same, the residues were combined, filtered, washed and the filtrate and washings combined. The analysis of the residue is given in Table LI. The filtrate and washings from the four bottles contained 12.89 g. of total solids, 7.7 g. of which was volatile on ignition and 9.0 g. of which was water soluble. The solids contained no pentosans, cellulose or lignin. The remaining four bottles of the series were permitted to run for 600 days, then their contents were combined, filtered, washed and analyzed. The filtrate and washings contained 9.1 g. of total solids, 5.0 g. of which was volatile on ignition, 9.0 g. being water soluble. The solids contained no pentosans, cellulose or lignin. From the data given in Table LI, it is apparent that 47% of the volatile matter was gasified in 136 days and 63% in 600 days. It is also noted that 54% and 57% of the pentosans and cellulose, respectively, disappeared in 136 days, while at the end of the 600-day period, 71 and 85% had been removed, respectively. Although the lignin content of the residue was 33% as compared with 25% in the original stalks, the total weight of lignin was reduced from 15.06 g. to 8.75 g. in 136 days and to 7.1 g. in 600 days.

An autoclave of 16 liters capacity was charged with 575 g. of chopped cornstalks and 12.5 liters of inoculum liquor from a cornstalk fermentation tank. The inoculum liquor and cornstalks were analyzed

TABLE LI.

TOTAL WEIGHTS OF MATERIALS ADDED TO AND REMOVED FROM CORNSTALK FERMENTATIONS AT 25-30°.

	Corn-stalks added, grams.	Totals added, grams.	Residue removed.				Fermented.			
			After 136 days.		After 600 days.		In 136 days.		In 600 days.	
			Corn-stalks.	Total. ^b	Corn-stalks.	Total. ^b	Total.	Per cent.	Total.	Per cent.
Total solids.....	60.0	69.0	26.11	39.0	21.4	30.5	30.0	44	38.5	56
Total volatile matter.....	57.8	63.2	25.67	33.4	13.7	23.7	29.8	47	39.5	63
Ash.....	2.40	5.8	0.44	5.6	2.7	6.8	0.2	3	1.0	17
Water soluble ^c	3.65	12.6	.74	9.7	1.8	10.9	2.9	23	1.7	14
Ether soluble.....	1.39	1.4	.17		0.2					
Ethyl alcohol-benzene soluble.....	3.92	3.9	1.74		.0					
Pentosans, total.....	12.66	12.7	5.93	5.9	3.7	3.7	6.8	54	9.0	71
Cellulose C. & B.....	22.32	22.3	9.61	9.6	3.4	3.4	12.7	57	13.9	85
Lignin.....	15.06	15.1	8.75	8.8	7.1	7.1	6.3	42	8.0	84
Protein.....	1.43	3.9	1.22		2.7					

^a Includes materials in inoculum liquor.^b Includes materials in filtered mother and wash liquors.^c Not corrected for ash.^d Analysis made according to Bray, *Paper Trade J.*, 87, 59 (1928).

separately as was also the residue and mother liquor left after the 644-day fermentation period (See Table XLIX). The gas generated during the fermentation was collected in the autoclave until a pressure of 25 to 35 pounds per square inch was reached, then it was withdrawn, measured and analyzed. As noted in Table XLIX, 416.1 g., or 75% of the total weight of volatile (organic) matter, was converted to gas during the anaerobic fermentation; 147.6 g. or 88% of the hemicelluloses, 167.8 g. or 93% of the cellulose added, and 58.5 g. or 52% of the total lignin added, were converted to gas. That no constituents other than those listed in Table LI were probably present at the end of the experiment is borne out by the fact that 101.1 and 101.2% of the materials were accounted for in the analysis.

Gas Recovery Data. The carbon in the 168 liters of carbon dioxide and the 182 liters of methane recovered amounts to 187 g. as compared with 182 g. of total carbon lost from the digestion mixture during the fermentation (see Table XLIX). These data further establish the fact that a quantitative account has been kept of all materials added and withdrawn and that the lignin lost from the digestion was recovered as gas.

DISCUSSION OF RESULTS.

Since a complete analysis of all the constituents added to and withdrawn from an anaerobic fermentation of cornstalks shows a material decrease in the weight of lignin present, it is apparent that a large portion of the lignin must have been materially altered or removed entirely from the substrate. That it was not altered so that it would not appear in the analysis is indicated by the fact that 101% of the materials present was accounted for in the analysis. The lignin lost in the fer-

mentation must have been recovered as gas because a carbon balance was obtained (Table XLIX). Furthermore, the fermentation of all the other constituents (less the 58.5 g. of lignin decomposed) could not account for the total volume and weight of gas formed. The production of 350 liters of carbon dioxide and methane, weighing 462 g., as the result of complete fermentation of 416 g. of material, represents a recovery of 1.11 g. of gas per gram of volatile matter fermented. One might have expected this latter figure (1.11) to have been a little higher, in view of the fact that the values for cellulose, pentosans and lignin are 1.11, 1.14 and 1.47, respectively^(32, 40), but careful consideration of the data shows that about four-fifths of the total gas was formed by the fermentation of cellulose and hemicelluloses which give the lower yields of gas per gram of material fermented.

CONCLUSIONS.

1. Isolated lignin ferments very slowly and incompletely under anaerobic conditions.

2. Complete analytical data show that appreciable quantities of lignin in its natural state in cornstalks, ferment anaerobically to carbon dioxide and methane.

D. PILOT UNIT STUDIES.

1. *In primary sewage digestion tank.* On September 25, 1929, the first pilot unit investigation was started. A sewage digestion tank of about 2,000 gallons capacity, constructed and equipped as shown in Figures 16 and 17, was used. This tank was seeded with well-digested sewage sludge and was then filled with raw sewage. As a rule the cornstalks were fed at a rate of from 15 to 20 pounds per day. Mechanical difficulties were encountered at once. After a few days it was found difficult to feed the cornstalks into the tank as the cornstalks within it collected at the top in a thick, firm mat. Use of the side and top circulators, which threw a strong stream of water on and into this mat, such method having been found to be efficient in breaking up scum and foam in sewage digestion tanks⁽³⁵⁾, failed to break up the mat. As the experiment progressed and the feeding continued this mat became thicker. Two electric driven propellers were set to operate in it, but the stirring effect was only local and did not break up the major portion of the mat. The liquor drawn from the bottom of the tank contained but little digested cornstalks as the entire amount present had collected at the top. The removal of the cover and breaking up of this mat released large volumes of gas. Therefore, it was apparent that a method for keeping the cornstalks submerged in the mother liquor and a method for releasing the entrapped gases were necessary. This line of research led to the "tip-top" bottle experiments already-referred to, which in turn led to the design and construction of a special drum digester to be used in the continuous anaerobic fermentation of fibrous materials.

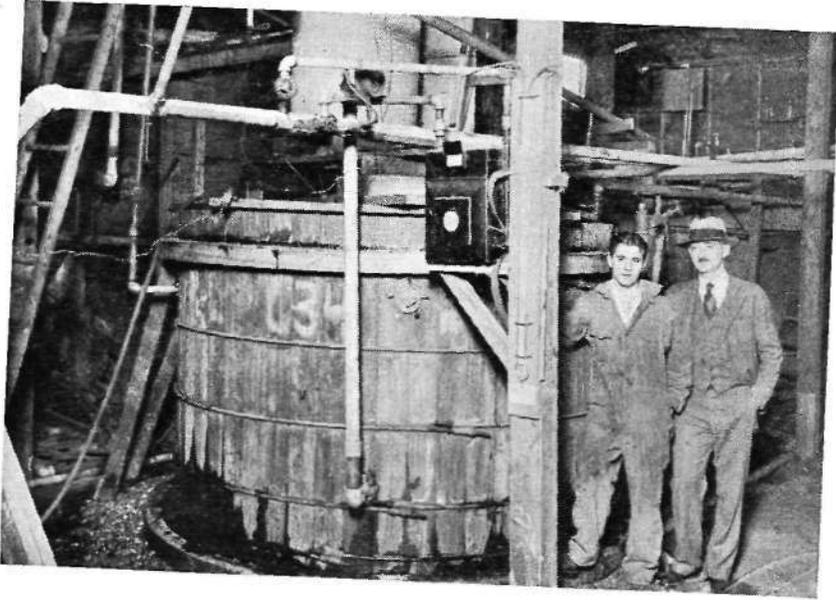


FIGURE 16.
Pilot Plant.

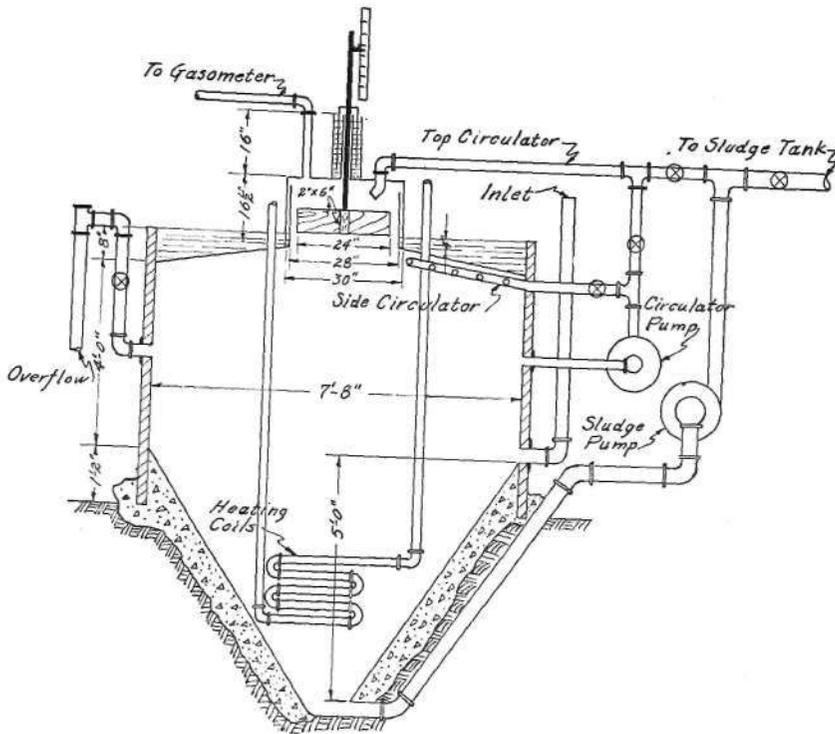


FIGURE 17.
Design of one of the Cornstalk Digestion Tanks.

The amount of gas formed per unit weight of cornstalks added to this first pilot unit (see Table LII) was less than that noted in laboratory investigations. During the 209 days, 2,536 pounds (dry weight) of cornstalks were added to the pilot tank. From this, 8,882 cubic feet of gas were collected, which, on the basis of an average composition of 41.7 per cent CO₂ and 50.9 per cent CH₄, weighed 658 pounds, or 26.0 per cent of the weight of cornstalks added*. An average of 3.5 cubic feet of gas was collected per pound of cornstalks added (7,000 cu. ft. per ton). The data presented in Table XXXVI show that in the tank C laboratory investigation, 30.3 per cent of the weight of the cornstalks was converted to gas. In this first pilot unit investigation, the 8,882 cubic feet of gas were formed in 209 days. This is an average of 42.5 cubic feet per day, or an average gas production of only 16 per cent of the tank volume per day. This figure is much lower than subsequent data collected from other pilot units. It is also lower than those noted in laboratory studies in which average volumes of gas, amounting to as much as 51 per cent of the volume of the tank, were collected daily.

These low gas production data are due mainly to the fact that the tank could not be fed and operated as desired because of the thick mat which collected at the surface of the digester. The tank was opened on an average of about every second or third day to break up the mat for examination, or disturbed in one way or another, in order to make certain observations or changes in methods of feeding, circulating, mixing, drawing of residue, etc. Although hard on gas recovery data these interruptions and observations are what led to some of the most valuable changes in the physical manipulations of the process.

On the basis of this pilot unit" experiment, and on the basis of all laboratory experiments of any size, it was apparent that a special type of digester would be necessary for the continuous fermentation of fibrous materials. Eudolfs and Heisig⁽²⁷⁷⁾ had reported similar difficulties in an attempt to anaerobically ferment and dispose of sewage screenings at the Milwaukee, Wisconsin sewage treatment plant. The special scum saturator which they used to overcome the formation of the mat was only partially successful, therefore, they abandoned this method of screenings disposal on the basis of the mechanical difficulties which confronted them. Nadermann and Strumpfel⁽²⁰⁶⁾ noted similar difficulties in the digestion of fine screenings from the Magdeburg, Germany sewage. They overcame it by violent circulation of the tank liquor through specially designed pumps⁽¹⁶⁾. They provided 0.35 cubic feet of tank capacity per capita of population for the treatment of their fine screenings.

* During the investigation small amounts of sewage and sewage sludge were added to the tank. Overflow liquors containing organic solids, etc. were also drawn and in some cases lost through natural overflow. In this consideration these additions and withdrawals (overflow) have been neglected.

TABLE LII.
OPERATION OF FIRST PILOT UNIT SEWAGE DISPOSAL TANK.

1. Duration of investigation.....	209 days.
2. Cornstalks fed, total dry weight.....	2,536 pounds.
3. Cornstalks withdrawn during run, dry weight.....	1,363 pounds.
4. Cornstalks removed from tank and end of run.....	270 pounds.
5. Total weight (dry) of cornstalks removed.....	1,633 pounds.
6. Weight of cornstalks digested (2-5).....	903 pounds.
7. Weight of CO ₂ , H ₂ and CH ₄ in gas drawn.....	658 pounds.
8. Volume of gas drawn, S. T. P.....	8,882 cubic feet. ^a
9. Volume of gas drawn per pound stalks fed.....	3.5 cubic feet.
10. Average composition of gas (weight of CO ₂ , H ₂ and CH ₄ equal 0.0742 pounds per cubic foot):	
CO ₂	41.7 per cent.
H ₂	1.8 per cent.
CH ₄	50.9 per cent.
N ₂	5.4 per cent.

^a Much gas lost through inefficient tank design and operation.

2. In Buswell-Boruff Digester.

(a) *Design and Operation.* Numerous special types of digesters were designed and the basic principle in a few were investigated in a small way in the laboratory. The method of gas release suggested by the "tip-top" bottle experiments, already described, seemed the most effective and economical to build and operate. On the basis of these experiments the writers designed and built the first drum type digester (Tank R) which was constructed essentially as shown in Figure 18.

The tank was rectangular in shape (4' x 4.3' x 10') and was divided into a relatively large digestion compartment and a smaller residue compartment by a transversely extending vertical baffle "A" spaced from one end.

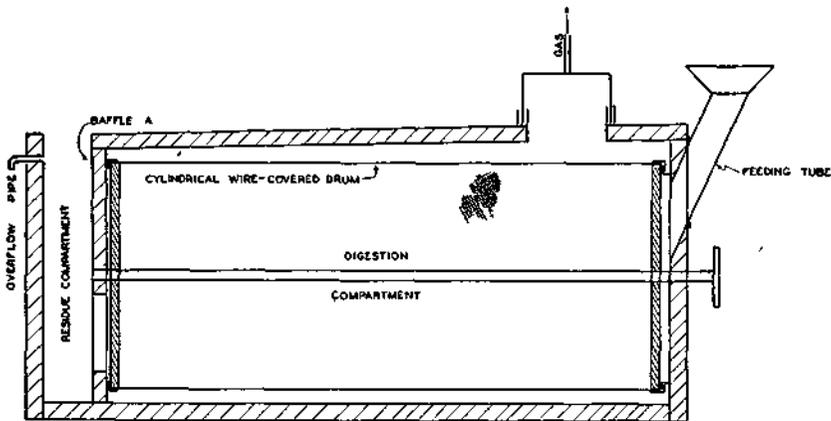


FIGURE 18.
Digester for Fibrous Materials.

A cylindrical drum, 3' in diameter by 9' long, was mounted horizontally within the digestion compartment upon a shaft journalled in bearings carried by the wall "A" and end wall of the tank. The other

large pilot unit (Tank B) which was built after investigations using this tank had been made, carried a 3'-6" drum. This drum was adapted to be rotated continuously or intermittently at any suitable speed. The frame of the drum was covered with a cylinder of wire screening (12 mesh). Each end of the drum was provided with a seal ring which was adjusted so as to prevent the escape of material from within the drum into the outer portions of the fermentation compartment.

The feeding end of the tank was provided with a spout extending through an opening in the tank end-wall within the circle formed by the seal ring. The material to be treated was fed directly into the open end of the drum adjacent to the feeding spout. A discharge opening was provided in the lower portion of the baffle wall "A" to permit digested material from the drum to pass out into the discharge compartment. Material discharged into this compartment was withdrawn.

The fermentation compartment was fitted with a gas-tight, galvanized iron cover having two gas vents with removable hoods resting on it. These hoods were sealed by carrying the tank liquor level about 6" above the galvanized iron cover. The water seal prevented the entrance of air into the digestion compartment where the fermentation was to take place. It also prevented the escape of gas. In addition, it served to prevent the building up of excessive pressures within the digestion chamber. A pipe from the collecting hoods served to draw the gas out into gas collectors.

As the material inside the drum fermented and collected gas bubbles within its mass, the expansion was taken care of by the liquor flowing up through the water-sealed pipes guiding the windlass cables into the tank, or it passed up through the residue' compartment and hence through a hole in the baffle wall into the space above the galvanized iron cover. Upon turning the drum over the entrapped gases were released and the liquor would flow back into the tank. At times when manures were fed, the liquor on top of the tank became very sour and offensive. For this reason the third and smallest unit, Tank C, was constructed in order to leave space inside the tank for this expansion. The greatest expansion ever noted during the operation of these tanks was 6 cubic feet during a 12-hour period when tank B was being fed cornstalks and not rotated. An expansion space of 7 cubic feet per 100 cubic feet of drum capacity would, therefore, seem sufficient to insure against the necessity of adding water to replace that liquor that might be lost during overnight periods when the drum was not rotated.

The windlass arrangement shown in Figure 18 was found to be a convenient method of intermittently rotating the drum. More rigid construction than that used in these first pilot unit tanks would permit the turning force to be applied at the outer end of the shaft carrying the drum.

In putting such a digester as this into operation, the authors have found a number of practices to be equally satisfactory. The particular one to be used in any case would depend on the materials available and the substance to be fermented. The digester is first filled with water or raw sewage. Well-digested sewage sludge has been found to serve well as a source of inoculum. If it is not available, but fibrous material

which has been through such a tank can be obtained at that time, even though it might be at some distant city, it could be dried, transported, and fed as the original inoculum material. Such fibrous residues seem to hold their inoculating power for some time. The organisms responsible for the fermentation undoubtedly pass into a spore stage which permits them to live in an aerobic environment. Fresh barnyard manures should also serve well as a starting material. The amount of inoculum added determines in a large manner the rate at which the digester can be started. In starting tanks of a thousand gallons capacity one should add approximately 50 gallons of well digested sludge. During the first week not over one-fourth to one-third of the maximum amount of material that such a tank can handle per day should be fed. During this time, and for two to four weeks' time after the digester has been started, the feeding schedule should be governed by the volatile acid content. As the volatile acids approach or pass certain concentrations, the amount of material fed should be decreased until the tank has fermented away the accumulated acids. This concentration for cornstalks, straw, sewage screenings, and such materials seems to be approximately 1,000 to 1,500 p.p.m. as acetic.

If the mother liquor, following inoculation and feeding, does not have an ammonia-nitrogen content of a least 100 p.p.m., (as N) then a water soluble source of nitrogen such as, NH_4OH , NH_4Cl or urine should be added. It is preferable not to add nitrates or sulfates as they furnish oxygen. Sulfates are reduced to, H_2S which gives the gas formed a strong stench. If such stench is desired then sulfates should be added. If such materials as packing-house wastes, barnyard manures, or other materials containing appreciable nitrogen (1% or greater) are fed, no other source of nitrogen need be added.

The raw fibrous material in a suitably comminuted form is introduced into the drum through the feeding spout at the front of the tank. Cornstalks and straw that have been through a silage cutter have been found suitable for feeding and for efficient tank operation. Packing-house or stable manures, sewage screenings, and such materials as these need no preliminary treatment. Although not absolutely necessary, it has also been found advisable to soak most of the air out of cornstalks prior to feeding them. This helps to maintain low oxygen concentrations within the tank. A fifteen minute soak is sufficient. Above all, the stalks should not be soaked in an open vat long enough to permit them to become sour as this reduces the readily available food material for the anaerobes, and the additional acids and aerobes introduced endanger the continuance of the desired fermentation. The introduction of the raw material may be either intermittent or continuous, and its rate of introduction, up to a certain amount, will depend upon the rate of gas formation desired. If such materials as sewage screenings or manures are to be fed, the rate of feeding will also be governed by the degree of anaerobic stabilization desired.

As the fermentation proceeds, the fermenting material entraps gas bubbles and the entire mass floats to the top of the drum. Continuous or intermittent revolution of the drum releases the gas bubbles, stirs the tank contents, and aids in moving the entire mass toward the residue

compartments. A complete revolution of the drum although it is not necessary seems to have a little advantage over turning the drum through a lesser arc. It should be turned at least half-way over each time.

Slow, continuous; or intermittent feeding, along with the revolution of the drum, gradually works the materials out through the opening in the lower part of the baffle into the residue compartment. If the drum (3' to 3'6" in diameter x 9' long) is kept from one-half to almost full of fermenting fibrous materials, there is no short circuiting, that is, no discharge of fresh material into the residue compartment. Short circuiting, while putting a tank into operation, is avoided by dropping a gate into the residue compartment so as to plug the opening in the baffle. The digested materials discharged into the residue compartment are still fermenting just enough to entrap gas bubbles in their structure. Such causes the entire mass to float to the top where it can be removed intermittently with a manure fork, or, if desired, an automatic apparatus could be arranged to handle it. The authors have found that in the fermentation of cornstalks, straw, farmyard and packing-house manures which contain considerable fibrous materials, the fibrous residue carries with it practically all of the other organic materials that may have been added with it or formed in the decomposition process. In other words, no need of a hopper-bottom has been noted for such materials. In the treatment of materials containing considerable fine or well-dispersed organic material in the presence of a lesser amount of fibrous waste, the authors have found that some sludge tends to collect in the bottom of the tank. These sludge deposits naturally also depend on the size of the opening in the screen or metal used to cover the drum. In all the investigations herein discussed, common galvanized iron or copper screen wire was used (12 mesh). The former is the better of the two but it lasted only a few months. It would, therefore, seem advisable to use monel screen or perforated metal plates as the covering material for the drums.

(b) *Operating data.* The respective rates at which the different materials were fed to Tanks E and B, as well as the volumes of gas recovered, are recorded in Table LIII and LIV. During the preliminary periods of operation of each of these tanks, there were frequent drawdowns and periods of interruption, due to remodeling, breaking of the light cables used at first to rotate the drum, and general interruptions due to inspection. These account for the fact that no quantitative data were kept on the amount and quality of the liquor drawn from Tank E during the first five months of operation. They also account for the large volumes of liquor drawn from Tanks E and B at other periods during their operation. Such drawdowns naturally reduced the amount of gas recovered from the materials fed.

(2) *Cornstalks.* From the 1,239.3 pounds dry weight of cornstalks fed to Tank E (drum capacity 63.7 cu. ft.) there were recovered 4,328 cubic feet of gas, of an average CH_4 content of 56 per cent. This amounts to an average gas production of 7,000 cubic feet per ton of cornstalks or 0.20 of a volume of gas per day per fermentation tank volume. The cornstalks were fed to this tank at an average rate of 10.1 pounds dry weight per day for the 123 days. The maximum rate

TABLE LIII.

PRELIMINARY PILOT UNIT DATA, TANK R.

Tank Capacity 172 cu. ft. (1,290 gallons). Drum 3' diameter x 9' long, 63.7 cu. ft. Started May 29, 1930. Temperature 25-30° C.

Material fed.	Corn-stalks.*	Wheat straw.†	Manure.‡	Green corn-stalks.	Manure.‡	Manure and artichokes.¶	Extracted artichokes.¶	Artichoke and whey.‡
Time operated, days.....	123.0	51.0	203.0	31.0	87.0	31.0	61.0	30.0
Total dry weight fed, lbs.....	1,239.3	289.0	3,951.7	427.3	1,367.3	236.2	577.9	484.5
Average dry weight fed per day, lbs.....	10.1	5.7	19.5	13.7	15.7	7.6	9.5	16.2
Dry weight fed per day, maximum for any one month, lbs.....	14.0		30.1		20.0		12.0	
Gas recovered:								
Total volume, cu. ft.....	4,328.0	1,065.0	13,350.0	1,150.0	3,745.0	1,812.0	6,809.0	2,740.0
Total weight, lbs.....	327.0	80.0	1,026.0	92.0	282.0	134.0	511.0	206.0
Average CH ₄ content, per cent.....	56.0	56.0	54.0	53.0	57.0	61.0	56.0	65.0
Average volume per day, cu. ft.....	35.2	20.8	65.7	37.1	43.1	58.4	108.0	91.0
Volume per day, maximum for any one month, cu. ft.....	43.1		72.4		46.5		153.0	
Cu. ft. per ton material fed.....	7,000.0	7,360.0	6,750.0	5,400.0	5,480.0		22,800.0	11,300.0
Volume of gas per tank volume per day.....	0.29	0.12	0.38	0.22	0.25	0.34	0.63	0.53
Residue withdrawn (dry basis):								
Weight, lbs.....	871.7	122.8	1,846.9	452.1	865.6	69.6	145.3	294.0
Average ammonia nitrogen, per cent.....	*	*	0.18	0.44	0.29	0.22		0.25
Average total nitrogen, per cent.....	*	*	2.0	2.4	2.6	1.9		2.3
Liquor and sludge withdrawn: ^b								
Volume, gallons.....	*	*	64,480.0	0.0	42,140.0	150.0	0.0	0.0
Total solids, lbs.....	*	*	507.8	0.0	93.6	23.1	0.0	0.0
Total nitrogen, lbs.....	*	*	35.7	0.0	8.3	1.4	0.0	0.0
Chemicals added.....								

* Three months of preliminary data not given. No quantitative data on liquors drawn.

† Unable to get long lengths of digested straw to discharge from drum.

^a Made up of 1,963.2 lbs. of chopped cornstalks (thru ensilage cutter) and 1,988.5 lbs. of cow droppings.^b Most of volume drawn necessary to make repairs, alterations or for general inspection.^c Tank being rotated often to remove digested materials. Such accounts for large weight of residue.^d Chopped straw bedding plus cow droppings. Tank drawn down and opened often for experiments with plunger feeder.^e Chopped straw bedding plus cow droppings (147.1 lbs.) with 89.1 lbs. dry weight of extracted Jerusalem artichokes.^f Unable to obtain enough extracted Jerusalem artichokes to feed tank at greater rate.^g Composed of 311 lbs. of extracted Jerusalem artichokes plus 173.5 lbs. dry weight of whey solids (290 gallons).^h Refilled with water or raw sewage.

TABLE LIV.
PILOT UNIT DATA, TANK B.

Tank Capacity 184 cu. ft. (1,380 gallons). Drum 3'6" Diameter x 9', 86.7 cu. ft. Started October 28, 1931. Temperature 25-30° C.

Material fed.	Cornstalks.	Green cornstalks.	Green cornstalks and straw.	Wheat straw.	Wheat straw and whey.
Time operated, days.....	303	31	30	121	93
Total dry weight fed, lbs.....	5,464.2	700.1	787.8	2,624.2	2,728.7
Average dry weight fed per day, lbs.....	18.0	22.6	26.3	21.6	29.4
Dry weight fed per day, maximum for any one month, lbs.....	28.5			23.0	37.0
Gas recovered:					
Total volume, cu. ft.....	22,707.0	1,600.0	2,030.0	6,635.0	7,040.0
Total weight, lbs.....	1,736.0	132.0	173.0	545.0	583.0
Average CH ₄ content, per cent.....	52.0	47.0	47.0	50.0	50.0
Average volume per day, cu. ft.....	75.0	52.0	68.0	55.0	76.0
Volume per day, maximum for any one month, cu. ft.....	102.0			62.0	82.0
Cu. ft. per ton material fed.....	8,309.0	4,570.0	5,150.0	5,070.0	5,150.0
Volume of gas per tank volume per day.....	0.41	0.28	0.37	0.30	0.41
Residue withdrawn (dry basis):					
Dry weight, lbs. ^a	3,179.7	509.8	352.7	1,821.3	2,158.4
Average ammonia nitrogen, per cent.....	0.1	0.1	0.1	0.1	0.06
Average total nitrogen, per cent.....	1.2	1.2	1.2	1.2	1.0
Total nitrogen, lbs.....	38.2	6.1	4.3	21.8	21.6
Liquor withdrawn:					
Volume, gallons ^d	2,250.0	0.0	350.0	0.0	1,380.0
Total solids, lbs.....	190.0	0.0	29.0	0.0	112.0
Ammonia nitrogen, lbs.....	3.23	0.0	2.01	0.0	4.40
Total nitrogen, lbs.....	8.31	0.0	1.09	0.0	1.52
Sewage sludge added:					
Volume, gallons.....	0.0	0.0	0.0	105.0	130.0
Total solids, lbs.....	0.0	0.0	0.0	52.0	65.0
Total nitrogen, lbs.....	0.0	0.0	0.0	1.72	2.39
Nitrogen added as NH ₄ Cl, lbs.....	4.65	0.92	2.33	3.10	1.15
Total nitrogen in cornstalks, etc., fed, lbs.....	39.84	5.15	6.70	21.37	23.13

^a 160.4 lbs. straw and 627.4 lbs. green cornstalks (dry weight).

^b 2,383.5 lbs. straw and 380 gallons or 343.2 lbs. whey solids from cheese manufacturers.

^c As drawn the residue averaged about 85 per cent moisture.

^d Liquor drawn for remodeling, repairs or inspection. No sludge noted. Refilled with water or raw sewage.

^e Added as NH₄OH.

^f Tank and drum emptied and cleaned out.

for any one month was 14.0 pounds per day. The average rate of gas production was 35.2 cubic feet per day. The maximum for any one month was 34.1 cubic feet per day.

During the 303 days that cornstalks were fed to Tank B, (total capacity, 184 cu. ft., drum capacity 86.7 cu. ft.) there were added 5,464.2 pounds dry weight of stalks. From this there was recovered 22,707 cubic feet of gas of an average CH_4 content of 52 per cent (B.t.u. about 500 to 520). This amounts to a gas production of 8,300 cubic feet per ton of stalks. The stalks were fed to this tank at an average rate of 18.0 pounds dry weight per day. The maximum rate for any one month was 23.5 pounds per day. The average rate of gas production was 75 cubic feet per day or 0.41 of the fermentation tank volume per day. The maximum rate for any one month was 102 cubic feet per day.

The greater gas yields and gas rates obtained from Tank B as compared with Tank R were mainly due to the fact that as the result of the studies made with Tank B, more was known concerning the mechanical operations involved in the continuous fermentation of these materials. The size of the drum in Tank B was also of greater capacity in relation to the total tank volume. The drum in Tank B (3'6" x 9') had a capacity of 86.7 cubic feet, or 47 per cent of the total tank volume, as compared with 63.7 cubic feet (3' x 9') or 37 per cent of the total tank volume for Tank R. The authors feel that it is largely the drum capacity that determines the digestion capacity of any tank. A cylindrical tank containing a drum with only about an inch of clearance would undoubtedly give greater yields of gas per total tank volume than would a rectangular tank. Ease and cost of construction should determine the type of tank built to enclose the drum. In any case, the drum should be made as large as the tank will handle.

At no time during the operation of Tank B was it pushed to its capacity. The volatile acids never got above 400 p.p.m. For at least one-fourth of the time, the ammonia nitrogen concentration was below 100 p.p.m. This is now known to be too low. The rest of the time the ammonia nitrogen concentration ranged from 100 to 150 p.p.m. which is the minimum concentration found suitable for optimum conditions. The pH never fell below 6.7. These data also contain those collected during a 30-day period when the tank was frequently (about every other day) treated with about a pound of slaked lime. No beneficial effect was noted on pH, volatile acid, or gas production data.

During the first half of the 303-day period that Tank B was fed cornstalks it was never fed at the maximum rate. That is, no particular difficulty was noted in feeding the tank through the feeding tube, and the volatile acid content, which has been found to be one of the best indicators, averaged less than 500 p.p.m. The ammonia nitrogen concentration during this period was maintained between 150 and 175 p.p.m. During the last five months of operation the tank was fed at a greater rate. At times the drum was so full that it was a little difficult to feed through the spout, but turning the drum over a few times always permitted the addition of the daily charge. The volatile acid content during this period of more intensive feeding averaged

about 800 p.p.m. At times it ran well over 1,000. When such occurred the daily portion of the accumulated acids was fermented away. Even during these high volatile acid periods no pH difficulties were ever encountered. As noted in laboratory investigations, the volatile acid test has been found to be the best indicator and guide for operation.

At times when organic acids are collecting in the tank the CO_2 content of the gas always increases. The amount of change is dependent on the rate of acid accumulation and the total amount and type of acids accumulating. At times when the acid content reached 1,000 to 1,200 p.p.m., the CH_4 content was reduced to 47 per cent. As stated above, reducing the amount of fresh material fed always fermented away these accumulated acids. This, in turn, always caused an increase in the CH_4 content of the gas. At such times the gas contained from 55 to 58 per cent CH_4 . The reason for this change in gas quality will be discussed later under the topic of "Hydrogen Ion Concentration and Accumulated Organic Acids."

Ammonia nitrogen regulation. As stated before, the minimum ammonia nitrogen concentration found suitable for the anaerobic fermentation of cellulosic materials is 100 p.p.m. Greater concentrations seemingly do not aid materially nor have they been found bacteriostatic. If the digester is originally filled with water or sewage this minimum concentration is not reached. In this case, the authors have found it advisable to add some cheap source of ammonia. Ammonium chloride has been found suitable and ammonium hydroxide, ammonium phosphate and ammonium sulfate have been used. The 11 pounds of ammonia nitrogen (as N) added to Tank B during the 485 days that it was being fed cornstalks and straw was necessary mainly to replenish the ammonia nitrogen. As noted in Table LV there were 73.06 pounds of total nitrogen in the cornstalks and straw added. The residues drawn contained an average of 1.2 per cent of 70.4 pounds of total nitrogen. Hence, it is noted that if the liquors had not been drawn for repairs, remodeling, inspection, etc., no ammonia nitrogen need have been added except that necessary to raise the initial ammonia nitrogen content of the tank to 100 p.p.m. or greater. The bacteria seemingly utilize the ammonia nitrogen to build up their own structure. Lysis of these bacteria, following death, releases the nitrogen which under anaerobic conditions is again reduced to ammonia nitrogen, thus completing the cycle.

TABLE LV.
NITROGEN BALANCE, PILOT UNIT TANK B.

Material being fed.	Days.	Nitrogen added as NH_4Cl , lbs.	Nitrogen cornstalks etc. added, lbs.	Nitrogen withdrawn in liquors, lbs.	Nitrogen in residue, lbs.
Cornstalks.....	303	4.65	5.31	39.84	38.2
Green cornstalks.....	31	.82	0.00	5.15	6.1
Cornstalks and straw.....	30	2.33	2.01	6.70	4.3
Wheat straw.....	121	3.10	0.00	21.37	21.8
Total.....	485	11.00	10.32	73.06	70.4

The residue removed from Tanks R and B was composed of the more resistant portions of the cornstalks, namely, the cortex, the fibrovascular bundles and the nodes (See Figure 19). All of the porous structured material was fermented away. As noted in the previously reported laboratory studies, the chemical composition of this residue was found, for all practical purposes, to be the same as the original cornstalks. It is largely the pithy fibers that are removed, although computations show that not all of the gas is obtained from this tissue alone. The residue is stable, that is, it does not cause a nuisance if piled in a wet condition. It composted in a year's time to a black fibrous humus. Addition of ammonium salts and phosphate accelerated materially the rate of composting. As the anaerobic fermentation of this material has removed all of the readily fermentable constituents, this residue could be distributed on farming land or garden plots without danger of producing sour soil or competition for the soil nitrogen.

In the manufacture of paper pulp and various wall and insulating boards it is advisable to remove the pithy tissue³²⁹. With this in mind, samples of the residue from this fermentation (digested cornstalks) were shipped to Prof. O. R. Sweeney (Ames, Iowa), who, with his assistants, compared its value with that of raw undigested cornstalks for the preparation of paper pulp and different cornstalk boards. Samples of paper as well as pressed board made from mechanical and cooked pulp were prepared. Their findings as reported may be summarized as follows:

"The cornstalks treated with the Buswell-Boruff process were unlike the regular cornstalks because the treatment they had received left them without pith and in a finely shredded state. Because of this, there was no need of rod-milling the pulp.

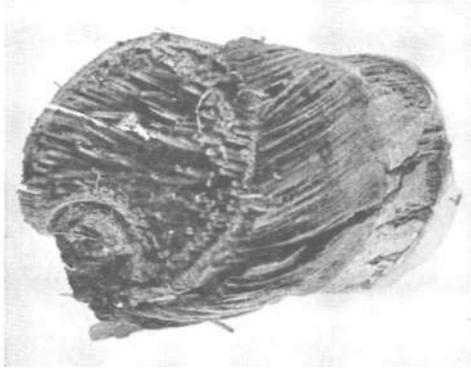


FIGURE 19.
A Node of Partially Decomposed Cornstalk.

"The board made from cooked stalks seemed to be very strong and of a comparative weight to other insulating boards. One noticeable difference in the board was the denseness. It seemed to be very compact, which undoubtedly helped to strengthen its structure.

"The mechanical board seemed to be rather spongy when it was run through the forming machine, and this is the reason that it has a thicker surface. Like the cooked pulp, the mechanical also made a very strong board in comparison with other insulating boards now on the market.

"The material gave a strong paper, difficult to bleach, but compared favorably with cornstalks for paper manufacture."

2. *Wheat straw.* Wheat straw was fed to both Tank E and Tank B. Uncut straw was fed to Tank R for 51 days at the rate of only 5.7 pounds dry weight per day. From this, an average of only 20.8 cubic feet of gas was recovered per day. The long straw fibers wound around the shaft of the drum, and in balls within it, thus making it impossible to get good elimination of the partially digested material. This accounts for the short experimental run and the low rate of feeding and gas recovery.

Wheat straw that was cut in moderately short lengths by passing it through an ensilage cutter was fed to Tank B for 121 days at an average rate of 21.6 pounds dry weight per day. From this, an average of 55 cubic feet of gas of a CH₄ content of 50 per cent was recovered per day. The straw produced 5,070 cubic feet per ton added or 0.30 volume of gas per fermentation tank volume per day. The residue from this fermentation was stable and according to experiments performed by Dr. O. R. Sweeney for the Central Fiber Products Company of Tama, Iowa, it made just as good a straw board as the undigested raw wheat straw.

3. *Wheat straw and whey waste.* At the same time that the above experiments were being made on straw, the authors were conducting small scale experiments on the disposal and utilization of creamery and cheese factory wastes⁴³. In order to determine the fate of whey waste in combination with cellulosic materials, the former was added along with the straw to Tank B and with extracted Jerusalem artichokes to Tank E. Whey solids increased the amount of gas obtained daily. The data are given in Tables LIII and LIV.

4. *Manure.* Realizing that farmers use an appreciable amount of bedding for horses and milk cows and that manures are preferably composted before being spread on land, the authors thought it advisable to determine the amount of gas that might be recovered in anaerobically composting fresh manures. During one investigation chopped cornstalks and in the other chopped wheat straw was used as bedding for one cow. Enough bedding was used to insure no absorption of the urine in the stalks and hence the ammonia nitrogen was largely recovered.

During the 203-day investigation on the gasification and composting of cornstalk-manure, 3,951.7 pounds dry weight of material were fed to Tank E (172 cu. ft. capacity). This was composed of 1,963 pounds of cornstalks and 1,988.3 pounds (dry weight) of cow droppings. This material was fed at an average rate of 19.5 pounds per day (0.11 lbs. per cu. ft. of tank capacity). The maximum rate for any one month was 72.4 cubic feet per day. At this rate of feeding, the material furnished 6,750 cubic feet of gas per ton dry weight of manure fed. The residue was not offensive, was largely composted and was used as a

fertilizer with success by workmen at the experimental plant. It contained an average of 0.18 per cent ammonia nitrogen and 2.0 per cent total nitrogen, dry basis.

The fresh manure weighed from 20 to 22 pounds per cubic foot. The residue as drawn weighed about 150 pounds but after draining and drying it for one day, it weighed only 100 pounds per cubic foot.

During the 87-day period that manure composed of chopped straw and cow droppings was being fed to Tank R, a total of 1,367.3 pounds dry weight of material was added. This amounts to an average feed of 15.7 pounds per day (0.09 lb. per cu. ft. of tank capacity). The maximum rate for any one month was 20.0 pounds per day. From this manure were recovered 3,745 cubic feet of gas of an average CH₄ content of 57 per cent. This is an average of 43.1 cubic feet per day. The residue from this fermentation was also well composted and contained 2.6 per cent of total nitrogen, dry basis.

On the basis of the above data, the bedding and droppings from one cow should furnish all the gas necessary for cooking and heating of water for a farm family of 2 to 3 people. The carrying out of this fermentation would involve only one additional handling of the manure, would require but little of the farmer's time, and for this time • and his small financial investment, would give him a well-composted manure and plenty of gas for heating, lighting or power purposes.

5. *Extracted Jerusalem artichokes.* For a time the authors were able to obtain sufficient extracted Jerusalem artichoke press-cake waste to make it worth while to feed it to one of the pilot unit digesters. (From Drs. Adams and Englis of the Chemistry Department of the University of Illinois.) Previous small-scale laboratory experiments had demonstrated that large volumes of gas could be recovered by the anaerobic fermentation of this waste pulp. At no time was sufficient pulp available to really feed the tank at a maximum rate. For 61 days it was fed intermittently to Tank R (172 cu. ft. capacity) at a rate that averaged 9.5 pounds dry weight per day. From the 577.9 pounds dry weight of material added there were recovered 6,600 cubic feet of gas, or an average of 108 cubic feet, per day. The maximum rate obtained during one month was 153 cubic feet per day. It will be noted that this is the greatest gas rate obtained during the operation of either of the two larger pilot units. Most of the gas from any one feeding was liberated within three days. At one time, 165 pounds dry weight of pulp were added all at once. During the first day 470 cubic feet of gas were formed. This amounts to 2.72 volumes of gas per fermentation tank volume. From the second to the fifth day, inclusive, 165, 83, 53, and 38 cubic feet were formed, respectively. The residue was fibrous, non-offensive and dried readily.

6. *Sewage screenings.* Small scale laboratory studies had shown that it was possible to anaerobically ferment and stabilize sewage screenings by the use of the "tip-top" bottle apparatus previously described. In one experiment, 20.4 grams dry weight of screenings were added to two digestion bottles. One was equipped so it could be inverted in order to release the entrapped gases while the other was stationary.

The "tip-top" digester gave 2.1 liters of gas in 7 days as com-

pared with the 1.4 liters collected from the stationary bottle. In 50 days the "tip-top" bottle had generated 5.8 liters as compared with one 4.6 liters for the stationary digester.

To further investigate this problem, the authors built a small (12.3 cu. ft.) pilot unit digester which was 22 inches wide, 23 inches tall, and 48 inches long (inside measurements). This small rectangular tank carried a drum that was 20 inches in diameter and 42 inches in length (7.64 cu. ft.).

TABLE LVI.
THE ANAEROBIC STABILIZATION OF SEWAGE (BAR SCREENINGS).
In Tank C Pilot Unit Digester, 12.3 cu. ft. Temperature 25-30° C.

	January to February data.	March to May data.
Time, days.....	60	92
Bar screenings fed:		
Wet weight, lbs.....	902	1,975
Dry weight, lbs.....	133.6	227.0
Average dry weight per day, lbs.....	2.2	2.5
Gas recovered:		
Volume, cu. ft.....	567	1,234
Average volume per day, cu. ft.....	9.3	13.4
Weight, lbs.....	43.7	92.5
Cu. ft. per lb. screenings.....		
Average CH ₄ content, per cent.....	56.0	59.0
Residue withdrawn:		
Wet weight, lbs.....	433.0	*749.0
Dry weight, lbs.....	56.5	*139.4
Sludge drawn from bottom of tank, gallons.....	0.0	0.0

Summary of Capacity Data.

(Per thousand cu. ft. of tank capacity.)

Screenings fed per day:		
Wet weight, lbs.....	1,221.0	1,745.0
Dry weight, lbs.....	181.0	200.0
Gas recovered per day, cu. ft.....	755.0	1,090.0

* Includes residue left in drum at end of experiment.

This tank was started (Oct. 22, 1931) by adding 20 gallons of thin digested sewage sludge. The remainder of the tank was filled with raw sewage. Bar screenings from the Urbana-Champaign sewage disposal plant were fed to the tank. At first it had a tendency to go sour, therefore, the amount of screenings being fed was reduced and residue from pilot unit Tank R, which at that time was being fed manure, was added. This established the desired fermentation. General preliminary investigations were conducted with the tank for two months during which time normal fermentation had been well established and the tank was ready for continuous operation. The data collected during the months of January and February and during March, April, and May are summarized in Table LVI. As noted in this table, screenings were fed during the first period at an average rate of 2.2

pounds dry weight per day, and during the second period at an average rate of 2.5 pounds dry weight per day. The bar screenings were characteristic of domestic sewage screenings. No sorting of the screenings was made prior to feeding. At the above rates of feeding there were recovered an average of 9.3 and 13.4 cubic feet of gas per day, respectively. The authors feel that the rate at which the tank was fed during this second period is about right for bar screenings, namely, 2.5 pounds for the 12.3 cubic foot digester, or 200 pounds dry weight per thousand cubic feet of digester capacity. At this rate of feeding the screenings are stabilized and in the order of 1,090 cubic feet of gas are generated per thousand cubic feet of digester capacity. This amounts to 5.45 cubic feet of gas per pound dry weight of screenings added.

This residue is not offensive, is fibrous and dries rapidly. A bucketful of this residue was brought into the office of the authors where it remained for two months. No odor was noted. This residue could be drawn on sludge beds by itself, or with digested sewage sludge and hence dewatered and disposed of in the usual manner, or it could be drawn and used without drying for filling purposes or piled until it dried sufficiently to be burned. When piled or drawn into the open it causes no nuisance and does not draw flies. In this respect it is much different from fresh screenings. By being able to leave it on top of the ground it can undergo further decomposition and ultimately be reduced to humus. This has experimentally been found to be the case. Fresh screenings must be buried under ground where they compost very slowly. Many cases have been reported where the uncovering of undigested screenings which had been buried for over a year showed them to be practically as foul as they were on the day they were originally covered over. Additional comments on the disposal of sewage screenings by the use of that type of digester as compared with other methods have been made elsewhere⁽²⁶⁾.

7. *Paunch manure.* Following the investigation on the digestion of sewage screenings the drum of the Tank C pilot unit was cleaned out and cow paunch manure, obtained from a slaughter-house in Danville, Illinois, was fed. The data obtained in this study, which was made in the laboratory⁽²¹⁾ are recorded in Table LVII.

TABLE LVII.

THE ANAEROBIC STABILIZATION OF COW PAUNCH MANURE (PACKING-HOUSE WASTE).
In Tank C, 123 cu. ft. Capacity. Temperature 25-30° C.

	Pounds.	Kilograms.
Paunch manure fed:		
Total wet weight.....	2,054	933
Total dry weight.....	311	141
Total dry volatile matter.....	280	127
Average dry weight fed per day.....	2.6	1.2
Minimum-maximum dry weight fed per day*.....	1.6-3.6	0.7-1.6
Total gas recovered.....	89	40
Residue removed, total dry weight.....	204	93
Sludge drawn, dry weight (30 gallons or 115 liters).....	9	4

TABLE LVII-Concluded.

	Cubic feet.	Cubic meters.
Gas recovered, S. T. P.:		
Total volume.....	1,178	33.3
Average volume per day.....	8.7	0.27
Minimum-maximum volume per day ^a	7.6-14.5	0.22-0.41
Volume per tank volume per day: ^b		
Average.....	0.79	
Minimum-maximum ^a	0.51-1.18	
Average CH ₄ content of gas, per cent.....	60.0	

^a Thirty-day averages.

^b Cubic feet of gas per cubic foot of tank capacity, or liters per liter of tank capacity.

TABLE LVIII.

PILOT UNIT DATA ON THE CONTINUOUS FERMENTATION OF CERTAIN CELLULOSIC MATERIALS, MONTHLY AVERAGES.

Material fed.	Duration of experiment, (months).	Rate fed (lbs. dry wt. per day per thousand cu. ft. tank capacity).		Per cu. ft. drum capacity.	Gas produced (cu. ft. per lb. material added).		Volume of gas produced, tank volume.		Per cu. ft. drum capacity.	Methane content of gas, (per cent).
		Ave.	Max.		Ave.	Max.	Ave.	Max.		
		Cornstalks.....	17		109	128	.27	4.0		
Straw.....	8	140	175	.37	2.5	2.9	0.35	0.46	1.0	46-50
Stable manure:										
1. Cornstalk bedding	8	116	175	.47	3.5	5.2	0.38	0.47	1.3	50-60
2. Straw bedding.....	2	112	127	.35	2.7	3.3	0.28	0.32	0.9	56-60
Sewage screenings.....	6	200	239	.39	5.9	7.0	1.0	1.2	1.9	56-61
Packingshouse wastes:										
1. Paunch manures.....	5	216	290	.47	3.5		.75	1.18	1.91	58-62

TABLE LIX.

ANAEROBIC FERMENTATION OF FARM AND INDUSTRIAL WASTES.
Capacity and Gas Recovery Data, 26-30° C.

Materials fed.	Rate fed per day.			Volume of gas per day.		Cu. ft. per lb., total solids added.
	Lbs. T.S./ft. tank capacity.	Lbs./ft. drum capacity.	Volume per tank volume.	Per tank volume.	Per drum volume.	
Cellulose.....	0.08 (1.25 gm./l)			1.1		13.7
Starch.....	0.04 (0.7 gm./l)			.5		12.5
Cracked corn.....	0.09 (1.5 gm./l)			1.0		11.1
Cornstalks.....	0.13	.27		.56	1.2	4.3- 5.0
Straw.....	0.18	.37		.46	1.0	2.6
Manure.....	0.18	.47		.47	1.3	2.6
Paunch manure.....	0.20	.47		1.18	1.9	4.1
Sewage screenings.....	0.20	.32		1.0	1.6	5.0
Beer slop.....	0.31-0.94		1/3-1/6	3.0-7.0		9.7- 7.5
Milk waste.....	0.14-0.23		1/20-1/30	1.6-2.7		11.4-11.7

FACTORS INFLUENCING THE FERMENTATION OF CELLULOSE AND FIBROUS MATERIALS.

Having presented data to illustrate the rate and amounts of gas that may be recovered in the anaerobic fermentation of cellulose and certain inefficiently utilized plant materials such as straw, cornstalks, and the like, the reader's attention will now be directed to those factors which seem to determine the nature and general rate of fermentation of these materials. The order of presentation is no indication of the relative importance of the various factors discussed. Of these various factors, the maintenance of strict anaerobic conditions (absence of air), regulation of rate of feeding so as not to cause the accumulation of too high an organic acid salt concentration, the maintenance of temperatures of from 25° to 30° C., and the pursuance of the fermentation in such a manner that the entrapped gases are liberated and the floating mat broken up, are considered the main and controlling factors.

A. TEMPERATURE.

In order to determine the effect of temperature on the rate of gas production from cellulose, a number of one-liter bottles were fitted up in the usual manner (Figure 8) and into each was placed 8.0 grams of filter paper, 25 cc. of digested sewage sludge, 600 cc. of settled sewage, and the remainder of the bottle filled with distilled water. Control bottles containing only the inoculum solution were also set up and incu-

TABLE LX.
EFFECT OF TEMPERATURE ON RATE OF FERMENTATION OF CELLULOSE.^b
Run 13.

Time—days.	Total gas produced, cc.*			
	20° C.	25° C.	32° C.	40-50° C.
5	35	387	541	250
8	65	888	891	510
13	180	1,277	1,216	1,164
23	554	1,477	1,514	1,471
35	992	1,477	1,477	1,471
43	1,037	1,577	1,586	1,531
79				

^a Not corrected for increase in CO₂ of mother liquor.

^b 8.0 grams of filter paper added.

bated at 20° C, 25° C, 32° C, and 40-50° C, as were also two bottles (duplicates) of the above described substrate-inoculum mixture for each temperature. None of the bottles gassed at the rate they should have. The duplicates checked very well.

The net volume of gas generated at 20° C. was only 65% per cent as much as that formed at 32° C. The gases formed at 25° C. and at 40-50° C. were 99 per cent and 97 per cent, respectively, of the volume of gas formed at 25° C. (See Table LX.) Under the conditions of the above experiment, one concludes that the digestion of cellu-

lose takes place readily in the range from 25° C. to 50° C, with slightly better results at 32° .

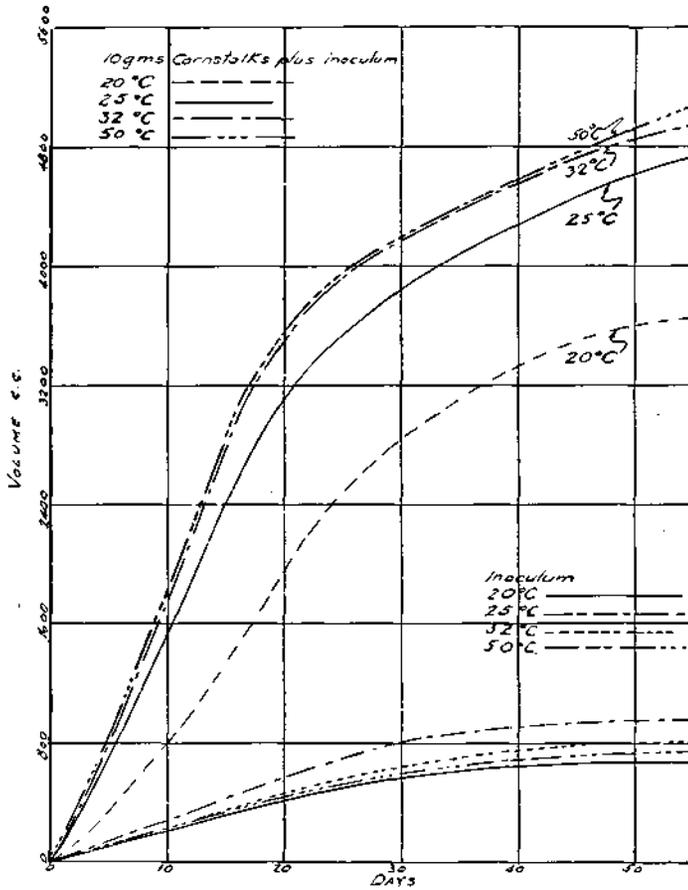
Other experiments by the writers indicate the optimum temperature range for the cellulose-fermenting mesophiles to be from 25-28° C. There is little doubt that the thermophiles in the sewage sludge added as the inoculum in the above experiment were in the minority and that a typical thermophilic fermentation was probably never developed. Many have reported the presence of thermophiles that will produce methane from cellulose but good qualitative gas data are scarce. On the basis of general knowledge concerning the rate of growth and fermentation of thermophiles, and more specifically the comparative rates noted by the present writers⁽²⁴⁾ while investigating the mesophilic and thermophilic fermentation of distillery wastes, it would seem reasonable to expect that if once a good thermophilic flora were established and habituated to cellulose, it should produce methane much faster than the mesophiles. A recent report (1931) of some research studies on thermophilic sludge digestions carried out by the City of Manchester, England⁽¹⁸⁴⁾ shows that at 52° -53° each gram of paper ferments anaerobically to produce about 630 cc. of gas in 5 days or 700 cc. in 10 days. Other studies of less quantitative nature have already been presented in the historical section of this bulletin.

It was also thought advisable to investigate the effect of certain temperatures on the rate of the fermentation of cornstalks. To start with, four large bottles containing raw sewage, a small amount of sludge, and a little powdered cornstalks were incubated at 20° C, 25° C, 32° C., and 50° C, respectively. After a week, these settled liquors were, in turn, used as the respective inoculum solutions for the second cornstalk fermentations to be carried out at these temperatures. It was thought that this procedure might lead to the development of a more selective flora. A control bottle, a bottle containing the inoculum solution, and 10 grams of chopped cornstalks were incubated at the above mentioned temperatures, namely, 20° C, room temperature (23-28° C.), 32° C. and 50° C. The gases were collected in the usual manner (over salt water). They were measured and a few samples were analyzed. There was no notable difference in the chemical composition of the gases drawn from the different cornstalk digestions. The different rates of gasification have been plotted in Figure 20. From these data it is apparent that it would not pay to try to keep a large digestion tank at a temperature much above 24° to 32° C. In fact, some indirect evidence has been collected which tends to show that at 30° there is more of a tendency for the accumulation of free organic acids. It is evident, however, that it would pay to insulate the tanks, and, if necessary, use part of the gas generated for heating in order to maintain temperatures above 20° C.

B. HYDROGEN ION CONCENTRATION AND ORGANIC ACIDS.

The optimum pH range for the anaerobic fermentation of cellulose and crude fibrous materials to CO₂ and CH₄ seems to be between 6.5 and 7.5. Values as great as 8.5 have been noted during the successful anaerobic digestion of certain industrial wastes containing cellulosic materials. As noted in experiments already cited, the writers have been

FIG 20
EFFECT OF TEMPERATURE ON RATE OF
FERMENTATION OF CORNSTALKS
(Run XXX B)



able to maintain cellulose and cellulosic fermentations on the alkaline side without the addition of alkalis or buffers. The liquors in many of the earlier small-scale bottle and tank experiments had a tendency to become slightly acid (pH 6.5-6.8). These low pH values (in exceptional cases 6.0) were due to the accumulation of organic acids. The failure of these acids to ferment to CO_2 and CH_4 as desired, can now be attributed to one or a number of controllable factors, such as too rapid feeding (Tank A on cellulose; tanks packed with cornstalks), insufficient or wrong source of nitrogen (Tank A, Bun II, first "tip-top" experiment), aeration (Tank A; c.f. Langwell patents), unfavorable environment (Bun XIII), or the lack of a sufficient number of the acid-fermenting anaerobes. Now and then a bottle or small tank that was not inoculated properly (see Inoculation below), would also go sour.

The accumulation of organic acids and their salts to the extent of 2,000 to 6,000 p.p.m., as acetic, overtaxes the buffer capacity of the medium. Increasing the buffer capacity by the addition of phosphate or carbonate seems to give no relief. In fact, it seems to aggravate the trouble. Liming to raise the pH has never met with success. In the Tank A investigation already discussed, phosphate, magnesium oxide, lime, potassium carbonate, and very dilute sodium hydroxide were all added at some time during the run but none of them gave permanent relief. Artificial pH regulation by chemicals has never been found necessary or advisable in any of the anaerobic fermentations investigated by the writers.

As far as the authors are aware all previous workers have used some alkaline buffer, usually CaCO_3 , in their fermentations. As noted in the historical, they all report the accumulation of acids in their methane fermentations. They also obtained much lower gas rates and yields than are herein reported. Langwell's patents, which deal specifically with the production of organic acids (acetic, butyric, and lactic) from cellulose and cellulosic materials, call for the addition of an alkaline buffer with aeration. He recovers small quantities of methane (0 to 8%) with large yields of CO_2 and organic acids.

Other examples, where the production and accumulation of organic acids are accelerated by neutralizing the free acids with alkalis, could be cited^(186, 377). In general, if the accumulation of acids and acid salts is desired, alkalis should be added. As the methane-producing organisms apparently work best in the absence of high concentrations of organic acid salts, the addition of alkalis should not be practiced if one is endeavoring to produce methane as the main end product. Some experiments by the present writers tend' to indicate that the addition of acids (HCl , H_3PO_4 and H_2SO_4) in sufficient amounts to change practically all organic acid salts to the free acids, produces better gasification of these substances. These results, however, may be due to the chemical changes brought about by the addition of the acids.

If the acid concentration can be kept below 2,000-3,000 p.p.m. (2.0-3.0 gms. per liter) the fermentation proceeds with ease, provided the acid-fermenting organisms are present and the buffer capacity of the medium is sufficient to maintain the pH above 6.5^(18, 327). The present

authors have been able to ferment milk wastes and accumulated acids to CO_2 and CH_4 where the total organic acid content at times was as high as 10,000-13,000 p.p.m. The pH in these cases, however, was 7.4 to 8.4⁽⁴³⁾. The continuous gasification of these wastes was more rapid and reliable, however, when the tanks were operated at a lower organic acid salt concentration. The fermentation of cellulose and crude plant residues proceeds most readily under conditions wherein the organic acids formed are not allowed to accumulate but are fermented to gaseous end-products as fast or about as fast as formed.

As stated previously, the authors have found the volatile acid test to be a good indicator as to the status of the digestion. If the acids accumulate to a concentration that has been found dangerous for the pursuance of the desired fermentation, then the operator should reduce the rate of feeding to such an extent that the bacteria will call upon the acids for food material and thereby reduce their concentration. In exceptional cases, it may also be advisable to withdraw part of the mother liquor and refill the bottle or tank with tap water or sewage. This practice dilutes the organic acids present, adds buffer, and as a rule, if the digestion is carefully handled following such a period, normal fermentation and gas production will be reestablished. If acid conditions prevail for too long a time it may be necessary to reinoculate with a medium known to contain the desired flora.

Three other factors besides the addition of alkalies have been found to lead to the accumulation of acids. These are aeration, excessive rates of feeding, and insufficient nitrogen. Any one or combination of these things seems to alter the normal state of fermentation to such an extent that the desired relationship between the various organisms responsible for the production of CO_2 and CH_4 is unbalanced. These various factors will be considered separately a little later in this bulletin.

It is somewhat difficult to establish the desired CO_2 - CH_4 fermentation when feeding starches or sugars or materials containing high percentages of these constituents. These materials are quickly converted to organic acids. If, however, the inoculum or mother solution has been developed to handle such substances and if the materials are fed slowly at the start and then the feed gradually increased, no difficulties should be encountered. As noted in experiments already presented, starch and cracked corn have been fed and successfully fermented to CO_2 and CH_4 at rates of 0.7 grams and 1.89 grams per day per liter of tank capacity, respectively.

Unless started properly, starches and sugars not only cause the accumulation of excessive concentrations of organic acids, but with most pure and mixed cultures, they give mainly CO_2 and H_2 and not CO_2 and CH_4 . In one such case in 35 days the writers obtained only 22.7 per cent of the starch added as gas (49% CO_2 , 37.2% H_2 , 10% CH_4 and 3.8% N_2) while 63 per cent was recovered as organic acids. The pH at the end of the run was 5.4.

The anaerobic digestion of organic acid wastes, such as alcohol beer-slop and citrus pulp, present interesting problems. Citrus pulp contains large amounts of organic acids (pH 4.0) and other acid-producing materials in proportion to the small amount of crude fiber present

(carbohydrates 6.88%, crude fiber 0.62%, moisture 91%). The total acidity of the citrus pulp studied was not determined but undoubtedly it was very high. That of one certain alcohol beer-slop has been found to be equivalent to 15 cc. of normal alkali per liter. The pH averaged about 5 and the volatile organic acids about 2,000 p.p.m. One acetone-butanol beer-slop investigated had a total volatile acid content of 1,400 p.p.m. and a pH of 5.2. This distillery slop fermented readily⁽²⁴⁾. The citrus pulp and alcohol beer-slop, however, have been found to be more difficult to handle. They can be fermented, but the maximum feeding rates are much lower (Ex. Heinz beer-slop, maximum rate of 2.6 gms. dry solids per day per liter of tank capacity as compared with 5.2 gms. for Commercial Solvent, acetone-butanol beer-slop). For a more complete practice in the anaerobic fermentation of fatty acids, the reader is referred to Neave and Buswell⁽²¹¹⁾ and Tarvin and Buswell⁽³³⁵⁾.

C. INOCULUM.

Sufficient and proper inoculation is necessary in any biological fermentation whether it be carried on by a pure or by a mixed culture. Previous workers have found it necessary to use appreciable quantities of sewage sludge, river slime, and similar substances to produce the desired methane fermentation of cellulose and related substances. This practice introduces the question as to the quantity and quality of gas that should be subtracted as coming from the organic matter in the inoculum. A control bottle containing only the inoculum gives a fair correction if the total solids are low, but if appreciable material is present, there is always the question as to whether the inoculum solids give the same quantity and quality of gas in the presence of the substrate being studied as they do in its absence. There are some who claim that inactive organic materials may again become active and produce additional gas if associated with fresh actively fermenting materials⁽²⁶³⁾. The question is important in batch experiments such as those carried out by certain of the earlier workers in which the volatile matter as inoculum amounted to as much as 30 per cent by weight of the amount of cellulose added. In feeding experiments, such as those described herein, the amount of inoculum solids becomes insignificant in comparison with the total amount of cellulose fed.

At first, in order to start their fermentations, the present investigators used, per liter, about 80 to 100 cc. of digested sludge with about 900 cc. of sewage or overflow liquor from an anaerobic sewage disposal tank. These inocula contained about 2.8 grams of volatile (organic) matter per liter. Later, by the use of overflow liquor and water or settled sewage, the authors were able to reduce this to 1.0 to 1.5 grams per liter provided only a small amount (2 to 5 gms./liter) of cellulose or cellulosic material was introduced at the start. Settled or raw sewage by itself was found unsatisfactory as an inoculum and mother liquor. Furthermore, it contains insufficient nitrogen. A piece of the actively digesting material with some of the liquor from a bottle or tank that was producing methane from this material was also found to be a good inoculum for small bottle or tank experiments. One part of cornstalk

residue removed from a digester was found to be sufficient inoculum for an original feed of 5 parts of fresh cornstalks.

D. NITROGEN REQUIREMENTS.

This laboratory has found ammonia nitrogen in the form of $(\text{NH}_4)_2\text{CO}_3$, NH_4Cl , or NH_4OH to be a suitable source of nitrogen for the anaerobes carrying out the methane fermentation of the mono-, di-, and polysaccharides as well as closely related compounds and fatty acids. Symons and Buswell⁽³³²⁾ have found that approximately 7 milligrams of ammonia nitrogen are required for each gram of substrate (carbohydrates and related compounds) decomposed. Most of this ammonia nitrogen may be recovered as organic nitrogen in the humus-like matter (possibly dead bacterial protoplasm) deposited in the fermentation vessel⁽³²¹⁾. The present writers have noted that no additional ammonia nitrogen need be added, if such liquors as overflow liquors from an anaerobic sewage tank, which contain in the order of 250 p.p.m. of ammonia nitrogen, or a portion of this liquor diluted with one quart of water is used as the original inoculum and mother liquor.

In one batch investigation (Run XVI) on the digestion of cornstalks which was run for 600 days without the addition of new materials, the final ammonia nitrogen concentration of the liquors in the various bottles ran from 147 to 159 p.p.m. as compared with 150 p.p.m. when originally started.

The nitrogen seemingly passes through a characteristic anaerobic cycle, for after being used by the organisms, it again reappears as ammonia nitrogen, due undoubtedly to lysis and decomposition of the dead bacteria (See Table LXII).

In continuous feeding studies on cornstalks, straw manures, sewage screenings, and other materials of this nature, one need not add ammonia nitrogen to the digester if the material being added contains sufficient ammonia or total nitrogen to maintain the ammonia nitrogen content at or above 100 p.p.m. As the materials mentioned above contain 0.7 per cent or greater of total nitrogen, no ammonia need be added with these materials except that necessary to bring the original mother liquor up to the 100 p.p.m. level, or to replace that withdrawn in liquors during drawdowns. The protein material present in these cellulosic materials readily decomposes to ammonia and fatty acids. The ammonia may be utilized by the bacteria. The fatty acids, if not present in too high a concentration, are further decomposed to CO_2 and CH_4 . Commercial peptone gives 563 to 653 cc. of gas per gram of material⁽²⁶⁾.

The effect of insufficient nitrogen in the mother solution on the rate of fermentation of cellulose and cellulosic materials has been noted repeatedly by the present writers. Some data have already been given. Those in Table LXI also show the effect of insufficient nitrogen. In their continuous feeding investigation, the temperature, low rate of feeding, and other variables were kept as constant as possible. The tank was operated for some time at a low ammonium nitrogen level, then ammonium chloride was added to increase the concentration of the ammonium ion. As noted in Table LXI during the 18-day period

immediately prior to the time ammonium chloride was added, the ammonia nitrogen concentration averaged 15.1 p.p.m. During this period an average of only 6.8 cubic feet of gas was produced daily, while during the 51 days immediately following, when the ammonia nitrogen concentration averaged 59.0 p.p.m., the gas production averaged 34.2 cubic feet per day. The greater daily gas volumes were produced while the ammonia nitrogen concentration was at or above 100 p.p.m.

The fact that little or no ammonia or organic nitrogen is lost from the digestion liquor as gaseous N_2 has already been referred to in this bulletin. The nitrogen data given in Table LXII will serve to illustrate. The gaseous nitrogen which is collected in the gases (about 1%) gets into the system as gaseous N_2 through the liquors and materials fed. Some may be admitted while collecting and analyzing the gases generated during fermentation.

TABLE LXI.
EFFECT OF AMMONIA NITROGEN CONCENTRATION ON THE RATE OF GASIFICATION OF CORNSTALKS.

(All other variables kept as near constant as possible.)

Volume of digestion tank, cu. ft.	140
Cornstalks fed per day, dry weight.....	6 to 10

During an 18-day period, low NH_3-N content.

	Maximum.	Minimum.	Average.
Ammonia nitrogen, p. p. m.	25.0	4.2	15.1
Volatile acids as acetic, p. p. m.	494.	275.	377.
Gas per day, cu. ft.	5.9	3.5	6.8

During a 51-day period, NH_3-N content regulated.

	Maximum.	Minimum.	Average.
Ammonia nitrogen, p. p. m.	124.	24.	59.
Volatile acids as acetic, p. p. m.	364.	95.	228.
Gas per day, cu. ft.	68.	24.	34.2

TABLE LXII.
NITROGEN DATA.
Run 4.

Fermentation of—	Ammonia N. at start, p. p. m.	Total N. at start, p. p. m.	Ammonia N. at end, p. p. m.	Total N. at end.	Apparent loss in total nitrogen, mg.	Weight of N_2 in gas drawn, mg.
Cellulose.....	266	336	255	308	74.5	738
Toilet paper.....	266	338	260	337	0.0	438

E. PRETREATMENT.

The effect of soaking pure cellulose (filter paper) in hot or cold water or in lime for 1 to 5 days did not manifest itself in the rate or degree of gasification of this material. Some slight advantage due to shredding has already been reported in this bulletin.

The digestion of a piece of cornstalk, the cortex layer of which was unbroken and the pith of which was enclosed between two nodes, was found to be extremely slow. However, if the cortex layer of a cornstalk is broken the pithy material is fermented away very rapidly. The completeness of the removal is noted in Figure 19. It has, therefore, been found advisable to put all cornstalks through an ensilage cutter or a shredder of some kind before submitting them to anaerobic fermentation. Additional treatment in a mill or pulverizer has been shown to produce but very little more gas (maximum of 5%) per unit weight in any given time.

The bacteria attack the cortex but the rate of gas production from this material is much less than that from the more open structured pith. The data given in Table LXIII will serve to illustrate.

TABLE LXIII.
COMPARATIVE RATES OF FERMENTATION OF CORNSTALK CORTEX AND PITH.

Time in days.	Total volume of gas per gram of material added.		
	Pith and vascular bundles, cc.	Cortex, cc.	Entire cornstalk, shredded, cc.
9	124	55	
45	401	238	
89	544	331	
13	262	200	205
24	426	320	348

Exogenous plant tissue has been found to ferment much more rapidly if reduced to the condition of excelsior, as used for packing, or to wood, flour, or mechanical pulp.

A short treatment (5 to 30 minutes) of shredded or chopped cornstalks in water to liberate the entrapped air has always been felt worth while. After a tank has been fermenting normally for some time one can omit this pretreatment if desired. If the fermentation has a tendency to turn sour then liberation of the entrapped oxygen (air) is advisable. Eference to the reactions given earlier in this bulletin and to part F, "Oxygen" will explain the reasons for this treatment.

Although certain other physical and some chemical treatments have been investigated in a preliminary manner, and although certain ones seem to give more gas per unit weight of material, their employment does not seem feasible, due to the extra time, tank capacity, and equipment

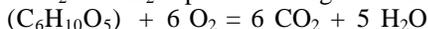
that would be necessary. These preliminary studies, unless otherwise stated, were all conducted under atmospheric pressure and at room temperatures. They have been summarized in Table LXIV.

TABLE LXIV.
EFFECT OF PRETREATMENT ON GASIFICATION OF SHREDDED CORNSTALKS.

Pretreatment.	Time of fermentation, days.	Volume of gas produced per gram of cornstalks added, cc.
None.....	25	87
1 hour H ₂ O boil.....	25	183
2 day H ₂ O soak.....	25	92
2 day lime soak; neutralized.....	25	173
None.....	109	367
1 hour H ₂ O boil.....	109	412
2 day H ₂ O soak.....	109	377
2 day lime soak; neutralized.....	109	411
None.....	52	286
2 hour H ₂ O boil.....	52	382
4 day H ₂ O soak.....	52	358
4 day lime soak; neutralized.....	52	575
None.....	65	264
1 hour H ₂ O boil.....	65	394
1 hour 2% H ₂ SO ₄ reflux.....	65	303

F. OXYGEN.

The introduction of air (oxygen) into a tank or bottle producing CO₂ and CH₄ from cellulose or related substances, increases the CO₂ content of gas and the quantity of organic acids present in the mother liquor. This was found to be the case in the Tank A investigation on pure cellulose already discussed, as well as in certain pilot unit crude-fibre fermentations already discussed. The Langwell and other similar patents which deal with the facultative fermentation of cellulose and cellulosic materials with the formation of acetic, lactic, and butyric acids and large quantities of CO₂, practically always describe methods of introducing air into the fermentation vessel. The direct biological oxidation of cellulose to CO₂ and H₂O produces large volumes of this gas.



The anaerobic or facultative fermentation of cellulose (C₆H₁₀O₅) to butyric acid (C₄H₈O₂), which is accelerated by small amounts of air, must be accompanied by the direct or indirect liberation of some highly oxygenated product such as CO₂ because the substrate cellulose, or its hydrolytic product, glucose, is more highly oxygenated than butyric acid. Acetic (C₂H₄O₂) and lactic (C₃H₆O₃) acids, two of the other products found in such fermentations, are equally as oxygenated as glucose (C₆H₁₂O₆). The other two products, ethyl alcohol (C₂H₆O) and hydrogen (H₂), like butyric acid, are less highly oxygenated, hence their formation should also lead to the production of CO₂. No highly oxygenated

product other than CO₂ has been isolated or proposed as a product of these cellulosic fermentations..

Therefore, the introduction of air (oxygen) into anaerobic cellulose fermentations directly, as well as indirectly, increases the CO₂ content of the gas and causes the accumulation of higher concentrations of organic acids. For these reasons it has been found best to keep as much air out of the cellulose and crude fibre fermentations as is possible. The experimental proof that the introduction of air and the accumulation of organic acids is always accompanied by an increase in the CO₂ content of the gases formed, has been noted repeatedly.

G. HYDROGEN.

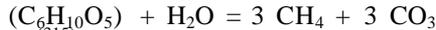
A number of the earlier workers thought that the immediate precursors of the CH₄ recovered in anaerobic fermentations might be H₂ and CO₂. If cellulose fermented to give two volumes of H₂ with every volume of CO₂, such as,



then the hydrogen biologically reacted with one-half the carbon dioxide, such as

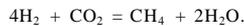


the resultant over-all reaction would be the same as written previously, namely,



In 1906, Sö hngen⁽³¹⁵⁾, using a formate culture, was able to recover 285 cc. of CH₄ as the result of the union of 1,191 cc. of H₂ with 300 cc. of CO₂. Lieske^(77, 78) and co-workers have more recently rediscovered the reaction. In order to further investigate these possibilities the writers arranged an apparatus so that hydrogen could be slowly introduced

TABLE LXV.
FORMATION OF METHANE FROM CARBON DIOXIDE AND HYDROGEN.



	Prelim. period. No H ₂ added.	Second period. Rubber connections.	Third period. All glass apparatus.
Digestion volume, liters.....	2.6	2.6	2.6
Time, days.....	4	44	150
Cellulose fed, grams.....	7	15	15
H ₂ fed, impure, S. T. P., liters.....	0		7.200
H ₂ fed, pure, S. T. P., liters.....	0	2.016	6.920
Gas recovered, S. T. P.:			
CO ₂ , liters.....	0.855	5.568	6.447
CH ₄ , liters.....	0.820	6.925	9.849
H ₂ , liters.....	0.020	0.381	0.139
CO ₂ reduced to CH ₄ , liters ($\frac{CH_4 - CO_2}{2} = A$).....		0.679	1.701
Theoretical H ₂ required, liters (A x 4).....		2.714	6.804
Actual H ₂ utilized, liters (Fed - recovered as H ₂).....		1.635	6.781

^a Original inoculum: 1.6 liters of thin cellulose fermenting culture plus 1.0 liter of overflow liquor from sewage tank.

^b Corrected for gain of 1,100 cc. per liter in dissolved CO₂.

into a culture which was fermenting cellulose to a 1:1 ratio of CO_2 and CH_4 . During the preliminary period (see Table LXV) no H_2 was added. When it was assured that the normal 1:1 gas ratio was being formed, hydrogen was added slowly through a porous crucible located at the bottom of the fermentation bottle. The first 44-day run, during which time 2.016 liters of H_2 were added to the 2.6 liter bottle, showed a greater recovery of CH_4 than could be accounted for by the loss in H_2 . This may have been due to a number of biological reasons or the fact that CO_2 was lost through the rubber connections. To overcome this latter possibility an all-glass apparatus was constructed. During 150 days operation of this apparatus there were fed 15 grams of cellulose and 7.2 liters of H_2 . From this was recovered 6.5 liters of CO_2 (corrected for gain in dissolved CO_2 in liquor) and 9.8 liters of CH_4 . To account for this additional volume of CH_4 , in accordance with the reactions given above, there should have been consumed 6.804 liters of H_2 . The actual disappearance of molecular H_2 was 6.781 liters. The data are all summarized in Table LXV.

These data further substantiate the belief that the immediate precursors of CH_4 could be CO_2 and H_2 . They also indicate a possible means of increasing the CH_4 content and hence the B.t.u. of gas formed in the anaerobic digestion of such materials as cellulose, crude fibre, etc.

H. MIXING.

In all of the earlier bottle experiments rubber connections were avoided because it was thought that over a period of time CH_4 or CO_2 might diffuse through the side walls of the tubing. No definite data, however, were collected to show that this was the case. With rigid construction it was impossible to mix the contents of the bottle. Later, mercury-sealed stirrers were inserted to break up the thick cellulose mat which collected at the top of the bottles or tanks, which aided materially in keeping the cellulose wetted, the gases liberated, and the fermentation proceeding at a uniform rate.

Following the above, bottles were fitted with rubber connections in order that the contents might be mixed and the mat broken at will. These, in turn, led to the "tip-top" set up shown in Figure 12. Mere inversion of the "tip-top" bottle, with the closing and opening of the proper pinch cocks, would break up the mat and release the entrapped gas bubbles. This type of apparatus was used in all the later studies and was found the most satisfactory set up for all laboratory investigations on fibrous materials. This apparatus served as the basis for the development of the special drum type digester advised for the large scale fermentation of cellulosic materials. By the use of this "tip-top" principle of mixing the bottle contents and releasing the entrapped gas, greater feeding capacities and gas production yield were noted.

The employment of motor-driven propellers placed in the floating fibrous material and the use of hydraulic mixing carried on by circulation of tank liquor drawn from below the floating mat and discharged over and into this mat were both found to be inadequate. Neither method would break up the floating mat.

I. RATE OF FEEDING AND CHEMICAL CONTROL.

The rate at which cellulose or fibrous plant materials can be fed and digested to CO_2 and CH_4 depends on many variables such as apparatus used, ammonia content, pH, volatile acid salt concentration, degree of anaerobiosis, etc. Granted that an apparatus such as the "tip-top" bottle set up or the drum digester is used and that the cellulose mat is kept broken up; granted also that the proper flora has been established and the ammonia nitrogen is between 100 and 500 p.p.m., the pH near neutrality, and the volatile acid content not over 1,000 p.p.m. as acetic, then cellulose by itself can be fed and digested at rates of from 0.75 to 1.25 grams dry weight per day per liter of tank capacity (0.047 to 0.078 lbs. per cu. ft.). At such rates of feeding the recovery has been from 0.5 to 1.15 liters of gas per day per liter of tank capacity. At slower rates of feedings greater yields are received per gram of cellulose fed but lesser volumes per tank volume per day. No definite rate of feeding can be established for cellulosic or crude plant materials because they differ so widely in physical structure and rate of fermentation (See Table LVIII). In the laboratory, cornstalks have been fed continuously at rates of 4 to 6 grams per day per liter of tank capacity (0.25 to 0.36 lbs. per cu. ft.). Pilot unit Tank B was fed cornstalks for 7 months at an average rate of 0.08 pounds per cubic foot of tank capacity. The greatest rate for any one month was 0.12 pounds per cubic foot.

Excessive rates of feeding, that is, rates somewhat above the maximum given for cellulose and cornstalks, bring about the gradual accumulation of organic acids, which in time overtaxes the buffer capacity of the medium which causes the pH to drop. For every industrial or agriculture waste studied, there seems to be a certain limit at which the fresh material can be fed⁽³⁷⁾. If this limit is exceeded for a short time, or if for some unknown reason the normal state of fermentation becomes unbalanced and organic acids start to collect, the authors have found that reduction of the amount of material being fed per day usually brings about the reestablishment of the desired conditions. If the acids continue to collect, the feeding should be stopped, and in exceptional cases, it has been found advisable to reinoculate the tank with material known to be undergoing the desired fermentation. Circulation of acid liquors from one tank into a second tank that is in good condition, with the return of the overflow from this second tank back into the first tank, has also been found helpful, provided the resultant dilution of the acids in the two or more tanks brings the concentration below the desired concentration, which is usually about 1,000 p.p.m. as acetic.

Treatment of batch and continuous-feeding cellulose and crude fibre fermentations with phosphate, lime, and other alkalies, cystine, ammonium sulfide, or hydroquinone have never been found to produce more effective or more rapid digestion. The importance of ammonia nitrogen control has already been considered.

V.
INDUSTRIAL WASTES.
ANAEROBIC FERMENTATION OF CITRUS PULP.

C. S. BORUFF.

The following preliminary investigation was made at the request of W. E. Baier of the research department of the California Fruit Growers Exchange, 616 East Grove Street, Ontario, California. This organization is interested in disposing of large quantities of citrus pulp waste in such a way as to render such disposal cheap and non-offensive.

Upon receipt of a five-gallon keg of sterile citrus pulp from Mr. Baier, bottles were set up to determine the anaerobic fermenting characteristics of the waste. Sixty-nine, 138, and 276 grams of the wet pulp were placed, respectively, in three 3,660 cc. dark glass bottles. To this undried pulp was added inoculum solution until the bottles were completely filled. Two of the bottles were also fitted with glass stoppered observation tubes so that pH determinations could be made at will without exposing the entire bottle to the air. The inoculum solution was composed of: 500 cc. distilled water, 100 cc. digested sludge, 3,000 cc. settled sewage, 50 cc. saturated lime water, 2 grams of CaCO_3 , and 0.5 gram of Na_2HPO_4 .

A partial analysis of the citrus pulp as furnished by Mr. Baier is as follows:

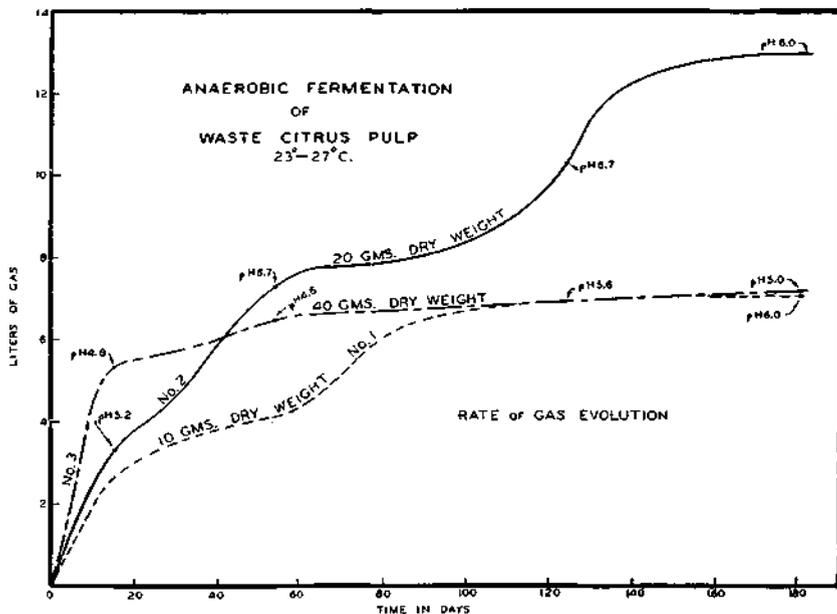


FIGURE 21

pH	4.0
Ash	0.34 per cent
Moisture	90.95 per cent

Further analysis of the pulp as received at Urbana gave the following results:

	Per cent	
Moisture	85.50	
Ash	0.46	
Ether extract	0.28	0.38
Crude fiber.....	0.62	0.84
Protein	0.95	1.28
Carbohydrates.....	6.86	9.38
Organic nitrogen	0.197	
Kjeldahl nitrogen	0.198	
Total nitrogen (salicylic)	0.208	
(All of above data are on a wet basis)		

GAS EVOLVED.

The rate of gas evolution may be noted from Figure 21. This figure also shows the pH readings as made at certain times during the investigation. A few cc. (5-10) of saturated lime water was added each time the pH was found to be below 6.5. The gas evolved during the digestion was not of a constant composition. At first it ran high in carbon dioxide (CO₂), but soon CO₂-H₂ fermentation gave way to the CO₂-CH₄ type. This change can be noted in the few gas analyses tabulated in Table LXVI. The gas always possessed a noticeable hydrogen sulfide odor.

TABLE LXVI.
REPRESENTATIVE GAS ANALYSES.
(Per cent by volume.)
Bottle No. 2 (20 grams pulp. Dry basis).

Components.	3rd day.	35th day.	152nd day.
CO ₂	53.8	39.4	25.6
H ₂	6.0	1.0	0.6
CH ₄	4.3	53.4	69.0
N ₂	5.9	5.8	13.8

Bottle No. 3 (40 grams pulp. Dry basis).

Components.	7th day.	152nd day.
CO ₂	77.3	44.6
H ₂	17.0	1.2
CH ₄	?	30.1
N ₂	3.0	14.1

* N₂ + CH₄ content totaled 3.0 per cent. Too small volume for differential analysis.

ODORS.

The gas as drawn always had an appreciable hydrogen sulfide (H₂S) and somewhat of a sour odor. The 5-10 cc. portions of liquor drawn

from the bottles for pH determinations, as well as bottle No. 3, when opened at the end of the experiment possessed the characteristic H_2S odor along with a more pronounced volatile acid odor which was similar to, if not identical with that of butyric acid.

VOLATILE ACIDS.

It may be drawn from the low pH values recorded during the fermentation, that at times high volatile acid concentrations were present in the digestion mixture. At the close of the experiment, however, bottles No. 1 and No. 2 possessed pH values of 6.0 and contained only 90 p.p.m. of volatile acid calculated as acetic. Bottle No. 3, however, contained 4,880 p.p.m. and possessed a pH of 5.0. It is noted in Figure 21 that the pH of the liquor of bottle No. 3 was down to 4.8 when the break in rate of gas evolution occurred. Prior to this break it had been producing on an average of 10.4 cc. of gas per gram of material per day.

SUMMARY.

A summary of the data is found in Table LXVII. These data, however, do not represent the optimum of conditions, and hence do not represent the maximum speed or degree of fermentation. It is known that acid conditions as found throughout this preliminary investigation greatly hinder digestion. If such acid production was taken care of and the digestion mixture kept stirred it is quite reasonable to expect that much more rapid and much more thorough digestion could be expected. The gases given off in the fermentation are of fuel value in that they run on an average from 50 to 60 per cent methane (CH_4). Anaerobic digestion as a means of citrus pulp disposal seems promising in that the gas collected in bottles No. 1 and No. 2 amounted in terms of weight to 84 and 78 per cent, respectively, of the total weight of the pulp added.

TABLE LXVII.

SUMMARY.

Bottle number.	1	2	3
Time, days.....	180	180	180
Weight of pulp added:			
(a). Wet (grams).....	69	138	273
(b). Dry (grams).....	10	20	40
Volume of gas evolved (cc.) N. T. P.....	7,045	12,955	7,105
Volatile acids as acetic at end (p. p. m.).....	90	90	4,880
pH at end.....	6.0	6.0	5.0

In order to further test out the possibility of commercially disposing of large volumes of citrus pulp by means of anaerobic fermentation the investigator advises a laboratory continuous feeding experiment, so arranged that the pulp may be fed daily and the inactive residue drawn off as seen fit. Such apparatus should be equipped with

a good mechanical or hydraulic stirrer and close chemical supervision should be given it.

THE ANAEROBIC FERMENTATION OF EXTRACTED JERUSALEM ARTICHOKE AND SUGAR BEET WASTES*

The danger of an invasion of Illinois by the European corn borer led to studies on the suitability of Jerusalem artichoke as a substitute crop. Since the use of this crop as a raw material in the manufacture of sugar would result in a waste of a rather offensive nature it seemed advisable to study the waste disposal problem. Sugar beet wastes and chicory wastes are similar in character and were studied at the same time.

A. GENERAL METHOD OF ATTACK.

The several preliminary studies made on the anaerobic fermentation of extracted Jerusalem artichoke, chicory, and sugar beet wastes were carried out in dark glass bottles. The bottles were filled with the digestion mixtures and connected to gas collectors which were filled with a saturated solution of salt. Other investigations were carried out in 2 liter bottles and still others in tanks of 6 to 7 liter capacity. In practically all investigations, analyses were made of all inoculum liquors. These inoculum liquors were usually obtained from an anaerobic sewage disposal plant as these liquors were known to contain large numbers of anaerobic bacteria and nitrogen of a suitable type. Analyses were also made of all liquors and sludges withdrawn during the period of the investigation.

B. THE FERMENTATION OF JERUSALEM ARTICHOKE.

Batch Experiments. The first studies on the digestion of extracted Jerusalem artichoke waste showed that 50 to 60 per cent of the dry weight of the material added could be recovered as gas in 63 days. These preliminary fermentations showed a marked tendency to become sour (pH 4 to 5) due to the accumulation of volatile organic acids. The gas formed contained from 55 to 60 per cent methane. These preliminary experiments were batch fermentations, that is, they were made by charging a 1-liter bottle with 10 grams (dry weight) or more of the extracted material and a suitable inoculum. Overflow liquor from a sewage disposal plant was used in this case.

As an additional preliminary investigation, each of three 1-liter bottles was filled with 5.87 grams (dry weight) of fresh artichoke waste and a suitable inoculum. Control bottles were used to correct the total gas volumes obtained from the bottles containing the artichoke waste and the respective inoculum. The volatile acid content of the mother liquor in the bottles was 214 parts per million and at the end of the experiment was only 21, 42, and 148 parts per million (as acetic) respectively, while the pH values were 7.4, 7.6, and 7.6 respectively. The data from these experiments show that in 18 days 82 to 87 per cent of the waste was recovered as a gas containing about 50 per cent methane.

* This work was done by G. B. Abbott and the results are taken from his B.A. Thesis, U. of Ill., 1933. The work was done under the direction of C. S. Boruff.

Continuous Feeding Experiments. These experiments were carried out in a tank of 7 liters capacity. The first tank, K₁, was started by using 7 liters of overflow liquor from an anaerobic sewage disposal tank, 3 grams of calcium carbonate, and 100 grams (wet weight) of extracted waste. The tank was operated for 22 days but gasification never reached that noticed in the preliminary bottle experiments. The volatile acids remained high from the start and the pH low. The construction of the fermentation tank did not permit circulation or stirring.

A second tank, K₂, was started in the same manner as K₁, except that no calcium carbonate was used. The contents of the tank were mixed periodically by shaking. The data collected during the 70-day duration of the experiment are summarized in Table LXVIII. During the second 20-day period, good rates of gasification were obtained. Upon continued operation, however, the volatile acids increased to around 1,500 parts per million and hence it was necessary to decrease the rate of feeding. The per cent gasification (weight of gas X 100 divided by weight of volatile matter fed) during this last 30-day period was high, namely, 116 per cent, but the average volume of gas obtained per day was only 2.73 liters as compared with 7.3 liters for the second 20-day period.

The third continuous feeding experiment, K₃, was started by adding 7 liters of a mixture of overflow liquor and sludge from a sewage disposal tank. This liquor contained 2.2 per cent total solids, 44.0 per cent of which was volatile. The pH of this liquor was 7.7, the volatile acids were 755 parts per million, the ammonia nitrogen 318 parts per million, and the total nitrogen 690 parts per million. To this tank was also added 100 grains of extracted Jerusalem artichoke waste (15.28 grams dry weight) and 3 grams of calcium carbonate. The data for this 100-day experimental run are summarized in Table LXIX. The gas production varied materially from day to day. The average for the 100-day investigation was 5.57 liters per day. The per cent gasification, that is, the weight of gas times 100 divided by the total volatile matter

TABLE LXVIII.
THE ANAEROBIC FERMENTATION OF JERUSALEM ARTICHOKE WASTE.
(Continuous Feeding Experiment, Tank K2, 26°-29° C.)*

	1st 20 days.	2nd 20 days.	Last 30 days.	Total.
Materials fed:				
Total dry weight, grams.....	90.29	193.0	87.3	370.59
Total volatile matter, grams.....	86.25	186.0	84.7	356.95
Average dry weight fed per day, grams.....	4.51	9.65	4.36	5.40
Total gas drawn, liters.....	54.33	145.90	82.01	282.24
Weight of gas drawn, grams.....	65.2	175.08	98.41	338.69
Per cent gasification.....	75.6	94.1	115.5	94.88
Greatest volume of gas for any one day, liters.....	5.3	17.2	11.0	17.2
Average volume of gas per day, liters.....	2.72	7.30	2.73	4.03
Average gas analysis:				
CO ₂	44.42	42.16	43.33
H ₂30	1.0056
CH ₄	51.44	53.76	52.31
N ₂	3.40	3.40	3.40
Air.....	.60	.6563

* Capacity of tank 7 liters.

TABLE LXVIII—Concluded.

At the end of 18 days was unable to obtain a new supply of waste when it was needed. Likewise, at the end of 40 days could obtain no waste. Tank was unable to respond after period of starvation.

TABLE LXIX.

ANAEROBIC FERMENTATION OF EXTRACTED JERUSALEM ARTICHOKES.

(Continuous Feeding Experiment, Tank K₃, 26°-28° C.*)

	1st 20 days.	2nd 20 days.	3rd 20 days.	4th 20 days.	5th 20 days.	Total 100 days.
Waste fed:						
Total dry weight, grams.....	208.7	483.05	158.93	121.76	105.56	1,078.0
Total volatile matter, grams.....	203.16	461.88	153.12	116.80	101.20	1,036.16
Average dry weight fed per day, grams.....	10.43	24.15	7.95	6.09	5.28	10.78
Sludge drawn:						
Liters.....		2.	5.	2.	2.	11.
Volatile matter, grams.....		44.8	145.58	36.6	54.0	280.98
Oxygen consumed p. p. m. per per cent of volatile matter.....			2,400.		3,000.	
5-day B. O. D., p. p. m. per per cent volatile matter.....			2,200.			
Gas data:						
Total gas, liters.....	133.60	218.95	90.80	54.00	59.72	557.07
Weight of gas, grams.....	157.2	271.8	109.6	61.6	70.7	676.9
Average volume per day, liters.....	6.88	10.95	4.54	2.70	2.99	5.57
Average volume per day per liter of tank volume, liters.....	1.0	1.6	0.7	0.4	0.4	0.8
Greatest volume for any one day, liters.....	11.4	21.5	7.8	6.8	10.03	21.5
Per cent gasification.....	77.	59.	72.	53.	70.	65.
Average gas analysis:						
CO ₂	43.9	44.9	40.7	35.0	39.2	41.5
H ₂	0.3	0.2	0.1	0.2	0.2	0.2
CH ₄	50.7	49.8	58.8	63.2	57.9	54.3
N ₂	5.1	5.1	2.4	1.6	2.7	4.0

* Volume of tank 7 liters.

fed, varied from 55.5 to 78.9. The average for the 100-day period was 64.5 per cent. The gasification of the organic matter found in sewage under similar conditions and periods of digestion, averages about 50 per cent, in cornstalks about 35 per cent, and in other cellulosic wastes from 30 to 60 per cent, depending upon the character of the material. The gasification obtained in this artichoke fermentation represents the removal of most of the readily fermentable material.

The oxygen consumed from KMnO₄ per per cent of volatile matter was found at one time to be 2,400 parts per million and at another 3,000 parts per million. The 5-day biochemical oxygen demand of a similar sludge drawn during the third 20-day period was 4,400 parts per million or 2,200 parts per million per per cent volatile matter. These data compare favorably with other stable (non-putrescible) wastes. This sludge could be lagooned, drawn to waste, or dried on sludge beds and hauled away. The 5-day biochemical oxygen demand of the liquor drawn from this digestion ranged from 100 to 500 parts per million. In this experiment the liquor was drawn for analytical purposes only. On the basis that the material fed would contain 15 per cent solids by weight and that 65 per cent of this would be converted to gas there should remain a sludge of about 6 per cent solids. Since the solids in the sludge ran from 2 to 3 per cent (probably 4 to 6 per cent in

a large scale plant), there was and should be no need for withdrawing liquor from the tank; in fact the addition of water should be and was found necessary in order to maintain the proper operating volume.

On the basis of the above data, namely, that a 7-liter fermentation tank can be fed at the rate of 11 to 24 grams (dry weight) of press cake per day, it would require a tank of from 3,500 to 7,630 gallon capacity (467 to 1,020 cu. ft.) to handle 100 pounds of this dry extracted Jerusalem artichoke waste. On the basis of 65 per cent gasification there could be recovered from this 100 pounds of material about 835 cubic feet of gas. At 50 cents per cubic foot such a tank should cost from \$233.00 to \$510.00 which at 12 per cent for interest, amortization, repairs, etc., would give a net gas cost of 9.17 to 20.07 cents per thousand cubic feet.

Tank M₄ was started by adding 6 liters of overflow liquor and sludge from a sewage disposal tank and one liter of sludge from tank K₃. The total solids in this mixture was 1.4 per cent of which 52 per cent were volatile. The volatile acids calculated as acetic was 890 parts per million, the ammonium nitrogen 255 parts per million, and the total nitrogen 505 parts per million. The pH was 7.2. This tank was fed almost daily for 20 days in spite of the fact that the volatile acids were gradually increasing. The volatile acid content at the end of the 20 day period was about 1,500 parts per million. Each day during the first 20 days, 500 cc. were removed and this volume made up by adding tap water. Such dilution has been found of material aid in other fermentations but it seemed to be of little value in this case. The average volume of gas drawn per day for the 20-day period was 5.22 liters. The per cent gasification was 52.5. The methane content of the gas averaged 52.1 per cent.

In order to determine the effect of stirring or agitation upon the fermentation, a 2-liter bottle was equipped with rubber connections so that it might be mixed at will. The other objective in this experiment was to determine the maximum rate at which the fermentation might be run. The average volume of gas produced per day during the 65-period of the experiment varied from .98 liters during the first 20-day period to 2.01 during the third period. The average was 1.6 liters of gas per day from the 2-liter digestion bottle. The per cent gasification varied from 52.2 to 63.7. The average was 58.4 per cent.

The volatile acids varied greatly from day to day. On days when the volatile acids were high no fresh material was fed. This procedure when accompanied by frequent mixing seemed to overcome the souring difficulty noted in many of the earlier tank experiments. The pH in some cases dropped as low as 6.3. No CaCO₃ or phosphate salts were added at the beginning of this experiment. The bottle was kept from one-third to one-half full of digesting and digested material. From time to time as the amount increased in the bottle, sludge (residual material) was removed. By virtue of the fact that this was a small scale experiment it was very difficult to draw sludge without getting considerable liquor with it. The amount of sludge drawn during the first period of 20 days was 250 cc. This represented 5.5 grams of volatile organic matter. During the second 20-day period 550 cc. of sludge

was drawn and this contained 7.15 grams of volatile organic matter. In the last period of 25 days no sludge was drawn. The total amount of sludge drawn was 800 cc. and contained 12.65 grams of volatile organic matter.

On the basis of the above data, namely, that an average of 2.0 grains (dry weight) of waste can be added per day per liter of tank volume and that from this an average of .93 liters of gas can be recovered per day, it would require a tank of 6,000 gallons capacity (802 cu. ft.) to handle 100 pounds (dry weight) of this waste per day. From this there could be recovered 748 cubic feet of gas per day. This gas would have a B.t.u. of almost 480. At 50 cents per cubic foot and 12 per cent interest this gas would cost 17 cents per thousand cubic feet. These figures are much of the same magnitude as those calculated from the data collected from tank K₃.

Thermophilic Investigations. Two investigations were made at thermophilic temperatures (53° C). Both tanks (7 liters) were started by using thin liquors from thermophilic tanks that were actively digesting Commercial Solvents beer-slop waste at 53° C. One tank failed to start properly. The other tank P, started. The data are summarized in Table LXX. The average volume of gas produced per day varied from 3.86 to 8.77 liters. The average for the 80-day period was 5.57 liters per day. The per cent gasification varied from 53.0 to 87.1. The average for the period was 66 per cent. The average methane content of the gas for the 80-day period was 64.5 per cent.

On the basis of this experiment there would seem to be no advantage in a thermophilic (50° to 55° C.) over a mesophilic (25° -30° C.) fermentation for the gasification and stabilization of this waste.

TABLE LXX.
THERMOPHILIC FERMENTATION OF EXTRACTED JERUSALEM ARTICHOKEs.

(Continuous Feeding Experiment, Tank P, 52° -55° C.*)

	1st 20 days.	2nd 20 days.	3rd 20 days.	4th 20 days.	Total 80 days.
Materials fed:					
Total dry weight, grams.....	361.55	150.75	205.97	129.20	847.47
Total volatile matter, grams.....	346.20	144.60	197.60	124.10	813.57
Average dry weight fed per day, grams.....	18.08	7.50	10.29	6.46	10.59
Sludge drawn:					
Liters.....	2.50	2.00			4.50
Volatile matter, grams.....	46.22	37.04			83.26
Gas data:					
Total gas drawn, liters.....	175.53	104.88	87.19	78.35	445.95
Weight of gas drawn, grams.....	210.63	125.85	104.63	94.02	535.14
Average volume of gas per day, liters.....	8.77	5.24	4.35	3.86	5.57
Greatest volume for any one day, liters.....	16.40	12.40	10.05	6.03	16.40
Per cent gasification.....	60.90	87.10	53.00	75.70	66.00
Average gas analysis:					
CO ₂	38.8	39.8	37.7	36.4	38.2
O ₂	0.2				
H ₂	2.5	1.6	1.8	.2	1.5
CH ₄	54.5	53.0	53.0	58.5	54.7
N ₂	5.8	5.6	8.0	4.9	6.1
Air.....	0.8				

* Tank capacity 7 liters.

C. FERMENTATION OF CHICORY.

Batch Experiment. A preliminary batch experiment was made using extracted chicory waste. This waste fermented much more rapidly than did the Jerusalem artichoke waste and gave a much higher yield of gas. In 10 days, 9,620 cc. of gas had been recovered from 13.3 grams (dry weight) of waste fed. At the end of 35 days, 9,750 cc. of gas had been recovered which represented a recovery of 90 per cent of the original volatile matter as gas. The methane content of this gas was 66.2 per cent.

Continuous Feeding Experiments. A 2-liter bottle equipped with rubber connections to permit shaking was charged with 1,500 cc. of overflow liquor and 500 cc. of sludge from a sewage disposal tank. The data obtained during the 55-day experimental period are summarized in Table LXXI. It was noted that the waste could be fed at a greater rate than could the Jerusalem artichoke waste, namely, 2.6 grams of waste (dry weight) per liter of tank volume per day, as compared to 1.7 grams of Jerusalem artichoke waste (dry weight) per liter of tank volume per day.

On the basis of the above data, namely, that an average of 2.6 grams (dry weight) of waste can be fed per day per liter of tank volume and that from this an average of 1.63 liters of gas can be recovered per day, it would require a tank of 4,600 gallons capacity (615 cu. ft.) to handle 100 pounds (dry weight) of this waste per day. From this, there could be recovered 1,000 cubic feet of gas per day. This gas would have a B.t.u. value of 450 to 490. At 50 cents per cubic foot

TABLE LXXI.
ANAEROBIC FERMENTATION OF EXTRACTED CHICORY.
(Continuous Feeding Experiment, 26°-29° C.*)

	1st 20 days.	2nd 20 days.	Last 15 days.	Total 55 days.
Materials fed:				
Dry weight, grams.....	58.2	134.5	96.6	289.3
Volatile matter, grams.....	57.8	133.5	95.9	287.4
Average volatile matter fed per day, grams.....	2.9	6.7	4.8	5.2
Gas data:				
Total gas, liters.....	35.5	84.4	58.8	178.7
Weight of gas, grams.....	42.6	101.3	70.6	214.5
Average volume per day, liters.....	1.8	4.2	3.5	3.3
Average volume per day per tank volume, liters.....	.88	2.11	1.77	1.63
Greatest volume for any day, liters.....	3.95	9.43	5.64	9.43
Per cent of volatile matter fed recovered as gas.....	73.7	76.1	73.6	74.6
Average gas analysis:				
CO ₂	47.7	-----	-----	-----
H ₂	1.1	-----	-----	-----
CH ₄	49.5	-----	-----	-----
N ₂	1.7	-----	-----	-----
Air.....	1.2	-----	-----	-----

* Digester volume, 2 liters.

and 12 per cent interest, this gas would cost 10.1 cents per thousand cubic feet. These cost figures are much lower than those noted for the fermentation of Jerusalem artichoke wastes.

D. FERMENTATION OF SUGAR BEET WASTE.

Continuous Feeding Experiment. In this experiment, a 2-liter bottle was equipped so it could be shaken and was charged with an inoculum of overflow liquor and sludge from a sewage disposal tank. The data obtained from this experiment are summarized in Table LXXII. As noted from the table, the per cent recovery was high (77%) but the rate of gasification compared with that of Jerusalem artichoke waste was low (1.54 grams, dry weight, per day per liter of tank volume). Due to the fact that it was not possible to obtain as much of the waste as was needed, the experiment was stopped at the end of 30 days.

On the basis of the data obtained, it would require a tank of 9,450 gallons (1,264 cu. ft.) capacity to handle 100 pounds (dry weight) of the extracted sugar beet waste. From this could be recovered 1,237 cubic feet of fuel gas which would cost 16.8 cents per thousand cubic feet, if you figure the cost of constructing the tank at 50 cents per cubic foot. This was the figure that has been used in calculating all the cost data.

TABLE LXXII.
FERMENTATION OF SUGAR BEET WASTE.
(Length of run, 30 days, temperature 26°-29° C.*)

Materials fed:	
Dry weight, grams.....	89.
Volatile matter, grams.....	76.
Average volatile matter fed per day, grams.....	2.53
Gas data:	
Total gas, liters.....	48.82
Weight of gas, grams.....	58.58
Average volume per day, liters.....	1.95
Average volume per day per tank volume, liters.....	.98
Per cent of volatile matter fed recovered as gas.....	77.0
Average gas analysis:	
CO ₂	36.3
H ₂3
CH ₄	59.6
N ₂	3.8
Air.....	4.8

* Digester volume, 2 liters.

CONCLUSIONS.

On the basis of the data obtained from the studies here reported, it will be noted that a 1,000-gallon tank (133.7 cu. ft.) can be fed at the rate of 12.8 pounds (dry weight) of extracted Jerusalem artichoke waste per day. This would give 106.7 cubic feet of fuel gas at a cost of 16 to 18 cents per thousand cubic feet. For chicory, a 1,000-gallon tank (133.7 cu. ft.) could be fed at the rate of 21.65 pounds (dry weight) of extracted chicory per day. The cost of this gas would be 10.1 cents per 1,000 cubic feet. Likewise, sugar beet waste could be fed at the rate of 10.6 pounds (dry weight) of the extracted waste per 1,000 gallons of tank capacity. This would give 131.0 cubic feet of gas per day. The cost of producing this gas would be 16.8 cents per 1,000 cubic feet.

These experiments also show that there was no advantage in using a thermophilic fermentation. It was, also, shown that the procedure of diluting the fermentation mixture with water each day did not prevent the tank from going sour as has been the case in some studies on these fermentations. Since completing these studies, a recirculation procedure has been developed by Buswell and Boruff which seems to overcome the troubles noted in all digestions which tend to go sour.

ANAEROBIC STABILIZATION OF MILK WASTE*

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Mixed flora and an operating technic have been developed for the successful anaerobic fermentation and stabilization of milk wastes. Such treatment is more economical than present standard methods. It removes 95 per cent of the pollution load. Additional treatment on filters could be used if desired. From 8.3 to 12.4 cubic feet of gas of a B.t.u. of about 550 can be recovered at a moderate cost from each pound (dry weight) of waste milk solids. This volume and B.t.u. could be increased by carburetion.

The wastes from milk-bottling plants, creameries, and cheese factories have, in the past, been found troublesome and difficult to handle. These wastes become sour, very offensive, destroy the normal life in streams, and upset the operation of sewage treatment plants. This is due mainly to the fact that they contain a high percentage of lactose which is quickly attacked by bacteria. If these wastes are highly diluted with other sewage, they can be handled by regular sewage treatment methods. If, however, they constitute an appreciable part of the flow, they must be treated separately and preferably at the site of the milk plant.

Treatment by aerobic oxidation in trickling filters (3.5 to 6 feet deep) dosed at rates of from 500,000 to 2,000,000 gallons per acre per day⁽³⁸⁰⁾, sand filters dosed at about 50,000 gallons per acre per day⁽¹⁴⁷⁾, or lath filters dosed at rates of from 250,000 to 2,250,000 gallons per acre per day⁽¹⁷²⁾, have been found the most successful. In this connection it should also be stated that filters are efficient only when dilute solutions of the waste are being treated (0.05 to 1.0 per cent solids). It has also been found advisable to remove grease and settleable solids prior to dosing⁽¹⁷¹⁾.

Filter methods are expensive and no by-product of the treatment is recovered. One treatment plant described by Kimberly⁽¹⁴⁷⁾, which was composed of a 10-foot slag filter and a final settling tank, cost \$3,000, or \$116.80 per pound of solids treated daily. Another plant using an 8-foot sand filter cost \$1,500, or \$292 per pound of solids to be treated daily. Activated sludge treatment has not been found practicable^(71, 171).

Disposal of milk wastes by broad irrigation at rates of about 6,000 gallons per acre per day is utilized by some plants⁽¹⁰¹⁾. Such a practice usually produces odors unless prechlorination is used. Disposal by dilution, without causing a nuisance, is impossible for most plants. Eldridge⁽⁷¹⁾ states that 2,000 gallons of unpolluted water of a high oxygen content is necessary for each pound of milk waste (wet weight).

* Reprinted from Industrial and Engineering Chemistry. Vol. 24, Page 1423, December, 1932.

Chemical precipitation with an acid or alkali, alone or accompanied with a heavy metal salt, such as iron or aluminum, has been investigated by many^(71, 147, 323), including Buswell and Neave of this laboratory (unpublished data). Such a method is not practical because it leaves in solution the soluble solids (largely lactose) which constitute the major portion of the pollution load. There is also left the problem of disposal of the precipitated sludge.

Early studies in this and many other laboratories, as well as in large scale-plants, have shown that handling of milk wastes by regular anaerobic sewage digestion methods always results in failure, because the waste becomes sour very rapidly, a condition which, in turn stops normal digestion and stabilization of the solids^(71, 147, 171). If septic or Imhoff tanks are used at all, they are built so that the wastes have a detention time of only 24 to 72 hours. The effluents from such tanks, which act mainly as settling basins and reduce the pollution load but very little, are then treated on filters.

Whittier and Sherman⁽³⁸⁹⁾ have investigated the possible utilization of whey for the production of propionic acid and ketones. A continuous lactic acid fermentation of whey has also been successfully worked out by Whittier and Rogers⁽³⁸⁸⁾. Buswell and Neave, in some of their unpublished mixed culture studies, were able to recover liquors containing as much as 1.7 per cent volatile acids. The gas formed was composed of carbon dioxide and hydrogen.

The combined wastes from milk-collecting and -bottling stations usually contain less than 1 per cent solids⁽⁷¹⁾. The combined wastes from creameries and cheese factories seldom contain as much as 4 per cent solids⁽⁷¹⁾. In the latter cases, the buttermilk and whey wastes could be separated from the other factory wastes and treated in an undiluted state. Such segregation is practiced at plants which recover buttermilk for cattle feed purposes. Owing to its acid condition and poor food quality, but little whey is salvaged at present. Government figures show that there are about 79 million pounds of buttermilk solids and 339 million pounds of whey solids thrown to waste yearly in the United States. Over 66 million pounds of whey solids are wasted in the State of Illinois yearly⁽³⁶²⁾. (All data in terms of dry weight.) These figures have been corrected for the amounts recovered for various purposes (buttermilk, 163 million pounds; whey, 18 million pounds). The handling losses may best be summarized by referring to a table compiled by Eldridge⁽⁷¹⁾ (Table LXXIII).

TABLE LXXIII.
MILK-HANDLING LOSSES.

Plant.	Milk received lost to sewer.		
	Minimum. Per cent.	Average. Per cent.	Maximum. Per cent.
Condensery.....	0.5	1.5	2.0
Creamery.....	0.5	0.8	1.2
Receiving station.....	0.35	0.5	0.7
Dry milk.....	1.2	2.0	2.5
Cream cheese (excluding whey).....		6.0	

Since Boruff and Buswell⁽²⁴⁾ have been successful in fermenting certain sour wastes anaerobically and converting these materials to carbon dioxide and methane, and Neave and Buswell⁽²¹¹⁾ have shown that a large number of the organic acids can be fermented to these same gases, it seemed probable that the milk waste problem might be solved by this method and a large portion of the solids recovered as power and fuel gas while the residual material was stabilized.

EXPERIMENTAL PROCEDURE.

Anaerobic fermentation tanks of from 3 to 10 liters capacity were fitted with feeding tubes and gas, liquor, and sludge withdrawal connections. To these tanks were added well-digested sewage sludge and asbestos fibers to about one-third the tank volume. The tanks were then filled with settled overflow liquor from an anaerobic sewage tank. The liquor and sludge were used as the initial medium and source of the anaerobic bacteria. The sludge could be largely replaced by asbestos fibers which were found to act admirably as the necessary contact material. The particular waste in question was then fed very slowly. Close checks of the pH, volatile acid content, and gas analysis were made regularly. If the tank showed a tendency to go sour, the daily feedings were omitted or reduced for a short time until the tank fermented the accumulated acids to carbon dioxide and methane. In this manner flora were developed which would rapidly ferment to carbon dioxide and methane the milk solids added without permitting the accumulation of organic acids. The accumulation of acids, with a corresponding decrease in gas production, was also noted at times when tanks were fed beyond their normal capacity. The acids in such cases were found to be mainly propionic and acetic with traces of lactic and formic. The greatest concentration of lactic ever found was 40 p.p.m. Such an acid condition could be overcome by reducing the feed or by the exchange of liquor between the acid tank and one operating normally.

A number of studies was conducted in two tanks which were operated in series. Back circulation at such times as referred to above always reestablished normal fermentation. Such a practice was first used by Boruff and Buswell⁽²⁴⁾ in the fermentation of beer-slop waste. One small second-stage tank could serve a number of primary digesters. For the most part the tanks were fed undiluted whey and a mixture of buttermilk and whey waste. Representative analyses of these raw wastes are given in Table LXXIV. Thermophilic studies (53° to 58° C.) as well as mesophilic studies (27° to 29° C.) were conducted, but because the former showed no advantage over the latter, only the results of mesophilic studies will be presented.

Fermentation and stabilization data covering 8 months of experimentation are given in Tables LXXV and LXXVI. These data are average representative figures taken from experimental runs of from 35 to 72 days' duration. During this time from 11 to 25 liters of waste were fed to each tank. From these data it is apparent that the un-

diluted whey or buttermilk-whey waste can be fed to anaerobic fermentation tanks at the rate of from 2.2 to 2.9 grams of volatile matter per day per liter of tank capacity. From this fermentation there can be recovered 1.6 to 2.4 volumes of gas per day per tank volume. No noticeable amount of sludge was formed in any of the experiments, including one tank that was operated continuously for 208 days. As a result of fermentation, the overflow liquor from these tanks con-

TABLE LXXIV.
ANALYSIS OF MILK WASTE (UNDILUTED).

	Whey.			Buttermilk.			Skim milk.		
	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.
pH.....	4.5	4.9	5.6	4.5	5.1	5.8	6.5	6.8	7.0
Total solids, grams/liter.....	57.0	67.3	80.4	66.8	78.4	88.0	98.0	107.6	140.0
Volatile matter, grams/liter.....	53.0	61.8	73.4	61.9	72.4	82.0	91.3	100.2	131.0
Ammonia nitrogen, p. p. m.....	35	100	195	65	130	210	65	85	100
Organic nitrogen, p. p. m.....	1,048	1,252	1,495	3,900	4,225	4,620	4,825	5,180	5,335
Volatile acids, p. p. m.....	985	1,525	2,905	510	1,265	2,055	25	630	1,920
Oxygen consumed, p. p. m.....	19,200	24,060	28,400	19,950	24,800	30,000	32,000	35,350	39,400
5-day b. o. d., p. p. m.....	32,000	35,000	38,000	32,000	35,000	38,000	55,000	59,000	62,000

TABLE LXXV.
ANAEROBIC FERMENTATION OF MILK WASTES.

(At 27° to 29° C; all data based on continuous feeding experiments in 7- to 10-liter tanks.)

	Whey.	Buttermilk and whey, 1-1 (undiluted).		Whey diluted with equal volume of water.		Buttermilk + whey + water (1 to 1 to 4).		Skim milk (undiluted).
	Single stage.	1st stage.	2nd stage.	1st stage.	2nd stage.	1st stage.	2nd stage.	Single stage.
Rate waste fed per day per liter tank volume: ^a								
Volume (undiluted basis), cc.....	34.2	43.5	b	30.6	b	48.5	b	15.7
Total solids, grams.....	2.4	3.1	-----	2.3	-----	3.5	-----	1.8
Volatile solids, grams.....	2.2	2.9	-----	2.0	-----	3.2	-----	1.7
Gas recovered per day per liter tank volume:								
Volume, liters.....	1.6	1.9-2.4	0.3	1.3	0.5	2.7	0.7	1.0
Weight, grams.....	2.1	2.2-2.8	0.3	1.7	0.5	3.3	0.7	1.2
Gas analyses, per cent:								
CO ₂	46.8	39.0	29.0	43.2	27.3	40.4	28.0	41.3
CH ₄	49.8	55.0	61.0	49.6	58.0	58.0	67.0	55.2
H ₂	0.5	0.5	0.5	0.2	0.2	0.2	0.0	0.6
N ₂	2.9	5.5	9.5	2.0	14.5	1.4	7.0	2.9
Volatile matter fed, recovered as gas, per cent.....	96	76-97	c86-107	85	c110	103	c125	71

^a Grams/liter x 0.0624 = lb./cu. ft.

^b Recirculation of 2 to 4 liters daily. Second tank fed overflow liquor from first tank.

^c Includes gas from first stage. Union of water in decomposition reaction accounts for recoveries over 100 per cent (40).

TABLE LXXXVI.
OVERFLOW LIQUOR, SANITARY CHEMICAL DATA.

	Whey (undiluted).	Buttermilk and whey, 1-1 (undiluted).		Whey diluted with equal volume of water.		Buttermilk + whey + water (1 to 1 to 4).		Skim milk (undiluted).
	Single stage.	1st stage.	2nd stage.	1st stage.	2nd stage.	1st stage.	2nd stage.	Single stage.
pH.....	7.3	7.4	7.5	7.0	7.3	7.0	7.4	7.5
Volatile acids, p. p. m.....	1,750	1,580	1,100	1,690	500	1,350	500	1,285
Total solids, grams/liter.....	4.7	5.3	4.5	3.6	3.4	3.5	3.4	5.3
Total solids reduction, per cent.....	93.4	92.6	93.7	95.2	95.4	95.1	95.2	95.2
Volatile matter, grams/liter.....	2.2	3.1	3.6	1.5	1.2	2.3	2.2	3.1
Volatile matter reduction, per cent.....	96.6	95.3	94.6	97.8	96.2	96.6	96.8	97.0
Organic nitrogen, p. p. m.....	131	202	196	103	110	153	171	361
Organic nitrogen reduction, per cent.....	89.9	91.7	93.8	92.0	91.5	94.3	94.0	94.0
Oxygen consumed, p. p. m.....	480	350	150	695	570	500	380	890
Oxygen consumed reduction, per cent.....	98.1	98.4	99.3	97.0	97.5	98.1	98.6	97.4
5-day b. o. d., p. p. m.....	2,425	5,500	2,900	4,250	2,620	4,650
5-day b. o. d., reduction, per cent.....	93	85.5	92.2	91.8	94.7	91.7

tains only about 3 to 5 per cent of the original volatile solids, and only from 8 to 10 per cent of the organic nitrogen added. The oxygen-consumed (potassium permanganate) values of these overflow liquors show a residual of from 350 to 460 p.p.m., or a reduction of 98 per cent. The 5-day biochemical oxygen demand of the whey overflow liquor averaged 2,425 p.p.m., a reduction of 93 per cent. The overflow from the second-stage buttermilk-whey tank showed an oxygen-consumed value of only 150 p.p.m., or an over-all reduction of 99.3 per cent.

The experiments on the fermentation of diluted whey (1 to 1 with water) showed only minor differences from those conducted on the concentrated waste. Those on the diluted buttermilk-whey mixture (1 volume of whey + 1 volume of buttermilk + 4 volumes of water) show that more grams of solids can be fed per day per liter of tank volume if such solids are diluted. Greater gas volumes are also recovered (2.7 volumes per day per tank volume as compared with 1.9 to 2.4). The most probable explanation of this fact is that the decomposition of buttermilk, which contains an average of 4,355 p.p.m. of total nitrogen, produces concentrations of toxic decomposition products which act as bacteriostats and reduce the activity of the organisms. High ammonium carbonate concentrations are known to be toxic to some bacteria. At times the ammonia nitrogen concentration, in tanks being fed buttermilk and whey, ran from 1,200 to 2,500 p.p.m. During such periods low fermentative action was always noted. Dilution of such tanks with water always restored normal rapid fermentation. Such dilution was also found necessary in certain other studies on the anaerobic fermentation of casein and peptone. This difficulty was never met in the fermentation of diluted buttermilk-whey mixtures or in the fermentation of whey alone. Whey contains only about 1,352 p.p.m. of total nitrogen. In a commercial plant such dilution could be accomplished by the addition of all or a part of the wash water.

The few experiments on the fermentation of undiluted skim milk that were run did not give as high gas yields and degrees of purifica-

tion as did the other studies. The tanks did not go sour. Here again the authors feel that the trouble was due to the accumulation of toxic protein decomposition products. There is no reason to believe that diluted skim milk would not ferment readily.

Although the sanitary data for the diluted as well as the undiluted waste fermentations (see Table LXXVI) show large percentage reductions in the pollution load, the liquors in most cases should be given additional treatment by adding them to slag, stone, lath, or sand filters, or by diluting by addition to city sewers, where they would ultimately be treated in the city treatment works, or by diluting in a nearby stream, provided sufficient flow is available. Local conditions would determine the practice adopted. As these liquors are not sour (pH 7.0 to 7.5, and volatile acids, as acetic, only 500 to 1,850 p.p.m.) nor highly putrescible and contain but little settleable solids, they should not interfere with the normal operations of sewage treatment works.

COST OF EQUIPMENT AND INSTALLATION.

Assuming that anaerobic digestion tanks can be built for 50 cents per cubic foot and figuring 12 per cent for interest, amortization, and repairs, such tanks cost 16.4 cents per thousand cubic feet of volume per day. On the basis that raw, unsettled, and untreated buttermilk and whey wastes can be fed at rates of from one-twentieth to one-thirtieth the fermentation tank volume per day (2.3 to 3.6 grams per liter) and produce 1.6 to 2.7 volumes of gas per day per tank volume, gas could be produced at costs ranging from 6.1 to 10.3 cents per thousand cubic feet. As compared with other methods of gas production or transportation, this is a moderate cost.

The B.t.u. of the fermentation gases average about 550. This B.t.u. and the total volume could be readily increased by carburetion through gasoline. A volume of 776 liters of fermentation gas, when bubbled through Phillips 66 gasoline at room temperatures, was found to be increased in volume to 840 liters with an increase in the B.t.u. to about 990. This was accomplished by the vaporization of 382 cc. of the original 700 cc. of gasoline added.

On the basis of an average feeding of one twenty-fifth of a volume of milk waste (undiluted basis) per day per tank volume, it would require a tank, or tanks if operated as a two-stage process, of 5.72 cubic feet capacity for the anaerobic fermentation of one pound dry weight of milk waste solids. At 50 cents per cubic foot, this amounts to only \$2.86 per pound of milk solids treated. This fermentation would remove at least 95 per cent of the pollution load. The remaining 5 per cent contained in the overflow liquor could be stabilized readily on filters. Assuming that this final treatment could be made at a cost similar to that given for filter treatment in the first portion of the paper, the total investment for complete treatment would be \$8.70 per pound of solids if trickling filters were used following the anaerobic digestion, or \$17.46 per pound if sand filters were used, as compared with \$116.80 per pound if trickling filters were used alone) or \$292 per pound if sand filters were used. The above figures are not given to show actual costs but rather relative costs of the two processes.

Thus the total treatment plant cost of anaerobic fermentation followed by aerobic filtration is only a small percentage of that reported⁽¹⁴⁷⁾ for the treatment of milk wastes by standard present day methods, which, incidentally, are merely treatment methods and give no valuable by-product. Anaerobic fermentation of milk wastes followed by secondary treatment—namely, filtration—will not only give efficient stabilization but will also produce 8.3 to 12.4 cubic feet of gas per pound of dry solids added.

STABILIZATION OF PAUNCH MANURES AND PACKING-HOUSE SCREENINGS*

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By the use of a special drum type of digester it has been found possible to digest and stabilize cattle and hog paunch manures and packing-house screenings fed continuously at rates of at least 4.5, 6.0, and 5.6 grams dry weight, respectively, per day per liter of tank capacity (0.28, 0.37, and 0.35 pound per cubic foot per day). The stabilization of such materials furnishes from 1.0 to 4.0 volumes of combustible gas per day per tank volume. The amount of gas depends on the rate of feeding and the type of waste treated. A stable and satisfactory residue is produced.

The consensus of opinion is that the problem of disposal of organic wastes is greatly simplified if these wastes can be handled in a concentrated form. In the packing industry the wastes that are readily separated in concentrated form include the paunch manures, pen manures, and the material that is or could be collected by passing the liquid waste or drainage through grease-skimming tanks and fine screens. Most packing-houses are keeping the greater portion of their paunch manures out of the sewers. Many plants are also passing their liquid wastes through coarse or fine screens (10 to 32 mesh). In most cases these materials are hauled away to a dump where they slowly dry and eventually may be burned. This is offensive and requires considerable waste land. Some plants use a portion of these wastes in the manufacture of fertilizer. The economics of such a practice depends entirely on the market. The present writer has considered the problem of the disposal of these manures and screenings and wishes to propose what seems to be a practical method for their treatment and utilization.

A considerable amount of work on the treatment of liquid-or water-carried packing-house wastes (sewage) has been done by other workers**. A consideration of this part of the general problem lies outside the purpose of this discussion.

CONCENTRATED WASTES.

At one prominent packing plant 5,000 pounds of coarse screenings of about 85 per cent moisture content are being recovered per day per million gallons of flow (0.6 kg. per cubic meter). At another plant, where an average of 150 cattle, 300 hogs, and 200 sheep are killed each day, there are recovered between 1,500 to 2,000 pounds (680 and 910 kg.) of wet screening's per day from the 32-mesh screen employed. These

* Reprinted from Industrial and Engineering Chemistry. Vol. 25, Page 703, June, 1933.

** References 19, 20, 48, 54, 76, 98, 115, 136, 173, 191, 196-8, 212, 215, 229, 280, 322.

screenings are dried and burned in a near-by ravine. Fine screens of 20-, 30-, and 40-mesh have been found to remove 368, 858, and 1,270 pounds dry weight, respectively, of packing-house screenings per million gallons (0.04, 0.10, and 0.15 kg. per cubic meter)⁽¹⁹⁷⁾. Nelson⁽²¹²⁾ reports the removal of 5,000 to 15,000 pounds of screenings of 80 to 85 per cent moisture per million gallons of waste (0.6 to 1.8 kg. per cubic meter). This laboratory and others⁽¹⁹⁷⁾ have found screenings to contain 85 to 95 per cent volatile matter, 1.5 to 7 per cent organic nitrogen, and 6 to 23 per cent ether-soluble substances. Fine screens remove from 9 to 19 per cent of the total settleable solids.

To these already large weights of screenings must be added the paunch manures. For cattle these amount to about 10 pound's (4.5 kg.) dry weight per animal. From a plant killing about 10,000 cattle, 20,000 sheep, and 25,000 hogs per week, one might expect to recover in the order of 200,000 pounds (96,000 kg.) dry weight, of paunch manure. On the basis that such a plant had a sewage flow of 7.5 million gallons per day (28,400 cubic meters), there could be recovered at least an additional 1,000 pounds (453 kg.), dry weight, of fine screenings per million gallons, or a total of 36,000 pounds (16,300 kg.), dry weight, of paunch manure and screenings per day. This would remove the greater portion of the fibrous materials from the sewage but would leave in it enough to aid in the settling of other solids in sedimentation tanks. Such a removal would materially lighten the total solids pollution load. Disposal of such a weight of paunch manure and screenings on waste land or by partial drying followed by incineration would be troublesome, offensive, and expensive⁽²⁶⁾. These methods have other disadvantages.

Investigations on the biological stabilization of fibrous materials, such as paunch manure and screenings, have met with operating difficulties^(20, 39). In the first place, if these materials are not properly inoculated they are liable to go sour; that is, volatile organic acids accumulate to such an extent that the pH drops below the optimum and the desired fermentation is arrested. By controlling carefully the rate of feeding in a manner that will be described later, and by assuring the presence of sufficient material that is undergoing the desired fermentation in the digester at, all times, this first difficulty has been overcome. Mixing has been found to aid the digestion of such materials. The second difficulty is due to the fact that gas bubbles become entrapped in the fermenting materials. This causes them to float and produce a thick scum or mat that becomes so firm that ordinary mechanical equipment cannot break it up^(26, 39). This condition prohibits continuous operation, as well as endangers the pursuance of the desired biological action. By the use of a digester designed by Buswell and Boruff^(26, 39, 41) to overcome this difficulty, the author has been able to ferment successfully and stabilize anaerobically hog, sheep, and cow paunch manures, as well as fine screenings recovered from the combined sewage of a local packing plant. Figure 18 shows the design of this new type of digester. The manures and screenings are fed automatically or manually through the feeding tube into the perforated drum inclosed in the rectangular tank. Both ends of the drum are equipped with seal rings. These allow

the drum to be turned in its bearings without the escape of fibrous material from the drum into the rest of the tank. The digester is filled with water or sewage until it runs out the overflow pipe. A gas-tight cover and water-sealed hood serve to keep out air and collect the gases formed during the digestion. Slow or intermittent revolution of the drum liberates the entrapped gas bubbles and breaks up the thick mat which collects at the top. Frequent charging of the tank with fresh waste, together with revolution or inversion of the drum causes the digested material to work itself out through the opening in the lower end of baffle *A* into the residue compartment. This digester material is still fermenting sufficiently to cause the entrapping of gas bubbles within its mass, which, in turn, causes it to float to the top of the residue compartment from which it can be removed periodically with forks or other suitable means. Detailed operation of this type of tank has been described elsewhere⁽³⁹⁾. The use of this particular type of tank has been patented⁽⁴¹⁾.

EXPERIMENTAL PROCEDURE.

Experiments on the treatment of paunch manures and packing-house screenings, using bottles of 10 to 14 liters capacity, and a small pilot unit digester (12.3 cubic feet or 0.35 cubic meter) have been made. The laboratory bottle digesters were equipped so that they could be operated in a manner similar to that of the special type digester. Feeding and residue withdrawal tubes were provided. The rubber gas hose which connected the bottle to the gasometer permitted the operator to invert the bottle partially from time to time. This operation corresponded to the periodic rotation of the drum of the special digester and served the same purpose—namely, to break up the fibrous mat that collected at the top and thus release the entrapped gases. A summary of much of the data obtained is given in Tables LXXVII and LVII. All digesters were operated at room temperatures—namely, 25° to 29° C. (77° to 84° F.). They were originally put into operation by being filled with liquors from an anaerobic sewage sludge digestion tank (1 volume of digester sludge with 9 volumes of overflow liquor).

At first all tanks were fed very slowly. The data for the 5- to 10-day preliminary feeding periods are not given, and hence the tables do not show quantitative solids balances. After being started, the rate of feeding was gradually increased in accordance with the volatile acid content and the gas analyses and yields obtained, these being indicators that the desired fermentation was taking place. If the volatile acid content rose above 2,000 p.p.m. (as acetic), the feeding was temporarily stopped, or at least the rate reduced. This always caused the gasification of the accumulated acids. No chemicals were ever added for pH regulation.

TABLE LXXVII.
ANAEROBIC STABILIZATION OF PACKING-HOUSE MANURES AND SCREENINGS.*
(Digestion temperature, 26° to 30° C.)

	Cow paunch manure.	Packing-house sewage screenings. ^b	Hog paunch manure.
Digestion volume, liters.....	10	10	10
Duration of run, days.....	120	80	150
Material fed:			
Total wet weight, grams.....	31,880	25,840	24,800
Total dry weight, grams.....	5,383	4,467	8,974
Total volatile matter, grams.....	4,958	3,961	8,328
Dry weight fed per day per liter tank volume:			
Average for entire period, grams.....	4.5	5.6	6.0
Minimum-maximum (30-day average), grams.....	3.3-5.5	4.2-7.4	3.5-8.2
Average dry weight fed per day, lb./M cu. ft. tank capacity.....	281	350	374
Gas recovered, S. T. P.: ^c			
Total volume, liters.....	1,500.5	1,595.6	4,683.8
Volumes of gas per tank volume per day:			
Average for entire period.....	1.3	2.0	3.1
Minimum-maximum (30-day average).....	1.0-1.7	1.8-2.3	1.9-4.0
Average volume per gram of volatile matter fed, cc.....	303	402	561
Total weight, grams.....	1,748	1,936	5,990
Representative analysis, per cent by volume:			
CH ₄	61.2	57.8	53.3
CO ₂	37.0	40.9	45.4
H ₂	0.3	0.3	0.3
N ₂	1.5	1.0	1.0
Volatile matter fed recovered as gas, per cent by weight.....	35	49	72
Routine control tests of tank contents:			
pH (average).....	7.3	7.3	7.0
Volatile acids (average), p. p. m. as acetic.....	1,220	1,000	1,400
Sludge drawn:			
pH.....	47.5	47.5	9.2
Total volume, liters.....	37.5	26.5	30.0
Total solids, average per cent.....	7.9	8.8	9.3
Total solids, grams.....	2,977	2,341	2,796
Total volatile matter, grams..... ^d	2,562	1,915	1,848
Average ammonia nitrogen, p. p. m.....	1,030	857	990
Average total nitrogen, p. p. m.....	2,790	3,244	4,715
5-day B. O. D. (limits), p. p. m.....	4,200-7,800	5,720-8,200	8,100-14,400
5-day B. O. D. (average), p. p. m.....	6,720	6,760	11,500
5-day B. O. D., p. p. m./per cent volatile matter (limits).....	933	936	1,360-2,500
Materials fed, analysis of:			
Average dry weight, per cent.....	16.9	17.3	36.2
Average ash content (dry basis), per cent.....	8.0	11.3	7.2
Ammonia nitrogen (dry basis), per cent.....	0.05-0.15	0.08-0.18	0.03-0.08
Total nitrogen (dry basis), per cent.....	1.11-3.79	1.61-2.09	1.48-2.15
5-day B. O. D., p. p. m./per cent volatile matter (limits).....	1,000-3,220	1,570-2,220	850-1,680
Water added to digester, liters.....	10	2	4

^a Digestions conducted in tall glass bottles fitted so they could be inverted for mixing as per drum in pilot tank (Figure 18).

^b Sewage contained all paunch and intestinal manures, blood plus other wastes common to packing-house sewage.

^c Standard temperature and pressure.

^d In all cases the sludge drawn was not odorous but was fibrous and dried readily.

COW PAUNCH MANURE.

As noted in Table LXXVII, cow paunch manure was fed to a 10-liter bottle digester for 120 days at average monthly rates of 3.3 to 5.5 grams, dry weight, per day per liter of tank capacity (0.21 to 0.34 pound per cubic foot). The average for the entire period was 4.5 grams (0.28 pound) per day. From the 31,880 grams wet weight

(5,388 grams dry weight) of paunch manure fed, 1,500 liters of gas were recovered. This amounts to 303 cc. per gram, or 4.9 cubic feet per pound, dry weight, of volatile matter added. The daily gas production ran from 1.0 to 1.7 volumes per day per fermentation tank volume. The average was 1.3 volumes. The gas contained 61.2 per cent methane and hence had a B.t.u. value of about 600 (151,000 calories per gram).

A small pilot unit digester (12.3 cubic feet, 0.35 cubic meter), constructed according to plans as illustrated in Figure 18. was operated for a period of a little over 5 months on cow paunch manure. During the 122-day period reported in Table LVII, this digester was fed a total of 2,054 pounds (934 kg.) wet weight of paunch manure. For experimental reasons the digester was fed at different rates each month. The monthly rates varied from 1.6 pounds (0.73 kg.) dry weight to 3.6 pounds (1.64 kg.), dry weight, of material per day (0.13 to 0.29 pounds, dry weight, per day per cubic foot of tank capacity). From this material a total of 1.178 cubic feet (33.3 cubic meters) of gas was recovered. The monthly averages varied from 7.6 to 14.5 cubic feet (0.21 to 0.41 cubic meter) per day, or from 0.61 to 1.18 volumes of gas per day per volume of digestion tank capacity. Differences in the rate of feeding caused this wide variation, which, as stated above, was not necessary as far as the digestion of the paunch manure was concerned, but was practiced to note the effect on the gas production and on the character of the residue withdrawn. The greater the amount of material fed per day, up to a certain maximum, the greater was the yield of gas per tank volume per day, but the lower the gas yield per unit weight of material fed. The residues produced during the periods of most rapid feeding were, for all practical purposes, about as stable as those produced at the much lower feeding rates.

The somewhat smaller daily volumes of gas recovered from this pilot unit tank (0.61 to 1.18 volumes per tank volume per day) as compared with that produced in the laboratory investigation (1.0 to 1.7 volumes) were due mainly to the lower rates of feeding. The average volume of gas produced per unit weight of volatile matter added to the laboratory investigation was 303 cc. per gram (4.9 cubic feet per pound); that recovered from the pilot unit investigation averaged 262 cc. per gram (4.2 cubic feet per pound). This difference can be attributed to the fact that the temperature varied from 26° to 30° C. in the laboratory studies, whereas it ran from 23° to 30° in the pilot unit. Small differences in temperatures in the 25° C. range have been found to have a marked effect on the rate of gasification of such materials as these. As the pilot unit was located in an unheated building, it was necessary to heat the tank intermittently. Furthermore, the manure fed to the pilot tank was obtained only twice a week from the packing house and was fed in four feedings per week, whereas the laboratory run was fed daily with the same material which was ground in order to make it easier to feed and withdraw. It was kept fresh by preserving on ice. Frequent small feedings have always been found by the writer to give greater gas yields per unit of weight than larger and less frequent feedings. Putting the material through a coarse grinder would also assist stabilization and gasification.

During the entire 122 days of operation only 30 gallons (114 liters) of sludge were drawn from the bottom of the pilot unit tank. These liquors averaged 3.6 per cent solids for a total of only 9 pounds (4.1 kg.) of material. In other words, only a small amount of material was passing through the 12-mesh screen covering the drum and collecting at the bottom of the digester. At the end of the run, the drum (7.6 cubic feet or 0.22 cubic meter) was found to contain 50 pounds (23 kg.) dry weight of digesting material.

The digester residue drawn from the laboratory investigation had an average pH of 7.5, an average total solids content of 7.9 per cent, a 5-day B. O. D. (biochemical oxygen demand) of 6,720, and a 5-day B.O. D. per per cent of volatile matter of only 983 as compared with 1,000 to 3,220 for the original cow paunch manure. Other data are given in Table LXXVII. The residues drawn from the laboratory and the pilot unit tanks were of approximately the same quality except that the pilot unit contained a higher percentage of total solids—namely, about 12 per cent as compared with 7.9. The residues were not odorous, but were very fibrous owing to the presence of undigested, but stabilized hay, straw, and other cellulosic constituents. They drained and dried well. As the residues contained a little higher moisture content than the paunch manure added, there were no overflow liquors. During the 120-day laboratory experiment, 10 liters of water were added to the digester.

PACKING-HOUSE SCREENINGS.

After the 120-day continuous run on cow paunch manure, the laboratory digester was fed packing-house screenings collected from the sewage of a local slaughter house by the use of a 30-mesh screen. As noted in Table LXXVII, these screenings were fed at rates of 4.2 to 7.4 grams dry weight of material per day per liter of tank capacity (0.26 to 0.46 pound per cubic foot). They produced from 1.8 to 2.3 volumes, or an average of 2.0 volumes of gas per day per fermentation tank volume, or 402 cc. per gram of volatile matter added (6.4 cubic feet per pound). The gas contained 58 per cent methane.

The residue drawn from this investigation was of the same good quality as that obtained from the paunch manure studies. The original 5-day B. O. D. of 1,570 to 2,220 p.p.m. per per cent of volatile matter was reduced to an average of only 936 p.p.m. The residue was stable and fibrous, and dried readily.

HOG PAUNCH MANURE.

A laboratory investigation was also made on the digestion of hog paunch manure. As noted in Table LXXVII, this material was fed at average monthly rates of 3.5 to 8.2 grams, dry weight, per day per liter of tank capacity. The average rate was 6.0 grams (0.38 pound per day per cubic foot). From the 24,800 grams, wet weight (8,974 grams, dry weight), of material fed, there were recovered 4,689 liters of gas containing 53 per cent methane. This amounts to 1.9 to 4.0 volumes of gas per day per fermentation tank volume (average of 3.1) or an average of

561 cc. of gas per gram., dry weight, of volatile matter added (9.0 cubic feet per pound). An average of 72 per cent of the weight of volatile matter added was recovered as gas. At the higher rates of feeding (7 to 8 grams per day per liter of tank capacity) the digester had a tendency to turn sour; that is, the volatile acid content would reach 3,000 p.p.m. or more, and the pH would drop as low as 6.6. The sludges drawn during the rapid feeding periods were not of good quality. The 5-day B. O. D. ran about 2,500 p.p.m. per per cent of volatile matter as compared with 1,360 p.p.m. during the more moderate feeding periods. The sludges with the high B. O. D. values possessed and retained a slight odor. The author would advise that hog paunch manure not be fed at rates over 4.5 grams per day per liter of tank capacity (0.28 pound per cubic foot). This is the same order of magnitude as found best for cow paunch manure and packing-house screenings.

As hog paunch manures usually contain considerable finely divided and partially digested corn residues and but little cellulosic material, an appreciable amount of the solids present would undoubtedly pass through the screen used to cover the drum of a digester built as shown in Figure 18. To meet this situation it would be advisable to construct the digester with a sludge withdrawal pipe and possibly a hopper bottom to permit the withdrawal of digested sludge from the bottom of the tank as desired and found necessary. The fibrous material would be withdrawn from the residue compartment as usual.

Thus one notes that, by the use of a digester of the type shown in Figure 18, paunch manures, as well as large weights of sewage solids removed by screens from packing-house wastes, can be stabilized with a minimum of handling and little cost. The average capacities noted in the above studies—namely, 4.5 to 6.0 grams per day per liter of tank capacity (0.28 to 0.37 pound per cubic foot)—are at least twice those capacities noted for the best of present-day primary sewage sludge digesters. Furthermore, standard type digesters are unable to handle solids containing an appreciable quantity of fibrous material because of the thick scum and mat that forms on the surface of the liquor⁽³⁹⁾. If grease-removal tanks are used, the skimmings can be added to the digester. This material digests very rapidly and furnishes a large quantity of gas of a high B.t.u. value⁽⁴⁰⁾. On the basis that commercial-size digesters could be built for 50 cents per cubic foot of tank capacity, and allowing 12 per cent for interest, amortization, and repairs (operation not considered because it would be very low), a thousand cubic feet of tank capacity would cost 16.4 cents per day. Using the average data obtained in the investigations presented, the gas generated in the stabilization of cow paunch manures fed at an average rate of 0.28 pound, dry weight, per day per cubic foot of tank capacity, would be produced for 13 cents per thousand cubic feet; that generated from screenings and hog paunch manures fed at average rates of 0.35 and 0.37 pound, dry weight, per day per cubic foot of tank capacity, respectively, would be produced for 8 cents and 5 cents per thousand cubic feet, respectively. These gas production costs are low when compared with prices paid for gas of an equivalent heat value. The 18 tons of manures and screenings that are

available at the packing plant referred to earlier in this paper should produce approximately 200,000 cubic feet of gas per day.

Screened packing-house sewage should be given additional treatment. The author has investigated the possibility of digesting the suspended and dissolved solids left in these liquors by passing them (unsettled) through standard type digesters in series. This gave a satisfactory effluent but would not be feasible, owing to the volume of digestion capacity required (twice the volume of waste). The treatment of these screened liquors warrants further study to determine if present-day practices are the most economical.

POWER AND FUEL GAS FROM DISTILLERY WASTES.*

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State Water Survey, Urbana, Ill.

Hot distillery wastes containing 3 to 4 per cent solids and 0.2 per cent organic acids may be fermented thermophilically to produce fuel gas (a mixture of methane and carbon dioxide) at very low cost. From an average daily volume of 1,500,000 gallons of this waste, 3,600,000 cubic feet of gas could be produced. A gasification of 58 to 72 per cent of the organic matter is accomplished in 2 to 6 days. A stable inoffensive sludge (residue) is formed, as well as a liquor that can safely be drawn to sewers.

Distillery wastes in the main always have been a liability. Methods of salvaging them for use as stock food or fertilizer base have been investigated and some are used by distilleries today. Methods of recovering the organic acids and glycerol have also been studied. The use of this material as a binder for fuels and for other purposes⁽²³⁴⁾ has also been proposed. None of these procedures, however, has netted a substantial profit.

The sanitary disposal of beer-slop wastes is a serious problem. Usually they are about 20 times as heavy as normal sewage and contain a very high percentage of organic matter. The literature indicates that treatment plants handling sewage which contains a moderate amount of this waste will operate with difficulty⁽¹⁹⁹⁾.

Danok⁽⁶²⁾ suggested a pure culture method of decomposing such wastes. He states that the success of his method depends on the use of a sterile waste and a pure culture of specific bacteria. A British patent⁽²⁾ outlines a process for the aerobic or anaerobic decomposition of such waste sby the addition of "betaine-destroying organisms." Neave and Buswell⁽²¹⁰⁾, in 1928, reported studies on the anaerobic stabilization of slops from an alcohol plant. In their batch experiments they found that slop diluted 1 to 4 still inhibited bacterial growth, but that when it was diluted 1 to 9 with sewage and inoculated with sewage sludge it fermented smoothly at 77-81° F. (25-27° C.), with the formation of carbon dioxide and methane, but with the destruction of only 55 to 65 per cent of the solids in 73 days.

Hatfield⁽¹¹⁶⁾, after studying the rate of settling and gasification of Commercial Solvent beer-slop waste, reported that when diluted with 6 to 13 parts of sewage the solids were easily settled out. He found that the sludge was readily digested with sewage solids and that it produced

* Reprinted from Industrial and Engineering Chemistry. Vol. 24, Page 33, January, 1932.

the same quality and quantity of gas as the organic matter from sewage.

The settling of beer-slop waste with sewage, however, removes only about 30 per cent of the total waste load, leaving the rest to be handled by activated sludge or trickling filter units. The supernatant liquor from a settling tank being treated with 1 volume of beer-slop to every 9 volumes of average sewage would still be 2 to 3 times as strong as average settled sewage. This would place an excessive load on the activated sludge or trickling filter units. "The cost of treating such a strong waste," Hatfield says, "makes its recovery as a by-product within the industry necessary and advisable." At the time of Hatfield's studies the Commercial Solvent Corporation was fermenting corn mash. The residue from this fermentation (1,250,000 gallon a day) had a total solids content of 11,196 p.p.m. Hatfield calculated the population equivalent of these wastes to be about 800,000. Since that time the Commercial Solvents Corporation has used rye and the solids content of its waste has been materially increased.

GAS YIELDS FROM FERMENTED WASTES.

On the basis of related studies⁽²³⁾ by the writers, it was thought advisable to see what gas yields could be obtained by the fermentation of the undiluted Commercial Solvent waste. As the temperature of the raw waste ran from 194° to 203° F. (90° to 95° C), it was decided to try a thermophilic digestion. Such fermentations have long been recognized as much more rapid than those conducted at ordinary mesophilic temperatures. Table LXXVIII gives an analysis of the waste used in this investigation. About half the solids were found to be filterable. This waste is much heavier than that used in the mesophilic studies by Neave and Buswell⁽²¹⁰⁾ and by Hatfield⁽¹¹⁶⁾.

Anaerobic fermentation tanks (1 to 2 gallon capacity) were constructed with tubes for feeding and withdrawing liquor, residue, and gas. The gas was collected in gasometers and analyzed frequently. These fermentation tanks were started by adding liquor and sludge from a thermophilic tank in which an active culture of gasifiers had been developed. All tanks were operated at 127° F. (53° C). The fermentation tanks, after they were well started, were fed at different rates.

TABLE LXXVIII.
ANALYSIS OF COMMERCIAL SOLVENT BEER-SLOP WASTE.

pH.....	4.5-5.3
Total solids, p. p. m.....	28,000-40,000
Volatile matter, p. p. m.....	26,000-36,000
Volatile acids (as acetic), p. p. m.....	1,800-2,400
Ammonia nitrogen, p. p. m.....	14-56
Total nitrogen, p. p. m.....	1,400-1,900
B. O. D., 5-day, p. p. m.....	17,000
Oxygen consumed, p. p. m.....	10,000-20,000
Temperature of waste as drawn, ° F.....	194-203
Total solids:	
Ash, per cent.....	10
Protein (org. N x 6.25), per cent.....	32
Fats and oils, per cent.....	8
Total carbohydrates (less cellulose) as glucose, per cent.....	21
Undetermined (crude fiber, etc.), per cent.....	29

For example, one tank was fed at the rate of one-half its volume of waste per day (tank volume = twice the volume of waste), and another at the rate of one-third its volume of waste per day (tank volume = 3 times volume of waste). These experiments extended over seven months. The first three months were devoted to a determination of the rate at which the sour slop liquor could be fed to the fermentation vessels without inhibiting the bacterial action. The data submitted were obtained from a subsequent uninterrupted 4-month run.

Figure 22 and Table LXXIX summarize the gas data. By feeding slops at the rate of 1 volume a day to a tank of twice this volume, 14 volumes of gas per volume of slop, in other words, 7 volumes of gas per unit of tank volume, can be obtained daily. If, on the other hand, the tank has a 4-volume capacity, giving the waste a longer detention period, 16.8 volumes of gas may be recovered each day from 1 volume of waste fed. If the same volume of waste is fed each day to a tank 6 times this volume, giving the waste a detention period of 6 days, 18 volumes of gas, or 3 times the fermentation tank volume per day, can be recovered. The gas recoveries for the different tank volume to waste volume ratios are given in Table LXXIX.

Heukelekian and Eudolfs⁽¹²⁶⁾ reported that they can feed thermophilic sewage digestion tanks at a rate as high as 77 pounds of volatile matter per day per 1,000 cubic feet of tank capacity (23.5 grams per day per 19-liter tank). From this digestion they were able to recover 1.2 volumes of gas per tank volume per day (Table LXXX). This has been considered a very rapid digestion. The writers have been able to feed 930 pounds of volatile matter per day per 1,000 cubic feet of tank capacity and to recover 7 volumes of gas per unit of tank volume per day. With the lower rate of feeding, whereby the waste is more thoroughly stabilized, an average of 3 volumes of gas per unit of tank volume can be recovered.

TABLE LXXIX.
GAS PRODUCTION DATA AND RELATED CALCULATIONS.

Tank volume (times volume of waste).	Yield of gas (volumes per tank volume per day).	Analysis of gas. ^a			Interest, ^b amortiza- tion and repair charges per 1,000 cu. ft. gas, cents.	Character of residual waste.
		CH ₄ , per cent.	CO ₂ , per cent.	B. t. u.		
2	7.0	55	43	550	2.34	Sludge fairly stable; overflow liquor unstable. ^c
3	5.2	58	40	550	3.15	Liquor unstable. ^d
4	4.2	58	40	550	3.90	Liquor unstable. ^e
6	3.0	53	40	550	5.46	Liquor fairly stable; but contains only 10 per cent of original solids; ^f sludge stable.

^a Contains 0.1 to 0.5 per cent H₂ and 1 to 3 per cent N₂.

^b Figured at 12 per cent on the investment.

^c Organic matter gasified = 58 per cent total added.

^d Organic matter gasified = 63 per cent total added.

^e Organic matter gasified = 67 per cent total added.

^f Organic matter gasified = 72 per cent total added.

TABLE LXXX.
COMPARATIVE RATES OF THERMOPHILIC DIGESTION OF FRESH SEWAGE SOLIDS
AND BEER-SLOP WASTE.

Material.	Rate of feeding (volatile matter per 1,000 cu. ft. tank volume per day), lbs.	Volumes of gas recovered per tank volume per day.
Sewage solids (124).....	77	1.2
Beer-slop waste.....	930 310	7.0 3.0

The gas from a tank having a volume twice that of the waste fed each day contains 55 per cent methane and hence has a B.t.u. of 550, which is about the same as that of coal gas (Table LXXIX). The gases from the fermentations carried out in larger tanks (4 to 6 times the volume of waste) contain a little higher percentage of methane (58). This difference in methane content is due to the fact that the volatile organic acids, which tend to remain high in a tank that is being fed at a high rate, are decomposed if held longer in the digestion tank. These acids give a higher ratio of CH_4 to CO_2 ⁽²¹¹⁾.

GAS PRODUCTION COSTS.

On the basis of 50 cents a cubic foot, a 1,000-cubic foot concrete digestion tank would cost \$500. Such a tank should last from 10 to 20 years. This investment, at 12 per cent, would mean a yearly cost of \$60, or 16.4 cents per day per 1,000 cubic feet of tank capacity. The 12 per cent should easily take care of interest, amortization, and repairs.

On this basis a tank of twice the volume of the waste to be treated, and capable of delivering 7 times its volume of gas per day, would yield gas of 550 B.t.u. at a cost of 2.34 cents per 1,000 cubic feet. In a tank having a volume 3 times that of the waste (Table LXXIX) more gas will be formed per unit of waste, but less gas per tank volume (5.2 volumes of gas per tank volume). This gas could be produced at a cost of 3.22 cents per 1,000 cubic feet. If the industry wanted to stabilize the waste and reduce the putrefactiveness to such an extent that the sludge could be drawn into a lagoon or fill and the liquor run into the sewer or river, it would be compelled to build a tank 6 times the volume of the waste. Then 3 volumes of gas per tank volume would be produced at a cost of only 5.48 cents per 1,000 cubic feet.

On a heat value basis the smaller tank would partially stabilize the waste and produce gas at a cost of 4.2 cents per 1,000,000 B.t.u. The larger tank would stabilize the waste and still deliver the fuel gas at a cost of only 9.4 cents per 1,000,000 B.t.u. These costs compare favorably with those of heat from soft coal, as well as with those of heat from natural gas piped from the southwestern states to Peoria, Ill. The cost of 1,000,000 B.t.u. of heat from soft coal have 12,000 B.t.u.,

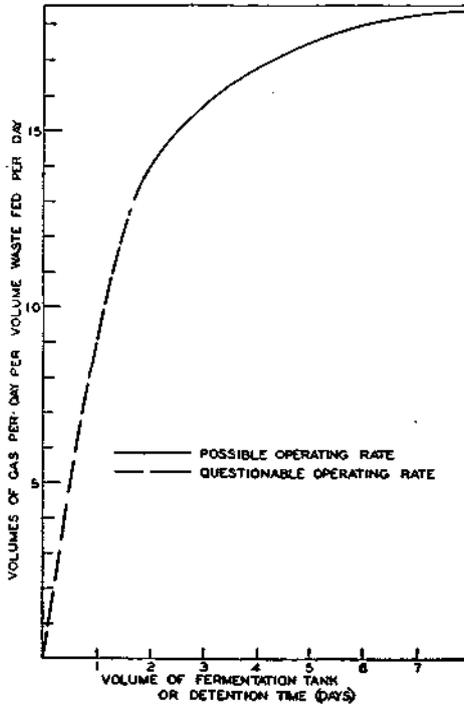


FIGURE 22.
Production of Fuel Gas from Beer-Slop Waste.

TABLE LXXXI.
COMPARATIVE COSTS OF COAL, NATURAL GAS AND DIGESTER GAS FROM BEER-SLOP WASTE.

Fuel.	Cost per 1,000,000 B. t. u., cents.
Soft coal (12,000 B. t. u.) at \$1.50 per ton.....	6.2
Natural gas (1,000 B. t. u.) at 15 cents per 1,000 cu. ft.....	15.0
Digester gas:	
From tank, twice volume of waste.....	4.2
From tank, 6 times volume of waste.....	9.4

at \$1.50 per ton is 6.2 cents (Table LXXXI). A gaseous fuel always has a decided advantage over a solid fuel.

The B.t.u. of gas from the fermenter could be readily increased by washing the gas with water (with or without alkali) under pressure, which would remove much or all of the carbon dioxide. This carbon dioxide could be recovered for synthetic purposes or for the production of dry ice. The residual gas (methane) could be burned or used in synthetic reactions.

WASTE STABILIZATION.

Table LXXXII gives representative sanitary chemical data collected during a 7-month small-scale continuous feeding experiment on the gasification and stabilization of Commercial Solvent beer-slop waste.

The sludge and overflow liquor from a tank having a volume twice that of the waste fed per day (2-day capacity) was still somewhat unstable, although 58 per cent of the organic matter had been gasified. The sludge drawn from the tank was very fibrous and contained 70 only per cent moisture after having drained 8 hours on sand. After a 3-day drying (indoors) it had a moisture content of 51 per cent. The dried sludge possessed only a slight odor. The sludge had a 1-day biochemical oxygen demand (B. O. D.) of only 615 mg. per 1 per cent of volatile matter. Rudolfs and Fischer⁽²⁷⁶⁾ state that a sludge of a 1-day B. O. D. of 1,000 to 1,500 mg. per 1 per cent of volatile matter is ready to be drawn and will not create a nuisance. The sludge as drawn produced only 44 cc. of gas per gram of volatile matter in 24 hours at 52° C. This is a lower volume than that noted for most well-digested mesophilic sewage sludges⁽²³⁶⁾.

The overflow liquor from this tank (2-day capacity) was too unstable to be drawn into the open air. It had an average B. O. D. of 5,700 p.p.m. and contained 2,000 p.p.m. of volatile organic acids.

TABLE LXXXII.

EFFECT OF VARIOUS DIGESTION TIMES (TANK CAPACITY) ON STABILITY OF WASTE.^a

	Original beer-slop waste.	From tank, twice volume of waste (2-day capacity). 58 ^b		From tank, 4 times volume of waste (4-day capacity). 67 ^b		From tank, 6 times volume of waste (6-day capacity). 72 ^b	
		Sludge.	Liquor.	Sludge.	Liquor.	Sludge.	Liquor.
pH.....	5.0	8.0	7.5	7.9	7.8	8.2	8.0
Total solids, p. p. m.....	33,000	45,000	5,000	40,000	4,000	35,000	4,000
Total volatile solids, p. p. m.....	30,000	39,000	4,200	33,000	3,600	28,000	3,200
Settleable solids, p. p. m.....	650	255	200
Volatile acids (as acetic), p. p. m.....	2,000	2,000	2,000	1,000	1,600	300	500
Ammonia nitrogen, p. p. m.....	30	650	650	650	600	750	600
Total nitrogen, p. p. m.....	1,600	3,000	1,000	3,000	1,000	3,000	850
Oxygen consumed, p. p. m.....	16,000	10,000	3,000	1,300	1,000
Immediate (30-min.) O ₂ demand, p. p. m.....	120	150	230
B. O. D., 3-day, p. p. m.....	17,000	7,900	5,700	4,600	3,700	3,200	3,000
B. O. D., 1-day, p. p. m.....	6,000	2,400	1,300	1,500	1,500	1,000	1,000
1-day B. O. D., mg. of O ₂ per cent volatile matter.....	2,000	615	454	357
Cc. gas per gram, volatile matter in 24 hours.....	44	28
Cc. gas per gram, volatile matter in 10 days.....	148	83
Moisture in sludge after draining 8 hours, per cent.....	70	80	84
Moisture in sludge after drying 3 days, per cent.....	51	66
Odor as drawn.....	a	d	e
Odor after 3 days.....	d	e	f
Volumes of sludge drawn per 100 volumes of waste fed.....	23	22	21

^a Average representative analysis.^b Percentage of organic matter gasified.^c Bad.^d Moderate.^e Slight.^f Humus.

Sludge drawn from a tank having a volume 6 times that of the daily volume of waste (6-day capacity) was very stable. It was not as fibrous as that from the smaller tank (2-day capacity). After an 8-hour draining on sand it had a moisture content of 84 per cent, but after a 3-day drying (indoors) this was reduced to 66 per cent. Its 1-day B. O. D. per 1 per cent of volatile matter was 357 mg. As drawn it had only a slight sewage sludge odor, and after drying this had entirely disappeared. The liquors from this tank still had a high 5-day B. O. D. (3,000 p.p.m.). Its organic content (volatile matter) was 3,200 p.p.m., but the volatile organic acids were only 200 p.p.m. This overflow liquor contained but 10 per cent of the original organic matter of the waste, and was so stable that it could be run into the sewer without jeopardizing the operation of the treatment works. This additional load would still keep the organic content of the combined waste within that of normal sewage.

In addition to the routine here reported, the fairly stable fibrous sludge could be drawn from a 2-day capacity tank and only the overflow liquor from this small tank given further treatment. This method would require a much smaller total tank volume for the complete treatment of the waste, as the sludge drawn would amount to 23 per cent of the volume of the original waste fed. The volume of the tank for the final 4-day detention of the 6-day period could be proportionately decreased.

THE TREATMENT OF "BEER SLOP" AND SIMILAR WASTES.**

By A. M. BUSWELL, PH.D., Chief of State Water Survey, Urbana, Ill.

The wastes from breweries and distilleries, commonly referred to as "beer-slop," are typical of a rather large group of industrial wastes for which a common method of treatment is in general applicable. In this group we would include wastes from breweries, distilleries, grain vinegar, cereal beverages (Ovaltine), starch manufacture, creameries, meat packing, citrus fruit juice, pea, bean and corn canneries, and beet sugar; in short most of the industries which produce food and drink in a "ready to use" condition.

Nearly all of these industries use water for three purposes, (a) process water, which is relatively small in amount but when discharged carries several per cent of organic matter; (b) wash water used to clean equipment, floors, etc., which is variable in quantity and may be of the approximate concentration of domestic sewage; and, (c) cooling or condenser water which is usually several times the volume of (a) and (b) but with proper piping it may be discharged in an uncontaminated condition.

This paper deals primarily with the treatment of "process water" or more properly "process by-product" in the case of certain industries such as creameries and citrus fruit juice bottlers. In the treatment proposed it is assumed that this concentrated waste (a) can be separated completely from (c) and more or less completely from the more dilute liquids composing the wash water (b).

*** Reprinted from Water Works and Sewerage, April, 1935.

The concentration of these process liquors as shown in the tables below range from 1 per cent to 6 or 7 per cent total solids, of which the major portion is organic matter in true solution. The inorganic matter (ash) and settleable solids run considerably under 50 per cent of the total, and the oxygen consuming substances are almost wholly in solution.

With material of this sort the usual expedient of sedimentation produces relatively little improvement. The addition of chemical coagulants is likewise of limited benefit as far as oxygen demand reduction is concerned. The reason for this failure is apparent when we stop to consider that much of the organic matter in these wastes is in a state of true solution (i. e., like a salt solution or a sugar solution) and cannot be removed by coagulation. In this respect they differ from domestic sewage, for it has recently been shown ⁽¹⁰⁾ that passage through an "ultra filter" (i. e., a filter which will remove true colloids) will reduce the B. O. D. of ordinary sewage to zero. Substances in true solution are not removed by "ultra filtration."

The aerobic methods of treatment, trickling filters or activated sludge, may be employed to remove dissolved organic matter but the loading limits for good results are very low⁽⁴³⁾. Rates of 10,000 to 50,000 gallons per acre per day are required for the undiluted wastes on trickling filters and aeration periods of 24 hours or more with high air consumption are required when the activated sludge process is used. The sludge produced tends to be light and feathery and settles poorly.

One of the most embarrassing characteristics of high organic wastes is the rapidity with which they become sour. Acetic, lactic and other organic acids develop in a few hours to give acidities of several thousand parts per million. In some cases these acids develop during the normal manufacturing process and the wastes as discharged are strongly acidic. Experience has shown that sour wastes cannot be treated by aerobic methods even at the low loadings given above. Neutralization must be employed and frequently dilution is necessary, water or domestic sewage being used if available. The necessary dilution ratios run from 1 to 10 to 1 to 100. Where the industry operates only a few months a year, broad irrigation or lagooning at the rate of 10,000 gallons per acre per day may be employed.

It is apparent then that ordinary sewage treatment methods applied to industrial wastes of this character require an outlay for plant and equipment which is a heavy burden on the industry. Some manufacturing plants, to handle their wastes in this way, would have to build treatment works capable of purifying the wastes from several million people.

Since the concentrations encountered are similar to those found in the sludge from sedimentation tanks it was early suggested that these wastes might be handled as sludge is handled, namely, by anaerobic or septic digestion. Attempts to accomplish this were unsuccessful due in most cases to the extensive and rapid production of acids which arrested all further bacterial decomposition. It was noted⁽²⁴⁾ that if the wastes were diluted ten times with sewage the acidity could be controlled and complete anaerobic decomposition of the organic matter brought about. Dilution, however, has the disadvantage that the total volume to be treated is increased, thereby necessitating extra tankage capacity.

In the case of wastes containing more or less fibrous material or grease, a firm scum or mat forms^(21, 26), at the top of the digesting tank, frequently to considerable depths. This further hinders the process by creating an acid zone and decreasing the effective volume of the tank.

CORRECTING INTERFERENCE WITH ANAEROBIC TREATMENT.

Limitations to the anaerobic method of stabilization have been under investigation in the laboratories of the Illinois State Water Survey for about ten years. The solutions proposed are as follows:

Control of Acid Production.—It has been shown by numerous investigations⁽³⁶⁾ that practically any kind of organic matter except mineral oils will decompose under the action of anaerobic bacteria to form methane and carbon dioxide. Fats, proteins, carbohydrates, alcohols, and even phenols are decomposable in this manner. The chemical reactions which take place in this decomposition occur in the main in two steps; the complex organic molecules combined with water to form the simpler organic acids (acetic, propionic, etc.) and then these acids decompose to methane and carbon dioxide. If an abundance of food material is available for the bacteria they tend to form acids more rapidly than such acids can be decomposed. It is well known that under these conditions the acid soon reaches a concentration at which all bacterial action is stopped. If on the other hand the amount of food material is properly limited the bacteria will decompose the acids as fast as they are formed, and the fermentation goes on smoothly and continuously. Fresh material may then be added regularly to the fermentation vessel in controlled amounts.

The Duclaux* method for total volatile acid is used to determine the rate of accumulation of acids during digestion. These organic acids are only slightly ionized and it is the acid radical not the hydrogen ion which must be transformed into CO_2 and CH_4 . For these reasons it has not been found possible to depend on the pH determination for control of the process.

The limit of acidity for smooth continuous fermentation has been found for most materials to be about 2,000 p.p.m. calculated as acetic. When the acidity of the liquor in a fermentation tank exceeds this value it may be reduced either by decreasing the rate of feed of raw material or by dilution. Decreasing the rate of feed would involve storing or by-passing untreated waste which would be objectionable. Dilution with water may be used but we have found it advantageous to carry out the fermentation in two or more tanks operating in series and use of the spent liquor for dilution. The rate of feed is adjusted so that the acidity in the first tank is kept near the upper limit in order to obtain maximum efficiency. The secondary tanks receive the overflow liquor from the first tank at a rate to keep acidity at a low value. To reduce the acidity the liquor from the secondary tanks may be pumped to the first tank allowing a like amount of liquor from the first tank to flow into the second tank. In other words the liquor in the two tanks is intermixed and the acidity reduced. Suppose in an installation with two tanks of equal size

* State Water Survey Bull. 30, p. 77 or standard texts on Organic Analysis.

the acidity in the first tank has increased to 2,500 p.p.m. while that in the second is 500 p.p.m. After back circulation of liquor from the second tank for a few minutes allowing that displaced from the first tank to flow to the second the acidity will be 1,500 p.p.m. in both tanks. This is below the tolerance limit and the feed of raw material to the first tank may proceed without interruption.

Scum and Foam Control.—Wastes of a greasy or gummy character frequently produce a scum over the top of the fermentation tank. If allowed to accumulate this scum becomes sour and impairs the general operation of the tank. In our experience it can be easily controlled by pumping liquor from well above the sludge line and allowing it to flow back onto the scum. A few minutes a day of this sort of circulation is sufficient to prevent any appreciable accumulation of floating solids. Some operators have suggested circulating sludge from the bottom of the tank over the scum. This is no doubt effective but it impairs the quality of the digested sludge.

Foam may be controlled in a similar manner but in this case the discharge at the top of the tank should be at a low velocity to avoid any jetting action. A high speed jet of water frequently produces foam.

Handling Fibrous Wastes.—Packinghouse wastes⁽²¹⁾, citrus fruit pulp, and sewage screenings⁽²⁶⁾ tend to form a fibrous mat over the top of the fermentation tank which cannot be controlled by circulation as described above. A special mechanical digester⁽²¹⁾ has been designed to solve this difficulty.

This digester consists of a perforated horizontal drum submerged in a covered tank. A feeding tube pierces the outer wall of the tank at a point within the circumference of the drum. The fibrous wastes are fed automatically or manually through the feeding tube into the perforated drum inclosed in the rectangular tank. Both ends of the drum are equipped with seal rings. These allow the drum to be turned in its bearings without the escape of fibrous material from the drum into the rest of the tank. The digester is filled with water or sewage until it runs out the overflow pipe. A gas-tight cover and water-sealed hood serve to keep out air and collect the gases formed during the digestion. Slow or intermittent revolution of the drum liberates the entrapped gas bubbles and breaks up the thick mat which collects at the top. Frequent charging of the tank with fresh waste, together with revolution or inversion of the drum causes the digested material to work itself out through the opening in the lower end of a baffle into the residue compartment. This digested material is still fermenting sufficiently to cause the entrapping of gas bubbles within its mass, which, in turn, causes it to float to the top of the residue compartment from which it can be removed periodically with forks or other suitable means.

For the treatment of beer-slop from distilleries and steep water from breweries the acid control is the most important operating factor. By taking advantage of this method of control it has been possible not only to treat these sour wastes but to handle them at loadings of 0.3 to 0.9 lbs. of organic matter per cubic foot of tank volume per day. These figures are to be compared with loadings of 0.05 to 0.08 lbs. per cubic

foot for domestic sewage sludge. Table LXXXIII summarizes data on a wide variety of wastes.

The gas production per unit of tank volume is of course increased proportionately by these heavy loadings. Three cubic feet of gas per cubic foot of tank volume are easily obtained and twice that rate is attainable. Since the principle cost of anaerobic fermentation is the installation charge it then decreases proportionately with increased loading rates. Gas production under these conditions is taken out of the waste disposal class and becomes a profitable manufacturing process. Calculations based on the data in Table LXXXIII indicate that a gas of 540 B.t.u. can be produced for a cost as low as three cents per thousand cubic feet, although ten cents would be the cost in many cases. As the waste to be treated becomes more dilute the costs naturally increase. The character of the wastes is also a factor, the fibrous wastes decomposing more slowly than the soluble by-products.

TABLE LXXXIII—FEEDING AND GAS PRODUCTION RATES
OBTAINED DURING THERMOPHILIC DIGESTION
OF VARIOUS INDUSTRIAL WASTES.

A. Commercial Solvents' Beer-Slop (Acetonic)

1. While fermenting rye

3.3% Total solids, 91% volatile

Rate of feed:

Volume basis— $\frac{1}{2}$ vol./day/tank volume

Solids basis—0.93 lbs. T.S./day/cu. ft. tank

Gas recovery:

Volume basis—7 vol. of gas/day/unit tank vol.

Solids basis—7.5 cu. ft. gas/lb. T.S.*

Rate of feed:

Volume basis—% vol./day/tank volume

Solids basis—0.62 lb. T.S./day/cu. ft. tank

Gas recovery:

Volume basis—4.2 vols. of gas/day/unit vol.

Solids basis—8.6 cu. ft. gas/lb. T.S.

Rate of feed:

Volume basis— $\frac{1}{6}$ vol./day/tank volume.

Solids basis—0.31 lb. T.S./day/cu. ft. tank

Gas recovery:

Volume basis—3.0 vol. of gas/day/unit tank vol.

Solids basis—9.7 cu. ft. gas/lb. T.S.

2. While fermenting corn

2.0% Total solids, 92% volatile

Rate of feed:

Volume basis— $\frac{1}{4}$ vol./day/tank volume

Solids basis—0.33 lb. T.S./day/cu. ft. tank

Gas recovery:

Volume basis—3.7 vol. of gas/day/unit tank vol.

Solids basis—11.0 cu. ft./lb. T.S.

B. Heinz Distillery Waste

1. 7.1 Total solids, 96% volatile pH 4.1

Feeding rate:

Volume basis— $\frac{1}{28}$ vol./day/unit tank vol.

Solids basis—0.16 lb. T.S./day/cu. ft. tank

Gas recovery:

Volume basis—2.0 vols. of gas/day/unit tank vol.

Solids basis—12.5 cu. ft. gas/lb. T.S.

* T. S.—Total Solids.

- C. Buttermilk and Whey Waste
 1. 7.0% Total solids, 91% volatile pH 5.0 High in sugars
 Feeding rate:
 Volume basis—1/29 vol./day/tank vol.
 Solids basis—0.15 lb. T.S./day/cu. ft. tank
 Gas recovery:
 Volume basis—1.6 vols. of gas/day/unit tank vol.
 Solids basis—10.7 cu. ft. gas/lb. T.S.
- D. Artichoke Waste
 Rate of feed:
 0.095 lbs./cu. ft./day
 Gas recovery:
 Volume basis—0.81 vol. gas./day/unit tank vol.
 Solids basis—8.35 cu. ft. gas/lb. T.S.
- E. Extracted Chicory
 Rate of feed:
 0.156 lbs./cu. ft./day
 Gas recovery:
 Volume basis—1.64 vol. gas/day/unit tank vol.
 Solids basis—10.5 cu. ft. gas/lb. T.S.
- F. Sugar Beet Waste
 Rate of feed:
 0.078 lbs./cu. ft./day
 Gas recovery:
 Volume basis—0.96 vol. gas/day/unit tank vol.
 Solids basis—12.3 cu. ft. gas/lb. T.S.
- G. Packing-house Paunch Manure
 Rate of feed:
 0.37 lb./cu. ft./day
 Gas recovery:
 Volume basis—3.1 vol. gas/day/unit tank vol.
 Solids basis—8.95 cu. ft./lb. T.S.

TABLE LXXXIV—PARTIAL ANALYSIS OF CITRUS PULP

	Per cent*
Ash	0.46
Ether extract	0.38
Crude fiber	0.84
Protein	1.28
Carbohydrates	9.38
Organic nitrogen	0.197
Kjeldahl nitrogen	0.198
Total nitrogen (salicylic)	0.208
pH	4.0
Moisture	85.5

* All of above data are on a wet basis.

TABLE LXXXV.

ANAEROBIC DIGESTION OF CITRUS PULP.

Bottle No.	1	2	3
Time, days	180	180	180
Weight of pulp added:			
(a) Wet (grams)	89	133	273
(b) Dry (grams)	10	20	40
Volume of gas evolved (cc.) N. T. P.	7,045	12,955	7,105
Volatile acids as acetic at end (p. p. m.)	90	90	4,880
pH at end	6.0	6.0	5.0
Per cent digested	84.0	73.0	

Table LXXXV summarizes three batch experiments with citrus pulp. In our experience batch fermentation requires about ten to twenty times as long a period for a given degree of gasification as is needed when the material is fed continuously in controlled amounts. It should be possible to treat citrus pulp on a large scale at about the rate that proved satisfactory with paunch manure.

EFFLUENT QUALITY.

The degree of purification accomplished varies from 75 to 90 per cent. Since these raw materials frequently have B. O. D. values ranging from 15,000 to 35,000 p.p.m. the effluent B.O. D. may run from 1,000 to 3,000 p.p.m. Aerobic methods may be applied to the effluent where necessary. Naylor, more than thirty years ago, showed that even a short septic action treatment of such wastes previous to trickling filters was highly advantageous. The volume of these wastes is so small in proportion to the clean water discharge that the dilution available from this source may be sufficient in some cases. Distilleries, for example, discharge about six times as much cooling water as "beer-slop."

In some cases with which we are familiar the domestic sewage flow is ten to twenty times the volume of heavy industrial wastes. With preliminary anaerobic treatment the effluent could be discharged into the sewers without producing an abnormal industrial load.

COMPARISON OF COSTS.

In so far as the anaerobic method is applicable it is decidedly the simplest and cheapest method of converting organic matter into in-offensive materials. Even in the conventional type of sewage treatment plant it is in the sludge digestion tank that 60 to 70 per cent of the organic matter is finally stabilized, the end products being insoluble combustible gas and humus.

A comparison of costs per pound of matter stabilized was worked out by the writer and Dr. C. S. Boruff for creamery wastes⁴³. The figures are worthy of serious consideration.

On the basis of an average feeding of one twenty-fifth of a volume of milk waste (undiluted basis) per day per tank volume, would require 5.72 cubic feet of tank capacity for the anaerobic fermentation of one pound (dry weight) of milk waste solids. At 50 cents per cubic foot, this amounts to only \$2.86 tank cost per pound of milk solids treated per day. This fermentation would remove at least 95 per cent of the pollution load. The remaining 5 per cent contained in the overflow liquor could be stabilized readily on filters. Assuming that this final treatment could be made at a cost similar to that given for filter treatment by various investigators, the total investment for complete treatment would be \$8.70 per pound of solids if trickling filters were used following the anaerobic digestion, or \$17.46 per pound if sand filters were used, as compared with \$116.80 per pound if trickling filters were used alone, or \$292 per pound if sand filters only were used. The above figures are not given to show actual costs but rather relative costs of the two processes.

RECOVERY OF STOCK FOOD.

It is not within the scope of this paper to discuss the evaporation of wastes for stock food recovery. It is doubtful whether this can be done with profit unless the solids run higher than 7 per cent. We are certain that with 1 to 3 per cent solids the net profit is in favor of anaerobic fermentation.

As a waste disposal process evaporation is not a complete solution to the problem since the "carry-over" from the evaporators amount to 400 to 1,000 p.p.m.

The data on which this paper is based were taken from publications prepared with the writer's former collaborator, Dr. C. S. Boruff.

Acknowledgment—The above paper was presented before a joint meeting of the California Sewage Works Assn. and the Public Health Eng. Section of the American Public Health Assn. at Pasadena, Calif.

COMPLETE TREATMENT OF DISTILLERY WASTES.*

A. M. BUSWELL AND M. LEBOSQUET,
State Water Survey, University of Illinois, Urbana, Ill.

Pilot plant results confirm laboratory data on the purification of distillery wastes by anaerobic fermentation. The effluent from anaerobic fermentation when diluted 1 to 5 with trickling filter effluent can be successfully stabilized on a trickling filter at the rate of 250,000 gallons of the undiluted digestion liquor per acre per day. The high nitrate content in the recirculated filter effluent prevents odor nuisance. The sludge is small in amount and contains the phosphate of the grain as magnesium and calcium ammonium phosphate.

Anaerobic fermentation under especially controlled conditions has been recommended for the treatment of heavy wastes such as distillery slop^(24, 43, 146), milk and whey^(11, 153, 200) packing-house wastes, etc.^(21, 26).

This process has two limitations, as previous authors have pointed out. Anaerobic fermentation cannot be economically used with wastes containing less than about 1 per cent dry weight of digestible organic matter. When the wastes contain 2 per cent or more, the methane recovered will carry most or all of the cost of treatment. In some cases this limitation of the process can be overcome by suitable changes in plant operation to avoid diluting the wastes with clean water (e. g., cooling water).

The second limitation of the process is that, although anaerobic treatment is capable of removing 80 to 90 per cent of the organic matter in the wastes, the remaining liquor is still about ten times as strong as domestic sewage. In many cases this degree of purification is sufficient—for example, where the effluent can be discharged into a sewer or large stream or where five or six volumes of cooling or condenser water are available for dilution. Where complete purification (i. e. to less than 100 parts per million B. O. D.) is required, some other method of treatment must be applied.

Early in the summer of 1935 the authors were asked to set up and operate a pilot plant to determine whether anaerobic digestion plus some subsequent treatment could be used for complete treatment of distillery wastes. Numerous earlier experiments with various chemical

* Reprinted from Industrial and Engineering Chemistry. Vol. 28, Page 795. July, 1936.

coagulants (not reported) had failed to produce any substantial improvement in the liquid remaining after anaerobic digestion. Since Naylor⁽²⁰⁸⁾ had shown that similar wastes after septic action could be treated on aerobic bacterial filters and Hoover⁽¹²⁹⁾ had successfully treated diluted wastes on trickling filters, this form of secondary treatment was chosen.

DIGESTION TANKS.

The major units of the demonstration plant, as shown on Figure 23, consisted of two steel digestion tanks, *A* and *B*, and one tank, 3, in which was located a trickling filter. Each of the digestion tanks, *A* and *B*, was 9 feet in diameter and 7.5 feet deep, with a capacity

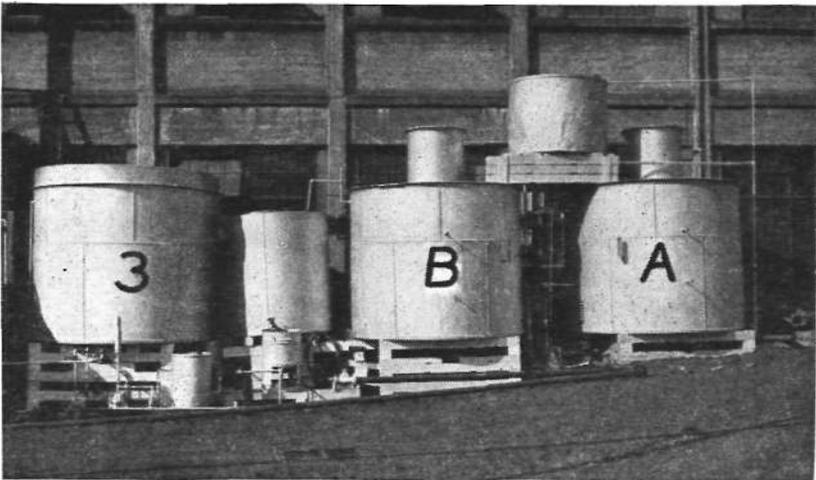


FIGURE 23.

Units of the Demonstration Plant.

of 3,600 gallons, making a total capacity of 7,200 gallons or 960 cubic feet. These tanks were already available and required considerable adaption in order to serve their purpose. Steam coils were placed in the center of the tanks at the bottom, and covers, with gas domes 3 feet in diameter and 3 feet high, were welded in place over the tanks.

The process consisted essentially of passing the slop through two digestion tanks in series and applying the digested effluent, diluted with a part of the finally purified waste, to a trickling filter.

The flow through the digestion tanks was as follows (Figure 24) :

The raw thin slop was pumped to the cooling and feed tank, 1. Although provision was made in this tank for cooling the slop to the proper temperature (130° F.), the cooling facilities were seldom used. The heat of the slop could ordinarily be utilized in heating the first tank, *A*. After a dose had been measured and sampled, it was allowed to flow into the primary digestion tank, *A*.

This tank was heavily loaded, accomplishing the greater part of the digestion and yielding most of the gas generated. From tank *A* the partially digested slop was forced by succeeding doses to secondary digestion tank *B* where a further digestion at a decreasing rate was accomplished.

A back-circulation pump, 5, made it possible to return liquid from secondary tank *B* to primary tank *A* for seeding and dilution. This is an important control measure, which, in conjunction with other control tests, made it possible to place on the primary tank, loadings of the order of ten times those used in common sewage-sludge digestion practices.

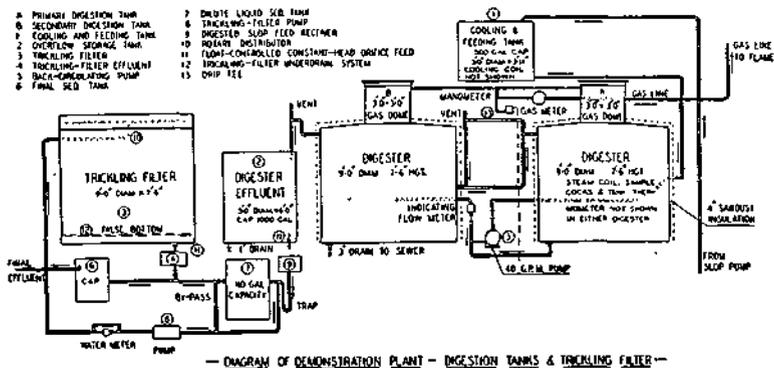


FIGURE 24.

A 1,000-gallon, digested liquid overflow tank, 2, made it possible to place the full 24-hour load on the filter in 8 hours, or during one shift. The trickling filter, 3, which required little or no attendance, was operated at a constant rate over the 24 hours; the necessary equalizing storage was furnished by overflow tank 2.

TRICKLING FILTER.

Dilution of some sort had to be provided in the design of trickling filter 3 in order to reduce the oxygen demand of the liquid supplied to the filter. If, at the same time, this dilution could result in a reduction of the odors, the filter could be operated without causing a serious nuisance.

Trickling filter 3 was constructed in a circular steel tank. The stone bed was 9 feet in diameter and 7 feet deep. Although the filter was of an experimental nature, its size would be sufficient for a small dairy and could almost be considered a plant-scale demonstration.

The effluent from trickling filter 3 was used to dilute the digester effluent fed to filter 11. This idea was used elsewhere in treating a concentrated industrial waste⁽²⁰⁸⁾. In order to accomplish the desired result, the effluent from the trickling filter was discharged into a small

box, 4, with two outlets. One outlet led to a final sedimentation tank, 6, the overflow from which was the final effluent. The other outlet led to a diluting liquid sedimentation tank, 7. The discharge line from this tank was conducted to the suction of trickling filter pump 8. Also connected to this suction was the digested distillery slop feed line, 9. By this arrangement pump 8 dosed filter 3 at a rate equal to a rate of raw feed, plus a quantity of trickling filter effluent. For example, when pump 8 was operated at a rate of 2 gallons per minute and digested distillery slop (2 and 11) was fed at a rate of 0.5 gallon per minute, the pump automatically made up the difference (1.5 gallons per minute) from diluting liquid sedimentation tank 7. With the arrangement used, feed 11 could be turned off entirely, in which case the pump would take its entire pumpage from diluting liquid sedimentation tank 7. A closed system would then result, the trickling filter effluent being pumped in its entirety back onto filter 3 again and again.

Sedimentation tank 7 was installed for the purpose of removing settleable solids from that portion of the trickling filter effluent which was used for deodorizing and diluting the feed of digested distillery slop. This was important in reducing the amount of suspended matter put on the trickling filter. As a consequence no troubles due to filter clogging were encountered.

Other details of the design included a distributor of the rotary type, 10, and an orifice, 11, to accomplish a constant rate of feed of digested liquid. A wooden false bottom, 12, was constructed in trickling filter tank 3 as an underdrain system. Adequate vents were supplied. The filter material used was 1.5-3 inch blast furnace slag.

The 9-foot trickling filter had an area of 63.6 square feet or 0.001462 acre. A rate of 1,000,000 gallons per acre daily, therefore, would be 1,462 gallons daily, or slightly over 1 gallon per minute.

DIGESTING TANK LOADINGS.

Difficulties were encountered during the early stages of the demonstration in obtaining high rates of feed on the digestion tanks. Since the digestion tanks were placed in operation less than 2 weeks after the decision to proceed, there was insufficient opportunity of preparing adequate seeding material. As a consequence it was necessary to use digesting sludge from a sewage treatment works for this purpose, where the digestion takes place at ordinary sewage temperatures, whereas the slop was digested within the thermophilic range or at 130° F. The first four weeks of operation elapsed before the sludge was conditioned sufficiently for appreciable amounts of slop to be fed. After this time (September 10) the feed had been increased to 100 gallons per day. This greater feed was continued until September 20 when it was possible to increase the feed until, by September 29, feed had been increased to 250 gallons per day.

At this point both of the tanks were covered with a proper insulating material to reduce the heat loss and, therefore, greatly reduce the number of times steam had to be applied. A 4-inch layer of sawdust was used. It was then possible to increase the feed further until, on

October 5, 350 gallons per day were being fed. This dose was maintained until October 17, at which time the volatile acids had risen so high that it was necessary to curtail the feed until, on October 24, only 100 gallons per day could be fed.

In previous experiments the sludge occasionally compacted during the earlier stages and required agitation until the fermentation was well established. Accordingly, arrangements were made to increase the circulation and agitate the sludge from time to time.

Following the agitation, it was possible to increase the feed from 100 to 550 gallons per day in the course of 6 days. This rapid rate of increase is significant as indicating the rate at which it is possible to start the tanks when the right type of installation is available.

GAS PRODUCTION.

The gas production confirmed results of previous laboratory-scale work and the work on a larger scale at the sewage treatment works of Peoria Sanitary District⁽¹⁵³⁾. A uniform rate of 11 cubic feet of gas was obtained per pound of volatile solids fed to the tank.

ANALYTICAL DATA.

It was not possible, with the personnel and equipment available, to make complete daily analyses. A large number of samples were analyzed at the plant, and several samples were brought to Urbana for check analyses. The raw screened slop ran from 3 to 4 per cent solids (80 per cent volatile) with a B. O. D. of 15,000 to 16,000 p.p.m. and an organic nitrogen content of 1,900. The effluent from the digestion showed a B. O. D. of 1,500 to 2,000 p.p.m. The effluent from the trickling filter, while operating at a rate of 250,000 gallons per acre per day (based on the undiluted digester effluent fed), had a B. O. D. of 138 p.p.m. (average of sixteen field samples). The four samples of trickling-filter effluent brought to Urbana for analysis showed B. O. D. values of 39, 98, 80, and 104 p.p.m., respectively. These samples showed nitrate nitrogen values ranging from 60 to 820 p.p.m. and nitrite nitrogen from 25 to 150 p.p.m. Tightly stoppered samples showed no putrefaction after several weeks. Kiby⁽¹⁴⁶⁾ reported 200 p.p.m. nitrate nitrogen and 70 p.p.m. nitrite nitrogen in the effluent from a trickling filter treating similar wastes.

ODORS.

Anaerobic fermentation always results in production of hydrogen sulfide and other odorous compounds. In this installation the escape of odors is avoided by completely sealing the fermentation tanks. The gas which carries the odors is burned for heat or power. Odors from the digester effluent as it discharged to the trickling filter were anticipated. However, as soon as nitrification had become established, the high nitrates in the recirculated filter effluent stabilized the digestion effluent so that no odor was produced when the usual 4 or 5 to 1 mixture of

the two liquids was sprayed onto the trickling filter. This stabilizing effect of nitrates has been known for many years and was once suggested as a means for treating raw sewage⁽¹⁶⁷⁾. An analytical method for determining oxygen demand by means of nitrates was once in

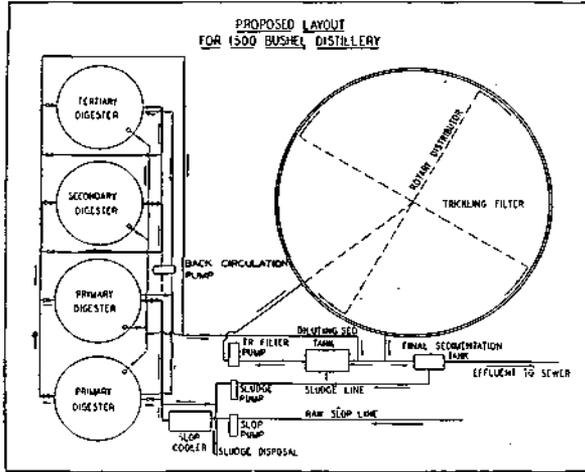


FIGURE 25.

use⁽¹⁶⁸⁾. This early work, together with the results of the present experiment, amply justify the conclusion that this process can be operated without odor nuisance.

SLUDGE.

Since the fermentation resulted in 90 per cent gasification, the amount of sludge produced was relatively small. Some sludge was carried over with the effluent so that actual accumulation of sludge was relatively negligible when compared with the sludge from other types of material. No accurate measure could be made within the period of this experiment. Practically all of the phosphorus of the grain was recovered as magnesium and calcium ammonium phosphate. One sample of sludge showed 3.3 per cent phosphorus and another 4.2 per cent on a dry basis.

VI. COST CONSIDERATIONS.

It is obvious from the data presented in this bulletin that a wide variety of materials can be fermented anaerobically to produce a gas of high fuel value. Such a process may be of importance for two purposes; first, for the production of fuel gas, and second, for the disposal of waste. Whether or not anaerobic fermentations are to be of any practical importance depends on the cost of the process as compared with other processes.

Inasmuch as the economics of the situation vary widely, depending on whether the fermentation is intended primarily for the production of fuel or for the disposal of waste, the two will be discussed separately.

PRODUCTION OF FUEL GAS.

The cost of gas produced by anaerobic fermentation will necessarily vary with the cost of the material fermented. This, in turn, will vary according to the location of the plant and the availability of cheap, easily fermented materials. In most cases it should be possible to use very cheap materials, including waste and organic matter which would otherwise not be utilized. It will be readily realized that the amount of waste cellulosic material in the form of stems and leaves of crop plants and cuttings such as sawdust shavings and brush from the production of lumber is very considerable, and that if it can be made to serve as a source of energy enough power can be produced to have a real effect on the world's power bill. Boruff and Buswell calculated that a ton of cornstalks will yield from 10,000 to 20,000 cubic feet of gas. Taking the lower figure, a ton of cornstalks would furnish gas for 400 people for one day, allowing 25 cubic feet per capita per day. From the data given by Webber⁽³⁸²⁾, for yields from regions where 30 per cent of the land is planted to corn, a circle with an 8-mile radius would produce enough cornstalks to supply a city of 80,000 inhabitants with gas. The residue after fermentation would be suitable for paper-making.

It is believed that small gas-producing units would be quite satisfactory for farms and ranches. Cornstalks, straw, manure, and other waste could be fermented. However, a more satisfactory procedure would be to ferment ground grain, cull fruit, and other materials which leave little or no residue upon digestion. This would obviate the necessity of removing bulky residues, and the unit would require very little attention.

An estimate has been made of the cost of a fuel gas plant capable of producing one million cubic feet of gas per day. The results are summarized below. Only the cost of production is considered, the cost of raw material being omitted from this estimate. It is to be noted that the following estimate is prepared on the basis of gas production. In other parts of the report data are given on the cost of using the process for stabilization of industrial wastes.

COST OF FUEL PLANT.

1,000,000 cu. ft./24 hours.

INVESTMENT.

	Unit price.		
Digestion Tanks:			
12—300,000 gallon steel tanks with fiat bottom and gas tight roof, erected.....	\$350	\$52,200	
Foundations.....		5,800	
Distribution manifolds in tanks, installed.....		2,400	
			\$60,400
Pumps:			
2—4-inch beer slop pumps with control installed.....	\$610	\$1,220	
2—6-inch recirculation pumps with control, installed.....	530	1,060	
2—4-inch cooling water pumps with control, installed.....	560	1,120	
			3,400
Coolers and Connections:			
3-inch x 2-inch double pipe cooler, 2,700 sq. ft. cooling surface.....		\$12,150	
Valves and piping.....		2,420	
Erection.....		3,430	
			18,000
Valves and Piping:			
For beer-slop, installed.....		\$4,600	
For sludge, installed.....		2,000	
For gas, installed.....		3,200	
			9,800
Gas Regulation and Meters.....		4,000	
Pump House and Pit.....		3,600	
Total.....		\$99,200	
Contingencies, Patents, Engineering and Construction Supervision (21%).....		20,800	
Total.....		\$120,000	
OPERATING COSTS.			
Daily Fixed Charges:			
6% daily.....			\$23.20
Land.....			
Additional piping.....			
Depreciation:			
Tanks, 5% (\$92,200 at 5%).....			\$14.80
Piping, 5%.....			
Pumps, 10% x 3,400.....			1.00
Pump house and pit, 5% x 3,600.....			.50
Total daily depreciation.....			\$16.30
Taxes*.....			
$99,200 \times 3.85 \times \frac{1}{100} = 1,909.60$ daily.....			\$6.20
Land.....			
Additional piping.....			
Insurance:			
Fire at 25 cents per \$100.....			
Tornado at 11 cents on \$99,200.....			\$0.28
Liability.....			.36
Total daily insurance.....			\$0.64
Attendance Labor:			
3 men at 50 cents per hour.....			\$12.00
Supervisor.....			2.00
Total daily attendance labor.....			\$14.00
Factory Overhead—10% Operating Costs:			
Labor.....	\$14.00		
Steam and water.....	very slight		
Power pumps.....	26.88		
	\$40.88 x 1/10 = daily		\$4.09
Maintenance:			
2% of initial cost—\$99,200.....			\$6.40
Daily Costs—100% Average Production:			
Cost of capital.....			\$23.20
Depreciation.....			16.30
Taxes.....			6.20
Insurance.....			.64
Attendance labor.....			14.00
Factory overhead.....			4.09
Power.....			26.88
Maintenance.....			6.40
Total.....			\$97.71
Income:			
1,000-M. cu. ft. gas (550 B. t. u.) at 10 cents.....			\$100.00
Profit.....			\$2.29

The cost of producing the gas is seen to be approximately ten cents per 1,000 cubic feet, excluding the cost of the raw material.

If crop surpluses make it desirable to use corn as a source of fuel, it would be more economical to convert it to methane than to ferment it to alcohol. This is readily demonstrated by the figures in Table LXXXVI.

TABLE LXXXVI.

Cost per Million B.t.u. from Shelled Corn

As Methane

1 pound of corn yields 12 cu. ft. gas, 53% CH ₄	
1 ton of corn yields 24,000 cu. ft. gas, 530 B.t.u. value =	
12,700,000 B.t.u./T.	
Cost of anaerobic fermentation, at 10c/1,000 cu. ft.=	
\$2.40/T.	
Exclusive of corn	= \$0.19/million B.t.u.
Plus corn at 25c per bu.	= 0.90/million B.t.u.
Plus corn at 50c per bu.	= 1.62/million B.t.u.

As Alcohol

1 bu. of corn yields 2.5 gal. (16.6 lbs.) of alcohol =	
592 lb./T.	
Assume 11,600 B.t.u./lb.	
1 ton of corn	= 6,800,000 B.t.u./T.
Assume manufacturing cost of 10c/gal.	
Cost of producing alcohol	= \$9.00/T.
Exclusive of corn	= 1.30/million B.t.u.
Plus corn at 25c per bu.	= 2.64/million B.t.u.
Plus corn at 50c per bu.	= 4.00/million B.t.u.

Although methane produced from corn has an economic advantage over alcohol as a fuel, it does not enjoy a similar advantage over other fuels. A comparison of the costs of various fuels shows that other fuels are cheaper at present. (Table LXXXVII.)

TABLE LXXXVII.

Comparative Costs of Various Fuels

Fuel	Cost per million B.t.u.
Soft coal (12,000 B.t.u.) at \$1.50 per ton.	\$0.062
Natural gas (1,000 B.t.u.) at 15c per 1,000 cu. ft.	0.150
Alcohol, from corn at 25c per bu.	2.640
Digester gas, from corn at 25c per bu.	0.900
Digester gas, from waste (zero cost).	0.190

Whereas the digester gas made from corn is more expensive than coal or natural gas, the gas produced from waste materials which are readily available at no cost compares rather favorably in cost with natural gas. A gaseous fuel always has a distinct advantage over a solid one, such as coal. It is not difficult to believe that digester gas will become economically more important as our natural deposits of fuels are depleted.

WASTE DISPOSAL.

The economics of anaerobic digestion as a means of waste disposal presents a somewhat different situation. Here the primary purpose is to dispose of offensive organic materials which might otherwise create a serious problem. To be of value the anaerobic method must be as economical as other waste treatment methods of equal efficiency. Actually, it is often a much cheaper method of treatment, as the fuel gas recovered is a valuable by-product. With certain types of wastes, enough gas is obtained to effect a fuel saving greater than the cost of treatment, thus showing a profit on the process.

Milk Waste.—Assuming that anaerobic digestion tanks can be built and installed complete for 50 cents per cubic foot and figuring 12 per cent for interest, amortization, and repairs, such tanks cost 16.4 cents per thousand cubic feet of volume per day. On the basis that raw, unsettled, and untreated buttermilk and whey wastes can be fed at rates of from one twentieth to one thirtieth the fermentation tank volume per day (2.3 to 3.6 grams per liter) and produce 1.6 to 2.7 volumes of gas per day per tank volume, gas could be produced at costs ranging from 6.1 to 10.3 cents per thousand cubic feet. As compared with other methods of gas production or transportation, this is a moderate cost.

On the basis of an average feeding of one twenty-fifth of a volume of milk waste (undiluted basis) per day per tank volume, it would require a tank, or tanks if operated as a two-stage process, of 5.72 cubic feet capacity for the anaerobic fermentation of one pound dry weight of milk waste solids. At 50 cents per cubic foot, this amounts to only \$2.86 per pound of milk solids treated. This fermentation would remove at least 95 per cent of the pollution load. The remaining 5 per cent contained in the overflow liquor could be stabilized readily on filters. The total investment for complete treatment would be about \$8.70 per pound of solids if trickling filters were used following the anaerobic digestion, or \$17.46 per pound if sand filters were used, as compared with \$116.80 per pound if trickling filters were used alone, or \$292 per pound if sand filters were used. The above figures are not given to show actual costs but rather relative costs of the two processes.

Thus the total treatment plant cost of anaerobic fermentation followed by aerobic filtration is only a small percentage of that reported⁽¹⁴⁷⁾ for the treatment of milk wastes by standard present-day methods, which, incidentally, are merely treatment methods and give no valuable by-product. Anaerobic fermentation of milk wastes followed by secondary treatment—namely, filtration—will not only give efficient stabilization but will also produce 8.3 to 12.4 cubic feet of gas per pound of dry solids added.

Distillery Wastes.—As has been pointed out elsewhere in this bulletin, the wastes from distilleries are typical of a rather large group of industrial wastes. Therefore, the cost of treating such wastes should be a good guide to the cost of treating any of a large variety of similar wastes. Accordingly, an estimate of the cost of treating beer slop has been carefully worked out and is summarized below.

These wastes are discharged at or near the boiling point and must be cooled to 50°-55° C. before fermentation is possible. For this reason

Cost of Gas Based on Operation Costs and Replacement of Plant
in Five Years.

Equipment costs per day.....	\$4.39
Operation, labor.....	1.20
Power, heat, water, etc.....	very small

Total.....\$5.59

51 M cu. ft. of gas per day for \$5.59 or 11 cents per M.

The above estimates apply to large plants located on large streams. Where complete treatment is required trickling filters will be needed. The following data are reproduced from a report on the cost of a plant for a small distillery located on a small stream.

DISTILLERY WASTE TREATMENT PLANT.

Anaerobic Fermentation
and

Trickling Filter.

(Based on 1,500 bu. Grind / Day.)

ESTIMATE OF COST.

	Unit price.	Total.
4—380,000 gallon steel tanks with flat bottom and gas tight roof, erected.....	\$4,200	\$16,800
4—Agitators, installed.....	1,700	6,800
20,140 sq. ft. heat insulation, installed.....	.40	8,100
4—Heating coils, installed.....	750	3,000
		<u>\$34,700</u>
Pumps:		
2—70 g. p. m. beer-slop pumps with float control, installed.....	\$170	\$340
2—200 g. p. m. trickling filter pumps with manual control, installed.....	220	440
2—250 g. p. m. back circulation pumps with manual control, installed.....	220	440
2—70 g. p. m. heating water pumps with manual control.....	140	280
1—20 g. p. m. open impeller sludge pump with manual control, installed.....	140	140
		<u>\$1,640</u>
Valves and Piping:		
For beer-slop, installed.....		\$1,500
For sludge and drains, installed.....		1,100
For gas, installed.....		1,000
For heating, including automatic control, installed.....		1,300
		<u>\$4,900</u>
Slop Cooler and Connection.....		1,900
Water Heater and Control.....		1,200
Gas Regulation and Meters.....		2,000
Pump House and Pit.....		1,000
		<u>\$47,340</u>
Total.....		9,470
Contingencies, Patents and Engineering (20%).....		
Total digestion equipment.....		\$56,810
Trickling Filter—Complete.....	\$26,400	
Contingencies and engineering (15%).....	3,960	
Total trickling filter.....		<u>30,360</u>
Grand total.....		<u>\$87,170</u>

The fermentation of grains to produce butanol and acetone is carried on under somewhat different conditions than exist in the alcohol fermentation, principally in respect to mash concentrations. The resulting beer-slop is therefore more dilute. Other differences especially in

acidity are encountered. For these reasons a cost calculation based on this particular waste is given below.

CALCULATIONS ON TREATMENT OF BEER-SLOP FROM
BUTANOL-ACETONE FERMENTATION BY ANAEROBIC
FERMENTATION.

All data on basis of 1,000 bu. grind of corn per day.

Volume of waste: 68,000 gallons

Total Solids: 11,300 lbs. or about 2.0%

Unscreened Waste

11,300 lbs. solids fed at rate of 0.25 lbs. solids per day
per cu. ft. of tank capacity

11,300

 = 45,200 cu. ft. of tank

0.25

Cost of tank at \$60 per M cu. ft.

46 M x \$60 = \$2,760

A 46 M cu. ft. tank would give a 5-day detention period.

Gas Produced:

11,300 lbs. solids at 10 cu. ft. per lb. = 113 M cu. ft.
of gas per day per 1,000 bu. corn ground.

Methane content of gas = 58%.

TOTAL INSTALLATION COST OF DIGESTION TANKS
TO HANDLE WASTES FROM 1,000 BU. CORN
GRIND PER DAY.

Unscreened Waste

Digestion tanks	\$2,760
(Erected)	
2 pumps with motors	500
Installation	25
Pipes and valves	750
Labor	400
Foundations and labor	400
Electric equipment	50
Labor	15
Pump house	75
Labor	50
Painting	75
Screen to shut out sun's rays (heat) if necessary	
Omissions and contingencies (5%)	255
Total	\$5,355

Daily Costs—Retirement in 5 Years

\$5,355

 = \$3.68 per day

4 x 365

Operation Costs:

3 shifts of 2 men each could take care of 20-25
M bu. disposal plant. Total labor at 50 cents per
hour = \$24 per day.

24/20 M bu. = \$1.20 per day per M bu.

Power costs very low. Using condenser water for
cooling if necessary.

Tanks \$3.68 per day

Labor 1.20

Total \$4.88 per day per M bu.

Gas Costs:

113 M cu. ft. of gas for \$4.88 = 4.32 cents per M
cu. ft.

CONCLUSION.

Under the present economic conditions, fuel gas produced by anaerobic fermentation is too expensive to compete with other kinds of fuel when the raw material must be purchased or transported any distance.

As a method of waste disposal, anaerobic digestion is quite economical because of the gas which is produced as a by-product. Under good conditions the process can be made to pay for itself.

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