The butyl-acetonic fermentation of the Jerusalem artichoke

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UMI®
THE BUTYL-ACETONIC FERMENTATION OF THE
JERUSALEM ARTICHOKE

by

Ray T. Wendland

A Thesis Submitted to the Graduate Faculty
for the Degree
DOCTOR OF PHILOSOPHY
Major Subject Biophysical Chemistry

Approved:

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INTRODUCTION

Of the common fermentations which have as their object the production of pure chemicals, the alcoholic fermentation and the butyl-acetonic have assumed positions of major significance in commerce. The tremendous development of the latter took place during the World War. At that time the British munitions industries found that they could not obtain sufficient acetone for the manufacture of Cordite, the favorite high explosive in the British ordnance, from the old processes of wood distillation, and pyrolysis of acetic acid in the presence of calcium hydroxide. The specter of shortage in this vital substance forced the government to cast about for any possible source of the necessary raw material. The butyl-acetonic fermentation was known to the bacteriologists, and a plant had just been erected (1913) to use the process, but no significant quantities of pure chemicals had yet been produced. But shortly after the British began their large scale military operations, an army of chemists and chemical engineers was commissioned by the government to investigate the process and to make possible the production of the huge quantity of acetone necessary. (Gabriel 1928) The result was that great plants were erected in England, Canada, India, and in the United States
for the butyl-acetonic fermentation of corn mash, which was found to be a highly satisfactory material.

Since the quantity of butyl alcohol produced was twice that of the acetone, and there was no immediate use for this product, a tremendous amount accumulated. Although at the time a burden, this accumulation became a source of great profit to the owners, following the development of the nitrocellulose spray lacquers in 1921. Volatile lacquer solvents were needed in great quantities and at low cost. Butyl acetate and other derivatives were used in train lots to supply the demand, and as soon as the war time excess was consumed, the butyl-acetonic fermentation was revived and brought to a new peak of development. Besides the liquid products, the hydrogen and carbon dioxide produced were recovered and converted to methanol to supply the ever increasing demand for a variety of volatile solvents. The war time operations and most of those following were covered by the Weizman patents. (1915, 1919, 1922). However, with the technological changes in the automotive industry and the economic collapse of 1929, the acetone-butyl alcohol producing industry suffered a severe setback. Recently its chief representative, the Commercial Solvents Corporation, has made a substantial recovery, and there is reason to believe that the butyl-acetonic fermentation will continue to be of considerable economic importance.
The best medium for the growth of the butyl-acetone organisms is gelatinized corn mash, and this has been used almost exclusively in commercial practise. The rapidity of the transformations brought about in this substrate by the organisms has made it seem likely that other carbohydrate sources should also be fermented with equal ease. However, this has been found not to be entirely true, the difficulty seeming to be that the organisms are very particular in their protein requirements. If the proper proteins or amino acids are lacking, the organisms will produce sour fermentations, if growth takes place at all. The acids formed are principally acetic and butyric, and these accumulate at the expense of the normal production of neutral solvents.

The problem of protein nutrition of the butyl organisms has been studied by Weinstein and Rettger (1933). They extended Robinson's study of carbohydrate utilization in synthetic sugar media to include an investigation of production of individual solvents. They found that in these media, containing a wide variety of sugars and peptone as the nitrogen source, acetone was always produced in amounts proportional to the consumption of sugar. But butyl alcohol was consistently low or absent. By supplementing these sugar media with various vegetable proteins, Weinstein and Rettger showed that the proteins belonging to the class of prolamines were necessary in the metabolism of the butyl acetone organisms if they are to produce butyl alcohol.
However, these results have not been substantiated by other workers, and the supposition was made that some other solvent stimulating factor was responsible for the variation in the results (Reynolds, Werkman, and Coile, 1934).

But with this information at hand one may proceed to supplement various carbohydrate sources in order to produce a medium satisfactory for the butyl-acetonic fermentation. A variety of sugar containing materials has been subjected to this fermentation with varying degrees of success. In Japan Tsuchiya (1932) found that molasses could not be fermented directly using a culture of Bacillus granulobacter pectinovorum. Fermentation did take place, however, after the addition of soy bean meal or soy bean cake. Arroyo (1935) in Puerto Rico isolated an organism from the roots of the sugar cane which produced satisfactory fermentation of molasses. Other reports concerning the fermentability of molasses by the butyl-acetone organisms may be found. The Jerusalem artichoke because of its high potential levulose content has been the object of several investigations. Thaysen and Green (1927) used the juice from the tubers in attempts to produce satisfactory yields of solvents. They found that the bulk of the carbohydrates present could not be fermented directly by the butyl-acetone organisms. But they report "a yield of 12 gals. of 'oil' or more per ton of fresh tubers can be obtained after hydrolysis and the mash is diluted to such an extent that the
reducing sugars can be prevented from exerting their inhibitory actions on the organisms and their enzymes.

The objections that arise in regard to this report are that no details whatever are given for the method of solvent analysis, or the separation of oil, including the determination of the individual components. The yield on the ton basis is 12 gals., and since the specific gravity of the solvent mixture (six parts butanol, three of acetone, and one of ethanol) is very close to 0.80, the weight yield is approximately 79 pounds. The fresh tubers may be assumed to contain 16 per cent available reducing sugar, hence the yield is only 25 per cent, which is quite low. In addition, no attempt was made to supplement the proteins present in the artichoke juices in order to increase the yield. Furthermore, the reason why the reducing sugars of the artichoke should exert any inhibiting effect upon the fermentation was not clear, since levulose makes up the bulk of the hydrolysis products. The fermentation of levulose in a semi-synthetic medium was shown by Underkoffler (1934) to give solvent yields of the same order as glucose and starch.

Reynolds and Werkman (1934) have also reported that the tubers of the Jerusalem artichoke are not readily available to attack by Cl. acetobutylicum,

In view of the facts that the levulose present in the hydrolyzates of the artichoke juices is a cheap source of fermentable carbohydrate, and that it should be easily possible to
provide the necessary protein supplement, further investiga-
tions in this field seemed to be in order. The primary purpose
of this investigation was to attempt to make the butyl-acetonic
fermentation of the Jerusalem artichoke a successful one. The
realization of this object seemed desirable, first, because fer-
mentation methods have shown great promise in the better util-
ization of the primary products of agriculture, and also in
the profitable conversion of agricultural wastes and by-pro-
ducts, and, second, because the great industrial importance of
the butyl-acetonic fermentation might be increased by the in-
roduction of another satisfactory raw material.

Accordingly, in carrying out the investigation it was
planned to include a study of the juices derived from the
tubers of the artichoke by various methods of treatment, and
then to develop a satisfactory artichoke substrate by the in-
clusion of various protein supplements which would insure the
maximum conversion of the levulose to the desired solvents.
HISTORICAL.

The butyl-acetonic fermentation is one that has attracted the interest of scientific investigators since the inception of organized bacteriology. Pasteur (1876) in his studies on the diseases of beer, observed the occurrence of butyl alcohol among the products formed by his butyric acid producing cultures to which he had given the name *Vibrio butyricus*. Prazmowski (1879), among the early workers, was concerned principally with the differentiation of the butyl organism from *Bacillus subtilis* on the one hand, and the various thermophilic cellulose fermenters on the other. By patient study of the morphological features of sporulation and regermination, he established the difference between *B. subtilis* and the butyl alcohol producing organism. In addition, he proved conclusively that his pure cultures of the latter would not attack cellulose, but that certain contaminants would give rise to a vigorous fermentation of cellulose, and readily outgrow the original butyl organisms. This fermentation in the presence of contaminants simulated that which was described by van Tieghem (1877) as a pure butyl alcohol producing fermentation. Thus Prazmowski invalidated any claims for the butyl organism as a cellulose fermenter.
In contrast to Frazmowski's methods were those of Fitz (1882) who was interested principally in the physiology of the transformations brought about by these butyl alcohol producing organisms. His contributions greatly extended the knowledge of the differentiation of these bacteria.

However, progress was slow in determining the chemical nature of the butyl fermentations. Iodoform producing substances were recognized, but acetone was not positively identified as a product of fermentation until 1905 when Schardinger proved its presence. Soon after ethyl alcohol was also shown to accompany the formation of butyl alcohol and acetone. In 1918 Fernbach and Strange patented the commercial use of one of their pure cultures. Since then numerous strains have been characterized, and scientific studies of the fermentation have been more prolific.

The organisms responsible for this fermentation yield two interesting products, butyl alcohol and acetone, in concentrations that would ordinarily be highly toxic to other common micro-organisms. The organisms show extraordinary proteolytic activity and the ability to bring about a very rapid hydrolysis of starch, without any appreciable accumulation of reducing sugar in the substrate. As might be expected from these properties, the organisms are markedly polyphagous, attacking many substances which would ordinarily be regarded as toxic end products, and transforming them into its own characteristic
products, butyl alcohol, acetone, and a small amount of ethyl alcohol.

These organisms have been isolated from the soil, cereal grains, etc., by various workers and developed to full activity by repeated subculturing in corn mash of about 5 per cent concentration, in which they propagate readily. They are persistent spore formers, and after sporulation the extraneous vegetative forms present may be killed off by "heat shocking", that is, immersion of the culture in boiling water for about two minutes. After cooling, the heat resistant spores will grow readily again in fresh corn mash. By this process the culture may be purified and its fermentative powers enhanced.

These organisms are classified by Bergey (1934) as Clostridium butyricum Prazmowski, and by Pribram (1933) as Clostridium saccharobutyricum. But a host of other names has been applied for their technical description, probably because of the failure of the workers concerned to characterize the organisms properly. Common in the literature are Bacillus granulobacter pectinovorurn, used by Speakman, Bacillus butylicus Fitz, Bacillus amylolacte van Tieghem, Granulobacter saccharobutyricum Beijerinck, Clostridium pastorianum Winogradsky, and Clostridium acetobutylicum. The last name was proposed by McCoy, Fred, Peterson, and Hastings, (1926) and it seems to enjoy the widest usage. Brown (1926) after
a thorough study of the butyric acid- butyl alcohol organisms, accepts *Clostridium acetobutylicum* as authentic. Those interested in the classification of these groups of bacteria should consult his thesis.

The butyl-acetone organism is anaerobic and Gram positive. Among the important physiological characteristics are the formation of acetic and butyric acids, (Reilly and Hickinbottom, 1919) butyl and ethyl alcohols, carbon dioxide, hydrogen, and small amounts of acetyl methyl carbinol (Wilson, Peterson, and Fred, 1927), from dextrose, levulose, maltose, sucrose, lactose, and starch in the presence of a suitable nitrogen source. It is non-pathogenic, and in the soil it appears to be able to fix small amounts of atmospheric nitrogen.

The fermentation in corn mash begins with a rapid proliferation of vegetative cell, and the typical changes in the substrate are soon manifested. Gas production is evident in three hours, and is sufficient in 14 to 20 hours to lift the suspended matter to the top of the vessel in the formation of the characteristic head. Hydrogen constitutes about 80 per cent of the gas evolved up to the sixth hour, after which carbon dioxide production becomes more rapid. At the end of the fermentation, the carbon dioxide evolved is 52.5 per cent of the total gas, hydrogen 47.5 percent (Speakman, 1920).

The starch present is hydrolyzed rapidly, but there is no
accumulation of reducing sugar in the medium because the sugar formed is rapidly converted to acids. Reilly (1919) found that soon after inoculation the ratio of acids is four to five parts $\text{AcOH} / 1$ part $\text{PrCOOH}$. At the period of maximum acidity the ratio has diminished so that $\text{PrCOOH} / \text{AcOH} = 1.1$ to $1.4/1$. There now occurs a rapid decrease in titratable acidity and the typical solvents begin to form. The organisms begin to lose motility and spindle shaped cells containing miniature endospores make their appearance. Gas evolution slows down but the production of solvents continues, and is complete after about 72 hours. Killefer (1927) and Gabriel (1928) describe the various plant operations involved in the commercial production of these solvents from corn. Coincident with the hydrolysis of the starch during the course of the fermentation is a very rapid degradation of the protein present (Peterson, Fred, and Domogalla 1924). The formation of the various protein cleavage products imparts to the solution sufficient buffering action so that the actual pH change is very small despite the large increase in titratable acidity. Two-thirds of the total conversion of protein to proteoses, peptones, and amino acids takes place in the period from the 12th to the 24th hour (Peterson and Fred 1932), and at the completion of fermentation practically no native protein remains.

Naturally, a certain amount of the amino acids is metabolized directly. Speakman (1926) showed that tyrosine is
deaminated within the cells, and the hydroxy derivative excreted. He believed that the ammonia catalyzed the oxidation of the simple carbohydrates after their absorption into the cell.

As mentioned previously, the principal products of this fermentation are butyl alcohol, acetone, ethyl alcohol, carbon dioxide, and hydrogen. In a study of the carbon balance, Reilly and others (1920) demonstrated that when starch is the only fermentable carbohydrate present in a medium that will support the normal activity of the butyl-acetone organisms, it alone is the source of the typical solvents. However, the mechanism of their formation from the intermediate reducing sugar is not well understood. It is certain that the quantity of free dextrose present is very small in the fermentation of starch, although when dextrose is added to a fermenting mash, it is consumed slowly, but completely. It is probable then that the organisms hydrolyze starch continuously and utilize the dextrose produced immediately.

In a comprehensive study of carbohydrate metabolism, Robinson (1922) demonstrated that the organism produces three exocellular hydrolytic enzymes, maltase, inulinase, and amylase. In addition it retains within the cell wall, sucrase and lactase. The medium which was employed for the study was made up of the inorganic salts, potassium acid phosphate, magnesium sulfate, sodium chloride and ferrous sulfate,
plus Bacto-peptone, and the carbohydrate which was under investigation.

Of the monosaccharides studied, dextrose, levulose, and mannose were utilized completely and produced acidity changes in the medium that were comparable to those observed in the maize mash fermentation.

The fermentation of galactose was incomplete. A high acidity was produced, and there was no appreciable decline from the acidity peak. It should be noticed in this connection that galactose differs essentially in its stereo-isomeric structure from dextrose, mannose, and levulose, all of which are inter-convertible by simple transformations.

According to Robinson, arabinose and xylose are utilized to the extent of only 50 per cent, but Peterson, Fred, and Schmidt demonstrated later (1924) that these could be utilized completely in Robinson's medium.

Of the disaccharides studied by Robinson, maltose, according to expectations is fermented as rapidly as glucose. Lactose and sucrose are utilized much more slowly, and the decrease in acidity from the maximum is gradual compared to that observed in the maize mash fermentations. Malibiose and trehalose are not fermented at all; the cultures ceased activity soon after inoculation.

Of the trisaccharides, melezitose is utilized to the extent of about 45 per cent. A high acidity is produced and the fermentation following the peak of acid production is very
slow. No monosaccharide reducing sugars could be detected in
the medium, therefore, it appears that melezitose was absorbed
directly but slowly into the cell, and hydrolysis followed.
The fermentation of raffinose was not vigorous, but there was
a gradual increase in the reducing sugar content of the medium.
The reducing sugar was identified as melibiose. Robinson ex-
plains these results in terms of the activity of sucrase within
the cell. Raffinose is absorbed and hydrolyzed to melibiose
and levulose. The levulose is utilized directly and a part of
the melibiose formed diffuses into the medium, and was identi-
fied as such by the osazone. The accumulation of the remainder
within the cell inhibits cellular activity and eventually stops
the fermentation.

Among the polysaccharides, starch, of course, is consumed
readily. Inulin, however, is used only to the extent of about
50 per cent, and a rather high acidity is produced in the fer-
mentation medium, without a corresponding decline such as that
characteristic of the starch fermentation. But Robinson felt
himself justified in assuming that inulinase is one of the en-
zymes produced by the butyl-acetone organisms.

Any chemical mechanisms proposed for this fermentation
must account for the formation from the sugars of the neutral
solvents, butyl and ethyl alcohols, and acetone, as well as the
production of carbon dioxide and hydrogen. But entirely satis-
factory explanations, based on quantitative chemical theory and
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substantiated by adequate experimental evidence, have not yet been offered. However, it is well known that the production of the neutral solvents is intimately linked with the earlier formation of the typical fermentation acids, acetic and butyric. Their precursors are not known, but the possible role of acetalddehyde should be considered. Speakman (1925) in a study of the biochemical production of acetone from sugars by *Pacillus acetoclyticum* proposed the following scheme:

\[
2\text{CH}_2\text{CHO} \rightarrow \text{CH}_3\text{CHOHCH}_2\text{CHO} \xrightarrow{\text{CH}_3\text{CHO} + \text{H}_2\text{O}} \\
\text{CH}_2\text{CHOHCH}_2\text{COOH} + \text{CH}_3\text{CH}_2\text{OH} \\
\text{CH}_3\text{CHOHCH}_2\text{COOH} + \text{CH}_3\text{CHOHCH}_2\text{CHO} \xrightarrow{\text{H}_2\text{O}} \\
\text{CH}_3\text{COCH}_2\text{COOH} + \text{CH}_3\text{CH}_2\text{OH} \\
\text{CH}_2\text{COCH}_2\text{COOH} = \text{CH}_3\text{COCH}_3 + \text{CO}_2
\]

In this particular fermentation the 2 to 1 ratio of ethyl alcohol to acetone is satisfactorily accounted for. This possible role of acetoacetic acid was studied further by Johnson, Peterson, and Fred (1933) and by Peterson and Johnson (1933) who transfused acetoacetic acid into active fermentations produced by *Cl. acetobutylicum*, and found that it was decarboxylated to form acetone. By a study of the rate of utilization of the acetoacetic acid, they concluded that the reaction was monomolecular. Transfusion of pyruvic acid resulted in the production mainly of acetic acid, acetone, and a significant amount of acetyl methyl carbinol.
Neuberg in his classical researches had shown how various biological processes could be diverted from their principal course by the interception of the inter-mediate acetaldehyde. The synthesis of the 4 carbon chain was demonstrated by Neuberg and Rosenthal (1924) by intercepting the acetaldehyde formed in various fermentations by the use of sodium acid sulfite. The formation of butyric acid, following the action of this "synthesizing" enzyme, which Neuberg called "carboligase", could then be possible.

Although acid formation is intimately linked with solvent production, excessive production of acid such as will reduce the pH of the fermenting medium to 3.9 or lower will result in failure of the fermentation to convert the acids formed to neutral solvents (Reynolds, Coile, and Werkman 1934). Wynne (1931) had also demonstrated that the addition of various mineral acids to the butyl-acetonic fermentation would result in complete inhibition if the pH had been reduced to 3.9 or lower. The effect of the aliphatic organic acids was the same at this pH level.

Seversen (1936) extended the investigation of acid utilization by \textit{Cl. acetobutylicum}. A general increase in solvent production was noted after the transfusion of the lower aliphatic acids. Acetic acid increased acetone production principally, while butyric acid increased butyl alcohol, as well as acetone and ethyl alcohol.
Bernhauer and Kürchner (1935) and Bernhauer and others (1936) report further studies on the role of intermediates in the butyl-acetonic fermentation. For a more complete study of the role of acid formation in various fermentations, and especially that of butyric acid, the extended discussion by Bredemann (1932) should be consulted.

The subject of nitrogen metabolism of the butyl-acetone organisms is quite obscure. It is certain that a complex source of nitrogen is required for satisfactory solvent production, and that the protein present in yellow corn is admirably suited for the purpose. Whether the presence of prolamine is the essential factor, as reported by Weinstein and Rettger (1932), has been thrown into some doubt by the later investigation of Reynolds, Coile, and Werkman (1934).

With respect to the role of certain amino acids, the issue has been clarified by recent work that demonstrated the importance of asparagine, aspartic acid, and glutamic acid in butyl alcohol production. Tatum, Peterson, and Fred (1934) first called attention to the fact that cultures of their butyric acid anaerobes would produce large quantities of butyl alcohol if the media were enriched by the addition of potato extract, corn steep, soy bean, cabbage, and lettuce extracts. Later, Tatum and Peterson (1935) demonstrated the same stimulation if asparagine were incorporated in the medium. They went farther and stated that the dibasic amino acids are the class of
nitrogen compounds that are responsible for the increased production of butyl alcohol by the butyric acid organisms. Finally, Tatum, Peterson, and Fred (1935) identified asparagine to be the substance present in the various extracts that stimulated the butyl alcohol production. Aspartic and glutamic acids were also shown to be responsible for the stimulation. Since the prolamines contain significant quantities of these dibasic amino acids, it may be that the effect of these noted by Weinstein and Rettger was a stimulation of this type.
PROCEDURES

A. Bacteriological

1. Cultures used.

The various cultures used in this investigation were obtained from Dr. L. A. Underkofler and Dr. L. M. Christensen of this department. In each case the cultures had been stored on dry sterile soil. The cultures were designated as A211, W36g, POS, X, and FBB; the last named culture was derived from one of Fernbach's original cultures. In subsequent work, the most vigorous subcultures derived from those above, were employed.

The general technique of isolation and characterization of the butyl-acetone organisms has been described by Underkofler (1934). The procedure for handling the cultures is described by Underkofler, Christensen and Fulmer (1936).

By the means of sterile pipets (3-4 mm. inside diameter) 0.3 to 0.5 g. of the soil are transferred to tubes of sterile 6 per cent corn mash. The tubes are plunged into boiling water, and after 2 minutes, are cooled quickly, and incubated at 37°. Transfers are made every 24 hours into fresh sterile mash.
After the second transfer, the contents of 1 or 2 tubes are emptied into 500 cc. of 6 per cent mash in an Erlenmeyer flask. As soon as the corn fermentation has headed up as a result of the vigorous gassing, the mash may be used for inoculating various substrates. If 500 cc. are insufficient for the experiment at hand, another transfer is made into larger amounts of corn mash. In order to secure vigorous growth in the subcultures, the best inoculation ratio is 1 - 25, but should not be less than 1 - 35. One is then assured of the heading of the cultures within 24 hours.

Since these organisms are anaerobes, it is important that the media in which they grow shall have as small a surface to volume ratio as can be conveniently arranged. The vigorous evolution of carbon dioxide and hydrogen will then maintain an anaerobic condition that will insure good growth of the organisms. Subcultures do well in the ordinary fermentation tubes of 1.5 x 25 cms. For the larger batches it was found that 500 cc. Kjeldahl flasks filled to the neck were very satisfactory. When Erlenmeyer flasks are used, the mash should reach a level as high as is consistent with proper handling in subsequent operations.

2. Media used.

The standard medium employed for subcultures and control fermentations is gelatinized corn mash of 6 to 7 per cent
concentration. It is prepared by adding a weighed amount of ground yellow corn to tap water in a flask. The flask is heated by steam, at atmospheric pressure, for about 1/2 hour with occasional shaking. It is then plugged with cotton and heated in the autoclave at 15 lbs. pressure for 1.5 hours. The pressure is reduced slowly, and the flask finally removed and cooled for future use. The fresh media prepared in this manner will give better fermentations than older ones, because the absence of dissolved oxygen allows a more rapid growth of the organisms. If the media are stored in the incubator at 37° C., uptake of oxygen is slow, but care must be taken to compensate for excessive loss of water by evaporation.

For preparation of test tube media, the mash after initial steaming, is transferred to fermentation tubes of about 1.5 x 25 cms. These tubes, containing 15 to 20 cc. of mash, are plugged and sterilized 1.5 hours at 15 lbs. pressure.

B. Chemical

1. Solvent analysis.

The character of these investigations necessitated a large amount of routine analytical work. Hence, it is important that the analyses for the various materials involved should be well standardized. The principal products of the fermentation, butyl alcohol, acetone, and ethyl alcohol, commonly called "solvents", 
are determined most frequently. The method employed for solvent analysis is that described by Christensen and Fulmer (1935) and is given here in detail.

a. Determination of butyl and ethyl alcohols, and acetone in fermentation liquors. 250 cc. of the fermented liquor are measured into a graduated cylinder, rinsed into a 500 cc. Kjeldahl flask, and 1 - 2 g. powdered calcium carbonate added. The liquor is distilled rapidly; the first 100 cc. are collected in volumetric flasks which are cooled in running water to which cracked ice is added.

A 10 cc. portion of this distillate is diluted to 100 cc. in a volumetric flask. Ten cubic centimeters from the latter are added to a mixture of 10 cc. of concentrated sulfuric acid and 10 cc. of 0.40 N potassium dichromate contained in a 2 x 22 cm. Fyrex test tube. A tight fitting rubber stopper bearing a short piece of capillary tubing is inserted, and the tube is whirled vigorously to obtain complete mixing of the solutions. It is then heated 10 minutes in a boiling water bath, after which it is cooled in running water. The contents are rinsed out into an Erlemmeyer flask, 300 to 400 cc. of water are added, and then 15 cc. of 20 per cent potassium iodide solution; the iodine liberated is titrated with 0.100 N sodium thiosulfate.

For the blank, 10 cc. of distilled water are added to the same amount of reagents, the whole being treated in the same manner as above. The difference between the volumes of 0.100 N
thiosulfate consumed in the two titrations constitutes \(m_1\).

For the determination of \(m_2\) a 20 cc. portion of the distillate is added to 40 cc. of C.P. carbon tetrachloride and allowed to stand for 2 hours at 25\(^\circ\) C. Large test tubes may be used for the purpose, or small Erlenmeyer flasks which may be shaken more readily, and also have the advantage of providing larger surface contact between the two liquids. A 10 cc. portion is then removed from the aqueous layer and diluted to 100 cc. Ten cubic centimeters of this solution are heated with the sulfuric acid-dichromate mixture in a manner identical to that for determination of \(m_1\). The difference between the iodine values of blank and in this titration constitutes \(m_2\).

In order to make these oxidations applicable to the calculation of individual solvents in the mixture, it is necessary to determine acetaldehyde directly. The method employed is the Goodwin (1920) modification of the Messinger titration (1889).

From the solution obtained by the 1 - 10 dilution of the original distillate, 10 cc. are pipetted into 25 cc. of N sodium hydroxide in a 250 cc. Erlenmeyer flask standing in cracked ice. Then 20 cc. of 0.20 N iodine solution in potassium iodide are added slowly from a buret with constant shaking. The flask is stoppered tightly and allowed to stand in the ice for 15 minutes, after which 25 cc. of N sulfuric acid are added. The iodine liberated is titrated with 0.100 N thiosulfate solution. The blank determination using the same
quantities of reagents without the solvent mixture is run in the identical manner. The difference between the two volumes of thiosulfate consumed is a measure of the acetone present.

The reaction involved is expressed by the equation,
\[ \text{CH}_3\text{COCH}_3 + 3\text{I}_2 + 4 \text{NaOH} = \text{CH}_3\text{I} + 3\text{NaI} + \text{CH}_3\text{COONa} + 3\text{H}_2\text{O} \]

From the equation it follows that acetone is expressed by the relation, acetone (in grams per 100 cc. of distillate)
\[ = 100 \left( \frac{128.93}{1000} \right) (\text{cc. N thiosulfate}) \times \text{CH}_3\text{COCH}_3 / 6 \text{ I} \]
\[ = 0.09675 (\text{cc. N thiosulfate}) \]
\[ = 0.09675 (\text{cc. N/10 thiosulfate}) \]

Haughton (1937) has examined the procedure critically, and demonstrated the presence of HCOONa in the products. He attributes its formation to the side reaction.
\[ \text{CH}_3\text{COCH}_3 + 10\text{I} + 5\text{NaOH} = \text{HCOONa} + 2 \text{CH}_3\text{I} + 4 \text{NaI} + 4\text{H}_2\text{O} \]

which he claims is responsible for a consistent error of +2%.

Langlykke (1937) has commented at length on the analytical complications involved by the presence of acetyl methyl carbinal in fermentation distillates. This compound is known to be present in small amounts among the products of the butyl-acetonic fermentation. However, its effect in the acetone determination, as well as Haughton's objection, are believed to be minimized in this work, because the acetone analysis was always performed in ice cold solution. Goodwin does not specify this condition.

With a knowledge of the acetone content of the distillates,
the calculations for butyl and ethyl alcohols can be made from the following equations:

\[
\text{BuOH (in grams per 100 cc. of distillate)} = 0.25 (m_1 - m_2) - 0.01m_1 - 0.07A + \text{Corr.}
\]

\[
\text{EtOH (in same units)} = .114m_1 - .788B - .0788A
\]

\[A = \text{g. of acetone per 100 cc. of distillate}\]

\[B = \text{g. of BuOH per 100 cc. of distillate}\]

\[\text{Corr.} = \text{correction factor.}\]

These equations together with the necessary correction factors are given by Christensen and Fulmer (1935)

b. Determination of total solvents by specific gravity.

It has been shown that the normal ratio of solvents from corn is 60 per cent butyl alcohol, 30 per cent acetone and 10 per cent ethyl alcohol, (although variable within rather narrow limits). Knowing the densities of these three constituents, and assuming the above ratio, one may calculate the total solvents present in various distillates by a careful determination of the specific gravities. The formula expressing the relationship is:

\[\text{grams per 100 cc. of distillate} = (1.000 - \text{SP. gr.}) \cdot 698\]

A Westphal chainomatic balance was used for the specific gravity measurements.

This method is useful in the rapid examination of a large number of distillates to detect maximum yields, or in case a poor fermentation is suspected, to determine whether the solvent
yield is sufficient to warrant detailed analysis for the principal constituents. However, too much reliance should not be placed upon the exact numerical values obtained, because the factor 698 will greatly amplify any small errors present in the specific gravities, and the distillates may easily be contaminated with small amounts of foreign materials.

2. Sugar Analysis.

Since the butyl-acetonic fermentation of corn mash may be regarded as the standard for the butyl organism, it is necessary to know the total amount of fermentable substrate present in the corn used for control fermentations. By determination of the carbon balance, Reilly (1920) has shown that when starch is the only carbohydrate material present in a substrate supporting the normal activity of the butyl organism, it alone is the source of the typical solvents.

Accordingly, starch analyses must be made by standard methods in order to account for various solvent yields on a comparable basis. Analyses were performed according to "Official Methods" (1931) with the following modification. The acid hydrolysis was used as prescribed, except that the ground corn, after weighing, was first extracted with ether and alcohol-water solution as directed for the enzymatic conversion of the starch. The hydrolyzate was then neutralized and the reducing sugar determined by the Shaffer-Hartmann method (1920),
using the sugar tables given by Stiles, Peterson, and Fred (1926).

The methods of acid and enzymatic hydrolysis were compared on the same samples of corn and gave results which differed usually by less than 0.5 per cent. Since the acid hydrolysis is more rapid than the enzymatic, it was used for most of the starch analyses.

The Shaffer-Hartmann procedure was employed also for determining the reducing sugars in the artichoke diffusion juices and hydrolyzates. The solutions to be tested were always filtered, clarified by lead acetate treatment, and diluted to a known volume.

3. **Acidity determination.**

Because of the marked proteolytic action of the butyl organisms, a buffering effect is produced in the various media which prevents any significant lowering of the pH due to formation of the typical fermentation acids. Consequently, pH measurements are of little value in the study of acidity changes during the fermentation, and base titration must be employed.

The changes in acidity due to the formation and conversion of the fermentation acids were followed by measurement of titratable acidity. A known volume, usually 10 cc., was withdrawn from the fermenting mash, heated just to boiling in an open test tube to remove carbon dioxide and then cooled quickly.
After dilution to a point where the color would not interfere, the solution was titrated with 0.10 N sodium hydroxide using phenolphthalein as the indicator.

However, with the introduction of artichoke juice into the fermentations, the use of ordinary indicators for acid-base titrations is impossible, because of the dark color of the media. pH measurements were made in a few cases to determine the initial and final acidity of the mash and maximum change in acidity during the active phase of the fermentation.

4. Separation and identification of the individual components of the solvent mixture.

The analytical procedures described above were used most often in the course of the experimental work that follows. However, the oxidative analysis is not specific for butyl and ethyl alcohols, and the acetone titration is in reality one for the determination of a certain class of carbonyl compounds. Therefore, the question sometimes arises whether these compounds actually exist in the various fermentation liquors. The only way to be certain of their presence is to separate and identify them according to standard methods. This procedure of separation and identification has been followed in several cases, and the technique used is described here in some detail.

The treatment usually involved 12 to 20 liters of fermented mash. The beer was divided between two 12 liter flasks which
were connected through long condensers with a 5 liter receiving flask which was cooled in an ice bath. The liquor in the large flasks, after the addition of 10 to 15 g. of powdered calcium carbonate, was distilled rapidly, and 5 to 6 liters of distillate were collected. The oily layer on the surface of the distillate was removed and saved. The distillate was saturated with sodium chloride and redistilled. This new distillate was again collected in an iced flask and the upper oily layer removed as before. The oily layers were combined and constituted nearly pure butyl alcohol in aqueous solution.

The distillate is saturated with anhydrous potassium carbonate and the upper layer removed and distilled fairly rapidly through a column packed with beads. The distillate in the iced receiver is homogeneous to 91°, and is reserved for separate treatment. At 91° the butyl alcohol solution first mentioned is put into the distilling flask, and the distillation is continued. The small amount boiling below 91° is collected with the previous fraction at that temperature. At 91° the binary mixture BuOH-H₂O distils across and the distillate separates into two phases until the temperature reaches about 94°. At this point the water is completely removed, and the temperature rises rapidly to 115°, at which point a separate fraction is collected. Pure butyl alcohol distils across from 115° to 118°, and the few cc. of residue above 116° are rejected.
All of the previous fractions are combined and to them added excess anhydrous sodium sulfate. The mixture was allowed to stand over night after which the salt sludge was filtered off by suction. The clear filtrate was then fractionated for individual solvents.

Derivatives of the various fractions obtained were prepared according to Kamm, "Qualitative Organic Analysis" 1931. The acetone was characterized as the 2,4 dinitro phenyl hydrazone, and the alcohols as the 3,5 dinitro benzoates.
THE EXPERIMENTAL INVESTIGATION

1. Materials used.

In order to determine the best conditions for the butyl-acetonic fermentation of the Jerusalem artichoke, several different procedures were investigated. These included use of the untreated artichoke chips, the juice obtained by extracting the dried chips with water, and the hydrolyzates obtained by acid treatment of both the chips and the aqueous extracts. Then, to provide essential constituents that may be lacking in any of these various artichoke substrates, additional nutrients were added in the form of corn mash, corn gluten, and soy bean meal.

The artichoke material available was that prepared by Eichinger and McGlumphy (1931) for the commercial production of levulose. Several tons of artichoke tubers had been chipped, dried, sealed up in sacks, and stored. This source constituted the principal supply of material used in the fermentations.

The juice used was that obtained by the hot water extraction of the dried chips, as carried out in the diffusion battery in the levulose plant. It was desirable to prepare a
large batch of juice at one time, rather than to operate the battery continuously. Since the juice would spoil easily, and sterilization was not feasible, the amount not used immediately was concentrated by vacuum evaporation.

After some experimentation an efficient apparatus was devised for rapid concentration of the juice. Two 12 liter round-bottom flasks were connected through large Pyrex tubing with a central receiving flask, also of 12 liter capacity. The latter was cooled by immersion in a large crock through which cold water was running. Each of the flasks had a siphon attachment; the siphons on the distilling flasks permitted recharging without dismantling the set, and that on the receiver allowed one to draw off the distillate periodically. The distilling flasks were immersed in hemispherical iron pans in which water was boiled to heat the flasks. The receiver was connected to the suction pump.

During the first operation about 80 liters of juice were submitted to evaporation, and yielded 18 liters of syrup after about 20 hours of treatment. The temperature during evaporation was not allowed to exceed 75° and during most of the time it remained at 50° to 55°. The water pump used was of the high vacuum type, and occasional trouble was encountered when some slight increase in water pressure would result in production of a vacuum that reduced the boiling point to 40° C. Violent boiling would take place for a time, and the resulting viscous
liquid at the lower temperature would bump rather badly. But if the boiling point were maintained at 60° to 65°, and about 1 cc. of castor oil added, there would be no trouble with foaming or bumping, even when the syrup became quite concentrated.

This apparatus was used for some time and proved satisfactory except that it was a little difficult to assemble. Finally, a distinct improvement was made by the use of steam heating. A single large Pyrex bottle of 16 liters capacity was set up in the autoclave, and connected by means of a copper condenser, 4 feet in length and of 12 mm. bore, to a 12 liter flask used as receiver. Cotton wadding wrapped in towels was used as packing around the neck of the bottle to prevent too much escape of steam. The suction pump was attached to the 12 liter receiver. This apparatus was easy to assemble and dismantle, and with a rapid stream of water running through the condenser, would evaporate 2.5 to 3 liters of water per hour.

The syrup produced by the concentration of the diffusion juice contains approximately 70 per cent total solids. The carbohydrate content consists largely of polysaccharides of levulose, although some free reducing sugar is always present. The first preparation yielded a syrup which contained, according to direct analysis, 15.7 g. of reducing sugar per 100 cc. After hydrolysis of the syrup by the method given below, thus converting the polysaccharides to reducing sugars, the concentration of reducing sugar was found to be 65.4 g. per 100 cc.
This value therefore represents the total available carbohydrate in the syrup. Even at this concentration there was a marked tendency toward mold formation on the surface of the syrup during storage. But if the air in the storage vessel is displaced by the introduction of carbon dioxide the syrup can then be preserved for long periods without spoilage.

2. Fermentation of untreated artichokes by cultures X and POS.

About 20 kgs. of the dried chips were granulated in the grinding mill, and then used in the following experiments.

a. Five 2 liter Erlenmeyer flasks of mash were prepared as follows:

1. 1500 cc. water plus 75 g. ground chips
2. 1500 cc. " " 90 g. " "
3. 1500 cc. " " 105 g. " "
4. 1500 cc. " " 120 g. " "
5. 1500 cc. " " 135 g. " "

The flasks were plugged and sterilized at 20 lbs. for 1 hour. After cooling each was inoculated with 100 cc. of the third transfer of culture X grown in 5 per cent corn mash. Gas evolution was fair after 24 hours, but there was little change in the mash except for the development of a sour odor and high acidity. The pH was 4.3 to 4.4. After distillation of the liquor, specific gravity measurements on the distillates indicated practically no solvents.

b. The above experiment was repeated using the same amounts of materials but the flasks were inoculated with POS
culture. The results were the same as with culture X. Gassing developed, but there was little change in the substrate except increase in acidity. These two experiments are in agreement with the findings of Reynolds and Werkman (1934).

3. Fermentation of juice obtained by hot water extraction of the dried chips in the diffusion battery.

A batch of juice was taken directly from the battery and diluted in 2 liter flasks so that the total solids ranged from 4 to 12 per cent. The flasks were sterilized for 1 hour at 10 lbs. pressure, then inoculated with culture X growing in 6 per cent corn mash. The fermentations resulting were very irregular, some showing appreciable gassing, others not. Whatever fermentative activity was shown usually disappeared within 24 hours, leaving the liquor with a sour odor. The pH was usually from 4.4 to 4.6 at this stage and remained at that level. After 84 hours the specific gravity measurement of the distillate showed the solvent yield to be inappreciable.

In a second series, various amounts of the diffusion juices were concentrated by vacuum evaporation. During the process, the amount of reducing sugar increased somewhat, so that when the resulting syrup was diluted and fermented as above, gassing was more active. But the solvent yields were very low, and further attempts to utilize the unhydrolyzed material were abandoned.
4. Fermentation of hydrolyzed artichokes.

The conversion of the polysaccharides of the Jerusalem artichoke into reducing sugars has been accomplished by acid hydrolysis, and by electrodialysis, which Hardy (1933) has employed in the preparation of levulose concentrates. In the present investigations, the method of acid hydrolysis as described by Eichinger (1931) was used. According to the tables presented by Eichinger, the maximum conversion of the polysaccharides, with minimum destruction of levulose, is obtained by adding sufficient sulfuric acid to reduce the pH of the juice to 1.5, maintaining at 80° for 1 hour, and neutralizing with calcium carbonate. If hydrochloric acid is used, the pH should be 1.75. The author mentioned presents data by means of which the apparent normality of acid required can be determined for juices of various concentrations.

a. Hydrolysis of the chips with sulfuric acid; neutralization by means of powdered calcium carbonate. 270 g. of the chips were stirred into 3900 cc. of water and hydrolyzed at 80° after the addition of 29 cc. of 18 N sulfuric acid. The solution was neutralized after one hour by the addition of 25 g. of calcium carbonate. The pH was then 5.7. The final volume was 4120 cc. and the reducing sugar content was 3.98 g. per 100 cc. 1200 cc. of juice were introduced into each of three 2 liter flasks. These were sterilized at 10 pounds pressure
for 20 minutes, cooled, and each inoculated with 100 cc. of 6 per cent corn mash subculture. The fermentations were fairly vigorous with gas formation persisting for about 24 hours. Solvent determinations were made by the specific gravity method. The analytical results are given in Table 1.

Table 1. Fermentation of chip sludge hydrolyzed with sulfuric acid.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sp. gr.</th>
<th>volume</th>
<th>Solvents: g. per 100 cc.</th>
<th>Total solvents: g. per flask</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>.9952</td>
<td>1420</td>
<td>2.28</td>
<td>19.0</td>
</tr>
<tr>
<td>1b</td>
<td>.9953</td>
<td></td>
<td>2.29</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>.9949</td>
<td>1300</td>
<td>2.57</td>
<td>18.4</td>
</tr>
<tr>
<td>2b</td>
<td>.9950</td>
<td></td>
<td>3.50</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>.9948</td>
<td>1320</td>
<td>3.64</td>
<td>19.2</td>
</tr>
<tr>
<td>3b</td>
<td>.9948</td>
<td></td>
<td>3.64</td>
<td></td>
</tr>
</tbody>
</table>

According to these data, the average yield of solvents is 36 per cent of the sugar, or 21.9 per cent of the total weight of chips used. Despite the fact that these solvent yields were quite good on the basis of specific gravity of the distillates, the direct titration of acetone showed this product to be low. The acetone content of the distillates was only 0.60 to 0.65 g. per 100 cc., whereas, the normal for corn mash varies from 1.1 to 1.25 g.

b. Fermentation of chip sludge hydrolyzed at lower acidity than the optimum. A chip sludge was prepared in the same manner
as above, except that the hydrolysis was carried out at pH 2.2, instead of at 1.50, as recommended for sulfuric acid hydrolysis. The yield of reducing sugar was only 42 per cent on the basis of the dry weight of chips, in contrast to the 60 per cent conversion obtained under optimum conditions.

The resulting fermentations carried out as above showed about the same degree of activity. Solvent analysis was performed, using the oxidation procedure, and showed the yield, on the basis of reducing sugar present to be about 31 per cent. On the basis of dry weight of chips used, the yield was only 12.9 per cent, thus showing again the inability of the organisms to attack the polysaccharides present.

The results of the above described studies show only moderate yields of solvents. Furthermore, the experiments cited represented only the best of a series of fermentations in which the materials were used as described above; other fermentations gave irregular results with far lower solvent yields. In most cases there was appreciable gassing within 24 hours which carried much of the sludge to the top of the flask. But the gassing would diminish abruptly, and further signs of fermentative activity disappeared. There was not much change in the color (a distinct brightening will be referred to later in connection with successful fermentations.) A sour odor would persist in contrast to the usual ethereal flavor of liquors rich in acetone and butyl alcohol. However, these sludges prepared by
sulfuric acid-calcium carbonate treatment showed more fermentative activity than the unhydrolyzed chips. It should be noted that the irregularity observed is probably due to a variation of factors concerned with protein degradation during the acid treatment. Another objection is that the use of calcium carbonate gives rise to troublesome and oftentimes uncontrollable frothing during the neutralization.

c. Chips hydrolyzed with hydrochloric acid; hydrolysate neutralized with sodium hydroxide. Ten liters of water were heated to 90° in a 12 liter round bottom flask, 750 grams of ground artichoke chips were added, followed by the addition of 145 cc. of 1:1 hydrochloric acid with vigorous stirring. The temperature was maintained at 80° for 1 hour and the acid was neutralized by the addition of sodium hydroxide solution to a pH of 6.0. The final volume was 10630 cc., the total reducing sugar 537.9 g., and the sugar per 100 cc., 5.06 g. 2500 cc. were put into each of four 4 liter Erlenmeyer flasks, and sterilized for 1/2 hour at 15 pounds pressure, then inoculated with 100 cc. of actively fermenting mash. Gas evolution was vigorous on the second day; after 84 hours the mash was analyzed. The results are given in Table 2.
Table 2. Fermentation of sludge hydrolyzed with hydrochloric acid and neutralized with sodium hydroxide.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sp. gr.</th>
<th>Solvents g. per 100</th>
<th>Final vol. cc.</th>
<th>Total solv. g. per flask</th>
<th>Sugar g. per flask</th>
<th>% yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>.9945</td>
<td>3.85</td>
<td>2710</td>
<td>41.7</td>
<td>131.5</td>
<td>32.2</td>
</tr>
<tr>
<td>1b</td>
<td>.9944</td>
<td>3.92</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>.9946</td>
<td>3.73</td>
<td>2750</td>
<td>41.6</td>
<td>131.5</td>
<td>31.5</td>
</tr>
<tr>
<td>2b</td>
<td>.9947</td>
<td>3.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>.9950</td>
<td>3.50</td>
<td>2790</td>
<td>39.1</td>
<td>131.5</td>
<td>30.5</td>
</tr>
<tr>
<td>3b</td>
<td>.9948</td>
<td>3.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>.9946</td>
<td>3.73</td>
<td>2730</td>
<td>41.0</td>
<td>131.5</td>
<td>30.6</td>
</tr>
<tr>
<td>4b</td>
<td>.9947</td>
<td>3.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In addition it may be noted that the acetone analyses of these distillates showed improved yields, ranging from 0.90 to 1.05 g. per 100 cc.

d. Fermentation of filtrate from acid hydrolyzed sludge.

One thousand g. of chips were hydrolyzed with hydrochloric acid in 9500 cc. of water. After neutralization, the sludge was poured onto four large Buchner funnels, and filtered. The juice was pressed out, and the residue washed with about 200 cc. of water. The filtrates were combined to make a total volume of 9160 cc. of which 3000 cc. were poured into each of three 4 liter Erlenmeyer flasks. These were plugged and sterilized 1/2 hour at 15 pounds. Inoculation was made with 150 cc. of actively fermenting X culture in 5 per cent corn mash. The sugar
per 100 cc. of hydrolyzate was 5.75 g.; total amount per flask 172.0 g. Gas production was very active on the second day and lasted until about the 40th hour. The results are given in Table 3.

Table 3. *Fermentation of filtrate from hydrolyzed chip sludge*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sp. gr.</td>
<td>g. per 100 g.</td>
<td>vol.</td>
<td>g. per: (aver.)</td>
</tr>
<tr>
<td>la</td>
<td>.9952</td>
<td>3.26</td>
<td>0.94</td>
<td>3100</td>
</tr>
<tr>
<td>lb</td>
<td>.9953</td>
<td>3.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>.9944</td>
<td>4.06</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>.9943</td>
<td>4.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>.9936</td>
<td>4.48</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>.9939</td>
<td>4.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Number 1 in this series became very slimy. Microscopic examination showed an abundance of long chained rods with tubular capsule formation. The fermentation did not give yields so high as in g., but the deficiency may be attributed to the removal of some desirable source of nutrient in the solid matter.

In general, all of these direct fermentations of artichoke juices prepared under different conditions were lacking in the vigor that characterizes the typical transformation of starch in corn into the usual solvents. As mentioned before, the yield of solvents in terms of glucose equivalent to the
starch should be 36 to 40 per cent, the values regularly observed in the corn mash fermentations.

5. Use of additional nutrients in the fermentation of artichoke juices.

Since it is apparent, from the discussion in the preceding pages, that the direct fermentation of chip sludge and diffusion juices, either in the hydrolyzed or unhydrolyzed forms, is not successful, the use of additional nutrients to increase the activity of the organism appeared desirable. Corn mash medium is the ideal substrate for the growth of the butylacetone organisms, and therefore, it seemed that if the various artichoke juices were supplemented by the addition of corn mash, a more vigorous fermentation would result. Accordingly, an experiment was devised to test this supposition.

a. Replacement of various amounts of corn by carbohydrate equivalents of unhydrolyzed artichoke juice. About 30 kgs. of corn meal were mixed uniformly and stored in bottles. Ether vapor was added to prevent mold growth. According to the "Official Methods" (1931), analysis by diastatic conversion showed the available dextrose to be 66.0 g. per 100 g. of corn.

The syrup used contained 15.7 g. reducing sugar per 100 cc. The total available reducing sugar was 65.7 g. per 100 cc.; hence, the replacement of 1 g. of corn in the mash by 1 cc.
of syrup maintained chemical equivalents of sugar in the liquor. For the experiment at hand, the syrup was diluted so that it could be measured more readily.

The media were prepared in 2 liter Erlenmeyer flasks using corn mash plus increasing amounts of artichoke syrup as described. The corn was gelatinized, the diluted syrup added, and the flasks plugged and sterilized. The total volume in each case was made up to about 1600 cc. Each flask was inoculated with 100 cc. of 36 g culture in 5 per cent corn mash. The fermentations were vigorous in gas evolution, and the corn in each flask was borne to the surface and displayed the typical head formation. The odor of the liquor was characteristic of the butyl-acetonic conversion. After 34 hours, analyses for the individual solvents were performed. The description of the media, and the solvent yields obtained are given in Table 4.
Table 4. Solvent yields obtained by the replacement of corn by carbohydrate equivalents of unhydrolyzed artichoke syrup.

<table>
<thead>
<tr>
<th>No.</th>
<th>Corn: Syrup g. per 100 ec. of</th>
<th>Solvents: g. per</th>
<th>Total: Solvents from corn and syrup g. per</th>
<th>flask</th>
<th>a</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>0.17</td>
<td>0.34</td>
<td>28.9</td>
<td>23.9</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>108</td>
<td>1.11</td>
<td>0.41</td>
<td>26.25</td>
<td>26.0</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>95</td>
<td>1.02</td>
<td>0.63</td>
<td>23.96</td>
<td>23.1</td>
<td>0.86</td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>0.98</td>
<td>0.61</td>
<td>22.17</td>
<td>20.2</td>
<td>1.97</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>0.90</td>
<td>0.62</td>
<td>19.95</td>
<td>17.3</td>
<td>2.65</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>1.38</td>
<td>0.75</td>
<td>18.09</td>
<td>14.45</td>
<td>3.63</td>
</tr>
</tbody>
</table>

*Column a is the yield of solvents from the corn mash in each flask; b is the amount corresponding to the difference between the total yield and that from the corn, in other words, the amount attributable to the syrup.

The data show that in each fermentation, where more than 10 per cent of the corn has been replaced by its equivalent of artichoke syrup, there is a progressive decrease in the solvent yield as the amount of replacement increases. This progressive yield may be due to either or both of the following factors: the syrup may contain substances which inhibit the fermentation of the carbohydrates which would otherwise take place; or, the complex polysaccharides are not hydrolyzed into fermentable sugars because of enzymatic deficiencies of the butyl organisms. In figures 1 to 4 are plotted data from Table 4.
Figs. 1 to 4. Solvent yields from replacement of corn by hydrolyzed artichoke syrup.
Figure 1 is a plot of the total yield of solvents against total sugar (actual amount of reducing sugar in the juice plus the dextrose equivalent of the starch in the corn); Figure 2 is a plot of the yield of solvents from the syrup (see b. in Table 4) against the amount of levulose in the syrup; Figure 3 is a plot of the total yield of solvents against the per cent replacement of corn mash by the unhydrolyzed syrup; Figure 4 is a plot of the per cent yield of solvents against the total sugar.

It is evident from these curves that the yield of solvents is dependent upon the quantity of reducing sugar present. If an inhibiting factor were present, as Thaysen and Green (1927) assumed, then there would have been a decrease in the percentage conversion of sugar to solvents, but the graph of Figure 4 eliminates this possibility. The yield from the levulose is somewhat irregular when small amounts are involved, but it rapidly builds up to an average of about 35 per cent conversion. The logical explanation then for the fall in total yield with increasing replacement of corn by the unhydrolyzed juice, is that the organisms are unable to convert the polysaccharides present in the juice into fermentable sugars. Because of this fact, the next step in the problem of producing a successful buty-acetonic fermentation of the Jerusalem artichoke is to hydrolyze these polysaccharides completely before the introduction of the organisms.
b. Replacement of corn mash by hydrolyzed syrup. A 400 cc. portion of syrup was diluted to 3200 cc. and acidified to pH 1.75 by the addition of 230 cc. of 2.0 N hydrochloric acid. The solution was heated for 1 hour at 80\(^\circ\)C, then neutralized by the addition of sodium hydroxide. The final volume was 3590 cc., and the reducing sugar concentration was 8.75 g. per 100 cc.

The corn used was analyzed for starch according to the "Official Methods". The glucose equivalent to the starch was 61.1 g. per 100 g.

Various proportions of the hydrolyzate and gelatinized corn mash were mixed in 4 liter Erlenmeyer flasks, and the media sterilized for 1.5 hours at 18 pounds pressure in the autoclave. After cooling, each flask of media was inoculated with 110 cc. of the third transfer of FOS culture growing in 5 per cent corn mash medium.

The compositions of the several media are as follows:

<table>
<thead>
<tr>
<th>Flask Number</th>
<th>Corn Mash (g)</th>
<th>Hydrolyzate (cc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>220</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>180</td>
<td>380</td>
</tr>
<tr>
<td>3</td>
<td>140</td>
<td>760</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>1140</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>1250</td>
</tr>
</tbody>
</table>

In each case the volume was adjusted to about 3300 cc. before sterilization. The fermentations were vigorous, gas formation was rapid, and the typical heads formed within 20 hours. After 84 hours, samples were drawn from each flask and complete solvent analyses were performed. The results are presented in Table 5.
Table 5. Solvent yields obtained by the replacement of corn by carbohydrate equivalents of hydrolyzed artichoke syrup.

<table>
<thead>
<tr>
<th></th>
<th>Flask numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

I. Solvents, g. per 100 cc. of distillate:

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. butanol</td>
<td>2.33</td>
<td>2.17</td>
<td>2.18</td>
<td>2.36</td>
<td>2.08</td>
</tr>
<tr>
<td>b. acetone</td>
<td>1.24</td>
<td>1.19</td>
<td>1.16</td>
<td>1.15</td>
<td>0.99</td>
</tr>
<tr>
<td>c. ethanol</td>
<td>0.45</td>
<td>0.53</td>
<td>0.53</td>
<td>0.45</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>3.89</td>
<td>3.87</td>
<td>3.96</td>
<td>3.53</td>
</tr>
</tbody>
</table>

II. Final volumes of fermented liquors:

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. butanol</td>
<td>23.25</td>
<td>23.90</td>
<td>24.00</td>
<td>23.45</td>
<td>24.00</td>
</tr>
<tr>
<td>b. acetone</td>
<td>1.24</td>
<td>1.19</td>
<td>1.16</td>
<td>1.15</td>
<td>0.99</td>
</tr>
<tr>
<td>c. ethanol</td>
<td>0.45</td>
<td>0.53</td>
<td>0.53</td>
<td>0.45</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>3.89</td>
<td>3.87</td>
<td>3.96</td>
<td>3.53</td>
</tr>
</tbody>
</table>

III. Total solvents:

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. butanol</td>
<td>31.0</td>
<td>24.10</td>
<td>16.85</td>
<td>14.40</td>
<td>7.71</td>
</tr>
<tr>
<td>b. acetone</td>
<td>16.5</td>
<td>13.25</td>
<td>10.05</td>
<td>7.00</td>
<td>3.68</td>
</tr>
<tr>
<td>c. ethanol</td>
<td>5.72</td>
<td>5.86</td>
<td>4.56</td>
<td>2.74</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>53.22</td>
<td>43.21</td>
<td>33.46</td>
<td>24.14</td>
<td>13.10</td>
</tr>
</tbody>
</table>

IV. Solvents from the corn (dextrose):

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. butanol</td>
<td>0</td>
<td>5.30</td>
<td>10.80</td>
<td>17.20</td>
<td>20.59</td>
</tr>
<tr>
<td>b. acetone</td>
<td>0</td>
<td>2.90</td>
<td>5.75</td>
<td>8.40</td>
<td>9.82</td>
</tr>
<tr>
<td>c. ethanol</td>
<td>0</td>
<td>1.32</td>
<td>2.62</td>
<td>3.29</td>
<td>4.54</td>
</tr>
<tr>
<td></td>
<td>9.52</td>
<td>19.17</td>
<td>23.89</td>
<td>34.95</td>
<td></td>
</tr>
</tbody>
</table>

V. Solvents from the syrup (levulose):

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. butanol</td>
<td>0</td>
<td>2.90</td>
<td>5.75</td>
<td>8.40</td>
<td>9.82</td>
</tr>
<tr>
<td>b. acetone</td>
<td>0</td>
<td>1.32</td>
<td>2.62</td>
<td>3.29</td>
<td>4.54</td>
</tr>
<tr>
<td>c. ethanol</td>
<td>0</td>
<td>1.32</td>
<td>2.62</td>
<td>3.29</td>
<td>4.54</td>
</tr>
<tr>
<td></td>
<td>9.52</td>
<td>19.17</td>
<td>23.89</td>
<td>34.95</td>
<td></td>
</tr>
</tbody>
</table>

VI. Dextrose from the corn:

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. butanol</td>
<td>134.4</td>
<td>109.97</td>
<td>85.54</td>
<td>61.10</td>
<td>36.66</td>
</tr>
<tr>
<td>b. acetone</td>
<td>0</td>
<td>28.1</td>
<td>56.2</td>
<td>84.3</td>
<td>92.20</td>
</tr>
<tr>
<td>c. ethanol</td>
<td>134.4</td>
<td>136.07</td>
<td>141.74</td>
<td>145.4</td>
<td>128.86</td>
</tr>
<tr>
<td></td>
<td>39.6</td>
<td>39.2</td>
<td>37.2</td>
<td>36.5</td>
<td>37.6</td>
</tr>
</tbody>
</table>

VII. Levulose from the syrup:

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. butanol</td>
<td>0</td>
<td>28.1</td>
<td>56.2</td>
<td>84.3</td>
<td>92.20</td>
</tr>
<tr>
<td>b. acetone</td>
<td>134.4</td>
<td>136.07</td>
<td>141.74</td>
<td>145.4</td>
<td>128.86</td>
</tr>
<tr>
<td>c. ethanol</td>
<td>39.6</td>
<td>39.2</td>
<td>37.2</td>
<td>36.5</td>
<td>37.6</td>
</tr>
<tr>
<td></td>
<td>39.6</td>
<td>39.4</td>
<td>39.1</td>
<td>39.5</td>
<td>35.8</td>
</tr>
</tbody>
</table>

IX. Per cent yield of solvents (2) from total sugar:

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. butanol</td>
<td>39.6</td>
<td>39.2</td>
<td>37.2</td>
<td>36.5</td>
<td>37.6</td>
</tr>
<tr>
<td>b. acetone</td>
<td>0</td>
<td>32.9</td>
<td>24.1</td>
<td>54.30</td>
<td>37.9</td>
</tr>
<tr>
<td>c. ethanol</td>
<td>39.6</td>
<td>39.4</td>
<td>39.1</td>
<td>39.5</td>
<td>35.8</td>
</tr>
<tr>
<td></td>
<td>39.6</td>
<td>39.4</td>
<td>39.1</td>
<td>39.5</td>
<td>35.8</td>
</tr>
</tbody>
</table>
The calculations listed under IV and throughout the remainder of the table are based on the assumption that the solvent yields from the starch in all cases are proportional to those observed in the control flask, and independent of the amount of levulose added in the form of the hydrolyzate. Once the total solvent yield is determined, that which may be attributed to the levulose is obtained by difference. In Fig. 5 the yields from the syrup in terms of individual solvents and total solvents (IV, V, VI, VII, and VIII in Table 5) are plotted against the quantity of levulose present in the substrate.

Again it is apparent that the solvent yields from the syrup are dependent only upon the quantity of reducing sugar present, provided that the proper nutrients are present. This fact, together with the data in III and IX of Table 5 shows that there is no significant lowering of the yield of solvents up to 72.5 per cent replacement of the corn by its carbohydrate equivalent in hydrolyzed artichoke syrup.

It should be noticed also that the solvents yield in the control flask, based on the dextrose equivalent of the starch, is in the upper part of the range mentioned as normal for a good fermentation of corn, namely, 36 to 40 per cent. The yields from the starch in the remainder of the flasks are on the average about 4 per cent higher than from the levulose.
Figure 5. Solvent yields from reducing sugars in the hydrolysate.
In a subsequent series in which corn mash was replaced by various amounts of the hydrolyzed syrup, the replacements ranged, in 10 per cent intervals, from 0 to 100 per cent. Throughout the series, up to and including 80 per cent replacement, the average percentage conversion of the carbohydrate was 37 per cent. With the 90 per cent replacement the conversion was 33 per cent, and with 100 per cent replacement, which was the direct fermentation of the hydrolyzed juice, the yield was only 25 per cent, as in the experiments described in 4 above. These results are in complete agreement with those in Table 5, and in addition show the point at which nutrients for the organisms begin to be deficient. From these results it appears that the essential nutrients for the butyl-acetonic fermentation of the Jerusalem artichoke are supplied when corn mash constitutes 10 to 15 per cent of the total available sugar in the medium.

6. Effect of time of addition of hydrolyzate to the fermenting corn mash. The following experiment was devised to determine whether better solvent yields would be obtained by adding the hydrolyzate to the corn mash before inoculating the medium, or by adding portions periodically during the course of the fermentation. The hydrolyzate used was prepared in the usual manner by the treatment of syrup with hydrochloric acid, and neutralization with sodium hydroxide. The juice contained 6.9 g. reducing sugar per 100 cc. Erlenmeyer flasks
of 6 liter capacity were used for the fermentations and contained the following amounts of mash.

1. 330 g. of corn plus 5 liters water
2. 165 g. of corn plus 3.5 liters water
3. 132 g. of corn plus 3 liters water

Each was plugged and sterilized, and after cooling, was inoculated with 200 cc. of POS culture growing in 6 per cent corn mash. Number 1 was the control fermentation; 2 and 3 received the following amounts of hydrolyzate at the times indicated.

<table>
<thead>
<tr>
<th>Flask No.</th>
<th>Volume of hydrolyzate added at the indicated times after inoculation</th>
<th>Amount of corn mash replaced by hydrolyzate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 hours</td>
<td>12</td>
</tr>
<tr>
<td>1 Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>500</td>
</tr>
</tbody>
</table>

These fermentations showed the acidity changes characteristic of good corn mash fermentations. Flask number 1 showed good head formation 12 hours after inoculation, while 2 and 3 showed heads after 24 hours. The flasks were removed from the incubator after 80 hours. After the final volumes were measured, two 250 cc. samples removed from each flask and distilled for solvent analysis. The analyses were performed in the usual manner. The results are given in Table 6.
mixture of the two cultures. However, it was believed that the
fore use of sterilized with the germinated corn mash after
previous experiments the hydrolyzate was always encountered be-
different sterilization of the hydrolyzate. In the
meant, which is, of course, much more convenient.
In fact, the result of adopted favor the latter method of treat-
which all of the juice is added before inoculation of the mash.
Resultation is concentrated to the yields in fermentations to
lyzed juice is added to the fermentation corn mash during the
no significant difference in the solvents yields when the hydro-

Comparison of this table with Table 2 shows that there is

<table>
<thead>
<tr>
<th>Per cent Yield</th>
<th>Total Sugar</th>
<th>20°Brix</th>
<th>160.8</th>
<th>62.20</th>
<th>67.40</th>
<th>69.65</th>
<th>67.60</th>
<th>72.00</th>
<th>72.60</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.9</td>
<td>22.1</td>
<td>198.2</td>
<td>62.5</td>
<td>62.6</td>
<td>67.4</td>
<td>69.65</td>
<td>67.60</td>
<td>72.00</td>
<td>72.60</td>
</tr>
</tbody>
</table>

Total Solvents G. 6. per 100 cc. Solvent

Table 6. Solvent Yields after Fermentation of Hydrolyzed

54
conditions of hydrolysis were such that the juice would be sterilized during the treatment. If the subsequent handling of the juice were carried out under conditions minimizing the introduction of contaminants, sterility sufficient for good fermentations would then be maintained. To test this assumption the following experiment was performed. A 12 liter round bottom flask was plugged with cotton and sterilized by heating in the autoclave for 1/2 hour at 20 lbs. pressure. In another flask 1 liter of syrup was diluted to 9 liters with water and the solution was heated to 80°. While the mouth of the sterile flask was being heated with a free flame, the diluted syrup was poured in, and sufficient hydrochloric acid was added to adjust the pH to 1.75. The flask was plugged and the contents maintained at 80° for 1 hour. The flask was then cooled a little, and 10 per cent sodium hydroxide was added from a sterile container to neutralize the acid. The mouth of the large flask was heated with a free flame during the addition of the alkali, and the liquids were thoroughly mixed by vigorous shaking. The amount of alkali used was the quantity calculated to neutralize only 9/10 of the acid originally added. The pH of the resulting solution was then 5.8 to 6.1, depending on the initial quantity of syrup.

To test the sterility of the solution, various amounts were removed by means of sterile pipets and added to tubes of freshly sterilized corn mash. These were incubated at 37° and
examined daily for signs of fermentation. The large flask was likewise incubated. After two weeks of incubation there was no noticeable liquefaction of the starch in the corn mash tubes, and gas production was likewise absent. The contents of the large flask were also unchanged after the two week period. After three weeks of incubation the corn mash tubes showed a few bubbles of gas which were, however, barely perceptible. On the surface of the solution in the large flask had appeared four or five tiny colonies which seemed to be mold growth. These were fished out and examined with the low power microscope. Slender filaments of mold mycelia were visible, but the amount of growth was quite small.

These results justified the use of the hydrolyzed juice directly without further sterilization, provided that transfers from the hydrolysis vessel were made under sterile conditions. Therefore, in the remainder of the experiments described in this section, whenever hydrolyzed artichoke syrup or chips are mentioned, they have been employed directly after hydrolysis without any further sterilization.

e. Fermentation of hydrolyzed juice in the presence of soy bean meal as protein supplement. Preliminary experiments in which the unhydrolyzed juice was fermented in the presence of various amounts of soy bean meal showed very low solvent yields. The reducing sugar already present in the juice was consumed, but the bulk of the polysaccharides was not attacked.
These results agreed with those obtained by using unhydrolyzed juice with the other nutrients; therefore, attention was turned to the fermentation of the hydrolyzed juice in the presence of various amounts of soy bean meal.

Various quantities of an aqueous suspension of soy bean meal were sterilized in a series of 1 liter Erlemeyer flasks. Artichoke hydrolyzate containing 3.5 g. of sugar per 100 cc. was then added to the flasks. The final concentration of sugar in each flask was 3 per cent; the soy bean meal content ranged from 0 to 3 per cent. The media were inoculated with actively fermenting POS subculture in corn mash, and after 3 days they were tested for solvents using the specific gravity method and also the acetone titration. The results of the fermentations are given in Table 7. The corn mash control flask contained 45 g. of corn of 62 per cent dextrose equivalent. Each of the other flasks received 650 cc. of hydrolyzate, equivalent to 22.75 g. of sugar, together with the amounts of soy bean meal indicated. The final volumes were about 800 cc.
Table 7. Influence of soy bean meal on the fermentation of artichoke hydrolyzate.

<table>
<thead>
<tr>
<th>No.</th>
<th>Soy bean content</th>
<th>Solvents: g. per 100 cc. of distillate: g. per total solvent: yield from sugar.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.51</td>
<td>1.61</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.37</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.62</td>
<td>0.95</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.25</td>
<td>1.09</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>1.50</td>
<td>1.11</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>1.98</td>
<td>1.09</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>2.50</td>
<td>1.14</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>3.10</td>
<td>1.01</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>2.70</td>
<td>0.97</td>
</tr>
<tr>
<td>10</td>
<td>Corn mash control</td>
<td>1.18</td>
<td>3.60</td>
</tr>
</tbody>
</table>

It will be seen that the solvent yields reached a maximum when the soy bean content was about 0.9 per cent. The yields were of the same order up to about 2.5 per cent soy bean concentration, after which they diminished slightly. This preliminary experiment showed that good solvent yields could be obtained from the artichoke hydrolyzate in the presence of soy bean meal.

f. Fermentation of hydrolyzed chip sludge in the presence of soy bean meal with sugar concentration varied. Another experiment was devised in which chip sludge was used and the concentration of sugar in the mash was varied slightly. The hydrolyzate was prepared by treating 450 g. of ground artichoke
chips in a 5 liter flask with sulfuric acid. After neutralization with sodium hydroxide, the total volume was 4460 cc., and sugar per 100 cc. was 5.67 g. Various amounts of hydrolyzate were added to a series of 2 liter Erlenmeyer flasks which contained sterile soy bean mash. In the previous experiment the minimum content of soy bean meal for successful fermentations was about 25 per cent of the reducing sugar present. In this experiment the same ratio was maintained. In addition, 2 flasks were prepared containing only sterile soy bean mash which was fermented to determine whether any appreciable solvents are produced from the soy beans alone. The compositions of the media in the several flasks are as follows:

<table>
<thead>
<tr>
<th>Flask No.</th>
<th>Composition of Substrate</th>
<th>Percent Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 g. of corn - 61.2 g. dextrose*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18 g. soy bean meal plus 1200 cc. hydrolyzate 4.28</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15 g. &quot; &quot; &quot; 1000 cc. &quot;</td>
<td>3.73</td>
</tr>
<tr>
<td>4</td>
<td>12 g. &quot; &quot; &quot; 800 cc. &quot;</td>
<td>2.94</td>
</tr>
<tr>
<td>5</td>
<td>60 g. &quot; &quot; &quot; no hydrolyzate</td>
<td>--</td>
</tr>
<tr>
<td>6</td>
<td>90 g. &quot; &quot; &quot; &quot;</td>
<td>--</td>
</tr>
</tbody>
</table>

*total amount of carbohydrate in terms of dextrose equivalent

The volume of mash in each flask was about 1600 cc. The contents of each flask were inoculated with about 80 cc. of POS subculture in 5 per cent corn mash. The corn mash control fermented normally. Also in flasks Nos. 2, 3, and 4 there was sufficient gassing to form typical heads from the
suspended artichoke material. The gassing continued about 30 hours at which time the color of the medium had become light brown in contrast to the dark muddy color before inoculation. There was some gas production in the flasks that contained only the soy bean meal, but the fermentative action was not of the vigorous character of the normal butyl-acetonic fermentation.

After 84 hours, samples were withdrawn and complete solvent analyses were performed. The results obtained are given in Table 8.

Table 8. Influence of sugar concentration on the fermentation of soy bean - artichoke sludge

<table>
<thead>
<tr>
<th>No.</th>
<th>Sugar conc.</th>
<th>Solvents</th>
<th>Total %</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.60</td>
<td>1.13</td>
<td>0.26</td>
<td>24.80</td>
</tr>
<tr>
<td>2</td>
<td>4.23</td>
<td>2.23</td>
<td>1.24</td>
<td>0.27</td>
</tr>
<tr>
<td>3</td>
<td>3.73</td>
<td>2.19</td>
<td>1.26</td>
<td>0.31</td>
</tr>
<tr>
<td>4</td>
<td>2.94</td>
<td>1.76</td>
<td>0.97</td>
<td>0.29</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>--</td>
<td>0.08*</td>
<td>--*</td>
</tr>
<tr>
<td>6</td>
<td>--</td>
<td>--</td>
<td>0.09</td>
<td>--</td>
</tr>
</tbody>
</table>

*When the very low acetone yields in Nos. 5 and 6 were noted, these distillates were rejected without further solvent determination.

This table offers additional proof that soy bean meal is a very good supplement in the fermentation of artichoke hydrolyzates by the butyl-acetone organisms.
The yields in the first four flasks were uniformly high except for No. 2 which had the highest sugar concentration, namely 4.28 per cent. Although the 35 per cent conversion obtained in this case is still quite good, it appears that for maximum conversion of the sugars to solvents, the concentration of reducing sugar in the mash should not greatly exceed 4 per cent.

**g. Large scale fermentation of artichoke hydrolyzate in presence of soy bean meal.** Following the demonstration that soy bean meal furnishes the necessary nutrients for solvent production in artichoke hydrolyzates, a large scale fermentation was planned to produce sufficient solvents for the actual separation and identification of the individual components. A 22 liter round bottom flask was used for the purpose, and after its preliminary sterilization, 1300 cc. of concentrated artichoke syrup were added, and diluted with water to 15 liters. Hydrolysis was accomplished with sulfuric acid which was afterwards neutralized with sodium hydroxide. The total amount of reducing sugar present was 920 g. A suspension containing 140 g. of soy bean meal in a liter of water was sterilized and added to the hydrolyzate in the large flask. The resulting concentration of soy bean meal in the medium was about 0.9 per cent. After standing over night in the incubator at 37°, the flask was heated to 100° for 15 minutes, then cooled to 37°, inoculated with 800 cc. of POS
subculture in 5 per cent corn mash, and incubated. Two corn mash control flasks containing 110 g. of corn in 1500 cc. of water were inoculated at the same time.

The control media headed up in 10 hours. After 12 hours the mash in the large flask was gassing vigorously, and continued so for 30 more hours. Gassing diminished slowly after that time and had finished by the 60th hour. On the surface of the medium there was a light head consisting of the residue from the corn mash inoculum and the soy bean meal. Samples were withdrawn from the controls and from the large vessel after 90 hours for complete solvent analysis.

The remainder of the liquor in the large vessel, about 17 liters, was distilled in the manner previously described for solvent separation. The yield of dry mixed solvents obtained was 255 g. The first fractionation yielded 55 g. at 55° to 56°, and 120° g. at 115° to 116°. Refractionation of the middle portion yielded an additional 14 g. at 55° to 56°, 24 g. at 115° to 116°, and about 3 g. at 78° to 79°. The rest of the liquid distilled through a range varying from 80° to 75°, and from 81° to 95°.

The fraction at 55° to 56° was identified as acetone by preparation of the 2, 4 dinitro phenyl hydrazone.

The melting point of the derivative obtained was 126° to 127°; the melting point of 2,4 dinitro phenyl hydrazones of acetone is 128°. The fraction boiling from 115° to 116° was
identified as butyl alcohol by preparation of the 3, 5 dinitro benzoate.

The melting point of the derivative obtained was 63° to 64°; the melting point of butyl 3, 5 dinitro benzoate is 64°.

The fraction boiling from 78° to 79° was not definitely characterized. A 3, 5 dinitro benzoate was obtained which melted at 76°. Ethyl 3, 5 dinitro benzoate melts at 92°, and a small amount was prepared for examination. The freshly prepared crystals were compared under the microscope with some of those which melted at 76°. Many needle shaped crystals from the latter bore a close resemblance to those of the known derivative. Hence, it was concluded that ethyl alcohol was present in the fraction boiling from 78° to 79°; although the difficulty of effecting a complete separation of the ethanol from the other solvents, and from the small amount of water which is always present, prevented the preparation of a satisfactory derivative.

The total amount of solvents recovered by this fractionation, as already mentioned, was 255 g. Since the total amount of sugar contained in the hydrolyzate was 920 g., the yield was 28.7 per cent. Obviously in such a method of recovery of solvents, there is considerable loss, and the standard method of solvent analysis showed a higher yield, amounting to 56.8 per cent. The results of the analyses for individual components
of the solvent mixture from this large scale fermentation, as well as the two corn mash controls, are given in Table 9.

Table 9. Solvent yields from the large scale fermentation of artichoke hydrolyzate in the presence of soy bean meal.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sugar content: g. per 100 cc. of distillate</th>
<th>Solvents: from g. per: the flask</th>
<th>BuOH</th>
<th>Acetone</th>
<th>EtOH</th>
<th>Total yield: %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68.2*</td>
<td></td>
<td>2.11</td>
<td>1.30</td>
<td>0.68</td>
<td>27.47</td>
</tr>
<tr>
<td>2</td>
<td>68.2*</td>
<td></td>
<td>2.10</td>
<td>1.31</td>
<td>0.83</td>
<td>27.10</td>
</tr>
<tr>
<td>3</td>
<td>920.0</td>
<td></td>
<td>2.81</td>
<td>1.39</td>
<td>0.63</td>
<td>339.10</td>
</tr>
</tbody>
</table>

*Corn mash controls using 110 g. of corn which is equivalent to 68.2 g. of dextrose.

It will be noticed that the sugar concentration in the large vessel was (920/17,550) 100, or 5.25 per cent. This experiment, as originally planned, called for a sugar concentration of about 4 per cent, as was specified for satisfactory yields in Table 8. However, a miscalculation was made in regard to the concentration of sugar in the syrup used. The result was that the medium, as finally prepared, had the higher sugar concentration of 5.25 per cent. However, inspection of Table 9 will reveal that the conversion of the sugar to solvents attained the very satisfactory figure of 36.8 per cent. Thus, the fermentation of substrate on this larger
scale resulted in a better solvent yield than could have been expected from the smaller scale operations. This observation is common in the fermentation industries where a successful plant operation usually produces a better yield of the desired products than can be obtained by the laboratory methods.

Further investigations were carried out to determine how high the sugar concentrations could be raised before there was an appreciable decrease in solvent yield. Two more fermentations were carried out in the 22 liter flasks. The media were prepared exactly as described except that the sugar concentration in the first fermentation was 6.5 per cent and 8.0 per cent in the second. The external evidence of fermentative activity was the same as with the lower concentration of sugar. There was abundant gassing, and the color of the media changed from the original dark coloration to a light brown. However, solvent analyses showed that the conversion of sugar to solvents in the first fermentation was only 28 per cent, and in the second, it was reduced to 22 per cent. After completion of the fermentations, there was a rather high residual sugar content, which amounted to 2.5 to 3.0 per cent. This decrease in the percentage yield of solvents was to be expected, and no further comment is necessary.

h. Fermentation of artichoke hydrolyzate in the presence of corn gluten. Underkofler (1934) was able to obtain satisfactory utilization of several sugars in synthetic media to
which corn gluten had been added. The use of corn gluten was therefore suggested as a possible protein supplement in the butyl-acetonic fermentation of the Jerusalem artichoke.

The following experiments were planned to determine whether satisfactory yields of solvents could be obtained from artichoke media supplemented by the addition of corn gluten. A series of five 4 liter Erlenmeyer flasks were prepared, each of which contained 2 liters of water. Corn gluten was added in the amount of 35 g., 40 g., 45 g., 50 g., and 55 g., respectively. Each flask was plugged and sterilized, and after cooling, each received 1000 cc. of sterile hydrolyzate, equivalent to 114 g. of reducing sugar. The final sugar concentration of the juice was then about 5.7 per cent. Each flask was inoculated with 100 cc. of corn mash FOS subculture and incubated at 37°. Gas production was only moderate, and the fermentative activity did not appear to be vigorous. After 84 hours, samples were withdrawn from each flask and tested for solvents by the specific gravity method. The specific gravities of the several distillates ranged, without any particular order, from 0.9985 to 0.9960. The corresponding yields of solvents were, then, 18.5 to 29.5 per cent. These yields are low, and, in fact, approximate those observed in the direct fermentation of artichoke hydrolyzates. Repetition of the experiment confirmed these results, although the yields from control fermentations
using corn mash were normal. The residual sugar present in the media after fermentation had ceased was quite high, the average remaining being about 50 per cent of the original. These results discouraged further attempts to produce satisfactory fermentations of the artichoke hydrolyzates using corn gluten as a supplementary nutrient.
SUMMARY AND CONCLUSIONS

The experimental work described in the previous section constituted a study of the solvent yields obtained by the fermentation, under various conditions, of the Jerusalem artichoke. Previously reported work had shown that levulose was fermentable by \textit{Cl. acetobutylicum}, yielding butyl alcohol and acetone. The levulose present in the artichoke hydrolyzates has likewise been shown in this investigation to be fermentable, and to produce yields of solvents of the same order as obtained by the fermentation of starch in corn mash.

The direct fermentation of unhydrolyzed artichoke juices was not successful. The direct fermentation of the hydrolyzed juices produced solvent yields of 20 to 30 per cent of the reducing sugar present.

The fermentation of media in which various amounts of corn mash were replaced by unhydrolyzed artichoke juice resulted in a progressive decrease in the solvent yields. However, when the corn mash was replaced by carbohydrate equivalents of hydrolyzed juice, the solvent yields were strictly proportional to the quantity of sugar present, up to the point of 85 to 90 per cent replacement of the corn mash. The per cent yields of the solvents were usually 38 to 40 per cent for the dextrose
equivalent to the starch in the corn mash, and 35 to 37 per cent for the reducing sugars present in the hydrolyzate. The mixture of hydrolyzate with corn mash, therefore, constitutes a satisfactory medium for the butyl-acetone fermentation of artichokes.

The addition of soy bean meal to artichoke hydrolyzates resulted in a notable increase in solvent yields. When the concentration of soy bean meal in the medium is 0.8 to 2.5 per cent, and the sugar concentration 2.8 to 4.0 per cent, the percentage conversion of sugar to solvents is 38 to 40 per cent. The fermentation of a large quantity of medium with a soy bean content of 0.9 per cent and sugar concentration of 5.25 per cent produced solvent yields equivalent to 36.8 per cent of the sugar. If the sugar content in the large fermentations was increased to 8.0 percent, there was a corresponding decrease in solvent yields. The mixed solvents produced in these large fermentations were recovered and identified as butyl alcohol and acetone by the preparation of appropriate derivatives.

The addition of corn gluten to artichoke hydrolyzates was not observed to produce an increase in the yield of solvents, as in the case of addition of corn mash or soy bean meal.

The conclusions to be drawn from these experiments are, that, under the proper conditions, the reducing sugars present in the artichoke hydrolyzates can be fermented by *Clostridium acetobutylicum* to produce normal yields of solvents. For this
purpose the hydrolyzates must be supplemented by the addition of such protein containing materials as corn mash or soy bean meal.

The bulk of the polysaccharides present in the unhydrolyzed juices cannot be utilized by the butyl-acetone organisms. The sugar present in the hydrolyzates is fermentable, but for its complete utilization, there must be added the various protein supplements mentioned. Contrary to the conclusions previously drawn by Thaysen and Green (1927), in this investigation the sugars present in the hydrolyzates were not observed to exert any inhibitory effect upon the fermentation up to a concentration of 5.25 per cent.

By the use of larger quantities of substrate, 15 to 20 liters, the sugar concentration may be increased somewhat without the considerable decrease in yield observed in the smaller scale operations.
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