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The chemical action of Aerobacter faeni on xylose and on sucrose

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THE CHEMICAL ACTION OF *AEROBACTER FAENI* ON XYLOSE AND ON SUCROSE

By

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A Thesis Submitted to the Graduate Faculty

for the Degree of

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TABLE OF CONTENTS

		Page
I.	Introduction	4
II.	Historical	7
III.	Description of Organism	9
IV.	Experimental	10
	A. Development of the Medium	10
	B. Qualitative Analysis	18
	1. Discussion	18
	2. The Identification of the Products formed from Xylose and Sucrose	20
	C. The Relative Amounts of the Products formed under Aerobic and Anaerobic Conditions from Xylose and Sucrose	31
	1. Introduction	31
	2. Procedure	31
	3. Methods of Analysis Used	35
	4. Results of Analysis	47
V.	Discussion	50
VI.	Summary	52
VII.	Literature Cited	53

I. INTRODUCTION

One of the fields of research now occupying the attention of various agencies is the practical commercial utilization of agricultural wastes such as the cornstalk, corncob, oat hulls and the like. One method of utilization holding promise is the production of chemicals by the fermentation of these products. The commercial production of chemicals by fermentation of agricultural products is old but until recently was practically limited to the chemicals ethyl alcohol and acetic acid from the grains and fruits. Later black strap molasses, a waste product from the cane sugar industry has become an important source of ethyl alcohol and glycerol. One of the most significant recent developments in the field has been the large scale production of butyl alcohol and acetone by the anaerobic fermentation of corn. One plant uses thousands of bushels of corn per day in this process.

The commercial processes mentioned deal for the most part with the fermentation of cellulose, disaccharides such as cane sugar and maltose, or monoraccharide hexoses such as dextrose.

The agricultural waste products, such as cornstalks, corncobs and oat hulls, contain a large proportion of

pentosans, which are carbohydrate materials yielding pentoses, especially xylose, upon hydrolysis. In the utilization of these materials it is necessary to deal with and develop the fermentation chemistry of the pentosans. Before proceeding directly with the pentosans it is necessary to know more about the chemism of organisms acting on a pure pentose such as xylose. Such was the purpose of this investigation. The ultimate goal would be to take a typical fermentation and make a complete study of the effect of physical and chemical environment on the kind and amount of substances produced, with a view to control of yields of valuable products.

Due to the complexity of the problem and more immediately to the unsatisfactory state of analytical methods in this type of work it has developed that this report is preliminary in nature, covering the qualitative analysis for the products produced by Aerobacter faeni on xylose and sucrose, and a preliminary study of the relative amounts of the products produced under aerobic and anaerobic conditions.

This problem involves a knowledge of both bacteriology and chemistry and as such is best advanced by the utmost in cooperation between the departments of chemistry and bacteriology. Hence the attack on this problem has involved

the collaboration of the bacteriologist, the plant chemist and the bio-physical chemist.

II. HISTORICAL

Comparatively few studies have been made of the fermentation of xylose, in which the dissimilation products have been adequately identified. The work which has been done is summarized in Table I. References where impure or mixed cultures were used are not included, nor are those where it was simply reported that the organism utilized xylose as a source of carbon.

TABLE I

DISSIMILATION PRODUCTS OF XYLOSE

Organism	Products	Authority
Friedlander's pneumobacillus	acetic ac., ethyl alc., l-lactic ac., succinic ac.	Grimbert, 1896.
Sorbose bacterium	xylonic ac.	Bertrand, 1898
Citromyces	citric ac.	Wehmer, 1913
Lactobacillus pentoaceticus	acetic ac., lactic ac.	Fred, Peterson and Davenport, 1919
B. acetoethylicum	acetone, ethyl alc.	Northrop, Ashe and Morgan, 1919
B. acetoethylicum	acetone, ethyl alc. formic ac.	Northrop, Ashe and Senior, 1919

TABLE I (Cont'd.)

DISSIMILATION PRODUCTS OF XYLOSE

Organism	Products	Authority
B. lactis aerogenes, B. para typhoid, B. typhosus	acetic ac., butyric ac., ethyl alc., formic ac., lactic ac., succinic ac.	Fred and Peterson, 1920
B. acetoethylicum	acetic ac., formic ac., lactic ac., ethyl alc., acetone, CO_2.	Arzberger, Peterson and Fred, 1920
Lactobacillus pentoaceticus	acetic ac., lactic ac.	Anderson, Fred and Peterson, 1920
Lactobacillus pentosus, Lactobacillus pentoaceticus	acetic ac., lactic ac.	Fred, Peterson and Anderson, 1921
B. granulobacter pectinovorum	acetic ac., acetone, butyl alc., butyric ac., lactic ac.	Speakman, 1923
Acetobacter xylinum, B. herbicola aureum, B. vulgatus.	acetone, CO_2, ethyl alc.	Fred, Peterson and Anderson, 1923
B. granulobacter pectinovorum	acetone, butyl alc., CO_2	Peterson, Fred and Schmidt, 1924
Sacc. cerevisiae	ethyl alc., CO_2, glyceric aldehyde (?)	Abbott, 1926
Asp. niger	citric ac., oxalic ac.	Amelung, 1927
Asp. niger	citric ac., oxalic ac.	Bernhauer, 1928
Propionibacterium pentosaceum	acetic ac., CO_2, propionic ac.	Werkman, Fulmer and Rayburn, 1929

III. DESCRIPTION OF THE ORGANISM USED

The organism finally decided upon is known as Aerobacter faeni, n.sp. which was isolated and identified by Burkey (1928) in his studies on bacteria attacking constituents of the cornstalk. This organism, among other advantages, will grow well in a medium containing inorganic nitrogen in the form of ammonium salts. It grows rapidly on xylose, produces gas, and is relatively easy to handle.

The description of the organism as given by Burkey follows:

> "Non-motile rods, 1.0μ broad and 1.0μ to 3.0μ long, conforming to the generic diagnosis. Acid and gas produced from the mono- and di-saccharides, including melezitose, from pentose sugars, raffinose, rhamnose, trehalose, salicin, aesculin, all the alcohols except erythritol, from glycogen, soluble starch, and pectin. No fermentation from amygdalin, inulin, or the pentosans. Acid and gas in litmus milk. Indol produced. Gelatin not liquified. Isolated from hay infusion."

IV. EXPERIMENTAL

A. Development of the Medium.

The medium used at first was arbitrarily made up and contained the usual inorganic components of a typical nutrient medium.

$K_2HPO_4 \cdot 3H_2O$	2.0 g.
NaCl	4.0 g.
$MgSO_4 \cdot 7H_2O$	0.2 g.
NH_4Cl	6.0 g.
$CaCO_3$	10.0 g.
sugar	10.0 g.

Made up to 1 liter with distilled water.

Later the use of NaCl and $MgSO_4 \cdot 3H_2O$ was discontinued with no apparent decrease in the growth of the organism. Experiments were undertaken to find whether there is an optimum concentration of the ammonium chloride or of the phosphate.

1. Effect of varying concentration of NH_4Cl on the amount of xylose utilized.

The medium consisted of,

Xylose	0.5 g.
$K_2HPO_4 \cdot 3H_2O$.	0.1 g.
NH_4Cl	varying amounts.

made up to 50 cc with distilled water.

Erlenmeyer flasks of 125 cc capacity, closed with cotton plugs, were used as containers. The media were sterilized at 15 lbs. pressure for 15 minutes, and after cooling were inoculated with 1 cc of a 24 hr. culture, and incubated at 37°C. After the fermentation had proceeded for 10 days, the contents of each flaskwere made up to 100 cc and analyzed for xylose by the phloroglucinal method as described in the Official Methods of the A.O.A.C. The results are given in Table II.

TABLE II.

EFFECT OF VARYING CONCENTRATIONS OF AMMONIUM CHLORIDE ON THE AMOUNT OF XYLOSE UTILIZED. pH 7.2-6.5

Flask Number	NH_4Cl in gm.	Growth	Unused xylose in gm.
1	0.000*	+	0.320
2	0.001	+	0.430
3	0.010	+	0.418
4	0.050	+	0.384
5	0.100	+	0.408
6 6	0.200	+	0.440
7	0.400	+	0.374
8	0.600	+	0.440
9	0.800	+	0.400

* Some NH_4Cl added in inoculum.

TABLE II. (Cont'd.)

Flask Number	NH_4Cl in gm.	Growth	Unused xylose in gm.
10	1.000	+	0.414
11	2.000	-	---
12	3.000	-	---

The results were inconclusive. It was thought that the acidity developed during the fermentation might have been a contributing factor so an experiment was made using a liter of medium containing

$K_2HPO_4.3H_2O$	2.0 g.
NH_4Cl	6.0 g.
Xylose	20.0 g.

made up to 1 liter with distilled water. The medium was divided into two equal portions and each placed in a 1 liter Erlenmeyer flask. To one flask were added 10 g. of precipitated $CaCO_3$. Both flasks were plugged with cotton and sterilized at 15 lbs. for 20 minutes.

The pH after sterilization was 7.1 as determined by the quinhydrone electrode.

Each flask was inoculated with 1 cc of a 24 hr. culture. After 5 days the evolution of gas in the flask without carbonate had practically stopped and the pH was found to be 4.2. Some 0.2 N sterile NaOH was added which brought the pH

close to neutrality, the next day the pH was down to 4.1 so more NaOH was added. After the medium had been neutralized 4 times in all the fermentation stopped, and analysis showed no xylose present.

Ten days after inoculation the evolution of gas in the flask containing carbonate had ceased, analysis showed only a trace of unfermented xylose, the pH of the solution was 5.8.

With this fact in mind another attempt was made to determine the optimum concentration of the ammonium chloride, and the phosphate. Erlenmeyer flasks of 125 cc capacity were used, containing 50 cc of medium and closed with cotton plugs. The following media were used:

(a) 0.1 g $K_2HPO_4.3H_2O$
4.0 g Xylose
2.0 g $CaCO_3$
Varying amounts of NH_4Cl
50 cc of distilled water

(b) 0.3 g NH_4Cl
4.0 g Xylose
2.0 g $CaCO_3$
Varying amounts of $K_2HPO_4.3H_2O$
50 cc of distilled water.

In these experiments an attempt was made to adjust all of the

flasks to the pH of 7. The method used was to make up twice the amount of the desired solutions and divide into two equal portions. To one portion of each solution was added a definite amount of rosolic acid and then 0.1 N KOH or 0.1 HCl was added until the color matched a standard solution of pH = 7 which contained the same amount of indicator. The proper amount of KOH or HCl was then added to the other half of the solution. (The purpose of this scheme was to get around using an internal indicator. However, it was found later that rosolic acid was very satisfactory as an internal indicator with this organism.) The flasks were sterilized 15 minutes at 15 lbs. pressure, inoculated with 1 drop of a 24 hour culture and incubated at 37°. At the end of 12 days the flasks were analyzed for unused xylose. The results are given in Table III.

TABLE III

(a) Effect of varying concentrations of NH_4Cl on Xylose utilized.

(b) Effect of varying concentrations of $K_2HPO_4.3H_2O$ on Xylose utilized.

Flask Number	NH_4Cl in gm.	Growth	Unused Xylose in gm.	
1	0.01	+	2.48	(a)
2	0.10	+	1.76	
3	0.30	+	1.50	

TABLE III. (Cont'd.)

Flask Number	NH_4Cl in gm.	Growth	Unused Xylose in gm.	
4	0.70	-	---	(a)
5	1.00	-	---	

Flask Number	$K_2HPO_4.3H_2O$ in gm.	Growth	Unused Xylose in gm.	
6	0.01	+	0.93	
7	0.05	+	1.15	
8	0.10	+	1.35	(b)
9	0.50	+	2.76	
10	0.70	+	2.94	

For some unknown reason growth did not occur in flasks 4 and 5. It was not due to the concentration of NH_4Cl however as shown in the previous experiment. The maximum utilization of sugar for NH_4Cl occurred in flask No. 3 containing 0.6% NH_4Cl. The maximum utilization of sugar for the phosphate occurred in flask No. 6 containing 0.02% $K_2HPO_4.3H_2O$.

In order to check the experiment with the phosphate, another experiment was made using the optimum concentration of NH_4Cl. The experiment was carried out as before. After 9 days of fermentation the flasks were analyzed for unused xylose. The results are given in Table IV.

TABLE IV.

EFFECT OF VARYING CONCENTRATIONS OF $K_2HPO_4.3H_2O$ ON THE AMOUNT OF XYLOSE UTILIZED.

Flask Number	$K_2HPO_4.3H_2O$ in gm.	Unused Xylose in gm.
1	0.005	1.68
2	0.010	1.49
3	0.050	1.20
4	0.100	1.73

From these experiments it appears that the optimum concentration of NH_4Cl is about 6 g. per liter, and that of $K_2HPO_4.3H_2O$ is about 1.0 g. per liter. It would appear that the maximum amount of xylose that can be utilized is about 70 g. per liter.

In one experiment in which a large volume of medium containing sucrose was used to prepare a larger amount of material for investigation, and tap water was employed instead of distilled water, there seemed to be a more rapid fermentation than with a similar flask where distilled water had been used. This suggested that perhaps $MgSO_4$ might be helpful. To test this experiments were performed using the following media:

Flask #1.

3.5 g xylose 3.5000 ?

0.6 g NH_4Cl

0.1 g $K_2HPO_4.3H_2O$

5 g $CaCO_3$

made up to 100 cc with distilled water.

Flask #2.

Same with addition of 0.1 g $MgSO_4$.

After 7 days the contents of the flasks were analyzed for xylose. Flask No. 1 contained 0.0482 gm unfermented xylose. Flask No. 2 contained 0.0398 gm unfermented xylose. While these results are too meager to be conclusive the advantage seems to be slightly in favor of the $MgSO_4$ being present. So in the later part of the work it was used in the proportion of 1 g. per liter. No attempt was made to find an optimum concentration because early in the work it had been found that $MgCO_3$ could be substituted for $CaCO_3$ as a buffer for the fermentation. In this connection it was found that the $MgCO_3$ must be sterilized separately and then added to the cold medium. If it is sterilized in the medium enough $MgCO_3$ dissolves to make the medium sufficiently alkaline to prevent growth.

The medium as finally adopted for the quantitative experiments had the following composition.

Medium A.

Sugar	20.0 g
NH_4Cl	6.0 g
$K_2HPO_4.3H_2O$	1.0 g
$Mg\ SO_4.7H_2O$	1. g

Made up to 1 liter with distilled water.

$CaCO_3$, precipitated, 10.0 g

The pH of this medium when made up was never far from 7.2, after sterilization it was found that the pH had consistently dropped about 0.2.

B. Qualitative Analysis.

1. Discussion.

Since nothing was known of the nature of the products produced by this organism, other than the results of the standard tests used in classifying it, it was necessary to make a qualitative analysis of the products resulting from the fermentation. As the result of a large number of tests on the mixtures the following substances have been found: Ethyl alcohol, 2-3-butylene glycol, acetylmethyl carbinol, acetic acid, formic acid, succinic acid, l-lactic acid, CO_2, H_2, and butyric acid. The general method used by the organic chemist in identifying the compounds in a mixture is to separate them by various physical and chemical procedures, to purify

them and then to identify them by elementary analysis, determination of physical constants and preparation of derivatives. In practice this method is somewhat modified in identifying the products formed in fermentation, since the solutions are diluted instead of concentrated and some of the products are present only in infinitesimal amounts. In this type of work sensitive specific tests are much to be desired. The compounds or groups of compounds produced are often characteristic of the species of organism used, in fact this is one basis of bacteriological classification. As a result the usual practice is to first make specific tests for those substances whose presence is suspected. The next step is to discover by analysis if all of the carbon is accounted for. If the amount not accounted for is larger than the error allowed by the methods of separation and analysis used a more systematic investigation is necessary. In this work it was not found feasible to ferment a large amount of medium and then make all the qualitative tests on its contents, for as soon as the flask has been opened it is subject to contamination from other bacteria and molds. Instead, the various fractionsresulting from separation, such as volatile acids, non-volatile acids etc., from a number of flasks were carefully examined. Finally when the various products were identified, and the methods of separation were developed, a qualitative examin-

ation was made for these products on the contents of a single flask.

At the time this work was inaugurated the price of xylose was listed at about $100 per pound. For this reason it was decided to use sucrose when it was desired to obtain a large amount of some product for closer investigation and development of analytical methods. It is well known that as a general rule the same products are produced by a given organism from any sugar which it will ferment. The ratio of the products produced may or may not vary. To make sure however that the same products were produced from both xylose and sucrose, a complete qualitative analysis was carried out on both sugars.

2. The Identification of the Products Formed from Xylose and Sucrose.

The medium used was composed as follows:

$K_2HPO_4.3H_2O$	2.0 g
NaCl	4.0 g
$Mg\ SO_4$	0.2 g
NH_4Cl	6.0 g
$CaCO_3$	10.0 g
Xylose	20.00g

Made up to 1000 cc with distilled water.

The medium was placed in a 2 liter Erlenmeyer flask,

closed with a cotton plug and sterilized 1/2 hr. at 15 lbs. pressure. After cooling to room temperature it was inoculated with 5 cc of a 24 hour culture and incubated at 37°.

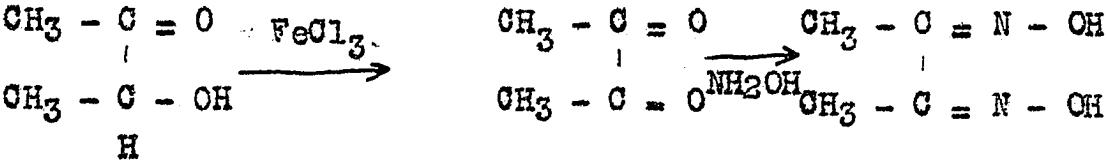
At the end of 8 days the fermentation had apparently ceased, so the contents were subjected to investigation. The tests were carried out as follows.

Xylose. A 25 cc portion was tested for unused xylose by means of the phloroglucinol method as described in the Official Methods of the A.O.A.C. This method is quantitative and is based on the transformation of the xylose to furfurol when treated with 12% H Cl. The furfural is then determined by precipitating it as the furfural phloroglucide which can be filtered and weighed. No xylose remained unfermented.

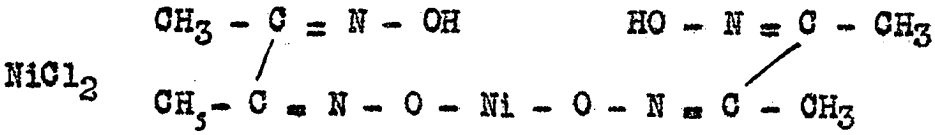
Acetylmethyl carbinol and 2-3-Butylene glycol. The solution was tested for the presence of acetylmethyl carbinal and 2-3-butylene glycol by the specific test devised by Lemoigne (1920) and modified by Kluyver, Donker and Visser't Hooft (1925). Both products were found to be present, the 2-3-butylene glycol apparently predominating in amount. The test was carried out as follows. A 15 cc sample of the neutralized solution was placed in a 500 cc distilling flask, connected to a water cooled condenser and mixed with 5 cc of a 45% ferric chloride solution and 1 cc of 0.5 N acetic acid. Heat was applied and 10 cc slowly distilled into a test tube. The distillate was

mixed with 1 cc of a 20% water solution of hydroxylamine hydrochloride, 2 cc of a 20% solution of sodium acetate and about 5 drops of 10% nickel chloride solution. The mixture was then boiled for a few minutes. A precipitate of fine red needles was formed if acetylmethyl carbinol was present in the original solution.

The test is due to the oxidation, by ferric chloride, of the acetylmethyl carbinol to the easily volatilized diacetyl which is distilled off. The diacetyl reacts with the hydroxylamine to form dimethyl glyoxime which in turn reacts with the nickel chloride to form the characteristic red insoluble nickel dimethyl glyoxime. These transformations can be symbolized as follows:



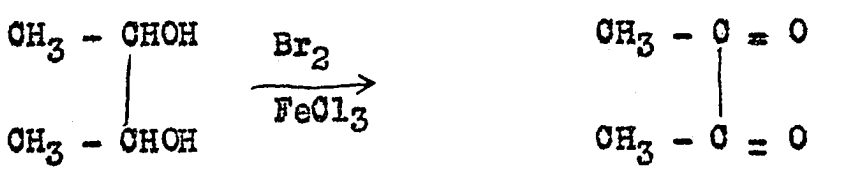
Acetylmethyl carbinol diacetyl dimethylglyomime



Nickel dimethylglyoxime

The ferric chloride oxidizes the acetylmethylcarbinol but not the 2-3-butylene glycol. Bromine, in the presence of iron salts, oxidizes 2-3-butylene glycol to diacetyl. The glycol can therefore be tested for in the residue from the acetyl-

methyl carbinol test. The glycol, having a low vapor pressure, is not appreciably lost when the diacetyl is distilled off. The test was carried out as follows. The residue in the distilling flask was mixed with 15 cc of water, 2 cc of bromine and 3 g. of solid sodium acetate and refluxed for 20 minutes on a water bath. The most satisfactory apparatus in which to carry out the bromine treatment was found to be a soil flask. This consists of an Erlenmeyer flask with a ground glass stopper in which is sealed a straight tube about 100 cm long. The water jacket of a condenser can be attached to it to make a water cooled reflux condenser. After cooling to room temperature the solution was decanted from any liquid bromine remaining. The bromine in the solution was exactly neutralized by means of a saturated solution of sodium thiosulfate, using starch potassium iodide test papers to determine the neutralization point. The solution was then slowly distilled, 10 cc were collected, neutralized to litmus with NaOH and tested for diacetyl as above.



2-3, butylene glycol diacetyl

Alcohol. The remainder of the solution was made distinctly alkaline to litmus with $Ca(OH)_2$, filtered from excess $CaCO_3$ and distilled, first at atmospheric pressure, then fi-

nally under reduced pressure (20 mm at 55° C) until less than 50 cc remained. This distillate was mixed with 50 g $NaHSO_3$ to hold back the acetylmethyl carbinol, and distilled until about 100 cc remained. This distillate which was slightly acid due to the presence of some SO_2, was made alkaline with NaOH and again distilled, using a short fractionating column. The distillation was continued until less than 100 cc of solution remained in the distilling flask. The distillate, which was neutral to litmus, was mixed with about 25 g of potassium dichromate and 50 cc of concentrated sulfuric acid and then refluxed for 15 minutes. After cooling to room temperature the solution was transferred to a distilling flask and distilled until about 200 cc remained. This residue was subjected to steam distillation until a 10 cc fraction of the distillate collected in a test tube did not decolorize one drop of phenolphthalin in 1 drop of 0.1 N NaOH. The acid distillate was made up to 1 liter and a 50 cc portion titrated with 0.0611 N $Ba(OH)_2$ using phenolphthalim as indicator. The amount required was 19.15 cc. A 400 cc portion, equivalent to 153.2 cc of 0.0611 N $Ba(OH)_2$, was then subjected to a Duclaux distillation as modified by Knetemann. (See description in the section on quantitative analytical methods.) The results are given in Table V.

TABLE V.

DISTILLING CONSTANTS OF VOLATILE ACID PRODUCED FROM ALCOHOL.

No. of cc Distilled from 400 cc.	cc 0.0611 N $Ba(OH)_2$ required	% Acid in Distillate:	% Acid for pure Acetic. (Knetemann).
50	12.8	8.3	8.2
100	13.6	17.2	16.8
150	14.3	26.6	26.1
200	15.2	36.5	36.1
250	16.3	47.2	46.9
300	18.0	58.9	59.1

The distilling constants, as shown in the table for the acid obtained by oxidation of the alcohol, agree very well with the distilling constants for pure acetic acid. Confirmation was obtained by neutralizing the remainder of the solution with NaOH, evaporating to ~~degrees~~ dryness and preparing the p- toluide as described by Mulliken (1904 pp. 81-2). The p-toluide melted sharply at 147°. The melting points of the p-toluides of the volatile acids are given by Mulliken as follows:

Acetic acid,	acet-p-toluide	m.p. 146°-7°
Propionic acid	propion-p-toluide	m.p. 123.5°- 124.5°
N. Butyric acid	butyr-p-toluide	m.p. 72.5° - 73.5°

This proves ethyl alcohol to be the only volatile alcohol present in appreciable quantities in the fermentation mixture.

Volatile Acids. The residue from the removal of the neutral volatile products was acidified to Congo-Red paper with

dilute H_2SO_4 and subjected to steam distillation. The distillation was discontinued when a fraction of more than 5 cc of distillate was required to decolorize 1 drop of phenolphthalein in 1 drop of 0.1N NaOH. The distillate, about 1500 cc, was made up to 2 liters and an aliquot portion titrated. It was found that 400 cc were equivalent to 105 cc of 0.0611 N $Ba(OH)_2$. A 400 cc portion was then subjected to a Knetemann distillation. The results are given in Table VI.

TABLE VI.

DISTILLATION CONSTANTS OF VOLATILE ACIDS

cc Distilled from 400 cc	cc.0611 N $Ba(OH)_2$ required	% Acid in Distillate	% Acid for Pure Formic (Knetemann)	% Acid Pure Acetic
50	7.5	7.1	4.7	8.2
100	8.2	14.9	10.0	16.8
150	8.4	22.9	16.0	26.1
200	9.2	31.7	22.6	36.1
250	10.1	41.3	30.5	46.9
300	11.5	52.3	40.1	59.1

The distilling constants in the table indicate that the volatile acids are largely formic and acetic, though the evidence is not conclusive since these results might conceivably be obtained by a mixture of formic with one of the higher volatile acids such as propronic or butyric. To clear up this point one half of the remaining acid solution was neutralized with

NaOH and evaporated completely to dryness. The salt obtained was tested for formates by dissolving part of it in water containing a little $HgCl_2$, and boiling. A white precipitate of Hg_2Cl_2 was obtained, which under the conditions used is a positive test for formic acid. A little of the salt was also boiled with $AgNO_3$. A black precipitate of metallic silver was obtained showing the pressure of formates. The salt also reduced alkaline potassium permanganate. (Mulliken 1904, p.83 and Allen I p. 486) The results obtained in the manner described are satisfactory proof of the presence of formic acid. The remainder of the sodium salt was examined to determine the nature of the other volatile acid radicals by preparing the p-toluides as described above. After several recrystallizations a p-toluide was obtained which melted at 148°, showing the presence of acetic acid in the fermentation mixture. These results show that the only volatile acids present in appreciable amounts are formic acid and acetic acid.

Non-Volatile Acids. The residue from the steam distillation of the volatile acids was extracted continuously with ether for 72 hours in an apparatus described later. The ether extract was added to about 400 cc of water and the ether removed by warming on a water bath. The solution was then boiled for a few minutes and titrated hot with 0.25 N $Ba(OH)_2$ using phenolphthalein as indicator. The solution, containing the barium salts of the non-volatile acids, was

then evaporated on a water bath to a volume of about 30 cc. It was then poured into 120 cc of 95% ethyl alcohol and allowed to stand for two days. The barium succinate is quite insoluble and precipitates out. Barium lactate is soluble under these conditions. The precipitate was filtered off and dissolved in about 50 cc of water. A slight excess of H_2SO_4 was added to precipitate the barium as $BaSO_4$ and the solution was filtered and extracted for 48 hours with ether. The extract was evaporated to dryness and the crystals obtained were treated with p-toluidine in the manner described by Mulliken (p. 86). The melting point of the p-toluide obtained was 256°. The melting point given by Mulliken for succinic acid p-toluide is 254.5° - 255.5°. The crystals of succinic acid when recrystallized from hot water, dried, and mixed with an authentic sample of succinic acid did not depress its melting point of 186°. These results prove the presence of succinic acid. The solution remaining after filtering off the barium succinate was tested for lactic acid by both the Uffelmann (1909), and Fletcher Hopkins (1907) tests. Both tests were positive.

The Uffelmann test was carried out as follows. A reagent was prepared by mixing 10 cc of a 4% solution of phenol with 20 cc of water and adding 1 drop of 1% $FeCl_3$. This forms a clear liquid of an amethyst color, which is turned yellow by a solution containing lactic acid. According to Uffelmann the test is sensitive to 1 part in 10,000 of lactic acid.

This test is not specific for lactic acid but is given also by tartaric, citric, malic, and oxalic acids. However, the presence of none of these other acids is to be expected in a fermentation of this type.

The Fletcher Hopkins test was carried out in the following manner. A few drops of the suspected solution, 5 cc of concentrated H_2SO_4, and a drop of a saturated $CaSO_4$ solution were heated in a test tube placed in a water bath for 2 hours. The tube was cooled and 2-3 drops of a dilute solution of thiophen (50 drops in 100 cc alcohol) were added. The presence of lactic acid was shown by the formation of a light cherry red color when the tube was again heated on the water bath. The reaction is due to the formation of acetaldehyde from the lactic acid. The color is the result of a product formed by reaction of the acetoldehyde with thiophen.

Both these tests, while giving a good indication of the presence of lactic acid, are not specific, so the zinc salt was prepared. The alcoholic solution of the barium lactate was evaporated on the water bath to remove the alcohol and the barium lactate was dissolved in about 100 cc of water. The barium was removed by adding the theoretical amount of 0.25 N H_2SO_4 to form $BaSO_4$ which was filtered off. The solution of lactic acid was then boiled with an excess of zinc carbonate and the excess zinc carbonate filtered off. The solution was decolorized by boiling with a few grams of

the decolorizing carbon known as Norit and allowed to evaporate at room temperature in the open air. After crystallization the salt was dried to constant weight over sulfuric acid and the percent moisture determined by heating for 3 hours at 103°-5°. It was found that the moisture content was 13%. The racemic zinc lactate has three molecules of water, or 18.17%, while the optically active form contains two molecules of water, or 12.9%. The salt when dissolved in water was found to be dextro rotatory when examined in a polarimeter. This shows that the free acid has a levo rotation.

Gaseous Products. A small portion of the medium containing xylose was placed in an ordinary fermentation tube, sterilized and inoculated with 1 cc of a 24 hour culture. After the evolution of gas had stopped, on the third day, the tube was filled with 25% NaOH and shaken. The volume of gas decreased from 9½ cc to 4½ cc. The gas remaining exploded in air when ignited. This indicates that the gaseous product was composed of carbondioxide and hydrogen in approximately equal parts by volume.

In examining the products formed from sucrose the fermentation and analysis was carried out in exactly the same way as described for xylose. The presence of unfermented sugar, however, was determined by the reducing power before and after inversion by hydrochline acid, as determined by the Shaffer Hartman (1921) method.

C. The Relative Amounts of the Products formed under Aerobic and Anaerobic Conditions from Xylose and Sucrose.

1. Introduction. Before an exact study of the effect of environmental change on the amounts of various fermentation products formed can be made, it will be necessary to have very exact methods of analysis for these products. If the study were limited to the quantitative determination of a few selected substances, such as carbon dioxide or unfermented xylose, the problem would be much simpler, for the analytical methods for these compounds are fairly precise. But with the analytical methods available at the present time an exact determination of all the various products which are present in a fermentation mixture, such as results from the action of Aerobacter faeni on sugar, is impossible.

In view of this fact it was decided to make a study of the relative amounts of the various products formed under conditions differing in environment as much as possible. Many organisms are quite sensitive to changes in chemical or physical environment and will not grow except under conditions varying within narrow limits. Aerobacter faeni, however, is a facultative organism growing almost equally well under both aerobic and anaerobic conditions. This suggested a study of this environmental change on the relative amounts of the various products formed.

2. Procedure. All of the fermentations were carried

out in the same type of apparatus. Aerobic conditions were established by passing air through the flask, and for anaerobic conditions hydrogen was used. The description of the apparatus is as follows. (See Fig. 1.)

A. The fermentation flask, a 2 l Erlenmeyer.

1. Gas inlet tube containing a plug of cotton at the outer end.

2. Gas outlet tube containing a glass stopcock.

3. Tube for introducing inoculum.

(a) Thick walled rubber tubing.

(b) Screw clamp.

(c) Cotton plug.

B. Vanier absorption bottle containing 40% KOH.

C. Absorption bottle containing 0.5 N KOH.

1. Short piece of glass tubing filled with beads.

2. Soda lime tube.

D, E. Gas washing bottles containing strong KOH.

F. Mercury trap.

1. Tube attached to gas source.

Medium A (See p. 18) was used throughout the experiment. Because of the decomposition of xyclose at high temperatures in solutions containing salts, separate sterilization was employed when this sugar was used as substrate. The fermentation flask containing the medium was placed in the autoclave and sterilized 30 min. at 15 lbs. pressure. During the process the glass stopcock was closed and the screw clamp open.

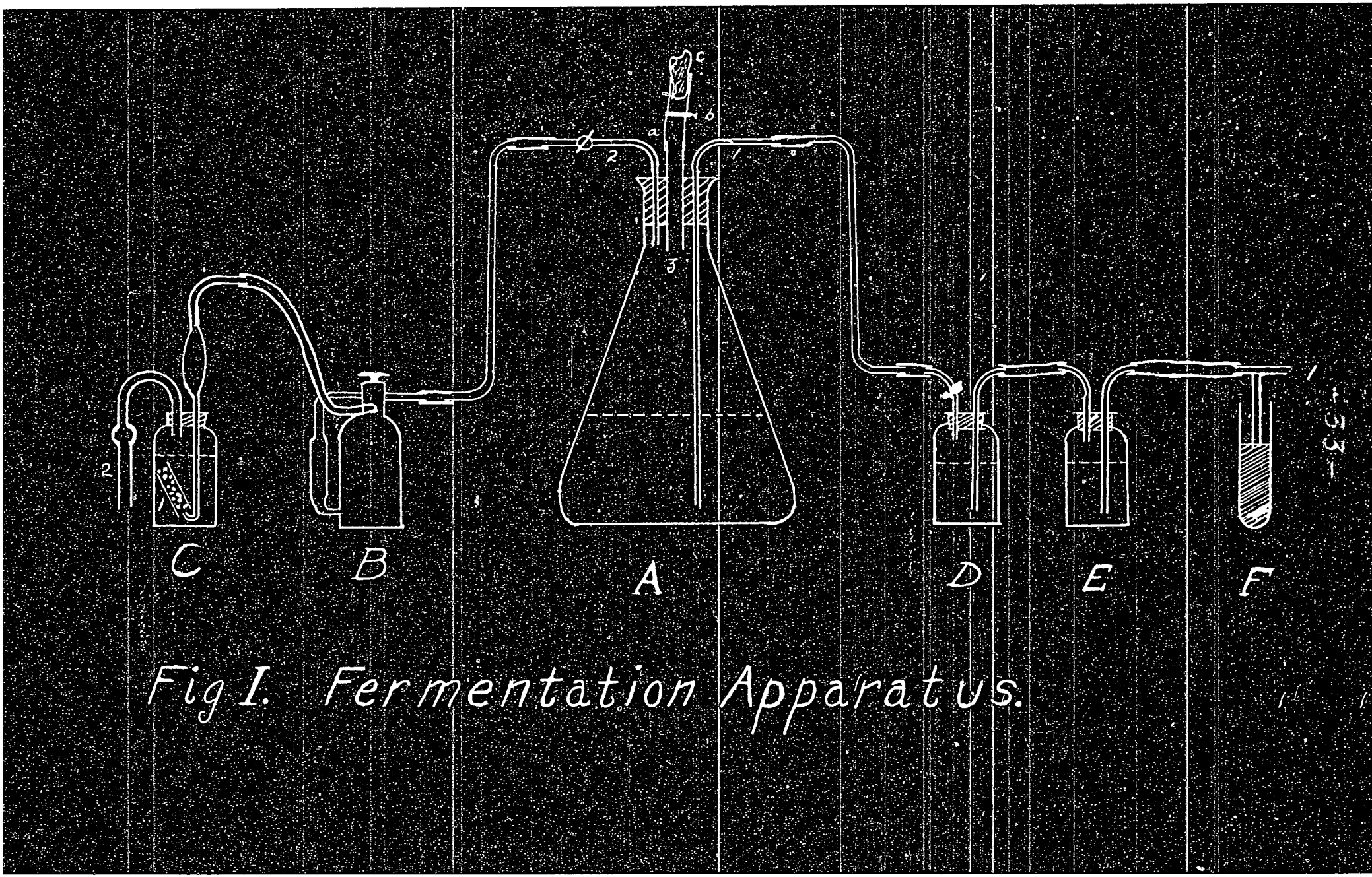


Fig I. Fermentation Apparatus.

After sterilization the flask was connected to the remainder of the apparatus, with the screw clamp closed and the stopcock open. A stream of gas was bubbled through the medium while it cooled so that it would be saturated with the gas. After reaching the temperature of the incubator, (37°C), the flask was inoculated with 5 cc of a 24 hr. culture. On the next day, and each day following, the gas was shut off at (a) by means of a screw clamp, forcing it to escape through the mercury trap. The stopcock was then closed and the vanier bottle removed and weighed to determine the amount of CO_2 absorbed. To insure the presence of excess alkali the solution was renewed after 6 grams of CO_2 had been absorbed. After about 8-10 days, when practically all of the CO_2 had been evolved, the aeration was stopped. The flask was then cooled to room temperature and 10 cc of 40% CO_2 - free NaOH added to it. The reason for adding the strong alkali was to make the solution sufficiently alkaline to prevent any further bacterial action. It had one unexpected but very satisfactory result. A flocculent precipitate was formed, probably magnesium ammonium phosphate, which, when settling carried down with it all of the suspended bacteria, leaving the solution perfectly clear. The solid material was filtered off and washed. The filtrate and washings were made up to 2 liters with CO_2 - free distilled water and analyzed by the methods described below.

3. Methods of Analysis used. Each different combination of products will require a slightly different method of approach in chemical analysis. In the methods described below only the substances, which have been found present by the qualitative analysis, are considered.

Carbon dioxide. The amount of CO_2 produced in the fermentation was calculated after determining the amount absorbed in the absorption train, the amount present in the medium and the amount in the solids which had been filtered off. From this total was subtracted the amount originally present in the 10 g. of $CaCO_3$. The difference was the amount produced from the sugar. As stated previously, the amount of CO_2 absorbed in the vanier bottle was determined by weight. The amount in the other gas absorption bottle was determined by titrating an aliquot portion with 0.5 N HCl, using phenolphthalein as indicator, after the addition of sufficient 2 N $BaCl_2$ to precipitate the carbonate. By knowing the value of the blank the amount of CO_2 absorbed could be calculated. The amount of CO_2 present in the fermentation mixture was calculated from the determination of CO_2 in a 200 cc aliquot portion. The apparatus used was that described by Heck (1929). The same apparatus was used to determine the CO_2 present in the solid material except that a vanier bottle was inserted between the absorption flask and the remainder of the apparatus to take care of the large amount of CO_2 evolved.

Acetylmethylcarbinol. Two methods were used in determining the amount of this compound present in the mixture. The first method was the Kluyver, Donker and Visser't Hooft (1925) modification of Lemoigne's (1920) method as adapted for an analytical procedure by Wilson, Peterson and Fred (1927). The determination was carried out as follows. A 200 cc aliquot portion of the fermentation mixture was made just acid to litmus with acetic acid and mixed with 20 cc of 40% $FeCl_3$. The solution was then slowly distilled until 100-125 cc of distillate had been collected. The distillate was mixed with 10 cc of 20% hydroxylamine hydrochloride, 20 cc of 20% sodium acetate and 5 cc of 10% nickel chloride, and refluxed for 15 minutes. The precipitate of nickel dimethylglyoxime was filtered in a Gooch crucible, dried and weighed. Grams of nickel dimethylglyoxime X 0.6097 = grams of acetylmethylcarbinol.

Apparently no work has been done to determine the completeness of the oxidation of acetylmethylcarbinol to diacetyl by $FeCl_3$. Wilson, Peterson and Fred (1927) found that by starting with pure diacetyl only 70-75% was recovered as the nickel dimethylglyoxime. Results obtained in the present investigation indicate that the values obtained for acetymethylcarbinol by this method are consistently about 25% lower than the values obtained by the second method, described below. By comparing these results with those obtained for pure diacetyl mentioned above, it would appear that the oxidation to

diacetyl is complete but that a consistent loss of about 25% of the diacetyl occurs during the distillation or subsequent treatment.

The second method for the determination of acetylmethylcarbinol is based on the fact, discovered by Pechmann and Dahl (1890), that this substance reduces Fehling's solution. Kling (1906) found that the reaction was quantitative, the only product of oxidation being acetic acid. He reported that 1 gram of acetylmethylcarbinol was equivalent to 2.85 grams of copper when oxidized by an alkaline solution of Cu O. Walpole (1911) used the method for the determination of acetylmethylcarbinol in fermentation mixtures, he reported that 1 cc Fehling's solution = 2.48 Mg. of acetylmethylcarbinol. This indicates that 1 gram of the carbinol = 3.556 gm. Cu. Pederson and Breed (1928) used the reduction method but used a calculated value based on the equation $CH_3CHOHCOCH_3 + Cu\ O = 2\ Cu_2\ O$ which gives the ratio between cuprous oxide and acetylmethylcarbinol as 1 to 0.2767 or 1 gram acetylmethyl carbinol = 3.25 g. Cu. They considered their results too low. Since the reduction value of acetylmethylcarbinol was not known for the method of reduction used in the Shaffer Hartmann procedure, the value obtained by Kling was arbitrarily chosen. The method was adapted to the present problem by determining the reducing power of an aliquot portion of the fermentation mixture using the Shaffer Hartmann method and making a cor-

reaction for the amount of sugar and formic acid present. It is possible that some acetylmethylcarbinol was lost by aeration during the fermentation.

2-3, Butylene Glycol. Attempts to determine 2-3, butylene glycol by oxidation to diacetyl with bromine gave very inconsistent results, confirming the experience of Donker (1926). The method adopted consisted in extracting the glycol and weighing it. The determination was carried out in the following manner.

A 500 cc aliquot portion of the fermentation mixture was evaporated under reduced pressure to a volume of about 100 cc, keeping the temperature below 45°. The solution was then saturated with NaCl and extracted for 72 hours with ether in the apparatus described below. (See Fig. 2.)

A. 250 cc Distilling flask.

B. Hopkins Condenser.

C. 250 cc Erlenmeyer flask.

D. Electric hot plate.

After the extraction was completed the extract was mixed with about 20 grams of anhydrous sodium sulfate and allowed to stand over night. The salt was then filtered out and washed five or six times with ether which had been dried over sodium. The filtrate and washings were evaporated to a small volume and transferred to a weighing bottle. The remainder of the ether was evaporated and the glycol allowed to attain constant weight

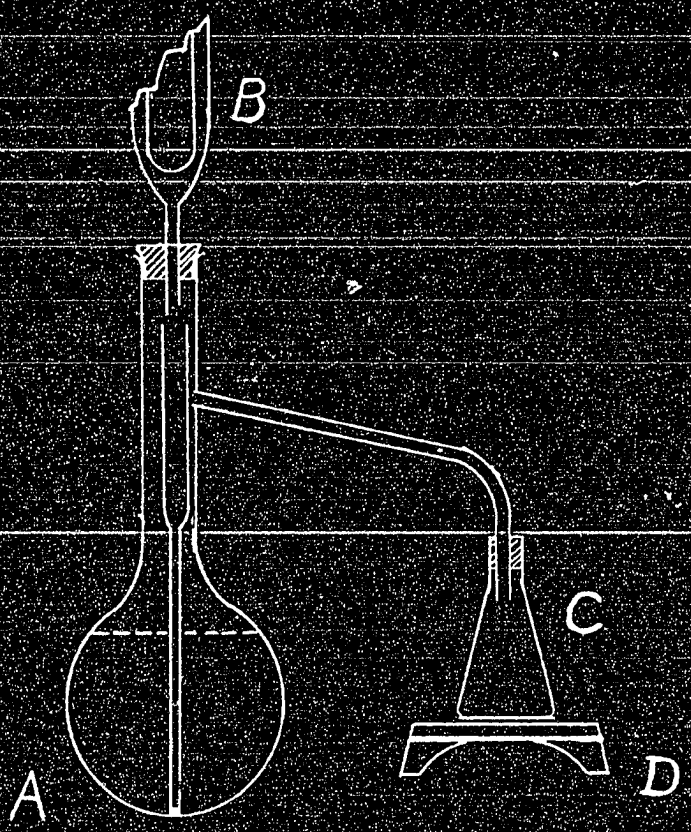


Fig.2. Ether Extraction Apparatus.

in a desiccator containing concentrated sulfuric acid. The amount of impurities in the glycol separated by this method is quite small, as was found when this fraction from a large amount of fermentation mixture was examined. However, it is necessary that the solution should be strongly alkaline as otherwise some acids will be extracted. When acetylmethylcarbinol was found to be present in the fermentation mixture, the amount of it present in the glycol extract was determined and the correction applied. The determination was made by dissolving the glycol in water, making up to 100 cc and examining an aliquot portion for reducing power.

Sugar. Xylose was determined in a 50 cc portion of the mixture by the phloroglucinol method. Sucrose was calculated from the difference in reducing power before and after inversion. Inversion was accomplished by mixing 50 cc of the mixture, which had been made just acid to litmus with HCl, with 5 cc of Conc. HCl and allowing it to stand 24 hours at room temperature. The solution was then made just alkaline to litmus with strong KOH, made up to 100 cc and an aliquot portion titrated by the Shaffer Hartmann method. In carrying out the reduction the method of heat control, using a manometer, which is described by Morrow (1927, - pp. 199-200) was used.

Ethyl Alcohol. Most of the methods in use for the determination of small quantities of ethyl alcohol are based

on its oxidation to acetic acid by means of $K_2Cr_2O_7$ and H_2SO_4. The usual procedure is to distill off the alcohol from a neutral solution, treat the distillate with a mixture of potassium dichromate and sulfuric acid and distill with steam. The acetic acid in the distillate is then titrated with alkali. Dox and Lamb (1916) modified the method so as to determine the alcohol in a small sample of solution. Their modification consisted in removing the alcohol from a dilute alcoholic solution saturated with ammonium sulfate, by passing air through it. The alcohol vapor was absorbed in conmentrated sulfuric acid. The acid containing the alcohol was then mixed with $K_2Cr_2O_7$ and steam distilled as described above. Tomoda (1929) modified the method of Dox and Lamb by saturating the solution with sodium bisulfite instead of ammonium sulfate, making possible the determination of ethyl alcohol in the presence of volatile aldehydes and ketones. This method did not prove satisfactory in the present investigation because the very small sample (5 cc out of 2000 cc) introduced a large error.

The method finally adopted was as follows. Two hundred cubic centimeters of the fermentation mixture were mixed with 35 g. NaCl and slowly distilled, using a fractionating column to keep back the 2-3, butylene glycol. A few pieces of porous plate were used to insure even boiling without superheating. About 125-150 cc of distillate were collected. The distillate was made up to a volume of 200 cc with CO_2 -

free distilled water, and an aliquot portion examined for the amount of acetylmethylcarbinol present. A 50 cc portion of the distillate was then placed in a 150 cc pressure bottle with 10 grams of $K_2Cr_2O_7$ and 30 cc of 50% H_2SO_4. The bottle was then placed in boiling water for 20 minutes. After cooling, the solution was subjected to steam distillation. Each 100 cc portion of distillate as collected was titrated with 0.0611 N $Ba(OH)_2$, using phenolphthalein as indicator. The distillation was discontinued when the 100 cc portion of distillate required less than 0.5 cc of alkali. This titration gave the total amount of acetic acid present. From this value was subtracted the amount of acetic acid resulting from the oxidation of the acetylmethylcarbinol. (1 Mol acetylmethylcarbinol = 2 Mols acetic acid). The interference of acetylmethylcarbinol in this determination is a factor that has been disregarded or overlooked by many workers in this field.

Volatile Acids. As the result of the qualitative analysis, it was found that the volatile acids to be determined were formic and acetic. Any method for these acids can be no more exact than the method used for separating them from the non-volatile acids. There have been several methods suggested for the determination of formic acid based on its reducing power, such as that of Klein (1906) in which the neutralized acid is titrated with $KMnO_4$, and the method of Fincke (1913) based on the reduction of $HgCl_2$ to Hg_2Cl_2 which

is filtered and weighed. These methods could not be used conveniently in the present investigation, due to the interference of acetylmethylcarbinol which reduces both $KMnO_4$ and $HgCl_2$.

The method adopted in this work was a combination of the modifications of the Duclaux distillation suggested by Knetemann (1928) and Virtanen and Pulkki (1928). The distillation constants and the method of manipulation were those of Knetemann, the method of calculating the relative amounts of the volatile acids present was that described by Virtanen and Pulkki. The original articles should be consulted for the theoretical treatment and the essential details of apparatus and technic. The method is fairly precise for two volatile acids but much less so for three.

The determination was carried out in the following manner. A 500 cc portion of the alkaline fermentation mixture was evaporated to a volume of about 200 cc, made acid to Congo Red paper with 25% H_2SO_4 and steam distilled. The indicator paper was left in the solution and if, as sometimes occurred, after a few minutes the paper turned red, sufficient acid was run in through the steam inlet tube to turn the paper blue again. The distillate was collected in a 2 liter flask. After 1200-1500 cc had distilled over, at intervals the flask was removed and the distillate collected in a test

tube containing 1 drop of 0.1 N alkali. When more than 5 cc of distillate were required to decolorize the indicator, the distillation was stopped. The distillate was then gently boiled for 15 minutes under a water cooled reflux condenser at least 70 cm. long, fitted at the top with a soda-lime tube, to drive out any dissolved CO_2. The contents of the flask were allowed to cool in the apparatus and then made up to 2 liters with CO_2-free distilled water. A 50 cc portion was then titrated with 0.0611 N $Ba(OH)_2$ to determine the total acidity, using phenolphthalein as indicator. A 400 cc portion of the acid solution was placed in the distilling flask with 2.5 g. of granular pumice stone, and distilled at the rate of about 50 cc in 12 minutes. Each 50 cc portion as collected was titrated with 0.0611 N $Ba(OH)_2$. The distillation values calculated for the pure volatile acids by Knetemann are given in Table VII.

TABLE VII.

DISTILLATION VALUES OF THE PURE VOLATILE ACIDS (KNETEMANN)

Distilled from 400 cc	% Acid in Distillate						
	Formic Acid	Acetic Acid	Propionic Acid	Butyric Acid	Valeric Acid	Caproic Acid	Caprylic Acid
50	4.7	8.2	15.9	23.0	33.8	42.1	69.1
100	10.0	16.8	31.0	42.7	58.6	69.0	91.9
150	16.0	26.1	45.6	60.0	76.4	85.4	98.3
200	22.6	36.1	59.3	74.0	88.3	94.2	99.8
250	30.5	46.9	72.1	85.2	95.2	98.2	100.0
300	40.1	59.1	83.5	93.3	98.6	99.7	-
350	53.7	73.9	93.3	98.3	99.8	100.0	-

The amount of each acid present is calculated by means of the following equations:

$$A + B = Z$$

$$\frac{a}{100} A + b/100\ B = Z_1$$

where A = No. cc of acetic acid

B = No. cc of formic acid

Z = total acidity of 400 cc portion in terms of the alkali used.

Z_1 = No. cc of alkali required for 200 cc.

a = distillation value for 200 cc pure acetic

b = distillation value for 200 cc pure formic

The distillation values obtained for each of the four fermentations are given in Table VIII.

TABLE VIII.

THE DISTILLATION VALUES OBTAINED FOR THE MIXTURES OF VOLATILE ACIDS PRODUCED BY FERMENTATION.

cc Distilled	Xylose		Sucrose	
	Aerobic	Anaerobic	Aerobic	Anaerobic
50	6.2	11.7	6.7	7.5
100	12.8	23.0	12.5	15.4
150	19.7	34.1	18.8	23.6
200	27.0	44.7	25.9	32.1

By comparing these values with the values for the pure acids

it is evident that the only series which does not lie between the values for formic and acetic acid is that obtained in the anaerobic xylose fermentation. The high values indicate the presence of some acid higher than acetic, and the characteristic odor of butyric acid was very evident. In this experiment the amount of butyric acid was calculated from the following equations:

$$A + B + C = Z$$

$$a'/100\ A + b'/100\ B + c'/100\ C = Z',$$

$$a/100\ A + b/100\ B + c/100\ C = Z,$$

where

A = No. cc of acetic acid.

B = No. cc of formic acid.

C = No. cc of butyric acid.

Z = total acidity of 400 cc portion in terms of the alkali used.

Z' = No. cc alkali required for 100 cc of distillate

Z = No. cc alkali required for 200 cc of distillate

a' = distillation value for 100 cc pure acetic.

b' = distillation value for 100 cc pure formic.

c' = distillation value for 100 cc pure butyric.

a, b, c = corresponding values for 200 cc.

Non-Volatile Acids. The residue from the steam distillation of the volatile acids was mixed with about 15 grams

of Na_2SO_4 and extracted with ether for 72 hours in the apparatus previously described (Fig. 2). The ether extract was then mixed with 400 cc of distilled water and the ether evaporated. The solution was then boiled for about 10 minutes and titrated, while hot, with 0.2487 N $Ba(OH)_2$ using phenolphthalein as indicator. The solution was then carefully evaporated to a volume of about 100 cc, and then mixed with 400 cc of 95% ethyl alcohol. After standing for 2 days the precipitate of barium succinate was filtered off in a weighed Gooch crucible, dried for an hour at 100°, cooled in a desiccator and weighed. One gram of barium succinate = 0.4657 grams of succinic acid. By knowing the total amount of $Ba(OH)_2$ required for the non-volatile acids, and the weight of barium succinate, the amount of lactic acid can be calculated by difference.

4. Results of analysis. The results obtained in the analysis are given in Table IX, which gives the amount of the substance produced in grams and the percent yield based on the carbon content. The values obtained from each sugar under aerobic and anaerobic conditions should be compared rather than the values for xylose and sucrose. The reason for this is that the two xylose fermentations were run parallel as were the two sucrose fermentations. It will be noted that no butyric acid was found in the anaerobic sucrose fermentation while 4.4% was obtained in the anaerobic xylose fermentation. This difference is believed to be due, not to an inherent dif-

TABLE IX.

THE RELATIVE AMOUNTS OF THE PRODUCTS FORMED UNDER AEROBIC AND ANAEROBIC CONDITIONS FROM XYLOSE AND SUCROSE.

	Xylose				Sucrose			
	Aerobic		Anaerobic		Aerobic		Anaerobic	
	Grams	%	Grams	%	Grams	%	Grams	%
CO_2	12.10	41.3	7.78	26.5	16.439	53.5	10.550	34.2
2-3, Butylene Glycol	2.11	14.1	2.73	18.2	3.024	19.2	3.331	21.0
Acetylmethylcarbinol	0.52	3.5	0.05	0.3	1.288	8.4	0.113	0.7
Ethyl Alcohol	1.26	8.2	2.58	16.8	2.252	14.1	3.863	23.9
Formic Acid	0.76	2.5	0.14	0.5	0.427	1.3	0.534	1.6
Acetic Acid	0.48	2.4	1.05	5.2	0.205	1.0	1.357	6.4
Butyric Acid	None	0.0	0.65	4.4	None	0.0	None*	0.0
Lactic Acid	0.23	1.1	2.53	12.6	0.455	2.2	2.858	13.6
Succinic Acid	0.34	1.7	0.09	0.5	0.332	1.6	0.534	2.6
Sugar Unused	0.00	0.0	0.00	0.0	0.095	0.5	0.000	0.0
		74.8		85.2		101.6		104.0

* This may have been due to a slight difference in technique. The medium was aerated for about three hours after cooling instead of during the process of cooling.

ference in the two fermentations but to a difference in technic used. In the xylose fermentation the medium was cooled while the hydrogen was passing through it, while in the su-

crose fermentation the solution was cooled before being aerated with hydrogen.

The results in the table show that the relative amounts of certain of the products are different when formed under aerobic and anaerobic conditions. Some of the differences are not believed to be of special significance because of analytical difficulties and variations in individual cultures. The last point has been particularly emphasized by Pederson and Breed (1928). Among the significant variations the following may be noted. Regardless of whether the sugar fermented is xylose or sucrose, anaerobic conditions decrease the production of CO_2 and acetylmethylcarbinol, while the yields of 2-3, butylene glycol, ethyl alcohol, acetic acid and lactic acid are increased. The increase in production of lactic acid is especially striking.

It is apparent that when organisms of the type of Aerobacter faeni are used in the large scale production of 2-3, butylene glycol and lactic acid, anaerobic conditions will give much higher yields.

V. DISCUSSION

The fact that only the substances mentioned in the preceeding sections have been identified does not preclude the possibility of other substances being present in very small amounts. Undoubtedly other chemicals are present, some, as end products of the fermentation, more, however, as intermediary compounds whose presence is transient. A study of the latter compounds is of utmost importance in explaining the mechanism of the fermentation process. However, a study of the mechanism of fermentation is beyond the scope of this investigation. It may be mentioned in passing that any theory as to mechanism to be acceptable must satisfactorily explain the variation in amounts of individual products with changes in environment such as has been found in the present investigation.

In connection with the qualitative analysis there are several points which should be subjected to further study. Walpole (1911) found that the 2-3, butylene glycol produced by Bacillus lactis aerogenes from sugar was a mixture of several isomeric modifications. His results led him to believe that the mixture was composed of 90% of an optically inactive form whose diphenyl urethane derivative melted at 199.5°, but his experiments gave no indication whether this inactive form was the meso- or racemic-isomer. From the other 10% of the mix-

ture he obtained another inactive form which gave a diphenyl urethane with a melting point of 157°. Since his glycol preparations were optically active, apparently some of the optically active isomer was present. Boeseken and Cohen (1928) made a very complete study of the configuration of the 2-3, butylene glycols. They examined a sample of the glycol produced by fermentation and found that it was composed of a large proportion of the meso-isomer and small amounts of the optically active and racemic forms. The nature of the 2-3, butylene glycol produced by Aerobacter faeni should be studied along similar lines. Another point to be cleared up is the optical activity of the acetylmethylcarbinol produced in this fermentation. A third problem to be investigated is the examination of a large amount of the lactic acid produced by Aerobacter faeni to discover whether or not the levo-isomer is the only one present.

In connection with the quantitative investigation, a determination which should be made is the ratio between the amounts of carbon dioxide and hydrogen, under both aerobic and anaerobic conditions. It was omitted from the present work because of the procedure which was used in maintaining anaerobic conditions.

VI. SUMMARY.

It has been shown that Aerobacter faeni grows well on a simple medium containing ammonium chloride as the sole source of nitrogen. A qualitative analysis has shown that the same products are formed from both xylose and sucrose. The products which have been identified are acetymethylcarbinol, 2-3, butylene glycol, ethyl alcohol, formic acid, acetic acid, butyric acid, l-lactic acid, succinic acid, hydrogen and carbon dioxide. The relative amounts of these products (except hydrogen) produced under aerobic and anaerobic conditions have been studied. The results show that the amounts of certain products do depend on these conditions. It would appear that when organisms of the type of Aerobacter faeni are used in the large scale production of 2-3, butylene glycol or lactic acid, anaerobic conditions will give much higher yields.

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