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# Quantitative studies on the formation of xylose from pentosan-containing materials

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14  
QUANTITATIVE STUDIES ON THE FORMATION  
OF XYLOSE FROM PENTOSAN-CONTAINING MATERIALS

BY

Loren Conrad Bryner <sup>124</sup>/<sub>44-7</sub>

A Thesis Submitted to the Graduate Faculty  
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject - Bio-physical Chemistry

**Approved**

Signature was redacted for privacy.

**In charge of Major work**

Signature was redacted for privacy.

**Head of Major Department**

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**Dean of Graduate College**

Iowa State College

1934

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## INTRODUCTION

Until recently, xylose has been classed as a rare sugar, commanding a price of 500 dollars a pound. It was prepared in small quantities in the laboratory and was chiefly employed in bacteriological investigations. Xylose is not known to occur free in nature, but its condensation product, xylan, occurs in large amounts as a constituent of various cellulosic materials. Such agricultural waste products as bagasse, cornstalks and cobs, cereal straws and hulls, peanut shells, cotton burs, cottonseed-hulls, and like materials, are excellent sources of xylose. Woods, and especially hardwoods, contain appreciable quantities, in fact, because it was first obtained from these materials it was given the name of "wood sugar". Most of these pentosan-containing materials yield from 30 to 40% reducing sugars, as xylose, and yields of crystalline xylose as high as 10 to 12% have recently been obtained in semi-commercial operation.

The tremendous amount of these cellulosic waste materials at hand is not generally appreciated. There are 150,000,000 tons of cornstalks and 20,000,000 tons of corn-cobs produced annually in the United States. It has been estimated that the cottonseed-oil plants can furnish yearly over 1,000,000 tons of cottonseed-hull bran, and that the peanut industry could readily collect annually 50,000 tons of hulls. The Quaker Oats Company, alone, use in normal times about 30,000,000 bushels of oats per year in the manufacture of rolled oats, with a yield



of about 400 tons a day of oat hulls.

Realizing the importance of the vast source of raw materials that could be converted into xylose, the Bureau of Standards has developed an improved method for the production of xylose from cottonseed-hulls (Hall, W. L., C. S. Slater, and S. F. Acree, 1930). The process is now being conducted on a semi-commercial scale with the production of 100 pounds of 95% pure xylose per day. The method comprises the following steps: (1) Wash the bran with hot water at 10 pounds steam pressure to remove gums and water soluble salts that are known to interfere with the crystallization of the xylose. (2) Wash with cold dilute (0.16N) sulfuric acid. (3) Digest for two hours at 10 pounds steam pressure with dilute (0.32 N) sulfuric acid to hydrolyze the xylan to xylose. (4) Neutralize with calcium carbonate to pH 2.4. (5) Treat with carbon and filter. (6) Evaporate the filtrate to a specific gravity of about 1.28, and filter to remove the calcium sulfate. (7) Evaporate to a specific gravity of 1.35 and cool. (8) Centrifuge to separate the crystalline xylose from the molasses.

Rockwood and Khorezian (1921) have shown that xylose is not readily assimilated by man but that animals with long intestines can tolerate a somewhat larger amount. Since xylose also has small value as a sweetening agent, it seems likely that it has little or no value as a food. The more promising outlet for this potential source of xylose seems to be its conversion into industrial chemicals. This might be accom-

plished by any of several fermentation processes.

Sugars are not required in crystalline form in fermentation processes, and by working out the optimum conditions for the hydrolysis of these pentosan-containing materials, xylose solutions of the desired concentration should be produced at a relatively low cost. In fact, this hydrolysis can be accomplished along with the sterilization, which is normally required in any industrial fermentation process. Considering the cost of cornstalks at five dollars per ton and that a 2 to 3% acid solution would produce a 7% crude xylose solution at a yield of 30%, based on the dry stalks; it is readily seen that xylose in large quantities can be produced at an exceedingly low cost.

The optimum conditions for the hydrolysis of these various pentosan-containing materials have not been determined and it is the purpose of this investigation to make a detailed study of the effect of steam pressure, acid concentration and other factors that influence the acid hydrolysis of these materials; and to correlate the copper number with the production of solvents by the butyl-acetonic fermentation. The oat hull was chosen for the detailed study chiefly because it is produced in large quantities as a by-product in the manufacture of oat meal, is already concentrated in several localities and yields considerable quantities (up to 40%) of reducing sugars, calculated as xylose.

## HISTORICAL SURVEY

Xylose can be prepared either by direct acid hydrolysis of the pentosan-containing material or by extracting the xylan from the cellulosic material with alkali (7% NaOH) and precipitation of the xylan by adding 95% ethyl alcohol, with subsequent acid hydrolysis of the xylan to xylose. Koch (1886) was first to make this observation that xylose could be produced by hydrolysis of wood gum (xylan) and called it "holz-zucker" or wood sugar. Since that time numerous papers have appeared describing the preparation of xylose from various raw materials, but all of the methods employed are only slight modifications of the original method worked out by Koch. Harding (1923) has given an extensive review on the history of xylose, its discovery and methods of preparation. For complete details on the preparation of xylose, reference may be made to the work of Hudson and Harding (1917), Monroe (1919), Morrow (1927), and others. Fred and Peterson (1923), in a series of articles on the utilization of agricultural waste products, have given directions for the preparation of xylose solutions from oat hulls and peanut hulls, and made studies on the fermentability of these solutions. A more recent contribution is the work by Hall and co-workers (1930) previously mentioned in the introduction of this thesis.

Considerable work has been done on the constitution of xylan. Haworth and co-workers (1931) have shown that xylan consists only of xylose units. LaForge and Hudson (1918) stat-

ed that the minimum acidity required for the hydrolysis of xylan was 1.57% sulfuric acid at a temperature of 130°C. Schorger (1926) in his book on the Chemistry of Cellulose and Wood has devoted considerable space to a discussion of the pentosans, their properties, sources and methods of preparation.

The hydrolysis of xylan can also be accomplished by enzymic action. Hawkins (1915) has shown that the fungus Glowarella cingulata is able to utilize xylose or xylan as a sole source of carbon and that the filtered extracts of the fungus mycelium are able to act on xylan under aseptic conditions to produce xylose. The name xylanase has been suggested for the enzyme. Berkely (1920) has shown that the giant kelp, Macrocystis pyrifera, contains enzymes which have the power to breakdown the pentosans and methyl pentosans contained in its own structure, as soon as it is cut; and that these enzymes are inactivated by prolonged contact with alcohol. Silage and sauerkraut in all probability contain enzymes of this type, which are produced by the bacteria which occur in such places.

## EXPERIMENTAL METHODS

### Analytical Methods

Oat hulls yield a mixture of sugars along with other soluble products when hydrolyzed with dilute acids. The chief sugar formed is xylose along with a small amount of glucose, the former resulting from the hydrolysis of xylan and the latter from the hydrolysis of starch which adheres to the hulls. Upon close examination of the hulls one can detect many fragments of broken kernels and occasionally a whole kernel that was not extracted by the commercial separation of the oat-kernels from the hulls.

Glucose and xylose are both reducing sugars and Bertrand (1906) has shown that xylose possesses practically the same reducing power as glucose toward alkaline copper reagents. Hawkins (1929) has also shown that equal weights of glucose and xylose possess the same reducing power for ferricyanide reagents. From these facts the conclusion can be drawn that xylose or a mixture of xylose and glucose can be determined quantitatively by employing a standard method for glucose.

It is evident that in selecting a method for the quantitative determination of these hydrolytic products it must possess the following characteristics: first, it must determine the total sugars formed; second, it should be rapid and easily carried out; and third, it must be accurate. Of the various methods employed for the determination of small amounts of re-

ducing sugars, that of Shaffer and Hartman (1920) seems to be the best suited for the above purpose. Their method is based upon the reduction of cupric copper (Fehling's Soxhlet solution) to the cuprous condition by the sugars, with subsequent oxidation to cupric copper by means of iodine and the determination of excess iodine by titrating with standard sodium thiosulphate solution.

The procedure was as follows: 49 cc. of water were measured into a 500 cc. Erlenmeyer flask; to this was added 1 cc. (accurate pipette) of the sugar solution containing from 20 to 200 mgm. of sugar. Next, 25 cc. of each of the two Fehling's Soxhlet solutions were added bringing the total volume to 100 cc. The flask was heated on an asbestos mat with the flame so adjusted as to bring the solution to boiling in four minutes. After boiling two minutes, the flask and contents were covered with a small beaker or crucible and allowed to stand in the sink under cold running water (three to four minutes). After cooling, 20 cc. or more of an iodate-iodide solution were added, depending upon the amount of cuprous oxide present. The flask and contents were shaken and 15 to 17 cc. of 5 N sulfuric acid were added rapidly from a graduated cylinder, with continuation of the gentle shaking until the cuprous oxide had been dissolved. There were then added 20 cc. of saturated potassium oxalate solution and the flask rotated to completely dissolve the cuprous iodide. The sample was immediately titrated with exactly 0.1 N sodium thiosulfate solu-

tion, using a few drops of starch solution near the end of the titration as an indicator.

The method of calculation is as follows: The titration of the sugar determination is subtracted from the blank titration, the remainder representing the iodine required for the oxidation of the cuprous salt. Multiply by the copper factor of the thiosulfate (1 cc. 0.1 N = 6.36 mg. Cu) and find the amount of sugar equivalent to the copper by reference to a Munson-Walker table, divide by the weight of sample, and multiply by 100 to get per cent xylose (reducing sugars).

Stiles, Peterson and Fred (1926) have worked out the values of glucose corresponding to the difference in titration between control and sample, which served very efficiently in making calculations of the many sugar determinations reported in this thesis. By adjusting the normality of the thiosulfate to exactly 0.1 and taking a one gram sample for analysis, the number of milligrams of xylose could be determined by reference to their table, which gave the per cent direct.

#### Apparatus and General Procedure

The apparatus consisted essentially of an especially built autoclave, arranged in such a manner that samples could be removed from the reaction medium for analysis without disturbing the temperature and pressure equilibrium. It was found during the development and construction of this apparatus that no

metal tubing or containers could be employed because of their neutralizing effect on the very dilute acids used in the hydrolysis studies. In view of this fact, all parts of the apparatus having direct contact with the reaction medium were constructed of Pyrex glass.

The essential parts of the digester are shown in figure 1. The reaction chamber was constructed from a five liter balloon flask by sealing in a small neck about one inch from the large neck to allow entrance for the sampling tube, and incidentally to hold it in place. The sampler was constructed of thick walled Pyrex glass tubing, one end of which was sealed and a small bulb blown, in which several small holes were made to act as a screen or filter to prevent solid materials from entering and blocking the tube.

It was found necessary to cool the samples before releasing the pressure and this was accomplished by means of a small six inch condenser with a Pyrex glass stopcock sealed on one end and the other end sealed to a thick walled tube of about one centimeter diameter outside and two millimeters diameter inside. The purpose of this heavy tubing was to withstand the pressure of the packing gland at steam pressures to 100 pounds per square inch, and to act as a support for the condenser. The other end of this thick walled tube is connected to the sampler by means of a short piece of rubber tubing about one inch in length. A mixture of finely powdered graphite and Lubriscal served very efficiently as a lubricant for the Pyrex



glass stopcock. It was found necessary to lubricate the stopcock and also fasten it in place by means of two or three rubber bands before each run to prevent leaking at the high pressures.

The mechanical stirrer was of the propeller type, driven (720 r.p.m.) by a quarter horse power motor that ran very constantly (1800 r.p.m.). The stirrer was constructed of a heavy glass rod and was fastened to a bronze coupling by means of a packing gland. The bronze coupling was in turn fastened to the Monel metal drive shaft by means of a small set screw. This shaft passed through a copper disc that served as a lid for the reaction flask, to prevent condensed steam from dripping down into the reaction medium. The shaft passed on through a packing gland to the outside of the digester and into the driving pulley.

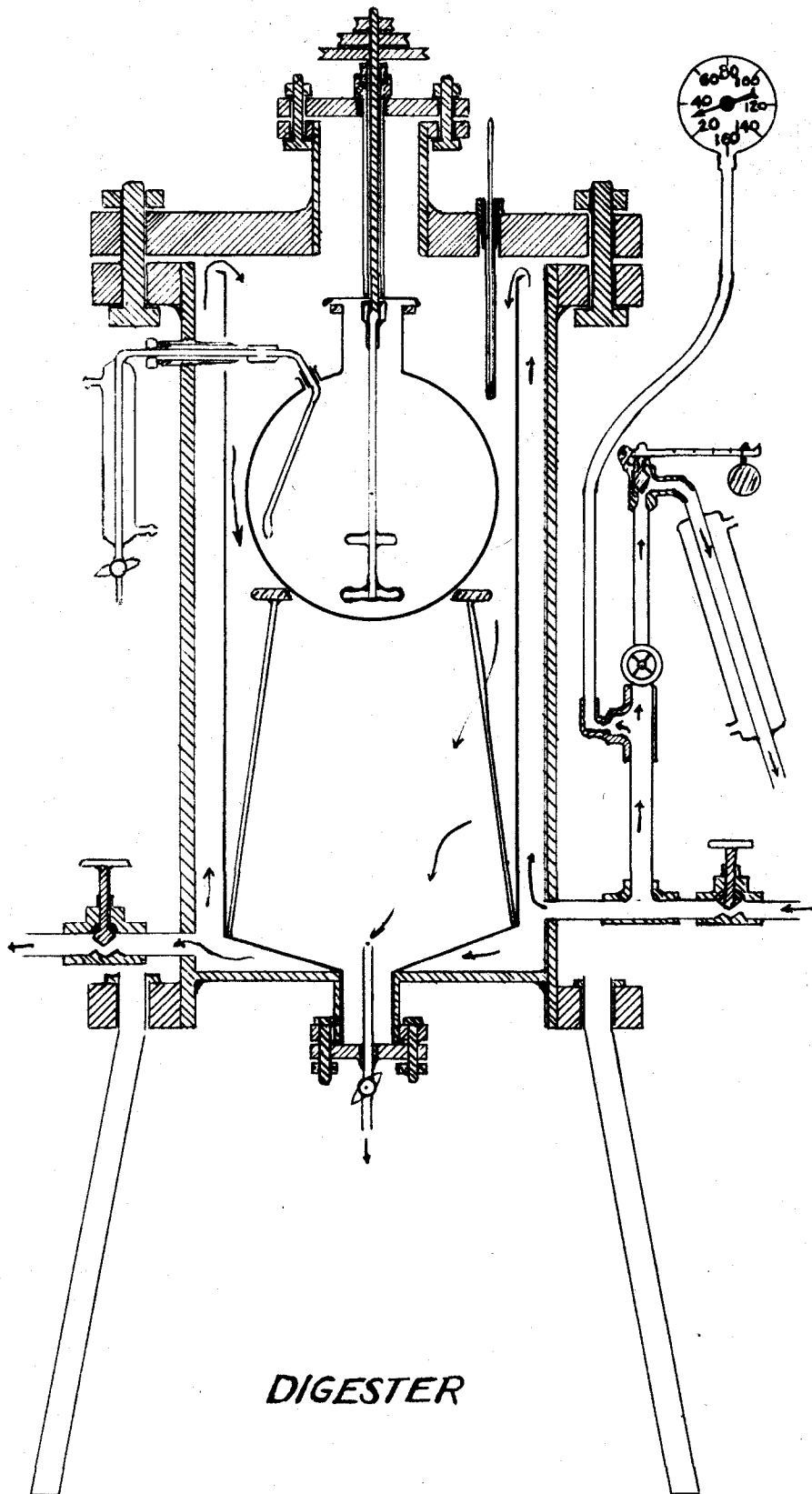
It was found necessary to remove all the air from the digester at the beginning of each run; this was accomplished by allowing the stopcock at the bottom of the digester to remain open for three minutes after the steam had been turned on. The arrows in the diagram indicate the flow of the steam and condensate. Fifteen minutes were allowed after the desired pressure had been reached for the temperature of the reaction medium to come to equilibrium. After the elapse of this time the reaction time was started.

Constant pressure was maintained by carefully regulating the steam inlet and outlet valves and setting the pop-off gauge

at the desired pressure. The mechanical stirrer was run continuously during the entire time of a cook. The first five cubic centimeters of liquid drawn at the time of sampling was discarded to allow for unhydrolyzed material contained in the sampler and condenser. Three cubic centimeters of solution were drawn for analysis and immediately cooled to room temperature by immersion in cold water.

A check run was made to determine the change in concentration and incidentally the change in volume of a dilute acid solution while enclosed in the digester under pressure in the presence of steam. Four liters of 0.06344 N hydrochloric acid were placed in the digester and the pressure was regulated to 60 pounds per square inch and held constant for twelve hours. Samples were removed at intervals of time and titrated with 0.1 N sodium hydroxide. The solution was stirred constantly during the entire time.

The titration results seem to indicate that there was no appreciable change in concentration or volume of the solution during the twelve hour run. However, upon cooling down, one would expect a decrease in volume of the acid, and this was found to be the case.



**DIGESTER**

*Figure 1.*

THE HYDROLYSIS OF OAT-HULLS  
WITH DILUTE ACID UNDER VARIOUS PRESSURES

Influence of Pressure and Acid Concentration

A series of cooks were made at each of the pressures; atmospheric in presence of steam, 20, 40, 60, 80, and 100 (steam pressure in pounds per square inch) with various acid concentrations. The procedure was as follows: 400 g. of clean air dried hulls and 4000 cc. of carefully standardized hydrochloric acid were placed in the digester, and all necessary manipulations previously described were carried out. Samples were removed from the reaction mixture and analyzed for reducing sugars according to the procedure given previously. The yields were all calculated to the oven dry basis.

The results of the hydrolysis are given in tables II to VII and are represented graphically in figures 2 to 7. An analysis of the data shows the following points.

(1) The rate of hydrolysis of the hulls is increased by an increase in acidity at a given temperature.

(2) There is an optimum acid concentration for each temperature.

(3) Above this optimum acid concentration, the sugars are decomposed (caramelized), some of the xylose being converted into furfural, as shown by the drop in the yield (see the curves).

(4) The rate of hydrolysis and yield is increased with increase in temperature to such an extent, that at a steam pres-

sure of 100 pounds per square inch, water alone will hydrolyze oat-hulls, with a yield of 20% reducing sugars, in 250 minutes.

(5) Measurements of the pH of the sugar solutions, by means of a quinhydrone electrode, show very clearly (table V) that there is an increase in acidity during the hydrolysis of oat-hulls with water alone.

(6) At the higher pressures (60, 80, 100 pounds) there was more destruction of the products of hydrolysis, showing a greater tendency to form furfural and to darken the oat-hull residue.

(7) The optimum conditions for hydrolysis of oat-hulls with hydrochloric acid at various pressures are presented in table I.

Table I

OPTIMUM CONDITIONS FOR HYDROLYSIS OF OAT-HULLS  
WITH HYDROCHLORIC ACID AT VARIOUS PRESSURES

Liquid-Hull Ratio	Pressure : Lbs./Sq.In.	HCl : Normality	Time of Heating : in Minutes	Reducing Sugars %
10 to 1	: Atmospheric	: 2.00	: 180	: 39.50
10 to 1	: 20	: 0.10	: 120	: 40.00
10 to 1	: 40	: 0.05	: 90	: 40.00
10 to 1	: 60	: 0.042	: 75	: 39.50
10 to 1	: 80	: 0.042	: 60	: 39.90
10 to 1	: 100	: 0.042	: 30	: 40.50
5 to 1	: 40	: 0.07	: 150	: 36.00

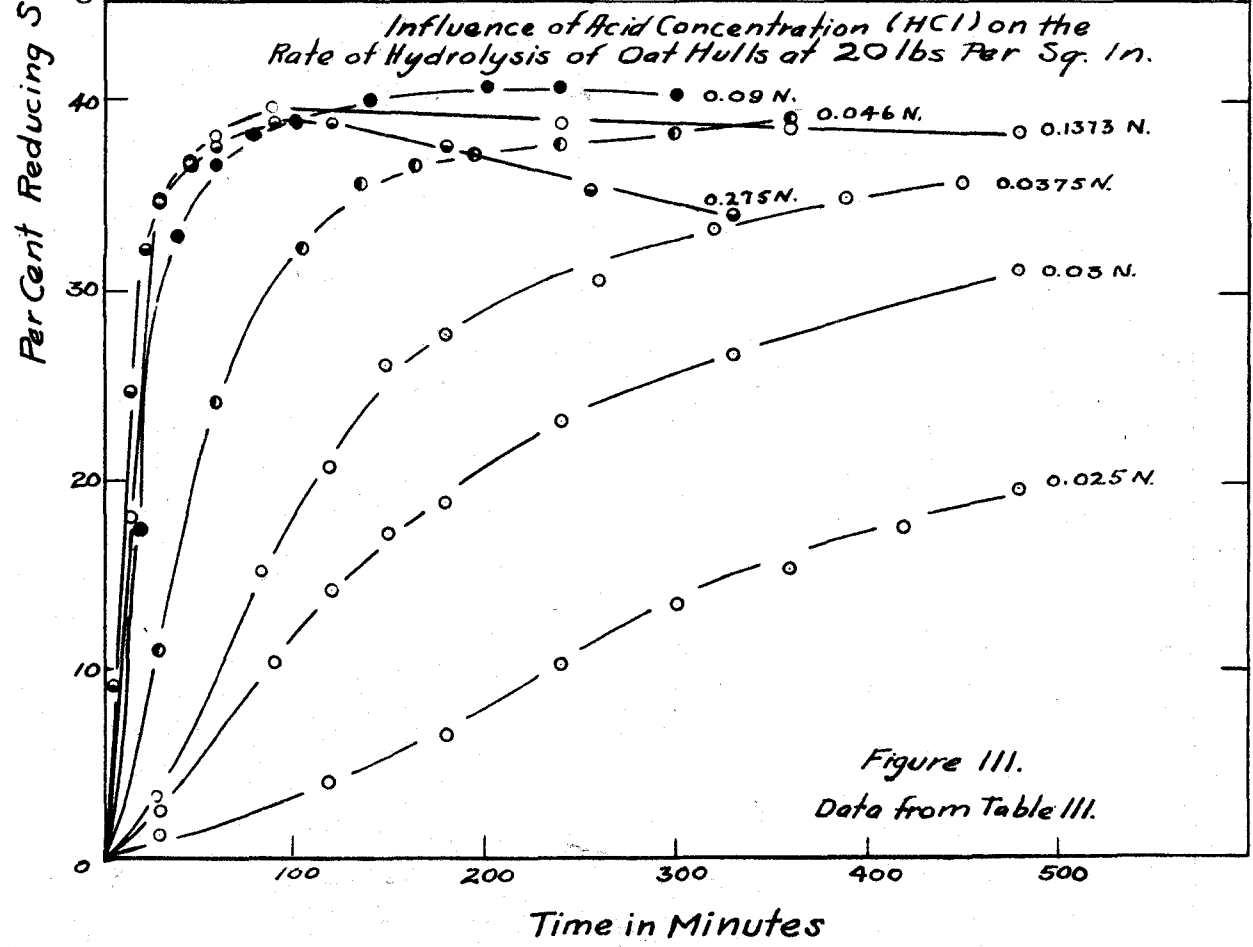
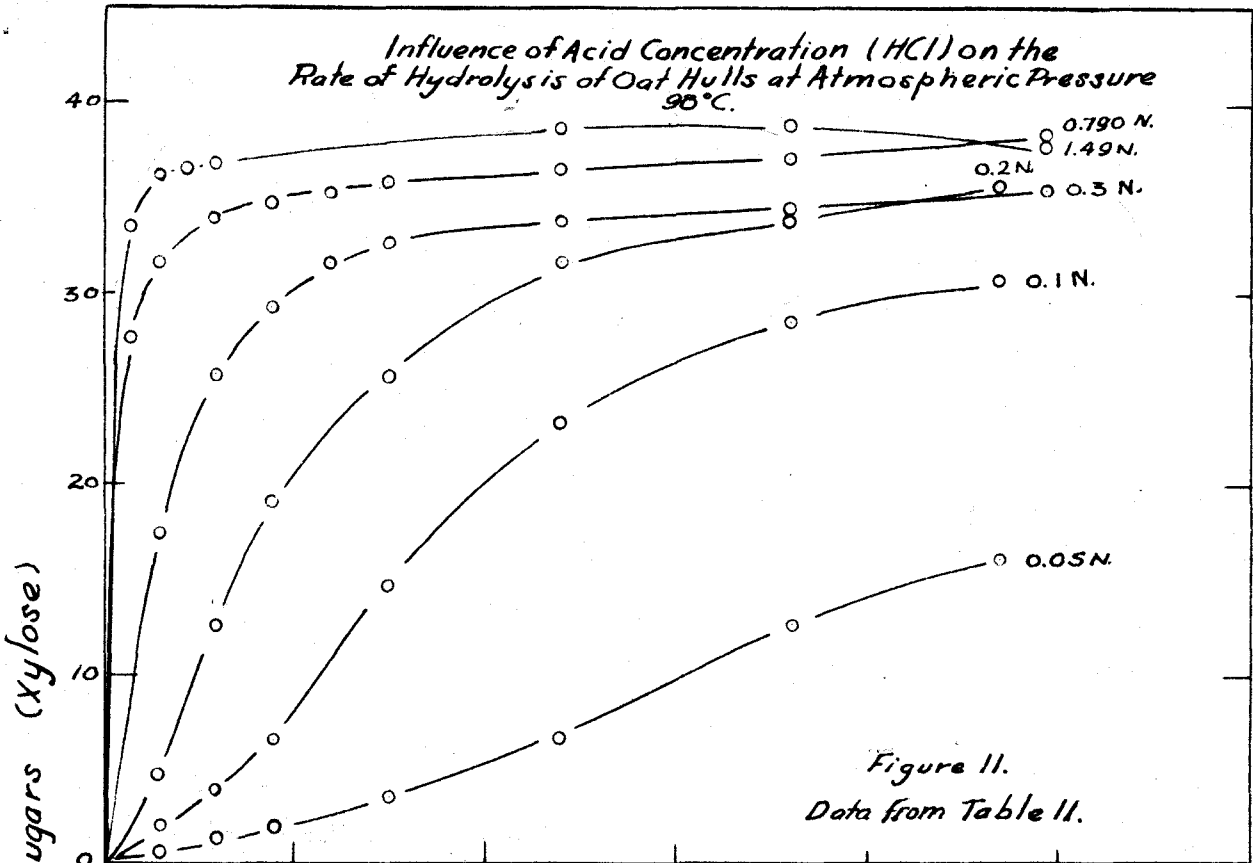


Table II

INFLUENCE OF ACID CONCENTRATION ON THE RATE OF HYDROLYSIS  
OF OAT-HULLS AT ATMOSPHERIC PRESSURE (98°C.)

HCl 0.05 N		HCl 0.098 N		HCl 0.198 N		HCl 0.295 N		HCl 0.790 N		HCl 1.49 N	
Time:	R - S	Time:	R - S	Time:	R - S	Time:	R - S	Time:	R - S	Time:	R - S
30	0.90	30	2.40	30	4.77	30	17.50	15	26.70	15	33.60
60	1.60	60	4.24	60	12.60	60	25.75	30	31.60	30	36.40
90	2.10	90	6.90	90	19.10	90	29.40	60	34.00	45	36.60
150	3.76	150	14.85	150	25.60	120	31.60	90	34.80	60	36.90
240	6.60	240	23.30	240	31.80	150	32.60	120	35.20	240	38.80
360	12.70	360	28.60	360	33.90	240	33.90	150	36.01	360	39.00
480	16.10	480	30.90	480	35.80	360	34.50	240	36.60	495	38.00
—	—	—	—	—	—	495	35.60	360	37.10	—	—
—	—	—	—	—	—	—	—	495	38.50	—	—

Note: Time in Minutes

R-S = Per cent Reducing Sugars, as Xylose, on Dry Basis.

Table III

INFLUENCE OF ACID CONCENTRATION ON THE RATE OF HYDROLYSIS  
OF OAT-HULLS AT 20 POUNDS PRESSURE PER SQUARE INCH

HCl 0.025N		HCl 0.03 N		HCl 0.0375 N		HCl 0.046 N		HCl 0.09 N		HCl 0.1573 N		HCl 0.275N	
Time	R - S	Time	R - S	Time	R - S	Time	R - S	Time	R - S	Time	R - S	Time	R - S
30	1.50	30	2.65	30	3.18	30	11.02	20	17.50	15	15.60	7.5	9.43
120	4.24	90	12.50	80	15.25	60	24.15	40	33.00	30	34.52	15	24.90
180	6.77	120	14.20	120	20.88	105	32.20	60	36.80	45	37.00	22.5	32.20
240	10.40	150	17.17	150	26.10	135	35.70	80	38.20	60	38.40	30	34.80
300	13.56	180	18.87	180	27.80	165	36.60	100	38.90	90	39.90	45	36.60
360	15.50	240	23.20	260	30.60	195	37.30	140	40.00	120	38.80	60	37.60
420	17.70	330	26.85	320	33.40	240	37.75	200	40.80	240	38.80	90	38.90
480	19.70	480	31.28	390	35.00	300	38.40	240	40.80	360	38.70	120	39.00
—	—	—	—	450	35.80	360	39.20	300	40.20	480	38.60	180	37.60
—	—	—	—	—	—	—	—	—	—	—	—	255	35.30
—	—	—	—	—	—	—	—	—	—	—	—	330	34.25

Note: Time in Minutes

R-S = Per cent Reducing Sugars, as Xylose, on Dry Basis.



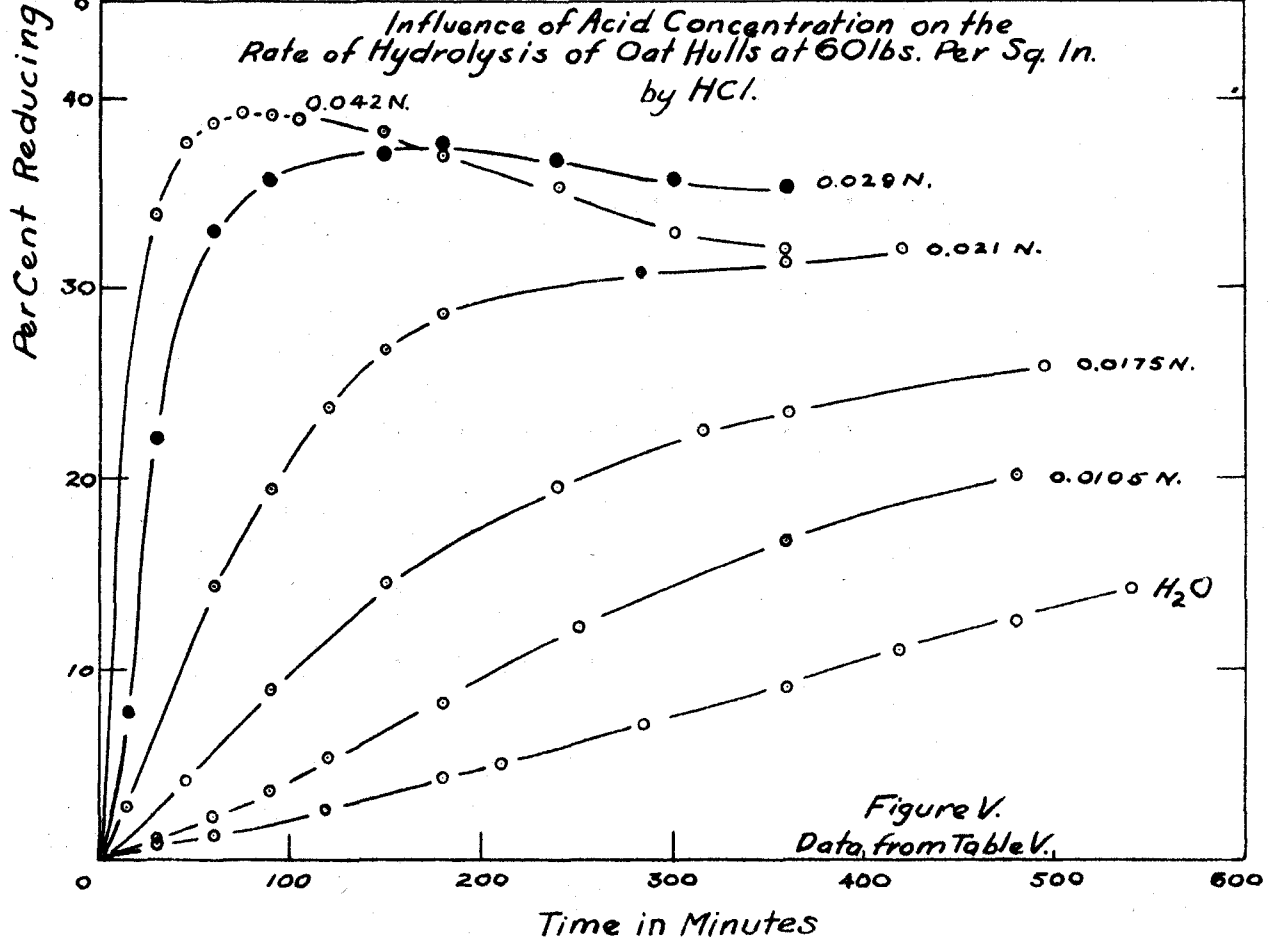
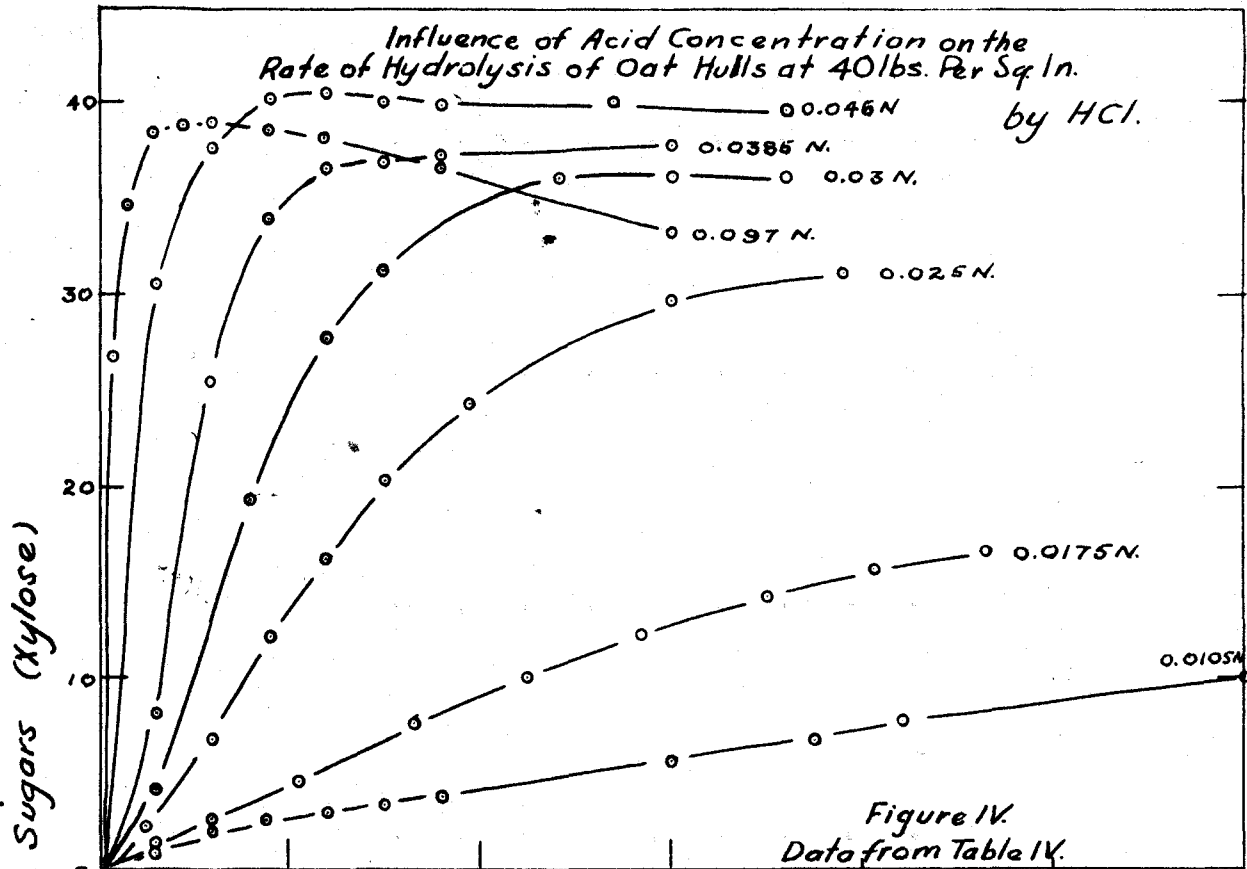


Table IV

INFLUENCE OF ACID CONCENTRATION ON THE RATE OF HYDROLYSIS  
OF OAT-HULLS AT 40 POUNDS PRESSURE PER SQUARE INCH

HCl 0.0105N		HCl 0.0175N		HCl 0.025N		HCl 0.03N		HCl 0.0385N		HCl 0.046N		HCl 0.087N	
Time	R - S	Time	R - S	Time	R - S	Time	R - S	Time	R - S	Time	R - S	Time	R - S
30	0.95	30	1.38	25	2.33	30	4.03	30	7.55	30	30.6	7.5	26.9
60	2.01	60	2.65	60	6.68	80	19.20	60	25.40	60	37.6	15	34.8
90	2.65	105	4.56	90	12.10	120	27.90	90	33.90	90	40.1	30	38.5
120	2.97	165	7.63	120	16.20	150	31.28	120	36.60	120	40.6	45	38.9
150	3.30	225	10.07	150	20.21	240	36.01	150	36.75	150	39.9	60	39.3
180	3.92	285	12.10	195	24.30	300	36.01	180	37.30	180	39.9	90	38.7
300	5.63	345	14.50	300	29.70	360	36.01	240	37.60	270	40.1	120	38.3
375	6.68	405	15.90	390	31.25	—	—	300	37.90	360	39.4	180	36.6
420	8.05	465	16.95	—	—	—	—	—	—	—	—	300	33.3
600	10.07	—	—	—	—	—	—	—	—	—	—	—	—

Note: Time in Minutes

R-S = Per cent Reducing Sugars, as Xylose, on Dry Basis.

10  
23

Table V

INFLUENCE OF ACID CONCENTRATION ON THE RATE OF HYDROLYSIS  
OF OAT-HULLS AT 60 POUNDS PRESSURE PER SQUARE INCH

H <sub>2</sub> O		HCl 0.0105N		HCl 0.0175N		HCl 0.021 N		HCl 0.029 N		HCl 0.042N	
Time:R - S	pH	Time:R - S	Time:R - S	Time:R - S	Time:R - S	Time:R - S	Time:R - S	Time:R - S	Time:R - S	Time:R - S	Time:R - S
30	4.90	45	1.27	4.1	15	2.97	15	7.9	30	34.00	
60	4.78	90	2.33	9.0	60	14.50	30	22.15	45	37.75	
120	4.55	90	3.60	14.5	90	19.50	60	33.00	60	38.70	
180	4.35	120	5.63	19.5	120	23.83	90	35.70	75	39.50	
210	4.27	180	8.37	22.6	150	26.85	150	36.80	90	39.20	
285	4.12	250	12.10	23.5	180	28.60	180	37.75	105	38.70	
360	4.05	360	16.80	26.0	285	30.60	240	36.80	150	38.50	
420	3.98	480	20.12	—	360	31.28	300	35.70	180	37.00	
480	3.94	—	—	—	420	32.00	360	35.70	240	35.30	
540	3.87	—	—	—	720	26.60	—	—	300	33.00	
660	3.77	—	—	—	—	—	—	—	360	32.10	

Note: Time in Minutes  
R-S = Per cent Reducing Sugars, as Xylose, on Dry Basis.

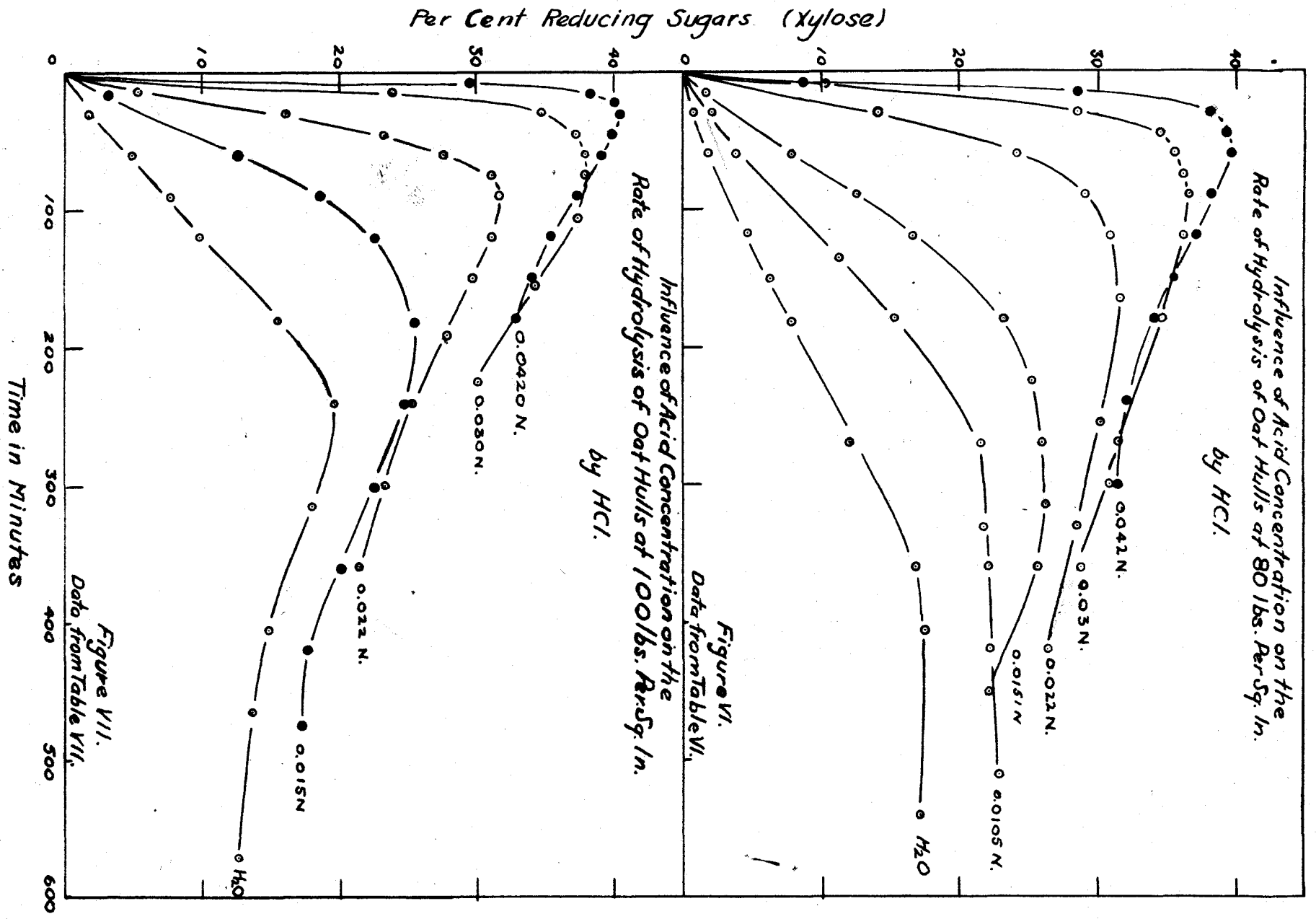


Table VI

INFLUENCE OF ACID CONCENTRATION ON THE RATE OF HYDROLYSIS  
OF OAT-HULLS AT 80 POUNDS PRESSURE PER SQUARE INCH

H <sub>2</sub> O	HCl 0.0105N	HCl 0.0151N	HCl 0.022N	HCl 0.03N	HCl 0.042N
Time:R - S	Time:R - S	Time:R - S	Time:R - S	Time:R - S	Time:R - S
30 : 0.74	30 : 2.12	30 : 1.59	30 : 14.10	10 : 10.30	0 : 1.50
60 : 1.80	60 : 3.80	60 : 7.90	60 : 24.15	30 : 28.60	7.5 : 8.80
120 : 4.88	135 : 11.34	90 : 12.60	90 : 29.10	45 : 34.52	15 : 28.60
150 : 6.35	180 : 15.15	120 : 16.67	120 : 31.00	60 : 35.70	30 : 38.20
180 : 7.70	270 : 21.60	180 : 23.30	165 : 31.80	75 : 36.40	45 : 39.40
270 : 11.98	330 : 21.80	225 : 25.20	255 : 30.25	90 : 36.80	60 : 39.90
360 : 16.95	360 : 22.25	270 : 26.00	330 : 28.60	120 : 36.40	90 : 38.40
405 : 17.60	420 : 22.45	315 : 26.20	420 : 26.50	150 : 35.70	120 : 37.40
540 : 17.26	510 : 23.00	360 : 25.75	— : —	180 : 34.80	180 : 34.25
— : —	— : —	450 : 22.25	— : —	270 : 31.50	240 : 32.10
— : —	— : —	— : —	— : —	300 : 31.00	300 : 31.50
— : —	— : —	— : —	— : —	360 : 28.90	— : —

Note: Time in Minutes  
R-S = Per cent Reducing Sugars, as Xylose, on Dry Basis.

INFLUENCE OF ACID CONCENTRATION ON THE RATE OF HYDROLYSIS  
OF OAT-HULLS AT 100 POUNDS PRESSURE PER SQUARE INCH

Table VII

H <sub>2</sub> O		HCl 0.015 N		HCl 0.022 N		HCl 0.03 N		HCl 0.042 N	
Time	pH	Time	pH	Time	pH	Time	pH	Time	pH
30	1.80	15	3.18	15	3.83	15	5.3	15	24.0
60	4.99	60	12.72	30	3.75	30	16.2	30	34.8
90	7.70	90	18.65	45	3.68	45	23.3	45	37.4
120	9.85	120	22.55	60	3.60	60	27.6	60	37.9
180	15.50	180	25.40	75	3.48	75	31.1	75	38.2
240	19.06	240	24.70	90	3.40	90	31.8	105	37.4
315	18.00	300	22.25	120	3.38	120	31.1	155	34.1
405	14.84	360	20.05	150	3.36	150	29.7	225	31.8
465	13.45	420	17.60	190	3.36	190	27.9	—	—
570	12.72	480	17.26	240	3.33	240	25.1	—	—
—	—	—	—	300	—	300	23.4	—	—
—	—	—	—	360	—	360	21.5	—	—

Note: Time in Minutes  
H-S = Per cent Reducing Sugars, as Xylose, on Dry Basis.

### Influence of the Liquid-solid Ratio

The studies on the influence of pressure and acid concentration were all carried out on the 10:1 ratio basis (10 cc. HCl solution to 1 g. air dried hulls). This ratio was chosen for the following reasons: (1) mechanical agitation is more efficient, (2) sampling from the large volume (about 4.5 liters) would have little effect on the yield, (3) simplified calculations. From a careful analysis of the data obtained from these studies, it was decided that the apparatus was more efficient at 40 pounds pressure per square inch and that an acid concentration of 0.05 N would be most satisfactory for the liquid solid ratio studies.

Fifty liters of hydrochloric acid approximately 0.05 N were prepared and standardized; and a series of cooks made using four liters of acid and various amounts of hulls, such as 200, 400, 600, 700, and 800 grams. The results of these hydrolysis studies are given in table VIII and represented graphically in figure 8. From a careful analysis of these data it is seen that with increase in concentration of hulls there is a drop in yield and the time lag increases. That is, with increase in concentration of hulls, the acid concentration must also be increased. For example, with a 5:1 ratio the optimum acid concentration at 40 pounds is 0.02 greater than that for a 10:1 ratio at the same temperature. The maximum yield for a 10:1 ratio was 40% xylose, and that for a 5:1 ratio was found to be 36%. The corresponding xylose concentrations are 4% and 7.2%, respectively.

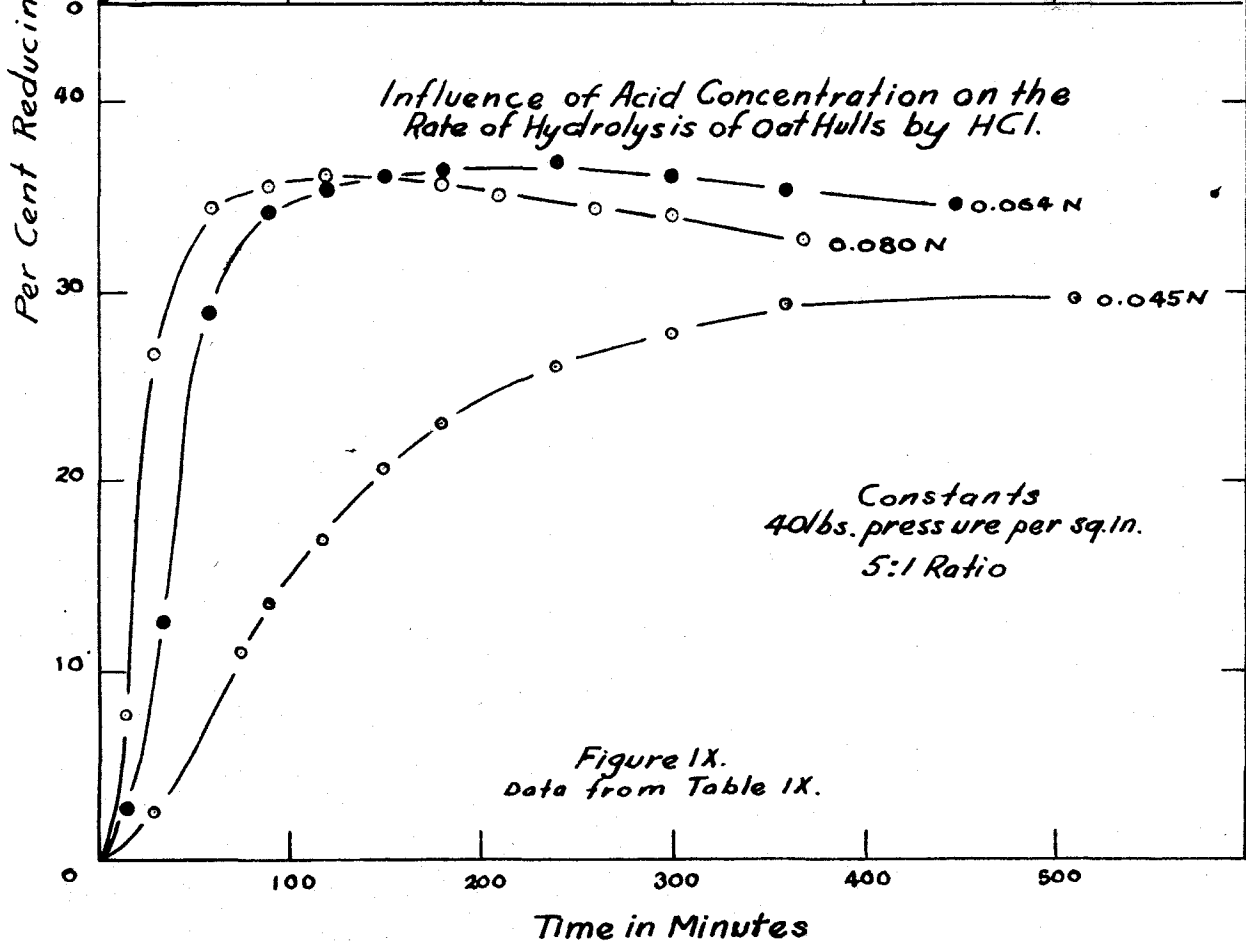
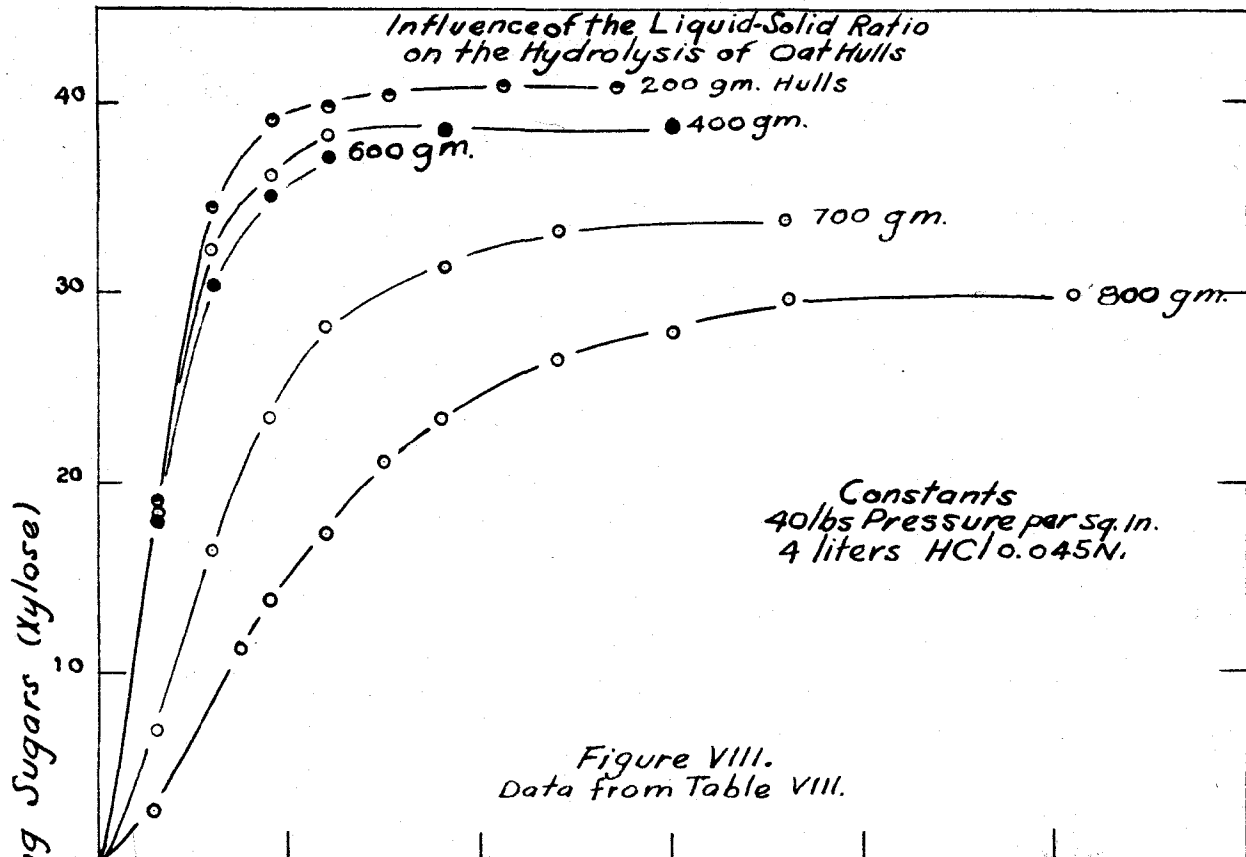




Table VIII

INFLUENCE OF LIQUID-SOLID RATIO ON THE HYDROLYSIS  
OF OAT-HULLS AT 40 POUNDS PRESSURE PER SQUARE INCH  
HCl 0.045 N

200 Grams	400 Grams	600 Grams	700 Grams	800 Grams
Oat-Hulls	Oat-Hulls	Oat-Hulls	Oat-Hulls	Oat-Hulls
Time: R - S	Time: R - S	Time: R - S	Time: R - S	Time: R - S
0: 0.35	0: 0.40	0: 0.45	0: 0.45	0: 0.40
30: 19.00	30: 18.25	30: 18.15	30: 6.90	30: 2.65
60: 34.25	60: 32.20	60: 30.20	60: 16.30	75: 11.10
90: 39.00	90: 36.01	90: 35.00	90: 23.30	90: 13.80
120: 39.60	120: 38.30	120: 37.00	120: 28.00	120: 17.10
150: 40.20	180: 38.50	— : —	180: 31.40	150: 21.10
210: 40.60	300: 39.00	— : —	240: 33.10	180: 23.30
270: 40.60	— : —	— : —	360: 33.60	240: 26.30
— : —	— : —	— : —	— : —	300: 27.90
— : —	— : —	— : —	— : —	360: 29.60
— : —	— : —	— : —	— : —	510: 29.90

Note: Time in Minutes

R-S = Per cent Reducing Sugars, as Xylose, on Dry Basis.

Table IX

INFLUENCE OF ACID CONCENTRATION ON THE RATE OF  
HYDROLYSIS OF OAT-HULLS WITH A 5:1 RATIO AT  
40 POUNDS PRESSURE PER SQUARE INCH

HCl 0.045 N		HCl 0.064 N		HCl 0.080 N	
Time	R - S	Time	R - S	Time	R - S
30	2.65	15	2.97	15	7.80
75	11.10	30	12.80	30	27.00
90	13.80	60	29.25	60	34.80
120	17.10	90	34.50	90	35.90
150	21.00	120	35.85	120	36.50
180	23.30	150	36.40	150	36.30
240	26.30	180	36.60	180	36.00
300	27.90	240	37.10	210	35.40
360	29.60	300	36.30	260	34.60
510	29.90	360	35.70	300	34.25
—	—	480	34.80	370	33.00

Note: Time in Minutes

R-S = Per cent Reducing Sugars, as Xylose,  
on Dry Basis.

Carbon Balance

This investigation was carried out to find the distribution of the decomposition products of the oat-hull after hydrolysis with dilute acid. The oat-hulls were analyzed for moisture, ether extract, ash, lignin, crude fiber, reducing sugars after hydrolysis, total solubles, and residue. This residue, from the hydrolysis, was analyzed for crude fiber, ash and lignin. The filtrate, containing the total solubles after hydrolysis, was analyzed by evaporating an aliquot portion to dryness. The ash content of this residue was also measured.

Clean finely ground oat-hulls were used in this investigation. They were furnished through the kindness of the Quaker Oats Company of Cedar Rapids, Iowa.

The analytical procedures are as follows:

Moisture: A five gram sample of ground air-dried oat-hulls was dried in the drying oven at 105°C. for 24 hours and the loss in weight calculated as per cent moisture.

Ash: A two gram sample of the oven dried oat-hulls was burned slowly, in a previously weighed crucible, in a muffle furnace. After ignition of the carbonaceous matter, the crucible and contents were brought to a red heat and held for two hours, allowed to cool and weighed.

Crude Fiber: A two gram sample of oven dry material

was refluxed for thirty minutes with 200 cc. dilute sulfuric acid (1.25 g.  $H_2SO_4$  per 100 cc.), filtered and washed well with boiling water, transferred back into the same flask and refluxed for another thirty minutes with 200 cc. dilute sodium hydroxide solution (1.25 g. NaOH per 100 cc.), filtered, by means of a gooch crucible washed well with hot water, dried in the oven at  $105^\circ C.$ , weighed and ashed in a muffle furnace. The weight of the ash was deducted to obtain the weight of the crude fiber and from this the per cent of crude fiber was calculated. This procedure for crude fiber is the official A. O. A. C. method.

Ether Extract: A ten gram sample of oven dry material was extracted with anhydrous ether in a Soxhlet extractor for eight hours, dried at  $105^\circ C.$  in the drying oven for 18 hours and weighed.

Lignin: The method of Peterson, Walde, and Hixon (1932) was followed. A two gram sample of oven dried material was placed in a 150 cc. beaker and treated with 30 cc. of 72% sulfuric acid. The beaker and contents were placed in a desiccator which was kept at ice box temperature, the mixture being stirred occasionally to aid the digestion. After eighteen hours the mixture was transferred to a two liter beaker and diluted to 1200 cc., boiled for two hours, allowed to cool and filtered through a gooch crucible, dried in the oven at

105°C., weighed and ashed. The ash was deducted and the per cent lignin calculated.

Residue and Total Solubles: One hundred grams of ground air-dried oat-hulls were treated with one liter of 0.1373 N hydrochloric acid in a two liter balloon flask. The flask and contents were placed in the digester (autoclave) for 90 minutes at 20 pounds pressure per square inch. After hydrolysis was complete the pressure was released very slowly and the flask and contents were allowed to cool, filtered on a buchner funnel and washed free from soluble materials. This oat-hull residue was carefully collected and dried in the oven at 105°C. for 24 hours, weighed and the per cent residue calculated. The filtrate and washings were combined, diluted to two liters and from this solution aliquots were taken for sugar analysis and total solubles.

The reducing sugars were determined by the Shaffer-Hartman method which is discussed in a previous section.

The total solubles were determined as follows: An aliquot of 500 cc. of the filtrate from the oat-hull hydrolysis was neutralized with ammonium hydroxide and concentrated slowly on the steam bath to about 50 cc. The remaining concentrate was carefully transferred to a weighed porcelain evaporating dish and was evaporated to dryness in a vacuum desiccator. Six weeks were required for the residue to become completely dry as shown by its constant weight. From this weight the per cent

of total solubles was calculated after deducting the calculated amount of ammonium chloride that was present in the residue. The ash was determined from this residue as follows: The dry residue was ground in an agate mortar and a one gram sample taken for analysis. This sample was first dehydrated in the drying oven at 105°C. in a weighed porcelain evaporating dish. After 24 hours drying the dish and melted sample was transferred to a muffle furnace and very slowly burned. These precautions were necessary to prevent loss of sample during the combustion. After the combustion was complete the muffle was heated to red heat and held for two hours, allowed to cool and the dish and contents weighed. From this weight the per cent ash was calculated. All analyses were run in duplicate and some in triplicate to ensure as near accurate results as possible.

The results of the analysis follow:

Table X

ANALYSIS OF OAT-HULLS, RESIDUE AND FILTRATE  
AFTER ACID HYDROLYSIS

Analyzed for	Oat-Hulls: Oven Dry Basis %	Unhydro- lyzed Residue %	Fil- trate %	Residue from Filtrate %	Loss Due to Hydrol- ysis %
Moisture (loss at 105°C.)....	5.60				
Ether Extract...	2.02				
Ash.....	7.32	*10.27		3.73	0.20
Crude Fiber....	36.04	*58.54			
Lignin.....	20.69	*30.75			0.08
Reducing Sugars: (Xylose).....	39.10		3.91		
Total Solubles (After Hydrol- ysis).....	*45.20				
Residue (Unhy- drolyzed)....	*52.60				
Total.....	*97.80	*99.56			

\*Values totaled.

These data show very clearly that some volatile products were lost during hydrolysis of the oat-hulls. This loss was 2.2%, as calculated by adding the weights of the two residues and subtracting from 100. These materials were probably volatile acids (acetic) and a small amount of furfural.

The distribution of the ash in the residue and filtrate is in good agreement with the value obtained from the original oat-hulls. The calculation was made as follows: the residue contained 10.27% ash which is equal to 5.43% (10.27 ÷ 52.6) of the original and the filtrate contained 3.73%, which is equal

to 1.69% (3.73 + 45.2) of the original; the addition of these two values (5.43 + 1.69), gives a percentage of 7.12 as compared with 7.32% from the oat-hulls.

These data show that a fraction of the lignin was lost (decomposed or dissolved) during hydrolysis. Of the 20.69% lignin in the oat-hull, 16.20% is recovered in the residue, leaving 4.49% unaccounted for. The residue from the filtrate was shown to contain 39.10% reducing sugars and 1.69% ash, calculated on the basis of the original oat-hulls. Deducting the total of these values (40.97) from the per cent total solubles (45.2), 4.41% is unaccounted for. The loss (4.49%) can be accounted for, upon the assumption that it represents dissolved or decomposed lignin.

The unhydrolyzed residue was shown to consist of the following: ash, 10.27%; crude fiber, 58.54%; and lignin, 30.75%. These values total 99.56%.



## HYDROLYSIS OF XYLAN

### Preparation and Analysis of Xylan

The xylan was extracted from finely ground oat-hulls, by adding them to a seven per cent solution of sodium hydroxide until a heavy viscous mixture was obtained. The mixture was stirred one hour by means of a motor stirrer, allowed to stand over night, stirred for another hour and filtered under suction. The filtration was made through a towel, supported on a Büchner funnel. The residue was washed with water and the washings were added to the filtrate. The filtrate was stirred while two volumes of 95% ethyl alcohol were added; after complete mixing, the sodium hydroxide was partly neutralized by means of hydrochloric acid. The precipitated xylan was allowed to stand over night and then collected on a filter, washed three times with ethyl alcohol, then twice with dry ether, dried in the air with occasional mixing to break up the lumps and finally passed through a forty-mesh sieve. The product was stored in a tightly stoppered bottle.

The xylan gave the following analysis:

Moisture (Loss at 105°C.) . . . . .	12.40%
Ash . . . . .	19.50%
Xylan (From Xylese). . . . .	60.10%
Lignins (By Difference). . . . .	8.00%

The per cent xylan was simply calculated from the optimum

yield of xylose obtained from hydrolysis at 20 pounds pressure per square inch, on the basis of the following equation:



This method is based on the yield of xylose from hydrolysis of xylan, whereas the so-called standard method is based upon the amount of furfural formed from the xylan by distillation with 12 per cent hydrochloric acid and precipitation of the resultant furfural with phloroglucinol. Kline and Acree (1932), in a report on methods for determining pentoses and pentosans has cast some doubt upon the accuracy of the distillation method and also the phloroglucinol method for furfural. The author is of the opinion that the xylose method is sufficiently accurate for the present studies, assuming (1) 100 per cent hydrolysis of xylan (2) that for the short time required for the hydrolysis of the xylan a nominal amount of furfural is formed (3) that the method used for the analysis of xylose is accurate.

#### Influence of Pressure and Acid Concentration

Forty grams of air dried xylan were added slowly to four liters of 0.07 N hydrochloric acid with constant stirring by means of a motor stirrer. The purpose of the vigorous stirring was to prevent the xylan from forming a heavy gummy-like mass. The mixture was allowed to stand over night and 10 cc. of the supernatant liquor were withdrawn with a pipette and titrated

with 0.1 N sodium hydroxide solution, to determine the change in acidity caused by the alkaline material present in the xylan. It was found that the acidity had been reduced to 0.044 normal. The xylan mixture was then placed in the digester and hydrolyzed. Two runs were made, one at atmosphere pressure in the presence of steam (98°C.) and the other at 20 pounds pressure per square inch. Samples of 10 cc. were removed at intervals and analyzed for xylose, according to the usual procedure given elsewhere in this thesis.

The purpose of this study was to make a comparison between the conditions required for the hydrolysis of xylan when in the free state and when present in the oat-hulls. The results are given in table XI and represented graphically in figure 10. These data indicate that the free xylan is hydrolyzed much more readily and under less drastic conditions than when present in the oat-hulls.

The optimum yield of xylose from the air dried xylan was 68.3 per cent. To calculate this value to the oven dry, ash free basis, the following assumption was made: that the 68.3 value would be 100% if the xylan was absolutely pure and on this basis the other values were calculated by multiplying by 100 and dividing by 68.3.

There was no time allowance made for the temperature of the reaction medium to come to equilibrium after the steam had been turned on, the reaction time being started at that instant. The short temperature lag is shown by the graph.

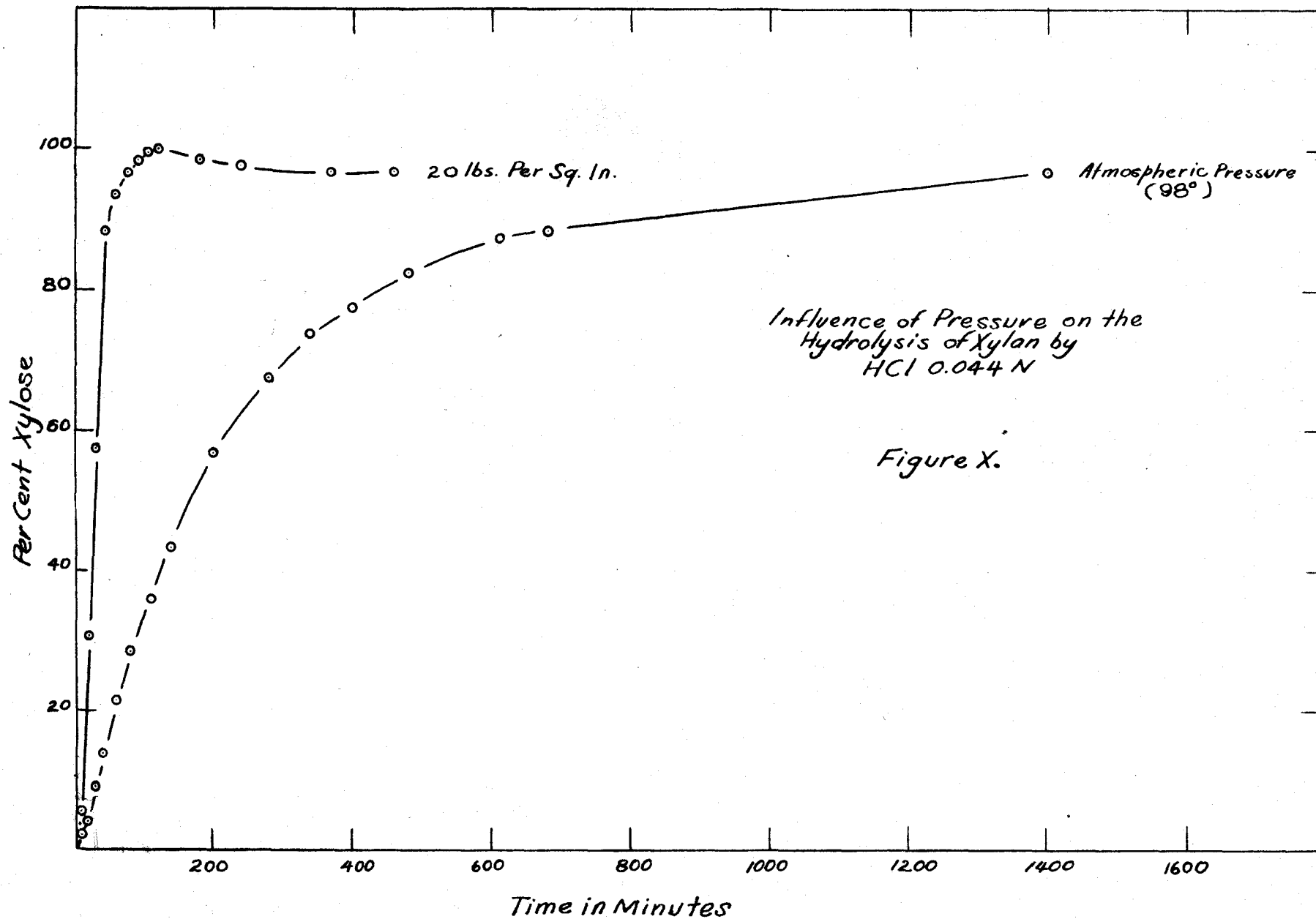


Table XI

INFLUENCE OF PRESSURE ON THE  
RATE OF HYDROLYSIS OF XYLAN

Atmospheric Pressure		:	20 Pounds Pressure per Square Inch	
Time in Minutes	% Xylose	:	Time in Minutes	% Xylose
10	2.20	:	10	5.85
20	4.40	:	20	30.30
30	9.95	:	30	57.50
40	13.90	:	45	88.20
60	21.20	:	60	93.60
80	28.50	:	75	96.80
110	36.70	:	90	98.20
140	43.20	:	105	99.60
200	56.70	:	120	100.00
280	67.30	:	180	98.50
340	74.00	:	240	97.60
400	77.60	:	370	96.80
490	82.50	:	460	96.80
610	87.10	:		
680	88.40	:		
1400	96.80	:		

CORRELATION OF COPPER NUMBER WITH THE PRODUCTION  
OF SOLVENTS BY THE BUTYL-ACETONIC FERMENTATION

These studies were carried out to correlate the yield of solvents, produced by the butyl-acetonic fermentation, with the copper number of various xylose solutions. The copper number is an index to the quantity of reducing sugars present in a given solution, but there is no reason to believe that it indicates the fermentable materials present.

The xylose solutions were prepared by hydrolyzing 800 g. of oat-hulls with four liters of 0.07 N hydrochloric acid (5:1 ratio) in the digester at 20 pounds pressure per square inch. After the desired treatment the steam was turned off and the apparatus was allowed to cool down slowly, about 45 minutes being required. After this time the oat-hull mixture was removed and filtered through a towel, about three liters of xylose solution were recovered. The xylose solution was next neutralized with saturated sodium hydroxide solution, to a pH of 5.0 and analyzed for reducing sugars (copper number). The xylose solution was then ready for fermentation. Three cooks were made (60, 90, and 150 minutes).

Culture W-36 was employed in the fermentations. This culture was isolated from wheat in this laboratory and was used by Underkofler (Thesis, 1934) in his studies on the butyl-acetonic fermentation of xylose. The standard technique employed in this laboratory was followed in the preparation of cultures, media, and analysis of the fermented xylose solutions.

The results of the fermentations are given in table XII.

Table XII

CORRELATION OF THE COPPER NUMBER WITH THE PRODUCTION OF SOLVENTS BY THE BUTYL-ACETONIC FERMENTATION

Time of Cook in Minutes	Yield of Reducing Sugar Weight % of Halls (also Xylose)	Solvents, Weight % of Xylose
60	23.3	32.60
90	29.7	32.80
150	36.5	5.33

These data indicate that the maximum yield of solvents is produced from solutions which have had somewhat less drastic treatment than is required for maximum reducing sugar yield. That is, the best results in fermentation will be obtained when the acid concentration, temperature, or time of hydrolysis are slightly less than those given for maximum reducing sugar yield. This result may be due to the formation of furfural or other toxic materials. It also seems possible that xylan or intermediates may also be fermentable by this organism.

### GENERAL DISCUSSION

The nature of the xylose-yielding substance in cellulosic materials is not exactly known. One group of investigators hold that xylose is formed from a  $C_6$  compound while others contend that it results from hydrolysis of a  $C_5$  compound. Some think xylan ( $C_5H_8O_4$ ) is an intermediate, others that it is the parent substance. Xylose does not occur in the free state, but only in the form of a polymer or other complexes.

During the hydrolysis of oat-hulls with dilute mineral acids, about 45%, by weight, of the oat-hull is dissolved to give a yield of 40% reducing sugars (xylose). A small amount of ash and about 4% of lignin are also dissolved. About 2.2% of the oat-hull was lost as volatile products (acetic acid) during hydrolysis and the remaining unhydrolyzed residue amounted to 52.6%. The acetic acid formed might be accounted for on the basis of the following equations:



On this basis there would be formed one mole of acetic acid for each two moles of xylose. Acetic acid may also be formed from lignin. The xylan was shown to be hydrolyzed much more readily than the oat-hulls, which is probably due to a surface



phenomenon. At high temperature and acid concentrations the xylose is decomposed to furfural. This conversion may be shown by the following equation:



The odor of volatile acids and furfural can be very easily detected in the vapor from the golden brown solution that results from the hydrolysis. At high acid concentrations and temperatures the oat-hulls are darkened and have a charred appearance, and are broken up into more finely divided particles.

In the utilization of the oat-hull as a source for xylose, it would not be practical to hydrolyze at pressures much above 40 pounds per square inch or to use acid concentrations that bring about the hydrolysis too rapidly. The oat-hull residue which is left after hydrolysis was shown to consist mainly of crude fiber and lignin. Considering this residue as a possible source for lignin, it would be advisable to conduct the hydrolysis in such a way that the lignin would be left intact. After removal of the lignin by alkali extraction, the crude fiber residue might be hydrolyzed to reducing sugars (glucose) and utilized along with the original xylose solution in some fermentative process for the production of industrial chemicals.

### SUMMARY

1. Detailed studies have been made on the influence of acid concentration on the hydrolysis of oat-hulls under the following pressures: atmospheric in presence of steam, 20, 40, 60, 80, and 100 pounds per square inch, steam pressure.

2. The rate of hydrolysis of oat-hulls by hydrochloric acid is markedly affected by temperature and acid concentration.

3. The optimum conditions for hydrolysis of oat-hulls have been established for a 10:1 ratio (10 cc. HCl solution to 1 g. of oat-hulls) as follows: atmospheric pressure in presence of steam 2.0 N for 180 minutes; 20 pounds pressure per square inch 0.1 N for 120 minutes; 40 pounds 0.05 N for 90 minutes; 60 pounds 0.042 N for 75 minutes; 80 pounds 0.042 N for 60 minutes; 100 pounds 0.042 N for 30 minutes. The concentration of acid should be increased for a 5:1 ratio, for example, at 40 pounds it is 0.02 greater than for a 10:1 ratio.

4. Xylan was prepared and shown to be more readily hydrolyzed in the free state than when present in the oat-hulls.

5. The oat-hull and products of hydrolysis have been analyzed for ash, crude fiber, moisture, ether solubles, reducing sugars, lignin, total solubles, and unhydrolyzed residue. The distribution of lignin and ash during hydrolysis has been well accounted for. The data indicate that xylose results from the hydrolysis of a  $C_6$  compound rather than from a  $C_5$  compound, and that volatile acids, particularly acetic, are form-

ed in appreciable amounts.

6. A correlation has been made between the copper number and the production of solvents by the butyl-acetonic fermentation. In general, somewhat lower acid concentration, lower temperature or shorter time of treatment than those optimum for reducing sugar yield, are desirable.

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