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THE QUANTITATIVE SACCHARIFICATION OF WOOD AND CELLULOSE

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THE QUANTITATIVE SACCHARIFICATION OF WOOD AND CELLULOSE[1, 2]

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Summary

A rapid analytical technique has been developed for the hydrolysis of cellulosic materials to reducing sugar in nearly quantitative yields. The method involves the treatment of the material with 72 percent sulfuric acid for 45 minutes at 30° C. followed by a secondary hydrolysis for 1 hour in a 15-pound autoclave or for 4-1/2 hours at the boiling point.

Data are presented to show how the yield of reducing sugar varies with the conditions used.

A table is given showing the "potential reducing sugar" content of 15 species of wood and the variation occurring within a species.

Introduction

In experimental work on the hydrolysis of wood, it is desirable to determine the percentage of total sugar and of fermentable sugar potentially available in a sample of wood or hydrolyzed wood residue. According to existing techniques, this can be done by means of a cellulose and a pentosan determination, both comparatively long and slow operations.

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[2]Presented before the American Chemical Society, New York City, September 11-15, 1944.

[3]Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

The quantitative saccharification of wood was investigated at the Forest Products Laboratory, in cooperation with the Office of Production Research and Development of the War Production Board, in an effort to devise a rapid and convenient technique for determining potential reducing sugars as sugar, rather than indirectly as cellulose, or by difference after a lignin determination. A certain amount of work has been done in this field using methods essentially similar to the Klason, or Willstätter, lignin determination (1, 8) except that the hydrolysate was analyzed for reducing sugar. Kiesel and Semiganovski (2) describe a method for quantitative saccharification using 80 percent sulfuric acid. Ritter, Mitchell, and Seborg made a study of factors affecting the conversion of an isolated wood cellulose to sugar (5). The work of earlier investigators, too, showed that high yields of sugar are obtainable from cellulosic materials (4, 9).

Description of Experiments

Conditions for Hydrolysis with Concentrated Acid

The present experiments on the quantitative saccharification of wood were undertaken using, as a basis, the sulfuric acid lignin analysis. This method, according to a widely accepted procedure (6), involves the mixing of 72 percent sulfuric acid with the cellulosic material at 20° C. for 2 hours, diluting to 3 percent acid, and hydrolyzing at the boiling point for 4 hours. The chief criterion for a correct lignin analysis is that lignin must be obtained in minimum yield. Conditions must be severe enough to remove all carbohydrates, but not sufficiently severe to cause their recondensation into insoluble materials. Workers in the field of lignin chemistry appear to be in general agreement that a sulfuric acid concentration of 72 percent is most satisfactory for lignin analysis from the standpoint of speed and accuracy. For this reason this concentration of sulfuric acid was used for experiments on saccharification.

To reduce the time required for analysis, it is desirable to have the temperature of the 72 percent sulfuric acid as high as is permissible without causing excessive decomposition of the cellulosic material. Ritter, Mitchell, and Seborg showed that the yield of lignin from wood treated with 72 percent sulfuric acid for a period of 1 hour showed a nearly constant low value when the temperature was varied from 15° to 30° C. (6). These same workers showed that with a 2-hour treatment with 72 percent sulfuric acid, Cross and Bevan cellulose showed a maximum yield of reducing sugars at 35° C. For these reasons it was considered well to begin experiments on saccharification of cellulose using a temperature of 30° C.

Conditions for Secondary Hydrolysis

According to the various methods of lignin analysis with strong acids, a secondary hydrolysis is conducted at the boiling point after dilution of the strong acid with water. By carrying out this secondary hydrolysis in an autoclave (sterilizer) at 15 pounds steam pressure, the time necessary for complete hydrolysis is reduced.

Before making this modification, however, a glucose solution was made, in 4 percent sulfuric acid, of such concentration as to be similar to the sugar concentration present during secondary hydrolysis. The decomposition of this solution was determined after heating for various lengths of time at 100° C. and at 121° C. in an autoclave. To avoid changes in volume during the treatment, the samples were sealed in glass bombs. After heating, the samples were shaken with excess chalk, and filtered and sugar analyses were made by the Shaffer and Somogyi method using their reagent 50 with a 30-minute heating period. The sugar concentrations in the original and in the heated samples were as shown in table 1.

The data in table 1 indicate that sugar decomposition in solutions held at 100° C. for 3 hours was comparable to that in solutions held at 121° C. for 1 hour. Although it was difficult to measure accurately such small changes in sugar concentration, the data given in table 1 show that there was no apparent disadvantage in conducting the secondary hydrolysis in an autoclave.

The Yield of Reducing Sugar from Wood and other Cellulosic Materials Hydrolyzed under Various Conditions

A series of experiments was performed in which 5 milliliters of 72 percent sulfuric acid was allowed to react at 30° C. for various lengths of time with 0.5 gram of Douglas-fir wood, ground to pass a 30-mesh screen. The samples were then diluted with different amounts of water and autoclaved at 15 pounds steam pressure for periods of 15, 30, and 60 minutes. Sugar analyses were made after neutralization with chalk. The results are shown in table 2.

From the data in table 2, it appears that there is considerable latitude in the choice of conditions for quantitative saccharification of wood. High yields of reducing sugar occurred with the following treatments:

(1) 40 minutes primary hydrolysis
60 minutes secondary hydrolysis with
4 percent H_2SO_4

(2) 85 minutes primary hydrolysis
60 minutes secondary hydrolysis with
4 percent H_2SO_4

(3) 120 minutes primary hydrolysis
60 minutes secondary hydrolysis with
2 percent H_2SO_4

Another experiment was conducted in an effort to define more precisely the conditions for quantitative saccharification of wood. No further work was done on the acid concentration for secondary hydrolysis since 4 percent acid appeared satisfactory. A different sample of wood was used for this experiment. The results are given in table 3.

The accuracy of analytical methods for determining reducing sugars is such that optimum conditions cannot be precisely chosen from the data in table 3. Primary hydrolysis for 45 minutes and secondary hydrolysis for 60 minutes appears to give sufficient latitude to insure reproducibility of the results within the limitations of the sugar analysis.

This method of saccharification was applied to extracted cotton, to a high-alpha pulp, and to glucose in an effort to determine how nearly quantitative the method was, and where the chief loss of glucose occurred.

A glucose sample when given a primary hydrolysis of 45 minutes and a secondary hydrolysis of 60 minutes showed a "potential sugar content" of 96.9 percent. A similar sample that was autoclaved with 4 percent sulfuric acid but was not treated with 72 percent acid showed the presence of 97.3 percent potential sugar, which indicates that the secondary hydrolysis is more destructive than the treatment with 72 percent acid. Several samples of wood cellulose and extracted cotton showed the presence of 105.0 to 108.0 percent potential sugar; the theoretical yield of reducing sugar from pure cellulose is 111.1 percent.

To compare this technique with other methods, the potential sugar present in two samples of Douglas-fir was determined by the proposed method and by a method developed by Kiesel and Semiganovski (2) and recommended by Luers (3), in which 80 percent sulfuric acid is used for the primary hydrolysis, and the secondary hydrolysis is conducted at the boiling point for 5 hours after dilution with 15 volumes of water. The values obtained were as follows:

	Kiesel and Semiganovski method	Proposed method
Sample I	68.3 percent	71.4 percent
Sample II	64.4 percent	68.0 percent

From these results it is apparent that the proposed method gives a higher yield of sugar than the Kiesel and Semiganovski method. While neither this method nor any other method for the saccharification of cellulose gives truly quantitative yields of reducing sugar, it is adequate for present purposes. If more accuracy is required, an experimentally determined factor can be used to correct for the sugar decomposed in the process.

Recommended Method for the Quantitative Saccharification of Cellulosic Materials

The method recommended for the quantitative saccharification of cellulosic materials is as follows:

The sample is ground to pass a 30-mesh screen. It is then air dried to a low moisture content, and the moisture is determined. A quantity of the material sufficient to contain approximately 0.35 gram of cellulose is weighed into a 30-milliliter shell vial. To this is added 5 milliliters of 72 percent sulfuric acid that has been cooled to 15° C. It is next mixed thoroughly with a stirring rod and put in a water bath at 30° C. This temperature is maintained for 45 minutes while the mixture is stirred at 5- or 10-minute intervals. Using wood it is best to add all of the 72 percent acid at once. With cotton or a pulp it is desirable to make a homogeneous mixture with 1 milliliter of 72 percent acid and then add the remaining 4 milliliters. After a total time of 45 minutes the mixture is washed from the vial into an Erlenmeyer flask with 140 milliliters of water. The diluted solution is autoclaved at 15 pounds steam pressure for 1 hour. At the end of this time the sample is cooled, diluted to exactly 250 milliliters, neutralized with excess chalk, filtered, and analyzed for sugar by the Shaffer and Somogyi method (7). While the use of an autoclave is preferred for the secondary hydrolysis, identical yields of reducing sugar have been obtained by boiling for 4-1/2 hours at atmospheric pressure. If it is desired, the fermentable sugar present can be determined in the final neutralized solution by the rapid yeast sorption method described in another report (10).

The Potential Reducing Sugar Content of Various Samples of Wood Determined by the Proposed Method

There are wide variations in the amount of sugar potentially available in samples of wood of different species, and even in different samples of the same species. Table 4 illustrates the differences occurring within a species. All percentages are given on an oven-dry unextracted basis.

In table 5 is shown the potential sugar in samples of representative hardwoods and softwoods. In all tests, samples of wood cut from three different logs were ground together to average partially the differences within the species.

The data in table 5 are intended only as a rough indication of the percentage of sugar available from the various species. It is obvious from the data in table 4 that large variations occur within a species; hence, only large differences can be considered of significance.

Literature Cited

(1) Klason, P., Cellulosechemie 4, 81 (1923).

(2) Kiesel, A. and Semiganovski, N., Ber. 60B, 333-8 (1927).

(3) Luers, H., Angew. Chemie 45, 369-76 (1932).

(4) Monier-Williams, C. W., J. Chem. Soc. 119, 803-5 (1921).

(5) Ritter, G. J., Mitchell, R. L., and Seborg, R. M., J. Am. Chem. Soc. 55, 2989-91 (1933).

(6) Ritter, G. J., Seborg, R. M., and Mitchell, R. L., Ind. Eng. Chem. Anal. Ed. 4, 202-4 (1932).

(7) Shaffer, P. A. and Somogyi, M., J. Biol. Chem. 100, 695 (1933).

(8) Willstatter, R. and Zechmeister, L., Ber. 46, 2401, (1913).

(9) Wohl, A. and Krull, H., Cellulosechemie 2, 1-7 (1921).

(10) Saeman, J. F., Harris, E. E., and Kline, A. A., FPL Mimeo. R1459

Table 1.--Sugar concentrations in samples of glucose determined after heating at 100° C. and 121° C. in an autoclave

Sample	Heating period	Temperature	Sugar concentrations
	Minutes	° C.	Milligrams per milliliter
1	0	0	1.58
2	2	121	1.58
3	8	121	1.55
4	30	121	1.56
5	60	121	1.54
6	0	100	1.58
7	5	100	1.56
8	15	100	1.56
9	60	100	1.56
10	180	100	1.55

Table 2.--Yield of reducing sugar from Douglas-fir hydrolyzed under various conditions

Time of primary hydrolysis at 30° C.	Time of secondary hydrolysis at 121° C.	Reducing sugar yield using 1 percent acid for secondary hydrolysis	Reducing sugar yield using 2 percent acid for secondary hydrolysis	Reducing sugar yield using 4 percent acid for secondary hydrolysis
Minutes	Minutes	Percent	Percent	Percent
40	15	27.7	39.7	50.5
	30	38.8	51.2	64.0
	60	53.0	63.4	68.0
85	15	36.9	45.0	53.7
	30	46.0	56.4	64.7
	60	56.4	65.2	66.8
120	15	42.3	48.1	57.0
	30	51.9	59.2	65.5
	60	58.0	68.0	64.5

Table 3.--Reducing sugar yield of samples of Douglas-fir wood subjected to different periods of primary and secondary hydrolysis

Time of primary hydrolysis at 30° C. with 72 percent acid	Time of secondary hydrolysis with 4 percent acid	Reducing sugar
Minutes	Minutes	Percent
30	30	68.3
30	60	68.6
30	120	67.0
45	30	67.9
45	60	68.6
45	120	67.0
60	30	68.7
60	60	68.9
60	120	66.4

Table 4.--Yield of potential reducing sugar from various samples of Douglas-fir

Sample No.	Potential reducing sugar	Description of sample
	Percent	
1	71.4	Young, rapid growth, 70 percent sapwood.
2	61.7	Old, slow growth heartwood.
3	68.3	Young, rapid growth heartwood.
4	68.0	Heartwood, 1.5-foot diameter tree.
5	69.3	Sapwood, same tree.
6	67.3	Mixture of chipped wood.
7	68.0	Mixture of chipped wood.
8	71.4	Young, rapid growth, two-thirds sapwood.

Table 5.--Yield of potential reducing sugar and fermentable sugar from samples of representative hardwoods and softwoods

Species	Potential reducing sugar	Fermentability by yeast sorption
	Percent	Percent
Hardwoods		
American beech	70.1	75.1
Aspen	75.1	76.3
Birch	69.9	67.8
Maple	68.2	71.0
Red oak	63.6	63.0
Sweetgum	66.4	73.8
Yellow-poplar	70.9	76.1
Softwoods		
Douglas-fir	66.6	86.2
Eastern white pine	66.5	86.3
Hemlock	66.1	88.2
Ponderosa pine	68.0	82.2
Redwood	52.4	77.1
Sitka spruce	70.1	85.3
Southern yellow pine	64.8	82.0
Sugar pine	64.3	82.4