Combination of Sulfuric Acid with Cellulose during the Hydrolysis with a Small Amount of Concentrated Sulfuric Acid

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(Received August 1, 1963)

In the usual process of wood saccharification with concentrated sulfuric acid, the first stage of the process involves removal of hemicellulose from wood by mild hydrolysis and is called prehydrolysis. Cellulose in the residue of the first process is dissolved and hydrolyzed to a soluble glucose polymer[1] with concentrated sulfuric acid in the second process called mainhydrolysis. Finally, the polymer is hydrolyzed to glucose by dilute acid hydrolysis called posthydrolysis. In the mainhydrolysis a large amount of sulfuric acid is consumed.[2-5] This is a disadvantage of the concentrated sulfuric acid process, although the process has advantages such as large hydrolysis rate[6] and little erosion problem[7].

In order to determine the minimum quantity of sulfuric acid required in the mainhydrolysis, a diagram of the equilibrium relations of the system, soluble glucose polymer - sulfuric acid - water, has been obtained.[8] The dissolving power of sulfuric acid was not so large; for example, 100 parts of 72% sulfuric acid could dissolve and hydrolyze only 49 parts of cellulose to form the soluble glucose polymer, and 100 parts of 84% sulfuric acid was required for 82 parts of cellulose. The formation of a sulfuric acid - cellulose compound was observed in the course of hydrolysis and this compound seemed to play some chemical role in dissolving and hydrolyzing the cellulose, the extent of the combination of sulfuric acid with cellulose being an important factor in determining the yield of the soluble glucose polymer. In order to confirm this point the present work was undertaken. It was also expected to find out the reason why such large amounts of sulfuric acid are consumed during the hydrolysis.

Material and Method

The experiments were carried out in a manner similar to that in the previous work, using the same material.[8]

Filter paper (Tôyô Roshi, No. 2), after being grated, was used as the cellulose sample. The cellulose flakes dried at 105°C (ca. 5 g.) were immersed in dilute sulfuric acid (20—50% concentration), and then filtered; the content of cellulose and sulfuric acid in the wet material was then calculated. The wet sample (ca. 1 g.) thus obtained was freeze-dried in a test tube for a few hours, until the weight loss reached the desired amount, and the test tube was then sealed under vacuum at low temperature. The weight of cellulose and sulfuric acid was calculated.

The sealed tube was heated in a water bath at 50°C for different periods of time. The tube was immediately cooled, the contents rinsed out with water, and centrifuged, and the insoluble sediment washed with water slightly acidified with hydrochloric acid; the centrifuging and the washing were repeated four times. The combined solution thus obtained was made up to 100 ml. with the requisite amount of hydrochloric acid to give a 2.5% hydrochloric acid concentration (solution I). A portion of solution I was heated at 100°C for 3 hr. to complete the hydrolysis of the soluble glucose polymer.[1]

Reducing sugar in solution I and in hydrolyzed solution I was determined by Somogyi's method.[9] From the amount of reducing sugar in hydrolyzed solution I, the weight of the soluble glucose polymer (as potential reducing sugar) dissolved in concentrated sulfuric acid was estimated. The value obtained by dividing the reducing power of hydrolyzed solution I by that of unhydrolyzed solution I was used as the average degree of polymerization of the soluble glucose polymer. Not only cellulose but also glucose polymer, both soluble and insoluble, are referred to as potential reducing sugars throughout this report.

Four determinations of sulfuric acid were carried out. (1) Sulfate ion in solution I; eqiuvalent to the amount of free sulfuric acid present in the concentrated sulfuric acid saturated with the soluble glucose polymer. (2) Sulfuric acid in the sediment decomposed by nitric acid; sulfuric acid combined with the insoluble glucose polymer.

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[1] T. Kobayashi and Y. Sakai, *Bull. Agr. Chem. Soc. Japan*, **22**, 117, 277 (1958).

[2] J. W. Dunning and E. C. Lathrop, *Ind. Eng. Chem.*, **37**, 24 (1954); Bureau of Mines Report of Investigations 4772, United States Department of Interior, Feb., 1951.

[3] T. Kuzuoka, *Mokuzai Tôka Shingikai Hôkoku* (*Report of the Wood Saccharification Discussion Committee*), No. 2, 1 (1953).

[4] T. Kobayashi and T. Ito, ibid., No. 4, 5 (1954).

[5] T. Kobayashi, Y. Sakai, K. Mitachi and K. Tsuyuzaki, *Ringyô Shidôsho Kenkyû Hôkoku* (*Report of the Hokkaido Forest Products Research Institute*), No. 15, 138 (1959).

[6] T. Kobayashi, *Mokuzai Tôka Shingikai Hôkoku* (*Report of the Wood Saccharification Discussion Committee*), No. 1, 27 (1952).

[7] T. Kuzuoka, *Bôshoku Gijutsu*, **4**, 79, 82 (1955).

[8] T. Kobayashi, Y. Sakai and K. Iizuka, *Bull. Agr. Chem. Soc. Japan*, **24**, 443 (1960).

[9] M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).

(3) Sulfuric acid in solution I after the destruction of organic matter; total sulfuric acid (free acid plus combined acid) in the soluble glucose polymer-sulfuric acid-water system. (4) Sulfuric acid in solution I which had been hydrolyzed in 2.5% hydrochloric acid at 100°C for 3 hr.; values agree with those of (3) (Table I, t-test⁽10⁾). The amount of sulfuric acid obtained by (3) or (4) plus sulfuric acid by (2) agreed with the amount of sulfuric acid found by weighing the cellulose-sulfuric acid mixture, although they showed some fluctuations, as shown in Table I (t-test⁽10⁾). That the combined sulfuric acid is present as ester sulfate is shown from these results.

The sulfuric acid determinations were carried out by Morgulis and Hemphill's iodometric micro method.[11] Combined sulfuric acid was determined according to Hoffpauir and Guthrie's method[12] involving decomposition of the organic material with nitric acid to which a small amount of perchloric acid had been added.

Results

The cellulose-concentrated sulfuric acid mixtures prepared by the freeze-drying process described above were heated at 50°C for different periods. Hydrolysis of the cellulose was accompanied by the formation of a cellulose-sulfuric acid compound. When the extent of hydrolysis of cellulose vs. time curve reached a plateau, the extent of sulfuric acid combination vs. time curve also leveled off. An example of the results is shown in Fig. 1. Soluble glucose polymer (SGP), sulfuric acid combined and average degree of polymerization of SGP in the equilibrium state were obtained from the straight lines in the figure. Table II lists the results of the reaction in different sulfuric acid concentrations. A higher extent of sulfuric acid combination was observed with higher concentrations of sulfuric acid. During the hydrolysis and the combination of sulfuric acid, water must have been consumed or formed. Therefore, the sulfuric acid concentration shown here is the initial one.

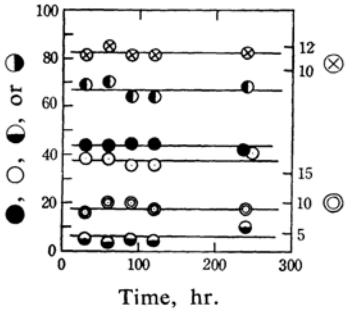

Fig. 1. Sulfuric acid combination during hydrolysis of cellulose in 89% sulfuric acid (H_2SO_4/Cellulose=0.5/1.0) at 50°C.
● SGP, g./100 g. Cellulose
○ Combined H_2SO_4, g./100 g. Total H_2SO_4
⊗ H_2SO_4 combined with SGP, g./g. H_2SO_4 combined with IGP
◐ Combined H_2SO_4, mol./100 mol. GU of SGP
◒ Combined H_2SO_4, mol./100 mol. GU of IGP
◎ Av. D. P. of SGP

Boundary between Concentrated and Dilute Sulfuric Acid in Cellulose Hydrolysis. — Table III shows that when cellulose was hydrolyzed in 60 or 57% sulfuric acid, no sulfuric acid combines with the soluble glucose polymer and monomer, and only a slight amount is combined with the insoluble glucose polymer. On the other hand, when the concentration was 65% or higher, a significant amount of sulfuric acid combined with the soluble and the insoluble glucose polymers (Table II). Although the hydrolysis product of cellulose in 57% sulfuric acid consisted chiefly of glucose monomer (Table III, Fig. 5), the product in sulfuric acid of 60% or higher concentration had a significantly higher degree of polymerization (Table II). The action of sulfuric acid at about 60% or higher concentrations appears to be different from that at lower concentrations. This is supported by another experiment

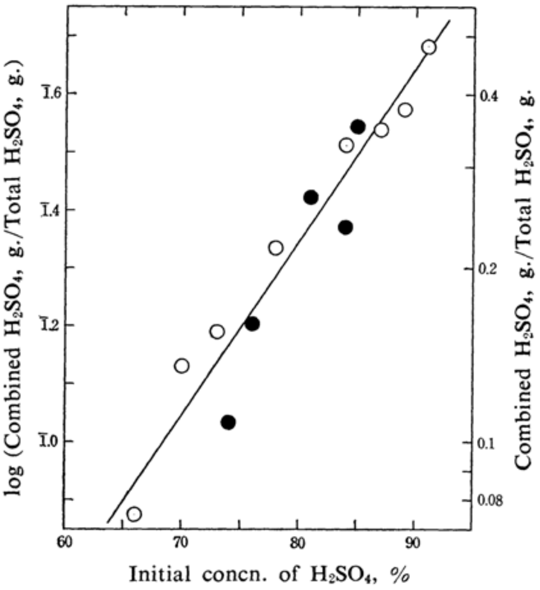

Fig. 2. Relation of the extent of sulfuric acid combination to sulfuric acid concentration in the hydrolysis of cellulose.
H_2SO_4/Cellulose: ● 1.0, ○ 0.5

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10) O. L. Davies, "Statistical Methods in Research and Production with Special Reference to the Chemical Industry," 2nd ed., London, Oliver and Boyd (1949), p. 51.

11) S. Morgulis and M. Hemphill, *J. Biol. Chem.*, **96**, 573 (1932).

12) C. L. Hoffpauir and J. G. Guthrie, *Ind. Eng. Chem., Anal. Ed.*, **16**, 391 (1944).

TABLE I. DETERMINATION OF SULFURIC ACID

Initial concn. of H_2SO_4 %	H_2SO_4 added to 100 mg. of cellulose mg.	H_2SO_4 in sol. part: Free H_2SO_4 by detn. (1) mg./100 mg. of H_2SO_4 added	H_2SO_4 in sol. part: Total H_2SO_4 by detn. (3) mg./100 mg. of H_2SO_4 added	H_2SO_4 in sol. part: Total H_2SO_4 by detn. (4) mg./100 mg. of H_2SO_4 added	H_2SO_4 in insol. part by detn. (2) mg./100 mg. of H_2SO_4 added	H_2SO_4 recovery: (3)+(2) mg./100 mg. of H_2SO_4 added	H_2SO_4 recovery: (4)+(2) mg./100 mg. of H_2SO_4 added
56	94	101	101	97	1	102	98
61	104	95	104	96	1	105	97
69	51	90	93	93	2	95	95
74	97	90	95	94	2	97	96
75	95	83	101	99	1	102	100
76	95	90	100	103	0.3	100	103
79	48	83	89	94	3	92	97
85	55	67	103	96	7	110	103
86	97	70	102	100	0.3	102	103
87	45	67	98	88	6	104	94
88	49	62	102	98	3	105	101
90	45	49	93	99	3	96	102

TABLE II. SULFURIC ACID COMBINATION IN EQUILIBRIUM STATE DURING HYDROLYSIS OF CELLULOSE IN SULFURIC ACID OF DIFFERENT CONCENTRATION AT 50°C

H_2SO_4/Cellulose	H_2SO_4 concn.	H_2SO_4 combined/H_2SO_4 total	H_2SO_4 combined with SGP/H_2SO_4 combined with IGP	H_2SO_4 combined/GU of SGP	Av. D P. of SGP
g./g.	%	g./100 g.	g./g.	M/100 M	
0.5	65	—	—	—	3.02
	66	7.50	4.31	20.5	—
	70	13.5	3.18	29.1	—
	71	—	—	—	5.02
	73	15.5	3.65	30.0	5.02
	78	21.5	6.94	47.0	6.02
	84	32.6	5.30	55.0	—
	87	34.5	—	57.0	20.9
	89	37.5	11.3	67.0	8.9*
	91	48.4	17.2	95.2	25.1
1.0	54	—	—	—	1.10
	60	—	—	—	1.59
	61	—	—	—	1.82
	74	10.8	34.7	—	—
	76	16.0	26.3	28.0	6.02
	81	26.5	—	47.5	8.91
	84	23.5	81.0	—	15.1
	85	35.0	86.4	62.0	10.0

* Fig. 1.

which indicated that the hydrolysis rate constant of pentosan in 60% sulfuric acid deviates from the relationship between rate constant and acid concentration obtained in 50% and lower concentrations.[13]

Effect of Sulfuric Acid Concentration on the Ratio of Combined Sulfuric Acid to Total Sulfuric Acid. — When an equilibrium was established in hydrolysis of the cellulose with sulfuric acid of 66% or higher concentration, as shown in Fig. 1 (89% sulfuric acid), the ratio of the combined sulfuric acid to the total sulfuric acid showed a definite value. When the ratios (Table II) are plotted against the "initial" concentration of sulfuric acid, Fig. 2 is obtained. The following empirical equation is obtained from Fig. 2,

$$\log x = -3.047 + 0.0299\,C$$

or

$$x = 0.000897 \times \exp(0.0689\,C) \qquad (1)$$

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[13] T. Kobayashi, Y. Sakai and S. Mutoh, *Agr. Biol. Chem.*, **26**, 378 (1962).

TABLE III. COMBINATION OF SULFURIC ACID WITH CELLULOSE DURING HYDROLYSIS IN 60°C AND 57% SULFURIC ACID (H_2SO_4: Cellulose=1.0)

Initial concn. of H_2SO_4	H_2SO_4 added to cellulose	H_2SO_4 in sol. part		H_2SO_4 in insol. part		Av. D. P. of sol. glucose polymer	Hydrolysis time at 50°C
		Free H_2SO_4	Combined. H_2SO_4	Combined H_2SO_4	Combined H_2SO_4 per one glucose unit		
%	mg.	mg.	mg.	mg.	mol./mol.		hr.
60	334	333	0	4	0.024	1.5	18
	331	326	2	4	0.024	1.4	48
	332	331	−1	4	0.024	1.8	48
57	334	332	−2	—	—	1.0	63
	333	336	7	5	0.030	1.3	89
	360	353	2	—	—	0.9	96

where C is "initial" concentration of sulfuric acid (%) and x, the ratio of combined sulfuric acid to total sulfuric acid. This equation indicates, for example, that when 100 g. of cellulose is hydrolyzed at 50°C in 72% sulfuric acid containing 50 g. of sulfuric acid, $50\times0.128=6.4$ g. of sulfuric acid reacts to form the cellulose-sulfuric acid compound.

Ratio of Sulfuric Acid Combined with Soluble Glucose Polymer to Sulfuric Acid Combined with Insoluble Glucose Polymer. — A part of the combined sulfuric acid is in combination with the soluble glucose polymer (SGP) and the other part with the insoluble glucose polymer (IGP). As shown in Fig. 1, the ratio of sulfuric acid combined with the SGP to that combined with the IGP is constant when the hydrolysis of cellulose is at equilibrium. This ratio (Table II) is shown in Fig. 3 against the "initial" concentration of sulfuric acid. The following empirical equation is obtained from the figure.

$$\log y = 0.0354\,C + 1.76\,r - 2.88$$

$$\text{or} \qquad y = 0.00132 \exp(0.0815\,C + 4.06\,r) \qquad (2)$$

where r is sulfuric acid: cellulose ratio and y, the ratio of sulfuric acid combined with SGP to that with IGP. This equation indicates, for example, that in the hydrolysis of cellulose in 72% sulfuric acid at 50°C, a proportion of $3.5/(3.5+1)$ to one of the combined sulfuric acid is in combination with SGP. So,

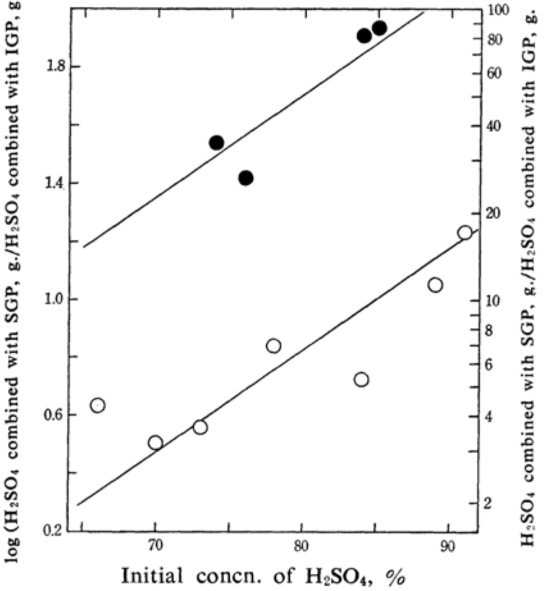

Fig. 3. Ratio of sulfuric acid combined with soluble glucose polymer to sulfuric acid combined with insoluble glucose polymer in different concentration of sulfuric acid.
H_2SO_4/Cellulose : ● 1.0, ○ 0.5

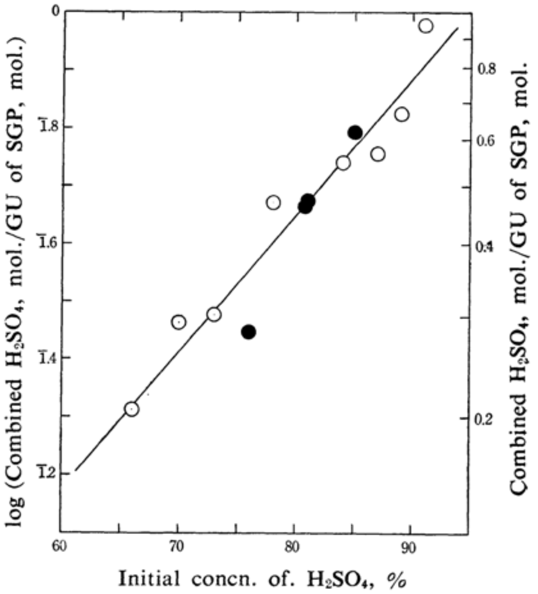

Fig. 4. Ratio of molecules of sulfuric acid combined with soluble glucose polymer (SGP) to glucose units (GU) of soluble glucose polymer.
H_2SO_4/Cellulose : ● 1.0, ○ 0.5

when 100 g. of cellulose is hydrolyzed at 55°C in 72% sulfuric acid containing 50 g. of sulfuric acid, $50\times0.128\times3.5/4.5=5.0$ g. of sulfuric acid is in combination with the SGP and $50\times0.128\times1/4.5=1.4$ g. is in combination with IGP.

Combined Sulfuric Acid Molecules per Glucose Unit of Soluble Glucose Polymer. — The number of sulfuric acid molecules fixed to one glucose unit (GU) of SGP showed a definite value when the hydrolysis of cellulose was at equilibrium, as shown in Fig. 1. This value depends on the "initial" concentration of sulfuric acid (Table II), as shown in Fig. 4. The following empirical equation is obtained from Fig. 4.

$$\log z = -2.247 + 0.0237\,C$$

$$z = 0.00566\exp(0.0546\,C) \qquad (3)$$

where z is the number of sulfuric acid molecules fixed to one GU of the SGP. The equation indicates, for example, that when 100 g. of cellulose is hydrolyzed at 50°C in 72% sulfuric acid containing 50 g. of sulfuric acid, 0.288 mol. of sulfuric acid is fixed to 1 mol. of GU of the SGP. Then, $50\times0.128\times(3.5/4.5)\times(180/98)\times(1/0.288)=32$ g. of cellulose is converted into the SGP.

Average Degree of Polymerization of Soluble Glucose Polymer.—When the conversion of cellulose to the SGP reached an equilibrium, the average degree of polymerization (av. D. P.) of the SGP showed a definite value (Fig. 1). Figure 5, a plot of logarithm of av. D. P. of the SGP (Table II) against the "initial" concentration of sulfuric acid, shows a straight line.

$$\log u = -2.218 + 0.0396\,C$$

$$u = 0.00605\exp(0.0911\,C) \qquad (4)$$

where u is av. D. P. of the SGP. In a ratio of sulfuric acid to cellulose ranging from 0.5 : 1 to 1.0 : 1, sulfuric acid of higher concentrations yields SGP of larger D. P., for example, 65% sulfuric acid gives SGP having av. D. P. 2.2 and 85% sulfuric acid 13.

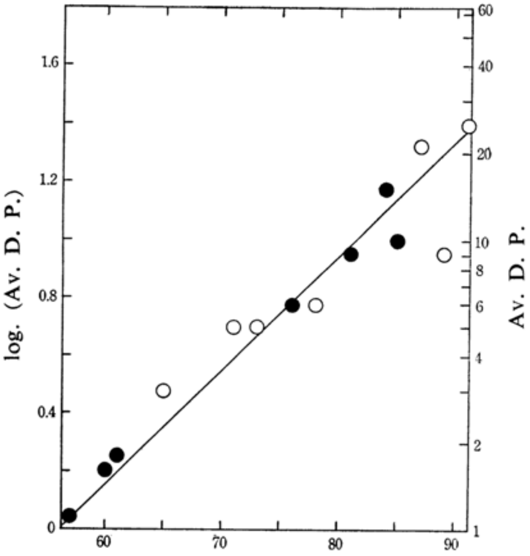

Fig. 5. Relation of average degree of polymerization of soluble glucose polymer to sulfuric acid concentration.
H_2SO_4/Cellulose : ● 1.0, ○ 0.5

Concentration of Aqueous Sulfuric Acid in the Cellulose - Sulfuric Acid - Water System. — On the assumption that water is consumed or formed during the hydrolysis according to the following equations,

$$C_6H_{10}O_5 + H_2SO_4 \rightarrow C_6H_9O_5SO_3H + H_2O$$

$$C_6H_{10}O_5 + H_2O \rightarrow C_6H_{12}O_6$$

the "corrected" concentration of sulfuric acid in the cellulose - sulfuric acid mixture was calculated by use of Eqs. 3 and 4. Figure 6 shows this value against the "initial" concentration of sulfuric acid. The "corrected" sulfuric acid concentration in the cellulose-sulfuric acid-water system can not exceed 75%, even when the initial concentration exceeds 85%.

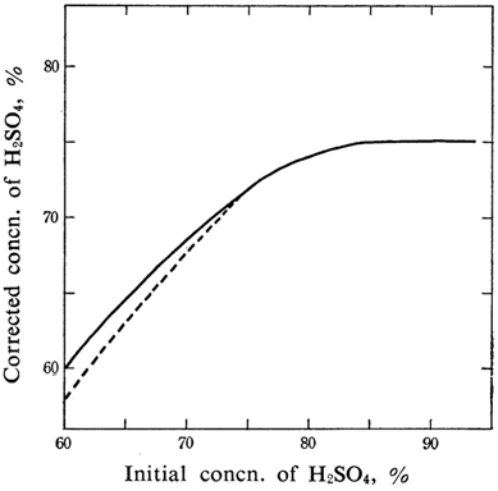

Fig. 6. Corrected concentration of sulfuric acid in cellulose - conc. sulfuric acid system.
H_2SO_4/Cellulose : —— 1.0, - - - 0.5

Yield of Soluble Glucose Polymer and the "Initial" Concentration of Sulfuric Acid and the Sulfuric Acid : Cellulose Ratio. — The amount of SGP (g.) produced when 100 g. of sulfuric acid is used is :

$$\mathrm{SGP(g.)} = 100\mathrm{(g.)}\left(\frac{\text{combined } H_2SO_4 \text{ (g.)}}{\text{total } H_2SO_4 \text{ (g.)}}\right)$$

$$\times\left(\frac{\text{H}_2\text{SO}_4\text{ combined with SGP (g.)}}{\text{combined H}_2\text{SO}_4\text{ (g.)}}\right)$$
$$\times\left(\frac{\text{GU of SGP (mol.)}}{\text{H}_2\text{SO}_4\text{ combined with SGP (mol.)}}\right)$$
$$\times\left(\frac{180\,(\text{g./mol.})}{98\,(\text{g./mol.})}\right)$$
$$=100\,(\text{g.})\,(x)\left(\frac{y}{1+y}\right)\left(\frac{1}{z}\right)\left(\frac{180}{98}\right)$$
$$=\frac{0.0384\times\exp(0.0958\,C+4.06\,r)}{1+0.00132\times\exp(0.0815\,C+4.06\,r)} \qquad (5)$$

The yield of the SGP produced from cellulose in sulfuric acid of various "initial" concentration is shown in Fig. 7 as a function of the sulfuric acid : cellulose ratio.

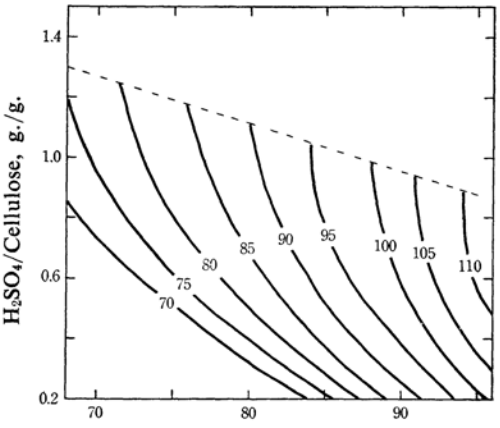

Fig. 7. Soluble glucose polymer yield expressed as a function of sulfuric acid concentration and H$_2$SO$_4$: cellulose ratio.

Discussion

In Table IV the data obtained from some pilot plant tests are compared with the values calculated from Eq. 5. This table shows that, in the ordinary concentrated sulfuric acid process, the reaction did not reach completion. It is considered that this can be attributed to the fact that diffusion of acid into the wood did not reach completion. Kuzuoka[3] has reported that when the mixture of saw dust and concentrated sulfuric acid was treated through the rolls, the larger the number of treatments the higher the yield of reducing sugar (Table IV). On the other hand, in Odincov's experiments,[14] the reducing sugar yield greatly exceeded the equilibrium value calculated from Eq. 5. This suggests that a mechanical force is effective in accelerating the diffusion of sulfuric acid into the wood and the hydrolysis of cellulose.

Summary

The combination of sulfuric acid with cellulose occurring during the hydrolysis of cellulose in a small amount of concentrated sulfuric acid has been investigated. The amounts of the soluble and the insoluble glucose polymers produced from cellulose, and the amount of sulfuric acid combined with them, have been estimated. To obtain an effective concentration of sulfuric acid on the cellulose, the concentration of the sulfuric acid must be greater than about 60%. The sulfuric acid concentration in the cellulose-sulfuric acid-water system can not exceed 75%, even when the initial concentration exceeds 85%. The amount of soluble glucose polymer (g.) produced by the action of 100 g. of sulfuric acid is given by 0.0384 $\exp(0.0958\,C+4.06\,r)/[1+0.00132\exp(0.0815\,C+4.06\,r)]$ (C is "initial" concentration of sulfuric acid (%) and r, sulfuric acid-cellulose ratio).

TABLE IV. COMPARISON OF DIFFERENT PROCESSES OF WOOD SACCHARIFICATION WITH CONCENTRATED SULFURIC ACID

$$\frac{\text{SGP yield per 100 g. of H}_2\text{SO}_4}{\text{Calcd. SGP yield per 100 g. of H}_2\text{SO}_4},\ \%$$

H$_2$SO$_4$/Cellulose	0.1	0.3	0.5	1.0	1.2
Concn. of H$_2$SO$_4$, %	75	75	80	80	80
Mixing in extruder (Dunning[2])			97	63	
Mixing in extruder (Kobayashi[5])			91	70	
Pressing through rolls (Kuzuoka[3])	(1 treatment)			87	88
	(3 treatments)			97	93
Vibration milling (Odincov[14])	458	144	(15 min. treatment)		
	336	123	(120 min. treatment)		
Calcd.*	40	57	80	90	90

* Calculated from experimental Eq. 5.

The author thanks Professor Tatsuyoshi Kobayashi for his interest and encouragement and also Mr. Takumichi Kono and Miss Wasako Kumagai for their assistance.

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[14] P. N. Odincov, I. I. Beinart and N. F. Murashchenko, *Gidroliz. i Lesokhim. Prom.*, No. 6, 6 (1960).