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# Hydrothermal Conversion of Cellulose to Glucose and Oligomers in Dilute Aqueous Formic Acid Solution

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Additional information is available at the end of the chapter

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## 1. Introduction

Many efforts have been made to convert various cellulosic materials into glucose for bio-ethanol production via enzymatic and acid hydrolysis [1-4]. Compared to common polysaccharides such as starch [5-7], guar gum [8], and pectin [9], cellulose is difficult to depolymerize because of the presence of inter- and intra-molecular hydrogen bonding. This bonding results in relatively longer reaction times for enzymatic hydrolysis, and lower yields of glucose from acid hydrolysis due to further decomposition of the products. In addition to these conventional methods, Adschiri et al. [10] and coworkers [11] employed supercritical water without any additives to hydrolyze cellulose in extremely short contact times. In this process, use of fine particles of the cellulosic material may be beneficial because accurate regulation of the contact times is needed. Unfortunately, because lignocelluloses include fibers, textiles, wood, and grass plants, which have widely varying compositions and structures, pulverizing and crushing are neither easy nor economical. From a practical point of view, with such cellulosic materials, relatively longer contact times, (i.e., on the order of several minutes) and mild temperatures (e.g., hydrothermal conditions) are attractive so that the conversion can be carried out in conventional reactors. Cellulose is in fact slowly degraded in water alone under hydrothermal conditions. Furthermore, the reaction temperature can be decreased to suppress decomposition of the desired products, and acid can be added to accelerate the reaction.

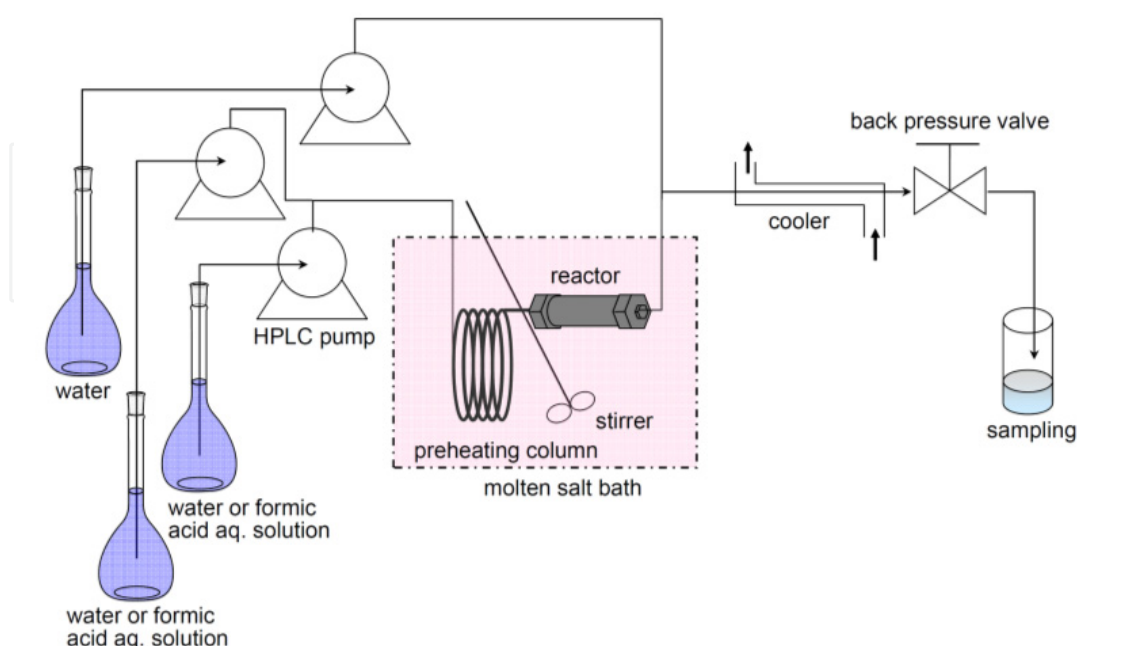
Many kinds of biomass, including wood, corn stover, and cotton fiber, have been pre-treated in dilute or concentrated acid solutions to moderately accelerate the hydrolysis of the samples [1-4, 12-26]. The most commonly used acid is sulfuric acid [12-22]. Organic acids such as formic acid and acetic acid are produced during the thermal treatment of

lignocellulosic samples, making these potential pre-treatment candidates as well. However, hydrolysis of lignocellulosic samples in an organic acid solution has been limited [23-26].

In this chapter, the effectiveness of dilute acid solution is demonstrated in hydrothermal saccharification of cellulose based on the experimental results described in our previous study [27], and the formation step of each product was studied by examining the relationship between product yields.

## 2. Experimental

A schematic diagram of the semi-batch reactor set-up used in this study is shown in Figure 1. The set-up is similar to that employed for the hydrolysis of starch or poly(galacturonic acid) under hydrothermal conditions [6, 7, 9]. The reactor, which was made of stainless-steel tubing 6.7 mm I.D., 8 cm long), was connected to a preheating column (1/8-inch stainless-steel tubing of 2.17 mm I.D., 2 m long). Stainless-steel tubings of 0.5 mm I.D. served as the reactor outlet (11 cm long) and cold water supply for quenching the eluted product solution. These tubes were joined with a T-union, and then the line was further connected to tubing (0.5 mm I.D., 44 cm long) equipped with a cooling jacket to quench the solution, followed by a back pressure regulator (Model 880-81, JASCO, Tokyo, Japan) capable of adjusting pressure fluctuations within  $\pm 0.1$  MPa using an electromagnetic high-frequency open-shut valve. The preheating column and the reactor were immersed in a molten salt bath whose temperature was maintained within  $\pm 2$  K. The cellulosic samples tested were cotton cellulose (dewaxed, standard sanitary cotton, Pharmacopoeia of Japan, Tokyo), filter paper (ashless, No. 7, Advantec, Tokyo, Japan) and microcrystalline cellulose powder (Avicel, Merck Japan).



**Figure 1.** Experimental set-up.

A 0.5 g room-temperature cellulose sample wrapped softly with quartz wool (0.05 g) was placed in the reactor. A frit disk (2- $\mu$ m pore size) was placed at the exit of the reactor to fix the quartz wool and cellulose sample, and the reactor and all of the lines were filled with ultrapure, degassed water supplied mainly at a constant flow velocity of 15 ml/min by two HPLC pumps. The reactor was then immersed in a molten salt bath maintained at a prescribed temperature. Reaction time was determined from the moment the reactor was immersed in the molten salt bath. It was found by measuring the inside reactor temperature with a thermocouple that the reactor temperature reached the prescribed value within two minutes. Room temperature ultrapure water provided at a flow rate of 5 ml/min and a pressure of 10 MPa by an HPLC pump at a constant flow mode was used to quench the eluted reaction solution. The product solution eluted from the back pressure regulator was collected at intervals from 1 to 10 min. The residence time of the fluid between the reactor inlet and the exit of the back pressure regulator was about 15 seconds [9]. Monogalacturonic acid as a tracer was pulse-injected by an HPLC injector (Rehodyne 7520, U.S.A.) with a 5  $\mu$ L sample loop, installed in the line upstream the preheating column only when the residence time measurements were carried out, to determine the residence time of fluid in the reactor.

Glucose monomer, fructose, oligomers with degree of polymerization (DP) up to 9, and 1,6-anhydroglucose (levoglucosan) were quantitatively measured using high-performance anion-exchange chromatography (HPAEC, LC30, Dionex, Tokyo, Japan) with an electrochemical detector using a CarboPac PA column (Dionex Tokyo, Tokyo Japan). Oligomer yields (DP <6) were calibrated using the ratio of glucose to cellopentaose (Sigma, Tokyo, Japan) as a standard, and the yields of oligomers with DPs higher than 6 with cellobiose (Sigma, Tokyo, Japan) and cellotriose (Sigma, Tokyo, Japan). Secondary decomposition products were also measured by HPLC. The recovered solution was analyzed for total organic carbon (TOC) content using a total carbon analyzer (Model 5000A, Shimadzu, Kyoto, Japan). Oligomers having various DP values were identified using a MALDI-TOF mass spectrometer (AXIMA-CFR, Shimadzu, Kyoto, Japan).

Product yield and the amount of TOC in the solutions were defined as:

$$\text{Product yield (\%)} = 100 \times \frac{\text{Carbon of product component (g)}}{\text{Carbon of initial cellulose sample (g)}} \quad (1)$$

$$\text{TOC (\%)} = 100 \times \frac{\text{Carbon of soluble component (g)}}{\text{Carbon of initial cellulose sample (g)}} \quad (2)$$

$$\text{Conversion } x (-) = \frac{\text{Yield (\%)} \text{ of glucose or total sugar}}{100} \quad (3)$$

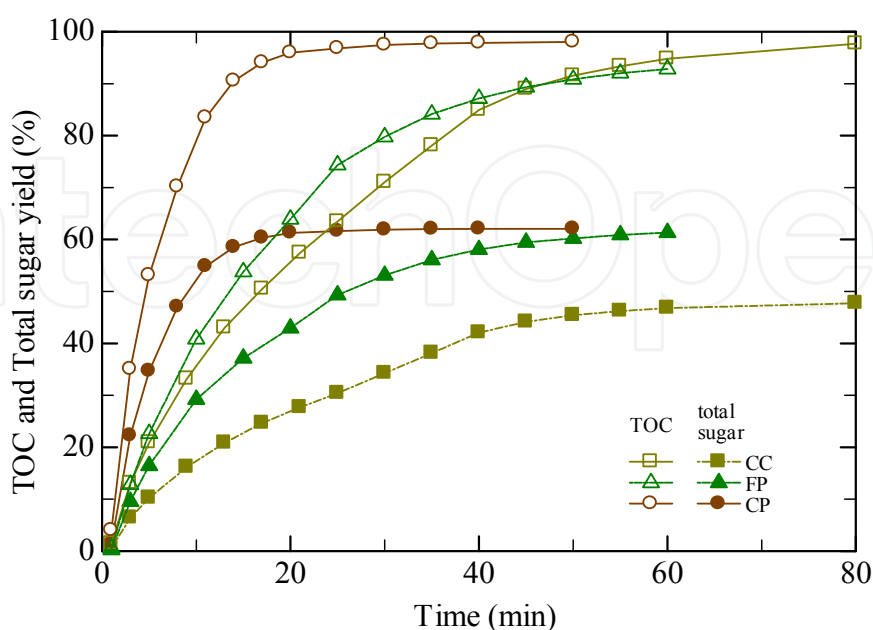
Total sugar yield (%) was defined as the sum of yields of glucose, fructose, and celooligosaccharides with DP = 2 to 9. Note that cellulose samples, cotton cellulose, filter paper, and microcrystalline cellulose powder, were assumed to be pure cellulose.

### 3. Results and discussion

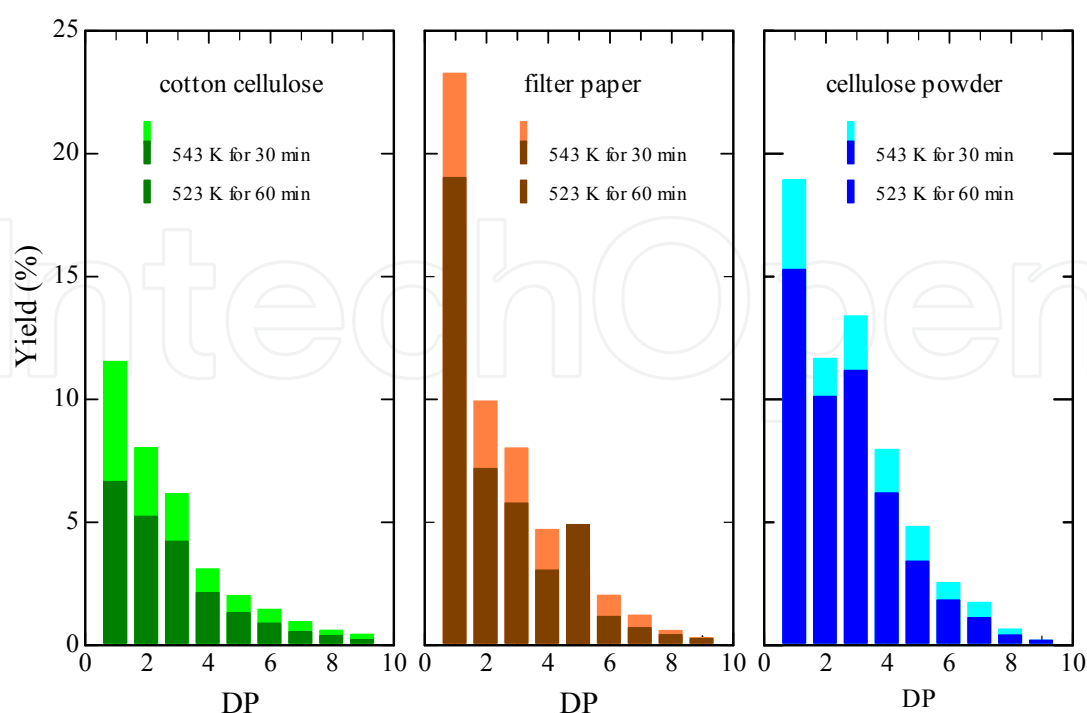
#### 3.1. Comparison of cellulose types with pure water

Figure 2 compares yields of TOC and total sugar, which includes glucose, oligomers with DPs up to 9, and fructose, for cotton cellulose (CC), filter paper (FP) and cellulose powder (CP) at 543 K and 10 MPa in pure water. The formation rates in TOC and total sugar yields increase in order for CC to FP to CP. The three celluloses were almost completely solubilized and the total sugar yields reached about 60 % for CP and FP, and 48 % for CC. Note that almost no residual solids were left for all celluloses in the reactor after reaction completion. Although the TOC yields over time for FP and CC were not very different, the increase rates of total sugar yields for FP were much faster than those for CC. The difference between TOC and total sugar yields could be ascribed to be oligomers with DPs higher than 10. After 24 h, white fine particles precipitated were observed in the product solution although the solution was transparent and no precipitation when it was recovered soon after reaction completion.

Figure 3 shows yields of glucose and oligomers with DPs = 2 to 9 for three celluloses in pure water at pressure of 10 MPa, and 543 K for 30 min and 523 K for 60 min. The yields of glucose and oligomers substantially decreased with increasing DP for three celluloses, but the yields depended on the cellulose types. Except for glucose the yields from CP were the highest, and those from CC were the lowest. Each glucose yield from filter paper at 523 K and 543 K was the highest among the three types of cellulose, as compared with yields of oligomers having DP higher than 2. The reason is not known, and further studies are required.



**Figure 2.** Comparison of cellulose types for TOC and total sugar yield at 543 K in water.



**Figure 3.** Comparison of cellulose types: cotton cellulose (CC), filter paper (FP), and cellulose powder (CP) for yields of monomer and oligomers with DP=2 to 9 at 543 K for 30 min and at 523 K for 60 min in water.

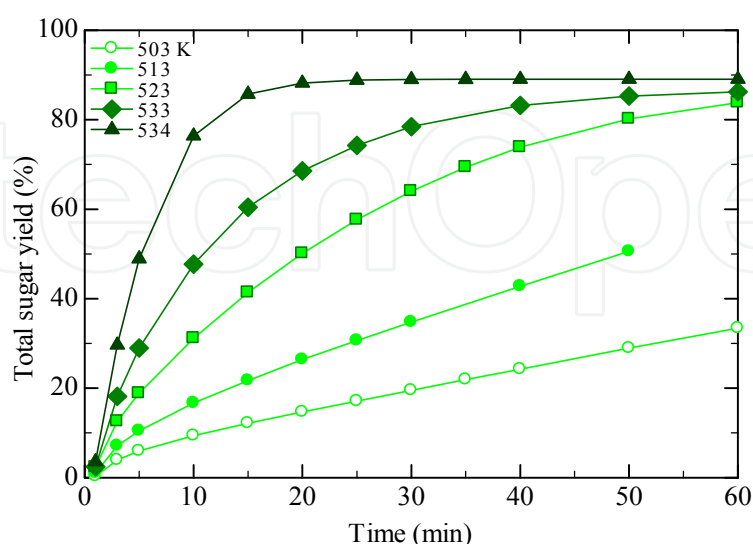
Acid	Formic	Acetic	None
Conc (wt%)	0.10	0.13	-
Temperature (K)	523	523	523
Time (min)	60	60	60
Yield (100×gC/gC of initial sample)			
Glucose	36.62	18.98	6.65
DP = 2	16.07	10.96	5.24
3	14.26	10.95	4.22
4	8.26	5.94	2.13
5	4.21	3.57	1.32
6	2.34	1.99	0.90
7	1.40	1.49	0.55
8	0.48	0.61	0.38
9	0.17	0.33	0.22
Fructose	0.21	0.21	0.36
Total sugar	84.0	55.0	22.0
1,6-Anhydroglucose	2.79	1.45	0.24
5-HMF	1.31	1.56	0.07

**Table 1.** Product yields from cotton cellulose with aqueous formic acid and acetic acid solutions, together with water alone at 523 K and 60 min [27].

### 3.2. The presence of formic acid

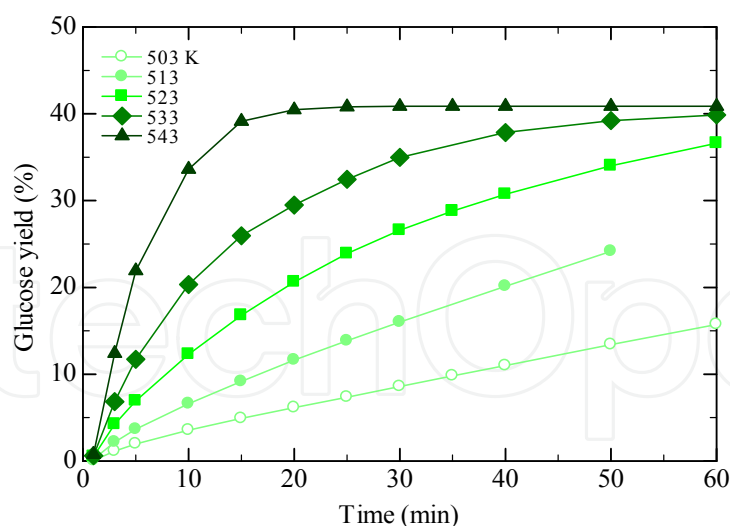
Product yields for the hydrolysis of cotton cellulose at 523 K for 60 min in 0.1 wt% aqueous formic acid, 0.13 wt% acetic acid solution, and water alone are listed in Table 1. The addition of the acids was effective for significantly increasing the yields of glucose and oligomers with lower degrees of polymerization. The yield of total sugar, defined as glucose, fructose and oligomers with a DP up to 9, reached 84.0 % with formic acid and 55.0 % with acetic acid, and significantly improved compared to the 22.0 % obtained in the absence of acid. Yields of fructose, which is believed to be generated via isomerization of glucose under hydrothermal conditions [28], are low. Yields of 1,6-anhydroglucose (levoglucosan), obtained from the dehydration of glucose, were slightly higher with formic acid than with acetic acid and water. 5-Hydroxymethylfurfural (5-HMF), a further undesirable decomposition product of glucose produced via dehydration [29] that acts as an inhibitor of the subsequent fermentation process [30,31], was found in 1 to 2 % yield with added acid, but only in a trace amount with water due to the lower conversion level. The results indicate that addition of formic acid is preferable to acetic acid because it provides higher yields of sugars and lower yields of decomposition products.

Figures 4 to 6 show yields of total sugar, glucose and cellobiose, respectively, over reaction time for CC in 0.1 wt% aqueous formic acid solution at 503 to 543 K and 10 MPa. The yields of the three components increased with increasing time and temperature. The maximum yield of 88 % for total sugar was attained after 20 min at the highest temperature (543 K). Above 523 K the total sugar yields seemed to reach almost 90 %, glucose and cellobiose yields did 40 % and 15 %, respectively, and both yields showed higher rates at higher reaction temperatures.

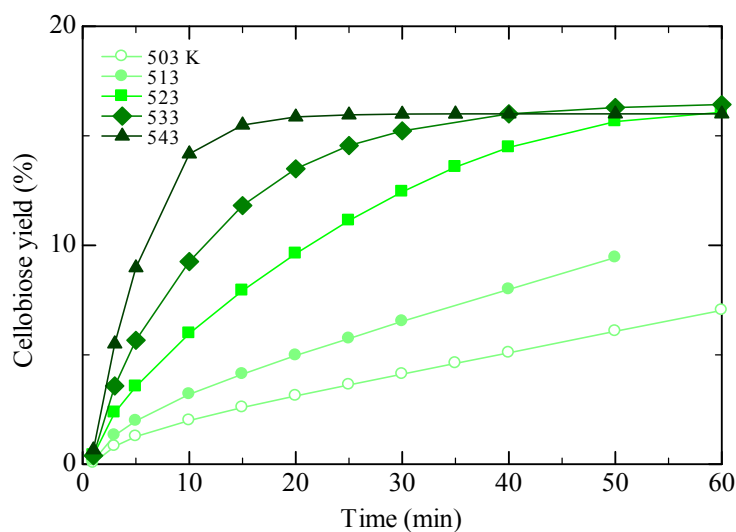


**Figure 4.** Total sugar yield over reaction time at temperatures from 503 to 543 K for cotton cellulose in 0.1 wt% aqueous formic acid solution [27].





**Figure 5.** Glucose yield over reaction time at temperatures from 503 to 543 K for cotton cellulose in 0.1 wt% aqueous formic acid solution [27].



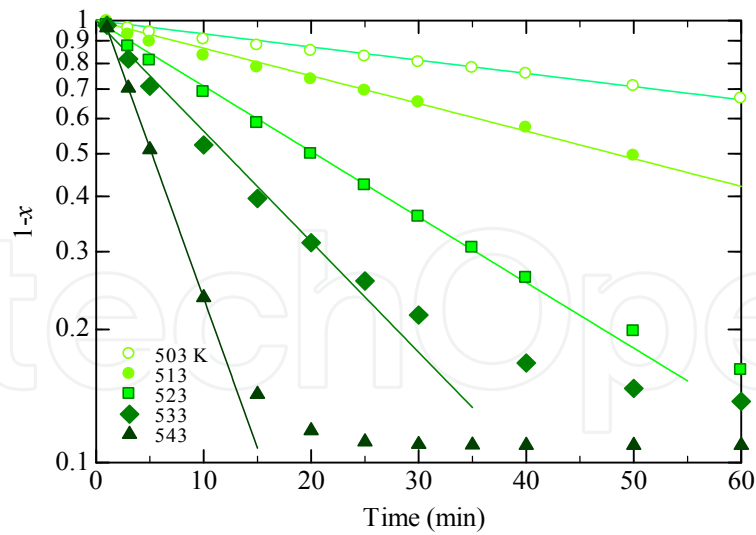
**Figure 6.** Cellobiose yield over reaction time at temperatures from 503 to 543 K for cotton cellulose in 0.1 wt% aqueous formic acid solution.

Figure 7 shows  $(1-x)$  over reaction time in the semi-logarithmic plot, where  $x$  is the conversion based on total sugar yield or glucose yield, and the data are the same as in Figs 4 and 5. As depicted, over the main conversion,  $(1-x)$  ranges higher than 0.2, the data based on total sugar yield and glucose (not shown in figure) were well represented by each straight line at each temperature, and those can be expressed by the first order reaction kinetics in eq(4).

$$\frac{dx}{dt} = k(1-x) \quad (4)$$

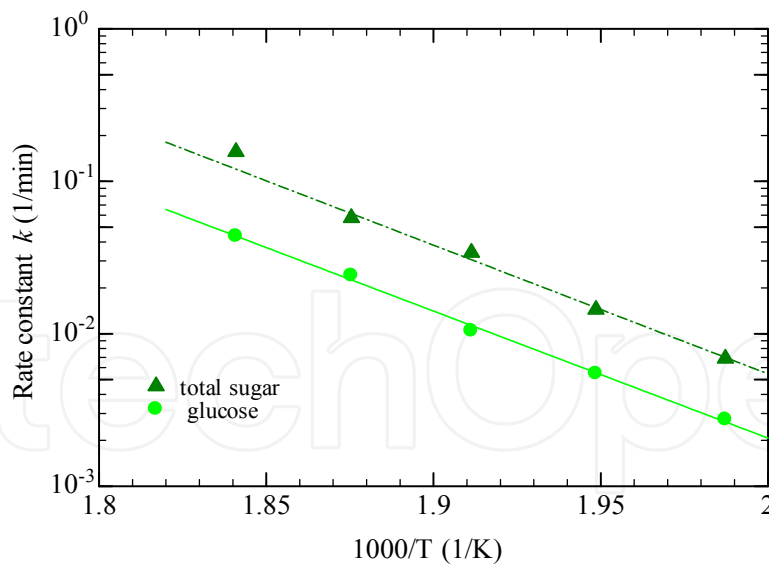
where  $x$  is the overall first order reaction rate constant.





**Figure 7.**  $(1-x)$  over reaction time for data shown in Figure 4.  $x$  is conversion based on total sugar yield [27].

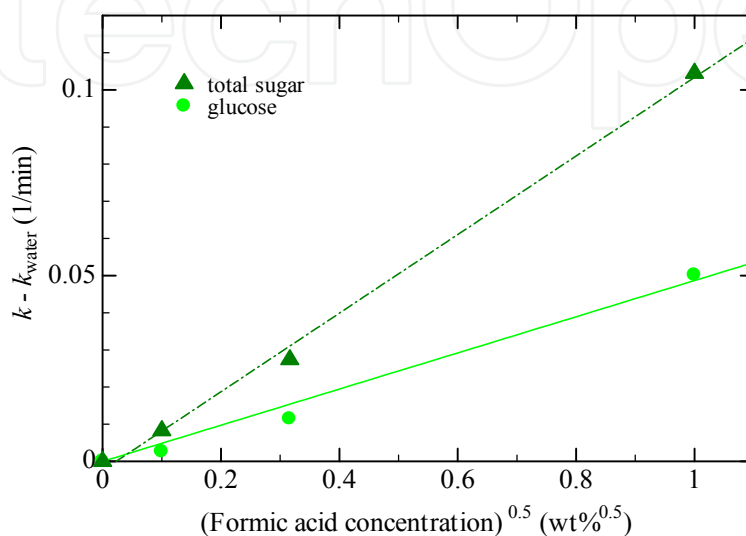
Figure 8 shows Arrhenius plots for first order rate constants for conversions based on yields of total sugar and glucose. The pre-exponential factor and the activation energy are  $4.157 \times 10^{14}$  1/min and 161.6 kJ/mol for total sugar, and  $9.656 \times 10^{13}$  1/min and 159.6 kJ/mol for glucose, respectively. The activation energies are almost the same, and the pre-exponential factors for total sugar were about three times higher than those for glucose.



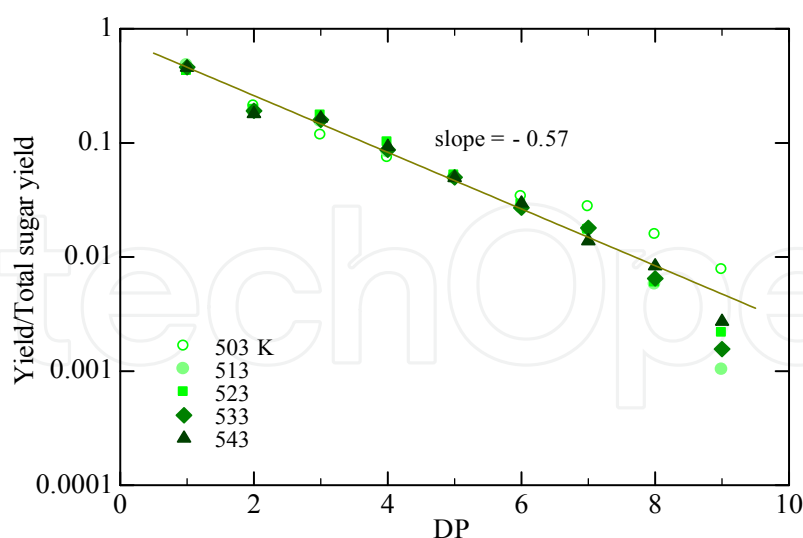
**Figure 8.** Arrhenius plots of rate constants  $k$  for conversions based on total sugar yield and glucose, respectively, in 0.1 wt% aqueous formic acid solution [27].

Figure 9 shows the differences in  $(k - k_{\text{water}})$  vs square root of formic acid concentration for total sugar and glucose. Since cellulose was hydrolytically decomposed under hydrothermal conditions without any additives, the contribution of formic acid on the rate may be expressed by the difference. Cellulose degradation reaction can be considered to be two

parallel reaction pathways: degradation in pure water with rate constant  $k_{\text{water}}$  and hydrolysis in an aqueous dilute acid solution with  $k$ . Up to formic acid concentration of 1 wt%, the maximum concentration studied, the difference rates ( $k - k_{\text{water}}$ ) for conversions based on both total sugar and glucose yields were proportional to the square root of the concentration. This relationship may result from the fact that the concentration of  $[\text{H}^+]$  is proportional to the square root of the acid concentration in a dilute solution.



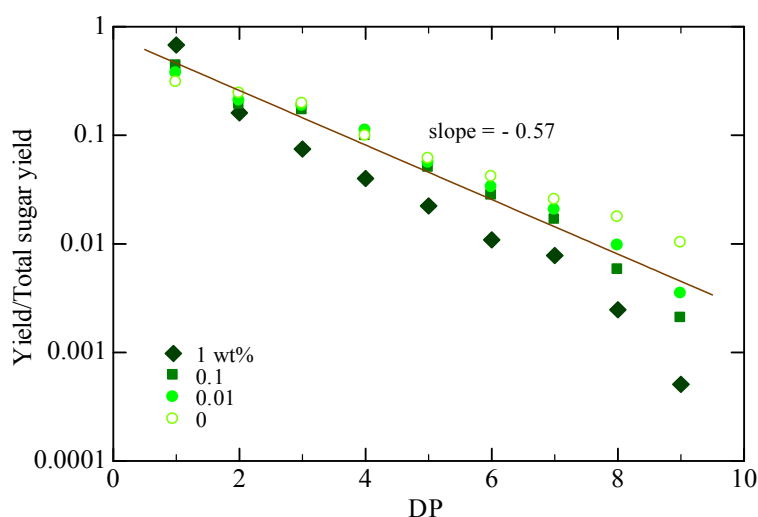
**Figure 9.** Rate constant difference ( $k - k_{\text{water}}$ ) vs. square root of formic acid concentration at 523 K for conversions based on glucose and total sugar, respectively [27].



**Figure 10.** Ratio of yield to total sugar yield over degree of polymerization of celooligosaccharides at various temperatures from 503 to 543 K for cotton cellulose in 0.1 wt% aqueous formic acid solution [27].

The yield ratio of product component to total sugar vs. degree of polymerization of celooligosaccharide at temperatures from 503 to 543 K for various reaction times in a 0.1

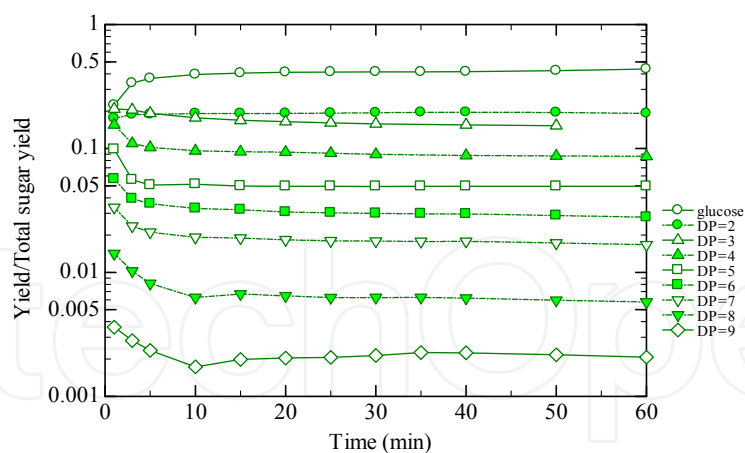
wt% aqueous formic acid solution can be seen in Figure 10. Yield ratios as a function of formic acid concentration (0 to 1 wt%) for reactions run at 523 K are also shown in Figure 11. It is interesting that in a semi-logarithmic plot, most of the data points at various reaction times are almost overlapped at each temperature and formic acid concentration, and those are represented by straight lines with a slope of  $-0.57$ . Only the ratios for oligomers with  $DP > 7$  at the lowest temperature of 503 K and at the highest acid concentration of 1 wt% at 523 K deviate from the line. The former could be due to lower conversion, whereas the latter may result from higher reaction rate at higher acid concentration. The fact that the ratios are expressed by a single straight line at various temperatures and concentrations, except under these two conditions, indicates that the hydrolytic depolymerization reaction could be controlled by the same reaction path or the same reaction stage in each case.



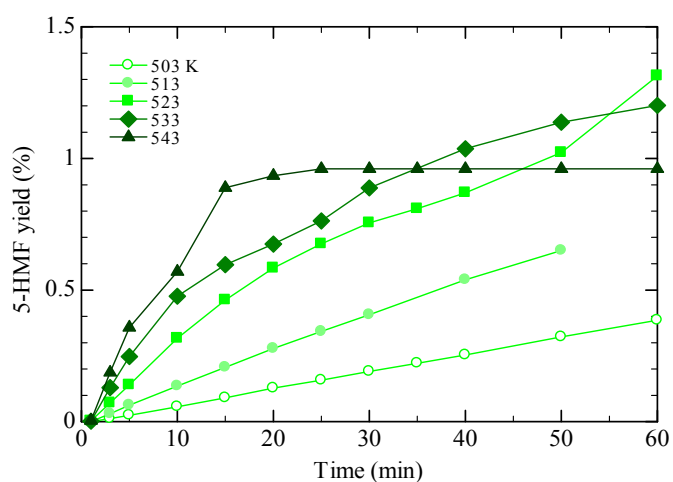
**Figure 11.** Ratio of yield to total sugar yield over degree of polymerization of monomer and oligomers at various acid concentrations at 523 K for cotton cellulose in aqueous formic acid solution [27].

The time change of the yield ratio of product components with a  $DP = 1$  to 9 is plotted in Figure 12. After the first 10 min, the yield ratios for components with different DPs are nearly independent of reaction time, indicating that the formation rate of each component is constant. Furthermore, the values decrease with DP, presumably because of the differing solubilities of the components.

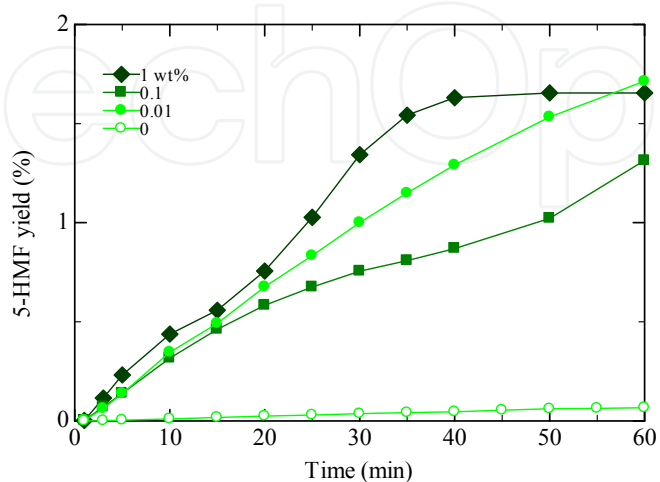
In Figures 13 and 14 the time changes of 5-hydroxymethylfurfural (5-HMF) yields are shown at temperatures from 503 to 543 K and formic acid concentrations from 0 to 1 wt%, respectively. Because 5-HMF can be produced from dehydration of the monosaccharide, it acts as an indicator of the further decomposition of the glucose produced in the reaction [6,7]. It is also undesirable because of its inhibitor effects on the proceeding fermentation process [30,31]. The yield of 5-HMF increased with increasing reaction time at most temperatures, but plateaued at 1 %, probably because of its further decomposition at the highest temperature. Acid concentration was also observed to have an effect on 5-HMF yield, which appeared to level off at 1.5 % at the highest acid concentration of 1 wt%.



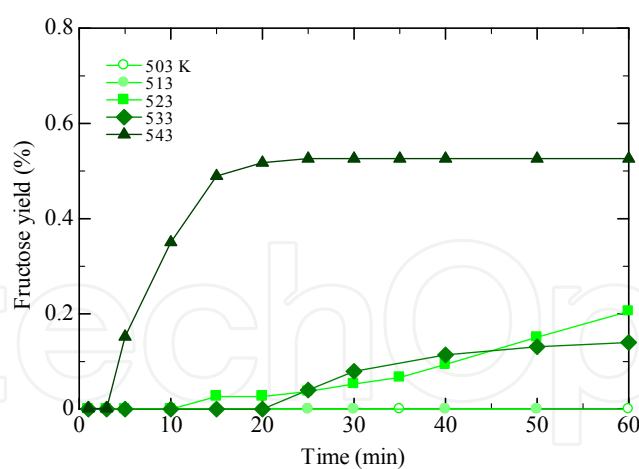
**Figure 12.** Ratio of each product yield to total sugar yield over reaction time for DP = 1 to 9 at 523 K in 0.1 wt% aqueous formic acid solution for cotton cellulose [27].



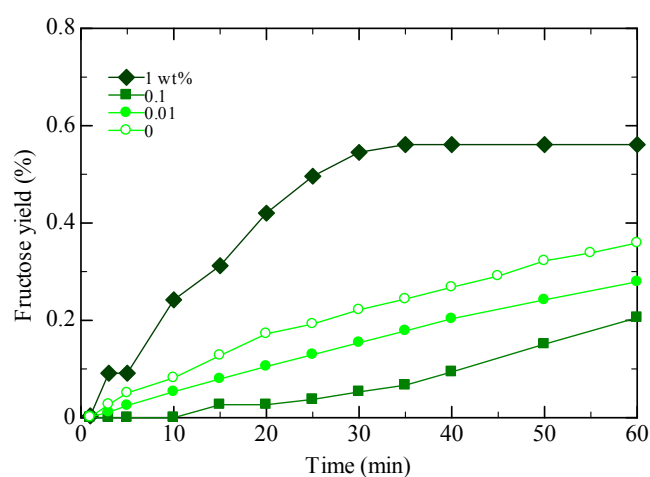
**Figure 13.** 5-HMF yield over reaction time at temperatures from 503 to 543 K in 0.1 wt% aqueous formic acid solution for cotton cellulose [27].



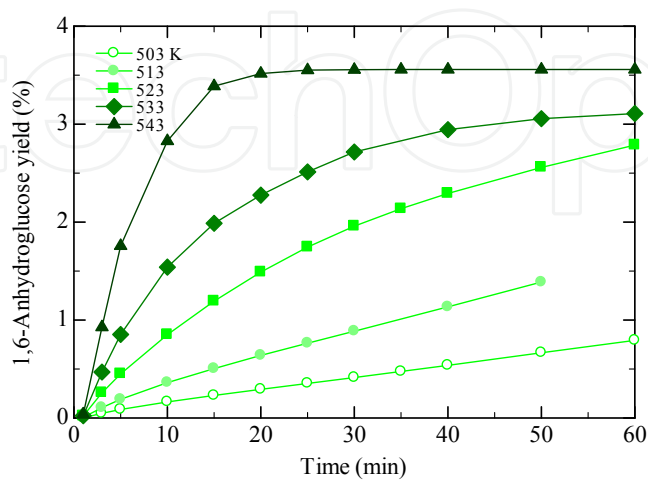
**Figure 14.** 5-Hydroxymethylfurfural yield over reaction time at 523 K and various formic acid concentrations up to 1 wt% for cotton cellulose [27].



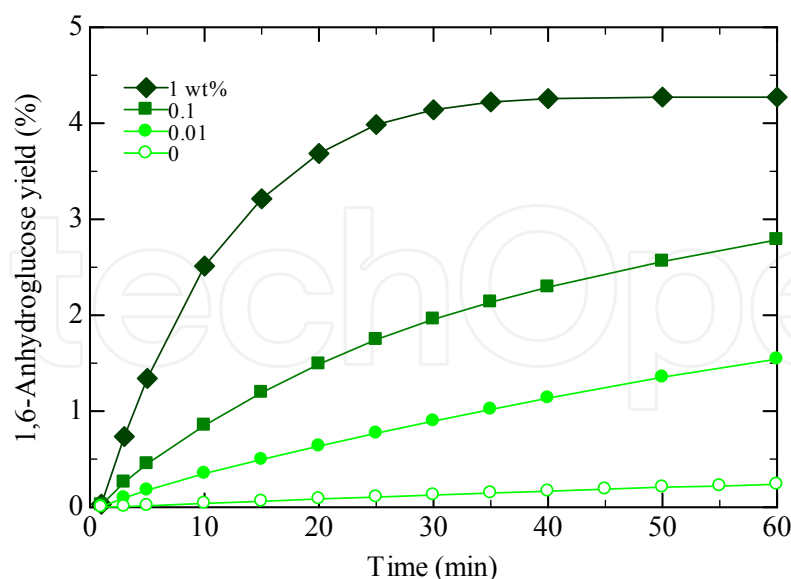
**Figure 15.** Fructose yield over reaction time at temperatures from 503 to 543 K in 0.1 wt% aqueous formic acid solution for cotton cellulose.



**Figure 16.** Fructose yield over reaction time at 523 K and various formic acid concentrations up to 1 wt% for cotton cellulose.



**Figure 17.** 1,6-Anhydroglucose yield over reaction time at temperatures from 503 to 543 K in 0.1 wt% aqueous formic acid solution for cotton cellulose.



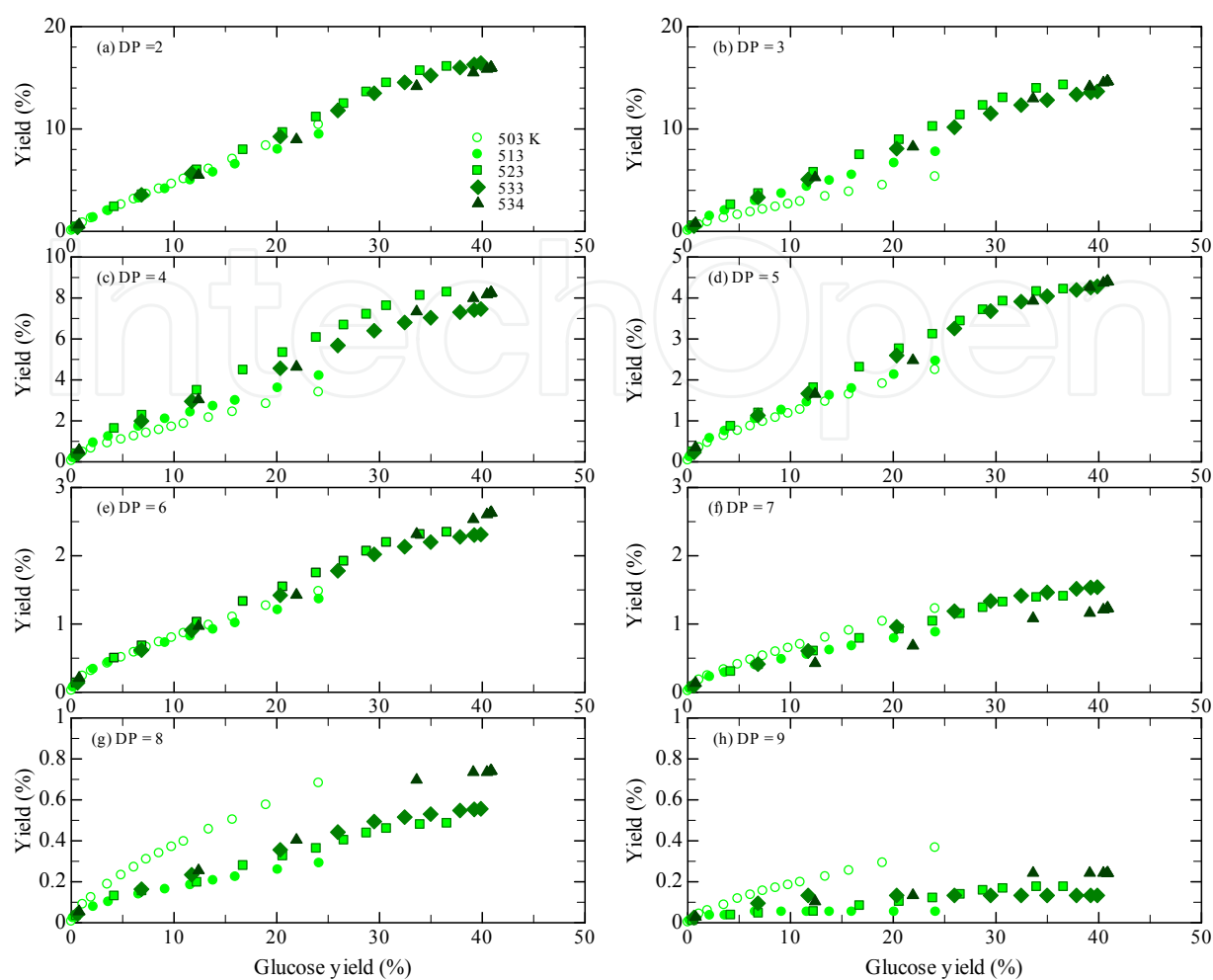
**Figure 18.** 1,6-Anhydroglucose yield over reaction time at 523 K and various formic acid concentrations up to 1 wt% for cotton cellulose.

Figures 15 and 16 show the effect of temperature at 0.1 wt% formic acid concentration and that of formic acid concentration at 523 K, respectively, on fructose yields over reaction time. Fructose yields were lower than 1 % at all conditions, and the yields did not increase with temperature and formic acid concentration. This may result from the formation via isomerisation, not direct hydrolysis of cellulose and/or its oligomers.

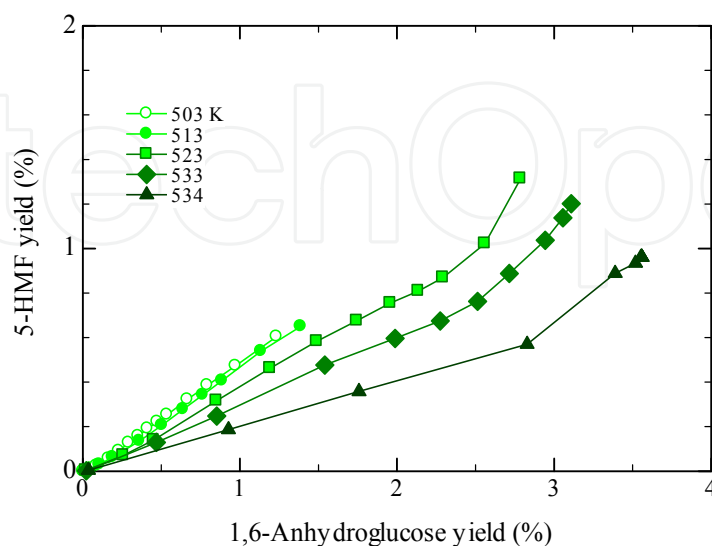
Figures 17 and 18 show the effects of temperature and formic acid concentration, respectively, on yield of 1,6-anhydroglucose, which is formed from dehydration of glucose. Differently from the time change in fructose yields, the yields at the highest temperature of 543 K and the highest formic acid concentration of 1 wt% simply increased with time, and then levelled off at each reaction condition. The yields also increased with temperature and formic acid concentration, as has been seen for glucose.

Figure 19 shows product yield vs. glucose yield at 503 to 543 K for various reaction times and 0.1 wt% formic acid concentration for oligomers with DP = 2 to 9. Although the yields of oligomers having different DPs were different, the yields were increasingly proportional to glucose yields, apparently, irrespective of reaction temperature and time. This may imply that the formation of each oligomer could be controlled by the same reaction step, not independently.

Figure 20 also shows cross yield plots of 5-hydroxymethylfurfural and 1,6-anhydroglucose. The yield of 5-HMF almost linearly increased with increasing 1,6-anhydroglucose yield except at higher yields of 1,6-anhydroglucose, and the slope was affected inversely by temperature. Both compounds were considered to be produced via dehydration reaction of glucose, and the yields of both compounds were dependent.



**Figure 19.** Oligomer yield vs. glucose yield at temperatures from 503 to 534 K for various reaction times at 0.1 wt% aqueous formic acid solution for cotton cellulose.



**Figure 20.** 5-HMF yield vs. 1,6-anhydroglucose yield at temperatures from 503 to 534 K for various reaction times at 0.1 wt% aqueous formic acid solution for cotton cellulose.



## 4. Conclusions

In a semi-batch reactor depolymerization of the three types of cellulosic samples was hydrothermally carried out at temperatures of 523 and 543 K and 10 MPa in pure water, and that of cotton cellulose at temperatures from 503 to 543 K and 10 MPa in a dilute aqueous formic acid solution at concentrations up to 1 wt%. The product yields and the rates were influenced by the cellulose types. The yields of major products such as glucose, fructose and oligomers having DPs up to 9, 5-HMF and 1,6-anhydroglucose were measured. The presence of a small amount of formic acid was significantly effective for increasing the yields and the reaction rates. The amount of unconverted material based on yields of glucose or total sugar (glucose, fructose and oligomers having DP up to 9) was represented by first-order reaction kinetics with nearly the same activation energies.

## Author details

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