Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 2365–2370
Carbohydrate RESEARCH

A kinetic model for production of glucose by hydrolysis of levoglucosan and cellobiosan from pyrolysis oil

Steve Helle,[a,*] Nicole M. Bennett,[b] Karen Lau,[b] Justin H. Matsui[b] and Sheldon J. B. Duff[b]

[a]*Environmental Science and Environmental Engineering, University of Northern British Columbia, 3333 University Way, Prince George, BC, Canada V2N 4Z9*
[b]*Department of Chemical and Biological Engineering, The University of British Columbia, 2360 East Mall, Vancouver, BC, Canada V6T 1Z3*

Received 16 April 2007; received in revised form 18 July 2007; accepted 24 July 2007
Available online 2 August 2007

Abstract—Anhydro sugars, produced during wood pyrolysis, can by hydrolyzed to sugars under acidic conditions. The acid hydrolysis of two common anhydro sugars in wood pyrolysis oils, levoglucosan (1,6-anhydro-β-D-glucopyranose) and cellobiosan (β-D-glucopyranosyl-(1→4)-1,6-anhydro-D-glucopyranose), was investigated. Levoglucosan hydrolysis to glucose follows a first-order reaction, with an activation energy of 114 kJ mol^{-1}. For cellobiosan hydrolysis, 44% of the cellobiosan is hydrolyzed initially via the β−(1→4) glycosidic bond to form levoglucosan and glucose. The remaining cellobiosan is hydrolyzed initially at the 1,6 anhydro bond to form cellobiose. Both reactions are first order with respect to cellobiosan, with an activation energy of 99 kJ mol^{-1}. The intermediate levoglucosan and cellobiose are hydrolyzed to glucose.

Keywords: Levoglucosan; Cellobiosan; Glucose; Hydrolysis; Pyrolysis oil

1. Introduction

Levoglucosan (1,6-anhydro-β-D-glucopyranose) and cellobiosan (β-D-glucopyranosyl-(1→4)-1,6-anhydro-D-glucopyranose or 1,6-anhydro-β-cellobiose) are among the organic compounds formed from the burning of biomass, along with the other derivatives from the breakdown of cellulose, aliphatic and oxygenated compounds, and terpenoids from vegetation biopolymers.[1] Levoglucosan and cellobiosan can be used as tracer compounds to determine smoke distribution.[2,3] Levoglucosan does not undergo acid-catalyzed hydrolysis under typical atmospheric conditions.[4]

Levoglucosan and cellobiosan are also present in biomass pyrolysis oils, which are produced from the degradation of biomass materials at high temperatures under nonoxidative conditions. Pyrolysis forms many products, broadly classified as char, oil, and gas.[5] The distribution of pyrolysis products depends on the reaction conditions, with maximum yields of pyrolysis oil obtained around 500 °C.[5] A major product in the pyrolysis oil is levoglucosan, formed from cellulose.[6,7] Under many pyrolysis conditions, a significant amount of cellobiosan is also produced, with yields as high as 30% of the levoglucosan yield.[8]

The pyrolysis of biomass provides an alternate method to acid or enzymatic hydrolysis to overcome one of the major challenges of producing renewable chemicals from biomass, namely the extraction and conversion of cellulose to glucose. The anhydro sugars formed during pyrolysis, for example, levoglucosan and cellobiosan, may be converted to sugar by dilute acid hydrolysis.[9,10] Previous work has shown that the addition of sulfuric acid to pyrolysis oil generated more glucose than could be accounted for by the amount of levoglucosan present.[9] While this extra glucose was attributed to unknown carbohydrate oilgomers such as cellobiosan and other sugars, little is known about the identity, relative proportions, and hydrolysis kinetics of these compounds.

* Corresponding author. E-mail: helle@unbc.ca

0008-6215/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2007.07.016

In this study, we have investigated the rates of levoglucosan and cellobiosan hydrolysis in water, sulfite pulp mill pulping liquor, and in pyrolysis oil extract. The effects of acid concentration and temperature on hydrolysis rates were measured. We present a kinetic model for the production of fermentable sugars from the acid hydrolysis of pyrolysis extracts.

2. Results and discussion

2.1. Levoglucosan hydrolysis kinetics

Levoglucosan hydrolysis is known to follow first-order kinetics.[10]

$$\text{Levoglucosan } (A) \xrightarrow[k_1]{} \text{Glucose } (D) \tag{1}$$

Assuming first-order kinetics, the rate of disappearance of levoglucosan and the rate of production of glucose are given in Eqs. 2 and 3,

$$\frac{dA}{dt} = -k_1 A \tag{2}$$

$$\frac{dD}{dt} = k_1 A \tag{3}$$

where A and D are the molar concentrations of levoglucosan and glucose, respectively, and k_1 is the first-order rate constant for levoglucosan hydrolysis. If the initial glucose and levoglucosan concentrations are zero and A_0, respectively, then Eqs. 1 and 2 integrate to the following:

$$\frac{A}{A_0} = e^{-k_1 t} \tag{4}$$

$$\frac{D}{A_0} = (1 - e^{-k_1 t}) \tag{5}$$

Sample data for levoglucosan hydrolysis in water are presented in Figures 1 and 2. Figure 1 demonstrates the effect of temperature on the hydrolysis rate of levoglucosan in dilute sulfuric acid, with the acid concentra-

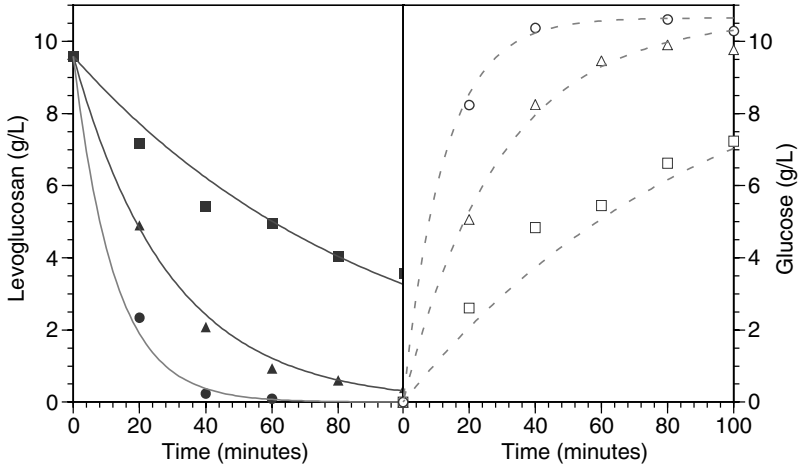

Figure 1. Effect of temperature on levoglucosan kinetics. Hydrolysis at 90 °C (■), 100 °C (▲), and 110 °C (●). All hydrolyses at 500 mM sulfuric acid.

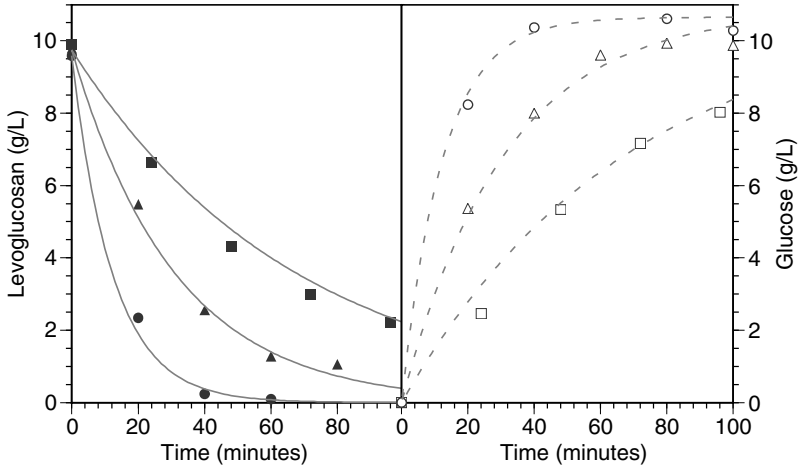

Figure 2. Effect of sulfuric acid concentration on levoglucosan kinetics. Hydrolysis at 120 mM H_2SO_4 (■), 250 mM H_2SO_4 (▲), and 500 mM H_2SO_4 (●). All hydrolyses at 110 °C.

tion held constant. For each hydrolysis condition, the first-order rate constant was determined by simultaneous nonlinear regression of the levoglucosan and glucose concentration data using Eqs. 4 and 5. The resulting first-order model curve fits are shown in Figures 1 and 2.

Increasing the temperature by 10 °C increments resulted in close to twofold increases in the hydrolysis rate (Fig. 1). Over the temperature range studied, the reaction rate increased exponentially with increasing temperature, following an Arrhenius relationship. Increasing the sulfuric acid concentration also increased the levoglucosan hydrolysis rate. For example, with the temperature held constant at 110 °C, increasing the acid concentration from 250 mM to 500 mM had approximately the same effect as increasing the temperature from 100 °C to 110 °C with sulfuric acid concentration held constant at 500 mM. Increasing the acid concentration from 110 mM to 500 mM had approximately the same effect as increasing the temperature from 90 °C to 110 °C. Over the ranges tested, a 10 °C change in temperature had approximately the same effect as a doubling (or halving) of the acid concentration. The following relationship was used to relate the rate constant, k_1 (s^{-1}), to temperature and acid concentration:

$$k_1 = [H_2SO_4]^n A e^{-E_1/RT} \quad (6)$$

where $[H_2SO_4]$ is the acid concentration in mmol L^{-1}, n and A are constants, R is the universal gas constant (8.314 J mol^{-1} K^{-1}), E_1 is the activation energy (in J mol^{-1}), and T is the temperature (in Kelvin). For levoglucosan hydrolysis in dilute sulfuric acid, the activation energy was 114 kJ mol^{-1} and A was 1.0×10^{10}. When the hydrolyses were carried out in aqueous matrices, the hydrolysis rate increased approximately linearly with increasing acid concentration ($n = 1$). Slight deviations from linearity were observed for hydrolysis in SSL and in SSL/extract. The rate constants for hydrolysis at 500 and 250 mM H_2SO_4 are presented in Figure 3, along with the Arrhenius model (Eq. 6). The activation energy and the exponent n obtained in this study were similar to the values of 97 kJ mol^{-1} and 1 found using hydrochloric acid and lower temperatures (25–50 °C).[10]

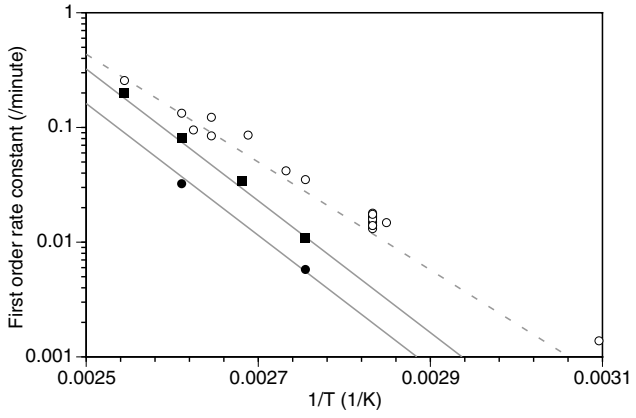

Figure 3. First-order levoglucosan hydrolysis rate constant at different sulfuric acid concentrations, and in different media. (●) 250 mM H_2SO_4 in water; (■) 500 mM H_2SO_4 in water; (○) 500 mM H_2SO_4 in SSL and/or pyrolysis oil extract.

First-order rate constants were calculated from a number of levoglucosan hydrolysis experiments performed in spent sulfite pulping liquor, water extract of pyrolysis oil, and spent sulfite pulping liquor extract of pyrolysis oil, with and without acid addition. Spent sulfite liquor contains the hemicellulose sugars and the lignin that are removed from the wood during the sulfite pulping process. Several sulfite pulp mills ferment the spent sulfite liquor to produce ethanol, although the process is limited by the low concentration of sugars. Spent sulfite liquor was used to extract levoglucosan from pyrolysis oil with the goal of increasing the sugar concentration in the pulping liquor prior to fermentation. When spent sulfite liquor (or water) is mixed with pyrolysis oil, the levoglucosan and other water-soluble compounds in the pyrolysis oil transfer to the spent sulfite liquor (or water), leaving a residue of the hydrophobic lignin compounds. For a given sulfuric acid concentration, the levoglucosan hydrolysis rate was greater in spent sulfite liquor (pure levoglucosan added to spent sulfite liquor), spent sulfite liquor pyrolysis oil extract, or water pyrolysis oil extract than the levoglucosan hydrolysis rate in dilute sulfuric acid. For hydrolysis in spent sulfite liquor or pyrolysis oil extract, the activation energy was found to be 87 kJ mol^{-1}, which is significantly lower than the activation energy for hydrolysis in dilute sulfuric acid. In addition, acid concentration had less of an effect on the hydrolysis rate in pyrolysis oil extract compared to hydrolysis in water (n was 0.67 for hydrolysis in pyrolysis oil extract compared to 1 for hydrolysis in water). The parameter A was 3.3×10^6. The difference between hydrolysis in sulfuric acid/distilled water and sulfuric acid/(pyrolysis oil extract or spent sulfite liquor) was greatest at low temperatures and high acid concentrations.

2.2. Cellobiose hydrolysis kinetics

Cellobiose is not normally listed as a component of pyrolysis oils, but it is a major intermediate for cellobiosan hydrolysis (see below). Cellobiose hydrolysis follows a first-order reaction similar to levoglucosan hydrolysis:[11]

$$\text{Cellobiose } (C) \xrightarrow[k_2]{} \text{Glucose } (D) \quad (7)$$

$$\frac{dC}{dt} = -k_2 C \quad (8)$$

$$\frac{dD}{dt} = 2k_2 C \quad (9)$$

where C is the cellobiose concentration (mol L^{-1}) and k_2 is the first-order cellobiose hydrolysis rate constant. Integrating from an initial cellobiose concentration of C_0 and an initial glucose concentration of zero yields the following:

$$\frac{C}{C_0} = e^{-k_2 t} \quad (10)$$

$$\frac{D}{C_0} = 2(1 - e^{-k_2 t}) \quad (11)$$

These equations were found to describe cellobiose hydrolysis well. At a given acid concentration and temperature, cellobiose hydrolysis was slower than levoglucosan hydrolysis. For example, at 110 °C and 500 mM sulfuric acid, k_1 was 0.00135 s^{-1}, while k_2 was 0.00062 s^{-1}. Sample data are presented in Figure 5b. The activation energy for cellobiose hydrolysis was found to be 103 kJ mol^{-1}, similar to the previously reported value of 110 kJ mol^{-1}.[11]

2.3. Cellobiosan hydrolysis kinetics

As measured by ion chromatography using a pulsed ampirometric detector, cellobiosan hydrolysis products included cellobiose, levoglucosan, and glucose. The mass balance closes within the error of analysis, indicating that there are no other major products formed. The proposed reaction scheme is presented in Figure 4. The initial hydrolysis reaction may be either the hydrolysis of the β-(1→4) glycosidic bond, producing levoglucosan and glucose, or the hydrolysis of the 1,6-anhydro bond to produce cellobiose. Cellobiose and levoglucosan are subsequently hydrolyzed to glucose (Eqs. 1 and 7).

With each hydrolysis step modeled as a first-order reaction, the rate equations developed from the pathway shown in Figure 4 are as follows:

$$\frac{dA}{dt} = -k_1 A + k_4 B \quad (12)$$

$$\frac{dB}{dt} = -k_3 B - k_4 B = -(k_3 + k_4)B = -k_5 B \quad (13)$$

$$\frac{dC}{dt} = k_3 B - k_2 C \quad (14)$$

$$\frac{dD}{dt} = k_1 A + k_4 B + 2k_2 C \quad (15)$$

where B is the cellobiosan concentration (mol L^{-1}), k_3 is the rate coefficient for cellobiosan hydrolysis to cellobiose and k_4 is the rate coefficient for cellobiosan hydrolysis to glucose and levoglucosan. The first-order rate coefficient for the removal of cellobiosan (k_5) is equal to $k_3 + k_4$. For the case with initial cellobiosan equal to B_0, and zero initial levoglucosan, cellobiose, and glucose, Eqs. 12–15 may be integrated to yield the following:

$$\frac{A}{B_0} = \frac{k_4}{k_3 + k_4 - k_1}\left(e^{-k_1 t} - e^{(-k_3 - k_4)t}\right) \quad (16)$$

$$\frac{B}{B_0} = e^{(-k_3 - k_4)t} \quad (17)$$

$$\frac{C}{B_0} = \frac{k_3}{k_3 + k_4 - k_2}\left(e^{-k_2 t} - e^{(-k_3 - k_4)t}\right) \quad (18)$$

$$\frac{D}{B_0} = -\frac{(k_4 - k_2)(k_4 - 2k_1) + k_3(k_4 - 2k_2)}{(k_3 + k_4 - k_1)(k_3 + k_4 - k_2)} e^{(-k_3 - k_4)t} - \frac{k_4}{k_3 + k_4 - k_1} e^{-k_1 t} - \frac{2k_3}{k_3 + k_4 - k_2} e^{-k_2 t} + 2 \quad (19)$$

Sample hydrolysis data for cellobiosan are presented in Figure 5c, along with hydrolysis of levoglucosan (Fig. 5a) and cellobiose (Fig. 5b) under the same conditions. For determining the model parameters, k_5 was calculated from the change in cellobiosan concentration with time, then k_3 and k_4 were calculated by simultaneous nonlinear regression of Eqs. 16–19 to the cellobiosan, glucose, levoglucosan, and cellobiose data using previously determined values of k_1 and k_2.

For the hydrolysis experiment presented in Figure 5, $k_1 = 0.000539$ s^{-1}, $k_2 = 0.000218$ s^{-1}, $k_3 = 0.000183$ s^{-1}, $k_4 = 0.000158$ s^{-1}, and $k_5 = 0.000342$ s^{-1}. The levoglucosan hydrolysis rate was two-and-a-half times greater than the cellobiose hydrolysis rate and one-and-a-half times greater than the cellobiosan hydrolysis rate. For cellobiosan, hydrolysis to cellobiose proceeded at a greater rate than hydrolysis to levoglucosan and glucose. The greater rate of cellobiose formation, coupled with the slower cellobiose hydrolysis reaction, resulted in a greater accumulation of cellobiose than levoglucosan. The amount of cellobiosan hydrolyzed through cellobiose can be calculated from the ratio of k_3 to k_4, which is equal to 1.2. This

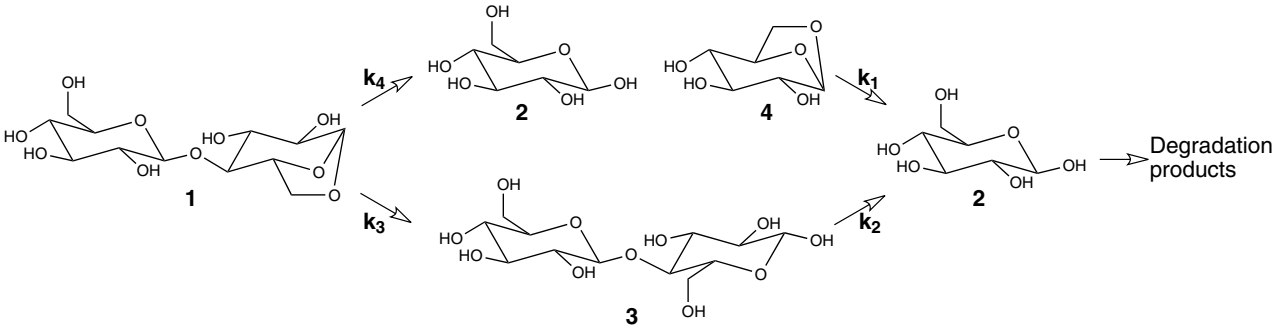

Figure 4. Cellobiosan (**1**) hydrolysis scheme. Cellobiose (**2**), glucose (**3**), and levoglucosan (**4**) are formed by acid hydrolysis.

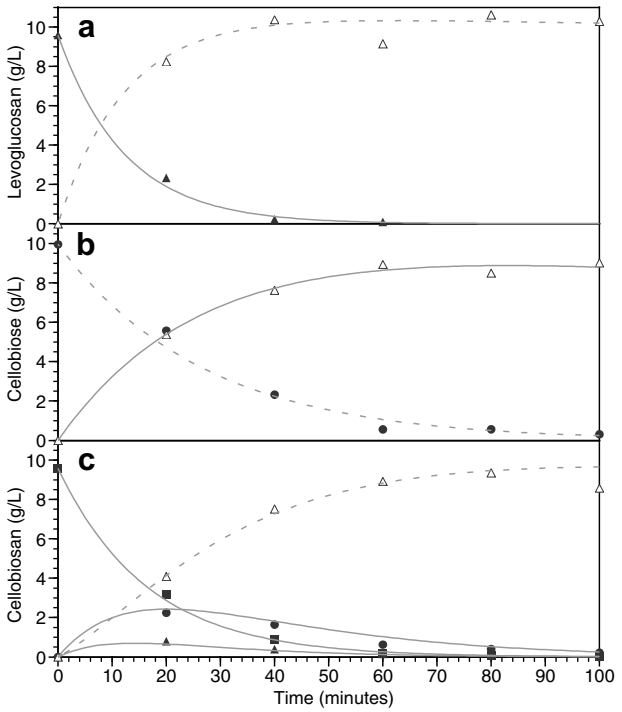

Figure 5. (a) Levoglucosan hydrolysis. Lines represent Eqs. 4 and 5. (b) Cellobiose hydrolysis. Lines represent Eqs. 10 and 11. (c) Cellobiosan hydrolysis. Lines represent Eqs. 16–19. $T = 110\ ^\circ C$, $[H_2SO_4] = 250$ mM.

indicates that 1.2 mol of cellobiose are formed for every mole of levoglucosan formed, or, 54% of the cellobiosan is hydrolyzed via cellobiose, and 46% via levoglucosan.

Eq. 6 was used to determine the relationship between the first-order rate constants and the temperature and acid concentration. The activation energies and the exponent n for k_1, k_2, k_3, and k_4 are listed in Table 1. The activation energies for k_2, k_3, and k_4 are similar, and lower than the activation energy for k_1. Acid concentration had a greater effect on k_2, k_3, and k_4 compared to k_1. On average the levoglucosan hydrolysis rate was 55% greater than the cellobiosan hydrolysis rate, and 140% greater than the cellobiose hydrolysis rate. The ratio of cellobiosan hydrolyzed via cellobiose remained constant at approximately 55% for all of the hydrolysis conditions tested.

Cellobiosan hydrolysis data, collected over a range of acid concentrations (from 110 to 750 mM sulfuric acid) and temperatures (from 80 to 120 °C), were normalized using Eqs. 20 and 21.

Table 1. Kinetic coefficients for cellobiosan hydrolysis

	E (kJ mol^{-1})	n
k_1	114	1.0
k_2	103	1.35
k_3	98	1.30
k_4	99	1.26

$$\text{Dimensionless time: } \tau = \frac{t}{1/(k_3 + k_4)} \tag{20}$$

$$\text{Dimensionless concentrations: } a = \frac{A}{B_0}; \quad b = \frac{B}{B_0}; \quad c = \frac{C}{B_0}; \quad d = \frac{D}{B_0} \tag{21}$$

As demonstrated in Figure 6, the hydrolysis pattern was similar over a range of hydrolysis conditions. This combined data set can be used to obtain an average activation energy and exponent for the effect of acid concentration. Using the combined data set, the activation energy for cellobiosan hydrolysis is 99 kJ mol^{-1}, which is similar to the activation energy obtained for k_2, k_3, and k_4. Levoglucosan hydrolysis will not have a great impact on the analysis due to its low concentrations compared to the other compounds. When using the entire data set, the effect of acid concentration was smaller, with $n = 1.1$, compared to a value of 1.3 for cellobiosan hydrolysis.

Acid hydrolysis of pyrolysis oil extracts often results in a greater than theoretical glucose yield based on initial levoglucosan.[9] The work presented here has several implications. First, for a given acid concentration, hydrolysis rates for anhydro sugars are significantly faster in pyrolysis oil extract and pulp mill wastewater compared to the rates in dilute acid. Secondly, this work has shown that other anhydro sugars that are typically present in pyrolysis oils also hydrolyze to glucose under typical dilute acid conditions, although at a slower rate compared to levoglucosan. If acid hydrolysis conditions are chosen based only on the levoglucosan concentration in the pyrolysis oil extract, the sugar yield will be lower than otherwise possible. Thirdly, the effect of pyrolysis conditions on the generation of cellobiosan and other oligomers should be studied if the end goal is to maximize the production of fermentable glucose from bio-oil.

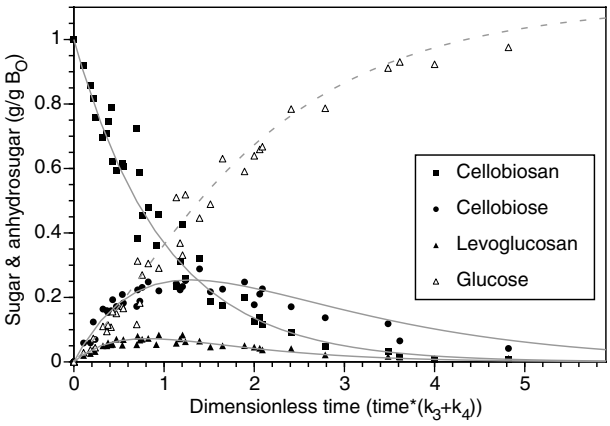

Figure 6. Normalized cellobiosan hydrolysis data for $[H_2SO_4] = 110$–750 mM and $T = 80$–120 °C.

3. Experimental

Levoglucosan was obtained from Sigma–Aldrich. Cellobiosan was from Sussex Research Laboratories, Inc. (Ottawa, Ontario). Pyrolysis oil was from VTT Processes, prepared from Scots Pine feedstock in August 2004. The pyrolysis oil was stored at a low pH (pH $\sim$ 2.5, as received) in glass containers at 4 °C with no light exposure. Softwood spent sulfite pulping liquor (SSL) was obtained from Tembec Inc., Temiscaming, Quebec. SSL (240 g/L solids) was shipped from Tembec Inc., Temiscaming Qc., and stored at a low pH (pH $\sim$ 2, as collected) at 4 °C.

The hydrolysis experiments were conducted in sealed glass test tubes placed in heating blocks. For levoglucosan, cellobiosan, and cellobiose hydrolysis, solutions were prepared in distilled water or spent sulfite liquor. Hydrolysis experiments were also performed using pyrolysis oil extract, prepared by adding pyrolysis oil to water, mixing vigorously for 5 min, then centrifuging to separate the aqueous extract from the residue. The solutions to be hydrolyzed were preheated prior to the addition of 72% sulfuric acid. After a set amount of time (5–240 min), the test tubes were removed from the heating block and placed in cold water to stop the reaction. A range of acid concentrations (0–0.5 M H_2SO_4) and temperatures (50–130 °C) were used. A minimum of five test tubes were used for each hydrolysis condition (acid concentration and temperature). All additions were measured using an analytical balance.

For experiments in water, levoglucosan, glucose, cellobiose, and cellobiosan were measured by ion chromatography, with detection by a pulsed amperometric detector. A Carbopac PA1 column was used, with a Dionex HPLC system equipped with an AS 50 auto-Sampler, GP50 gradient pump and ED50A electrochemical detector. The mobile phase was distilled and deioinized water (Milli-Q Ultra-pure water purification system, Millipore, MA, USA). Sodium hydroxide was added postcolumn to maintain a pH of 10.4 in the detector. The injection volume of the sample was 25 μL and the column temperature was a constant 35 °C. The levoglucosan calibration curve was nonlinear, and a minimum of four standards were run spanning the concentration range used. The other calibration curves (glucose, cellobiose, and cellobiosan) were linear. For samples that contained pyrolysis oil or spent sulfite liquor, levoglucosan, and glucose were measured as O-peracetylated aldononitrile derivatives by gas chromatography.[12]

References

1. Gao, S.; Hegg, D. A.; Hobbs, P. V.; Kirchstetter, T. W.; Magi, B. I.; Sadilek, M. *J. Geophys. Res.* **2003**, *108*, 8491–8506.
2. Simoneit, B. R. T.; Schauer, J. J.; Nolte, C. G.; Oros, D. R.; Elias, V. O.; Fraser, M. P.; Rogge, W. F.; Cass, G. R. *Atmos. Environ.* **1999**, *33*, 173–182.
3. Decesari, S., 1; Fuzzi, S.; Facchini, M. C.; Mircea, M.; Emblico, L.; Cavalli, F.; Maenhaut, W.; Chi, X.; Schkolnik, G.; Falkovich, A.; Rudich, Y.; Claeys, M.; Pashynska, V.; Vas, G.; Kourtchev, I.; Vermeylen, R.; Hoffer, A.; Andreae, M. O.; Tagliavini, E.; Moretti, F.; Artaxo, P. *Atmos. Chem. Phys.* **2006**, *6*, 375–402.
4. Fraser, M. P.; Lakshmanan, K. *Environ. Sci. Technol.* **2000**, *34*, 4560–4564.
5. Bridgwater, T. *J. Sci. Food Agric.* **2006**, *86*, 1755–1768.
6. Bridgwater, A. V.; Czernik, S.; Piskorz, J. In *Progress in Thermochemical Biomass Conversion*; Bridgwater, A. V., Ed.; Blackwell Science, Ltd: Oxford, UK, 2001; pp 977–997.
7. Li, L.; Zhang, H. *Energy Sources* **2004**, *26*, 1053–1059.
8. Piskorz, J.; Majerski, P.; Radlein, D.; Vladars-Usas, A.; Scott, D. S. *J. Anal. Appl. Pyrolysis* **2000**, *56*, 145–166.
9. Yu, Z.; Zhang, H. *Bioresour. Technol.* **2003**, *90*, 95–100.
10. Vidrio, E. *McNair Scholars J* **2004**, *5*, 90–103.
11. Mosier, N. S.; Ladisch, C. M.; Ladisch, M. R. *Biotechnol. Bioeng.* **2002**, *79*, 610–618.
12. Helle, S. S.; Murray, A.; Lam, J.; Cameron, D. R.; Duff, S. J. B. *Bioresour. Technol.* **2004**, *92*, 163–171.