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Supporting Information  
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## **Soluble cello-oligosaccharides produced by carbon catalyzed hydrolysis of cellulose**

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## Experimental Methods

### Materials

Microcrystalline cellulose (Avicell PH-101) was purchased from Sigma Aldrich. Activated carbon (denoted AC) was supplied from Ajinomoto Fine-Techno. Distilled water and ethanol (99.5%) were purchased from Wako Pure Chemical Industries. Granular SiO<sub>2</sub> (Q30, 100 – 200 mesh) was supplied from Fuji Silysia Chemical Ltd. Cellotriose (95%), cellotetraose (95%), cellopentaose (95%) and cellohexasose (94%) were purchased from Megazyme. Glucose and cellobiose were purchased from Kanto Chemical Industries. D<sub>2</sub>O and DMSO-d<sub>6</sub> for proton nuclear magnetic resonance (<sup>1</sup>H NMR) analysis was obtained from Sigma Aldrich.

### Air-oxidation and characterization of carbon catalyst

For the preparation of carbon catalyst, activated carbon AC (4.0 g) was spread on a Pyrex dish of diameter 130 mm with a uniform thickness. The sample was then heated in an electric furnace under air with the following program: heating from 298 K to 393 K at a rate of 10 K min<sup>-1</sup> and then maintaining at 393 K for 2 h to remove the physisorbed water. Followed by heating to 698 K at a rate of 4 K min<sup>-1</sup> and then maintaining at 698 K for a further 10 h. The obtained oxidized carbon catalyst was denoted as AC-Air (1.9 g, yield: 47.5%). Specific surface area of carbon catalyst was determined by N<sub>2</sub> adsorption-desorption measurement (BEL Japan, BELSORP-min) after vacuum drying at 393 K for 3 h. Fourier transform infrared spectroscopy (FT-IR) spectra of carbon catalyst was measured by making a KBr pellet containing 0.1 wt. % of sample, followed by using a PerkinElmer Spectrum 100 FT-IR Spectrometer.

### Ball-milling

Mix-milling: 5.0 g substrate (microcrystalline cellulose, paper or eucalyptus) and 0.77 g AC-Air were milled together in an alumina pot with 200 g alumina balls of 5 mm diameter at 500 rpm in a Fritsch P-6 planetary ball mill. One milling cycle included 10 minutes of milling followed by 10 minutes of pause to allow dissipation of heat. Unless explicitly mentioned, all samples were milled for 6 cycles to give a total milling time of 1 h. After milling, the mixture was separated by sieving, followed by drying under vacuum at 393 K for 3 h. Mix-milled cellulose is denoted as MMC (S/C = 6.5). Single milling: 5.0 g microcrystalline cellulose was milled under same condition for 1 h without the addition of carbon catalyst.

### Hydrolysis experiment

Hydrolysis experiments were done in a semi-flow reactor shown in Figure S2. The reactor was made from stainless steel tube with length of 52 mm and diameter of 40 mm, and it was housed in a heater. A piston sensing back-pressure regulator was used to maintain the system pressure at 3 MPa. For a typical reaction, a mixture of substrate (MMC or ball-milled cellulose) and Granular SiO<sub>2</sub> (Q30, 100 - 200 mesh) was prepared by physically mixing 0.175 g substrate and 0.175 g SiO<sub>2</sub> (0.553 g and 0.952 g MMC in the case of space velocity (SV) of 20 h<sup>-1</sup> and 13 h<sup>-1</sup>, respectively, to reduce the pressure drop across the reactor. Then the mixture was packed into the reactor and attached to the semi-flow system. The setup was pressurized and purged with water (0.75 mL min<sup>-1</sup>) at room temperature for 10 min. After purging, heating was started, and the reactor was allowed to reach the desired temperature in about 20 min. The reaction was continued for another 20 min at this temperature and the product was collected for the whole 40 min reaction. The product was filtered with a 0.2 micron PTFE filter paper before analysis. Oligosaccharides yield was calculated as follows (equation S1):

$$\text{Yield of oligosaccharides (\%)} = \frac{\text{Moles of carbon in oligosaccharides}}{\text{Moles of carbon in cellulose}} \times 100 \quad (\text{S1})$$

### Precipitation of cello-oligosaccharides

The product solution was added dropwise to a beaker containing twice the volume of ethanol. This mixture was stirred for 1 h and then allowed to stand for another 2 h. The precipitate was recovered by centrifugation and washed with 5 ml of pure ethanol, followed by drying under ambient condition for 24 h and then vacuum drying for further 4 h at room temperature.

### Characterization of cello-oligosaccharide mixture

Freshly obtained product solution was analyzed using high performance liquid chromatography (HPLC) systems equipped with a refractive index detector (Shimadzu LC 10-ATVP). A Shodex Sugar SH-1011 column (ø 8 × 300mm; eluent, water at 0.5 mL min<sup>-1</sup>; 323 K), a Phenomenex Rezex RPM-Monosaccharide Pb++ column (ø 7.8 × 300 mm, eluent: water at 0.6 mL min<sup>-1</sup>, 343 K) and a Shodex Asahipak NH 2P-40E 30 column (ø 3 × 250 mm; eluent, water/acetonitrile (2:3 by vol.) at 0.3 mL min<sup>-1</sup>; 318 K) were used for the detection of cello-oligosaccharides. Matrix assisted laser desorption / ionization – time of flight mass spectroscopy (MALDI-TOF MS) analysis of the freshly obtained product solution was performed using a Bruker Dal-tonics Autoflex-III smartbeam instrument. Sample was prepared by placing a 1 µL mixture of cello-oligosaccharides solution and matrix (2,5-dihydroxybenzoic acid solution) onto the MTP 384 Massive Target T plate followed by drying. FT-IR spectra of precipitated oligosaccharides and cellulose was measured by making a KBr pellet containing 0.1 wt. % of sample. The pellet was analyzed by using a PerkinElmer Spectrum 100 FT-IR Spectrometer. <sup>1</sup>H NMR measurements were performed using a JEOL ECX-600, <sup>1</sup>H 600 MHz instrument. NMR sample was prepared by dissolving 5 mg of precipitated sample in 1.1 mL of DMSO-d<sub>6</sub> containing 0.1 mg of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) and sonicating for 20 minutes.

### Separation and characterization of individual cello-oligosaccharides

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The separation of individual cello-oligosaccharides from the product solution was performed by using an HPLC equipped with a fraction collector (Shimadzu). Separation of cello-oligosaccharides up to  $G_6$  was obtained by using three Shodex OHpak SB-802.5 HQ columns in series ( $\varnothing$  8 × 300mm; eluent, water at 0.5 mL min<sup>-1</sup>; 328 K). The separated cello-oligosaccharides solutions were evaporated to remove water followed by addition of 1.1 mL of D<sub>2</sub>O containing 0.1 mg of DSS and measuring the <sup>1</sup>H NMR spectrum in a JEOL ECX-600, <sup>1</sup>H 600 MHz instrument.

### Supplementary Text

#### HPLC analysis of cellulose hydrolysis product

Cello-oligosaccharides were obtained by hydrolyzing cellulose adsorbed on carbon catalyst in the semi-flow reactor that extracted the soluble products as they were formed. HPLC analysis of a typical products solution using a Shodex Asahipak NH-2P-40 3E column showed distinct peaks of glucose, cellobiose ( $G_2$ ), cellotriose ( $G_3$ ), cellotetraose ( $G_4$ ), cellopentaose ( $G_5$ ) and cellohexaose ( $G_6$ ) (Figure S9A). Celloheptaose ( $G_7$ ) and cellooctaose ( $G_8$ ) were also detected in small amounts. During analysis, part of cello-oligosaccharides precipitated in the organic mobile phase and a linear calibration curve could not be obtained for quantification. Analysis using a Shodex Sugar SH-1011 column and a Phenomenex Rezex RPM-Monosaccharide Pb++ column with water as the mobile phase showed peaks of glucose, cellobiose and cellotriose. Remaining cello-oligosaccharides appeared as a single overlapped peak (Figure S9B,C). The total yield of cello-oligosaccharides was calculated from these data according to the area of the peaks as the calibration factor showed a linear relationship with the degree of polymerization (DP) of cello-oligosaccharides (Figure S10). To avoid the influence of overlap of peaks, the glucose yield was calculated using the Phenomenex Rezex RPM-Monosaccharide Pb++ column and the cellotriose yield was calculated using the Sugar SH-1011 column.

#### Time course

In order to have a better understanding of hydrolysis process of cellulose in the semi-flow reactor, we investigated the evolution of products during the reaction. Formation rate of products during the hydrolysis at 453 K was plotted against reaction time, as shown in Figure S11. The hydrolysis of cellulose started when the temperature reached 423 K. Then the rate of cello-oligosaccharides formation gradually increased and reached the maximum at 20 min. Likewise, cellobiose and glucose formation rates also followed the same trend. After 30 min, the formation of all products reduced simultaneously and the reaction was complete within 40 min.

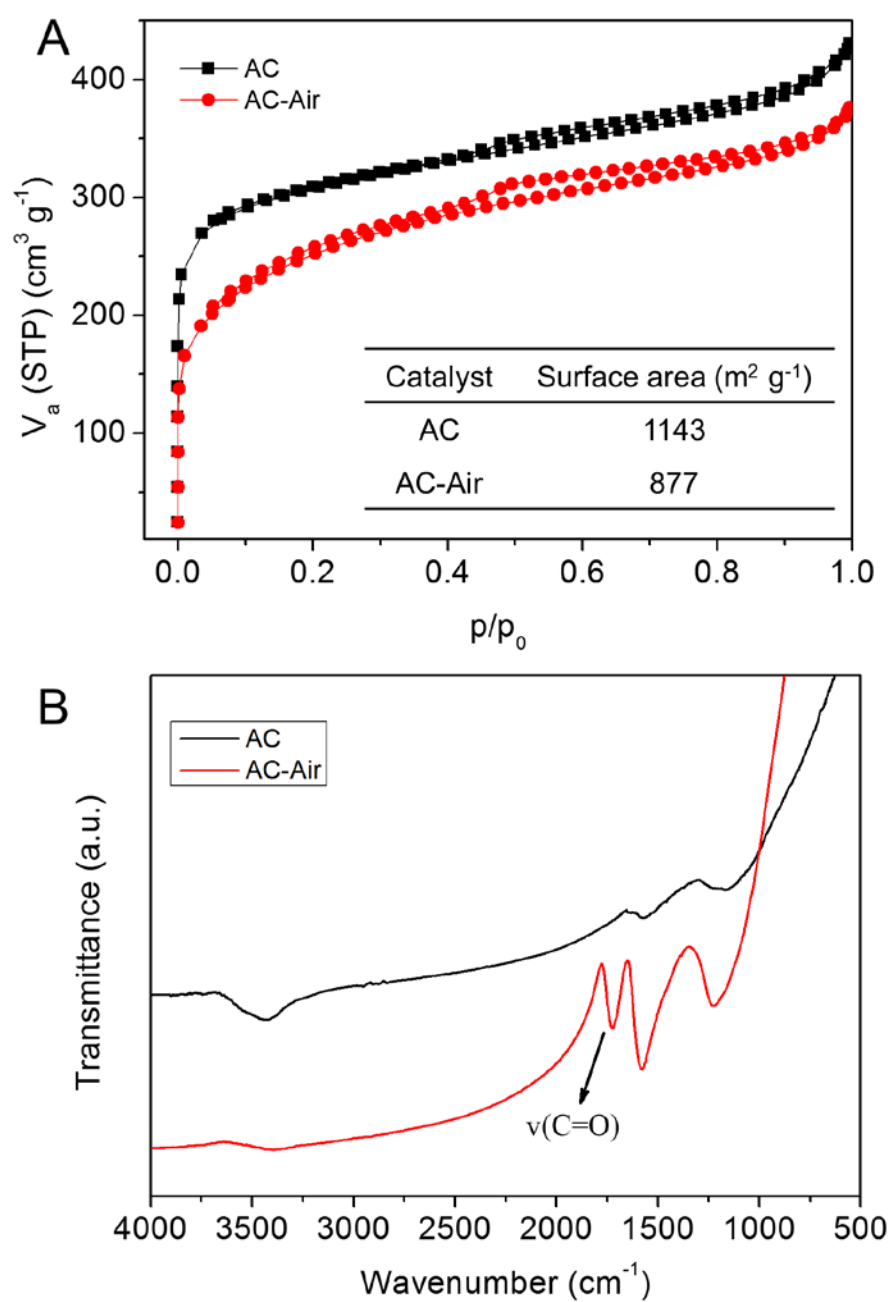
#### Hydrolysis of real biomass

Carbohydrate compositions of recycled paper and eucalyptus were determined by analysis protocols set by National Renewable Energy Laboratory (NREL/TP-510-42618),<sup>S1</sup> as shown in Table S2. The yield of cello-oligosaccharides from different substrates is shown in Figure S5 and it was calculated based on the initial glucan content of the substrate.

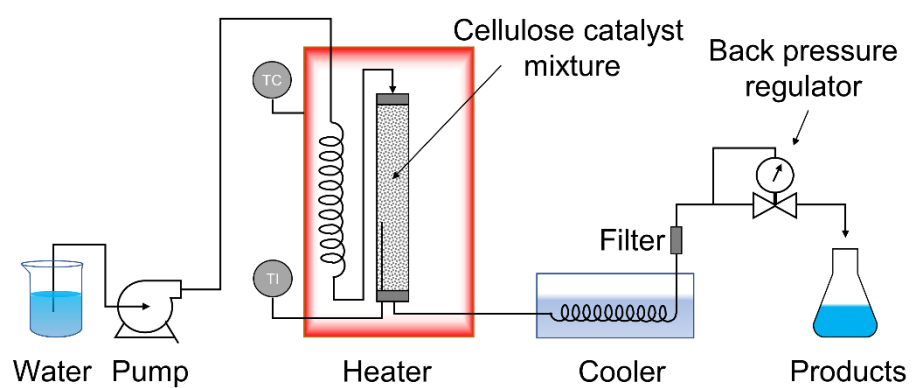
#### FT-IR and <sup>1</sup>H NMR analysis of cello-oligosaccharides

For further characterization, the cello-oligosaccharides obtained under optimized conditions (Table S1 entry 8) were separated from the solution by precipitation in ethanol. The FT-IR spectra of precipitated cello-oligosaccharides is shown in Figure S12 and compared with parent cellulose. Peaks located at 3413 cm<sup>-1</sup> and 2903 cm<sup>-1</sup> were attributed to the stretching of O-H groups and aliphatic saturated C-H, respectively. The peak at 1639 cm<sup>-1</sup> was assigned to the bending vibration of O-H. The peaks at 1465 cm<sup>-1</sup> and 1380 cm<sup>-1</sup> were attributed to the in-plane bending vibrations of C-H groups. The peaks at 1250 – 1000 cm<sup>-1</sup> were assigned to the stretching vibration of C-O-C in pyranose ring of glucose. The peak of 897 cm<sup>-1</sup> was associated to the  $\beta$ -glycosidic linkage between monomer units. A comparison of FT-IR spectrum of cellulose and cello-oligosaccharides showed no additional peaks confirming the absence of any undesired modification in the cello-oligosaccharides structure.

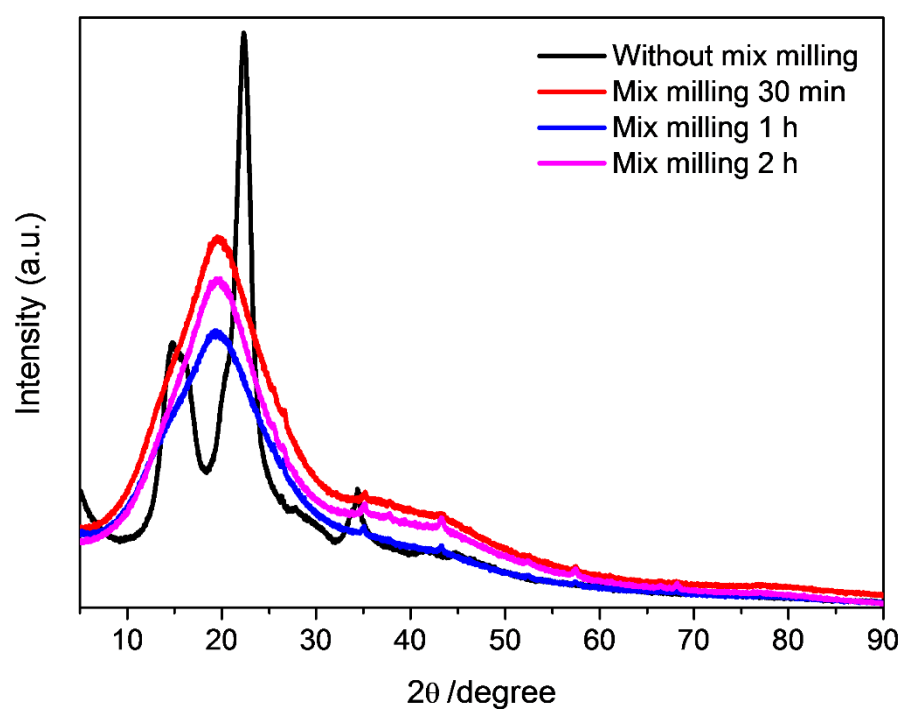
In addition, we used <sup>1</sup>H NMR spectroscopy to investigate the nature of glycosidic linkages present in cello-oligosaccharide mixture. The comparison of <sup>1</sup>H NMR spectra of cello-oligosaccharides and cellopentaose, used as reference, suggested that the cello-oligosaccharide was chemically similar to cellopentaose and no additional peaks were observed (Figure S13). The dominant doublet at  $\delta_H$  4.22 ( $\delta_H$  denotes <sup>1</sup>H chemical shift in ppm) in the expanded anomeric region was assigned to the  $\beta$ -1,4 linked monomers with a non-reducing end. The peak for anomeric protons of  $\beta$ -1,4 internal linkages and  $\alpha$  reducing ends were detected at  $\delta_H$  4.30 and  $\delta_H$  5.25, respectively. The  $\alpha$ -1,6 linkages ( $\delta_H$  4.9) characteristic of branched oligosaccharides produced by glycosylation reaction were not detected.



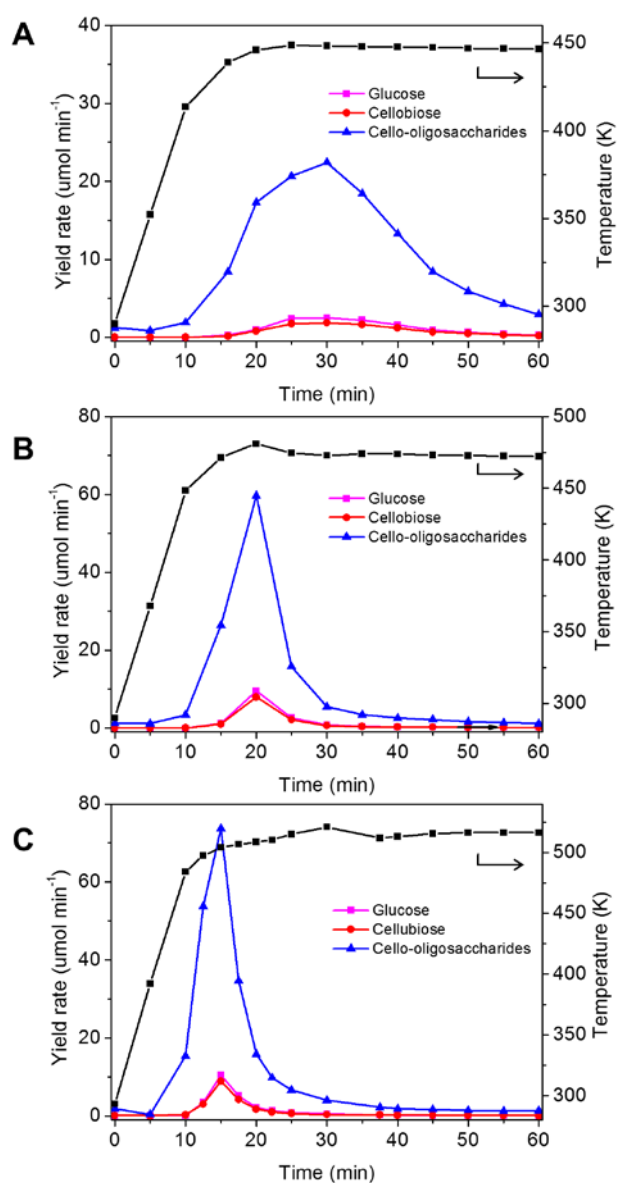
**Figure S1.** Characterization of AC and AC-Air, (A) N<sub>2</sub> adsorption isotherms, (B) FT-IR spectra.



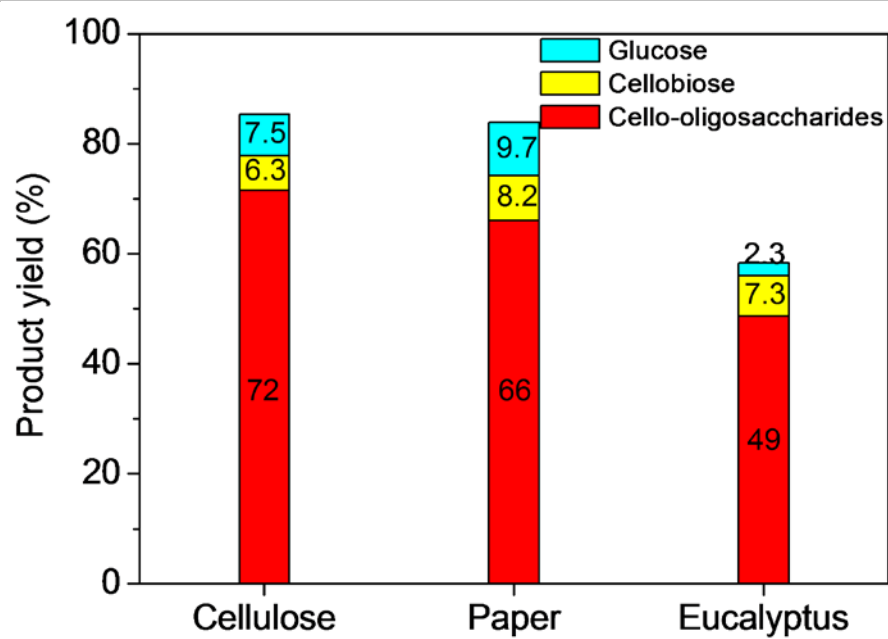
**Figure S2.** Scheme showing the design of semi flow process for hydrolysis of cellulose.



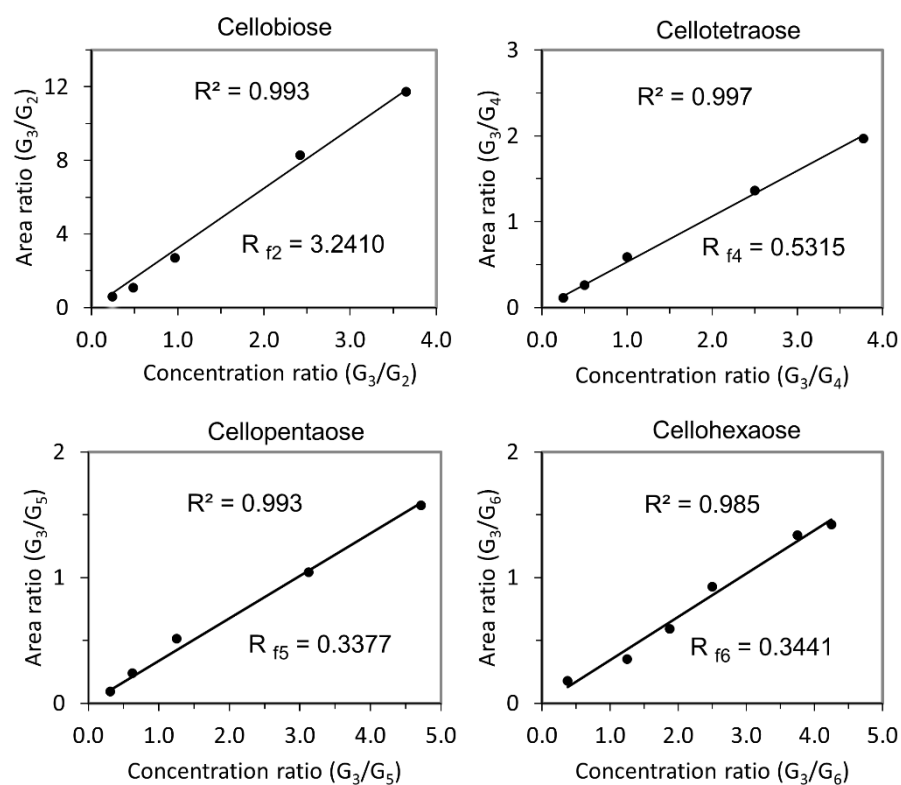
**Figure S3.** XRD patterns of mix-milled samples containing cellulose and AC-Air. S/C = 6.5.



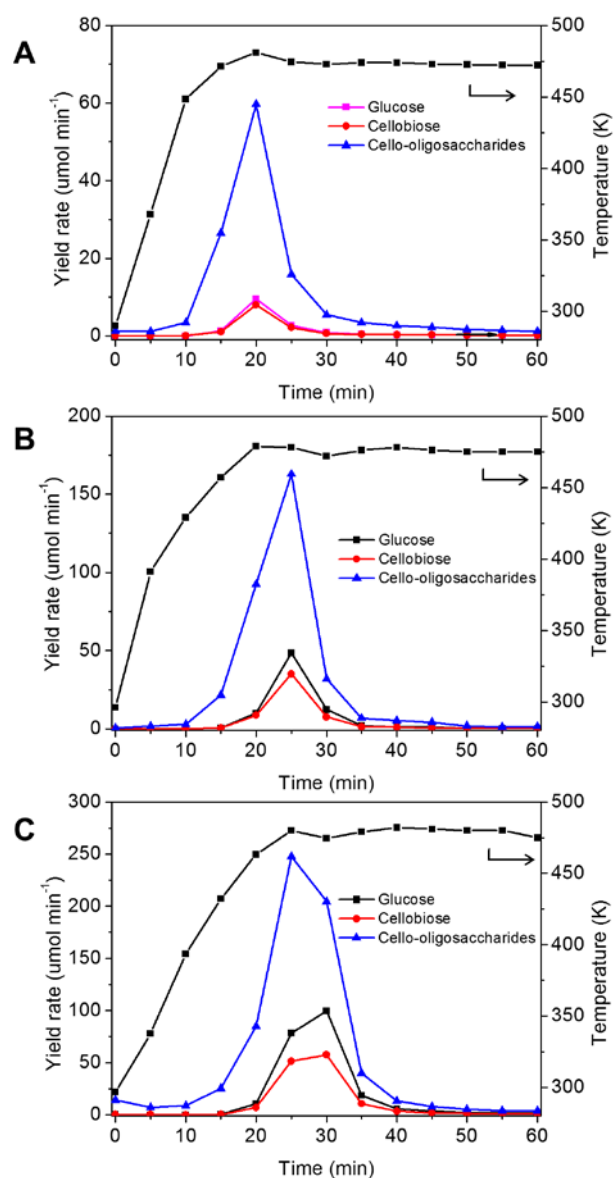
**Figure S4.** Time course of hydrolysis products at different temperatures: (A) 448 K; (B) 473 K; (C) 513 K. Reaction conditions: 0.175 g MMC (S/C = 6.5), 0.75 mL min<sup>-1</sup> water, 1 h mix milling.



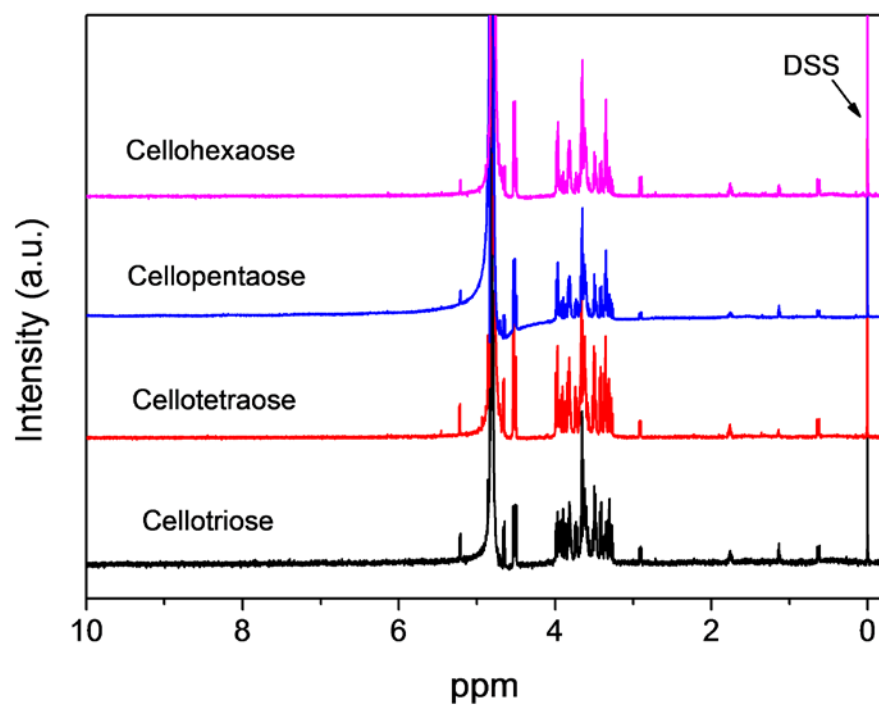
**Figure S5.** Product yields for real biomass hydrolysis. Reaction conditions: 0.175 g mix-milled substrate (S/C = 6.5), 473 K, 40 min, 0.75 mL min<sup>-1</sup> water, 1 h mix milling.



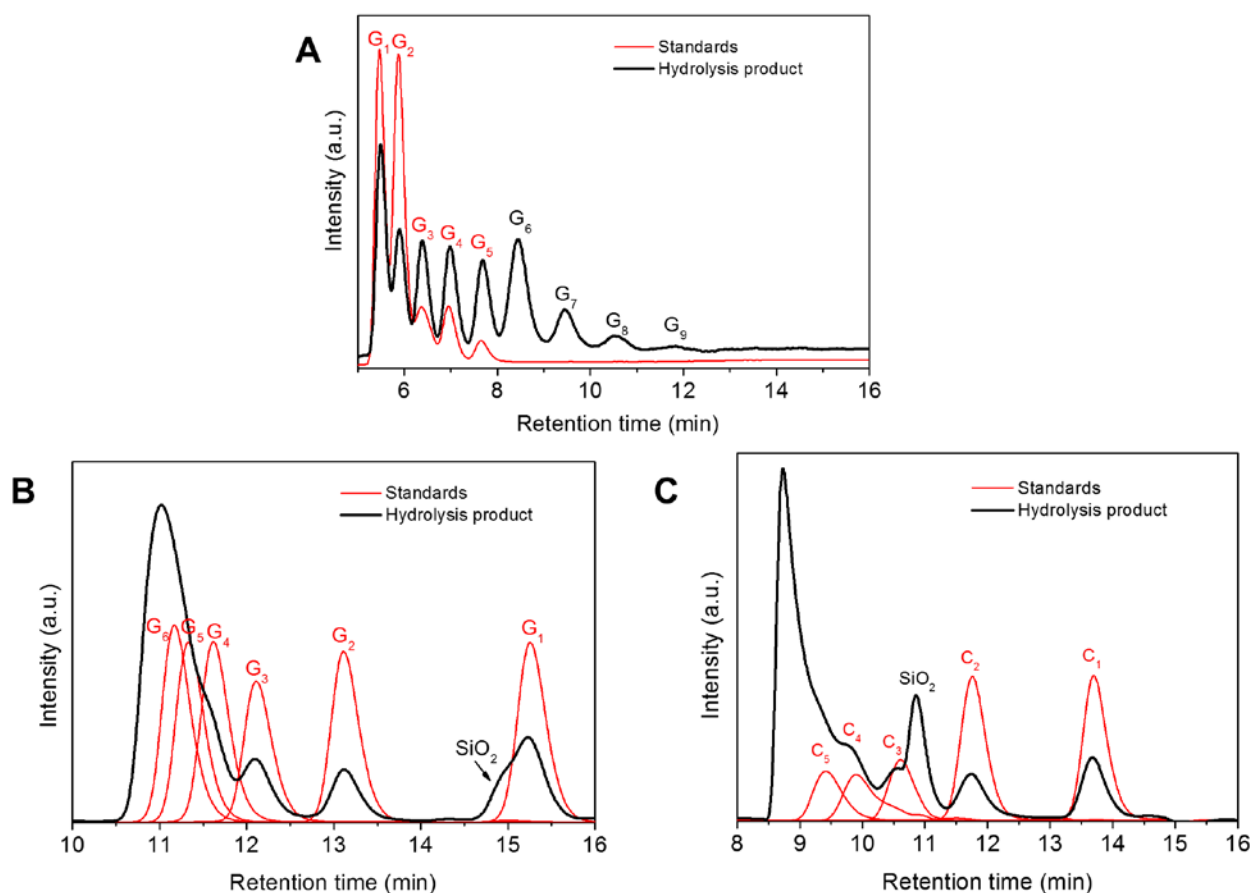
**Figure S6.** Relationship of area ratio of cellotriose and other cello-oligosaccharides against concentration ratio based on MALDI-TOF MS.



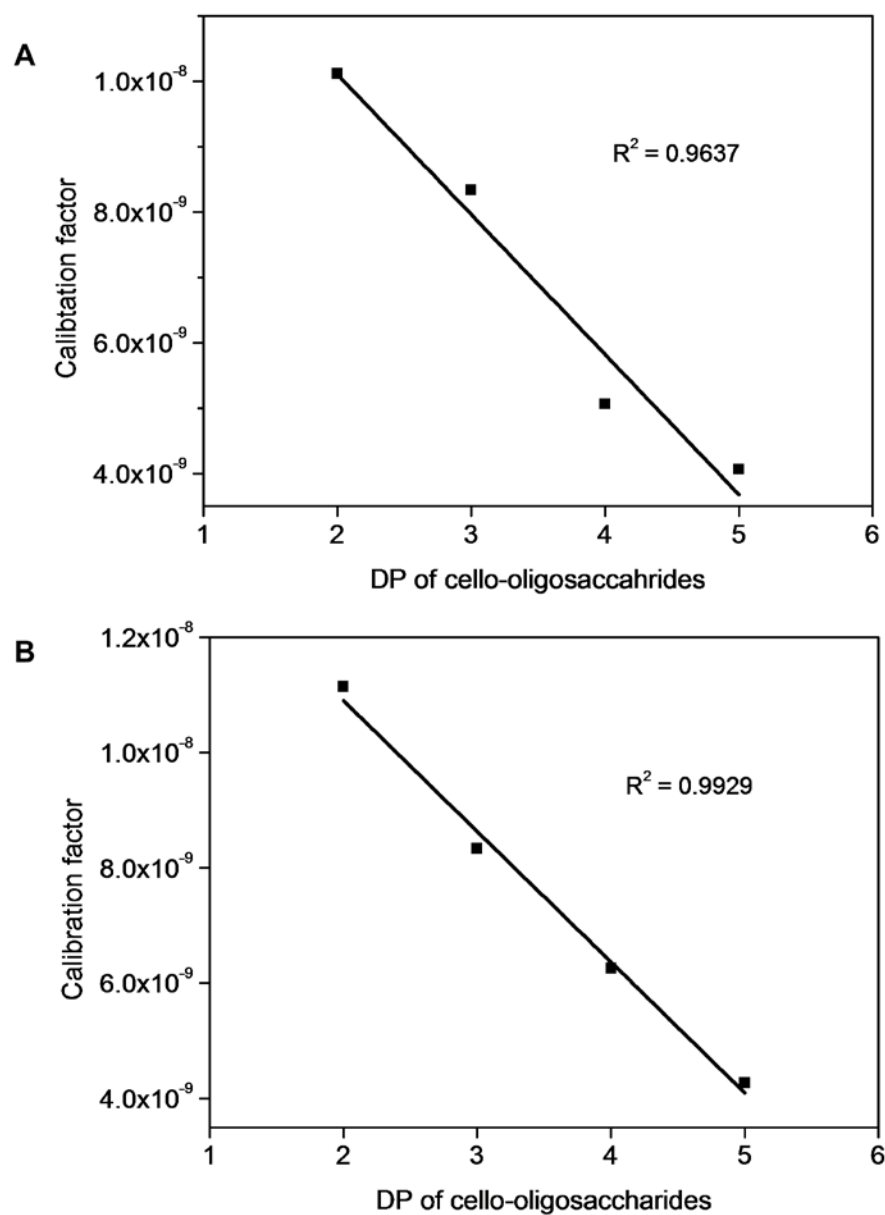
**Figure S7.** Time course of hydrolysis products with different space velocity: (A)  $70 \text{ h}^{-1}$ ; (B)  $20 \text{ h}^{-1}$ ; (C)  $13 \text{ h}^{-1}$ . Reaction conditions: MMC ( $\text{S/C} = 6.5$ ),  $473 \text{ K}$ ,  $0.75 \text{ mL min}^{-1}$  water,  $1 \text{ h}$  mix milling.



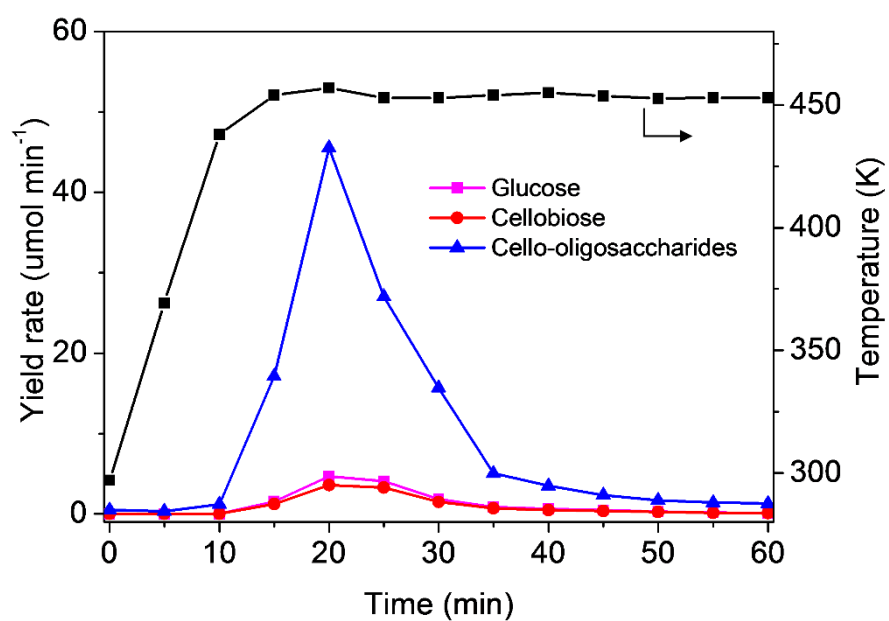
**Figure S8.**  $^1\text{H}$  NMR spectra of full region of individual cello-oligosaccharide in  $\text{D}_2\text{O}$ .



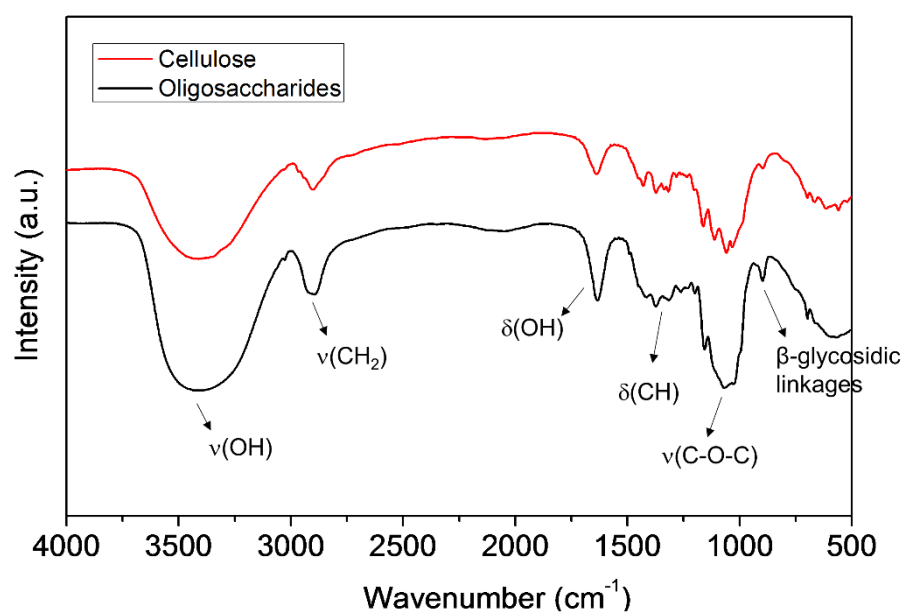
**Figure S9.** HPLC chromatogram of hydrolysis products and  $\beta$ -1,4 linked cello-oligosaccharide standards. (A) Column: Amino NH 2P-40 3E; (B) Column: Sugar SH-1011; (C) Column: Phenomenex Rezex RPM-Monosaccharide Pb++. Reaction conditions: 0.175 g MMC (S/C = 6.5), 40 min, 0.75 mL min<sup>-1</sup> water, 1 h mix milling.



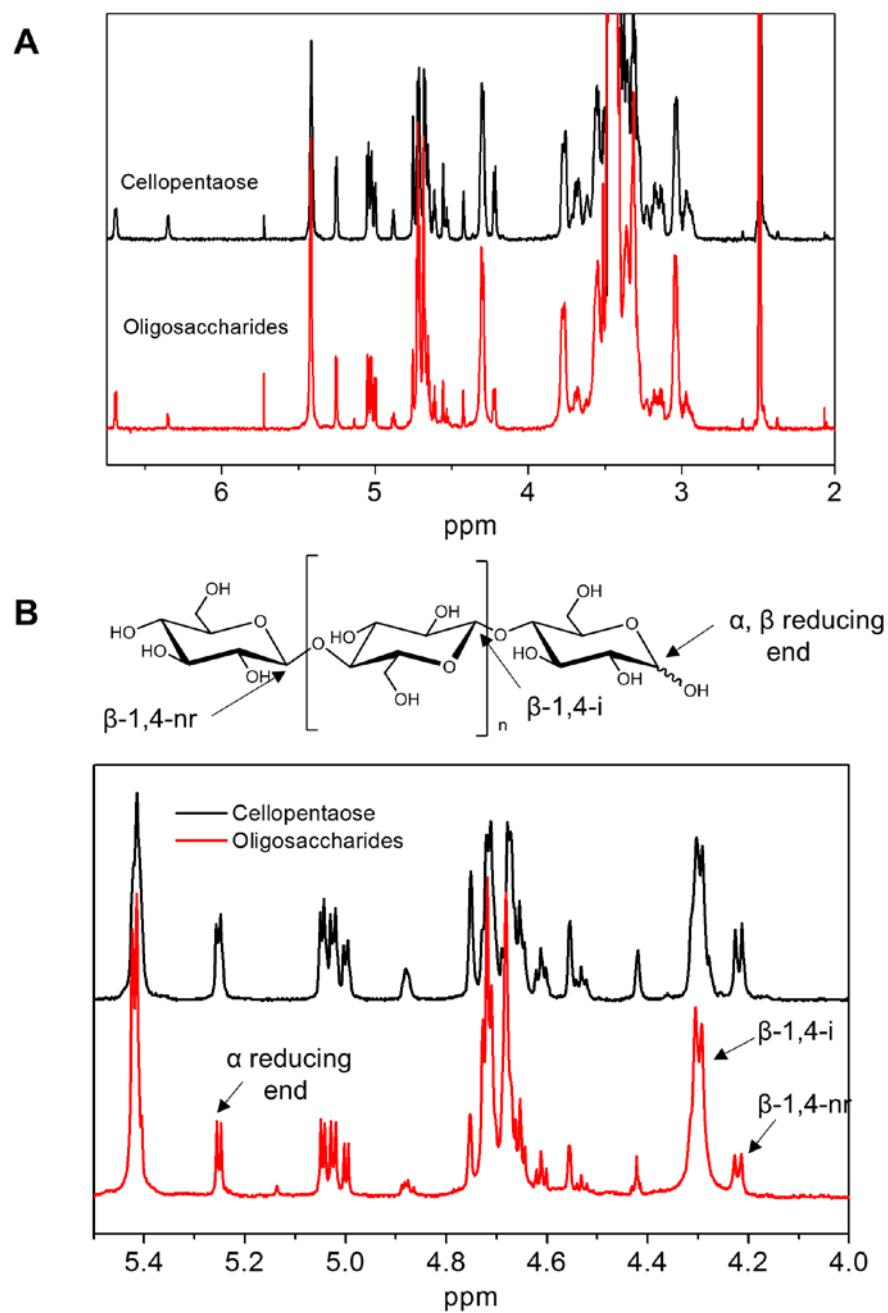
**Figure S10.** A linear relationship was observed between DP of cello-oligosaccharides and their calibration factor in HPLC using (A) Sugar SH-1011 column and (B) Phenomenex Rezex RPM-Monosaccharide Pb++ column.



**Figure S11.** The evolution of products during the course of cellulose hydrolysis. Reaction conditions: 0.175 g MMC (S/C = 6.5), 453 K, 0.75 mL min<sup>-1</sup> water.



**Figure S12.** Comparison of FT-IR spectra of precipitated cello-oligosaccharides and cellulose.



**Figure S13.**  $^1\text{H}$  NMR spectra of oligosaccharide mixture and cellopentaose in  $\text{DMSO-d}_6$ . (A) the complete spectra, (B) the expanded anomeric region.

## SUPPORTING INFORMATION

**Table S1. Hydrolysis of cellulose under different conditions.**

| Entry | Mix-milling duration (h) | T (K) | Yield based on carbon (%)     |            |         |        |       |
|-------|--------------------------|-------|-------------------------------|------------|---------|--------|-------|
|       |                          |       | Oligosaccharides <sup>a</sup> | Cellobiose | Glucose | Others | Total |
| 1     | 0                        | 453   | 11                            | 3.2        | 4.3     | 0.2    | 19    |
| 2     | 0.5                      | 453   | 53                            | 4.0        | 4.4     | 0.2    | 62    |
| 3     | 1                        | 453   | 69                            | 5.0        | 6.8     | 0.3    | 81    |
| 4     | 2                        | 453   | 71                            | 5.6        | 6.8     | 0.2    | 83    |
| 5     | 1                        | 413   | 15                            | 0.4        | 0.7     | 0.0    | 16    |
| 6     | 1                        | 428   | 26                            | 1.3        | 1.9     | 0.1    | 29    |
| 7     | 1                        | 443   | 46                            | 3.7        | 4.8     | 0.2    | 55    |
| 8     | 1                        | 473   | 72                            | 6.3        | 7.5     | 0.3    | 86    |
| 9     | 1                        | 493   | 67                            | 6.0        | 7.2     | 0.2    | 80    |
| 10    | 1                        | 513   | 67                            | 5.4        | 6.4     | 0.5    | 80    |
| 11    | 1                        | 523   | 59                            | 5.4        | 6.4     | 0.3    | 71    |

Conditions: 0.175 g MMC (S/C = 6.5); 40 min, water flow rate: 0.75 mL min<sup>-1</sup> (SV = 70 h<sup>-1</sup>); <sup>a</sup> cello-oligosaccharides with degree of polymerization more than 3.

## SUPPORTING INFORMATION

**Table S2.**

**The compositions of cellulose materials measured by NREL/TP-510-42618 method.<sup>S1</sup>**

| Substrate  | Glucan | Xylan | Acid insoluble lignin |
|------------|--------|-------|-----------------------|
| Paper      | 69.7   | 11.8  | 11.0                  |
| Eucalyptus | 41.4   | 12.8  | 21.3                  |

### References

- [S1] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, "Determination of structural carbohydrates and lignin in biomass" (Tech. Rep. 510-42618, NREL, 2011)