J Wood Sci (2009) 55:367–375
DOI 10.1007/s10086-009-1040-6

ORIGINAL ARTICLE

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Two-step hydrolysis of Japanese beech as treated by semi-flow hot-compressed water

Received: February 23, 2009 / Accepted: May 15, 2009 / Published online: August 4, 2009

Abstract A two-step hydrolysis of Japanese beech (*Fagus crenata*) was conducted by semi-flow treatment with hot-compressed water. The first treatment stage was conducted at 230°C/10 MPa for 15 min and the second at 270°C/10 MPa for 15 min. Hemicellulose and lignin were found to be hydrolyzed in the first stage, while crystalline cellulose was hydrolyzed in the second stage. The treatment solubilized 95.6% of the Japanese beech wood flour into water with 4.4% remaining as water-insoluble residue, which was composed mainly of lignin. Hydrolysis products from the first stage were xylose and xylo-oligosaccharides, glucuronic acid and acetic acid from *O*-acetyl-4-*O*-methylglucuronoxylan, and hydrolyzed monomeric guaiacyl and syringyl units and their dimeric condensed-type units from lignin. Products from the second hydrolysis stage were glucose and cello-oligosaccharides from cellulose. The dehydrated products levoglucosan, 5-hydroxymethylfurfural (5-HMF), and furfural, as well as fragmented products glycolaldehyde, methylglyoxal, and erythrose, were recovered in the first stage from hemicellulose, and to a greater extent in the second stage from cellulose. Furthermore, organic acids such as glycolic, formic, acetic, and lactic acids were recovered in both stages. Based on these lines of evidence, decomposition pathways of *O*-acetyl-4-*O*-methylglucuronoxylan and cellulose are independently proposed.

Key words Japanese beech · Hot-compressed water · Hemicellulose · Cellulose · Lignin

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This study was presented in part at the 17th Annual Meeting of the Japan Institute of Energy, Tokyo, August 2008, and at the 55th Annual Meeting of the Japan Wood Research Society, Matsumoto, March 15–17, 2009

Introduction

Due to the foreseeable depletion of fossil fuels and the environmental issues of global warming, the utilization of lignocellulosic materials as renewable resources has been highlighted for biofuel production over the past two decades.[1–3] Lignocellulosic biomass, of which 10–50 billion tons is produced each year globally, are renewable and available for biofuel production.[4] However, lignocellulosic biomass is a complex mixture of natural polymers, and is composed of hemicellulose, cellulose, and lignin, which are difficult to separate and dissolve in water or other solvents. Cellulose is a crystalline homopolysaccharide composed of D-glucopyranose linked together by (1→4)-glycosidic bonds. It makes up about 40%–50% of the dry wood mass. Hemicellulose is, on the other hand, known as an amorphous polysaccharide. It is composed of a variety of monosaccharides, such as mannose, glucose, galactose, xylose, arabinose, and glucuronic acid, while lignins are aromatic three-dimensional polymers that are dehydrogenatively synthesized but not readily degraded by microbial systems.[5,6]

Both cellulose and hemicellulose could be utilized by microorganisms if they could be hydrolyzed to monosaccharides.[5] Therefore, to degrade cellulose and hemicellulose to fermentable saccharides, biological, chemical, and/or physical methods have been applied and developed for fractionation or hydrolysis of lignocellulosic materials.[7] Among these methods, the use of hot-compressed water, which refers to water in subcritical or supercritical state, or at sufficiently high pressure and high temperature, can hydrolyze and thus fractionate lignocellulosic materials.[8–11] Therefore, due to the increased amount of ionized products in water under the hot-compressed conditions, ether and ester linkages could be hydrolyzed with water, playing an important role as a solvent, reactant, and catalyst.[12]

The hot-compressed water treatment system can be categorized into batch-type and flow-type arrangements.[13] Because significant decomposition reactions occur in batch-type treatment, flow-type hot-compressed water treatment

was preferred to hydrolyze and fractionate lignocellulosic materials.[7,10,14–16] It is reported that all hemicellulose, 35%–60% of lignin, and 4%–22% of cellulose was hydrolyzed by hot-compressed water (200°–230°C, 34.5 MPa, 15 min) with flow-type treatment.[7,10] It is generally known that cellulose is much more resistant to hydrolysis than hemicellulose, so it is reasonable that different treatment temperatures be used for hemicellulose hydrolysis and cellulose hydrolysis. In fact, Ando et al.[17] studied the hydrolysis of bamboo, chinquapin, and Japanese cedar when treated by a two-step process with hot-compressed water at 180°C and 285°C. As a result, more than 95% of bamboo and chinquapin could be hydrolyzed. However, quantitative analysis of hydrolyzed saccharides was not performed and only qualitative evaluation of various saccharides was made without discussion of the decomposition products of saccharides.[17] Moreover, the hydrolysis process as treated by hot-compressed water greatly depends on the variety of biomass studied.[7]

The aim of this work was to quantitatively analyze all products from Japanese beech when treated by a semi-flow type two-step method with hot-compressed water and to establish the decomposition pathways of hemicellulose and cellulose from the hydrolysis and decomposition products.

Materials and methods

Material and chemicals

Wood flour of Japanese beech (*Fagus crenata*) passing through an 18-mesh screen was prepared for hot-compressed water treatment. Wood flour was extracted with ethanol–benzene (1:2 v/v) and dried at 105°C for 24 h before experiment. All chemicals used were of reagent grade without purification.

Hot-compressed water treatment

The semi-flow hot-compressed water treatment was conducted using the apparatus shown in Fig. 1. Approximately 1 g of wood flour was placed in a reaction cell. Two preheating units (Heater-1 and Heater-2) were used to reach the two temperatures of 230°C and 270°C for the two-step process at 10 MPa pressurized by Pump-1, which was controlled by a back-pressure regulator. Heater-3 at the base of the reaction cell was used to control the temperature within the reaction cell at 230°C or 270°C for each step of the treatment. When the temperatures at Heater-1, Heater-2, and Heater-3 reached the designated temperatures, Pump-1 was turned on for the first step at 230°C with needle valve N2 closed. After the first step of the reaction with needle valve N1 closed, needle valve N2 was opened for the second step of the treatment. Thermocouples (T3 and T4) showed the temperatures of the inlet and outlet of the reaction cell when the experiment was running. After the hot-compressed water passed through the reaction cell, the water-soluble portion was cooled down immediately by the cooling system to terminate all reactions. Samples were then collected with a fraction collector every 1 min. To completely quench the reaction of the whole system, cold distilled water in Water tank-2 was pumped into the reaction cell by Pump-2. Both steps of the process used a flow rate of 10 ml/min.

Analytical methods

The residue that remained in the reaction cell was collected, filtered, and dried at 105°C to determine the oven-dried weight. The residue was also studied by X-ray diffractometry (RINT2000 diffractometer, Rigaku Denki) to examine the crystallographic structure of cellulose under Cu-Kα radiation (λ = 0.1542 nm) using a Kα filter, operated at 40 keV and 30 mA, integrating five times. Residual lignin was measured as Klason lignin.

The yield of water-soluble product was calculated by the following equation:

$$\text{Water-soluble yield} = \frac{\text{Initial wood flour mass} - \text{Residue mass}}{\text{Initial wood flour mass}} \times 100\% \quad (1)$$

The water-soluble portion was filtrated by 0.45-μm filter for high-performance liquid chromatography (HPLC), high-performance anion-exchange chromatography (HPAEC),

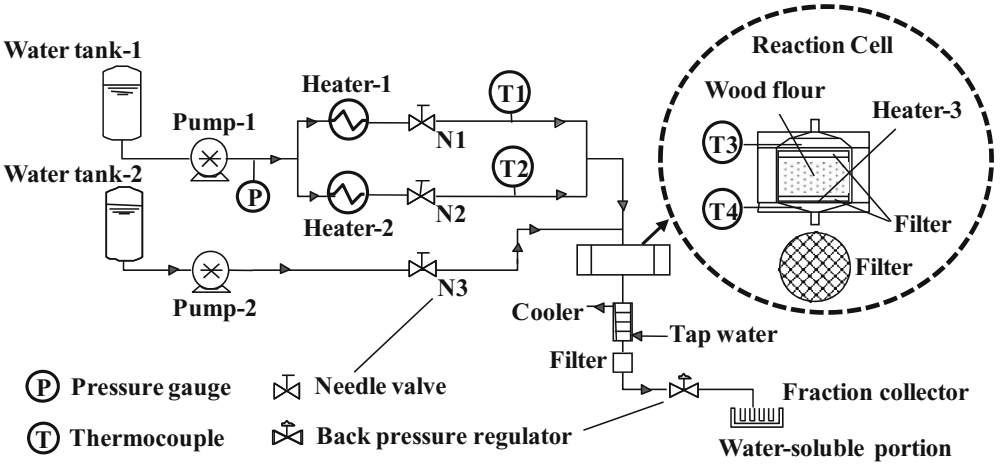

Fig. 1. Experimental setup for the two-step semi-flow hot-compressed water system used for treatment of Japanese beech wood flour (230°C/10 MPa for 15 min, 270°C/10 MPa for 15 min)

and capillary electrophoresis (CE). The HPLC system (Shimadzu, LC-10A) was equipped with a Shodex Sugar KS-801, Ultron PS-80P or HPX-87H column and refractive index detector/ultraviolet-visible (UV-Vis) detector. Distilled water was used as the eluent at a flow rate of 1.0 ml/min and oven temperature was set at 80°C for the KS-801 and Ultron PS-80P column. For the HPX-87H column, 5 mM H_2SO_4 was used as eluent at a flow rate of 0.6 ml/min with the oven temperature set to 45°C.

The HPAEC system (Dionex ICS-3000 system) was equipped with a CarboPac PA-1 column (4 × 250 mm) and pulsed amperometric detection was employed and operated at 35°C and flow-rate of 1.0 ml/min under the helium atmosphere. HPAEC was used for detection of monosaccharides, cello-oligosaccharides, and xylo-oligosaccharides in the water-soluble portion. The mobile phase was a gradient-programmed mixture of three eluents: distilled water, 0.2 M sodium hydroxide, and 1.0 M sodium acetate. The eluents were contained in three separate reservoirs and were degassed by an aspirator and subsequently purged with helium to prevent absorption of CO_2.

The CE (Agilent; HP3D) was used to assay the low-molecular weight organic acids. A fused-silica capillary (Agilent; 75 µm diameter, 104 cm total length, 95.5 cm effective length) was used at 15°C.

Gas chromatography-mass spectrometry (GC-MS; Hitachi M7000s and M9000 series) with a fused-silica capillary column (Varian, CP-Sil 8 CB; 30 m × 0.25 mm × 0.25 µm) was used to analyze lignin-derived monomeric and dimeric products in the water-soluble portion. The low molecular weight lignin-derived products were trimethylsilylated prior to GC-MS analysis. Apart from the lignin-derived monomeric and dimeric products, its trimeric and higher products were estimated by subtracting the monomeric and dimeric products from the total soluble lignin: lignin-derived trimeric and higher products (wt%) = soluble lignin (wt%) – lignin-derived monomeric and dimeric products (wt%). The soluble lignin was calculated from the Klason lignin determined on the original wood minus that on the residue after hot-compressed water treatment.

Quantification of products in water-soluble portion

Concentrations of the products in the water-soluble portion were calculated based on the peak areas on chromatograms obtained from HPLC, HPAEC, CE, and GC-MS. A set of standards with known concentrations, containing the compounds that were to be identified both quantitatively and qualitatively, was prepared and analyzed together with the samples by using the relevant analytical equipment as mentioned above. To quantify each product contained in one sample, the concentration was determined via a calibration curve made from its corresponding standards at two concentrations. The obtained concentration was then multiplied by its fraction volume (approximately 10 ml per fraction) to calculate the product weight. The product percentages based on the oven-dried weight of wood flour are in Table 1.

Results and discussion

To explore a relationship between treatment temperature under pressurized conditions (10 MPa) and the degree of hydrolysis of hemicellulose and cellulose, semi-flow hot-compressed water treatments were conducted at various temperatures as preliminary experiments. It was consequently found that maximum xylo-oligosaccharides were obtained at 230°C, while 270°C proved optimal for cello-oligosaccharides. In addition, X-ray diffractometry revealed that the crystalline structure of cellulose residue disappeared when the temperature reached 270°C or higher.[18] Based on these lines of evidence, the temperature of 230°C was set as the first stage to hydrolyze hemicellulose without extensive decomposition of cellulose,[16] and 270°C was selected as the second stage to hydrolyze crystalline cellulose. The obtained temperature profile for such a two-step hot-compressed water treatment is shown in Figs. 2–8.

Hydrolysis of major cell wall components by hot-compressed water

In the two-step hot-compressed water treatment (230°C/10 MPa for 15 min and then 270°C/10 MPa for 15 min), 95.6% of the wood flour was hydrolyzed into water-soluble product with 4.4% remaining as water-insoluble residue. The hydrolyzed products obtained from hemicellulose and cellulose are shown in Fig. 2.

From the first treatment step (230°C/10 MPa for 15 min), xylo-oligosaccharides including xylose, xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose were detected. In addition, glucuronic acid and acetic acid were found. Therefore, they must have originated from a major hemicellulose of the hardwood, *O*-acetyl-4-*O*-methylglucuronoxylan.

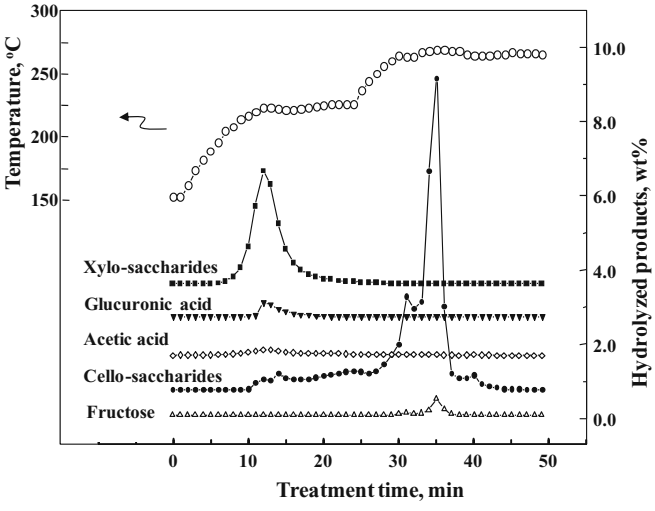

Fig. 2. Hydrolyzed products produced from Japanese beech by two-step treatment with hot-compressed water at 10 MPa (230°C for 15 min, 270°C for 15 min). Left axis corresponds to temperature (*open circles*); right axis corresponds to product levels

Table 1. Yields of the compounds produced from hemicellulose, cellulose, and lignin after two-step treatment of Japanese beech wood flour with hot-compressed water at 10 MPa (230°C for 15 min, 270°C for 15 min)

Compound	Yield (%)				
	First step		Second step		Total
	Hemicellulose	Lignin	Lignin	Cellulose	
Hydrolyzed products[a]	**20.6**	**–**	**–**	**30.8**	**51.4**
Xylo-saccharides	14.1	–	–	0.0	14.1
Glucuronic acid	1.3	–	–	0.0	1.3
Arabinose	0.1	–	–	0.0	0.1
Galactose	0.2	–	–	0.0	0.2
Rhamnose	0.1	–	–	0.0	0.1
Mannose	0.0	–	–	0.0	0.0
Cello-saccharides	4.8[b]	–	–	29.6[b]	34.4
Fructose	0.0	–	–	1.2	1.2
Dehydrated products	**0.4**	**–**	**–**	**7.5**	**7.9**
Levoglucosan	0.0	–	–	1.3	1.3
5-HMF	0.0	–	–	3.1	3.1
Furfural	0.4	–	–	3.1	3.5
Fragmented products	**0.8**	**–**	**–**	**3.8**	**4.5**
Methylglyoxal	0.2	–	–	1.7	1.9
Glycolaldehyde	0.6	–	–	1.2	1.7
Erythrose	0.0	–	–	0.9	0.9
Products from lignin	**–**	**15.0**	**8.7**	**–**	**23.7**
Sinapyl alcohol	–	0.8	0.0	–	0.8
Coniferyl alcohol	–	0.2	0.0	–	0.2
Sinapyl aldehyde	–	0.1	0.0	–	0.1
Coniferyl aldehyde	–	0.1	0.0	–	0.1
Syringaldehyde	–	0.1	0.0	–	0.1
Vanillin	–	0.1	0.0	–	0.1
Diarylpropane-type dimmer	–	2.6	0.3	–	2.9
Pinoresinol-type dimmer	–	0.6	0.1	–	0.7
Biphenyl-type dimmer	–	1.1	0.1	–	1.2
Trimeric and higher products	–	9.3	8.2	–	17.5
Organic acids	**1.6**	**–**	**–**	**0.7**	**2.3**
Acetic acid	1.3	–	–	0.3	1.6
Glycolic acid	0.0	–	–	0.2	0.2
Formic acid	0.2	–	–	0.2	0.4
Lactic acid	0.1	–	–	0.0	0.1
Total	18.6[b]	15.0	8.7	47.6[b]	89.9
Unknown					5.7
Water-insoluble residue					4.4

5-HMF, 5-Hydroxymethylfurfural
[a] Hydrolyzed products from hemicellulose and cellulose
[b] Cellosaccharides in the first step considered as a part of the cellulose-derived products

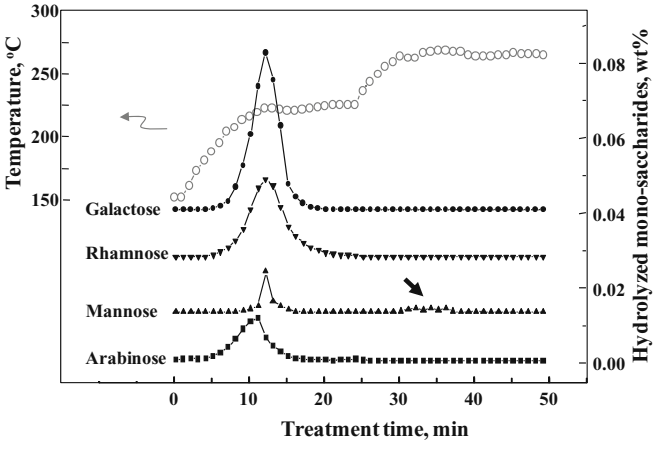

Fig. 3. Hydrolyzed monosaccharides produced from Japanese beech by two-step treatment with hot-compressed water at 10 MPa (230°C for 15 min, 270°C for 15 min). Left axis corresponds to temperature (*open circles*); right axis corresponds to product levels. *Bold arrow* indicates presence of mannose

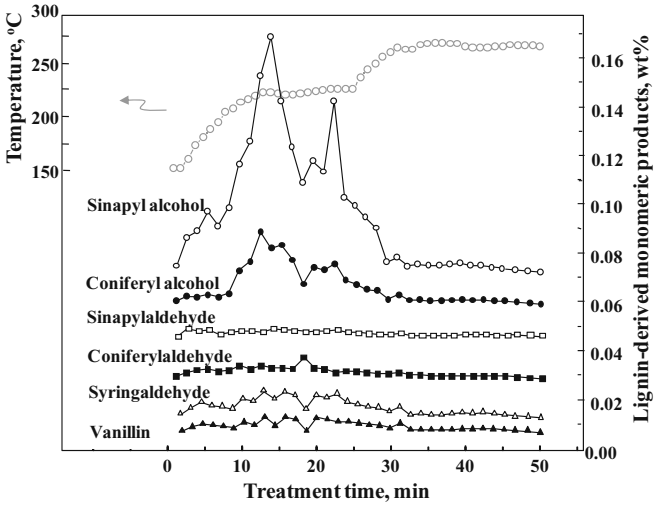

Fig. 4. Lignin-derived products (monomeric compounds) from Japanese beech by two-step treatment with hot-compressed water at 10 MPa (230°C for 15 min, 270°C for 15 min). Left axis corresponds to temperature (*open circles*); right axis corresponds to product levels

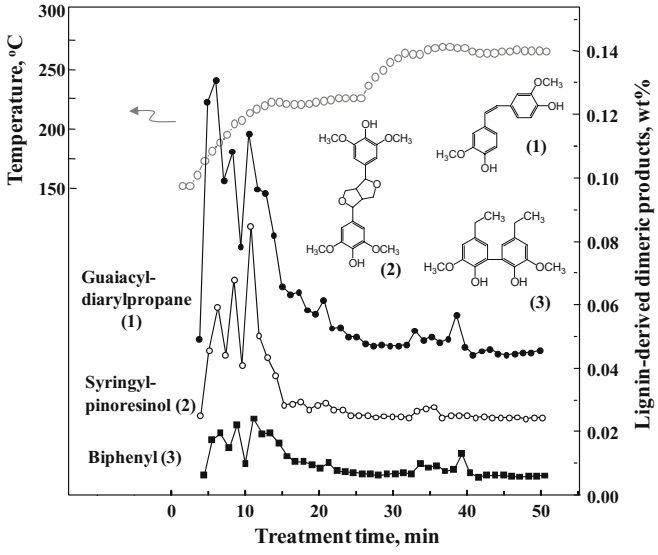

Fig. 5. Lignin-derived products (dimeric compounds) from Japanese beech by two-step treatment with hot-compressed water at 10 MPa (230°C for 15 min, 270°C for 15 min) Left axis corresponds to temperature (*open circles*); right axis corresponds to product levels

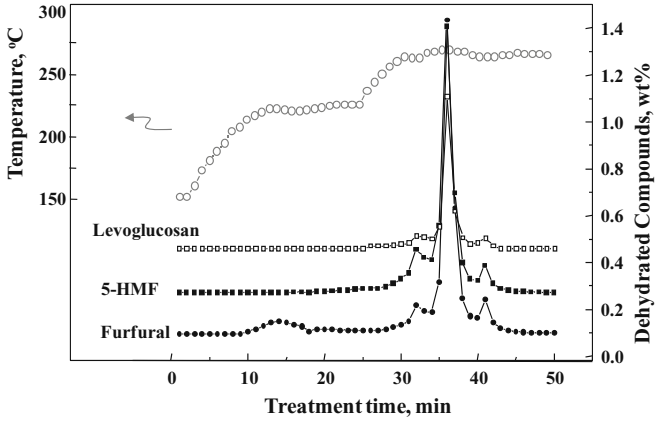

Fig. 6. Dehydration products from Japanese beech by two-step treatment with hot-compressed water at 10 MPa (230°C for 15 min, 270°C for 15 min). Left axis corresponds to temperature (*open circles*); right axis corresponds to product levels. *5-HMF*, 5-hydroxymethylfurfural

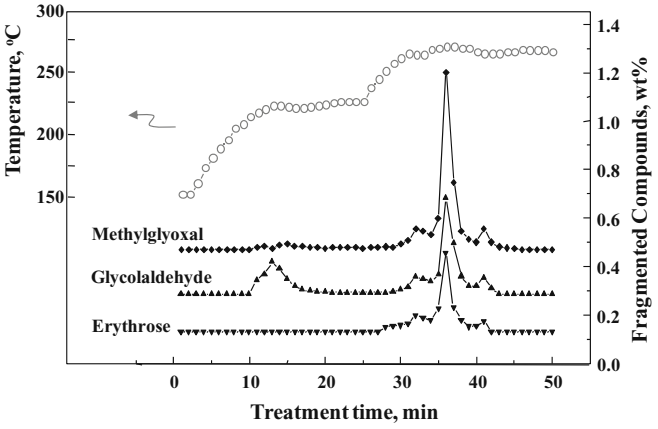

Fig. 7. Fragmentation products from Japanese beech by two-step treatment with hot-compressed water at 10 MPa (230°C for 15 min, 270°C for 15 min). Left axis corresponds to temperature (*open circles*); right axis corresponds to product levels

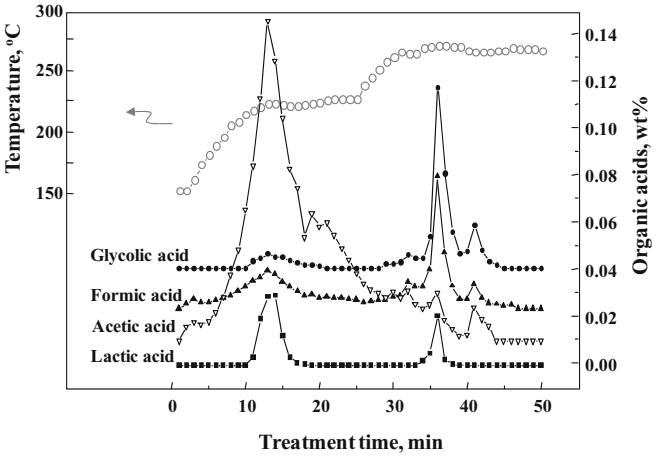

Fig. 8. Organic acids produced from Japanese beech by two-step treatment with hot-compressed water at 10 MPa (230°C for 15 min, 270°C for 15 min). Left axis corresponds to temperature (*open circles*); right axis corresponds to product levels

However, acetic acid from acetyl residues seems to have appeared at a lower temperature than glucuronic acid. This difference is more evident in a larger scale figure as seen later (Fig. 8), which shows that acetic acid was already produced at about 150°C, while glucuronic acid started to appear around 230°C. Such a difference in the hydrolysis temperature indicates that the linkage between xylose and glucuronic acid in the hemicellulose is more stable than the one between acetyl residue and xylose.[19]

Other sugar constituents of hemicellulose found in the first stage of the treatment included galactose, rhamnose, mannose, and arabinose, as shown in Fig. 3. A small amount of mannose was observed from the second stage (Fig. 3). Considering that hemicellulose is mostly hydrolyzed in the first stage, the observed mannose is probably from another source. It is possible that it may be an isomerized form from glucose hydrolyzed from cellulose in the second stage.[20,21] This isomerization to mannose was confirmed by the treatment of D-glucose under the same hot-compressed water condition.

For recovery of cello-oligosaccharides including glucose, cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexaose, the retention time was from 10 to 45 min. However, the main recovery from the first stage (10–20 min retention time) was mainly from hemicellulose such as glucomannan, while that from the second stage (270°C/10 MPa for 15 min) was from cellulose, because in the preliminary experiment the crystalline structure of cellulose remained unchanged at temperatures around 230°C.[18] Therefore, cello-oligosaccharides that emerged between the first and second stages (20–25 min retention time) may be from paracrystalline cellulose. The observed fructose is not a sugar component in Japanese beech, but it may be isomerized from glucose after hydrolysis from cellulose.[20,22]

From the results shown in Figs. 2 and 3, this two-step process is efficient in hydrolyzing hemicellulose and crystalline cellulose separately in the respective steps. Moreover, these two important chemical components can be successfully targeted for hydrolysis independently.

Lignin was unexpectedly hydrolyzed starting at a low temperature of 160°C, as shown in Figs. 4 and 5. Among the monomeric compounds, sinapyl alcohol and coniferyl alcohol were found as hydrolysis products from the first step, together with their aldehydes, sinapyl aldehyde and coniferyl aldehyde. In addition, their fragmented products, syringaldehyde and vanillin, were recovered as the main monomeric products. For dimeric compounds, guaiacyldiarylpropanes, syringyl-pinoresinols, and biphenyls were recovered. Interestingly, such products are all condensed-type linkages. These results are in good agreement with the previous study using supercritical water,[11] indicating that the condensed-type linkages in lignin cannot be hydrolyzed, but that ether linkages in lignin can be preferentially hydrolyzed.

Decomposition of hydrolyzed products from hemicellulose and cellulose

After hydrolysis of hemicellulose and cellulose, the hydrolyzed products are further decomposed if the treatment with hot-compressed water is prolonged. Generally, they are further decomposed by dehydration and fragmentation reactions.[23] Because the decomposed products would inhibit the later fermentation process for ethanol production,[24] it is important to know the decomposition pathway.

Figure 6 shows the production of the dehydrated products, such as levoglucosan, 5-hydroxymethylfurfural (5-HMF), and furfural. It is quite apparent that levoglucosan and 5-HMF are not produced in the first step, while furfural is produced in both steps. Generally, furfural is considered as a dehydrated product from pentose such as xylose and arabinose.[15,24] However, it was also produced during the second treatment step. The dehydrated products from the first step were all removed from the system and collected as the water-soluble portion by the fraction collector, as depicted in Fig. 1. Therefore, the result indicates that furfural was not only produced from pentose but also from hexose such as glucose. The formation of furfural without pentose is possibly via a five-carbon ketose pathway as proposed in the literature.[25]

The formation of 5-HMF and levoglucosan from hexose in the second treatment stage indicates that the formation of these two dehydrated products is temperature dependent. In fact, glucose and mannose were produced from glucomannan in the first step (230°C) as shown in Figs. 2 and 3, but 5-HMF and levoglucosan were not detected (Fig. 6).

Figure 7 shows that methylglyoxal and glycolaldehyde were produced in both treatment stages, while erythrose was formed only in the second step. Therefore, in the first step, it is likely that pentose such as xylose and arabinose from hemicellulose was decomposed to glycolaldehyde and glyceraldehyde. Glyceraldehyde was then dehydrated to methylglyoxal as observed in the glyceraldehyde pathway of hexose fragmentation.[26]

In the second stage, on the other hand, glycolaldehyde and erythrose were formed via retro-aldol condensation[21,27] in the glycolaldehyde/erythrose pathway, while methylglyoxal was produced via the glyceraldehyde/dihydroxyacetone pathway for hexose fragmentation.[21,28] However, under the conditions applied, glyceraldehyde and its dihydroxyacetone isomer in the glyceraldehyde pathway were not detected in either stage. This is because the dehydration reaction of glyceraldehyde to methylglyoxal and/or organic acids occurs quickly.[26]

Production of organic acids

Organic acids are considered as decomposition products of the dehydrated and fragmented compounds.[21] The produced organic acids included acetic acid, glycolic acid, formic acid, and lactic acid (Fig. 8). The acetic acid in the first stage, however, mostly came from hydrolysis of acetyl groups in *O*-acetyl-4-*O*-methylglucuronoxylan, while acetic acid in the second stage must be a result of the decomposition of cellulose and/or lignin.[29] Glycolic acid, formic acid, and lactic acid were produced in both stages. Thus, decomposition of dehydrated and fragmented compounds took place in both stages. Some of the acetic acid was also produced in the first stage by decomposition of dehydration and fragmentation products. Acrylic acid and levulinic acid were not detected under the conditions applied.

Overall products produced from hemicellulose, cellulose, and lignin

Table 1 summarizes the yields of the compounds produced from hemicellulose, cellulose, and lignin in a two-step treatment with hot-compressed water at 230°C/10 MPa for 15 min and 270°C/10 MPa for 15 min. The results clearly shows that the water-soluble portion contained 51.4% hydrolyzed compounds in saccharide forms (15.8% from hemicellulose and 35.6% from cellulose), 14.7% decomposition products (dehydrated and fragmented products, plus organic acids), and 23.7% lignin-derived products. In addition, 5.7% of the water-soluble portion was considered as unknown. On the other hand, the water-insoluble residue was only 4.4% and contained 2.8% Klason lignin.

The hydrolyzed saccharide forms from hemicellulose included xylose, xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose. In addition, glucuronic acid, arabinose, galactose, rhamnose, and mannose were detected. On the other hand, the saccharide forms from cellulose included glucose, cellobiose, cellotriose, cellotetraose, cellopentaose, cellohexaose, and fructose isomerized from glucose.

Given that the chemical composition of Japanese beech is 28.7% hemicellulose, 44.8% cellulose, and 26.5% lignin, about 55.1% of hemicellulose was hydrolyzed in

the first stage, while 79.5% of cellulose and 89.4% of lignin were hydrolyzed in the first and second stages. However, only 1.4% of hemicellulose contributed to the dehydrated products, while the figure was 16.7% for cellulose. The trend for fragmented products was similar with 2.8% of hemicellulose and 8.5% of cellulose leading to fragmented products.

For organic acids, 1.6% of wood flour resulted in organic acids from hemicellulose, but this figure includes acetic acid from acetyl residues. Considering organic acids other than acetic acid, 1.0% of hemicellulose and 1.6% of cellulose resulted in organic acids. The analysis of lignin-derived products will be discussed in more detail elsewhere.

From these lines of evidence, it can be concluded that under the given treatment conditions, hemicellulose is more readily hydrolyzed with less dehydrated, fragmented, and organic acid products than cellulose itself.

Decomposition pathway of hemicellulose and cellulose

With consideration of the decomposition products discussed above, the two-step process may involve two different hydrolysis processes and decomposition mechanisms. Furfural and 5-HMF are considered as typical products of acid hydrolysis rather than alkaline hydrolysis. However, fructose, a typical Lobry de Bruyn-Alberda van Ekenstein isomerization product from glucose, is formed only in alkaline hydroylsis.[19] In the two-step process, only furfural was produced in the first step. Therefore, hydrolysis at the first stage might predominantly proceed by acid catalysis by acetic acid formed from acetyl residues and glucuronic acid from hemicellulose and other organic acids.

Correlation analysis between xylo-oligosaccharides and total decomposed products during xylo-oligosaccharide formation indicated that they are significantly correlated to each other (correlation coefficient: 0.97, calculated by SPSS 11.0). This indicates that the decomposed products in the first step must be derived from xylo-oligosaccharides. The same correlation analysis made between cello-oligosaccharides and total decomposed products during cello-oligosaccharide formation resulted in a correlation coefficient of 0.87. Thus, it could be inferred that even though these decomposed products were mainly derived from cello-oligosaccharides, some of them possibly came from another source, possibly lignin.

Cellulose hydrolysis by treatment with hot-compressed water has been well studied,[15,30] although no hemicellulose hydrolysis pathway has been reported in detail. Based on the observations in this study, however, a pathway for the hydrolysis of the major hemicellulose in hardwood, *O*-acetyl-4-*O*-methylglucuronoxylan, is proposed in Fig. 9, as treated with hot-compressed water. The hydrolysis, dehydration, and fragmentation reactions could be confirmed because dehydrated product (furfural) and fragmentation products (glycolaldehyde and methylglyoxal) were detected in the first step, which supports the pathway of hydrolysis of *O*-acetyl-4-*O*-methylglucuronoxylan.

For glucomannan, a minor hemicellulose in hardwood, it is reported that dehydration and fragmentation always occur in parallel under hot-compressed water treatment, even with a short reaction time.[21,31] Because dehydrated products of hexose such as 5-HMF and levoglucosan were not detected in the first step, the six-carbon carbohydrates might be stable or decomposition might not occur at the lower temperature of this step. The other evidence was that the fragmentation product erythrose, which is generated together with glycolaldehyde from hexose, was not detected in the first step. Therefore, only hydrolysis of glucomannan occurred.

The results of the second step (see Fig. 10) added more evidence to support the previous work by our laboratory on the pathway of cellulose hydrolysis by treatment with hot-compressed water.[15] The isomerization of glucose to mannose should be added to the proposed pathway.

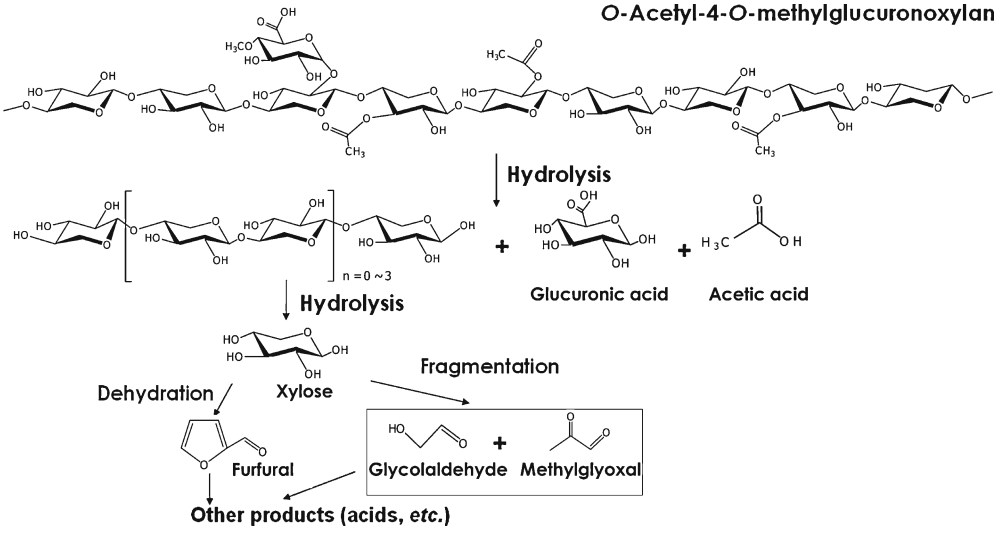

Fig. 9. Proposed pathway for *O*-acetyl-4-*O*-methylglucuronoxylan decomposition in hot-compressed water at 230°C/10 MPa for 15 min

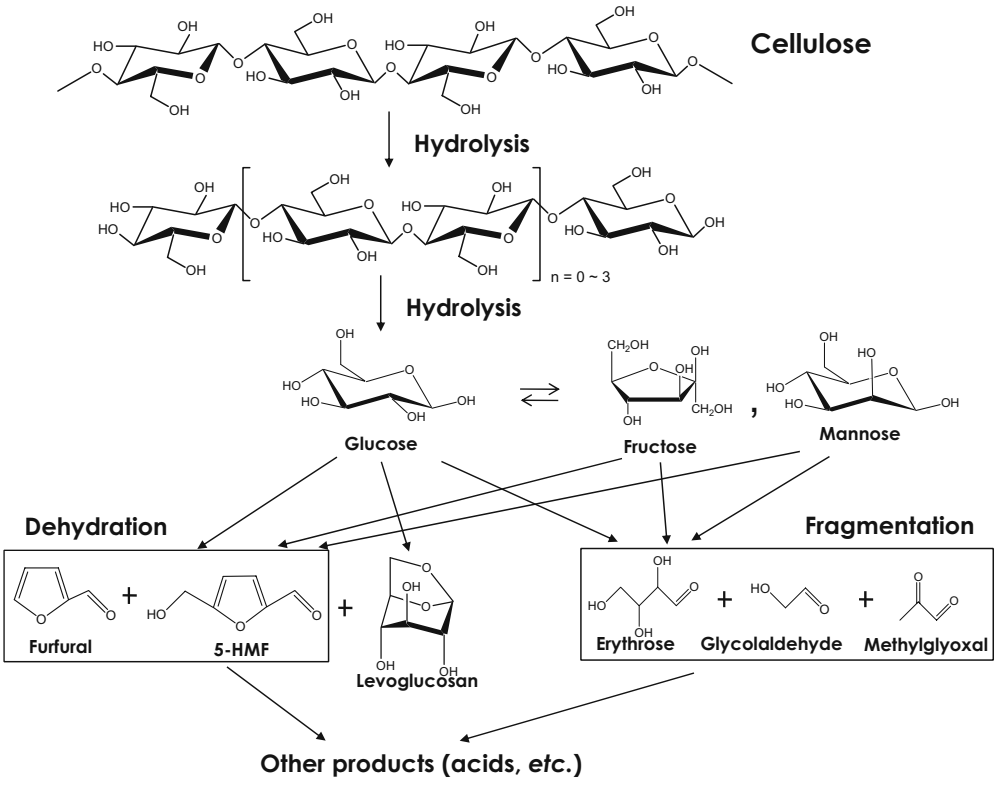

Fig. 10. Proposed pathway for cellulose decomposition in two-step treatment with hot-compressed water (230°C/10 MPa for 15 min, 270°C/10 MPa for 15 min)

Conclusions

A two-step semi-flow hot-compressed water treatment was applied to Japanese beech and it was found that, compared with a one-step method, the two-step method is very effective in providing hydrolysis products from hemicellulose and cellulose as separate product streams. In addition, this two-step method could be used as a powerful tool to study the behaviors of the respective major wood components during hot-compressed water treatment. Of particular note, the semi-flow character of the two-step treatment method allowed not only qualitative assessment but also quantitative analysis of the production of various products to gain better insights into the distribution of products produced from Japanese beech. As a result, it was elucidated that *O*-acetyl-4-*O*-methylglucuronoxylan (xylan) can be, as expected, hydrolyzed to xylose and xylo-oligosaccharides, glucuronic acid, and acetic acid. It was noted that the acetyl residues were hydrolyzed at 150°C rather than at 230°C when glucuronic acid was formed, indicating that the linkage between xylose and glucuronic acid in hemicellulose is more stable than the linkage to the acetyl residue. It was also remarkable that paracrystalline regions of cellulose were hydrolyzed readily at 230°C/10 MPa, whereas crystalline regions were hydrolyzed at 270°C/10 MPa. In addition, glucose, the hydrolysis product of cellulose, was found to be isomerized to mannose during the treatment at 270°C/10 MPa.

Lignin was unexpectedly hydrolyzed at a relatively low temperature of about 150°C to produce sinapyl alcohol and coniferyl alcohol, and the corresponding aldehydes. However, the dimeric products from lignin were all linked by condensed-type linkages, which is in good agreement with our previous study.[11] The production of 5-HMF was found to be temperature dependent and was produced only from hexose at 270°C/10 MPa, while furfural was produced from hexose and pentose. Finally, acetic acid was produced not only by hydrolysis of xylan but also from decomposed products and the propyl side chain of lignin.[29] These results shed new light on the behaviors of the wood cell wall components, and provide important information for efficient use of biomass resources for fuels and chemicals.

Acknowledgments This work was conducted as part of the NEDO project (FYs 2007–2010) "Eco-ethanol production from lignocellulosics with hot-compressed water treatment followed by acetic acid fermentation and hydrogenolysis."

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