#### **ORIGINAL ARTICLE**

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# High pressure facilitates delignification of Japanese cedar in supercritical methanol

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#### Abstract



Supercritical methanol is a tool for decomposing woody biomass and dissolving the products under relatively mild conditions. Thus, the use of supercritical methanol—in combination with catalytic hydrogenolysis—in industrial production of biochemicals has been widely studied. However, the contribution of specific methanol properties to wood decomposition remains unclear. In this study, the effect of the pressure on the decomposition of Japanese cedar in supercritical methanol was investigated in a semi-flow reactor. Delignification progressed better at higher pressure, attributable to the ability of methanol to dissolve high molecular weight, lignin-derived oligomers at high pressure. This solubility was strongly correlated with the methanol density. Methanol pressure also affected the product yields, with higher pressure resulting in higher coniferyl alcohol and sugar yields. This is attributable to the fact that the higher pressure facilitated rapid dissolution of the decomposition products and recovery from cell walls, protecting the products from further degradation. The findings of this study will contribute to the establishment of supercritical methanol in industrial production of biochemicals.

Keywords Woody biomass, Japanese cedar, Supercritical methanol, Semi-flow reactor, Methanolysis

#### Introduction

Woody biomass is one of the most promising alternatives to fossil resources, and wood-derived saccharides as well as aromatic compounds can be precursors for value-added chemicals [1]. However, the strong and complex structure of wood, which is composed of cellulose, hemicelluloses, and lignin, renders efficient conversion difficult. Dry pyrolysis is an excellent method for decomposing wood without catalysts but involves complex reactions that produce a variety of gaseous, liquid, and solid products, rendering product formation minimally selective [2, 3]. In contrast, decomposition of wood in solvents, such as supercritical fluid, has the potential to rapidly dissolve the target products and protect them from over-degradation or recondensation [4-6].

Supercritical fluids at high temperatures and pressures can decompose wood without catalysts as well as dissolve the products because of such fluids' high diffusivity (as in a gas) and solubility (as in a liquid) [7]. Supercritical fluids can change the dielectric constant and ionic product by changing the temperature as well as the pressure, enabling tuning of solvent properties [8]. Supercritical water (critical point [CP]: 374 °C and 22.1 MPa) initially attracted attention as an inexpensive and environmentally friendly solvent, but because of its high reactivity it has resulted in undesirable product degradation [9–11]. Alcohols with lower critical temperatures and pressures might be preferable for decomposing woody biomass. Alcohols with intermediate polarity provide higher solubility for aromatics than water and therefore dissolve lignin-derived products better than water, thus facilitating delignification [6, 12, 13].

Methanol (CP: 239 °C and 8.09 MPa) is a solvent with low environmental impact [14]. Supercritical methanol



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can achieve excellent wood liquefaction because of its high solubility and diffusivity [12, 13, 15, 16]. Ishikawa and Saka reported that the degradation of microcrystalline cellulose in supercritical methanol proceeded well at temperatures > 300 °C by methanolysis in a manner that produced methyl glucosides [17]. Minami and Saka reported that Japanese cedar and beech were > 90% liquefied in supercritical methanol at 350 °C for 30 min, and coniferyl alcohol as well as its  $\gamma$ -methyl ether were mainly obtained as lignin-derived monomers [12].

In recent years, biomass conversion that combines the excellent liquefaction capability of supercritical methanol with catalytic hydrogenolysis has been widely studied [18–21]. In this strategy, woody biomass is first degraded and solubilized in supercritical methanol, and then converted into saturated aliphatic-rich and low-oxygen products by catalytic hydrogenolysis. For example, Matson et al. degraded organosolv lignin in supercritical methanol at 300 °C and then hydrogenated the lignin-derived products into C2-C6 aliphatic alcohols and their methylated derivatives by using Cu-doped porous metal oxide as a catalyst at 300 °C to 320 °C and 16–22 MPa [18]. However, most of these studies focused on the mechanism and optimization of the catalytic reactions. Methanol might play an important role in depolymerization, solubilization, and interactions with products, but researchers' understanding of such solvent effects remains limited.

Unlike batch reactors, semi-flow reactors enable woodderived products to be quickly removed from the reaction system, protecting them from undesirable decomposition and recondensation [22, 23]. Semi-flow reactors are suitable for evaluating the solvent effects of methanol because they can be used to independently control the temperature, pressure, and flow rate. There are not many studies on wood decomposition with semi-flow reactors, most of which were conducted by Saka's group in subcritical water [22, 23], and there are no studies on supercritical methanol. In this study, therefore, Japanese cedar was treated with supercritical methanol in a semi-flow reactor; the effects of the pressure are discussed in detail. The treatment temperature was set at 270 °C, which mainly degrades matrix components (lignin and hemicelluloses), and cellulose to a lesser extent [12]. The present authors place special emphasis on the relationship between delignification and the methanol pressure.

#### **Materials and methods**

#### Materials

Sapwood flour of Japanese cedar (*Cryptomeria japonica*) was purchased from Nakawood (Tokushima, Japan), sieved to obtain a fraction between 100 and 35 mesh (approximately 0.15–0.5 mm in size), extracted with acetone for 4 h with a Soxhlet apparatus, and dried at

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105 °C overnight to prepare extractive-free wood flour for supercritical methanol treatment. Holocellulose was prepared from cedar wood flour by repeating the Wise method [24] seven times, and subjecting the product to supercritical methanol treatment. This holocellulose was used as a comparison to study the effect of delignification on the degradation of polysaccharides in wood cell walls. Methanol (>99.8%, guaranteed reagent grade) was purchased from Nacalai Tesque Inc. (Kyoto, Japan) and used without purification.

#### Semi-flow supercritical methanol treatment

The supercritical methanol treatment was conducted in a semi-flow reactor (Fig. 1). Approximately 150 mg of the extractive-free wood flour (or 100 mg of holocellulose) was placed in a particulate filter (SS-4TF-2, 2 µm nominal pore size; Swagelok Co., Solon, OH, USA) that was used as a sample holder. Methanol was supplied with a plunger pump (NP-KX-500; Nihon Seimitsu Kagaku Co., Ltd., Tokyo, Japan) at a flow rate of 5 mL/min; the pressure in the reaction system was controlled at 10, 20, or 30 MPa with a back-pressure valve. A coil heat exchanger (inner diameter, 1.4 mm; length, 5.9 m) to preheat the methanol was connected to the inlet side of the sample holder, and a thermocouple to measure the methanol temperature (defined as the reaction temperature) was connected to the outlet side, all of which were heated in an electric furnace (ARF-100KC; Asahi-Rika Co., Ltd., Chiba, Japan). The furnace was powered with a temperature controller (DSS83-30P085; Shimaden Co., Ltd., Tokyo, Japan). The reaction temperature was feedbackcontrolled to increase from room temperature to 270 °C at approximately 8 °C/min and then maintained at 270 °C for 30 min.

During the treatment, decomposition products from the sample dissolved in methanol flowed out of the holder; this methanol-soluble fraction was cooled in a straight heat exchanger (inner diameter, 3.0 mm; length, 390 mm) and collected at the outlet of the back-pressure valve. After the treatment, the furnace was opened and separated from the reaction system, and the sample holder was quenched by increasing the methanol flow rate to 30 mL/min. The solid residue in the holder was then collected, dried in an oven at 105 °C, and the constant mass was determined as the methanol-insoluble residue. All experiments except for holocellulose treatment were conducted in duplicate and the average of the results was reported.

#### Analytical methods

The lignin content in the insoluble residue was determined by the Klason method [25] as the sum of Klason lignin and acid-soluble lignin. The contents of cellulose





Fig. 1 Scheme of the semi-flow reactor

and hemicelluloses in the insoluble residue were estimated based on the constituent monosaccharides in the hydrolysates, obtained by lignin determination. The glucose/mannose ratio of glucomannan was assumed to be 1/4 [26]. The hydrolysates were analyzed by highperformance anion-exchange chromatography (HPAEC) with a Prominence system (Shimadzu Corp., Kyoto, Japan) and an electrochemical detector (DECADE Elite, Antec Scientific, Zoeterwoude, The Netherlands) under the following conditions: column, CarboPac PA-1 (250mm analysis column connected with a 50 mm guard column; Thermo Fisher Scientific Inc., Waltham, MA, USA); eluent, 30 mM aqueous sodium hydroxide; flow rate, 1.0 mL/min; column temperature, 35 °C.

Because the methanol-soluble fraction contained methylated mono- and oligosaccharides (as discussed below), the saccharide yield was reported as the monosaccharide yield after hydrolysis. This yield was determined by HPAEC analysis after methanol was evaporated, 4 wt% aqueous sulfuric acid was added, and the result was hydrolyzed in an autoclave at 121 °C.

Gel permeation chromatography (GPC) and highperformance liquid chromatography (HPLC) were performed with an LC-20A system (Shimadzu Corp.) for the methanol-soluble fraction under the following conditions. GPC: column, Shodex KF-803, KF-802.5, KF-802, and KF-801 (Resonac Holdings Corp., Tokyo, Japan) in series; flow rate, 0.6 mL/min; eluent, tetrahydrofuran; detector, ultraviolet (UV), 280 nm; column temperature, 50 °C. HPLC: column, Cadenza CD-C18 (Imtakt Corp., Kyoto, Japan); eluent, methanol/water=20/80 to 100/0 (50 min); flow rate, 1.0 mL/min; detector, UV, 280 nm; column temperature, 40 °C. Heteronuclear single quantum coherence (HSQC) spectroscopy was performed with a Varian AC-400 spectrometer (400 MHz, Varian Medical Systems, Inc., Palo Alto, CA, USA) to analyze the lignin-derived oligomers in the methanol-soluble fraction. To remove the monomeric compounds, methanol was evaporated from the soluble fraction, which was fractionated with ethyl acetate and water. Next, the ethyl acetate was evaporated from the ethyl acetate fraction, washed with *n*-hexane, dissolved in deuterated dimethyl sulfoxide, and then subjected to HSQC analysis.

Gas chromatography–mass spectrometry (GC–MS) was conducted with a QP2010 Ultra (Shimadzu Corp.) for the methanol-soluble fraction after trimethylsilylation under the following conditions: column, CPSil 8CB (Agilent Technologies Inc., 30 m length, 0.25 mm diameter, 0.25  $\mu$ m thickness); injection temperature, 250 °C; split ratio, 10/1; column temperature, 100 °C (2 min), 4 °C/min to 220 °C, 220 °C (2 min), 15 °C/min to 300 °C, 300 °C (2 min); carrier gas, hydrogen; flow rate, 37.4 mL/min. For trimethylsilylation, the soluble fraction was dried under vacuum after addition of 1,3-diphenoxybenzene as an internal standard and treated with hexamethyldisilizane (150  $\mu$ L), trimethylchlorosilane (80  $\mu$ L), and pyridine (100  $\mu$ L) at 60 °C for 30 min.

Matrix-assisted laser desorption ionization-timeof-flight mass spectrometry (MALDI-TOF/MS) was performed to analyze oligosaccharides in the methanolsoluble fraction with an AXIMA Performance (Shimadzu Corp.) under the following conditions: linear mode; acceleration voltage, 20 kV; pulsed laser, 200  $\mu$ J per shot. Matrix and sodium solutions were prepared in accordance with the present authors' previous study [27].

#### **Results and discussion**

#### Decomposition behavior of cell wall components

Figure 2 shows the composition of the methanol-insoluble residue after supercritical methanol treatment of Japanese cedar at 270 °C in comparison with untreated wood. Cellulose was the most stable cell wall component, with ca. 80% or more of the original cellulose remaining in the residue; its degradation was most suppressed at 30 MPa. Hemicelluloses were degraded and solubilized to some extent, but ca. 60% remained in the residue with little pressure dependence. Lignin was the most degraded and solubilized of the three components. There was more delignification at higher pressures, with ca. 2/3 of the lignin decomposed and solubilized at 30 MPa. Because cellulose remained the highest at this pressure, the resulting residue was rich in polysaccharides (82%).

In the hot-compressed water treatment of Japanese cedar by Takada et al. [28], hemicelluloses were almost degraded and solubilized, whereas lignin tended to remain in the residue; this showed the opposite trend to the treatment using supercritical methanol. This might be because methanol better solubilizes lignin-derived products than water, whereas the reactivity of polysaccharide methanolysis is lower than that of hydrolysis. Note that the Hildebrand solubility parameters [29] for water, methanol, and coniferyl alcohol were estimated with a process simulator (Pro/II 2022; Schneider Electric SE, Rueil-Malmaison, France) to be 23.4, 14.5, and 12.7, respectively. Because compounds with close solubility parameters tend to dissolve in each other, it is obvious that methanol can dissolve lignin-derived products more readily than water.

In addition, our previous study with a batch reactor reported that supercritical methanol treatment of Japanese cedar at 270 °C and 27 MPa yielded similar polysaccharide-rich residues [12], although the pressure could not be controlled. The current experiments with the semi-flow reactor indicate that this favorable delignification is because of the high pressure of ca. 30 MPa.

Figure 3 shows GPC chromatograms of the methanolsoluble fractions. The chromatograms were acquired with a UV (280 nm) detector and thus approximate the molecular weight distribution of lignin-derived products that undergo UV absorption. The molecular weights were distributed from the monomer range to > 3000 in polystyrene equivalents, with the distribution shifting to the high molecular weight side as the methanol pressure increases. This result suggests that the higher delignification at higher pressure is because of the dissolution of higher molecular weight, lignin-derived products. The physical property of methanol that is directly related to pressure is its density. Methanol densities at 270 °C were reported to be 157, 453, and 524 kg/m<sup>3</sup> at 10, 20, and 30 MPa [30], respectively; indicating that methanol



**Fig. 3** GPC chromatograms of the methanol-soluble fractions after supercritical methanol treatment of Japanese cedar at various pressures (UV detection at 280 nm)



Fig. 2 Composition of the methanol-insoluble residue after supercritical methanol treatment [room temperature to 270 °C (8 °C/min) and at 270 °C for 30 min] of Japanese cedar at various pressures (values in parentheses are percentages of the composition of untreated wood)

is gaseous at 10 MPa and nearly liquid at 20–30 MPa. The present authors assumed that the higher density of methanol dissolved and transported more lignin-derived products outside the cell walls, and this will be discussed further in a subsequent paragraph.

For comparison, Fig. 4 shows the results for Japanese cedar holocellulose. In the untreated holocellulose, 4.6% lignin remained, and, probably because of its small quantity, the trend of lignin degradation was not clear. As mentioned above, methanolysis of polysaccharides in supercritical methanol is slower than hydrolysis in subcritical water, and therefore we expected less degradation of polysaccharides even in delignified samples. However, cellulose and hemicelluloses in holocellulose degraded and solubilized significantly better than those in wood. This difference is probably not only because of the effect of delignification; there was also some delignification in wood during supercritical methanol treatment (Fig. 2). In addition, Ishikawa and Saka reported that temperatures >300 °C were required for efficient degradation of cellulose (microcrystalline cellulose, Avicel) in supercritical methanol [17]. Therefore, the higher degradation of polysaccharides in holocellulose than in wood at 270 °C might be because of chemical changes induced by oxidative delignification with sodium chlorite (NaClO<sub>2</sub>) in the Wise method. For example, Ono et al. reported that NaClO<sub>2</sub> treatment increased the number of carboxyl groups in the residual lignin fraction [31]. Although working under slightly different conditions, Matsuki et al. reported that aqueous sodium hypochlorite (NaClO) treatment produced carboxyl groups on the surface of cellulose crystals by oxidative cleavage at the C2 and C3 positions [32]. There might have been such structural changes during preparation of the holocellulose and might have contributed to the degradation of polysaccharides by supercritical methanol treatment, but at present there are no data to enable full discussion of this point. The use of holocellulose with chemical modifications may not be appropriate to discuss the effects of delignification and cell wall nanostructures.

#### Characterization of lignin-derived products

As described in the experimental method section, the methanol-soluble fraction is the products that have passed through the filter of the sample holder under high temperature and high pressure conditions. Therefore, precipitates may form at room temperature and ambient pressure. In the case of subcritical water, lignin-rich precipitates quickly formed in the soluble fraction [28]. However, in the case of supercritical methanol in this study, no precipitates were observed. Figure 5 shows the HSQC spectra of lignin-derived oligomers in the methanol-soluble fraction obtained from Japanese cedar. In these spectra,  $\beta$ -O-4, pinoresinol, and phenylcoumaran structures (A, B, C in Fig. 6, respectively) were identified; these are also found in milled wood lignin [33]. There was little difference between the spectra at 10 and 30 MPa, indicating that the favorable delignification at 30 MPa was simply because of the dissolution of high molecular weight products.

Our study reported that the yield of alkaline nitrobenzene oxidation products from insoluble residues decreased with increasing supercritical methanol treatment time [12]. In dimeric lignin model compounds, the condensed-type bonds were reported to be stable, whereas the phenolic  $\beta$ -ether bond was homolytically cleaved to yield coniferyl alcohol [34, 35]. The nonphenolic  $\beta$ -ether bond was also cleaved, but was less reactive



Fig. 4 Composition of the methanol-insoluble residue after supercritical methanol treatment [room temperature to 270 °C (8 °C/min) and at 270 °C for 30 min] of holocellulose of Japanese cedar at various pressures (values in parentheses are percentages of the composition of untreated holocellulose)

#### (a) 10 MPa



Fig. 5 HSQC spectra of lignin-derived oligomers obtained from supercritical methanol treatment of Japanese cedar at 10 and 30 MPa

than the phenolic bond [34]. These previous studies and the present results suggest that lignin depolymerization proceeds mainly by cleavage of the  $\beta$ -ether bonds, and then, of the resulting oligomers, those soluble in supercritical methanol leach from cell walls. Because the dissolved oligomers were rapidly transported out of the reaction system, further  $\beta$ -ether bond cleavage was probably infrequent. Thus, lignin oligomers that have retained relatively original lignin structures A, B, and C are thought to be obtained.

However, the present authors detected unique structures that were generated in supercritical methanol. One of them is structure A', in which the  $\alpha$ -hydroxyl group is replaced with a methoxy group. This structure was also reported in supercritical methanol treatment of the nonphenolic  $\beta$ -*O*-4 dimer model [34]. Similarly, substitution of the  $\alpha$ -hydroxyl group with an ethoxy group was reported in studies of organosolv lignin with ethanol [36, 37]. The present authors also detected structure F, in which the  $\gamma$ -hydroxyl group is methylated at the coniferyl alcohol units (structure E). Tsujino et al. reported formation of  $\gamma$ -methyl ether from free coniferyl alcohol in supercritical methanol and explained this conversion via the quinonemethide pathway [34].

Regarding the hydroxyl groups in the lignin-derived oligomers, methylation was only at the benzyl (C $\alpha$ ) and conjugated allyl (C $\gamma$  in the coniferyl alcohol units) positions. These results provide insight into the methylation mechanisms by S<sub>N</sub>1-type substitution because of the carbocation stability of these conjugated carbons (Fig. 7). Such S<sub>N</sub>1 reactions would be favored by the acidic nature of supercritical methanol. However, there is no direct evidence to support this hypothesis in the current study.

Figure 8 shows GC/MS chromatograms of the methanol-soluble fractions. The major peaks are coniferyl alcohol **15** and its  $\gamma$ -methyl ether 7. Coniferyl alcohol is the primary product of the homolytic  $\beta$ -ether cleavage of lignin, which can be easily converted into  $\gamma$ -methyl ether in supercritical methanol [12]. The  $\gamma$ -methyl ether might also have been formed prior to  $\beta$ -ether cleavage



Fig. 6 Substructures of lignin-derived oligomers determined by HSQC-NMR analysis



**Fig. 7** Possible reactions for the formation of  $\alpha$ - and  $\gamma$ -methyl ether structures

(Fig. 6). Typical pyrolysis products of coniferyl alcohol (such as isoeugenol **2**, dihydroconiferyl alcohol **9**, and coniferyl aldehyde **10**) were also detected [**38**, **39**], but at lower levels. Because of rapid transport of products out of the reaction system in the semi-flow reactor, the major monomers formed from lignin were coniferyl alcohol and its  $\gamma$ -methyl ether, whereas further degradation of these was limited.

Monomers derived from cellulose and hemicelluloses were also detected, and all were methylated monosaccharides except for D-xylose, indicating that the depolymerization of polysaccharides in the supercritical methanol treatment was mainly by methanolysis, as reported in previous studies [12, 17]. However, the quantities of these monomers were small, indicating that most of the polysaccharide-derived products were recovered as oligosaccharides in the case of the semi-flow reactor because of the short residence time.

### Changes in molecular weight distribution with treatment time

The semi-flow reactor enabled real-time collection of the methanol-soluble fractions during the supercritical methanol treatment. Figure 9c shows the GPC chromatograms of methanol-soluble fractions (mainly ligninderived products as per the UV detector) collected at 3-min intervals during supercritical methanol treatment of Japanese cedar at 10 and 30 MPa. Figure 9a and



Fig. 8 GC–MS chromatograms of the methanol-soluble fractions obtained from supercritical methanol treatment of Japanese cedar at various pressures

b shows changes in temperature and methanol density, respectively, over this time.

At 150 °C, lignin degradation already started and signals were observed in the low molecular weight region, but there was no difference between 10 and 30 MPa until ca. 220 °C (No. 1–3). During this period, the density difference of methanol between 10 and 30 MPa was small (Fig. 9b). When the temperature exceeded 220 °C, a difference in molecular weight distribution was evident, and the 30 MPa treatment tended to contain larger molecular

weight products (No. 4–5). With increasing temperature, the difference became larger, especially in the high molecular weight region; and when the temperature reached 270 °C, the 30 MPa treatment dissolved additional high molecular weight products (No. 6–7). The difference in methanol density began to increase from ca. 220 °C, and the difference at 270 °C was large. This density change closely corresponded to the change in molecular weight distribution, strongly suggesting that the higher density corresponded to higher solubility for



Fig. 9 a Change in methanol density, b change in treatment temperature, and c GPC chromatograms of the methanol-soluble fractions after supercritical methanol treatment of Japanese cedar at 10 and 30 MPa

high molecular weight products. Lignin degradation continued after the temperature reached 270  $^{\circ}$ C (No. 8–16), and the intensity of the high molecular weight region was always stronger at 30 MPa compared with 10 MPa. A similar plot was made for the yields of major ligninderived monomers (coniferyl alcohol, its  $\gamma$ -methyl ether, and isoeugenol) (Fig. 10). Coniferyl alcohol and its  $\gamma$ -methyl ether increased rapidly from ca. 200 °C



Fig. 10 Changes in the 1-min yields of major lignin-derived monomers from supercritical methanol treatment of Japanese cedar at various pressures

and 230 °C, respectively; whereas, isoeugenol was evident after the temperature reached 270 °C. Presumably, the cleavage of phenolic  $\beta$ -ether bonds (the most reactive) began at ca. 200 °C in a manner that formed coniferyl alcohols; their conversion to  $\gamma$ -methyl ethers requires slightly higher temperatures, and the pyrolysis of coniferyl alcohol and its  $\gamma$ -methyl ether to isoeugenol requires ca. 270 °C.

The internal volume, *V*, from outside the sample holder filter to the inlet of the cooling heat exchanger was 0.91 mL (Fig. 1). The average residence time, *t*, from the solubilization of the products to the cooler was calculated by using the methanol flow rate, *F* (5 mL/min); the ordinary methanol density,  $\rho_0$  (792 kg/m<sup>3</sup>); and the methanol density in the supercritical state,  $\rho_s$  (157 kg/m<sup>3</sup> for 10 MPa and 524 kg/m<sup>3</sup> for 30 MPa at 270 °C), as follows: paragraphs, the lower solubility at 10 MPa probably enabled these monomers to remain in the cell walls long time, resulting in more coniferyl alcohol degradation. The 20 and 30 MPa conditions exhibited relatively similar results because of the small difference in methanol density.

Note that if UV-absorbing furfural and 5-hydroxymethylfurfural were formed from saccharides, it would affect the results of this GPC analysis. We believe that the effect of these furan compounds would be limited because only trace amounts of furfural and 5-HMF were detected by HPLC. This is probably due to the rapid recovery of the products in the semi-flow reactor, which can suppress the over-degradation of saccharides.

$$t = \frac{V}{F \times \frac{\rho_0}{\rho_s}} = 0.036 \min (10 \text{ MPa}) \text{ or } 0.12 \min (30 \text{ MPa}) \text{ at } 270 \,^{\circ}\text{C}.$$

The 10 MPa condition had a shorter residence time in the reaction system, but in both cases the residence time was brief. In previous studies at 270 °C, formation of  $\gamma$ -methyl ether from coniferyl alcohol required 2–3 min [34], yet formation of isoeugenol required ca. 5 min [40]; thus, these would not be produced during such short residence times. It is possible that some of the produced coniferyl alcohol, as well as the lignin oligomers, was not immediately dissolved in methanol and remained in cell walls where degradation continued. Comparing 10 and 30 MPa,  $\gamma$ -methyl ether and isoeugenol were evident slightly earlier at 10 MPa. This can be explained by the delayed dissolution of coniferyl alcohol because of its low solubility at 10 MPa.

Figure 11 summarizes the total yields of these ligninderived monomers. At 10 MPa, the yield of coniferyl alcohol was the lowest and those of  $\gamma$ -methyl ether and isoeugenol were the highest. As discussed in previous

#### Characterization of polysaccharide-derived products

The typical monosaccharide products were methyl glycosides, such as methyl xylosides (Fig. 8). However, 270 °C was somewhat low for polysaccharide methanolysis, resulting in large quantities of polysaccharides remaining in the insoluble residue (Fig. 2). The yields of methyl glycosides were low and typical pyrolysis products, such as levoglucosan and furfural, were not detected. Therefore, it is likely that the semi-flow reactor with a short residence time did not enable degradation into monomers and the products were mainly oligosaccharides.

MALDI-TOF/MS analysis was attempted to detect the oligosaccharides, but this method was not easy, unlike oligosaccharides produced by hot-compressed water treatment [27]. Whenever detected, oligosaccharides were observed that seemed to be methylated. As an example, Fig. 12 shows a MALDI-TOF/MS spectrum of the methanol-soluble fraction from Japanese cedar



Fig. 11 Yields of major lignin-derived monomers from supercritical methanol treatment of Japanese cedar at various pressures



Fig. 12 MALDI/TOF-MS spectrum of oligosaccharides obtained from supercritical methanol treatment of holocellulose of Japanese cedar at 30 MPa

holocellulose, with possible oligosaccharide structures based on mass-to-charge ratios. Note that oligosaccharides were detected as sodium-added ions (+23) because sodium solution was used.

The signals in the 132 m/z interval with 32 (OCH<sub>3</sub>+H) and 23 (Na) added were considered to be pentosans methylated at the reducing end. The signals of methyl glucuronic acid (+190) or methyl ester of methyl glucuronic acid (+204) added to the methyl pentosans were also observed. These oligosaccharides might be derived from arabino-4-O-methylglucuronoxylan as per methanolysis, and some methyl glucuronic residues were methyl esterified. Methylated hexosans from cellulose or acetylgalactoglucomannan, which are thought to be similarly produced, were not detected.

The methanol-soluble fractions were hydrolyzed with dilute sulfuric acid; Fig. 13 shows the resulting yields of the constituent neutral sugars. Because hexoses were detected, it is likely that hexosans were also present in the methanol-soluble fraction, although they were not detected by MADLI-TOF/MS. Regarding Japanese cedar (Fig. 2), ca. 10% to 20% of the cellulose and ca. 40% of the hemicelluloses were degraded and solubilized with little pressure dependence, but the sugar yield (Fig. 13) increased with increasing pressure. The reason for this result might be that, as with the lignin-derived products, higher methanol pressure resulted in higher solubility and faster recovery of oligosaccharides, thus suppressing over-degradation of sugars. However, sugar yields were low at all pressures, and most polysaccharides remained in the residue because of low reactivity for methanolysis at 270 °C. Holocellulose was degraded and solubilized better than wood (Fig. 4), with ca.  $\geq$  50% of the cellulose and  $\geq$  80% of the hemicelluloses degraded, and thus the sugar yield tended to be ca. twice that of wood. Regarding holocellulose, the percentages of galactose and arabinose decreased compared with wood. Because there was



Fig. 13 Yields of constituent neutral sugars in the methanol-soluble fractions obtained by supercritical methanol treatment of Japanese cedar and its holocellulose

no substantial difference in the constituent sugar composition of Japanese cedar and its holocellulose samples, it is possible that the galactose and arabinose residues were more susceptible to over-degradation in supercritical methanol, but the reason of this over-degradation is unclear. Sugar yield also increased with increasing pressure for holocellulose, but there was no substantial difference between 20 and 30 MPa.

#### Conclusions

This study, by using a semi-flow reactor, reveals the effect of pressure on the decomposition of wood in supercritical methanol. First, higher methanol pressure resulted in better delignification. Correspondingly, higher pressure resulted in higher molecular weight lignin-derived oligomers dissolved in methanol. The change in molecular weight distribution with treatment time indicates that the difference in molecular weight distribution with pressure corresponded well to the change in methanol density. From these facts, this study concludes that higher density methanol can dissolve higher molecular weight oligomers, resulting in favorable delignification.

Second, methanol pressure also affected the yield of lignin-derived monomer products, with higher pressure yielding more coniferyl alcohol and lower pressure yielding more coniferyl alcohol  $\gamma$ -methyl ethers as well as their degradation products. This fact was explained by the higher pressure enabling more coniferyl alcohol to be rapidly dissolved and released from the cell walls, protecting the coniferyl alcohol from further

degradation. Cellulose and hemicelluloses were degraded by methanolysis, and recovered mainly as methylated oligosaccharides in the semi-flow reactor. The higher pressure tended to increase the sugar yield, which was also explained by the rapid dissolution as well as the ligninderived products.

In conclusion, pressurizing methanol to increase its density in the semi-flow reactor facilitated dissolution and recovery of the products, and could suppress undesirable over-decomposition.

#### Abbreviations

<u>I</u> P	Critical point
HPAEC	High-performance anion-exchange chromatography
GPC	Gel permeation chromatography
HPLC	High-performance liquid chromatography
JV	Ultraviolet
ISQC	Heteronuclear single quantum coherence
GC–MS	Gas chromatography-mass spectrometry
MALDI-TOF/MS	Gas chromatography-mass spectrometry

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#### Author contributions

YY and EM designed this study, designed and fabricated the semi-flow reactor, and evaluated the experimental data; YY performed the supercritical methanol treatment experiments and chemical analyses of the products, and drafted the manuscript; EM and HK supervised this study and reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

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#### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

#### Declarations

#### **Competing interests**

The authors declare that they have no competing interests.

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