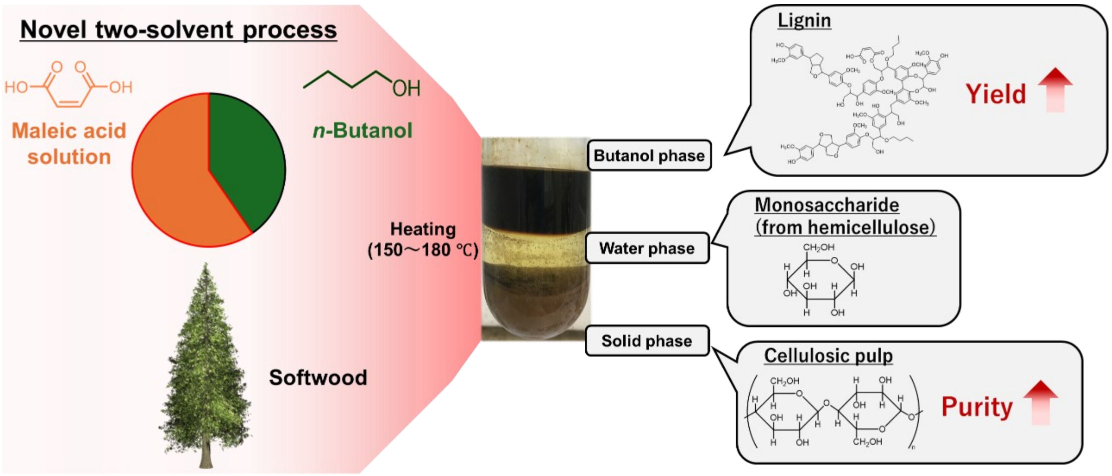
Fractionation of Softwood Lignin and Polysaccharides By *n*-Butanol/Maleic Acid Two-Solvent Process

Takumi Yamada, Yuki Tokunaga, and Hiroshi Nonaka,*

**Corresponding author: nonaka@bio.mie-u.ac.jp*

DOI: 10.15376/biores.20.4.9653-9666

GRAPHICAL ABSTRACT



Fractionation of Softwood Lignin and Polysaccharides By *n*-Butanol/Maleic Acid Two-Solvent Process

Takumi Yamada, Yuki Tokunaga, and Hiroshi Nonaka,*

The organosolv method, using water and *n*-butanol with acid catalysts, separates biomass into three phases: *n*-butanol, aqueous, and solid, which primarily contain lignin, hemicellulose, and cellulose, respectively. In this study, maleic acid was applied as a catalyst in the water/*n*-butanol method to avoid using inorganic acids. Comparable or better component separation was achieved relative to using sulfuric acid as a catalyst. Furthermore, by using a high concentration of maleic acid, instead of a catalytic amount, lignin was extracted from softwood with a high yield (more than 97 wt.% lignin extracted) under the condition at 180 °C for 1 hour with approximately 10 wt% maleic acid. Additionally, the reaction temperature and time were varied to achieve better lignin fractionation and functionalization. The highest total fractionation extent and well-carboxylated lignin were obtained at 150 °C. The chemical properties of lignin were also analyzed, and their structure and molecular weight were investigated.

DOI: 10.15376/biores.20.4.9653-9666

Keywords: Lignin extraction; Maleic acid; Organosolv method; Lignin; n-Butanol; Pulp

Contact information: Graduate School of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie 514-8507, Japan; **Corresponding author: nonaka@bio.mie-u.ac.jp*

INTRODUCTION

Humanity must reduce its dependence on limited fossil fuel resources, which will eventually be depleted (Langeveld *et al*. 2011). The use of wood, which is primarily composed of cellulose, hemicellulose, and lignin, is essential for building a sustainable society. Numerous studies have focused on separating these components to enable their effective utilization.

The organosolv method has potential as a pretreatment for biorefinery applications, as it allows high-yield recovery of cellulosic pulp and lignin, as well as enabling relatively easy recycling of solvents (Vaidya *et al*. 2022). In the organosolv method, hemicellulose is generally decomposed, and lignin is depolymerized and solubilized in the solvent while protecting its structure. The organosolv method uses a variety of solvents, concentrations (30 to 100 wt.%), reaction temperatures (100 to 250 °C), and times (30 to 60 min), with or without catalysts (Vaidya *et al*. 2022; Rabelo *et al*. 2023). Among these, low-boiling-point alcohols such as methanol and ethanol are the most commonly used due to their low energy requirements for recovery and complete miscibility with water (Zhao *et al*. 2009). For example, treatment with 60 to 80% methanol-water and a hydrochloric acid catalyst has been reported to remove approximately 75% of lignin from pine wood (softwood) and up to 90% from beech wood (hardwood) (Zhao *et al*. 2009). Those methanol or ethanol systems have efficient delignification in hardwood and herbaceous plants. However, delignification of softwood remains challenging (Kalogiannis *et al*. 2020; Vaidya *et al*. 2022; Rabelo *et al*. 2023). For that problem, a recent study using the ethanol/water treatment of softwood with iron (III) chloride hexahydrate as a catalyst, a cellulose purity of 85% and a lignin yield of 74% have been achieved (Parot *et al.* 2022). However, these methods have challenges such as lignin redeposition onto the pulp surface due to incomplete separation of major biomass components, which reduces enzymatic saccharification efficiency (Teramoto *et al*. 2008; Amiri *et al.* 2015; Donohoe *et al.* 2008).

n-Butanol uniformly mixes with water during the high temperature reaction to form a lignin extraction solvent and then separates from water at room temperature (Kawamata *et al.* 2021). In addition, *n*-butanol is a sustainable chemical because it can be produced from glucose *via* fermentation by *Clostridium acetobutylicum* (Ezeji *et al.* 2007). Extraction methods using *n*-butanol typically separate lignin in the upper *n*-butanol phase from sugars in the lower water phase, including non-catalytic, acid catalytic, and microwave treatments (Kawamata *et al.* 2019, 2021; Schmetz *et al.* 2019; Rivas *et al.* 2021; Ishimaru *et al.* 2024). Since *n*-butanol separates from water, unlike low-boiling-point alcohols, it is expected to inhibit lignin redeposition. However, these methods have a limitation in that lignin separation is less effective in softwoods (approximately 80% of lignin remains in the residue). The corrosive properties and strong microbial inhibitory effects of inorganic acid are also concerns with its use as a catalyst.

Various studies have investigated the use of organic acids as catalysts with low corrosivity. For example, oxalic acid is reported to exhibit lignin extraction efficiency comparable to or greater than that of sulfuric acid when used as a catalyst in ethanol-based organosolv treatment (Sar *et al*. 2022). Maleic acid cooking has also gained attention as a way to effectively promote biomass delignification (Ma *et al.* 2022). Other study compared the effectiveness of weak acid hydrolysis using sulfuric acid, citric acid, maleic acid, lactic acid, succinic acid, acetic acid, and malic acid and found that, maleic acid was particularly effective (Risanto *et al.* 2023). These acids are not only known to cleave the β-*O*-4 bond in lignin but also to form ester bonds with the hydroxyl groups at the Cα and Cγ positions of lignin (Villaverde *et al.* 2009). For example, the treatment of lignocellulose with high concentrations of maleic acid has been reported to enhance the enzymatic saccharification efficiency of pulp and promote mechanical fiberization for production of microfibers and nanofibers (Su *et al*. 2021). This effect is attributed to the esterification of hydroxyl groups in cellulose and lignin by maleic acid, which induces a negative charge in both lignin and cellulose, thereby inhibiting the non-productive adsorption of cellulase onto lignin or generating electrostatic repulsion between fibers (Su *et al*. 2021; Bian *et al*. 2017). The important point is that maleic acid is a bivalent organic acid and can effectively aid in the extraction of lignin.

A recent study reported a pretreatment method for enhancing the enzymatic hydrolysis of cellulose, involving maleic acid and *n*-butanol, and succeeded in removing a high amount of lignin (Postiaux *et al*. 2025). However, the extracted lignin has not yet been focused on. This study combined *n*-butanol and maleic acid to develop novel two organic solvent-based processes. First, maleic acid was used as the acid catalyst in the water/*n*-butanol process, including high concentrations of maleic acid (> 10 wt.%) for the effective extraction and functionalization of softwood lignin. Second, the reaction temperature and time were varied to achieve optimal lignin fractionation and functionalization. The fundamental properties of the extracted lignin, including functional groups, molecular weight distribution, and chemical structure, were used to assess its potential for valorization.

EXPERIMENTAL

Materials

Japanese cedar wood (*Cryptomeria japonica*) flour (178 µm pass) as a softwood was purchased from NAKAWOOD Co., Ltd. (Tokushima, Japan) (Table 1). Maleic acid, *n*-butanol, diethyl ether, D(-)-ribose, D(+)-glucose, D(+)-xylose, D(+)-mannose, D(+)-galactose, D(-)-arabinose, L(+)-arginine, tetrahydrofuran (THF, stabilizer free), phosphoric acid, acetone-d_6 (99.9 %, 0.05 vol% TMS), and dimethyl sulfoxide (DMSO)-d_6 (99.9%, 0.05 vol% TMS) were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). All reagents were special grade, except D(+)-glucose, which was reagent grade. Sulfuric acid (72%) was prepared from 95% sulfuric acid (Wako, Special Grade), saturated barium hydroxide solution (EXTRA PURE) was prepared from barium hydroxide octahydrate (Nacalai Tesque, Inc., Kyoto, Japan, EXTRA PURE REAGENT), bisphenol A was obtained from Tokyo Chemical Industry Co., Ltd. (TCI, Tokyo, Japan), polystyrene standard (M_W 2,000) was obtained from Pressure Chemical Co. (Pennsylvania, U.S.), polystyrene standard (M_W 25,000, 50,000, 200,000, and 400,000) was obtained from Chemco Scientific Co., Ltd. (Osaka, Japan), polystyrene standard (Shodex STANDARD M_W 3,790 and 7,000) was obtained from Resonac Holidings Crop. (Tokyo, Japan), and mono-disperse polystyrene standard (M_W 100,000) was obtained from AMCO Inc. (Tokyo, Japan).

Table 1. Composition of Cedar Wood Flour Used in this Study (wt.%)

Lignin	Glucan	Mannan	Xylan	Galactan	Arabinan
34.7	41.4	7.3	4.6	0.9	0.9

Two-solvent Treatment

Twelve grams of cedar wood flour (air-dried) were placed in a stainless-steel pressure-resistant container (TPR1 TVS-N2, Taiatsu Techno Corp., Tokyo, Japan). *n*-butanol (80 mL) and maleic acid (2.4, 16, or 32 g dissolved in 80 mL of deionized water) were added to TPR1. The *n*-butanol-to-water ratio was based on the method reported by Kawamata *et al*. (Kawamata *et al.* 2021). The mixture was heated to 150, 160, 170, or 180 °C from room temperature while stirring and held for 0.25, 0.5, or 1 h (Table 2). During the reaction, a homogeneous phase of mixed solvents was formed because of stirring and boiling mixed solvents under reaction temperatures. The solution was allowed to cool to room temperature. The mixture was centrifuged at 2330 g for 5 min, and the *n*-butanol phase was recovered using a pipette in an eggplant flask for vacuum concentration. The concentrated *n*-butanol phase was added dropwise to diethyl ether with cooling and stirring, and the insoluble fraction was recovered as lignin. The lignin was then washed with diethyl ether until the washing solution became clear. The water phase and solid phase were separated *via* filtration using glass fiber filter paper (Whatman^{TM} GF/A, Cytiva, Tokyo, Japan), and the solid residue was washed with 400 mL of deionized water and dried at 105 °C over 12 h. The extraction process is illustrated in Fig. 1. The yield of the solid residue from the raw biomass was calculated using Eq. (1). The weight of the solid residues was used in their absolute dry states, and the weight of the initial biomass was converted to absolute dry states using the water content. These reactions were performed two times, and the results are presented as the mean value. As a control, the same procedure was carried out using 80 mL of 1.5 wt% sulfuric acid aqueous solution and 80 mL of *n*-butanol.

$$\text{Yield of solid residue (\%)} = \frac{\text{Weight of solid residues}}{\text{Weight of initial biomass}} \times 100 \qquad (1)$$

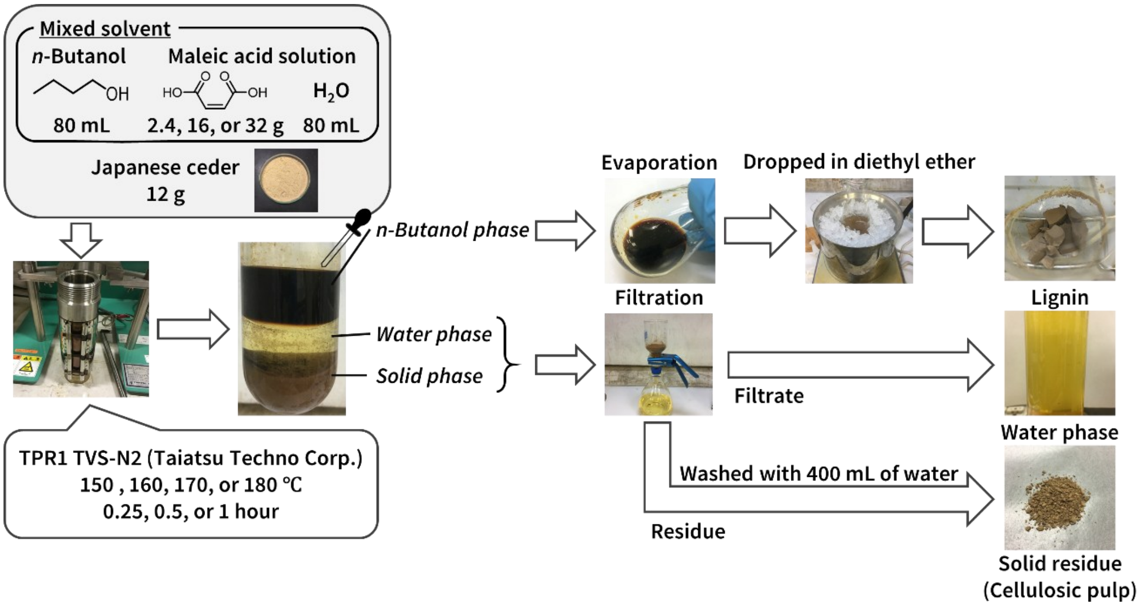


Fig. 1. Three-component fractionation with *n*-butanol/maleic acid two-solvent process

Table 2. Experimental Conditions

Experiment (Exp.)	Amount of Maleic acid (g)	Reaction Temperature (°C)	Reaction Time (h)
0	1.2 (H_2SO_4)	180	1
1	2.4	180	1
2	16	180	1
3	32	180	1
4	16	150	1
5	16	160	1
6	16	170	1
7	16	180	0.25
8	16	180	0.5

※ Water 80 mL and *n*-butanol 80 mL were used in every experiment.

Compositional Analysis of Solid Residues and Water Phase

The sugar contents of raw biomass (Table 1) and solid residues (Table 4) were determined using a two-step sulfuric acid hydrolysis method (72% and 4%) based on the protocol published by NREL (Sluiter *et al.* 2012). First, 72% sulfuric acid was added to 0.3 g of the dry sample and allowed to react for 1 h at 30 °C. Next, the reaction mixture was diluted to 4% sulfuric acid with deionized water and reacted in an oil bath at 121 °C for 1 h. After hydrolysis, 0.03 g of ribose was added as an internal standard, and the reaction mixture was filtered. The residue was quantified as acid-insoluble lignin using Eq. (2). The lignin extracted extent was calculated using the lignin content in the solid residue, the yield of the solid residue, and the lignin content in the initial biomass, as shown in Eq. (3).

$$\text{Acid insoluble lignin (\%)} = \frac{\text{Weight of acid insoluble fraction}}{\text{Weight of initial solid residue}} \times 100 \qquad (2)$$

$$\text{Lignin extraction extent (\%)} = \left(1 - \frac{\text{Yield of solid residue} \times \text{Lignin content in solid residue}}{100 \times \text{Lignin content in initial biomass}}\right) \times 100 \qquad (3)$$

Sugars in the filtrate (glucose, mannose, xylose, galactose, and arabinose) were quantified using an internal standard method. The filtrate was neutralized with a saturated barium hydroxide solution and subjected to post-column fluorescence detection of reducing sugars using high-performance liquid chromatography with arginine (Mikami *et al.* 1983). Decomposition of sugars has not been corrected.

Fourier Transform Infrared Spectroscopy (FT-IR) of Lignin

The FT-IR spectra (650 to 4000 cm^{-1}) of the lignin fractions were collected using an FT/IR-4X (JASCO Corp., Tokyo, Japan) equipped with an ATR Diamond (Specac Ltd., Orpington, UK). The measurement mode was transmittance, and 16 scans were collected. The samples were vacuum-dried before analysis.

Solubility of Lignin in THF

THF (3 mL) was added to 50 mg of lignin sample and stirred with a vortex shaker (VORTEX Genius 3, IKA Works, NC, USA) and filtered with a glass microfiber filter (Whatman^{TM} GF/A, particle retention capacity 1.6 µm), and washed with 17 mL THF. The filter was air-dried and oven-dried at 105 °C overnight. The solubility of the lignin in THF was calculated based on the weight of the insoluble fraction. The filtrate was used for size-exclusion chromatography (SEC).

SEC of Lignin

Lignin dissolved in THF was prepared for the solubility tests. SEC was performed on a Shimadzu (Kyoto, Japan) Class LC-20 system with a UV detector (280 nm) and KF801, KF802, KF803, and KF804 in series (Shodex, Resonac Corp., Tokyo, Japan), using THF (0.015 mol/L phosphoric acid) as the eluent at a flow rate of 1.0 mL/min. A calibration curve was obtained using polystyrene standard (M_w = 400,000, 200,000, 100,000, 50,000, 25,000, 7,000, 3,790, and 2,000), bisphenol A (M_w = 228), and *p*-cresol (M_w = 108) (Shiraki *et al.* 2021).

Nuclear Magnetic Resonance (NMR) Spectroscopic Analysis of Lignin

The 2D ^{1}H-^{13}C heterogeneous single quantum correlation (HSQC) NMR spectra of the lignin samples were analyzed using a JNM-EC500R (500 MHz NMR, JEOL Co., Ltd., Tokyo, Japan). Three representative lignin samples from experiments (Exps.) 1, 2, and 4 were dried under reduced pressure, and approximately 60 mg of each was dissolved in 700 µL of acetone-d_6 (Exp. 1) or DMSO-d_6 (Exps. 2, 4).

RESULTS AND DISCUSSION

Tables 3 and 4, respectively show the yields of sugars in water phase and the solid residue to initial biomass, and the composition of solid residue in each reaction condition. Figure 2 shows the yields of solid residue and lignin extraction extents. For control, the result for the reaction using sulfuric acid (1.2 g) as a catalyst is also shown (H_2SO_4). In addition, second-order regression curves are provided to illustrate the trends of yield and lignin extraction extent in Fig. 2 (a), (b), and (c).

Table 3. Yields of Sugars in Water Phase and Solid Residue in Each Reaction Condition (wt%)

Experiment	Glucose	Mannose	Xylose	Galactose	Arabinose	Solid Residue
0	1.4 ± 1.1	0 ± 0.0	0.1 ± 0.1	0 ± 0.0	0 ± 0.0	37.8 ± 0.0
1	2.8 ± 1.7	3.5 ± 0.2	2.4 ± 0.1	0.9 ± 0.0	0.6 ± 0.0	44.9 ± 0.2
2	13.2 ± 4.2	1.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	26.8 ± 0.1
3	12.3 ± 0.9	1.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	16.3 ± 1.3
4	5.3 ± 2.2	5.9 ± 1.6	5.5 ± 2.0	1.6 ± 0.5	1.2 ± 0.3	60.9 ± 0.6
5	5.1 ± 0.1	4.5 ± 0.0	3.1 ± 0.1	1.1 ± 0.0	0.7 ± 0.0	46.5 ± 1.5
6	5.8 ± 2.1	2.7 ± 1.1	1.0 ± 0.9	0.5 ± 0.4	0.4 ± 0.1	36.6 ± 1.4
7	6.8 ± 0.4	3.5 ± 0.1	2.0 ± 0.0	0.8 ± 0.1	0.5 ± 0.0	34.4 ± 0.7
8	8.4 ± 0.1	2.7 ± 0.2	1.2 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	31.4 ± 0.3

Table 4. Composition of Solid Residue in Each Reaction Condition (wt%)

Experiment	Glucan	Mannan	Xylan	Galactan	Arabinan	Lignin
0	64.7	0.2	0.0	0.0	0.0	29.8
1	72.3	0.8	0.6	0.0	0.0	9.0
2	78.2	0.9	0.7	0.0	0.0	3.3
3	60.9	0.4	0.6	0.0	0.0	17.9
4	65.1	1.2	0.6	0.0	0.0	25.3
5	68.7	0.0	0.0	0.0	0.0	14.3
6	83.8	0.7	0.2	0.0	0.0	7.6
7	62.7	0.0	0.0	0.0	0.0	12.3
8	82.9	0.7	0.2	0.0	0.0	7.4

In reactions using sulfuric acid catalysts, the lignin extraction extent was approximately 67%, which was higher than the results reported by Schemtz *et al*. (2019) using sulfuric acid catalysts in water/*n*-butanol treatment. The improvement of lignin extraction extent may be attributed to the increased proportion of *n*-butanol (Schemtz *et al*. 2019; Kawamata *et al*. 2021). Compared to sulfuric acid, maleic acid resulted in a higher lignin extraction extent (88.4%) (Fig. 2 (a)) and higher purity of cellulose, as shown in Table 4. A recent study, focused on the delignification of softwood, reported a maximum cellulose purity of 85% under the most favorable conditions (Parot *et al*. 2022). In contrast, the present study has already exceeded this value under conditions using only a catalytic amount of maleic acid. This is attributed to the synergistic effect of maleic acid and *n*-butanol as two-solvent.

Then, the effect of maleic acid concentration was examined at 180 °C and 1 h, as shown in Table 4, where less hemicellulose remained in the solid residue regardless of the maleic acid concentration. Table 3 shows that the yield of hemicellulose-derived sugars, such as mannose and xylose, decreased with increasing maleic acid concentration, suggesting that higher acid strength leads to the decomposition of monosaccharides. The maximum glucose yield in the aqueous phase was at 16 g of maleic acid (Exp. 2), indicating that further increases in maleic acid concentration resulted in reduced yields (Exp. 3), as the enhancement of the loss was due to monosaccharide decomposition and structural changes bonding with maleic acid or *n*-butanol rather than an increase due to cellulose hydrolysis (Lancefield *et al*. 2017). The extraction extent of lignin was enhanced by increasing the amount of maleic acid from 2.4 g to 16 g. This was due to cellulose hydrolysis, which enabled the extraction of lignin present in deeper internal regions, and the cleavage of β-*O*-4 bonds in lignin also made it easier to extract (Lee *et al.* 2014). However, increasing the amount of maleic acid to 32 g decreased the lignin extraction extent. This indicates that 16 g of maleic acid was sufficient for lignin extraction, consistent with the solid residue yield reported for maleic acid/*n*-butanol pretreatment (Postiaux *et al.* 2025). Using 16 g of maleic acid led to a lignin content of only 3.3% in the solid residue, which is remarkably low compared to previous studies (Kawamata *et al.* 2021; Schmetz *et al.* 2019).

Next, regarding the effects of reaction temperature (Table 3, Table 4, and Fig. 2 (b)) and time (Table 3, Table 4, and Fig. 2 (c)), higher reaction temperatures and longer reaction times led to increased hydrolysis of cellulose, decomposition of monosaccharides, and enhanced lignin extraction. This was consistent with the general tendency of the organosolv treatments (Agnihotri *et al*. 2015; Vaidya *et al*. 2022). At 150 °C (Exp. 4), hemicellulose was sufficiently hydrolyzed, and the decomposition of monosaccharides was slow, resulting in the highest yield of hemicellulose-derived sugars in the aqueous phase. The resulting pulp had a lignin content of 25.3%, which was still relatively high, but the yield was 60.9% (Table 3), indicating that most of the cellulose was recovered in the solid. Lignin was extracted at an extent of 55.2%.

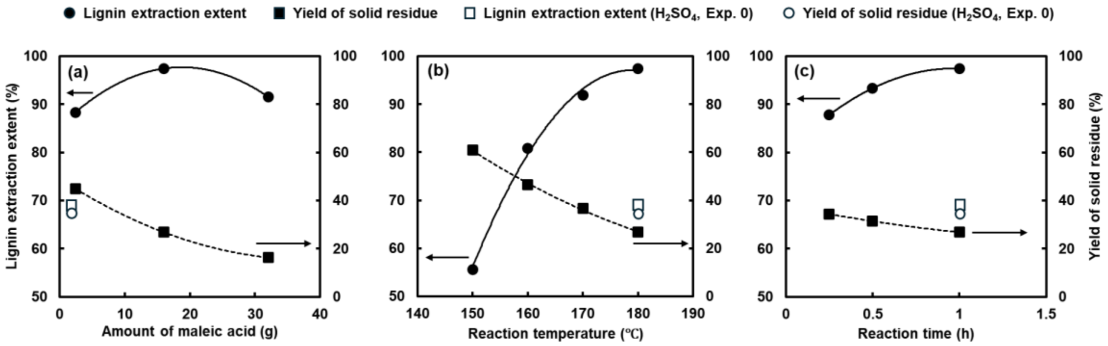


Fig. 2. Yields of solid residues and lignin extraction extent in each reaction condition. Effect of (a) maleic acid concentration (180 ℃, 1 h), (b) reaction temperatures (maleic acid 16 g, 1 h), and (c) reaction times (maleic acid 16 g, 180 ℃)

The FT-IR analysis of each lignin fraction showed a peak corresponding to the C=O stretching vibration in carbonyl or ester groups at approximately 1720 cm^{-1} (Yang *et al.* 1996; Ma *et al.* 2022) in most samples (Fig. 3). These peaks were shifted toward the longer wavelength side compared to the C=O stretching of maleic acid, indicating that they were esterified. These peaks were particularly strong for lignin samples of Exps. 3 and 4. This may indicate that the esterification of lignin and maleic acid was the most promoted under these two conditions. In Exp. 3, the amount of used maleic acid was high, suggesting that maleic acid was introduced to a greater extent. In contrast, analysis of Exp. 4 demonstrated that ester bonds in lignin are less susceptible to hydrolysis at 150 °C than at 180 °C or other temperatures and that the ester bonds formed are more likely to be maintained. The peak near 1150 cm^{-1} corresponds to the C-O bond of the ethers (Hu *et al.* 2014). This is relatively large in Exp. 1 and small in Exp. 3. This was due to the relative retention of ether bonds, such as the β-*O*-4 linkage of lignin under low acid concentration conditions, while higher acid concentrations promote their cleavage.

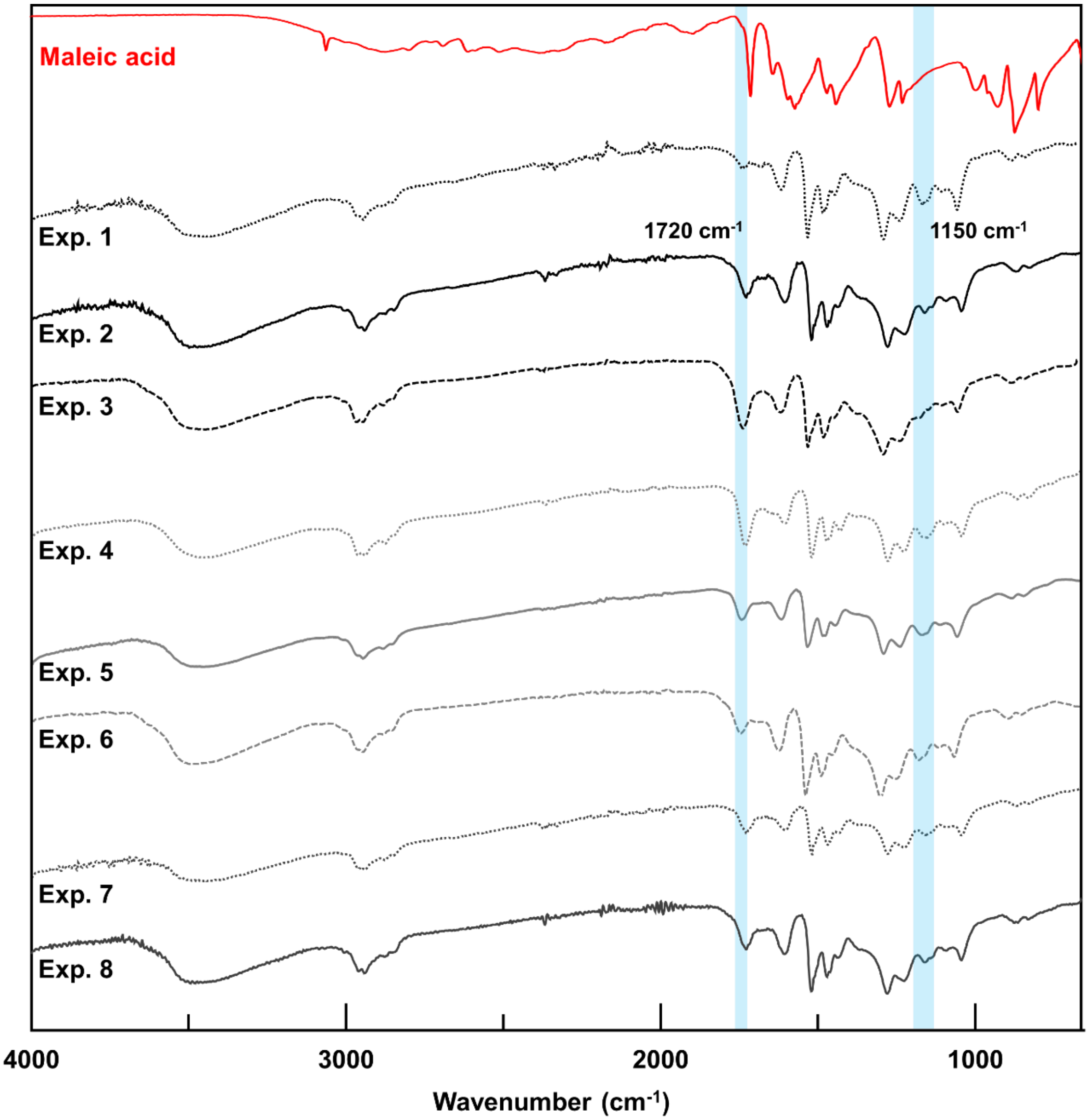


Fig. 3. FT-IR spectra of maleic acid and lignin recovered in each reaction condition

Most of the lignin obtained in this study was more than 96% dissolved in THF. The weight average molecular weight (M_w), number average molecular weight (M_n), and yield of ether insoluble lignin are provided in Fig. 4. First, the effect of different maleic acid concentrations on lignin was examined; a lower concentration of maleic acid (Exp. 1) resulted in a lower lignin molecular weight of approximately M_w = 3,000 (Fig. 4 (a)) and lower polydispersity index (PDI), M_w/M_n = 1.7. This is because the high molecular weight fraction was not extracted, and there was less self-condensation, due to the low acid concentration. In Exp. 2, after reaction at 180 °C for 1 h with 16 g of maleic acid, the delignification extent reached 97.5% (Fig. 2), suggesting that high-molecular-weight lignin was extracted. Furthermore, it is possible that self-condensation increased the molecular weight. When more maleic acid (32 g) was added (Exp. 3), the M_w decreased to approximately 4,000. According to the FT-IR results, this reduction can be attributed to the extensive cleavage of ether bonds (*e.g.* β-*O*-4) induced by high maleic acid concentrations.

Regarding the effect of different reaction temperatures, the relatively high molecular weight observed at lower reaction temperatures, such as from Exp. 4, was due to the preservation of ester and ether linkages, as indicated by the FT-IR results. This was in agreement with the findings reported by Grojzdek *et al*. for organosolv treatment using ethanol (Grojzdek *et al*. 2025). No clear relationship exists between the reaction time and molecular weight of the obtained lignin.

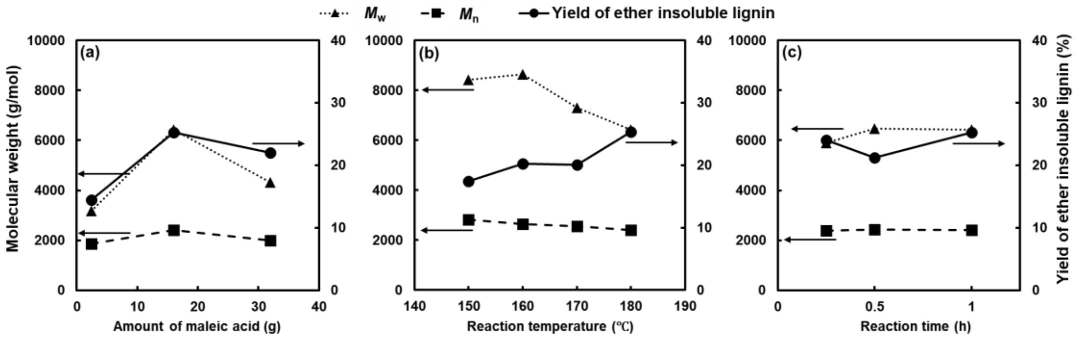


Fig. 4. Calculated weight average molecular weight (M_w), number average molecular weight (M_n) of lignin using SEC analysis, and yield of ether insoluble lignin. Effect of (a) maleic acid concentration (180 ℃, 1 h), (b) reaction temperatures (maleic acid 16 g, 1 h), and (c) reaction times (maleic acid 16 g, 180 ℃)

Figure 5 shows the 2D HSQC NMR spectra of the lignin samples of Exps. 1, 2, and 4. Exp. 1 uses the catalytic amount of maleic acid, Exp. 2 was selected as representative because it had the highest lignin extraction extent, and Exp. 4 was chosen for its highest degree of carboxylation. The spectra are shown in three regions: (I) $\delta_\mathrm{C}/\delta_\mathrm{H}$ = 40.0-110.0/2.0-8.0, (II) $\delta_\mathrm{C}/\delta_\mathrm{H}$ = 100.0-140.0/6.0-8.0, and (III) $\delta_\mathrm{C}/\delta_\mathrm{H}$ = 0.0-50.0/0.0-2.0.

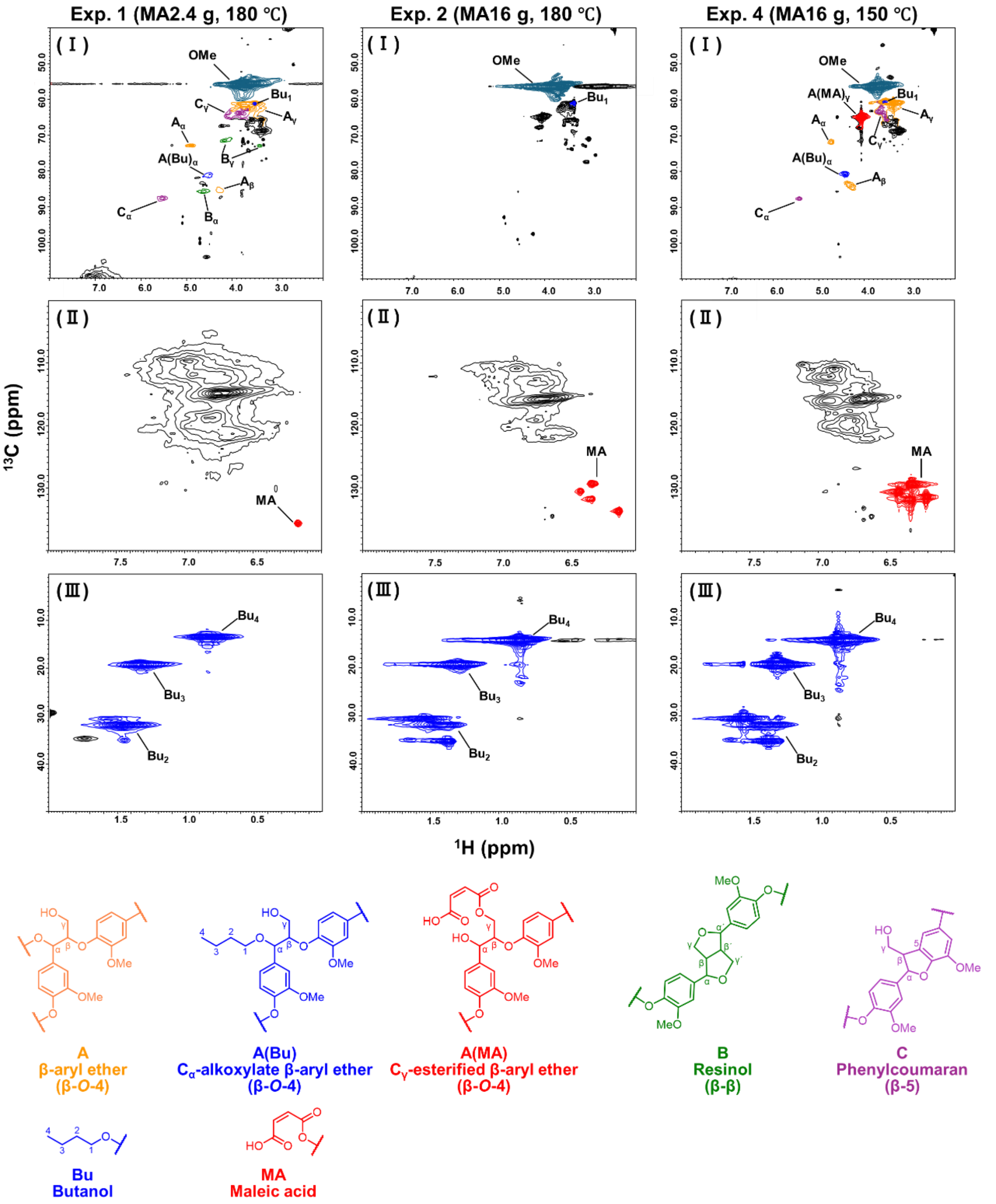


Fig. 5. ^{1}H-^{13}C 2D HSQC NMR spectra of lignin sample from Exps. 1, 2, and 4. (I) δ_C/δ_H = 40.0–110.0/2.0–8.0, (II) δ_C/δ_H = 100.0–140.0/6.0–8.0, (III) δ_C/δ_H = 0.0–50.0/0.0–2.0)

First, in the aliphatic side chain region (I), signals corresponding to β-*O*-4 type structures are apparent in Exp. 1 (δ_C/δ_H = 73.1/4.9 ppm, α-position; δ_C/δ_H = 85.4/4.3 ppm, β-position; δ_C/δ_H = 61.4/3.5 ppm, γ-position) and Exp. 4 (δ_C/δ_H = 71.9/4.7 ppm, α-position; δ_C/δ_H = 84.0/4.3 ppm, β-position; δ_C/δ_H = 60.5/3.5 ppm, γ-position), but not in Exp. 2. This suggests that the β-*O*-4 linkages are mostly cleaved under high maleic acid concentration and high reaction temperature, approximately 180 °C. Other signals of β-β (δ_C/δ_H = 85.8/4.6

ppm, α-position; δ_C/δ_H = 71.5/4.1 and 72.6/3.4 ppm, γ-position) and β-5 (δ_C/δ_H = 87.7/5.5 ppm, α-position; δ_C/δ_H = 63.9/3.8 ppm, γ-position) structures are seen in Exp. 1. In addition, signals corresponding to the alcoxylated β-*O*-4 type α position are seen in Exp. 2 (δ_C/δ_H = 80.9/4.5 ppm) and Exp. 4 (δ_C/δ_H = 80.7/4.4 ppm), indicating that *n*-butanol was introduced at the α-position of β-*O*-4 type structures (Wu *et al*. 2014). In Exp. 4, a signal corresponding to the esterified γ-position of the β-*O*-4 structure (δ_C/δ_H = 65.0/4.1 ppm) is strongly observed, suggesting that the ester bond is difficult to break once introduced in the reaction at 150 °C (Su *et al*. 2021). In contrast, as seen in Fig. 5 Exp. 2, most of the signals could not be assigned, indicating that the lignin was highly denatured under Exp. 2.

Next, in the region of aromatic and unsaturated hydrocarbon (II), the signals for the aromatic (G-unit) of lignin and unsaturated C=C binding sites of maleic acid were identified in all, and the strongest signal intensity of maleic acid is seen in Exp. 4. This indicates that more maleic acid was introduced into the lignin under Exp. 4.

Finally, in the aliphatic region related to *n*-butanol (III), signals of *n*-butanol ($Bu_{2,3,4}$) can be seen in all. As mentioned above, under Exps. 1 and 4, *n*-butanol was introduced at the α-position of β-*O*-4. In contrast, under Exp. 2, the signal of β-*O*-4 could not be confirmed, but the signal of *n*-butanol was also present in (III) under Exp. 2, so *n*-butanol was introduced at an undetermined point. Understanding the structures of these processed lignin remains a topic of continued scientific interest.

CONCLUSIONS

1. In organosolv processes, sulfuric acid is typically used as the acid catalyst. The water/*n*-butanol method is equally or more effective at extracting lignin from softwood when using maleic acid as the acid catalyst, compared to using sulfuric acid.
2. The lignin extraction extent was highest, approximately 97%, using an increased concentration of maleic acid (16 g of maleic acid, 80 mL of *n*-butanol, and 80 mL of water) at 180 °C for 1 h, indicating that this new two organic-solvent system with *n*-butanol/maleic acid was efficient for delignification and lignin recovery.
3. When this solvent combination is used with a reaction temperature of 150 °C, the lignin extraction extent is lower than at 180 °C, but lignin with a high esterification extent can be recovered. Due to the slow decomposition of sugars, monosaccharides and pulp can be recovered in high yields.
4. The recyclability of the solvent system warrants further investigation to enhance the feasibility of industrial implementation.

ACKNOWLEDGMENTS

This research was funded by the Konno and Restar Foundation.

REFERENCES CITED

Agnihotri, S., Johnsen, I. A., Bøe, M. S., Øyaas, K., and Moe, S. (2015). "Ethanol organosolv pretreatment of softwood (*Picea abies*) and sugarcane bagasse for biofuel and biorefinery applications," *Wood Sic. Technol.* 49, 881-896. DOI: 10.1007/s00226-015-0738-4

Amiri, H., and Karimi, K. (2015). "Improvement of acetone, butanol, and ethanol production from woody biomass using organosolv pretreatment," *Bioprocess Biosyst. Eng*. 38, 1959-1972. DOI: 10.1007/s00449-015-1437-0

Bian, H., Chen, L., Dai, H., and Zhu, J. Y. (2017). "Effect of fiber drying on properties of lignin containing cellulose nanocrystals and nanofibrils produced through maleic acid hydrolysis," *Cellulose* 24, 4205-4216. DOI: 10.1007/s10570-017-1430-7

Donohoe, B., Decker, S. R., Tucker, M. P., Himmel, M. E., and Vinzant, T. B. (2008). "Visualizing lignin coalescence and migration through maize cell walls following thermochemical pretreatment," *Biotechnol. Bioeng.* 101(5), 913-925. DOI: 10.1002/bit.21959

Ezeji, T. C., Qureshi, N., and Blaschek, H. P. (2007). "Bioproduction of butanol from biomass: from genes to bioreactors," *Curr. Opin. Biotechnol.* 18, 220-227. DOI: 10.1016/j.copbio.2007.04.002

Grojzdek, E. J., Kozmelj, T. R., Tofani, G., Segers, B., Nimmegeers, P., Billen, P., Pogorevc, R., Likozar, B., and Grilc, M. (2025). "Design of organosolv lignin fractionation: Influence of temperature, antisolvent, and source on molecular weight, structure, and functionality of lignin fragments," *ACS Sustainable Chem. Eng.* 13, 3452-3466. DOI: 10.1021/acssuschemeng.4c08125

Hu, J., Shen, D., Wu, S., Zhang, H., and Xiao, R. (2014). "Composition analysis of organosolv lignin and its catalytic solvolysis in supercritical alcohol," *Energy & Fuels* 28, 4260-4266. DOI: 10.1021/ef500068h

Ishimaru, H., Yoshikawa, T., Higuchi, Y., Nakasaka, Y., Yoshida, A., Sonoki, T., and Masuda, T. (2024). "Direct extraction of biphasic organosolv lignin for producing aromatic aldehydes *via* alkaline oxidation," *J. Taiwan. Inst. Chem. E.* 158, article 105009. DOI: 10.1016/j.jtice.2023.105009

Kalogiannis, G. K., Karnaouri, A., Michailof, C., Tzika, M. A., Asimakopoulou, G., Topakas, E., and Lappas, A. A. (2020). "OxiOrganosolv: A novel acid free oxidative organosolv fractionation for lignocellulose fine sugar streams," *Bioresour. Technol.* 313, article 123599. DOI: 10.1016/j.biortech.2020.123599

Kawamata, Y., Yoshikawa, T., Aoki, H., Koyama, Y., Nakasaka, Y., Yoshida, M., and Masuda, T. (2019). "Kinetic analysis of delignification of cedar wood during organosolv treatment with a two-phase solvent using the unreacted-core model," *Chem. Eng. J.* 368, 71-78. DOI: 10.1016/j.cej.2019.02.103

Kawamata, Y., Yoshikawa, T., Koyama, Y., Ishimaru, H., Ohtsuki, S., Fumoto, E., Sato, S., Nakasaka, and Y., Masuda, T. (2021). "Uniqueness of biphasic organosolv treatment of soft- and hardwood using water/1-butanol co-solvent," *Ind. Crops. Prod.* 159, article 113078. DOI: 10.1016/j.indcrop.2020.113078

Lancefield, C. S., Panovic, I., Deuss, P. J., Barta, K., and Westwood, N. J. (2017). "Pre-treatment of lignocellulosic feedstocks using biorenewable alcohols: Towards complete biomass valorisation," *RSC* 19, 202-214, DOI: 10.1039/c6gc02739c

Langeveld, H., Sanders, J., and Meeusen, M. (2011). "The biobased economy: Materials and chemicals in the post-oil era," *Fuel* 90, article 2076.

Lee, A. R., Berberi, V., Labranche, J., and Lavoie, J. M. (2014). "Lignin extraction - Reassessment of the severity factor with respect to hydroxide concentration," *Bioresour. Tech.* 169, 707-712. DOI: 10.1016/j.biortech.2014.07.038

Ma, S., Chen, B., Zeng, X., Li, Z., Tang, X., Sun, Y., Lin, L., and Zeng, X. (2022). "Chemical structure change of lignin extracted from bamboo biomass by maleic acid," *Int. J. Bio. M.* 221, 986-993. DOI: 10.1016/j.ijbiomac.2022.09.002

Mikami, H., and Ishida, Y. (1983). "Post-column detection of reducing sugars in high performance liquid chromatography using arginine," *BUNSEKI KAGAKU.* 32, E207-E210. DOI: 10.2116/bunsekikagaku.32.6_E207

Parot, M., Rodrigue, D., and Stevanovic, T. (2022). "High purity softwood lignin obtained by an eco-friendly organosolv process," *Bioresource Technol. Rep.* 17, article 100880. DOI: 10.2139/ssrn.3942524

Postiaux, A., Putra, F. J. N. P., Kahar, P., Richel, A., and Ogino, C. (2025). "Maleic acid-butanol pretreatment to enhance cellulose accessibility for enzymatic hydrolysis and ethanol production from oil palm empty fruit bunch," *ACS Environ.* 5, 76-85. DOI: 10.1021/acsenvironau.4c00045

Rabelo, C. S., Nakasu, S. Y. P., Scopel, E., Araújo, F., M., Cardoso, H. L., and Costa, D. C. A. (2023). "Organosolv pretreatment for biorefineries: Current status, perspectives, and challenges," *Bioresource Technol.* 369, article 128331. DOI: 10.1016/j.biortech.2022.128331

Risanto, L., Adi, D.T.N., Fajriutami, T., Teramura, H., Fatriasari, W., Hermiati, E., Kahar, P., Kondo, A., and Ogino, C. (2023). "Pretreatment with dilute maleic acid enhances the enzymatic digestibility of sugarcane bagasse and oil palm empty fruit bunch fiber," *Bioresource Technol.* 369, article 128382. DOI: 10.1016/j.biortech.2022.128382

Rivas, S., López, L., Vila, C., and Parajó, J. C. (2021). "Organosolv processing of vine shoots: Fractionation and conversion of hemicellulosic sugars into platform chemicals by microwave irradiation," *Bioresource Technol.* 342, article 125967. DOI: 10.1016/j.biortech.2021.125967

Sar, T., Arifa, V. H., Hilmy, M. R., Ferreira, J. A., Wikandari, R., Millati, R., and Taherzadeh, M. J. (2022). "Organosolv pretreatment of oat husk using oxalic acid as an alternative organic acid and its potential applications," *Biomass Convers. Biorefin.* 10, DOI: 10.1007/s13399-022-02408-1

Schmetz, Q., Teramura, H., Morita, K., Oshima, T., Richel, A., Ogino, C., and Kondo, A. (2019). "Versatility of a dilute acid/butanol pretreatment investigated on various lignocellulosic biomasses to produce lignin, monosaccharides and cellulose in distinct phases," *ACS Sustainable Chem. Eng.* 7 (13), 11069-11079. DOI: 10.1021/acssuschemeng.8b05841

Shiraki, Y., Goto, T., and Nonaka, H. (2021). "Concentrated sulfuric acid hydrolysis of softwood with *t*-butyl alcohol," *Biomass Conv. Bioref.* 11, 937-941. DOI: 10.1007/s13399-019-00594-z

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2012). *Determination of Structural Carbohydrates and Lignin in Biomass* (NREL/TP-510-42618), National Renewable Energy Laboratory, Golden, CO, USA.

Su, C., Hirth, K., Liu, Z., Cao, Y., and Zhu, J.Y. (2021). "Acid hydrotropic fractionation of switchgrass at atmospheric pressure using maleic acid in comparison with *p*-

TsOH: Advantages of lignin esterification," *Ind. Crops. Prod.* 159, article 113017. DOI: 10.1016/j.indcrop.2020.113017

Su, C., Wang, X., Hirth, K., Arvanitis, M., Cao, Y., Fang, G., and Zhu, J. Y. (2025). "Bioactive lignin from maleic acid hydrotropic fractionation: Revealing the structural-bioactivity relationship," *Int. J. Biol. Macromol.* 302, article 140519. DOI: 10.1016/j.ijbiomac.2025.140519

Teramoto, Y., Lee, S. H., and Endo, T. (2008). "Pretreatment of woody and herbaceous biomass for enzymatic saccharification using sulfuric acid-free ethanol cooking," *Bioresour. Technol*. 99(18), 8856-8863. DOI: 10.1016/j.biortech.2008.04.049

Vaidya, A. A., Murton, D. K., Smith, A. D., and Dedual, G. (2022). "A review on organosolv pretreatment of softwood with a focus on enzymatic hydrolysis of cellulose," *Biomass. Convers. Bior.* 12, 5427-5442. DOI: 10.1007/s13399-022-02373-9

Villaverde, J. J., Li, J., Ek, J., Ligero, P., and Vega, A. D. (2009). "Native lignin structure of *Miscanthus* x *giganteus* and its changes during acetic and formic acid fractionation," *J. Agric. Food Chem*. 57, 6262-6270. DOI: 10.1021/jf900483t

Wu, M., Pang, J., Zhang, X., and Sun, R. (2014). "Enhancement of lignin biopolymer isolation from hybrid poplar by organosolv pretreatments," *Int. J. Polym. Sci.* 2014, article 194726. DOI: 10.1155/2014/194726

Yang, C. Q., Xu, Y., and Wang, D. (1996). "FT-IR spectroscopy study of the polycarboxylic acids used for paper wet strength improvement," *Ind. Eng. Chem. Res.* 35, 4037-4042. DOI: 10.1021/ie960207u

Zhao, X., Cheng, K., and Liu, D. (2009). "Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis," *Appl. Microbiol. Biotechnol.* 82, 815-827. DOI: 10.1007/s00253-009-1883-1

Article submitted: April 28, 2025; Peer review completed: May 30, 2025; Revisions accepted: September 5, 2025; Published: September 15, 2025.
DOI: 10.15376/biores.20.4.9653-9666