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Characterization of Eucalyptus Lignin Fractionation from a MIBK-Based Solvothermal Process

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Abstract

In this study, the effects of sulfuric acid on cellulose yield, lignin removal, and lignin recovery in solvothermal fractionation of eucalyptus (EC) were studied. An acid concentration of 0.04 M sulfuric acid (H_2SO_4), temperature of 180 °C and residence time of 30 min resulted in maximum lignin removal from the solid phase, at 87.7 %. Lignin recovery in the organic phase under optimum conditions was 84.6 %. It should be noted that H_2SO_4 was the best catalyst in the optimal solvothermal process, and it increased the cellulose yield to 95.2 % in the solid phase. Additionally, the physicochemical and structural properties of the extracted lignin were analyzed using FTIR, TGA, elemental analysis, GPC, and Py-GCMS methods. Thermal degradation analysis showed that recovered lignin is primarily composed of syringyl, guaiacyl and p-hydroxyphenyl units cross-linked by C–C, inter-unit α-O-4, β-O-4 linkages. The weight average molecular weight (Mw) analysis of recovered lignin demonstrated a low molecular weight for recovered lignin (2.19 g/mol). However, the main phenolic derivatives in the extracted lignin obtained from EC were S-units (i.e., syringol, 4-methylsyringol, 4-vinylsyringol). In addition, G-units (4-vinylguaiacol, 4-methylguaiacol, phenol, 2-methylphenol, and 4-methylphenol) were obtained after release from H-units. Py-GCMS analysis showed the predominance of G-units (32.8 %) over S-units (57.4 %). This work demonstrated the potential of fractionated lignin in the production of valuable chemicals in biorefineries.

Keywords: Eucalyptus, Biomass material, Lignin fractionation, Solvothermal process, Optimization

Introduction

Owing to its ready availability, biomass is a promising bioresource in that it can be converted into biomaterials, biofuels, and biochemicals [1]. Generally, biomass materials from agricultural waste residues or economic crops consist mainly of 35 - 50 % cellulose, 20 - 35 % hemicellulose, and 10 - 25 % lignin [2]. Typically, the chemical content of lignin is of the softwood type (24 - 33 %), hardwood type (19 - 28 %), and grass type (15 - 25 %). In terms of structure, lignin can comprise a 3-dimensional amorphous polymer and an amorphous aromatic polymer. The natural structure of lignin consists of aliphatic and aromatic hydroxyl groups and 3 basic phenylpropanoid monomers, such as the p-coumaryl alcohol unit, coniferyl alcohol unit, and sinapyl alcohol unit [3]. In addition, the structure of lignin has various cross-links, including those involving aryglycerol-β-ether dimer (β-O-4), aryglycerol-α-ether dimer (α-O-4), siaryl ether (4-O-5), resinol (β-5), diphenylethane (β-1), phenylcoumaran (β-β′), phenylcoumaran (β-β), and biphenyl (5–5) [4].

Some lignin content is converted into different value-added products, such as phenol, aromatic sources, and vanillin. Moreover, most phenolic compounds can be produced via lignin extraction from biomass materials. In addition, phenolic compounds can also be used as precursors for the plastic, bioplastic, and phenol-formaldehyde production processes in the chemical and petrochemical industries [5]. The previously studied organosolv fractionation process was selected to fractionate eucalyptus (EC) wood chips with the ternary phase solvent mixture containing methyl isobutyl ketone (MIBK)/methanol/water (25:42:33 v/v%) with 0.008 M sulfuric acid (H_2SO_4) promoter at 180 °C for 60 min [6]. The results showed that a lignin solubility in the organic fraction of up to 218 % was obtained, along with a high purity of 92 % [7]. Lignin fractionation by the organosolv fractionation process from

bagasse, pararubber wood sawdust, palm fiber, and cassava fiber was studied. Under optimal conditions, the solvent mixture consisted of methyl isobutyl ketone:ethanol:water (0.25:0.42:0.33 v/v%). It should be noted that an H_2SO_4 concentration of 0.025 M showed the highest (89.8 %) lignin recovery in the organic phase, and a 16.9 % fraction of a phenolic compound was identified through Py-GCMS. Moreover, the highest lignin removal efficiencies, 88.2, 67.3 and 71.7 %, were found for bagasse, pararubber wood sawdust, palm fiber and cassava fiber, respectively.

This research is designed to isolate lignin from fractionation of EC using a solvothermal fractionation-based organosolv process under optimal conditions. Moreover, solid residue and isolated lignin were characterized using different techniques. Characterization techniques used to investigate the basic structure and physicochemical and thermal decomposition properties included Fourier transform infrared spectrophotometry (FTIR), thermogravimetric analysis (TGA), elemental analysis (CHONS), gel permeation chromatography (GPC), X-ray diffraction analysis (XRD, scanning electron microscopy (SEM), and pyrolysis gas chromatography-mass spectrometry (Py-GCMS); the results were used for qualitative and quantitative analyses of lignin recovery, and the properties were compared to those of commercial lignin under stable conditions. In this work, we focused on optimization of the maximized fractionation of lignocellulosic materials for the utilization of EC in integrated biorefinery applications.

Materials and methods

Materials

Eucalyptus (EC) was collected from Mae Jan, Chiang Rai, Thailand. The raw material was dried at 70 °C for 24 h in a hot oven, cut using a Retsch ZM200 mill and sieved to a particle size of approximately 0.5 - 0.85 cm. The final moisture content of the milled EC was 7 %, which was established by drying it in the oven at 105 °C to constant weight for 4 h. The processed EC was stored in sealed plastic bags and kept at room temperature for further experimentation. The chemical composition of EC (cellulose, hemicellulose, lignin, and ash content) was analyzed using standard laboratory analytical procedures of the U.S. National Renewable Energy Laboratory (NREL) [8]. Briefly, a multiprocess analytical method was used to determine chemical compositions. The first step involved acid hydrolysis with 72 % sulfuric acid for 60 min at 30 °C. After that, an appropriate amount of DI water was added to dilute the concentration to 4 %. The slurry was sealed and placed in an autoclave at 121 °C for 1 h to complete the hydrolysis step. In the liquid phase, structural carbohydrates were quantified in the form of monomeric compounds. In the solid phase, the residue was placed in a muffle furnace at 575 °C for 24 h, and then the portion of lignin and ash was calculated as the relative dried weight. In terms of chemical composition, EC was mainly composed of cellulose (42.3 %), hemicellulose (20.3 %), lignin (32.1 %), and 5.3 % other components (e.g., ash and extractives).

Solvothermal fractionation process for lignin extraction

The clean fractionation process was performed in a 600 mL stainless-steel reactor. Subsequently, the reactor was heated in a temperature-controlled vertical shaking system to carry out optimal mixing and a thermocouple was used for internal temperature measurement (Parr Reactor 4560, Parr instrument, Moline, IL, USA). The initial standard reaction contained 10 g of EC in 150 mL of a ternary solvent mixed-phase system. The ternary mixture comprises methyl isobutyl ketone (MIBK), ethanol, and water (35, 25 and 40 % v/v, respectively). Sulfuric acid (H_2SO_4) was selected as the homogeneous catalyst with a concentration of 0.020 - 0.040 M and residue times were 30, 60 or 90 min. The reaction was heated to the desired temperature within the range 180 - 200 °C, and the initial pressure was set to 25 bars under nitrogen gas (N_2). Inside the reactor, the mixture of solvents and biomass were stirred at 100 rpm to retain the homogeneous catalyst of the system. After establishing the desired condition, the reactor was immersed in a water bath for 15 min. The fractioned solid was separated from the liquid phase and washed with MIBK and DI water (1:2 v/v). The liquid phase was collected, combined with the rinsate and placed in a separatory funnel. In the aqueous-organic mixture, water was added until an aqueous-organic phase clearly separated. The chemical composition of isolated solid and native EC was determined using the NREL method. Cellulose yield, cellulose purity, hemicellulose removal, lignin removal, and lignin recovery were calculated using the following equations;

$$\text{Cellulose yield (\%)} = \frac{\text{(cellulose remaining in solid pulp)}}{\text{(cellulose content in raw corn stover)}} \times 100 \quad (1)$$

$$\text{Cellulose purity (\%)} = \frac{\text{(cellulose remaining in solid pulp)}}{\text{(total content in solid pulp)}} \times 100 \quad (2)$$

$$\text{Hemicellulose removal (\%)} = \frac{(\text{hemicellulose content in raw corn stover}) - (\text{hemicellulose remaining in solid pulp})}{(\text{hemicellulose content in raw corn stover})} \times 100 \quad (3)$$

$$\text{Lignin removal (\%)} = \frac{(\text{lignin content in raw corn stover}) - (\text{lignin remaining in solid pulp})}{(\text{lignin content in raw corn stover})} \times 100 \quad (4)$$

$$\text{Recovered lignin (\%)} = \frac{(\text{weight of recovered lignin from organic phase})}{(\text{lignin content in raw corn stover})} \times 100 \quad (5)$$

Experimental design and optimum parameters for lignin recovery using response surface methodology (RSM)

Response surface methodology (RSM) and statistical analysis were chosen to identify the optimization parameters of 3 variables (i.e., concentration, temperature, and time) for cellulose yield, lignin removal, and lignin recovery. To estimate the model coefficients, 3 modified Box-Behnken designs were implemented (15 experiments). The 3 factors involve reaction concentration (X_1, 0.020 - 0.040 M), reaction temperature (X_2, 180 - 200 °C), and residence time (X_3, 30 - 90 min) with 3 coded levels for each factor (−1, 0, 1). The variance in each assessed factor was partitioned into offset term, linear, interaction, and quadratic components, and all predictive parameters were written as the following second-order polynomial equation;

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X^2_1 + \beta_{22} X^2_2 + \beta_{33} X^2_3$$

here, Y is the predicted response; X_1, X_2, and X_3 are the independent variables; β_0 is a constant term; β_1, β_2, and β_3 are the linear coefficient terms; β_{12}, β_{13}, and β_{23} are the interaction coefficients; and β_{11}, β_{22}, and β_{33} are the quadratic coefficients. A fitted quadratic polynomial was used to generate 3D surface plots of the correlation between the independent variables and the response.

Characterization of the solid residue after fractionation

X-ray diffraction* (*XRD analysis)

Crystallinities of the native and isolated solid fractions were determined by X-ray diffraction (XRD) using an X'Pert PRO diffractometer (Panalytical, Almelo, Netherlands). The samples were scanned in the range of 2θ = 10 - 30 ° with a step size of 0.02 ° at 500 kV and 30 mA and with Cu Kα radiation (λ = 1.54 Å). Crystallinity was calculated using the following equation;

$$\text{CrI} = \frac{\text{I}_{002} - \text{I}_{\text{amorphous}}}{\text{I}_{002}} \times 100$$

here, I_{002} is the intensity of the crystalline signal for biomass (i.e., cellulose) at 2θ = 22.4, and $I_{amorphous}$ is the peak for the amorphous portion (i.e., cellulose, hemicellulose, and lignin) at 2θ = 18.0.

Scanning electron microscopy (SEM)

The microstructures of the native and isolated solids were analyzed using scanning electron microscopy (JSM-6301F, JEOL, and Japan) with an electron beam energy of 5 - 20 kV. The EC samples were dried and coated with gold before SEM analysis.

Analysis of the aqueous phase

The soluble product profiles in the aqueous fraction were analyzed with high-performance liquid chromatography (LDC Model 4100, Shimadzu, Kyoto, Japan) equipped with refractive index and UV-Vis detectors, and an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). The oven operated at 65 °C, the mobile phase contained 5 mM H_2SO_4 and the flow rate was 0.5 mL/min. The number of oligosaccharides was determined according to the NREL method.

Characterization of lignin recovery after fractionation

Elemental compositions analysis

The elemental compositions of commercial lignin and recovered lignin were determined using the elemental analyzer CHNS-628 (LECO, Saint Joseph, MI, USA). Commercial lignin and recovered lignin were dried at 60 °C in a vacuum evaporator with a pressure of 20 mbars to remove moisture. Subsequently, 0.1 g of commercial lignin or recovered lignin were encapsulated in the container to determine the carbon, hydrogen, and nitrogen contents in the sample. For sulfur analysis, 0.2 g of commercial lignin and lignin recovery was placed into a ceramic boat furnace. Incineration at 1,350 °C using sulfur IR cells was performed to detect the amount of sulfur.

Fourier-transform infrared spectroscopy (FT-IR)

The chemical structure of commercial lignin and lignin recovery was characterized using Fourier-transform infrared spectroscopy analysis (FTIR). Analysis was performed using an FTIR instrument (Perkin Elmer, Waltham, MA, USA), and samples were prepared using the KBr pellet method. The region between 4000 and 400 cm^{-1} was recorded with a resolution of 4 cm^{-1} and 32 scans. Peaks for lignin were compared with those of standard functional groups.

Thermogravimetric analysis (TGA)

The thermal stabilities of commercial lignin and recovered lignin were measured by heating specimens at a rate of 7 °C per min under a nitrogen gas flow of 20 - 35 mL/min. The weight of the specimen was monitored as a function of temperature using a TA Instruments Inc., Model TA Q50 system. Approximately 30 - 50 mg of commercial lignin and recovered lignin were weighed in an aluminum pan and heated to a temperature of approximately 1,000 °C at a rate of 5 °C/min to determine the total mass loss. A plot of weight-loss versus temperature was prepared from the data obtained. A derivative of this curve (DTG) was produced to indicate the temperatures at which maximum rates of weight loss occurred.

Gel permeation chromatography (GPC)

Precipitated samples of commercial lignin and recovered lignin were subjected to gel permeation chromatography (GPC) to determine average molecular weight (M_w) and polydispersity indexes (M_w/M_n) using a Jasco instrument equipped with an interface (LC-NetII/ADC) and a UV detector (254 nm). In this process, 0.1 g of lignin was dissolved in tetrahydrofuran solution, tests conducted with 2 PolarGel-M803 columns (300×7.5 mm^2) and a PolarGel-M guard column (50×7.5 mm^2), and tetrahydrofuran (THF) was selected as the mobile phase. The flow rate was 0.5 mL/min, and the column temperature was 40 °C. Calibration was performed using polystyrene standards (Sigma-Aldrich) with a range of 55,000-266 g/mol.

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS)

Pyrolysis-GC/MS was used to characterize the chemical composition of the recovered lignin used in this study. Pyrolysis of the recovered lignin was performed at 500 °C in an EGA/PY-3030D microfurnace pyrolyzer (Frontier Laboratories Ltd., Fukushima, Japan) connected to a GC 7820A (Agilent Technologies, Inc., Santa Clara, CA) and an Agilent 5975 mass-selective detector (EI at 70 eV). The dimensions of the column used were 30 m × 0.25 mm i.d., and it had a 0.25 μm film thickness (DB-1701, J&W Scientific, Folsom, CA). The oven temperature was increased from 50 °C (1 min) to 100 °C at 20 °C min^{-1} and then to 280 °C (5 min) at 6 °C min^{-1}. Helium was the carrier gas (1 mL min^{-1} flow rate). The released compounds were identified through comparison of their mass spectra with those of the Wiley and NIST libraries and those reported in the literature [9] and through comparison with the retention times and mass spectra of authentic standards, when possible. The molar peak area was calculated for each released lignin degradation product. The total areas were normalized, and the data for 2 replicates were averaged and expressed as percentages [10].

Table 1 Effect of reaction factors on the solid composition of the solvothermal fractionation process for isolated lignin using the response surface method (RSM).

	Factors			Responses (%)		
Run no.	**T (°C)**	**Time (Min)**	**Concentration (M)**	**Cellulose Yield (%)[a]**	**Lignin Removal (%)[b]**	**Lignin recovery (%)[c]**
1	190	60	0.03	91.20	77.30	73.40
2	190	90	0.04	84.13	64.33	63.50
3	200	60	0.02	90.13	83.00	78.60
4	180	90	0.03	59.60	65.63	63.85
5	190	30	0.04	73.88	87.63	84.50
6	180	60	0.02	85.38	57.80	53.30
7	190	30	0.02	90.48	84.40	81.20
8	200	30	0.03	66.40	81.60	76.50
9	190	60	0.03	90.50	76.60	72.40
10	200	60	0.04	83.53	51.40	48.10
11	190	60	0.03	90.50	76.60	74.50
12	180	30	0.03	82.40	87.23	83.25
13	200	90	0.03	88.55	77.88	75.75
14	180	60	0.04	78.00	72.00	69.12
15	190	90	0.02	80.91	84.65	80.70

[a]Based on the relative content of cellulose in the remaining pulp
[b]Based on the relative content of lignin in solid pulp compared with lignin content in raw material
[c]Based on the weight of lignin in the organic phase

Results and discussion

Chemical composition of the raw material and solid fractions from solvothermal fractionation

As reported, the chemical components of the raw eucalyptus (EC) residue include cellulose (35 %), hemicellulose (20 %), lignin (36 %), ash (5 %), and extractive (4 %), with all contents expressed as wt%. Experimental optimization with 15 runs is shown in **Table 1**. The influence of the reaction factors concentration, temperature, and residence time on cellulose yield, lignin removal, and lignin recovery was investigated using response surface methodology (RSM). The results were found to lie in the ranges 59.6 - 91.2, 51.4 - 87.6 and 48.1 - 84.5 %, respectively. The resulting optimization of the solvothermal fractionation process was analyzed using analysis of variance (ANOVA) and conditions of acid concentration (0.020 - 0.040 M), temperature (180 - 200 °C), and time (30 - 90 min) were chosen to maximize cellulose yield, lignin removal, and lignin recovery. The calculated regression equation for fractionation optimization highlighted cellulose yield (Y_1, %), lignin removal (Y_2, %), lignin recovery (Y_3, %) and gave fractional weights to acid concentration (X_1, M), reaction temperature (X_2, °C), and residue time (X_3, min). It was found that a second-order polynomial multiple regression equation represented cellulose yield, lignin removal, and lignin recovery, as in Eqs. (6) - (8).

$$\text{Cellulose yield (\%)} = -2149 - 2190X^1 + 25.70\,X^2 - 6.385\,X^3 + 8161\,X^2_1 - 0.07293\,X^2_2 - 0.010225\,X^2_3 + 1.94\,X_{12} + 16.510\,X_{13} + 0.037458\,X_{23} \quad (6)$$

$$\text{Lignin removal (\%)} = -2789 + 25086\,X1 + 27.29\,X2 - 3.473\,X3 - 43073\,X^2_1 - 0.06476X^2_2 + 0.008582\,X^2_3 - 114.50\,X_{12} - 19.625\,X_{13} + 0.014896\,X_{23} \quad (7)$$

$$\text{Lignin recovery (\%)} = -2992 + 25149\,X^1 + 29.40X^2 - 3.541\,X^3 - 41329\,X^2_1 - 0.07020\,X^2_2 + 0.009083\,X^2_3 - 115.80\,X_{12} - 17.08\,X_{13} + 0.01471\,X_{23} \quad (8)$$

The predicted values of cellulose yield, lignin removal, and lignin recovery in pretreated EC showed that equations for cellulose yield, lignin removal, and lignin recovery were statistically significant at the 95 % confidence interval for the optimization of EC fractionation, as the R-squared values of all responses were higher than 0.90. In addition, the model accurately predicted cellulose yield (98.4 %), lignin removal (98.8 %), and lignin recovery (98.4 %) (**Figures 1(A) - 1(C)** and **Table 2**).

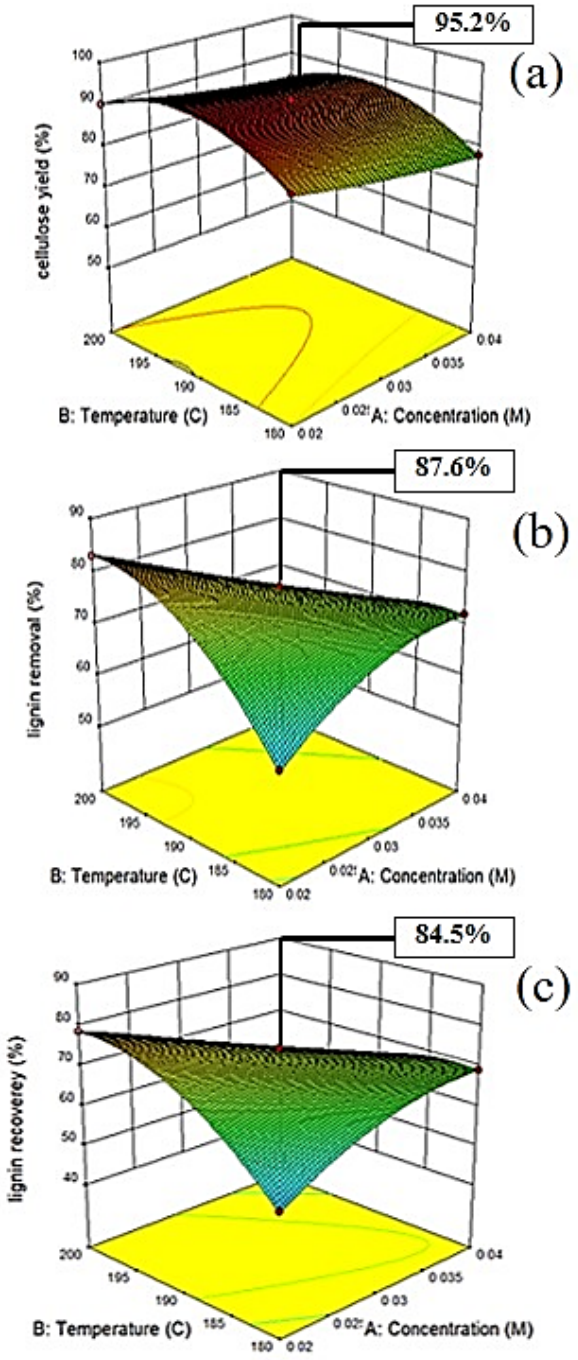

Figure 1 Response surface plot for the solvothermal fractionation process: (a) Effect of reaction concentration (0.020 - 0.040 M) and temperature (180 - 200 °C) on cellulose yield in the solid fraction with varying formic acid concentrations (0.020 M); (b) Effect of reaction time (30 - 90 min) and temperature (180 - 200 °C) on lignin removal in the solid fraction with varying formic acid concentrations (0.040 M); (c) Effect of reaction time (30 - 90 min) and temperature (180 - 200 °C) on the amount of lignin recovered in the solid fraction with varying formic acid concentrations (0.040 M).

Table 2 Resultant regression models for responses based on ANOVA.

Source	p-value[a]		
	Cellulose yield	**Lignin removal**	**Lignin recovery**
Model			
Linear			
concentration	0.0000	0.0000	0.0000
temperature	0.0000	0.0010	0.0070
time	0.9820	0.0000	0.0000
Quadratic			
concentration*concentration	0.0000	0.0000	0.0000
temperature * temperature	0.0350	0.0000	0.0000
time*time	0.0000	0.0000	0.0000
2-Way Interaction			
concentration * temperature	0.5090	0.0000	0.0000
concentration *time	0.0000	0.0000	0.0000
temperature * time	0.0000	0.0000	0.0000

[a]Nonsignificant p-values are highlighted

Optimization of cellulose yield, lignin removal, and lignin recovery from eucalyptus

The model equations for all target responses (Eqs. (6) - (8)) showed the influences of acid concentration, reaction temperature, and residence time on cellulose yield, lignin removal, and lignin recovery in EC fractionation. The results indicated that the highest predicted cellulose yield of 95.63 % would be obtained with 0.020 M acid at a maximum temperature of 189.2929 °C with a residence time of 50.6061 min. Maximum lignin removal (95.91 %) and lignin recovery (91.77 %) were predicted for an acid concentration of 0.040 M at 180 °C for 30 min.

The best conditions, according to the optimization, would lead to cellulose yield > 90 %, lignin removal > 80 %, and lignin recovery > 80 %. However, in the regression analysis, these results were predicted for optimal conditions with an acid concentration of 0.040 M, a temperature of 180 °C, and a residence time of 30 min with a solvent mixture of methyl isobutyl ketone (MIBK)/ethanol/water (35:25: 40 v/v%). Under these conditions, the experimental studies provided the indicated results for maximum cellulose yield (95.2 %), lignin removal (87.7 %), and lignin recovery (84.6 %). It was found that the experimental results met the criteria under the optimal conditions.

SEM and XRD characterization of native eucalyptus and solid remaining after solvothermal fractionation

The microstructures of the native EC and solid isolated under the optimal conditions are shown in **Figure 2**. The surface of the native EC was smooth with embedded with matrix components of the material. The solid obtained under optimal conditions exhibited cavities and cracks on the surfaces due to the removal of lignin and modification of the hemicellulose structure, which resulted in modifications leading to enhancement of the available surface area for the internal cellulose structure [11].

In addition, the crystallinity indexes (CrI) of native EC and isolated solid were identified using X-ray diffraction (XRD) techniques, and results are shown in **Figure 3**. According to analysis of the XRD diffraction patterns, CrI decreased to 62.8 % in isolated solid from 74.3 % in native EC. From these results, it can be seen that the decrease in CrI was due to elimination of hemicellulose and the external structure of lignin under the optimal conditions, resulting in a decrease in CrI. According to a previous study, sugarcane bagasse components were isolated during the organosolv process. The CrI of the solid isolated from fractionation exhibited a decrease in CrI (67.09 %) compared to that of native sugarcane bagasse (72.83 %) [12]. Furthermore, a decrease in CrI after the fractionation process under the organosolv process was also seen.

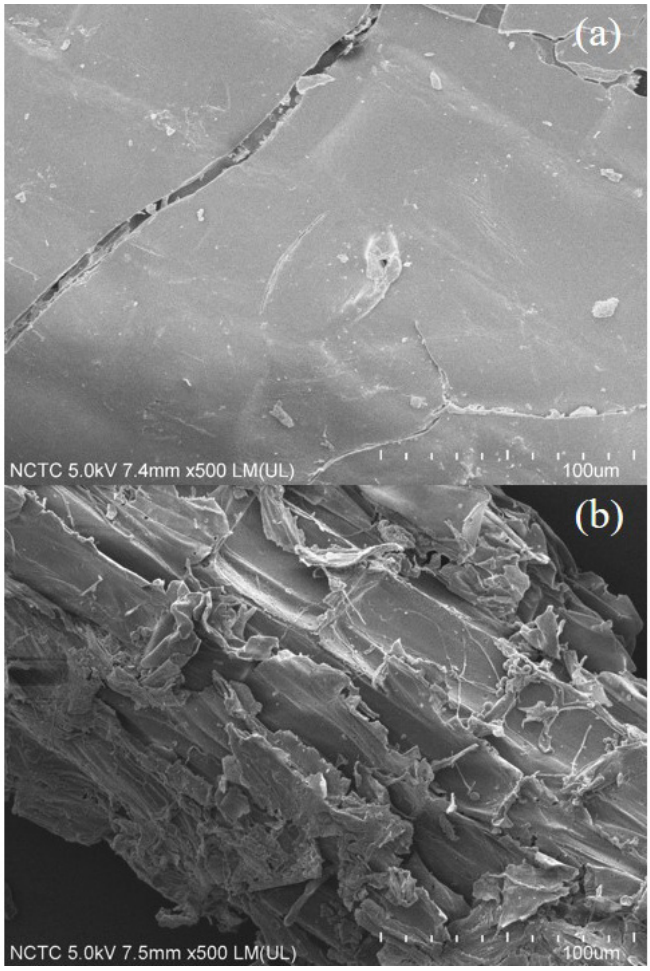

Figure 2 Scanning electron microscopy images of (a) native eucalyptus and (b) isolated solids after fractionation, which were obtained under optimal conditions involving a temperature of 180 °C and a 30 min residence time with a solvent mixture comprising MIBK/ethanol/water (35%:25%:40% v/v%).

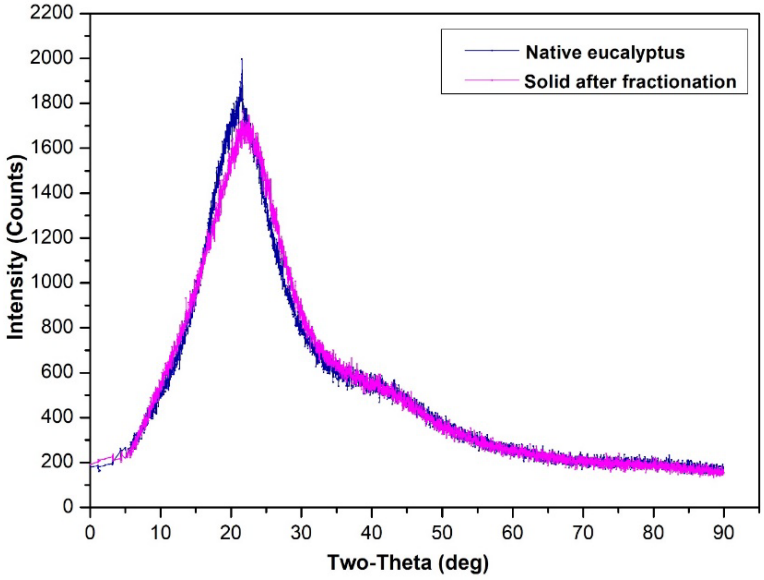

Figure 3 XRD analysis of native eucalyptus and isolated solids after fractionation.

Characterization of lignin fraction by Fourier transform infrared spectroscopy (FTIR) analysis of recovered lignin and original lignin

Fourier-transform infrared (FTIR) spectroscopy analysis of the recovered lignin and original lignin is shown in **Table 3**. The results showed C–H stretches in the methyl absorption region (2922 cm^{-1}) and those of methylene groups (2850 cm^{-1}). The intense peak at 1720 - 1728 cm^{-1} was related to C=O stretches of carbonyl groups, while the peak at 1600 was assigned to aromatic compounds (phenolic hydroxyl groups; OH). The absorption at 1460 - 1500 cm^{-1} corresponds to aromatic groups, and the frequencies of aromatic skeleton vibration in combination with C-H vibrations were found at 1422 - 1428 cm^{-1}. The peaks at 1332 cm^{-1} were found to represent of syringyl (S) units. The absorption peaks at 1221 and 1230 cm^{-1} were associated with C–O and C=O stretching vibrations of guaiacyl (G) units. The peak at 1220 cm^{-1} corresponds to coniferyl aromatic rings, while the vibrations shown at 1120 - 1121 cm^{-1} were related to aromatic C–H groups. Similarly, bands occurring from 1031 to 1034 cm^{-1} were observed for aromatic C–H groups of syringyl and G units. In addition, signals for C–H and CH_3 groups of G units were at 816 and 830 cm^{-1}, respectively [13-15].

Table 3 Functional groups based on FTIR analysis of recovered lignin and commercial lignin under.

Order	Commercial lignin	Lignin recovery	Bend	Functional group
1	2922	2922	C–H stretch	Methyl group
2	2850	2850	C-H stretch	Methylene groups
3	1720	1728	C=O stretching	Carbonyl groups
4	1600	1600	OH stretch	Phenolic hydroxyl group
5	1461,1500	1460,1500	Aromatic	Aromatic carbon atoms group
6	1422	1428	C–H stretch in (G, S)	Aromatic skeleton
7	1332	1332	C–H deformation	Syringyl (S) units
8	1230	1221	C-O stretch and C=O vibrations	Guaiacyl (G) units
9	1220	-	C–O vibrations	Coniferyl aromatic rings
10	1120	1121	C–H	Aromatic C-H group
11	1031	1034	aromatic C-H	Synapyl and Guaiacyl unit
12	816	830	C-H and C-H_3 groups	Guaiacyl unit

Molecular weight analysis of recovered lignin and original lignin

Molecular weight analyses for recovered lignin and commercial lignin are shown in **Table 4**. The weight average molecular weights (M_w), number average molecular weights (M_n) and polydispersity indexes (PDI) were determined using gel permeation chromatography (GPC). The results showed that the molecular weight of recovered lignin under the optimal conditions exhibited a lower average M_w and M_n than commercial lignin. The M_w values of recovered lignin and commercial lignin were 20,812 g/mol and 21,200 g/mol, respectively. The PDIs of recovered lignin and commercial lignin were 2.19 and 2.46, respectively. These result indicate similar particle sizes for the recovered lignin before and after the fractionation process. As previously reported, lignin isolated from EC with an alkali-acid purification process using a ternary mixture solvent of MIBK/methanol/water for organosolv fractionation exhibited PDI values for the extracted lignin of 2.0 - 2.10 g/mol, which was not significantly different from the results of this study [16].

Table 4 Weight-average molecular weight (Mw) of recovered lignin and commercial lignin.

	Sample name	Mw[a] (g/mol)	Mn[b] (g/mol)	Mw/Mn (PDI)[c]
1	Lignin recovery	20812	9500	2.19
2	Commercial lignin	21200	8600	2.46

[a]Weight-average molecular weight (Mw)
[b]Number-average molecular weight (Mn)
[c]Polydispersity index (PDI)

Table 5 Elemental analysis of recovered lignin and commercial lignin.

	Proximate analysis (wt%, d.b%)				Elemental analysis				
Sample	Volatile materials (d.b%)	Fixed carbon (d.b%)	Moisture (%)	Ash (d.b%)	C	H	O	N	S
Lignin recovery	68.2	29.9	0.3	1.6	61.3	5.50	30.1	0.01	1.2
Commercial lignin	62.5	36.8	0.20	0.5	60.9	5.78	29.8	0.04	0.78

Effect on proximate analysis and elementals composition

The proximate analysis of recovered lignin and commercial lignin obtained under the optimal conditions is shown in **Table 5**. The results showed that the proximate analysis of recovered lignin mainly indicated volatile materials with abundances in the range of 62.5 - 68.2 wt%, fixed carbon in the range of 29.9 - 36.8 wt%, moisture in the range of 0.2 - 0.3 wt%, and ash in the range of 0.5 - 1.7 wt%. However, recovered lignin showed a higher ash content than commercial lignin, as a high amount of sulfur was used in the catalyst for the solvothermal fractionation process [17]. In addition, the elemental content of recovered lignin and commercial lignin showed that the sulfur content in the recovered lignin was 1.2 % based on dry weight, which is slightly higher than that of commercial lignin (0.78 %) due to the use of concentrated sulfuric acid (H_2SO_4) as the catalyst in the EC fractionation process [16].

Thermal decomposition of recovered lignin and commercial lignin

Lignin thermal stability was determined using thermogravimetric analysis (TGA) (**Figure 4**). Lignin decomposition occurred in 3 stages. The first stage was observed at low temperatures ranging from 50 to ~ 150 °C, which corresponds to the loss of water [18]. The second decomposition stage occurred in the high-temperature range 160 - 400 °C and was due to the decompositions involving volatile substances, degradation of β-O-4, α-O-4 bonds, phenolic hydroxyl linkages, and organic products including CO, CH_4, and OH groups [19]. In this stage, the maximum decomposition rates for recovered lignin and commercial lignin occurred at temperatures of 350 and 365 °C, respectively. This indicates that aliphatic side chains might break down and split from aromatic rings with the loss of C–C linkages, inter-unit β-O-4 linkages (the most abundant type), and β-β and β-5 lignin bonds [20,21]. In the final stage, at temperatures in the range 400 - 550 °C, the pyrolytic degradation of lignin, and decomposition and condensation of aromatic rings occurred [22].

Pyrolysis-GCMS of recovered lignin and commercial lignin

The identities and relative molar abundances of the commercial lignin and lignin-derived compounds released with relative abundance from the H, G, and S-lignin units and the S/G ratio were determined using pyrolysis gas chromatography-mass spectrometry (Py-GCMS) (**Table 6**). These results showed that commercial lignin contains S-units almost exclusively, with only small numbers of G-units and H-units. S/G ratios of 1.93 and 1.75 were obtained for commercial lignin and recovered lignin, respectively. The pyrograms highlighted the compounds derived from the syringyl (S), guaiacyl (G), and p-hydroxyphenyl (H) lignin units, with guaiacol (1), phenol (2), 4-methylphenol (4), 4-methylguaiacol (5), 4-ethylguaiacol (6), 4-vinylguaiacol (8), syringol (12), cis-isoeugenol (13), 4-methylsyringol (16), 4-

vinylsyringol (22), and trans-propenylsyringol (27) showing high abundances; compounds such as phenol (2), 2-methylphenol (3), and 4-methylphenol (4), could be detected after the pyrolysis of the youngest wood (alfalfa and bromegrass). Moreover, some of these compounds can also be derived from abundant phenolic compounds. However, a significant number of compounds were derived from H-lignin units, other polysaccharides, such as hemicellulose and cellulose [23].

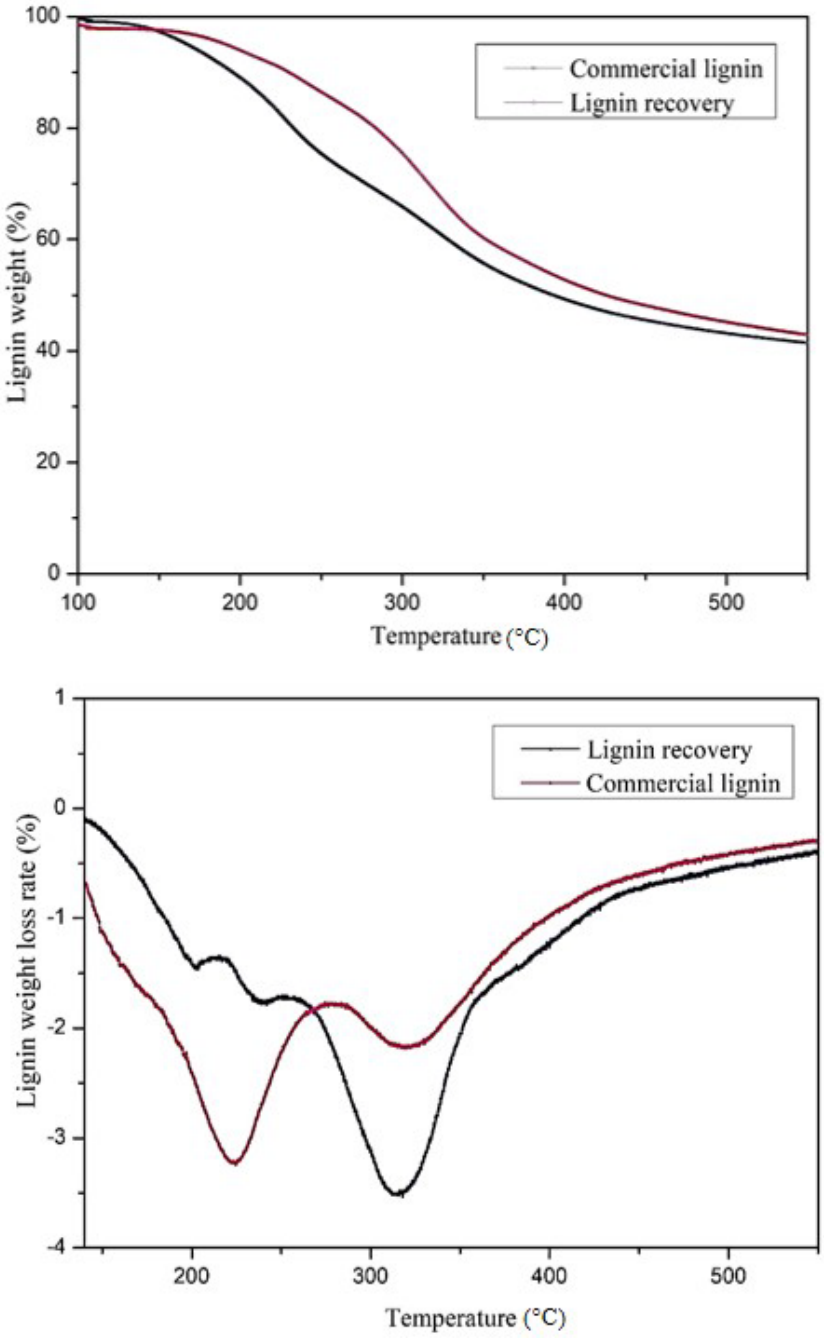

Figure 4 Thermal decomposition of recovered lignin and commercial lignin.

Table 6 Results of Py-GCMS analysis for the identification of commercial lignin and recovered lignin and relative molar abundances (%).

No.	Compound	Origin	relative molar abundance (%)	
			Commercial lignin	Lignin recovery (This study)
1	Guaiacol	G	2.2	2.3
2	Phenol	H	7.5	7.2
3	2-Methylphenol	H	0.6	0.5
4	4-Methylphenol	H	2	2.1
5	4-Methylguaiacol	G	2.9	4.4
6	4-Ethylguaiacol	G	3.2	3.3
7	4-Vinylphenol	G	1.7	2.1
8	4-Vinylguaiacol	G	8.9	8.72
9	Guaiacyl vinyl ketone	G	0.1	0.3
10	Eugenol	G	0.8	0.6
11	4-Propylguaiacol	G	0.1	0.4
12	Syringol	S	26.5	25.4
13	cis-Isoeugenol	G	2.24	1.86
14	Syringic acid methyl ester	S	0.5	0.4
15	trans-Isoeugenol	G	1.88	0.47
16	4-Methylsyringol	S	4.2	8
17	Vanillin	G	0.7	2.5
18	Homovanillin	G	0.1	1.35
19	4-Ethylsyringol	S	2.8	3
20	4-Allylguaiacol	G	1.8	1.3
21	Acetovanillone	G	0.9	1.4
22	4-Vinylsyringol	S	8.58	8.8
23	Guaiacylacetone	G	0.9	1
24	4-Allylsyringol	S	2.1	0.6
25	4-Propylsyringol	S	0.7	0.5
26	cis-Propenylsyringol	S	1.8	1.4
27	trans-Propenylsyringol	S	4.7	3
28	Syringaldehyde	S	1.6	4.5
29	Dihydroconiferyl alcohol	G	0.1	0.3
30	Homosyringaldehyde	S	0	0.3
31	Syringyl-3-oxo-propanal	S	0	0.8
32	Acetosyringone	S	1.5	2.8
33	trans-Coniferyl alcohol	G	2	0
34	Coniferaldehyde	G	0.2	0.5
35	Syringylacetone	S	2.4	2.3
36	Propiosyringone	S	0.2	0.5
37	Syringyl vinyl ketone	S	0.2	0.6
38	Syringic acid	S	0.2	0.9
39	trans-Sinapyl alcohol	S	1.2	1
40	trans-Sinapaldehyde	S	0	0.6
	Total H (%)		10.1	9.8
	Total G (%)		30.72	32.8
	Total S (%)		59.18	57.4
	S/G ratio		1.93	1.75
	Total (H, G, S)		100	100

Conclusions

This study was aimed at determining the optimal conditions for a modified solvothermal fractionation (SF) process converting lignocellulosic eucalyptus (EC) into chemicals and value-added products. A modified single-step organosolv fractionation method was investigated for fractionation of lignin from EC using mean optimum conditions for the solvothermal fractionation process that would enhance lignin removal (87.7 %) from the solid phase. The optimal conditions for lignin recovery in the organic phase involved 0.035 M sulfuric acid, a reaction temperature of 180 °C, a residence time of 40 min and the use of a mixed solvent comprising methyl isobutyl ketone (MIBK):ethanol:water (35:25:40 v/v%); these conditions resulted in a lignin recovery efficiency of 84.6 %, indicating the efficacy of the conditions. While the cellulose yield and hemicellulose removal yield were both > 90 %, thermal degradation analysis showed that the maximum decomposition of recovered lignin resulted from the degradation of β-O-4, α-O-4, phenolic hydroxyl linkages and loss of CO, CH_4, OH groups at temperatures within the range 160 - 400 °C, indicating a low molecular weight for recovered lignin.

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