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Saksit Imman

University of Phayao

Punjarat Khongchamnan

University of Phayao

Wanwitoo Wanmolee

NSTDA: National Science and Technology Development Agency

Navadol Laosiripojana

KMUTT: King Mongkut's University of Technology Thonburi

Torpong Kreetachat

University of Phayao

Nopparat Suriyachai (✉ nopparat.suri26@gmail.com)

University of Phayao

Research

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Fractionation and characterization of lignin from sugarcane bagasse using a sulfuric acid catalyzed solvothermal process

Saksit Imman,^a, Punjarat Khongchamnan^b, Wanwitoo Wanmolee^c, Navadol Laosiripojana^{d,e},
Torpong Kreetachat^a, Nopparat Suriyachai^{a,*}

^aIntegrated Biorefinery excellent Center (IBC), School of Energy and Environment,
University of Phayao, Tambon Maeka, Amphur Muang Phayao 56000, Thailand

^bSchool of Energy and Environment, University of Phayao, Tambon Maeka, Amphur Muang
Phayao 56000, Thailand

^cNational Nanotechnology Center (NANOTEC), National Science and Technology
Development Agency (NSTDA), Pathum Thani 12120, Thailand

^dThe Joint Graduate School for Energy and Environment (JGSEE), King Mongkut's
University of Technology Thonburi, Prachauthit Road, Bangmod, Bangkok 10140, Thailand

^eBIOTEC–JGSEE Integrative Biorefinery Laboratory, National Center for Genetic
Engineering and Biotechnology, Innovation Cluster 2 Building, Thailand Science Park,
Khlong Luang, Pathumthani 12120, Thailand

*Corresponding author: Tel: +66-5446-6666

E-mail: nopparat.suri26@gmail.com (Nopparat Suriyachai)

Abstract

Background: Conversion of lignocellulosic residue to bioenergy and biofuel is a promising platform for global sustainability. Fractionation is an initial step for isolating lignocellulosic components for subsequent valorization. The aim of this research is to develop the solvothermal fractionation of sugarcane bagasse to produce high purity lignin. Physico-chemical structure of isolated lignin from this process was determined.

Results: In this study, a central composite design-based response surface methodology (RSM) was used to optimize an acid promoter for isolating lignin from sugarcane bagasse using a solvothermal fractionation process. The reaction was carried out with sulfuric acid, in the concentration of 0.01-0.02 M and a reaction temperature of 180-200°C for 30-90 min. The optimal conditions for the experiment were obtained at the acid concentration of 0.02 M with a temperature of 180°C for 90 min in methyl isobutyl ketone (MIBK)/methanol/water (35%:25%:40% v/v%). The results showed that 87.1% of lignin recovery was conducted in the organic phase, while 88.0% of lignin removal was done in the solid phase. Furthermore, the change in the physico-chemical characteristics of solid residue and lignin recovery was recorded using various techniques. A GPC analysis of recovered lignin from the organic fraction showed a low average Mw/Mn (1374/785 g/mol) and lower polydispersity index (1.75) compared to commercial organosolv lignin. The maximum lignin degradation temperatures of recovered lignin and commercial organosolv lignin were estimated to be 310°C and 334°C, which can point to potential relationships with the degradation of β -O-4 cross-links. The results indicated that recovered lignin is mostly cross-linked by the β -O-4 cross-links.

Conclusions: The development of sulfuric acid catalyzed solvothermal process in this study provides the efficient extraction of high-value organosolv lignin from sugarcane bagasse and the production of recovered lignin in organic phase with low contamination from other

contents. The lignin characteristic data can contribute to the development of lignin valorization in value-added applications.

Keywords: chemical compound, lignin fractionation, optimization, sugarcane bagasse, sulfuric catalyst, solvothermal process.

Background

Currently, the energy crisis due to the decrease in fossil fuels and the atmospheric pollution caused by the combustion of fossil fuels is an important issue worldwide [1]. In addition, the increase in population and industrial development resulted in increased energy demand and a decrease in fossil fuel reserves. For this reason, many countries have tried to find alternative energy resources to replace fossil fuels. Lignocellulosic biomass is the one alternative renewable energy source that can be considered. Raw materials such as bioethanol and biomaterials are required to produce this alternative renewable energy [2]. Agricultural biomass mainly consists of cellulose (30-50%), hemicellulose (20-40%), lignin (10-30%), and other extractive products [3]. Cellulose is an organic compound that strengthens plants. It consists of a polysaccharide unit linked together with beta 1-4 glycosidic bonds and has great importance in the chemical industry [4]. The natural structure of hemicellulose is one of a number of heteropolymers and generally consist of five-carbon sugars, such as pentose sugar, arabinose sugar, and xylans sugar. Generally, the lignin composition of the softwood type is 24-33%, 19-28% in hardwood type, and 15-25% in the grass type [5]. The typical structure of lignin consists of three basic phenylpropanoid monomers, i.e., p-hydroxyphenyl alcohol (H-unit), coniferyl alcohol (G-unit), and sinapyl alcohol (S-unit) [6]., and the most common linkages are namely, arylglycerol- β -ether dimer (β -O-4), arylglycerol- α -ether dimer (α -O-4), diaryl ether (4-O-5), resinol (β -5), diphenylethane (β -1), phenylcoumaran (β - β'), phenylcoumaran (β - β), and biphenyl (5-5) [7]. Moreover, the lignin content can be converted

into different value-added products, such as phenol, aromatic sources, and vanillin using various conversion technologies [8].

The clean fractionation (CF) process is one of the most promising technologies for lignocellulosic material component fractionation-based biorefineries. Nowadays, there are available studies about the one-step clean fractionation process utilizing a ternary solvent mixture phase (i.e., organic solvent, water and alcohol) in order to carry out one-step isolation of agricultural components efficiently. In recent years, the organosolv fractionation process for the degradation and isolation of lignin was considered to be one of the most promising processes for bioproduction, bioenergy, and biofuel [9, 10]. We have previously examined lignin fractionation by the organosolv fractionation process from sugarcane bagasse, para-rubber wood sawdust, palm fiber, and cassava fiber. The previously-studied combination of hydrothermal and organosolv process was selected to fractionate sugarcane bagasse using the solvent of methyl isobutyl ketone (MIBK):water in the ratio 80:20 with 5%v/v formic acid promoter at 180°C for 80 min. The results showed that a cellulose yield of 64.69% was obtained with less contamination from other components. Moreover, the recovered lignin obtained from the organic phase was 65.36% [11]. Chutikan et al., 2018 reported the solvent mixture consists of methyl isobutyl ketone:ethanol:water (25%:42%:33% v/v) with an H₂SO₄ concentration of 0.025 M. In addition, the highest lignin removal efficiencies of 88.2%, 70.6%, 67.3%, and 71.7% were found for bagasse, para rubber wood sawdust, and palm fiber and cassava fiber, respectively. Lignin recovered through the organic phase was identified as containing amounts of phenolic compounds. The highest lignin recovery of 89.8% was obtained from the organic phase, and 16.9% of phenolic compounds were identified through PY-GCMS. However, a less studied S/G ratio was obtained from bagasse after the fractionation process using a ternary mixture of MIBK:water:methanol using H₂SO₄ as promoter [12].

This research focuses on the optimization of isolated lignin from sugarcane bagasse yield obtained using the solvothermal fractionation process under optimal conditions. Lignin isolation was carried out with the ternary mixture phase solvents (i.e. MIBK:methanol:water) and using sulfuric acid to enhance the recovery of lignin from the sugarcane bagasse. Moreover, various characterized approaches including klason lignin determination, elemental analysis, Fourier transform infrared spectroscopy analysis (FTIR), Thermogravimetric analysis (TGA), Gel permeation chromatography (GPC), XRD, Brunauer, Emmett, and Teller (BET), scanning electron microscope (SEM), Nuclear magnetic resonance spectroscopy (2DNMR-HSQC), and Pyrolysis-gas chromatography–mass spectrometry (Py-GC-MS) were adopted to document the S/G ratio and the changes in solid fraction and lignin recovery. The solvothermal fractionation process for improving isolation lignin efficiency was investigated for the conversion of lignin into high yield products and high purity products for application in integrated biorefineries.

Results and discussion

The interactive effects of residue time, reaction temperature, and acid concentration on the cellulose yield, lignin removal, and lignin recovery

In this work, all responses were evaluated based on the quality and quantity of the solid phase and liquid phase, which depends on the degree of independent factors in the solvothermal fractionation process. The stable conditions for the cellulose yield, removed lignin, and recovered lignin were generated by feeding the experimental results into the RSM method to study the interactive effects of the process parameters on the cellulose yield, removed lignin, and recovered lignin.

The effect of the three parameters, which includes residue time, reaction temperature, and acid concentration on the major components of sugarcane bagasse, were investigated

using the Design–Expert software. The designs of 15 experiments are shown in Table 1. For optimizing the fractionation condition, the residue time 30–90 min, reaction temperature 180–200 °C, and acid concentration from 0.01 to 0.02 M were assessed for maximum cellulose yield, lignin removal, and lignin recovery. The results showed that the cellulose yield, removed lignin, and recovered lignin after the solvothermal fractionation process varied in the range of 70.1-86.1%, 72.7-87.2%, and 71.5-86.2%, respectively. From the results of the experiment, the results of reaction parameters (temperature, time, and acid concentration) include enhanced lignin removal from native sugarcane bagasse into the liquid phase, while the cellulose yield in the remaining solid decreased under high temperature and concentration conditions. An increase in acid concentration resulted in a marked decrease in the solid fraction.

The results obtained after the solvothermal fractionation process were evaluated. It was found that the evaluated regression equations for the optimization of cellulose yield (Y_1 , %), lignin removal (Y_2 , %), and lignin recovery (Y_3 , %) were the functions of the residue time (X_1), reaction temperature (X_2), and acid concentration (X_3). The predicted equation was fit to the multiple second-order polynomial regression, which is defined by Equations 1-3.

$$\text{Cellulose yield (\%)} = Y_1 = -271 + 2.072 X_1 + 2.87 X_2 + 120 X_3 - 0.004292 X_1^2 - 0.00763 X_2^2 - 204500 X_3^2 - 0.00675 X_{12} - 12.83 X_{13} + 38.50 X_{23} \quad (1)$$

$$\text{Lignin removal (\%)} = Y_2 = 54 + 1.498 X_1 + 0.36 X_2 - 4722 X_3 - 0.002139 X_1^2 - 0.00200 X_2^2 - 54000 X_3^2 - 0.006417 X_{12} + 4.50 X_{13} + 36.00 X_{23} \quad (2)$$

$$\text{Lignin recovery (\%)} = Y_3 = 44.4 + 1.478 X_1 + 0.427 X_2 - 4736 X_3 - 0.001793 X_1^2 - 0.00214 X_2^2 - 48792 X_3^2 - 0.006354 X_{12} + 2.67 X_{13} + 36.00 X_{23} \quad (3)$$

A variance analysis (ANOVA) of the quadratic regression model provided the equations of the cellulose yield (Y_1), lignin removal (Y_2), and lignin recovery (Y_3) responses. It was proved to be a statistically significant model for determining the optimal conditions of

sugarcane bagasse. All quadratic models were statistically significant at the confidence interval of 95% for the optimization of sugarcane bagasse under optimal conditions, as the R-square values of all the responses were higher than 0.90, as shown in Table 2. In addition, this consistency indicates the high accuracy of the model based on a comparison of the predicted and experimental values, which resulted in cellulose yield (99.31%), lignin removal (99.49%), and lignin recovery (99.63%), as shown in Figures 1 A–C.

Optimization of reaction parameters obtained from solvothermal fractionation process

The utilization of isolated lignin from sugarcane bagasse using the solvothermal fractionation process and the subsequent lignin recovery from the organic fraction is an expensive method for lignocellulose treatment. Therefore, the pretreatment conditions need to be optimized for maximum lignin recovery.

The model equations for all the target responses (Equations 1-3) showed the influence of residue time, reaction temperature, and acid concentration on cellulose yield, lignin removal, and lignin recovery in the sugarcane bagasse fractionation process. From the analysis, it was found that the optimal conditions for maximum cellulose yield of 87.4%, the process had to be performed at 0.0173 M of sulfuric acid at a high reaction temperature at 200 °C for a high residue time of 58.4 min. In addition, the maximum lignin removal from the solid fraction (88.6%) and lignin recovery from the organic fraction (87.8%) was achieved at 0.02 M, 180 °C for 90 min.

In addition, from the regression model analysis, it was found that these were predicted to have taken place under optimal conditions at an acid concentration of 0.02 M and a temperature of 180°C for 90min in methyl isobutyl ketone (MIBK)/methanol/water (35%:25%:40% v/v). The optimal conditions obtained from the experimental results gave the

reaction efficiency of maximum cellulose yield (86.1%), lignin removal (88.0%), and lignin recovery (87.1%).

Characterization of the native sugarcane bagasse and solid residue under the optimal conditions of solvothermal fractionation process

The analysis of the physio-chemical properties of the solid residue after the solvothermal fractionation process under the optimal conditions was compared with that of the native sugarcane bagasse using various techniques, such as a scanning electron microscope and XRD analysis.

The morphological appearance of the native sugarcane bagasse and solid residue obtained after solvothermal fractionation are shown in Figure 2a and 2b. The results indicate that the native sugarcane bagasse was covered with a wax layer and had a smooth surface. It is evident that the cellulose fibers have relatively uniform morphology. Figure 2b shows the disrupted sugarcane bagasse surface due to the effect of hemicellulose and lignin removals on the surface morphology, resulting in highly pure cellulose fibers. In addition, the surface area and crystallinity index of naïve sugarcane bagasse and solid residue after fractionation under the optimal conditions were examined using XRD patterns (Table. 3). Native sugarcane bagasse showed increased crystallinity from 68.8% (native) to 78.5% (isolated) due to harsh conditions that could induce the removal of hemicellulose and lignin. This would result in higher crystallinity of cellulose fiber.

Fourier transform infrared spectroscopy (FTIR) analysis of commercial organosolv lignin and recovered lignin

In this present work, the physio-chemical properties of lignin recovery in the organic phase under the optimal conditions were analyzed in comparison to that of commercial organosolv

lignin. The FTIR spectra of recovered lignin and commercial organosolv lignin are shown in Figure 3. The recovered lignin and commercial organosolv lignin were characterized according to C-H stretching in CH₂ and CH at 2928 and 2936 cm⁻¹ [13]. The absorbance at 2930 and 2898 cm⁻¹ was associated with the vibrations of the C-H group in methyl and methylene group [14]. The vibrations at 1711 cm⁻¹ were related to C=O stretching of carbonyl groups' absorption region in recovered lignin and commercial organosolv lignin. The signal at 1665 cm⁻¹ corresponds to the carbonyl's vibrations in the aromatic absorption region [15]. The intensity at 1505 cm⁻¹ showed C=C stretching in the aromatic ring absorption region [16]. The peak at 1457 and 1461 cm⁻¹ was assigned to C-H bending vibration. The absorbance was at 1425–1426 cm⁻¹, which corresponds to the bending vibrations of the aromatic skeletal group. The intensity of the signal at 1367 cm⁻¹ of the O-H group of the phenolic hydroxyl groups' absorption region [17]. The absorbance at 1229–1230 cm⁻¹ corresponds to C-O stretch in the syringyl unit (S). The absorbance bands at 1120–1126 cm⁻¹ are related to the out of plane C-H vibration of the syringyl unit. Whereas, the absorption intensity of 1035–1030 cm⁻¹ was associated with the C-H and C-O vibrations in the guaiacyl units (G) [18]. In addition, the intensity at 918 to 829 cm⁻¹ represented the C-H bending group in the guaiacyl units and syringyl unit [19].

Molecular weight distribution of commercial organosolv lignin and recovered lignin

To investigate the effect of lignin extraction after solvothermal fractionation process on the molecular weights of recovered lignin, the molecular weight distributions and polydispersity (*M_w/M_n*) of recovered lignin were compared with those of commercial organosolv lignin, and the results are presented in Table 4. The molecular weight (*M_w*), number average (*M_n*), and PDI were determined using gel permeation chromatography (GPC). It was found that the absolute *M_w* of commercial organosolv lignin was higher than that of the recovered lignin.

The average Mw/Mn of commercial organosolv lignin was 1,558/733 (g/mol). After lignin isolation through the solvothermal fractionation process, it was seen that the Mw/Mn of recovered lignin was 1,374/785 (g/mol). However, the recovered lignin shows low molecular weight and low PDI (1.75), which is a little different from that of commercial organosolv lignin (2.12). As previously reported, lignin isolation from bagasse was carried out under optimal conditions using ethanol-water fractionation. It was found that the PDI of lignin extraction after ethanol-water fractionation process was 1.58, which is slightly lower than that of alcell, which is 2.18 [20].

Elemental analysis of commercial organosolv lignin and recovered lignin

The chemical composition of the lignin recovered was calculated using elemental content analysis (CHON and S) determination (Table 5). The composition content of the lignin samples included the carbon element (C), hydrogen element (H), oxygen element (O), and ash content (wt%). The lignin recovery and commercial organosolv lignin appear to have the same range of elements. The elemental analysis of recovered lignin demonstrated high amounts of carbon content (62.4–63.72 wt%), hydrogen content (5.21–5.30 wt%), oxygen content (29.7–31.4 wt%), nitrogen content (0.13–0.78 wt%), and sulfur content (0.13–0.40 wt%). The sulfur content in the recovered lignin was found to be slightly higher than that of commercial organosolv lignin; the sulfur element in the lignin fraction may have been derived from the sulfuric acid promoter used during the solvothermal fractionation process [21].

Thermal decomposition of commercial organosolv lignin and recovered lignin

The thermal decomposition of the recovered lignin and commercial organosolv lignin samples was conducted using thermogravimetric analysis (TGA) under optimal conditions

(Figure 4). The results show that the thermal degradation of recovered lignin and commercial organosolv lignin can be divided into four stages. Initially, the first stage was observed at ~50–150 °C due to moisture loss from the lignin sample [22]. The second stage, showing the decomposition of volatile substances (i.e., CO, CO₂, CH₄, and b'-O-4' linkages due to the cleaving of the side chains) was observed at 150–380°C [23]. The maximum lignin degradation temperatures of the recovered lignin and commercial organosolv lignin were estimated to be 310°C and 334°C, respectively, which can indicate a potential relationship with the degradation of the b'-O-4' linkages [24]. The third stage was observed to be from 400 to 450 °C; the decomposition of the aromatic rings and the calving of the C-C linkages and 5-5' linkages of the lignin sample took place in this stage [25]. In the final stage, the degradation of the aromatic ring and demethylation or the demethylation reaction occurred above 450 to 550 °C [26].

Structural differences between bagasse lignin and commercial organosolv lignin ascertained through 2D-HSQC NMR

To further investigate the structural features of the lignin fraction obtained, all lignins including bagasse lignin (BGL) and commercial organosolv lignin (COL) were comparatively studied by 2D NMR. It should be mentioned that the detailed assignment of the correlation signals in the spectra (Table 6) is based on previous publications [27]. Figure 5(1) shows the side-chain (δ_C/δ_H 50.0–90.0/2.5–6.0) and aromatic/unsaturated side-chain regions (δ_C/δ_H 100.0–150.0/6.0–8.0) of the HSQC spectra of the lignins. The main substructures found are also depicted in Figure 5(2). In the side-chain region of the HSQC spectra, the most prominent correlation signals represent the methoxyl groups (δ_C/δ_H 55.7/3.71) in both BGL and COL. In addition to the methoxyl groups, the signals for C_α/H_α correlations of the β-O-4 aryl ether linkages (A) structures at δ_C/δ_H 71.3/4.81 were also

observed in all lignins, though they were weaker in COL. This result is in agreement with Moghaddam et al.[27] who reported that this signal could be detected when mild extraction conditions were employed. Besides, the C_γ/H_γ correlations of β -O-4' structures (A) at 59.9/3.61 were also observed in the lignins; these are commonly present in significant amounts in the HSQC spectra of native lignin from bagasse [28]. The signals from the C_γ/H_γ correlations of γ -acylated lignin structures (A) in the lignins arise from *p*-coumarates acetylating at γ -OH of the lignin side-chain, as already published by del Río et al [29]. There is only one interunit linkage visible corresponding to the $C_{\beta(S)1}/H_{\beta(S)1}$ and $C_{\beta(S)2}/H_{\beta(S)2}$ correlations at δ_C/δ_H 85.9/4.10 and 86.2/4.08, respectively, in COL; However, these were absent in the spectrum of BGL. Signals for β - β' resinol substructures (B) were also observed in the HSQC spectra.

The ^{13}C - ^1H correlations in a range of δ_C/δ_H 100.0–150.0/6.0–8.0 (Figure 5(1) A and C) correspond to the aromatic/unsaturated side-chain regions of *p*-hydroxyphenyl (H), syringyl (S), and guaiacyl (G) lignin units, as well as the *p*CA and FA pendant units that are associated with the lignin. Prominent signals at δ_C/δ_H 103.7/6.67 were present in all lignin samples due to $C_{2,6}$ - $H_{2,6}$ correlations in S units. Strong signals at δ_C/δ_H 111.3/6.96 and 115.4/6.78 corresponding to C_2 - H_2 and C_5 - H_5 correlations in G units were also observed in all the lignins, while the signal at δ_C/δ_H 118.6/6.84 related to C_6 - H_6 in G-units was only observed for COL (Figure 5(2), substructure 3). Notably, the $C_{3,5}$ - $H_{3,5}$ correlations of *p*-coumarates (*p*CA) overlapped with the C_5 - H_5 correlations of G units and were observed for both COL and BGL. The signals for H-lignin units at δ_C/δ_H 127.7/7.21 associated with $C_{2,6}$ - $H_{2,6}$ correlations (Figure 5(2), substructure 5) were also observed in all samples, including COL and BGL. Strong C_α - H_α and C_β - H_β correlation signals between *p*CA and ferulates (FA) at δ_C/δ_H 144.0/7.48 and 116.3/6.38 were also presented by all samples (Figure 5(2), substructure 6 and 7). In addition to the signals of the *p*CA units, minor C_6 - H_6

correlation signals in ferulates (FA) at δ_C/δ_H 123.3/7.11 were only presented by COL. Besides, BGL shows relatively strong signals between the regions of δ_C/δ_H 10.0–50.0/0.5–3.0 (Figure 5(1) E and F); these can be attributed to methyl groups attached to methyl-esters as well as propyl and ester groups of phenolic monomers, indicating the formation of new functional groups of lignin during the acid-catalyzed MIBK pretreatment. In conclusion, given the location in the HSQC spectra of these signals, the absence of oxidized S ($C_{\alpha}=O$) corresponding to $C_{2,6}$ – $H_{2,6}$ (S') correlations and pCA_{β} and FA_{β} clearly indicate changes in lignin substructures during the pretreatment [29]. The results of the phenolic monomers attribution using the HSQC spectra were further confirmed by the Py-GCMS.

The identification of relative mass compounds of commercial organosolv lignin and recovered lignin using Py-GC-MS

The relative molar abundances of compounds of the recovered lignin and commercial organosolv lignin were analyzed under optimal conditions using Py/GCMS (Table 7). The results show that commercial organosolv lignin was composed of 30.53% of H-units, 44.42% of G-units, and 25.05 of S-units, while the recovered lignin from the organic fraction shows a predominance of G-unit, which accounted for 42.19% and is composed of 32.69% of H-units and 25.12% of the S-type. However, the S/G ratio of lignin recovery was approximately 0.60 and 0.56 for commercial organosolv lignin and recovered lignin, respectively. As a result, it was found that the major products obtained from lignin recovery were guaiacol (18.9%), syringol (17.2%), and 4-ethyl phenol (14.2%).

Moreover, the pyrograms compound showed the products derived from *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin. The results showed that recovered lignin contained mainly aromatic hydrocarbon groups, alkoxy groups, and phenolic compounds. Recovered Lignin comprised mainly of the guaiacol types (G), such as guaiacol

unit (1), 4-methylguaiacol (8), and 4-vinylguaiacol (11) or products in the hydroxylphenyl group (H), consisting of phenol (2), 4-ethyl phenol (7), and 4-vinylphenol (12). However, low amounts of syringol (13) and acetosyringone (18) compounds were derived from Syringol type (S).

Conclusions

This study aimed to find the optimal conditions for the modified solvothermal fractionation (SF) process of sugarcane bagasse (BG) for conversion into chemical compounds and value-added products. As per the results, the optimal conditions were acid concentration of 0.02 M at a temperature of 180 °C for 90 min using the organosolv fractionation process. It was observed that 87.1% of recovered lignin and 88.0% of lignin was removed from the solid phase. The maximum lignin decomposition (150–380°C) obtained from the TGA analysis due to the cleavage of β -aryl structures (β -O-4), resinol (β - β), and phenylcoumaran (β -5). The GPC analysis showed a low molecular weight of recovered lignin (1.76 g/mol) compared to commercial organosolv lignin (2.12 g/mol). Furthermore, the highly purified recovered lignin after the fractionation process was used for feedstock and was further converted into high value-added products in industrial biorefineries.

Materials and methods

Materials

Sugarcane bagasse was obtained from Mae Chan, Chang rai, Thailand. The sugarcane bagasse was dried at 70 °C for 24 h in a hot oven. The dried sugarcane bagasse was ground and sieved to obtain the range of particles from 0.5 to 2.0 mm with 0.25–0.5 cm thickness. The final moisture content of the milled sugarcane bagasse was 5%, determined using the loss of weight after oven drying at 105°C for a constant weight (5 h). The processed

sugarcane bagasse was stored in sealed plastic bags at room temperature for the experiments. The chemical composition was determined according to the analytical laboratory procedures of the National Renewable Energy Laboratory [30]. The chemical composition of the sugarcane bagasse included cellulose (37.2%), hemicellulose (22.1%), lignin (26.5%), ash (8.2%), and extractives (6 %).

Procedure of isolation of lignin from sugarcane bagasse using the solvothermal fractionation process

Organosolv fractionation of sugarcane bagasse was performed in a 600 mL stainless steel reactor heated by an electric jacket with an inside thermocouple to measure temperature (Parr Reactor 4560, Parr instrument, Moline, IL, USA). Initially, the reaction contained 15 g of screened sugarcane bagasse mixed with 150 mL of the single-phase solvent mixture comprising MIBK/methanol/water (35%:25%:40% v/v) with various concentrations of H_2SO_4 catalyst (0.01-0.02 M). The reaction temperature and time were 180–200°C and 30-90 min, respectively. Nitrogen gas (N_2) was purged from the reactor, and the initial pressure was adjusted to 20 bar. The reaction was stirred at 100 rpm to maintain a homogeneous system. After the desired temperature and time were reached, the reaction was immediately stopped in a water bath. The solid cellulose-enriched fraction was separated by filtration and washed with MIBK and water (1:2 v/v) mixture. The liquid fraction was combined and placed into a separatory funnel. distilled water was added to the organic phase until the phase separation was done. The mixture was stirred and then incubated at room temperature for 20 min to complete phase separation. The liquid phase containing hemicellulose and soluble products was recovered. The separated organic phase was dried at 105 °C to obtained isolated lignin. The yield of the isolated solid was determined on a weight basis. The chemical composition

of the isolated solid was determined using the National Renewable Energy. Laboratory (NREL method). The cellulose yield, cellulose purity, hemicellulose removal, lignin removal, and recovered lignin were calculated using the following equations:

$$\text{Cellulose yield (\%)} = \frac{(\text{cellulose remaining in solid pulp})}{(\text{cellulose content in raw sugarcane bagasse})} \times 100 \quad (4)$$

$$\text{Cellulose purity (\%)} = \frac{(\text{cellulose remaining in solid pulp})}{(\text{total content in solid pulp})} \times 100 \quad (5)$$

Hemicellulose removal (%) =

$$\frac{(\text{hemicellulose content in raw sugarcane bagasse}) - (\text{hemicellulose remaining in solid pulp})}{(\text{hemicellulose content in raw sugarcane bagasse})} \times 100 \quad (6)$$

$$\text{Lignin removal (\%)} = \frac{(\text{lignin content in raw sugarcane bagasse}) - (\text{lignin remaining in solid pulp})}{(\text{lignin content in raw sugarcane bagasse})} \times 100$$

(7)

$$\text{Lignin recovery (\%)} = \frac{(\text{weight of recovered lignin from organic phase})}{(\text{lignin content in raw sugarcane bagasse})} \times 100 \quad (8)$$

Statistical design and optimization for recovered lignin using response surface method (RSM)

The experimental design and statistical analysis were performed according to Box's response surface design method using the Design-Expert software (version 10.0.1). The design of the experiment was explored with three independent factors based on the three-level Box-Behnken design (BBD). The three factors comprised reaction time (X_1 , 30–90 min), reaction temperature (X_2 , 180–200 °C), and concentration (X_3 , 0.01–0.02 M) with three coded levels for each factor (-1, 0, 1). A total of 15 experiments with three replications at the center point were run in random order. The assessed variance of each factor was partitioned into offset, linear, interaction, and quadratic components. All parameters were evaluated using a second order polynomial equation as follows.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2$$

$$+ \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (9)$$

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

403

404 **Characterization of the isolated solid fraction**

405 *X-ray diffraction spectroscopy (XRD)*

406 Crystallinities of the native sugarcane bagasse and isolated solid fractions were determined
407 by XRD using an X'Pert PRO diffractometer (Panalytical, Almelo, the Netherlands). The
408 samples were scanned in a range of $2\theta=10^\circ-30^\circ$ with a step size of 0.02° at 500 kV and 30
409 mA and Cu K α radiation ($\lambda=1.54 \text{ \AA}$). Crystallinity was calculated according to the following
410 equation.

411

$$CrI = \frac{I_{002} - I_{amorphous}}{I_{002}} \times 100$$

412

413 where I_{002} is represent the intensity of the crystalline portion of the biomass (i.e.,
414 cellulose) at $2\theta = 22.4$ and $I_{amorphous}$ is the peak of the amorphous portion (i.e., cellulose,
415 hemicellulose and lignin) at $2\theta = 18.0$.

416 *Scanning electron microscope (SEM)*

417 The microstructure of the sugarcane bagasse samples and solid fraction in the optimization
418 experiments was analyzed by scanning electron microscope (JSM-6301F, JEOL, Japan) with
419 electron beam energy of 5-20 kV. The sugarcane bagasse samples were dried and coated with
420 gold for SEM analysis.

BET surface area measurement

The total surface area of the raw material and solid residues obtained by fractionation was determined using the method proposed by Brunauer, Emmett, and Teller (BET) on a Belsorp-max TPD pro (BEL Japan, Tokyo, Japan) equipped with a thermal conductivity detector (semidiffusion type, 4-element WeRe filament).

Analysis of aqueous phase

Soluble product profiles of the aqueous fraction were analyzed using a high-performance liquid chromatograph (LDC Model 4100, Shimadzu, Kyoto, Japan) equipped with refractive index and ultraviolet–visible spectroscopy detectors and an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operating at 65 °C with 5 mM H₂SO₄ as the mobile phase at a flow rate of 0.5 mL/min. The number of oligosaccharides was determined according to the NREL method.

Characterization of lignin fraction

Elemental analysis

The elemental composition of lignin recovery and commercial organosolv lignin was estimated using an elemental analyzer CHNS-628 (LECO, Saint Joseph, MI, USA). Lignin samples were dried at 60 °C in a vacuum evaporator at 20 bars to remove moisture. Then, 0.1 g of lignin sample was encapsulated in a container to determine the amount of carbon, hydrogen, and nitrogen in the sample. Then, for sulfur analysis, 0.2 g lignin was placed in a ceramic boat furnace. The sample was incinerated at 1,350 °C using a sulfur IR cell to calculate the amount of sulfur.

Fourier transform infrared spectroscopy analysis (FTIR)

The chemical structure of lignin recovered from the organic phase was determined using Fourier transform infrared spectroscopy analysis (FTIR). FTIR analysis was performed using an instrument from PerkinElmer (Waltham, MA, USA) and the samples were prepared by using the KBr pellet method. The region between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} at 32 scans was recorded. The peaks made by lignin were compared with the peaks of the standard functional groups.

Thermogravimetric analysis (TGA)

The thermal stability of the isolated and native samples of lignin recovery was measured by heating the specimens at a rate of 7 $^{\circ}\text{C}$ per minute under the flow of nitrogen gas at 20-35 mL/min. The weight of a specimen was monitored as a function of temperature using an instrument from TA Instruments Inc. (Model TA Q50). Approximately 30-50 mg of recovered lignin was weighed in an aluminum pan and heated at a rate of 5 $^{\circ}\text{C}/\text{min}$ from ambient temperature to approximately 1000 $^{\circ}\text{C}$ for determination of the total loss of mass loss. A curve depicting the weight loss against temperature was constructed from the obtained data. A derivative of this curve (DTG) was calculated to determine the temperature corresponding to the maximum rate of weight loss.

Gel permeation chromatography (GPC)

The precipitated lignin samples and lignin recovery during the reaction were subjected to gel permeation chromatography to determine the average molecular weight (M_w) and the polydispersity index (M_w/M_n) using a Jasco instrument unit equipped with an interface (LC-NetII/ADC) and a UV detector (254 nm). Lignin (0.1 g) was dissolved in tetrahydrofuran (THF) and the test was carried out using two PolarGel-M 803 columns (300 mm $\times$ 7.5 mm) and a PolarGel-M guard (50 mm $\times$ 7.5 mm); the tetrahydrofuran was used as a mobile phase. The flow rate was 0.5 mL/min at 40 $^{\circ}\text{C}$. The column temperature was 40 $^{\circ}\text{C}$. Calibration was performed using polystyrene standards (Sigma-Aldrich) ranging from 266 to 55,000 g/mol.

Nuclear magnetic resonance spectroscopy (2DNMR-HSQC)

The molecular structure of the recovered lignin was determined using two-dimensional heteronuclear single quantum coherence. The experiment began with the determination of ^1H - and ^{13}C -dimensions at 10-0 ppm and 220-5 ppm, respectively; 0.5 mg of lignin was dissolved in 0.5 mL of DMSO- d_6 and compared with the commercial organosolv lignin and lignin recovery samples using 2DHSQC-NMR analysis (Bruker Advance 600 spectrometer, Bruker, Germany).

Pyrolysis-gas chromatography–mass spectrometry (Py-GCMS)

Pyrolysis-GC/MS was used to characterize the chemical composition of the recovered lignin used in this study. The pyrolysis of recovered lignin was performed at 500 °C in an EGA/PY-3030D microfurnace pyrolyzer (Frontier Laboratories Ltd., Fukushima, Japan) connected to a GC 7820A system (Agilent Technologies, Inc., Santa Clara, CA) and an Agilent 5975 mass-selective detector (EI of 70 eV). The A DB-1701 column was 30 m $\times$ 0.25 mm, 0.25 μm film thickness (J&W Scientific, Folsom, CA). The oven temperature was programmed to increase 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 used as the carrier gas (1 mL min^{-1}). The released compounds were identified by comparison of their mass spectra with those provided by the Wiley and NIST libraries, and with the spectra reported in the literature [31]. and, whenever possible, with the standard retention times and mass spectra. Molar peak areas were calculated for each released lignin degradation product. The summed areas were normalized, and the data for two replicates were averaged and expressed as percentages [32].

Abbreviations

BET Brunauer, 2DNMR-HSQC: two-dimensional-nuclear magnetic resonance spectroscopy heteronuclear single quantum coherence two-dimensional; Emmett, and Teller; FT-IR:

Fourier transform infrared spectroscopy analysis; GPC: Gel permeation chromatography; Py-GC-MS: Pyrolysis-gas chromatography–mass spectrometry; RSM: Statistical design and optimization for recovered lignin using response surface method; SEM: Scanning electron microscope; TGA: Thermogravimetric analysis; XRD: X-ray diffraction spectroscopy.

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Authors' contributions

Nopparat Suriyachaie: review and editing, Conceptualization, Methodology; Punjarat Khongchamnan: Writing - original draft, Formal analysis; Wanwitoo Wanmoleeb: Formal analysis, Writing; Navadol Laosiripojana; Visualization. Torpong Kreetachata; Validation. Saksit Imman: Investigation, Supervision, Formal analysis, Visualization, Writing - review and editing.

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Availability of data and materials

We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for noncommercial purposes, without breaching participant confidentiality.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Written informed consent for publication was obtained from all participants.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Figures

Figure 1

Response surface plot of the solvothermal fractionation process: Effect of residue time (30-90 min) and reaction temperature (180-200 °C) on (a) cellulose yield in solids after fractionation at 0.02 M using H₂SO₄ as the promoter, (b) lignin removal in the solid fraction at 0.02 M using H₂SO₄ as the promoter, and (c) lignin recovery in the organic fraction at 0.02 M using H₂SO₄ as the promoter

Figure 2

Scanning electron microscope images of (a) native sugarcane bagasse and (b) solid residue obtained after the solvothermal fractionation process under the optimal conditions at 0.02 M acid, 200 °C, and 60 min.

Figure 3

The functional groups based on FTIR analysis of lignin recovery and commercial organosolv lignin after the solvothermal fractionation.

Figure 4

(a). The TGA curves of lignin recovery and commercial organosolv lignin after the solvothermal fractionation.

Figure 5

(1). The 2D HSQC NMR spectra of commercial organosolv lignin (A, C, and E) and bagasse lignin (B, D, and F) extracted with MIBK.

Figure 6

(2). The lignin substructures observed in the HSQC spectra of the lignins: (A) β -O-4' linkages, (B) resinol structures formed by β - β , (S) syringyl unit, (G) guaiacyl unit, (H) p-hydroxyphenyl unit, (pCA) p-coumarate, (FA) ferrulate, and (F) p-hydroxycinnamyl alcohol.