Understanding the Effects of Ethylene Glycol-Assisted Biomass Fractionation Parameters on Lignin Characteristics Using a Full Factorial Design and Computational Modeling

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ABSTRACT: Contributing to recent lignin valorization efforts, this study uses an integrative approach to explore the effects of fractionation parameters on lignin characteristics. The following reaction parameters are explored: water content of the water–organic solvent mixture, reaction temperature, and sulfuric acid content. Ethylene glycol (EG) was selected as the fractionation solvent because of its promising lignin solubility and extractability. This study takes a novel approach in conducting EG-assisted biomass fractionation; instead of removing lignin from the biomass, lignin was extracted and characterized. Lignin characteristics involving recovery and linkages were analyzed. A maximum of 27 wt % lignin recovery was achieved at a low water content (25%) and high reaction temperature (180 °C) in the presence of sulfuric acid (1 wt %). From NMR analysis, aryl–ether linkages, which are important to preserve for lignin valorization, were decomposed as a result of relatively high temperature and the presence of sulfuric acid. Statistical analysis showed that all individual parameters and their interactions had significant effects on lignin recovery. Computational analysis revealed that hydrogen bonding between the EG and lignin macromolecules greatly decreased with an increasing amount of water.

INTRODUCTION

In response to the rapidly increasing demand for energy and materials, there is a need for sustainable solutions more than ever. Bioenergy is one such solution because it is abundant and renewable. While the cellulose in lignocellulosic biomass is commonly used to produce bioethanol,1 lignin in biomass tends to be viewed as a byproduct and is commonly discarded.2 Although annual world lignin generation exceeds 50 million metric tons,2,3 it is still underutilized as low-grade fuel4 and animal feed.5

However, from a more recent technoeconomic and environmental standpoint, lignin has many practical applications that can reduce costs and waste in the long run. Novel lignin valorization efforts have focused on lignin’s potential to produce low molecular weight building block chemicals5,10,12 these versatile chemicals have industrial applications, such as the synthesis of polymers,13 commodity chemicals,5 surfactants, dyes, adhesives, and fertilizers.14,15

High-quality lignin is defined to be lignin with abundant aryl–ether linkages, including β-O-4 linkages, because such linkages are proportional to the yield of low molecular weight phenolic compounds.16 These low molecular weight compounds act as building block chemicals that are versatile in the polymer industry. Organosolv fractionation is employed to recover lignin from biomass in a way that minimizes harm to the environment.17 While organosolv fractionation has traditionally been used to remove lignin, it can also be used to recover lignin. In this novel approach toward lignin extraction, organosolv fractionation extracts lignin from biomass into a liquid fraction, and the lignin can be recovered through precipitation followed by filtration. Because an acidic environment facilitates lignin precipitation,18,19 it can be used in the fractionation process. However, employing acid catalysts in the fractionation process often causes significant structural changes of lignin. For instance, acids can readily cleave aryl–ether linkages, decompose major lignin interunit linkages in biomass, and induce repolymerization with aromatic units by forming condensed aromatic C–C bonds.20,21 Therefore, the use of acid catalysts potentially influences the quality of recovered lignin.

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In a typical organosolv process, low boiling point solvents including methanol, ethanol, and isopropyl alcohol are used to fractionate biomass components due to relatively low cost and easy solvent recovery. Ethylene glycol (EG) is reported as an effective organic solvent for lignin fractionation because of its relatively high lignin extraction capability compared to other organic solvents.\textsuperscript{25,26} EG has a high lignin solubility relative to other solvents such as ethanol and methanol. This high lignin solubility can be explained by a Hansen solubility parameter; because EG has a relatively large hydrogen bonding component, lignin is highly soluble in EG.\textsuperscript{26} Although high boiling point polyols such as EG and glycerol are commonly criticized for being difficult to recycle after biomass pretreatment, the low equipment requirement and inexpensive cost are favorable from an economic perspective.\textsuperscript{25} In this study, the effects of three reaction parameters including water content (defined as the volumetric percentage of water in a water–organic solvent mixture), reaction temperature, and sulfuric acid content on lignin recovery were explored. Softwood was selected as the feedstock in this biomass fractionation study because it is known to be the most recalcitrant lignocellulosic biomass due to its high lignin content.\textsuperscript{26} Water is used along with EG for technoeconomic and environmental purposes. The use of water decreases organic chemical (EG) use in upstream processes and reduces water and energy consumption for EG removal from biomass in downstream processes. Furthermore, water decreases the viscosity of fluid to facilitate high biomass loading in industrial applications.\textsuperscript{27} Although water addition has many advantages as described above, it possibly reduces lignin recovery because of the hydrogen bonding between water and EG molecules.\textsuperscript{28} For this reason, the water–EG ratio was selected as the primary parameter of interest in this study. Temperature is a well-known reaction parameter in biomass fractionation processes. For instance, an insufficient reaction temperature could not effectively extract lignin from biomass, while a severe temperature significantly modified the intact lignin linkages.\textsuperscript{5} Similarly, the addition of sulfuric acid increased lignin recovery,\textsuperscript{16,19} but it could also break β-O-4 linkages in lignin. It is necessary to understand the effects of each parameter on lignin recovery, and further optimization is needed to develop a sustainable lignin fractionation process.

This study aims to (1) explore effects of fractionation parameters on lignin characteristics including quantity and physicochemical properties of lignin recovered from softwood and to (2) determine the significance of individual and interactive parameters’ effects on lignin recovery using a full factorial design. Density functional theory (DFT) is used to calculate bond lengths and interaction energies in lignin–EG−water mixtures to better understand mechanistic interactions. To achieve these aims, EG-assisted fractionation was conducted on softwood and recovered lignin was characterized.

**RESULTS AND DISCUSSION**

**Lignin Recovery.** EG has relatively high lignin solubility compared to other organic solvents;\textsuperscript{25,26} thus, it can facilitate effective biomass fractionation and recover a relatively substantial amount of lignin.\textsuperscript{27} The maximum lignin recovery was 27 wt % at 25% water content and 180 °C with 1 wt % sulfuric acid. On the other hand, the minimum lignin recovery was 0.4% at 75% water content and 160 °C without sulfuric acid. Figure 1 shows lignin recovery values obtained from each of the eight fractionation experiments. Significantly, different lignin recoveries were observed between experiments depending on the water contents under the same reaction temperatures and the same sulfuric acid contents. A lower water content resulted in a higher lignin recovery because more lignin is extracted from biomass by EG. Sulfuric acid loading affected the lignin recovery because it catalyzed the hydrolysis reaction of aryl–ether linkages and made fragmented lignin soluble in EG.\textsuperscript{18,19} At a higher reaction temperature, bonds between lignin and other biomass components were easier to break, making the lignin more accessible and readily dissolved in EG. In a similar study, sugarcane bagasse was fractionated by an EG−water mixture (10% water content) with 1.2% sulfuric acid at 130 °C for 30 min, and it resulted in 35.8% lignin recovery.\textsuperscript{27} Even though several parameters were different from the ones explored in this current study, the fractionation procedure was similar. It is speculated that this higher lignin recovery is attributed to using an EG−water mixture with a very low water content and a specific type of biomass. Generally, it is easier to separate biomass components from herbaceous biomass compared to woody biomass due to woody biomass having higher lignin content and more rigid structures.

**Lignin Characterization.** Lignins fractionated from spruce, pine, and fir (SPF) softwood under different reaction conditions were characterized using two-dimensional (2D) heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) analysis as shown in Figure 2. Among several experiments, three fractionation experiments (180 °C, 0 wt % sulfuric acid, and 25% water content; 180 °C, 1 wt % sulfuric acid, and 25% water content; 160 °C, 1 wt % sulfuric acid, and 25% water content) were selected for physicochemical property analyses of lignin by gel permeation chromatography (GPC) and NMR techniques. The structural information of the recovered lignins was studied using NMR analysis. Both aromatic (δC/δH 105−140/6.0−8.5) and aliphatic regions (δC/δH 50−90/3.0−5.5) were investigated for the information of lignin subunits and interunit linkages. The assignments of HSQC NMR spectra were determined as reported in a previous study.\textsuperscript{29} According to the HSQC NMR results of the three samples (180 °C, 0 wt % sulfuric acid, and 25% water content; 180 °C, 1 wt % sulfuric acid, and 25% water content; 160 °C, 1 wt % sulfuric acid, and 25% water content), higher temperature and sulfuric acid content loading in the fractionation process led to more decomposition of lignin interunit linkages. In particular, the contours of β-O-4

![Figure 1. Effect of the water content and sulfuric acid content (wt %) at 160 and 180 °C on lignin recovery (%).](Image 324x608 to 564x749)
linkages, indicated as \( A_\alpha \) and \( A_\beta \), were clearly found in lignin resulting from 180 °C without sulfuric acid as well as from 160 °C and 1 wt % sulfuric acid to a lesser extent. However, in the fractionation experiment with 180 °C and 1 wt % sulfuric acid, there were no contours of these aryl–ether bonds. This observation also suggests that the combined effect of sulfuric acid and temperature on the quality of recovered lignin is much more significant than that of the individual parameters.

The same trend occurs with phenylcoumaran (B) and resinol substructures (C); such linkages were cleaved at 180 °C with 1 wt % sulfuric acid. These observations indicate that the presence of sulfuric acid significantly affects the cleavage of major lignin linkages to a greater extent than reaction temperature.

While condensed guaiacyl units were observed from the extracted lignin at 180 °C and 1 wt % sulfuric acid and 160 °C with 1 wt % sulfuric acid, the condensed aromatics were not detected from the lignin recovered at 180 °C and 0 wt % sulfuric acid. These results indicate that an acidic environment promoted lignin condensation reactions by forming reactive benzyl carbocations,\(^3\) which resulted in condensed aromatics. It was also noted that the softwood lignin was more susceptible to condensation reactions because guaiacyl units have a large amount of unsubstituted aromatic carbons at the C5 position.\(^5\)

Recently, it was reported that 1,4-butanediol can effectively fractionate reactive lignin in a lignin-first biorefinery approach.\(^2\) As a common diol solvent along with EG, it showed a high capability of dissolving the lignin and efficiently separated lignin streams with a high amount of \( \beta\)-O-4 linkages. This suggests that diols, including EG, are effective in preserving \( \beta\)-O-4 linkages in fractionated lignin.

Molecular weights and polydispersity index values for the three recovered lignin samples from EG-assisted fractionation along with milled wood lignin (MWL) extracted from the raw material are presented in Table 1. As shown, the \( M_n \) of MWL, a representative native lignin, was found to be 8752 g/mol. In the presence of an acid catalyst, the extracted lignin has a \( M_w \) value of 1689 g/mol, which is 68% lower compared to that without the acid catalyst (5271 g/mol). These results align with NMR results; the addition of acid during the biomass fractionation effectively catalyzes the cleavage of aryl–ether bonds, resulting in the production of lignin fragments and a subsequently high lignin recovery.

**Statistical Analysis.** The second-order polynomial of the best fit is shown below

\[
y = -133.193 + 1.660A + 0.881B + 23.010C - 0.011AB - 0.202AC - 0.047BC
\]

The \( R^2 \) value of the model is 0.9999, while the adjusted \( R^2 \) value is 0.9999; because these values are identical, the model is a good fit for the experimental data.

Table 2 demonstrates the use of analysis of variance (ANOVA) to determine the significance of the models. The null hypothesis of the ANOVA test states that the means of the levels of factors A, B, and C are the same and that there are interactions between factors A and B, factors A and C, and factors B and C. According to Table 2, the probability of obtaining an \( F \)-value greater than the calculated one is less than 0.05 at a 95% significance level. As a result, there is sufficient

![Figure 2. 2D HSQC NMR spectra of (A) lignin from 180 °C, 0 wt % sulfuric acid, 25% water content; (B) lignin from 180 °C, 1 wt % sulfuric acid, 25% water content; (C) lignin from 160 °C, 1 wt % sulfuric acid, 25% water content; (D) main structures present in lignin from SPF wood.](image)

| Reaction Conditions | \( M_n \) (g/mol) | \( M_w \) (g/mol) | PDI |
|---------------------|------------------|------------------|-----|
| MWL                 | 4312             | 8752             | 2.0 |
| A (180, 0, 25)      | 2013             | 5271             | 2.6 |
| B (180, 1, 25)      | 1236             | 1689             | 1.4 |
| C (160, 1, 25)      | 1705             | 2705             | 1.6 |

\(^a\)Reaction conditions are in parenthesis: temperature (°C), \( \text{H}_2\text{SO}_4 \) amount (wt %), and water content (%).
recovery. Equation 5 is used as a formula to predict lignin reaction temperature, and sulfuric acid content on lignin better understand the interactive effects on lignin recovery. According to Table 3, all parameters and interactions are significant because their p-values are all less than 0.05.

Table 3. Parameter Estimates of Intercept, Individual Parameters, and Interactions

| term                        | estimate | std error | t ratio | p > |f|
|-----------------------------|----------|-----------|---------|-----|---|
| intercept                   | 8.68125  | 0.00125   | 6945    | <0.0001 |
| water content               | -7.71375 | 0.00125   | -6171   | 0.0001 |
| reaction temperature        | 3.08375  | 0.00125   | 2467    | 0.0003 |
| sulfuric acid content       | 2.48875  | 0.00125   | 1991    | 0.0003 |
| water content × reaction temperature | -2.74625 | 0.00125 | -2197 | 0.0003 |
| water content × sulfuric acid content | -2.52125 | 0.00125 | -2017 | 0.0003 |
| reaction temperature × sulfuric acid content | -0.23375 | 0.00125 | -187 | 0.0034 |

*p > |f| refers to the p-value.

Evidence that means of the factor levels are not the same and that there are interactions between factors A and B, factors A and C, and factors B and C. It proceeds that all individual parameters and their interactions have significant effects on lignin recovery. To determine the significance of each coefficient in Equation 5, a statistical t-test is employed for each parameter. The t-test results show whether main effects and interactions effects are significant on lignin recovery. The null hypothesis is that the true value of each parameter is zero, thereby having no significant effect on lignin recovery. According to Table 3, all parameters and interactions are significant because their p-values are all less than 0.05.

Table 2. ANOVA for the Second-Order Model Based on Experimental Data

| DF  | SS         | MS         | F ratio | p > F |
|-----|------------|------------|---------|-------|
| model | 6          | 713.26848  | 118.878 | 9      | 510 246 | 0.0002 |
| error | 1          | 0.0000125  | 1.25 × 10^-5 |       |
| total      | 7          | 713.26849  |         |       |

*DF = degrees of freedom, SS = sum of squares, MS = mean square. p > F refers to the probability of obtaining an F-value greater than the calculated one at a 95% significance level.

It is noted that there are only two levels at each factor in this design. More levels at each factor achieve a broader range of values for an improved design.

Effect of Water Content on Lignin Recovery. While it is widely known that EG is highly soluble in water,32,33 the effect of adding water to organic solvents on lignin recovery has seldom been explored. As a result, an experiment investigating the solubility of lignin in various EG–water mixtures was conducted. Figure 4 depicts the colors of organosolv lignin dissolved in EG–water mixtures with varying water contents. Organosolv lignin dissolved in water forms a slightly cloudy yellow solution, while organosolv lignin dissolved in EG forms a transparent dark brown solution. The color of the lignin dissolved in the solvent mixture with 25% water content is significantly darker than that in the mixture with 75% water content, suggesting greater lignin solubility in the solvent mixture with 25% water content.

Quantitative data of the experiment investigating lignin solubility supports the qualitative data in that a smaller water content results in greater lignin solubility. To make sense of the absorbance readings collected from the experiment, the highest absorbance reading was normalized to a value of 1; all other absorbance readings were taken relative to that value. Assuming absorbance readings to be proportional to lignin solubility, Figure 4 shows that normalized absorbance readings are inversely proportional with the water content, and this trend roughly aligns with data from fractionation experiments conducted (see Figure 1). An EG–water mixture with 25% water content containing lignin had a normalized absorbance reading of 0.459, which is 6.9 times more compared to the water mixture with 25% water content.

As expected, high lignin recovery is associated with low water content, high reaction temperature, and high sulfuric acid content. Furthermore, from plots A and B, the interactive effects of the water content and temperature as well as the water content and sulfuric acid content on lignin recovery were similar. Temperature and sulfuric acid content have a similarly lesser effect on lignin recovery than that of the water content.

This trend is further demonstrated in plot C, where lignin recovery increases at a similar rate with respect to sulfuric acid content and temperature. It is noted that there are only two levels at each factor in this design. More levels at each factor achieve a broader range of values for an improved design.

Figure 3 consists of surface plots that were constructed to better understand the interactive effects of water content, reaction temperature, and sulfuric acid content on lignin recovery. Equation 5 is used as a formula to predict lignin recovery values for the surface plots. To obtain the maximum lignin recovery within the scope of parameter values used in this study, the following reaction condition should be employed: 25% water content, 180 °C, and 1% sulfuric acid. As expected, high lignin recovery is associated with low water content, high reaction temperature, and high sulfuric acid content. Furthermore, from plots A and B, the interactive effects of the water content and temperature as well as the water content and sulfuric acid content on lignin recovery were similar. Temperature and sulfuric acid content have a similarly lesser effect on lignin recovery than that of the water content.

Figure 3. Surface plots of lignin recovery vs different pairs of parameters: (A) water content and temperature; (B) water content and sulfuric acid content; (C) sulfuric acid content and temperature.
Qualitative and quantitative results support the hypothesis that an increased water proportion in an EG–water solvent mixture leads to a lower lignin recovery because of the relatively low lignin solubility during fractionation. This could be attributed to two reasons or a combination of the reasons: (a) hydrophobicity of lignin or (b) EG–water hydrogen bonds. It is also important to note that these reasons do not conflict with each other but can rather be two valid reasons for decreasing lignin solubilities as the water content increases.

The trend observed in Figure 4 could also be attributed to lignin’s hydrophobic properties. In a similar way that water is used to precipitate lignin out during the lignin precipitation stage of the fractionation experiments, the addition of water to EG in the lignin solubility experiments could simply result in less lignin dissolving in the solvent mixture. Such hydrophobic interactions between lignin and water could be a reason why an increasing water content would result in decreasing lignin recovery. However, such a speculation merits more evidence.

Interactions between a lignin dimeric compound, an EG molecule, and a water molecule were investigated using a constructed DFT model. Figure 5 depicts the different hydrogen bond lengths between the same oxygen atom of EG and same hydrogen atom of lignin in two arrangements. Hydrogen bond lengths between the lignin dimer and EG are consistently longer in mixtures with water. For instance, hydrogen bond a has a length of 1.7892 Å, while the hydrogen bond d has a length of 1.8378 Å. This implies that the addition of water weakens the hydrogen bonds between the lignin dimer and EG. Because hydrogen bonds between the hydroxyl groups in EG and lignin contribute to the lignin dissolution in EG, hydrogen bond lengths from the DFT model suggest that the addition of water in organic solvents used for fractionation decrease lignin recovery.

Studies reporting strong hydrogen bonding interactions between the hydroxyl groups of water and EG support the DFT model. A study employing near-infrared spectroscopy found that in EG–water mixtures, small water clusters surround the OH groups of EG; this results in strong hydrogen bonds between the hydroxyl oxygen atoms in EG and the water hydrogen atoms. These conclusions support the varying hydrogen bond lengths in Figure 5 because the presence of water molecules causes interactions between water and EG; this results in significantly fewer available oxygen atoms of EG hydroxyl groups to form hydrogen bonds with certain hydrogen atoms in the lignin dimer. At a higher water content, the small water clusters that could surround the hydroxyl groups of EG molecules reduce the chances of hydrogen bond formation between lignin and EG.

In addition to hydrogen bonding, interaction energies can be analyzed to develop a better understanding of interactions between lignin, EG, and water. A study on energy decomposition analysis shows that the interaction energy between EG and water increases each time a water molecule is added to the same number of molecules of EG. The DFT models in Figure 5 align with this analysis; structure A has an interaction energy of 13.91 kcal/mol, while structure B has an interaction energy of 39.12 kcal/mol. These results may indicate that the water molecules form stable structures with EG molecules, leading to fewer interactions between lignin molecules and EG molecules.

Although the experiment conducted on lignin solubility and the constructed DFT model provides mechanistic insights on EG–lignin interactions to understand the effect of the water content on lignin recovery, there are several limits to the DFT model. Conclusions drawn from the simple model can develop, rather than ensure, an understanding of interactions between EG, water, and lignin. Besides the lignin model compound used in the study, there are other compounds in lignin that interact with EG and water.

### CONCLUSIONS

EG-assisted biomass fractionation resulted in 27 wt % lignin recovery, but it also significantly cleaved aryl–ether bonds in lignin during the process. The use of the sulfuric acid catalyst at a high temperature during the fractionation cleaved most

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**Figure 4.** Qualitative observations of organosolv lignin dissolved in EG–water mixtures with varying water contents from 0 to 100 (A). The lignin concentration in each solution was 5 g/L. Quantitative observations in exploring the effect of the water content on normalized absorbance readings (B).

**Figure 5.** Optimized geometry of (A) a lignin model dimer and EG and (B) a lignin model dimer, EG, and two water molecules. The following pairs of hydrogen bonds represent the bonds between the same hydrogen atom and oxygen atom: bonds a and d, bonds b and e, and bonds c and f. Hydrogen bond lengths are as follows: 1.7892 Å for a, 2.8397 Å for b, 2.8967 Å for c, 1.8378 Å for d, 3.3774 Å for e, and 2.9369 Å for f.
aryl–ether linkages in lignin, which are important to preserve for lignin valorization. Statistical analysis shows that all individual effects and interactive effects were significant on lignin recovery. DFT results imply that the addition of water to EG decreased lignin recovery due to strong EG–water hydrogen bonds and the resulting decrease in the intermolecular interaction between EG and lignin.

**EXPERIMENTAL SECTION**

**Materials.** A softwood mixture of SPF was purchased from Okanagan Pellet Company (Kelowna, BC, Canada) and ground to a size of less than 250 μm. The moisture content of the air-dried SPF was 5.8 wt %. Table 4 shows the chemical composition of the raw material that was analyzed according to the NREL protocol. Sulfuric acid (96.5 wt % assay) and EG were purchased from Fisher Scientific and Sigma-Aldrich, respectively, and they were used without further purification.

**EG-Assisted Fractionation of Biomass.** In the fractionation process, SPF wood was added to an EG–water mixture and heated at a target temperature of 160 or 180 °C to separate biomass components. Approximately, 10 g of SPF wood was loaded with 100 mL of the EG–water mixture (water content of 25 or 75%) to a 460 mL Parr batch reactor (Parr Instrument, US). Sulfuric acid (0 or 1 wt % of biomass loading) was also added to the mixture. During the reaction, the mixture was treated at the target temperature with 300 rpm stirring for 1 h; the temperature inside the reactor was kept within ±5 °C of the target temperature. Once the reaction completed, the reactor was cooled to room temperature in an ice bath. The mixture was filtered, and the solid cake was washed with an EG–water mixture (the same water content as that of the reaction solvent). The solid cake was washed a second time with distilled water, so that the residual EG was removed from the solid cake.

A volume of water (∼3 times the volume of the filtrate) was added to the filtrate to facilitate lignin precipitation. If no sulfuric acid was added during pretreatment, sulfuric acid (1% of wood loading mass) was added to facilitate lignin precipitation. The mixture was placed in a fridge for 24 h to further facilitate lignin precipitation. The lignin precipitates were filtered and washed with distilled water; the washed lignins were dried and stored at 50 °C in a vacuum oven for further analysis.

Lignin recovery was calculated as a percentage of the experimentally recovered lignin mass (g) to the theoretical lignin mass (g)

\[
\text{Lignin recovery (\%) = } \frac{\text{experimentally recovered lignin mass (g)}}{\text{theoretical lignin mass (g)}} \times 100\%
\]

where theoretical lignin mass indicated the actual mass of lignin that can be extracted from the wood mass used in the experiment, as determined by the NREL protocol.

Theoretical lignin mass (g) = dry raw wood mass (g) \times raw biomass lignin content (%)

**Lignin Characterization Techniques.** GPC analysis was conducted to determine the molecular weight of the lignin samples. Prior to the analysis, lignin samples were acetylated in an acetic anhydride/pyridine mixture (1:1, v/v) at 25 °C for 24 h. Acetylated lignins were dissolved in tetrahydrofuran (THF) for GPC analysis. Lignin molecular weight analysis was conducted using the Agilent GPC Security 1200 system equipped with three Waters Styragel columns (HR1, HR2, and HR6) and an ultraviolet detector. The analysis was conducted with THF as a mobile phase at 1.0 mL/min. Polystyrene standards were used for calibration. Polymer Standards Service WinGPC Unity software was used for data collection and processing.

A 2D 1H–13C HSQC NMR analysis was conducted to understand the structural changes of lignin during fractionation. The recovered lignin was dissolved in a 5 mm NMR tube with DMSO-d6. The NMR experiment was conducted at 300 K using a Bruker AVANCE 800 MHz spectroscopy equipped with a TCI CryoProbe with the following conditions: spectral width of 12 ppm in F2 (1H) with 1024 data points and 220 ppm in F1 (13C) with 512 data points; 32 scans (NS) and 1.5 s interscan delay (D1).

**Computational Analysis.** The mechanistic interactions between lignin, EG, and water were explored by conducting computational analysis. Considering that the structure of lignin is very complex, it is difficult to conduct a mechanistic study with the lignin macromolecule. Instead, lignin model compounds are typically employed for computational simulations. In this work, (1-(4-hydroxyphenyl)-2-phenoxypropane-1,3-diol), a lignin model dimer, was used to represent the lignin macromolecule. Two molecular models with varying elements were produced: one consisted of the lignin dimer and an EG molecule, while the other consisted of the lignin dimer, an EG molecule, and a water molecule. Bond distances between the hydrogen atoms of the lignin dimer and the oxygen atoms of the EG molecule were compared between the two models. The geometry optimization of the lignin dimeric model compound, EG, and water were performed using DFT with the B3LYP and the 6-31+G(d,p) basis set. In addition, frequency calculations were conducted to verify that the optimized structures corresponded to energy minima. The most stable structure of the lignin dimer, EG, and water was used to calculate interaction energy (IE) using the supermolecular approach

\[
\text{IE} = -\{E_{\text{complex}} - (E_{\text{dimeric lignin}} + E_{\text{EG}} + E_{\text{2(H, O)}})\}
\]
Lignin Solubility in Varying EG-Water Mixtures. To elucidate the effect of the water content on lignin recovery, a simple experiment was conducted. Organosolv lignin (0.1 g) extracted from SPF wood was put in 20 mL EG-water mixtures with the five following water contents: 0, 25, 50, 75, and 100%. The contents of the vials were mixed using an ultrasonic bath for 1 h. The solid content was filtered using Whatman 1001-110 Grade 1 qualitative filter paper. The filtrate was put in a centrifuge (Cole-Parmer) to remove any insoluble lignin and transferred to a cuvette for UV spectrophotometer (1600 PC, VWR) analysis at 240 nm, the wavelength to detect soluble lignin. EG-water mixtures (20 mL) at the five aforementioned water contents without lignin were used as blank solutions. The absorbance reading of each sample with lignin dissolved at varying water contents was taken relative to that of the corresponding water content without lignin.

Statistical Design. A statistical full factorial design was implemented with three factors that have two levels each, as shown in Table 5. This resulted in eight experiments conducted in a random order without replication. The three factors, water content, reaction temperature, and sulfuric acid content were represented by the variables, A, B, and C, respectively; the response was lignin recovery.

The second-order polynomial equation below is used to predict lignin recovery values, y, based on collected experimental data, where $\alpha_i$ represents regression coefficients:

$$y = \alpha_0 + \alpha_A A + \alpha_B B + \alpha_C C + \alpha_{AB} AB + \alpha_{AC} AC + \alpha_{BC} BC$$

(5)

Regression coefficients were determined based on experimental data using JMP software. The model used the standard least-squares personality with an emphasis set at effect screening. ANOVA was used to determine the significance of each parameter and their interactions. Response surfaces were constructed to visually explore the interactions between factors and their combined effects on lignin recovery.

Table 5. Full Factorial Design for Fractionation Experiments

| factor                        | low level | high level |
|-------------------------------|-----------|------------|
| water content (%)             | 25        | 75         |
| reaction temperature (°C)     | 160       | 180        |
| sulfuric acid content (wt %)  | 0         | 1          |

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Notes

The authors declare no competing financial interest.

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