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Reductive Catalytic Fractionation of Corn Stover Lignin

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S Supporting information

ABSTRACT: Reductive catalytic fractionation (RCF) has emerged as an effective biomass pretreatment strategy to depolymerize lignin into tractable fragments in high yields. We investigate the RCF of corn stover, a highly abundant herbaceous feedstock, using carbon-supported Ru and Ni catalysts at 200 and 250°C in methanol and, in the presence or absence of an acid co-catalyst (H₃PO₄ or an acidified carbon support). Three key performance variables were studied: 1) the effectiveness of lignin extraction as measured by the yield of lignin oil, 2) the yield of monomers in the lignin oil, and 3) the carbohydrate retention in the residual solids after RCF. The monomers included methyl coumarate/ferulate, propyl guaiacol/syringol, and ethyl guaiacol/syringol. The Ru and Ni catalysts performed similarly in terms of product distribution and monomer yields. The monomer yields increased monotonically as a function of time for both temperatures. At 6 h, monomer yields of 27.2 and 28.3% were obtained at 250°C and 200 °C, respectively, with Ni/C. The addition of an acid co-catalysts to the Ni/C system increased monomer yields to 32% for acidified carbon and 38% for phosphoric acid at 200 °C. The monomer product distribution was dominated by methyl coumarate regardless of the use of the acid co-catalysts. The use of phosphoric acid at 200°C or the high temperature condition without acid resulted in complete lignin extraction and partial sugar solubilization (up to 50%) thereby generating lignin oil yields that exceeded the theoretical limit. In contrast, using either Ni/C or Ni on acidified carbon at 200°C resulted in moderate lignin oil yields of ca. 55%, with sugar retention values >90%. Notably, these sugars were amenable to enzymatic digestion, reaching conversions >90% at 96 h. Characterization studies on the lignin oils using 2D HSQC NMR and GPC revealed that soluble oligomers are formed via solvolysis, followed by further fragmentation on the catalyst surface via hydrogenolysis. Overall, the results show that clear tradeoffs exist between the levels of lignin extraction, monomer yields, and carbohydrate retention in the residual solids for different RCF conditions of corn stover.

KEYWORDS: Lignocellulose, biomass, lignin, pretreatment

INTRODUCTION

Lignin is an alkyl-aromatic heteropolymer commonly found in cell walls of terrestrial plants, where it serves multiple physiological roles in plant structure, water and nutrient transport, and defense against microbial attack.¹⁻³ Lignin in plant cell walls is typically linked via C-O and C-C linkages, the distribution of which is driven by the composition of the monolignol pool during plant cell wall biosynthesis. Lignin accounts for up to 28% of the mass in lignocellulosic biomass.⁴ In typical biomass conversion processes, lignin is often considered a major hindrance to accessing and depolymerizing cellulose and hemicellulose to soluble sugars for conversion into renewable fuels and chemicals. As such, many biomass pretreatment strategies have been designed to overcome the physical and chemical barrier of lignin through biomass fractionation or lignin redistribution, often with little to no concern for the fate of the lignin as a usable substrate.⁵⁻⁹

In parallel to sugar conversion to fuels and chemicals, lignin valorization has long been examined, but the inherent heterogeneity and recalcitrance of lignin has made its

conversion to value-added compounds a major technical barrier that has not been overcome to date. As a prime example, in the pioneer second generation ethanol biorefineries being constructed and brought online over the last several years, the residual lignin following saccharification and ethanol fermentation is routed for combustion to produce heat and power or for co-firing with coal, leading to poor valuation of lignin in lignocellulosic biorefining. Indeed, most pretreatment and fractionation techniques being scaled today yield lignin that is even more recalcitrant than native lignin, as pretreatment often relies on the use of mineral acids or hot water, both of which can readily cleave C-O bonds to form reactive intermediates that condense to more refractory C-C bonds.¹⁰⁻¹²

Driven by the critical need to improve biorefinery economics and sustainability, conversion strategies aimed at upgrading lignin into biofuels and biochemicals have recently experienced a substantial resurgence. New catalytic and biological approaches offer promise to overcome the primary barriers found in lignin valorization.^{2-3, 13-14} Of particular interest is the work from Pepper *et al.*¹⁵, demonstrating that a

mild reductive catalytic treatment, dubbed Reductive Catalytic Fractionation (RCF) by Schutyser *et al.*, can generate a narrow set of lignin-derived products at high yields (often >40%) from hardwoods.¹⁵⁻²⁰ Most RCF studies employ late transition metal catalysts with hydrogen gas or a hydrogen donor (e.g., methanol, isopropanol, or formic acid) to cleave aryl-ether bonds and stabilize the resulting reactive species via reductive pathways. Subsequent studies have demonstrated that parameters such as catalyst type, biomass feedstock, solvent, and reaction conditions, profoundly impact product yield, selectivity, and distribution.²¹⁻²² Two common observations across different studies converge on the critical roles of the solvent in rapidly cleaving the abundant β -O-4 bonds in lignin at mild temperatures (~200-250°C) and of the heterogeneous catalyst in stabilizing fragments even without being in direct contact with the solid biomass.

RCF is very effective on hardwoods because these feedstocks typically exhibit high syringyl-to-guaiacyl (S/G) monolignol ratios, which translate into high proportions of easily cleavable β -O-4 linkages relative to the more refractory C-C linkages. In contrast, feedstocks with lower S/G ratios, such as softwoods or herbaceous biomass, feature a higher proportion of C-C linkages, making RCF more challenging. In particular, herbaceous feedstocks exhibit low S/G ratios (0.62) and higher content of the hydroxycinnamic acids (HCAs), *p*-coumaric and ferulic acids, as represented in Figure 1.²³⁻²⁷ The *p*-coumaric acid moieties are typically pendant to the syringol moieties in lignin, whereas ferulic acid polymerizes through a broad suite of linkages both internal and pendant to the lignin polymer, as well as via covalent attachment to hemicellulose. Schutyser *et al.* reported lignin monomer yields of ca. 50%, 21%, and 27% after the RCF of birch (a hardwood), pine/spruce (a softwood mixture), and miscanthus (a grass), respectively.^{16, 28} Indeed, herbaceous feedstocks such as corn stover, switchgrass, wheat straw, sugarcane bagasse, and miscanthus represent a large fraction of the biomass available in the global bioeconomy.²⁹ As such, new RCF approaches for herbaceous feedstocks need to be developed to selectively activate a more diverse suite of linkages for effective

delignification and subsequent upgrading of the resulting stream of lignin fragments.

Here, we investigate the RCF of corn stover—an abundant feedstock in lignocellulose conversion, especially for North America.³⁰ Corn stover lignin is composed of up to ~20% *p*-coumaric acid and ~10% ferulic acid and a low S/G ratio of 0.62.²⁶⁻²⁷ We present a comprehensive study on the role of reaction conditions, catalyst type, mineral acid co-catalysts, and the acidity of the catalyst support to gain insight into the underpinnings of solvolysis and fragment stabilization. The use of mineral acid co-catalysts has been demonstrated for hardwood lignin, but has not been explored under reductive conditions for the extraction of herbaceous biomass that contain a considerable amount of ester linkages.³¹ As with previous studies, the primary objectives for the overall process are extent of delignification, lignin product yields, carbohydrate retention in the residual solids, and the susceptibility of the polysaccharides to enzymatic digestion. Comprehensive characterization of the resulting solubilized lignin stream and the residual solids was used to gain insight into the primary catalytic and solvolytic mechanisms that occur during RCF, and to provide guidance for the development of methods to improve the RCF process.

EXPERIMENTAL

Catalyst synthesis and characterization: Acidified activated carbon was synthesized via nitric acid oxidation. Specifically, 100 mL of concentrated nitric acid was added to 50 g of Darco activated carbon (Sigma-Aldrich) and stirred for 12 h. The slurry was then added to 400 mL of deionized water. The supernatant was decanted and additional washes were performed until the pH of the supernatant was within a range of 3-4. The slurry was then filtered and the cake was washed with additional deionized water rinses until the pH of the filtrate was constant.

The 5 wt% Ni/C catalysts were synthesized by wet impregnation. Briefly, 1.27 g of nickel nitrate hexahydrate (Sigma-Aldrich) was dissolved in 8 mL of deionized water and added to 4.75 g of Darco carbon (Sigma-Aldrich). The slurry

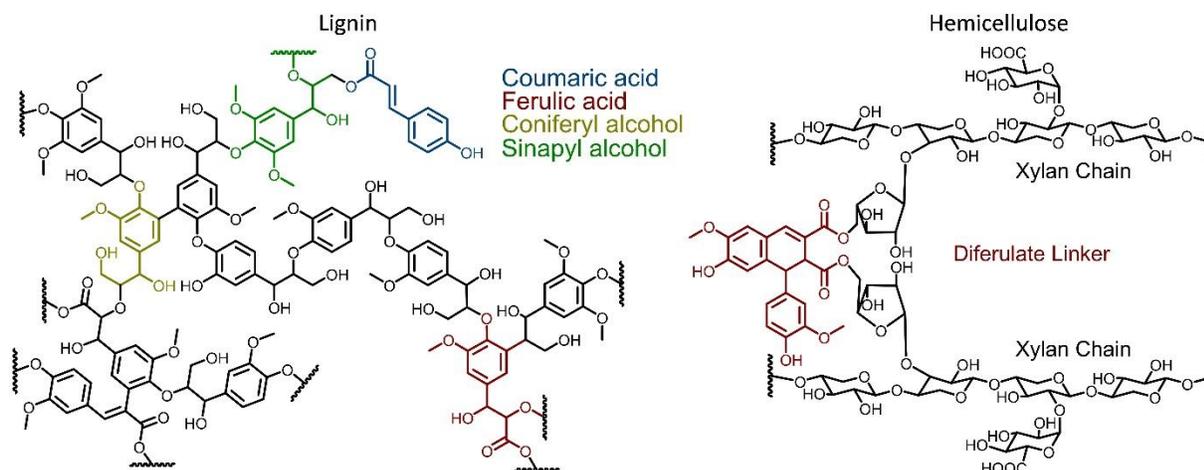


Figure 1: Hypothetical structures of lignin and hemicellulose modified by ferulic acid linkers in herbaceous biomass.

was stirred for 24 h to allow the nickel solution to fully wet the carbon. Next, the catalyst was dried in an oven held at 120°C overnight. The catalyst was reduced in a tube furnace by treating in nitrogen gas flowing at 100 mL min⁻¹ at 450°C for 2 h with a ramp rate of 7°C min⁻¹. Commercial 5 wt% Ru/C was purchased from (Sigma Aldrich) and was used as received.

The total number of acid sites was measured with ammonia temperature programmed desorption (TPD) on an Altamira Instruments AMI-390 system. Samples (~100 to 200 mg) were packed into a quartz tube and heated to 600 °C at 10 °C min⁻¹ in Ar flowing at 25 sccm and held for 1 h to pre-treat the carbon. The samples were then cooled to 120 °C, flushed with 25 sccm He for 10 min, then saturated with ammonia by flowing 25 sccm of 10% NH₃/He over the samples for 30 min at 120 °C. Excess ammonia was removed by flushing with 25 sccm He for 10 min. The samples were then heated to 600 °C in 25 sccm He at 30 °C min⁻¹, holding at 600 °C for 30 min, and the desorbed ammonia was measured with a thermal conductivity detector (TCD). The TCD was calibrated after each experiment by introducing 7 pulses of 10% NH₃/He from a 5 mL sample loop into a stream of 25 sccm He. Acid site quantification was performed assuming an adsorption stoichiometry of one ammonia molecule per site

Biomass reactivity studies: Reactions were performed in batch mode using a parallel reactor system (Parr 5000 series, 75 mL). Typical reactions used 1 g of corn stover (≤2 mm, S/G 0.62), 100 mg of catalyst containing 5 wt% of either ruthenium (Ru/C) or nickel (Ni/C) on carbon, and 30 mL of methanol. The reactors were stirred at 1,000 RPM using magnetic stir bars. The reactors were sealed and purged three times with 30 bar of helium. Then purged an additional 3 times with 30 bar of hydrogen. Reactions were run for 1, 3 and 6 h at temperatures of 200 or 250°C. The heating ramp took 20-30 min depending on the final temperature of the reaction. For reactions using a homogeneous acid, 170 μL of phosphoric acid (85 wt%, Sigma-Aldrich) was added resulting in an acid concentration of 0.1 M. To stop the reaction, the reactors were quenched in a room temperature water bath until the internal temperature equilibrated. The reactor contents were filtered with a 0.2 μm polyethersulfone filter paper. The solid biomass residue isolated after the reaction was washed with approximately 15 mL of methanol. To separate the dissolved sugars from the organic soluble lignin products, a liquid-liquid extraction was performed with water and dichloromethane (DCM). The methanol was removed by vacuum evaporation and then dissolved in a biphasic solution consisting of 10 mL water and 10 mL DCM. The DCM layer was separated and the water was extracted two additional times with 10 mL of DCM. The DCM layers were combined and extracted once with 5 mL of water. The DCM was then removed by vacuum evaporation to yield the DCM soluble lignin oil. Solid retention and lignin oil yields were calculated using the following equations:

$$Yield_{oil} = \frac{oil\ mass - extractive\ free\ oil\ mass}{dry\ corn\ stover\ mass \times lignin\ content} \quad (1)$$

$$Solids\ retention = \frac{solid\ residue\ mass}{corn\ stover\ mass} \quad (2)$$

Biomass compositional analysis: Pre-reaction compositional analysis was performed by the standard NREL Laboratory Analytical Procedure (LAP).³²⁻³³ Briefly, the extractives such as fatty acids, salts and free sugars were first removed using high temperature and pressure extractions with water and ethanol.³⁴ The extractive-free biomass was digested in 72 wt% sulfuric acid for 1 h at 30°C. Water was then added to the slurry to dilute to a final acid concentration of 4 wt%. Next, the slurry was heated in an autoclave at 121°C for 1 h. The insoluble lignin fraction, known as Klason lignin, was determined gravimetrically by filtering the remaining residue after acid digestion. The soluble lignin fraction was determined by UV/Vis spectroscopy (Thermo Scientific Nanodrop 8000 spectrophotometer), using absorbance measurements at 320 nm with an extinction coefficient of 3.0. The sugar content was determined by high performance liquid chromatography (HPLC, Agilent 1100 HPLC) using a refractive index detector (RI) kept at 85°C. A Shodex Sugar SP0810 column equipped with a guard column was used for analysis at 85°C with a flow rate of 0.6 mL min⁻¹ of HPLC grade water as the mobile phase.

Carbohydrate characterization: Post-reaction solid carbohydrate residues were dried at room temperature for 24 h. The dry solids were then sieved with a 104 μm metal sieve to remove residual catalyst pellets. The acid digestion for sugar quantification was performed as outlined in the LAP.³³

Lignin characterization: The monomer products were analyzed by gas-chromatography - mass spectroscopy (GC-MS, GC-Agilent 7890A and MS-Agilent 5975C). GC-MS samples were prepared from the DCM-soluble lignin oil. The lignin oil was dissolved in methanol to a concentration of 5 mg/mL. The GC oven parameters used were as follows: hold at 50°C for 1 min, heat at 10°C min⁻¹ until 250°C and then ramp at 25°C min⁻¹ to 300°C. The flame ionization detector (FID) was calibrated with propyl guaiacol and ethyl phenol (Sigma-Aldrich), as well as with methyl coumarate and methyl ferulate that were synthesized from the parent acids (Sigma-Aldrich) by esterification with methanol. The monomer yield and selectivity calculations were performed on a weight basis using the following equations:

$$Yield = \frac{\sum\ mass\ of\ each\ monomer}{Initial\ corn\ stover\ mass \times Total\ lignin\ content} \quad (3)$$

$$Selectivity_{alkenes} = \frac{\sum\ mass\ of\ each\ alkene}{\sum\ mass\ of\ each\ monomer} \quad (4)$$

The degree of depolymerization was determined by gel permeation chromatography (GPC, Hewlett Packard, Ti Series 1050). The GPC samples were prepared by dissolving 5-15 mg of DCM-soluble lignin oil in 0.5 mL of anhydrous pyridine

(Sigma-Aldrich) and then acetylated with 0.5 mL of acetic anhydride (Sigma-Aldrich) at 40°C for 24 h. Samples were then quenched with 1 mL of methanol and dried by flowing nitrogen at room temperature. The solids were dissolved in tetrahydrofuran (THF) to a concentration ranging between 1–2 mg mL⁻¹ and the solution was filtered through a 0.2 μm syringe filter. Sample volumes of 20 μL were injected into three PLgel 7.5x300 mm columns connected in series: 10 μm x 50 Å, 10 μm x 10³ Å and 10 μm x 10⁴ Å (Agilent). An isocratic profile (45 min) was used with THF as a mobile phase at a flow rate of 1 mL min⁻¹ at room temperature. Products eluting from the column were monitored with a diode array UV detector at 260±40 nm. The system was calibrated with polystyrene standards which range in size of 1 MDa to 580 Da. Toluene was also used as a standard at 92 Da.

Nuclear Magnetic Resonance: Heteronuclear Single Quantum Coherence (HSQC) NMR spectra for extracted lignin oil samples were recorded at 25°C on a Bruker 400 MHz nuclear magnetic resonance (NMR) spectrometer with a 5 mm BBO probe with a Z gradient. Each lignin oil sample (94–222 mg) was dissolved in deuterated chloroform (1 mL). Spectra were acquired with a sweep width of 15 ppm in the F₂ (¹H) dimension and 239 ppm in the F₁ (¹³C) dimension, respectively. A total of 400 scans were performed with 1024 data points in the ¹H dimension over 256 increments in the ¹³C dimension. An acquisition time of 80 ms was used for ¹H and 5.3 ms for ¹³C. A relaxation delay of 1.0 sec was used for each spectrum. The solvent peak of chloroform was used as an internal reference (δ_H 7.26, δ_C 77.36 ppm).

Enzymatic hydrolysis of residual solids: Enzymatic saccharification reactions of biomass were carried out according to NREL's LAP.³⁵ CTec3 and HTec3 (Novozymes) were applied to an AKTA FPLC protein chromatography system (GE) using a HiPrep 26/10 Sephadex (GE) desalting column to remove stabilizers and other additives that interfere with the bicinchoninic acid (BCA) protein assay and HPLC sugar quantification. Protein concentration was measured by BCA (Pierce). Enzymatic hydrolysis reactions with CTec3 and HTec3 were loaded in a ratio of 35 mg/g CTec3 and 5 mg/g HTec3 (40mg/g) and incubated at 50 °C in 20 mM sodium acetate at pH 5.0. Digestions contained 1% solids (10 mg of biomass per mL) and were conducted in sealed 2 mL vials with continuous mixing by inversion at 10–12/min intervals. Enzymes were loaded as milligram of protein per gram of glucan in 1.0 mL reaction volumes. Slurry samples were withdrawn from well-mixed digestion mixtures at selected time points during the digestions. Released cellobiose, glucose, and xylose in the diluted samples were then measured by HPLC analysis (same as compositional analysis). The sum of the concentrations of anhydro-glucose, anhydro-cellobiose, and anhydro-xylose is equivalent to the weight concentration of the carbohydrate chain that was hydrolyzed to produce the soluble sugars. The polysaccharide conversion was calculated with the following equation:

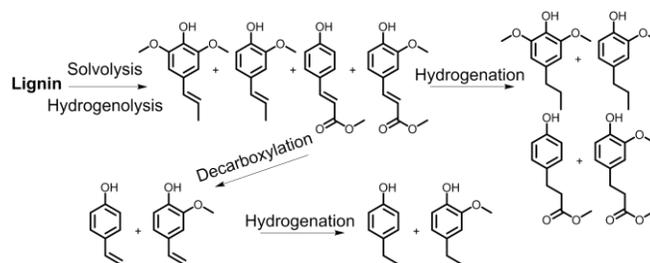


Figure 2: Proposed reaction scheme for RCF illustrating the observed monomer product distribution and potential reaction pathways.

$$\text{polysaccharide conversion} = \frac{\text{mass of soluble sugar}}{\text{initial polysaccharide mass}} \quad (5)$$

RESULTS

Product distribution of corn stover RCF: The primary monomeric products obtained from RCF are all derived from the 4 monolignols listed in Figure 1 and are shown in Figure 2. The monomeric products can be further grouped into three categories: propenyl phenols, hydroxycinnamates, and ethylene phenols. The propenyl phenols are generated from the direct cleavage of β-O-4 bonds and subsequent deoxygenation of the γ-hydroxyl group. Hydroxycinnamic acids such as coumaric and ferulic acid are primarily bound through ester bonds to the lignin and therefore can be liberated from lignin by transesterification with methanol. Methyl ferulate can be incorporated into the lignin structure as an ether, ester, or C-C linked species.²⁵ Therefore, methyl ferulate could also be produced by β-O-4 cleavage. Finally, the ethylene phenols are decarboxylation products produced from the hydroxycinnamates. The production of the monomeric species initially produces the unsaturated alkyl chain in the para position relative to the aromatic hydroxyl group, but is reduced to an alkane by the catalyst over the course of the reaction.

Table 1 is an illustrative example of the responses measured for each RCF extraction of corn stover showing the monomer, organic soluble oil, and residual solid yields for a 3 h extraction at 200°C with Ni/C. These responses are quantified for each extraction. Monomer yield is an important metric because it directly represents the useable lignin fraction for chemicals and fuels production. The total organic-soluble oil yield can be used as a measure of the extent of lignin extraction. In conjunction with this result the yield of residual carbohydrate solids indicates the degree of polysaccharide solubilization.

Table 1: Example product distribution obtained with a 3 h extraction at 200°C with Ni/C. Organic soluble oil yield, solids carbohydrate retention, and the selectivity to alkenes are shown.

Products	Yield (wt%)
Ethyl phenol	2.6
Propyl guaiacol	3.4
Propyl syringol	2.5
Methyl coumarate	8.7
Methyl ferulate	7.2
Other	0.4
Total	24.5
Selectivity to alkenes	55
Organic soluble oil	49
Solid carbohydrate retention	76

For the representative run shown in Table 1, a total yield of 24.5 wt% of monomers was observed, with a selectivity to aromatic alkenes of 55%. Hydroxycinnamates, namely methyl coumarate and methyl ferulate, accounted for 15.9 wt%. The next largest group of monomers observed were propenyl/propyl guaiacol and syringol totaling 5.9 wt%. Vinyl/ethyl phenol and guaiacol account for 2.5 wt%. This general trend in product distribution is common to all extractions performed in this work.

The residual solids constitute the fraction of the total biomass that was insoluble in methanol after the reaction. These solids largely consist of polysaccharides. Since the catalyst is difficult to separate from this solid mixture, the catalyst weight was subtracted from the total weight of dried solids. In the example illustrated in Table 1, the solids retention was 76 wt% based on the total mass of corn stover. The compositional analysis of corn stover shown in the Supporting Information (SI) indicates that the theoretical solids retention value is 71 wt%, assuming all the extractives, acetyl groups, and lignin are removed. The organic oil yield was used to assess the degree of delignification. The mass of oil obtained after the liquid-liquid extractions was corrected by the mass of DCM soluble extractives. The mass of DCM soluble extractives was determined from experiments performed with extractive-free corn stover. The lignin oil yield was then calculated by normalizing the corrected oil mass to the mass of total lignin in the corn stover. The lignin oil yield was 49 wt%. The dimer and oligomer yield for these conditions therefore is 50% because the monomers account for 24.5 wt% of the total lignin in the corn stover.

Depolymerization results for two different RCF catalysts:

The results for reactions carried out at the two temperature conditions are shown in Figure 3. Figure 3a displays the monomer yields and the degree of saturation of the propene chain of the aromatic alkenes at different times. The same six monomers were produced in each extraction as those described in Table 1. The individual values are shown in the SI. As shown in Figure 3a, the extractions performed at 200°C demonstrate that the monomer yield increased for both catalysts as the reaction time increased. The final monomer yield at 24 h is ~28 wt%. As seen for the reactions with Ni, there was little change in the monomer yield from 6 to 24 h. Additionally, the monomer yields from the two catalysts were similar at all times. The selectivity to alkenes, however, decreased at longer reaction times, resulting in nearly complete saturation of the propenyl chain at 24 h. Ru/C had a higher rate of saturation than Ni, suggesting that Ru was a more active hydrogenation catalyst. This trend also correlates with the degree of decarboxylation of the methyl esters in the reaction. Extractions with Ru/C result in half the yield of decarboxylation products as those obtained with Ni and have a correspondingly higher amount of coumarate and ferulate. The thermal decarboxylation of conjugated acids requires the presence of a double bond.³⁶ Thus, the faster saturation of double bonds in the case of Ru/C improves the methyl ester stability.

The delignification efficiency is shown in Figure 3c. Organic-soluble lignin oil was ~40 wt% yield at short times, and increased to ~60 wt% at 6 h, remaining constant up to 24 h. Oil yields were similar for both heterogeneous catalysts. The solid residue initially decreases from 76 wt% and remained constant up to 24 h for Ni/C. Reactions with Ru/C showed a marginal decrease in solids retention from 72 wt% at 1 h to 66 wt% at 24 h. No new compounds were observed in the organic oil for these reactions and the oil yield remained constant across these conditions. As such, it is presumed the decrease was due to the release of water soluble sugars or reduction products from sugars.

The results for supercritical methanol are shown in Figure 3b. In these reactions, higher monomer yields were reached at short times as compared to 200°C reactions. The highest monomer yield under these conditions occurred at 3 h. Ru/C produced a slightly higher monomer yield than Ni/C. The primary difference in the product distribution was the yield of methyl coumarate and methyl ferulate and their corresponding decarboxylation products. The reaction with Ru had a 4.5 wt% higher yield of the methyl esters and 2.7 wt% lower yield of decarboxylation products. The higher rate of decarboxylation observed with Ni/C directly accounts for the lower yield of monomers. When accounting for the mass lost as CO₂, the monomer yields differ by less than 0.5 wt%. At 6 h, a slight decrease in the yield of monomers was observed for

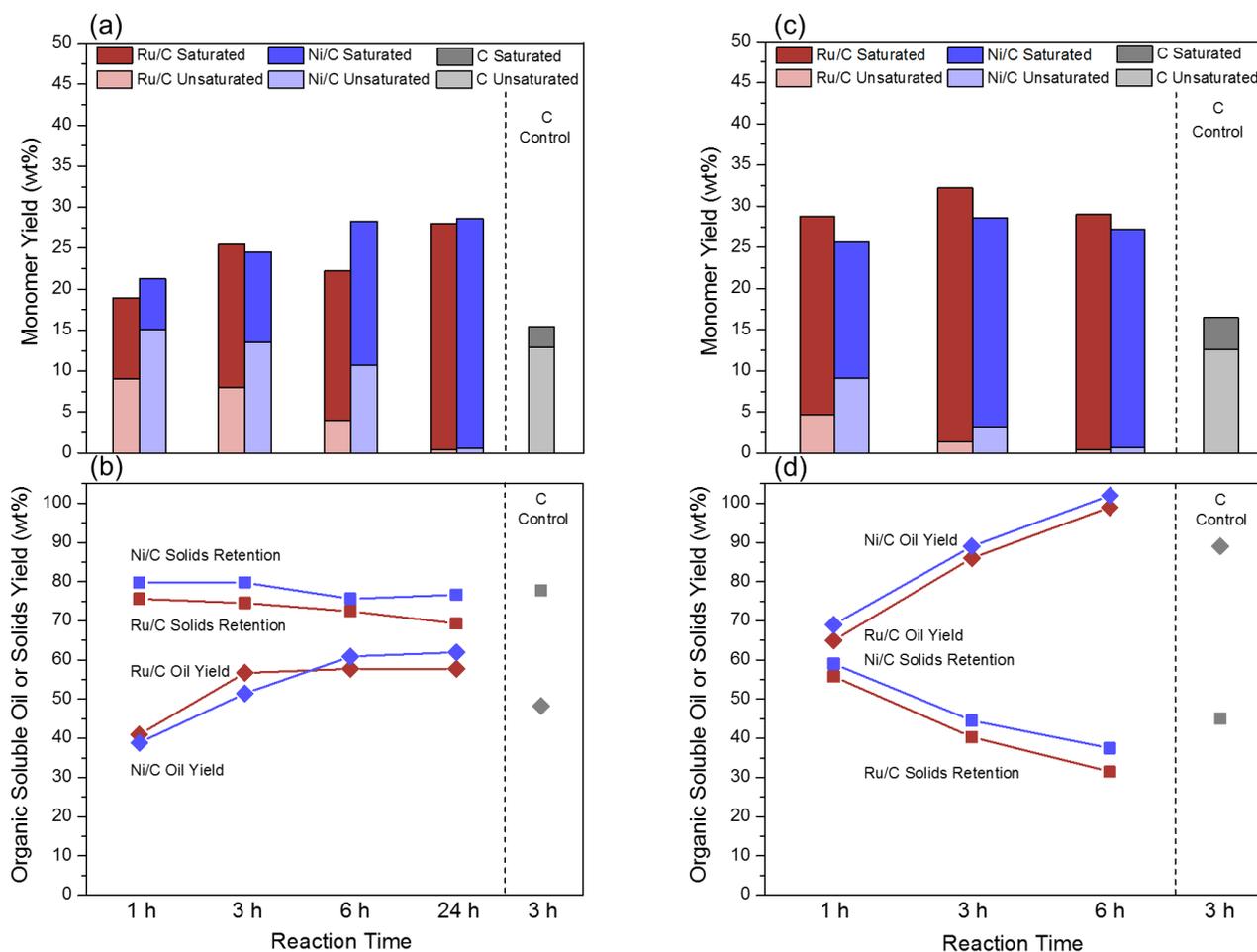


Figure 3: RCF of corn stover with Ru/C and Ni/C catalysts. The panels display the monomer yields, overall oil yields, and the solids retention for each catalyst at various reaction times. Panel (a) shows the monomer yields for 1, 3, 6, and 24 h at 200°C with Ru/C (red), Ni/C (blue), and Carbon (grey). The light and dark colors in the bars show the wt% yield of unsaturated propenyl aromatic monomers, whereas the dark color represents the yield of saturated propyl aromatics. Panel (c) shows the monomer yields at 250°C. Panel (b) and (d) display the yield of organic soluble lignin oil extracted from corn stover and the retention of insoluble carbohydrates after the extraction at 200 and 250°C, respectively.

both catalysts. Small amounts of dealkylation products were observed, such as guaiacol and syringol. In all reactions, lower selectivity to unsaturated products were observed.

As shown in Figure 3d, supercritical conditions result in a much higher delignification and lower solids retention for all reaction times. For example, at 1 and 6 h, 65 and 98 wt% of the lignin had been removed, respectively. Although much of the lignin was removed under these conditions, the solid retention was much lower in comparison to the 200°C reactions. Solid retention was below 60 wt% at 1 h and continued to decrease to about 37 wt% by 6 h. New products were also observed in the organic soluble oil fraction, consisting primarily of furan derivatives generated from the dehydration of solubilized carbohydrates. Additionally, a number of short-chain glycols was observed in the methanol fractions before the liquid-liquid extraction was performed. The production of glycols and other sugar decomposition

products have been observed for other high temperature hydrogenolysis process of woody biomass and sugar treatments.³⁷⁻³⁹ The physical appearance of the solid residues obtained from the two treatments was also different. Solids isolated after the 200°C treatment retained a similar size and shape to the untreated, pre-reaction corn stover particles; whereas at the high temperature, the particle size was smaller (figure S5).

To decouple solvolysis and hydrogenolysis, reactions were performed only with activated carbon at the two temperatures of interest. The monomer yields for both reactions were 16 wt% with slightly different monomer compositions. As expected, the primary products produced were methyl coumarate and methyl ferulate, which are produced via transesterification with the solvent. Interestingly, propenyl guaiacol and syringol were observed at concentrations of 1 and 1.5 wt% at 200 and 250°C, respectively.

Decarboxylation products were observed for both cases indicating that thermal decarboxylation can occur without a catalyst. The products observed from the control reactions were completely unsaturated except 65% of the vinyl phenol had reacted with methanol to form the methyl ether at 200°C. Some saturation of the vinyl phenol was also observed to ethyl phenol at 250°C. Organic oil yields and solids retention for the control reactions were nearly identical to those observed for the corresponding conditions with both catalysts.

RCF with an acid co-catalyst: An acid co-catalyst was added to improve the rate of lignin solvolysis and to accelerate the cleavage of the ester bonds to liberate coumarate and ferulate. A homogeneous or heterogeneous acid was used as a co-catalyst for lignin extraction. In the case of homogeneous phosphoric acid, a 0.1 M solution in methanol was used, whereas the heterogeneous acid was created by oxidizing the carbon support with nitric acid resulting in a solid with 32 μmol of H^+ /g as determined by ammonia TPD.

Results of acid co-catalyst RCF reactions are shown in Figure 4 and are compared to the base case where no acid was present. Binned monomer groups shown in Figure 4 contain both the saturated and unsaturated form of each compound. Monomer yields increased with reaction time up to 6 h. The final monomer yields with a homogeneous acid, a heterogeneous acid, and in the absence of an acid co-catalyst were 37.9, 31.9, and 28.6 wt%, respectively. Although each monomer type was observed in higher yields with acid, methyl coumarate was the largest contributor to the increased yield. Ether bond cleavage was also faster for reactions performed with a homogeneous acid. The yield of propyl guaiacol and syringol was nearly constant at ~ 10 wt% at all times, but increased over time for the other two cases. The heterogeneous acid resulted in a similar saturation as the base case with a 30% selectivity to alkenes, but the homogeneous acid maintained a 54% selectivity to alkenes after 6 h of extraction.

Figure 4b displays the organic oil yields and the solid retention for acid co-catalyzed experiments. The lignin oil yield with a homogenous acid approached 130 wt% of the expected value. This value was accompanied by a low solid retention of 39 wt% at 6 h. Due to the low solid retention, the increase in organic oil is likely due to acid catalyzed dehydration products from the carbohydrates. Dehydration of sugars with acid can produce furans, which can further react to form polymeric humins.⁴⁰ Organic oil yields and solid retention for the heterogeneous catalyst were similar to the base case, generating an oil yield of 54 wt% and a solid retention of 69 wt%.

Control experiments performed with acid and in the absence of Ni yielded ca. 16 wt% of monomers. The primary products observed were methyl coumarate and methyl ferulate. A distinct difference in the product distribution was observed when phosphoric acid was used as an acid catalyst when compared to the other two controls. The yield of coumarate

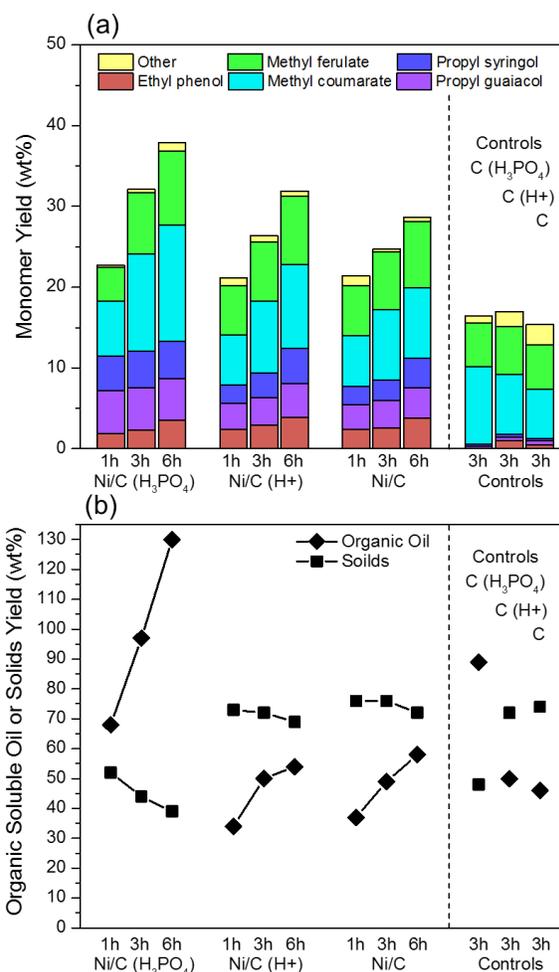


Figure 4: Comparison of monomer yields, lignin oils yields and solid retention from extractions performed with and without an acid co-catalyst at different times. Panel (a) displays the specific monomers and their corresponding yield for each different catalyst type which are the 4 separated clusters of bars. Increasing extraction time is shown for each catalyst type going from left to right. Control extractions with different acids are displayed after the dotted line. Panel (b) shows the oil and solids yield for each catalyst type with increasing time from left to right.

was higher, but the yield of propenyl guaiacol and syringol was halved compared to the other two controls. The decrease in yield of these compounds is likely due to the reactive nature of β -O-4 acid cleavage products in the absence of a reductive pathway for the stabilization of highly reactive intermediates.¹⁰⁻¹¹

Characterization of the oils and solids obtained from RCF: A critical component of biomass processing is the maximum utilization of both the lignin and carbohydrate fractions. Therefore, in lignin extraction from whole biomass via RCF, a key aim is to understand the chemical composition of the resulting lignin oil and the residual solids. The fractions

obtained from the 3 different RCF systems were further analyzed with two techniques at short and long times. Specifically, we used a combination of GPC and HSQC 2D NMR to gain insight into the high molecular weight species in the lignin oil. The residual solids were analyzed by compositional analysis to understand how much of the polysaccharides were solubilized during the extraction. Additionally, the solids were enzymatically treated to confirm the polysaccharides were readily digestible after RCF.

GPC: The lignin oils were analyzed by GPC to assess the reduction in molecular weight of lignin. The lignin oils were acetylated prior to analysis to ensure complete solubility in THF. The responses for each run were normalized to the monomer peak at 280 Da and scaled by the monomer yield obtained by GC. Figure 5 shows a comparison of the molecular weight distributions of the lignin oils when using Ni/C and Ru/C as catalysts at different reaction times at 200°C (note plots are displayed in a log scale). The features of each chromatogram are similar, containing 6 different peaks. Peaks at 210 and 280 Da correspond to monomers such as ethyl phenol and methyl coumarate, respectively. Small oligomer peaks observed at molecular weights of 550, 800 and 1,000 Da, are consistent with dimer, trimer and tetramer molecular weights. The largest oligomer peak can be observed at 2,800 Da, which corresponds to approximately 10 monomer units.

A clear trend can be observed where longer reaction times result in high oligomer yields. The primary change is observed in the largest oligomer peak at 2800 Da. At short times, the area of this peak is largest relative to other peaks within the sample chromatograph, but decreases as the reaction time increases. Additionally, the control experiment where

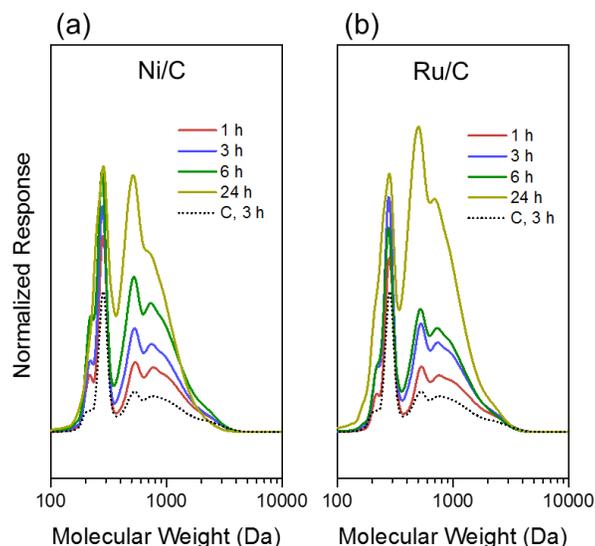


Figure 5: GPC data of the RCF of corn stover lignin using supported Ni and Ru catalysts at 200°C. Panel (a) show extractions performed with Ni/C. Panel (b) show extractions performed with Ru/C. A 3 h extraction with carbon is shown as a dotted line for comparison. Note MW is on a log scale.

hydrogenolysis cannot occur shows the lowest amount of small oligomers and highest amount of large oligomers. Comparing the results between Ni and Ru show that nearly all of the spectra are identical at the same reaction conditions. A similar trend can be observed for 250°C, but the peaks are much broader due to the harsher conditions used (see Figure S4).

Figure 6 shows the molecular weight distribution of the oil formed with acid co-catalysts. Similar to the reactions without acid, 6 peaks were observed in the chromatograms. However, new features were observed when phosphoric acid was used. Specifically, small peaks were observed at molecular weights under 200 Da that likely correspond to furan derivatives, as suggested by the GC data shown in the SI (Figure S3). Additionally, a large tail extending out to 7,000 Da was observed in the spectrum obtained for the 6 h reaction, potentially corresponding to a mixture of polyfurans or humins.

NMR: Lignin oil samples at different extraction times were investigated by HSQC 2D-NMR to gain structural information about the oligomeric fragments in the oils. Figure 7 shows the zoomed in NMR spectra from extractive free corn stover, a 1 h reaction with only carbon, and the 1 and 6 h reactions performed with Ni/C. An example of an entire spectra is shown in the SI (see Figure S9). The 1 h samples were run at concentrations of 180 mg/mL whereas the 6 h sample was run at 240 mg/mL. A peak corresponding to the β -O-4 ether bond is highlighted in the spectra, showing that more ether linkages are present with the carbon only run in comparison

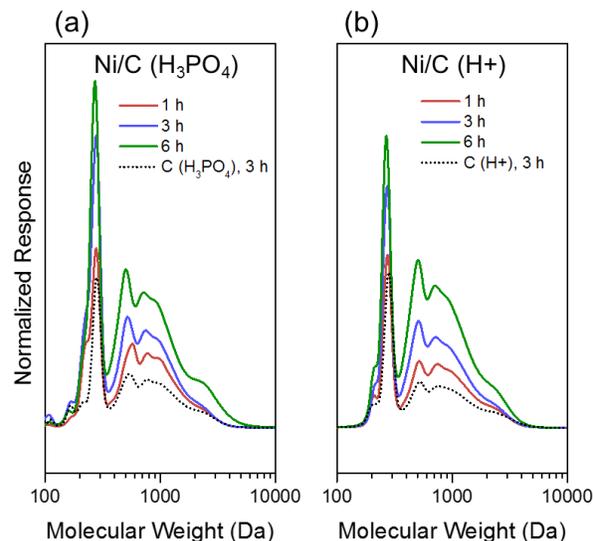


Figure 6: GPC data of the RCF of corn stover lignin using supported Ni with an acid co-catalyst. Panel (a) displays extractions performed with phosphoric acid at different times. Panel (b) displays extractions performed with acidified carbon. The respective controls are shown as dotted lines. Note MW is on a log scale.

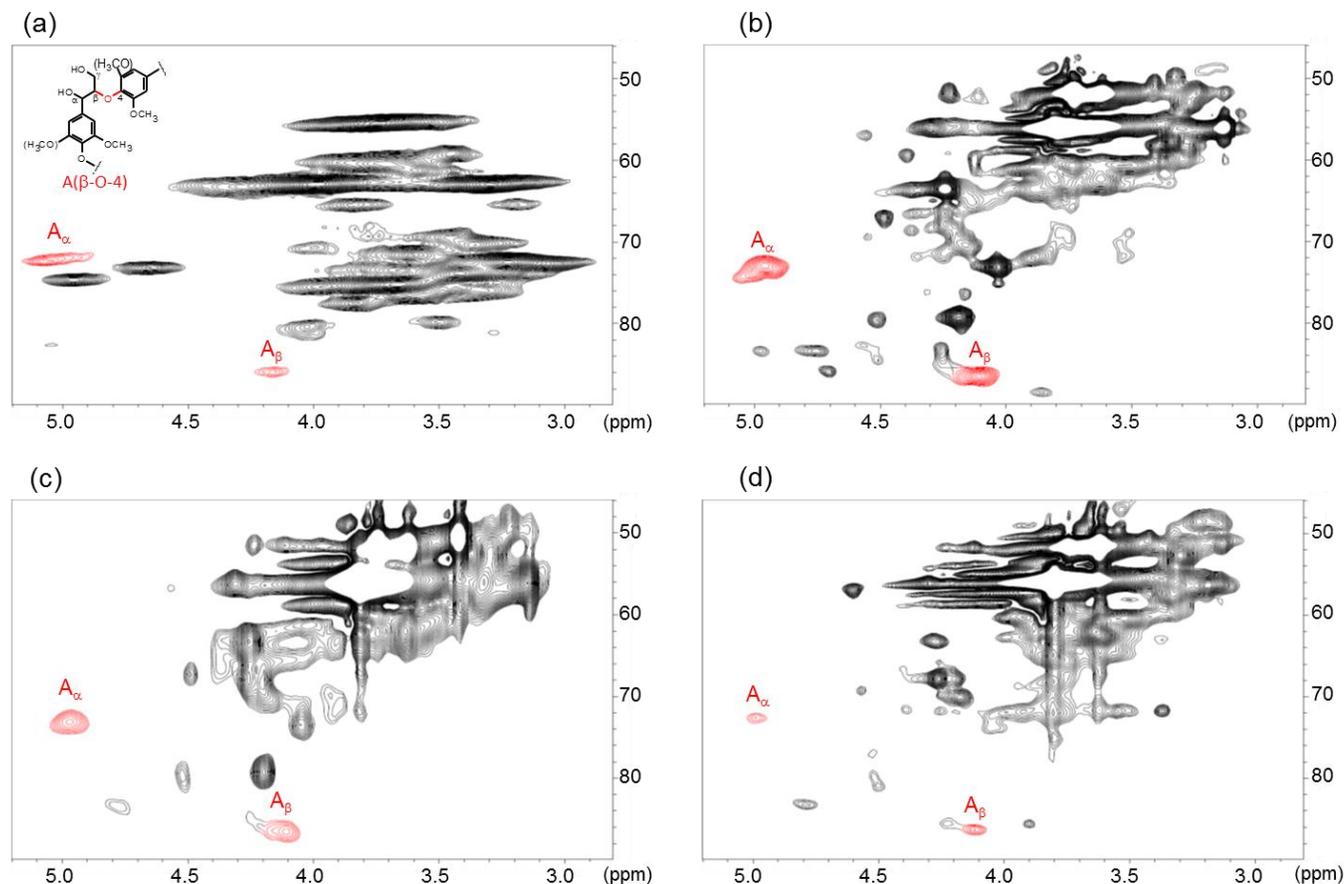


Figure 7: HSQC 2D-NMR of extractive-free corn stover and post extraction oils at 200°C. Panel (a) shows extractive-free corn stover. Panel (b) shows lignin oil from a 1 h extraction with only carbon. Panel (c) shows lignin oil from a 1 h extraction with Ni/C. Panel (d) shows lignin oil from a 6 h extraction with Ni/C.

to reactions performed with Ni/C at 1 h. The presence of this ether bond is further diminished at 6 h.

Sugar retention and digestion: Compositional analysis was performed post reaction to assess the polysaccharide content retained in the residual solids (Figure 8). The cellulose fraction describes the percent retention of C₆ glucans and the hemicellulose fraction represents a combination of retained C₅ and C₆ sugars including xylose, arabinose, and galactose. The sugars liberated during RCF were also quantified as aqueous sugars. Note that the aqueous fraction from the DCM-water extractions were treated with dilute acid before analysis (4 wt% sulfuric acid at 121°C for 1 h). This additional step was performed to convert methylated sugars back to the parent sugars.³¹

Total sugar retentions of 97% were observed for reactions performed with Ni/C at 200°C for both reaction times. When acidified carbon was used, the sugar retention was 94% at 1 h and decreased slightly to 91% at 6 h. The use of the phosphoric acid co-catalyst resulted in rapid hemicellulose dissolution. At 1 h, a sugar mass balance of 98% was obtained, while at 6 h the mass balance decreased to 71%. We attribute the loss of sugars to the dehydration and polymerization of furans, in

agreement with the GPC data (vide supra). Supercritical methanol conditions resulted in poor sugar retentions of 85 and 52% at 1 and 6 h, respectively. Interestingly, supercritical methanol was indiscriminate in the deconstruction of C₆ or C₅ sugars as evidenced by the similar rate of decomposition of both cellulose and hemicellulose.

The high sugar retentions for Ni/C and Ni/C (H⁺) prompted us to investigate if the sugars were digestible by cellulose (CTec3) and hemicellulose (HTec3) enzyme cocktails (Figure 9). The results of the 96 h digestion are shown in Figure 8. All of the residual solids showed comparable digestibility, producing glucan conversions of 80 to 90%. Conversion of xylan was slightly higher with 90 to 100% conversion. A control experiment using untreated biomass showed that without the RCF treatment, polysaccharides in corn stover were less prone to enzymatic degradation resulting in a glucan and xylan conversions of 45 and 61%, respectively.

Discussion

To be an effective pretreatment strategy for fuels and commodity chemical production from biomass, RCF will be required to achieve high levels of lignin extraction and

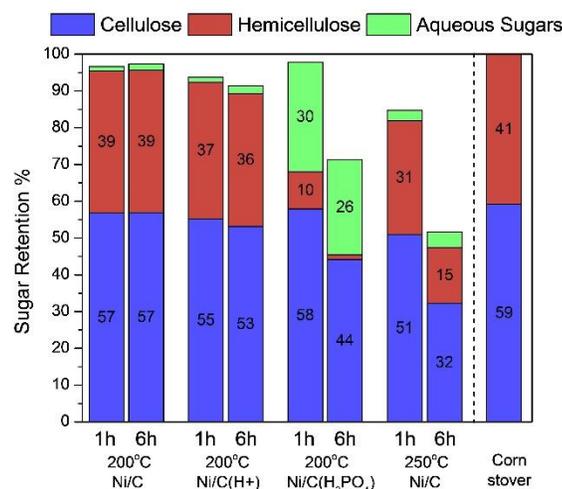


Figure 8. Compositional analysis of the residual solids. Each set of data displays the retention of each type of sugar at short and long times. Whole corn stover is also shown for comparison

depolymerization while minimizing sugar dissolution and decomposition. A clear tradeoff between these parameters exists based on the data shown in the present study. Reactions performed with super critical methanol resulted in moderately high monomer yields, but low cellulose and hemicellulose retention. The use of phosphoric acid increased monomer yields, but resulted in rapid dissolution of hemicellulose and eventual decomposition of the soluble sugars. Renders et al also observed high monomer yields and rapid hemicellulose dissolution when performing RCF of poplar co-catalyzed with phosphoric acid under similar conditions with a 3 h extraction.³¹ The sugar retention observed in this case was ~90% which falls in between those observed for corn stover at 1 and 6 h. Low temperature conditions with Ni/C and Ni/C (H⁺) result in moderate lignin extraction and depolymerization, but offer high solid retention and optimal polysaccharide enzymatic digestion. Thus, low temperature treatments offer a more direct path to total biomass utilization – a key driver for the RCF process.^{16, 20, 28} Additionally, the 200°C extractions have more amenable processing conditions such as operating pressures of 6 MPa as opposed to 13 MPa and a less corrosive reaction mixture when compared to phosphoric acid.

Tradeoffs between lignin extraction and depolymerization, and carbohydrate retention illustrate a clear relationship between biomass structure and the severity of the extraction conditions used. This relationship can be seen across the sets of experiments at 200°C with Ni/C performed with and without acid at 6 h. Experiments using Ni/C extracted 58% of the lignin with a monomer yield of 28%, while retaining >95% of the carbohydrates. Experiments using Ni/C on acidic carbon resulted in 54% lignin extraction with monomer yields of 32% and 90% carbohydrate retention. In comparison, when phosphoric acid was used, complete lignin extraction was obtained and monomer yields of 38% were obtained. The tradeoff for the higher yield was a low carbohydrate retention

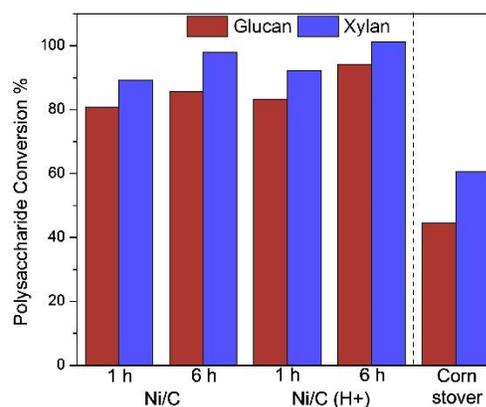


Figure 9: Enzymatic digestion performed on residual solids at a digestion time of 96.

of 45%. Interestingly, when a 24 h extraction was performed with Ni/C at 200°C the lignin extraction remained at 59% with monomer yields of 28% and a carbohydrate retention of >95%. These results imply that the maximum yield achieved with phosphoric acid cannot be obtained simply by extending the extraction time. The primary difference between these extractions is the retention of hemicellulose, which was completely removed with phosphoric acid. Therefore, the presence of carbohydrates, primarily hemicellulose, influences the efficacy of lignin solvolysis. The physical interpretation of this result, is that lignin and hemicellulose are intertwined in cell wall, which renders portions of lignin inaccessible to solvolysis and RCF as a whole. Therefore, complete delignification, based on this study, cannot occur without some dissolution of carbohydrates.

Recently, Luo et al. showed RCF could be performed when the metal catalyst is physically separated from the biomass using a catalyst basket.²⁸ From these data, solvolysis of lignin was shown to be essential for the extraction of soluble fragments or monomers that can then adsorb and react at the surface of the catalyst. In agreement with this study, we observed the direct solvolysis of lignin linkages when operating in the absence of a reduction catalyst. This effect was observed over multiple control reactions where a measureable amount of propenyl guaiacol and syringol was produced at 3 h. Additionally, the total mass of lignin oil obtained with only the carbon support was identical to extractions where a metal catalyst was used, implying solvolysis is solely responsible for lignin extraction.

The GPC results of the lignin oils also show similar peaks between the control and Ni/C reactions. Therefore, we can conclude that solvolysis is responsible for the range of small oligomers found in the extracted lignin oil. The largest fragment observed in the GPC was about 2800 Da, which corresponds to moieties composed of approximately 10 monomer units. Song et al. also investigated the oligomer size produced from the reductive treatment of birch by Maldi-

TOF mass spectroscopy, where the largest fragments had an m/z of 1500.¹⁹ We note that the actual size of the oligomers could be larger since multiple ionization points exist on a lignin oligomer. This observation supports the hypothesis that small oligomers are liberated by solvolysis from the bulk lignin polymeric structure embedded within raw corn stover and then undergo further depolymerization in solution. The existence of oligomers is further evidenced by the existence of β -O-4 bonds present in the extracted lignin oils as confirmed by NMR. These bonds are observed in samples with and without a hydrogenolysis catalyst. As expected, the relative amount of ether linkages is lower with a reduction catalyst as compared to the control using only the carbon support. β -O-4 bonds were also present in the 6 h extraction sample although in significantly reduced amounts. The presence of ether bonds in large soluble lignin fragments could explain the increase in lower molecular weight species observed by GPC between 6 and 24 h while no appreciable gains in total oil or monomers yields were observed. In this case, maximum lignin extraction had occurred by solvolysis and the hydrogenolysis of the remaining ether bonds likely produced C-C linked dimers and trimers. Taken together, these data suggest that large lignin fragments are extracted from biomass by solvolysis, which then migrate to the catalyst surface where hydrogenolysis of ether bonds occurs producing monomers and smaller oligomers.

Conclusion

Reductive catalytic fractionation of corn stover was investigated at 200 and 250°C in methanol using supported Ni and Ru catalysts in the presence of homogeneous and heterogeneous acid co-catalysts. It was found that although high monomer yields can be obtained with homogeneous acid or supercritical methanol conditions, poor sugar retention and decomposition at these conditions makes lignin depolymerization sub-optimal compared to milder treatments. Indeed, the use of an acidic support for the metal catalyst increases monomer yields, and also results in >90% carbohydrate retention. It was also shown that the residual polysaccharides from the extraction can be enzymatically deconstructed to sugars in high yields allowing for a holistic biomass utilization approach for further processing. Analysis of the lignin oils produced from RCF offered further insight into the underlying mechanism of how lignin fragments are liberated from whole biomass by solvolysis and then are reductively cleaved into monomers.

ASSOCIATED CONTENT

Supporting information

Contains additional figures and tables referenced in the above text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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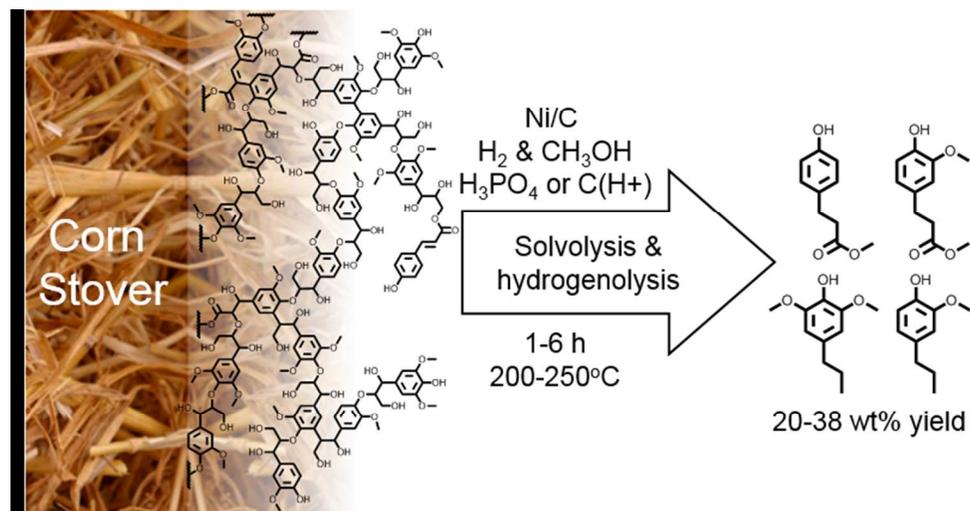
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Reductive Catalytic Fractionation of Corn Stover Lignin_{T T}

26 Eric M. Anderson, Rui Katahira, Michelle Reed, Michael G. Resch, Eric M. Karp, Gregg T. Beckham*, Yuriy Román-Leshkov*

27 Reductive catalytic fractionation (RCF) results in high extraction and subsequent depolymerization of corn
28 stover lignin affording a source of renewable aromatic chemicals.

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