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Reductive Catalytic Fractionation of C-Lignin

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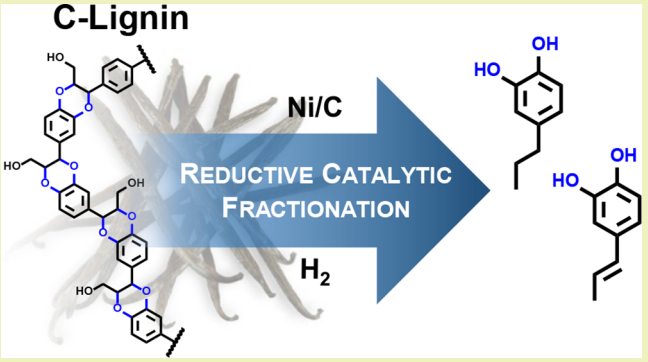
Supporting Information

ABSTRACT: Lignin composed solely of caffeyl alcohol units, or C-lignin, was recently discovered in the seed coats of a number of vanilla orchid and cactus species. The caffeyl alcohol monomer polymerizes into a highly uniform benzodioxane backbone, making C-lignin a promising substrate for lignin valorization, where heterogeneity is a key challenge. In this study, we used reductive catalytic fractionation (RCF) on vanilla seeds to investigate the depolymerization of naturally grown C-lignin. To overcome challenges associated with the high extractive content and poor sugar retention in vanilla seeds, the ratio of monomer yield to total lignin yield was used to isolate the depolymerization efficiency of C-lignin from the extraction efficiency of lignin from seeds. This approach allowed us to compare extents of depolymerization across lignin types and biomass feedstocks. C-Lignin RCF generated extents of depolymerization akin to those of hardwoods, despite observing incomplete benzodioxane cleavage due to catalyst deactivation caused by the seed extractives. In addition, depolymerization of C-lignin produced a favorable monomeric product distribution consisting of only propyl and propenyl catechol. These promising results suggest that genetic modification of other plant species to incorporate C-lignin has the potential to yield a single, valuable catechol product via RCF.



KEYWORDS: *Biomass conversion, Catechol, Benzodioxane, Vanilla seed, Lignin first, Hydrogenolysis, Solvolysis, Depolymerization*

INTRODUCTION

Lignin is the largest natural source of aromatics and comprises 15−30 wt % of biomass. Its conversion into fuels and chemicals is an ongoing and relevant challenge to reduce both humanity's collective carbon footprint and demand for fossil fuels, as well as to improve the economic viability of biorefining.[1−5] Most conversion strategies hinge on either reductive or oxidative pathways to cleave the ether linkages naturally present in the lignin polymer, resulting in a wide product distribution and limiting the yields of valuable monomers.[6−13] The prohibitive cost of complex separations is a main factor for the lack of an efficient process for valorizing lignin. Promisingly, a naturally occurring lignin that could potentially overcome these challenges was recently discovered in the seed coat tissues of a number of vanilla orchid and cactus species.[14,15] Known as C-lignin, this unique oxoaromatic polymer structure is polymerized from a single monomeric unit, caffeyl alcohol, almost exclusively connected through benzodioxane linkages (Figure 1).[14] This unique arrangement contrasts with the lignin structure found in most plant species (hereafter referred to as GS-lignin) containing a phenylpropanoid monomer combination of coniferyl alcohol (guaiacyl unit), sinapyl alcohol (syringyl unit), and *p*-coumaryl (hydroxyphenyl unit) in hardwood and softwood lignins, in addition to hydroxycinnamates, *p*-coumarate, and ferulate, in herbaceous lignins.[16] Tobimatsu et al. showed that the highly selective formation of the caffeyl alcohol monomer from which C-lignin is generated is associated with the loss in activity of the CCoAOMT and COMT enzymes responsible for converting hydroxyl groups to methoxy groups in the monolignol pathway, thus eliminating the formation of S and G units.[17] The stereochemistry of the benzodioxane backbone of C-lignin, consisting of a cis−trans isomeric mixture, led Chen et al. to conclude that C-lignin likely polymerizes through the same enzyme-initiated, kinetically controlled, radical recombination pathway as GS-lignin.[14,16] In GS-lignin, this radical recombination pathway results in the formation of several interunit linkages, including the predominant *β*−O−4 ether linkage, and a variety of carbon−carbon bonds such as *β*−5, 5−5, and *β*−*β*. However, in C-lignin, the hydroxyl group

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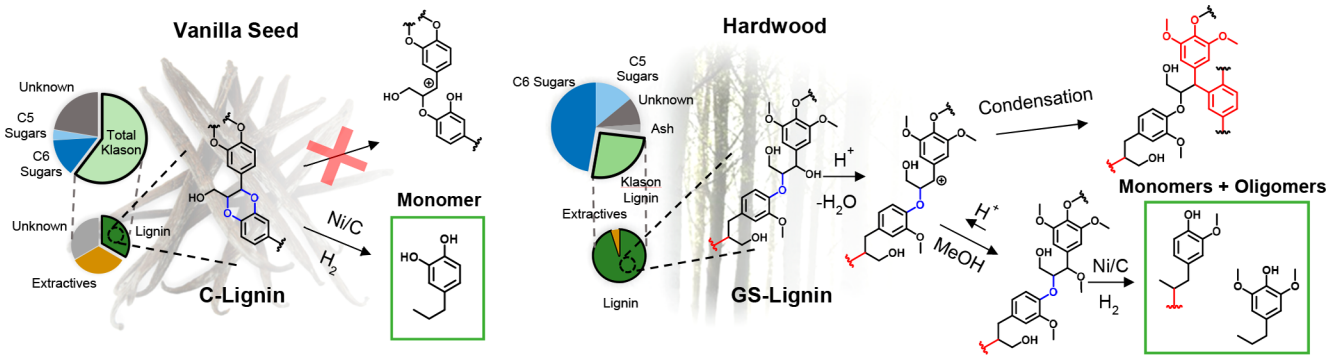

Figure 1. Schematic for the depolymerization and recondensation pathways for a benzodioxane linkage in C-lignin (shown on the left) and a β–O–4 linkage in GS-lignin (shown on the right). The benzodioxane linkage is hypothesized to prevent carbocation formation, which leads to condensation. Red bonds/molecules correspond to C–C linkages and blue bonds correspond to cleavable C–O bonds. The C–C linkage in the β-position of the guaiacol unit on GS-lignin is representative of the naturally occurring C–C bonds in GS-lignin. Pie charts showing the solids compositional analysis of seeds and poplar are included to illustrate the differences between the biomass types and the misleading Klason analysis on seeds.

on the 5-position of caffeyl alcohol enables a kinetically favored intramolecular ring closure, which drives the high selectivity toward benzodioxane linkages and prevents the formation of carbon–carbon bonds.[18] As depicted in Figure 1, these benzodioxane linkages should offer a unique opportunity for depolymerization processes to generate a smaller product distribution by increasing the percentage of easily cleavable ether bonds and eliminating a primary unwanted condensation pathway.

In addition to avoiding costly separations by having a smaller product distribution, the catechol products that could be produced from C-lignin are central intermediates in the biological conversion of aromatic monomers derived from lignin. In processes aimed to biologically produce high-value chemicals, such as adipic acid,[19] the coumarates, ferulates, phenols and guaiacols derived from RCF must first be metabolized by microbes to produce catechol,[20] a necessary step before enzymatic ring-opening can occur to form muconate (a platform chemical with a number of known processes to produce value added products).[21−24] If catechols can be directly produced from lignin, the upstream microbial step in the biological upgrading process could be completely bypassed. Furthermore, for C-lignin to become industrially relevant, it must be incorporated into more high-volume plant species. Though the genetic modification of model plant species to incorporate C-lignin has yet to be demonstrated in the open literature, there is hope for overcoming this challenge due to the plasticity of the lignin biosynthetic pathway.[25] Lignin plasticity has been shown through a variety of other genetic modifications to, for example, incorporate ferulic acid[26] or hydroxycinnamaldehydes[27,28] into the lignin polymer, to increase or decrease the guaiacol content,[29] or to make lignin with shorter chains.[30] Although genetic manipulations of lignin have been shown to produce plants with defects, reduced growth or other susceptibilities,[31] not all modified lignins cause these issues and the diverse number of lignin structures found in nature have reinforced the possibility of success in this area.[25]

Despite its potential, little is known about the depolymerization of C-lignin. Of the many lignin depolymerization techniques, reductive catalytic fractionation (RCF) has recently emerged as an effective strategy to selectively cleave the ether bonds of native lignin and stabilize the resulting fragments extracted from diverse biomass sources, including hardwoods (e.g., poplar[32−34] and birch[35−38]), softwoods (e.g., spruce[39] and pine[40]), and herbaceous feedstocks (e.g., corn stover[41] and Miscanthus[42]) both in batch and flow configurations.[43−45] RCF operates by solvolytically extracting lignin with a polar protic solvent and then reductively cleaving the ether linkages present in the soluble oligomers with a supported transition metal catalyst (e.g., Ni,[38,41,46,47] Pd,[34,48,49] Ru[35,50]) to create a narrow slate of oxygenated aromatic monomers and small oligomers. Generally, these monomers are consisting of substituted guaiacol, syringol and phenol moieties when RCF is performed on hardwood, softwood, and herbaceous feedstocks, with one case found in the literature from Barta et al. of catechols produced from candlenut lignin.[51] On the basis of the wide range of applications in the literature, we surmised that RCF would be an ideal platform to gain insights into the depolymerization of C-lignin. In this study, we developed tailored experimental protocols and metrics to investigate the depolymerization of C-lignin in vanilla seeds via RCF.

RESULTS AND DISCUSSION

Several metrics are used to quantify RCF performance: monomer yields benchmark the overall efficacy of the process; lignin oil yields (i.e., monomers + oligomers) measure the total lignin extraction (or % delignification); and the extent of monomer side chain saturation mirrors catalyst activity and can be used to track catalyst deactivation. Monomer yields are generally normalized by the initial lignin content in the biomass feedstock as determined by Klason analysis, which uses sulfuric acid to remove cellulose and hemicellulose and counts the remaining acid-insoluble fraction as lignin. Therefore, Klason lignin is a measure of the total acid insoluble, nonpolysaccharide content in the biomass as opposed to just lignin (Figure 1). Monomer yields are representative of the overall efficacy of the process because they take into account two important factors: the extent to which the lignin was extracted, and the extent to which the lignin was depolymerized. The extraction extent is based only on the solvolysis step of RCF, and depends on the chosen solvent, temperature, reaction time, reactor configuration, and importantly, the lignin-carbohydrate linkages and compartmentalization of lignin within the biomass. In contrast, the depolymerization

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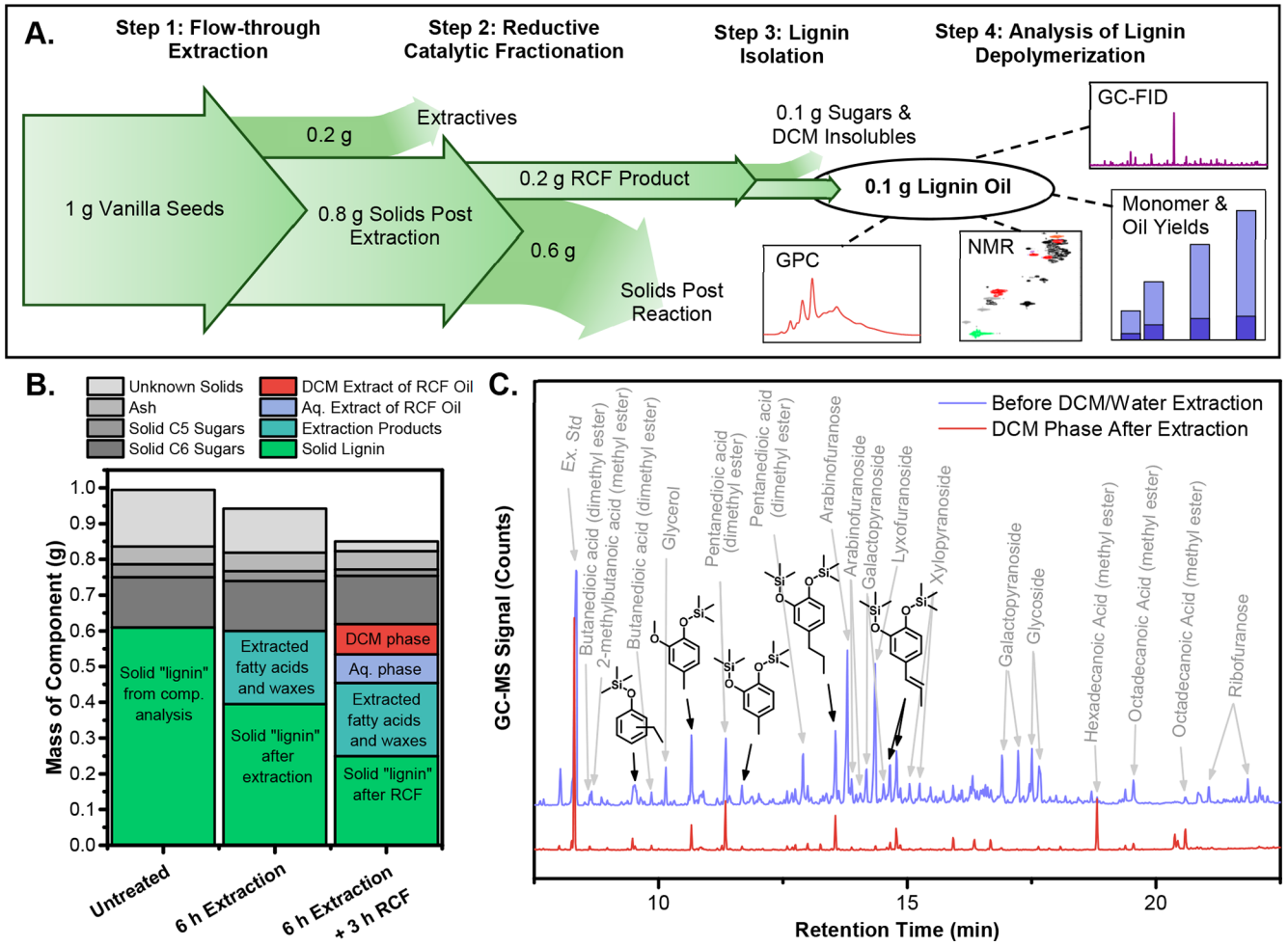

Figure 2. Experimental schematic and mass balance: (A) Diagram showing the experimental procedure and tracking the mass loss at each step. The RCF yields shown here are representative of the 3 h flow-through experiments: higher RCF extractions are obtained in batch reactions at higher temperatures or longer times. (B) Mass balance accompanying the diagram shown in panel A. Compositional analysis procedure and results are summarized in the SI. (C) Exemplary GC−MS trace of the RCF product oil before DCM extraction (blue) and the lignin oil after DCM extraction for the low-catalyst flow-through reaction at 0.5 h.

extent depends primarily on the turnovers of the catalyst and the lignin structure and chemistry.[45] Vanilla seeds feature a high Klason lignin content value of ca. 60 wt %,[14] making this lignin, at first glance, an attractive candidate for RCF. However, seeds serve a different role in the plant than traditional lignocellulosic material, and thus contain a much larger fraction of fatty acids and waxes, as well as a much smaller fraction of carbohydrates.

Compositional analysis of untreated vanilla seeds, solids after 6 h extraction, and solids after RCF demonstrate the difficulty of isolating lignin content from extractives content in vanilla seeds using traditional compositional analysis techniques, which merits caution when evaluating metrics to benchmark RCF performance. To demonstrate this, compositional analysis was performed on isolated vanilla seeds (*Vanilla planifolia*) obtained from a natural vanilla processing factory to determine Klason content both before and after an extraction pretreatment step. This pretreatment step was necessary to prevent the large fraction of extractives from poisoning the catalyst and artificially enhancing the lignin oil yields (see Figure S1 for RCF product without pre-extraction). In our optimized pretreatment, the milled seeds (<0.25 mm) were treated with a 6 h flow-through extraction (80 °C, 60 bar, and 0.5 mL/min of methanol) to extract fatty acids and waxes that accounted for approximately 20% of the overall weight (other pretreatment experiments and extractive characterization are shown in Figures S2−S4). Next, flow-through or batch RCF was performed on the pre-extracted seeds (200−250 °C, 15 wt % Ni/C, H_2, methanol) yielding another 20−40% of the initial solids by mass as RCF products. However, purification of the lignin fraction through a DCM/water extraction revealed that approximately half of the products extracted during RCF were aqueous soluble sugars and acids (Figure 2C), leaving 10−20% recovery of lignin oil as normalized by the initial seed mass. Importantly, Figure 2B shows that nearly all of the 0.4 g extracted in the overall process was removed from the initial solid "lignin" fraction, but only a small fraction of that was actually lignin. Therefore, it is clear that the Klason method grossly overestimated the true lignin content in vanilla seeds, even after a flow-through extraction step, because the large fraction of fatty acids and waxes present in the seeds was included as part of the acid insoluble fraction typically considered as "lignin". This outcome is expected, because the Klason method was developed for traditional lignocellulosic substrates with low extractive contents. Furthermore, our analysis of the large fatty acid fraction in vanilla seeds was

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corroborated with recent work by Barsberg et al., who confirmed the presence of lipids in orchid seed tissues by ATR-FTIR.[15]

To enable a more rigorous analysis of C-lignin, we propose using an alternative quantification metric: the monomer-to-oil ratio. The monomer-to-oil ratio is a representation of the extent of depolymerization and can be defined as follows:

$$\text{Monomer-to-oil ratio} = \frac{\text{Total monomer yield (g)}}{\text{Total lignin oil yield (g)}} \quad (1)$$

The monomer yield is determined from gas chromatography with a flame ionization detector (GC-FID) quantification of propyl catechol and propenyl catechol, and the oil yield is determined from the total mass of the dried DCM extract of the RCF products. By isolating the depolymerization step, it becomes possible to directly compare the depolymerization efficiency (or monomer-to-oil ratio) between C-lignin and GS-lignin despite their vastly different structures and compositions. Additionally, using the monomer-to-oil ratio as a metric does not require measuring the initial lignin content in the solid, thus alleviating the issue of poor lignin quantification resulting from the high extractive content in the seed. Theoretically, at the limit of complete ether bond cleavage with no recondensation, the ideal C-lignin structure with 100% benzodioxane linkages would achieve a monomer-to-oil ratio of 1. In contrast, the theoretical limit for hardwood lignin, which contains a variety of carbon−carbon linkages that prevent monomer formation, would be approximately 0.4−0.5.[40,52]

To study the role of the catalyst in the depolymerization of C-lignin, a catalyst loading study was performed using a dual-bed flow-through RCF system (details in SI). For each experiment, the upstream biomass reactor (solvolysis reactor) was loaded with 1 g of vanilla seed and the downstream catalytic reactor (reduction reactor) was loaded with either high (0.3 g), low (0.1 g), or no loading of 15 wt % Ni/C (pelletized to 100−200 mesh by mixing 50/50 with silica). After pre-extraction, RCF was performed for 3 h at 60 bar, with the solvolysis reactor at 210 °C and the reduction reactor at 190 °C, flowing 0.5 mL/min methanol and 50 mL/min H_2, and taking cumulative samples at 0.5, 1, 2, and 3 h. The lignin oil was purified through a DCM extraction, weighed, and derivatized for GC-FID analysis. Figure 3A shows that, as expected, the total lignin oil yield was very similar for each catalyst loading, because the solvolysis conditions were identical for each case. However, as catalyst loading was increased, the 3 h cumulative monomer yields (propyl catechol + propenyl catechol) increased from 0 wt % (normalized by the pre-extracted seed mass) with no catalyst to 1.5 and 1.9 wt % with low and high catalyst loading, respectively, indicating that the catalyst is necessary and responsible for cleavage of benzodioxane linkages. The increased monomer yields with catalyst loading correlated with increased cumulative molar selectivity toward propyl catechol (versus propenyl catechol, see eq S1) from 50% with low catalyst to 60% with high catalyst, revealing another role of the catalyst in saturation of the side chain, as has been shown in the literature for RCF of other biomass feedstocks.[46]

The relatively low overall monomer yields and side-chain saturation selectivities can be better understood by analyzing the transient aspect of the flow-through data shown in Figure 3A,B. Over the course of the 3 h reaction, the saturation

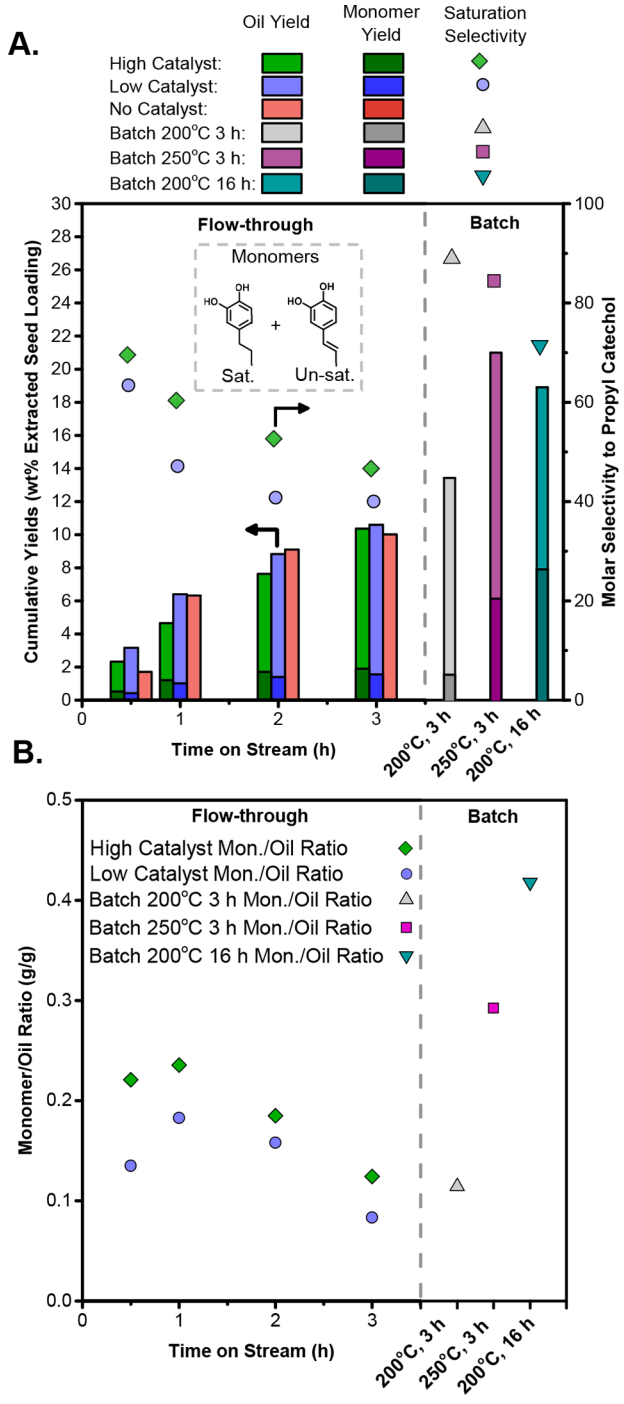

Figure 3. C-Lignin RCF data: (A) Monomer yields (dark bars), lignin oil yields (light + dark bars) and selectivity to propyl catechol (relative to propenyl catechol) (symbols). (B) Monomer-to-oil ratios. Conditions: Flowthrough: 0.3, 0.1, or 0 g 15 wt % Ni/C (50/50 SiO_2 100−200 mesh), 210 °C solvolysis, 190 °C reduction, 0.5 mL/min MeOH, 50 mL/min H_2. Batch: 30 bar H_2, 50 mL MeOH, 700 rpm, 250 or 200 °C, 3 or 16 h, 0.77 or 0.30 g pre-extracted vanilla seeds, 0.30 or 0.063 g 15 wt % Ni/C.

selectivity steadily decreased from 69% to 46% for high catalyst loading and from 64% to 40% for low catalyst loading, a clear indication of catalyst deactivation, as has been demonstrated previously on flow-through RCF with poplar.[43] Interestingly, the saturation selectivity for low catalyst loading is very similar to that of high catalyst loading, despite having three times less

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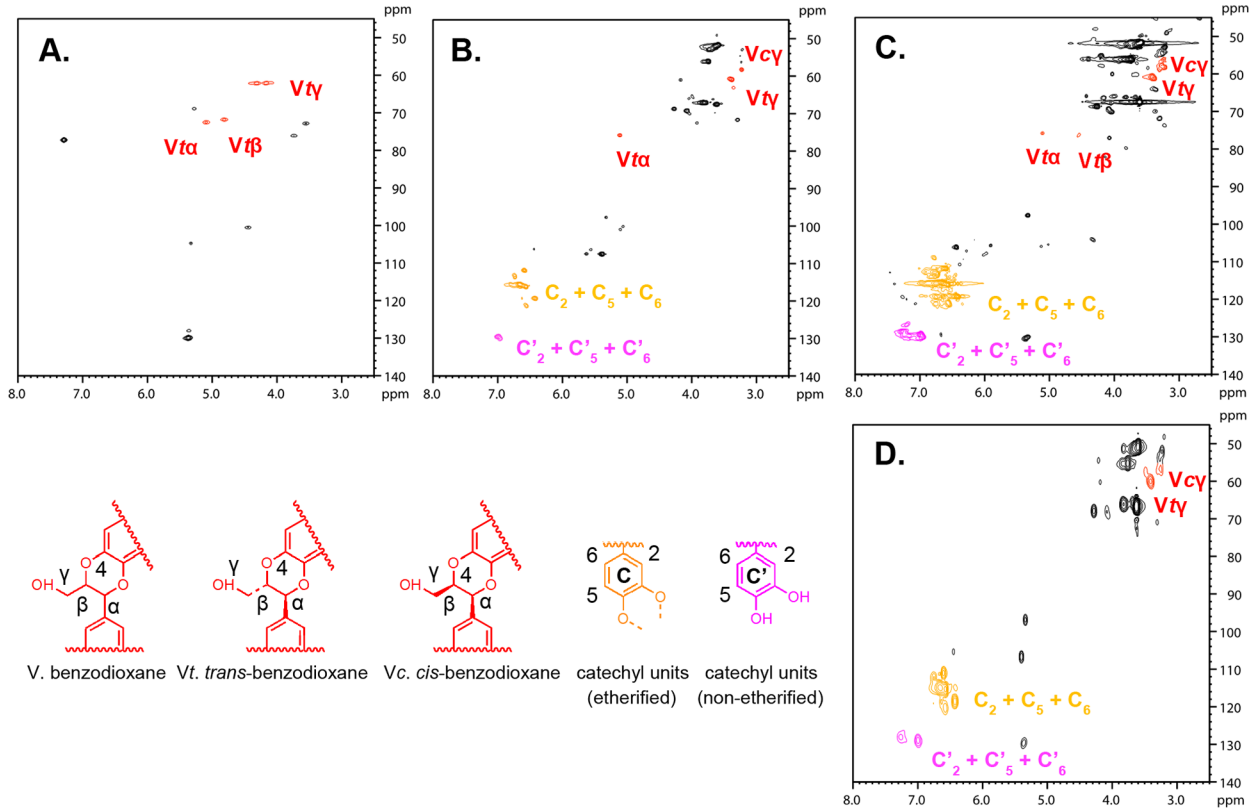

Figure 4. Heteronuclear single quantum coherence (HSQC) NMR spectra for (A) acetylated vanilla seed. (B) Flow-through RCF oil produced with high catalyst loading at 0−0.5 h on stream. (C) Batch RCF oil run at T = 250 °C, 30 bar H_2, and 3 h. (D) Batch RCF oil run at T = 200 °C, 30 bar H_2, and 16 h.

catalyst by mass, meaning the catalyst surface is being deactivated rapidly. This trend is also shown in Figure 3B with decreasing monomer-to-oil ratios as a function of time. Under complete conversion, the monomer-to-oil ratio should remain nearly constant throughout the 3 h reaction, as it would be completely dependent on the bond distribution in the lignin and not the extraction rates. Furthermore, 2D-HSQC NMR spectra were obtained on each of the oils, revealing that uncleaved benzodioxane linkages remain after RCF (Figures 4 and S5). Growing oligomer tails seen through gel permeation chromatography (GPC) and shifting product distributions by GC-FID as a function of time give further support of catalyst deactivation (Figures S6 and S7). The fast catalyst deactivation seen in the flow-through experiments can be explained by the product distributions in Figure 2B,C. The blue GC−MS trace in Figure 2C shows a large distribution of sugars and acids, and the blue aqueous phase bar in Figure 2B reveals that these components make up a significant fraction of the solvolysis effluent stream that is in contact with the catalyst. It has been previously shown in the literature that sugars lead to deactivation of RCF catalysts.[46,53,54]

To achieve higher lignin conversion despite catalyst poisoning, batch reactions were used to increase lignin−catalyst contact time. We studied three conditions in the batch reactor: 200 °C for a duration of 3 h (for comparison with flow results), 250 °C for 3 h, and 200 °C for 16 h, all using 15 wt % Ni/C, pre-extracted vanilla seeds, methanol solvent, and 30 (STP) bar of H_2 gas (see SI for complete experimental details). The loading of 15 wt % Ni/C was chosen to maximize reactivity while minimizing lignin adsorption to the support−above a catalyst loading of 0.4:1 catalyst:biomass the monomer yields decreased due to strong, preferential adsorption of the lignin to the catalyst (Figure S8). The 3 h 200 °C experiment achieved similar results as the flow-through reactions, with a monomer yield of 1.5 wt % (normalized to extracted seed loading), an oil yield of 13.4 wt % and a monomer to oil ratio of 0.11. However, the selectivity toward side chain saturation was much higher (89%). This result indicates that the longer contact time in batch reactions enables higher hydrogenation selectivity, but the monomer yields are still limited by the temperature and time of the reaction in both flow and batch reactor configurations. The experiment executed at 250 °C was in methanol's supercritical region, which has been shown to extract more lignin while also solubilizing a fraction of the sugars.[41] Although the higher extraction and solubilization of chemical components of the vanilla seeds led to a higher total lignin oil yield of 21 wt % normalized to pre-extracted seed loading, it also generated a lower monomer-to-oil ratio of 0.29 likely due to poisoning of the catalyst with nonlignin compounds and dilution of the lignin oil fraction with DCM soluble nonlignin extractives. A 250 °C 3 h batch reaction with no catalyst was performed as a control experiment, producing only traces of monomeric products and confirming that the catalyst is necessary for benzodioxane cleavage in supercritical methanol conditions (GC-FID chromatogram shown in Figure S9). Reducing the temperature to 200 °C and allowing for a longer reaction time of 16 h resulted in a slightly lower overall lignin oil yield of 19 wt %, but an increased monomer-to-oil ratio of 0.42. This effect is likely due to decreased extraction of nonlignin compounds and increased contact time with the catalyst to cleave a higher fraction of the benzodioxane linkages. The monomer-to-oil ratio increased to 0.51 when

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calculated on a molar basis, using UV−vis calibrated with catechol to determine the total aromatic content in the lignin oil (see Figures S10−S12 for calibration and supporting data, and section S.2 for calculation). The GC-FID trace of the batch lignin oil (Figure S13) showed a highly favorable monomer distribution consisting of primarily propyl catechol with a small fraction of propenyl catechol. A propenyl chain hydrogenation selectivity of 85% for 3 h, 250 °C and 72% for 16 h, 200 °C indicated catalyst deactivation in both batch conditions. In the 3 h 250 °C experiment, HSQC NMR showed uncleaved benzodioxane linkages after reaction (Figure 4); however, the 16 h, 200 °C experiment showed only traces of α- and β-benzodioxane linkages, with no traces of γ-benzodioxane linkages. Yet, GPC showed nearly identical molecular weight distributions of aromatic compounds (UV Signal measured at 280 nm) containing large molecular weights, indicating that recondensation also occurs at these conditions (Figure S14). The recondensation of unstabilized C-lignin was confirmed with an experiment in which the lignin extraction and the lignin reduction steps were separated into two batch reactions. First, a no-catalyst batch extraction in methanol and hydrogen was performed at 200 °C, and the lignin oil generated was then used in another batch reaction in the presence of catalyst, methanol solvent, and hydrogen gas at 250 °C. The composition of the oil was very similar before and after the catalytic reduction reaction, with only a slight decrease in the molecular weight of the oligomer fraction and very little monomer formation (Figures S15 and S16). This result indicates that the lignin likely condensed or restructured during the initial extraction and purification, a result that would be expected for GS-lignin, but that had not yet been shown for C-lignin containing the hypothetically more stable benzodioxane linkages.

The experimentally observed monomer-to-oil ratio of 0.51 (molar basis) was well below the theoretical maximum of 1 for C-lignin. Two known causes of this depressed monomer-to-oil ratio are the incomplete monomer stabilization caused by catalyst deactivation and the dilution of lignin oil with extractives, but other factors could contribute as well, such as recondensation routes before the lignin fragments reach the catalyst. However, despite these limitations, the monomer-to-oil ratios observed in the 16 h 200 °C batch reaction are comparable to those found in the literature for hardwood lignin (birch and poplar). In the case of hardwoods, low extractive content and high catalyst activity enables extents of depolymerization that approach the theoretical maximum at reaction conditions that enable complete β−O−4 cleavage. Our previous work in flow-through RCF of poplar[43] showed a monomer-to-oil ratio of 0.39 (mass basis) when operating at 50% lignin extraction and complete reductive ether bond cleavage. In batch configuration operated at near complete lignin extraction (93% and 86% delignification), Van den Bosch et al. demonstrated monomer-to-oil ratios of 0.43 for poplar and 0.46 for birch on a wt/wt basis, calculating monomer yields based on syringol and guaiacol derivatives (complete conditions and calculation of ratio included in SI).[35] Hardwoods, which can exhibit high S/G ratios and therefore higher ether content, are typically thought to set the upper bound for depolymerization efficiency for naturally occurring GS-lignins. Furthermore, the monomer yield from C-lignin was calculated by adding only propyl catechol and propenyl catechol, as compared to the monomer yields from other lignin types that consist of propyl guaiacol, propanol guaiacol, propyl syringol, propanol syringol, as well as hydroxycinnamic acid derivatives for herbaceous feedstocks.

CONCLUSIONS

The theoretical maximum depolymerization efficiency has been achieved for GS-lignins, with the monomer distribution set by the monolignols present in the polymer.[26] In this work, we have shown that similar depolymerization efficiencies can be achieved with C-lignin, but generating only propyl and propenyl catechol products. The differences between the theoretical and experimentally achieved monomer-to-oil ratios in this work suggest that the process could be improved by maintaining higher conversion at the catalyst and slowing down catalyst deactivation by further purifying the lignin oil. These impurity-caused limitations could be overcome by moving away from vanilla seeds as a substrate and incorporating C-lignin into more common, well understood, model plant species that could eventually become a high-volume lignocellulosic feedstock. Undoubtedly, plant engineering to incorporate C-lignin into other species would provide an opportunity to optimize RCF for maximum benzodioxane cleavage, and ultimately enable a process with high monomer yields of a single product.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.8b02741.

Description of experimental apparatus, reaction procedure, and analytical procedures. Calculations relevant to the data presented in the manuscript. Tables containing raw compositional analysis and product yield data. Figures of extraction experiments and characterization; additional HSQC-NMR, GPC and GC-FID chromatograms for flow-through experiments; catalyst loading study; raw spectra, calibration and molar conversion using UV−vis; GC-FID and GPC for batch reactions; separate extraction and reduction experiments; and NMR of propyl catechol standard (PDF)

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Notes

The authors declare no competing financial interest.

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