Green Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Green Chem.*, 2017, **19**, 5131

Predicting lignin depolymerization yields from quantifiable properties using fractionated biorefinery lignins†

Thanaphong Phongpreecha,[a] Nicholas C. Hool,[a] Ryan J. Stoklosa,[b] Adam S. Klett,[c] Cliff E. Foster,[d] Aditya Bhalla,[d,e] Daniel Holmes,[f] Mark C. Thies[c] and David B. Hodge *[g,h]

Lignin depolymerization to aromatic monomers with high yields and selectivity is essential for the economic feasibility of many lignin-valorization strategies within integrated biorefining processes. Importantly, the quality and properties of the lignin source play an essential role in impacting the conversion chemistry, yet this relationship between lignin properties and lignin susceptibility to depolymerization is not well established. In this study, we quantitatively demonstrate how the detrimental effect of a pretreatment process on the properties of lignins, particularly β-O-4 content, limit high yields of aromatic monomers using three lignin depolymerization approaches: thioacidolysis, hydrogenolysis, and oxidation. Through pH-based fractionation of alkali-solubilized lignin from hybrid poplar, this study demonstrates that the properties of lignin, namely β-O-4 linkages, phenolic hydroxyl groups, molecular weight, and S/G ratios exhibit strong correlations with each other even after pretreatment. Furthermore, the differences in these properties lead to discernible trends in aromatic monomer yields using the three depolymerization techniques. Based on the interdependency of alkali lignin properties and its susceptibility to depolymerization, a model for the prediction of monomer yields was developed and validated for depolymerization by quantitative thioacidolysis. These results highlight the importance of the lignin properties for their suitability for an ether-cleaving depolymerization process, since the theoretical monomer yields grows as a second order function of the β-O-4 content. Therefore, this research encourages and provides a reference tool for future studies to identify new methods for lignin-first biomass pretreatment and lignin valorization that emphasize preservation of lignin qualities, apart from focusing on optimization of reaction conditions and catalyst selection.

Received 5th July 2017,
Accepted 27th September 2017

DOI: 10.1039/c7gc02023f

rsc.li/greenchem

Introduction

Technologies for the conversion of renewable, non-food plant biomass to fuels, chemicals, polymers, and materials have the potential to displace fossil sources of carbon while simultaneously contributing to rural agricultural economies and lowering CO_2 emissions.[1] While technologies for the production of cellulosic biofuels are beginning to be commercialized in the U.S., Europe, and Brazil, substantial technical, logistical, and economic challenges still remain.[2] In particular, one economic challenge is that current processes produce low-value, high-volume biofuels that require substantially higher capital costs per unit of biofuel produced relative to starch- or sucrose-derived biofuels. This is a consequence of plant cell wall recalcitrance, whereby the polysaccharides that may serve as the feedstock for biological conversion to biofuels are embedded in the plant cell wall matrix. This cell wall matrix can be considered as a composite material comprising polysac-

[a]*Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI 48824-1226, USA*
[b]*Sustainable Biofuels and Co-Products Research Unit, Eastern Regional Research Center, USDA, ARS, 600 East Mermaid Lane, Wyndmoor, PA, 19038, USA*
[c]*Department of Chemical and Biomolecular Engineering, Clemson University, Clemson, SC 29634-0909, USA*
[d]*DOE Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI 48824-1601, USA*
[e]*Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824-6407, USA*
[f]*Department of Chemistry, Michigan State University, East Lansing, MI 48824-1322, USA*
[g]*Department of Chemical and Biological Engineering, Montana State University, Bozeman, MT 59717, USA. E-mail: david.hodge3@montana.edu*
[h]*Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, SE-971 87 Luleå, Sweden*
†Electronic supplementary information (ESI) available. See DOI: 10.1039/c7gc02023fd

charides (cellulose and hemicelluloses) and lignin that presents a challenge for "deconstruction" and conversion.[3]

Diversifying the product portfolio of lignocellulose-to-biofuels processes to include renewable, bio-based chemicals is widely recognized as an opportunity both to improve the economics of these processes and to buffer against market fluctuations. Thus, there is an obvious and compelling need to develop integrated processing approaches that enable multi-product biorefineries. Despite comprising a substantial portion of plant cell walls (as much as one third of the mass of lignocellulosic biomass) and representing the second-most abundant naturally occurring biopolymer after cellulose, the chemical functionality in lignins remains largely unutilized as a source of renewable carbon for the production of bio-based chemicals, polymers, and materials. As such, there is an opportunity for technologies that can be integrated with conversion processes employing a biomass pretreatment/fractionation strategy, whereby it may be possible to utilize all or a fraction of the process-modified lignins for purposes other than as a low-value solid fuel.[4] Commercialization of co-products from wood lignins derived from Kraft pulping have long proved challenging, but it should be understood that lignin origin (*e.g.*, hardwood, softwood, or grass) and processing history (*e.g.*, alkaline pulping, organosolv pulping, sulfite pulping, dilute-acid pretreatment, *etc.*) have an enormous impact on the structure, properties, and suitability of the lignin for a target application, which this work will investigate.

Although lignin valorization has increasingly been the subject of substantial research, significant challenges still remain.[5,6] General strategies for lignin utilization include thermochemical conversion to heat and power or syngas, utilization of (potentially modified) polymeric lignins in applications such as resins for application as adhesives or coatings,[7] and lignin depolymerization to generate sets of aromatic compounds that can serve as bio-based alternatives to petroleum-derived aromatic platform chemicals.[8] Notably, a recent DOE report has highlighted the critical need for utilizing lignin-derived compounds as potential platform chemicals as well as the challenge in achieving this due to the difficulty of generating a limited range of aromatic compounds from lignin at high yields and selectivities that can act as renewable aromatic monomer cognates to existing petrochemicals.[9] This challenge is derived both from the random nature of the lignin polymer as a consequence of its synthesis *via* free radical-mediated oxidation and its propensity for modification during conversion or extraction processes. Strategies for the depolymerization of lignin include catalytic cracking/pyrolysis,[10,11] oxidation[12] and hydrogenolysis-hydrodeoxygenation,[13,14] and base-catalyzed depolymerization.[15] As examples, the depolymerization of lignin using solid acid-catalyzed hydrolysis has been reported to yield primarily 4-propenyl guaiacol/syringol products, while base-catalyzed depolymerization can yield additional aromatic products.[15,16] Oxidative cleavage of lignin in many studies aims to achieve a defined set of products including vanillin, vanillic acids, and their syringyl counterparts.[17–19] A number of studies have investigated molecular oxygen or hydrogen peroxide as an oxidant in the presence of a transition metal-based homogeneous catalysts including Cu, Fe, or V.[20–23] Other oxidative approaches include using a combination of formic acid and TEMPO catalysts, or polyoxometalates.[24,25] Lignosulfonates derived from sulfite pulping of wood have been utilized commercially as a feedstock for the production of vanillin since the 1940s using catalyzed and/or uncatalyzed alkaline oxidation with vanillin yields of up to 15% reported.[12,26,27] Vanillin production from Kraft lignin using these approaches has been extensively explored, although yields are typically lower compared to lignosulfonates since Kraft lignins may be more condensed.[28] Approaches for reductive cleavage, particularly hydrogenolysis, have received substantial recent attention, and a number of heterogeneous catalysts have been proposed including ligand-less Ni,[29] Ni alloys,[30] NiAu,[31] Ni/SiO_2,[32] or Pd/C.[33] The products differ depending on reaction conditions. A mild liquid reaction of range ~200 °C will predominantly produce guaiacol or syringol with the alkyl side chain intact at the 4-position. The amount of substitution of this alkyl side chain with hydroxyl groups can vary depending on reaction conditions and catalyst.[34,35] Under more severe conditions, ring hydrogenation and hydrodemethoxylation can occur to yield saturated compounds such as 4-propylcyclohexanol or 4-propylcyclohexane, which are suitable for fuel applications.[36,37]

For many depolymerization approaches, enormous effort has been invested in the study of reaction conditions, particularly selection of catalysts and solvents.[38–41] More importantly, these conditions were also often used as a benchmark to determine the effectiveness of the depolymerization approach. On the other hand, the structural features of lignin used in the reaction are often not considered, despite several studies reporting significantly lower yields when the same methods performed on lignin dimer models were applied to real lignin.[30,42] Specifically, there has not been conclusive evidence demonstrating how these structural features are fundamentally related, especially after lignin-modifying extraction or pretreatment, and how it would affect lignin's susceptibility towards depolymerization. Previous studies have suggested that native lignin in biomass with higher syringyl/guaiacyl unit ratios (S/G ratios) give higher monomer yields from quantitative thioacidolysis, which is due to the higher natural β-O-4 content.[43,44] Many lignin-modifying pretreatments have been investigated,[45–47] with each of these imparting changes to the physical and chemical properties of lignin through different mechanisms (for example by the cleavage of C–O bonds). However, many of these pretreatments can also result in the formation of new C–C linkages which hinder approaches to generate aromatic monomers.[48–50] Knowledge of how these changes impact a lignin's susceptibility to depolymerization could help in the integration of strategies for lignin conversion into biorefining processes.

Therefore, the objective of this study is to develop a better understanding of the relationship between lignin properties and to model their effect on lignin susceptibility to depolymerization through cleavage of ether linkages. This will be

achieved through solubility-based fractionation of a hardwood lignin from an alkaline pretreatment liquor in order to generate lignin fractions exhibiting diverse properties. These fractions will be characterized and subjected to three depolymerization approaches. Correlations amongst the lignin properties and between obtained yields will be established in order to develop a methodology for assessing lignin quality for its suitability for depolymerization.

Results and discussion

Lignin fractionation

The lignins used in the first part of this study were obtained from the fractional precipitation of pretreatment liquors following the mild alkali pretreatment of hybrid poplar at a final temperature of 150 °C at a total time of 3 h as performed in our previous work in order to minimize lignin modification and to maximize xylan retention in the pretreated biomass.[51] Both native and process-modified lignin are highly heterogeneous which hinders accurate characterization and may limit their value for applications requiring a homogeneous, well-defined starting material. For this work, alkali-solubilized lignins are subjected to fractionation in order to both obtain more homogeneous lignin fractions and to enrich or deplete these fractions in certain properties that can yield insight into property differences that impact the conversion process chemistry.

A number of approaches for the recovery and fractionation of lignin from alkaline pulping or pretreatment liquors have been explored in the past. These approaches include ultrafiltration or approaches based on precipitation due to altering the properties of the solvent by acidification with a weak or strong acid and the use of organic solvents.[52–55] Of these approaches, CO_2 acidification has been employed in a number of recent commercial or pilot studies, including LignoBoost™, LignoForce™, and of particular interest to this study, the Sequential Liquid Recovery and Purification™ (SLRP™) process.[56–58] This pH-based fractionation by CO_2 has previously shown the ability to generate lignin fractions with different properties from Kraft black liquor of softwood biomass.[59] For the present work, lignin fractionation was performed using two approaches to sequentially acidify and fractionally recover lignins from this alkaline pretreatment liquor. The first approach utilized sequential acidification and precipitation with CO_2 at room temperature and atmospheric pressure, while the other (*i.e.*, the SLRP™ process) was performed at elevated temperature and pressure as in our previous work.[59] The mass yields for the lignin fractions obtained from these processes are presented in Table 1. This shows that for recovery at the same pH range, the room temperature recoveries were more than double from the SLRP™ process. This may be due to the higher solubility of the lignin at higher temperature. These fractions, hereafter, will be referred to as named in Table 1 throughout the study. Furthermore, it should be understood that these fractions do not represent 100% of the recoverable lignin, but were generated in order to better understand how diversity in lignin properties impact depolymerization aromatic monomer yields.

Table 1 Percent recovery of each fraction through CO_2 acidification at room temperature and at 115 °C (SLRP™). The pH 2 fraction was obtained by acidification with H_2SO_4

Room temperature (RT) fractionation		
Precipitation pH	Fraction name	Percent recovery
11	RT-1	8.42
10.6	RT-2	20.79
10	RT-3	13.95
9	RT-4	12.59
2	RT-5	26.07
Sequential liquid-lignin recovery and purification (SLRP)		
10.5	SLRP-1	4.05
10	SLRP-2	7.95

Properties of fractionated lignins

Next, we characterized the properties of the different lignin fractions by applying a number of characterization techniques. These techniques included determination of S/G ratios as measured by both 2D ^1H–^{13}C-correlated HSQC NMR and quantitative thioacidolysis, the fraction of linkages between monolignols by HSQC NMR, the β-O-4 contents by quantitative ^{13}C-NMR, the phenolic and aliphatic hydroxyl content as determined by ^1H-NMR, and the molecular weight using gel permeation chromatography (GPC). All results are available in the ESI.† It was found that all of these properties, with the exception of the aliphatic hydroxyl content, exhibited obvious trends across the various fractions obtained by pH-based lignin fractionation.

The HSQC spectra of fraction SLRP-1 is shown in Fig. 1 as a representative spectrum. From this, it can be observed that in the aromatic region a minor fraction (3%) of the syringyl units are oxidized (S′). Also, it can be observed that there is no significant increase in the peak corresponding to *p*-hydroxyphenyl, which implies that there was no or negligible demethoxylation occurring during the alkali pretreatment and fractionation process. The S/G ratios from the HSQC spectra were determined as the relative integration areas of the $S_{2,6}$ and $S'_{2,6}$ peaks to the G_2 peak. The results (Fig. S1†) indicate a distinct trend that lignin precipitated at higher pH, particularly from the SLRP process, showed higher values of S/G ratios with the highest (S/G = 1.99) from fraction SLRP-1 and lowest (S/G = 1.80) at pH 2 (RT-5). It should be noted that the SLRP fractions exhibit higher S/G ratios relative to room-temperature fractions. This trend is in a good agreement with S/G ratios obtained from thioacidolysis, which are all slightly lower than those from HSQC, ranging from 1.95 in SLRP-1 fraction down to 1.73 in sample RT-5.

Three types of linkages in the aliphatic region from HSQC spectra were detected, including β-O-4, β-5 and β-β (Fig. 1). From both regions of the spectra, no other peaks corres-

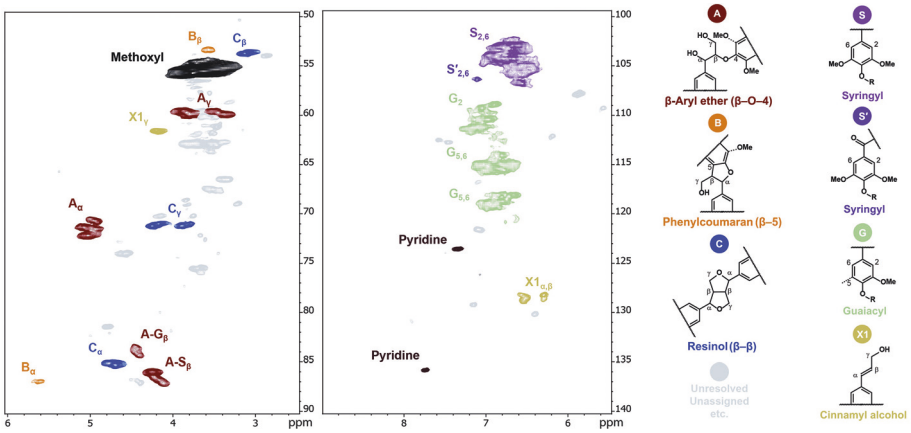

Fig. 1 2D $^{1}H-^{13}C$ HSQC NMR spectra showing lignin fraction SLRP-1 composition and linkages. The contours in aliphatic region (left) are used to quantify the fraction of inter-unit linkages, while those in the aromatic region (right) are used for S/G ratios.

ponding to possible linkages from alkali pretreatment, such as stilbene and diarylmethane, were detected. It should be noted that there could be some linkages as a result of condensation that would not be detectable through HSQC NMR such as 5-5′ linkage. Therefore, the assessment of linkages from HSQC NMR was semi-quantitative, *i.e.* percentage of detected linkages. The percentage trend of linkages is obvious and is shown in Fig. S3,† which indicates that lignin at higher pH fractions, particularly SLRP fractions, contained a higher proportion of the β-O-4 and β-5 linkages, while the relative abundance of the β–β linkage increases as recovery pH decreases. It should be noted that adding standards for absolute quantification was not performed due to several prohibitive factors that originate from the lignin; primarily the error caused by varying T_2 relaxation and resonance offsets in HSQC NMR which affect the signal intensity.[60] Instead, quantitative ^{13}C-NMR was used to directly determine the amount of β-O-4 linkages per 100 aryl units. A similar trend was observed, in which the fractions recovered at higher pH contained higher β-O-4, and the increase was particularly pronounced in SLRP fractions (Fig. S1†). Notably the fraction with highest β-O-4 content, SLRP-1, was shown to have double the β-O-4 content relative to the lowest.

Next, we investigated the amount of phenolic and aliphatic hydroxyl groups by application of ^{1}H-NMR of the acetylated lignin fractions. The results indicated that the amounts of aliphatic hydroxyl groups remain nearly constant across all fractions with the exception of the pH 2 fraction (Fig. S2†). The amount of phenolic hydroxyl groups, on the other hand, demonstrated a clear trend in which higher pH fractions, especially the SLRP fractions, had a lower content of phenolic hydroxyl groups. We have previously identified this same trend in SLRP-fractionated lignins derived from a softwood Kraft black liquor.[59] Lastly, the results from GPC for molecular weight also demonstrated a trend across the fractions with the high-pH fractions exhibiting the highest molecular weights (Fig. S3†).

In summary, we identified trends in almost all properties of lignin except aliphatic hydroxyl group. Higher values for β-O-4 content, S/G ratios, and molecular weight were observed in higher pH fractions with the effect more pronounced in SLRP fractions. The phenolic hydroxyl group showed an opposite trend where its amount is lower in higher pH fraction and increases as pH fraction decreases. Apart from S/G ratios, these trends from the hardwood used in this study are similar to those reported earlier from softwood by Stoklosa *et al.*[59]

Depolymerization of fractionated lignins

As it was shown that there are trends in structural features corresponding to precipitated pH, this section aims to investigate how the difference in these features affects their susceptibility to depolymerization. This was examined by quantifying yields of monomers generated from lignin following depolymerization utilizing three different conversion chemistries. These included catalytic oxidation utilizing molecular oxygen and a homogeneous copper catalyst under alkaline conditions and elevated temperature. This approach is comparable to what has been employed commercially in the past to produce vanillin from lignosulfonates.[61] The second approach employed hydrogenolysis using ethanol as both a solvent and a hydrogen source catalyzed by Ni on an inert support at elevated temperature and pressure. The third approach employed quantitative thioacidolysis. Although each approach shows different monomer-product distributions, we chose to focus on the total yields (Fig. 2). Similar to the lignin properties across the different fractions, clear trends were observed for monomer yields following depolymerization. We must stress that the yields, by necessity, must be based on the total content of lignin. There is not a clear consensus on how aromatic monomer yields from lignin should be defined, especially lignin that has been chemically modified where its composition may be altered beyond definition of lignin.[62] Therefore in order to be consistent, the present study has reported all yields from these reactions on a basis of Klason

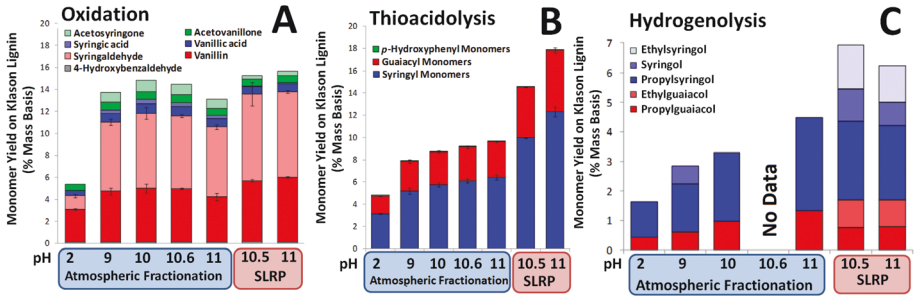

Fig. 2 Trends in monomer yields from different depolymerization techniques, including (A) Cu-catalyzed oxidation, (B) quantitative thioacidolysis, and (C) Ni/C-catalyzed hydrogenolysis.

lignin, which does not consider acid-soluble lignin and may contain contaminants such as proteins.[63]

Prior to performing oxidation on the different lignin fractions, a kinetic study of monomer yields and selectivities was performed on SLRP-2 (Fig. S4†) in order to determine optimal reaction conditions since oxidation products have been reported to repolymerize under these conditions.[64] Although optimal yields may differ from fraction to fraction, the optimal yields for sample SLRP-2 were obtained at 7 min which was chosen as the reaction time used for all other pH fractions. The results show that monomer yields from oxidation generally increased as the recovery pH increased (Fig. 2A). It should be noted that previous work using alkali poplar lignins for Cu-catalyzed oxidation were able to achieve yields of around 10%, which is roughly an average of all the RT samples combined.[21] The differences in yields were not apparent, especially in fractions recovered at higher pH. There was also a discrepancy in sample RT-1 and RT-2, which fell out of trend. The ambiguity in trends is likely due to the intrinsic mechanism of oxidative cleavage in alkali solution, wherein depolymerization and condensation reactions share similar intermediates.[65] Moreover, the desired product itself, vanillin, was previously shown to be able to condense to form dimers.[64] The concurrence of these reactions makes the trend of lignin-monomer yields through oxidation not as obvious as we would expect compared to the trend from quantified properties.

We next employed quantitative thioacidolysis to assess the yields across the lignin fractions. Thioacidolysis cleaves exclusively ether linkages within lignin and yields stable products. The results from quantitative thioacidolysis show a clear trend whereby fractions recovered at higher pH demonstrated higher yields (Fig. 2B). Similar to the trends observed for lignin properties, the results from the SLRP fractions are particularly notable when compared to the fractions recovered at ambient temperatures and pressures. Additionally, lower yields were observed for these fractions than were obtained from oxidation, which may be attributed to the less selective nature of oxidation reaction, which was shown to also cleave certain C–C linkages, in addition to ether linkages in presence of Cu^{2+} or other catalysts to yield aldehydes and ketones.[22,23,66] The results were not entirely unexpected as both ^{13}C-NMR and HSQC NMR show that higher pH fractions contain more β-O-4 linkages, which is the target for thioacidolysis. However, the monomer yields have demonstrated how differences in β-O-4 content affect lignin depolymerization. From Fig. 2B a five-fold difference in yields can be observed between the two extreme fractions that correspond to a two-fold difference in β-O-4. The topic of how β-O-4 content can be related to these depolymerization yields will be investigated in subsequent sections.

Lastly, the lignin fractions were screened for monomer yield by hydrogenolysis catalyzed by carbon-supported Ni. This approach has been used in many recent studies[34,41,67,68] as it has been demonstrated to produce high monomer yields (>40%) with excellent selectivity.[69] It was also shown by HSQC NMR to selectively cleave only ether linkages at a reaction temperature of 200 °C in ethanol.[35] When subjected to hydrogenolysis the fractionated alkali lignins, however, showed that the highest yield achieved was 7% (Fig. 2C). Unlike the approach using oxidation, the monomer products are stable under the conditions used for hydrolysis and a clear trend can be observed within the fractions from alkali-pretreated lignin. The samples from higher pH fractions, particularly SLRP, produced more monomers.

We have shown that lignin properties have a strong impact on its susceptibility toward cleavage of ether linkages. Particularly, the trends are clear in thioacidolysis and hydrogenolysis as it was not complicated by other side reactions that may have occurred during oxidation. Nonetheless, general trends from any of the depolymerization techniques point toward higher monomer generations in the fractions recovered at higher pH, notably in the SLRP fractions, with significant gaps as high as four times the yield from thioacidolysis between the highest pH SLRP fractions and the lowest pH atmospheric fraction. Therefore, lignin properties can greatly affect the outcome of depolymerization reactions.

Correlating lignin properties and monomer yields for fractionated alkali lignins

Several obvious trends were identified among the fractionated alkali lignins, namely that properties of lignin co-vary and that these properties can be linked to the potential for generation of aromatic monomers from lignin. In this section, these

results are correlated in order to gain deeper insight into how these trends are connected. The trends among almost all properties from the previous section imply that the properties of lignin, even after the alkali pretreatment, were still fundamentally related. Correlations among some of the quantified properties, as summarized by the Pearson's Correlation Coefficient (R), are presented in Fig. 3. This shows that a number of strong correlations can be observed between many properties. Importantly, S/G ratios, molecular weight, phenolic hydroxyl groups, and β-O-4 content, were all found to have R values of greater than 0.95, with the exception of the correlation between S/G and β-O-4 content, which is still above 0.9. It has been shown that in native lignin, S/G ratios are correlated with β-O-4 content as high S/G ratios result in more β-O-4 linkages as the extra methoxyl group in syringyl monomers prevents formation of C–C linkages at the 5-C position during lignin biosynthesis.[44] In our pretreated lignin fractions, this same correlation still exists.

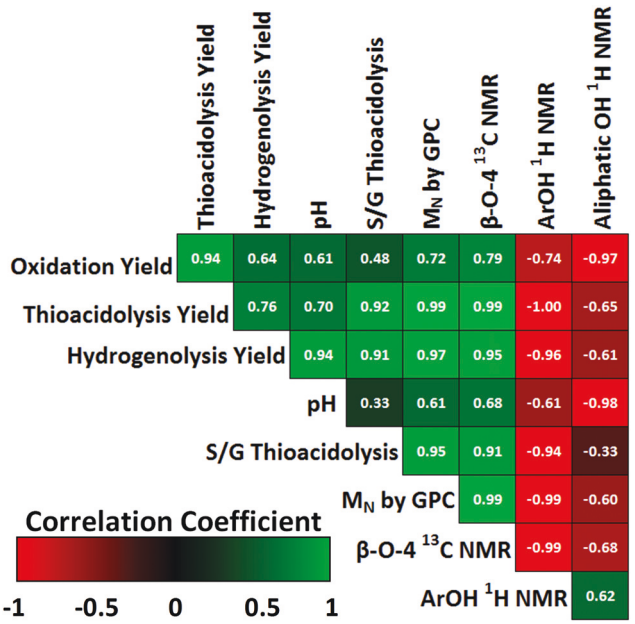

Fig. 3 Correlation map for fractionated alkali lignin properties and monomer yields demonstrating strong correlations between most lignin properties and its susceptibility to depolymerization *via* β-O-4 cleavage.

Given that the original native lignin possesses the highest β-O-4 content and that during pretreatment these linkages are broken, the fractions depleted in β-O-4 content signify more cleavage and are correlated to lower molecular weight. Cleavage of the β-O-4 linkages then result in the formation of new phenolic hydroxyl groups giving negative correlations between phenolic hydroxyl groups, β-O-4 linkages, and molecular weight (Fig. 4A). The difference in the amount of phenolic hydroxyl groups is likely the factor that allows fractionation based on pH in this study to be possible because in alkaline solutions one of the factors that promotes lignin solubility is the deprotonation of phenolic hydroxyl groups.[70]

Similar to the trends observed among the lignin properties, trends can be observed across these lignin fractions subjected to depolymerization with different techniques. Monomer yields from thioacidolysis and hydrogenolysis exhibited strong correlations to a number of lignin properties, while oxidation yields were much less strongly correlated (Fig. 3). Specifically, thioacidolysis yields showed a linear correlation for many of the lignin properties (Fig. 4B & C). However, it should be noted that this linearity could be the result of the small range of β-O-4 content inherent in processed lignin. It may not be extrapolated to a wider range of β-O-4 content as will be shown in later sections. Nonetheless, the results indicate that the diverse structural properties of the lignin are fundamentally correlated and affect the depolymerization reaction accordingly. Consequently, a single property can be used as a descriptor of lignin susceptibility to depolymerization.

Prediction of monomer yields from β-O-4 content

We have established that lignin properties, even after the alkaline pretreatment performed in this work, are still fundamentally inter-dependent and they also correlate well with the yield of monomers generated by three different catalytic depolymerization chemistries. This section aims to further analyze the effect of lignin properties beyond just correlation. We aim to validate a generalizable prediction model based on a quantifiable lignin property. This would yield a tool to assess the potential of lignin to yield aromatic monomers. Since most of

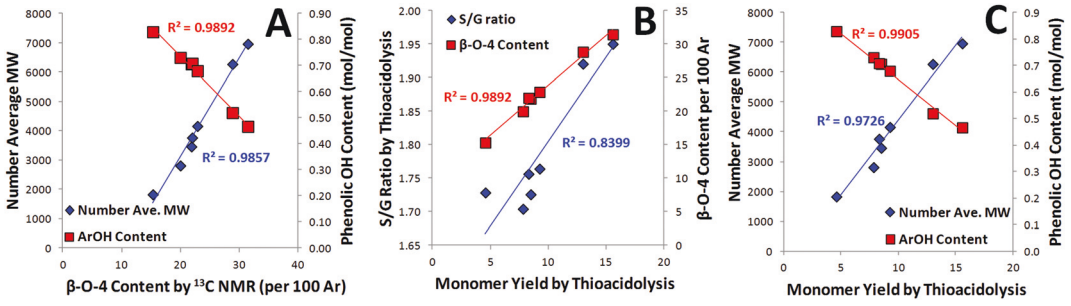

Fig. 4 Selected linear correlation between fractionated lignin properties and monomer yields, demonstrating that (a) β-O-4 content is directly correlated to molecular weight and inversely correlated to phenolic hydroxyl content, that (b) monomer yields from thioacidolysis (g/g) were correlated to S/G ratios and β-O-4 contents, as well as (c) molecular weight and phenolic hydroxyl contents.

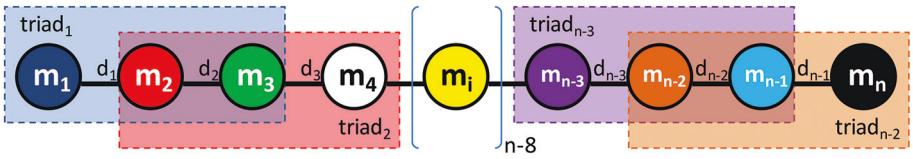

Fig. 5 Lignin chain model of chain length n used for formulating prediction rationale. The i^{th} monomer unit, n^{th} linkages of lignin are represented by m_i and d_n. A triad is composed of three monomer units.

these properties are related, this implies that a single property can be selected to represent the other properties, and serve as a descriptor for the depolymerization of lignin utilizing approaches that target inter-unit ether linkages. To do so, we chose the amount of β-O-4 content as a property to predict lignin susceptibility to depolymerization, *i.e.* maximum possible yields, because it is relatively easy to establish a rationale for the predicting formula.

For a monomer to be generated from the cleavage of β-O-4 linkages, a unit in a triad as shown in Fig. 5 must either have two adjacent β-O-4 linkages or be at the chain end connected by a β-O-4 linkage. For example, for m_2 in Fig. 5 to be released as a monomer, both d_1 and d_2 must be β-O-4 linkages because otherwise a dimer or oligomer would be generated instead if one of the connecting linkages was a C–C bond. Likewise, for a m_1 to be released as a monomer, d_1 must be an ether linkage. As a lignin polymer is assumed to be synthesized by the free radical mediated oxidative polymerization mechanism, the distribution of linkages throughout the polymer can be assumed to be random. Therefore, the quantified content of a lignin property, such as β-O-4 content, can be considered to represent the probability that linkage within the polymer is a β-O-4. With this assumption, the prior rationale can be applied to yield an expression predicting the probability that any monomer within the lignin polymer can be released by cleaving a β-O-4 bond as:

$$P(A \cap B) = P(A)P(B) \tag{1}$$

where $P(A)$ is the probability of a β-O-4 linkage existing on one end of a triad and $P(B)$ is the probability of a β-O-4 linkage occurring at the other end of the triad. Likewise, the probability that a monomer will be released from a polymer chain end can be considered as $P(C)$, represents the probability of a β-O-4 linkage occurring as the terminal linkage at the end of a polymer chain. This methodology is based on the simplification that lignin is a linear polymer without branches or cross-links between polymers. Recent work has provided evidence that at least some native lignins may be primarily linear oligomers.[71,72] Based on this rationale and assuming that any of the monomer-yielding events are mutually exclusive, the monomer yield can be estimated based on the expected values of β-O-4 content, which is:

$$E[\text{Yield}] = \frac{1}{n}\left[\sum_{i=1}^{n-2} E[P(A_i)P(B_i)] + \sum_{i-1}^{2} E[P(C_i)]\right] \tag{2}$$

Assuming the β-O-4 content is randomly distributed throughout the polymer chain (*i.e.*, the probabilities that any bond is a β-O-4 bond are equal), this expression simplifies to:

$$E[\text{Yield}] = \frac{(n-2)\times(\beta\text{-O-4 Content})^2}{n} + \frac{2\times(\beta\text{-O-4 Content})}{n} \tag{3}$$

where n is the number of monomers within the chain. The general form of this same equation has been previously derived for predicting distributions of polymer depolymerization products by Montroll and Simha in 1940.[73] For the case where the lignin chain is very long (*i.e.*, $n \gg 100$), eqn (3) can also be truncated to:

$$E[\text{Yield}] = (\beta\text{-O-4 Content})^2 \tag{4}$$

This simplified model relating lignin β-O-4 content to monomer yields was first proposed in the work of Yan *et al.*[69]

Based on the empirical model developed in the Experimental section and eqn (3), the predicted trends for monomer yield *versus* β-O-4 content for a lignin with a chain length of 100 and 25 units with the β-O-4 content exhibiting a Gaussian distribution were generated (Fig. 6). It can be observed that the trends do not vary significantly within this window of chain length indicating that the difference in number of end units is still relatively insignificant.[74] In Fig. 6, the red circles represent thioacidolysis yields from different alkali-pretreated fractions. These yields followed the predicted trend very well, where twice the amount of β-O-4 content in the fraction SLRP-1 produced almost three times the monomer amount compared to RT-5. However, the range of β-O-4 content within these frac-

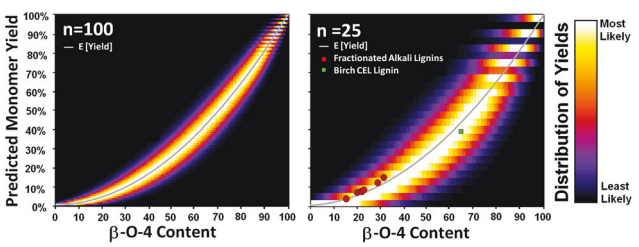

Fig. 6 Predicted monomer yields as a function of β-O-4 content for lignin polymer populations of chain length of 100 monomers (left) and 25 monomers (right) with yields (mol/mol) from quantitative thioacidolysis of fractionated poplar lignin and native birch lignin (CEL lignin) included for validation.

tions are still limited. Therefore, a lignin from dioxane extraction was generated with a 38% extraction yield. Application of ^{13}C-NMR and quantitative thioacidolysis to this lignin showed a β-O-4 content of 65% and a monomer yield of 38% (Fig. 6, green square). The results conformed to the predicted trend as well as asserting the importance of obtaining a lignin enriched in β-O-4 content if the goal is to generate high monomer yields.

Theoretically, if the β-O-4 content is reduced during alkali pretreatment, this should not cause its thioacidolysis yields to be inferior to those from extracted lignin as the loss in ether linkages would be compensated by an increase in end-chain units. However, the fact that the monomer yields from alkali lignin fractions are significantly lower could be derived from two factors. Namely, the potential formation of C–C bonds by condensation reactions during the pretreatment, and, second, that β-O-4 units cleaved during pretreatment yield monomers and short chain oligomers that were not recovered during lignin recovery by pH fractionation.

A closer look to the prediction results further emphasizes the significance of the quality of lignin. With lower β-O-4 contents in the pretreated biomass, regardless of reaction optimization or catalyst selection, high monomer yields are not achievable from these lignins. The correlation in eqn (4) not only explains the low yields from the alkali lignin fractions in this study, it also provides justification for performing lignin depolymerization prior to significant lignin modification. For example, certain studies employed catalytic reductive cleavage of lignin using whole biomass as a substrate with the reaction conditions and solvents chosen to limit undesirable condensation reactions.[75,76] With high quality lignin substrate and limited condensation, yields have been demonstrated in the range 20–50% from a variety of catalysts and biomass sources for β-O-4 contents that were expected to range from 50–70%,[34,68,77,78] while a high-syringyl content transgenic poplar containing primarily β-O-4 bonds could achieve monomer yields as high as 78% monomers.[79] Likewise, high monomer yields were also observed in oxidative cleavage approaches that used isolated lignin and mild reaction conditions.[80] Therefore, from the predicted results and mentioned examples, it was clear that lignin quality was crucial for achieving high monomer yields. This can be contrasted to the hydrogenolysis yields obtained from alkali hardwood lignin in this study, which used similar reaction conditions (Fig. 2).

Monomer yields of pretreatment-derived lignins from oxidation and hydrogenolysis are also plotted against the prediction model (Fig. 7) and show that unlike the yields from thioacidolysis that show good agreement with the model prediction, the yields from the oxidation reaction do not. As the model prediction is based solely on the cleavage of β-O-4 bonds, this discrepancy can be hypothesized to be due to the less selective nature of the oxidation reaction as well as potentially condensation of the monomer products. The yields for hydrogenolysis, on the other hand, seem to follow the trend. This should be expected as previous work has shown that only peaks corresponding to ether regions on HSQC NMR disappear after the hydrogenolysis reaction. Moreover, a hydrogenolysis reaction conducted on β-5 dimer did not produce any monomers.[35] However, it can be seen in Fig. 7 that although the trend follows the prediction line, the values are lower than what is predicted for thioacidolysis. This could be due to a number of factors such as incomplete conversion for the reaction time used, or incomplete solubilization of the pretreatment-derived lignins in the solvent. Either of these assertions is supported by the finding that a considerable amount of lignin residue remained after the reaction; however, accurate measurement of the residue was not possible due to the limited amount of available samples.

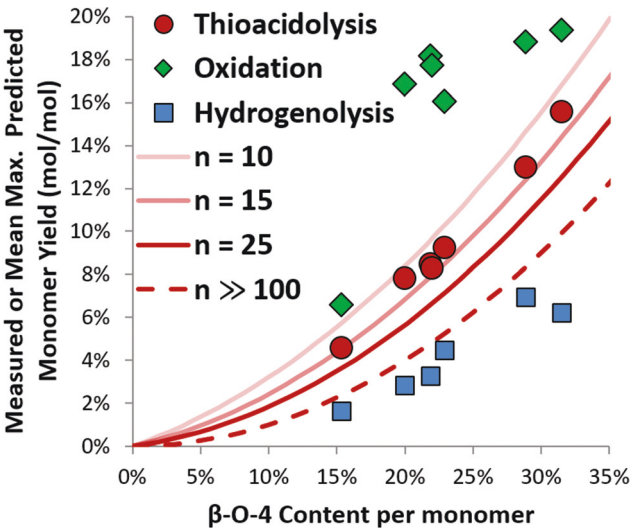

Fig. 7 The effect of chain length on predicted monomer yields (mol/mol) and yields from thioacidolysis, oxidation, and hydrogenolysis of fractionated lignin *versus* β-O-4 content. The monomer yields from all three reactions were corrected to a "per mol" basis from a "per mass" basis.

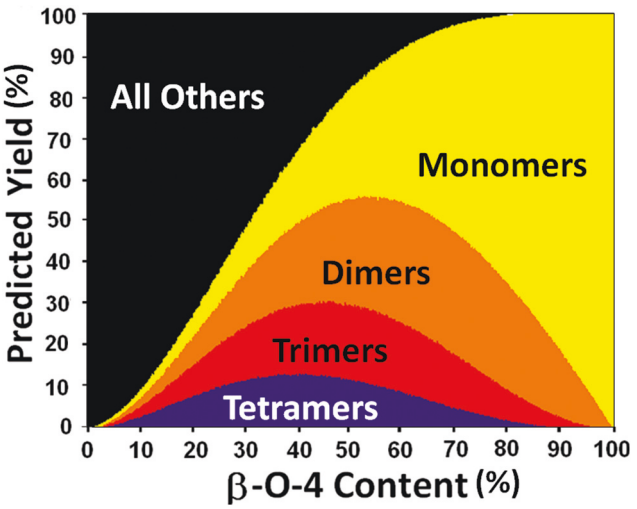

Fig. 8 Predicted yield distributions for monomers, dimers, and oligomers from the cleavage of β-O-4 in a population model of lignin polymers of n = 100 over a range of β-O-4 contents.

Besides monomer yields, this approach can be extended to predict the yield of oligomeric products by employing a similar rationale to that used for monomer generation. As an example, to yield a dimer, three monomers must be linked by two non-adjacent β-O-4 linkages. These results, as shown in Fig. 8 presented as cumulative yields, show that for a β-O-4 content of 50%, cleavage of all β-O-4 linkages would be predicted to yield approximately 10% tetramers, 17% trimers, 25% dimers, 27% monomers, and 21% all other oligomeric products. From this plot it can be observed that as β-O-4 content increases above *ca.* 55%, the yield of monomers dominates over other oligomeric products (Fig. 8). Again, this emphasizes the importance of enhancing and preserving β-O-4 content of lignin, preferably above 55% or more, if high monomer yields are the goal. In fact, recent studies tried to achieve this through so called lignin-first biomass pretreatment and isolation processes.[81–83]

Conclusions

The present work has demonstrated through characterization of hardwood lignins generated by pH-based fractionation of alkaline pretreatment liquors that many lignin properties exhibited strong correlations with each other. Specifically, lignin fractions with higher S/G ratios were found to have higher β-O-4 contents and molecular weights with lower phenolic hydroxyl contents. More importantly, the differences in these properties among fractions, specifically β-O-4 content, were shown to greatly impact the achievable monomer yields through three different lignin depolymerization approaches. Furthermore, a model was developed to explain the correlation between β-O-4 content and monomer yield. The prediction model was validated experimentally by ^{13}C-NMR quantified β-O-4 content and quantitative thioacidolysis yields from the fractionated lignins as well as "native" extracted hardwood lignin. As the model predicts that the maximum achievable yield is the β-O-4 content squared, it highlights the importance of preserving high β-O-4 contents in the lignins during pretreatment or isolation.

Experimental

Lignin fractionation

Pretreatment was performed in an M/K Systems, Inc. (Peabody, MA, USA) single vessel pulp digester with a capacity of 10 L using debarked wood chips of 20-year old hybrid poplar (*Populus nigra* × *maximowiczii* cv. NM6) obtained from Dr Raymond Miller (Michigan State University Forest Biomass Innovation Center, Escanaba, Michigan). Pretreatment was performed at a liquor-to-wood ratio of 4 : 1 using conditions identical to those in our previous work for an *H*-factor of 166.[51] These mild pretreatment conditions were utilized to preserve solubilized lignin quality. Lignin was fractionally precipitated from this liquor through two sequential pH reduction approaches. The first one employed sequential acidification and precipitation at using CO_2 at room temperature and atmospheric pressure. For room temperature fractionation, pH was decreased stepwise as shown in Table 1 until a pH of 9.0 was achieved and yielding 4 fractions. A final fraction was generated by further acidification to a pH of 2 by addition of 1 M H_2SO_4. The second lignin fractionation approach employed the Sequential Liquid-Lignin Recovery and Purification (SLRP) process using CO_2 acidification at elevated temperature (115 °C) and pressure (6.2 bar) as reported in our previous work.[84] For this, the SLRP fractions represent the lignin recovered by acidification between a pH of 11.0 of 10.5 (SLRP-1) and the pH increment of 10.5 to 10.0 (SLRP-2) as shown in Table 1. The Klason lignin content of each pH fractions, as determined by NREL standard procedure (NREL/TP-510-42618), was used as a basis for subsequent characterization and monomer yield determination.

Lignin extraction from birch

To assess the impact of lignin depolymerization on a "native" lignin, lignin was extracted from debarked silver birch (*Betula pendula* Roth.) grown in northern Sweden and supplied Curt Lindström (Smurfit-Kappa Kraftliner AB, Piteå, Sweden). The lignin extraction was performed according to the method proposed by Gu *et al.*[85] In short, the biomass was cryogenically ball-milled in a TissueLyser II (Qiagen, Hilden, Germany) for a total of 4 h with 15 min interval for cooling in liquid nitrogen. The ball-milled sample was dissolved in 8% LiCl/DMSO at a concentration of 5% by weight and stirred at 25 °C for 48 h then at 50 °C for 24 h. The biomass was precipitated as a gel by dropwise addition of the LiCl/DMSO solution into deionized water. This gel was thoroughly washed with deionized water until no Cl remained in the gel as determined by addition of $AgNO_3$. The decrystallized biomass sample was then lyophilized before undergoing enzymatic hydrolysis for 72 h (20 mg protein per g biomass; CTec II and HTec II from Novozymes in a 2 : 1 ratio at 50 °C with in pH 5.25, using 0.05 M Na-citrate buffer). Next, the sample was washed with excess water and lyophilized again. The solid residue was then subjected to dioxane extraction for 6 h with 50 mL 96% aqueous (v/v) dioxane.

Lignin acetylation

Acetylated lignin samples were used for ^{1}H NMR and GPC analyses. The procedure was adopted from previous study with slight modification.[59] Essentially, 12.5 g L^{-1} of lignin solution in 1 : 1 pyridine/acetic anhydride was prepared. Fractions that were harder to solubilize were subjected to sonication for 10–20 min. The completely dissolved solutions were left in an incubator at room temperature and 180 rpm for 24 h before applying washing steps as in the previous study.

NMR analysis

^{1}H-NMR spectra were acquired on a 500 MHz NMR spectrometer (Agilent 500/54) equipped with a OneNMR probe. Acetylated lignin was dissolved in $CHCl_3$-d_6 at a concentration of 44 mg mL^{-1}. Pentafluorobenzaldehyde (PFB) was used as an

internal standard with tetramethylsilane (TMS) as a peak reference. The spectra were acquired from −2 to 14 ppm with a 30° pulse, a recycle delay of 4 s, and a total of 800 scans. Aliphatic and aromatic hydroxyl content were determined on a per aromatic basis (mol mol^{-1}) by using the methoxyl content corrected for the thioacidolysis-determined S/G ratio. ^{13}C-NMR spectra were acquired on a 500 MHz NMR spectrometer (Varian Inova) equipped with a double-resonance broadband probe. Proton decoupling was applied only during acquisition period (*i.e.*, decoupling-NOE). A sample of lignin (300 mg) was dissolved in 0.6 mL DMSO-d_6 along with 2.0 mg chromium(III) acetylacetonate as a relaxation reagent. Sonication was used to facilitate dissolution. The spectra were acquired from −15 to 235 ppm with a 90° pulse, a recycle delay of 1.7 s, and an acquisition time of 1.2 s. A total of 10 000 scans were collected. Peak assignments were based on the previous literature.[86,87] The β-O-4 content was determined on a per 100 aromatic monomer basis (mol/mol) by using the methoxyl content corrected for the thioacidolysis-determined S/G ratio. The gradient 2D ^{13}C–^{1}H-correlation (HSQC) experiments (Bruker standard pulse sequence 'hsqcedetgpsisp2.2'; phase-sensitive ge-2D multiplicity-edited HSQC using PEP and adiabatic inversion and refocusing pulses with gradients in back-inept) were performed on a 900 MHz NMR spectrometer (Bruker Avance) equipped with a TCI triple-resonance inverse detection cryoprobe. A sample of lignin (100 mg) was dissolved in 0.6 mL DMSO-d_6. The solvent peak (δ_C 39.5, δ_H 2.49 ppm) is used as an internal reference. The spectra were acquired from 0 to 9 ppm in F_2 (^{1}H) dimension by using 3234 data points for an acquisition time (AQ) of 200 ms, and from −15 to 155 ppm in F_1 (^{13}C) dimension by using 512 increments (AQ of 6.66 ms). The number of scans per t1 increment was 32, and the recycle delay was 1 s. The total experimental time was 337 min. Peak assignments were based on the prior literature.[25,88–91]

Gel permeation chromatography

Gel permeation chromatography (GPC) was performed as in our previous work.[59] Briefly, acetylated lignin (2 mg mL^{-1}) was dissolved in THF. The samples were analyzed by HPLC (Agilent 1100) equipped with a Waters Styragel HR 4 column (Milford, MA, USA) at a flow rate of 0.5 mL min^{-1}, temperature of 40 °C, and detection by UV absorbance at 280 nm. Polystyrene standards of molecular weight 1, 10, 50, 100, 200 kDa were used for calibration.

Thioacidolysis

Quantitative thioacidolysis utilizing derivatized monolignol standards was performed as described in our previous work.[43] For this, three 2 mg replicates of dried and isolated lignin sample were weighed into glass vials and heated with a mixture of dioxane, ethanethiol, and boron trifluoride diethyl etherate to liberate the lignin monomers. The extracted thioether derivatized monomers were subsequently silylated with *N*,*O*-bis-trimethylsilyl-acetamide (BSA) and quantitated using GC-MS analysis (Agilent 7890A/5975C MS).

Catalytic oxidation

The catalytic oxidation method was adapted from the literature.[17] For this, 10 mg of each lignin sample and 5% (w/w) $CuSO_4$ were dissolved in 5 mL of 2 M NaOH. Samples were sonicated for 10 min to ensure complete dissolution. The solution was then poured into a 50 mL passivated stainless steel reactor and pressurized to 10 bar with O_2 gas (>99.99% purity, Airgas) following evacuation of the reactor headspace by vacuum. The reactors were submerged in a fluidized sand bath (Techne, Cole-Palmer) preheated to 160 °C for a specific period of time. When the reaction was complete, the reactor was submerged in an ice bath for 10 min. The pH of the final solution was reduced to 2.0 by addition of 6 M HCl and centrifuged to isolate the solid residue. The products in the liquid fraction were extracted four times by chloroform. The solution was then evaporated under vacuum at 40 °C and re-dissolved in 10 mL of 1 : 1 methanol/water with 1% (v/v) formic acid. Quantification of the products was performed by HPLC (Agilent 1100) using 1 : 4 methanol/water with 1% formic acid as a mobile phase and a C18 column (Atlantis T3 100 Å, 3 μm, 3.0 mm × 150 mm; waters). Standards for product quantification included vanillin, vanillic acid, acetovanillone, syringaldehyde, syringic acid, acetosyringone, 4-hydroxybenzaldehyde, and 4-hydroxybenzoic acid. All standards were purchased from Sigma Aldrich and monomer yields were determined on a per mass Klason lignin basis.

Catalytic hydrogenolysis

Catalytic hydrogenolysis of lignin was performed according to previous work.[41] In short, 10% metal loading Ni/C was synthesized by incipient wetness. Typically, 2.76 g of $Ni(NO_3)_2{\cdot}6H_2O$ (Sigma-Aldrich) was added to 8 g deionized water and stirred until well mixed. The solution was then added dropwise to 5 g of activated carbon (Darco® G-60, Aldrich, water absorption capacity of 2.1 mL g^{-1}). The mixture was kept wet at room temperature for 24 h before drying overnight at 100 °C. Then it was calcined at 400 °C under nitrogen atmosphere for 4 h, and reduced at 400 °C under 10% H_2 in an Ar atmosphere, both of which used at a temperature ramp of 6.25 °C per min. The finished catalysts were stored and weighed in an anaerobic glove box. For the hydrogenolysis reaction, 40 mg of lignin, 30 mg of catalyst, and 4 mL of ethanol were charged to a 10 mL passivated stainless steel reactor while in the anaerobic glove box. Reactors were submerged into a fluidized sand bath set at 220 °C for 6 h. The resulting liquid was collected, filtered, and injected into GC/MS (Agilent 7890A/5975C) using Agilent DB-WAXETR column (30 m × 0.250 mm × 0.25 μm) with 1 : 1000 split for product. The temperature program holds at 60 °C for 3 min before ramping up at 40 °C min^{-1} to 260 °C and held for 2.5 min. Standards were run for quantification. Yields were determined as mass products per mass Klason lignin using standard curves constructed from pure monomers including 4-propyl guaiacol, 4-propyl syringol, 4-ethyl guaiacol, 4-ethyl syringol, and syringol. All standards were purchased from Sigma

Aldrich except 4-propyl syringol, which was synthesized according to Parsell *et al.*,[67] and 4-ethyl syringol, which was estimated using the same response factor as 4-propyl syringol. These products comprise all peaks that were detected.

Model development

Computational prediction of monomer yields was performed in MATLAB (MathWorks, Inc., Natick, MA). For this approach, populations of lignin polymers of length n with $n-1$ bonds between the n monomers were generated over a range of expected values for β-O-4 contents (Fig. 5) on a per mol total interunit linkages (*i.e.*, $n-1$ bonds). The assignment of the identity of each of the $n-1$ bonds between dyads as a β-aryl ether was made by choosing a threshold value corresponding to the expected value for β-O-4 content for a given population using the '*randn*' function in MATLAB. This was done to generate populations of lignin polymers of constant chain length with randomly distributed β-O-4 bonds at a set average content of β-O-4 bonds. This methodology was applied to determine monomer yields from populations of 5000 polymers of desired chain length n over a range of 0 to 100% over 2500 intervals for the probability that any bond is a β-O-4 bond, where a monomer can be generated only from two adjacent β-O-4 bonds within a triad or a β-O-4 bond at a chain end. This methodology assumes that lignin is a linear polymer and that no cross-linking or aryl–aryl bonds are present in the polymer. For comparing calculated theoretical yields to quantified yields, the experimental yields by thioacidolysis, hydrogenolysis, and oxidation were converted to a mol/mol yield by assuming a molar mass of 196 g mol^{-1} for a guaiacyl monomer and 226 g mol^{-1} syringyl monomer within the lignin polymer.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank Dr Li Xie in the Max T. Rogers NMR facility at Michigan State University for his advice and assistance in performing NMR characterization. This work was supported in part through funding by the Northeast Sun Grant Initiative.

References

1 G. M. Souza, R. Victoria, C. Joly and L. Verdade, *Bioenergy & Sustainability: Bridging the gaps*, SCOPE, Paris, 2015.
2 B. H. Davison, C. C. Brandt, A. M. Guss, U. C. Kalluri, A. V. Palumbo, R. L. Stouder and E. G. Webb, *Biofuels, Bioprod. Biorefin.*, 2015, **9**, 454–467.
3 M. E. Himmel, S. Y. Ding, D. K. Johnson, W. S. Adney, M. R. Nimlos, J. W. Brady and T. D. Foust, *Science*, 2007, **315**, 804–807.
4 P. C. A. Bruijnincx and B. M. Weckhuysen, *Nat. Chem.*, 2014, **6**, 1035–1036.
5 A. J. Ragauskas, G. T. Beckham, M. J. Biddy, R. Chandra, F. Chen, M. F. Davis, B. H. Davison, R. A. Dixon, P. Gilna, M. Keller, P. Langan, A. K. Naskar, J. N. Saddler, T. J. Tschaplinski, G. A. Tuskan and C. E. Wyman, *Science*, 2014, **344**, 1246843.
6 J. H. Lora and W. G. Glasser, *J. Polym. Environ.*, 2002, **10**, 39–48.
7 N. E. E. Mansouri and J. Salvadó, *Ind. Crops Prod.*, 2006, **24**, 8–16.
8 J. Zakzeski, A. L. Jongerius, P. C. A. Bruijnincx and B. M. Weckhuysen, *ChemSusChem*, 2012, **5**, 1602–1609.
9 J. E. Holladay, J. F. White, J. J. Bozell and D. Johnson, *Top Value-Added Chemicals from Biomass - Volume II—Results of Screening for Potential Candidates from Biorefinery Lignin*, Pacific Northwest National Laboratory, Richland, WA, 2007.
10 Y. Zheng, D. Chen and X. Zhu, *J. Anal. Appl. Pyrolysis*, 2013, **104**, 514–520.
11 T. Yoshikawa, T. Yagi, S. Shinohara, T. Fukunaga, Y. Nakasaka, T. Tago and T. Masuda, *Fuel Process. Technol.*, 2013, **108**, 69–75.
12 H. Werhan, J. M. Mir, T. Voitl and P. Rudolf von Rohr, *Holzforschung*, 2011, **65**, 703–709.
13 P. Kelley, S. Lin, G. Edouard, M. W. Day and T. Agapie, *J. Am. Chem. Soc.*, 2012, **134**, 5480–5483.
14 T. H. Parsell, B. C. Owen, I. Klein, T. M. Jarrell, C. L. Marcum, L. J. Haupert, L. M. Amundson, H. I. Kenttämaa, F. Ribeiro, J. T. Miller and M. M. Abu-Omar, *Chem. Sci.*, 2013, **4**, 806–813.
15 M. R. Sturgeon, M. H. O'Brien, P. N. Ciesielski, R. Katahira, J. S. Kruger, S. C. Chmely, J. Hamlin, K. Lawrence, G. B. Hunsinger, T. D. Foust, R. M. Baldwin, M. J. Biddy and G. T. Beckham, *Green Chem.*, 2014, **16**, 824–835.
16 S. Jia, B. J. Cox, X. Guo, Z. C. Zhang and J. G. Ekerdt, *ChemSusChem*, 2010, **3**, 1078–1084.
17 G. Wu, M. Heitz and E. Chornet, *Ind. Eng. Chem. Res.*, 1994, **33**, 718–723.
18 R. Behling, S. Valange and G. Chatel, *Green Chem.*, 2016, **18**, 1839–1854.
19 A. Azarpira, J. Ralph and F. Lu, *BioEnergy Res.*, 2014, **7**, 78–86.
20 K. Stärk, N. Taccardi, A. Bösmann and P. Wasserscheid, *ChemSusChem*, 2010, **3**, 719–723.
21 G. Wu and M. Heitz, *J. Wood Chem. Technol.*, 1995, **15**, 189–202.
22 Y. Y. Jiang, L. Yan, H. Z. Yu, Q. Zhang and Y. Fu, *ACS Catal.*, 2016, **6**, 4399–4410.
23 B. Sedai, C. Díaz-Urrutia, R. T. Baker, R. Wu, L. A. "Pete" Silks and S. K. Hanson, *ACS Catal.*, 2011, **1**, 794–804.
24 J. M. Hoover and S. S. Stahl, *J. Am. Chem. Soc.*, 2011, **133**, 16901–16910.
25 B. Bujanovic, R. S. Reiner, S. A. Ralph and R. H. Atalla, *J. Wood Chem. Technol.*, 2011, **31**, 121–141.
26 M. B. Hocking, *J. Chem. Educ.*, 1997, **74**, 1055.

27 M. J. W. Dignum, J. Kerler and R. Verpoorte, *Food Rev. Int.*, 2001, **17**, 119–120.
28 C. Fargues, Á. Mathias and A. Rodrigues, *Ind. Eng. Chem. Res.*, 1996, **35**, 28–36.
29 A. G. Sergeev, J. D. Webb and J. F. Hartwig, *J. Am. Chem. Soc.*, 2012, **134**, 20226–20229.
30 J. Zhang, J. Teo, X. Chen, H. Asakura, T. Tanaka, K. Teramura and N. Yan, *ACS Catal.*, 2014, **4**, 1574–1583.
31 J. Zhang, H. Asakura, J. van Rijn, J. Yang, P. Duchesne, B. Zhang, X. Chen, P. Zhang, M. Saeys and N. Yan, *Green Chem.*, 2014, **16**, 2432–2437.
32 J. He, C. Zhao and J. A. Lercher, *J. Am. Chem. Soc.*, 2012, **134**, 20768–20775.
33 J. A. Onwudili and P. T. Williams, *Green Chem.*, 2014, **16**, 4740–4748.
34 Q. Song, F. Wang, J. Cai, Y. Wang, J. Zhang, W. Yu and J. Xu, *Energy Environ. Sci.*, 2013, **6**, 994–1007.
35 S. V. den Bosch, W. Schutyser, R. Vanholme, T. Driessen, S. F. Koelewijn, T. Renders, B. D. Meester, W. J. J. Huijgen, W. Dehaen, C. M. Courtin, B. Lagrain, W. Boerjan and B. F. Sels, *Energy Environ. Sci.*, 2015, **8**, 1748–1763.
36 K. Barta and P. C. Ford, *Acc. Chem. Res.*, 2014, **47**, 1503–1512.
37 K. Barta, T. D. Matson, M. L. Fettig, S. L. Scott, A. V. Iretskii and P. C. Ford, *Green Chem.*, 2010, **12**, 1640–1647.
38 S. N. Sun, M. F. Li, T.-Q. Yuan, F. Xu and R. C. Sun, *Ind. Crops Prod.*, 2013, **43**, 570–577.
39 W. Schutyser, S. V. den Bosch, T. Renders, T. D. Boe, S. F. Koelewijn, A. Dewaele, T. Ennaert, O. Verkinderen, B. Goderis, C. M. Courtin and B. F. Sels, *Green Chem.*, 2017, **19**, 2561–2570.
40 X. Wang and R. Rinaldi, *ChemSusChem*, 2012, **5**, 1455–1466.
41 Q. Song, F. Wang and J. Xu, *Chem. Commun.*, 2012, **48**, 7019–7021.
42 F. Gao, J. D. Webb, H. Sorek, D. E. Wemmer and J. F. Hartwig, *ACS Catal.*, 2016, **6**, 7385–7392.
43 F. Yue, F. Lu, R. C. Sun and J. Ralph, *J. Agric. Food Chem.*, 2012, **60**, 922–928.
44 W. Boerjan, J. Ralph and M. Baucher, *Annu. Rev. Plant Biol.*, 2003, **54**, 519–546.
45 P. Alvira, E. Tomás-Pejó, M. Ballesteros and M. J. Negro, *Bioresour. Technol.*, 2010, **101**, 4851–4861.
46 M. G. Aguayo, A. Ferraz, J. P. Elissetche, F. Masarin and R. T. Mendonça, *Holzforschung*, 2013, **68**, 623–629.
47 G. Brodeur, E. Yau, K. Badal, J. Collier, K. B. Ramachandran and S. Ramakrishnan, *Enzyme Res.*, 2011, **2011**, e787532.
48 G. Gellerstedt, A. Majtnerova and L. Zhang, *C. R. Biol.*, 2004, **327**, 817–826.
49 T. Nakamura, H. Kawamoto and S. Saka, *J. Wood Chem. Technol.*, 2007, **27**, 121–133.
50 K. Shimada, S. Hosoya and T. Ikeda, *J. Wood Chem. Technol.*, 1997, **17**, 57–72.
51 R. J. Stoklosa and D. B. Hodge, *BioEnergy Res.*, 2015, **8**, 1224–1234.
52 A. Toledano, L. Serrano, A. Garcia, I. Mondragon and J. Labidi, *Chem. Eng. J.*, 2010, **157**, 93–99.
53 S. I. Mussatto, M. Fernandes and I. C. Roberto, *Carbohydr. Polym.*, 2007, **70**, 218–223.
54 A. S. Klett, P. V. Chappell and M. C. Thies, *Chem. Commun.*, 2015, **51**, 12855–12858.
55 T. Saito, J. H. Perkins, F. Vautard, H. M. Meyer, J. M. Messman, B. Tolnai and A. K. Naskar, *ChemSusChem*, 2014, **7**, 221–228.
56 M. A. Lake and J. C. Blackburn, *Process for recovering lignin U.S. Patent*, 9260464, 2016.
57 P. Tomani, *Cellul. Chem. Technol.*, 2010, **44**, 53–58.
58 L. Kouisni, P. Holt-Hindle, K. Maki and M. Paleologou, *J. Sci. Technol. For. Prod. Processes*, 2012, **2**, 6–10.
59 R. J. Stoklosa, J. Velez, S. Kelkar, C. M. Saffron, M. C. Thies and D. B. Hodge, *Green Chem.*, 2013, **15**, 2904–2912.
60 L. Zhang and G. Gellerstedt, *Magn. Reson. Chem.*, 2007, **45**, 37–45.
61 W. L. Faith, D. B. Keyes, R. L. Clark, F. A. Lowenheim and M. K. Moran, in *Industrial Chemicals*, Wiley, New York, 3rd edn, 1965, pp. 796–799.
62 G. Brunow, K. Lundquist and G. Gellerstedt, in *Analytical Methods in Wood Chemistry, Pulping, and Papermaking*, ed. E. Sjöström and R. Alén, Springer, Berlin, Heidelberg, 1999, pp. 77–124.
63 R. Hatfield and R. S. Fukushima, *Crop Sci.*, 2005, **45**, 832–839.
64 S. Constant, M. Robitzer, F. Quignard and F. Di Renzo, *Catal. Today*, 2012, **189**, 123–128.
65 V. E. Tarabanko, D. V. Petukhov and G. E. Selyutin, *Kinet. Catal.*, 2004, **45**, 569–577.
66 B. Sedai, C. Díaz-Urrutia, R. T. Baker, R. Wu, L. A. "Pete" Silks and S. K. Hanson, *ACS Catal.*, 2013, **3**, 3111–3122.
67 T. Parsell, S. Yohe, J. Degenstein, T. Jarrell, I. Klein, E. Gencer, B. Hewetson, M. Hurt, J. I. Kim, H. Choudhari, B. Saha, R. Meilan, N. Mosier, F. Ribeiro, W. N. Delgass, C. Chapple, H. I. Kenttämaa, R. Agrawal and M. M. Abu-Omar, *Green Chem.*, 2015, **17**, 1492–1499.
68 S. V. den Bosch, W. Schutyser, S. F. Koelewijn, T. Renders, C. M. Courtin and B. F. Sels, *Chem. Commun.*, 2015, **51**, 13158–13161.
69 N. Yan, C. Zhao, P. J. Dyson, C. Wang, L. Liu and Y. Kou, *ChemSusChem*, 2008, **1**, 626–629.
70 E. I. Evstigneev, *Russ. J. Appl. Chem.*, 2011, **84**, 1040–1045.
71 C. Crestini, F. Melone, M. Sette and R. Saladino, *Biomacromolecules*, 2011, **12**, 3928–3935.
72 F. Yue, F. Lu, S. Ralph and J. Ralph, *Biomacromolecules*, 2016, **17**, 1909–1920.
73 E. W. Montroll and J. Simha, *J. Chem. Phys.*, 1940, **8**, 721–727.
74 E. Feghali, G. Carrot, P. Thuéry, C. Genre and T. Cantat, *Energy Environ. Sci.*, 2015, **8**, 2734–2743.
75 T. Voitl and P. Rudolf von Rohr, *ChemSusChem*, 2008, **1**, 763–769.
76 X. Huang, T. I. Korányi, M. D. Boot and E. J. M. Hensen, *Green Chem.*, 2015, **17**, 4941–4950.

77 E. M. Anderson, R. Katahira, M. Reed, M. G. Resch, E. M. Karp, G. T. Beckham and Y. Roman-Leshkov, *ACS Sustainable Chem. Eng.*, 2016, **4**, 6940–6950.
78 M. V. Galkin and J. S. M. Samec, *ChemSusChem*, 2014, **7**, 2154–2158.
79 L. Shuai, M. T. Amiri, Y. M. Questell-Santiago, F. Héroguel, Y. Li, H. Kim, R. Meilan, C. Chapple, J. Ralph and J. S. Luterbacher, *Science*, 2016, **354**, 329–333.
80 A. Rahimi, A. Ulbrich, J. J. Coon and S. S. Stahl, *Nature*, 2014, **515**, 249–252.
81 J. S. Luterbacher, A. Azarpira, A. H. Motagamwala, F. Lu, J. Ralph and J. A. Dumesic, *Energy Environ. Sci.*, 2015, **8**, 2657–2663.
82 S. V. den Bosch, T. Renders, S. Kennis, S.-F. Koelewijn, G. V. den Bossche, T. Vangeel, A. Deneyer, D. Depuydt, C. M. Courtin, J. M. Thevelein, W. Schutyser and B. F. Sels, *Green Chem.*, 2017, **19**, 3313–3326.
83 R. Rinaldi, R. Jastrzebski, M. T. Clough, J. Ralph, M. Kennema, P. C. A. Bruijnincx and B. M. Weckhuysen, *Angew. Chem., Int. Ed.*, 2016, **55**, 8164–8215.
84 J. Velez and M. C. Thies, *Bioresour. Technol.*, 2013, **148**, 586–590.
85 F. Gu, W. Wu, Z. Wang, T. Yokoyama, Y. Jin and Y. Matsumoto, *Ind. Crops Prod.*, 2015, **74**, 703–711.
86 R. El Hage, N. Brosse, L. Chrusciel, C. Sanchez, P. Sannigrahi and A. Ragauskas, *Polym. Degrad. Stab.*, 2009, **94**, 1632–1638.
87 M. Balakshin and E. Capanema, *J. Wood Chem. Technol.*, 2015, **35**, 220–237.
88 H. Kim and J. Ralph, *Org. Biomol. Chem.*, 2010, **8**, 576–591.
89 Z. Li, N. Bansal, A. Azarpira, A. Bhalla, C. H. Chen, J. Ralph, E. L. Hegg and D. B. Hodge, *Biotechnol. Biofuels*, 2015, **8**, 1–12.
90 S. A. Ralph, J. Ralph and L. L. Landucci, *NMR Database of Lignin and Cell Wall Model Compounds*, 2009. Available at URL http://www.glbrc.org/databases_and_software/nmr-database/.
91 T. M. Liitiä, S. L. Maunu, B. Hortling, M. Toikka and I. Kilpeläinen, *J. Agric. Food Chem.*, 2003, **51**, 2136–2143.