

Reductive Catalytic Fractionation of Lignocellulosic Biomass- new approaches and chemical insights to Process Debottlenecking

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Abstract

Over recent years, Reductive Catalytic Fractionation (RCF) has given traction as a potential method for lignocellulosic biomass valorisation. Based on the active stabilisation of reactive lignin intermediates, the process produces a carbohydrate-rich pulp and lignin oil- a mixture of lignin-derived monophenols, oligomers, polymers, and carbohydrate derivatives. For the RCF process industrial implementation, homogeneous monophenol streams and the faith of oligomeric and polymeric lignin fractions are among the key factors. Following this reasoning, the current thesis aims to provide new chemical insights into the polymeric lignin fractions and introduce new approaches to steer the RCF lignin oil composition. Accordingly, a solvent fractionation strategy is first developed to produce distinctive lignin oil fractions, enabling deconvoluting analytical data. As a result, new non-native reduced β -O-4 linkages are discovered, demonstrating an additional lignin stabilisation mechanism that also incorporates polymeric lignin moieties. Deconvoluting the spectral data also showed that the carbohydrates in lignin oil are oligoxylan alcohols. Additionally, a new Structural Index metric is introduced to assess the chemical nature of oligomeric and polymeric lignin with a simple GPC/UV-VIS analysis. To steer the process outcomes, mechanocatalytic pretreatments are introduced prior to the RCF. It is discovered that adding acetic acid to ball-milling before the RCF process increases monophenol yields and carbohydrate retention in pulp. In addition, a semi-batch RCF strategy is introduced with incremental temperature increases. It is demonstrated that process conditions have an immense effect on monophenols distribution, and at low temperatures, lignin can be partially extracted with complete carbohydrate retention in pulp. In a broader context, the current thesis provides new insights into the chemical nature of lignin-derived oligomers and polymers, which can open new avenues for these lignin fractions' modification and utilisation. Additionally, the introduced RCF approaches provide new ways to steer the process outcomes, creating further options in the process design considerations.

Declaration of Originality

I, Raul Rinken, hereby declare that the work described in this Thesis is my own and any other work was appropriately referenced.

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1. Introduction

Lignocellulosic biomass is the most abundant renewable organic material on Earth, produced through photosynthesis by terrestrial plants.^{1,2} It forms the structural framework of plants and is primarily composed of three biopolymers: cellulose, hemicellulose, and lignin. Cellulose is a crystalline polysaccharide and forms the primary scaffold of plant cell walls. Hemicellulose, a heterogeneous mixture of branched polysaccharides, surrounds the cellulose microfibrils and provides flexibility and cross-linking capacity. Lignin is a complex, amorphous aromatic polymer, which enwraps the polysaccharide matrix and imparts mechanical strength, hydrophobicity, and resistance to microbial degradation.^{3,4} While the total global annual biomass production through photosynthesis is assessed to be 100-200 billion tons, the lignocellulosic part forms roughly a quarter of it.^{1,5,6} Among the lignocellulosic biomass, it is assessed that 3-5 billion tons of it would be technically accessible for industrial use without compromising the current utilisation patterns.⁶⁻⁸ This, on the other hand, is in the same order of magnitude as annual oil (~5 billion tons)⁹ and gas (~3 billion tons)¹⁰ production in 2024 and significantly exceeds the annual production of primary petrochemicals (~0.5 billion tons) and petrochemical-derived materials (~1.2 billion tons).¹¹

As such, lignocellulosic biomass is abundantly available and presents a viable alternative to fossil resources for the production of chemicals, materials, and biofuels. Accordingly, its efficient utilisation and conversion can play an important role in tackling the challenge of anthropogenic climate change. While biomass is central to renewable carbon strategies, it is important to recognise that other non-fossil carbon sources also hold promise. These include captured CO₂, waste plastics, aquatic biomass, and industrial organic residues, among others.¹²⁻¹⁴ Rather than being seen as competitors, these alternative carbon sources should be seen as complementary approaches, each capable of replacing specific segments of the fossil-based value chains. Among these options, biomass stands out due to its broad availability and inherently renewable nature. Moreover, lignocellulosic biomass possesses a high degree of intrinsic chemical functionality, which can be leveraged for atom-efficient conversion strategies. Upgrading its components for the chemicals industry not only offers a sustainable

alternative to petrochemical routes but also enables potentially more cost- and resource-efficient production.¹⁵

The valorisation of the individual biopolymers within lignocellulosic biomass fundamentally depends on effective fractionation into cellulose, hemicellulose, and lignin streams that can be independently upgraded. Historically, biomass fractionation was developed primarily to support the pulp and paper industry, with the Kraft and sulphite pulping processes emerging as dominant technologies in the early 20th century.¹⁶ These two processes continue to account for the vast majority of global pulp production today with approximately 120 million tonnes produced annually via the kraft process and 10 million tonnes via the sulphite process, out of a total of roughly 200 million tonnes.¹⁷ Both rely on the chemical removal of lignin from wood: the kraft process uses sodium hydroxide and sodium sulfide under alkaline conditions, while the sulphite process employs acidic conditions with sulphite salts. Although these methods are efficient at isolating cellulose, the lignin fraction undergoes significant structural degradation and condensation during pulping and is primarily burned to recover energy and process chemicals. In the case of the sulphite process, lignosulfonates—sulfonated lignin derivatives—have been isolated commercially since the 1930s and used primarily as dispersants, binders, and in vanillin production.¹⁸ Similarly, lignin can be recovered to varying extents from kraft pulping, and recent decades have seen the introduction of new separation technologies that enable partial recovery of dissolved kraft lignin.¹⁹ Nevertheless, the high sulphur content and highly condensed nature of these technical lignins continue to limit their suitability for high-value applications, ultimately hindering the economic feasibility of recovering lignin at larger scales.²⁰

In addition to traditional wood fractionation processes, a variety of new biorefining concepts have emerged over time, aiming to achieve complete biomass valorisation while avoiding the incorporation of sulphur into the lignin structure. The organosolv process, developed in the late 20th century, uses organic solvents to extract lignin—sometimes along with hemicelluloses—under relatively mild, sulphur-free conditions.²¹ Although several pilot-scale demonstrations have been built, the process has yet to become widely adopted at industrial scale. In parallel, the past few decades have seen a renewed interest in soda pulping, a method first introduced in the mid-19th century that uses sodium hydroxide to extract lignin. While

once largely sidelined, soda pulping has found modern applications in the fractionation of non-woody biomass and the production of bioethanol, leading to the construction of new facilities in recent years.²² In addition, advancements in sulphite process technologies over the past two decades have enabled a broader product portfolio and pushed the process closer to complete biomass valorisation.¹⁸ More recently, new biorefineries have been established that rely on acid hydrolysis for wood fractionation. These industrial and demonstration-scale plants typically combine chemical pretreatment with biological upgrading of the carbohydrate fractions into value-added chemicals.^{23,24} Although such approaches can achieve near-complete biomass utilisation, the harsh acidic conditions often cause lignin to undergo condensation reactions, thereby limiting its use to a narrower range of applications.

To avoid the lignin condensation and unleash the lignin's full potential as a functional aromatic molecule, Lignin-First biorefining (LFB) has emerged in recent years as a method considering lignin valorisation already in the process design phase. LFB is an umbrella term for methods that are based on the active stabilisation of the reactive lignin species released during its solvolysis.²⁵ Accordingly, the LFB methods avoid lignin condensations and thereby render lignin streams with great potential for upgrading. To date, the LFB is an umbrella term for three different fractionation methods (Figure 1-1).²⁵

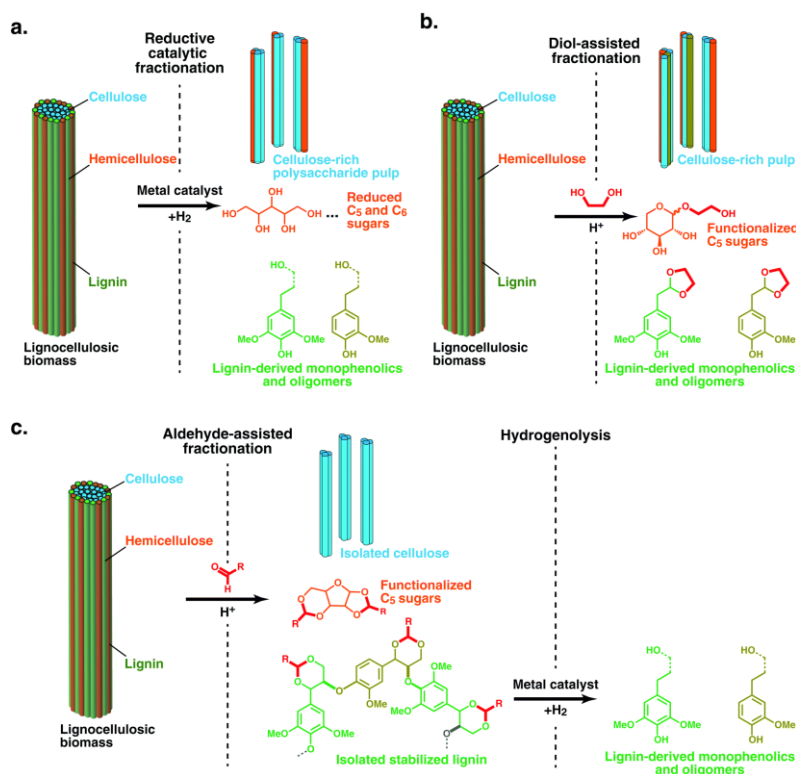


Figure 1-1. Three lignin-first strategies reported to date using solvolysis and catalytic stabilization of reactive intermediates to stable products or protection-group chemistry and subsequent depolymerisation. Reproduced from reference²⁵ licensed under [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/).

The most widely studied lignin-first approach is Reductive Catalytic Fractionation (RCF), which employs a heterogeneous catalyst in the presence of either molecular hydrogen or a hydrogen-donor solvent to stabilise reactive lignin intermediates as they are released during solvolysis. Notably, RCF under H_2 pressure was already explored in the early 1940s as an analytical method for studying lignin structure, although it was never commercialised.²⁶ The concept gained renewed attention in the early 2010s, when a new generation of research began to position RCF as a viable biorefining strategy.^{27–31} RCF produces a lignin oil containing a mixture of lignin-derived monophenolic compounds, which serve as versatile, functionalised building blocks for both bulk and fine chemicals. In addition to monomers, the oil typically contains varying amounts of oligomeric and polymeric lignin fractions, as well as hemicellulose-derived compounds, depending on the process conditions.²⁵ These higher molecular weight fractions- largely free from non-native condensed structure- could offer promising opportunities for materials and polymer applications. The process also yields a carbohydrate-rich pulp that is amenable to downstream upgrading. The second LFB approach,

known as diol-assisted fractionation, was introduced in 2015 and relies on the utilisation of diols in acidic media to trap the reactive carbonyl-containing lignin fragments into acetals.³² As such, readily upgradable lignin-derived monomers can be isolated along with the cellulose-rich pulp.³³ In 2016, aldehyde-assisted fractionation was first introduced. It involves introducing aldehydes prior to lignin extraction, which selectively protect reactive sites on the lignin structure and prevent undesired degradation and condensation.³⁴ The resulting lignin can either be used directly or further depolymerised using RCF-like methods.

While the RCF process have been studied for almost a decade by now, further understanding of it as well as various technical aspects like catalyst recycling must be resolved before it can reach full industrial feasibility. In addition, the very nature of RCF to avoid condensation reaction is also a tool to develop a better understanding of lignin itself, aiding biomass valorisation in a more general sense. As such, the current thesis focuses on advancing the RCF concept by expanding the understanding of the processes occurring during fractionation and by introducing new approaches to better steer process outcomes. The experimental work builds on previous research conducted in our group and employs poplar wood as the biomass feedstock, Raney Ni as the catalyst, and a 2-propanol/water mixture as the hydrogen-donor solvent system for the RCF process.

The thesis begins with a literature review that introduces the chemical foundations and current understanding of lignocellulosic biomass composition. From there, it follows the path of lignin from its release during biomass fractionation to its stabilisation and depolymerisation under reductive catalytic conditions, highlighting both chemical transformations and physicochemical processes. The chapter concludes with a discussion of techno-economic aspects related to RCF implementation. Chapter 3 of the thesis introduces a solvent fractionation strategy of RCF lignin oil for molecular profiling of different molecular weight fractions. During this process, an additional lignin stabilisation mechanism during the RCF process is discovered via the formation of non-native reduced β -O-4 linkages. The chapter also explores the chemical nature of carbohydrate-derived components present in the lignin oil and introduces a new UV-Vis-based metric for lignin characterisation. Chapter 4 investigates the effect of mechanocatalytic pretreatment using various additives prior to the RCF process. The results demonstrate that incorporating acetic acid during ball milling

enhances monomer yields and improves pulp quality. In Chapter 5, a semi-batch temperature-stepping RCF strategy is introduced, emphasising the importance of process conditions, and not necessarily the catalyst alone, in determining the product portfolio and monophenols selectivity.

2. Literature review

To facilitate the insights into the RCF process, the current literature review aims to cover the journey of lignin molecules, starting with the release from the lignocellulosic matrix to the stabilisation and further degradation on the catalyst surface (Figure 2-1). As the growing knowledge of lignin structure and the lignocellulosic matrix itself goes hand-in-hand with the understanding and possible outcomes of the RCF process, the first subchapter focuses on the lignin and other cell wall constituents' structure along with their placement in the lignocellulosic matrix. From the second sub-chapter, the lignin journey begins the detachment from the lignocellulosic matrix. Solvent properties for lignin dissolution and cleavages in the biopolymers are looked at in detail. The third sub-chapter covers the lignin processes taking place during the diffusion from the release from the lignocellulosic matrix to the bulk liquor. Accordingly, further degradation of lignin and carbohydrate moieties, condensation of reactive species, and the diffusion itself are touched. After lignin moieties reach the bulk liquor, the fourth chapter focuses on the catalytic processes on the heterogeneous catalyst. Therefore, the effects of solvents on it and the reductive processes that take place are covered. The subchapter ends with a tabulated summary of reaction conditions and catalysts utilised in RCF processes. The fifth sub-chapter gives a brief overview of the process impact on holocellulosic pulp focusing on biopolymers retention and upgradability of the pulps. The sixth and final subchapter considers the techno-economic aspects of the process. Accordingly, an overview of recent techno-economic analysis and life-cycle assessments of the process are given, and the downstream upgrading of the lignin fraction is discussed. The section finishes with the research objectives.

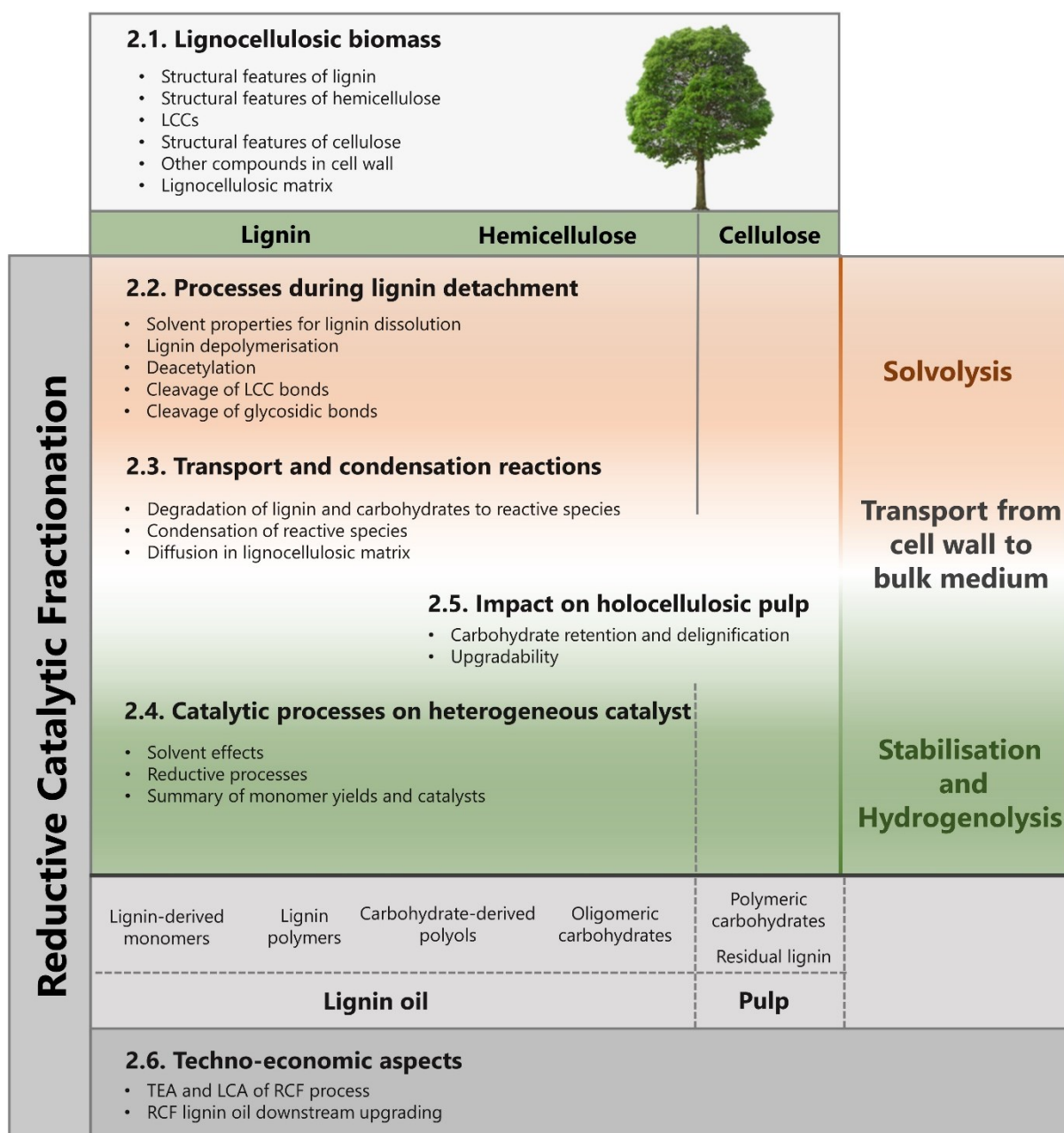


Figure 2-1. Visual overview of the literature review.

2.1. Lignocellulosic biomass

Lignocellulosic biomass encompasses a diverse range of plant-based materials, which can be divided into three broad categories: grasses, hardwood, and softwood. Figure 2-2 depicts a breakdown of softwood from the cellular level to the nanoscale, highlighting different parts of cell walls and major biopolymers present in secondary cell wall structures.³⁵ A plant cell wall is arranged in layers comprising primary and secondary cell walls and middle lamella. The secondary cell wall is further divided into outer (S1), middle (S2), and inner (S3) layers, which

deviate in the orientation of microfibrils present in corresponding layers. As the secondary cell wall forms more than 95% of the wall thickness in STEM wood cells, the lignocellulosic biomass section focuses primarily on the composition and architecture of secondary cell walls' main components- cellulose, hemicellulose and lignin.³⁶ This subchapter provides an overview of the chemical origin and molecular structure of each biopolymer, along with the intermolecular interactions and supramolecular structure, to facilitate the later discussion on chemical and physical processes during reductive catalytic fractionation.

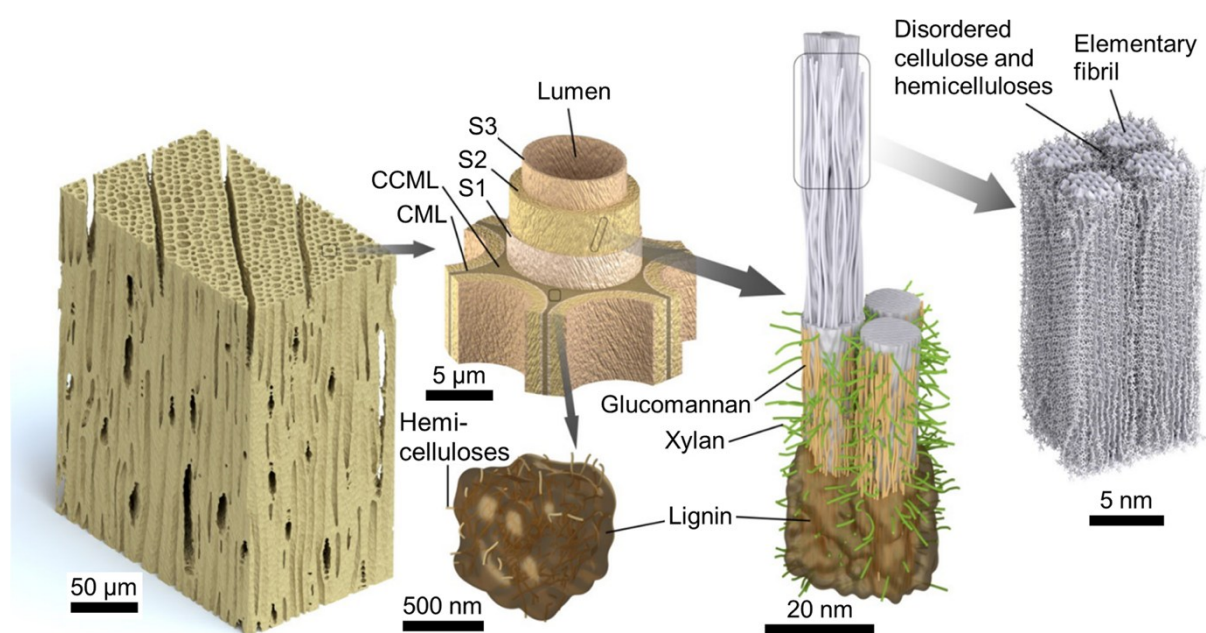
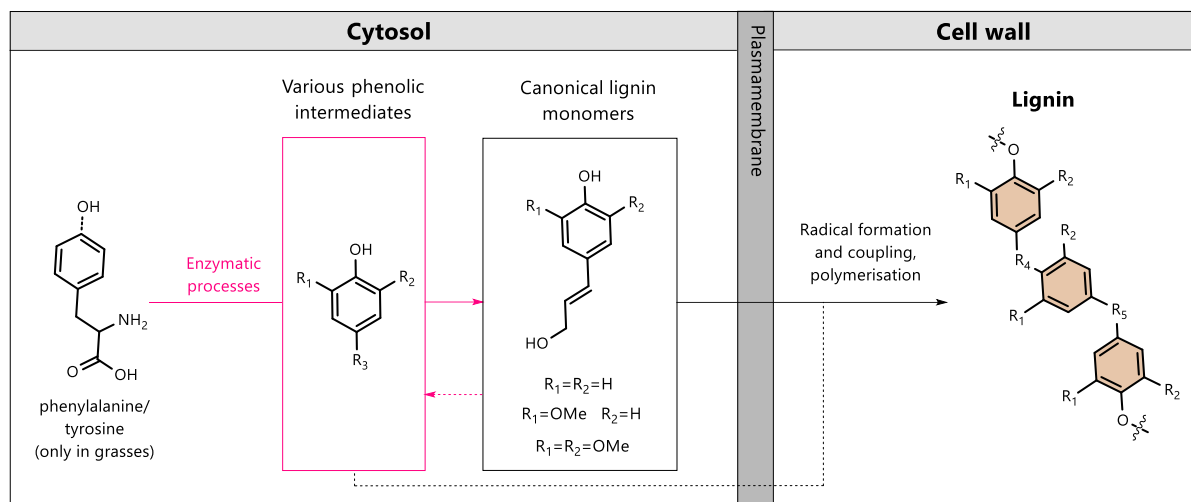


Figure 2-2. Schematics illustrating the breakdown of softwood from the cellular level to the nanoscale. Lumen is a void space in developed wood occupied by cell organelles during cell development. The primary cell wall in woody biomass is very thin and is located between the S1 layer and the compound middle lamella (CML). The larger volume of adhering CML in the corners of multiple cells is termed corner CML (CCML). Reproduced from reference ³⁵ licensed under [CC BY 4.0](#).

2.1.1. Structural features of lignin

Lignin derives from aromatic monomer units, which are synthesised via enzymatic pathways from phenylalanine (and from tyrosine in grasses) in the cell's cytosol. The majority of the monophenolic compounds that are transported to the cell wall are formed by three canonical lignin monomers- p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The key enzymatic steps towards the three canonical monolignols along with the intermediates and additional phenols forming in cell's cytosol are recently summarised by Vanholme *et al.* and could be found in Appendix Figure A-1.³⁷ After transport to the cell wall, laccase and peroxidase

enzymes generate monolignol radicals, which undergo coupling and polymerisation to form lignin.³⁸ Accordingly, Scheme 2-1 summarises the cell wall lignification process, emphasising that any phenol transported to the cell wall can undergo oxidative radical coupling and can be incorporated into the lignin structure.³⁹



Scheme 2-1. Graphical representation of the space where lignin formation takes place. Lignin monomers are synthesised in the cytosol, which, after transport to the cell wall, can undergo a lignification process.

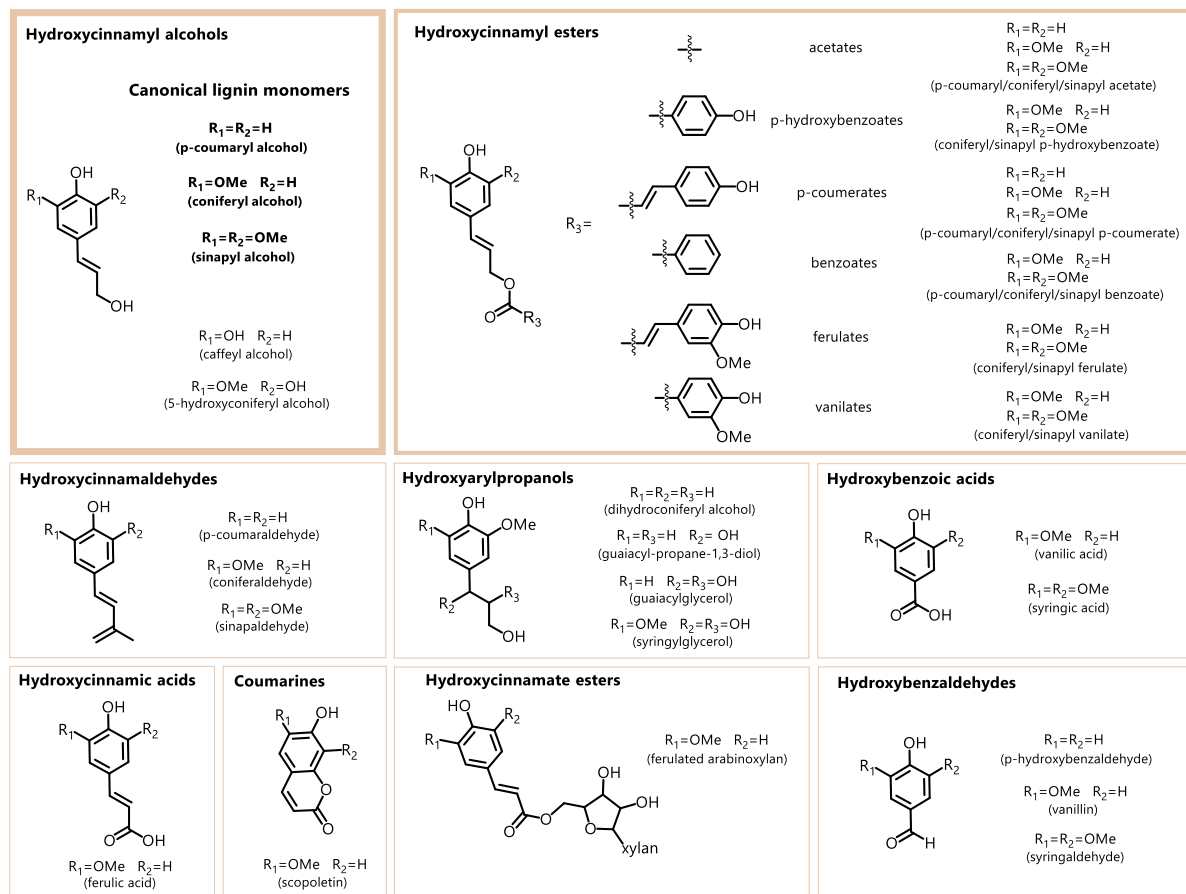
In the lignin structural features subchapter, different lignin monomeric units found from lignin to date are first summarised. This is followed by a discussion on the formation of lignin linkages and lignification. Finally, lignin structure is discussed in light of lignification theory and experimental data.

2.1.1.1. Lignin monomers

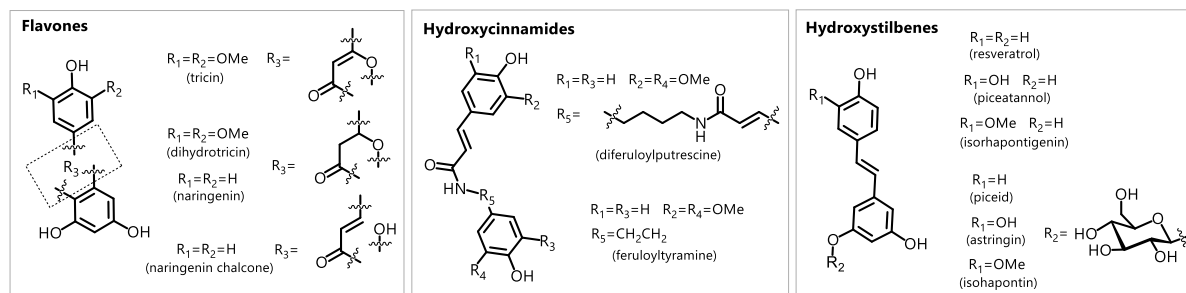
Lignin is often described as being formed from three canonical monolignols (p-coumaryl, coniferyl, and sinapyl alcohols) by oxidative radical coupling to form the corresponding H, G- and S-units in lignin.⁴⁰ While the three monolignols usually make up the bulk of the lignin, a variety of other phenolic compounds from the monolignol biosynthetic pathway as well as other routes beyond the canonical pathway, can be incorporated into the structure. Depending on the plant species, environmental conditions, and growth stage, the ratio of canonical monolignols and the extent of incorporation of non-canonical lignin monomers into lignin can vary significantly. Scheme 2-2 summarises the lignin monomers that have been detected in plant lignin structures to date. In total, 46 different monomers in 12 classes have been detected, with 34 monomers in 9 classes from the canonical monolignol biosynthesis pathway

and 12 monomers in 3 classes from biosynthetic pathways beyond the canonical monolignol.^{37,41} Many of the lignin monomers displayed on Scheme 2-2 have only been discovered in recent years, and it is likely that the list will further expand in the near future.

Lignin monomers from canonical monolignol biosynthetic pathway



Lignin monomers from beyond monolignol biosynthetic pathway



Scheme 2-2. Summary of the 46 different lignin monomers in 12 different classes found to date in natural lignins. The grouped monomers arising from the canonical monolignol pathway are boxed in light brown, with the most abundant group of hydroxycinnamyl alcohols highlighted in wide bold and another abundant class- the hydroxycinnamyl esters highlighted in bold. The monomer classes arising from biosynthesis pathways beyond the canonical monolignol are boxed in grey.

In hardwoods, lignin is primarily formed from coniferyl and sinapyl alcohols, with a minor inclusion of *p*-coumaryl alcohol. Softwood lignin is mainly constructed from coniferyl alcohol along with small amounts of *p*-coumaryl alcohol. In grasses, both coniferyl and sinapyl alcohols play a significant role, and slightly higher levels of *p*-coumaryl alcohol incorporation compared to hard- and softwoods can take place^{38,42}. Along with widespread canonical hydroxycinnamyl alcohols, two other monomers in this class have been found in native lignins. Caffeyl alcohol has been found to be the dominant monomer in vanilla seed coats as well as in some cactus seeds.⁴³ 5-hydroxyconiferyl alcohol has been found in small amounts (<1%) in the lignin of some grasses.^{44,45}

Besides canonical lignin monomers, hydroxycinnamyl esters are the most widespread non-canonical lignin monomers and can be found in many plants. Acetylation of lignin is a result of monolignol acetates' incorporation during lignification.^{46–52} Acetylated lignin species could be found at substantial levels in various grasses and at lower levels in several hardwoods. In Agave leaf fibres, up to 52% of the lignin units are acetylated, while the acetylation of lignin in rice culm can reach up to 2.5%.^{53–55} From hardwoods, aspen lignin incorporates around 1% of acetylated lignin monomers, while in hornbeam, 45% of the S-units and 3% of the G-units are acetylated.⁵³ In most plants, acetylation occurs mainly on S-units, and softwoods like pine and spruce, rich in G-type lignin, completely lack acetylated lignin units.^{41,53} Monolignol *p*-hydroxybenzoates are common in hardwood lignins (e.g., poplars, aspens, and willows), where they can comprise 10–15% of the total lignin.^{48,56–58} They have also been identified in grasses at substantial levels and have been shown to occur mainly on S-units.^{59,60} Monolignol *p*-coumarates are typical in grasses, where they can be prevalent (around 50% of the units are *p*-coumaroylated in elephant grass and papyrus) or only be found in trace amounts in others.^{61–65}

While acetates, *p*-hydroxybenzoates, and, to some extent, *p*-coumarates are common lignin monomers in many plants, other hydroxycinnamyl esters like ferulates, benzoates, and vanilates have only been found in trace amounts in native lignins. Monolignol ferulates have been found in small quantities (usually <0.1%) from various grasses.⁶⁶ Monolignol benzoates have shown a tendency to occur simultaneously with *p*-hydroxybenzoates, and it has been

found in trace amounts from poplar and palm tree lignin.^{67–69} To date, monolignol vanilates have only been described in trace amounts in Canary Island palms.⁶⁷

Hydroxycinnamaldehydes could be found at low levels in the lignin of most normal plants.^{70,71} As a direct precursor for canonical monolignols, the downregulation of the cinnamyl alcohol dehydrogenase enzyme responsible for the reduction has led to significant incorporation of the aldehydes into the lignin structure.^{71–75} Out of the hydroxyarylpropanols, dihydroconiferyl alcohol and guaiacylpropane-1,3-diol have been detected in small amounts in many softwoods as well as in some hardwoods.^{75–78} The hydroxyphenylglycerols have also been found in trace amounts from woody biomass lignin. However, it is unclear whether they derive enzymatically in the cell's cytosol or are a result of radical oxidation reactions during lignification in the cell wall and could, therefore, not be accounted for as lignin monomers.^{75,76,79,80}

Ferulic acid has been shown to be present in trace quantities in grasses as well as in hardwood lignins.^{45,81} In grasses, ferulates acylate polysaccharides, particularly arabinose residues at the C-5 in arabinoxylans.^{82–84} Coumarin scopoletin has recently been discovered in trace amounts in the lignin of some grasses, along with the enzyme responsible for its production from ferulates.^{85,86} Hydroxybenzaldehydes and hydroxybenzoic acids have been found in small quantities in the native lignin of grasses.⁸⁷

Besides phenolic compounds arising from the canonical monolignol biosynthetic pathway, recent years have also uncovered lignin monomers beyond the canonical monolignol pathway. In 2012, triclin was reported as the first monomer of this type being incorporated into the lignin of wheat straw.⁶⁵ The research followed has indicated triclin to be present in the lignins of a large variety of grasses.^{88–91} In the latter years, other flavonoids (e.g., naringenin, naringenin chalcone, and dihydrotriclin) have been discovered from the grass lignins.^{64,92} Another class of compounds derived from beyond the canonical monolignol biosynthetic pathway is hydroxystilbenes. Resveratrol, piceatannol, and isorhapontigenin have been found in the lignins of fruit endocarps.^{68,93} Hydroxystilbene glycosides have been recently reported in the lignin of Norway spruce bark.^{94,95} Ferulic acid amides are also derived from beyond the monolignol biosynthetic pathway. Ferulotyramine has been described as part of lignin in

tobacco plants as well as in several other plants,^{96–99} and diferuloylputrescine has been found in the lignin of maize kernels.¹⁰⁰

Along with the natively occurring monolignols, a significant amount of research has been dedicated to genetically modify plants to alter the occurrence of enzymes responsible for monolignol synthesis in plants. For example, overexpression of ferulate 5-hydroxylase (F5H), which is responsible for the methoxylation of coniferaldehyde and coniferyl alcohol, led to poplar containing 98% S-units.^{34,56,101} In turn, knocking out the F5H enzyme produces plants solely with G-lignins.¹⁰¹ A recent comprehensive overview of key efforts in monolignol engineering is provided by Ralph *et al.*, covering the engineering of conventional monolignol production as well as engineering monolignol pathway intermediates into lignin polymers.¹⁰²

Although the bulk of the lignin in plants is derived from the three canonical monolignols as well as from hydroxycinnamyl esters, research on the incorporation of other metabolites from the canonical lignin biosynthetic pathway and beyond that has significantly broadened the understanding of this biopolymer. Evolving efforts on lignin characterisation, as well as the examination of a wider range of plants, will likely lead to further expansion of the lignin monomers list in the upcoming years.

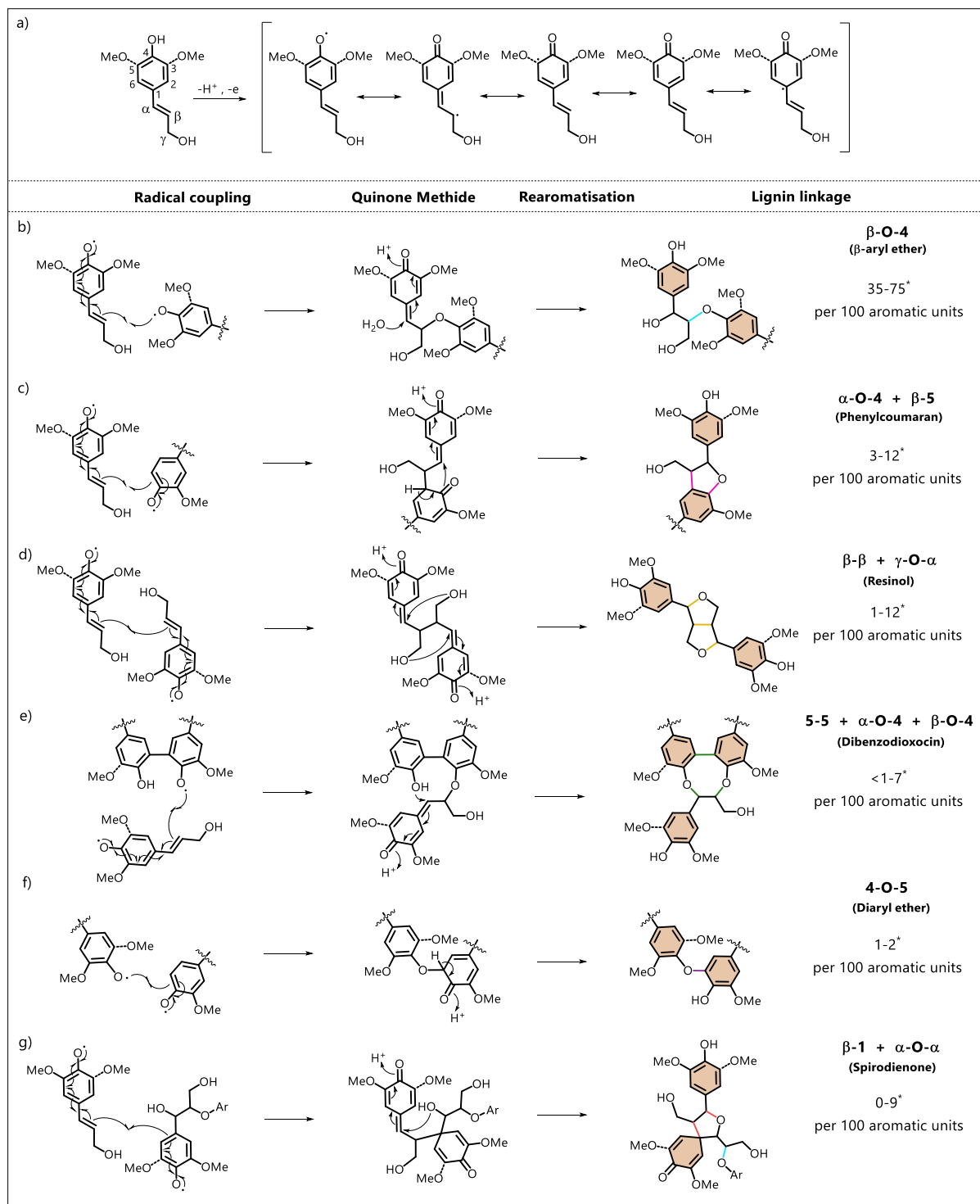
2.1.1.2. Radical coupling and formation of lignin linkages

After monolignol transport from cytosol to the cell wall, they undergo peroxidase/laccase-induced oxidative radical formation. Once the radicals are formed, they couple and cross-couple randomly, forming quinone methide intermediates. In an aqueous medium, the species undergo rearomatisation and thereby give rise to linked lignin moieties racemic in their nature.^{20,37,102} Accordingly, Scheme 2-3 summarises the lignin polymerisation process starting with resonance stabilised monolignol radical, formation of quinone methide intermediate and rearomatisation to form lignin.¹⁰³ Linkages arising from canonical monolignols are described as they make up the majority of lignin monomers and, therefore, participate in most linkages formation.

β -aryl ether is the most common lignin linkage prominent in all lignocellulosic biomasses. The number of β -O-4 linkages per 100 aromatic units can range from 35 in Maple to 60 to 73 in birch.^{104,105} In softwoods, the β -O-4 content is slightly lower than in hardwoods, usually ranging

from 40-50 linkages per 100 aromatics.^{20,104-106} ²⁰It can be formed via two monolignols radical coupling or, more commonly, via monomer-oligomer cross-coupling.¹⁰³ The rearomatisation occurs under kinetic and not thermodynamic control, giving rise to a racemic mixture of *anti/syn* isomers.¹⁰⁷⁻¹⁰⁹ Like β -O-4 linkage, phenylcoumaran can be formed via coupling of two monomeric units or oligomer coupling with a monomer. The produced quinone methide intermediate rearomatises by internal trapping with available phenol, resulting in *trans*-selective ring closure and formation of five-membered ring.^{40,103} The abundance of phenylcoumaran linkages varies from 3-12% in different plants. The third common lignin linkage in plants is resinol, contributing 1-12% of the total lignin linkages.²⁰ Unlike β -aryl ether and phenylcoumaran moieties, it can only be formed via the coupling of two monolignol units. Like the quinone methide intermediate for phenylcoumaran formation, the β - β coupled quinone methide is internally trapped. However, this time by hydroxyl groups at C_v, and the rearomatisation produces only a single isomeric form.^{103,110}

Along with the three common lignin linkages, other bonding motifs could be found in many plants. The oligomer-oligomer coupling of G-units producing biphenyl linkages almost exclusively undergoes another coupling to form a cyclic ether structure- the dibenzodioxocin.^{111,112} Importantly, evidence only for free phenolic versions of dibenzodioxocin has been found to date, implementing that the linkage does not create a branching point in lignin.¹⁰² Dibenzodioxocin linkages are common in G-unit-rich softwoods (5-7%) but less prominent in hardwoods (<1%) and grasses.²⁰ Similarly to dibenzodioxocin, diaryl ethers arise from the coupling of two oligomeric moieties, and only free phenolic forms have been evidenced.^{113,114} Therefore, the 4-O-5 linkage does not create a branching point either.¹⁰² The diaryl ether has been identified to link approximately 1-2% of the lignins in plants.^{20,102} Spirodieone moieties are derived from monolignol coupling with an existing phenolic end of β -aryl ether.^{115,116} Importantly, one of its phenolic groups remains non-etherified, implying that the spirodieone linkages do not present a branching point in the lignin structure.¹¹⁶ The occurrence of the moiety usually remains between 1-2% but can reach also higher levels in selected hard- and softwoods.^{20,102}



Scheme 2-3. Summary of the lignin linkages formation process from prototypical monolignols along with their relative abundance in different types of lignocellulosic biomass. a) Enzymatic oxidation and H-abstraction of monolignol to form resonance stabilised radical. b) Formation of β -aryl ether linkage. c) Formation of phenylcoumaran linkage. d) Formation of resinol linkage. e) Formation of dibenzodioxocin linkage from 5-5' linked oligomer. f) Formation of diaryl ether linkage from lignin oligomers. g) Formation of spirodienone linkage. *The content of lignin interunit linkages is aggregated from references ^{20,104-106}

The six main structures deriving from canonical monolignols form the backbone of the lignin. Importantly, the phenolic acyl groups in common lignin monomers like *p*-hydroxybenzoates and *p*-coumarates preferentially undergo radical transfer reactions instead of radical coupling, indicating that the given acyl groups mostly remain free phenolics. Therefore, *p*-hydroxybenzoates and *p*-coumarates are pendant groups in lignin structure.^{59,117,118} If an acylated monolignol undergoes β - β' radical coupling with another canonical monolignol or its ester, the resinol structure cannot be formed, and a tetrahydrofuran-like structure found in small amounts in some lignins is formed instead.^{47,55,58,119} Other unconventional lignin monomers usually follow similar coupling patterns to canonical monolignols, with some exceptions. The aromatic 3-hydroxyl group of caffeyl alcohol and 5-hydroxyconiferyl alcohol causes internal trapping of quinone intermediate after β -O-4 coupling and gives rise to benzodioxane moieties. Lignins solely synthesised from caffeyl alcohol and containing only benzodioxane moieties are called "C-lignins" and have been found in seed coats of vanilla and cactuses.^{43,120,121} Tricin have been shown to only appear at the starting end of the lignin chain and has been suggested to play a role as a nucleation site in grasses during lignification.¹²² An extensive overview of unconventional lignin monomers and their incorporation mechanism into lignin during lignification is given by del Rio *et al.* in a recent review.⁴¹

2.1.1.3. Lignin structure

One of the most intriguing discussions to date in the lignin research community remains the very nature of native polymeric lignin. Historically, lignin has often been stated to be a cross-linked polymer. However, the lignification theory and NMR studies on native lignin structures tend to point towards a more linear structure than usually thought.^{102,123} Three main points of disagreement exist, which essentially determine how branched and cross-linked the lignin is.

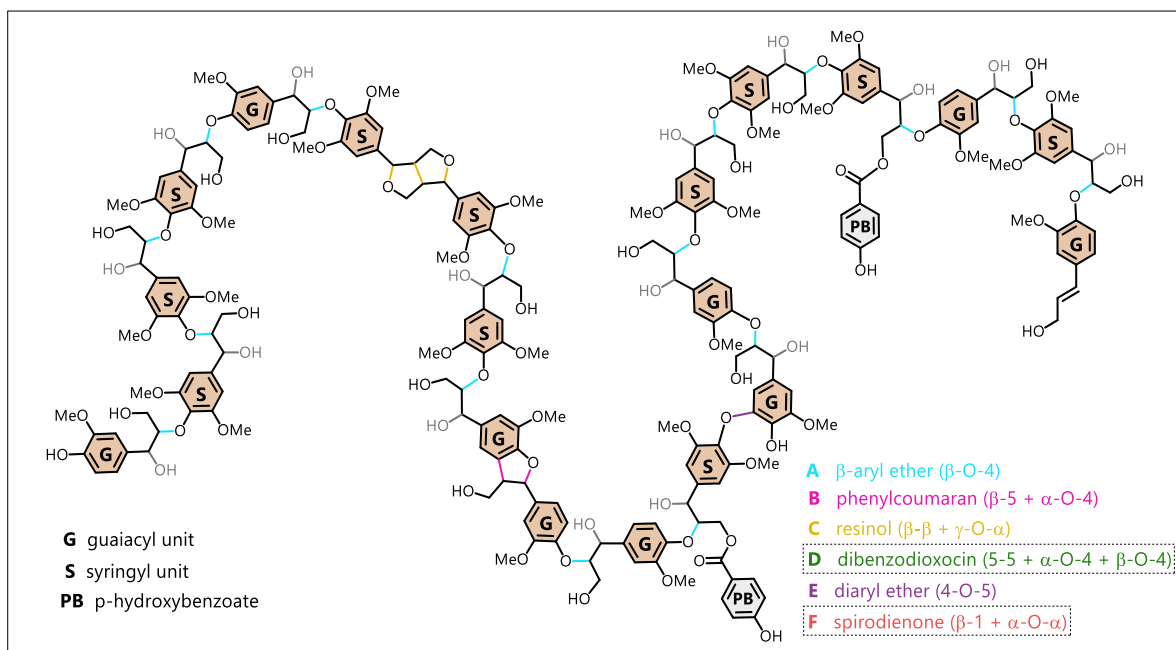
- The nature of 5-5' (diphenyl) structures. It has been stated that 5-5'-linked units are almost exclusively forming dibenzodioxocin linkages, in which the linked monomer remains as a free phenolic, resulting in a "U"-type structure instead of "Y"-type.^{20,102,124} This is challenged by the research on softwood milled-wood lignin, which, based on lignin derivatisation, NMR, and UV-Vis analysis, has concluded that most of the 5-5'-linked units are not dibenzodioxocin units, but rather etherified from both phenolic

ends forming "H"-type cross-linking point. The dibenzodioxocin moieties present are also etherified, giving a "Y"-type branching point.^{125,126}

- Etherification of 4-O-5 (diaryl ether) units. Only the free-phenolic form of the diaryl ether has been evidenced in native lignins, resulting in "U"-type structures instead of "Y"-type.^{102,113,114} Etherification of the phenol, often referred to in literature, would result in a "Y"-type structure and consequently a branching point in lignin structure.¹²⁵
- Etherification of α - and γ -positions in alkyl chain. NMR studies have revealed that although present in some synthetic lignins, there is little evidence of α -aryl ethers in plants, and the α -position is occupied by the hydroxyl group arising from water addition to quinone methide intermediate in β -O-4 moieties.^{102,127,128} It has also been suggested the presence of α - and γ -alkyl ethers which would give rise to "Y"-type branching points. However, no direct evidence or identification is known to date.¹²⁵ Formation of α -alkyl ester would require hydroxypropyl aromatic (γ -OH) unit addition to quinone methide intermediate during the β -O-4 bond formation, which is unlikely in cell walls rich in water and carbohydrates.

It is important to emphasise that the lignin structure arising from radical coupling is inherently non-deterministic due to the variety of monolignols and linkages as well as the racemic nature of the linkages. Therefore, any representation or proposed model of a "lignin structure" is merely a rough approximation of an average lignin molecule in the cell wall. It has been estimated that, on average, lignin contains 20-30 monomeric units with a considerable polydispersity, therefore also containing lignin molecules from only several monomers up to more than 100.^{125,129,130}

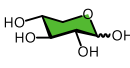
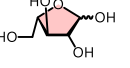
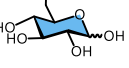
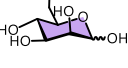
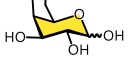
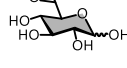
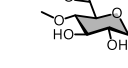
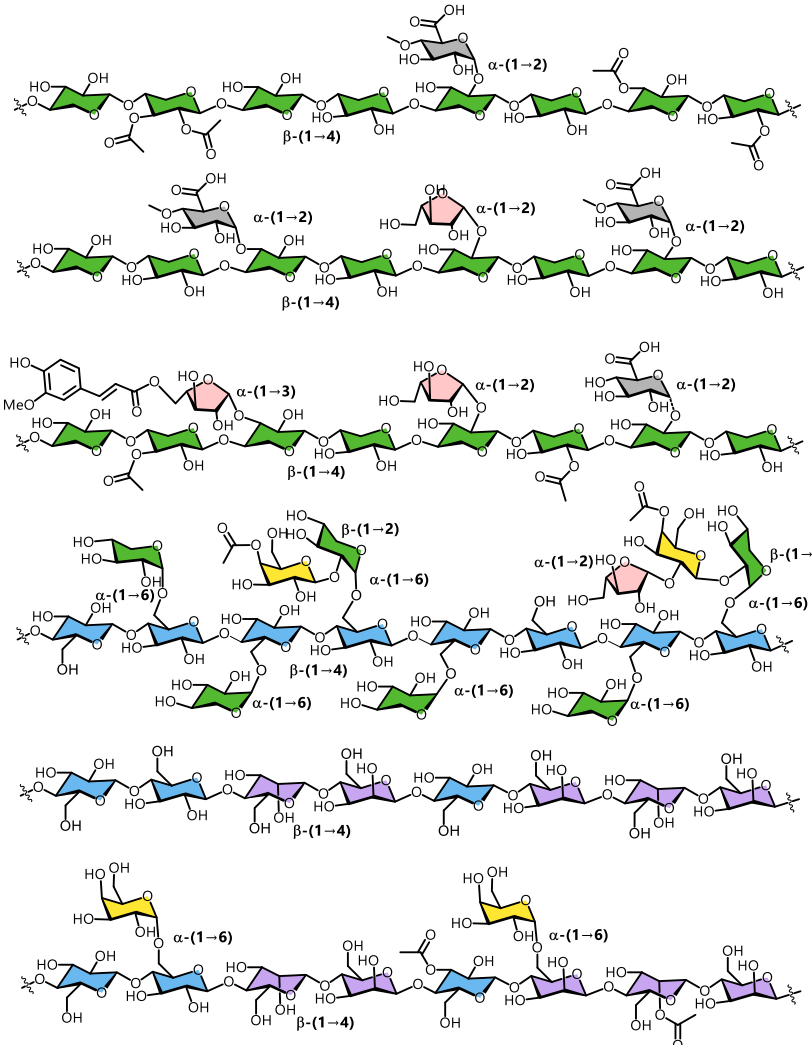
Moving forward, 5-5'-linked units are considered to be in dibenzodioxocin linkages and contain, along with 4-O-5 linkages, a free phenolic end. While in hardwoods and grasses, the 5-5'- and 4-O-5-linked units only make up a minor fraction (<2%), the prevalence of 5-5' linked units in softwood makes it important to keep in mind the ambiguity about the very nature of the 5-5'-linked units. Scheme 2-4 depicts a hardwood lignin structure proposed by Ralph *et al.* in 2019 based on the current lignification theory and NMR studies. The lignin molecule results from the polymerisation of 20 monolignol units and contains the estimated amounts of corresponding monolignols and lignin linkages.



Scheme 2-4. A lignin model for hardwood lignin derived by Ralph et al.¹⁰² The dibenzodioxocin and spirodienone linkages are not present in the current snapshot but could be found in small quantities in hardwood lignins. Light grey hydroxyl groups are derived from water addition during quinone methide rearomatisation. The lignin monomers in the backbone are marked in black, while the formed lignin linkages are colorised according to the legend of the figure.

2.1.2. Structural features of hemicellulose

Hemicelluloses are heterogeneous polysaccharides, which comprise 15-35% of the weight of biomass. Unlike lignin which is synthesised in the cell wall via radical coupling, hemicelluloses are enzymatically synthesised in the Golgi apparatus from corresponding nucleotide sugars.^{131,132} The synthesised polysaccharides are transported into the cell wall, where they can potentially undergo enzymatic remodelling. The main monomeric units in hemicelluloses are pentoses (D-xylose, L-arabinose), hexoses (D-mannose, D-galactose, D-glucose) and uronic acids (D-glucuronic and 4-O-methyl-D-glucuronic acid).¹³³ Along with the major monosaccharides, hemicelluloses can also contain L-rhamnose, L-fucose, and other uronic acids. Importantly, many hemicelluloses are also acetylated via O-2 and/or O-3 position, and the acetyl groups can account for 3-5% of the hardwood and 1-2% of softwood composition.¹³⁴ Scheme 2-5 summarises the main types of hemicelluloses along with their relative abundance in different types of biomass's primary and secondary walls.

Pentoses			Hexoses			Uronic acids	
							
D-xylose	L-arabinose		D-glucose	D-mannose	D-galactose	D-glucuronic acid	4-O-methyl-D-glucuronic acid
Glucuronoxylans (GXs)							
	Primary wall	Secondary wall					
Hardwoods	-	20-25 wt%					
Softwoods	-	-					
Grasses	-	-					
Arabinoglucuronoxylans (AGXs)							
	Primary wall	Secondary wall					
Hardwoods	-	-					
Softwoods	2 wt%	5-15 wt%					
Grasses	-	-					
(Glucurono)arabinoxylans (GAXs and AXs)							
	Primary wall	Secondary wall					
Hardwoods	5 wt%	-					
Softwoods	-	-					
Grasses	20-40 wt%	40-50 wt%					
Xyloglucans (XGs)							
	Primary wall	Secondary wall					
Hardwoods	20-25 wt%	-					
Softwoods	10 wt%	-					
Grasses	2-5 wt%	-					
Glucomannans (GMs)							
	Primary wall	Secondary wall					
Hardwoods	3-5 wt%	2-5 wt%					
Softwoods	-	-					
Grasses	2 wt%	0-5 wt%					
Galactoglucomannans (GGMs)							
	Primary wall	Secondary wall					
Hardwoods	-	0-3 wt%					
Softwoods	-	10-30 wt%					
Grasses	-	-					

Scheme 2-5. The main types of hemicelluloses, along with the most common pentoses, hexoses and uronic acids found in corresponding hemicelluloses. The content of the hemicelluloses in hardwoods, softwoods, and grasses is based on reference ¹³⁵.

Glucuronoxylans (GXs) are the main component of hardwood hemicelluloses.^{136,137} Its D-xylopyranose (Xylp) backbone is linked via β -(1 $\rightarrow$ 4) glucosidic linkages, with the 4-O-methyl-D-glucuronic acid (4-O-MeGlcA) attached via α -(1 $\rightarrow$ 2) bond to the linear backbone. On average, 3-7 xylose units out of 10 are acetylated at O-2 and/or O-3 positions, and the degree of polymerisation (DP) ranges from 100-200.^{136,138} Arabinoglucuronoxylans (AGXs) could be found mainly from softwoods.^{133,139} AGXs share the same backbone and 4-OMeGlcA units with

GXs, but AGX backbone is not acetylated and contains α -(1 $\rightarrow$ 3)-linked L-arabinofuranose (Araf) residues.¹⁴⁰ Arabinoxylans (AXs) and Glucuranoarabinoxylans (GAXs) are the main hemicelluloses in grasses and can also be found in the primary walls of hardwoods. They share the same xylan backbone with GXs and AGXs, and are substituted with α -(1 $\rightarrow$ 2)- or α -(1 $\rightarrow$ 3)-linked Araf units.^{133,141,142} The arabinosyl unit can be further acylated with ferulic acid from O-5 position, which in turn is responsible for cross-linking hemicelluloses and lignin.^{117,143} Beside arabinosyl units, AGX backbone is substituted with D-glucuronic acid (GlcA) and 4-O-MeGlcA via α -(1 $\rightarrow$ 2)-linkages and can contain O-2 and/or O-3 linked acetyl groups.¹⁴⁴

Xyloglucans (XGs) can be found in the primary cell walls of all lignocellulosic plants. XGs backbone is formed by β -(1 $\rightarrow$ 4)-linked D-glucopyranose (Glc_p), and on average, 6-8 units out of 10 are substituted with Xyl_p at O-6 via α -(1 $\rightarrow$ 6) linkages. The xylose side units can be further substituted with D-galactopyranose (Gal_p) via β -(1 $\rightarrow$ 2)-linkage and Araf via α -(1 $\rightarrow$ 2)-linkage, and also contain acetyl groups.^{145–147}

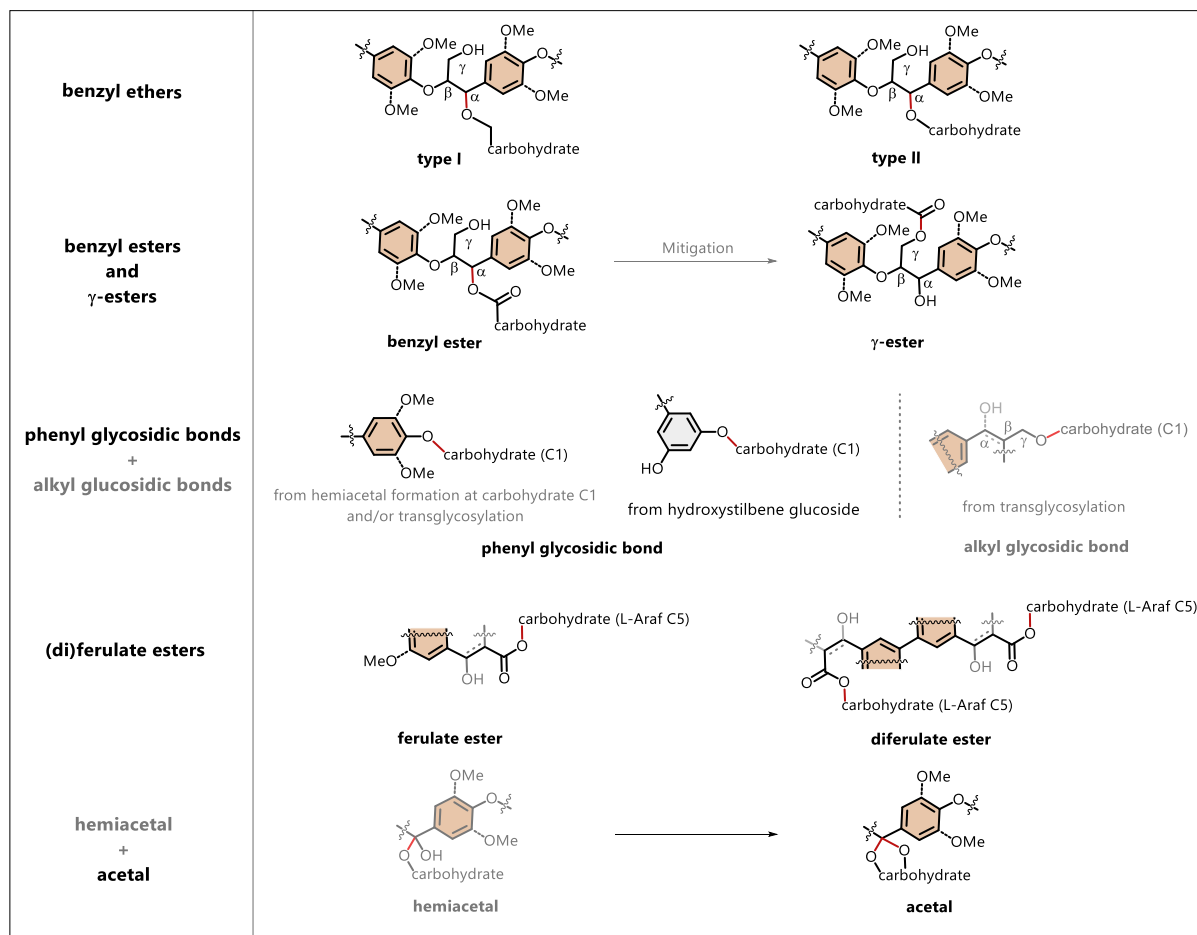
Galactoglucomannans (GGMs) are the main hemicelluloses in softwoods. They can account for up to 30 wt% of the secondary cell wall. GGM backbone consists of D-mannopyranose (Man_p) and D-Glc_p linked via β -(1 $\rightarrow$ 4) bonds, with Man_p being predominant over Glc_p. The GGM backbone is substituted by α -(1 $\rightarrow$ 6) linked Gal_p units and is partially acetylated (2-3 units out of 10) at O-2 and/or O-3 positions.^{148,149} Glucomannans (GMs) share the same backbone as GGMs but do not contain Gal_p side units. They can be found in both hard- and softwoods, where they form up to 5 wt% of the biomass.¹³⁵

Besides the aforementioned hemicelluloses, β -(1 $\rightarrow$ 3,1 $\rightarrow$ 4) linked glucans could also be considered hemicelluloses and can form 2-15 wt% of the grasses.¹⁵⁰ Other polysaccharides such as arabinans, arabinogalactans and galactans are occasionally added to the hemicellulose group. However, they are better suited to be part of pectin molecules as they do not share the equatorial β -(1 $\rightarrow$ 4) linked backbone with common hemicelluloses.¹³⁵

2.1.3. Lignin-carbohydrate complexes

In plant cell walls, lignin moieties can be covalently linked to carbohydrates, forming lignin-carbohydrate complexes (LCCs), with most carbohydrates involved being hemicelluloses.^{151,152} It has been shown that all lignin moieties in softwoods and 47-66% of lignin moieties in

hardwoods are bound in LCCs.^{153,154} Lignin-carbohydrate bonds arise during the biosynthesis of lignin in the cell wall and are usually assumed to result from nucleophilic addition of carbohydrate to quinone methide intermediate arising from lignin radical coupling.¹⁵⁵ Scheme 2-6 summarises the main types of LCCs bonds described to date.



Scheme 2-6. Main types of lignin-carbohydrate linkages found in LCCs.

Benzyl ethers are thought to arise from carbohydrate hydroxyl group nucleophilic addition to C_{α} in quinone methide intermediate from β -O-4 lignin linkage formation. Dependant if the hydroxyl group is primary or secondary in the carbohydrate, C6 hydroxyl groups in hexoses and C5 hydroxyl group in L-arabinofuranose form type I benzyl ethers, while nucleophilic addition from any secondary OH-group results in type II benzyl ether.¹⁵⁶ In softwoods, the number of LCC linkages per 100 aromatic units has been measured to vary between 3.8-6.1, while in hardwoods, the corresponding number of linkages is up to 4.^{151,157-160}

Benzyl esters are described to arise via a similar mechanism to benzyl ethers, but instead of pentose or hexose hydroxyl group addition to quinone methide intermediate, the nucleophilic addition takes place from the carboxylic OH-group from uronic acid.¹⁵⁶ Formed benzyl ester is proposed to further migrate to C_γ in lignin, giving rise to thermodynamically more stable γ-esters.^{161–163} The number of benzyl and γ-ester linkages per 100 aromatic units have been measured to be in the proximity of 5 in both soft- and hardwoods.¹⁵⁸

Phenyl glycosidic LCCs are abundant in woody biomass with 4.4–6.8 linkages per 100 aromatic units in softwoods and 5.0–7.9 in hardwoods.^{151,157–159} Nonetheless, phenyl glycosidic LCC bond formation remains ambiguous to date. One proposed mechanism is based on the acid-catalysed hemiacetal formation at the reducing end of hemicellulose with a phenolic hydroxyl group, resulting in phenyl glycosidic linkage.^{156,164,165} Recently, Rencoret *et al.* demonstrated the presence of hydroxystilbene glucoside monolignol in softwood bark, which gives rise to phenyl glycosidic LCCs after lignification.⁹⁵ In another recent study by Miyagawa *et al.*, in vitro experiments were carried out with monolignol 4-O-glucoside with and without the enzyme β-glucosidase responsible for transglycosylation in the cell wall in the presence of peroxidase responsible for initiating lignin radical coupling. The direct addition of the monolignol 4-O-glucoside via quinone intermediate was determined as the primary mechanism for incorporating the phenyl glycosidic bond. However, evidence of glucose migration to phenolic ends via transglycosylation was also found, supporting the hypothesis that phenyl glycosidic bonds could not only form via radical coupling of monolignol glucoside with monolignol or oligomeric lignin moiety but could also form between hemicellulose and phenolic moieties in cell wall while not involving quinone methide intermediates. The same study also found evidence of the formation of monolignol γ-O-glucosides via transglycosylation, giving rise to alkyl glycoside that can be incorporated into the lignin structure after radical coupling.^{166 117,123–}

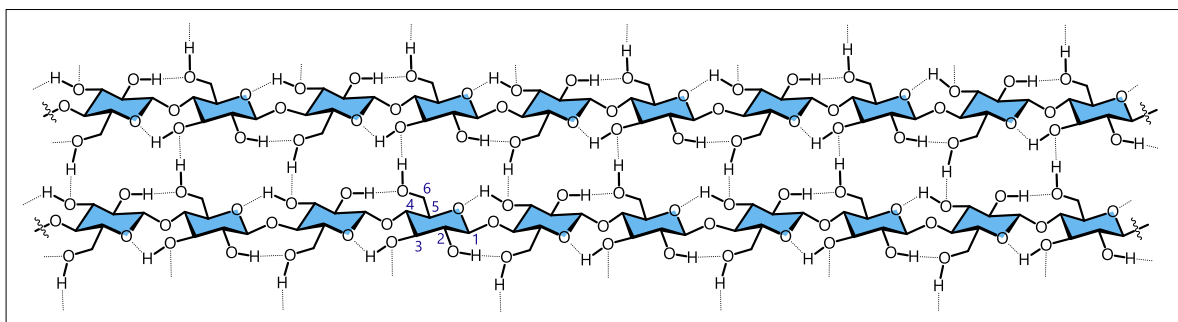
125

In grasses, ferulated carbohydrates (Scheme 2-2 and Scheme 2-5) participate in lignification via oxidative radical coupling, resulting in ferulate ester LCCs if the ferulated carbohydrate is coupling with monolignol or lignin oligomer or in diferulate LCC if two ferulated carbohydrates couple with each other.^{84,167–169} Another group of LCCs are acetals and hemiacetals, which can originate from the addition of hydroxyl groups from carbohydrates to carbonyl groups present

in some less common monolignol units (Scheme 2-2), forming hemiacetal and acetal type LCC bonds.^{151,170–172}

2.1.4. Structural features of cellulose

Cellulose is the main component of lignocellulosic biomass, forming 40-50 wt% of hard- and softwoods and 35-75 wt% of grasses.¹⁷³ Unlike lignin and hemicellulose, cellulose is a homopolymer formed by repeating units of β -(1 $\rightarrow$ 4)-linked D-glucopyranose. Its DP ranges from 1,000-30,000, making it 1-2 orders of magnitude more polymerised compared to other biopolymers in cell wall.¹⁷⁴ Cellulose can form different intra- and intermolecular hydrogen bonding patterns depending on its origin and treatment, resulting in several crystal structures.¹⁷⁵ In its native form, cellulose forms intermolecular hydrogen bonding between the oxygen atom at C3 and the hydroxyl group at C6, and intramolecular hydrogen bonding between ether-oxygen and hydroxyl group at C6 as well as between the oxygen atom at C6 and hydroxyl group at C2 (Scheme 2-7).^{176,177} This is termed cellulose I with two different crystal phases. Triclinic I $_{\alpha}$ is common in microorganisms, while monoclinic I $_{\beta}$ is the dominant form of cellulose in all higher plants.¹⁷³



Scheme 2-7. Molecular structure of cellulose along with the hydrogen bonding patterns in cellulose I.

Cellulose is synthesised at the plasma membrane by large cellulose synthase (CESA) complexes, producing glucan chains arranged into elementary fibrils containing 18 individual chains.^{178–181} The elementary fibrils are further organised into microfibrils 10-20 nm in width, which can additionally form microfibrillar bands in the order of 100 nm in width and up to a few microns in length.^{173,182}

2.1.5. Other compounds in cell walls

Besides the three biopolymers cellulose, hemicellulose, and lignin forming a large majority of lignocellulosic biomass, different parts of the cell wall can also contain varying amounts of other components which do not fall under the classification of any of these three. Pectins are similarly to hemicelluloses heteropolysaccharides, but are mainly present only on the primary cell wall and middle lamella.^{183,184} As the two parts of the cell wall make up only 5% of the whole cell wall in woody biomass, the amount of pectins in wood is only up to a few per cent.³⁶ Pectins are considerably more acidic compared to hemicelluloses as they mostly contain α -(1→4) linked D-galactopyranosyl acid backbone with large side chains containing L-arabinofuranose, L-rhamnopyranose, D-xylopyranose, and D-galactopyranose units.¹⁸³

Another component present in the cell wall is termed extractives. The term extractives could be defined as compounds that are extractable from biomass with various neutral solvents, and the extraction procedure and solvent(s) define the yield and composition of the extractives. Water and ethanol extractives can contain non-structural sugars, organic acids, inorganic material, waxes, nitrogenous materials and other minor components.^{185–187} The lipophilic components more soluble in solvents of low polarity (e.g. hexane, diethyl ether) include fats and fatty acids, steryl esters and sterols, terpenoids, and waxes.³⁶ In hard- and softwoods, the amount of extractives varies between 0.5-5%, while grasses can contain considerably higher amounts of extractives.¹⁸⁸ In addition, ash refers to inorganic residue remaining after burning the organic matter and accounts for the various elements present in lignocellulosic biomass. It mainly contains O, Ca, K, Si, Mg, Al, S, Fe, P, Cl, Na, Mn, and Ti, and usually contributes 0.1-2% of the biomass weight.^{189,190}

2.1.6. Lignocellulosic matrix

In recent years, there has been a significant boost in the methods used to determine the supramolecular structure of lignocellulose. In this effort, isotope-labelled plants grown in $^{13}\text{CO}_2$ atmosphere have been analysed with solid-state NMR combined with dynamic nuclear polarisation (DNP) methods, and density functional theory (DFT) calculations along with computer simulations have been employed to unveil the structural and chemical principles underlying the ultrastructure.^{35,191–198}

Unlike lignin and hemicellulose, the supramolecular structure of cellulose has a long history, and extensive research has been done over the years. Although cellulose forms a crystalline structure, it is established that the fibrillar bands of cellulose also contain amorphous regions.¹⁹⁹ The models describing amorphous parts of cellulose have evolved over the years. The first proposed model of crystalline fibril with an amorphous surface layer failed to explain cellulose mechanical properties and reduction in DP during hydrolysis.^{199–201} As the neutron scattering revealed that amorphous regions are situated within the microfibrils instead of their surface, amorphous-crystalline model where crystalline regions alternate with smaller amorphous regions along the fibril was proposed.^{199,202–205} The model has been further refined to mesomorphous-crystalline model, where long crystalline areas alternate with short amorphous regions and paracrystalline structure covers the fibril surface.¹⁹⁹

Hemicelluloses are known to have a strong interaction with cellulose fibrils. In solution, xylans are present in a thermodynamically more stable three-fold structure, where one 360° twist occurs every third glycosidic bond.^{195,206} In the lignocellulosic matrix, it has been recently shown that xylans closely interact with cellulose hydrophilic surfaces via hydrogen bonding, forcing otherwise thermodynamically less favoured two-fold helical screw confirmation on the fibril surfaces (Figure 2-4).^{192–195,207–209}

In 2022, Kirui *et al.* utilised solid-state 2D ¹³C-¹³C NMR correlation spectra of isotope-labelled plants grown in ¹³CO₂ atmosphere to determine the identity of intermolecular interactions in the secondary cell wall. Figure 2-3 depicts the measured interactions between surface (s) and interior (i) cellulose, 2- and 3-fold xylans along with their acetyl groups, and S- and G-type lignin and their methoxy groups attached to the aromatic ring.¹⁹³ Along with the previous study in 2019 by the same group performed on grasses utilising the same methodology, an extensive correlation between G/S units with xylose ring carbons as well as carbonyl and methyl carbons in acetyl groups was determined, validating the principal role of extensive xylan-lignin interaction in lignocellulose supramolecular structure stabilisation.^{192,193} Furthermore, a widespread association of mannan and lignin in softwood was determined. While the interactions between cellulose and lignin in grasses were limited, wood lignin exhibited extensive interactions between cellulose and lignin. This feature, at least in part, is associated with the mechanical strength of wood stiff stems.¹⁹³

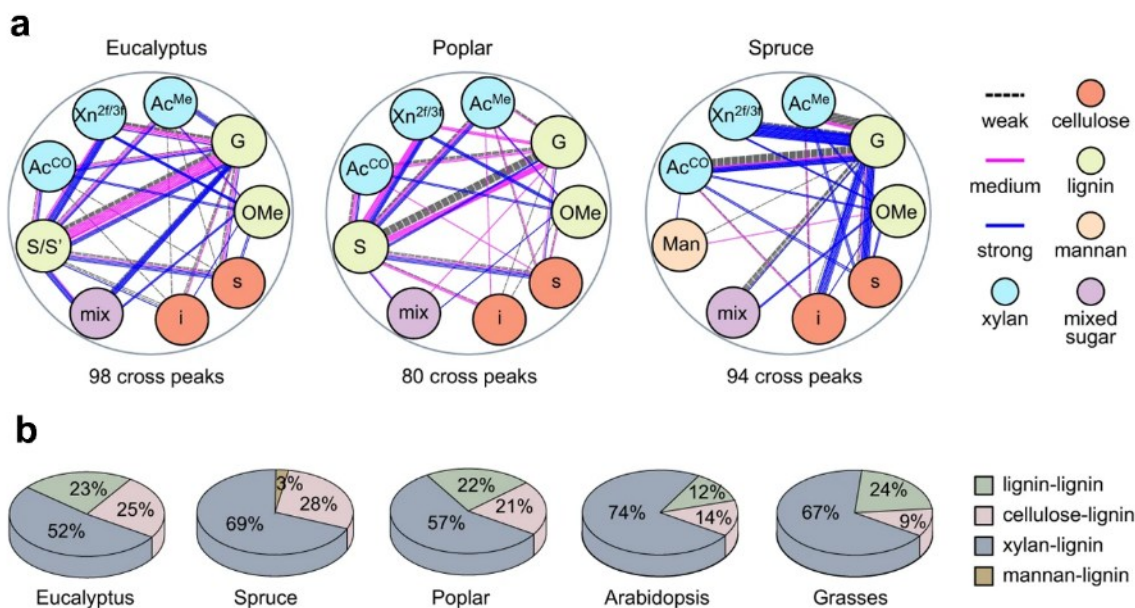


Figure 2-3. a) Summary of 272 intermolecular interactions identified in eucalyptus, poplar, and spruce. Cellulose, lignin, mannan, and mixed sugars are colour-coded in red orange, light green, light orange, and purple, respectively. b) The count of lignin–lignin, cellulose–lignin, and xylan–lignin interactions in eucalyptus, poplar, spruce, Arabidopsis, and grasses (maize, rice, and switchgrass). Reproduced and cropped from reference ¹⁹³ licensed under [CC BY 4.0](#).

Based on the interactions determined by NMR and accompanying DFT calculations, the authors depicted the nature of cellulose, hemicelluloses, and lignin spatial distribution in secondary cell walls based on their interactions (Figure 2-4).¹⁹³ In grasses, lignin and hemicelluloses are spatially more separated, and lignin is likely to form hydrophobic nanodomains with binding to three-fold xylans.^{192,193,197} In hardwoods, lignin and cellulose become more colocalised, and the molecular mixing of hemicelluloses and lignin becomes much more prevalent, with lignin binding all xylan conformers. In softwoods, lignin and cellulose contacts become even more prevalent, and lignin and hemicelluloses are well-mixed on the nanoscale.¹⁹³ Although the thermodynamic driving force for hemicellulose and lignin molecular mixing is not clear yet, the ability of the aromatic part to effectively retain and immobilise water molecules points to it in woody biomass.^{35,191,193,210}

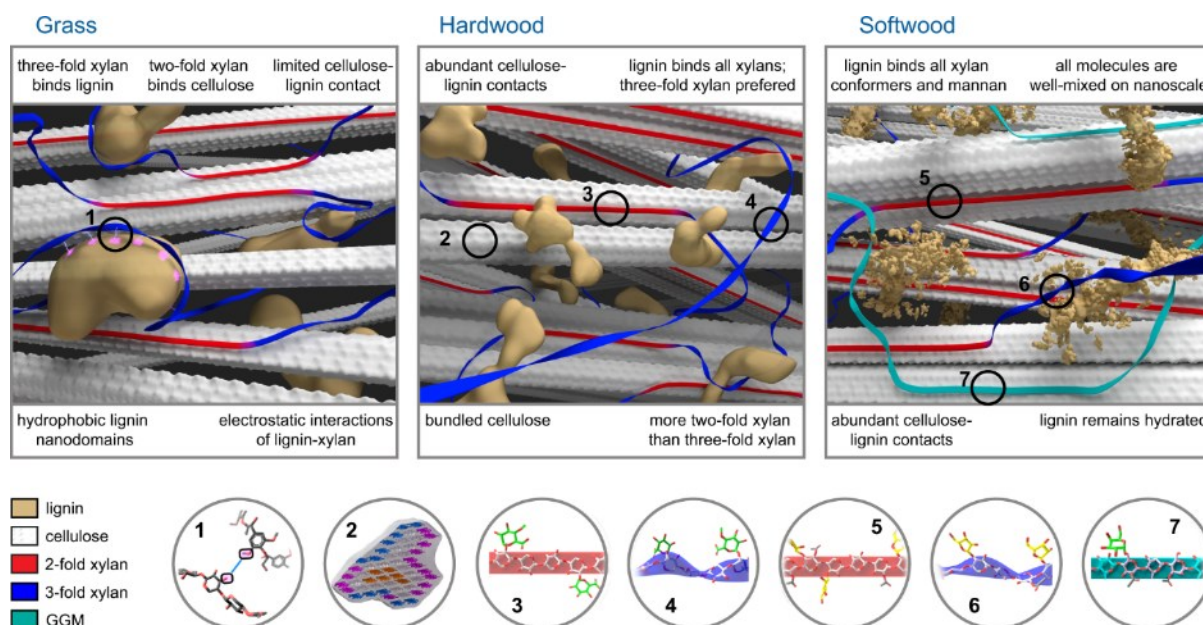


Figure 2-4. The figure shows the spatial arrangement of lignin (yellow), cellulose (white fibrils), two-fold xylan (red flat ribbons), three-fold xylan (blue twisted ribbons), mixed forms of xylan (magenta), and mannan (GGM; green) in secondary plant cell walls. Numbered spheres highlight the structural features of (1) lignin-xylan interaction in all plants, (2) cellulose bundles in woody plants, (3–6) two-fold and three-fold xylan with different sidechains, and (7) galactoglucomannan. The molecular fraction of polysaccharides is considered in the depiction, but the illustration may not be strictly to scale. Lignin and carbohydrates are much better mixed in woody plants than in grass, resulting in the binding of lignin to both three-fold and two-fold xylan as well as to cellulose microfibrils. The structural assembly of woody cell walls is thus different from the domain-separation scheme of grass cell walls. Reproduced from reference ¹⁹³ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

While Figure 2-4 depicts the interactions between different macromolecules in secondary cell walls of lignocellulosic biomasses, it does not present spatial crowding of the molecules. Recently, Sarkar *et al.* carried out diffusion modelling in the intact secondary cell wall of woody biomass.¹⁹¹ In this effort, they created an atomistic model containing four 18-chain cellulose I_β bundles, lignin structure illustration by Ralph *et al.* (Scheme 2-4) and glucuronoxylans containing acetyl groups compressed in a box to reach the experimental density of 1.5 g cm⁻³ in the dry secondary cell wall (Figure 2-5).^{102,191,211} The model depicts the molecular mixing between hemicellulose and lignin, close contact of lignin and cellulose, and dense packing of molecules in woody biomass.

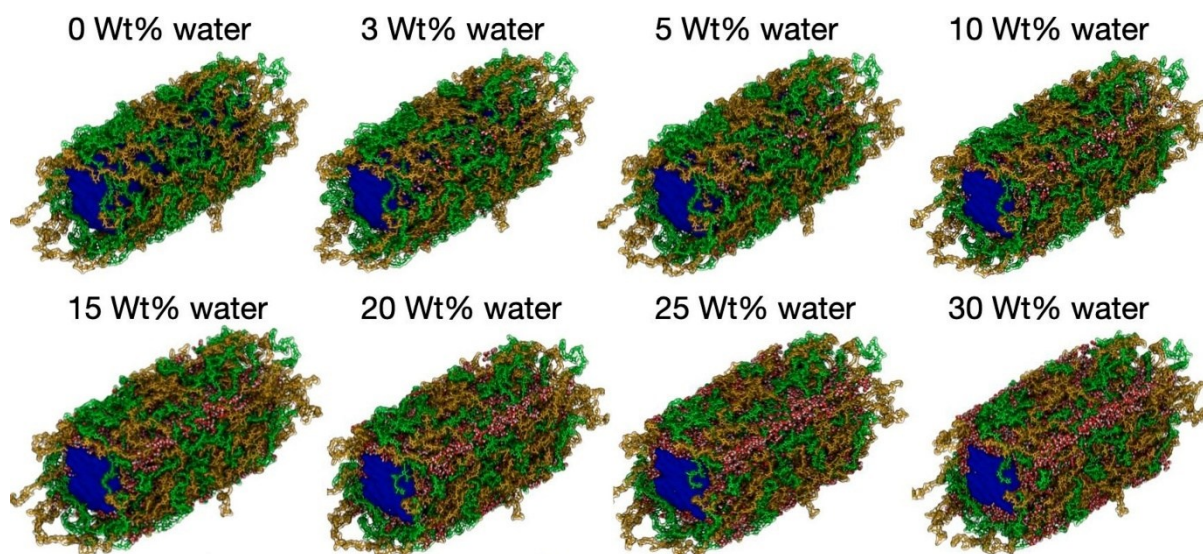


Figure 2-5. Atomistic representation of secondary cell wall in plants at different moisture levels. In this representation, blue is used for the central cellulose fibril, orange for lignin polymers, and green for hemicellulose. Atoms within a water molecule are colour-coded, with red for oxygen and white for hydrogen. The range 0–30% of water weight per cent represents low to high moisture content in wood. A box with dimensions 7.9x7.9x21.1 nm contains 120000-190000 atoms. Reproduced from reference ¹⁹¹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

The lignocellulosic biomass subchapter gave an overview of the chemical nature of the key components present in the lignocellulosic biomass cell wall, along with the key steps in their formation and interaction with each other. It is important to emphasise that the composition of the macromolecules and consecutive interactions with each other is strongly species and environment-dependent and, therefore, can vary significantly depending on the source of the lignocellulosic biomass. On the other hand, the research on the structural features of cellulose over the past century and on hemicellulose and lignin over the past decades, coupled with the research on their interactions over the past years, have laid a solid foundation for having a relatively comprehensive understanding of the molecular and supramolecular architecture of lignocellulosic biomass. Upcoming years will likely bring further refinement in the understanding of the factors governing the architecture of lignocellulosic biomass, especially in terms of thermodynamic factors governing the ultrastructure and, correspondingly, the cellulose, hemicellulose, and lignin structure developing from the evolutionary standpoint of a plant. Based on the chemical and structural framework of lignocellulose in this subchapter, the following sections will concentrate on the processes during reductive catalytic fractionation.

2.2. Processes during lignin detachment from lignocellulosic matrix

The journey of lignin in the reductive catalytic fractionation process starts with its release from the intact lignocellulosic matrix. This is accompanied by the processes on hemicelluloses and, to a lesser extent, on cellulose. The RCF process is commonly carried out under acidic conditions, either as being a result of the deacetylation of hemicellulose during the process itself resulting in an acidic medium (pH 4-5)²¹²⁻²¹⁶, or direct addition of acid to the process.²¹⁷ Accordingly, the depolymerisation of the biopolymers will be here considered in acidic medium. Figure 2-6 summarises the key processes during the lignin detachment from the lignocellulosic matrix along with the activation energies of C-O bond cleavages under acidic conditions. Importantly, the bond dissociation energies of various lignin-lignin C-O and C-C bonds (220-490 kJ mol⁻¹)²¹⁸⁻²²² are significantly higher compared to the apparent activation energy for the acidolysis of β -aryl ether bonds (13-46 kJ mol⁻¹)^{223,224}. Therefore, the solvothermal decomposition of lignin, as well as other lignocellulose components, can be considered negligibly slow under the conditions of the RCF process (140-250°C).

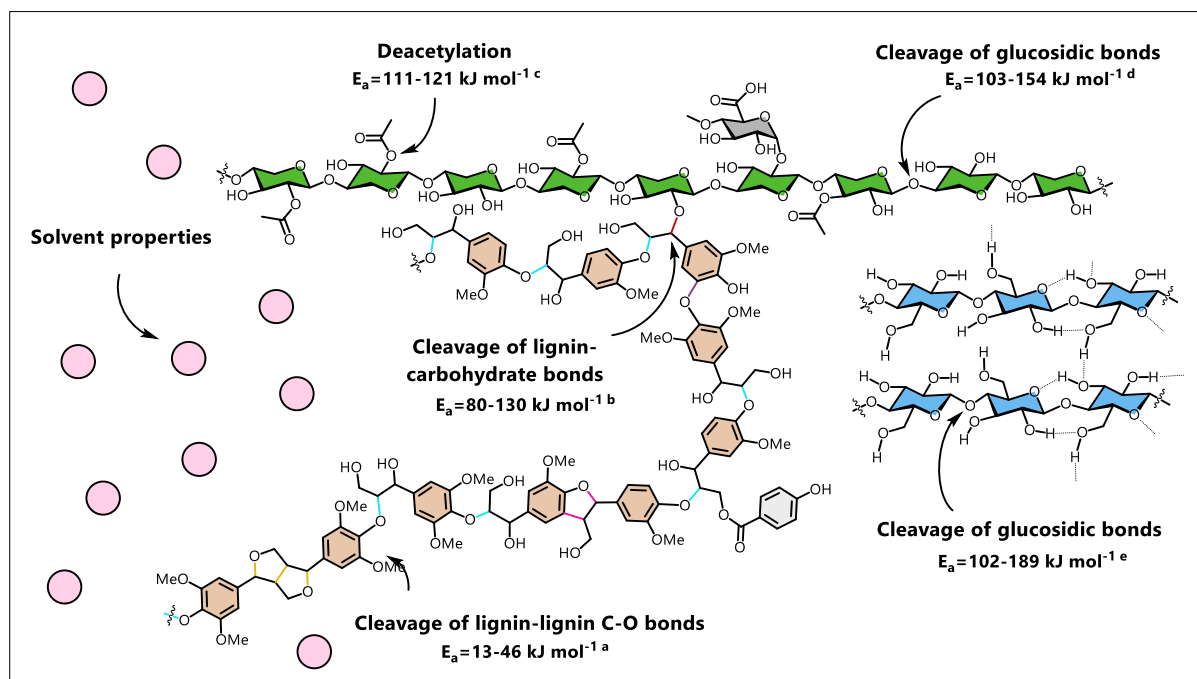


Figure 2-6. Key processes in the lignocellulosic matrix for lignin (and carbohydrates) dissolution during reductive catalytic fractionation. a) cleavage of β -O-4 linkages in lignin. Activation energy obtained from reference ²²³. b) Cleavage of lignin-carbohydrate bonds (benzyl ethers). Activation energy obtained from references ²²⁵⁻²²⁷. c) Deacetylation of hemicellulose responsible for medium acidification. Activation energy obtained from reference ²¹⁴. d) Hydrolysis of hemicelluloses. Activation energies obtained from reference ²¹⁴. e) Hydrolysis of cellulose. It is important to emphasise that only amorphous regions of cellulose can undergo hydrolysis under RCF conditions, as the crystalline

part hydrolysis requires the presence of strong acids ($pK_a < -3$) to proceed at appreciable rates.²²⁸ Activation energies obtained from references ^{224,229–234}.

Due to the poor contact between the heterogeneous catalyst and lignocellulosic matrix, RCF can be considered to share the same processes with Organosolv pulping at this very stage of the two processes.²²⁸ Therefore, the conducted research on solvent parameters is interchangeable between the two processes, and the hydrolytic dissolution mechanism could also be considered very similar. In this section, the solvent properties necessary for lignin dissolution are first discussed. This is followed by the discussion on the hydrolytic cleavage mechanism of the C-O bonds under the RCF conditions- namely deacetylation and cleavage in lignin-lignin, LCC, and glycosidic bonds.

2.2.1. Solvent properties for lignin dissolution

To solubilise a molecule, the intermolecular interactions between the solvent molecules must be in the same order of magnitude as the intermolecular interactions of solute molecules.²³⁵ The first theory of solubility parameters was described by Hildebrand and Scott in the 1950s. It was based on cohesive energy between the molecules in pure compounds, which, for liquids, is equal to the energy of vaporisation.²³⁶ The derived Hildebrand parameter is defined as the square root of cohesive energy density, and the two substances sharing similar Hildebrand parameters should, therefore, have favourable interactions.²³⁶ The methodology was soon improved by Hansen, who divided the total cohesion energy utilised by Hildebrand into three contributing forces- nonpolar interactions (London dispersion forces), polar interactions (between permanent dipoles), and hydrogen bonding.²³⁷ The corresponding energy contributions add up to the total cohesive energy, and correspondingly, Hansen solubility parameters (HSP) are linked with the Hildebrand parameter as

$$\delta^2 = \delta_D^2 + \delta_P^2 + \delta_H^2$$

where δ corresponds to the square root of total cohesive energy density (Hildebrand parameter), δ_D to the square root of the cohesive energy density of dispersion interactions, δ_P to the square root of the cohesive energy density of polar interactions and δ_H to the square root of the cohesive energy density of hydrogen bonding.²³⁷ Therefore, the Hildebrand solubility parameter corresponds to the length of a vector in 3-dimensional space, with the Hansen parameters acting as coordinates.²³⁸ In the case of a solvent mixture (m), the Hansen

parameters are commonly linearly additive according to the volume fractions ϕ_i and the vector coordinates $\delta_{D,m}$, $\delta_{P,m}$, and $\delta_{H,m}$ can be expressed as

$$(\delta_D^m, \delta_P^m, \delta_H^m) \equiv \left(\frac{\sum_i \phi_i \delta_{D,i}}{\phi_i}, \frac{\sum_i \phi_i \delta_{P,i}}{\phi_i}, \frac{\sum_i \phi_i \delta_{H,i}}{\phi_i} \right)$$

Consequently, a mixture of different solvents can result in combined solubility parameters much closer to solute than individual solvents alone. According to Hansen solubility theory, the solubility of a polymer (pol) is determined by a sphere with radius R_0 , with the centre on polymers' Hansen parameters $\delta_{D,pol}$, $\delta_{P,pol}$, and $\delta_{H,pol}$ (Figure 2-7a). The ratio between the length of solvent (solv) vector from the sphere centre and R_0 is termed as relative energy difference (RED), and could be expressed as

$$RED = \frac{R_a}{R_0}$$

where

$$R_a = \sqrt{4(\delta_D^{pol} - \delta_D^{solv})^2 + (\delta_P^{pol} - \delta_P^{solv})^2 + (\delta_H^{pol} - \delta_H^{solv})^2}$$

Therefore, any RED value less than 1 falls inside the sphere in Figure 2-7a, and the solvent could be considered good, while RED values greater than 1 usually result in poor solubility of the solute in the corresponding solvent(s).²³⁹

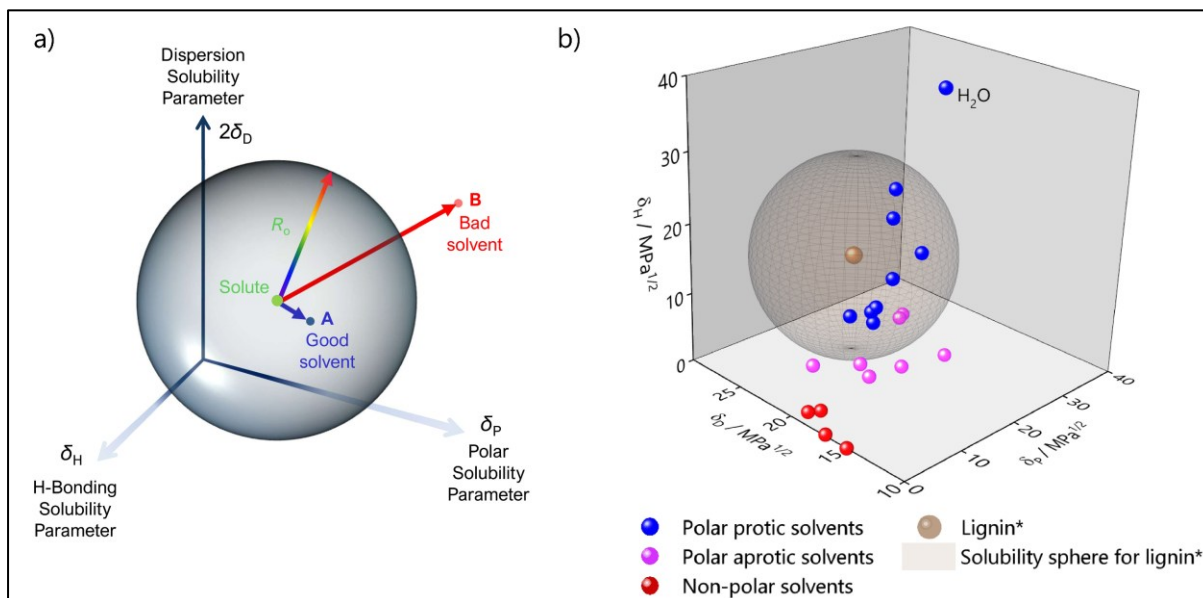


Figure 2-7. a) The 3-dimensional space of Hansen solubility parameters. A sphere centred at the Hansen parameters of a solute and with radius R_0 governs the solubility in the given solvent. If the relative energy difference (RED) of the solvent and solute falls in the sphere ($RED < 1$, ratio of the length of blue vector and R_0), the solvent can be considered good. However, if it falls outside the sphere ($RED > 1$, ratio of the length of red vector and R_0), the solvent can be considered poor for the given solute. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#). b) The 3-dimensional space of Hansen solubility parameters along with lignin and various solvents. *Lignin solubility parameters and R_0 are based on the determination by Hansen and Björkman ($\delta_D=21.9 \text{ MPa}^{1/2}$, $\delta_P=14.1 \text{ MPa}^{1/2}$, $\delta_H=16.9 \text{ MPa}^{1/2}$, $R_0=13.7 \text{ MPa}^{1/2}$).²³⁸ Polar protic solvents depicted are water, methanol, ethanol, 2-propanol, 1-butanol, 2-butanol, ethylene glycol, glycerol, and 1,1,1,3,3,3-hexafluoro-2-propanol. Water is highlighted as having a hydrogen bonding parameter considerably higher than other solvents. Polar aprotic solvents are acetone, ethyl acetate, dimethyl sulfoxide, dimethylformamide, acetonitrile, tetrahydrofuran, and 1,4-dioxane. Non-polar solvents include hexane, cyclohexane, benzene and toluene. The HSP values for solvents are obtained from reference ²³⁵, and the corresponding numerical values can be found in Appendix Table A-1.

While Figure 2-7a depicts the general requirement for solvent selection based on HSP, Figure 2-7b illustrates the HSP of polar protic, polar aprotic, and non-polar solvents along with lignin HSP ($\delta_D=21.9 \text{ MPa}^{1/2}$, $\delta_P=14.1 \text{ MPa}^{1/2}$, $\delta_H=16.9 \text{ MPa}^{1/2}$) in the sphere with $R_0=13.7 \text{ MPa}^{1/2}$.²³⁸ The extracted wood lignin HSP parameters and solubility sphere were first determined by Hansen and Björkman in 1998, when they correlated experimental solubility data of milled-wood lignin (MWL) in 82 different solvents.²³⁸ Utilising a larger set of solvent data and genetic optimisation algorithms, the same parameters were confirmed by Vebber *et al.* in 2014.²⁴⁰ The obtained parameters have since been used to rationalise the solvent selection for lignin extraction and fractionation, usually resulting in relatively good agreement of lignin solubility parameters proposed by Hansen and Björkman.^{238,239,241–248}

However, as discussed in the previous section, lignin structure is strongly dependent on the species and environment in which the plant grows. Therefore, the inherent nature of lignin varying in monomer composition, polymer linking, and molecular weight makes it infeasible to assume a singular set of HSP values across all lignins. Accordingly, Table 2-1 summarises HSP values for different lignins, adapting references from a recent review by Ragauskas and coworkers while differentiating the lignin source and method used.²⁴⁶ While the dispersion parameter δ_D deviates a little between different studies (16.1-21.9 MPa^{1/2}), the polar interactions parameter δ_P (4.9-18.4 MPa^{1/2}) and hydrogen bonding parameter δ_H (10.2-21.8 MPa^{1/2}) experience significant divergence. Therefore, the source and isolation method of lignins strongly determines the corresponding polar and hydrogen bonding parameters, and for an appropriate solvent selection, tuning these parameters of the solvent mixture can be a key to facilitating lignin dissolution.

Table 2-1. Hansen solubility parameters of lignin measured in different studies utilising a variety of different lignins and methods. Adapted from Meng *et al.*²⁴⁶ with permission (Elsevier).

Biomass	Method	δ (MPa ^{1/2})	δ_D (MPa ^{1/2})	δ_P (MPa ^{1/2})	δ_H (MPa ^{1/2})	R_0 (MPa ^{1/2})	Reference
Hard- and softwood	MWL empirical solubility	31.0	21.9	14.1	16.9	13.7	Hansen & Björkman ²³⁸
General	Combined data, computational methods	31.0	21.7	14.2	16.9	13.5	Vebber <i>et al.</i> ²⁴⁰
General	Combined data, computational methods	30.7	21.6	13.9	16.9	13.2	Weng ²⁴⁹
Pine	Combined data, computational methods	25.3	19.4	8.1	14.0		Barana <i>et al.</i> ²⁵⁰
Pine	Kraft lignin, computational method based on functional groups	24.6	16.7	13.7	11.7		Thielemans & Wool ²⁵¹
	Acetylated Kraft lignin, computational method based on functional groups	22.5	16.1	12.0	10.2		
Pine	Kraft lignin, computational method based on functional groups	24.5	20.3	4.9	12.8		Isnard <i>et al.</i> ²⁵²
Eucalyptus	Kraft lignin empirical solubility	28.3	17.8	18.4	12.1	13.5	Ribeiro <i>et al.</i> ²⁵³
Poplar	Lignin, computational methods based on solubility in ionic liquids	24.7	21.8	5.4	10.2		Huang <i>et al.</i> ²⁵⁴
Wheat straw	Soda lignin (Protobind®) empirical solubility	26.2	18.0	9.4	16.6		Adjaoud <i>et al.</i> ²⁵⁵
	Esterified Soda lignin (Protobind®) empirical solubility	24.8	17.0	13.1	12.5		

Beech	Organosolv lignin empirical solubility	23.8	17.6	11.8	10.8	8.0	Badorrek <i>et al.</i> ²⁵⁶
Sugar cane bagasse	Organosolv delignification in different solvents	31.7	21.4	8.6	21.8	13.6	Novo & Curvelo ²⁵⁷

While HSP values are a useful tool to understand polymer solubility by capturing forces pertinent to solvation, the limited number of parameters the model contains is insufficient to capture the full complexity of polymer-solvent interactions.²⁵⁸ In their recent study, Venkatram *et al.* utilised a dataset of 25 polymers to determine the accuracy of the Hansen solubility parameters, which was 67% for solvents and 76% for non-solvents.²⁵⁸ The limitations of HSP are especially important to address when considering lignin solvolysis from the lignocellulosic matrix. In this setting, factors like temperature, local concentration of lignin in the densely packed lignocellulosic matrix, polymer molecular weight, heterogeneity of lignin and intermolecular interactions with other polymers play crucial roles in determining the extent of lignin solvolysis.

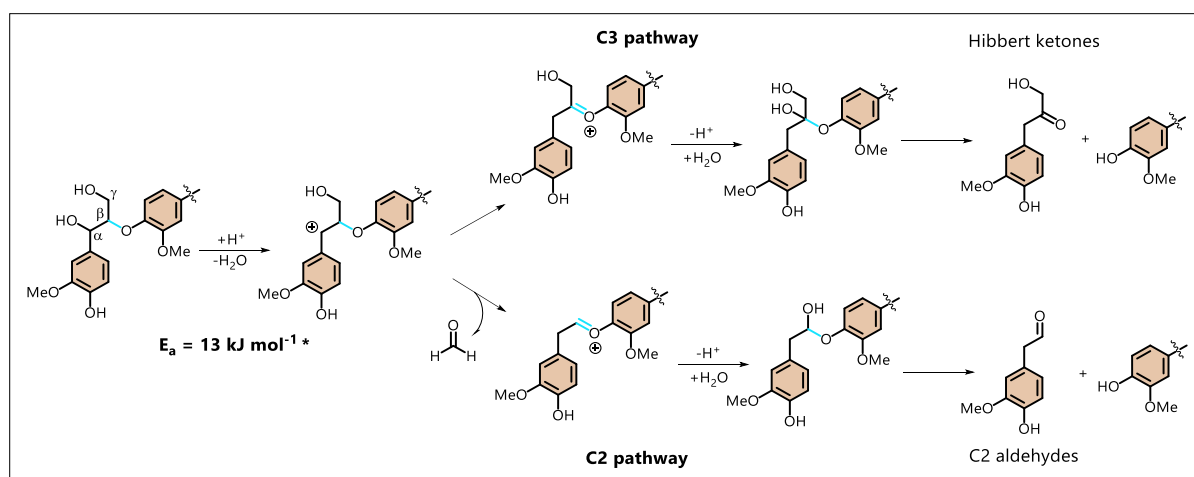
In the pursuit to determine HSP values more suitable for lignin solvation from the lignocellulosic matrix, Novo and Curvelo recently performed Organosolv delignification of sugar cane bagasse at 125°C for 1 h in a variety of organic solvents with the addition of 10 vol% 0.05 M HCl aqueous solution.²⁵⁷ Due to the relatively poor fit between the delignification extent and established solubility parameters by Hansen and Björkman, they calculated a new solubility sphere to match the delignification results with the HSP parameters of $\delta_D=21.4$ MPa^{1/2}, $\delta_P=8.6$ MPa^{1/2}, $\delta_H=21.8$ MPa^{1/2}, and $R_0=13.6$.²⁵⁷ In comparison to Hansen and Björkman HSP values, lower polarity parameter (-5.5 MPa^{1/2} in δ_P) and higher hydrogen bonding parameter ($+4.9$ MPa^{1/2} in δ_H) were determined.²⁵⁷ Accordingly, the solvent or solvent mixture's ability to form hydrogen bonds appears to play a more important role in lignin extraction from the lignocellulosic matrix compared to the solvation of isolated lignin. Hypothetically, the hydrogen bonding ability can play a role when considering the coexistence of lignin with hemicellulose and cellulose, helping to untangle the polymers as well as coextracting the hemicelluloses to make the lignocellulosic matrix more accessible. Further research pinpointing solvent parameters for lignin extraction from different lignocellulosic matrices is much needed to shed further light on this matter.

Hansen solubility parameters can be a useful tool for understanding the underlying principles of lignin dissolution. In general, polar protic solvents with the exception of pure water, can be considered good solvents for lignin. This also holds for polar aprotic solvents, especially when combined with water or another polar protic solvent to tune the mixture's parameters. When selecting solvent(s) for lignin extraction from the lignocellulosic matrix, empirical data considering HSP as a starting point should be obtained to facilitate the lignin extraction from the matrix and not the solvation of isolated lignin alone. In the context of RCF, a variety of solvent parameters other than the solvent's ability to dissolve lignin also become crucial. Parameters like H_2 solubility, ability for H-transfer, catalyst inhibition and lignin conformation in the solution will be discussed in Section 2.4 when catalytic processes in the RCF processes are discussed. Apart from the physical and chemical properties of the solvent, the green solvent rating, the price, and the availability of the solvent should be considered when developing the RCF concept for an industrial setting.

2.2.2. Lignin depolymerisation in lignocellulosic matrix

Lignin is predominantly linked by β -O-4 linkages, which cleavage is highly condition-dependent. In this regard, two mechanistic proposals exist that govern the lignin depolymerisation in the lignocellulosic matrix. Under the more standard RCF conditions employing small alcohols as the solvent, solvolysis of the β -O-4 linkages is considered to be the predominant pathway.^{25,259–262} In this regard, nucleophilic substitution of the solvent alcohol to the C_α is the first step of the process, mediating the dissolution of the lignin moiety as well as inducing the cleavage of the C-O bond in β -O-4 linkages.^{25,259} However, the mechanism of the solvent-mediated cleavage event remains unknown. When carrying out an NMR analysis of the lignin products resulting from evolving lignin solvolysis with methanol, Van den Bosch *et al.* reported the formation and decay of coniferyl/sinapyl alcohol structures in extracted lignin.²⁵⁹ This, on the other hand, would mean that the depolymerisation event in the lignocellulosic matrix must include a reductive step. Solvent-mediated depolymerisation is accompanied by the reductive catalytic processes outside the lignocellulosic matrix, which is discussed in Section 2.4.

In addition to solvolytic cleavage, the β -O-4 linkages in lignin can undergo acidolysis to varying degrees depending on the process conditions. The mechanism can be mediated either by weak acids resulting from the lignocellulosic biomass degradation or the direct addition of acid to the reaction mixture. Unlike the solvolytic cleavage of the β -O-4 linkages, acidolysis of the ether bonds in lignin is relatively well understood, especially in aquatic systems.^{223,263–269} Accordingly, the first step is the elimination of α -OH and formation of benzylic carbocation. The formed reactive carbocation can result in β -O-4 cleavage in two pathways (Scheme 2-8). In the first, C3 pathway, two C-O cleavage events take place, yielding the family of Hibbert ketones. The second, C2 pathway, proceeds via deformylation and sequential rehydration, resulting in the formation of C2 aldehydes.

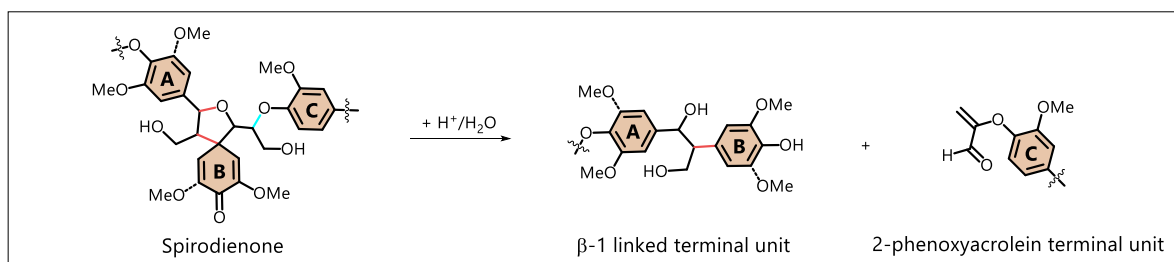


Scheme 2-8. The two pathways for β -O-4 linkage acidolysis. The proposed activation energy for the formation of benzylic carbocation is obtained from reference ²²³.

The extent of the two pathways is strongly dependent on the reaction conditions and the type of acid utilised. Accordingly, the Japanese community have done pioneering work over the years to pinpoint the key factors influencing the outcomes of β -aryl ethers acidolysis.^{264–267} As such, the Bronsted acid choice alone (H_2SO_4 vs HCl and HBr) can determine if the reaction proceeds predominantly via C2 or C3 pathway.^{263,265,267} In the context of Lignin-First biorefining, Barta's research group have utilised diols to trap the reactive carbonyl compounds. Accordingly, they have carried out extensive studies to maximise the C2 pathway by catalyst choice optimisation (triflates) and reaction conditions, facilitating the high yields of C2 acetals from the process.^{268–270} In 2014, Beckham and coworkers carried out an experimental and

theoretical study on the acid-catalysed cleavage of β -O-4 linked model compounds to determine the effect of groups present in native lignin on the acidolysis kinetics. It was found that the reaction barrier for the β -O-4 acidolysis is considerably lower next to phenolic groups, indicating that the acid-mediated depolymerisation is more likely to proceed via end groups of lignin. In addition, the activation energy for the formation of benzylic carbocation was proposed to be as low as 13 kJ mol^{-1} under the studied conditions, making it a highly favourable event among the lignocellulose deconstruction.

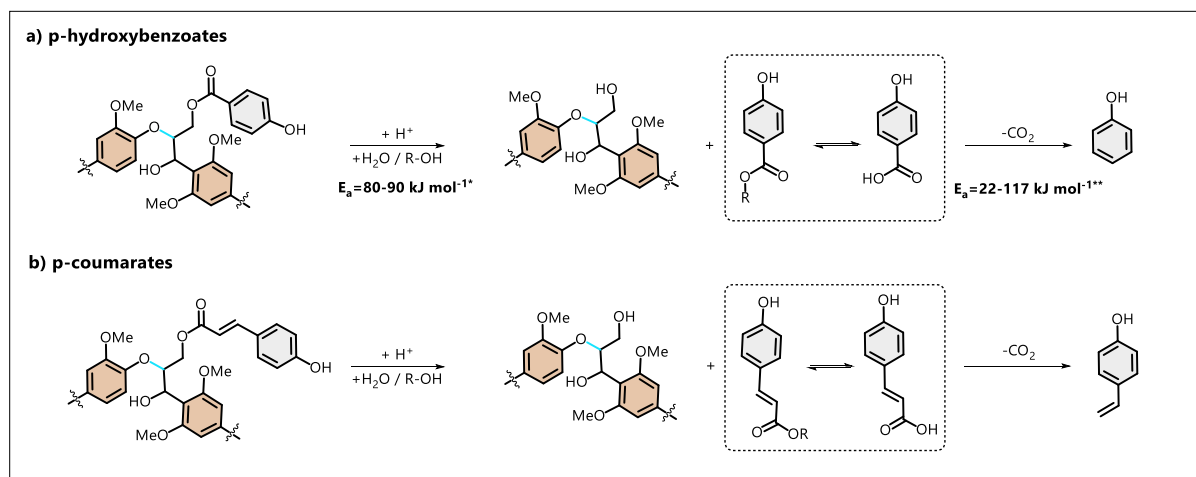
Phenylcoumaran and resinol linkages containing β -5 and β - β carbon-carbon bonds respectively have shown to be reasonably resistant to degradation in Organosolv processes.²⁷¹ Dibenzodioxocin structures are acid-labile and can undergo α -O-4 and β -O-4 cleavage in the lignocellulosic matrix.^{272,273} However, as it cleaves a free phenolic monomer while the 5-5 linkage remains intact, the cleavages do not cause depolymerisation of the lignin backbone. Diaryl ether linkages are not cleaved under the solvolysis pathway in the lignocellulosic matrix and can be considered to remain unmodified at this stage of the process.²⁷⁴ In contrast, spirodienone structures are prone to undergo ring opening under mildly acidic conditions, resulting in chain cleavage and the formation of β -1 linkage and phenoxyacrolein at the terminals of the formed oligomers (Scheme 2-9).^{20,115}



Scheme 2-9. Acid hydrolysis of spirodienone linkages.¹¹⁵

In the same timeframe and space with the cleavage of the lignin backbone in the lignocellulosic matrix resulting in the depolymerisation, the pendant groups in lignin can also undergo hydrolysis and transesterification. To date, the acid-catalysed hydrolysis and transesterification of lignin pendant ester groups in the context of RCF have seen little attention. At the same time, research on “clipping off” the aromatic pendant groups under base-catalysed conditions has emerged in recent years.^{275–278} Accordingly, the fate of the pendant groups in the context

of RCF is likely to gain further attention in the future years as well- both in the context of removal by pretreatments prior to the RCF as well as their fate during the process once untreated biomass is utilised. As an endeavour to acknowledge the γ -esters cleavage under RCF conditions in the lignocellulosic matrix, Scheme 2-10 summarises the potential fate of the most abundant γ -ester pendant groups along with the indicative activation energies for the transformations based on similar processes. Generally, ester hydrolysis under alkaline conditions is a fast process proceeding already at low temperatures ($<100^{\circ}\text{C}$), while hydrolysis in dilute acids often requires elevated temperatures.²⁷⁹ Under the mildly acidic conditions of RCF and the presence of alcohol, the γ -esters can also undergo transesterification. The extent of transesterification is dependent on the solvent composition as well as the properties of alcohol utilised for the process.



Scheme 2-10. a) Hydrolysis/transesterification of p -hydroxybenzoate pendant groups followed by decarboxylation of p -hydroxybenzoic acid under the conditions of reductive catalytic fractionation. b) Hydrolysis/transesterification of p -coumarate pendant groups followed by decarboxylation of p -coumaric acid under the conditions of reductive catalytic fractionation. *Obtained from references.^{280,281} **Obtained from references^{281,282}.

Pendant p -hydroxybenzoates are common in hardwoods and assemble closely to the structure of parabens, a widespread preservative in cosmetic and pharmaceutical products. The hydrolysis of parabens under the conditions of RCF (pH 4-5) has shown to proceed at appreciable rates already at 130°C with the activation energy for the hydrolysis in the range of $80-90 \text{ kJ mol}^{-1}$.^{280,281} Importantly, the formed p -hydroxybenzoic acid is prone to undergo decarboxylation. In a recent study, Castada *et al.* determined the apparent activation energies for the acid decarboxylation in different natural oils between $22-82 \text{ kJ mol}^{-1}$ just by heating

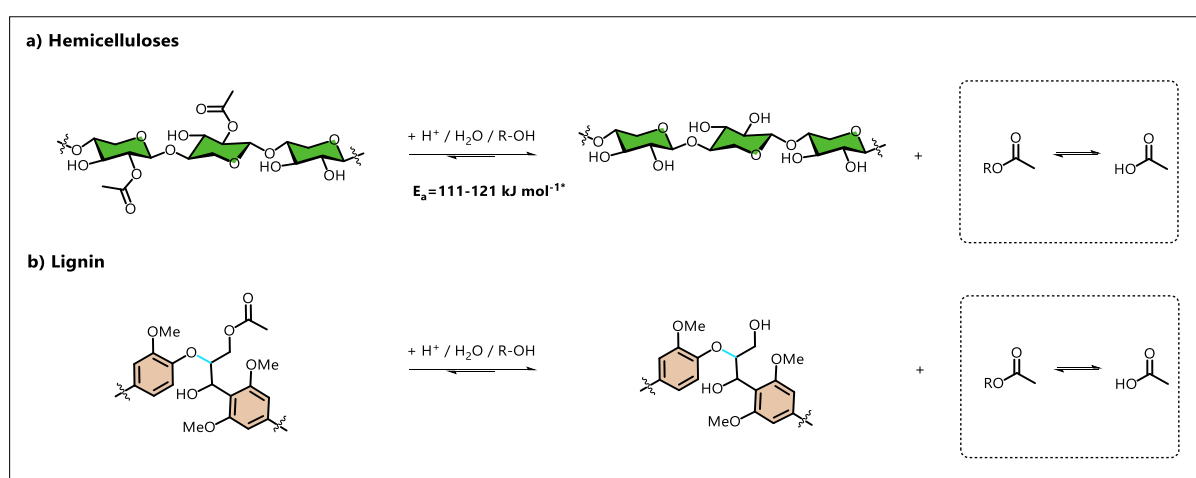
the oils.²⁸² In a separate study focusing on parabens hydrolysis and sequential decarboxylation in an aqueous medium, the activation energies of 117 kJ mol⁻¹ at pH 1.3 and 110 kJ mol⁻¹ at pH 6.6 were determined, while the decarboxylation rate varied a little in the pH range of 1-10.²⁸¹ Accordingly, the hydrolysis as well as decarboxylation of *p*-hydroxybenzoates in lignin is likely to be slower than the hydrolytic cleavage of β -O-4 linkages while still proceeding at appreciable rates similar to hemicellulose hydrolysis and deacetylation.

P-coumarates are another common pendant aromatic group in biomass abundant in many grasses. Recently, Saulnier *et al.* monitored the hydrolysis of *p*-coumarates in corn stover in an acidic aqueous medium at 180°C.²⁸³ In the study, the initial content of *p*-coumarate groups in the biomass dropped to half in 2.4 h at the pH range of 2.9-3.4 following the first-order kinetics. Accordingly, it is reasonable to assert that the hydrolysis of *p*-coumarates proceeds at rates similar to *p*-hydroxybenzoates and should be considered under the RCF conditions. Similarly to *p*-hydroxybenzoic acid, *p*-coumaric acid can undergo decarboxylation, resulting in *p*-vinylphenol. The decarboxylation of hydroxycinnamic acids is well studied in the presence of metal or organic catalysts,²⁸⁴⁻²⁸⁶ however, the rate in an acidic aqueous medium is ambiguous. Under pyrolytic conditions, *p*-coumaric acid decarboxylation displays activation energy of 79 kJ mol⁻¹.²⁸⁷ Recently, Wu *et al.* utilised Organosolv bagasse lignin to determine the fate of residual *p*-coumarates when treated with different catalysts in methanol at 155°C for 2 h.²⁸⁸ No added catalyst resulted in methyl *p*-coumarate yield of less than 0.5% and *p*-vinylphenol yield of 1.6%, while under acidic conditions, the ester yield was 1.3-2.2% and decarboxylation product yield 0.2-1.9%.²⁸⁸ Therefore, the rate of hydrolysis and solvent-dependant transesterification is comparable to sequential decarboxylation, and both processes can proceed at appreciable rates under the RCF conditions.

While the hydrolytic depolymerisation of lignin and hydrolysis of the pendant groups initiates in the lignocellulosic matrix, it is important to emphasise that the same reactions also govern lignin depolymerisation during its diffusion out from the lignocellulosic matrix. Once diffused out of the matrix, the hydrolytic processes continue to proceed simultaneously with the processes on the heterogeneous catalyst. The stability of the formed species described in this section will be discussed in Section 2.3, where condensation reactions are considered.

2.2.3. Deacetylation of lignocellulosic matrix

Hemicelluloses in lignocellulosic biomass are often acetylated, and the acetyl groups can comprise up to 5% of hardwood mass.¹³⁴ Besides the acetylated hemicelluloses, hydroxycinnamyl acetates can be incorporated into the lignin structure and can be found in various types of grass and at low levels from hardwoods.^{53–55} Under the conditions of RCF, acetyl groups in both lignin and hemicelluloses can be considered similar and are prone to undergo hydrolysis and transesterification, consequently acidifying the reaction medium (Scheme 2-11).



Scheme 2-11. a) Deacetylation of hemicelluloses resulting in acetic acid or solvent alcohol ester. The activation energy is obtained from reference ²¹⁴. b) Analogous γ -acetate hydrolysis/transesterification of lignin.

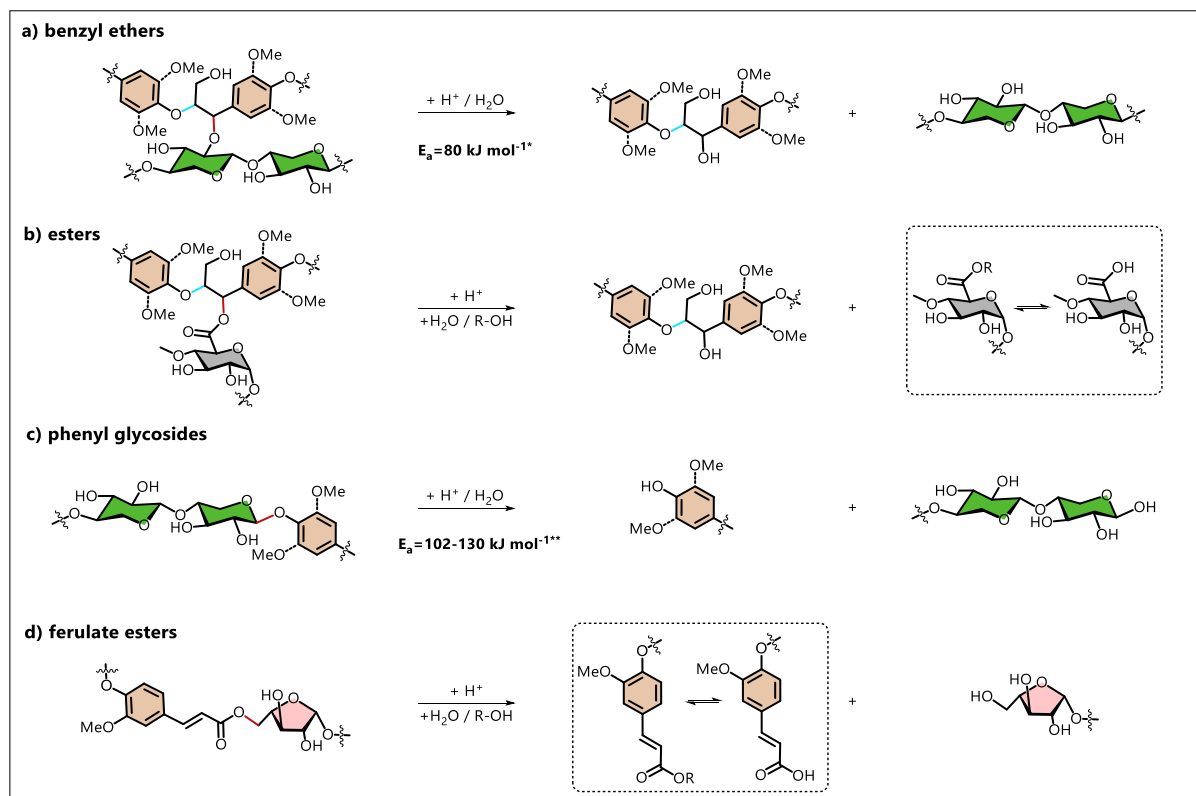
The apparent activation energy for hemicellulose deacetylation has been measured to be 121 kJ mol^{-1} in hardwoods and 111 kJ mol^{-1} in corncobs in an aqueous medium with no added acid or base.²¹⁴ Accordingly, deacetylation in the lignocellulosic matrix proceeds at a perceptible rate under RCF conditions and is comparable to lignin pendant groups hydrolysis. In a recent study, Graça *et al.* monitored the concentration of acidic acid in RCF process with poplar wood utilising a mixture of 2-PrOH/ H_2O (7:3 V:V) with Raney Ni at 200°C for 6 h.²¹⁵ While the concentration of acidic acid increased for the first two hours, it levelled off for the remaining process. The phenomena was attributed to the reached acetic acid/acetate equilibrium as free acetic acid ($pK_a=4.8$) suppresses further hydrolysis of acetate groups.²¹⁵ In another recent study, Arts *et al.* performed RCF experiments with birch wood utilising methanol with Pd/C catalyst and H_2 at 220°C for 2 h.²⁸⁹ As a result of the process, 3.7g of acetyl groups in 100g of

wood resulted in 5.3g methyl acetate, while 0.7g of acetyl groups remained in the resulting pulp. Therefore, more than 80% of the initial acetyl groups in wood resulted in transesterification product with methanol.

The extent of deacetylation, as well as the distribution of the deacetylation products (acid vs ester), appears to be strongly dependent on the RCF conditions and corresponding solvent mixture. Considering the abundance of acetyl groups in lignocellulosic biomass, future years are likely to bring further focus on deacetylation research in the context of RCF, as the potential industrial process would result in significant amounts of deacetylation products.

2.2.4. Cleavage of lignin-carbohydrate bonds

In woody biomass, lignin and hemicelluloses are covalently linked in three main classes of lignin-carbohydrate complexes- benzyl ethers, esters, and phenyl glucosides. In grasses, hemicelluloses and lignin are further linked by ferulate esters. Although kinetic data on lignin-carbohydrate bonds cleavage remain scarce, the following section attempts to summarise the cleavage of common lignin-carbohydrate bonds in the lignocellulosic matrix under the conditions of RCF process (Scheme 2-12).



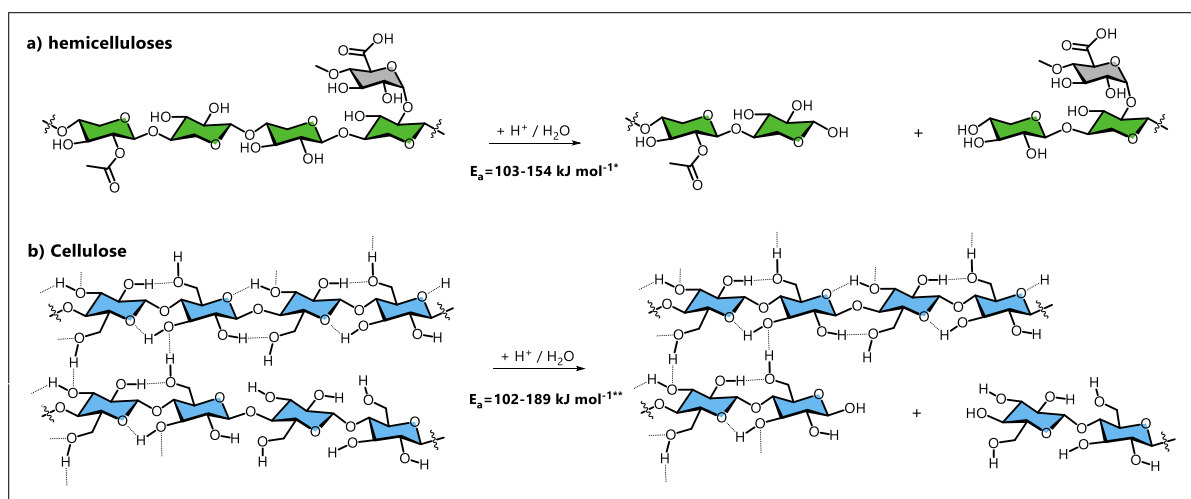
Scheme 2-12. Cleavage of common lignin-carbohydrate bonds in the lignocellulosic matrix in acidic medium. a) Hydrolysis of benzyl ethers. * Activation energy obtained from reference ²²⁷. b) Hydrolysis/transesterification of benzyl and γ -esters. c) Hydrolysis of phenyl glycosides. Activation energy obtained from references ^{226,290}. d) Hydrolysis/transesterification of ferulate esters.

Acid-catalysed hydrolysis of benzyl ethers has indicated activation energy of 80 kJ mol^{-1} in Organosolv process with aqueous methanol in the temperature range of $130-210^\circ\text{C}$.²²⁷ Accordingly, the cleavage of the LCC can be expected to proceed at a substantial rate in the RCF process. The reaction mechanism is likely to form carbocation at C_α , and consequently also result in β -O-4 cleavage or nucleophilic attack to the site (by water/alcohol or any other nucleophile). Type I benzyl ether LCCs involving primary alcohol could also undergo ether cleavage via $\text{S}_{\text{N}}2$ mechanism due to the presence of primary alcohol in the corresponding carbohydrate. The cleavage of ester-linked LCCs (either benzyl esters or mitigated γ -esters), as well as the ferulate esters sparse in grasses, are likely to proceed at rates in the same order of magnitude with the hydrolysis/transesterification of γ -ester pendant groups in lignin. Accordingly, the cleavage is likely to proceed at appreciable rates under the RCF conditions. The phenyl glycoside model compound has shown an activation energy of 102 kJ mol^{-1} in $5\text{mM } p\text{-TsOH}$ solution in methanol.²²⁶ In aqueous 10 mM HCl solution ($\text{pH}=2$), the same model

compound displayed activation energy of 130 kJ mol^{-1} .²⁹⁰ Lawoko *et al.* simulated different pre-treatment effects on phenyl glycoside model compound and reported 96% of the initial phenyl glycoside bonds cleaved at 170°C in aqueous acetic acid solution (13 mg mL^{-1}) after 90 minutes.²⁹¹ Accordingly, extensive cleavage of the phenyl glycosidic bonds is expected under the conditions of an RCF process. The cleavage mechanism is established to proceed via the unimolecular mechanism by the formation of carbocation at anomeric carbon.²⁹²

2.2.5. Cleavage of carbohydrate-carbohydrate glycosidic bonds

Sugars in hemicelluloses and cellulose are linked via glycosidic bonds, which are acid-labile and can undergo cleavage under the conditions of reductive catalytic fractionation. Scheme 2-13 summarises hydrolytic cleavage of glycosidic bonds in lignocellulose carbohydrates, ultimately resulting in the dissolution of the hemicellulose and cellulose fragments.



Scheme 2-13. a) Hydrolysis of hemicelluloses under acidic conditions. *Activation energy obtained from reference ²¹⁴. b) Hydrolysis of amorphous regions of cellulose under acidic conditions. ** Activation energy obtained from references ^{224,229-234}.

When considering hemicellulose hydrolysis in the lignocellulosic matrix, it is essential to outline the framework of what is considered under the hydrolysis term. In the context of reductive catalytic fractionation, the hemicellulose hydrolysis kinetics is considered in the context of dissolution from the lignocellulosic matrix. While the approximate degree of polymerisation (DP) in hardwood's glucuronoxylans is 100-200,^{136,138} the xylans only become water-soluble at an approximate $\text{DP} < 30$, with the oligomers being well soluble in an aqueous medium.²⁹³ Accordingly, hydrolytic cleavage towards the end of the polymer or multiple

hydrolytic cleavages are necessary to detach a hemicellulose fragment from the lignocellulosic matrix. Garrote *et al.* investigated the kinetics of xylan release from a variety of lignocellulosic materials under mild autohydrolysis conditions in the temperature range of 150-220°C, conditions closely reassembling the ones in the lignocellulosic matrix during an RCF process.²¹⁴ Obtained apparent activation energies were in the range of 103-154 kJ mol⁻¹, which is considerably higher compared to the cleavage of β -O-4 linkages in lignin and in the similar range to deacetylation and cleavage of LCC bonds. Therefore, the extraction of hemicelluloses under RCF conditions is expected to proceed at a slower pace than lignin.

Unlike hemicelluloses, cellulose is assembled into crystalline domains in the lignocellulosic matrix and is intrinsically less accessible for hydrolysis.²²⁹ Therefore, the glycosidic bonds in amorphous regions cleave 1000-5000 times faster compared to the crystalline areas.^{231,234} When considering cellulose hydrolysis in the context of an RCF process, the same principles apply as for hemicelluloses. Therefore, hydrolysis in the current context should refer to hydrolytic detachment and dissolution of cellulose fragments from the lignocellulosic matrix. Literature on cellulose hydrolysis kinetics in acidic medium includes a wide spread of apparent activation energies in the range 102-189 kJ mol⁻¹.^{224,229-234} With the consideration above, comprehensive studies considering cellulose release from various lignocellulose sources under the conditions RCF are much needed to pinpoint the parameters of cellulose hydrolytic dissolution. As a generalisation, crystalline domains can be expected to remain intact during the RCF process, while hydrolytic release of cellulose fragments from amorphous regions is expected to proceed at rates in the same order of magnitude as hemicellulose hydrolysis.

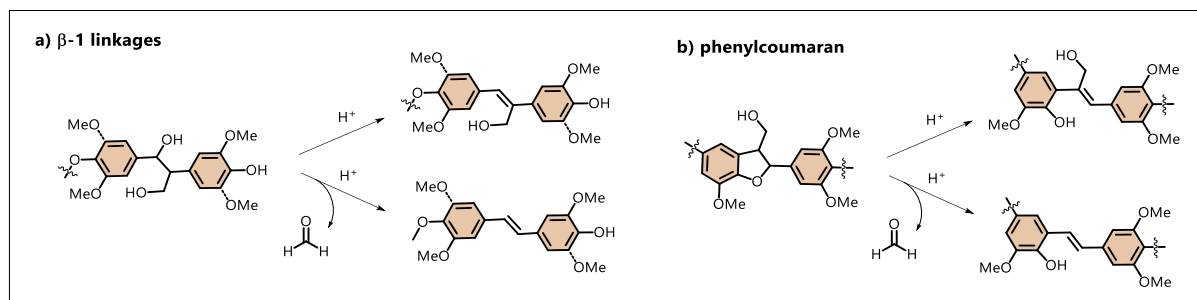
The apparent activation energy for carbohydrate fragments release from hemicellulose is considerably higher compared to the cleavage of β -O-4 moieties in lignin. Owing to the crystalline structure of cellulose fibres, the favourability of lignocellulose's main components in hydrolytic dissolution is expected to be lignin>hemicelluloses>cellulose under the RCF conditions. Accordingly, Chapter 5 demonstrates the favourability of the extraction of the three main components under the RCF conditions and lays down a strategy to exploit the differences in the release rate.

2.3. Processes after solvolysis- transport to bulk medium and condensation reactions

After the dissolution of lignocellulose moieties from the lignocellulosic matrix, the same degradation processes highlighted in the previous Section 2.2 continue to proceed. Besides the summarised hydrolytic dissolution processes, further degradation of the lignin and carbohydrate species can give rise to additional reactive species. This subchapter starts with a look into these processes and consequently summarises the reactive species present in the lignocellulosic matrix along with the possible condensation routes. In the final part of the section, the diffusion of the species out from the lignocellulosic matrix will be discussed.

2.3.1. Further degradation of lignin and carbohydrates to reactive species

Under RCF conditions, the lignin species can further degrade in a manner that does not result in depolymerisation. Therefore, lignin moieties linked by carbon-carbon bonds (phenylcoumaran, resinol, dibenzodioxocin, spirodienone) can undergo transformations resulting in moieties with modified structures. In Section 2.2.2, it was summarised that dibenzodioxocin is acid labile and can form a 5-5 linked structure with free-phenolic ends, while spirodienone is prone to ring opening resulting in β -1 linked terminal monomeric unit (Scheme 2-9).^{20,115,272,273} While the formed 5-5 structure could be considered recalcitrant to further degradation, β -1 linkages can undergo further degradation under acidic conditions. Water elimination from C_α results in a conjugated structure, while the same process coupled with the elimination of formaldehyde results in the formation of a stilbene-type structure (Scheme 2-14a). Utilising β -1 linked model compound containing γ -OH group, Minami *et al.* demonstrated almost complete conversion to stilbene (and formaldehyde detachment) after 20 minutes in supercritical methanol at 270°C.²⁹⁴ Although the conditions in an RCF process are milder and proceed in a liquid medium, the study demonstrates the favourability of formaldehyde detachment and stilbene formation from β -1 linked moieties.

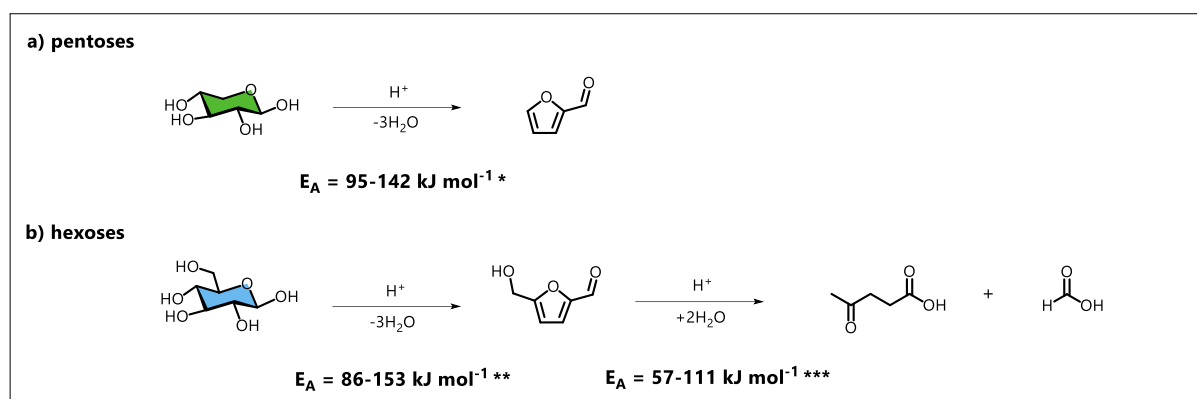


Scheme 2-14. a) Further degradation of β -1 linkages derived from spirodienone moieties to form stilbene-type structures. b) Degradation of phenylcoumaran moieties resulting in stilbene-type structures.

Similar elimination to β -1 linked moieties can also proceed in phenylcoumaran linkages, but the two units remain linked via β -5 linkage instead (scheme 2-14b). Li and Lundquist studied phenylcoumaran model compounds in an acidified mixture of dioxane and water, and observed stilbenes yield up to 38% under the reflux (100°C) in 10 h.²⁹⁵ In the same study, the elimination of only γ -OH was also observed, yielding a conjugated system between the two aromatic units. Stilbenes formation during the hydrothermal processes has been also associated with homolytic cleavage of β -O-4 linkages, which is followed by radical coupling and loss of γ -methylol group as formaldehyde.^{271,296} Although the origin of the stilbene structures can not be attributed to one singular process, they have been reported in various Organosolv lignins obtained under acidic conditions.^{271,297,298} Evidently, elimination reactions resulting in the formation of unsaturated moieties conjugated with aromatic rings and separation of water or formaldehyde can proceed in the lignocellulosic matrix under the RCF conditions. This further broadens the range of reactive species in the medium, and the formed species should accordingly be considered as participants in condensation reactions potentially resulting in the formation of new carbon-carbon bonds.

Reactive species prone to condensation can also be formed from carbohydrates. Once the dissolved oligomeric carbohydrate species have been hydrolysed to monomeric sugars, they can undergo further degradation by dehydration (Scheme 2-15). Accordingly, monomeric pentoses can form furfural under acidic conditions, which is prone to undergo condensation reactions. The apparent activation energy for xylose dehydration has been reported in the range of 95-141 kJ mol⁻¹,²⁹⁹⁻³⁰³ with the reaction in 0.5 M acetic acid media proceeding with an apparent activation energy of 109 kJ mol⁻¹.³⁰⁰ Therefore, the formation of furfural can

proceed at appreciable rates under the RCF conditions once the monomeric pentoses have been formed. Similarly to pentoses, hexoses released from hemicellulose and cellulose hydrolysis can undergo dehydration. The reported activation energy of glucose dehydration to 5-(hydroxymethyl)furfural (HMF) is in the range of 86-153 kJ mol⁻¹.^{304–307} Formed HMF can undergo rehydration, resulting in levulinic and formic acids with an apparent activation energy of 57-111 kJ mol⁻¹.^{305–309}

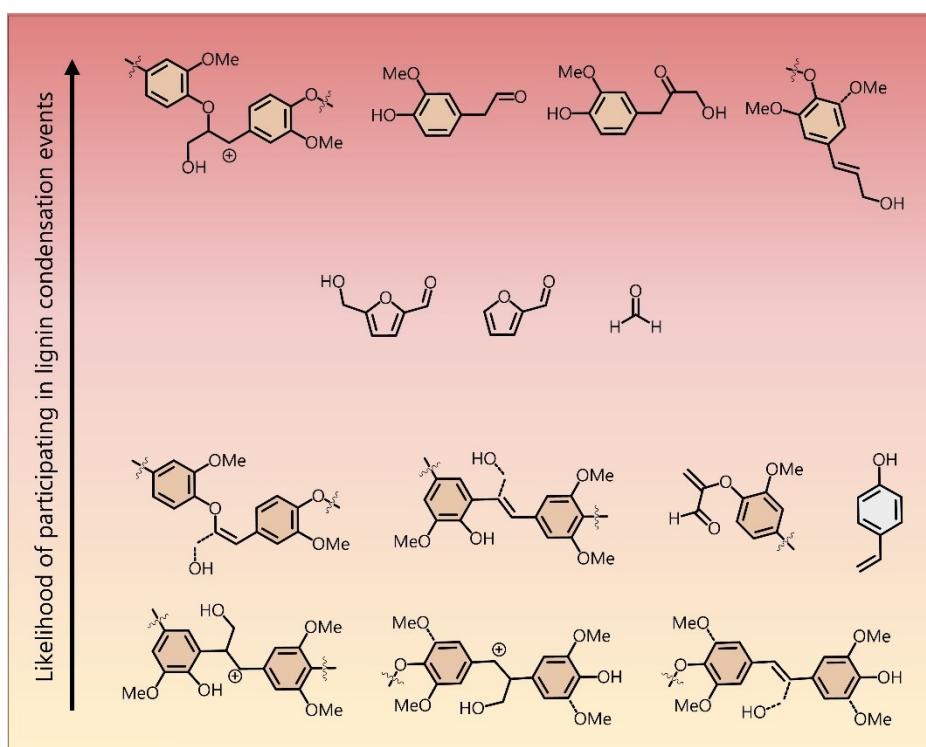


Scheme 2-15. a) Dehydration of pentoses (D-xylose on the scheme) to furfural. *Activation energy for xylose dehydration is obtained from references ^{299–303}. b) Dehydration of hexoses (D-glucose on the scheme) to 5-(hydroxymethyl)furfural and further dehydration to levulinic and formic acids. ** Activation energy obtained from references ^{304–307}. *** Activation energy obtained from references ^{305–309}.

2.3.2. Condensation of reactive species

To this point, the degradation of lignocellulose components has been discussed while dismissing the potential reactions between the derived molecules. Before diffusing out from the lignocellulosic matrix and reaching the heterogeneous catalyst, the lignin- and carbohydrate-derived reactive species can undergo condensation reactions between each other as well as with polymers still retained in the lignocellulosic matrix. In fact, the RCF and, more broadly, lignin-first strategy is based on the active stabilisation approach to avoid these condensation reactions that lead to recalcitrant lignin polymers.²⁵ While the term condensation itself usually refers to a process combining two molecules into one larger one with the elimination of a small molecule, a condensation reaction in the context of lignin extraction can be considered as the formation of new carbon-carbon bonds recalcitrant to catalytic cleavage under RCF conditions. Accordingly, condensation reactions resulting in new C-O bonds are not considered here as, although prevalent during the process, they can be cleaved either by hydrolytic or catalytic processes in the temperature ranges utilised in RCF.

In an attempt to categorise the reactive species resulting from lignin and carbohydrate degradation and potentially participating in C-C linkage forming condensation reactions, Scheme 2-16 displays the key reactive species forming in lignocellulosic matrix grouped based on their expected likelihood to participate in condensation reactions. In this regard, moieties arising from β -O-4 depolymerisation (benzylic carbocations, C2 aldehydes, Hibbert ketones and unsaturated moieties from solvolysis) are both abundant and reactive and are likely to extensively participate in condensation reactions. Additionally, carbohydrate dehydration products like furfural and HMF can be present in considerable amounts and, therefore, could also participate in condensation events to a considerable degree. In addition to the aforementioned more abundant reactive species, the lignocellulosic matrix can also contain a variety of reactive species that are less prevalent (e.g. enol ethers, vinyl compounds, stilbenes, acroleins) but can also be part of the lignin condensation.



Scheme 2-16. A selection of reactive species prone to undergo condensation reactions in the lignocellulosic matrix under the conditions of reductive catalytic fractionation. The species depicted on top are more abundant and, therefore, likely to participate in condensation events, while the species at the bottom are present in a much lesser degree.

Fragments depicted in Scheme 2-16 can couple in a large variety of different combinations, and accordingly, the condensed lignin lacks structural homogeneity and can also be

considered branched and cross-linked. Due to the complex structure of the condensed lignins, it remains a challenge to pinpoint the exact structural modifications taking place during the condensation. In a recent review by Gong and Shuai, lignin condensation was categorised into four main classes of reactions- benzylic carbocation-induced diarylmethane (DAM) condensation, aldehyde-induced DAM condensation, aldehyde-induced aldol condensation and radical-induced coupling reactions.³¹⁰ Following this rationale, but not limiting the carbocation-induced reactions only to DAM formation, the four reaction classes are discussed in the context of conditions in lignocellulosic during an RCF process.

Benzylic carbocation is a strong electrophilic centre prone to a nucleophilic attack and can accordingly form new carbon-carbon bonds when reacting with an electron-rich carbon moiety. Good nucleophiles in the medium include lignin's phenolic moieties, which can undergo electrophilic aromatic substitution to form α -6 linked condensed lignin moieties reassembling DAM structure.³¹¹ Similarly, carbohydrate-derived furfural and HMF can undergo electrophilic aromatic substitution with the benzylic carbocation, however, the electron-withdrawing aldehyde group reduces their reactivity to S_EAr .³¹² New carbon-carbon bonds from benzylic carbocation can also be formed during the nucleophilic attack by carbonyl compounds (Hibbert's ketones and C2 aldehydes) enol equilibrium products to the site. A similar nucleophilic attack could also proceed from any other non-aromatic C-C double bonds (eg. *p*-vinylphenol, stilbenes), resulting in a new condensed lignin structure.

Aldehyde-induced DAM condensation assembles the mechanism of acid-catalysed formation of phenol-formaldehyde resins and can accordingly link two lignin aromatic moieties via methylene bridge.^{313,314} Formaldehyde is the least sterically hindered aldehyde in the reaction mixture and could, therefore, be considered prone to undergo DAM condensation. Similar condensation reactions could also be induced by other aldehydes in the mixture (furfural, HMF, C2 aldehydes from β -O-4 cleavage), however, the reactivity can be considered lower due to the steric hindrance.³¹⁰

Aldol condensation can be induced between any two carbonyl compounds in the reaction mixture, resulting in a carbon-carbon linked conjugated enone product. In this fashion, Deuss *et al.* demonstrated with ¹³C-labelled β -O-4 model compounds that the aldol-condensation

product readily forms under acidic conditions at 140°C between the C2 aldehydes produced during the β -O-4 linkage acidolysis.³² Aldol condensation is also considered the main reaction type responsible for the formation of humins- a carbonaceous material forming in an acidic medium as a result of condensation reactions between furfural, HMF, and monomeric carbohydrates degradation products.^{315,316}

Radical-induced coupling reactions can be another pathway leading to condensation reactions in the medium. Although the extent of homolytic cleavage of covalent bonds could be considered negligible compared to heterolysis under the conditions of an RCF process (Chapter 2.2), the radicals can also be formed by the molecular oxygen present in the reactor.³¹⁰ Accordingly, the coupling between two radicals can result in a new C-C bond and condensed lignin moiety. The amount of oxygen in the reactor could be drastically decreased by purging the reactor with inert gas (e.g. N₂) prior to the process, therefore preventing oxygen-induced radical formation.

Different strategies have been developed over the years to hinder the release of reactive species and condensation reactions. Diol-assisted and aldehyde-assisted fractionations, the two other current lignin-first strategies apart from the RCF, rely on the stabilisation of reactive intermediate species already in the lignocellulosic matrix.²⁵ During diol-assisted fractionation, the formed carbonyl species, particularly C2 aldehydes, are bound into acetal structures by the diol, protecting them from condensation and resulting in acetal monophenolics and modified oligomers.^{32,317-319} Aldehyde-assisted fractionation utilises aldehyde to create an acetal bridge with C α -OH and C γ -OH, and accordingly prevents the formation of benzylic carbocation during the lignin extraction. After the extraction of stabilised lignin, monomers can be produced by hydrogenolysis with a heterogeneous catalyst.^{34,320-322} Besides the other two current lignin-first strategies, a variety of physical protection strategies have been developed to minimise the exposure time of reactive species in the medium during biorefining and are summarised in a recent review by Luo *et al.*³¹¹

It is important to emphasise that lignin condensation is not necessarily limited only to the four categories of reactions summarised above and can potentially involve more complex condensation pathways. To date, condensation reactions and corresponding products during

biorefining remain at least partially poorly understood. Similarly, the nature of residual lignin in pulp remains unclear as it is being influenced by the condensation reactions. Regardless of the exact nature of condensation reactions, minimising the extent of potential condensation pathways remains of paramount importance for lignin valorisation. In the context of RCF, the stabilisation relies on heterogeneous catalysis, and in order to minimise the extent of condensation, the time reactive species spend in the lignocellulosic matrix must be minimised. In the next subsection, a closer look is taken at the mass transport in the lignocellulosic matrix, an important factor determining the space-time of reactive species in the matrix.

2.3.3. Diffusion of dissolved species in lignocellulosic matrix

Based on the intrinsic structure of lignocellulose particles, the diffusion of dissolved species can be considered in two distinct domains. Firstly, diffusion through the cell wall itself, and secondly, diffusion in void cell lumen and vessel elements. Figure 2-8 depicts the two necessary mediums the substrates released from the lignocellulosic matrix must diffuse through before reaching the bulk solution outside the particle. A Cell wall itself is a densely packed medium (Figure 2-5), and accordingly, the diffusion rate in the cell wall can be considered much slower compared to the diffusion rate in the cell lumen and vessel elements.

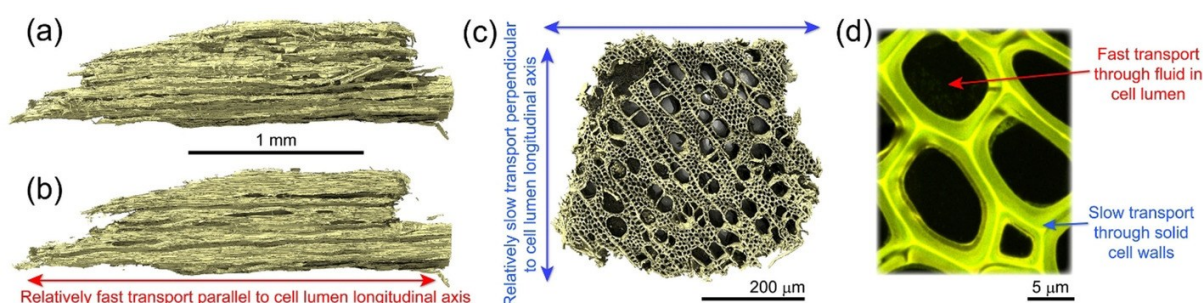


Figure 2-8. Visualisation of the structure of a milled poplar wood particle and its modes of internal mass transport. *a) Full X-ray computed tomography (XCT) reconstruction of a poplar particle. b) Longitudinal XCT cross-section, which illustrates high-aspect ratio cell lumen (pores) aligned with the major axis of the particle. c) Transverse XCT cross-section, which illustrates the distribution of vessel elements (large pores) and fibre cells (small pores). d) Optical micrograph of fibre cells in a transverse cross-section. Reproduced from reference ³²³ with permission (John Wiley and Sons).*

Depending on the biomass, the cell wall thickness usually ranges from 1-3 µm, which is the distance from the middle lamella to the lumen.^{323,324} Relevantly, the lignin is not uniformly distributed in the cell wall, and while the lignin content in wood's secondary cell walls can range between 20-30 wt%, the middle lamella contains 40-50 wt% of lignin and the cell corners

60-80 wt% of lignin.³⁶ Accordingly, the lignin species released from the high lignin concentration areas (middle lamella and cell corners) experience the longest diffusion path out from the cell wall, and the direction of diffusive flux is always towards the lumen. The void cell lumen usually has a diameter in the range of 10 μm and the vessel cell lumen in the range of 50 μm .^{323,324}

When considering small molecule diffusion in lignocellulosic particles, the axial diffusion along the lumens is usually estimated to be two orders of magnitude faster compared to the radial diffusion through cell walls.³²⁵ However, when considering oligomeric and polymeric moieties released from the lignocellulosic matrix, their dimensions are in the same order of magnitude as the mesoporous medium between cellulose fibres (3-10 nm).³²⁶ Accordingly, Knudsen's contribution should be accounted for when considering lignin (and carbohydrate) species diffusion in cell walls.

Transport phenomena in industrial pulping processes are well-studied and rely on complex transport models concentrating mainly on solvent and reactants penetration to the wood chips.^{323,325} However, when considering diffusion in the context of an RCF process, it is paramount to acknowledge that fast lignin transport out from the lignocellulosic matrix carries high importance due to the necessity to suppress reactive species and retain lignin upgradability. In the pursuit of establishing a reaction-diffusion model for wood particles under conditions similar to an RCF process, Thornburg *et al.* recently carried out pioneering work combining empirical data with computer simulations.³²³ In their study, poplar particles were sieved into different fractions followed by an unstirred Organosolv process in methanol with rapid heating to 220-250°C. Based on the physical characteristics of the particles and the kinetics of Organosolv extraction of lignin, hemicellulose and cellulose, they created a reaction-diffusion model to evaluate mass transport limitations inside the particles. The reaction model was based on pseudo-first-order reversible reactions, and the different particle sizes were correlated against the Thiele modulus (ϕ). Thiele modulus is calculated as the square root of reaction and diffusion rates ratio, and accordingly, low Thiele modulus (<0.4) corresponds to a kinetically limited regime while the system with high Thiele modulus (>4) is entirely diffusion limited.³²⁷ Based on the developed model, a more detailed overview of the diffusion in the two distinct domains, cell wall and lumen, is given.

Based on the empirical data fitted into the model, the reaction between methanol and lignin for the dissolution process in the cell wall is entirely limited by the reaction rate as the methanol diffusion is considered fast compared to the lignin release ($\varphi \sim 0.01$). Once the fragment is released, the diffusion rate of the lignin is comparable to its redeposition rate. In this manner, Thiele moduli of $\varphi = 0.61$ for tetrameric lignin compounds and $\varphi = 1.05$ for lignin decamers were calculated at 220°C under the given conditions. Importantly, elevating temperature is proposed to increase the Thiele modulus as the effect of faster lignin release and consequent redeposition surpasses the increased diffusion rate (corresponding Thiele moduli were $\varphi = 0.79$ and $\varphi = 1.43$). The authors consider lignin species up to 10 lignin monomeric units, however, the released lignin species can also exhibit considerably higher MW. With the increasing lignin dimensions, the mean free path for lignin molecules will likely decrease fast in the narrow pores of the cell wall, thereby increasing the Thiele modulus significantly. Importantly, the diffusion rate is also affected by the lignin configuration in the selected solvent, and over the course of delignification, the pore size in the cell wall increases, thereby lowering the Thiele modulus.^{326,328}

While the cell wall thickness is intrinsically in the range of a few microns and can not be easily reduced, the particle size is a parameter subject to manipulation. Accordingly, Thornburg et al. modelled the diffusion in wood lumens of different-sized particles. Figure 2-9 depicts lignin redeposition's Thiele modulus in a particle based on the particle characteristic lengths (L).

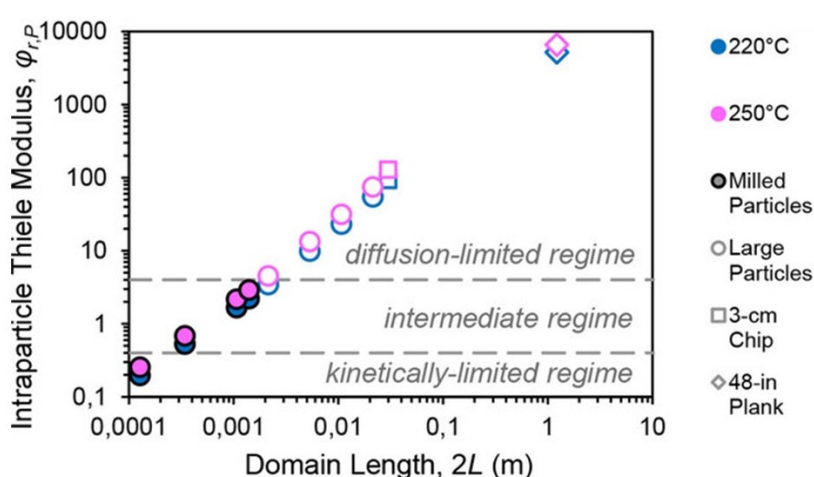


Figure 2-9. Relationship between intraparticle Thiele moduli for lignin redeposition ($\varphi_{r,p}$) and average particle domain length (2L) evaluated at 220°C and 250°C. Reproduced and cropped from reference ³²³ with permission (John Wiley and Sons).

While lignin redeposition dominates in particles with $2L > 2\text{mm}$, the diffusion and redeposition are expected to proceed at comparable rates for particles with $200\mu\text{m} < 2L < 2\text{mm}$. Particles with domain length $2L < 200\mu\text{m}$ are expected to proceed in a kinetically-limited regime, where lignin diffusion out from the particle greatly exceeds the extent of redeposition. As for the diffusion in cell walls, lowering temperature is expected to hinder the redeposition rate more than the diffusion rate. Accordingly, a process carried out at a lower temperature is likely to experience a lower Thiele modulus, and therefore, larger particles can also operate in kinetically-limited regime when the process temperature is lowered. In a separate study, Anderson *et al.* demonstrated that the RCF process with particle sizes ranging from 0.075-1mm was mass transfer limited when operated in a stirred batch mode, while the same process in the flow-through reactor was not.²⁶¹ As a result, the bulk solution lignin concentration appears to strongly influence the intraparticle effective diffusion rates.

In the Thornburg *et al.* investigation, hemicellulose and cellulose fragments extraction during the non-stirred Organosolv process levelled off right after the beginning of the process. Therefore, more than 90 wt% of the carbohydrates were retained in the pulp. However, in an Organosolv or RCF processes involving H_2O as a (co)solvent, lower xylan retention is usually observed, likely owing to the water's better ability to hydrolyse carbohydrates compared to methanol.³²⁹ Accordingly, future models in different solvents should also consider carbohydrate diffusion, as the extraction process is not entirely dominated by lignin in these mediums. Another consideration for future models includes the charged particles. It is known that in cell walls, positively charged cations diffuse more slowly compared to anions due to the strong interactions between hemicellulose's carboxylate groups retarding cations free diffusion.^{35,191} Accordingly, the diffusion rate of dissolved lignocellulose components is likely not only to depend on molecular weight and structure but also on the charge.

With the reaction-diffusion modelling in a framework applicable to an RCF process, Thornburg *et al.* demonstrated that intraparticle lignin extraction is diffusion-limited once the particle size exceeds a few millimetres, and particle sizes lower than a few hundred microns allow diffusion rates considerably higher compared to redeposition. Accordingly, researchers are recommended to utilise particles smaller than $200\mu\text{m}$ when evaluating heterogeneous catalysts in an RCF process to avoid feedstock-specific mass transfer limitations. In cell walls,

the released molecules' diffusion is predicted to compete at the same time and length scales with the redeposition, and the very nature of the released species strongly determines the diffusion rate out from the cell wall. Once the molecule has escaped the lignocellulosic matrix, the mass transfer in bulk solution becomes facilitated by advection. In the next section, the eyes are turned outside the lignocellulosic matrix, and a closer look is taken at the heart of an RCF process- the heterogeneously catalysed reductions.

2.4. Catalytic processes on heterogeneous catalysts

A variety of considerations must be taken into account to encapsulate the key parameters for an effective catalytic process. Accordingly, a closer look is first taken at the solvent effects on the reductive process. This is followed by a description of the key catalytic processes that take place on the catalyst surface. The subchapter finishes with a summary of key results and conditions from various RCF processes performed to date.

2.4.1. Solvent effects

While Chapter 2.2.1 concentrated on the solvent properties necessary to facilitate lignin dissolution, a variety of other solvent properties can affect the heterogeneously catalysed reductive process, hence influencing the outcomes of an RCF process. Accordingly, factors like H₂ solubility, lignin structural conformation, the solvent's ability for H-transfer, and the potential inhibition of the catalyst should be considered.

2.4.1.1. H₂ solubility in different solvents

H₂ solubility in solvent becomes important if the RCF process is carried out in the presence of hydrogen gas. A useful metric describing gas solubility in solvents is Henry's parameter, which can be expressed in numerous ways.³²⁷ In a simplified form, hydrogen molar fraction in a solvent, x_{H_2} , can be expressed as

$$x_{H_2} = \frac{y_{H_2} P_{total}}{H_{H_2}}$$

where the numerator corresponds to H₂ partial pressure in the gas phase and the denominator H_{H_2} to hydrogen Henry's volatility in the given solvent. For practical purposes and considering wide temperature ranges, Henry's volatility can be calculated by Harvey's equation, which

utilises solvent properties along with empirical constants obtained for each solvent.^{330,331}

Harvey's equation is expressed as

$$\ln H_{H_2} = \ln P_S^g + \frac{1}{T_r} \left[a + b(1 - T_r)^{0.355} + cT_r \left(\frac{1}{T_r} - 1 \right)^{1.5} \right]$$

where P_S is the solvent's saturation pressure at a given temperature, $T_r = T/T_c$ with T_c marking the critical temperature of the given solvent, and a , b , and c denote empirical constants. Utilising Harvey's equation, Trinh *et al.* recently investigated hydrogen solubility at temperature ranges that are compatible with RCF processes.³³² In this pursuit, they utilised experimental data along with molecular simulations, and the parameters for Harvey's equation were obtained in various solvents suitable for biomass catalytic valorisation. Based on the obtained parameters from Trinh *et al.* study,³³² Figure 2-10 depicts Henry's volatility parameter as a function of temperature up to 240°C, or up to a limit the correlation holds valid for the solvent.

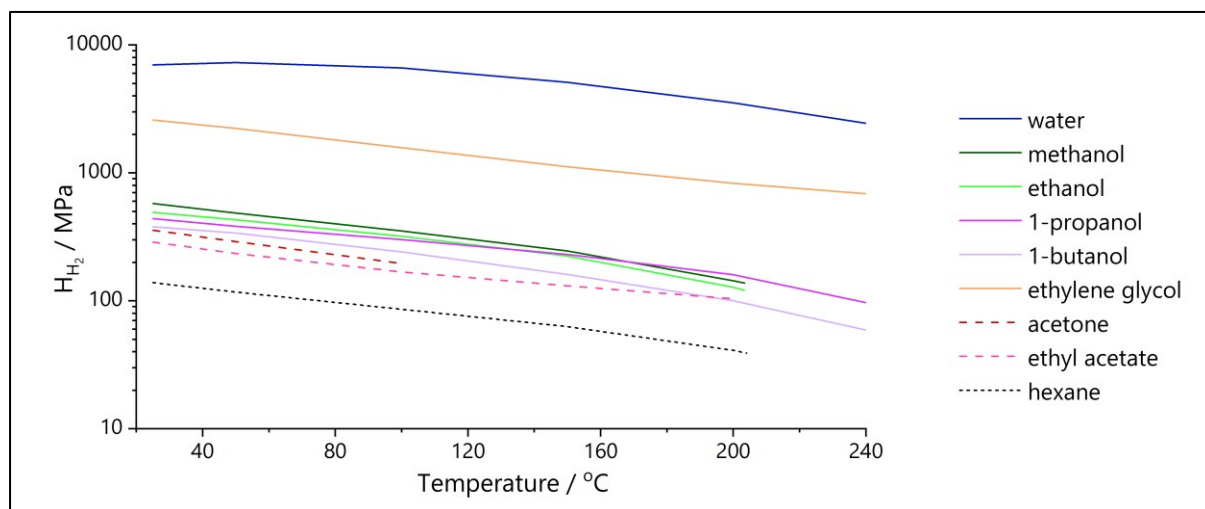


Figure 2-10. Henry's volatility parameter of H_2 in different solvents. Henry's parameter is based on Harvey's equation and corresponding coefficients determined by Trinh *et al.*³³² The H_{H_2} is plotted in the temperature range where the Harvey equation coefficients hold with up to an absolute average standard deviation of 5%.

As the denominator in determining the H_2 molar fraction in the solvent, a lower H_{H_2} value favours hydrogen solubility in the liquid phase. Accordingly, hydrogen is less soluble in solvents with high polarity (water, ethylene glycol) and more soluble in nonpolar solvents (hexane).³³³ In the context of an RCF process, utilising alcohol can significantly increase the hydrogen solubility compared to pure water, as the hydrogen solubility in methanol is more

than an order of magnitude higher than in pure water at 200°C. However, as we witnessed in Chapter 2.2.1, adding water to the solvent mixture can help to tune the solvent mixture parameters to facilitate lignin dissolution itself. Therefore, hydrogen solubility should be viewed as an additional parameter for picking a solvent for an RCF process and not a factor solely determining the solvent selection. It is also important to emphasise that solutes added to the reactor or released from the lignocellulosic matrix can affect the hydrogen solubility in the medium. Sugars and sugar alcohols have been found to slightly increase the hydrogen solubility in water, while ionic species dramatically reduce the H₂ solubility in a medium.^{333–336} When considering reactor design from a practical standpoint for effective hydrogen transport from the reactor headspace to the liquid, the impeller type is another important variable. As a general rule, turbine- and fluidfoil-type impellers facilitate the best gas distribution in liquids. However, in the context of reductive catalytic fractionation, the axial mixing component is also important for solids distribution.^{327,337}

2.4.1.2. Lignin configuration in different solvents

Depending on the solvent interaction with the lignin molecules, the lignin in solution follows a thermodynamically most favoured configuration. Recently, Vermaas *et al.* carried out molecular simulations to study the secondary structure of lignin in different solvents.³³⁸ Accordingly, Figure 2-11 depicts a linear lignin molecule configuration in various solvents.

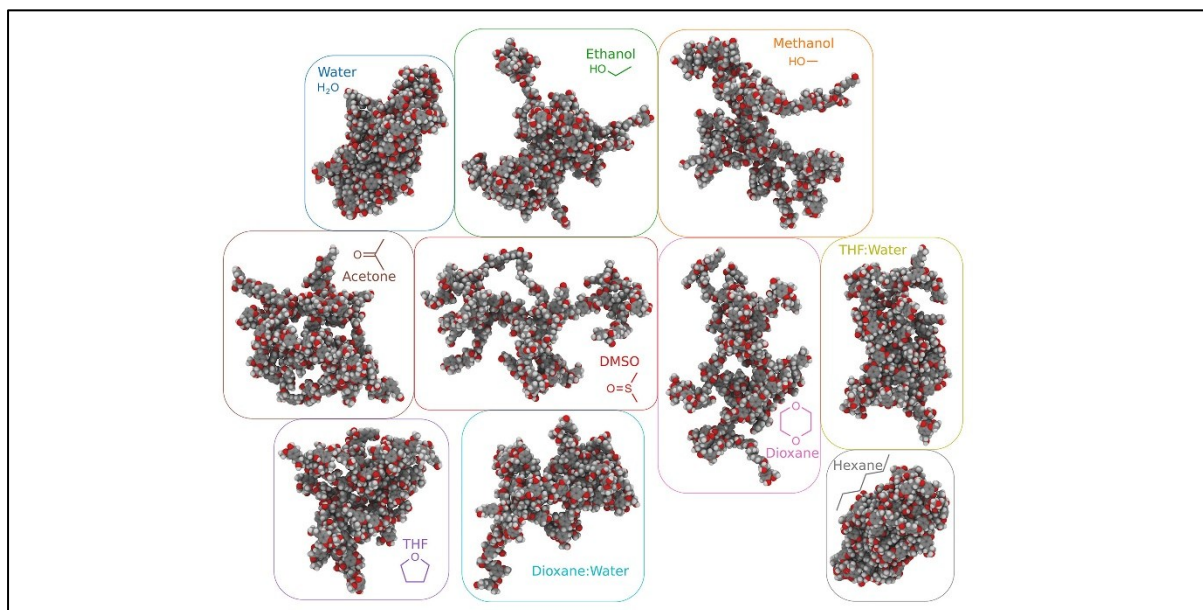


Figure 2-11. Representative snapshots of what a single polymer from the spruce looks like at the end of equilibrium simulation in different organic solvent environments. The borders, labels, and chemical structures indicate what the 10 different solvent environments are, although, for clarity, solvent molecules are omitted from the visualisation. Within the individual space-filling representations for the polymers, carbons are coloured grey, oxygens are coloured red, and hydrogens are coloured white. Reproduced from reference ³³⁸ with permission (American Chemical Society).

In the extended lignin configurations, the strength of lignin interactions with the solvent exceeds its intramolecular interactions. Accordingly, solvents that allow the largest extension of the lignin molecule also tend to be the best solvents for dissolving lignin.³³⁸ Hexane and water alone are poor solvents for lignin (Chapter 2.2.1) and, accordingly, form globular molecular configurations. In contrast, good lignin solvents like DMSO, dioxane and alcohols allow extensive unfolding of the lignin molecule. The lignin molecule configuration is especially important when considering lignin interaction with the catalyst for reductive processes. While reactions taking place on the catalyst surface can be considered to mainly involve oligomeric and monomeric lignin moieties, where intramolecular interactions are less prominent, we have recently shown that polymeric lignin species with high molecular weight also interact with the catalyst (Chapter 3).²³⁹ Therefore, understanding lignin configuration in solvent becomes particularly important when considering the high molecular weight lignin fractions and their potential catalytic modification and valorisation.

2.4.1.3. Solvent interactions with heterogeneous catalysts

Solvent direct interaction with the heterogeneous catalyst is another factor that can influence the outcome of the reductive processes on the catalyst surface. While the solvent interactions

with the catalyst are commonly limited to weak specific (H-bonding) and/or weak nonspecific (van der Waals) interactions, some solvents can also interact with the catalyst's active sites via chemisorption.³³³ Accordingly, the solvent molecules that have a strong interaction with the catalyst may compete with the substrate for the catalytic sites.³³³

Surface studies of Ni(111), Ni(211), and Ni(100) have shown that primary alcohols like methanol and ethanol readily bind with the catalyst surface through dissociative cleavage of the O-H bond.^{339–342} Accordingly, the chemisorption of methanol and ethanol leads to methoxyl/ethoxyl groups and H adatoms on the catalyst surface. This, in turn, can at least partially inhibit the catalyst's activity and lower the number of active sites available for substrate adsorption. In contrast, secondary alcohols readily interact with the catalyst from multiple atoms at low temperatures. For example, besides the scission of the O-H bond, 2-propanol also goes through the scission α -C-H bond already at 50°C on Ni(111) surfaces.³⁴³ As a result, acetone is released into the gas phase while two hydrogen atoms remain adsorbed on the catalyst. In this manner, secondary alcohols can act as hydrogen donors for hydrogenation or hydrogenolysis reactions already at low temperatures.^{344–346}

In 2012, Wang and Rinaldi performed Raney Ni catalysed reactions in different solvents with diphenyl ether model compound in the presence of H₂ at 80°C.³⁴⁷ Figure 2-12a displays the conversion and product distribution obtained in various solvents, while Figure 2-12b pictures a correlation between the solvent's Lewis basicity described by donor number (DN) and the conversion achieved. Significantly, conversion correlated strongly with the donor number with the complete or near complete conversions achieved in protic solvent displaying no Lewis basicity (hexafluoro-2-propanol) and in aprotic nonpolar solvents (methylcyclohexane, decaline, and n-heptane). During the conversion, both extensive hydrogenolysis and hydrogenation took place, resulting in complete or almost complete hydrogenation of the aromatic rings.

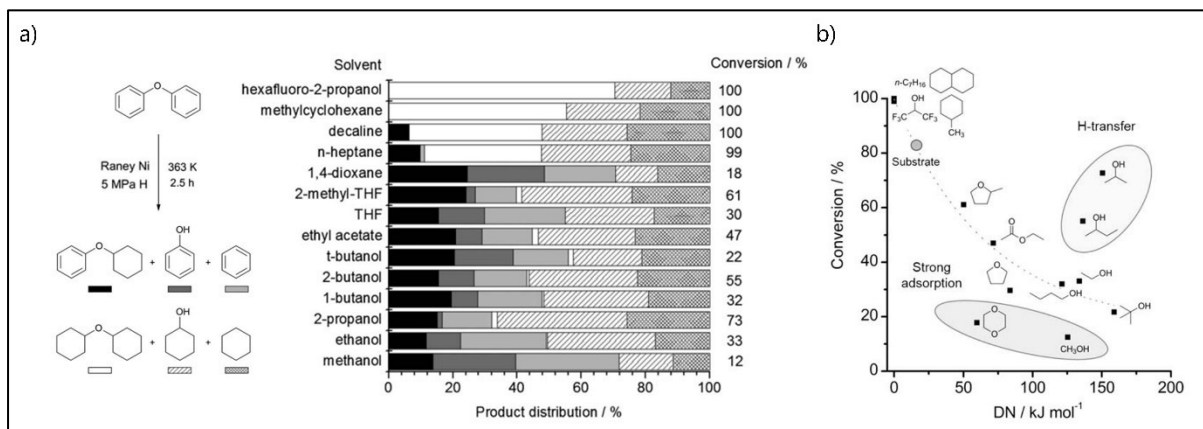


Figure 2-12. a) Results from the conversion of diphenyl ether with Raney Ni in various solvents. b) Lewis basicity of the solvents described by donor number correlated against the conversion of diphenyl ether. Reproduced from references ³⁴⁷ and ³³³ with permissions (John Wiley and Sons and Royal Society of Chemistry).

On the other hand, protic solvents with high Lewis basicity (1-butanol, ethanol, *t*-butanol) resulted in low conversion (up to 33%) and retained largely the aromaticity. Four solvents utilised in the study fell off from the general trend. Utilising 2-propanol and 2-butanol resulted in higher conversion than expected based on their Lewis basicity. This is attributed to the ability of H-transfer already at low temperatures, which in turn resulted in more extensive hydrogenolysis and hydrogenation. On the other hand, dioxane and methanol resulted in lower conversion at the given conditions. As 1,4 dioxane can bind with both O-sites on the catalyst surface, it effectively blocks many catalytic sites. At the same time, methanol can cover the catalyst surface with methoxyl groups, therefore hindering the adsorption of the substrate.

While the H₂ solubility in the given solvents can affect the catalyst performance, the study demonstrates that solvent adsorption on the catalyst surface is an important parameter when considering solvent selection for reductions on heterogeneous catalysts. Accordingly, solvents displaying similar hydrogen solubility result in very different conversions of diethyl ether under the studied conditions. N-heptane (H₂ solubility 4.7 mol m⁻³ at 1 bar and 25°C) results in 99% conversion, ethyl acetate (3.5 mol m⁻³) in 47% conversion, and methanol (3.5 mol m⁻³) in 12% conversion.^{347,348} Therefore, the interactions between the solvent itself and the catalyst prove to be another essential parameter when considering solvents for an RCF process.

The study above and the examples based on Nickel catalysts illustrate the catalyst's direct interaction with the solvent. Importantly, the interactions between O-atoms and the catalyst

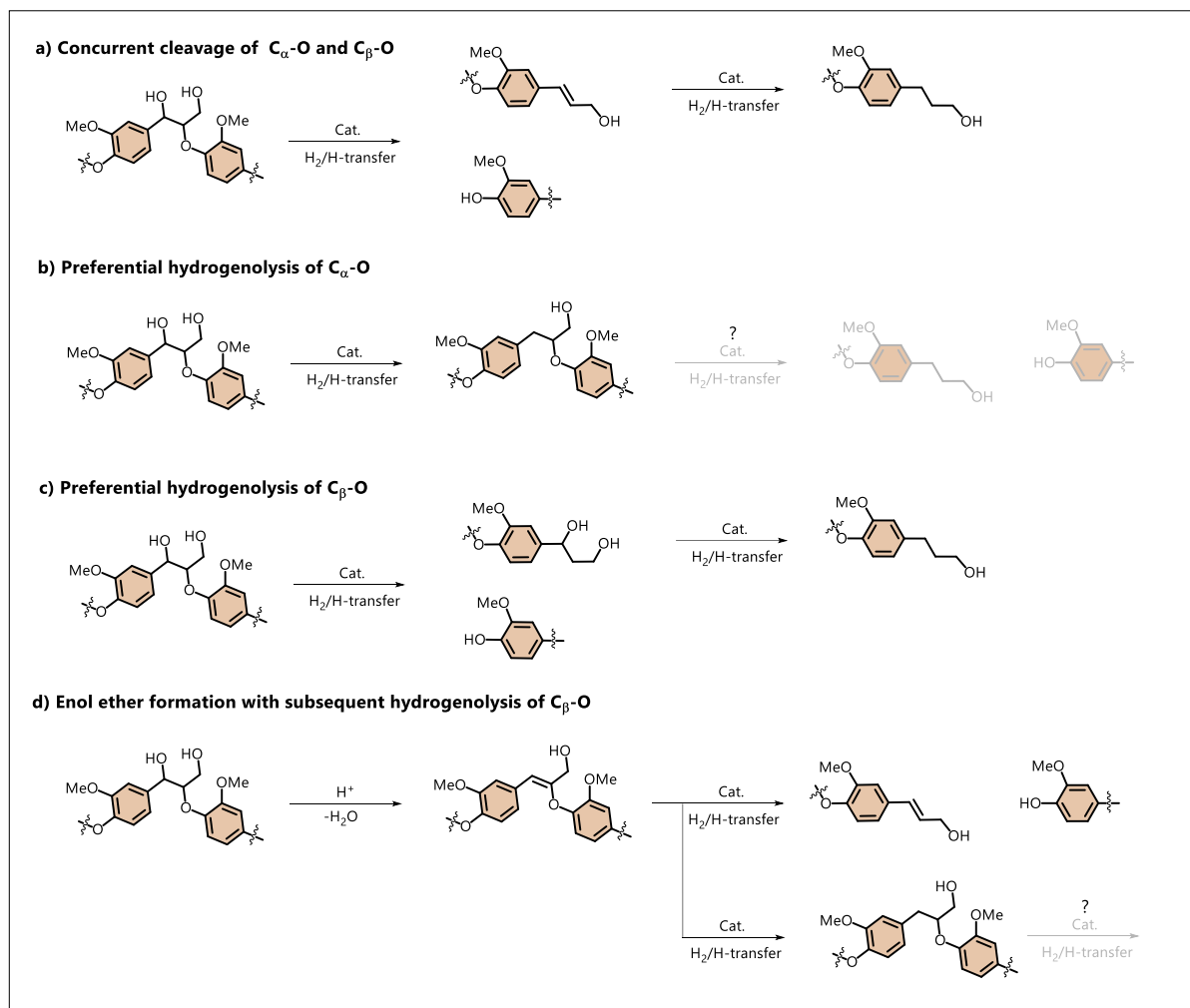
play an important role in the catalyst's effectiveness, which becomes further relevant when considering the carbohydrate fragments released from the lignocellulosic matrix and lignin fragments themselves. In light of different catalysts, it is important to emphasise the inherent differences between the active sites. Therefore, establishing a comprehensive understanding of the interaction between the selected catalyst and the solvent is paramount to steer the outcomes of the process itself.

2.4.2. Reductive processes- hydrogenolysis, hydrodeoxygenation, and hydrogenation

Reductive processes of lignin moieties on the surface of the heterogeneous catalyst are the key for lignin fragments stabilisation and production of lignin-derived monomers. Once chemisorbed on the catalyst surface, the lignin can undergo hydrogenolysis, hydrodeoxygenation (HDO), and hydrogenation. Following, a closer look is taken at the reductive processes based on the very nature of the lignin species in solution- lignin moieties still intact in their native form and lignin fragments altered during the hydrolytic processes.

2.4.2.1. Hydrodeoxygenation and hydrogenolysis of retained lignin linkages

Still natively linked lignin moieties undergo two main reductive processes on the catalyst surface- HDO and hydrogenolysis. As the dominant linkage in native lignin, the reductive degradation mechanism of lignin β -O-4 linkages has been considered in a variety of studies with multiple proposed mechanisms.³⁴⁹⁻³⁵¹ Accordingly, Scheme 2-17 summarises the key proposed routes for the reductive degradation of β -O-4 moieties.



Scheme 2-17. Possible routes for reductive degradation of β -O-4 moieties in lignin. a) Concurrent hydrogenolysis of C_{α} -O and C_{β} -O. b) Preferential hydrogenolysis of C_{α} -O leading to more stable reduced β -O-4 lignin linkage. c) Preferential hydrogenolysis of C_{β} -O, leading to depolymerisation and formation of alkyl chain with two hydroxyl groups. d) Acid-catalysed formation of enol ether, which is followed by hydrogenolysis of C_{β} -O leading to depolymerisation or hydrogenation leading to more stable reduced β -O-4 lignin linkage. As the possibility for cleaving the C_{β} -O after C_{α} -O would have been cleaved remains unknown under the RCF conditions, it is marked in light grey along with a question mark.

Recently, Li and Song carried out the hydrogenolysis of deuterium-incorporated dimeric and polymeric β -O-4 model compounds in order to pinpoint the mechanism behind the hydrogenolysis.³⁵¹ Utilising Ru/C and Pd/C catalyst in MeOH with 30 bar H_2 at the temperature range of 150-250°C, they concluded that the cleavage of C_{β} -O occurs simultaneously with the cleavage of C_{α} -O bond (Scheme 2-17a). Significantly, carrying out the experiment at lower temperatures resulted in a substantial amount of HDO product by the cleavage of C_{α} -O without the hydrogenolysis of C_{β} -O bond (40% yield at 150°C, 26% at 180°C, and 14% at 220°C)(Scheme 2-17b). When carrying out the RCF under the same conditions at 220°C and

250°C utilising the model compound with reduced $C_{\alpha}-H_2$ in β -O-4 linkage, no hydrogenolysis $C_{\beta}-O$ occurred with almost complete (96%) recovery of the model compound. Therefore, it can be concluded that HDO of $C_{\alpha}-OH$ proceeds at less severe conditions compared to the concurrent hydrogenolysis of $C_{\alpha}-O$ and $C_{\beta}-O$. Once the β -O-4 linkage has undergone the HDO at C_{α} , the linkages become significantly more recalcitrant to the hydrogenolysis of $C_{\beta}-O$ bond.

We have recently demonstrated the presence of the reduced β -O-4 moieties with $C_{\alpha}-H_2$ in lignin oils obtained in the RCF process with poplar chips and Raney Ni at 200°C for 3 h.²³⁹ Furthermore, reduced β -O-4 moieties also occur in the highest molecular weight fractions of lignin oil ($M_w=17000$ Da), and comprise up to 22% of the still retained β -O-4 linked moieties in oligomeric fraction ($M_w=500$ Da). Accordingly, the HDO of $C_{\alpha}-OH$ also occurs on the surface of Raney Ni and with the utilisation of actual lignocellulosic biomass. Furthermore, lignin interactions with the catalyst are not limited to oligomeric species, and polymeric lignin moieties also interact with the catalyst. However, the study does not allow pinpointing if the once-formed reduced β -O-4 moieties with $C_{\alpha}-H_2$ undergo further (catalytic) degradation or accumulate in the reaction medium over time.

In their study, Li and Song also determined the retention of deuterium atoms in different positions of the alkyl chain after reductive degradation of the β -O-4 model polymer. While the deuterium atoms were fully retained at C_{β} and C_{γ} at all studied temperatures after the cleavage of $C_{\alpha}-O$ and $C_{\beta}-O$ resulting in 4-(3-hydroxypropyl)guaiacol, the deuterium retention at C_{α} was 76% at 180°C dropping to 29% at 250°C. Therefore, only C_{α} experiences H/D exchange on the Pd/C surface at the given conditions and is therefore in closer proximity to the catalyst surface compared to C_{β} and C_{γ} . This further supports the preferential adsorption of the $C_{\alpha}-OH$ or C_{α} itself with the catalyst compared to C_{β} and C_{γ} and explains the ease of cleaving the $C_{\alpha}-OH$ bond. Importantly, different catalysts interact differently with the lignin. For example, both Pd/C and Raney Ni adsorb the aromatic ring of lignin. However, Raney Ni has a higher affinity for oxygen compared to Pd/C and, therefore, preferentially interacts with the oxygen atom, resulting in different reaction mechanism for the $C_{\alpha}-OH$ cleavage (Figure 2-13).³⁵²⁻³⁵⁴ While Raney Ni and Pd/C have both indicated the presence of independent $C_{\alpha}-OH$ bond cleavage, it is therefore important to emphasise that different catalysts can interact differently with the lignin molecules.

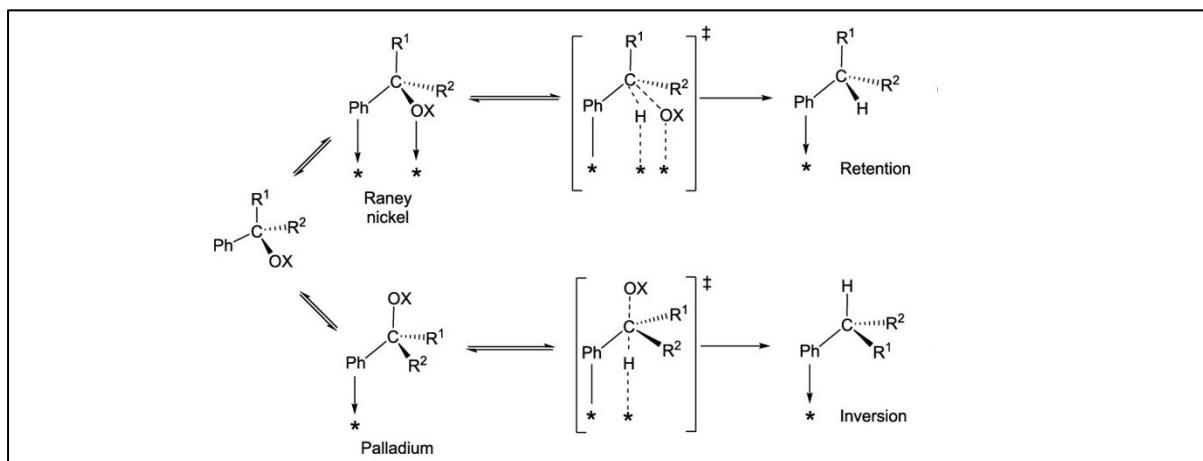


Figure 2-13. Hydrogenolysis mechanism of the benzylic hydroxyl group on Raney Ni and Palladium. Raney Ni having a strong affinity towards oxygen results in the retention of benzylic position stereochemistry, while the reaction on Pd results in the inversion of the chiral centre. Reproduced modified figure from reference ³⁵³ with permission (American Chemical Society) and permission from the original graph in reference ³⁵⁴ (Oxford University Press).

In order to cleave the β -O-4 linkage, the process could potentially also proceed only via the hydrogenolysis of the C_β -O bond, retaining the C_α -OH intact (scheme 2-17c).^{349,350} However, this contradicts the previous argument of the preferential interaction of C_α -OH with the catalyst.³⁵¹ Furthermore, monomeric lignin-derived compounds are exclusively reported without the hydroxyl group at C_α , therefore making the mechanism unlikely in the presence of the common catalysts utilised in RCF processes. Another potential mechanism for the β -O-4 linkage cleavage on the catalyst involves the initial elimination of the hydroxyl group at C_α and formation of enol ether as for the acid-catalysed β -O-4 cleavage (scheme 2-8), which is followed by hydrogenolysis of the C_β -O bond (scheme 2-17d). However, the complete retention of deuterium at C_β during the hydrogenolysis of the deuterated β -O-4 lignin model compound indicates that this is not the dominant path in the degradation of β -O-4 linkage in the presence of heterogeneous catalyst with hydrogen.³⁵¹ Accordingly, the concurrent cleavage of C_α -O and C_β -O can currently be considered as the main pathway of catalytic degradation with simultaneous occurrence of the cleavage of C_α -O only as a side reaction.³⁵¹ While this holds for Pd/C and most likely for Raney Ni, it is again important to remark that different heterogeneous catalysts can interact differently with the substrate, and other reaction mechanisms might be feasible under different conditions.

Apart from the cleavage of β -O-4 linkages, 4-O-5 linked lignin moieties can undergo hydrogenolysis on the catalyst surface. Ralph and coworkers recently utilised 4-O-5 dimeric

model compound with native-like structures and reported the cleavage on Pd/C to proceed exclusively via the scission of C₅-O bond, resulting predominantly in two phenolic monomers along with minor rearrangement products after the C₅-O scission.³⁵⁵ At the same time, many model compound studies have been carried out with diaryl ether model compounds, resulting in hydrogenation of the aromatic ring(s).^{346,347,356-358} Therefore, the phenolic hydroxyl group in the linkage appears to play an important role during the 4-O-5 bond cleavage by preventing the hydrogenation of the aromatic ring. While the literature on realistic 4-O-5 model compounds cleavage is very limited, it is plausible to assume that the cleavage of 4-O-5 bond proceeds similarly on other catalysts as it does on Pd/C, as the scission of C₄-O would result in non-phenolic moieties not reported from the RCF studies with various catalysts.

2.4.2.2. Stabilisation of reactive moieties

As seen in Chapters 2.2 and 2.3, lignin degradation results in various reactive species prone to undergo condensation reactions. Therefore, reductive catalytic processes stabilising the released fragments are the key to avoiding the formation of recalcitrant lignin and maximising the production of lignin-derived monomers. Broadly, the species stabilisation can be looked at in two main categories- reduction and HDO of carbonyl compounds and their equilibrium products and hydrogenation of the unsaturated lignin fragments (Scheme 2-16).

The catalytic reduction of aldehydes and ketones results in primary and secondary alcohols, which can undergo further HDO via C-O cleavage. Carbonyl groups in the benzylic position (C_α) have indicated to undergo complete conversion to alcohol and HDO product already at low temperatures (80°C) in the presence of Raney Ni,³⁴⁶ as well as with Cu/Al₂O₃ (90°C), Pd (60°C), and MoCoO_x/C (150°C).³⁵⁹⁻³⁶¹ Similarly, carbonyl groups at C_β are prone to reduction, resulting in complete conversion under mild conditions. Accordingly, full conversion of C_β ketone or aldehyde to alcohol or HDO product was achieved with Raney Ni (80°C) and Pd/C (50°C).^{346,362} Therefore, the catalytic conversion of the Hibbert ketones and C2 aldehydes released during the acidolysis of β-O-4 bonds can be considered a favourable process under the RCF conditions and can easily proceed once the substrate reaches the catalyst. Other aldehydes like furfural and HMF derived by dehydration of carbohydrates can similarly undergo catalytic reduction during an RCF process. However, complete conversion of aldehyde to alcohol, HDO or reduction of aromatic ring usually proceeds at more severe conditions.

While the reduction of lignin-derived aldehydes yielded complete conversion in the presence of Raney Ni after 3 h in 2-PrOH at 80°C, the same process at 120°C resulted in 44% conversion of furfural and 42% conversion of HMF.³⁴⁶ A complete conversion of furfural in the presence of Raney Ni was reported by Xu *et al.* at 180°C (MeOH, 4 h).³⁶³ On the Pd/C catalyst, a complete conversion of furfural has been reported at 150°C (H₂O, 1 h and EtOH, 1 h).^{364,365}

Similarly to the reduction of carbonyl compounds forming from the lignocellulosic biomass, hydrogenation of unsaturated lignin compounds acts as a stabilisation mechanism hindering condensation reactions. Notably, the hydrogenation of non-aromatic double-bonds in aliphatic regions readily proceeds at mild conditions in the presence of common RCF catalysts. Experiments with syringyl and guaiacyl model compounds with unsaturated alkyl chains resulted in complete hydrogenation of the double bonds with Raney Ni at 80°C and substantial hydrogenation of the aromatic ring and demethoxylation.³⁴⁶ In the temperature ranges of 200-250°C, catalytic reduction of eugenol on Pd, Ru and Pt on both carbon and Al₂O₃ supports has resulted in complete conversion with extensive hydrogenation of aromatic ring and HDO of methoxy and phenolic OH groups.^{366,367}

In the context of reductive catalytic processes taking place on the catalyst surface, it is important to emphasise that when performing an RCF process with actual biomass, a large variety of additional factors can influence the chemistry on the catalyst surface. Lignin's inherent molecular structure, configuration in the solvent, and surface crowding by solvent or other substrates released from the lignocellulosic matrix can have an impact on the processes on a catalyst surface.³⁶⁸ Therefore, an RCF process with actual biomass can often result in different monomer characteristics compared to the reactions with just model compounds.

2.4.3. Catalysts and monomer yields in Reductive Catalytic Fractionation

To this point, the discussion has mainly focused on the key processes during the reductive catalytic fractionation. In the following subsection, a tabulated summary of RCF results obtained with different biomasses under various conditions is given. The different results are compiled based on the utilised catalysts, and the monomer yields are listed based on the initial lignin content in the biomass.²⁵ The section finishes with additional considerations from the catalysts' perspective in RCF processes.

2.4.3.1. Nickel catalysts in RCF processes

Nickel has been used in different hydrogenation reactions since the very late 19th century and is widely applied in industrial processes due to its relatively low price (7-47 \$/kg between 2013-2023) and good hydrogenation performance.^{369–371} Accordingly, nickel has been widely explored as a potential catalyst for biomass conversion. Table 2-2 summarises a selection of RCF processes utilising Nickel catalysts, which are carried out in batch mode in various conditions and biomasses.

Table 2-2. Reductive catalytic fractionation of various biomasses with Nickel catalysts in batch mode.

Feedstock			Reaction conditions						Results ^a			Ref.
Biomass	Lignin content (wt%)	Particle size (mm)	Catalyst	Solvent (v:v)	T (°C)	P (bar)	H-source	Time (h)	Solvent: Biomass: Catalyst (mL:g:g)	Mono-phenolics yield (%)	Main product	
Poplar	30	5	Raney Ni	2-PrOH/H ₂ O (7:3)	160	Auto	Solvent	3	8.5 : 1 : 0.6	12.2	P(OH)S	372
					180					15.4	P(OH)S	
					200					16.3	P(OH)S	
					220					30.8	ES	
Sugarcane bagasse	22.5	N/I	Raney Ni	2-PrOH/H ₂ O (7:3)	180	Auto	Solvent	3	20 : 1 : 0.7	25.8	DHCHPA	373
Beech	21	<0.15	Ni/C (10%)	MeOH	200	20	H ₂	5	20 : 1 : 0.1	39.3	P(OH)S	374
				MeOH/H ₂ O (6:4)						51.4	P(OH)S	
Miscanthus	13	<0.4	Ni/C (11%)	MeOH	225	35	H ₂	12	45 : 1 : 0.15	68	PG	375
						35				65	PG	
						35				56	PG	
						10				29	MeFer	
						5				30	MeFer	
Birch	16	<0.4	Ni/C (11%)	MeOH	200	35 (N ₂)	Auto (2 bar N ₂)	6	20 : 1 : 0.10	27	MeFer	376
										20 : 1 : 0.05	P(ene)S	
										20 : 1 : 0.10	PS	
										20 : 1 : 0.05	P(OH)G	
										20 : 1 : 0.10	P(ene)S	
Eucalyptus	24	<2	Ni/C (15%)	MeOH	225	30	H ₂	3	96 : 1 : 0.16	16	P(ene)S	377
										28	P(ene)S	
Poplar	26	<2	Ni/C (5%)	MeOH	225	Auto 30	Solvent H ₂	6	15 : 1 : 0.05	6	pHBA/MP	378
										19.9	P(ene)G	
Corn stover	14	<0.2	Ni/C (5%)	MeOH	200	30	H ₂	1	30 : 1 : 0.10	21.3	MeFer	30
								3		24.5		
								6		28.3		
								24		28.6		
Pine	23	0.1	Ni/C (1%)	MeOH	200	30	H ₂	3	40 : 1 : 0.10	14.8	PG	379
Poplar	21	<0.25	Ni/SiO ₂ (25%)	MeOH	230	30	H ₂	3	25 : 1 : 0.10	26.5	P(OH)S	380
				EG	185	Auto	Solvent	3	20 : 1 : 0.20	9.6	P(ene)S	
Eucalyptus	27	0.5-1	Ni/ZIF-8 (14%)	MeOH	220	30	H ₂	12	20 : 1 : 0.10	42	P(OH)S	381
				EtOH						32.7	P(OH)S	
				2-PrOH						30.4	P(OH)S	
				MeOH				4		20.8	P(OH)G	
Birch	20	0.25-0.5	Ni/Al ₂ O ₃ (21%)	MeOH	250	30	H ₂	0.5	20 : 1 : 0.10	30.4	P(OH)S	259
								3		44.2		
								6		51.2		

Poplar	N/I	0.25-0.4	Ni/Al ₂ O ₃	MeOH	240	10	H ₂	3	20 : 1 : 0.15	25.9	P(ene)S	³⁸²
Birch	20 ^b									36.9 ^b	PS	
Poplar	15 ^b									32.4 ^b	P(OH)	
Ashtree	18 ^b	<0.4	Ni-W ₂ C/C (4%)	H ₂ O	235	60	H ₂	4	100 : 1 : 0.40	40.5 ^b	PS	³⁸³
Beech	25 ^b									26.1 ^b	PS	
Bagasse	14 ^b									32.9 ^b	P(OH)S	
Pine	34 ^b									10.1 ^b	PG	
			Ni/C(5%)			15				10.5	P(ene)G	
			Ni/C(15%)							19.5	PS	
Poplar	26	0.08-0.25	Ni/C(5%)	MeOH	200		H ₂	1	50 : 1 : 0.20	17.3	PS	²⁶¹
			Ni/C(15%)			30				19.8	PS	
		0.5-1.0	Ni/C(15%)							15.1	PS	

^aMonophenols yield is given based on the initial total lignin content in the biomass. P(OH)S- 4-(3-hydroxypropyl)syringol. PS- 4-propylsyringol. P(ene)S- 4-propenylsyringol. ES- 4-ethylsyringol. P(OH)G- 4-(3-hydroxypropyl)guaiacol. PG- 4-propylguaiacol. P(ene)G- 4-propenylguaiacol. MeFer- methylferulate. pHBA- p-hydroxy benzoic acid. MP- methyl paraben. DHCHPA- 3,4-dihydroxycyclohexanepropanoic acid ^bThe composition of biomass was measured by Van Soest method with lignin content deviating considerably compared to the standard NREL method for biomass composition determination.^{384,385} Accordingly, the monophenols yield deviates based on the determined biomass composition.

2.4.3.2. Pt, Pd, Rh, Ru, and Ir in RCF processes

Besides Nickel-based catalysts, a variety of other transition metal catalysts have been employed in different RCF processes. However, the price of Platinum (20 000-50 000 \$/kg in 2013-2023), Palladium (23 000-94 000 \$/kg in 2013-2023), Rhodium (20 000-970 000 \$/kg in 2013-2023), Ruthenium (1 300-26 000 \$/kg in 2013-2023) and Iridium (13 000-200 000 \$/kg in 2013-2023) is considerably larger compared to the Nickel catalyst.³⁸⁶ Accordingly, any advantages produced by utilising the catalysts above must well overdrive the three orders of magnitude price difference, even when considering that the metal content ranges between 1-10% on the catalyst support. Table 2-3 summarises a selection of RCF processes utilising Pt, Pd, Rh, Ru, and Ir catalysts that are carried out in batch mode in various conditions and biomasses.

Table 2-3. Reductive catalytic fractionation of various biomasses with Pt, Pd, Rh, Ru, and Ir catalysts in batch mode.

Feedstock			Reaction conditions							Results ^a		
Biomass	Lignin content (wt%)	Particle size (mm)	Catalyst	Solvent (v:v)	T (°C)	P (bar)	H- source	Time (h)	Solvent: Biomass: Catalyst (mL:g:g)	Mono-phenolics yield (%)	Main product	Ref.
Poplar	26	<2	Ru/C	MeOH	225	Auto 30	Solvent H ₂	6	15 : 1 : 0.05	14.7	P(ene)G	378
			Pd/C			Auto 30	Solvent H ₂			23.0	PS	
			Pt/C			Auto 30	Solvent H ₂			28.1	ES	
						Auto 30	Solvent H ₂			28.4	P(OH)S	
Pine	28	<2	Pd/C (5%)	EtOH/H ₂ O (1:1)	195	Auto	H ₂ / solvent	1	10 : 1 : 0.13	26.8	P(ene)S	31
Birch	20									29.6	PS	
Poplar	19	<0.4	Pd/C+ ZnCl ₂	MeOH	225	34	H ₂	12	30 : 1 : 0.1	54	PS	29
Birch	16									52	PS	
Eucalyptus	24									49	PS	
Pine	31									19	PG	
Birch	19	0.25-0.50	Ru/C (5%)	MeOH	250	30	H ₂	3	20 : 1 : 0.15	50.0	PS	28
Poplar	21									43.9	PS	
Softwood	27									20.5	PG	
Birch	21									0.25-0.50	Ru/C (5%) Pd/C (5%)	
		47.8	P(OH)S									
Birch	21	0.25-0.5	Pd/C (5%)	H ₂ O	200	30	H ₂	3	20 : 1 : 0.10	43.8	P(OH)S	329
				MeOH						28.1	P(OH)S	
				EtGly						26.9	P(OH)S	
				EtOH						17.4	P(OH)S	
Birch	22	Chips	Pd/C (5%)	EtOH/H ₂ O (1:1)	210	Auto	Solvent	15	20 : 1 : 0.10	35	PS	388
Poplar	24									22	PS	
Spruce	27									12	EG	
Pine	26									7	EG	
Eucalyptus	23	0.25-0.50	Ru/C (5%)	H ₂ O/ n-butanol (1:1)	200	30	H ₂	2	20 : 1 : 0.10	48.8	P(OH)S	389
			Pd/C (5%)							50.2	P(OH)S	
			Pt/C (5%)							49.3	P(OH)S	
			Rh/C (5%)							46.9	PS	
Birch	20	Sawdust	Pt/Al ₂ O ₃ (1%)	MeOH/ H ₂ O (1:1)	230	30 (N ₂)	Solvent	3	20 : 1 : 0.25	46.1	PS	390
Eucalyptus	27	0.25-0.40	Pd/C(10%)	MeOH	200	30	H ₂	4	40 : 1 : 0.10	26.4	P(OH)S	391
				EtOH	240					49.8	P(OH)S	
				2-PrOH						42.2	P(OH)S	
										40.0	P(OH)S	
Birch	20	0.20-0.40	Ru/C (5%)	MeOH/ H ₂ O (7:3)	250	20	H ₂	8	50 : 1 : 0.20	41.4	PS	392
			Pd/C (5%)							34.1	PS	
			Pt/C (5%)							38.8	PS	
Poplar	21	<0.25	Ru/C (5%)	MeOH	230	30	H ₂	3	25 : 1 : 0.05	37.8	PS	380
				EtGly	185	Auto	Solvent		20 : 1 : 0.10	8.1	P(ene)S	
Beech	22	<0.4	Ru/C (5%)	MeOH	250	10	H ₂	3	50 : 1 : 0.15	44.1	PS	393
Pine	25	Sawdust	Pt/C (5%)	MeOH/ H ₂ O (1:1)	230	30	H ₂	3	20 : 1 : 0.05	15	PG	394
Poplar	N/I	0.25-0.50	Ru/C Pd/C	MeOH	240	10	H ₂	3	20 : 1 : 0.15	46.4	PS	395
										21.3	DMHPP	
Pine	23	0.1	Pd/C (1%)	MeOH	200	30	H ₂	3	40 : 1 : 0.10	15.0	PG	379
			Pt/C (1%)							14.4	PG	
			Ru/C (1%)							12.5	PG	

^aMonophenols yield is given based on the initial total lignin content in the biomass. P(OH)S- 4-(3-hydroxypropyl)syringol. PS- 4-propylsyringol. P(ene)S- 4-propenylsyringol. ES- 4-ethylsyringol. P(OH)G- 4-(3-hydroxypropyl)guaiacol. PG- 4-propylguaiacol. P(ene)G- 4-propenylguaiacol. DMHPP- 3-dimethoxy-4-hydroxypropiofenone.

2.4.3.3. On the catalyst choice in RCF processes

In addition to the aforementioned catalysts commonly utilised in RCF processes, a variety of other catalytic systems have been employed over recent years. Zinc has been utilised as a co-

catalyst in Zn/Pd/C, in RuN/ZnO/C, and in ZnMoO₄/MCM-41 catalysts,^{29,349,396,397} while Copper has been used in Cu₂₀PMO, CuO/C, Cu/ZnO/Al₂O₃, and Cu/CeO₂ catalysts.^{390,398–400} Acidic sites containing zeolites have been utilised as a co-catalyst or by themselves (although in this case, it becomes ambiguous to classify it as an RCF process due to the lack of active stabilisation),^{381,401–403} while a large variety of triflate salts have been added to the reaction mixture to serve a similar purpose.^{404–410} In addition, mineral acids like H₃PO₄, H₂SO₄, and HCl in liaison with Pt/C, Pd/C, and Ni/C have been added to the reaction mixtures to promote depolymerisation,^{30,407,411–414} while the addition of NaOH has resulted in lower monomer yields.^{216,411,415,416} Exploration of modified and new catalytic systems in the context of the RCF process is ever continuing with every year bringing new research with new catalysts.

Considering the wide range of monomer yields and selectivity obtained with the same catalysts at the different reaction conditions (Tables 2-2 and 2-3), it transpires that process conditions play a very significant role in determining the outcome of the RCF process. Accordingly, while the catalyst plays an important role in the stabilisation process and its configuration influences the selectivity of the monomers, the reaction conditions like temperature, time, and solvent are at least as important. It is in line with the discussion and overview given in the previous subchapters, where lignin moieties' journey prior to reaching the catalyst was summarised. Accordingly, factors like lignin solubility and configuration in the solvent dependant on the temperature and solvent, the extent of lignin depolymerisation in the matrix and bulk liquor dependent on the chemical environment and temperature, the extent of lignin condensation in the matrix dependent on particle size and temperature as well as multiple more factors affect the outcomes with the monomers yield at least to the same extent as the catalyst itself.

When considering the catalyst for the RCF process, recyclability is another important factor determining the process scalability and operation cost. Accordingly, several studies have considered the utilisation of the catalyst over several RCF cycles. Rinaldi and Ferrini found that in the RCF process with Raney Ni, the catalyst could be reused at least 8 times in the batch process without significantly affecting lignin oil properties.²⁷ Liu *et al.* demonstrated up to 10% relative decrease in monomer yield when utilising RuN/ZnO/C catalyst for up to five runs.³⁹⁶ Their results implied that catalyst deactivation might be mainly caused by the fouling of metal/support, and regeneration of the catalyst did not result in a significant loss of catalytic

performance.³⁹⁶ Van den Bosch *et al.* utilised Ni/Al₂O₃ catalyst in the RCF process and observed a subsequent decrease in monomers yield from the first to the fifth run.²⁵⁹ However, a reductive treatment on the spent catalyst restored the availability of the Ni sites and the same monomer yield was achieved as during the first run with a fresh catalyst.²⁵⁹ In the flow-through RCF process, Lan *et al.* showed a 50% reduction in monomer yield after flowing 1.2 g acetal-stabilised lignin over 0.125 g Ni/C catalyst. At the same time, Anderson *et al.* observed a 10% decrease in monomer yield after processing four 1 g biomass beds over 0.15 g Ni/C catalyst.^{417,418} Accordingly, catalyst recyclability and the ability to recover its activity are major factors when considering RCF process upscaling, as the complex pool of lignocellulose moieties in reaction liquor renders the decrease in catalyst activity over time.

Considering the different RCF catalysts highlighted and discussed in this section, the present work will consistently employ Raney Ni as the heterogeneous catalyst, based on three main considerations. Firstly, the use of a single catalyst across all experiments facilitates direct comparison between different process configurations and variables. Secondly, nickel is significantly more affordable than other transition metal catalysts commonly used in RCF, such as palladium, ruthenium, or platinum. This cost advantage enhances the feasibility of industrial implementation, reducing capital expenditure (CAPEX) and making operational expenditure (OPEX) less sensitive to uncertainties around catalyst lifetime and regeneration. Thirdly, Raney Ni has an extensive track record in RCF literature, with various studies exploring its performance, stability, and recyclability under varied process conditions.^{27,215,216,346,372,419} Building on this established knowledge base allows the current study to position itself within a well-characterized framework and expand on prior insights. In addition, the general framework of new approaches regarding the process conditions could be expanded to other catalysts with additional studies as the Raney Ni shares the principle of being a heterogeneous catalyst facilitating reductive processes with other catalysts in RCF processes.

It is important to highlight that while Raney Ni offers various advantages in the RCF process like high hydrogenation, hydrodeoxygenation and hydrogenolysis activity along with high surface area and self-supporting structure,^{420,421} it also presents some practical disadvantages. Accordingly, Raney Ni is pyrophoric in nature when dry, which necessitates careful handling and limits ease of use in scaled-up or continuous processes.⁴²² Additionally, although Raney

Ni is generally less expensive than noble metal catalysts, it is often more costly than supported Ni catalysts (e.g., Ni/C or Ni/Al₂O₃), particularly on a per-use basis due to its higher nickel content and complex preparation.^{260,262,422}

2.5. Impact on holocellulosic pulp

While the focus of this literature review is on the lignin journey through the Reductive Catalytic Fractionation, the process also results in holocellulosic pulp rich in cellulose. Accordingly, a brief overview of the pulp properties, along with the effect of the process conditions on it, is given in this section. The upgradability of the pulp resulting from the RCF process is visited in the second part of this subchapter.

2.5.1. Retention of carbohydrates and delignification

In 2016, Ferrini *et al.* carried out a thorough investigation on pulp characteristics from the RCF process in comparison with the Organosolv process.⁴¹⁹ Accordingly, RCF and Organosolv processes utilising poplar wood with and without Raney Ni catalyst were carried out in 2-PrOH/H₂O mixture for 3 h in the temperature range of 160-220°C. As a result, the glucan content in RCF pulp increased from 57% at 160°C to 84% at 220°C, while in the Organosolv pulp, the glucan content increased from 48% to 81%, respectively. Accordingly, the RCF process presented consistently higher glucan retention in the pulp compared to the Organosolv process. The same trend was also observed for the xylans. The extent of RCF pulp delignification increased from 53% at 160°C to 87% at 220°C, while the Organosolv pulp delignification increased from 33% to 77%, respectively. Therefore, the RCF process results in pulps of superior quality and a higher degree of delignification compared to the Organosolv pulps obtained at the same process conditions without the catalyst.⁴¹⁹ The ability of the catalyst to decompose the carboxylic acids renders the liquor less acidic compared to the Organosolv process, thereby hindering the carbohydrate hydrolysis. Accordingly, the RCF pulps also display a higher degree of polymerisation for the cellulose in the pulp. At the same time, the passivation of the lignin moieties decreases the likelihood of lignin redeposition, resulting in a higher degree of pulp delignification compared to the Organosolv process.⁴¹⁹

In general, RCF processes commonly result in a high degree of cellulose retention (>80%) and a high extent of pulp delignification (>80%).^{27,395,396,411,423-426} At the same time, the

hemicellulose retention in the pulp can significantly vary from almost complete removal to near complete retention.^{392,427} Accordingly, the reaction conditions utilised and the inherent nature of different biomass types strongly influence the fate of hemicellulose in the RCF process. As a common rule, acids promote hemicellulose hydrolysis while bases suppress it. Accordingly, Renders *et al.* demonstrated that the addition of H_3PO_4 to an RCF process utilising poplar with Pd/C catalyst and MeOH results in an increase of monophenol yields and a decrease in hemicellulose retention, while the addition of NaOH drastically decreases the monophenol yield while resulting in high hemicellulose retention.⁴¹¹ Similarly, Chesi *et al.* reported a significant increase in xylans retention when NaOH, NaHCO_3 , and Na_2CO_3 were individually added to the RCF reaction mixture.²¹⁶

2.5.2. Upgradability of the RCF pulps

The cellulose-rich pulp from the RCF process can be upgraded in multiple ways. Due to the mass transfer limitations described in Chapter 2.3, small chips or sawdust are utilised for the process. This, in turn, hinders RCF pulp usage in the traditional pulp and paper industry, which usually requires long cellulose fibres. At the same time, the pulp could be upgraded by enzymatic hydrolysis to sugars and fermentation to ethanol or applied to produce speciality celluloses. To assess the yield of enzymatic hydrolysis to sugars from RCF pulps in comparison with Organosolv pulps, the pulps in the above-described work by Ferrini *et al.* were subjected to enzymatic hydrolysis with Cellulast® enzyme.⁴¹⁹ Accordingly, Figure 2-14 displays the glucose yield from RCF pulps and Organosolv pulps obtained at different temperatures.

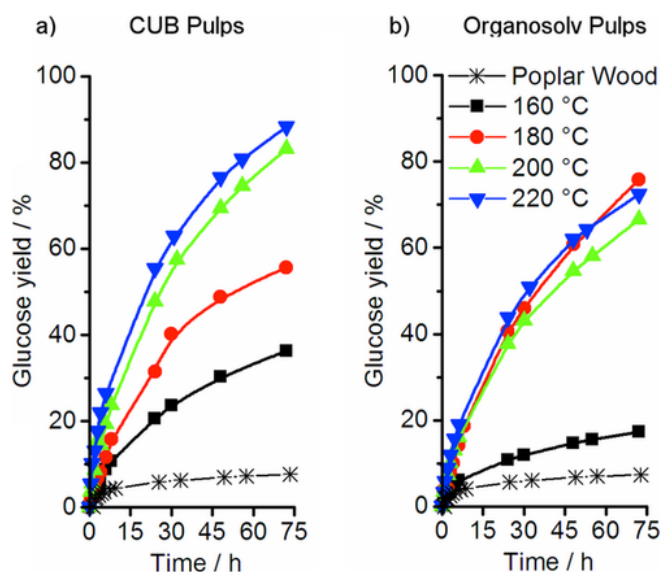


Figure 2-14. Glucose yields from the enzymatic hydrolysis of poplar wood. a) CUB pulps and b) Organosolv pulps obtained at varying temperatures. Reaction conditions: substrate (equivalent to 1g of glucans), Celluclast® (350U g⁻¹ substrate), pH 4.8 (acetate buffer), 45°C. Note: Catalytic Upstream Biorefining (CUB) is now known as Reductive Catalytic Fractionation (RCF). Reproduced from reference ⁴¹⁹ with permission (John Wiley and Sons).

Figure 2-14 indicates that the pulps obtained by the RCF process result in higher glucose yields compared to the Organosolv pulp counterparts. As transpires from the same study, the higher glucose yield is primarily achieved due to the lower content of residual lignin in RCF pulps, as the glucose yield was found to decay exponentially with the increasing residual lignin content at different enzymatic hydrolysis times.⁴¹⁹ Similarly, very high (>90%) glucose yields from enzymatic hydrolysis of RCF pulps have been reported in several studies.^{413,419,428,429} Obtained glucose from RCF pulps could be further fermented into bioethanol. Accordingly, Cooreman *et al.* recently reported an ethanol yield of 78% from enzymatically hydrolysed pulp when utilising RCF pulp from a process using *n*-BuOH/H₂O system in the presence of Ru/C catalyst, while the pulp obtained by the RCF process with only *n*-BuOH resulted in 60% ethanol yield respectively.⁴³⁰

In addition to enzymatic hydrolysis strategies, the cellulose-rich pulp could potentially be upgraded to speciality celluloses. While the production of microcrystalline cellulose (MCC) is yet to be demonstrated utilising RCF pulps, the strategy has been successfully explored in several studies utilising the pulps from the Organosolv process.^{431–433} Accordingly, while moving towards the industrial implementation of the RCF process, potential valorisation strategies for the pulp fraction have to be carefully assessed. However, it has become

universally recognised that RCF pulps present high-quality properties and could be more easily upgraded than their Organosolv counterparts.

2.6. Techno-economic aspects of RCF

The final section of the literature review gives a brief overview of the RCF process's techno-economic aspects. Firstly, the potential industrial production of RCF lignin oil is looked at from the techno-economic and environmental impact aspects based on the recent preliminary and still limited research in the field. The second part of this subchapter discusses potential routes for upgrading the RCF lignin oil downstream.

2.6.1. Techno-economic analysis and Life-Cycle Assessment for the production of RCF lignin oil

In 2020, Tschulkow *et al.* conducted a thorough techno-economic analysis (TEA) of the potential industrial-scale RCF process.⁴³⁴ The TEA was based on the RCF process utilising Ru/C catalyst with methanol and hydrogen gas with birch, poplar, pine, and waste wood. The process was designed to produce a pulp stream along with separate streams of lignin monomers and oligomers obtained by solvent extraction with hexane. With lignin monomers and oligomers fixed selling price of 1.75 € kg⁻¹, the production capacity was evaluated in terms of net present value (NPV) and internal rate of return (IRR). With a production capacity of 150 kt year⁻¹ of wood, the use case of waste wood presented the highest IRR of 26% with positive NPV, while the utilisation of pine, birch, and poplar at the same scale resulted in the IRR between 8-11% and negative NPV. The break-even annual capacities for the modelled RCF biorefineries were 80 kt for waste wood and between 191-234 kt for poplar, birch, and pine.⁴³⁴ While the study was based on a broad range of assumptions, it indicates an industrial-scale RCF plant would be a viable option when the lignin fraction's selling price is 1-2 € kg⁻¹.

In 2021, Bartling *et al.*, involving several group leaders from the RCF field, performed TEA and a life cycle assessment (LCA) of a potential industrial RCF process.⁴³⁵ In the study, the RCF processes were evaluated based on the minimum selling price (MSP) of RCF lignin products for an IRR of 10% over the 30-year lifetime of the biorefinery to produce an NPV of zero. The evaluated processes were based on the capacity to process 2 kt of dry hybrid poplar feedstock per day, and the pulp was enzymatically hydrolysed to sugars and fermented to ethanol with

the selling price of 1.65 \$ gallon⁻¹ (0.44 \$ L⁻¹). In the base case process, 9 L of methanol:H₂O 9:1 (V:V) mixture and 10 L of H₂ (STP) per minute were utilised per kg of dry biomass feed. 15 wt% Ni/C catalyst was used at 1:10 ratio to the dry biomass by weight. The proposed RCF process was carried out at 200°C and 60 bar. As the functional unit for LCA analysis, 1 kg of the lignin fraction in RCF lignin oil was utilised. Accordingly, Figure 2-15 lays out the different plant sections' contribution to the MSP as well as the global warming potential (GWP) and cumulative energy demand (CED) of the corresponding sections.

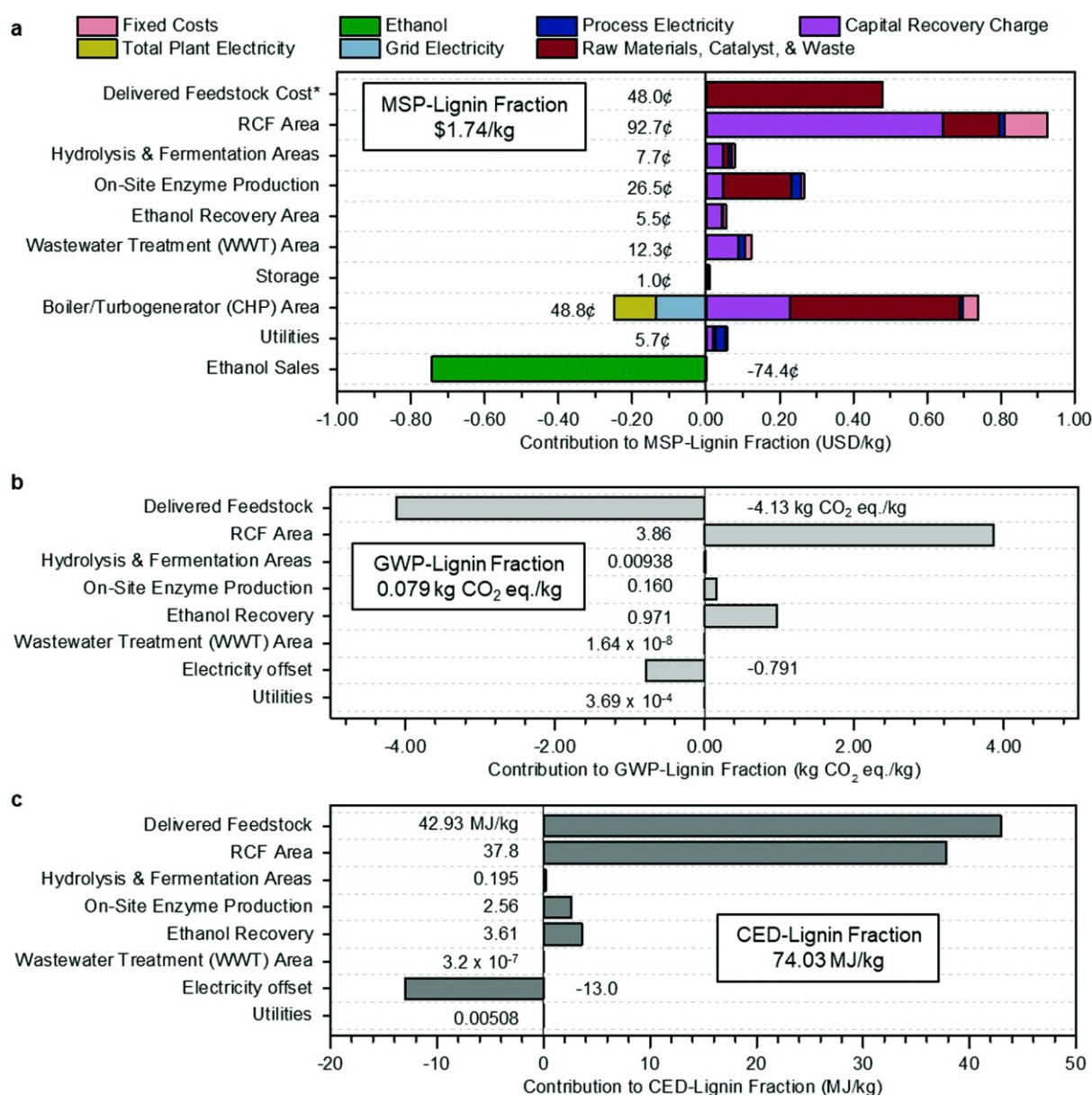


Figure 2-15. RCF lignin oil production base case economics, GWP, and CED utilising 2kt of hybrid poplar, 9L methanol:H₂O 9:1 (V:V) solvent mixture per 1kg of biomass, 15wt% Ni/C catalyst with 1:10 catalyst to biomass ratio, 200°C for 3h with the H₂ flow rate of 10L (STP) per minute per 1kg of biomass. (a) Contributions to the MSP of the

*lignin portion of RCF oil (MSP-lignin fraction). *Cost of feedstock includes all upstream feedstock logistics, handling, and pre-processing steps up to the reactor. (b) Breakdown of contributions to GWP-lignin fraction. The results shown are normalised to the production of 1 kg lignin fraction and allocated according to mass (c) Breakdown of contributions to CED-lignin fraction. Results shown are normalised to 1 kg lignin fraction and allocated according to mass. A thorough list of considerations can be found in the corresponding article.⁴³⁵ Reproduced from reference ⁴³⁵ licensed under [CC BY 3.0](#).*

The base case of the study resulted in an MSP of 1.74 \$ kg⁻¹ for the lignin fraction in RCF oil. The MSP for whole RCF lignin oil, including the carbohydrate-derived moieties, was estimated to be 1.13 \$ kg⁻¹, while the MSP for lignin-derived monomer fraction would be 3.63 \$ kg⁻¹.⁴³⁵ The largest cost driver for the plant was determined to be the capital cost for the RCF equipment (mostly reactors) required to withstand the high-pressure operation. From the GWP standpoint, the largest contributor was also the RCF area due to the high heat demand for solvent recovery, resulting in 0.079 kg CO₂ eq. per 1 kg of produced lignin fraction. The high heat demand of the RCF area was also one of the two large contributors to the CED next to the energy input required by the farming process, with the CED for the process assessed to be 74 MJ per 1 kg of produced lignin fraction.⁴³⁵ Based on the TEA and the sensitivity analysis of the process parameters, the authors recognise two key cost drivers. Firstly, the reduction in process pressure would significantly reduce the capital cost for the required RCF reactors. Secondly, lowering the solvent loading and the extent of decomposition would reduce the recovery's energy requirement and thereby significantly reduce the CED and GWP. The catalyst contribution to the MSP was assessed to exhibit only minimal impact on the MSP of the lignin fraction.⁴³⁵ However, the authors assume that catalyst replacement would correspond to approximately 26000 kg of processed wood per 1 kg of the catalyst. This is 2-3 orders of magnitude higher compared to the studies done in the lab scale, in which the catalyst has seen sintering, leaching, and poisoning, decreasing the monomer yield from the process.^{417,418} Accordingly, the assumption would only hold valid if effective catalyst regeneration measures were added to the process. While the catalyst cost contribution to the MSP is small when decreasing the catalyst lifetime 12 times, a 2-3 order of magnitude decrease compared to the assumption would already mean that the catalyst cost would be the main cost driver of the obtained RCF oil lignin fraction. Accordingly, additional research on the catalyst deactivation during the process and potential reactivation is required to justify the assumption made in the TEA and LCA analysis.

In addition to the base case, Bartling *et al.* also looked at three additional cases, where instead of methanol/water mixture as a solvent, ethanol:H₂O 85:15 (V:V) mixture with H₂, ethanol:H₂O 1:1 (V:V) mixture without H₂, and ethylene glycol:H₂O 99:1 (V:V) with H₂ were utilised. The reactor pressures in the given processes were 50, 30, and 6 bar, respectively. The ethanol case with hydrogen resulted in the lignin fraction MSP of 1.88 \$ kg⁻¹, while the GWP was net negative with -0.18 kg CO₂ eq. and CED of 77 MJ per 1 kg of lignin. The ethanol case without hydrogen resulted in MSP of 1.34 \$ kg⁻¹ for lignin fraction with GWP of -0.02 CO₂ eq. and CED of 75 MJ per 1 kg of lignin. However, as the monomer fraction was significantly decreased, the MSP of the monomer fraction alone became 7.58 \$ kg⁻¹. The TEA analysis of the ethylene glycol case resulted in a lignin fraction MSP of 1.51 \$ kg⁻¹ with GWP of 0.39 CO₂ eq. and CED of 78 MJ per 1 kg of lignin. As transpires from the analysis of different RCF cases, the MSP of the lignin fraction remains in the same order of magnitude with each case. The ethanol cases with and without hydrogen also provided examples of negative GWP, indicating that an RCF design with net negative GWP is well achievable.

In 2023, Arts *et al.* introduced an additional approach, where RCF products, along with the solvent, are partially recycled to the reactor.²⁸⁹ In their design, Pd/C catalyst with the assumed lifetime of 2 years was used with a reaction temperature of 220°C, utilisation of gaseous H₂ and a plant capacity of 150 kt of wood per year. While the same considerations for the catalyst regarding its recycling hold as for the work by Bartling *et al.*,⁴³⁵ the partial recycling of the process liquor resulted in a considerable decrease in MSP of the RCF lignin fraction.²⁸⁹ Accordingly, Figure 2-16 depicts the nearly 2-fold reduction in RCF lignin MSP when implementing the liquor recycling strategy. Additional reactor cost optimisation, along with liquor recycling, managed to bring the MSP of the lignin fraction to 0.7 € kg⁻¹. Along with the decreasing MSP of the lignin fraction, liquor recycling also allowed a three-fold decrease of the process GWP with 1.5 kg CO₂ eq. per 1 kg of refined lignin oil.

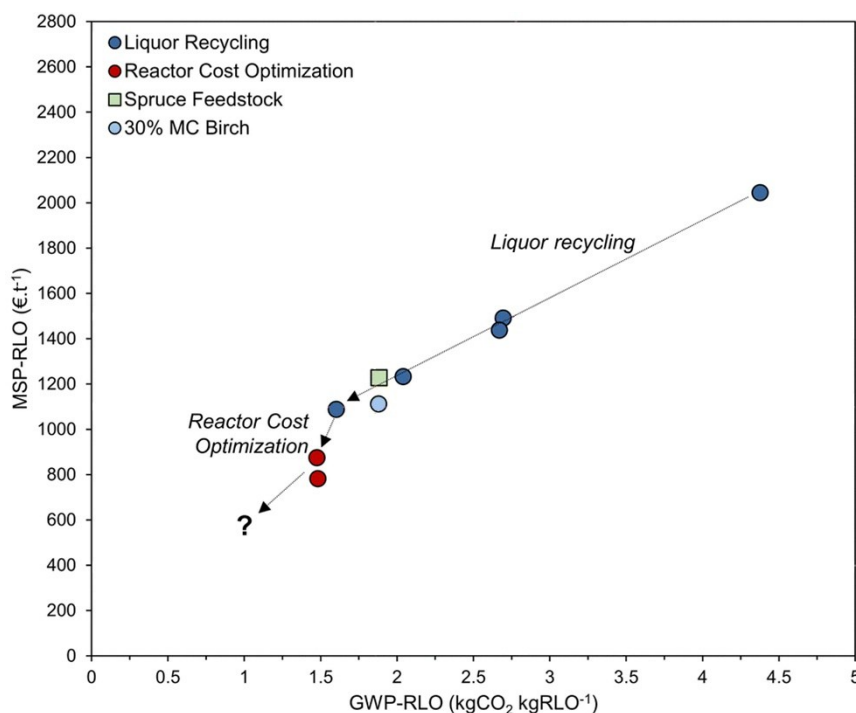


Figure 2-16. Global warming potential (GWP-RLO) vs. minimum selling price (MSP-RLO) of the lignin oil for all cases examined in the work in reference ²⁸⁹. The GWP-RLO represents the scenario where no renewable energy vectors (i.e., renewable hydrogen and electricity) were used in the RCF biorefinery, and the carbon neutrality principle was not applied. This allows to better compare the gains achieved with liquor recycling and reactor cost optimisation compared to the GWP of fossil-based chemicals. GWPs are on average 2 to 4 times smaller when renewable energy resources are used and decrease by $\sim 5 \text{ kgCO}_2 \text{ kgRLO}^{-1}$ under the carbon neutrality principle. Reproduced and modified from reference ²⁸⁹ licensed under [CC BY 3.0](#).

2.6.2. RCF lignin oil downstream upgrading

Obtaining RCF lignin oil at a conceivable price and quantity is one part of the equation for the process industrial implementation. The second part of the equation is finding compatible downstream routes for the products suitable for the market requirements. In this regard, two different strategies could be pursued. Firstly, the lignin-derived monomers could be defunctionalised to make them compatible raw materials for the established synthesis routes in the petrochemical industry. Secondly, the existing functionality in the lignin-derived monomers can be substituted or built on to facilitate highly atom-efficient production of fine chemicals.

Utilising the first approach, Liao *et al.* recently demonstrated the conversion of the lignin-derived methoxylated and alkylated phenols in RCF lignin oil into phenol and propylene.⁴²⁷ In doing so, the lignin-derived monomers were first hydroprocessed over Ni/SiO₂ at 285°C to produce a stream rich in n-propylphenols and n-ethylphenols. The obtained mixture was then

funnelled into a dealkylation process with a ZSM-5 (Z140-H) catalyst. As a result of the two additional catalytic processes, 20 wt% phenol and 9 wt% propylene yields were achieved based on the initial lignin content in birch wood. Based on the experimental results of printing ink production from the oligomeric lignin fraction in RCF lignin oil, the authors carried out TEA and LCA for a potential industrial plant producing 100 kt of bio-based phenol annually. The proposed case resulted in a high IRR of 23% with a payout time of only four years.⁴²⁷ Similarly to the discussion in the previous subsection, the authors indicate the capital cost of pressure reactors as the highest driver of the production price. The GWP of both phenol and propylene production was estimated to be considerably lower compared to the current petrochemical routes. Accordingly, the proposed phenol production resulted in 0.74 kg CO₂ eq. (vs. 1.73 kg CO₂ eq. on petrochemical route) and propylene in 0.47 kg CO₂ eq. (vs 1.47 kg CO₂ eq. respectively).⁴²⁷

While the study by Liao *et al.* paved a potential pathway for the production of phenol and propylene from the RCF process,⁴²⁷ the paradigm of lignin (and hemicellulose) derived monomers defunctionalisation to fill in the established petrochemical synthesis routes can be expanded to a more extensive range of commodity chemicals. In the previously introduced study by Bartling *et al.*, the MSP of RCF lignin oil and its fractions were compared with a variety of commodity chemicals potentially obtainable from the products of RCF lignin oil. Accordingly, Figure 2-17 displays the production volume and price of the commodity chemicals that could be potentially derived from the RCF process. As transpires from the comparison as well as from the study by Liao *et al.*,⁴²⁷ the lignin- and hemicellulose-derived streams from the RCF process can be competitively priced in the very high volume markets. However, further advances in downstream processing strategies are much needed to facilitate a step-change in the implementation of the RCF process in this paradigm.

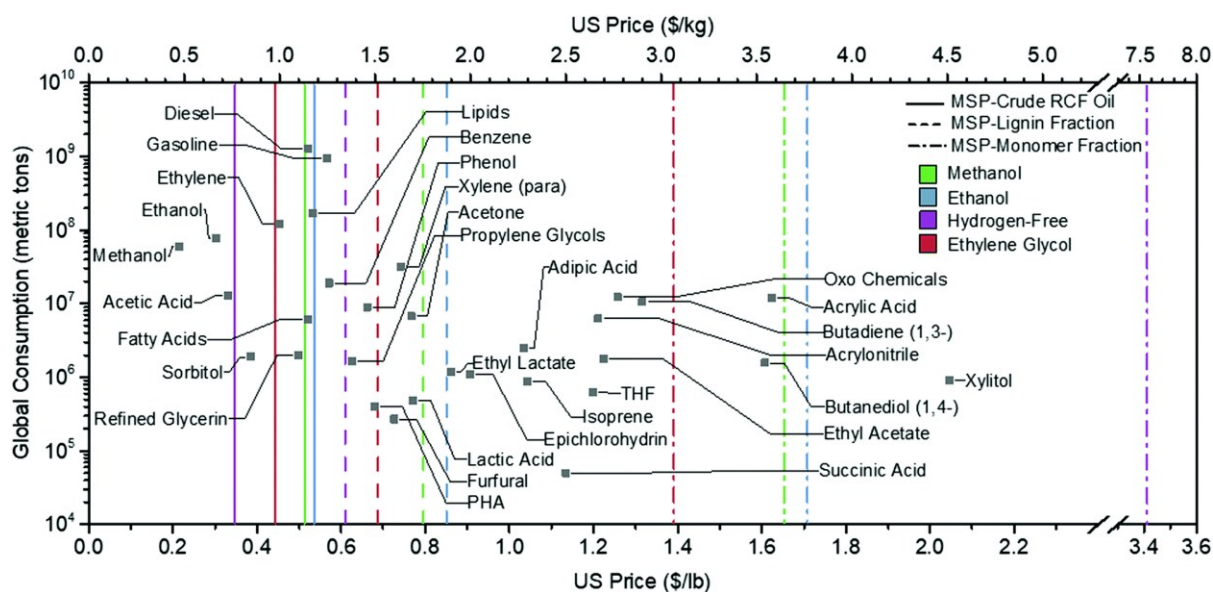


Figure 2-17. Minimum RCF oil selling prices with the U.S. price and global consumption of various chemicals for reference. The U.S. Price and Global Consumption data were sourced from a 2016 report by Bidy et al. and are 3–5 year averages.⁴³⁶ Tabulated data are summarised in the supplementary information of the article. Reproduced from reference⁴³⁵ licensed under [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/).

The second approach for downstream upgrading is utilising lignin-derived monomers' inherent functionality to provide an atom-efficient platform for fine chemical and polymer synthesis. In a recent study by Afanasenko *et al.*, several synthesis pathways leading to biologically active agents are highlighted from C2 ethylene glycol stabilised guaiacols obtained by diol-assisted lignin-first biorefining.⁴³⁷ As the lignin-derived aromatic compounds incorporate structural moieties often found in biologically active compounds due to their common biosynthetic origin, the study demonstrates the strategy's feasibility for producing active pharmaceutical ingredients.⁴³⁷ Concerning the lignin-derived monomers from the RCF process, Wu *et al.* recently introduced Raney Ni catalysed amination of phenolic alcohols, rendering a production route for γ -aminated phenols.⁴³⁸ Accordingly, a precursor for several pharmaceuticals was obtained. In addition, phenolic alcohols that are the primary monomeric product in the Raney Ni catalysed RCF process under the conditions utilised throughout this work can undergo functionalisation to form intermediates useful in synthesising heterocyclic compounds, which are known for their pharmacological activities.^{439–444} While the fine-chemicals routes from the RCF process lignin-derived monomers are still largely unexplored, the strategy has the potential to valorise biomass components order(s) of magnitude higher compared to the defunctionalisation of lignin-derived monomers to existing platform

chemicals. In addition to the fine chemicals, the lignin-derived monomers from the RCF process have been shown to serve as a successful precursor for polymer synthesis, indicating that a paradigm where the starting point is a more functionalised molecule could serve as a viable technology.^{397,443,445,446}

Herein, it is important to highlight that for the complete valorisation of the biomass, oligomeric (and polymeric) lignin fractions resulting from the RCF process should also be emphasised in the context of the product portfolio. Due to the suppression of lignin condensation during the RCF process, the resulting higher molecular weight lignin fraction is structurally different from the current technical lignins. Accordingly, the polymeric fraction has the potential to be utilised in materials and polymers that are yet to be explored.

2.7. Research objectives

In the journey of lignin molecules through the reductive catalytic fractionation process, a complex list of factors, each influencing the outcomes of reductive catalytic fractionation, was witnessed. As a result, understanding the processes taking place during an RCF process for controlling its outcomes poses a vast challenge to current researchers. At the same time, the large variety of independent and dependent process variables also offers an opportunity to develop novel routes to replace current oil-based chemical production. In addition, an entirely new class of platform chemicals and polymers could be obtainable. Considering the large number of variables affecting the result of an RCF process, the current work aims to enhance the understanding of the chemical transformation taking place during the RCF process. On top of that, new approaches aiming to enhance the process outcomes are introduced.

Accordingly, Chapter 3 introduces a solvent fractionation strategy for molecular profiling of different molecular weight lignin fractions in RCF lignin oil. In this process, an additional lignin stabilisation mechanism is discovered, which also incorporates polymeric lignin moieties. In addition, the chemical nature of oligomeric carbohydrates in the lignin oil is determined, and a new metric based on the UV-VIS absorption for the lignin is introduced. Chapter 4 explores the effect of mechanocatalytic pretreatment with different additives prior to the RCF process. Due to the need for biomass comminution before the RCF process, it is discovered that adding acetic acid to the ball-milling process enhances monomer yields and improves the pulp quality.

In Chapter 5, a semi-batch temperature-stepping RCF strategy is introduced, emphasising the importance of process conditions, and not necessarily the catalyst alone, in determining the product portfolio and monophenols selectivity.

3. Solvent fractionation strategy for detailed insights into lignin stabilisation and carbohydrate chemical nature in the H-transfer RCF process

Some of the results presented in this chapter are published in the article "Lignin Stabilization and Carbohydrate Nature in H-transfer Reductive Catalytic Fractionation: The Role of Solvent Fractionation of Lignin Oil in Structural Profiling" and can be found under the reference ²³⁹.

3.1. Introduction

Reductive catalytic fractionation of lignocellulosic biomass produces lignin oil containing lignin-derived monoaromatic phenolics as the primary products along with oligomeric and polymeric lignin fragments and carbohydrate-derived molecules. Alongside the lignin oil, delignified high-quality pulp is isolated.^{413,419,447} Dependant on the RCF process conditions, lignin oils obtained under the low severity conditions (e.g. H-transfer RCF using Raney Ni and 2-propanol/water at <200°C and <6h) can contain a substantial amount of lignin oligomers and polymers with a significant amount of heavy lignin fractions (>2000 Da).^{27,448}

Despite the rapid advancements in RCF strategies over the past decade, there is still very little information about the chemical nature of the lignin oligomers/polymers as well as the carbohydrates in the literature. Given the inherent complexity of the lignin oil matrix, a fractionation strategy can potentially reduce the complexity and thereby aid in deconvolving complex data (e.g., HSQC NMR measurements). This, in turn, would enable the elucidation of the structural features of lignin oligomers/polymers and carbohydrates in lignin oils. Furthermore, obtaining lignin oil fractions in different molecular weight ranges would facilitate determining the structural heterogeneity across the entire molecular weight distribution of lignin. Structural profiling is also essential for answering the pending research question about whether lignin stabilisation on the catalyst encompasses all lignin species in bulk medium or is only limited to low-MW species. Accordingly, facile and environmentally friendly methods for lignin oil fractionation are needed to further accelerate the progress in RCF research.

Solvent fractionation serves as a potential pathway for lignin oil separation into various fractions. Accordingly, solvent fractionation techniques have been extensively investigated on

technical lignins. Recently, Gigli and Crestini published a comprehensive review on the industrial technical lignins fractionation.⁴⁴⁹ They outlined that solvent fractionation encompasses 85% of all fractionation processes, while membrane-based technologies account for 15%.^{449–455} Concerning solvent fractionation, Gosselink *et al.* created a method for technical lignin fractionation that utilises lignin powder packed with inert particles in a column, where a series of increasing polarity solvents were used to solubilise different lignin fractions.⁴⁵⁶ A fractionation method using expanded solvent systems (2-methyltetrahydrofuran/CO₂) was recently reported by Leitner group.⁴⁵⁷ Adjusting the CO₂ pressure in the expanded solvent system, the method proved highly selective in separating lignin fractions in different MW ranges.

In RCF research, solvent fractionation methods have been primarily employed for the isolation of low-MW lignin-derived products. Sels group utilised ethyl acetate (EtOAc) and n-heptane in the work-up of lignin oil from pine wood, thereby facilitating significant progress in characterising lignin dimers and trimers.^{458,459} However, despite the reported use of solvent fractionation as a work-up method of lignin oil to separate the low-MW fraction of lignin oil, it has not yet been deployed as a method for the structural profiling of RCF lignin oil oligomeric/polymeric fractions.

In this chapter, phenomenological analysis of the solvent fractionation of RCF lignin oil obtained from poplar wood in the presence of Raney Ni and 2-propanol as the H-donor (2-PrOH/H₂O 7:3, v/v) at 200°C for 3 h is reported. It was found that utilising environmentally friendly solvents methanol (MeOH), acetone, and EtOAc allows for the production of lignin oil fractions in different MW ranges. Furthermore, the solvents can also be used in sequence to further broaden the range of different lignin oil fractions. This outcome allowed the structural heterogeneity of lignin products to be addressed and distinctive structural features imprinted by Raney Ni on lignin products and carbohydrates in the H-transfer RCF process to be elucidated. Accordingly, this chapter's results and discussion section contains four main parts. The first part presents a phenomenological analysis of solvent fractionation of RCF lignin oil with the three solvents. The second part expands the concept to sequential solvent fractionation with the same three solvents. The third part examines the fractions thoroughly using high-resolution HSQC NMR spectroscopy and elucidates the structural features created

by reductive processes of Raney Ni on lignin fragments and carbohydrates. The final fourth part discusses the Raney Ni role in lignin fragments stabilisation during the H-transfer RCF process.

3.2. Results and discussion

The current study began with a serendipitous finding, when during an attempt to dissolve RCF lignin oil in anhydrous acetone, a pale beige precipitate and yellow solution were obtained. This was somewhat surprising as acetone and its mixtures with polar solvents like alcohols and water are well known to have excellent lignin-extracting properties recently explored in several Organosolv process variants.^{460–462} However, as precipitate was formed due to the fractional dissolution of the RCF lignin oil, anhydrous acetone proved to dissolve monomeric and oligomeric lignin products while precipitating mid- and high-MW lignin fragments along with carbohydrates.

Table 3-1. Hildebrand solubility parameters (δ) along with Hansen parameters (δ_D , δ_P , δ_H) for lignins, lignin-derived monophenols, and selected solvents. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

Substance	$\delta(\text{MPa}^{0.5})$	$\delta_D(\text{MPa}^{0.5})$	$\delta_P(\text{MPa}^{0.5})$	$\delta_H(\text{MPa}^{0.5})$
Lignins (Chapter 2.2.1)	22.5-31.7	16.1-21.9	4.9-18.4	10.2-21.8
Lignin-derived monophenols ^{235,463}	22.1-26.7	17.0-19.3	4.3-8.2	12.5-17.3
MeOH	29.6	15.1	12.3	22.3
Acetone	19.9	15.5	10.4	7.0
EtOAc	18.2	15.8	5.3	7.2
Water	47.8	15.5	16.0	42.3

Motivated by the finding, Hansen solubility parameters discussed in detail in Chapter 2.2.1 were considered to select other potentially feasible solvents to obtain different cuts of low-or high-MW lignin fragments in RCF lignin oil. While δ_D and δ_H for lignins and lignin-derived monophenols are similar, the δ_P values for lignin-derived monophenols are considerably lower than those for lignins (Table 3-1). Accordingly, it is reasoned that δ_P could be a useful parameter for selecting the solvents to separate lignin fractions in different MW ranges. Therefore, anhydrous ethyl acetate and anhydrous methanol were selected along with

anhydrous acetone for the lignin oil solvent fractionation (Table 3-1). Importantly, water presents a high δ_P value (16.0 MPa^{0.5}) and can contribute to levelling the δ_P values of MeOH, acetone, or EtOAc to match the δ_P values of lignins. In such case, the dissolution extent of oligomeric/polymeric lignin fractions in RCF lignin oils would increase. Accordingly, anhydrous solvents and dried RCF lignin oil are necessary for reproducible results.

3.2.1. Phenomenological analysis of solvent fractionation

Figure 3-1 summarises the RCF lignin oil fractionation results, displaying the visual appearance of the isolated fractions using MeOH, acetone, and EtOAc. The RCF lignin oil presented the highest solubility in MeOH ((88±1% in MeOH vs. 56±1% in acetone vs. 50±1% in EtOAc), resulting in the darkest solution out of the three solvents. After drying, the MeOH-insoluble fraction was recovered as a dark reddish-brown solid corresponding to 4±1% of the lignin oil in weight. The solvent fractionation with acetone and EtOAc resulted in yellow solutions, with the acetone solution darker in shade. The acetone-insoluble fraction constituted 35±3% of the RCF lignin oil in weight, while the EtOAc-insoluble fraction did 51±1%. Both fractions were recovered as pale beige-coloured hygroscopic solids. Once exposed to humidity, they can turn back to a highly viscous oil.

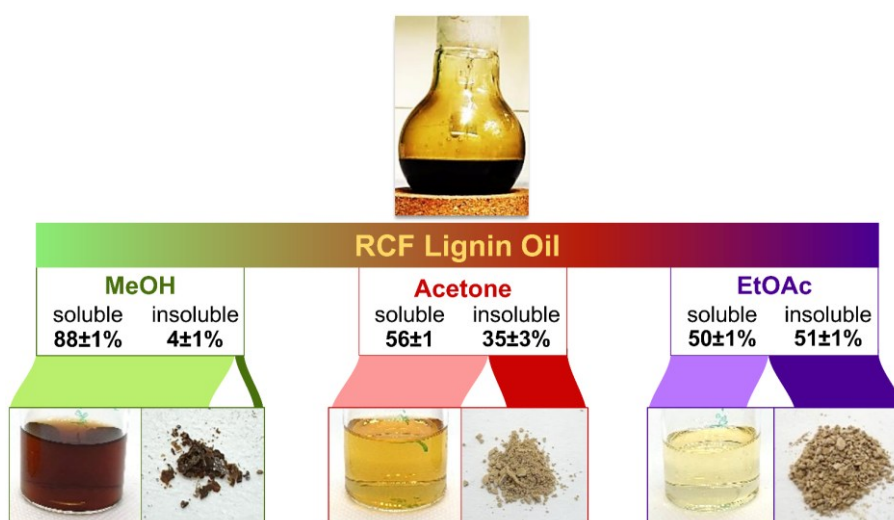


Figure 3-1. Initial observations of the solvent fractionation of RCF lignin oil in MeOH, acetone, or EtOAc. The pictures present the visual appearance of the soluble and insoluble fractions. Pictures of the soluble fractions were taken from the supernatant solution. The initial mass of RCF oil dispersed in each solvent was identical. Losses of volatile components (carried by solvent removal via rotoevaporation) are associated with an overall recovery below 100%. In all experiments, the overall recovery was higher than 90%. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

To examine the selectivity of the solvent fractionation, the apparent MW distribution of the fractions was obtained by gel permeation chromatography (GPC). Figure 3-2 displays the GPC chromatogram traces of different fractions, while Table 3-2 summarises the values for GPC trace onset and the apparent weight-average molecular weight M_w . The fraction insoluble in MeOH shows an apparent M_w value of 16900 Da, and chromatogram signal narrower than the RCF lignin oil. The comparison between acetone- and EtOAc-insoluble fractions reveals that mid-MW lignin fragments (from 1800-12800 Da) were less abundant in acetone-insoluble fraction, implying that mid-MW lignin was more soluble in acetone than in EtOAc. As a result, the acetone-insoluble fraction presented a higher M_w value than the EtOAc-insoluble fraction (6500 Da vs. 4800 Da, Table 3-2). As determined by gas chromatography (GC), all three insoluble fractions contained only trace amounts of monomer products (<0.1 wt% in total).

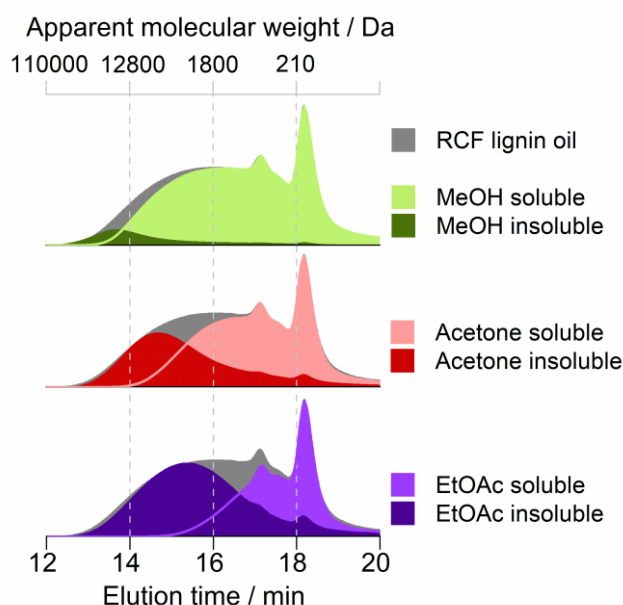


Figure 3-2. GPC traces for RCF lignin oil and its fractions insoluble or soluble in MeOH, acetone, or EtOAc. The traces were recorded at a wavelength of 270 nm. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

Table 3-2. Apparent values of M_w and GPC trace onset for the RCF lignin oil and its insoluble and soluble fractions in MeOH, acetone, and EtOAc. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

Fraction	GPC trace onset (Da)	Apparent M_w (Da)
RCF Lignin oil	36600	3400
MeOH-insoluble	60700	16900
MeOH-soluble	20500	2300

Acetone-insoluble	32000	6500
Acetone-soluble	8700	1200
EtOAc-insoluble	29700	4800
EtOAc soluble	3000	500

The selective removal of heavy species decreased the GPC trace onsets from 36600 Da for the RCF lignin oil to 20500 Da for the MeOH-soluble fraction, 8700 Da for the acetone-soluble fraction, and 3000 Da for the EtOAc-soluble fraction (Figure 3-2). Therefore, the apparent M_w values decreased from 3400 Da for the RCF lignin oil to 2300 Da for the MeOH-soluble fraction, 1200 Da for the acetone-soluble fraction, and 500 Da for the EtOAc-soluble fraction (Table 3-2). Importantly, the ability of acetone, and particularly EtOAc, to selectively dissolve low-MW lignin-derived species implies that the soluble fractions become proportionally enriched in monophenol products. Accordingly, the monophenol content increased from 11% in RCF lignin oil to 26% in the EtOAc-soluble fraction (Figure 3-3). The selectivity of EtOAc to dissolve low-MW species in RCF lignin oil agrees well with the similarity in HSP values between lignin-derived monomers and EtOAc. The δ_p parameter for EtOAc ($5.3 \text{ MPa}^{0.5}$) agrees more closely with those of the monomer products ($4.3\text{-}8.2 \text{ MPa}^{0.5}$) than those reported for lignins ($4.9\text{-}18.4 \text{ MPa}^{0.5}$) as indicated in Table 3-1. As EtOAc presents HSP much more distant from those reported for lignins, it is less suitable for dissolving lignin oligomers and polymers.

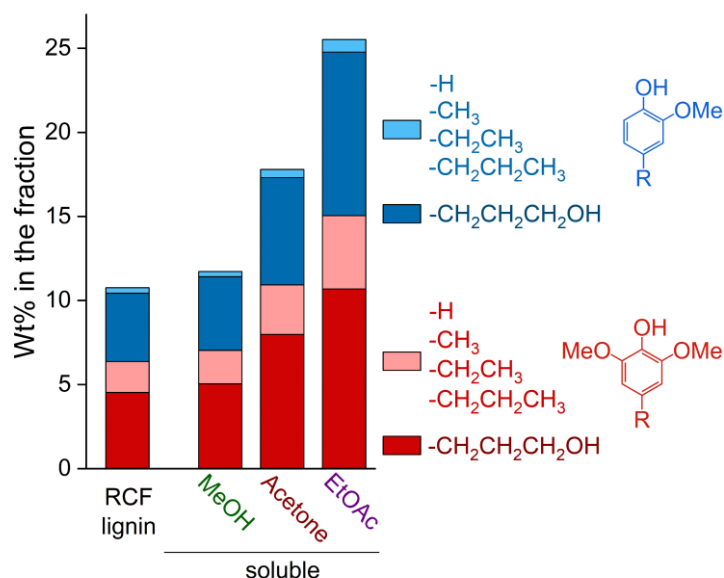


Figure 3-3. Content (wt%) of different lignin monomers in the RCF lignin oil and MeOH-, acetone-, and EtOAc-soluble fractions as determined by GC-FID. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

In addition to utilising a single wavelength to depict lignin molecular weight distribution near phenolic species absorption maxima, the photodiode array (PDA) detector also allows to obtain information in a broader spectral range. In native lignin linkages formed from canonical lignin monomers, lignin monomeric units are not conjugated with each other, except for 5-5' linkages in dibenzodioxocin moieties. However, the content dibenzodioxocin moieties in hardwoods is very low (<1%)²⁰. In addition, lignin *p*-hydroxybenzoate groups decorating lignin backbone as pendant groups in hardwoods contain α -carbonyl group conjugated with the aromatic ring. Nevertheless, parabens with a very similar structure to the pendant groups do not absorb light above 300nm.⁴⁶⁴ Therefore, it is suitable to state that hardwood lignin in its native form does not have significant absorption in wavelengths above 320nm.⁴⁶⁵

During the lignin extraction from the biomass, reactive lignin intermediates are prone to undergo condensation (and oxidation), thereby forming lignin domains with a greater extent of conjugation. Further delocalisation of π -electrons narrows the energy band between π and antibonding π^* orbitals, thereby causing a redshift in more conjugated lignin spectra.⁴⁶⁶ Utilising the rationale, a new metric for lignin characterisation- Spectral Index (SI), is hereby introduced. Considering that the absorption maxima of lignin is in the proximity of 280nm as for phenol in most solvents, Structural Index is defined as:

$$SI_{xxx} = 1 - \frac{A(xxx \text{ nm})}{A(280 \text{ nm})}$$

where A denotes the absorbance at 280 nm and at the selected wavelength. Additional work in our research group not in the scope of the current thesis utilises different isolation methods with the same eucalyptus biomass to lay a broader foundation for the Structural Index metric. Accordingly, it is demonstrated that Kraft and Organosolv processes result in low structural indexes (for Kraft lignin, the average $SI_{320}=0.46\pm0.03$ and $SI_{350}=0.73\pm0.04$, and for Organosolv lignin 0.42 ± 0.11 and $SI_{350}=0.65\pm0.02$ respectively), while the lignin obtained by RCF process displays considerably higher SI values ($SI_{320}=0.72\pm0.11$ and $SI_{350}=0.86\pm0.07$). Therefore, the relative abundance of condensed and/or oxidised species is higher in Kraft and Organosolv lignins compared to the RCF lignin and is well in line with established knowledge on the isolation methods. A full spectra of the isolated lignins along the molecular weight distribution, as well as the SI_{320} , SI_{350} , SI_{400} , and SI_{425} values at different apparent molecular weights for the study, can be found in Appendix Figures A-2 and A-3.

The Structural Index approach based on the PDA detector response in GPC analysis was also applied in the current study. Accordingly, Figure 3-4 displays the SI_{350} values for the unfractionated RCF lignin oil and the fractions soluble and insoluble in the selected solvents. Further considerations for the data processing and plotting for the SI index can be found in the Experimental section.

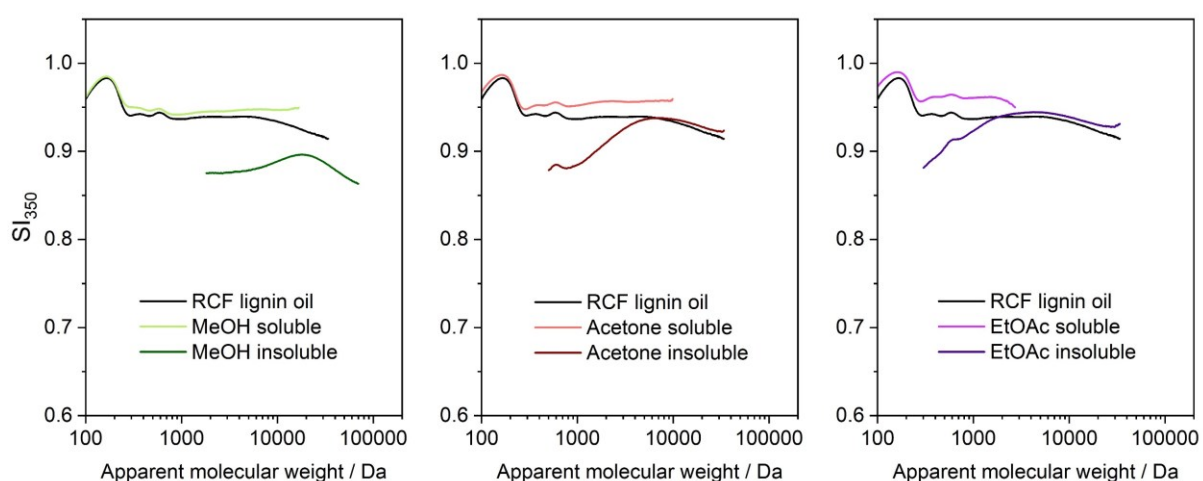


Figure 3-4. Structural Index at 350nm (SI_{350}) for the RCF lignin oil and fractions soluble and insoluble in MeOH, acetone, and EtOAc across the apparent molecular weight distribution from the column exclusion limit of 100 Da.

The unfractionated RCF lignin oil displayed a very high average SI_{350} value of 0.94 ± 0.01 , with a slight decline in SI_{350} towards the higher MW values. With decreasing M_w value of the soluble fractions from MeOH to EtOAc, the average SI_{350} values increased from 0.95 ± 0.01 for MeOH-soluble fraction to 0.97 ± 0.01 for EtOAc-soluble fraction. The same trend holds for the insoluble fractions, where the fraction insoluble in MeOH displayed the lowest SI_{350} value (0.88 ± 0.01) and the EtOAc-insoluble fraction the highest (0.93 ± 0.01). Therefore, the higher MW lignin species present in RCF lignin oil tend to have a lower SI value compared to the low MW counterpart. Importantly, solvent fractionation does not result in a “hard cut” of different MW fractions, and both soluble and insoluble fractions contain species that share the same apparent molecular weight (Figure 3-2). A comparison of the SI_{350} values at shared apparent MWs reveals that the oligomeric lignin species in insoluble fractions display considerably lower SI values than the soluble counterparts with the same apparent MW (Figure 3-4). Accordingly, solvent fractionation not only allows obtaining lignin fractions at different molecular weight ranges but also separates lignin fractions with different chemical natures sharing the same apparent MW.

To assess the impact of solvent fractionation on the elemental composition of the lignin oil fractions, elemental analysis results were arranged in a Van Krevelen diagram with the corresponding H/C and O/C values (Figure 3-5). The H/C ratio of the insoluble fractions ranged between 1.22-1.38 and the O/C ratio between 0.44-0.69. Notably, the H/C and O/C ratios of MeOH-insoluble fraction matched well to those estimated for theoretical hardwood lignin (actual vs. estimated value: H/C 1.22 vs. 1.25, O/C 0.44 vs. 0.44, for details, refer to Appendix Figure A-4). In contrast, the H/C and O/C values for acetone- and EtOAc-insoluble fractions became more distant from MeOH insoluble fraction and were shifted towards hemicellulose (H/C~1.60 and O/C~0.80) and cellulose (H/C~1.67 and O/C~0.83). This result indicates that both acetone- and EtOAc-insoluble fractions contain considerable amounts of carbohydrates.³⁷² The RCF lignin oil and the MeOH-soluble fraction presented a similar positioning on Van Krevelen diagram owing to the high solubility of lignin oil in MeOH. Acetone- and EtOAc soluble fractions displayed O/C values lower than those expected for lignin (0.38 vs. 0.44) and H/C values higher than expected for lignin (1.52 and 1.60 vs. 1.22). The high H/C ratio can partly be attributed to the large share of HDO products in low MW

lignin as a result of the RCF process. The monophenolic products presented the alkyl sidechains extensively hydrodeoxygenated (i.e., full removal of O-functionalities on C $_{\alpha}$ and C $_{\beta}$ and, to a much lesser degree, on the C $_{\gamma}$ position). Furthermore, the hemicellulose-derived diols and polyols,^{27,389,467} also contributed to increasing the H/C and O/C values.

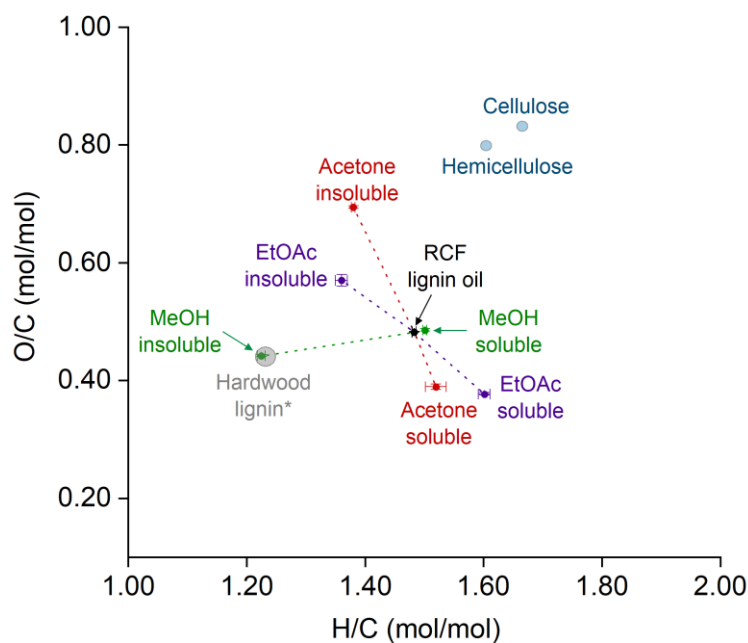


Figure 3-5. Van Krevelen diagram arranging the RCF lignin oil and its different fractions according to their H/C and O/C values. The grey circle region is centred on the O/C and H/C hypothetical values for hardwood lignins (for details, refer to Appendix Figure A-4). The blue circles present the regions for the H/C and O/C coordinates of cellulose and hemicellulose. The dashed lines guide the eye to the soluble and insoluble fractions obtained by fractionation in each solvent. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

3.2.2. Sequential solvent fractionation

Solvent fractionation with MeOH, acetone, and EtOAc demonstrated that the three solvents are suitable for dissolving lignin fractions in different MW ranges. Furthermore, acetone and EtOAc also precipitated carbohydrates present in RCF lignin oil. Considering the different lignin cut-off MW for dissolving in the three given solvents, a sequential solvent fractionation strategy was developed (Figure 3-6). In the first step, RCF lignin oil was fractionated with MeOH as before, resulting in the dissolution of 88 wt% of the lignin oil and precipitation of 4 wt%. The MeOH-soluble fraction is fractionated with acetone in the second step, resulting in acetone-soluble (SSF) fraction (59 wt% of RCF lignin oil, 67 wt% of MeOH-soluble fraction) and pale beige precipitate as acetone-insoluble (SSF) fraction (29 wt% of RCF lignin oil, 34 wt% of

MeOH-soluble fraction). The 'SSF' in brackets stands for sequential solvent fractionation to distinguish the fractions from the ones obtained by fractionation with a single solvent. In the third step, the obtained acetone-soluble (SSF) fraction was further fractionated with EtOAc, resulting in EtOAc-soluble (SSF) fraction (54 wt% of RCF lignin oil, 92 wt% of acetone-soluble (SSF) fraction) and EtOAc-insoluble (SSF) fraction (5 wt% of RCF lignin oil, 9 wt% of acetone-soluble (SSF) fraction). In a hypothetical case, where intermolecular interactions among lignin-derived molecules and carbohydrates are negligible compared to the interactions with the solvent, the acetone-soluble (SSF) fraction obtained from the MeOH-soluble fraction should match in yield with the acetone-soluble fraction yield obtained from RCF lignin oil. By the same reasoning, the EtOAc-soluble (SSF) fraction yield should match the yield of the EtOAc-soluble fraction obtained by fractionating lignin oil with EtOAc alone. However, the yield of the acetone-soluble (SSF) fraction obtained in sequential fractionation exceeds the soluble fraction yield obtained by fractionation only with acetone (59 wt% vs 56 wt%), and the yield of EtOAc-soluble (SSF) fraction exceeds the soluble fraction yield obtained by fractionation only with EtOAc (54 wt% vs 50 wt%). The discrepancy between the soluble fraction yields can be a result of interactions between high-MW and mid-MW lignin species, as the presence of high-MW species in RCF lignin oil favours further precipitation of mid-MW lignin species during fractionation only with acetone and EtOAc. However, it can also be a result of experimental uncertainty. As the focus of this study is the demonstration of the concept of RCF lignin oil solvent fractionation along with the structural characterisation of different lignin fractions, the hypothesis is retained for future studies.

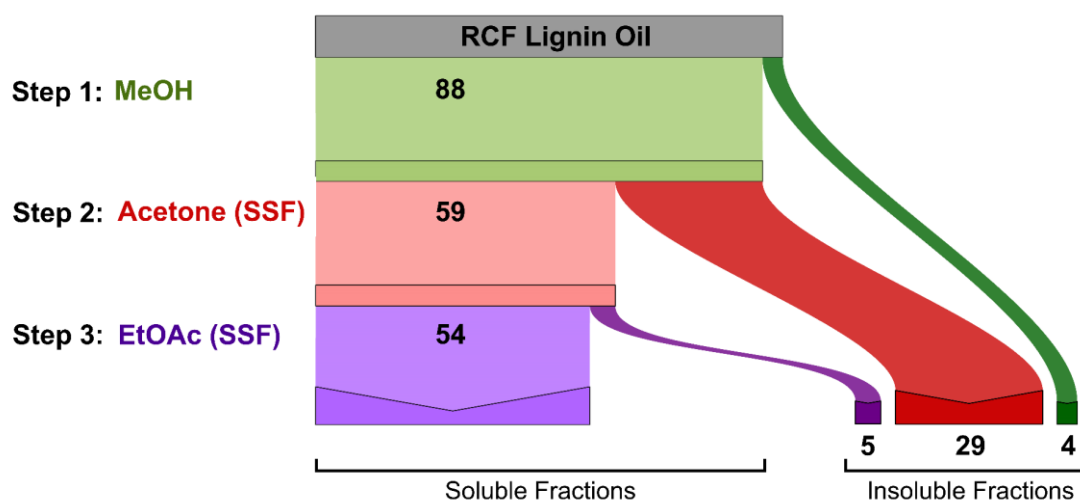


Figure 3-6. Overview and mass balances for sequential solvent fractionation strategy. The numbers are given as wt% of the initial RCF lignin oil. The 'SSF' in brackets stands for sequential solvent fractionation to distinguish the fractions from the ones obtained by fractionation with a single solvent.

To assess the lignin molecular weight distribution in the fractions obtained by sequential solvent fractionation, GPC analysis was carried out as for the single solvent fractionation. Accordingly, Figure 3-7 displays the chromatogram traces of different fractions obtained after each step, and Table 3-3 summarises the GPC trace onset values and the apparent weight-average molecular weight M_w .

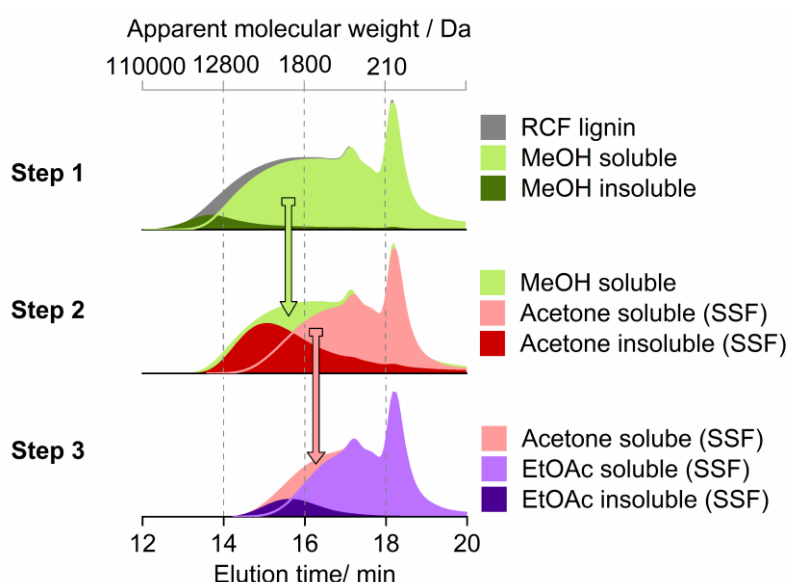


Figure 3-7. GPC traces for RCF lignin oil and its fractions obtained by sequential solvent fractionation. The traces were recorded at a wavelength of 270 nm.

Table 3-3. Apparent values of M_w and GPC trace onset for the RCF lignin oil and its various fractions obtained by sequential solvent fractionation with MeOH, acetone, and EtOAc.

Fraction	GPC trace onset (Da)	Apparent M_w (Da)
RCF Lignin oil	36600	3400
MeOH-insoluble	60700	16900
MeOH-soluble	20500	2300
Acetone-insoluble (SSF)	18600	4100
Acetone-soluble (SSF)	7200	1000
EtOAc-insoluble (SSF)	9200	2800
EtOAc soluble (SSF)	3900	700

Due to the removal of the highest-MW lignin fraction by fractionation with MeOH, the trace onset for acetone-insoluble (SSF) decreased along with apparent M_w compared to insoluble fraction obtained by RCF lignin oil fractionation just with acetone (Onset: 18600 Da vs. 32000 Da, M_w : 4100 Da vs. 6500 Da). The acetone soluble fractions from SSF and direct RCF lignin oil fractionation with acetone displayed somewhat similar GPC characteristics (Onset: 7200 Da vs. 8700 Da, M_w : 1000 Da vs. 1200 Da). By fractionating the acetone-soluble (SSF) fraction with EtOAc, the formed EtOAc-insoluble (SSF) fraction trace onset falls to 9200 Da due to the preceding solvent fractionation with MeOH and acetone. Similarly to fractionations with acetone, EtOAc-soluble (SSF) fraction and EtOAc-soluble fraction obtained directly from RCF lignin oil share similar GPC characteristics (Onset: 3900 Da vs. 3000 Da, M_w : 700 Da vs. 500 Da). Importantly, GPC analysis reveals that sequential solvent fractionation can produce a distinctive mid-MW (800-9200 Da) lignin fraction (EtOAc-insoluble (SSF)) from Acetone-soluble (SSF) fraction.

The previously introduced Structural Index metric was also applied to the fractions obtained by sequential solvent fractionation. Accordingly, Figure 3-8 displays SI_{350} values for the different fractions across the molecular weight distribution. The unfractionated RCF lignin oil along with MeOH-soluble and insoluble fractions are already displayed in Figure 3-4, but are given for reference as the MeOH-soluble fraction is used as a starting material for fractionation with acetone.

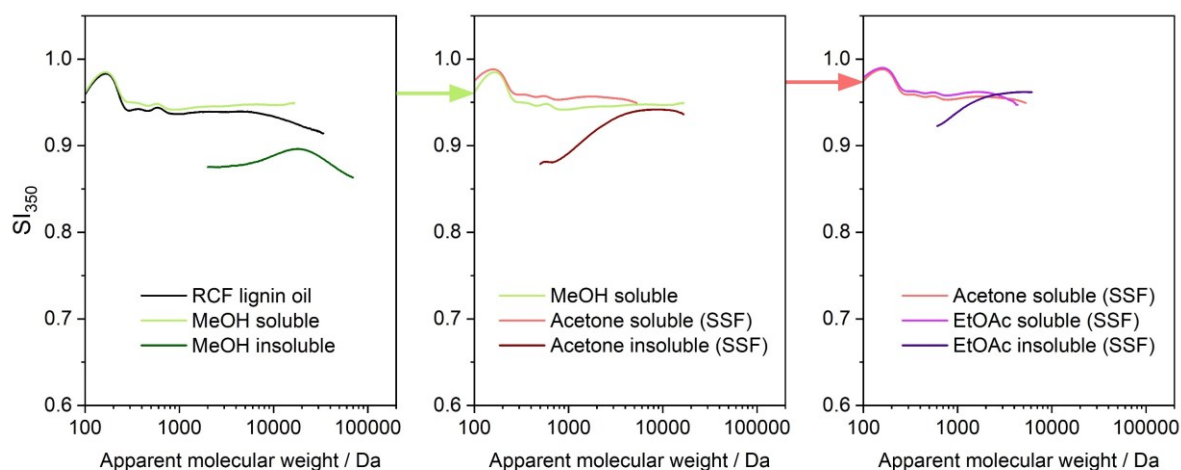


Figure 3-8. Structural Index at 350nm (SI_{350}) for the RCF lignin oil and the lignin oil fractions obtained by sequential solvent fractionation strategy. The apparent molecular weight is plotted from the column exclusion limit of 100 Da.

The fractionation of MeOH-soluble fraction with acetone resulted in identical average SI_{350} values for the acetone-soluble (SSF) and acetone-insoluble (SSF) fractions compared to the RCF lignin oil fractionation with acetone only ($SI_{350}=0.96\pm0.01$ and $SI_{350}=0.92\pm0.01$ respectively). Similarly, further fractionation of the acetone-soluble fraction (SSF) with EtOAc resulted in EtOAc-soluble fraction (SSF) displaying the same SI_{350} value as obtained by single solvent fractionation (0.97 ± 0.01). However, due to the prior removal of MeOH-insoluble and acetone-insoluble fractions, the EtOAc-insoluble fraction (SSF) displayed a very high SI_{350} value (0.95 ± 0.01). Accordingly, sequential solvent fractionation enables obtaining oligomeric lignin fraction with a very low content of condensed and/or oxidised lignin species.

To examine the elemental composition and nature of the different fractions obtained by sequential solvent fractionation, the elemental analysis results were plotted on the Van Krevelen diagram (Figure 3-9). During the fractionation of MeOH-soluble fraction with acetone, the obtained acetone-soluble (SSF) fraction displays almost identical H/C and O/C ratios compared to the soluble fraction obtained by direct RCF lignin oil fractionation with acetone (H/C 1.53 vs. 1.52, O/C 0.39 vs. 0.39). Similarly, soluble fractions from EtOAc fractionations stand very closely on Van Krevelen diagram (EtOAc-soluble (SSF) vs. EtOAc-soluble, H/C 1.55 vs. 1.60, O/C 0.40 vs. 0.38). As the MeOH-insoluble fraction isolated from RCF lignin oil is relatively small, both the acetone-insoluble (SSF) fraction and the acetone-insoluble fraction isolated directly from lignin oil display similar H/C and O/C ratios (H/C 1.36 vs. 1.38,

O/C 0.67 vs. 0.69). Accordingly, the acetone-insoluble (SSF) fraction contains the carbohydrates present in RCF lignin oil. In contrast to the aforementioned fractions from sequential solvent fractionation that closely reassemble the ones obtained from direct fractionation of RCF lignin oil, the EtOAc-insoluble (SSF) fraction is a unique cut of mid-MW lignin compounds. The H/C and O/C ratio of the fraction closely matches the one estimated for theoretical hardwood lignin (H/C 1.25 vs. 1.25, O/C 0.46 vs. 0.44), indicating that the fraction contains polymeric lignin and is carbohydrate free.

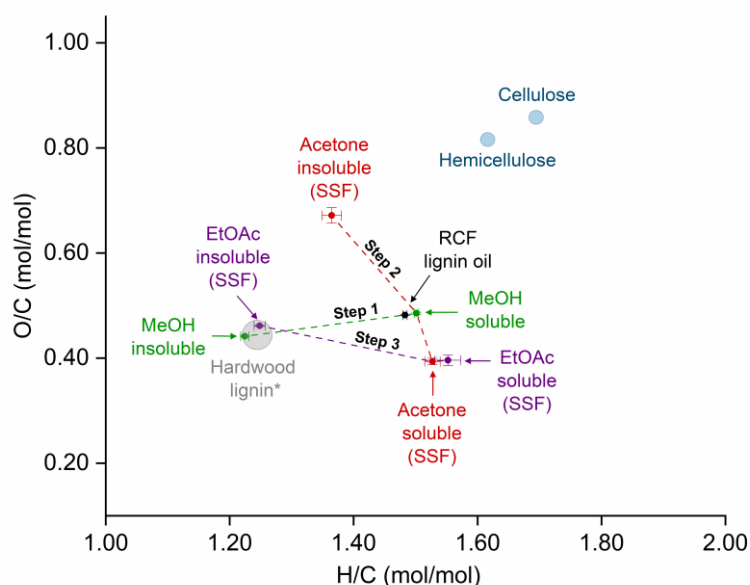


Figure 3-9. Van Krevelen diagram arranging the RCF lignin oil and fractions obtained by sequential solvent fractionation according to their H/C and O/C values. The grey circle region is centred on the O/C and H/C hypothetical values for hardwood lignins (for details, refer to Appendix Figure A-4).

3.2.3. Structural features of carbohydrates in RCF lignin oil

To address the structural features of lignin in different RCF lignin oil fractions, 2D HSQC NMR analysis in DMSO- d_6 was carried out. An 800 MHz NMR spectrometer equipped with a triple-resonance cryoprobe was utilised for the measurements. In the following sections of this chapter, HSQC NMR spectra of the fractions obtained by single solvent fractionation from RCF lignin oil are compared in selected regions. The HSQC NMR lignin linkages quantification for the fractions obtained by sequential solvent fractionation (acetone-soluble/insoluble (SSF), EtOAc-soluble/insoluble (SSF) can be found in Appendix (Table A-2) and are not included in the following analysis as the key features of different lignin fractions in different MW cuts as well as the carbohydrates can be described utilising the RCF lignin oil fractions obtained by

single solvent fractionations. This helps to reduce the amount of results displayed without compromising the key findings. Each soluble fraction is presented in blue and is overlaid with the corresponding insoluble fraction in red. HSQC NMR spectra in Figures 3-10 and 3-12 are presented at a threshold aiding HSQC spectra at low the identification of the main spectral features, while the HSQC spectra at a low threshold can be found from Appendix (Figures A-5- A-11).

To this date, little information on the chemical nature of carbohydrates in lignin oil is available in the current RCF literature.^{27,389,467} Accordingly, the key structural features of the carbohydrate fraction are detailed in this section to shed light on their chemical nature. Figure 3-10 displays the HSQC spectra in the oxygenated aliphatic region ($\delta\text{C}/\delta\text{H}$ 58-85/2.8-4.1 ppm) and anomeric centre region ($\delta\text{C}/\delta\text{H}$ 95-110/3.5-5.0 ppm). The carbohydrate signals on the spectra are indicated by 'X#_{NR/I/R}' and 'Ara#', where 'X' stands for xylopyranose unit, 'Ara' for anhydroarabinose unit, '#' for the carbon number labelling, and 'NR/I/R' for non-reducing-end/internal/reducing-end position of the xylopyranose units. The HSQC spectra in these regions confirm the observations made based on elemental analysis. Namely, the MeOH-insoluble fraction contains no ^{13}C - ^1H correlation signals characteristic of carbohydrates and the signals present can solely be associated with structural features of lignin (*vide infra*). At the same time, the MeOH-soluble fraction carried over the carbohydrates along with lignin fragments. Likewise, the acetone- and EtOAc-insoluble fractions HSQC spectra displayed ^{13}C - ^1H correlation signals close to those expected for (arabino)xylans, as will be discussed in more detail later. Despite the ^{13}C - ^1H correlation signals for the (arabino)xylans, the signal for the xylans' reducing-end terminal was missing from the spectra. This observation implies that the reducing end of the oligoxylans was converted into their corresponding alcohols during the RCF process catalysed by Raney Ni. Even though this information is unprecedented in the current RCF literature, the formation of oligo(arabino)xylan alcohols is plausible under the reductive conditions of RCF.

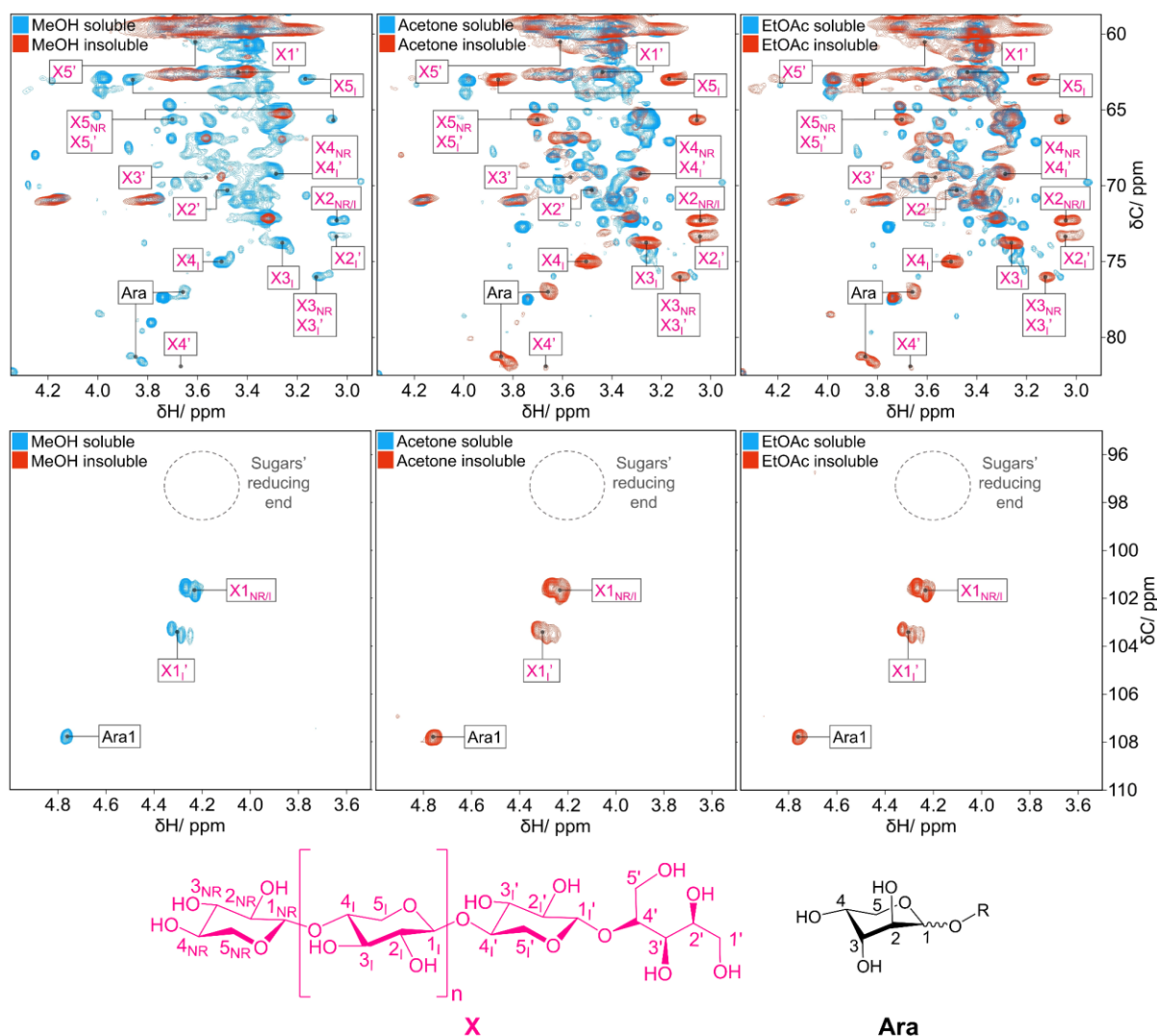


Figure 3-10. HSQC NMR spectra of lignin oil fractions highlighting the sugar signals in the oxygenated aliphatic region (top) and anomeric centre region (bottom). For each solvent, the soluble fraction spectrum (in blue) is overlaid on the insoluble fraction spectrum (in red). The dotted circle region indicates the missing signals for the anomeric centres at the oligosaccharide reducing end. 'X#NR/I' and 'Ara#' identify the carbohydrate signals in the spectra; the numeral '#' stands for the carbon atom number labelling. 'XNR' and 'XI' indicate the ^{13}C - ^1H correlation signals for non-reducing xylopyranose and internal xylopyranose units, respectively. 'Ara' denotes the ^{13}C - ^1H correlation signals for the anhydroarabinose unit. The oligosaccharide structure solely outlines key structural features in the backbone of the oligoxylan alcohols. Importantly, it does not intend to represent the actual structure of the oligo(arabino)xylan alcohols. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

HSQC NMR measurements, along with the GPC analysis (Figure 3-2), indicated that besides the carbohydrates, acetone- and EtOAc-insoluble fractions also contained mid-to high-MW lignin fragments. While postulating a hypothesis that the carbohydrates in RCF lignin oil were not covalently bonded to mid-to high MW lignin fragments, the carbohydrates could be extracted by hot water. To shed light on the connectivity of between lignin fragments and carbohydrates, the acetone-insoluble fraction was washed with hot water (80°C), a condition

much milder in comparison to RCF conditions (200°C, 3 h). Both soluble and insoluble fractions were collected, dried and analysed by HSQC NMR. The spectra of the water-soluble products in acetone-insoluble fraction showed to be composed of only carbohydrates with no trace of lignin fragments (Figure 3-11, the full spectra can be found in Appendix Figure A-12). In turn, the water-insoluble precipitate was a carbohydrate-free lignin fraction (Appendix, Figure A-13). Importantly, the carbohydrates dissolved in water from the acetone-insoluble fraction presented identical spectral features to the untreated acetone-insoluble fraction. Therefore, it is concluded that the carbohydrates in RCF lignin oil were not covalently bonded to lignin fragments.

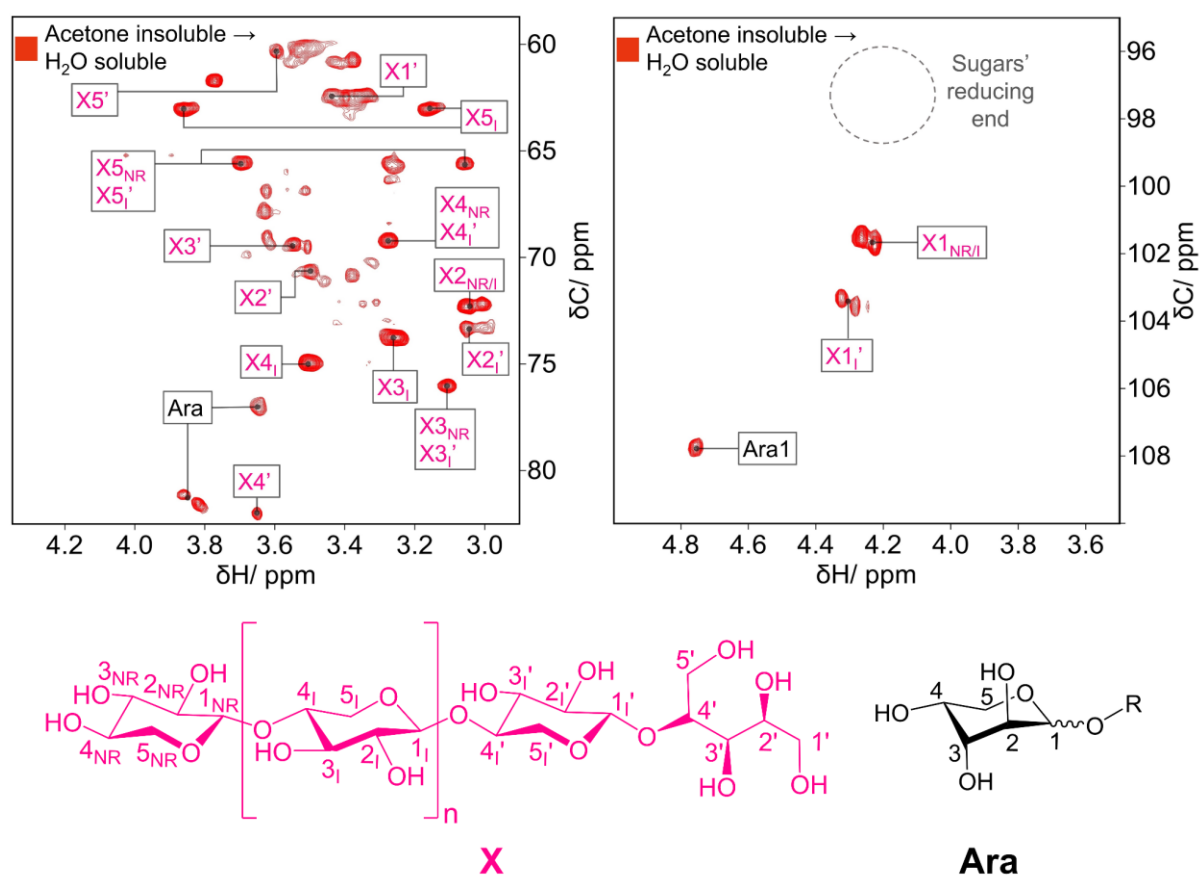


Figure 3-11. HSQC NMR spectrum of the carbohydrates in RCF lignin oil obtained by the hot water extraction of RCF lignin oil fraction insoluble in acetone and highlighted in the oxygenated aliphatic region (left) and anomeric centre region (right). The oligosaccharide structure solely outlines key structural features in the backbone of the oligoxyran alcohols. Importantly, it does not intend to represent the actual structure of the oligo(arabino)xylan alcohols.

Isolation of the carbohydrate fraction allowed the HSQC NMR spectra much less convoluted compared to those reported for the whole plant cell HSQC NMR spectra in pioneering work by John Ralph's group.^{468,469} In Figures 3-10 and 3-11, the anomeric region displays only two

sets of ^{13}C - ^1H correlation signals along with an additional single correlation signal at 107.81/4.76 ppm. The latter signal was associated with the anomeric centre of arabinose (Ara1) found in 4-O-methylglucuronoarabinoxylans.⁴⁷⁰ The first set of ^{13}C - ^1H correlation signals at 101.47/4.26 ppm and 101.82/4.22 ppm were associated with ^{13}C - ^1H pair on the anomeric carbon of non-reducing (X1_{NR}) and internal xylopyranose (X1_{i}) units in the β -(1 $\rightarrow$ 4)-D-xylopyranose backbone, as determined in the HSQC spectra of xylan⁴⁶⁹ and xylobiose (Appendix, Figure A-14). The second set of ^{13}C - ^1H correlation signals at 103.41/4.32 ppm and 103.58/4.25 ppm overlapped with the correlation signal of the anomeric centre from β -(1 $\rightarrow$ 4)-D-Glcp in the cellulose's non-reducing-end.⁴⁶⁹ However, when considering the HSQC NMR spectra cellobiose and cellobiitol (Appendix, Figure A-15), no other signals corresponding to the ^{13}C - ^1H pairs of glucopyranose were observed in the oxygenated aliphatic region of the spectra. This observation indicated that carbohydrates in the RCF lignin oil did not contain glucopyranose units.

Motivated by the current evidence, xylobiitol as a model was synthesised by xylobiose reduction with NaBH_4 under ambient conditions⁴⁷¹ to verify whether the second set of the correlation signals is related to the oligoxylan alcohol backbone. Comparison of the spectra from Figures 3-10 and 3-11 against the HSQC NMR spectra of xylobiose and xylobiitol (Appendix Figure A-15) confirmed that the second set of peaks unequivocally corresponds to the ^{13}C - ^1H pair on the $\text{C1}'$ of the penultimate xylopyranose unit of the oligoxylan alcohols. This finding also allowed estimating the average degree of polymerisation of the β -(1 $\rightarrow$ 4)-D-xylopyranose backbone. From the volume integrals of ^{13}C - ^1H correlation signals of the anomeric centre, the average β -(1 $\rightarrow$ 4)-D-xylopyranose backbone appeared to contain four xylopyranose units with an average occurrence of arabinofuranose of 0.5 unit relative to the total xylopyranose units (calculation method is given in Experimental section).

An important feature of poplar hemicellulose is the acetylation of 4-O-methylglucuronoxylan on the C2 and C3 positions of xylopyranose units (Chapter 2.1.2). Previous HSQC NMR studies for whole cell poplar samples utilising DMSO-d_6 /pyridine- d_5 (4:1, v/v) demonstrated that the correlation signals for the ^{13}C - ^1H pair at C2 ($\sim$ 73.5/4.64 ppm in 2-O-Ac- β -D-Xylp) and at C3 ($\sim$ 75.0/4.94 ppm in 3-O-Ac- β -D-Xylp) serve as a clear structural signature for the presence of acetylated xylans.⁴⁷⁰ However, the carbohydrate fraction in lignin oils (Figures 3-10 and 3-11)

is missing the correlation signals for 2/3-O-Ac- β -D-Xylp, indicating that the carbohydrates in the RCF lignin oil are not acetylated. In fact, deacetylation by the liquor solvents (2-PrOH/H₂O, 70:30, v/v) under the RCF conditions is expected to take place, thereby being the factor responsible for the absence of acetyl groups in the isolated carbohydrate fractions.⁴⁶⁷

3.2.4. Structural features of lignin fragments

Similarly to the carbohydrates, overlaid HSQC NMR spectra from the soluble and insoluble fractions were examined to investigate the main structural features of lignin species. In Figure 3-12, the overlaid spectra in: C—H aliphatic region (δ H/ δ C 0.5-3.0/5-50 ppm); (ii) oxygenated aliphatic region (δ H/ δ C 2.5-5.5/50-90 ppm); and (iii) aromatic region (δ H/ δ C 6.0-8.0 ppm) is displayed. A comparison of the soluble fractions (blue) and insoluble fractions (red) in the aliphatic C-H region reveals a stark difference between the two. The spectra of the soluble fractions show a very complex set of ¹³C-¹H correlation signals due to the presence of monomer and oligomer products with deoxygenated alkyl sidechains. In contrast, the spectra of the insoluble fractions contain only a few ¹³C-¹H correlation signals. Accordingly, the observation demonstrates that much less extensive HDO of the alkyl sidechain occurred during the RCF process on the mid- and high-MW lignin fragments compared to the low-MW lignin products.

Both soluble and insoluble fractions presented the ¹³C-¹H cross signals characteristic of the canonical linkages found in native lignins (A: β O-4 linkages, B: phenylcoumaran, and C: resinol interunit bonding motifs). Conventionally, the ¹³C-¹H pair for syringyl (S) and guaiacyl (G) units were the prevalent signals in the aromatic region for all fractions. As a secondary feature, signals for PB_{2/6} and PB_{3/5} from *p*-hydroxybenzoate (PB) were detected as they decorate the main lignin backbone as pendant groups.¹⁰² PB groups were more prevalent in high-MW lignin fractions as the abundance of the pendant groups relative to combined G- and S-units decreased from 7.8 $\pm$ 0.3% (16900 Da, MeOH-insoluble fraction) to 1.9 $\pm$ 0.1% (500 Da, EtOAc-soluble fraction)(Appendix, Figure A-16). Notably, the ¹³C-¹H correlation pairs characteristic of H_{2/6} and H_{3/5} from coumaryl (H) units were only detected in low-MW lignin fractions (~0.3% relative to combined H-, G-, and S-units). Considering that Nickel is active for the demethoxylation of guaiacols under the H-transfer conditions, it seems plausible that the

demethoxylation of G-type products contributes at least partially to the small amount of H-type products found in the low-MW fractions of the RCF lignin oil.^{346,472,473}

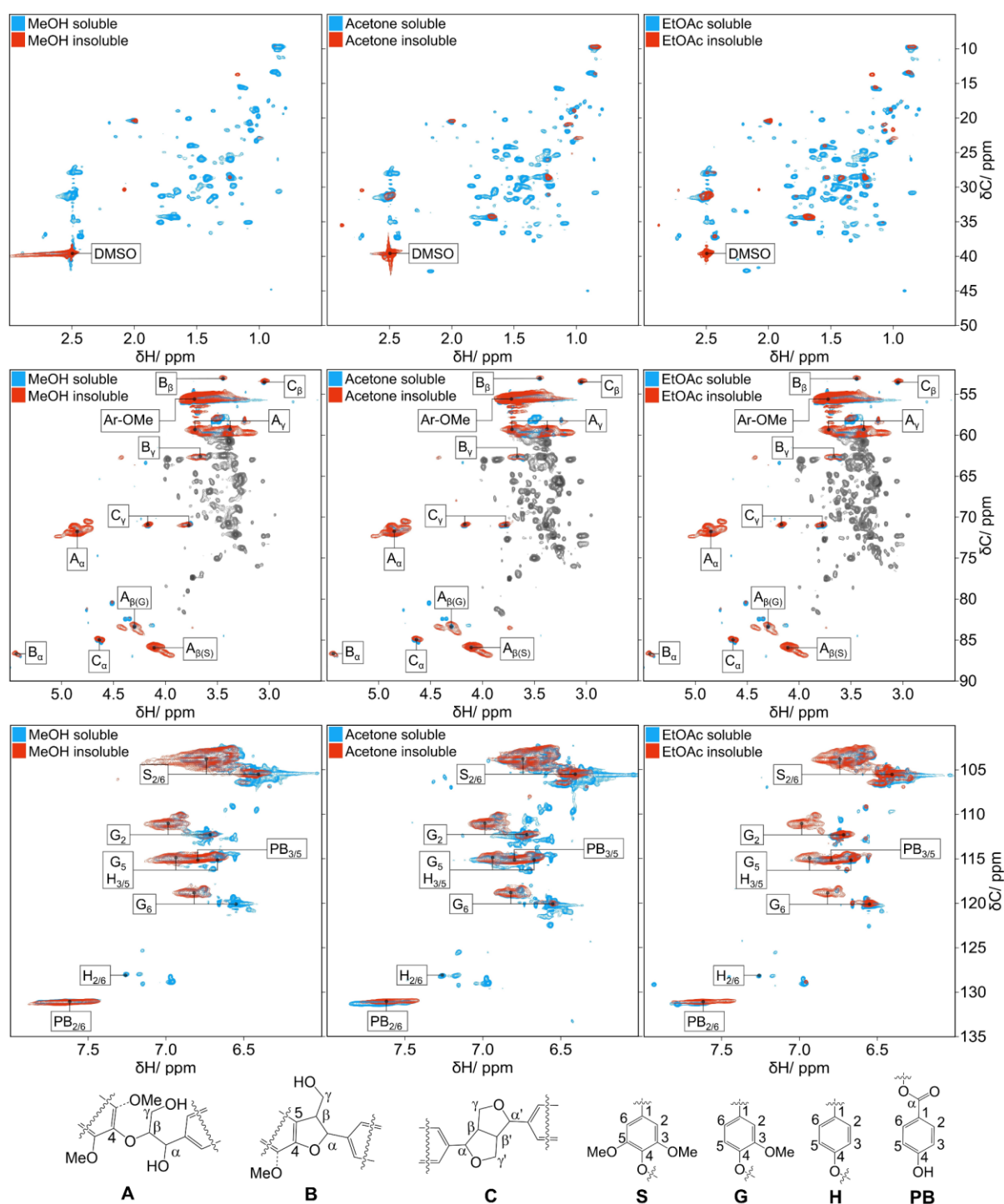


Figure 3-12. HSQC NMR spectra of lignin oil fractions zoomed on the aliphatic, oxygenated aliphatic, and aromatic regions. The soluble and insoluble fractions are superimposed to outline the difference between the two spectra. For clarity, carbohydrate ^{13}C – ^1H correlation signals are greyed out to emphasize the signals for the canonical lignin linkages. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

A closer look and careful analysis of the spectra at a low threshold revealed the presence of a non-native lignin linkage in all separated RCF lignin oil fractions created by HDO process on the β -O-4 interunit bonding motif during the RCF process. Accordingly, the C_α position contains a methylene group in the non-native reduced β -O-4 linkage. Figure 3-13 displays the highlighted ^{13}C - ^1H correlation signals attributed to the reduced β -O-4 linkage noted as $A_{X(\text{CH}_2)}$, where 'x' indicates the α , β , or γ position. The assignment is in good agreement with previous reports on β -O-4 lignin models and lignans presenting a methylene group at the C_α position.^{474–477} Notably, the feature could not be found in the reported HSQC NMR spectra of the lignin oils obtained from the RCF process in the presence of $\text{Ni}/\text{Al}_2\text{O}_3$ catalyst under H_2 pressure.²⁵⁹ The absence of these signals can also be a result of a low signal-to-noise ratio in the HSQC NMR measurements of the RCF lignin oil or due to the choice of a high threshold for the presentation of the spectral data, thus not acknowledging less intense signals. However, the formation of reduced β -O-4 linkages follows the chemoselectivity pattern found for HDO in experiments with model compounds containing O-functionalities at the benzylic position (e.g., CH_2OH , CHO , ether, or ketone group) when utilising Raney Ni and 2-PrOH as the H-donor.^{346,472}

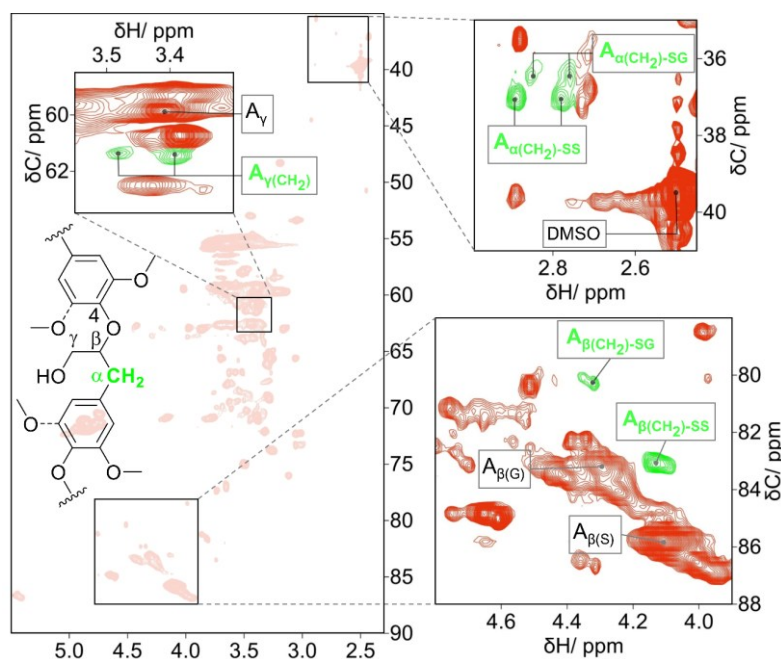


Figure 3-13. HSQC NMR spectrum of the EtOAc-insoluble fraction dissolved in $\text{DMSO}-d_6$. The insets present zoomed spectral regions at a threshold lower than that chosen in Figures 3-10, 3-11, and 3-12 (for clarity). The ^{13}C - ^1H correlation signals attributed to the reduced β -O-4 linkages are highlighted in green. Reproduced from reference²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

In Figure 3-13, the signals at 37.06/2.90 ppm and 37.06/2.78 ppm were assigned to $A_{\alpha(\text{CH}_2)}$ for the linkage between two S-units ($A_{\alpha(\text{CH}_2)\text{-SS}}$). Additionally, the less intense signals at 36.53/2.86 and 36.53/2.78 ppm were attributed to the reduced β O 4 linkage between an S-unit and an etherified G-unit ($A_{\alpha(\text{CH}_2)\text{-SG}}$). The ^{13}C - ^1H correlation signals at 83.32/4.10 ppm and 80.56/4.30 ppm were assigned respectively to $A_{\beta(\text{CH}_2)\text{-SS}}$ and $A_{\beta(\text{CH}_2)\text{-SG}}$. The signals for $A_{\gamma(\text{CH}_2)}$ were detected in a region convoluted by multiple overlapping signals. However, the signals for the corresponding ^{13}C - ^1H correlation could be identified at 61.34/3.48 and 61.34/3.39 ppm. The detected chemical shifts agree well with those reported for $A_{\gamma(\text{CH}_2)\text{-SS}}$ in lignin models.^{474–477} Despite the semiquantitative nature of the HSQC NMR data, the measured $A_{\gamma(\text{CH}_2)}$ -to- $(A_{\beta(\text{CH}_2)\text{-SS}} + A_{\beta(\text{CH}_2)\text{-SG}})$ ratio of 2 suggests that the $A_{\gamma(\text{CH}_2)\text{-SS}}$ and $A_{\gamma(\text{CH}_2)\text{-SG}}$ signals are likely to overlap in Figure 3-13.

To assess the structural homogeneity in different fractions of RCF lignin oil, the linkage counts of A, $A_{(\text{CH}_2)}$, phenylcoumaran (B), and resinol (C) per 100 aromatic units were estimated using the HSQC NMR spectral data (see Experimental section for further information). In Figure 3-14, the linkage count is correlated against the apparent M_w of the fractions from the GPC analysis. Both axes are presented on the logarithmic scale to facilitate the data visualisation. The linkage count determined for the unfractionated RCF lignin oil was lower than expected from the trend lines at the measured corresponding apparent M_w . However, due to the high polydispersity of unfractionated RCF lignin oil containing also monomer products, this shift is fully expected. Amongst the lignin oil fractions, the MeOH-insoluble fraction contained the highest total linkage count per 100 aromatic units (82 ± 2), followed by acetone- and EtOAc-insoluble fractions (64 ± 2 and 51 ± 2). All three soluble fractions presented lower linkage counts compared to their insoluble counterparts, with the MeOH-soluble fraction containing 29 ± 1 , acetone-soluble fraction 20 ± 1 , and EtOAc-soluble fraction 9 ± 1 described lignin linkages per 100 aromatic units.

Out of the canonical lignin linkages, the β -O-4 linkages were the most prevalent ones, followed by resinol and phenylcoumaran bonding motifs. The individual linkage counts for all canonical lignin linkages decreased with the reduction in MW. Due to the chemical nature of β -O-4 linkages, the decrease in linkage count is directly related to lignin depolymerization. In the case of phenylcoumaran and resinol, the decreasing linkage count is the result of

hydrogenolysis of C-O bond(s) in the corresponding linkages. However, the hydrogenolysis of the corresponding linkages does not result in depolymerization since the aromatic units remain connected by an alkyl chain linker.^{458,459} The reduced β -O-4 linkages present a trend different from canonical lignin linkages. The linkage count increases between the apparent M_w values of 500 Da to 1200 Da from 1.18 ± 0.08 to 1.73 ± 0.04 . At this point, the trend presents a plateau at about 1.8 linkage count per 100 aromatic units until 4800 Da, from where the linkage count drops to 0.48 ± 0.02 per 100 aromatic units at 16900 Da.

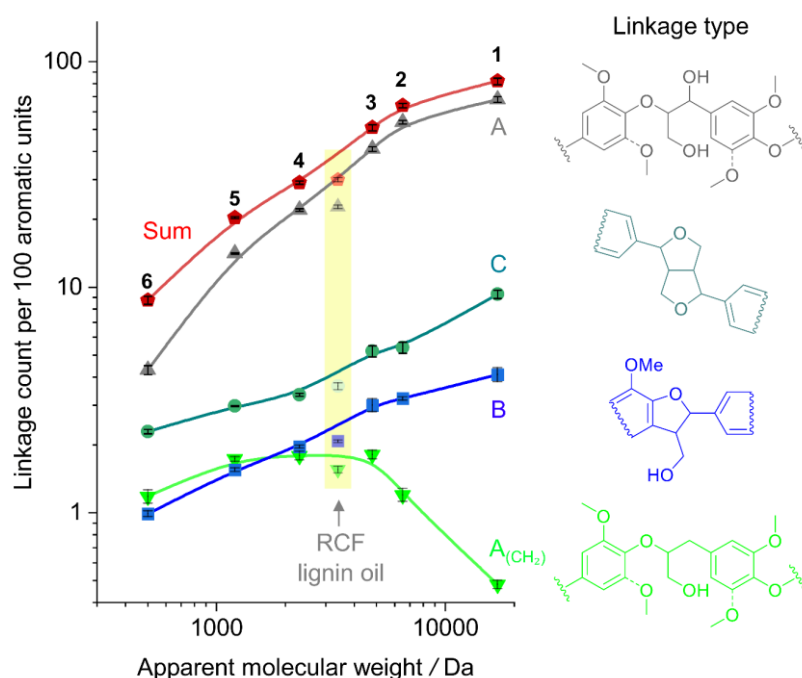


Figure 3-14. Distribution of estimated linkage counts per 100 aromatic units in the fractions relative to their apparent M_w values. The axes present the values on a logarithmic scales for clarity. The inset in yellow indicates the linkage count values for the RCF lignin oil relative to its apparent M_w . The numerals identify the fractions: 1. MeOH-insoluble, 2. Acetone-insoluble, 3. EtOAc-insoluble, 4. MeOH-soluble, 5. Acetone-soluble, and 6. EtOAc-soluble. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

The relative abundance of S- and G-type lignin in hardwood is one of the key factors determining the portfolio of lignin-derived products from the RCF process. Recently, Beckham and Román-Leshkov research groups demonstrated that the inherent S/G ratio in wood does not govern the monomer yields.⁴⁷⁸ They proposed that the distribution of interunit bonding motifs containing C—O or C—C bonds is defined by the lignin monomer transport during lignin biosynthesis. Several studies have indicated that during the course of the RCF process, the S/G ratio among the monomer products increases over time.^{396,478} Accordingly, more

extensive extraction of G-type lignin during the initial stages of the process is proposed to take place, being attributed to uneven distribution of S- and G-type lignin in cell walls with the G-type lignin domains being more accessible compared to S-type counterparts.^{396,478,479}

To assess the S- and G-unit distribution in the different fractions of RCF lignin, Figure 3-15 correlates the relative abundance of S- and G-units against their apparent M_w values to reveal a key feature of the RCF process. The occurrence of S-type units in the products increases gradually with the increase in the M_w of lignin. From the GC characterisation, the S- and G-type monomer products corresponded to $60\pm1\%$ and $40\pm1\%$, respectively. At the same time, the HSQC spectral data indicated an increase in S-units from $61.5\pm0.6\%$ to $69.1\pm0.8\%$, while the abundance of G-units decreased from $38.5\pm0.6\%$ to $30.9\pm0.8\%$ with the increasing MW.

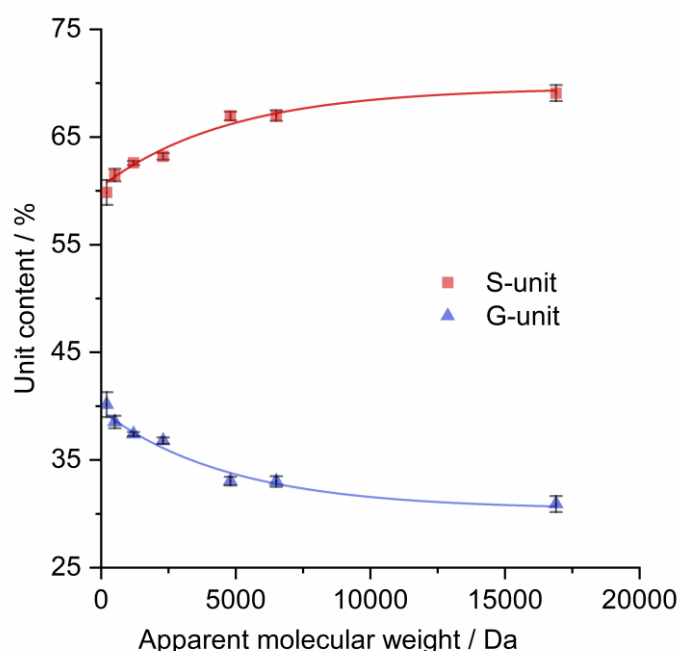


Figure 3-15. Correlation between the relative abundance of S- and G-type units in the lignin fractions and their apparent value of M_w . The first point (~ 200 Da) corresponds to the S- and G-type volatile products determined by GC-FID. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

Previous reports have provided strong evidence that lignin fragments rich in G-units were released at the early phase of RCF (<1 h).^{396,478} Taking this into consideration, the space time of lignin fragments rich in G-type is longer in the liquor when the RCF process is performed in a batch reactor. This also implies that lignin fragments rich in G-units have more time to undergo depolymerisation and to be converted into low-MW products. The data also confirms this

working hypothesis. Accordingly, the low-MW products (200-1000 Da) were enriched in G-units compared to the mid- and high-MW lignin products (Figure 3-15). In turn, the shorter space time of S-type rich lignin fragments in liquor resulted in less extensive depolymerisation and conversion to low-MW. As a result, mid- to high-MW lignin fragments became enriched in S-units. Considering Figure 3-13 and the discussion above, it becomes apparent that the 'history' of the lignin-releasing processes from the lignocellulosic matrix and the conversion in the liquor was preserved in the RCF lignin oil composition.

3.2.5. New insights into the stabilisation of lignin

As discussed in Chapter 2, the stabilisation of released lignin fragments constitutes the essential feature of the lignin-first biorefining concept. In the RCF process, the lignin fragments are stabilised by selective HDO of Hibbert ketones against electrophilic aromatic substitution. In other lignin-first approaches, the stabilisation is achieved by the use of protection agents (e.g., formaldehyde or other aldehydes,^{34,322} primary alcohols,^{480,481} and diols^{32,317}). Accordingly, the stabilisation of lignin fragments inhibits lignin condensation, which would otherwise degrade the native lignin structures due to the formation of non-native random C-C bonding motifs among the lignin fragments. In this context, it is now widely accepted that lignin condensation forms lignin-derived recalcitrant polymers with a low valorisation potential.^{20,25}

Regardless of the current consensus on the importance of lignin fragments stabilisation for the positive outcomes in RCF processes,^{25,224} not much is known about whether the stabilisation encompasses all lignin species in liquor or is only limited to low-MW lignin fragments. In this respect, the Dyson group recently reported a study on the catalyst role in the RCF process.⁴⁸² Utilising a designed catalyst having Rh nanoparticles embedded in the interior of hollow porous carbon nanospheres, they carried out RCF with birch under H₂ pressure. As a result, it was found that the nanospheres could sieve reactive lignin monomer intermediates from polymeric lignin fragments in the liquor. Additionally, supported by additional evidence obtained from model reactions using polystyrene or toluene as the substrate, it was proposed that the lignin stabilisation via reductive processes occurs primarily on the monomer products.⁴⁸²

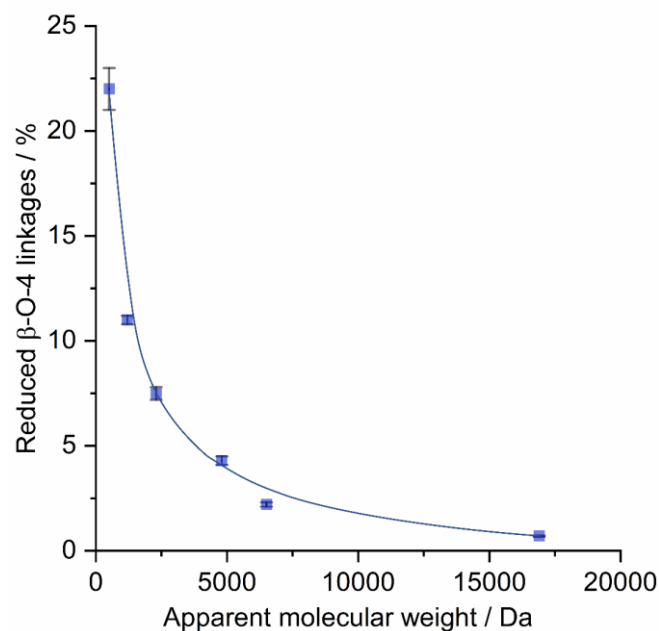
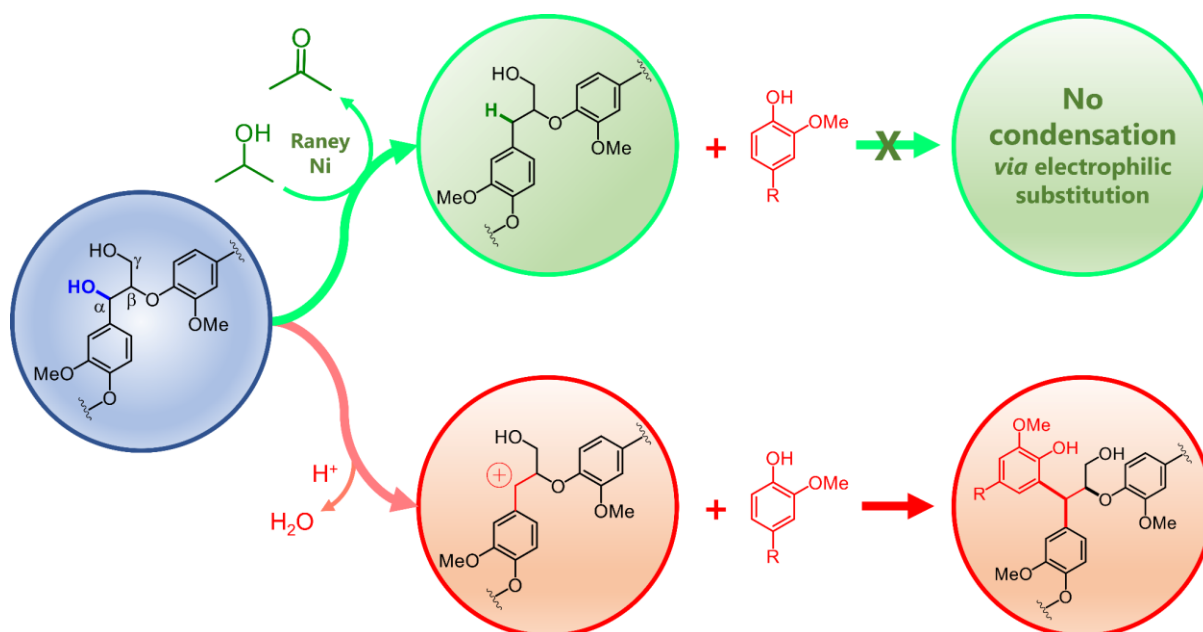


Figure 3-16. Correlation between the population of reduced β -O-4 linkages with the apparent value of M_w of the fractions isolated by solvent fractionation of the RCF lignin oil. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

In the current study, lignin fragments containing reduced β O-4 linkages formed via selective HDO of the C_α position of native β -O-4 linkages were found in the entire population of the lignin fragments. Accordingly, Figure 3-16 displays the relative abundance of reduced β -O-4 linkages among all β -O-4 linkages as a correlation against the apparent M_w . The population of reduced β -O-4 linkages decreased exponentially from 21% to 0.7% with the increase of the fractions apparent M_w from 500 Da to 16900 Da. Although at a smaller extent, the presence of reduced β -O-4 bonding motifs in mid- and high-MW RCF lignin oil fractions demonstrates that this stabilisation mechanism is not only limited to low-MW lignin fragments but also low- and high-MW lignin fragments interact with the catalyst and undergo reductive processes. The occurrence of a CH_2 group at the C_α position eliminates the possibility of the formation of benzylic carbocation, thus effectively impeding lignin condensation at the reduced β -O-4 bonding motifs (Scheme 3-1).



Scheme 3-1. Route proposed for stabilisation of lignin fragments via selective HDO of the C_α site. This reaction converts the C_α-OH group into a methylene group. This impedes electrophilic aromatic substitution, thus suppressing lignin condensation. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

3.3. Conclusion

In the current chapter, RCF lignin oil fractionation was demonstrated using environmentally friendly solvents (MeOH, acetone, EtOAc). Accordingly, lignin fractions in different molecular weight ranges were rendered either by RCF lignin oil fractionation with a single solvent or sequential solvent fractionation. Additionally, solvent fractionation techniques allowed the separation of carbohydrates from the RCF lignin oil. The separation of different lignin fractions allowed for determining the structural heterogeneity of lignin products across the entire molecular weight distribution in the RCF lignin oil. By the detailed characterisation of the fractions by HSQC NMR analysis, the formation of reduced β-O-4 linkages with methylene group at the C_α position was demonstrated to take place during the RCF process. Although it is generally accepted that the stabilisation process primarily occurs on monomeric and oligomeric intermediates, and the claim holds, the finding of reduced β-O-4 linkages establishes a new additional route for lignin stabilisation taking place during the RCF process. This process occurs to varying degrees in the entire population of lignin species in liquor.

Separation of carbohydrates by solvent fractionation allowed to deconvolute the HSQC NMR spectrum and determine their chemical nature. Accordingly, it was determined that the

carbohydrate fraction in RCF lignin oil is composed of oligo(arabino)xylan alcohols. The carbohydrates present were not covalently bonded to lignin fragments. Therefore, they could be removed from the fractions containing mid- and high-MW lignin by hot water extraction with no apparent degradation.

In the broad context, the findings presented in this chapter have far-reaching implications for lignin valorisation. In this respect, the stabilisation imparted by reductive processes during RCF preserves the structure of high-MW in a more native-like state. For instance, the heaviest lignin fraction (MeOH-insoluble, M_w of 16900 Da) presented an estimated total linkage count of 82 ± 2 per 100 aromatic units. Consequently, heterogeneous catalysis can be crucial in producing mid- and high-MW lignin products with controllable structural properties, which has thus far only been possible through the rational bioengineering of lignin in plants.²⁰ However, the structural features inserted by bioengineering are often lost in the lignin extraction process. Therefore, the evolution of the RCF concept toward the production of polymeric lignin products with controllable structural properties presents itself as an attractive new option in the lignin-first concept and, more broadly, in lignin valorisation in general.

In addition, the formation of reduced β -O-4 linkages and the population difference across different lignin fractions raises the question of whether lignin fractions with all β -O-4 linkages in reduced form could be produced. Accordingly, future experiments targeting specifically maximising the content of reduced β -O-4 linkages would be essential to assess this type of lignin's properties and potential applications. Additionally, profiling the RCF fractions obtained in this work by aliphatic and phenolic OH content by ^{31}P NMR would allow further assessment of the difference between the lignin oil fractions, especially in terms of different applications utilising lignin functionalisation.

4. Soft Mechanocatalytic Pretreatments to Control and Enhance the Outcomes of Reductive Catalytic Fractionation

4.1. Introduction

In Chapter 2, it was summarised that two independent, consecutive steps govern biomass conversion in RCF processes. At first, lignin and hemicellulose (and cellulose) species are solvolysed from the lignocellulosic matrix by hydrolytic processes. This step is governed by the solvent, the biomass morphology, and the reaction conditions within the reactor. Once dissolved and diffused into the bulk liquor medium, the reductive catalytic processes on the catalyst allow species stabilisation and eventual production of lignin-derived monoaromatic products. While the catalytic reduction step has been extensively researched for different catalyst and reaction conditions, lignin solubilisation along with diffusion has only been more emphasised in recent years. Accordingly, Anderson *et al.* conducted experiments in flow-through and batch reactors to isolate the limiting regimes for lignin solvolysis and catalytic reduction in the RCF process.²⁶¹ As a result, they found that under common RCF operating conditions in batch mode, lignin solvolysis is the rate-limiting step in the overall process.²⁶¹ Consequently, Thornburg *et al.* developed mesoscale reaction-diffusion models for the RCF process.³²³ They concluded that solvolysis is completely diffusion-limited in particles with characteristic lengths greater than 2 mm. To mitigate the mass transfer limitations from solvolysis, the particle size should not exceed 200 μm .³²³ Therefore, biomass comminution is an essential step to mitigate mass transfer limitations during a lignin-first process, especially when conducting experiments to assess the catalyst's performance.^{323,483} Considering the aim of the RCF to produce lignin streams of low recalcitrance and high potential for valorisation, the future industrial setting will need efficient mass transfer and, therefore, biomass comminution to allow dissolved lignin species fast transport to a catalyst and consequent catalytic stabilisation and conversion.

In order to reduce the particle size of lignocellulosic biomass, mechanical energy input is necessary. In this context, ball milling combined with an additive has given rise to mechanocatalytic depolymerisation of lignocellulose.^{484–495} H₂SO₄ impregnated wood undergoing mechanocatalytic pretreatment has rendered a complete conversion of biomass into water-soluble products, with carbohydrates being readily upgradable via enzymatic hydrolysis.^{491,496,497} On the other hand, the lignin structure has been shown to become more recalcitrant as a result of the process.⁴⁸⁷ Calvaruso *et al.* carried out experiments where lignin obtained by mechanocatalytic depolymerisation of H₂SO₄-impregnated wood was introduced to the RCF process.⁴⁸⁷ As a result, monophenol yields decreased at least four times compared to native biomass- a phenomenon attributed to lignin condensation during the mechanocatalytic pretreatment. Recently, Cheng *et al.* explored hemicellulose hydrolysis prior to the RCF utilising hydrothermal pretreatment with FeCl₃.⁴⁹⁸ As a result, 25% of the lignin, 86% of hemicellulose, and 12% of cellulose was removed during the pretreatment. However, the pretreatment negatively impacted the lignin structure and stability, resulting in lower pulp delignification and monoaromatic yields.

Considering that the release of lignin from lignocellulosic materials depends on the hydrolytic process on both lignin and hemicellulose, it is here reasoned that the energy used for the comminution process could be better employed in a tandem process depolymerising the lignocellulosic matrix to the extent that the substrate would remain insoluble under the RCF conditions. However, the interactions between lignin and hemicellulose would be perturbed by a 'soft' mechanocatalytic process. To the best of our knowledge, this approach is novel and has not been reported before.

In this chapter, 'soft' mechanocatalytic pretreatments are demonstrated to enhance the outcomes of the RCF process via H-transfer reactions in the presence of Raney-Ni and employing 2-propanol as a part of the lignin-extracting liquor composition. For the 'soft' mechanocatalytic treatment, two weak acids (acetic and oxalic acid) and two bases (NaOH and Na₂CO₃) were selected as additives for the solvent-free pretreatment of Poplar wood. In addition, two control experiments were introduced. In the first one, ball milling with no additive was performed prior to the RCF process. In the second one, untreated poplar chips were used for the RCF process under the same conditions. Based on the results with different additives,

acetic acid was outlined as an additive that enhances the RCF outcomes the most. Accordingly, the effect of acetic acid concentration as well as the kinetic energy dose with acetic acid during the mechanocatalytic pretreatment, were further screened.

The Results and Discussion section of this chapter is divided into five segments. The first segment gives an overview of the 'soft' mechanocatalytic pretreatment process with mass balances utilising different additives. The second and third segments concentrate on the characteristics of lignin oils and pulps obtained with different additives prior to the RCF. The fourth and fifth sections consider the effect of acetic acid concentration and kinetic energy dose on the lignin oil and pulp properties, respectively.

4.2. Results and discussion

4.2.1. 'Soft' mechanocatalytic pretreatments

Planetary ball milling of sulfuric acid-impregnated beechwood (1 mmol g^{-1}) has previously been shown to produce 99 wt% of water-soluble products when implying a kinetic energy dose of 81 kJ g^{-1} in a linear regime, where no 'cake layer' is formed on the jar wall.⁴⁹⁷ Following the rationale, the experiments with different additives in this study were carried out at the additive concentration of 2 mmol g^{-1} to compensate for the diprotic nature of sulphuric acid. To limit the depolymerisation of cellulose during the pretreatment, a kinetic energy dose of 10 kJ g^{-1} was utilised to avoid extensive depolymerisation of lignin and ensure the recovery of a holocellulosic pulp after the RCF process.

As a first step of the process, poplar chips were milled in the planetary ball mill with the corresponding additive. To prevent the additives and water extractives from interfering with the following RCF process, the milled wood was washed by suspending the processed material in water and separating the material by filtration with washing until it reached a neutral pH. The resultant solid, hereafter referred to as 'processed wood', and the water-soluble fraction termed as 'extractives' were dried and isolated. Notably, the extractives accounting for 2-7 wt% of the poplar wood chips introduced to ball milling may contain non-incorporated additives from the pretreatment. The percentage of processed wood recovered from ball milling and subsequent washing varied from 94% when no additive or acetic acid was used up

to 98% with oxalic acid pretreatment. Accordingly, Figure 4-1 provides a schematic overview of the process along with the corresponding mass balances.

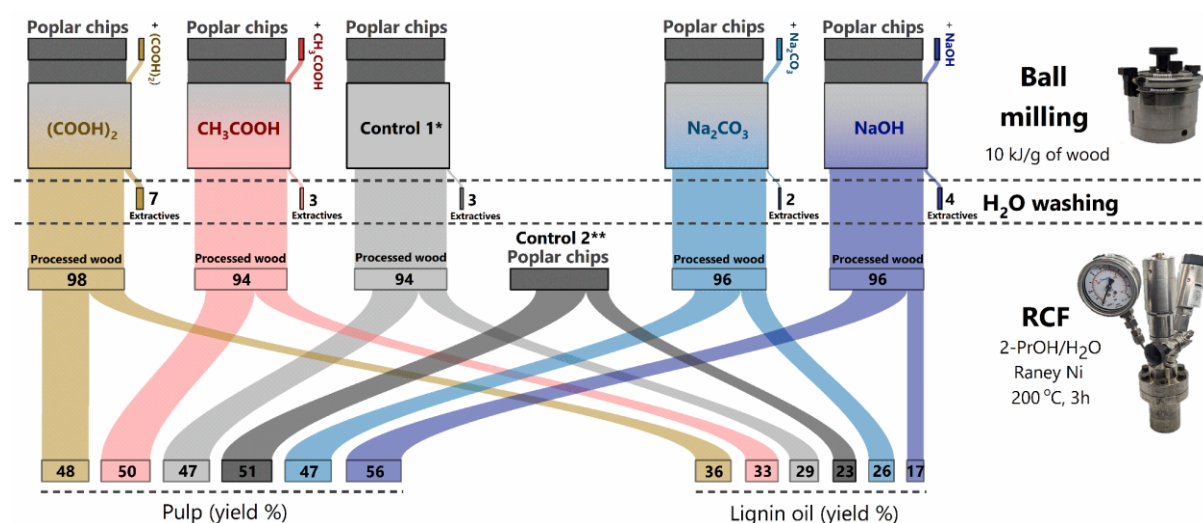


Figure 4-1. The scheme describing the process of soft mechanocatalytic pretreatment with different additives prior Reductive Catalytic Fractionation (RCF). *Control 1- ball-milled poplar with no additive introduced during the pretreatment. **Control 2- RCF starting with poplar chips.

To examine the particle size distribution of processed wood and poplar chips before RCF, we performed digital microscope imaging. Out of the 6000 processed wood particles measured, 94% exhibited a maximum diameter of 20 µm or less, while all the particles had a maximum diameter of fewer than 200 µm (Appendix Figure A-17). At the same time, the poplar chips introduced to the ball milling had approximate dimensions of 2 x 5 x 6 mm. To circumvent stock-specific mass transfer limitations between different lignocellulose sources during the lignin-first fractionation, the particle size should not surpass 200 µm.³²³ As all the processed wood particles obtained from the mechanocatalytic pretreatments with different additives measured below it, the variances observed during the RCF processes with processed wood can be mainly attributed to modifications in the lignocellulosic matrix, and pore diffusion mass transfer limitations have been effectively eradicated. The variance between control 1 (ball-milled sawdust) and control 2 (poplar chips) can, on the other hand, be attributed to different particle sizes and consequent mass transfer limitations in poplar chips.

The RCF process was performed in a batch mode utilising a mixture of 2-propanol/H₂O (7:3 v:v) as an H-transfer solvent in the presence of Raney Ni at 200°C for 3 h. Obtained pulps and lignin oils were isolated and evaluated for the effect of the prior soft mechanocatalytic

pretreatment. The pulp yields from RCF with oxalic acid (48%) and Na_2CO_3 (47%) pretreated wood were comparable to the control experiment 1 with no additive (47%). However, acetic acid pretreatment resulted in an elevated pulp yield of 50%, with the most significant increase observed for NaOH-pretreated wood at 56%. The control experiment with poplar chips (control 2) resulted in a pulp yield of 51%, which is higher than for ball-milled poplar. Notably, both acidic pretreatments boosted the yield of lignin oil relative to the control (29%), with yields of 33% for oxalic acid pretreatment and 36% for acetic acid. Conversely, both basic pretreatments resulted in lower lignin oil yields. Na_2CO_3 pretreated wood yielded 26% lignin oil, and NaOH pretreated wood resulted in 17% lignin oil yield. Significantly, the RCF process with poplar chips (Control 2) resulted in a considerable drop in lignin oil yield (23%) compared to its ball-milled counterpart (29%, Control 1).

4.2.2. RCF lignin oils characterisation

Following the scope of this study, a characterisation of the obtained lignin oils and pulps was carried out to assess the effect of different additives in the soft mechanocatalytic pretreatment on the outcomes of the RCF process. Accordingly, the obtained RCF lignin oils were analysed by GPC equipped with a PDA detector to determine the molecular weight distribution. Additionally, based on the Structural Index introduced in Chapter 3, the SI_{320} and SI_{350} values were calculated. Accordingly, Figure 4-2 displays the molecular weight distribution at 280nm as well as SI_{320} and SI_{350} values across the molecular weight distribution.

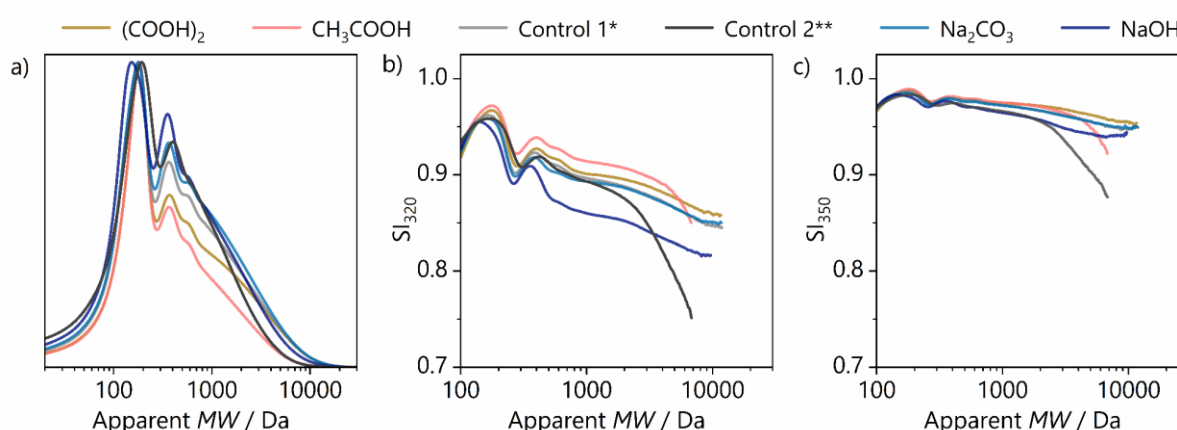


Figure 4-2. a) GPC traces of RCF lignin oils at 280 nm normalised by the absorbance of monomers. b) Structural Index at 320 nm across the apparent molecular weight of the RCF lignin oils from the column exclusion limit of 100 Da. c) Structural Index at 350nm for the RCF lignin oils. *Control 1 refers to ball-milled wood with no additive used to carry out the RCF process. ** Control 2 refers to poplar chips used directly for the RCF process.

All the RCF lignin oils exhibit a molecular weight distribution shape characteristic of lignin oils, indicating that the fundamental chemical characteristics of lignin were preserved during the soft mechanocatalytic pretreatments. Moreover, the GPC trace of lignin oils from wood pretreated with sodium carbonate and oxalic acid closely mirrors that of the control sample, each having a weight-average molecular weight (M_w) of 900 Da. In contrast, acetic acid and sodium hydroxide treatments reduced the apparent weight-average molecular weight of the resulting lignin oils, yielding 600 Da and 700 Da, respectively. Starting the RCF process with poplar chips resulted in M_w of 700 Da, which is 200 Da less compared to the ball-milled counterpart.

In Appendix Figure A-3, Eucalyptus Kraft and Organosolv lignin displayed SI_{320} in the range of 0.4-0.5 and SI_{350} between 0.6-0.7, while the eucalyptus RCF lignin oil displayed the corresponding average values of 0.72 and 0.86. The RCF lignin oils obtained in the current study display SI_{320} values of 0.87-0.92 and SI_{350} values between 0.95-0.97, indicating a low presence of oxidised and/or conjugated lignin species. The acetic acid pretreatment resulted in the highest average SI_{320} (0.92 ± 0.03) and SI_{350} (0.97 ± 0.01), while the lowest average SI_{320} was recorded for NaOH pretreatment (0.87 ± 0.04) and lowest SI_{350} for Control 2 with poplar chips (0.95 ± 0.03). Average SI values for every lignin oil are tabulated in Appendix Table A-6. While the average SI values for the different lignin oils remain within the boundaries of statistical error, comparing the SI lines reveals some differences in the polymeric lignin characteristics. The acetic pretreatments display the highest SI values for polymeric fraction compared to no additive Control 1. At the same time, acetic acid displays a downward gradient change in the SI values in the apparent MW proximity of 5000 Da. The pretreatment with Na_2CO_3 resulted in similar SI lines compared to no additive Control 1, while the NaOH lowered the SPI values for polymeric lignin, especially once considering the SI at 320 nm. Control 2 with poplar chips exhibits a different behaviour from the ball-milled samples. While the monomeric and oligomeric fraction SI is identical to its ball-milled counterpart (Control 1), the polymeric fraction SI drops rapidly near the apparent MW of 3000 Da, falling to 0.75 for SI_{320} and 0.88 for SI_{350} at the apparent MW of 6800 Da.

As a result of GPC and the consequent SI analysis, oxalic acid and Na_2CO_3 pretreatments displayed very similar characteristics to no additive Control 1. Acetic acid pretreatment lowered

the weight average molecular weight of lignin, while the oligomeric lignin SI increased and polymeric lignin SI dropped in comparison to Control 1. Therefore, the relative abundance of low molecular weight species is higher, while the properties of oligomeric and polymeric lignin vary more than for Control 1. NaOH pretreatment lowered the M_w while displaying lower SI values across the oligomeric and polymeric lignin fractions. Notably, the monomer peak is shifted to lower apparent MW values compared to other lignin oils, while the trimers peak already follows the rest of the RCF lignin curves. Therefore, the lower Mw value is attributed to the shift in the monomers and dimers' peaks. The lower SI values of NaOH pretreatment for the oligomeric and polymeric fraction indicate the presence of oxygenated and/or conjugated species and potentially the presence of condensed lignin species. The sudden drop in the SI values for Control 2 with poplar chips compared to the ball-milled poplar counterpart in Control 1 can indicate the mass transfer limitations for the chips. Due to the longer characteristic length for diffusion from the cell wall to the reductive medium with the hydrogenation catalyst, condensation of the dissolved lignin species can occur, resulting in SI drop and more recalcitrant polymeric lignin fraction.

To quantify the lignin-derived monophenols, the obtained RCF lignin oils were analysed with GC-FID. The monophenols yield from the RCF process is calculated based on the lignin content in processed wood after mechanocatalytic pretreatments. Accordingly, Figure 4-3 displays the total yield of S- and G-type monophenols along with individual yields of different monophenols in corresponding categories. Additionally, Figure 4-3a shows the relative abundance of S-type monophenols out of all S- and G-type lignin-derived monomers.

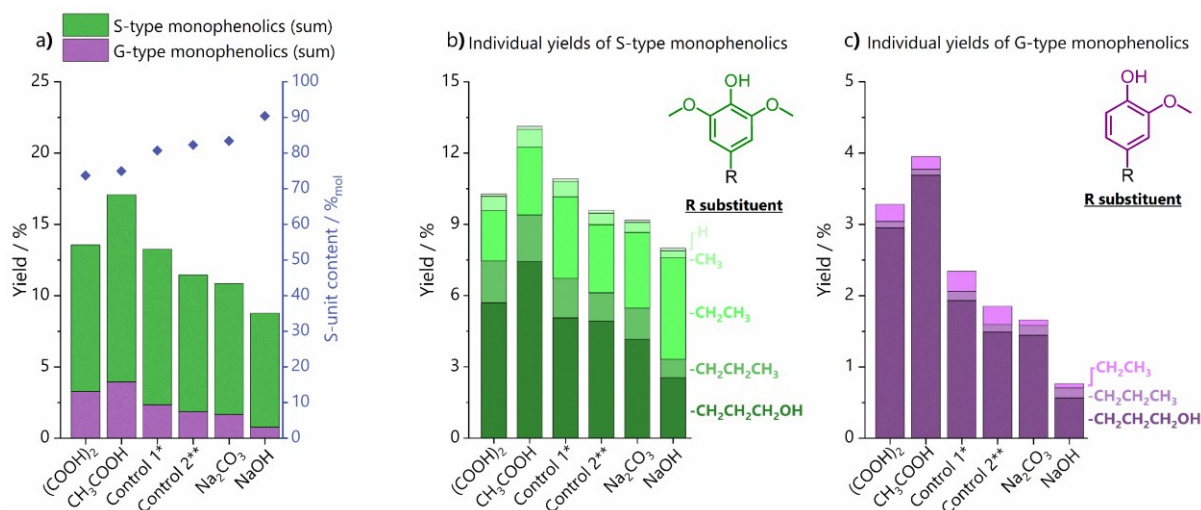


Figure 4-3. Comparison of a) total yields of S- and G-type monophenolics along with relative molar ratio of S-units among the S- and G-type monophenolics; b) and c) individual yields of S- and G-type monophenolics obtained from RCF. *Control 1 refers to ball-milled wood with no additive used to carry out the RCF process. ** Control 2 refers to poplar chips used directly for the RCF process.

H-type monophenols contributed less than 0.6 wt% to each lignin oil and were therefore omitted from the subsequent analysis. As a general trend, acidic pretreatments resulted in a higher total yield of S- and G-type monophenolics. Specifically, wood pretreated with oxalic acid and acetic acid yielded 13.6% and 17.1% monophenolics, respectively, compared to the 13.3% yield from Control 1. Notably, these pretreatments considerably elevated the yield of guaiacols from 2.3% in Control 1 to 3.3% and 4.0% for oxalic acid and acetic acid, respectively. Oxalic acid pretreatment decreased the yield of syringyl-type monophenols to 10.3%, while acetic acid boosted the yield to 13.1% (10.9% for Control 1). As the yield of G-type monophenolics for acidic pretreatments was boosted, the relative molar ratio of S-type monophenolics decreased to 74% for oxalic acid and 75% for acetic acid, in contrast to 81% for Control 1. Unlike the acidic additives, utilised base pretreatments led to a decline in monophenol yields from the RCF process. Wood pretreated with Na₂CO₃ and NaOH showed monophenol yields of 10.8% and 8.8%, respectively. The yield of syringols decreased to 9.2% for Na₂CO₃ and to 8% for NaOH, while the production of G-type monophenolics was further suppressed to 1.7% and 0.8%, respectively. Consequently, the relative molar ratio of S-type monophenolics increased to 83% for Na₂CO₃ and 90% for NaOH. The control experiment with poplar chips (Control 2) displayed a monophenolics yield of 11.4%, a 1.9% decrease in the yield compared to the ball-milled Control 1 counterpart. A drop in both S- and G-type monophenolics occurred- the yield of

syringols decreased from 10.9% for Control 1 to 9.6% for Control 2, while the guaiacols yield reduced from 2.3% to 1.8%, respectively. The RCF with poplar chips (Control 2) resulted in 82% relative molar ratio of S-type monophenols.

In addition to the monophenol yields, different additives during the pretreatment also affect the monophenols product distribution. P(OH)G molar fraction in G-type monophenols increases from 70% for NaOH up to 92% for acetic acid (80% for control). Similarly, there is a surge in the molar fraction yield of P(OH)S among S-type monophenols, rising from 29% for NaOH to 53% for acetic acid, compared to 43% under control conditions. The majority of the S-type monophenols in RCF lignin oil obtained from NaOH pretreatment are present as ES (56%), which is considerably higher than for control (34%) and for acetic acid pretreatment (24%). Accordingly, the pretreatment with acidic acid promotes the retention of C_γ-OH, while the pretreatment with NaOH favours the cleavage. RCF process with poplar chips (Control 2) displayed a similar monophenols distribution to ball-milled poplar (Control 1), with P(OH)G forming 78% of the G-type monophenols (80% in Control 1) and P(OH)S forming 48% of the S-type monophenols (43% in Control 1).

As highlighted in Chapters 2 and 3, past studies have also shown that during the early stages of the RCF process (<1h), G-type lignin is predominantly extracted, while the S/G ratio gradually increases as the process proceeds.^{396,478} This pattern can be attributed to the differential distribution of S- and G-lignin in hardwood cells - S-type lignin is more abundant near the middle lamella, while cell edges contain relatively more G-type lignin.^{396,478,479} In Chapter 3 it was demonstrated that different molecular weight fractions preserve the "kinetic history" of the RCF process, with G-type lignin being more prominent in low molecular weight fractions and S-lignin in high molecular weight fractions, lining with the uneven S/G distribution in hardwood cell walls.²³⁹ Considering the results obtained in this study, the known uneven distribution of S- and G-type lignin in hardwood cell walls, and the factors governing the monophenol yields, we can formulate that during the mechanocatalytic pretreatment, the changes in lignocellulosic matrix ultrastructure and potential chemical modifications have a substantial effect on the yield and distribution of monophenols from the RCF process.

During the mechanocatalytic pretreatment, the cell wall's outer portion is more exposed to the additive than the middle lamella. This holds especially true for solid additives (oxalic acid, Na_2CO_3 , and NaOH) where diffusion is highly limited. A prior study by Kleine *et al.* has demonstrated that β -O-4 linked dimeric model compounds and lignin moieties in hard- and softwood lignin can undergo cleavage during ball milling in the presence of NaOH .⁴⁹⁵ It is suggested that the cleavage initiates with deprotonation of the aliphatic proton at C_γ , followed by intramolecular cleavage of the β -ether. Given that the intermediate species are not stabilised during the ball milling process and the complexity of native lignin compared to models, the lignin can be susceptible to condensation reactions that inhibit its cleavage during the subsequent RCF process. This, along with other potential modifications, can partially explain the high selectivity of S-type monophenols and low yield for base pretreatments, as the lignin in the outer parts of G-lignin rich cell wall could be partially condensed during the pretreatment. Although Kleine *et al.* did not determine any cleavage of the β -O-4 model compounds when Na_2CO_3 was utilised as a reagent during the ball milling,⁴⁹⁵ utilising the more complex lignocellulosic matrix displays similar trends to NaOH with a lesser extent. Therefore, the pretreatment with Na_2CO_3 could be considered a 'light' version of NaOH pretreatment.

The acidic pretreatments promoted the monophenols yield with the lower S-type monophenols selectivity compared to Control 1. However, it must be considered that while oxalic acid is solid at room temperature, glacial acetic acid is liquid and consequently able to diffuse through the lignocellulosic matrix. Thus, the argumentation for acetic acid needs to involve the whole matrix, while oxalic acid is likely to primarily interact with the outer parts of the cell walls. Therefore, the potential reasoning behind higher monophenol yields for acidic pretreatments and base pretreatments, will be revisited in the pulp characterisation section.

Acetic acid and NaOH demonstrated the most significant and opposite impacts on monophenol yields, as well as on GPC attributes, pulp, and lignin oil yields. To further explore the influence of these pretreatments on the RCF process, 2D HSQC NMR analysis of the corresponding lignin oils and Control 1 was performed in DMSO-d_6 . Accordingly, Figure 4-4 displays the HSQC spectra zoomed in the oxygenated aliphatic region ($\delta\text{C}/\delta\text{H}=52\text{--}90/25\text{--}5.6$ ppm), anomeric centre region ($\delta\text{C}/\delta\text{H}=95\text{--}110/3.5\text{--}5.0$ ppm), and aromatic region ($\delta\text{C}/\delta\text{H}=100\text{--}135/6.0\text{--}8.0$ ppm).

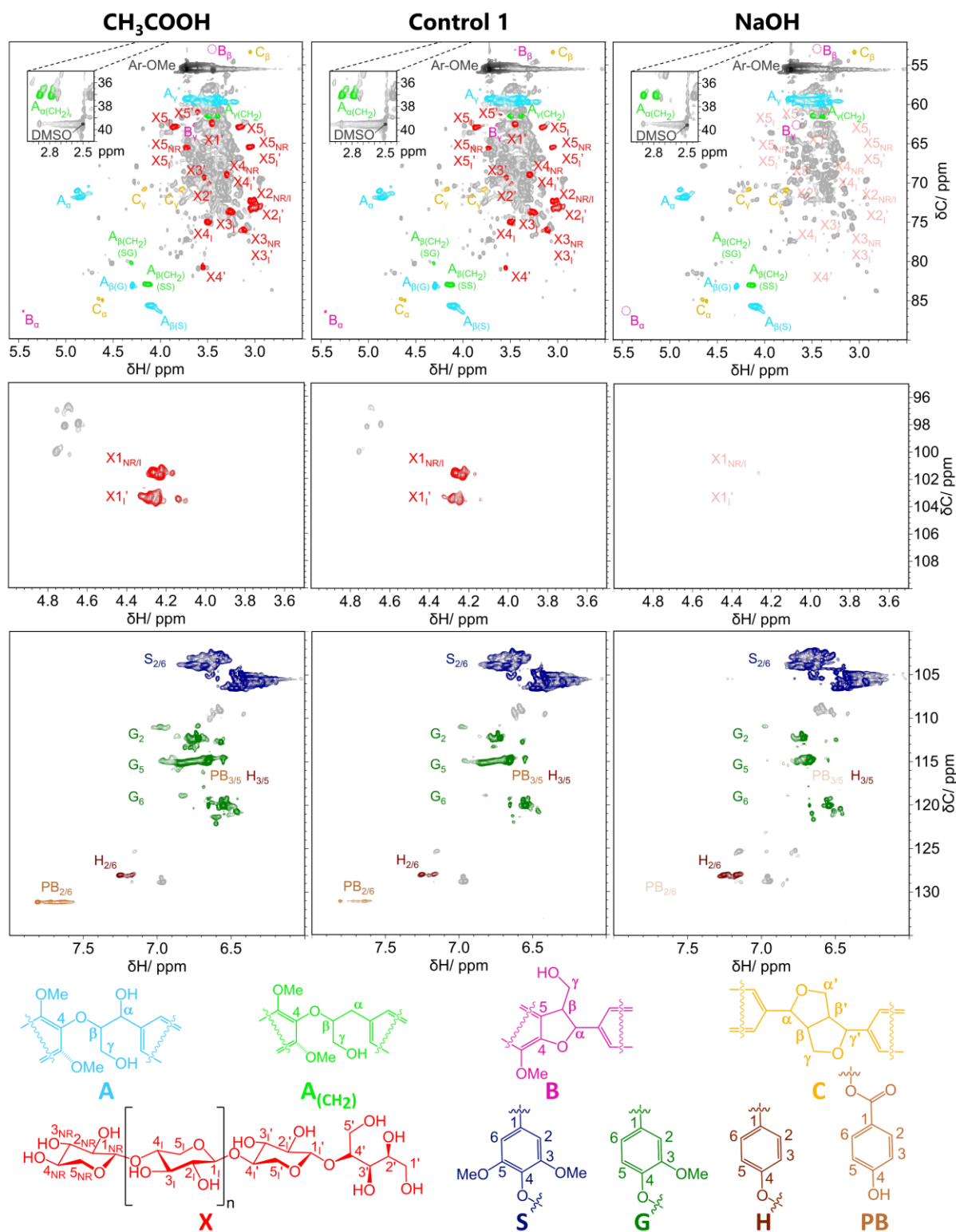


Figure 4-4. HSQC NMR spectra of RCF lignin oils obtained from pretreated wood with acetic acid, no additive (Control 1), and NaOH zoomed on the aliphatic, oxygenated aliphatic, and aromatic regions. The circled peaks appear in the spectrum but are below the presented threshold. *The signal for A_γ is convoluted, and the whole proximity is therefore highlighted.

In Chapter 3, a new set of signals was identified as reduced β -O-4 linkage containing methylene group at C_{α} , acting as an additional stabilisation mechanism for lignin (highlighted in green).²³⁹ Additionally, it was demonstrated that carbohydrates present in the lignin oil are in the form of oligoxylan alcohols (highlighted in red).²³⁹ Accordingly, besides the canonical lignin linkages, these new features are considered in the RCF lignin spectra analysis. Table 4-1 lists the number of canonical lignin linkages along with reduced β -O-4 linkages per 100 aromatic units, as well as the relative abundance of S-units among the S-and G-units.

Table 4-1. Number of lignin linkages per 100 aromatic units in RCF lignin oils obtained from pretreated wood with the corresponding additive. *Control 1 refers to ball-milled wood with no additive used to carry out the RCF process.

Linkage	CH ₃ COOH	Control 1*	NaOH
β -O-4 (A)	10.8 $\pm$ 0.3	11.9 $\pm$ 0.2	11.1 $\pm$ 0.1
Reduced β -O-4 (A _{CH2})	4.1 $\pm$ 0.2	3.8 $\pm$ 0.2	3.1 $\pm$ 0.1
β -O-4 total	14.9 $\pm$ 0.4	15.7 $\pm$ 0.3	14.2 $\pm$ 0.1
Reduced β -O-4 out of total β -O-4 / %	27.5 $\pm$ 0.5	24.4 $\pm$ 0.9	22.0 $\pm$ 0.3
Phenylcoumaran (B)	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.3 $\pm$ 0.1
Resinol (C)	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1
S-unit content / %	74.9 $\pm$ 0.5	77.9 $\pm$ 0.1	83.7 $\pm$ 0.4

Oxygenated aliphatic and anomeric regions display similar spectra for RCF lignin oils obtained from pretreated wood with acetic acid and no additive (Control 1). Although the reduced carbohydrates are present in both lignin oils, the anomeric carbon region highlights the larger abundance of oligoxylan alcohols for acetic acid. In contrast, the corresponding carbohydrate signals are much less pronounced in RCF lignin oil obtained from processed wood with NaOH, indicating a less extensive release of carbohydrates. Importantly, the lack of carbohydrates in NaOH RCF lignin may also explain the more extensive HDO of monophenol compounds observed in the previous section. It has previously shown that hemicellulose-derived carbohydrates reduce the activity of Raney Ni in the RCF process, resulting in much less extensive hydrogenation of the aromatic ring.²¹⁶ As a result, the higher proportion of

alkylphenol compounds in the RCF lignin oil obtained from NaOH-processed wood could be a result of a more active catalyst in the reaction liquor.

The lignin linkages quantification per 100 aromatic units demonstrated a similar count between different lignin oils, with the standard β -O-4 linkages fluctuating between 10.8-11.9 linkages per 100 aromatic units, phenylcoumaran between 0.3-0.6, and resinol between 0.6-0.7 linkages per 100 aromatic units. However, the relative abundance in reduced β -O-4 varies between different pretreatments. In the RCF lignin oil extracted from NaOH-processed wood, 22% of the β -O-4 linkages were reduced, while the corresponding fraction for no additive Control 1 was 24.4% and for acetic acid 27.5%. Furthermore, the reduced β -O-4 features associated with G-type lignin ($\delta C/\delta H=36.53/2.86$ ppm; $\delta C/\delta H=36.53/2.78$ ppm; $\delta C/\delta H=80.56/4.30$ ppm) are fading out for NaOH processed wood. Quantitatively, out of the reduced β -O-4 linkages, $86\pm 1\%$ are associated with two linked S-units for NaOH, compared to $82\pm 2\%$ in no additive Control 1 and $76\pm 2\%$ in acetic acid. When assuming a more active catalyst for the NaOH RCF lignin oil with lower carbohydrate content, the lower extent of reduced β -O-4 linkages present implies that the extent of HDO at C_α is not dominated by the catalyst activity, but rather the lignin spatial configuration and structure determining its ability to interact with the catalyst.

In the aromatic region, a clear distinction between the characterised lignin oils is the abundance of p-hydroxybenzoate (PB) pendant groups, which decorate the lignin backbone.¹⁰² While for the acetic acid and no additive control, there are 1.2 and 1.1 PB-units present per 100 aromatics, the group is absent from the lignin oil obtained from NaOH-processed wood. Furthermore, the NaOH spectra contain a group of extra ^{13}C - ^1H correlation signals between $\delta C/\delta H=125-130/6.7-7.2$ ppm, which may refer to lignin condensation products. The distribution of S-type lignin among the dissolved lignin species mimicked the pattern observed in lignin monomers. Nevertheless, there were discrepancies in the relative abundance of S-units when comparing monomers to all aromatic molecules, particularly for the control and NaOH samples. Acetic acid pretreatment manifested a consistent S-unit content of 75%, matching the ratio observed in monophenols. However, in the control experiment with no additives, the overall S-unit abundance was 3% lower (78%) in comparison to monophenols (81%). Similarly, wood processed with NaOH yielded a relative S-units

abundance of 84% for all lignin species, which was 6% lower than the ratio for lignin-derived monomers (90%).

4.2.3. Processed wood and pulps characterisation

Analytic saccharification based on the NREL protocol was carried out to assess the composition of processed wood after mechanocatalytic pretreatment and pulp after RCF.³⁸⁴ Accordingly, Figure 4-5 displays glucans, xylans, and lignin retention in the pulp after the RCF process with processed wood. The individual composition of each processed wood and pulp is tabulated in Appendix (Table A-7).

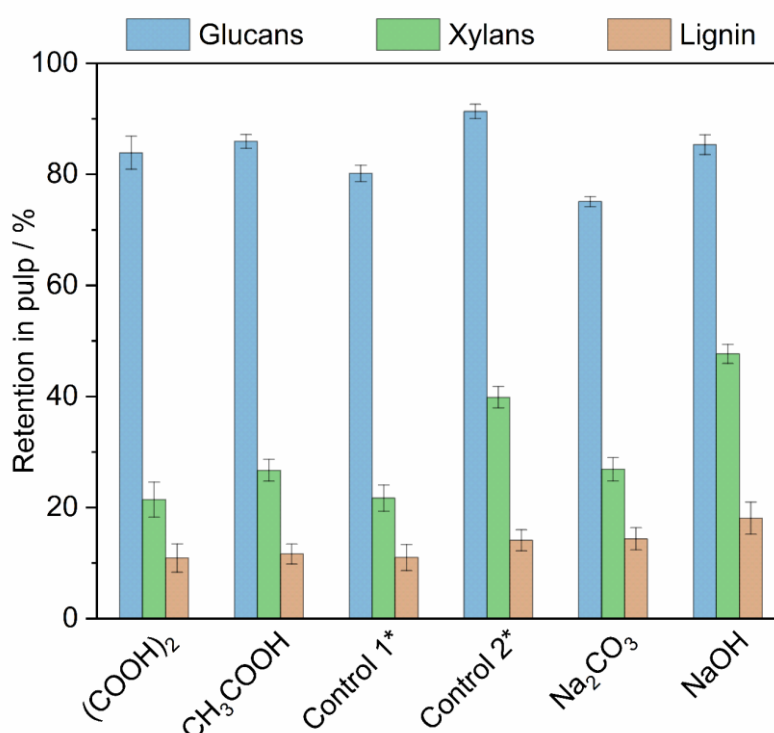


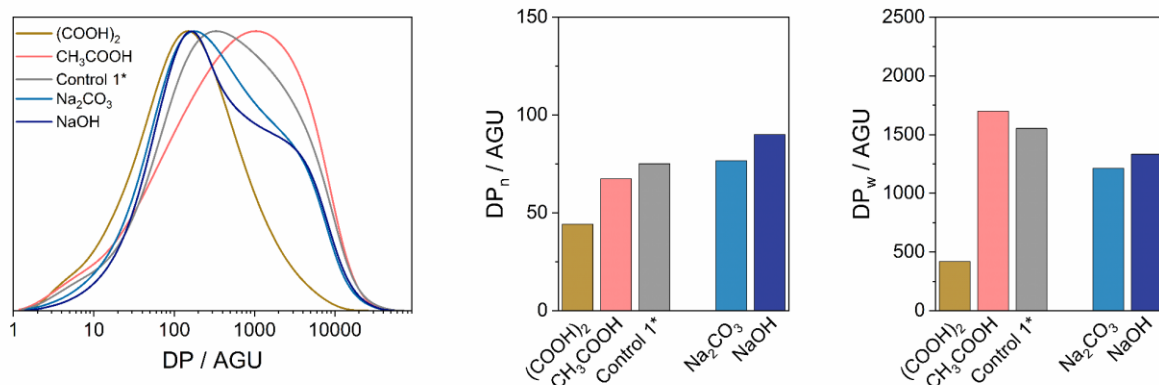
Figure 4-5. Retention of glucans (cellulose), xylans (hemicellulose), and lignin during the RCF process with processed wood obtained by mechanocatalytic pretreatment with different additives. *Control 1 refers to ball-milled wood with no additive used to carry out the RCF process. ** Control 2 refers to poplar chips used directly for the RCF process. The retention is based on the components' initial mass in processed wood.

In terms of glucans retention, oxalic acid (84%), acetic acid (86%), and NaOH (85%) pretreatments marginally outperformed the control (80%). Conversely, Na₂CO₃ pretreatment decreased glucan retention to 75%. Importantly, the poplar chips (Control 2) displayed considerably higher glucan retention than the ball-milled counterpart, with 91% of the glucans retained. Xylan retention with oxalic acid was on par with the control (21% vs 22%), while acetic

acid and sodium carbonate enhanced xylan retention to 27%. Notably, NaOH-pretreated wood demonstrated the highest xylan retention at 48%. Similarly to glucans, poplar chips retained considerably more hemicellulose compared to ball milling with no additive, with 40% of xylans retained. The acid pretreatments did not influence lignin retention in pulp, with 11% of the initial lignin retained in pulp after the RCF process. In turn, pretreatment with Na₂CO₃ resulted in 14% of the lignin retained, while NaOH-processed wood retained 18% of the initial lignin. Starting the RCF process with untreated poplar chips (Control 2) retained 14% of the initial lignin, which is higher compared to no additive ball-milled counterpart (Control 1).

To characterise the effect of mechanocatalytic pretreatments on cellulose degree of polymerisation (DP) in wood and pulp, the corresponding samples were derivatised to cellulose tricarbanilate and analysed by GPC.⁴⁹⁹ Accordingly, Figure 4-6 displays DP distribution based on anhydroglucose units (AGU) as well as the DP based on number average values (DP_n) and weight average values (DP_w) for the processed woods before RCF and pulp after the RCF process. It is important to emphasise that DP_n is a sensitive metric towards low DP values. Accordingly, the low DP part of the distribution carries a high weight. In turn, the DP_w evaluates the distribution less sensitively toward the low DP values. Therefore, the DP_w values are used in the following discussion, while the DP_n values serve as a reference.

Processed wood (before RCF)



Pulp (after RCF)

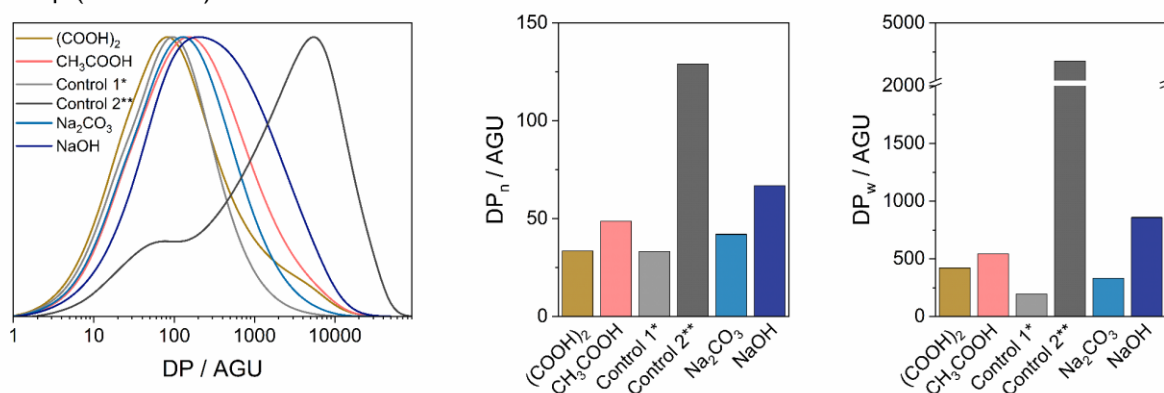


Figure 4-6. Distribution curves of the apparent degree of polymerisation for processed wood and pulps given in anhydroglucose units (AGU). On the right, the corresponding number average values (DP_n) and weight average values (DP_w) are displayed.

As a result of ball-milling, all processed woods experienced a significant drop in cellulose DP compared to native wood, indicating ball-milling induced cellulose depolymerisation. The ball-milling without any additive (Control 1) resulted in DP_w value of 1550 AGU, which is higher compared to the process with base additives. Accordingly, the processed wood obtained with Na_2CO_3 additive displayed DP_w of 1210 AGU and NaOH pretreated wood of 1330 AGU. The mechanocatalytic pretreatment with oxalic acid was most severely depolymerising the cellulose, resulting in DP_w of 420 AGU. In contrast to oxalic acid, the addition of the acetic acid increased the DP of processed wood in comparison to no additive ball-milling (Control 1), resulting in DP_w of 1700 AGU.

As a result of the RCF process, the no additive processed wood resulted in the lowest DP_w value (190 AGU), while Na_2CO_3 processed wood resulted in a pulp with DP_w of 330 AGU.

Notably, the RCF process with oxalic acid pretreated wood resulted in pulp displaying the same DP as the processed wood (420 AGU). The DP_w of the acetic acid treated processed wood dropped to 540 AGU in pulp, while the NaOH pretreated wood experienced a smaller drop with the pulp DP_w of 860 AGU. Importantly, and in contrast to the ball-milled processed woods, starting the RCF process with untreated poplar chips (Control 2) resulted in an order of magnitude higher DP_w (4670 AGU). The result illustrates that while the pretreatment additive has an effect on the DP of cellulose after the RCF process, the ball-milling itself is the main factor in reducing the cellulose DP. This aligns with established knowledge that mechanical energy from ball-to-ball and ball-to-wall collisions transferred to cellulose during the ball-milling reduces cellulose crystallinity, breaks interchain hydrogen bonds in crystalline domains and causes cellulose depolymerisation.^{497,500–505}

It has been established that, as a general rule, the RCF process yields a pulp with substantial cellulose retention and a high degree of delignification.^{20,25} Over the course of the process due to the deacetylation of hemicellulose, the reaction liquor becomes acidic, thereby facilitating further hemicellulose hydrolysis.^{215,216} Notably, alkaline conditions tend to amplify acetic acid release, while acidic conditions suppress it.^{134,215,216} The structural and biological functions of acetate substitution in hemicellulose continue to remain ambiguous.⁵⁰⁶ One theory suggests that the acetate groups in hemicellulose point towards the lignified region of the cell wall, thereby strengthening the cohesion between the lamellae.¹⁹⁵ Accordingly, Johnson *et al.* demonstrated that a lower extent of hemicellulose acetylation can lead to more processable poplar wood.⁵⁰⁶

In the current investigation, hemicellulose retention varied significantly between different pulps. It is important to emphasise that after ball milling with additive, processed wood was washed with water until neutral pH, meaning no unbound acid or base was carried over to the RCF process. Consequently, the changes in hemicellulose retention can be attributed to the modifications in the lignocellulosic matrix during the pretreatment. With the alkaline pretreatment, it is likely that partial deacetylation of pulp in the presence of moisture in wood took place, and the resulting sodium acetate was washed away prior to the RCF process. Less acetylated hemicellulose decreased the extent of acetate groups available for hydrolysis during

the RCF process, consequently keeping the liquor pH higher and hindering the hydrolysis of glycosidic bonds.

The pretreatment with oxalic acid did not influence the xylans retention or the extent of delignification, while at the same time, it slightly increased the monophenol yields and considerably lowered the relative S-unit abundance among the monophenols. Therefore, it is likely that during the pretreatment with solid oxalic acid, a disruption in the lignin ultrastructure takes place via partial reversible deacetylation of hemicellulose, especially at the outer proportion of the G-lignin rich cell walls. Consequently, hydrolytic release of lignin moieties becomes more favoured, leading to higher G-type monophenol yields compared to the control experiment with no additive.

While oxalic acid, Na_2CO_3 , and NaOH are all solids at room temperature, glacial acetic acid is a liquid and can diffuse well in the cell walls. Due to the nature of acetic acid, reversible deacetylation of existing acetyl groups, as well as further acetylation of the hemicellulose, can take place during the ball milling. Concurrently, the lignocellulosic matrix ultrastructure is disrupted across the whole cell wall, thereby enhancing the hydrolytic release of lignin moieties. At the same time, further acetylation of hemicellulose during the pretreatment could obstruct glycosidic bond hydrolysis as the removal of more acetylated species from the secondary cell wall has proven to be more challenging.⁵⁰⁶

The mechanism of how different additives act on the lignocellulose during the mechanocatalytic pretreatment remains largely ambiguous at this point, with a hypothesis formulated for each additive. However, as a result of the screening with different additives, it becomes clear that out of the two acidic and two base additives utilised, acetic acid provides the most favourable treats to enhance the RCF process outcomes. Accordingly, the pretreatment with acetic acid increases the monophenols and RCF lignin oil yield compared to ball milling without additive (Control 1). In addition, it also results in higher pulp yield with higher xylans and glucans retention and higher DP of cellulose in the pulp. Therefore, the next two sections concentrate on the acetic acid pretreatment to evaluate the effect of concentration and milling time on the process outcomes.

4.2.4. Acetic acid concentration and kinetic energy dose influence on lignin oils

To evaluate the effect of the amount of acetic acid added during the pretreatment, a set of experiments was carried out varying the added acetic acid amount from 0- 4 mmol g⁻¹ of wood. At the added acetic acid amount of 2 mmol g⁻¹ also used in the previous sections for screening, three additional experiments were carried out with ball-milling time of 30-100 min, resulting in kinetic energy doses from 4.3-14.3 kJ g⁻¹ of wood. Accordingly, Figure 4-7 displays the RCF lignin oil yield dependant on the amount of acetic acid utilised during the mechanocatalytic pretreatment as well as on the kinetic energy dose.

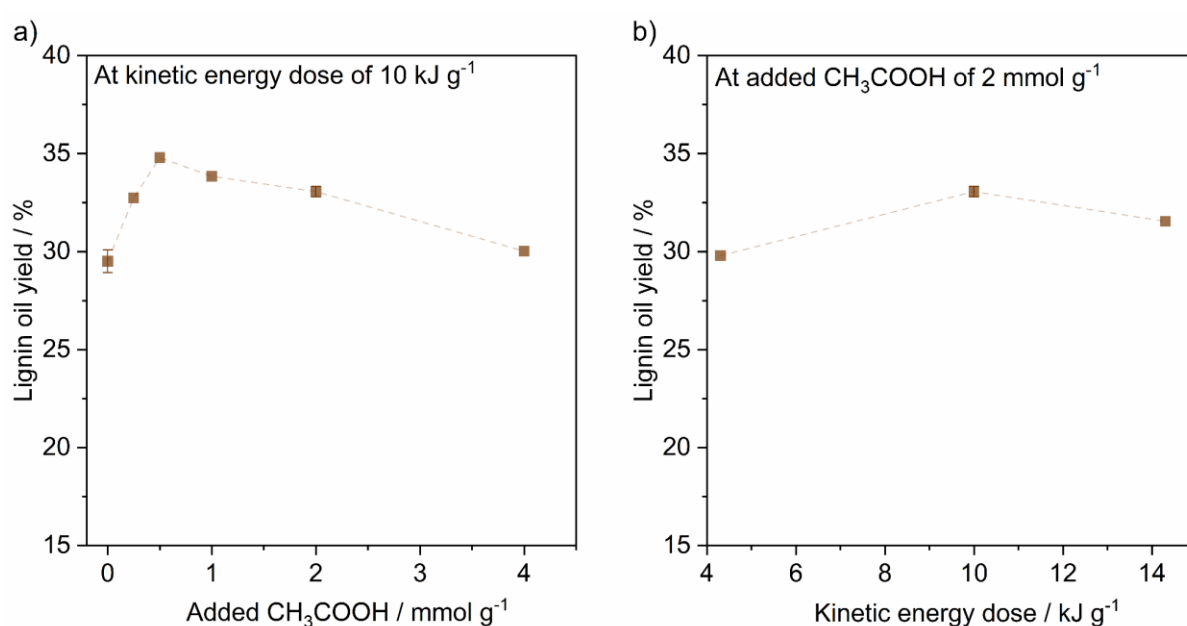


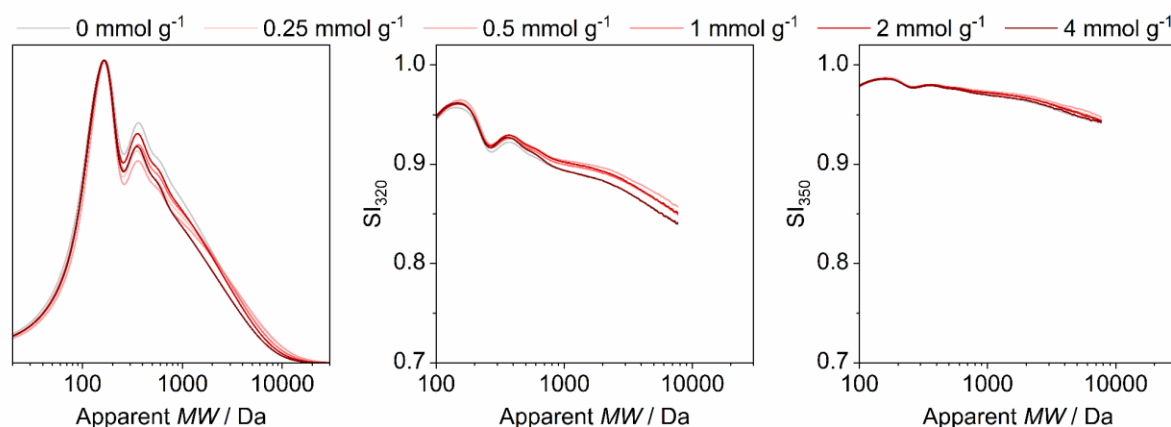
Figure 4-7. a) RCF lignin oil yield from initial poplar wood with varying amounts of acetic acid added during the pretreatment. All the mechanocatalytic pretreatments were carried out with the kinetic energy dose of 10 kJ g⁻¹. b) RCF lignin oil yield from initial poplar wood with varying kinetic energy dose during the pretreatment. All the mechanocatalytic pretreatments were carried out with the added acetic acid amount of 2 mmol g⁻¹.

At fixed kinetic energy dose, the RCF lignin oil yield presented a maximum in the added acetic acid range of 0-4 mmol g⁻¹. Accordingly, the lignin oil yield increased between 0 to 0.5 mmol g⁻¹ acetic acid added from 29.5% to 34.8%. From the highest lignin oil yield measured at 0.5 mmol g⁻¹, the yield gradually decreased, reaching 30.0% at the added acetic acid amount of 4 mmol g⁻¹. Similarly, at the fixed added acetic acid amount (2 mmol g⁻¹), the obtained RCF lignin oil yield presented a maximum. However, the deviation between different kinetic doses was small compared to varying acetic acid amounts. Accordingly, at the kinetic energy dose of

4.3 kJ g⁻¹, 29.8% of the initial wood resulted in lignin oil. At the kinetic energy dose of 10 kJ g⁻¹, 33.1% yield was achieved, while 14.3 kJ g⁻¹ resulted in a lignin oil yield of 31.5%.

As for the screening with different additives, the RCF lignin oils obtained from pretreatment with acetic acid were analysed by GPC and evaluated with the Structural Index introduced in Chapter 3. Accordingly, Figure 4-8 displays the molecular weight distribution at 280 nm as well as SI_{320} and SI_{350} values across the molecular weight distribution. Regardless of the acetic acid concentration or the kinetic energy dose, all the RCF lignin oils display a very similar molecular weight distribution. Similarly, the SI_{320} and SI_{350} distribution curves are very alike despite the acetic acid concentration or kinetic energy dose. In this manner, all the lignin oils obtained with acetic acid pretreatment (as well as the no additive 0 mmol g⁻¹) have the average SI_{320} between $0.90\text{--}0.91 \pm 0.03$, and the same average SI_{350} value of 0.97 ± 0.01 . As the oligomeric and polymeric fractions of the lignin oils display very similar Structural Index characteristics, it can be concluded that the acetic acid pretreatments do not have a strong influence on the chemical nature of oligomeric and polymeric lignin moieties in the RCF lignin oils.

a) Varying added CH_3COOH at kinetic energy dose of 10 kJ g^{-1}



b) Varying kinetic energy dose at added CH_3COOH of 2 mmol g^{-1}

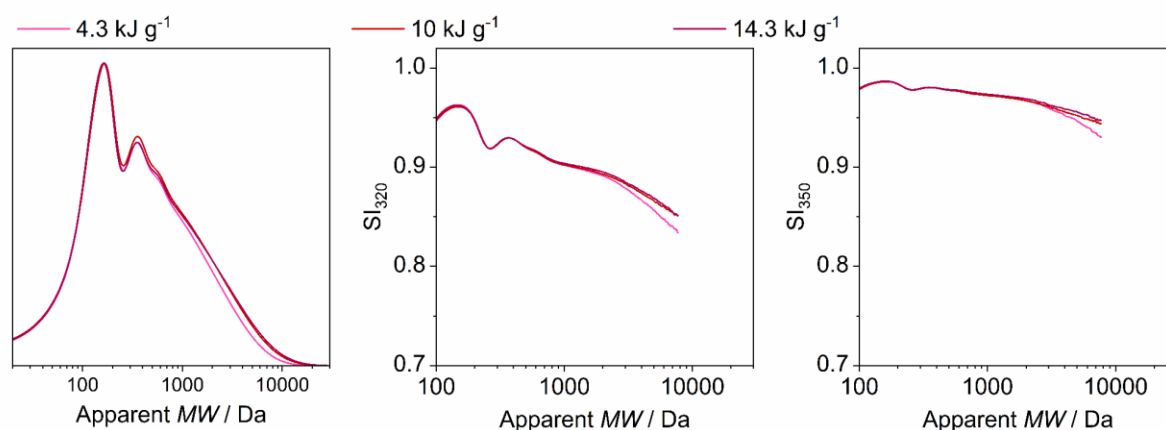


Figure 4-8. GPC traces of RCF lignin oils at 280 nm normalised by the absorbance of monomers, Structural Index at 320 nm and 350 nm across the apparent molecular weight of the RCF lignin oils from the column exclusion limit of 100 Da. a) Varying acetic acid amount during the pretreatment at the constant kinetic energy dose of 10 kJ g^{-1} . b) Varying kinetic energy dose during the pretreatment at the added acetic acid amount of 2 mmol g^{-1} .

To assess the acetic acid amount and kinetic energy dose effect on the lignin-derived monophenols yield, the lignin oils were characterised by GC-FID. Figure 4-9 summarises the S- and G-type lignin-derived monophenols yield from the initial lignin in processed wood. Similarly to the lignin oil yield, the monophenol yields present a maximum in the studied range of added acetic acid. The total monophenols yield increased between 0 and 1 mmol g^{-1} from 13.4% to 15.9%, following a gradual decrease to 13.2% at 4 mmol g^{-1} of acetic acid added. The trend of S- and G-type monophenols also followed the total monophenol yields individually, with the syringols yield growing from 11.5% to 12.8% ($0\text{--}1 \text{ mmol g}^{-1}$) and gradually decreasing to 11% after that (4 mmol g^{-1}). The guaiacols yield increased 2.0% to 3.1% ($0\text{--}1 \text{ mmol g}^{-1}$) and gradually decreased to 2.2% afterwards (4 mmol g^{-1}). The monophenols yield with changing

kinetic energy dose varied less compared to the changing amount of acetic acid added but still presented a maximum in the studied range. Accordingly, a kinetic energy dose of 10 kJ g^{-1} resulted in the highest total yield (14.8%), with syringols yield of 12.3% and guaiacols yield of 2.5%. A kinetic energy dose of 4.3 kJ g^{-1} resulted in a total yield of 14.3% (syringols 11.9% and guaiacols 2.4%), and 14.3 kJ g^{-1} resulted in a total yield of 13.7% (syringols 11.3% and guaiacols 2.4%).

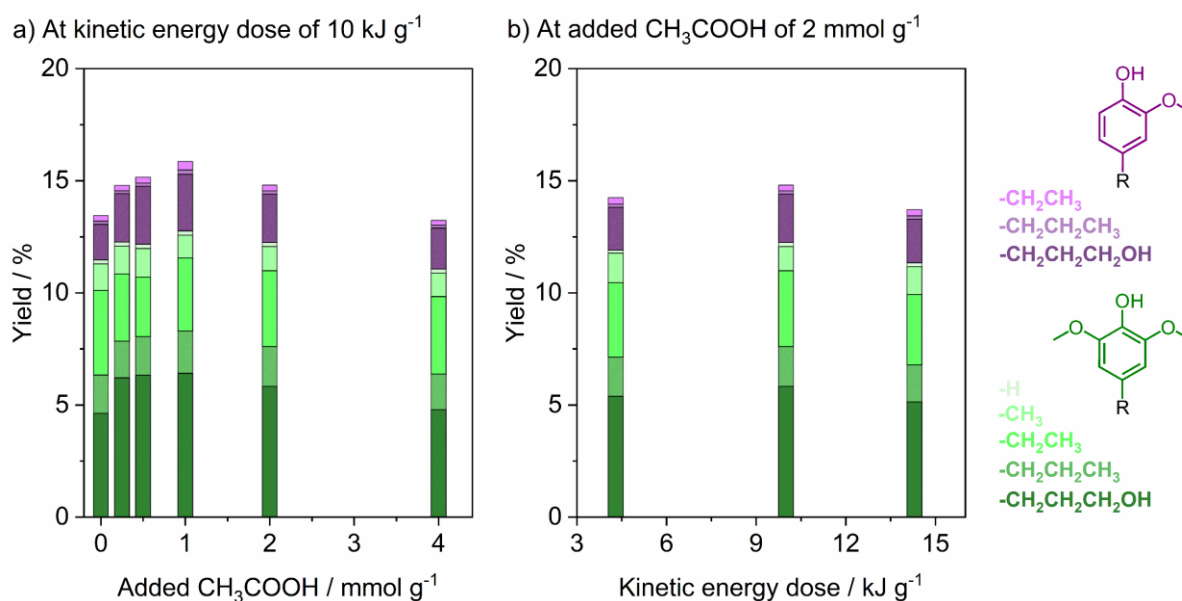


Figure 4-9. a) S- and G-Type monophenols yields from lignin in processed wood with varying amount of acetic acid added during the mechanocatalytic pretreatment. Fixed kinetic energy dose of 10 kJ g^{-1} was utilised. b) S- and G-Type monophenol yields with varying kinetic energy dose during the mechanocatalytic pretreatment. Fixed amount of acetic acid was added (2 mmol g^{-1}).

Besides the monophenol yields, the product distribution among the monophenols also varied with the changing amount of acetic acid added. While the P(OH)S formed 40%_{mol} of the S-type monophenols when no acetic acid was utilised, adding $0.25\text{--}1 \text{ mmol g}^{-1}$ acetic acid increased the relative abundance of P(OH)S to 50–52%_{mol}. With further increasing the amount of added acetic acid, the relative abundance P(OH)S started to decrease again with 48%_{mol} at 2 mmol g^{-1} and 43%_{mol} at 4 mmol g^{-1} . At the same time, the relative abundance of P(OH)G among the guaiacols varied only between 81–86%_{mol} with changing amounts of acetic acid. The changing kinetic energy dose presented very little effect on the monophenols distribution, with the relative abundance of P(OH)S varying 45–48%_{mol} among syringols and P(OH)G varying between 81–84%_{mol} among guaiacols.

Based on the lignin oils analysis, it can be concluded that changing acetic acid concentration helped to increase the monophenols yield and relative abundance of P(OH)S compared to no additive ball milling, while the properties of oligomeric and polymeric lignin remained largely unaltered based on the Structural Index analysis. When performing the mechanocatalytic pretreatment with 2 mmol g⁻¹ added acetic acid, but changing the kinetic energy dose, the lignin oil and monomers yield as well as the monomers distribution changed very little.

4.2.5. Acetic acid concentration and kinetic energy dose influence on pulps

To examine the effect of varying amount of acetic acid added and kinetic energy dose during the mechanocatalytic pretreatment on the processed wood and pulps, corresponding samples were characterised by analytical saccharification. Similarly to the experiments with different additives, the processed wood and pulp samples were derivatised to cellulose tricarbanilate and analysed by GPC to determine the corresponding degree of polymerisation. Firstly, however, Figure 4-10 displays the pulp yield from processed poplar wood after the RCF process.

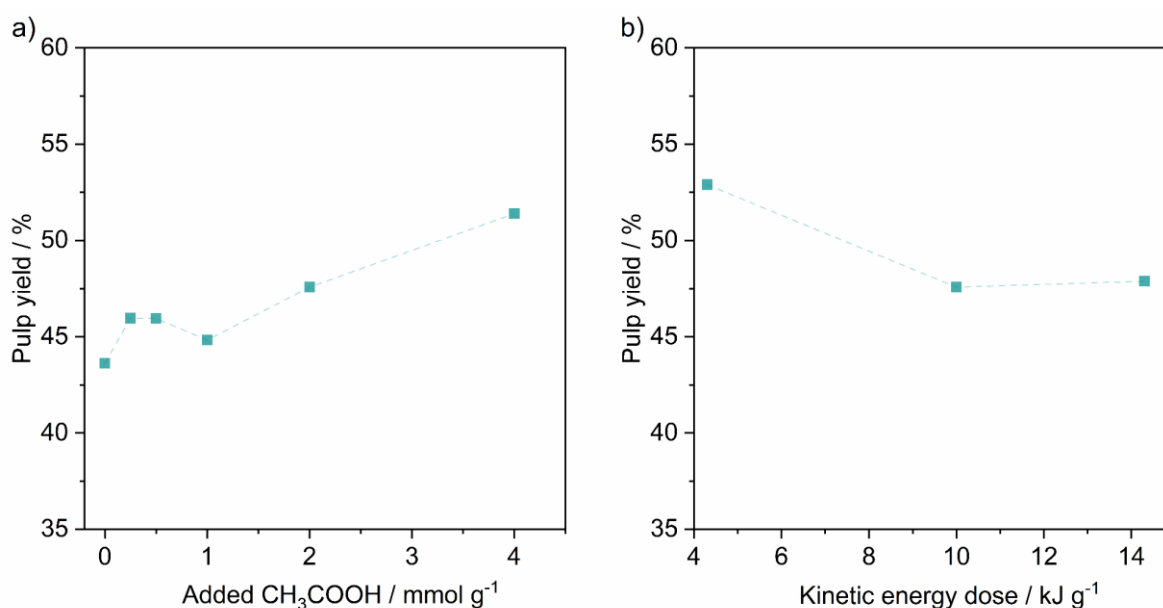


Figure 4-10. a) pulp yield from processed poplar wood with varying amounts of acetic acid added during the pretreatment. All the mechanocatalytic pretreatments were carried out with the kinetic energy dose of 10 kJ g⁻¹. b) Pulp yield from processed poplar wood with varying kinetic energy dose during the pretreatment. All the mechanocatalytic pretreatments were carried out with the added acetic acid amount of 2 mmol g⁻¹.

Increasing the amount of acetic acid added during the pretreatment presents an upward trend in pulp yield. Accordingly, the pulp yield increased from 43.6% for no added acetic acid to

51.4% when 4 mmol g⁻¹ acetic acid was added during the ball milling. At the same time, a lower kinetic energy dose at an added acetic amount of 2 mmol g⁻¹ resulted in higher pulp retention, with the pulp yield decreasing from 52.9% at the kinetic energy dose of 4.3 kJ g⁻¹ to 47.9% at 14.3 kJ g⁻¹. The pulp yields were combined with the results from analytical saccharification to obtain each main component retention in the pulp after the RCF process. Accordingly, Figure 4-11 displays glucans (cellulose), xylans (hemicellulose), and lignin retention in the pulp from the processed poplar wood. The top part presents the retentions with varying amounts of acetic acid at a fixed kinetic energy dose (10 kJ g⁻¹), while the bottom part displays the retentions with varying kinetic energy dose at a fixed amount of acetic acid added (2 mmol g⁻¹). Individual processed wood and pulp compositions can be found in Appendix Tables A-8 and A-9.

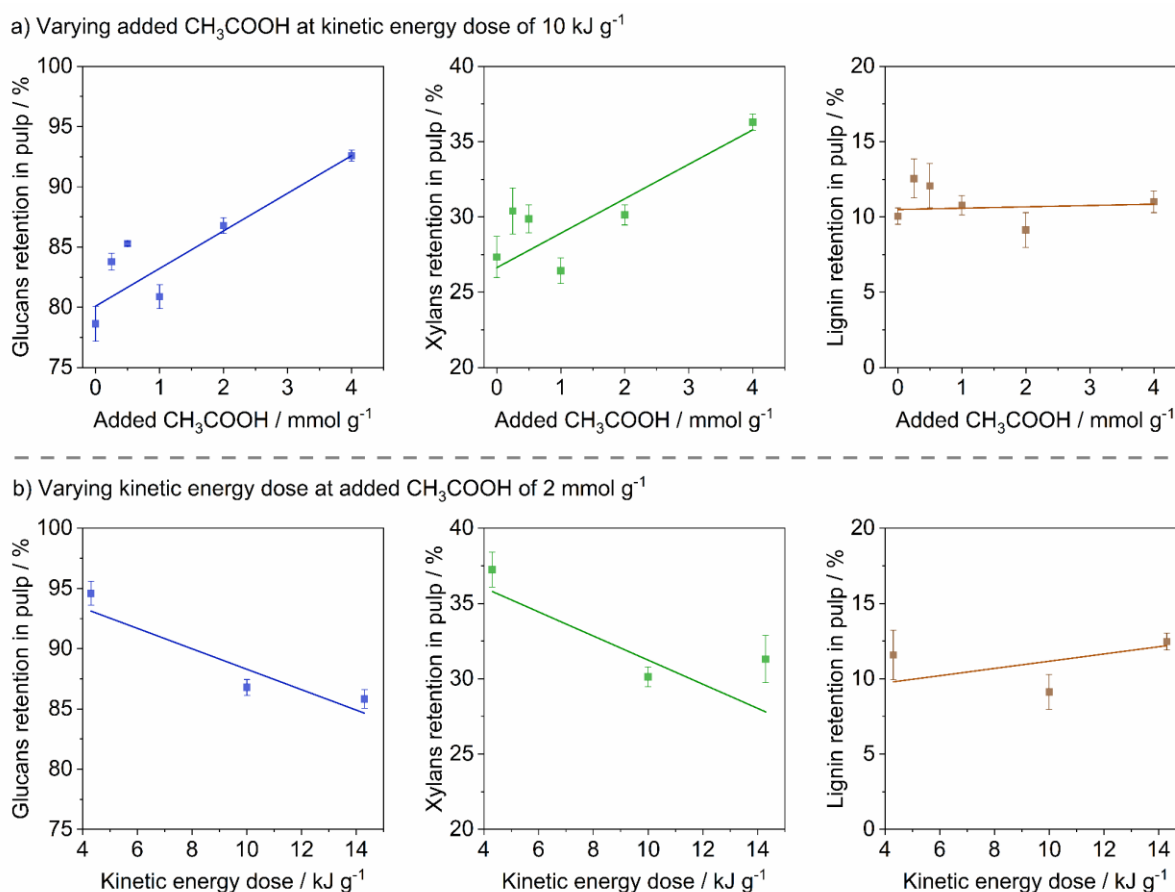
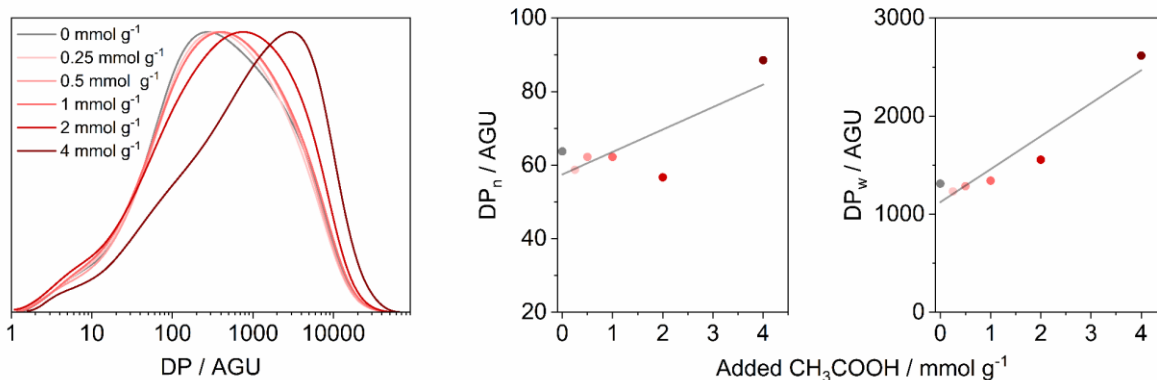


Figure 4-11. Retention of glucans (cellulose), xylans (hemicellulose), and lignin during the RCF process with processed wood obtained by mechanocatalytic pretreatment with different additives. a) Varying added acetic acid amount at a fixed kinetic energy dose of 10 kJ g⁻¹. b) Varying kinetic energy dose at a fixed amount of added acetic acid (2 mmol g⁻¹).

Increasing the amount of acetic acid added during the mechanocatalytic pretreatment strongly enhanced the amount of glucans retained in the pulp. Accordingly, the glucans retention increased from $79\pm1\%$ with no acetic acid added to $92.6\pm0.2\%$ at the added acetic acid amount of 4 mmol g^{-1} . Similarly, adding more acetic acid increased the xylans retention from $27\pm1\%$ to $36.3\pm0.6\%$, respectively. Varying the acetic acid amount during the pretreatment did not strongly affect lignin retention in pulp (varying between 9-12% in the studied range). Changing the kinetic energy dose lowered the glucans retention from $95\pm1\%$ at 4.3 kJ g^{-1} to $85.2\pm0.8\%$ at 14.3 kJ g^{-1} . As for the varying acetic acid amount, xylans presented a matching trend with glucans when the kinetic energy dose was varied. Accordingly, the xylans retention decreased from $37\pm1\%$ at 4.3 kJ g^{-1} to $31\pm2\%$ at the highest kinetic energy dose of 14.3 kJ g^{-1} . The lignin retention varied a little with changing kinetic energy dose, ranging between 9-12%.

From the glucans and xylans retention, it becomes clear that adding acetic acid during the mechanocatalytic pretreatment hinders the cellulose and hemicellulose hydrolysis in the RCF process. During the screening of different additives pretreatment in the previous section, it was evaluated that ball milling itself causes extensive depolymerisation of cellulose in wood, which aligns with the established knowledge.^{497,500–505} Accordingly, a question arises as to whether added acetic helps to preserve the cellulose structure during the pretreatment, resulting in higher retention during the RCF process. To assess the DP of processed wood after mechanocatalytic pretreatment and pulp after the RCF process, Figure 4-12 displays DP distribution based on anhydroglucose units (AGU) as well as the DP based on number average values (DP_n) and weight average values (DP_w). As before, DP_w values are used for discussion as being less sensitive towards the low DP values, while DP_n values on the graph serve as a reference.

a) Processed wood (before RCF)



b) Pulp (after RCF)

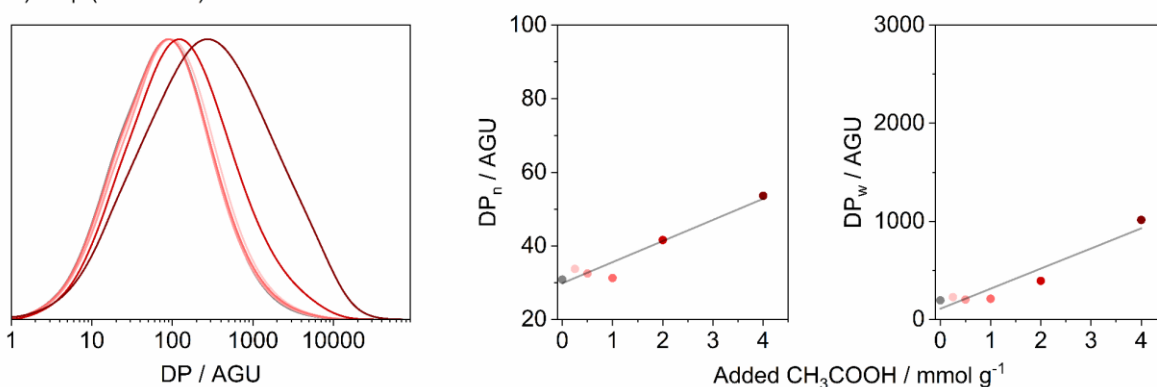


Figure 4-12. Distribution curves of the apparent degree of polymerisation for processed wood (a) and pulps (b) given in anhydroglucose units (AGU) and obtained by varying added acetic acid amount during the mechanocatalytic pretreatment at fixed kinetic energy dose (10 kJ g^{-1}). On the right, the corresponding number average values (DP_n) and weight average values (DP_w) are displayed.

Comparison of the DP distribution at different added acetic acid amounts reveals one of the key effects of the acetic acid during mechanocatalytic pretreatment. While varying the added acetic acid amount between $0\text{--}1 \text{ mmol g}^{-1}$ has little effect on the DP distribution, 2 mmol g^{-1} acetic acid shifts the distribution toward higher DP values and 4 mmol g^{-1} even further. Consequently, the DP_w value ranges between $1230\text{--}1340 \text{ AGU}$ for the pretreatments with $0\text{--}1 \text{ mmol g}^{-1}$ acetic acid, while 2 mmol g^{-1} increases the DP_w to 1560 AGU and 4 mmol g^{-1} to 2620 AGU . Importantly, the same trend carries over to the DP in pulps. Accordingly, the DP distribution for 2 and 4 mmol g^{-1} acetic acid is shifted towards the higher DP values. In the added acetic acid range $0\text{--}1 \text{ mmol g}^{-1}$, the DP_w only ranges between $190\text{--}230 \text{ AGU}$, while 2 mmol g^{-1} presents DP_w of 390 AGU and 4 mmol g^{-1} has a DP_w of 1010 AGU in pulp.

Based on the DP of processed wood and resulting pulp, it can be stated that the increasing glucans and xylans retention observed with an increasing amount of acetic acid added follows the trend of cellulose DP in processed wood. Accordingly, adding acetic acid during the pretreatment facilitates the preservation of cellulose (and hemicellulose) structure and consequently hinders their hydrolysis during the following RCF process. This, in turn, resulted in higher glucan and xylan retention during the RCF process.

In terms of kinetic energy dose at the fixed added acetic acid amount (2 mmol g^{-1}), lower mechanical energy input resulted in higher glucans and xylans retention. Similarly to the varying acetic acid amount during the mechanocatalytic pretreatment, the processed woods obtained with changing kinetic energy dose as well as the corresponding pulps, were derivatised to cellulose tricarbanilate. Accordingly, Figure 4-13 displays DP distribution based on anhydroglucose units (AGU) and corresponding DP_n and DP_w values.

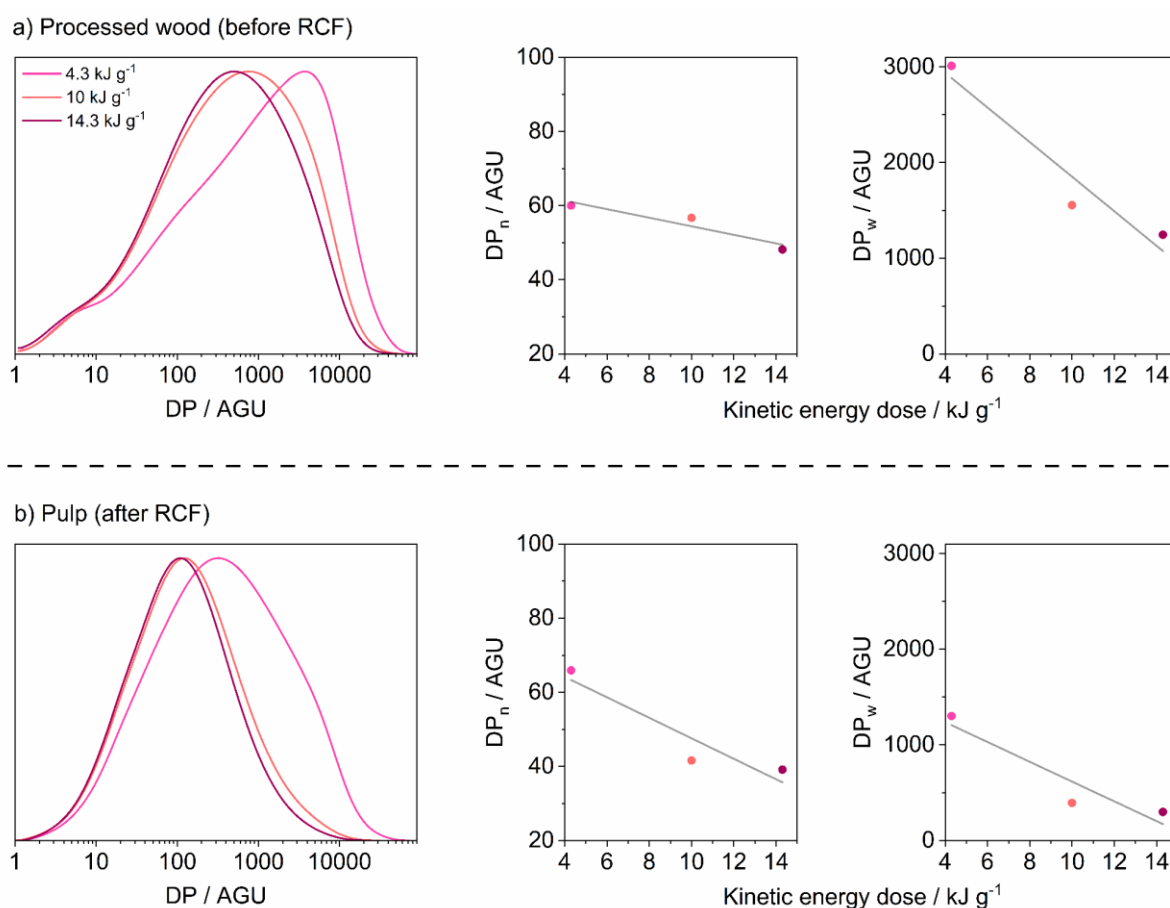


Figure 4-13. Distribution curves of the apparent degree of polymerisation for processed wood (a) and pulps (b) given in anhydroglucose units (AGU) and obtained by kinetic energy dose during the mechanocatalytic pretreatment at fixed

amount of acetic acid added (2 mmol g⁻¹). On the right, the corresponding number average values (DP_n) and weight average values (DP_w) are displayed.

The DP distribution of the processed woods reveals that increasing kinetic energy dose during ball-milling shifts the distribution curve towards lower DP values. This result aligns well with the fact that a higher kinetic energy dose results in more extensive cellulose degradation during the ball-milling.⁴⁹⁷ Accordingly, the kinetic energy dose of 4.3 kJ g⁻¹ resulted in DP_w of 3010 AGU in processed wood, while 10 kJ g⁻¹ reduced the DP_w to 1560 AGU and 14.3 kJ g⁻¹ to 1240 AGU. As for the varying amounts of acetic acid, the pulp obtained during RCF followed the same DP trend as the processed wood. The pulp from processed wood obtained at the kinetic energy dose of 4.3 kJ g⁻¹ displayed considerably higher DP compared to 10 and 14.3 kJ g⁻¹, with the DP_w values of 1300 AGU, 390 AGU, and 300 AGU, respectively.

4.3. Conclusion

Due to the need for the comminution of biomass prior to the RCF to avoid mass transfer limitations inside the lignocellulosic matrix, the current work explored an option to exploit the available mechanical energy to perform 'soft' mechanocatalytic pretreatment prior to the RCF with different additives. The control experiments, where the first one utilised no additive during the ball-milling, and the second one used poplar chips directly with no pretreatment for the RCF process, indicated that the RCF process with poplar chips is indeed mass transfer limited and the ball-milled wood resulted in higher lignin oil and lignin-derived monomers yields. Out of the two acids used for the pretreatment, acetic acid increased the total monophenols yield, while oxalic acid displayed little effect on monophenols yield. At the same time, acetic acid increased the DP of cellulose compared to the control experiment during the ball milling, resulting in higher glucans retention after RCF, while oxalic acid had the opposite effect. Two base pretreatments (Na₂CO₃ and NaOH) both lowered the monophenols yield. However, NaOH pretreatment resulted in very high S-unit selectivity among the lignin-derived monomers. Additionally, pretreatment with NaOH resulted in a significant increase in glucans and xylans retention.

Out of the screened additives, acetic acid presented the most desirable properties to enhance the outcomes of the RCF process. Accordingly, different acetic acid amounts in the range of 0-4 mmol g⁻¹ of wood and different kinetic energy doses at fixed acetic acid amounts (2 mmol

g⁻¹) were screened. The highest monophenols yield was achieved at 1 mmol g⁻¹ acetic acid added, while kinetic energy dose did not strongly affect monophenols yield in the studied range (4.3-14.3 kJ g⁻¹). The pulp analysis revealed that increasing the amount of acetic acid during the pretreatment helped preserve the cellulose DP, consequently increasing glucans and xylans retention and pulp yield. At the same time, utilising a lower kinetic energy dose resulted in significantly higher cellulose DP in both processed wood and pulp.

In a wider context, the current chapter demonstrates a new approach for biomass pretreatment prior to the RCF process. As a result, the method showed that by utilising different additives during the mechanocatalytic pretreatment, the outcomes of the RCF process can be tuned in the required direction. In this manner, acetic acid pretreatment increased both the lignin oil and lignin-derived monomers yield along with a boost in pulp yield- features that are desirable in the potential RCF industrial setting.

5. Semi-batch Temperature-stepping RCF Strategy for Controlling the Process Outcomes

5.1. Introduction

In Chapter 2, it was discussed that cellulose, hemicellulose, and lignin present very different apparent activation energies for depolymerisation under the RCF conditions. Accordingly, the degradation of β -O-4 linkages in lignin is expected to start at lower temperatures, hemicellulose hydrolysis requires higher temperatures, and cellulose hydrolysis even harsher conditions.^{214,223,224,229–234} Accounting for the difference in biopolymers' inherent nature to depolymerise under the RCF conditions, it is reasonable to argue that by running the RCF process with increasing temperature steps, the fractions with considerably different depolymerisation products and nature could be obtained.

In a traditional setting where the RCF process is carried out in batch mode, the process results in a single lignin oil and pulp. Accordingly, the fluctuations and changes in process conditions over the course of the process are also retained in the properties of single lignin oil and pulp. Therefore, the release of moieties from the lignocellulosic matrix cannot be separately controlled based on the lignin and carbohydrates' inherent characteristics, and cumulative process conditions determine the process outcome.

In addition to the classical batch mode, flow-through (FT) systems have been implied for RCF processes in recent years since its first demonstrations for RCF process in 2017.^{413,417} In an FT RCF setup, the pressurised solvent is first passed through a packed biomass column to extract lignin, and the obtained solution is passed through a catalyst containing second column along with the H_2 to achieve lignin stabilisation and further breakdown. As the temperature of both columns can be controlled separately, the FT setup allows the collection of different dissolved material fractions, which could also be obtained at different temperatures.

Besides the traditional batch setting and recently introduced FT setting for Reductive Catalytic Fractionation, we herein propose a third, semi-batch mode to carry out an RCF process. In semi-batch mode, dissolved species are removed from the batch reactor after each process

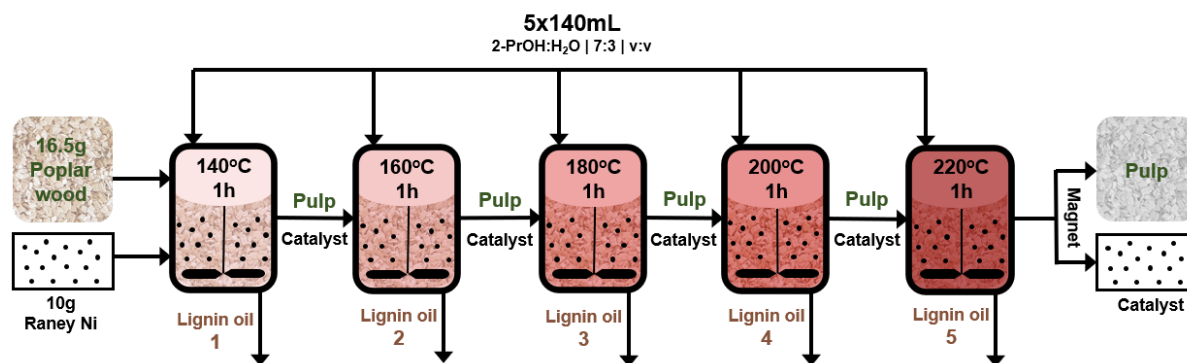
step, and the fresh solvent is added for the consequent step. Accordingly, multiple lignin oils can be obtained along with the pulp from the final step. In addition to the opportunity to obtain discrete lignin oils from the different stages of the traditional batch process, a semi-batch strategy also allows the application of different process conditions for each step to further amplify the differences between the obtained lignin oil. Accordingly, increasing temperature during each process step would allow exploiting the difference in the biopolymers' susceptibility to undergo depolymerisation.

In the current chapter, a semi-batch temperature-stepping RCF concept is explored for the first time. Five process steps are utilised with the temperature steps of 140°C, 160°C, 180°C, 200°C, and 220°C, resulting in five lignin oils and one final pulp. The first part of the chapter provides an overview of the semi-batch T-stepping process along with the mass balances. In the second part, the monomer yields in each step are quantified, and the obtained lignin oils are characterised by GPC and 2D HSQC NMR. In the final third part, the pulp properties change after each step is monitored, and the residual lignin in the pulp after each step is imaged by state-of-the-art fluorescence microscopy method.

5.2. Results and discussion

5.2.1. Isolation strategy and mass balances

The semi-batch process was performed in 5 consecutive 1 h steps, with the poplar chips and the Raney Ni catalyst carried over to the next step. The liquid phase with extracted lignin (and carbohydrates) fragments was separated from the solids after each step, and the fresh solvent was added prior to the next step. During each step, the temperature was increased stepwise from 140°C to 220°C with 20°C increments. Accordingly, Scheme 5-1 summarises the process, resulting in 5 different lignin oils obtained from the same poplar wood and in holocellulose pulp after the final step at 220°C. In addition to the semi-batch temperature stepping strategy, a regular RCF process at 220°C for 5 h with the same wood, solvent mixture, and catalyst was carried out for reference.



Scheme 5-1. Overview of the semi-batch temperature-stepping RCF process. While the solids (pulp and catalyst) are carried over after each step, the liquid phase is separated after each step with the fresh solvent mixture added.

As discussed in Chapter 3, EtOAc is a suitable solvent to solubilise lignin-derived monomers and oligomers, while the mid-and high MW lignin along with the carbohydrates in lignin oil, precipitate. Accordingly, the obtained lignin oils were fractionated with EtOAc to allow further insights into their chemical nature. Figure 5-1 displays the obtained lignin oil and pulp yield from initial oven dry wood (ODW) (after every single step and cumulative lignin oil yield) along with mass balances of lignin oils solvent fractionation with EtOAc.

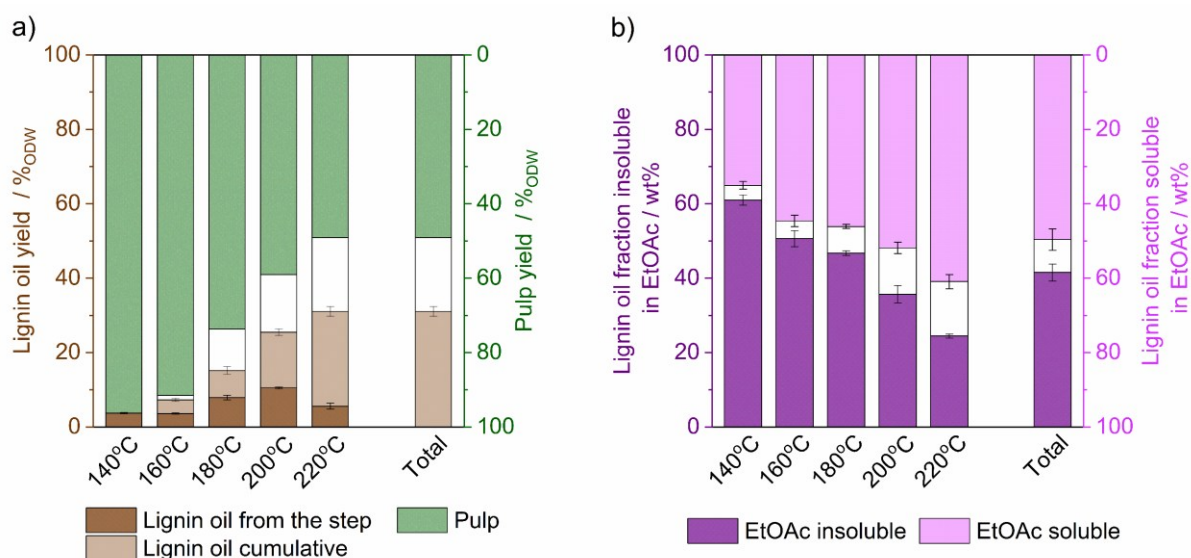


Figure 5-1. a) Pulp and lignin oil yields from poplar wood during each step of the T-stepping semi-batch process. The white void bar represents the missing mass attributed to the formation of gaseous products. b) The proportion of EtOAc soluble and insoluble fractions of the lignin oils obtained after each step.

During the first 1 h process at 140°C, 3.7% of the initial lignocellulose was converted into Lignin oil 1, while the rest resulted in pulp and was carried over to the second step at 160°C, which resulted in a lignin oil yield of 3.6%. At 180°C, the lignin oil yield from the step increased to

7.9%. Further increase in temperature during the fourth step to 200°C resulted in most lignin oil obtained by one step with the lignin oil yield of 10.5%. The final 1 h step at 220°C yielded 5.6% lignin oil, resulting in a total lignin oil yield of 31.1 % across the five steps. As a result of the five-step process, 49% final pulp yield was obtained. With the increasing temperature, the EtOAc-insoluble fraction gradually decreased from 61% in the lignin oil obtained at 140°C to 24% in the lignin oil obtained at 220°C. In turn, the EtOAc-soluble fraction increased from 35% in the lignin oil obtained at 140°C to 61% at 220°C.

5.2.2. Extracted lignin characteristics after each step

To assess the lignin-derived monomers yield during each step of the semi-batch T-gradient RCF process, the obtained RCF lignin oils were analysed by gas chromatography. Figure 5-2 displays the different monomer yields during every step and the total cumulative yield of all steps. Additionally, the lignin-derived monomers yield from single step RCF batch process at 220°C for 5 h with the same amount of wood and catalyst is displayed alongside. Alkylsyringol and alkylguaiacol species are combined to simplify the graph, and the individual yields of these species can be found in Appendix Table A-10.

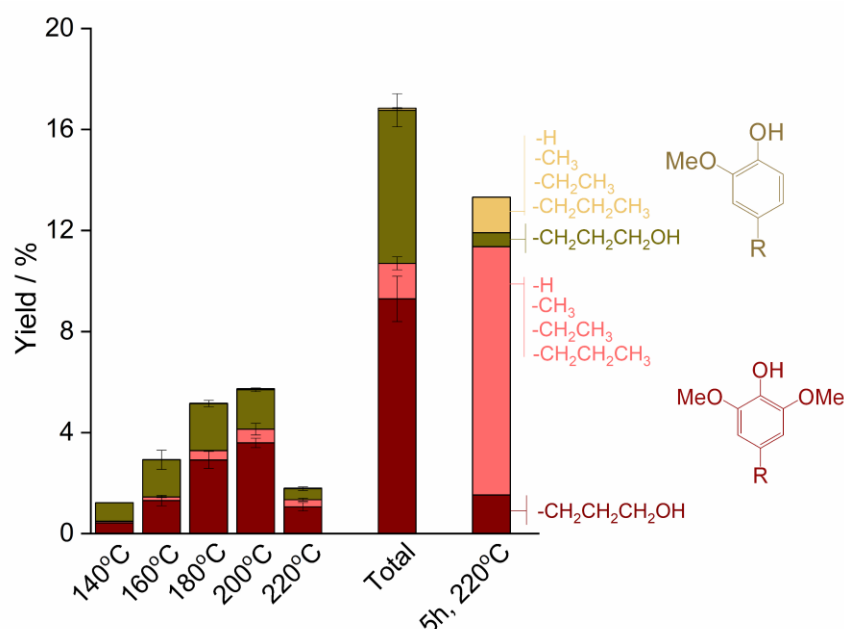


Figure 5-2. S- and G-type lignin-derived monomers yield from initial lignin weight in poplar wood during each step of the semi-batch T-stepping RCF process. Additionally, a single-step batch RCF process at 220°C for 5 h with the same amount of wood and catalyst is displayed for comparison.

At 140°C, the majority of the lignin-derived monomers in RCF lignin oil were G-type (63%) and consisted only 4-(3-hydroxypropyl)guaicol (P(OH)G) species (0.73% yield) with no alkylguaiacols detected. Similarly, the majority of the S-type monoaromatics were 4-(3-hydroxypropyl)syringol (P(OH)S) species (0.42% yield) with a minor amount of alkylsyringols (0.07% yield). The yield of P(OH)G achieved maxima at 180°C (1.86% yield), followed by a decline to 0.44% at 220°C. At the same time, the yield of alkylguaiacols did not exceed 0.04% during any of the steps. The amount of P(OH)S extracted increased until the step at 200°C (3.59% yield), followed by the decline to 1.06% at 220°C. The yield of alkylsyringols followed a similar trend, reaching a maximum at 200°C (1.62% yield) and dropping to 1.24% yield at 220°C.

The combined lignin-derived monophenols yield from the five steps was 16.8%, with P(OH)G yield of 6.1% and P(OH)S yield of 9.3%. In addition, the combined yield of alkylsyringols was 1.4%, with the total yield of 4-propylsyringol (PS) of 0.77% and 4-ethylsyringol (ES) of 0.61%. While the semi-batch T-stepping process preserved the hydroxyl group at C_γ (91% of the S- and G-type monophenols were P(OH)G and P(OH)S), the single step RCF at 220°C for 5 h displayed a steep contrast in monophenols distribution. With a total yield of 13.3%, the majority of the monophenols were alkylsyringols (9.8% yield). At the same time, the yield of P(OH)S was 1.5%. Similarly, for G-type monophenols, the yield of P(OH)G was 0.6%, while the alkylguaiacols displayed a yield of 1.4%.

The results described above illustrate well the importance of process conditions not only on the total yield of monophenols during an RCF process but also on the product distribution among the monophenols. While the selection of a catalyst remains a crucial factor in determining the lignin-derived monomers yield and distribution, the stark contrast between the monophenols distribution between the semi-batch T-stepping process and single-step RCF further underlines the importance of process conditions on the outcomes of RCF. Additionally, the semi-batch T-stepping RCF strategy allows for an increase in the overall monomer yield and the acquisition of P(OH)G and P(OH)S species with high selectivity. Due to the higher amount of G-type monophenols during the first steps at lower temperatures and the higher amount of S-type monophenols at higher temperatures, the strategy also allows for selectivity between the two types, thus potentially lowering the downstream separation

costs. The S- and G-type lignin distribution will be revisited later in the section to include oligomeric and polymeric lignin species in the discussion.

To study the molecular weight distribution of the five RCF lignin oils obtained after each step, the lignin oils and their EtOAc-soluble and insoluble fractions were analysed by GPC. Accordingly, Figure 5-3 depicts the corresponding apparent molecular weight distributions alongside the SI_{320} and SI_{350} values across the different molecular weights. Unfractionated RCF lignin oils are displayed as grey, while the EtOAc-soluble fraction corresponds to light purple and the EtOAc-insoluble fraction to dark purple. The average SI_{320} and SI_{350} values across the MW distribution are summarised in Appendix Table A-11.

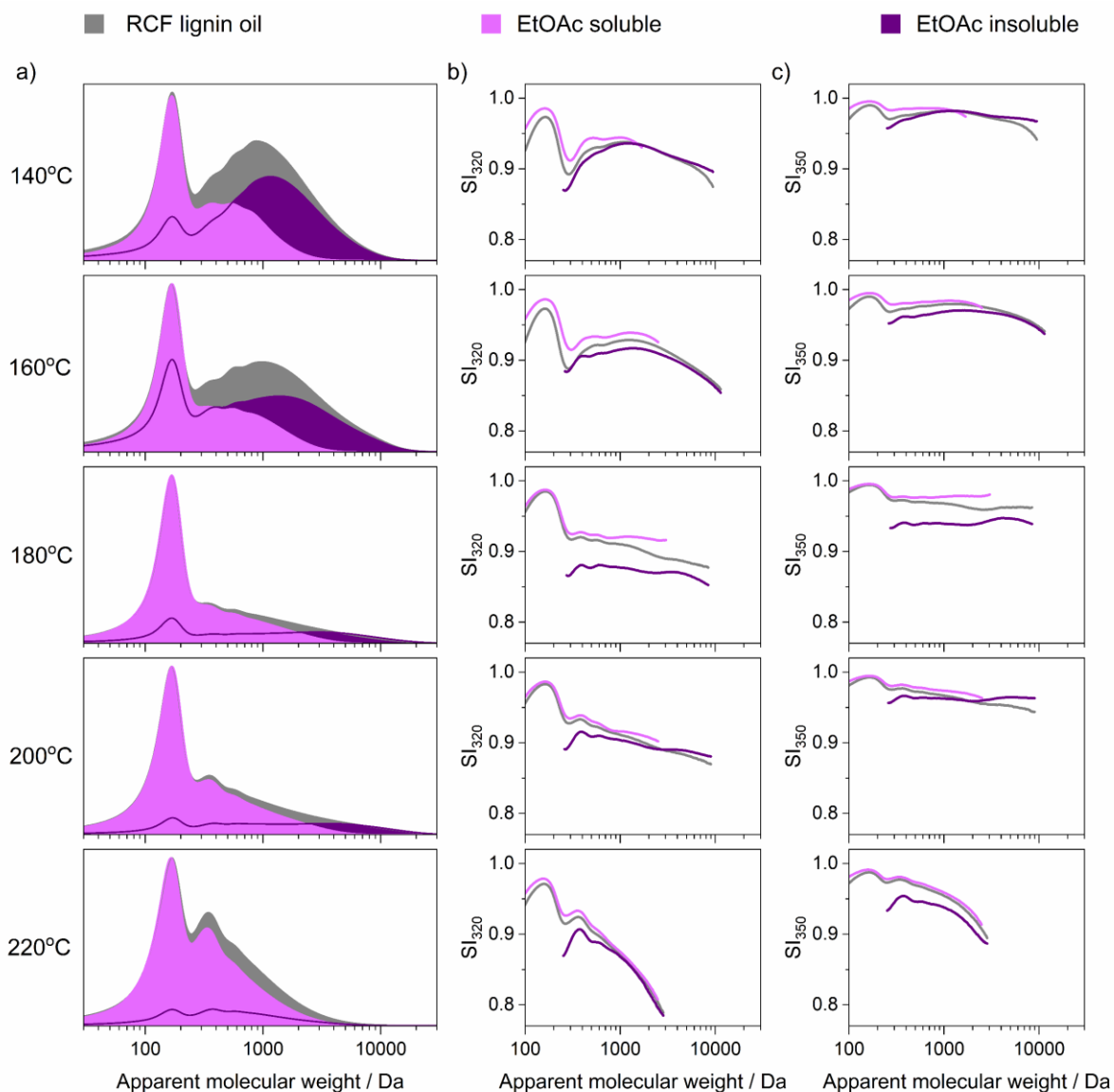


Figure 5-3. a) Apparent molecular weight distribution of RCF lignin oils and their EtOAc-soluble and insoluble fractions after each step. The signal is measured at 280nm. b) SI_{320} of the corresponding unfractionated lignin oils and EtOAc-soluble and insoluble fractions. c) SI_{350} of the corresponding unfractionated lignin oils and EtOAc-soluble and insoluble fractions.

RCF lignin oil extracted during step 1 (140°C) contains a significant amount of the oligomeric and polymeric lignin fractions. Importantly, both the lignin monomers/oligomers containing EtOAc-soluble fraction and oligomers/polymers containing EtOAc-insoluble fraction present very high average SI_{320} (0.93 ± 0.02 and 0.92 ± 0.02) and SI_{350} (0.99 ± 0.01 and 0.98 ± 0.01) values, indicating very low presence of oxidised and conjugated lignin species. Lignin oil extracted during the second step (160°C) displays similar apparent MW distribution characteristics to the lignin oil obtained during the first step with also high SI_{320} (0.94 ± 0.02 and 0.90 ± 0.02) and

SI_{350} (0.98 ± 0.01 and 0.96 ± 0.01) values for EtOAc-soluble and insoluble fractions respectively. During the third step (180°C), the apparent MW distribution changes significantly compared to the first two steps as a decrease in the proportion of mid- and high MW species occurs. While the lignin in the EtOAc-soluble fraction displays the same SI_{320} (0.94 ± 0.02) and SI_{350} (0.98 ± 0.01) values as the two previous EtOAc-soluble fractions, the average SI values for the EtOAc-insoluble fractions decreased ($SI_{320} = 0.87 \pm 0.01$ and $SI_{350} = 0.94 \pm 0.01$). The MW distribution of the RCF lignin oil obtained after the fourth step (200°C) showed a similar profile with the lignin oil from the third step with the same SI values for the low-MW EtOAc-soluble fraction. The SI values for mid- and high MW lignin species in the EtOAc-insoluble fraction displayed the same values as the EtOAc-insoluble fraction from step 2 (160°C). During the last fifth step at 220°C , the obtained lignin oil contains low- and mid-MW lignin species. Notably, the SI values show a drastic drop from the dimeric species for both the EtOAc-soluble and insoluble fractions. The resulting average values for EtOAc-soluble fraction ($SI_{320} = 0.90 \pm 0.05$ and $SI_{350} = 0.97 \pm 0.03$) are lower than for the rest of the obtained lignin oils with higher dispersity due to the steeper decline in SI towards the higher MW values. Similarly, the EtOAc-insoluble fraction ($SI_{320} = 0.86 \pm 0.03$ and $SI_{350} = 0.93 \pm 0.02$) displays a decrease in SI values with increasing MW.

In a broader view, all RCF lignin oils displayed very high average SI values ($SI_{320} = 0.93$ - 0.89 and $SI_{350} = 0.96$ - 0.98), indicating that the high efficiency of reductive catalytic processes in liquor prevented the presence of oxidated and conjugated species in RCF lignin oil. At the same time, the decrease in SI with increasing molecular weight at the final step at 220°C indicates some occurrence of condensed and/or oxidised lignin oligomers and polymers in the corresponding lignin oil. The observation can be a result of multiple factors. Firstly, due to the mass transport limitation in poplar chips, the released lignin fragments are prone to redeposit in the lignocellulosic matrix. With increasing temperature, the species containing condensed fragments resolubilise and become part of the lignin oil. Secondly, while the increasing temperature also promotes lignin depolymerisation and reductive processes on the catalyst, it also accelerates the lignin condensation within the lignocellulosic matrix and in liquor. The nature of residual lignin in pulp after each step will be revisited in the next subsection, where the pulp characteristics are discussed.

To characterise the chemical nature and bonding in lignin oils obtained after each step, the EtOAc-soluble and insoluble fractions were analysed by 2D HSQC NMR. The canonical lignin bonding motifs were quantified as a number of linkages per 100 aromatic units. Importantly, only S- and G-type unit signals in the aromatic region were accounted for linkages quantification due to the low abundance of H-units in poplar wood. Additionally, the reduced β -O-4 lignin linkages described in Chapter 3 were quantified. Accordingly, Figure 5-4 displays the abundance of the lignin linkages in RCF lignin oils EtOAc-soluble and insoluble fractions obtained after the semi-batch T-stepping process step. The spectra of each lignin oil fraction in aliphatic, oxygenated aliphatic, anomeric carbon, and aromatic regions can be found in the Appendix (Figures A17- A20).

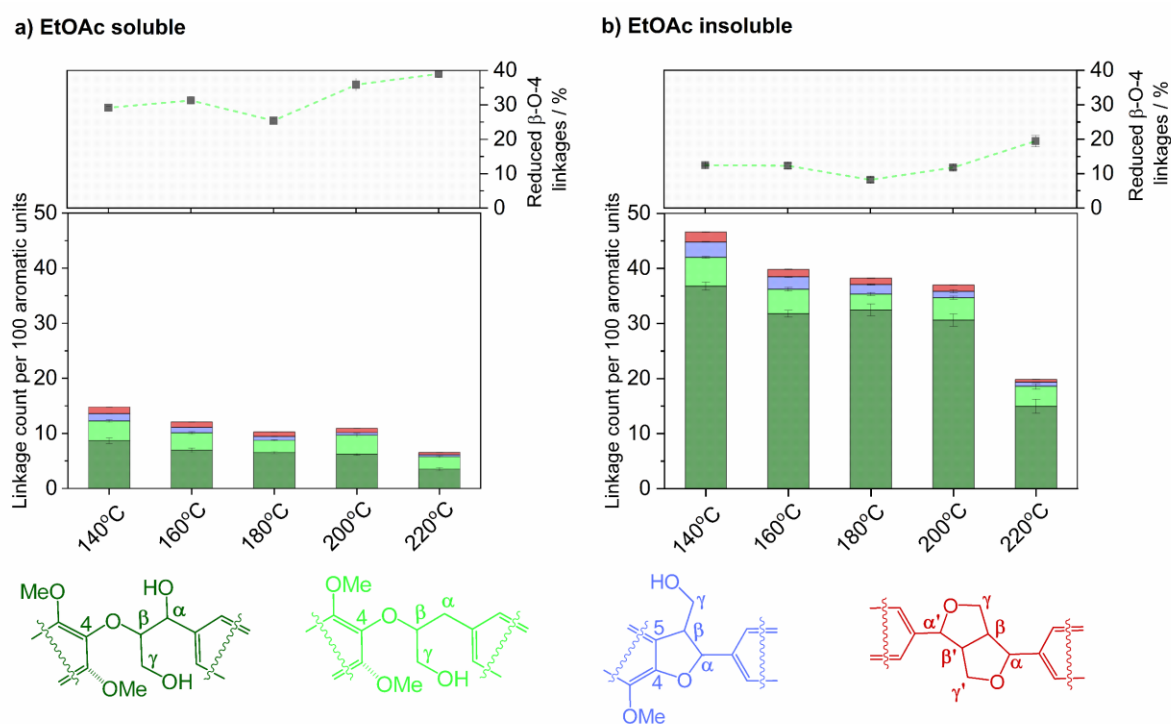


Figure 5-4. a) Relative abundance of reduced β -O-4 linkages among all β -O-4 linked lignin motifs in EtOAc-soluble fractions (top). The number of corresponding lignin linkages in EtOAc-soluble fractions in each corresponding RCF lignin oil obtained during the step (bottom). b) Relative abundance of reduced β -O-4 linkages among all β -O-4 linked lignin motifs in EtOAc-insoluble fractions (top). The number of corresponding lignin linkages in EtOAc-insoluble fractions in each corresponding RCF lignin oil during the step (bottom).

With increasing temperature during the steps, the number of β -O-4 linkages in the EtOAc-soluble fraction of lignin oils decreased from 8.7 ± 0.5 at 140°C to 3.5 ± 0.3 at 220°C. Similarly,

in the EtOAc-insoluble fractions containing mid-and high MW lignin fractions, the β -O-4 linkage count per 100 aromatic units fell from 37 ± 1 at 140°C to 15 ± 1 at 220°C . At the same time, the absolute number of reduced β -O-4 linkages fluctuated between 2.2 and 3.6 linkages per 100 aromatic units in the EtOAc-soluble fractions and between 2.9 and 5.2 in the EtOAc-insoluble fractions. When observing the relative abundance of the reduced β -O-4 linkages, the same trend for EtOAc-soluble and insoluble fractions emerges. While the relative abundance slightly increases from 140°C to 160°C (EtOAc-soluble from $29.1 \pm 0.4\%$ to $31.3 \pm 0.9\%$; EtOAc-insoluble $12.4 \pm 0.2\%$ and $12.3 \pm 0.9\%$), it drops at 180°C in both lignin oil fractions ($25.4 \pm 0.5\%$ and $8.2 \pm 0.7\%$ respectively). From 180°C onwards, the relative abundance of reduced β -O-4 linkages increases, reaching $39.0 \pm 0.9\%$ for EtOAc-soluble fraction and $19 \pm 1\%$ for EtOAc-insoluble fraction in the RCF lignin oil obtained at 220°C .

While the drop in linkage count of regular β -O-4 moieties with increasing temperature is expected due to the more extensive depolymerisation at higher temperatures, the observation on the relative abundance reduced β -O-4 moieties dropping in the RCF lignin oil obtained at 180°C is not intuitive. However, when looking at this in light of the retention of xylans and glucans presented in the next subchapter, a working hypothesis can be postulated for the decline. While the first two steps at 140°C and 160°C fully retain the xylans and glucans pulp, the third step at 180°C sees a decline in carbohydrate retention. Accordingly, the depolymerisation products of hemicellulose (and cellulose) start to become a part of the liquor and compete for the catalyst's active sites. Previously, it has been shown that small diols inhibit the catalytic activity while further increasing the number of OH substituents diminished the catalytic active of Raney Ni more severely at 130°C .³⁶⁸ Accordingly, the carbohydrates and polyols formed by the catalytic reduction of carbohydrate species from 180°C make the catalytic sites less accessible for lignin species. As the reduced β -O-4 moieties can only be formed by the catalytic HDO process, the drop in relative abundance at 180°C is potentially a result of carbohydrates entering the liquor at that temperature. Further increase in the relative abundance of reduced β -O-4 bonds at 200°C and 220°C indicates that with increasing temperature, the catalytic HDO of C α in β -O-4 linkages becomes more prominent while the depolymerisation of regular β -O-4 linkages accelerates. This result is plausible when considering that formed reduced β -O-4 linkages are unable to undergo depolymerisation via

conventional acid-catalysed route. The relatively high total count of reduced β -O-4 linkages in RCF lignin oils obtained during the first two steps at 140°C and 160°C postulates an intriguing research question for future studies. Namely, by further lowering the process temperature and prolonging the processing time, is it possible to produce lignin fractions with very high reduced β -O-4 linkages content by suppressing the conventional β -O-4 cleavage route due to the low temperature.

Besides the regular β -O-4 linkages in RCF lignin oils, both phenylcoumaran and resinol moieties linkage count decreased in every step of the semi-batch T-stepping RCF process. The number of phenylcoumaran linkages per 100 aromatic units decreased from 1.35 ± 0.02 at 140°C to 0.33 ± 0.02 at 220°C in EtOAc-soluble fractions and from 2.79 ± 0.07 to 0.75 ± 0.01 between the corresponding temperatures in EtOAc-insoluble fractions. Similarly, the abundance of resinol linkages in EtOAc-soluble fractions decreased from 1.19 ± 0.04 linkage per 100 aromatic units at 140°C to 0.45 ± 0.01 at 220°C, and in EtOAc-insoluble fraction from 1.82 ± 0.05 to 0.49 ± 0.04 respectively. As the lignin moieties in phenylcoumaran and resinol are partially bonded by C-C- linkages, these linkages' degradation does not result in depolymerisation in the given temperature ranges. However, the decline in the number of linkages with increasing temperature indicates that the ether-linked parts of the two canonical lignin linkages can undergo degradation.

In addition to the number of lignin linkages in the obtained lignin oils, the relative abundance of S-and G-type lignin was evaluated based on the aromatic region of HSQC NMR analysis. Furthermore, the number of H-units and PB-units per 100 S-and G-units was quantified in each lignin oil's EtOAc-soluble and insoluble fractions (Figure 5-5).

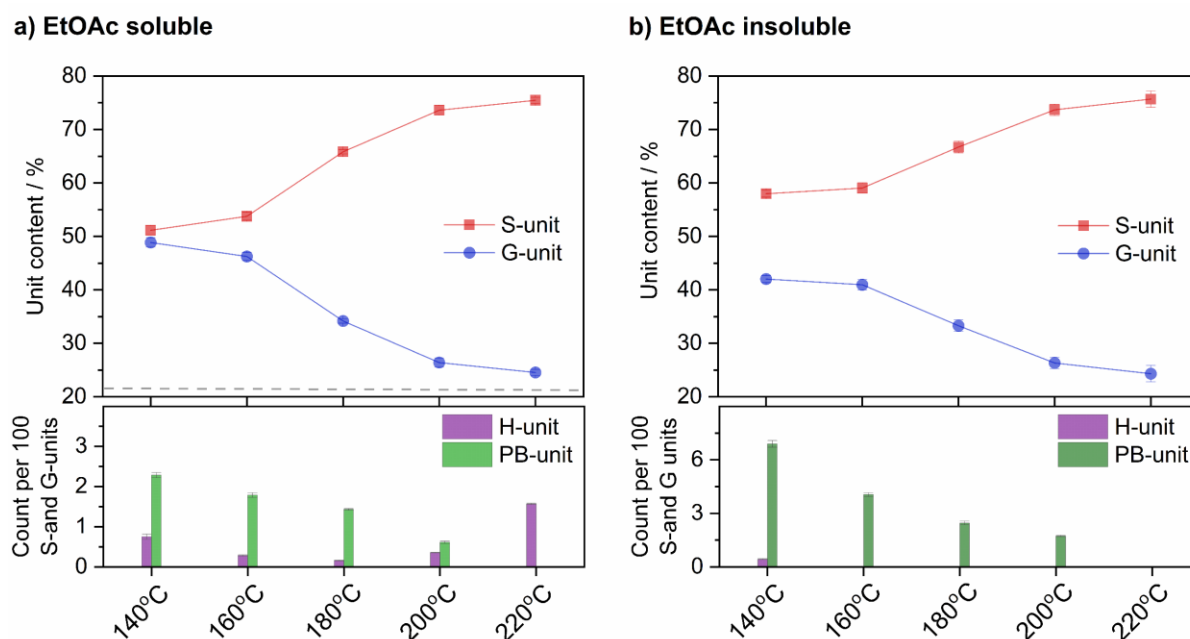


Figure 5-5. a) Relative abundance of S- and G-units in EtOAc-soluble fractions of RCF lignin oil obtained after each step of the semi-batch T-stepping process (top). Number of H- units and PB-units per 100 S- and G-units in EtOAc-soluble fractions (bottom). b) Relative abundance of S- and G-units in EtOAc-insoluble fractions of RCF lignin oil obtained after each step of the semi-batch T-stepping process (top). Number of H- units and PB-units per 100 S- and G-units in EtOAc-insoluble fractions (bottom).

Both EtOAc-soluble and insoluble fractions display a similar trend in S- and G-unit distribution. While the RCF lignin oil fractions obtained during the first steps contain relatively more G-units, the lignin oils obtained during the later steps are more rich in S-type lignin. Accordingly, the abundance of S-units in the EtOAc-soluble fractions increases from $51.1 \pm 0.9\%$ in the first step at 140°C to $75.5 \pm 0.6\%$ in the final step at 220°C . Similarly, the relative amount of the S-units in the EtOAc-insoluble fractions increases from $58.0 \pm 0.6\%$ to $76 \pm 1\%$, respectively. This result is in line with the uneven distribution of S- and G-units in hardwood cell walls, with the G-type lignin domains being more accessible than their S-type counterparts.^{396,478,479} Accordingly, previous reports have presented strong evidence during the early phase of an RCF process with hardwood, lignin fragments rich in G-units are released.^{396,478} Similarly, the higher relative amount of G-units extracted during the first two steps indicate that the G-type lignin is more accessible in hardwood cell wood and is preferentially extracted during the first stages of the RCF process.

The PB-units present as a pendant group in poplar lignin present a steady downward trend with every proceeding step and increasing temperature. The PB-units count declined from

2.28±0.06 during the first step at 140°C to 0.61±0.03 during the fourth step at 200°C in EtOAc-soluble fractions and from 6.9±0.2 to 1.73±0.04 in EtOAc-insoluble fractions respectively. In the RCF lignin oil obtained during the fifth step at 220°C, no PB-units were detected in EtOAc-soluble or insoluble fractions. The result indicates that the PB-units present as ester groups in lignin are more retained in higher MW lignin species. The observation is likely a result of two contributing factors. Firstly, lignin oligomers result from the depolymerisation of polymeric lignin moieties, therefore spending more time in solution since the dissolution of lignin species from the lignocellulosic matrix. Secondly, the larger lignin molecules experience more extensive intramolecular interactions, which can partially hinder the esters' hydrolysis/transesterification. The decline in PB content from steps 1 to 4 and the complete absence in RCF lignin oil obtained from step 5 also indicates that the cleavage of ester bonds in PB readily takes place already under RCF conditions considered very mild (140°C-160°C).

The abundance of H-units in the RCF lignin oils presented a peculiar trend. In the EtOAc-insoluble fractions, the H-units were only detected in the lignin oil obtained during the first step at 140°C (0.43±0.03 units per 100 S-and G-units). On the other hand, the H-units content in the EtOAc-soluble fraction decreased from 0.75±0.06 units per 100 S-and G-units in the first step to 0.16±0.01 units in the third step at 180°C. From the third step, the abundance of H-units started to increase, reaching 1.57±0.02 units during the fifth step at 220°C. The decrease in H-units during the first three steps indicates that the small amount of the H-type lignin units present in hardwood is very accessible and can, similarly to G-units, be extracted during the early stages of an RCF process. This is further supported by the lack of H-units in the EtOAc-insoluble fractions, indicating that the increase of H-units content in lignin oils obtained at higher temperatures is not a result of inherent H-unit content in lignin but rather a result of catalytic demethoxylation of G-units. Accordingly, the results indicate that in low MW lignin fractions, the demethoxylation of phenolic moieties becomes more and more prevalent with increasing temperature (since 200°C).

To depict different RCF lignin oil fractions S-and G-distribution after every semi-batch T-stepping RCF step, Figure 5-6 displays the relative abundance of S-units in lignin-derived monomers (measured by GC), in lignin-derived monomers and oligomers (EtOAc-soluble fraction), and in oligomers and polymers (EtOAc-insoluble fraction). A comparison of the three

trends further confirms the preferential extraction of G-type lignin during the early phase of RCF process. Additionally, as the space time of monomeric lignin moieties in liquor is on average longer than oligomeric and polymeric species, the higher G-unit abundance (and lower S-unit abundance in turn) of low MW species compared to mid- and high MW confirms the 'kinetic history' of lignin depolymerisation retained in different MW lignin fractions and reported in Chapter 3 (Figure 3-13).²³⁹

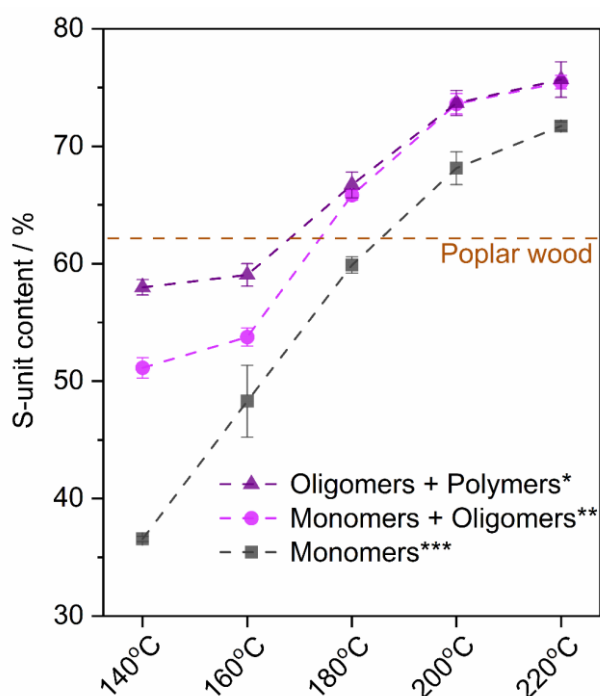


Figure 5-6. Relative content of lignin S-units in different fractions of RCF lignin oils obtained by semi-batch T-stepping RCF process. * EtOAc-insoluble fraction of lignin oils (determined by 2D HSQC NMR). ** EtOAc-soluble fraction of lignin oils (determined by 2D HSQC NMR). *** Lignin-derived monomers in lignin oils (determined by GC-FID)

Besides the lignin-derived species, RCF lignin oils also contain carbohydrate-derived moieties and carbohydrates. In Chapter 3, it was demonstrated that carbohydrates present in lignin oil are insoluble in EtOAc. Accordingly, Figure 5-7 displays NMR spectra of the EtOAc-insoluble fractions in the anomeric carbon centre region ($\delta\text{C}/\delta\text{H}$ 95-110/3.5-5.0 ppm). Importantly, the EtOAc-soluble fractions did not display ^{13}C - ^1H correlation signals in the anomeric carbon centre region, confirming that the carbohydrates present were indeed not soluble in EtOAc (Appendix Figure A-20).

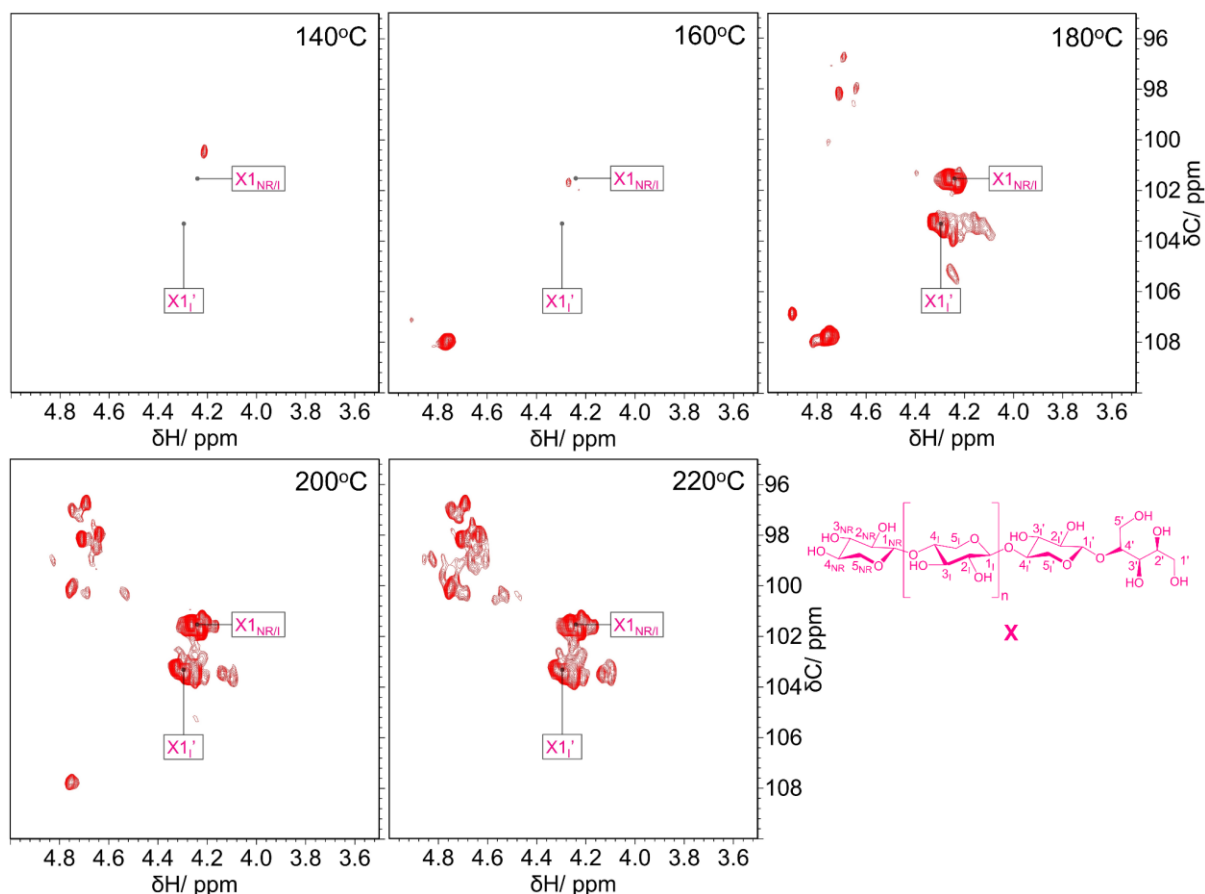


Figure 5-7. HSQC NMR spectra in the anomeric centre region for the EtOAc-insoluble fractions in the RCF lignin oils obtained by semi-batch T-stepping RCF and presented at the same threshold. The oligosaccharide structure solely outlines key structural features in the backbone of the oligoxyalan alcohols. Importantly, it does not intend to represent the actual structure of the oligoxyalan alcohols.

The lignin oil obtained during the first step at 140°C did not present ^{13}C - ^1H correlation signals characteristic to xylan moieties, while during the second step at 160°C, a faint signal for xylan moieties appeared (lower threshold figure can be found in Appendix Figure A-20). Since the third step at 180°C, the lignin oils contained distinctive correlation pairs for oligoxyalan alcohols, which was first reported in Chapter 3.²³⁹ By comparing the volume integrals from the signals for $\text{X1}_{\text{NR/I}}$ and the $\text{X1}_{\text{I}'}$ next to the reduced end, a similar degree of polymerisation was determined for all three lignin oils containing xylan species (for calculation method, see Experimental section). Accordingly, the average oligoxyalan backbone DP ranged between 3.1 and 3.5 during steps 3-5, indicating that hemicellulose-derived carbohydrates presence in RCF lignin oil is largely hindered by their solubility in process liquor as the lignin oils contain only oligomeric carbohydrates.

In addition to the oligoxylan alcohols peaks, a group of peaks starts appearing in the range of $\delta\text{C}/\delta\text{H}$ 96-101/4.5-4.9 ppm from the third step at 180°C. Along with the increasing temperature, the signal intensities for the given peaks increase. However, the chemical nature of the ^{13}C - ^1H correlation signals remains unknown at this point. While considering the δC and δH shifts of the signals, they can potentially be associated with hexoses present in poplar hemicelluloses.

5.2.3. Pulp Characteristics after each step

As pulp fraction was carried over to the next step in the semi-batch T-stepping RCF process, the process results in a single pulp after the fifth step at 220°C. To obtain pulp samples after each process step for characterisation, five separate semi-batch T-stepping processes were carried out, each stopping at the corresponding step. This allowed the obtaining of a pulp sample after each process step. The pulps composition was first determined by analytical saccharification following the NREL protocol.³⁸⁴ To compare the three main lignocellulose components extraction during each process step, Figure 5-8 displays the glucans, xylans, and lignin retention after each step from the initial poplar wood.

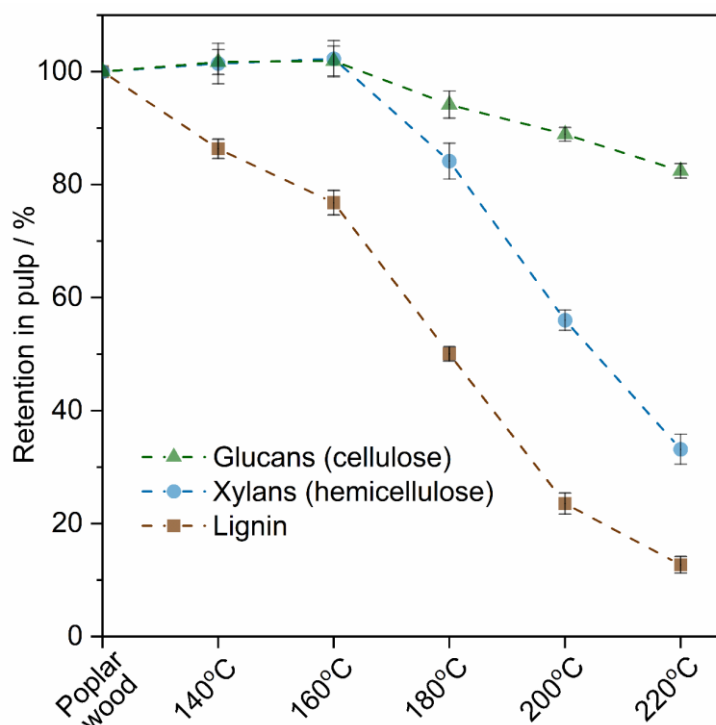


Figure 5-8. Retention of glucans (cellulose), xylans (hemicellulose), and lignin after each step of semi-batch T-stepping RCF. The retention is based on the components' initial mass in poplar wood.

Importantly, both glucans and xylans display complete retention during the first two steps of the semi-batch T-stepping process at 140°C and 160°C. Since the third step at 180°C, the glucans retention gradually decreased from $94\pm 2\%$ to $82\pm 1\%$ after the fifth final step at 220°C. In a similar trend, xylans retention decreased from $84\pm 3\%$ at 180°C to $33\pm 3\%$ after the step at 220°C. In turn, the lignin extraction started from the first step, as is evident by obtaining a lignin oil during it. After the first step, $86\pm 2\%$ of the initial lignin was retained in the pulp and after the second step $76\pm 2\%$, respectively. During the third and fourth steps of the process, the majority of the lignin initially present was extracted, with $50\pm 1\%$ of the lignin retained in pulp after the third step and $24\pm 2\%$ after the fourth step. After the final step at 220°C, $13\pm 1\%$ of the initial lignin remained in pulp, with a final delignification extent of $74\pm 3\%$. Individual pulp compositions after each step can be found in Appendix Table A-12.

The trends presented in Figure 6-8 align well with the apparent activation energies for different component cleavage highlighted in Chapter 2. Accordingly, the apparent activation energy for the cleavage of β -O-4 linkages for lignin depolymerisation is in the range of $13\text{--}46\text{ kJ mol}^{-1}$,²²³ resulting in lignin extraction already at 140°C. In turn, the apparent activation energy for the hydrolysis of glycosidic bonds in hemicellulose is in the range of $103\text{--}154\text{ kJ mol}^{-1}$.²¹⁴ Therefore, the hemicellulose is less prone to depolymerisation under the RCF conditions and is only partially extracted since the third step at 180°C. Similarly to hemicellulose, the apparent activation energy for hydrolysis of glycosidic bonds in cellulose is between $102\text{--}189\text{ kJ mol}^{-1}$.^{224,229–234} However, it remains important to emphasise that only amorphous regions of cellulose can undergo hydrolysis under RCF conditions, as the hydrolysis of the crystalline domains requires the presence of strong acid ($\text{pK}_a < -3$) to proceed at appreciable rates.²²⁸ Accordingly, cellulose moieties extraction also starts since the third step at 180°C, but is much less extensive in comparison with xylans in hemicellulose.

To investigate the nature of residual lignin in pulp after each process step, the corresponding pulp samples were imaged by fluorescence microscopy. Accordingly, cryostat microtome was utilised to facilitate the cutting of the softening pulp chips after each semi-batch T-stepping RCF step. The resulting $10\mu\text{m}$ cuts were mounted on slides and first analysed by live cell widefield epifluorescence. In this process, the samples were sequentially excited with 405nm, 488nm, 515nm, and 638nm lasers. The corresponding fluorescence images can be found in

the Appendix (Figure A-22). To quantify the fluorescence intensity with different excitation lasers, 20x objective multicolour Z-stacks was maximum intensity projected and whole image intensity summed. For the intensity quantification, at least three separate areas of each cut were utilised. To compare different pulp samples, the ratio of fluorescence intensities between 488/405nm, 515/405nm, and 638/405nm was taken as a unity for poplar wood in each case, and the corresponding ratios were compared to untreated poplar wood in each case. Accordingly, Figure 5-9 displays the three intensity ratios normalised to the intensity ratio in poplar wood.

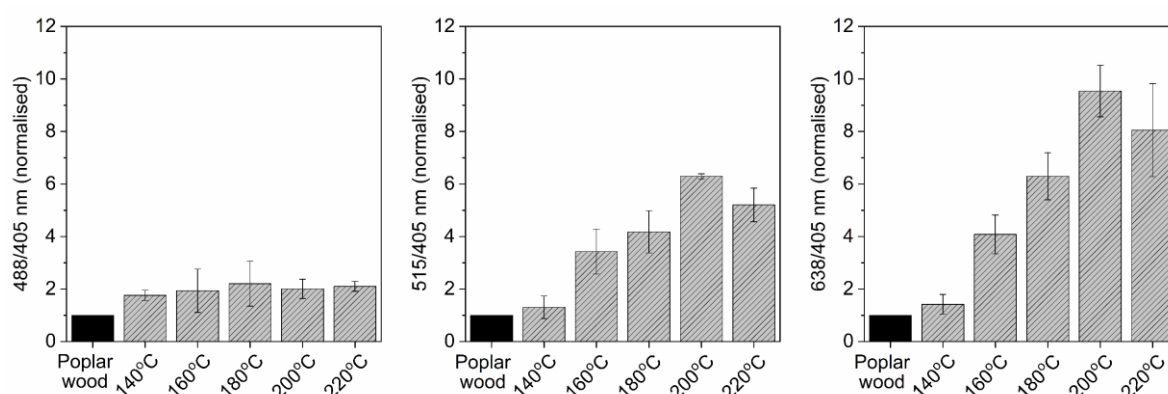


Figure 5-9. Relative fluorescence intensities between 488/405nm excitation lasers, 515/405nm excitation lasers, and 638/405nm excitation lasers each normalised to the corresponding ratio in untreated poplar wood. Representative images can be found in Appendix Figure A-22.

From Figure 5-9, it becomes clear that the nature of lignin retained in pulp changes with each process step. The fluorescence intensity ratio between 488nm and 405 nm excitation lasers increases two-fold during the first step at 140°C, and remains at that level throughout the rest of the steps. At the same time, the fluorescence intensity ratio between 515nm and 405 nm excitation lasers only grows 1.3 times during the first step compared to poplar wood, while being already 3 times higher at 160°C and 6 times higher at 200°C. The fluorescence intensity ratio between 638 nm and 405 nm excitation lasers displayed the starkest change in fluorescence intensity ratios compared to poplar wood. Accordingly, while the ratio changed a little compared to native wood during the first process step, the pulp obtained at 200°C displayed a 10-fold increase in the corresponding ratio.

As the lignin autofluorescence shifts towards higher wavelengths with each process step, the relative abundance of conjugated species in the retained lignin increases. Accordingly, the

lignin retained in the pulp matrix undergoes potential condensation and/or oxidation, thereby resulting in a higher proportion of conjugated moieties. This aligns with the established knowledge that unstabilised lignin fragments are prone to undergo condensation reactions. As the heterogeneous catalyst is spatially located outside the lignocellulosic matrix, the lignin species retained in the pulp do not undergo catalytic stabilisation.

To assess the different fluorophores (lignin moieties) distribution in the cell wall after each step, total internal reflection fluorescence (TIRF) microscopy was carried out on the pulp cuts. Additionally, a super-resolution radial fluctuations (SRRF) analytical approach was applied, allowing resolutions beyond the light diffraction limit. The analytical procedure is described in detail in the Experimental section.^{507,508} While a wide range of fluorescence microscopy methods have been utilised for lignin analysis,⁵⁰⁹ the SRRF approach itself was only established in 2016.^{510,511} Best to our knowledge, lignin in cell walls have only been imaged one time by SRRF in 2022, when Douglas fir cell walls were imaged.⁵¹² Additionally, Paäs *et al.* utilized the super-resolution stimulated emission depletion (STED) technique for lignin imaging in poplar cell walls, going beyond the limits of confocal microscopy.⁵¹³ Therefore, to the best of our knowledge, the current study presents a second imaging of lignin in plant cell walls by the SRRF technique and the first time that changes in pulp are followed in the RCF process by this technique. Accordingly, Figure 5-10 displays the combined SRRF images obtained by excitation with the four lasers described above. 60x magnification of each pulp is presented on the top image, along with a highlighted area of the cell wall on the bottom.

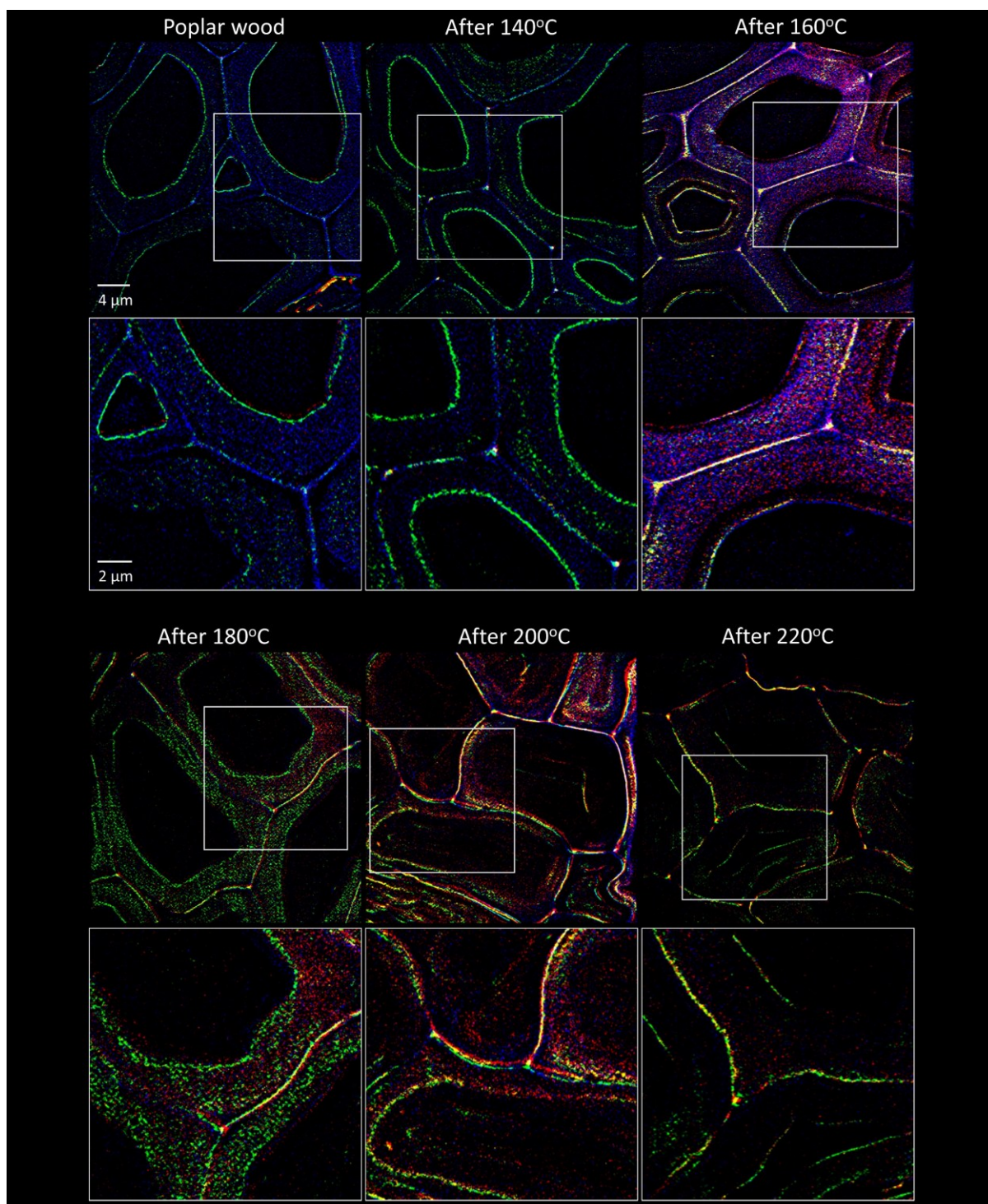


Figure 5-10. Fluorescence microscopy images obtained by super-resolution radial fluctuations (SRRF) of untreated poplar wood and pulp chips after each semi-batch T-stepping RCF step at corresponding temperatures. The top image represents a wider region containing a whole plant cell, while the bottom image is a highlighted area of the top picture displaying individual cell walls. The total lignin content in wood and pulps and determined by analytical saccharification were corresponding: poplar wood $28.2 \pm 0.5\%$, after the first step (140°C) $25.4 \pm 0.2\%$, after the second step (160°C) $24.0 \pm 0.4\%$, after the third step (180°C) $19.2 \pm 0.1\%$, after the fourth step (200°C) $11.3 \pm 0.3\%$, and after the final fifth step (220°C) $7.3 \pm 0.2\%$.

The SRRF images reveal that the nature of residual lignin in plant cell walls changes very ununiformly. The untreated poplar wood displays a relatively uniform blue emission with high concentration of lignin in the middle lamella, while the secondary wall lining cell lumen (S3) shows green emission. As a result of the first step at 140°C, the blue emission becomes dimmer while the S3 region strongly emits green. While the first two images display somewhat similar features, the pulp obtained after the second step at 160°C displays already a stark contrast. Accordingly, while the blue emission remains, the cell wall includes a relatively large proportion of moieties emitting red light from 638 nm excitation laser. The distinctive S3 layer fades away, while the lignin in the middle lamella starts emitting yellow light. The pulp obtained after the third step at 180°C starts to present a distinctive feature not present in poplar wood nor in the pulp after RCF at 140°C and 160°C. Accordingly, the firm structure of the cell wall starts to disintegrate, and the middle lamella, along with the cell walls, starts to become distorted. This result is in line with the retention of cell wall components, as during this step, cellulose and hemicellulose retention starts to decrease, and more extensive delignification takes place (Figure 5-8). In addition, the emission becomes more uniformly green with red-emitting regions. After the fourth step at 200°C, the cell wall becomes increasingly distorted, and the residual lignin emits yellow and red in large proportions. The lignin content in pulp has significantly decreased, and some regions of the cell wall already present very low lignin content. After the final step at 220°C, the cell walls are highly distorted, with a little lignin retained in the pulp. The emission contains green, yellow, and red regions, with the majority of the lignin being retained in the middle lamella region.

When comparing the SRRF images displayed in Figure 5-10 with the Structural Index analysis from lignin oils GPC data (Figure 5-3), a consistent trend emerges. The pulps obtained after 160°C and 200°C contain a significant amount of red-emitting moieties with a high level of conjugation. At the same time, the RCF lignin oil obtained at the third step (180°C) and fifth step (220°C) utilising the pulps from the second step (160°C) and the fourth step (200°C) display a drop in both SI_{320} and SI_{350} values for the high MW fractions of lignin oils. Accordingly, the species displaying fluorescence at higher wavelengths and have undergone modification by condensation and/or oxidation are extracted during the corresponding steps, resulting in a drop of SI_{320} and SI_{350} as also absorbing light at higher wavelengths.

5.3. Conclusion

In the current chapter, the semi-batch T-stepping RCF concept was introduced for the first time. Accordingly, poplar chips were utilised to obtain 5 different lignin oils at different temperatures along with pulp. GC analysis of the lignin oil revealed that a higher total yield of monophenols and a substantially higher proportion of P(OH)G and P(OH)S among the monophenols were obtained in comparison to the batch process with the same total reaction time at 220°C. The β -O-4 linkages content in lignin oils decreased with every consequent step in both oligomeric lignin fractions and higher MW lignin fractions. At the same time, all lignin oils presented a very high relative abundance of reduced β -O-4 linkages, with the reduced β -O-4 linkages forming 40% of all β -O-4 linkages in oligomeric fraction of the lignin oil obtained during the final step at 220°C. The HSQC NMR analysis also revealed that the semi-batch T-gradient strategy allows extraction of high proportions of G-type lignin during the first steps, while the final steps become rich in S-type lignin. Additionally, it was demonstrated that PB pendant groups, along with the native H-units present in lignin, can be extracted at low severity under the RCF conditions.

Pulp analysis revealed that xylans and glucans are fully retained in the pulp after the first two steps of the process at 140°C and 160°C. Following the rationale of apparent activation energy for lignin, hemicellulose, and cellulose depolymerisation, the pulp analysis demonstrated that lignin can be extracted prior to the extensive depolymerisation of hemicelluloses and cellulose. In addition to the analytical saccharification, the residual lignin in pulp after each step was imaged by state-of-the-art fluorescence microscopy utilising SRRF algorithms. The imaging revealed the residual lignin fluorescence shift towards the red with increasing temperature, therefore indicating lignin condensation in the pulp. Additionally, cell wall distortion was notified to start during the third step at 180°C.

In a broader context, the semi-batch T-stepping RCF strategy demonstrates the importance of reaction importance on the process outcomes, providing a new strategy to control the monophenols' yield and distribution. Preferential extraction of S-or G-type lignin, narrower monophenols distribution, and the deflection of hemicelluloses release at lower temperatures help to tackle the downstream separation cost in potential industrial RCF settings. At the same

time, an option to obtain multiple lignin oils with different properties while utilising single biomass facilitates the different future applications of RCF lignins.

6. Summary and Outlook

The current thesis provided new chemical insights into the polymeric lignin fractions and introduced new approaches to steer the RCF lignin oil composition. Following the scope of the thesis, the literature review demonstrated a complex set of processes taking place during the RCF process. After introducing the current knowledge on lignocellulosic biomass's chemical composition and architecture, the lignin journey from the release from the lignocellulosic matrix until the catalytic conversion was followed. In addition, the process's influence on holocellulosic pulp was discussed, and the techno-economic aspects of the RCF process were covered.

To facilitate the characterisation of oligomeric and polymeric fractions in RCF lignin oil, a solvent fractionation strategy was developed to obtain different molecular weight cuts of the lignin fraction. This feature allowed determining the structural heterogeneity of lignin products across the entire molecular weight distribution. Detailed characterisation of the 2D HSQC NMR spectra revealed the formation of non-native reduced β -O-4 linkages containing methylene group at the C_α position. While it is generally accepted that the stabilisation process in RCF occurs primarily on lignin monomer intermediates, the finding shows an additional stabilisation mechanism preventing lignin condensation. Importantly, the reduced β -O-4 linkages occur to varying degrees across the entire molecular weight distribution.

Utilising lignin's aromatic nature and the principle that further conjugation leads to UV-VIS spectra shifting towards higher wavelengths, a new Structural Index metric based on the absorbance ratio at different wavelengths was introduced. Coupling it with a GPC analysis utilising a PDA detector revealed that lignin moieties sharing the same apparent molecular weight in soluble and insoluble fractions display different chemical natures. Accordingly, solvent fractionation does not only separate lignin fractions based on their molecular weight but also on their chemical nature. The solvent fractionation strategy also allowed the separation of the carbohydrate fraction of the RCF lignin oil. Accordingly, it was determined that the carbohydrate fraction is composed of oligo(arabino)xylan alcohols, which were not chemically connected to lignin moieties in lignin oil.

In a broader context, the mid- and high MW lignin findings have far-reaching implications for lignin valorisation. Accordingly, the stabilisation imparted by reductive processes preserves the structure of high-MW lignin fraction in a more native-like architectural state. For example, the heaviest MeOH-insoluble fraction with M_w of 16900 Da showed an estimated total linkage count of 82 ± 2 per 100 aromatic units. Therefore, heterogeneous catalysis can be vital in producing mid- and high-MW lignin products with controllable structural properties, which thus far has only been possible through bioengineering of lignin in planta. However, such features inserted by bioengineering are often lost during the lignin extraction from the biomass. Consequently, the evolution of the RCF concept toward the production of lignin polymer products with controllable structural properties presents itself as an attractive 'new' option in the lignin-first paradigm.

To steer the process outcomes, it was argued that due to the need for biomass comminution prior to the RCF to avoid mass transfer limitations, the available mechanical energy could be used in tandem with different additives to perform 'soft' mechanocatalytic pretreatments. By screening two acids and two bases along with control experiments, acetic acid was found to increase the monophenol yields and carbohydrate retention in the pulp. At the same time, mechanocatalytic pretreatment with NaOH lowered the monophenol yields but provided very high selectivity towards S-type monophenols (>90%). Further screening with varying amounts of acetic acid during the pretreatment showed that a higher concentration of acetic acid further increases the carbohydrate retention and DP in pulp, while the monophenol yields showed optimum in the screened range at 1 mmol of acetic acid added per gram of wood.

In addition to the 'soft' mechanocatalytic pretreatment prior to the RCF, a semi-batch T-stepping RCF process was introduced to control the process outcomes. As a result, 5 distinctive lignin oil streams were obtained from the single starting material. The semi-batch T-stepping strategy demonstrated that very different monophenol fraction compositions can be achieved when using the same catalyst, biomass, and solvent, indicating that the process conditions have a huge role in monophenols distribution. In addition, the obtained lignin fractions presented a very high relative abundance of reduced β -O-4 linkages (up to 40% of all β -O-4 linkages) and considerably different S- and G-distributions. The pulp analysis revealed that carbohydrates were fully retained during the first two steps of the process at 140°C and 160°C.

In addition, the pulp was imaged by state-of-art fluorescence microscopy after each process step, allowing to follow the lignin characteristics change in space over the course of the semi-batch process with an indication of lignin condensation in the pulp matrix.

In a wider context, the two introduced approaches provide new ways to steer the process outcomes, thereby creating further options in the process design considerations when transitioning towards the process industrial implementation. Accordingly, the mechanocatalytic pretreatment strategy with acetic acid provides a way to enhance the lignin extraction and carbohydrate retention in pulp, while the semi-batch strategy emphasises the importance of process conditions and allows RCF lignin oils with narrower monophenols distribution.

The current thesis opens several avenues for further research to facilitate lignin valorisation. The discovery of reduced β -O-4 linkages and its high abundance in RCF lignin oils obtained by semi-batch T-stepping strategy raises a question of whether lignin streams where all β -O-4 are reduced could be obtained by lowering the process severity. This could be explored by either using raw biomass or already separated high-MW fractions of RCF lignin oil. As the absence of the C_α hydroxyl group makes the β -O-4 linkages potentially very resistant, this could open completely new directions for lignin-based materials design. Additionally, as the preliminary demonstration of mechanocatalytic pretreatments proved to enhance the process outcomes, a mechanistic study of the process would facilitate the rational design of the biomass pretreatments more generally. In this way, well-targeted disruptions in the lignocellulosic matrix can have a large effect on the valorisation strategies economics. The SRRF imaging of the pulp during the semi-batch RCF process demonstrated the method's feasibility in following changes in lignin's chemical nature. Accordingly, the approach could be expanded to various other lignin-extraction methods and utilised to monitor spatial condensation in various lignin industrial applications (e.g., adhesives).

7. Experimental

7.1. Materials

Raney Ni (Raney ®2800 slurry in H₂O) was purchased from Sigma-Aldrich. Poplar chips were obtained from premium animal bedding (Rettenmaier & Soehne). All solvents (HPLC grade, VWR) were utilized as purchased. DMSO-*d*₆, D-cellobiose, D-xylobiose, and the reagents in Chapter 4 (oxalic acid, acetic acid, Na₂CO₃, NaOH) were purchased from Sigma-Aldrich and used as received.

7.2. Mechanocatalytic pretreatment (Chapter 4)

Poplar wood chips (6.4g) were placed in a planetary ball mill along with the given additive (12.8 mmol, 2 mmol g⁻¹ of wood) and 34 stainless-steel grinding balls (10 mm diameter). In addition, a control setup was also introduced, where the ball mill was loaded without the presence of any additive. The components were milled together at 500 rpm for 70 minutes, after which the milled wood was washed on a 0.45 µm nylon filter with water until neutral pH for complete removal of the unbound additive and dried in a vacuum oven (40°C, 24 h). The moisture content of the processed woods was recorded, and the water phase was rotaevaporated under reduced pressure (50°C, 50 mbar) to obtain the mass of extractives. For varying kinetic energy dose experiments, 30 and 100-minute milling times at 500 rpm were utilised to achieve the 4.3 and 14.3 kJ g⁻¹ kinetic energy dose with acetic acid mechanocatalytic pretreatment.

7.3. Reductive Catalytic Fractionation (Chapters 3, 4, and 5)

7.3.1. 9 L scale (Chapter 3)

Poplar wood chips (704 g) and Raney Ni (331 g, wet) were suspended in a 2-PrOH/H₂O mixture (4140 mL, 70:30 v:v) in a 9 L stainless steel batch reactor (Parr Instruments & Co, model number: 4552). The suspension was stirred at 75 rpm for 30 min at room temperature. Then, the reactor was heated to 200°C for 1 h (3°C min⁻¹) under stirring (700 rpm). The H-transfer RCF process proceeded under autogenic pressure for 3 h. In sequence, the mixture was allowed to cool down to room temperature. The liquor was separated from the solids (Raney Ni in conjunction with the holocellulosic pulp) by filtration through a glass fibre filter (GF6, Whatman). The lignin

oil stream was isolated from the liquor by removing the solvent mixture using a rotoevaporator at 45°C under reduced pressure and further dried in a vacuum oven at 40°C.

7.3.2. 250 mL scale (Chapter 5)

7.3.2.1. Semi-batch T-stepping RCF process

Untreated poplar wood chips (16.5 g, moisture=8.0±0.3%) were placed in a 250ml autoclave (stainless steel, cylindrical, ID=60mm, H=90mm, unbaffled) along with Raney Ni (10g, wet) and solvent mixture (140 mL, 2-PrOH/H₂O 7:3 v:v). The reaction mixture was heated from RT to 140°C in 20 minutes and held at 140°C for 1 h (500rpm stirring, double axial impeller, D=28mm, 35mm distance between the impellers). After the 1 h, the reactor was quenched in cold water. The reaction mixture was filtered (0.45µm), and the pulp and catalyst were washed three times with 30 mL of the solvent mixture. The solids were placed back in the autoclave, 140 mL of fresh solvent mixture was added, and the process was repeated with the same parameters at 160°C, 180°C, 200°C and 220°C. The five extracted lignin streams were rotaevaporated at 45°C under reduced pressure and further dried in a vacuum oven at 40°C to obtain the five different lignin oils. After the final step at 220°C, the catalyst was magnetically separated from the pulp by dispersing the pulp and catalyst mixture in the solvent in a beaker on the top of the magnet. In addition, a series of RCF experiments were carried out using the same procedure to obtain pulps after each step for characterisation.

7.3.2.2. Batch RCF process

A reference batch reaction with the same amount of wood, catalyst and solvent mixture was carried out at 220°C. The reaction mixture was heated from RT to reaction temperature in 20 minutes, held there for 5 h, and consequently quenched in cold water. The same separation sequence as for the semi-batch T-stepping RCF was utilised, rendering an RCF lignin oil and a pulp.

7.3.3. 45 mL scale (Chapter 4)

For the RCF processes, the processed wood or poplar chips (3.3 g) and Raney Nickel catalyst (2 g) were placed in a 45 mL autoclave batch reactor with 2-PrOH/H₂O mixture (28 mL, 7:3 v/v). The RCF process was carried out under autogenous pressure at 200°C for 3 h (following a ramp from room temperature to the reaction temperature over 30 min) with 500 rpm stirring. The

resulting mixture was filtered through 0.45 μm nylon filter, washed 3 times with 10 mL of the solvent mixture, rotaevaporated at 45°C under reduced pressure, and further dried in a vacuum oven at 40°C to produce the lignin oil. The catalyst and pulp were magnetically separated, and the pulp fraction dried in a 40°C vacuum oven overnight. The moisture content in the starting materials and resulting pulps were recorded.

7.4. Solvent fractionation (Chapters 3 and 5)

7.4.1. Fractionation of RCF lignin oil (Chapters 3 and 5)

Anhydrous solvent (acetone, EtOAc, or methanol, 10 mL) was added to 0.4000 g lignin oil and sonicated for 30 min. The mixture was centrifuged at 5000 RCF for 10 min, and the supernatant was separated. The solid was redispersed in the solvent (10 mL), sonicated for 30 min, and centrifuged at 5000 RCF for 10 min. This procedure was repeated one more time. Next, the supernatants were combined and rotaevaporated at 40°C. The isolated fractions were dried under reduced pressure (50 mbar) in a vacuum oven at 40°C for 24 h. The mass of both fractions was recorded. The fractionation was repeated up to 5 times for each solvent, and the deviation is reported as the standard deviation for the repeats.

7.4.2. Extraction of carbohydrates with hot water (Chapter 3)

To extract carbohydrates from the acetone-insoluble fraction, the acetone-insoluble fraction was suspended in hot distilled water (80°C, 10 mL) and sonicated for 10 min. The mixture was centrifuged at 5000 RCF for 10 min, and the supernatant was separated. The solid was redispersed in the hot water (10 mL), sonicated for an additional 30 min and centrifuged at 5000 RCF for 10 min. This procedure was repeated one more time. The supernatants were combined and rotaevaporated at 50°C. The isolated fractions were dried under reduced pressure (50 mbar) in a vacuum oven at 40°C for 24 h.

7.5. Gel Permeation Chromatography (Chapters 3, 4, and 5)

7.5.1. Lignin analysis (Chapters 3, 4, and 5)

All lignin samples (10 mg) were dissolved in the mobile phase (1 mL, 0.1 wt% LiBr/DMF). The samples were analysed on a Shimadzu Prominence HPLC equipped with an autosampler (model: SIL-20A HT), oven (model: CTO-20A), PDA (model: SPD-M20A), and RI detector

(model: RID-20A). The separation was performed on a set of M and L PolarGel columns (300 mm × 7.8 mm, Agilent) operating at 60°C with a mobile phase flow of 1 mL/min. Apparent molecular weight calibration was performed against polystyrene standards (Agilent S-L-10, S-M-10) dissolved in the mobile phase. The chromatograms were analysed using the Shimadzu GPC software suite (version 5.98). The molecular weight distribution was analysed at the wavelengths of 270 nm and 280 nm. Note: When applied to product mixtures obtained from lignin, quantitative information regarding the content of species cannot be retrieved from a PDA or RI detector, as the detector response is not universal. Despite this limitation, the GPC technique coupled with UV-Vis spectroscopy provides valuable information on the apparent molecular weight distribution.

Structural Index was defined as:

$$SI_{xxx} = 1 - \frac{A(xxx \text{ nm})}{A(280 \text{ nm})} \quad 7 - 1$$

where *A* refers to PDA response at the given wavelength with a reference correction at 720±50 nm (average signal between 670-770 nm is subtracted from the signal at 280 nm and given wavelengths). The lower cut-off molecular weight for SPI analysis was 100 Da, corresponding to the lower exclusion limit of the PolarGel L column, and the upper molecular weight cut-off molecular weight corresponded to 2% of the maximum detector response at 280 nm. The average SI for each analysed lignin was calculated based on the detected SI values in the determined ranges along with the standard deviation. The reference correction with 720±50 nm was performed at Shimadzu GPC software suite (version 5.98), and the corrected chromatogram data at desired wavelengths (bandwidth ±4 nm) was exported for SPI analysis and plotting to OriginLab software.

7.5.2. Cellulose degree of polymerisation (Chapter 4)

For the GPC analysis of derivatised pulps, CTC (4 mg) was dissolved in THF (2 mL). The same Shimadzu Prominence HPLC instrument setup as for the lignin GPC analysis was utilised, now equipped with 3 mixed-bed columns in series (2 TSKgel "SuperH2M-M", 4.6 mm ID x 15.0 cm and 1 TSKgel "SuperHZ1000", 6 mm ID x 15.0cm in series). The analysis was performed at the columns temperature of 35°C and THF flow rate of 0.8 mL min⁻¹. The PDA detector response

at 236 nm was used for CTC detection, and the system was calibrated utilising polystyrene standards in the range of 400- 2×10^6 Da. The degree of polymerisation was calculated by dividing the apparent molecular weight values by the mass of the anhydroglucose tricarbanilate unit (519 g mol^{-1}).

7.6. Gas chromatography (Chapters 3, 4, and 5)

An aliquot of each sample (50.0 mg) was dissolved in MeOH containing internal standard *n*-decane at 1 mg/mL and filtered (membrane filter 0.45 μm). The sample solutions were analysed by a gas chromatograph (GC2010 Plus, Shimadzu) coupled to a flame ionization detector (FID) and a mass spectrometer detector (MSD) equipped with a capillary column DB-1MS (30 m, 0.25 mm ID, df 0.25 μm). The following oven temperature program was used: an isothermal step for 5 min at 40°C, then the temperature was ramped up at $5.2^\circ\text{C min}^{-1}$ to 320°C and kept for 5 min. Quantification of selected components was performed by using the FID response and authentic product standards with their effective carbon numbers.⁵¹⁴

7.7. Elemental Analysis (Chapter 3)

Elemental analysis was performed on an Elementar VarioMICRO Cube CHNS/O elemental analyser. The combustion and reduction tubes were operated at 1150°C and 850°C, respectively, and the oxygen flow was kept for 80 s. 1.5-2.5 mg of sample was weighted for each analysis, and the analysis of each sample was repeated at least three times. The oxygen content in the sample was determined by subtraction. The deviation was calculated as standard deviation relative to H/C and O/C mole fractions.

7.8. HSQC NMR measurements (Chapters 3, 4, and 5)

All samples described in the thesis were prepared by dissolving a sample (50 mg) in DMSO- d_6 (1 mL). The samples were analysed with an 800 MHz Bruker spectrometer equipped with inverse triple resonance cryoprobe with ATM module (5mm $^1\text{H}/^{13}\text{C}/^{15}\text{N}/\text{D}$ Z-GRD). All measurements were performed at 25°C. A matrix with 1024 x 2048 points was collected over four scans with Bruker *hsqcetgpsi* pulse program utilised. All data were processed using MestreNova software (version 14.3.0).

The lignin linkages count per 100 aromatic units, the relative abundance of reduced β -O-4 linkages and the content of different aromatic units were estimated by the following equations:

tions S1-S7:

$$\text{Linkage count per 100 aromatic units (\%)} = \frac{V(C_{\alpha})_{\text{linkage}}}{0.5V(S_{2/6}) + V(G_2)} \times 100 \quad 7-2$$

$$\text{Reduced } \beta\text{-O-4 linkages (\%)} = \frac{V(C_{\beta(\text{CH}_2)(\text{SS})}) + V(C_{\beta(\text{CH}_2)(\text{SG})})}{V(C_{\beta(\text{S})}) + V(C_{\beta(\text{G})}) + V(C_{\beta(\text{CH}_2)(\text{SS})}) + V(C_{\beta(\text{CH}_2)(\text{SG})})} \times 100 \quad 7-3$$

$$\begin{aligned} \text{Reduced } \beta\text{-O-4 linkage count per 100 aromatic units} = \\ \frac{V(C_{\alpha})_{\beta\text{-O-4}}}{0.5V(S_{2/6}) + V(G_2)} \times \frac{[\text{Reduced } \beta\text{-O-4 linkages (\%)}]}{100 - [\text{Reduced } \beta\text{-O-4 linkages (\%)}]} \times 100 \end{aligned} \quad 7-4$$

$$\text{S-unit (\%)} = \frac{0.5V(S_{2/6})}{0.5V(S_{2/6}) + V(G_2)} \times 100 \quad 7-5$$

$$\text{G-unit (\%)} = \frac{V(G_2)}{0.5V(S_{2/6}) + V(G_2)} \times 100 \quad 7-6$$

$$\text{H-unit (\%)} = \frac{0.5V(H_{2/6})}{0.5V(S_{2/6}) + V(G_2) + 0.5V(H_{2/6})} \times 100 \quad 7-7$$

$$\text{PB-unit (\%)} = \frac{0.5V(\text{PB}_{2/6})}{0.5V(S_{2/6}) + V(G_2)} \times 100 \quad 7-8$$

where V denotes the corresponding volume integrals. The degree of polymerisation of the oligoxylyan backbone in oligo(arabino)xylan alcohols, and the count of arabinose units decorating the backbone were estimated by the following equations:

$$\text{DP(oligoxylyan alcohol backbone)} = \frac{2V(X1'_I) + V(X1_{\text{NR/I}})}{V(X1'_I)} \times 100 \quad 7-9$$

$$\text{Arabinose unit in oligo(arabino)xylan alcohol} = \frac{V(\text{Ara1})}{2V(X1'_I) + V(X1_{\text{NR/I}})} \times 100 \quad 7-10$$

where $V(X1'_I)$ denotes the volume integral for the ^{13}C - ^1H pair of the anomeric center of the penultimate xylopyranose unit, and $V(X1_{\text{NR/I}})$, the combined volume integral for the ^{13}C - ^1H pair of the anomeric centres of the xylopyranose unit at the non-reducing end and the internal xylopyranose units occurring in the oligoxylyan alcohol backbone. $V(\text{Ara1})$ stands for the volume integral of the anomeric centre of the arabinose unit decorating the main backbone. To account for the xylitol end unit, $V(X1'_I)$ was multiplied by 2.

In all calculations, the volume integral for the indicated ^{13}C - ^1H pairs was measured five times on different occasions to determine the standard deviation associated with the HSQC NMR spectra measurements.

Following are given some additional notes on the HSQC NMR analysis:

1. The $^1J_{\text{CH}}$ dependence of polarization transfer in HSQC experiments is not entirely suppressed in regular HSQC pulse sequences (e.g., Bruker *hsqcetgpsi*).²⁵ As a result, the response for ^{13}C - ^1H pairs is not entirely uniform in the entire spectral range. Despite this, regular HSQC NMR measurements allow for the estimation of semiquantitative information for the characterization and comparison of lignins when ^1H - ^{13}C pairs in a similar chemical environment are chosen (e.g., C_α - H_α signals for the side-chain of lignin units or the C_2 - H_2 and C_6 - H_6 pairs in the G- and S-lignin units). In this case, the $^1J_{\text{CH}}$ values for the ^1H - ^{13}C pairs are similar. Nonetheless, a systematic deviation owing to the non-universal response for ^{13}C - ^1H pairs will invariably be present in each set of estimates (i.e., for each type of linkage). Despite this, the estimated values are consistent, thus, allowing to use the data for comparison in a series.
2. In Equation 7-3, it was decided to use the volume integral of C_β in the determination of the percentual of reduced β -O-4 linkages in order to mitigate any effect of $^1J_{\text{CH}}$ on the signal response uniformity. Since native and reduced β -O-4 linkages present identical neighbouring atoms connected on the C_β atom, $^1J_{\text{CH}}$ values should be identical. As a result, the signal response is reasonably similar for the ^{13}C - ^1H pairs in the HSQC NMR spectrum.
3. For the same reason as in 'Note 2', in Equations 7-9 and 7-10, it was decided to multiply $V(\text{X1})$ by a factor of 2 to account for the xylitol end unit.
4. In Equations 7-2, 7-5, 7-6, and 7-8, the contribution of $\text{H}_{2/6}$ was excluded from the denominator since the H-units signal seems to come from products of guaiacol demethoxylation and is hardly present in the utilised poplar wood.

7.9. Analytical saccharification (Chapters 4 and 5)

The biomass and pulp composition was determined by analytical saccharification according to NREL Laboratory Analytical Procedure (LAP).³⁸⁴ In short, 300 mg of pulp was dispersed in 3 mL

72% H₂SO₄ at 30 °C for 1 h. 84 ml of DI water was added, and the mixture was autoclaved for 1 h at 121°C. The resulting suspension was filtered through 0.45 µm nylon filters, and the Klason lignin residue was dried in a 105 °C convection oven overnight and weighed. The resulting solution was analysed by HPLC using a Perkin Elmer Series 200 HPLC at 80°C with a Bio-Rad Aminex HPX-87H Column (300 x 7.8 mm) column. A 5 mM H₂SO₄ solution with a flow rate of 0.6 mL min⁻¹ was used as the mobile phase. The amount of acid-soluble was detected based on the UV-absorbance at 205nm according to the protocol.

7.10. Fluorescence microscopy (Chapter 5)

Imaging was carried out with the microscopy setup as described in reference ⁵⁰⁸. Briefly, live cell widefield epifluorescence and HILO were conducted using an inverted microscope built around a Till iMIC body (Till Photonics/FEI; Munich, Germany), equipped with UPLSAPO 20 × oil (NA 0.85) and TIRF APON 60 × oil (NA 1.49) objective lenses (Olympus Corp., Tokyo, Japan). The samples were sequentially excited with 405nm (150 mW), 488nm (100 mW), 515 nm (80 mW) and 638 nm (150 mW) PhoxX laser diodes (Omicron-Laserage; Rodgau, Germany) combined in the SOLE-6 light engine (Omicron-Laserage). Excitation light was launched into Yanus scan head, which along with a Polytrope galvanometric mirror (Till Photonics/FEI; Munich, Germany) was used to position the laser for widefield epifluorescence or HILO illumination. Excitation and emission light were spectrally separated with the imaging filter cubes containing a 390/40 BrightLine exciter filter (Semrock, NY, USA), a zt 405 rdc 2.0mm thick beamsplitter (Chroma Technology Corp, Vermont, USA), and a ET 460/50 emitter filter (Chroma) for 405 nm laser, or containing a zt 488 rdc 2.0mm thick beamsplitter (Chroma) and a 525/45 BrightLine single bandpass emission filter (Semrock; West Henrietta, NY, USA) for 488 nm laser, or containing zt 514/640 rpc 2.0 mm thick polychromatic beamsplitter and a 577/690 Brightline dual-bandpass emission filter for 515 nm and 638 nm lasers. The electron-multiplying charge-coupled device Ultra 897 camera (Andor Technology; Belfast, UK) was mounted to a microscope through a TuCam adapter with 2 × magnification (Andor Technology). The camera was cooled down to – 100°C with the assistance of a liquid recirculating chiller Oasis 160 (Solid State Cooling Systems; Wappingers Falls, NY, USA). All imaging experiments were performed on 10 µm thick sample cuts with a microtome (Cryostat Microtome CM 1950) and mounted on slides with Ibbidi mounting media (Ibbidi, Munich,

Germany). At least 3 selected areas per sample were captured as 16-bit OME-TIFF multicolour Z-stacks (100 frames, with 200nm piezo-focusing increment), with 100ms exposure time and EM gain 300 in the case of widefield epifluorescence imaging or multicolour time-stacks (200 frames at 30Hz) in the case of SRRF experiments. The SRRF images were reconstructed with GPU of NVIDIA Quadro P4000 video card, using Fiji ImageJ 1.53i with NanoJ-SRRF plugin 1.14⁵⁰⁷ (200 frames per time point; temporal analysis settings: Temporal Radiality Auto-Correlations; radiality magnification: 5; symmetry axes: 8, ring radius 0.5). For intensity quantification, 20x objective multicolour Z-stacks were maximum intensity projected and whole image intensity summed.

7.11. Microscopy for particle size distribution (Chapter 4)

The milled wood was analysed using a Keyence VHX 5000 microscope at 300x magnification. Sample slides were prepared by dispersing the powdered wood on a glass slide. The particles were identified by the Keyence software, and their size was reported using the maximum diameter of the particle. Different areas of the samples (around 10 pictures per sample) were analysed to guarantee the reproducibility of the findings. The particle size distribution was determined with pictures including at least 2000 particles in total.

7.12. Synthesis (Chapter 3)

7.12.1. Synthesis of xylobiitol (Chapter 3)

Xylobiose (0.7 mmol) was dissolved in deionized H₂O (2.5 mL) in a 10 mL round-bottomed flask. NaBH₄ (1.5 mmol) was dispersed in deionized H₂O (0.8 mL) for 1 min and added to the xylobiose solution right after. The reaction mixture was stirred (600 rpm) at room temperature for 1 h. Pre-washed Amberlite IR120(H) resin was added to the reaction mixture until the gas separation stopped (2 mL in total), and the mixture was stirred for an additional 10 min. A 3-cm column was packed on the P3 glass filter with fresh resin dispersed in water (10 mL), and the reaction mixture with the added resin was poured into the column. After gravitational flow had removed most of the water, the resin was washed three times with deionized H₂O (20 mL). The filtrate was rota-evaporated to dryness (at 40°C under 30 mbar). MeOH (5 mL) was added to the residue, shaken for 10 min, and rota-evaporated to dryness (at 40°C under 200 mbar). This process was repeated two more times. The remaining residue was placed in a vacuum

oven for 24h (at 40°C under 50 mbar). The crude product was further purified by recrystallization. MeOH (8 mL) was added to the solid, boiled to half volume, slowly cooled to room temperature, and further cooled down in an ice bath. The remaining solvent was removed by decantation. The final product was dried in a vacuum oven under 50 mbar at 40°C for 24 h, resulting in a white solid (63% yield). The product was characterized by 2D HSQC NMR in DMSO-*d*₆, indicating no impurities (Figure A-15). The ¹³C-¹H correlation signals assignment of the product is given in Table A-5.

7.12.2. Synthesis of cellobiitol (Chapter 3)

Cellobiose (2.9 mmol) was dissolved in deionized H₂O (11.5 mL) in a 25 mL round-bottomed flask. NaBH₄ (6.1 mmol) was dispersed in deionized H₂O (1.5 mL) for 1 min and then added to the cellobiose solution. The reaction mixture was stirred (600 rpm) at room temperature for 1 h. Cellobiitol was isolated using the same work-up procedure as for xylobiitol. The final product was dried in a vacuum oven under 50 mbar at 40°C for 24 h, resulting in a white solid (71% yield). The product was characterized by 2D HSQC NMR in DMSO-*d*₆, indicating no impurities (Figure A-15). The ¹³C-¹H correlation signals assignment of the product is given in Table A-5.

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enzymatic steps evidenced by at least in vitro activities, dashed arrows represent suggested pathways. PAL, PHENYLALANINE AMMONIA-LYASE; TAL, TYROSINE AMMONIA-LYASE; C4H, CINNAMATE 4-HYDROXYLASE; 4CL, 4-COUMARATE:CoA LIGASE; HCT, *p*-HYDROXYCINNAMOYL-CoA:QUINATE/SHIKIMATE *p*-HYDROXYCINNAMOYLTRANSFERASE; C3H, *p*-COUMARATE 3-HYDROXYLASE; CSE, CAFFEYOYL SHIKIMATE ESTERASE; CC_oAOMT, CAFFEYOYL-CoA O-METHYLTRANSFERASE; CCR, CINNAMOYL-CoA REDUCTASE; F5H, FERULATE 5-HYDROXYLASE; COMT, CAFFEIC ACID O-METHYLTRANSFERASE; CAD, CINNAMYL ALCOHOL DEHYDROGENASE; HCALDH, HYDROXYCINNAMALDEHYDE DEHYDROGENASE; PER, PEROXIDASE; FMT, FERULOYL-CoA MONOLIGNOL TRANSFERASE; PMT, *p*-COUMAROYL-CoA MONOLIGNOL TRANSFERASE; CHS, CHALCONE SYNTHASE; CHI, CHALCONE ISOMERASE; FNS, FLAVONE SYNTHASE; F3'H, FLAVONOID 3'-HYDROXYLASE; F5'H, FLAVONOID 5'-HYDROXYLASE; THT, HYDROXYCINNAMOYL-CoA:TYRAMINE N-HYDROXYCINNAMOYLTRANSFERASE; PHT, HYDROXYCINNAMOYL-CoA:PUTRESCINE HYDROXYCINNAMOYL TRANSFERASE; STS, STILBENE SYNTHASE. Reproduced from reference³⁷ with permission (Elsevier).

Table A-1. Individual Hildebrandt and Hansen solubility parameters of a variety of polar protic, polar aprotic, and non-polar solvents were obtained from reference²³⁵. The first two lines represent the Hansen solubility parameters and sphere radius of lignin determined by Hansen and Björkman²³⁸ and Novo and Curvelo.²⁵⁷ RED values represent the corresponding vector length radiuses described in Chapter 2.2 of the different solvents with respect to Lignin 1 (Hansen and Björkman²³⁸) and Lignin 2 (Novo and Curvelo²⁵⁷).

Solute		δ	δ_D	δ_P	δ_H	R_0	Reference
Lignin 1			21.9	14.1	16.9	13.7	Bjorkman ²³⁸
Lignin 2			21.4	8.6	21.8	13.6	Curvelo ²⁵⁷
Solvent		δ	δ_D	δ_P	δ_H	RED 1 (Lignin 1)	RED 2 (Lignin 2)
Polar protic	water	47.8	15.5	16.0	42.3	2.08	1.83
	methanol	29.6	15.1	12.3	22.3	1.08	0.97
	ethanol	26.5	15.8	8.8	19.4	0.99	0.85
	2-propanol	23.6	15.8	6.1	16.4	1.07	0.94
	1-butanol	23.2	16.0	5.7	15.8	1.06	0.94
	2-butanol	22.2	15.8	5.7	14.5	1.10	1.01
	ethylene glycol	33.0	17.0	11.0	26.0	1.00	0.74
	glycerol	36.2	17.4	12.1	29.3	1.13	0.85
	HFIP	23.1	17.2	4.5	14.7	0.99	0.87
Polar aprotic	Acetone	19.9	15.5	10.4	7.0	1.21	1.40
	EtOAc	18.2	15.8	5.3	7.2	1.31	1.38
	Acetonitrile	24.4	15.3	18.0	6.1	1.28	1.62
	DMSO	26.7	18.4	16.4	10.2	0.73	1.12
	DMF	24.9	17.4	13.7	11.3	0.77	1.05
	THF	19.5	16.8	5.7	8.0	1.16	1.24
	1,4-dioxane	20.5	19.0	1.8	7.4	1.21	1.23
Non-polar	Hexane	14.9	14.9	0	0	1.90	1.98
	Cyclohexane	16.8	16.8	0	0.2	1.76	1.84
	Benzene	18.5	18.4	0	2	1.58	1.65
	toluene	18.2	18	1.4	2	1.54	1.63

9.2. Chapter 3

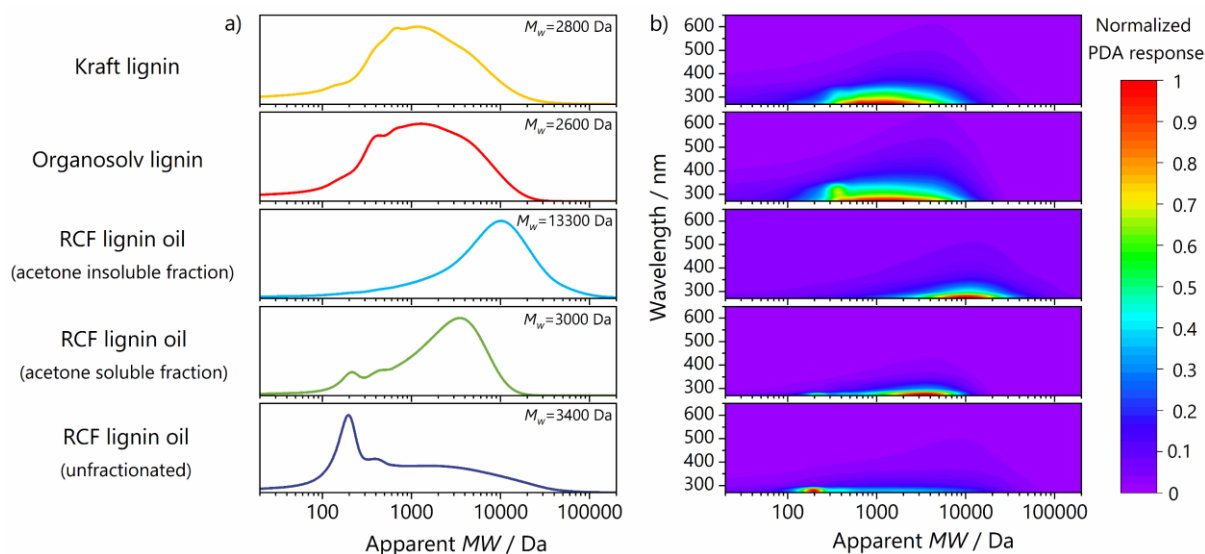


Figure A-2. a) Normalised PDA response at 280 nm along with weight average molecular masses of lignin samples obtained from Eucalyptus wood with the corresponding treatment in the apparent MW range of 20-200 000 Da. b) Normalised PDA response from 270-650 nm in the apparent MW range of 20-200 000 Da

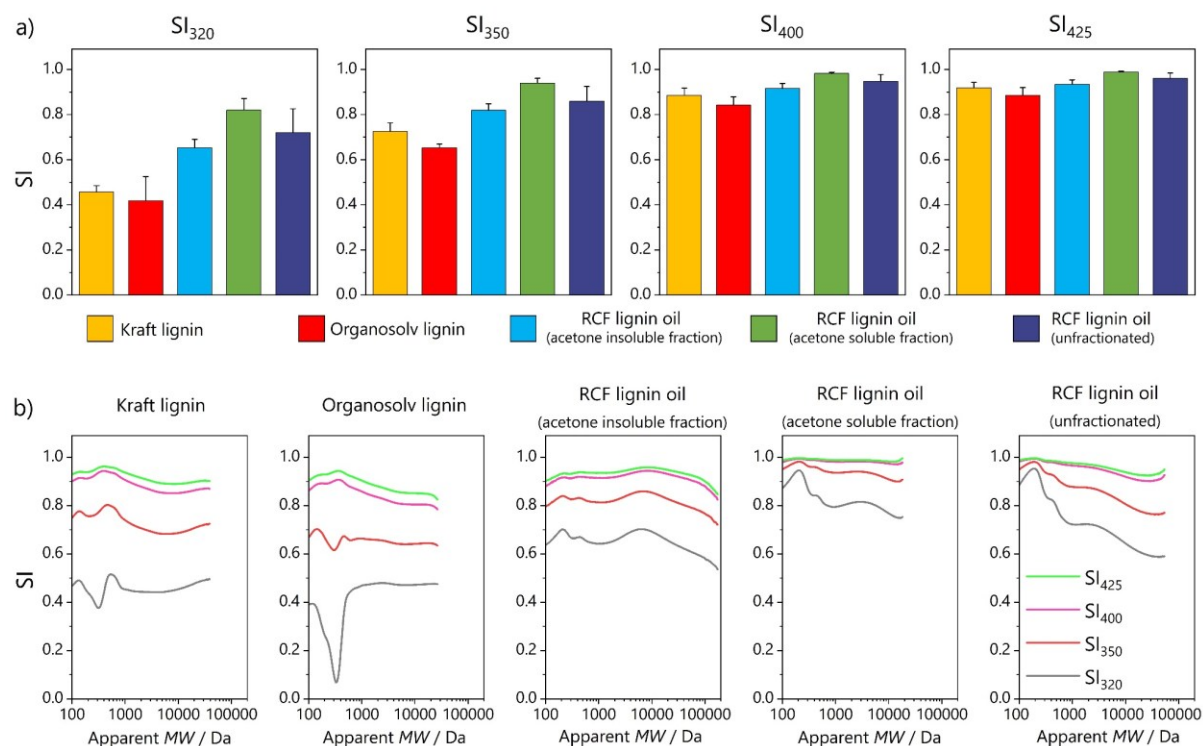


Figure A-3. a) Structural Index (SI) values of the different lignin fractions along with standard deviation to the distribution average value. b) SI distribution of different lignin fractions across the apparent MW range of 100-200 000 nm.

Calculation of theoretical H/C and O/C values for hypothetical hardwood lignin

Hypothetical hardwood lignin made of β -O-4 linkages was considered to determine the effect of S/G/H ratio on the theoretical values for H/C and O/C ratios. An aromatic unit linked on both ends has 11 carbon, 5 oxygen, and 14 hydrogen atoms for S-unit; 10 carbon, 4 oxygen, and 12 hydrogen atoms for G-unit; 9 carbon, 3 oxygen, and 10 hydrogen atoms for H unit. A degree of polymerization above 10 is assumed, allowing to neglect the impact of the end groups on the theoretical values for H/C and O/C molar ratios. The molar ratios were estimated as

$$H/C = \frac{14 \times (S_{\text{unit}}) + 12 \times (G_{\text{unit}}) + 10 \times (H_{\text{unit}})}{11 \times (S_{\text{unit}}) + 10 \times (G_{\text{unit}}) + 9 \times (H_{\text{unit}})} \#$$

$$O/C = \frac{5 \times (S_{\text{unit}}) + 4 \times (G_{\text{unit}}) + 3 \times (H_{\text{unit}})}{11 \times (S_{\text{unit}}) + 10 \times (G_{\text{unit}}) + 9 \times (H_{\text{unit}})}$$

where S_{unit} , G_{unit} and H_{unit} represent the fraction of each unit present in hardwood. For poplar containing 62% of S-type lignin ($S_{\text{unit}}=0.62$) and 38% of G-type ($G_{\text{unit}}=0.38$), the theoretical values for H/C ratio and O/C ratio are 1.25 and 0.44, respectively. Figure A-3 displays corresponding H/C and O/C molar ratios for different S/G/H ratios under the same assumptions, with the highlighted spot for poplar used in this study. For hypothetical S-G lignin structures with G-unit fraction between 0.25 and 0.50, the values for H/C and O/C were 1.25 ± 0.02 and 0.44 ± 0.02 , respectively.²³⁹

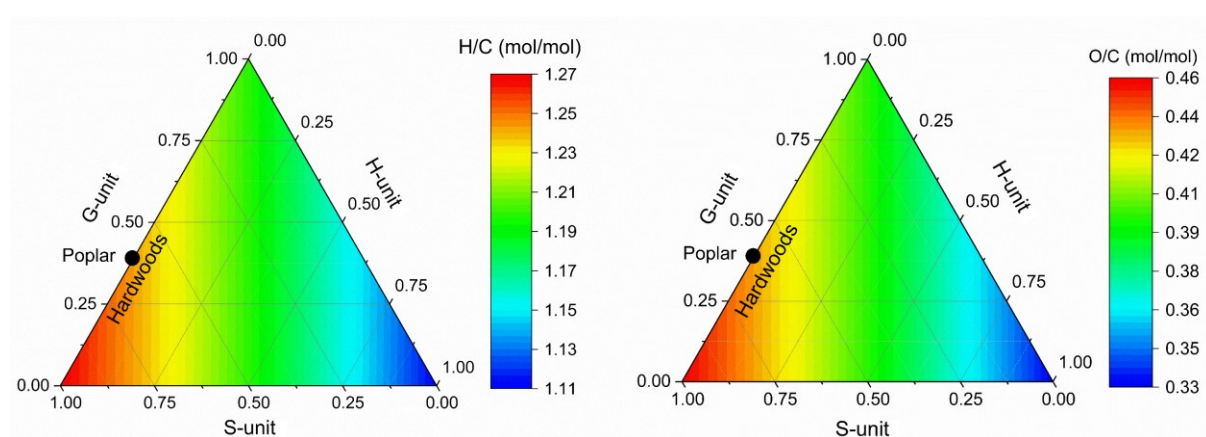


Figure A-4. Ternary diagram showing the distribution of theoretical values of H/C and O/C in the whole range of S/G/H ratios. Values were calculated for hypothetical linear lignin structures connected by β -O-4. The dot indicates the hypothetical H/C and O/C molar ratio values considered as the theoretical values for the Poplar lignin in this study. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

Table A-2. Common lignin linkages quantification per 100 aromatic units in the RCF lignin oil fractions obtained by sequential solvent fractionation (SSF). In addition, the count of reduced β -O-4 linkages and relative abundance of S-units is displayed.

Linkage	Acetone insoluble (SSF)	Acetone soluble (SSF)	EtOAc insoluble (SSF)	EtOAc soluble (SSF)
(A) β -O-4	53.4 $\pm$ 0.7	12.8 $\pm$ 0.3	56 $\pm$ 1	8.2 $\pm$ 0.1
(A _(CH₂)) Reduced β -O-4	2.2 $\pm$ 0.2	1.8 $\pm$ 0.1	1.8 $\pm$ 0.3	1.5 $\pm$ 0.1
(B) Phenylcoumaran	3.7 $\pm$ 0.2	1.6 $\pm$ 0.1	4.0 $\pm$ 0.3	1.3 $\pm$ 0.1
(C) Resinol	5.4 $\pm$ 0.1	3.2 $\pm$ 0.1	5.1 $\pm$ 0.1	2.8 $\pm$ 0.1
S-unit content (S/(S+G) (%))	68.7 $\pm$ 0.7	62.5 $\pm$ 0.1	66.2 $\pm$ 0.4	62.2 $\pm$ 0.2

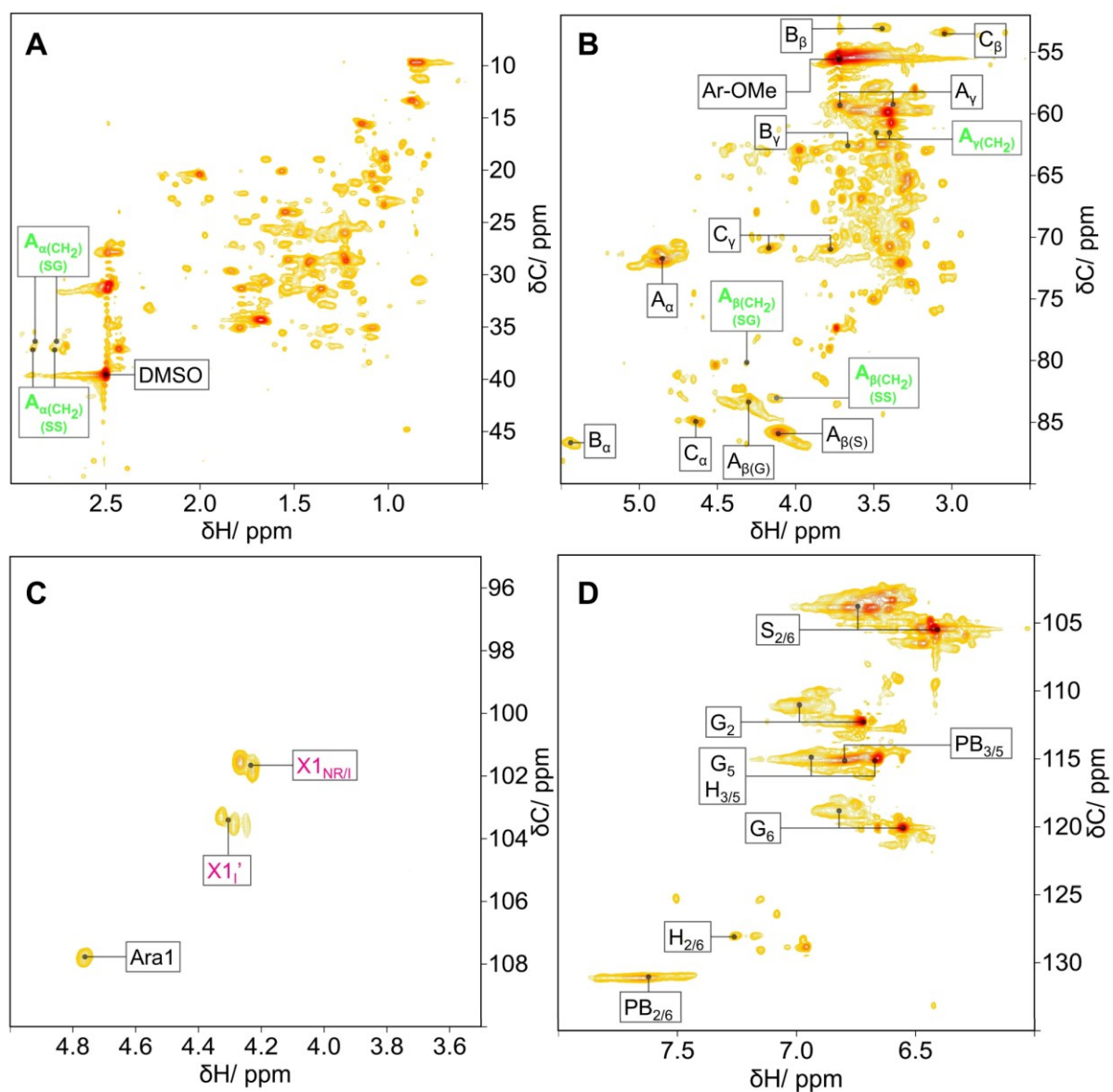


Figure A-5. . HSQC NMR spectrum of RCF lignin oil zoomed in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, and (D) aromatic region (solvent: DMSO- d_6). Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

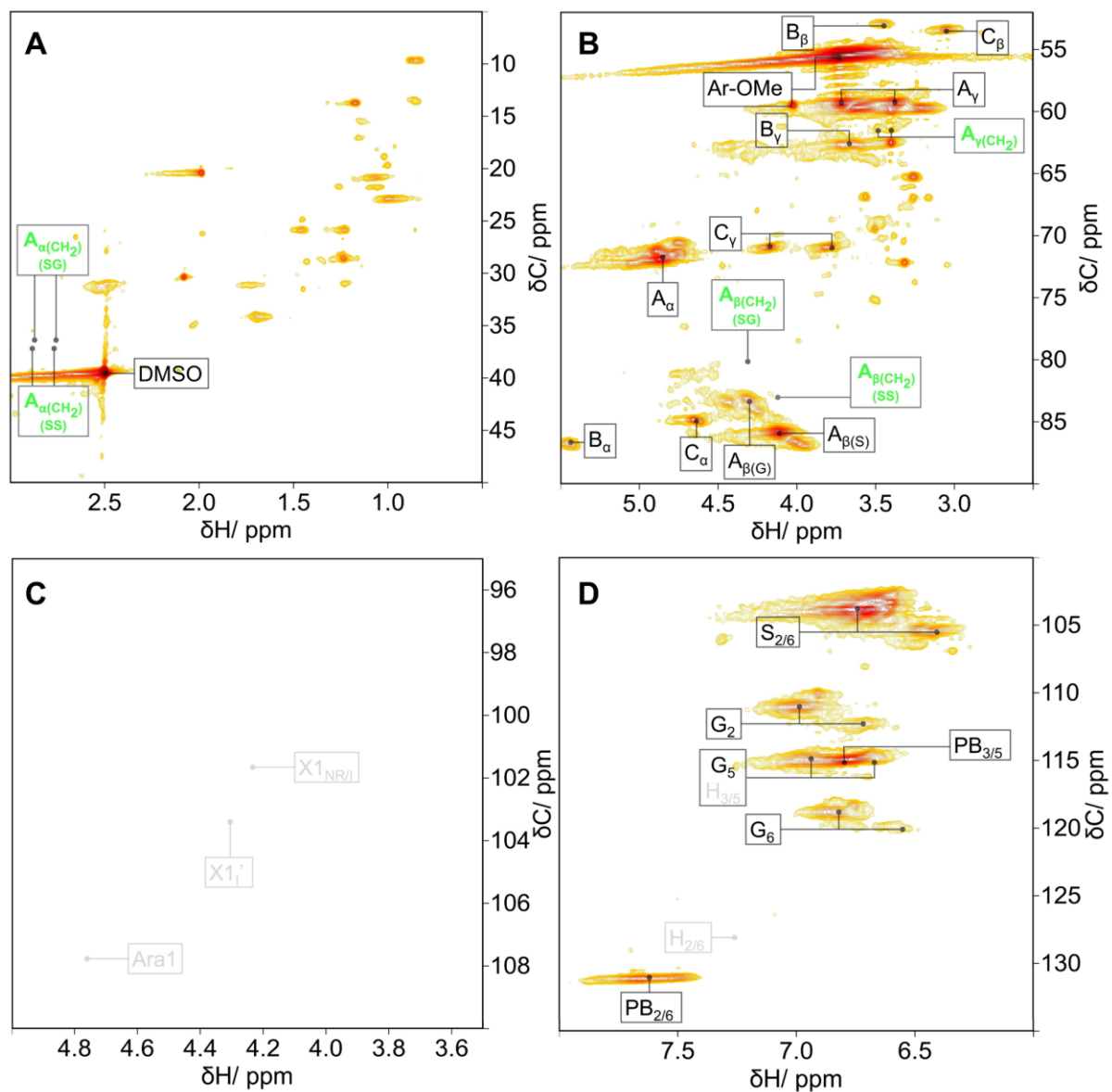


Figure A-6. HSQC NMR spectrum of RCF lignin oil methanol-insoluble fraction zoomed in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, and (D) aromatic region (solvent: DMSO- d_6). Note: The ^1H - ^{13}C correlation signals for the $A_{(\text{CH}_2)}$ linkages are present below the threshold chosen to present the HSQC NMR spectral data. Captions for signals not detected were greyed out. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

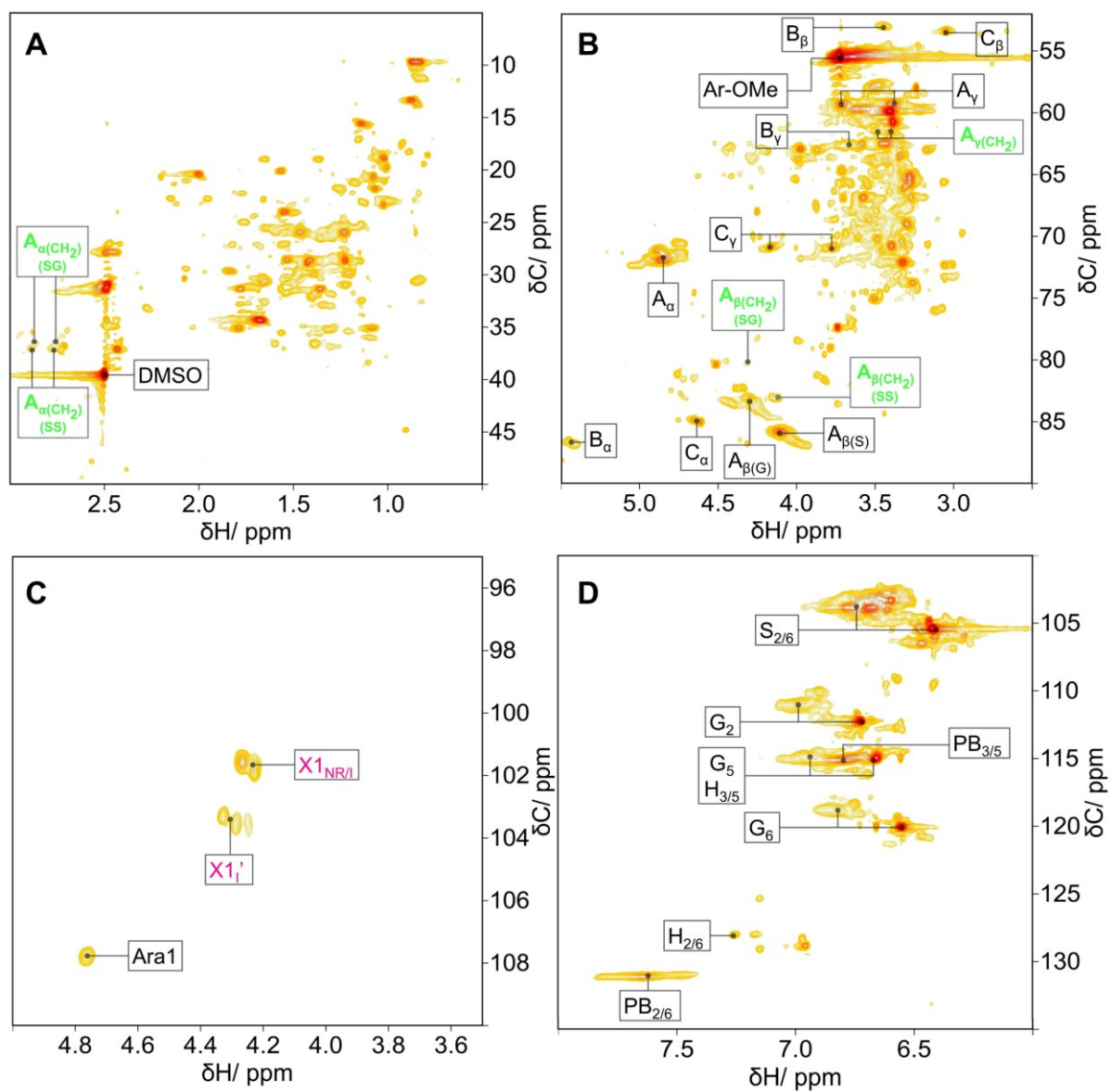


Figure A-7. HSQC NMR spectrum of RCF lignin oil methanol-soluble fraction zoomed in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, and (D) aromatic region (solvent: DMSO- d_6). Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

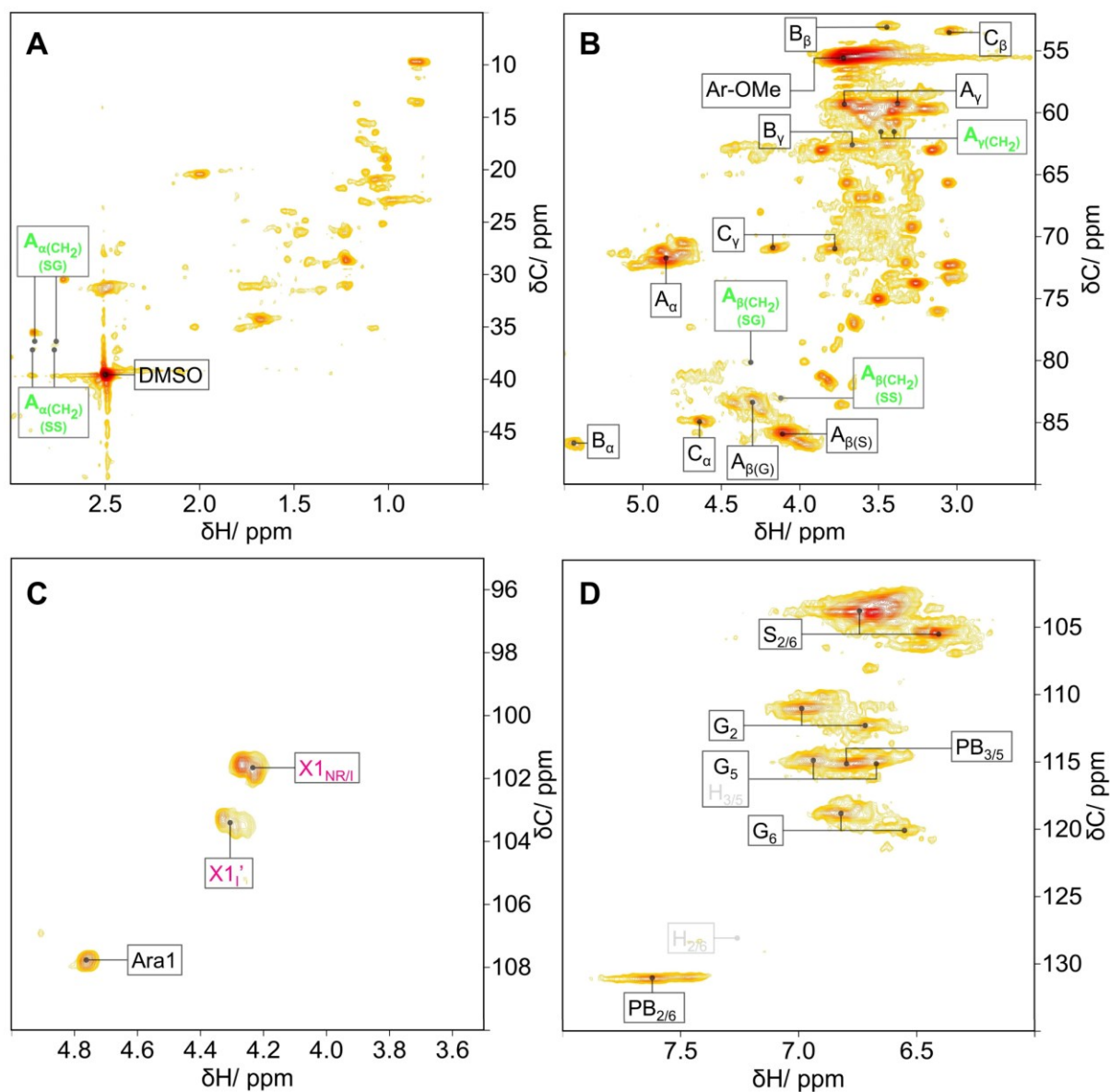


Figure A-8. HSQC NMR spectrum of RCF lignin oil acetone-insoluble fraction zoomed in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, and (D) aromatic region (solvent: $\text{DMSO}-d_6$). The ^1H - ^{13}C correlation signals for the $\text{A}_{\alpha}(\text{CH}_2)$ are present below the threshold chosen to present the HSQC NMR spectral data. Captions for signals not detected were greyed out. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

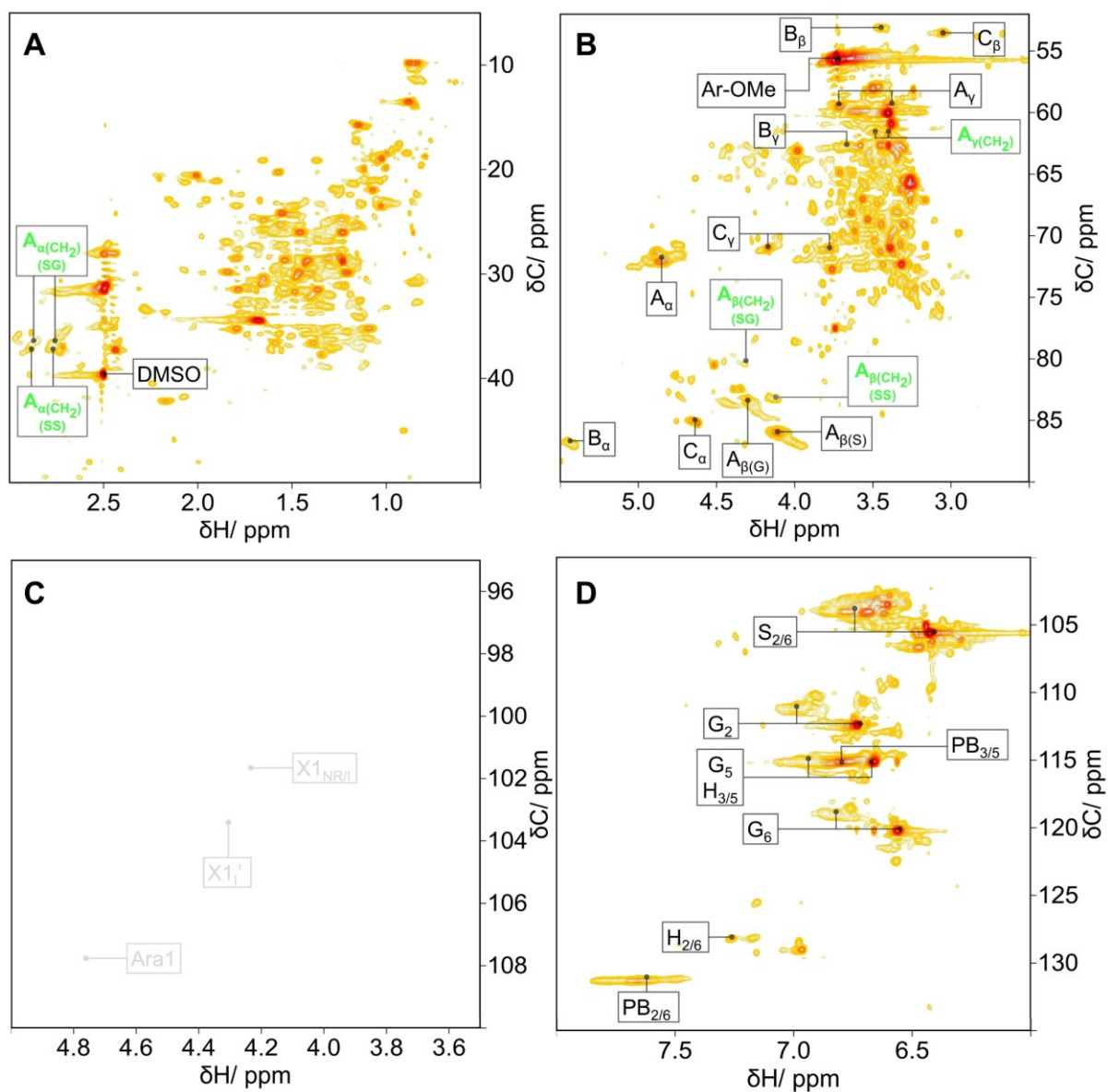


Figure A-9. HSQC NMR spectrum of RCF lignin oil acetone-soluble fraction zoomed in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, and (D) aromatic region (solvent: DMSO- d_6). Captions for signals not detected were greyed out. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

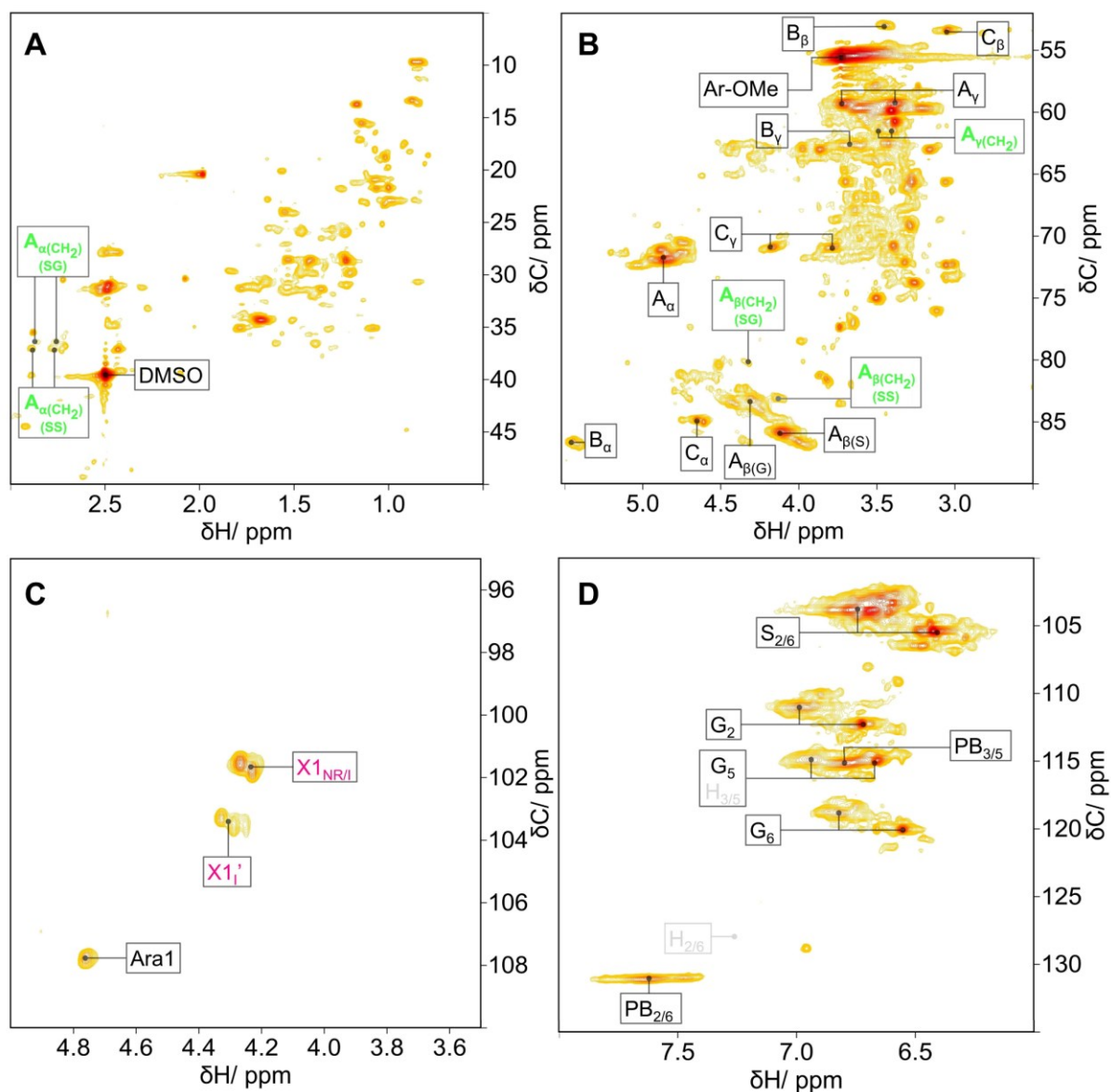


Figure A-10. HSQC NMR spectrum of RCF lignin oil EtOAc-insoluble fraction zoomed in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, and (D) aromatic region (solvent: DMSO- d_6). Captions for signals not detected were greyed out. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

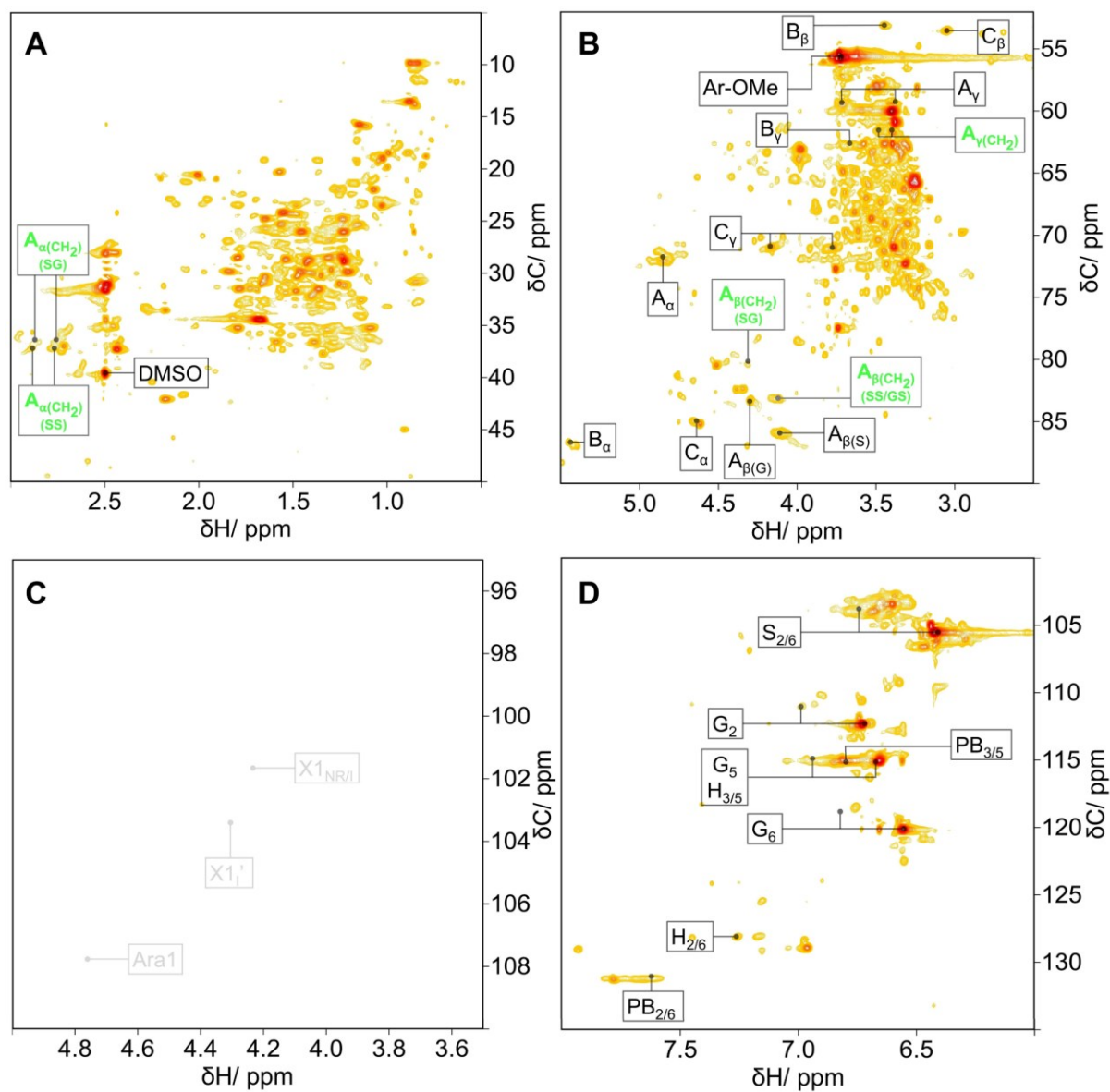


Figure A-11. HSQC NMR spectrum of EtOAc-soluble fraction of RCF lignin oil zoomed in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, and (D) aromatic region. (solvent: DMSO- d_6). Captions for signals not detected were greyed out. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

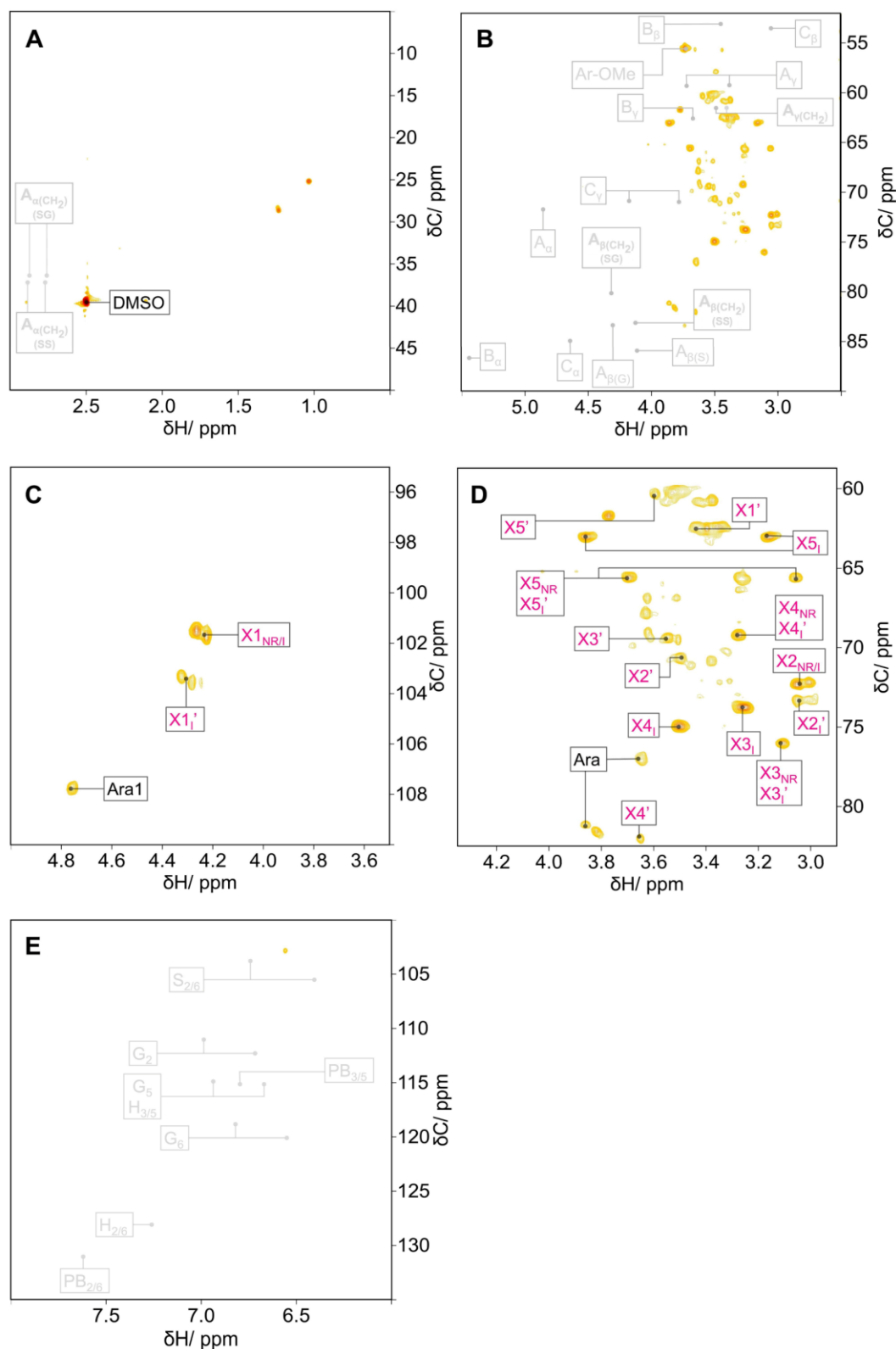


Figure A-12. Results from the hot water extraction carried out on the acetone-insoluble fraction of the RCF lignin oil. HSQC NMR spectrum of hot water extractives presented in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, (D) oxygenated aliphatic region focused on carbohydrates, and (E) aromatic region (solvent: DMSO-*d*₆). Captions for signals not detected were greyed out. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

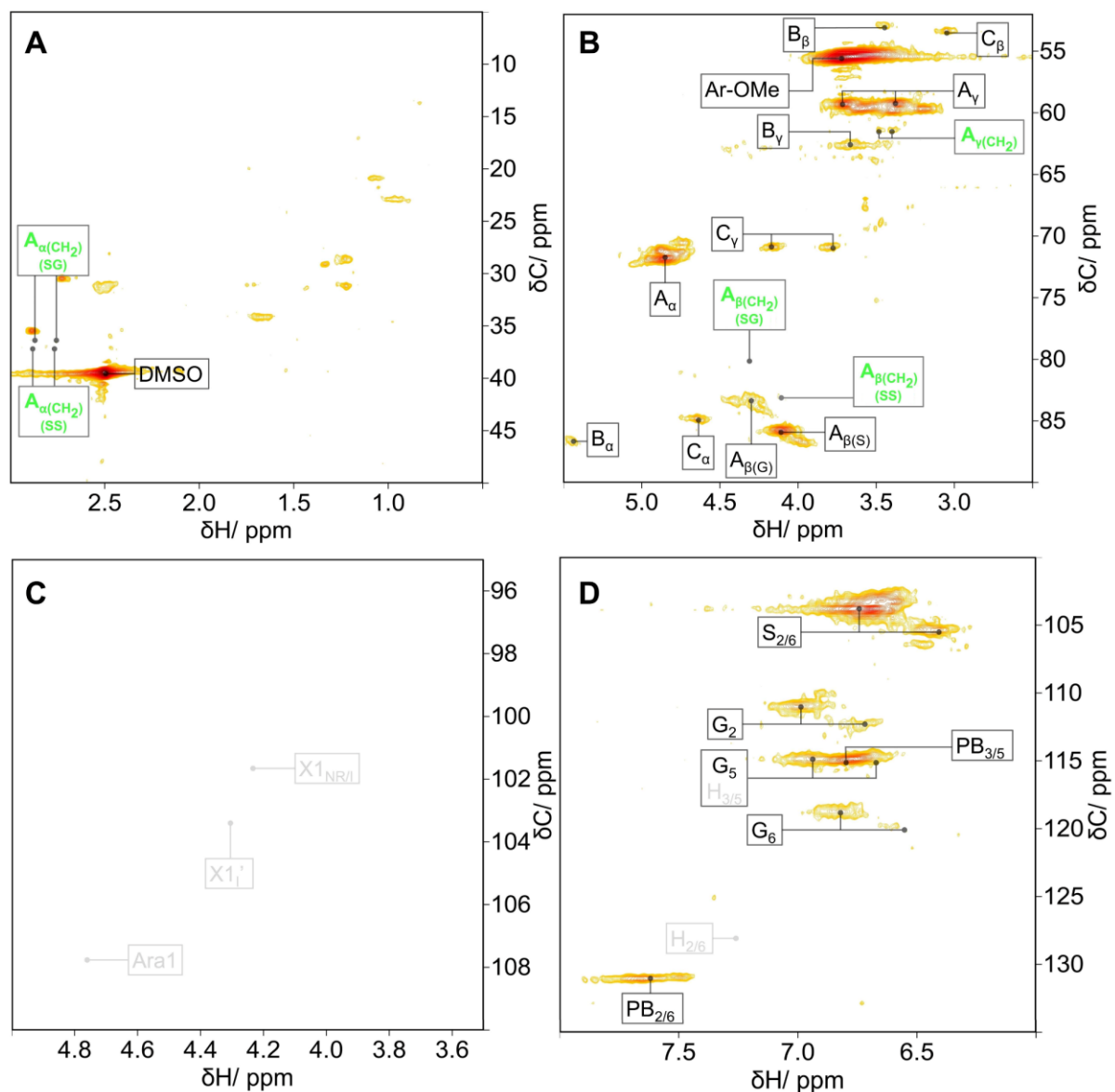


Figure A-13. Results from the hot water extraction carried out on the acetone-insoluble fraction of the RCF lignin oil. HSQC NMR spectrum of hot water-insoluble fraction presented in (A) aliphatic region, (B) oxygenated aliphatic region, (C) anomeric region, and (E) aromatic region (solvent: DMSO- d_6). The ^1H - ^{13}C correlation signals for the $A_{(\text{CH}_2)}$ linkages are present below the threshold chosen to present the HSQC NMR spectral data. Captions for signals not detected were greyed out. Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

Table A-3. List of ^{13}C - ^1H pairs with their respective $\delta\text{C}/\delta\text{H}$ values (in ppm) obtained from the spectra in Chapter 3 (solvent: DMSO- d_6). Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

Label	$\delta\text{C}/\delta\text{H}$ (ppm)	Description
A$_{\alpha}$	71.90/4.86	C $_{\alpha}$ -H $_{\alpha}$ in β -O-4 lignin linkages
A$_{(\text{CH}_2)\alpha}$ (SG)	36.53/2.86 36.53/2.78	C $_{\alpha}$ -H $_{\alpha}$ in reduced β -O-4 lignin linkages containing S and G units (with etherified G unit)
A$_{(\text{CH}_2)\alpha}$ (SS)	37.06/2.90 37.06/2.78	C $_{\alpha}$ -H $_{\alpha}$ reduced β -O-4 bonding motif formed between two S units
A$_{(\text{G})\beta}$	83.34/4.27	C $_{\beta}$ -H $_{\beta}$ in β -O-4 bonding motif (with etherified G unit)
A$_{(\text{S})\beta}$	85.81/4.10	C $_{\beta}$ -H $_{\beta}$ in β -O-4 bonding motif (with etherified S unit)
A$_{(\text{CH}_2)\beta}$ (SG)	80.56/4.30	C $_{\beta}$ -H $_{\beta}$ in reduced β -O-4 bonding motif formed between S and G units (with etherified G unit)
A$_{(\text{CH}_2)\beta}$ (SS)	83.32/4.10	C $_{\beta}$ -H $_{\beta}$ in reduced β -O-4 bonding motif formed between S units
A$_{(\text{CH}_2)\gamma}$	61.34/3.39 61.34/3.48	C $_{\gamma}$ -H $_{\gamma}$ in reduced β -O-4 bonding motif
A$_{\gamma}$	59.23/3.38 59.23/3.71	C $_{\gamma}$ -H $_{\gamma}$ in β -O-4 bonding motif
B$_{\alpha}$	86.69/5.45	C $_{\alpha}$ -H $_{\alpha}$ in phenylcoumaran bonding motif
B$_{\beta}$	53.07/3.45	C $_{\beta}$ -H $_{\beta}$ in phenylcoumaran bonding motif
B$_{\gamma}$	62.75/3.68	C $_{\gamma}$ -H $_{\gamma}$ in phenylcoumaran bonding motif
C$_{\alpha}$	85.28/4.62	C $_{\alpha}$ -H $_{\alpha}$ in resinol bonding motif
C$_{\beta}$	53.60/3.05	C $_{\beta}$ -H $_{\beta}$ in resinol bonding motif
C$_{\gamma}$	70.85/3.87 70.85/4.16	C $_{\gamma}$ -H $_{\gamma}$ in resinol bonding motif

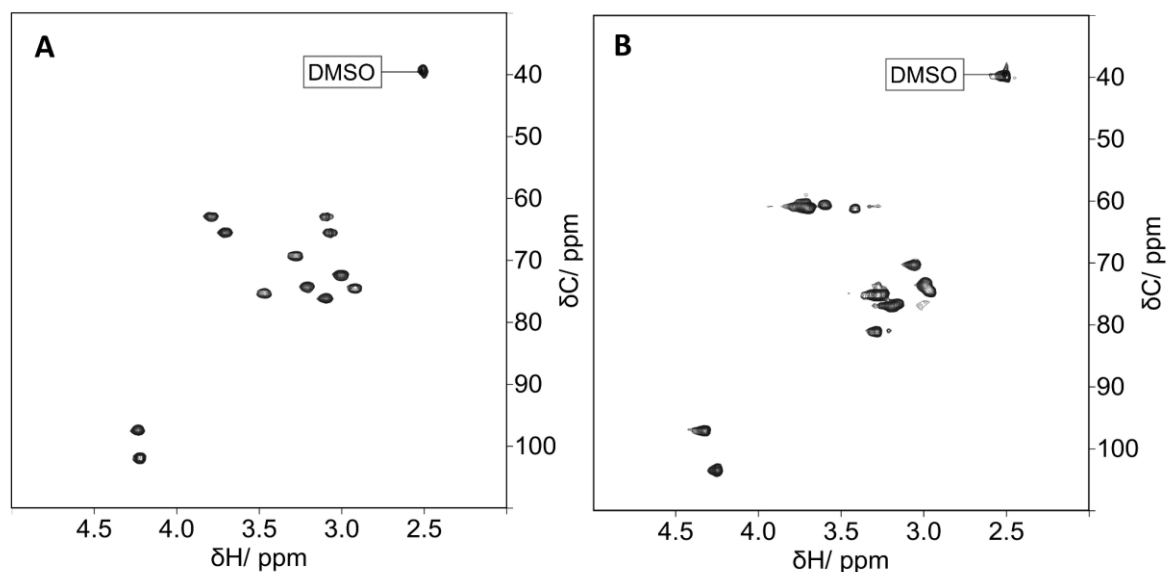
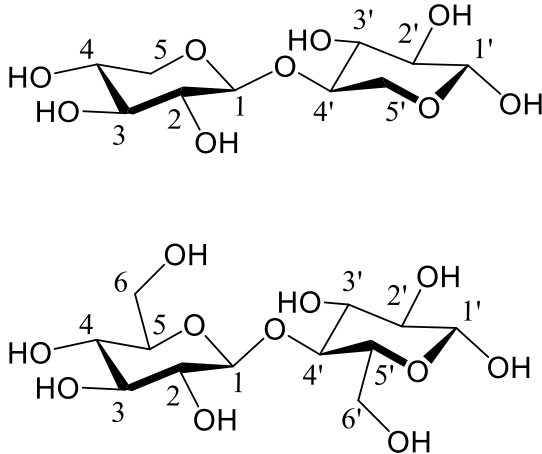


Figure A-14. HSQC NMR spectra (A) xylobiose and (B) cellobiose. (solvent: DMSO- d_6). Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

Table A-4. List of ^{13}C - ^1H pairs with their respective $\delta\text{C}/\delta\text{H}$ values (in ppm) obtained from the spectra of xylobiose and cellobiose in this study (solvent: DMSO- d_6). Reproduced from reference ²³⁹ licensed under [CC BY 4.0](#).

Carbon atom	Xylobiose	Cellobiose
	$\delta\text{C}/\delta\text{H}$ (ppm)	$\delta\text{C}/\delta\text{H}$ (ppm)
1	101.56/4.22	103.56/4.25
2	72.21/3.00	73.54/2.99
3	76.21/3.09	76.88/3.16
4	69.54/3.28	70.21/3.06
5	65.54/(3.06/3.71)	76.88/3.16
6	N/A	61.30/(3.41/3.70)
1'	97.56/4.23	96.89/4.32
2'	74.21/2.92	74.88/2.95
3'	74.21/3.21	75.54/3.27
4'	75.54/3.47	80.88/3.28
5'	62.87 3.09/3.80	75.54 3.27
6'	N/A	60.87/(3.60/3.71)



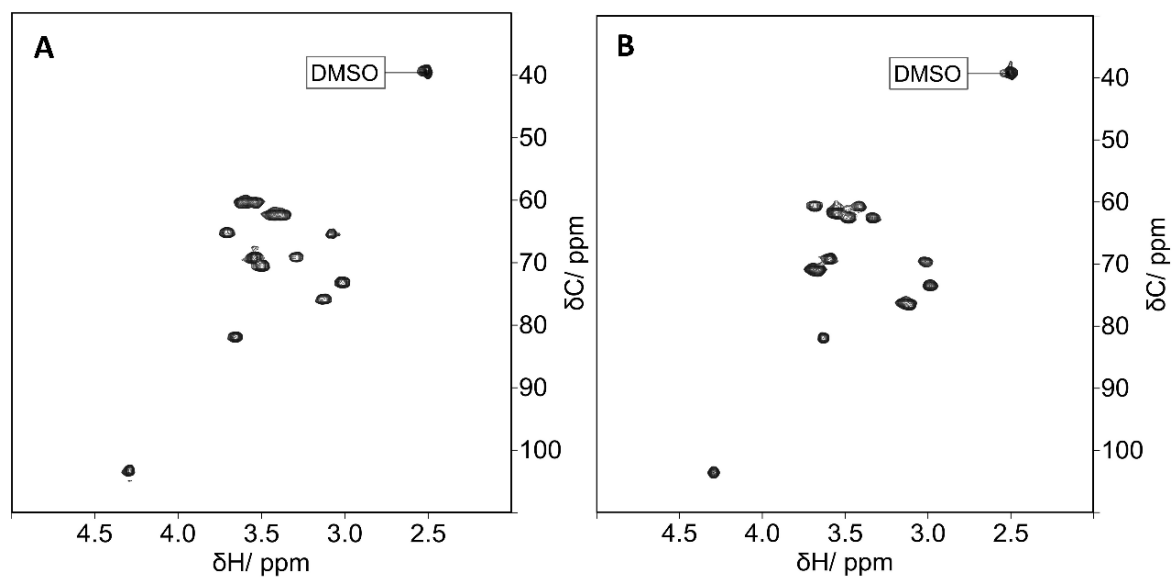


Figure A-15. HSQC NMR spectra (A) xylobiitol and (B) cellobiitol (solvent: DMSO- d_6). Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

Table A-5. List of ^{13}C - ^1H pairs with their respective $\delta\text{C}/\delta\text{H}$ values (in ppm) obtained from the spectra of xylobiitol and cellobiitol in this study (solvent: DMSO- d_6). Reproduced from reference ²³⁹ licensed under [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).

Carbon atom	Xylobiitol	Cellobiitol
	$\delta\text{C}/\delta\text{H}$ (ppm)	$\delta\text{C}/\delta\text{H}$ (ppm)
1	103.56/4.29	103.56/4.30
2	72.88/3.02	73.54/2.98
3	76.21/3.13	75.80/3.14
4	68.87/3.29	69.54/3.02
5	65.54/ 3.09/3.71	76.21/3.13
6	-	60.87/ 3.41/3.68
1'	62.20/3.43	62.87/ 3.33/3.48
2'	70.87/3.50	70.87/3.68
3'	69.54/3.54	68.87/3.59
4'	82.22/3.65	81.55/3.63
5'	60.20/3.62	70.87/3.68
6'	-	61.54/3.55

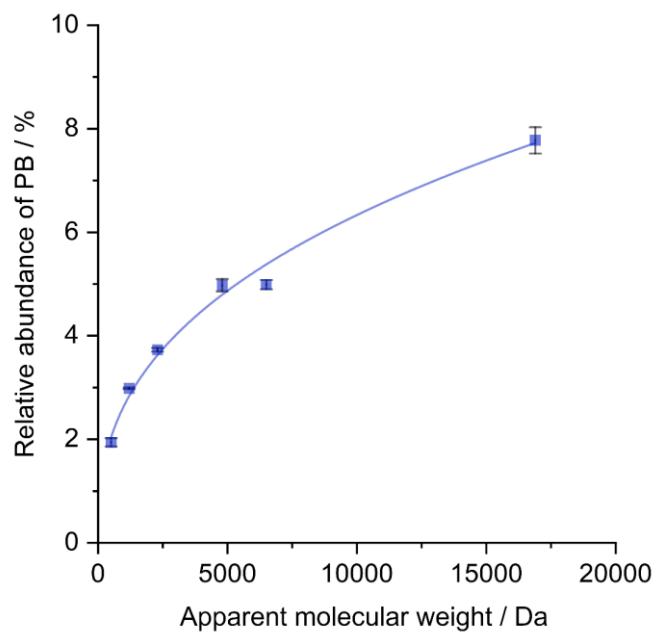


Figure A-16. Correlation between the %PB units relative to combined G- and S-unit content and the apparent weight-average molecular weight of the fractions isolated by solvent fractionation of the RCF lignin oil.

9.3. Chapter 4

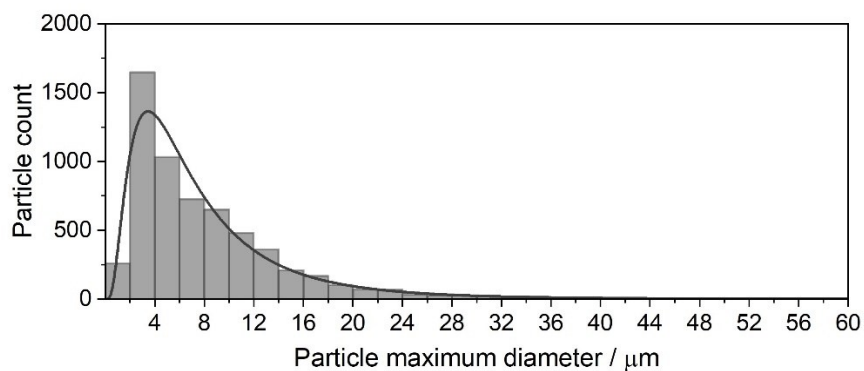


Figure A-17. Particle size distribution of poplar wood after the mechanocatalytic pretreatment in the ball mill. The distribution is measured with digital microscope imaging.

Table A-6. Structural Index values at 320 nm and 350 nm for RCF lignin oils obtained from processed wood with different additives.

Pretreatment additive	SI ₃₂₀	SI ₃₅₀
(COOH) ₂	0.90±0.03	0.97±0.01
CH ₃ COOH	0.92±0.02	0.97±0.01
Control 1 (no additive)	0.89±0.03	0.97±0.01
Control 2 (Poplar chips)	0.88±0.05	0.95±0.03
Na ₂ CO ₃	0.89±0.03	0.97±0.01
NaOH	0.87±0.04	0.96±0.01

Table A-7. Processed wood and pulp composition along with the glucans, xylans, and lignin retention for the mechanocatalytic pretreatments with different additives.

Additive	Sample	Glucans	Glucans retention	Xylans	Xylans retention	Total lignin	Lignin retention	Delignification
(COOH) ₂	Processed wood	46±2	84±3	15.8±0.8	21±3	27.8±0.8	11±3	77±4
	Pulp	81±2		7.1±0.3		6.4±0.3		
CH ₃ COOH	Processed wood	47±1	86±2	16.2±0.4	27±2	28.0±0.2	12±2	77±3
	Pulp	80±1		8.6±0.2		6.5±0.2		
Control 1 (no additive)	Processed wood	46±1	80±1	15.5±0.7	22±2	27.0±0.5	11±2	77±4
	Pulp	78±1		7.2±0.2		6.4±0.3		
Control 2 (Poplar chips)	Poplar	46±1	91±1	14.6±0.4	40±2	28.1±0.5	14±2	72±3
	Pulp	81±1		11.4±0.3		7.8±0.3		
Na ₂ CO ₃	Processed wood	48±1	75±1	16.1±0.6	27±2	28.1±0.6	14±2	70±3
	Pulp	76±2		9.2±0.2		8.6±0.3		
NaOH	Processed wood	50±1	85±2	14±0.3	48±2	25.4±0.8	18±3	68±3
	Pulp	77±2		11.9±0.3		8.2±0.3		

Table A-8. Processed wood and pulp composition along with the glucans, xylans, and lignin retention for the mechanocatalytic pretreatments with varying amounts of added acetic acid.

Added CH ₃ COOH / mmol g ⁻¹	Sample	Glucans	Glucans retention	Xylans	Xylans retention	Total lignin	Lignin retention
0	Processed wood	46±1	79±1	12.9±0.4	27±1	27.0±0.5	10.1±0.5
	Pulp	82±1		8.1±0.1		6.2±0.3	
0.25	Processed wood	44.9±0.6	83.8±0.7	13.0±0.3	30±2	27.0±0.2	13±1
	Pulp	81.9±0.5		8.6±0.2		7.4±0.7	
0.5	Processed wood	45.0±0.1	85.3±0.2	12.9±0.1	30±1	26.1±0.8	12±2
	Pulp	83.6±0.3		8.4±0.2		6.8±0.8	
1	Processed wood	45.9±0.1	81±1	13.3±0.1	26.4±0.9	27.6±0.3	10.8±0.6
	Pulp	83±2		7.8±0.2		6.6±0.3	
2	Processed wood	45.5±0.6	86.8±0.7	13.4±0.2	30.1±0.7	27.4±0.4	9±1
	Pulp	84.6±0.2		8.7±0.1		5.4±0.6	
4	Processed wood	46.0±0.1	92.6±0.5	13.6±0.1	36.3±0.6	27.1±0.2	11.0±0.7
	Pulp	82.9±0.7		9.6±0.1		5.8±0.3	

Table A-9. Processed wood and pulp composition along with the glucans, xylans, and lignin retention for the mechanocatalytic pretreatments with varying kinetic energy dose at a fixed amount of acetic acid added (2 mmol g⁻¹)

Kinetic energy dose/ kJ g ⁻¹	Sample	Glucans	Glucans retention	Xylans	Xylans retention	Total lignin	Lignin retention
4.3	Processed wood	46.1±0.6	95±1	13.5±0.2	37±1	27.5±0.6	12±2
	Pulp	83±1		9.5±0.2		6.0±0.8	
10	Processed wood	45.5±0.6	86.8±0.7	13.4±0.2	30.1±0.7	27.4±0.4	9±1
	Pulp	84.6±0.2		8.7±0.1		5.4±0.6	
14.3	Processed wood	45.6±0.6	85.8±0.8	13.4±0.3	31±2	27.7±0.4	12.5±0.6
	Pulp	81.7±0.8		8.8±0.2		7.2±0.3	

9.4. Chapter 5

Table A-10. Monophenol yields from independent steps of semi-batch T-gradient RCF. For reference, a regular batch process utilising the same biomass, catalyst and solvent for 5 h at 220°C is given for reference. P(OH)S-4-(3-hydroxypropyl)-syringol, PS- 4-propylsyringol, ES- 4-ethylsyringol, P(OH)G- 4-(3-hydroxypropyl)-guaiacol, PG- 4-propylguaiacol, EG- 4-ethylguaiacol.

Step temperature	P(OH)S	PS	ES	P(OH)G	PG	EG
140°C	0.42±0.01	0.06±0.01	0.012±0.002	0.73±0.01	ND	ND
160°C	1.3±0.2	0.11±0.02	0.032±0.002	1.5±0.4	0.007±0.001	0.004±0.001
180°C	2.9±0.3	0.25±0.02	0.11±0.01	1.9±0.1	0.003±0.001	0.006±0.001
200°C	3.6±0.2	0.28±0.04	0.3±0.1	1.6±0.1	0.018±0.005	0.022±0.003
220°C	1.1±0.2	0.07±0.02	0.21±0.04	0.44±0.07	0.007±0.001	0.015±0.001
Total	9.3±0.9	0.77±0.06	0.6±0.1	6.1±0.6	0.04±0.01	0.05±0.01
1x5h @220°C	1.5	1.6	7.8	0.6	0.2	1.2

Table A-11. Average SI_{320} and SI_{350} values for the RCF lignin oils and EtOAc soluble and insoluble fractions obtained after each step at a given temperature.

Step temperature	Fraction	SI_{320}	SI_{350}
140°C	RCF lignin oil	0.93±0.02	0.98±0.01
	EtOAc soluble	0.95±0.02	0.99±0.01
	EtOAc insoluble	0.92±0.02	0.98±0.01
160°C	RCF lignin oil	0.92±0.02	0.97±0.01
	EtOAc soluble	0.94±0.02	0.98±0.01
	EtOAc insoluble	0.90±0.02	0.96±0.01
180°C	RCF lignin oil	0.91±0.03	0.97±0.01
	EtOAc soluble	0.94±0.02	0.98±0.01
	EtOAc insoluble	0.87±0.01	0.94±0.01
200°C	RCF lignin oil	0.91±0.03	0.97±0.01
	EtOAc soluble	0.94±0.03	0.98±0.01
	EtOAc insoluble	0.90±0.01	0.96±0.01
220°C	RCF lignin oil	0.89±0.05	0.96±0.02
	EtOAc soluble	0.90±0.05	0.97±0.02
	EtOAc insoluble	0.86±0.03	0.93±0.02

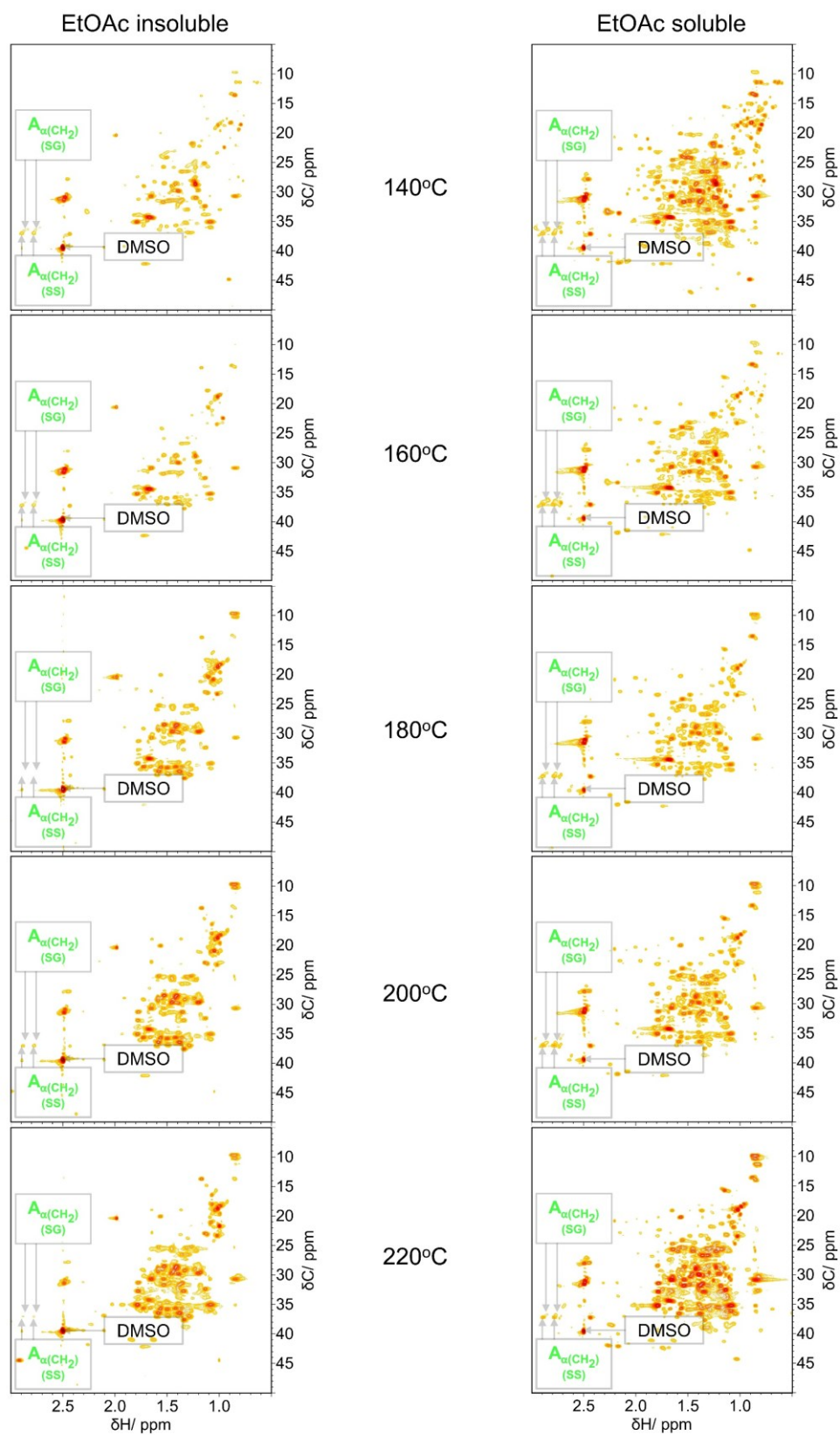


Figure A-18. HSQC NMR spectrum of EtOAc-insoluble and EtOAc-soluble fractions in RCF lignin oils obtained after each step of semi-batch T-stepping process in the aliphatic region (solvent: DMSO- d_6).

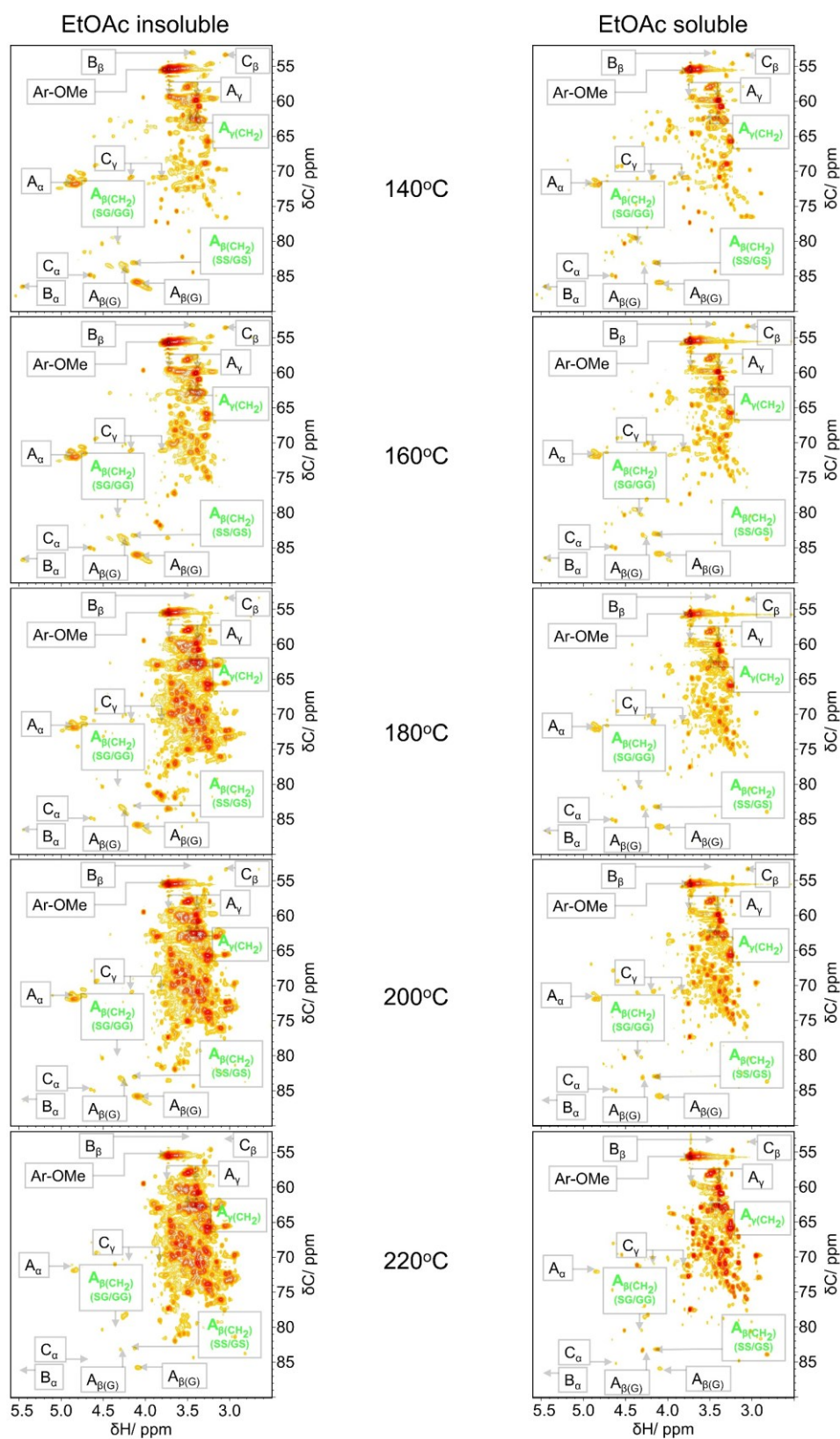


Figure A-19. HSQC NMR spectrum of EtOAc-insoluble and EtOAc-soluble fractions in RCF lignin oils obtained after each step of semi-batch T-stepping process in the oxygenated aliphatic region (solvent: DMSO- d_6).

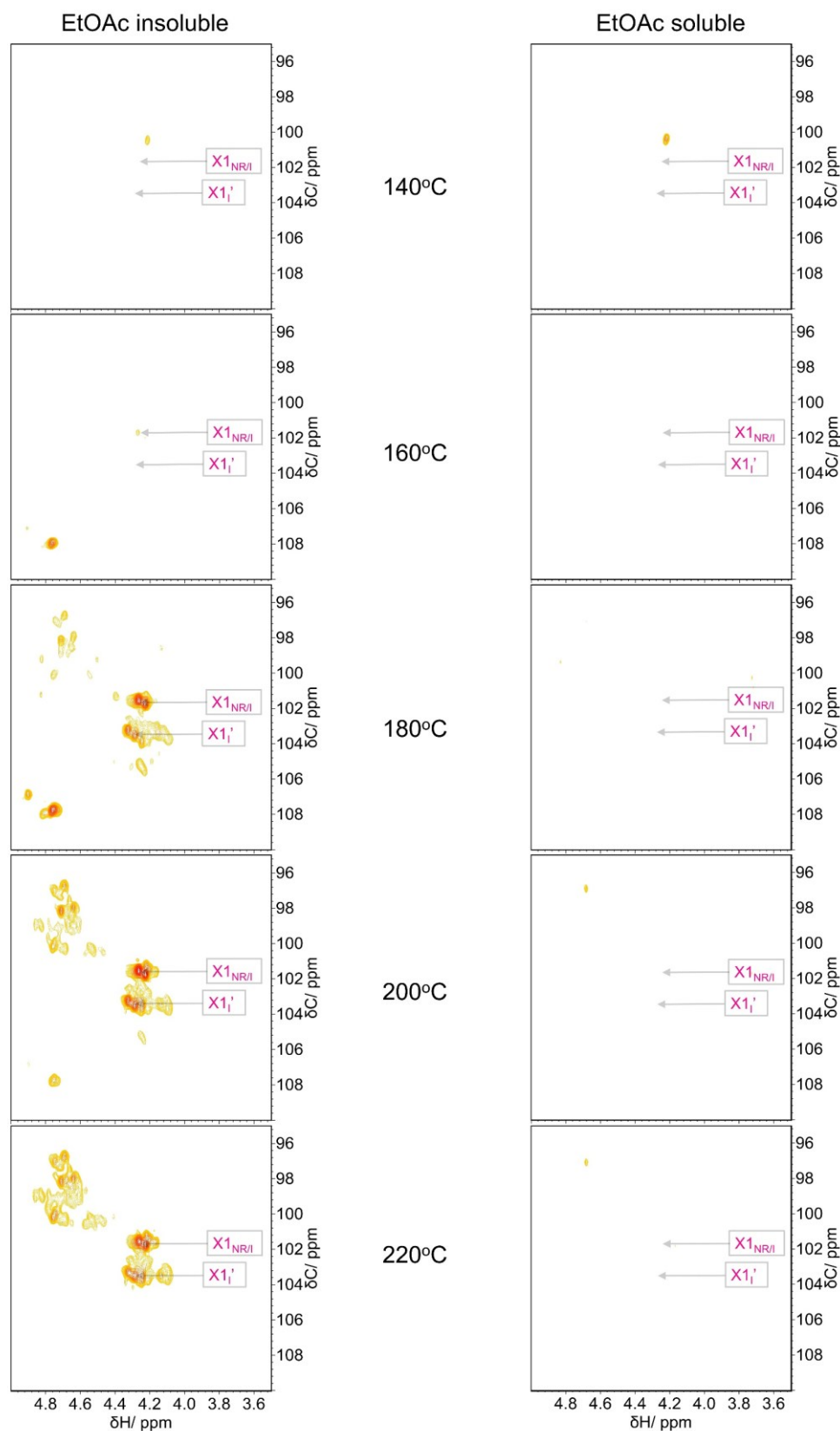


Figure A-20. HSQC NMR spectrum of EtOAc-insoluble and EtOAc-soluble fractions in RCF lignin oils obtained after each step of semi-batch T-stepping process in the anomeric carbon centre region (solvent: DMSO- d_6).

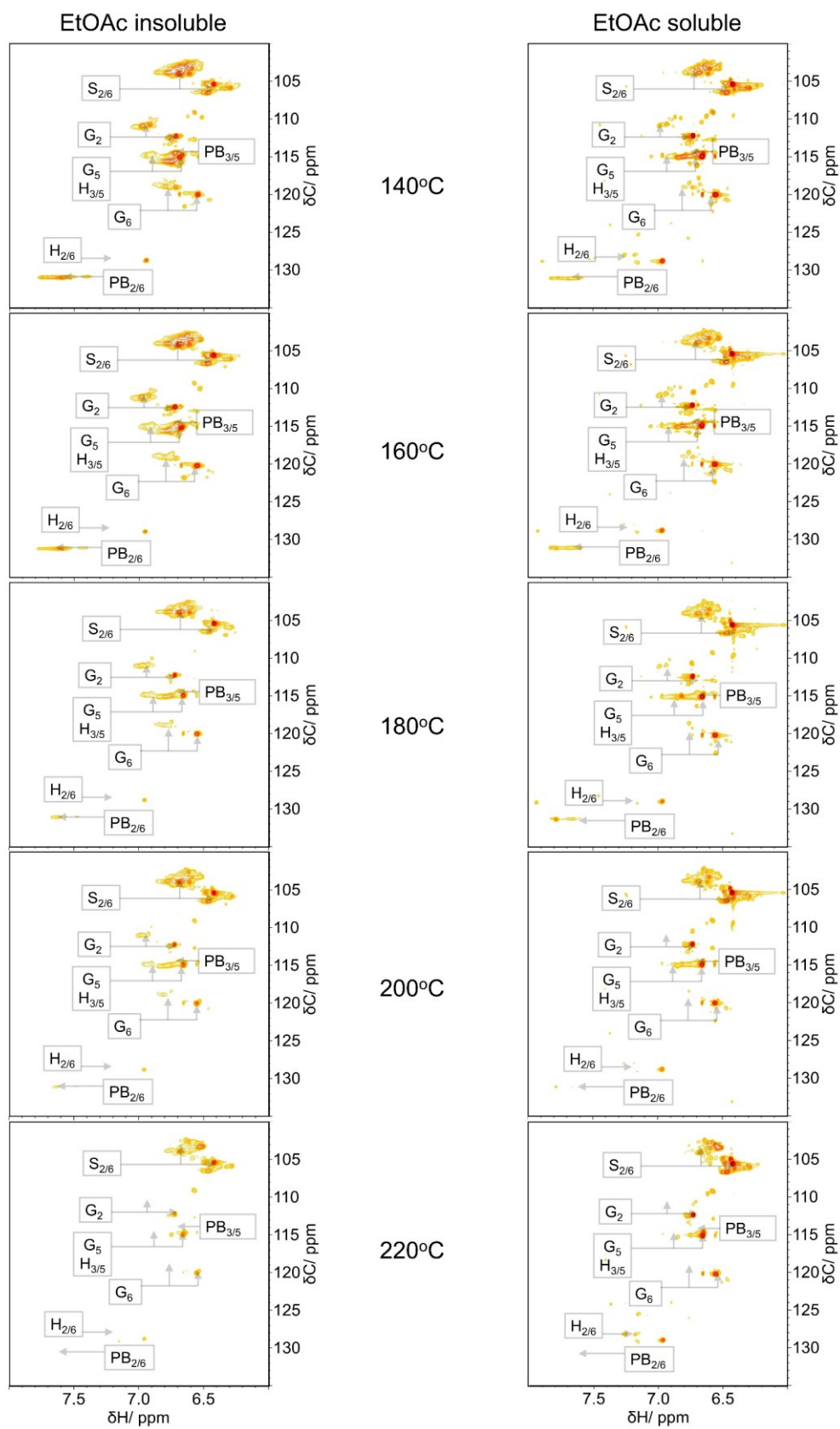


Figure A-21. HSQC NMR spectrum of EtOAc-insoluble and EtOAc-soluble fractions in RCF lignin oils obtained after each step of semi-batch T-stepping process in the aromatic region (solvent: DMSO- d_6).

Table A-12. *Pulpp composition along with the glucans, xylans, and lignin retention after each step of the semi-batch T-stepping RCF process.*

Step	% glucans	% Glucans retention	% xylans	% Xylans retention	% Klason Lignin	%ASL	%Total lignin	% lignin retention	% delignification
Poplar	45.6±0.9	NA	14.6±0.4	NA	26.3±0.4	1.84±0.06	28.2±0.5	NA	NA
140°C	48.4±0.5	102±2	15.5±0.3	101±3	23.7±0.2	1.68±0.07	25.4±0.2	86±2	10±2
160°C	52±1	102±3	16.6±0.3	102±3	22.3±0.4	1.72±0.04	24.0±0.4	77±2	15±2
180°C	58±1	94±2	16.7±0.5	84±3	17.9±0.1	1.30±0.01	19.2±0.1	50±1	32±2
200°C	68.9±0.3	89±1	13.9±0.1	56±2	10.5±0.3	0.83±0.02	11.3±0.3	24±2	60±3
220°C	77±1	82±1	9.9±0.4	33±3	6.7±0.2	0.58±0.01	7.3±0.2	13±1	74±3
1x5h @220°C	87±2	87±2	5.3±2	41±2	3.7±0.2	0.60±0.02	4.3±0.2	7±2	85±4

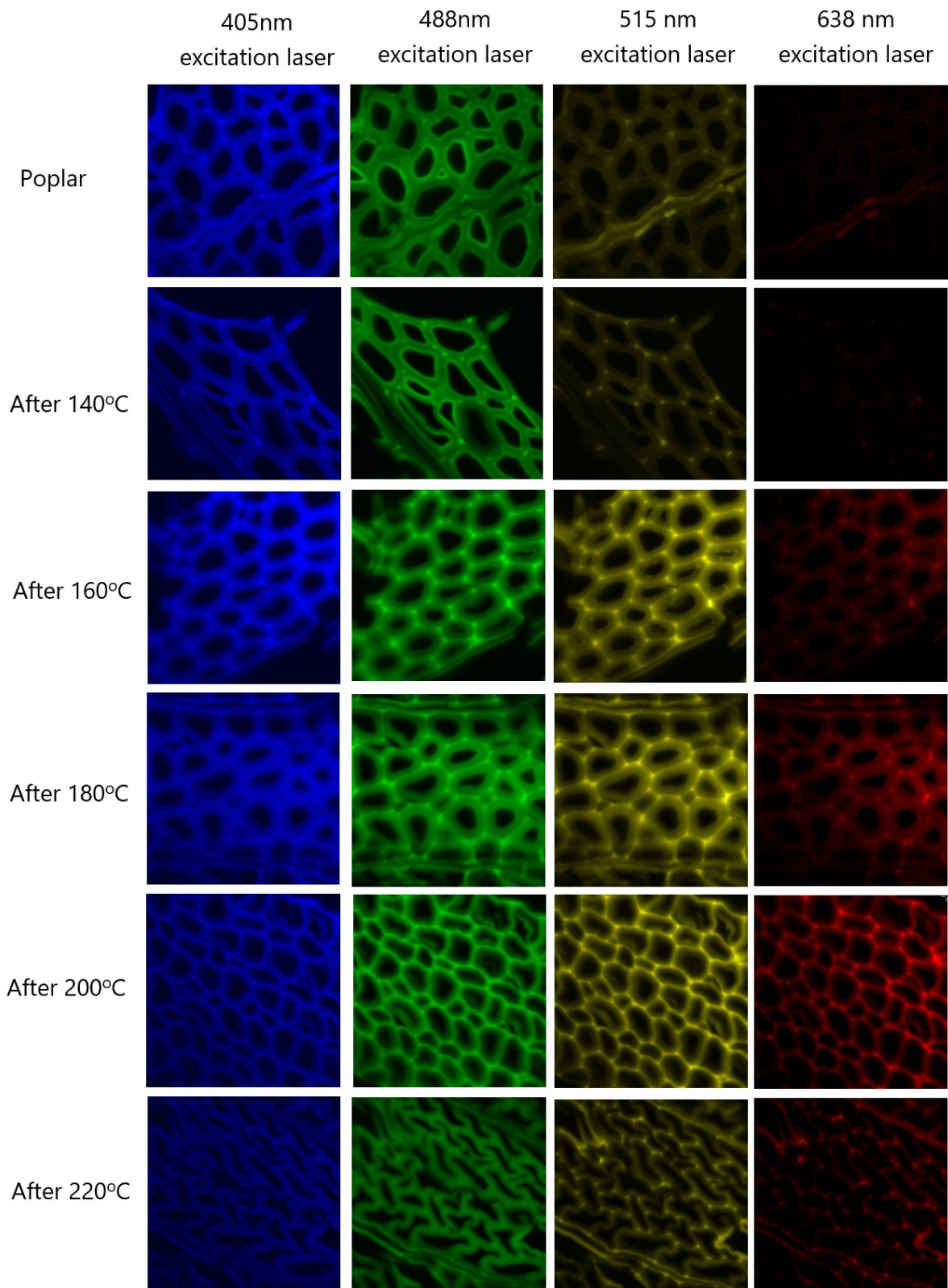


Figure A-22. Live cell widefield epifluorescence images obtained by corresponding excitation lasers.

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Publication: ACS Sustainable Chemistry & Engineering

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