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A fundamental study on the processes underpinning the lignin-first biorefining of lignocellulose

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Declaration of Originality

This dissertation is a description of the work carried out by Claudio Chesi in the Department of Chemical Engineering, Imperial College London, under the supervision of Dr. Roberto Rinaldi. Except where acknowledged, the content is the original work of the author.

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Abstract

Lignocellulosic biomass represents a valuable candidate for the replacement of fossil resources in the production of value-added chemicals, materials and biofuels, thanks to a diverse composition rich in carbohydrates (i.e. cellulose and hemicellulose) and aromatics (i.e. lignin). However, the success of lignocellulose as the feedstock of tomorrow relies on the valorisation of its full content, which requires appropriate methodologies for fractionation into its components and, particularly, the preservation of lignin reactivity. The lignin-first biorefining based on Early-stage Catalytic Conversion of Lignin (ECCL) is among the most promising approaches for catalytic fractionation of biomass. It deconstructs biomass in organic solvents in the presence of a hydrogenation catalyst, enabling the recovery of a high-quality carbohydrate fraction together with a highly functionalised lignin product. This dissertation investigates the mechanistic aspects underpinning the Catalytic Upstream Biorefining process (CUB) based on the ECCL concept, employing Raney Ni as the hydrogenation catalyst and 2-PrOH 7:3 v/v as a lignin-extracting liquor and H-donor. Finally, the biomass structure played a crucial role in the transport of the released lignin fragments from the lignocellulosic matrix to the reaction medium, where catalytic reactions can occur, as higher deconstruction achieved in CUB experiments performed in more severe conditions enhanced the degrees of lignin depolymerisation. In the context of biomass deconstruction, sugars derived from hemicellulose degradation in CUB were found to have a relevant impact on Raney Ni's catalytic selectivity, which could be shifted from phenols to cyclohexanols by enhancing the preservation of hemicellulose. An investigation on the time evolution of the physicochemical processes occurring in CUB highlighted the relevance of the first

50 min of the process, during which the extent of delignification is the highest. Importantly, the activity of Raney Ni in limiting repolymerisation reactions on the released lignin fragments is maintained throughout the time range 0-300min. Through a Design of Experiment study, the interplay of process parameters (i.e. wood loading, catalyst loading, solvent volume) on the performances of CUB (i.e. pulp delignification, hemicellulose retention in pulp, ratio low-MW/high-MW species in lignin, total content of monophenols in lignin, content of hydroxypropyl phenols in lignin) was assessed in detail, revealing that the amount of catalyst employed and the concentration of lignin species in the reaction medium relevantly affect biomass deconstruction and lignin depolymerisation. Based on the acquired knowledge, optimum conditions for maximising the performances of CUB were designed and tested, showing promising results for the application of the process to a large scale.

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List of abbreviations

2-PrOH	2-propanol
ACN	Acetonitrile
C	Catalyst loading
CUB	Catalytic Upstream Biorefining
DAD	Diode Array Detector
daf	Dry and ash-free
DMF	*N,N*-dimethylformamide
dn/dc	Refractive index increment
DoE	Design of Experiment
ECCL	Early-stage Catalytic Conversion of Lignin
ELSD	Evaporative Light Scattering Detector
EtOH	Ethanol
FID	Flame Ionisation Detector
G-unit	Guaiacyl-unit
GC	Gas Chromatography
GPC	Gel-permeation Chromatography
H-unit	Hydroxyphenyl-unit
HDO	Hydrodeoxygenation
ISTD	Internal Standard
IV	Intrinsic Viscosity
LCC	Lignin-Carbohydrate-Complex
MeOH	Methanol
MS	Mass Spectrometry

MW	Molecular Weight
M_n	Number average molecular weight
M_w	Weight average molecular weight
M_z	Z-average molecular weight
RID	Refractive index detector
S	Solvent volume
S-unit	Syringyl-unit
SCB	Sugar Cane Bagasse
THF	Tetrahydrofuran
VSD	Viscometry detector
W	Wood loading

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1. Introduction

The utilisation of fossil resources for the production of materials commonly used every day (e.g. fuels, plastics, chemicals) has produced a negative impact on the environment, which created a global climate change crisis.[1] Over the last decades, worldwide effort has been devoted into bringing about a global change to reduce the dependence of the society on the fossil resources. In this perspective, several renewable resources proved themselves as viable alternatives to fossil resources for the generation of energy and the production of chemicals and materials.[2] Among the most attractive renewable feedstocks is lignocellulosic biomass, constituted by three biopolymers: cellulose, hemicellulose and lignin. Current large-scale processes for the production of paper, sugars and bioethanol are centred on the valorisation of cellulose and to a lesser extent, hemicellulose.[3] In turn, lignin is normally isolated as a by-product with limited applications for the industry, due to its high recalcitrance.[3b, 4]

Lignin possesses a unique structure that makes it the most abundant resource of aromatic compounds available in nature on a large scale. For this reason, in the recent years lignin has been under the spotlight, as increasing research interest was focused on its use as a source for the sustainable production of fuels, materials and chemicals.[5] The fractionation of lignocellulosic biomass into its three main components largely benefitted from the development of novel approaches aimed at the recovery and utilisation of the full content of holocellulose and lignin composing the feedstock.[6] Under this umbrella, the lignin-first biorefining based on Early-stage Catalytic Conversion of Lignin (ECCL) constitutes an innovative approach for the effective extraction and direct conversion of lignin.

In 2014, Ferrini and Rinaldi reported a lignin-first biorefining in which catalytic hydrogen-transfer reactions allow the recovery of lignin as a mixture of phenolic compounds whose peculiar functionalisation opens up new horizons for the production of value-added products.[7] Most importantly, this approach is proposed to limit the extent of condensation reactions, which typically generate recalcitrant lignin products in conventional fractionation processes. Based on the ECCL concept, the Catalytic Upstream Biorefining process (CUB) has emerged as a promising candidate for lignocellulose biorefinery, as it employs a catalytic system that reduces the costs and safety restraints normally associated with other ECCL methodologies. In this dissertation, the intricate network of physicochemical processes underpinning the CUB process was examined, aiming to gain the in-depth insight required for the design of experimental conditions allowing the valorisation of its full content.

Chapter 2 presents a brief description of the structure of lignin, an overview of the conventional and novel fractionation processes for extracting lignin from biomass, and their effects on the properties of the lignin products generated. The ECCL concept is analysed in detail, and the advantages of the CUB process are discussed in comparison to other lignin-first approaches.

Chapter 3 addresses the effect of neutral to weakly alkaline conditions on the catalytic fractionation. As these conditions prevent the degradation of hemicellulose typically occurring upon fractionation, the influence of hemicellulose on the catalytic activity and the chemical properties of the lignin products is discussed.

Chapter 4 examines the CUB process in weakly acidic conditions, aiming to study the relation between the acetylation levels of different lignocellulosic feedstocks and their performances in CUB through the use of acidic conditions. The role of the

lignocellulosic structure was also assessed through experiments performed with more severe conditions, aimed to enhance the deconstruction of lignocellulose.

Chapter 5 presents a study on the time evolution of the processes underpinning the extraction of lignin from the lignocellulosic matrix and its depolymerisation to aromatic monomers.

In Chapter 6, the interplay of wood, catalyst and solvent in dictating the performances of the CUB process are assessed through a Design of Experiment approach. The obtained results led to the design of two sets of experimental conditions, aimed to (1) maximise biomass deconstruction and lignin conversion, and (2) test the CUB process on a large scale. The conditions were tested in CUB experiments and discussed.

Finally, Chapter 7 provides a summary of the main findings presented in this dissertation, and presents possibilities for future experiments that might increase the understanding of ECCL, and help to engineer the process in the perspective of the full conversion of the whole lignocellulose biomass.

2. State of the art

In facing the depletion of fossil resources and the environmental issue arisen from their overexploitation, lignocellulosic biomass constitutes a viable and sustainable alternative source to supply the global demand of fuels, chemicals and materials.[5c, 8] Indeed, lignocellulose grows in a CO_2-neutral process widely over the planet, and importantly does not compete with crops aimed at food supply.[9] Hence, the utilisation of lignocellulose circumvents the ethical difficulties related to the "Food vs. Fuel" debate normally associated with employing farmland for biofuels production.[10] Thanks to its complex structure made of three highly functional biopolymers (i.e. cellulose, hemicellulose, lignin),[3b] lignocellulose offers a broad array of possibilities for the production of bio-derived materials and energy.[5b]

The intricate architecture of lignocellulose also entails a resistance to fractionation into its main components,[11] which constitutes a challenging obstacle for the biorefinery strategies aiming at the deconstruction of biomass and subsequent synthesis of value-added products. Currently, the main biorefinery approaches available, such as paper mills or the fermentative production of bio-ethanol, are only able to valorise the carbohydrate fraction of lignocellulose (i.e. cellulose and hemicellulose content), which is effectively isolated from biomass at good yields and high purity.[3a, 12] Conversely, the lignin fraction is isolated as a complex material with high recalcitrance, which prevents its effective conversion into useful products. Consequently, lignin is usually regarded in these processes as a waste product with limited applications,[4a] and mainly employed as low-grade fuel for the recovery of the inorganics employed in the paper mills,[3b, 4b] or for minor commercial applications such

as the production of vanillin,[13] dispersing and binding agents,[14] resins,[15] polyurethane films,[16] corrosion inhibitors,[17] additives in animal feeds.[3b]

This sub-utilisation of lignin prevents the capitalisation of the full content of biomass. However, combining lignin valorisation with the effective utilisation of the carbohydrate fraction derived from lignocellulose deconstruction would further increase the economic viability and sustainability of biorefineries, enhancing the overall appeal of these strategies.[3b, 5c, 18] Indeed, lignin is a rich natural resource of aromatic compounds, with a diverse composition of highly-functionalised monomeric units that hold great potential for the synthesis of fine chemicals.[5b, 9, 18a] Therefore, expanding the focus of scientific research on lignin valorisation is crucial for the role of biomass in replacing fossil resources globally.

2.1 Lignin structure in biomass

Lignin is one of the three biopolymers composing lignocellulose, together with cellulose and hemicellulose.[18a, 19] In the plant cell wall (Figure 2-1), lignin covers the cellulosic and hemicellulosic fibres, acting as a binder for the whole lignocellulosic structure. As commented by Rinaldi,[18b] this particular structure of the lignocellulosic matrix resembles a “core-shell” system, where a core made of cellulosic fibres is covered with a shell of lignin and hemicellulose. In this arrangement, cellulose acts as the backbone of the plant cell wall thanks to its crystallinity and resistance against depolymerisation. Hemicellulose physically and chemically connects the cellulosic fibres to lignin, conferring strength and rigidity to the plant cell wall, which is then protected from the attacks of microorganisms. Additionally, due to its lipophilic nature,

lignin prevents the permeation of water through the plant tissue, thus facilitating the transport of aqueous solutions of nutrients through the xylem tissue.[4b, 19-20]

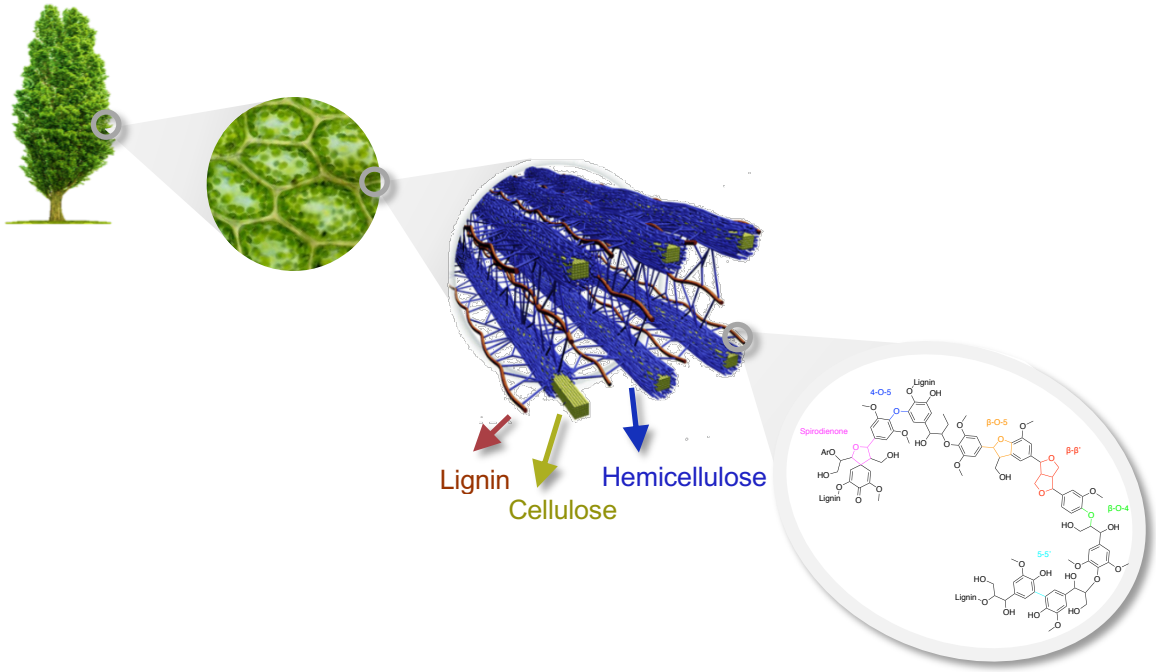


Figure 2-1 – Schematic representation of the structure of lignocellulosic biomass. Adapted from Ref.[18a, 21]

The lignin content can greatly vary depending on the type and part of the biomass, and normally accounts for 10-40 wt% of the total weight of biomass, with hard tissues (e.g. stems, shells) containing more lignin than soft tissues (e.g. leaves).[12b, 22] The native structure of lignin found in biomass is mainly composed of three phenolic monomers (i.e. monolignols): *p*-coumaryl, *p*-coniferyl and *p*-sinapyl alcohol, also defined respectively as *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, according to the functionalisation of their phenolic ring (shown in Figure 2-2).[4b, 18a, 22a] Alongside, other monolignols can be found in the structure of lignin, such as: acylated monolignols, monolignol acetates, *p*-hydroxybenzoates and *p*-coumarates.[5b, 23]

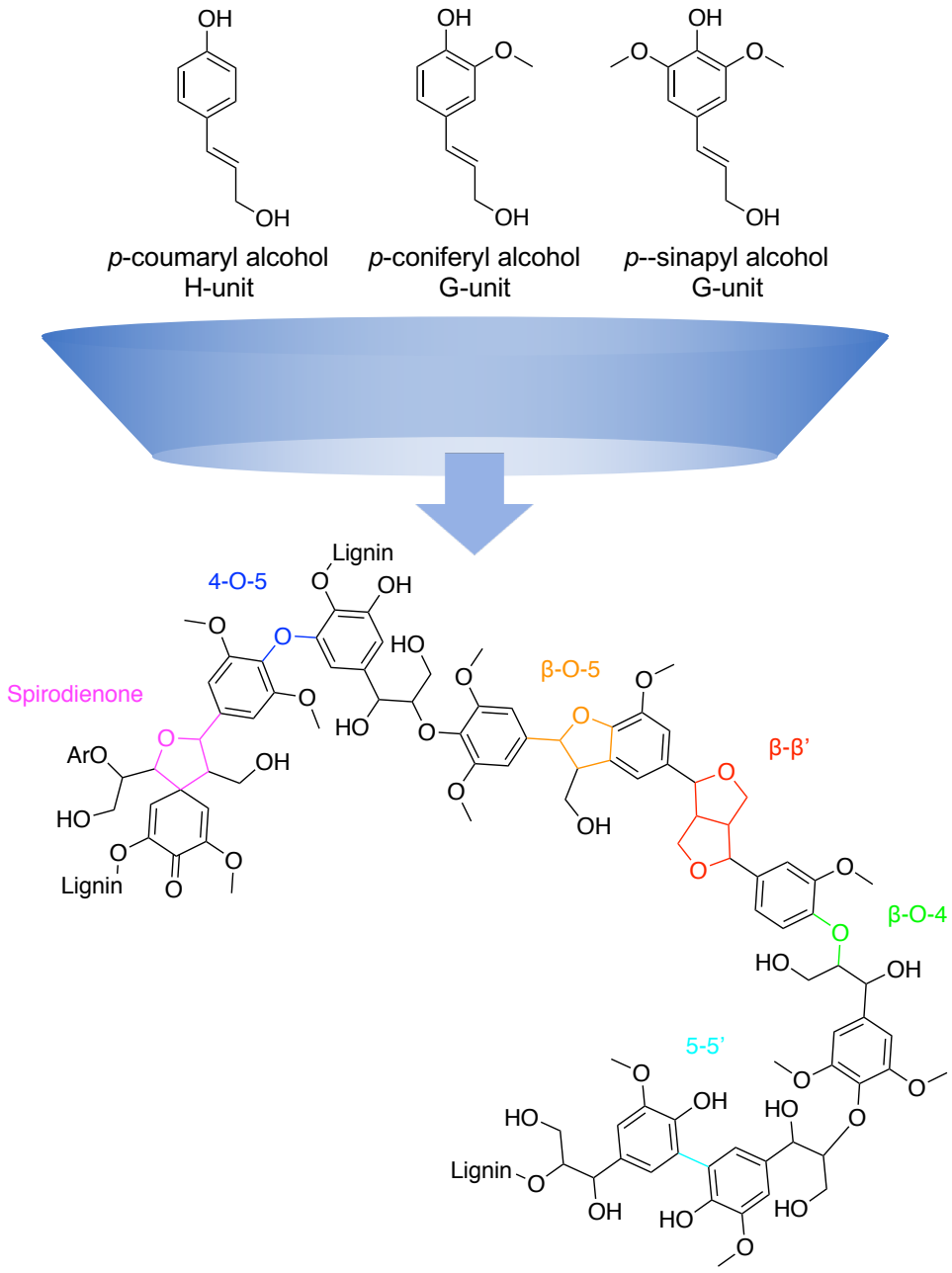
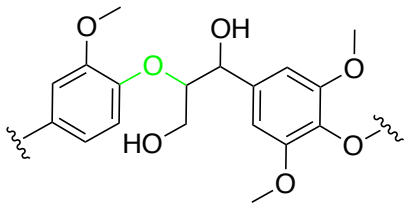
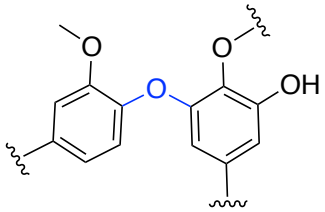
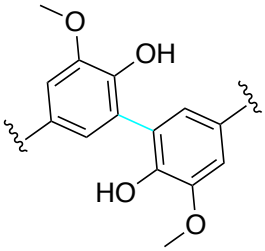
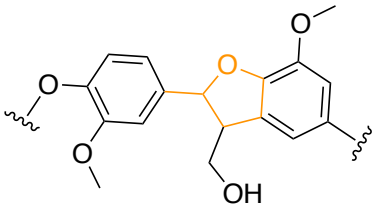
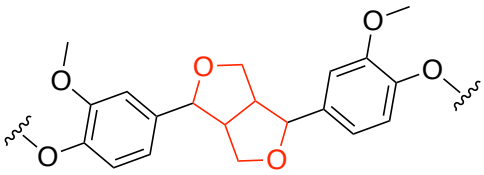


Figure 2-2 – Phenolic monomers in lignin, and schematic representation of a hardwood lignin structure, with most common linkages.[18a]

The content of these monomers in lignin depends on the type of wood, as the composition of softwood lignin is usually dominated by coniferyl alcohol units, and is therefore referred to as G-lignin, while hardwood lignin, also referred to as S/G-lignin, usually presents comparable amounts of coniferyl and sinapyl alcohols. Finally, grass

lignin contains roughly equal amounts of all three phenolic units, and is therefore also named H/G/S-lignin.[4b, 18a, 22a] Following the biosynthesis of monolignols, the polymerisation of lignin (i.e. lignification) is initiated by laccase and peroxidase enzymes, which convert the monolignols into the corresponding phenolic radicals.[5b] As such species present various sites available for polymerisation, the following step of uncontrolled radical-radical coupling generates different carbon-carbon and carbon-oxygen bonds connected in a cross-linked network of phenolic species.[5b, 22b] The resulting linkages are reported in Figure 2-2. The relative amount of such linkages in lignin depends on the type of wood. Normally, *β-O-4* is the most common linkage, as it usually accounts for ca. 45-60% of the total linkages in native lignin from different types of wood.[18a, 24] Other lignin linkages include 4-O-5 (C-O bond), 5-5' and *β*-1 (C-C bonds), spirodienone, *β-β*" and *β*-5 (resinol and phenylcoumaran structures, formed through intramolecular cyclisation).[25] The high abundance (45-60%)[18a, 24] and low bond dissociation energy (215 kJ/mol)[26] of the *β*-O-4 linkage makes it the ideal target for lignin depolymerisation in the perspective of lignin valorisation.[27]

Table 2-1 - Most common lignin linkages and their estimated bond dissociation energy.

Linkage	Structure	Bond dissociation energy (kJ/mol)
β-O-4		(C*β*-O) 225-300
4-O-5		325-345
5-5'		470-495
β-O-5		(C*α*-O) 200-280
β-*β*’		(C*α*-O, C*α*-C*β*) 280-284 (C*β*-C*β*’) 340

2.2 Technologies for lignin fractionation

The choice of the fractionation step, namely “upstream” biorefining process, plays a crucial role in biorefinery, as it defines the type of chemical modifications dictating the structure and characteristics of the isolated lignin product, which define the range of “downstream” treatments available for its conversion into value-added products. In this manner, the choice of an appropriate fractionation approach is crucial in the perspective of lignin valorisation. Along with the isolation of high-quality products, the viability of the fractionation method also requires an applicability to a wide array of substrates, while also being economically, energetically and environmentally sustainable. Conventional fractionation treatments of biomass are discussed in Section 2.2.1. This class of methods include a wide variety of fractionation treatments based on physical, physicochemical, chemical or biological processes.[28] In sequence, the novel fractionation processes developed with the aim of a full utilisation of biomass are discussed in Section 2.2.2. Among these, a new emerging trend for the catalytic upstream biorefining of biomass based on the Early-stage Catalytic Conversion of Lignin concept, is discussed in detail in Section 2.2.2.1.

2.2.1 Conventional fractionation processes

2.2.1.1 Pulping processes

Pulping processes aim at the generation of strong and highly-pure cellulosic fibres for the production of paper.[3] Among these processes, Kraft pulping is the dominant fractionation approach for the production of paper. In this process, biomass is treated at 150-180°C in an aqueous solution of sulphide with strongly alkaline pH.[3b] During

the process, the phenolic groups in β-O-4 lignin linkages are converted into quinone methide groups, which react with the HS-groups available in the liquor to cleave the bond and yield depolymerised lignin fragments.[22a, 29] Hence, lignin fragments with molecular weight (MW) of 1-3 kDa are isolated from the cellulosic fibres as they are released in the extraction medium (i.e. liquor).[3a, 30] As the lignin fragments are insoluble in acidic conditions, the lignin fraction is precipitated and recovered from the liquor by lowering its pH with the addition of carbon dioxide or mineral acids.

The success of the Kraft process is mainly related to the applicability to a wide array of wooden biomass, together with the high extraction efficiency of lignin, which leads to the isolation of highly pure cellulosic fibres required for paper production. However, the lignin product thus isolated has limited applicability aside from burning for energy recovery. Indeed, the process incorporates sulphur into the structure of lignin (1-2 wt% of the final lignin product), hence requiring sulphur-tolerant catalysts for its downstream treatment. In addition, further depolymerisation of lignin requires harsh conditions for the cleavage of C-C bonds with high dissociation energy[4b] formed upon repolymerisation fractions occurring during fractionation.

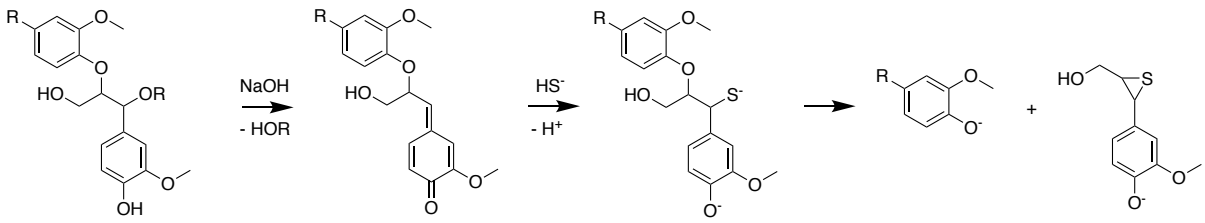


Figure 2-3 – Mechanism of Kraft pulping.[22a]

Sulfite pulping, widely employed for pulp and paper production, involves cooking biomass at 125-150 °C in an aqueous solution of HSO_3^- or SO_3^{2-} (pH 1-5), or

in alkaline solutions of ammonia sulfite.[3b, 22a, 31] Under these conditions, the *α*-carbon on the side chain of the phenolic subunits composing lignin becomes sulfonated, which makes the lignin soluble in water throughout almost the entire pH range.[3b] In this manner, the degraded lignin is separated from the solid carbohydrate pulp through filtration. The *β*-O-4 linkage is cleaved in alkaline conditions to yield depolymerised lignin fragments, while it is stable in acid conditions.[22a] During the process, aldol condensation also takes place on the lignin fragments, resulting in the formation of stable C-C bond which increase the recalcitrance of the released lignin products. Furthermore, depolymerisation occurs to a lesser extent compared with Kraft. As a result, the final lignin product from sulphite process usually presents a higher MW than Kraft lignin.[22a] Similarly to the Kraft pulping, sulphite process also entails the incorporation of a high amount of sulphur in lignin (8 wt% of the final lignin)[31] which, together with a high fraction of impurities, makes the catalytic downstream processing of this lignin difficult.

The economic and energetic feasibility of both Kraft and sulfite pulping requires the use of the isolated lignin as a fuel for the process itself. Therefore, using the lignin products for further downstream treatments aimed at its valorisation would imply the use of alternative energy resources to feed these processes.[3b, 14]

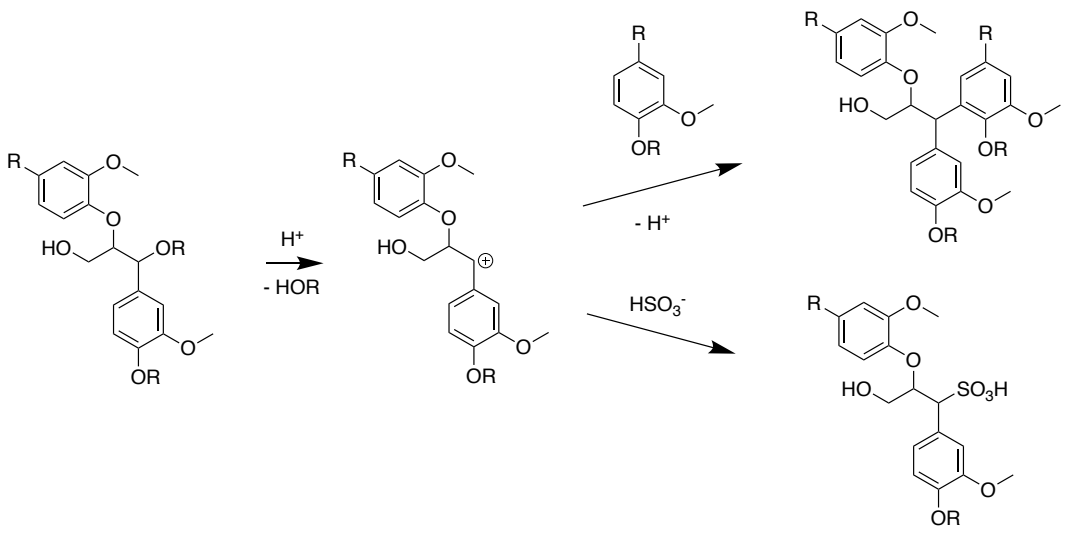


Figure 2-4 – Mechanism of sulfite pulping.[22a]

2.2.1.2 *Physical processes*

Physical processes for fractionation imply the application of mechanical forces or radiation on the biomass feedstock. Mechanical processes (i.e. mills, grinders, extruders) target the reduction in particle size and crystallinity of cellulose in biomass, hence increasing the surface area of the lignocellulosic fibres. Although the application of mechanical forces makes these fibres more prone to enzymatic hydrolysis for the production of sugars,[32] they do not remove lignin from the lignocellulosic matrix, which can negatively affect the activity of the enzymes by direct binding or by limiting their accessibility to the pulp.[33] Furthermore, the process is energy-intensive,[28b] and would require the combination with other pretreatments to make the fractionation of biomass more effective and the overall process economically viable.[12b]

Irradiating the biomass feedstock with gamma rays, electron beams or microwaves promotes the degradation of the cellulosic fibres into weaker fibres and oligosaccharides, which are more prone to enzymatic hydrolysis than the initial feedstock.[28b, 32] However, irradiating processes exhibit several drawbacks. As

observed for the mechanical processes, irradiation is not able to remove lignin from the lignocellulosic matrix, hence promoting a decrease in the enzymatic activity for the downstream production of monosaccharides from cellulose. Furthermore, positive effects of the irradiating pretreatment in improving the enzymatic hydrolysis of cellulose were only observed for substrates with high amounts of lignin, hence limiting the applicability of this technology.[28b] Finally, the high costs and difficulties of adaptation to industrial applications make these processes unsuitable for their use on a large scale.

2.2.1.3 Physicochemical methods

Pretreatments combining both chemical and physical processes include thermochemical technologies (e.g. pyrolysis), and explosions methods (i.e. steam explosion, ammonia fibre explosion, CO_2 explosion).

Pyrolysis is used on a large scale for the thermochemical conversion of biomass into fuels,[34] and consists in treating lignocellulose under an inert atmosphere at temperatures above 300°C, with short residence times. Lignin and carbohydrates are both decomposed to yield a liquid fraction (i.e. pyrolysis oil or bio-oil, mainly composed of lignin-derived products, and cellulose- and hemicellulose-derived products as minor components) accounting for 75% of the whole biomass content, together with a solid fraction (i.e. char, a carbonaceous material) and gaseous products (typically CO, CO_2, CH_4, and H_2).[35] Cellulose- and hemicellulose-derived sugars are also found in the bio-oil, hence leading to a loss of sugars.[3b, 36] Although pyrolytic bio-oils have lower oxygen content than the native feedstock, their use as fuel additives requires downstream treatments for further removal of oxygen.

Furthermore, bio-oils are not stable, as they can undergo repolymerisation upon storage.[35]

Explosion methods involve the pressurisation of lignocellulose with steam, liquid ammonia or supercritical carbon dioxide at high temperatures for short periods of time (few seconds to several minutes), followed by rapid depressurisation which produces explosive decompression of the substrate.[28b, 32] Although these methods produce depolymerisation of lignin, they often lead to cellulose depolymerisation, which is undesirable in the perspective of paper production as it entails sugar losses. The production of toxic derivatives (e.g. phenols, furans), detrimental for the activity of enzymes for downstream hydrolysis,[32, 37] and the high costs associated with the explosion processes limit their applicability.

2.2.1.4 Chemical methods

Chemical treatments involve acidic or alkaline solutions (similarly to Kraft and sulphite pulping), organic solvent or oxidising agents for the fractionation of biomass. The most used alkaline processes are performed with sodium hydroxide, calcium hydroxide or ammonia at low temperatures and long contact times.[32] These processes promote lignin removal from the lignocellulose matrix. However, they are usually ineffective on softwoods.[32] The use of hydroxides entails partial removal of hemicellulose, which is desirable in the perspective of paper production. Nonetheless, it also entails the incorporation of inorganic salts in the biomass.[32] In turn, ammonia treatments often lead to consumption of this additive, which increases the costs associated with the process.[32]

Ozonolysis can be employed for the oxidative treatment of biomass, which effectively depolymerises lignin to yield oxidised low-MW products at room temperature.[28b, 32] However, ozonolysis is expensive because of the large amount of ozone required for the process.[12c]

Acid processes involve the use of diluted solutions of sulphuric acid (the most common), hydrochloric acid, phosphoric acid, nitric acid or organic acids for the treatment of biomass at temperatures above 100°C.[28b, 32, 38] Under these conditions, both hemicellulose and lignin are depolymerised and dissolved in solution,[38] together with furans and levulinic acid as by-products of sugar decomposition, which can inhibit the activity of enzymes for downstream processing.[37] Furthermore, the use of acidic conditions requires facilities built with materials which can resist to corrosion, in addition to neutralisation of the solution obtained from the process, leading to an increase in the costs of the process.[28b, 39]

Among treatments with organic solvents, Organosolv involves cooking wood at 100-250 °C in organic solvents, sometimes in presence of water and acid or base additives, to yield a cellulosic pulp and a liquor containing hemicellulose and lignin.[32, 40] The latter can be isolated by increasing the concentration of water in the liquor, hence leading to the isolation of three separated fractions which can be upgraded individually.[18a]

Upon cooking biomass in Organosolv conditions, the hemicellulose fraction undergoes deacetylation releasing acetic acid into the liquor. Another chemical process occurring in Organosolv pulping is the decomposition of part of the hemicellulose sugars released in the liquor generating furfurals, which can be decomposed to formic acid and levulinic acid.[41] These carboxylic acids can act as catalysts for hemicellulose hydrolysis[41g, 42c, 43] and lignin depolymerisation,[41f, 42]

whose initial steps are described in Figure 2-5. In these conditions, the benzylic hydroxyl group involved in a *β*-O-4 linkage on the polymeric chain of lignin is protonated, initiating a step chain leading to hydrolysis of the linkage (**1** in Figure 2-5) to yield lignin-bound Hibbert ketones, i.e. L(HK). The acid hydrolysis of the resulting *β*-O-4 linkage (**2**), now a terminal unit on the lignin chain, yields free monophenols (Hibbert ketones and the corresponding equilibration products, **3-8**). An alternative pathway is represented by the protonation of the hydroxyl group on the γ-C of **2**, which produces enol-ethers (**2'**) by eliminating formaldehyde. Such species are therefore prone to hydrolysis to yield monophenols (C_2 side chain, **10-12**), which can eliminate a second molecule of formaldehyde yielding C_1-side-chain monophenols (**13**).

Interestingly, Organosolv conditions may allow for the preservation of a large fraction of *β*-O-4 linkages on lignin, thus rendering a lignin product resembling its native form.[44] Differently from Kraft and sulphite lignin products, Organosolv lignin is sulphur-free, hence preventing the formation of sulphurous by-products during combustion for energy recovery or downstream processing.[18a, 45] However, Organosolv conditions also promote condensation reactions on the reactive sites of Hibbert ketones, yielding recalcitrant products rich in stable C-C bonds with an overall MW distribution lower than Kraft and sulphite lignin products.[46]

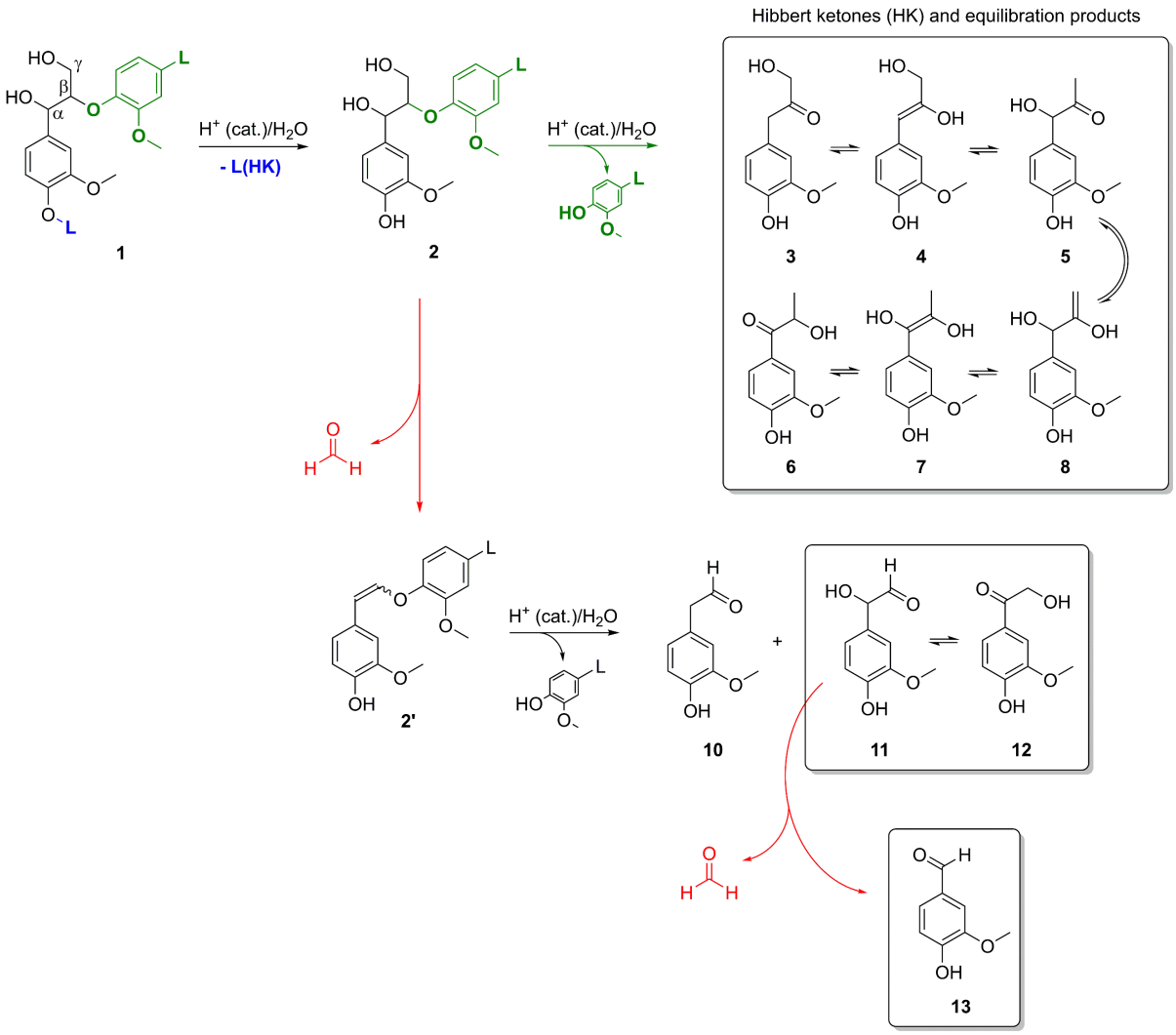


Figure 2-5 – Reaction scheme for the depolymerization of lignin during Organosolv process. Reproduced from Ref. [29].

A wide array of organic solvents has been employed for Organosolv treatments, such as alcohols, esters, ketones, glycols, organic acids, phenols and ethers,[28b, 47] often combined with alkaline[41a, 48] or acidic conditions.[49] The Organosolv process was demonstrated on a commercial scale for the fractionation of hardwood biomass in ethanol (Alcell process).[50] However, the low economic feasibility associated with the high price of solvent and the need for its removal and recovery has so far prevented the commercial success of the Organosolv process.[3b]

2.2.1.5 *Biological methods*

In biological treatments, microorganisms such as fungi and enzymes are used for biomass deconstruction under low-severity conditions. Rot fungi promote the degradation of both the lignin and carbohydrate fraction through enzymes (e.g. laccase, lignin peroxidase and manganese peroxidase) produced by fungi.[51] In turn, enzymes designed for selective carbohydrate degradation (e.g. cellulases) can be used on biomass pretreated to increase the accessibility of cellulosic and hemicellulosic fibres.[52] When low-severity pretreatments are employed, lignin is left unaltered from the enzymatic process. In this manner, it is commonly used as a model for native lignin.[53] However, biological treatments usually require long residence times.[12c, 54]

2.2.2 Novel fractionation processes

The conventional methods for biomass fractionation discussed in the previous section employ severe conditions for the deconstruction of the lignocellulose matrix into cellulosic fibres easily accessible for enzymatic hydrolysis. These processes often require high consumption of energy and isolate lignin as a sub-utilised waste stream. With the aim of improving the valorisation of the full content of biomass, research attention has focused on the development of methods employing milder conditions for biomass fractionation.[18b] These novel approaches enable the isolation of high-purity cellulose fibres through controlled removal of lignin. Some of these approaches produce sugars produced in a one-pot process, hence making the enzymatic downstream treatment for carbohydrate hydrolysis redundant. Alongside, they

typically recover lignin as a stream amenable for further upgrade aimed at the production of value-added materials.[18b]

In 2014, Rinaldi proposed a categorisation of the novel fractionation approaches, according to the type of deconstruction they promote on the lignocellulose architecture resembling a core-shell system,[18b] as previously discussed in Section 2.1. In "deep depolymerisation" methods,[42a, 49b, 55] acid catalysis is employed to perform extensive deconstruction of the whole lignocellulosic structure in a non-selective manner. As a result, the oligosaccharides from holocellulose depolymerisation and the oligomers from lignin depolymerisation are dissolved in the extraction medium. The latter can then be recovered by precipitation through chemical reactions or addition of an antisolvent. "Core leaching" approaches[56] require an appropriate solvent to selectively remove the holocellulose "core" from the lignocellulose matrix. However, this treatment also involves the collapse of the remaining matrix, with the lignin "shell" undergoing partial degradation. In turn, the "shell peeling" methods[57] involve an approach opposite to the "core leaching". In fact, they target the lignin "shell" to be removed from the holocellulose "core" of the lignocellulosic matrix through solvolytic processes. As a result, lignin fragments are released in the reaction solution, together with sugars derived from partial degradation of the hemicellulose fraction, while the cellulosic fraction is mostly unaltered after the process and remains behind as a solid residue.[18b]

Among the "deep depolymerisation" approaches is the γ-valerolactone (GVL) - catalysed saccharification of biomass demonstrated by Dumesic et al.[42a, 49b, 55] This methodology involves a flow-through reactor including a packed bed of lignocellulosic biomass through which an acidic solution of GVL:H_2O (80:20 w/w, with 0.05-0.1 mol/L H_2SO_4) is flown. In this configuration, the two carbohydrate fractions in biomass (i.e.

hemicellulose and cellulose) can be selectively released by the application of a temperature gradient. Indeed, the hemicellulose fraction can be hydrolysed to monosaccharides at lower temperature (150-180°C), and removed from the system. Subsequently, cellulose can be released at higher temperatures (180-220°C). After removing GVL through phase separation with liquid CO_2, the sugars were recovered in an aqueous solution with a concentration of 127g/L, demonstrating that the process performances are close to those of enzymatic hydrolysis of polysaccharides, typically isolating aqueous solutions of sugars of 150-200g/L.[49b] The lignin fraction can be extracted as a soluble stream after treatment with the aqueous solution of GVL at 120°C. A two-step upgrading procedure was also demonstrated for this lignin product, involving hydrogenolysis catalysed by Ru/C in presence of H_2, followed by extraction in heptane. In this way, almost 50% of the original carbon content in lignin was converted to monophenols.[55]

Deep depolymerisation of lignocellulose can also be achieved through a mechanocatalytic approach.[6e, 58] Indeed, the application of mechanical forces on acid-impregnated substrate in a ball mill results in extensive saccharification of biomass, which leads to the isolation of sugars in high yields (88-92% glucose, 93-98% xylose). The lignin isolated through precipitation from the aqueous solution of sugars closely resembles the structure of lignins obtained by Organosolv fractionation (Section 2.2.1.4).[6e, 58a-d] Notably, solvent-free impregnation can be achieved by using p-toluenesulfonic acid (solid) or HCl (gaseous) for the acidification of biomass, hence providing a more energy efficient and environmentally friendly alternative to the wet impregnation performed with H_2SO_4.[58a, 58c] Importantly, further studies on this mechanocatalytic approach on a 1g- and 1kg-scale process demonstrated that increasing the scale results in a decrease of the overall energy consumption, hence

suggesting that the process might be energetically and economically applicable on a larger scale,[6e] contrarily to the conventional processes for biomass deconstruction employing mechanical forces (Section 2.2.1.2).

A “core-leaching” example for biomass fractionation is the dissolution of cellulose using dialkylimidazolium ionic liquids with different counterions at 175-195°C.[56] Lignin is thus separated from cellulose through regeneration with acetone/water. However, the residual cellulosic stream experiences sugar losses (66% yield relative to the original content of carbohydrate in biomass) and still contains high amounts of lignin (16wt%). Furthermore, the extraction efficiency of the process decreases upon recycling the ionic liquid, hence limiting its desirability in lignocellulose fractionation.

OrganoCat process[57a, 59] (a “shell peeling” process) removes lignin and hemicellulose from the lignocellulosic matrix in a biphasic system composed of water and 2-methyltetrahydrofuran (2-MeTHF). This process is performed at mild temperatures (80-140°C) with oxalic or formic acid as catalyst and isolates the hemicellulose-derived sugars into the aqueous fraction, while lignin fragments are recovered in the organic phase by an *in-situ* extraction. In turn, cellulose is recovered as a solid easily separated through filtration, and shows performances in enzymatic hydrolysis comparable to commercial cellulose. Hence, the three main components of biomass can be selectively recovered and further treated through downstream processes. Importantly, the acid catalyst can be recovered from the aqueous solution and recycled in the process.

Biomass deconstruction performed by cooking the substrate in aqueous mixtures of protic ionic liquids (e.g. 1-butyl-3-methylimidazolium hydrogen/methyl sulfate, mono/di/triethylammonium hydrogen sulfate) demonstrates another approach

for “shell peeling”.[60] Indeed, by treating the lignocellulose at 150-180°C in such ionic liquids with short residence time (15-30min), hemicellulose is effectively removed from the lignocellulosic matrix together with lignin, which is depolymerised through cleavage of its β-O-4 linkages,[61] and precipitated by the addition of water, along with insoluble humins derived from hemicellulose. As cellulose is insoluble in these ionic liquids, it can be easily recovered through filtration after the process. The pulp thus obtained is amenable to enzymatic saccharification with high yields (75% of theoretical maximum of sugars). These novel ionic liquids require an inexpensive synthesis, and can be easily recovered and recycled after the fractionation process,[60d] hence abating the main barriers typically associated to the use of ionic liquids in past applications.[62]

Within the most promising technologies for lignin valorisation in biorefinery, the “Lignin-first” strategy, based on the Early-stage Catalytic Conversion of Lignin (ECCL), promotes effective biomass deconstruction through “shell peeling”.[7a, 63] This approach constitutes the core of the studies presented in this dissertation, and is, therefore, introduced in detail in the following section.

2.2.2.1 Lignin-first biorefining

The “Lignin-first” approach for lignocellulose deconstruction is based on the Early-stage Catalytic Conversion of Lignin concept (ECCL). Although the catalytic conversion of lignin was already employed in the last century,[64] only in 2014 it was applied for the first time on the fractionation of lignocellulose.[7a]

Through hydrogen-transfer processes catalysed by heterogeneous catalysts, lignin fragments released upon fractionation through solvolysis are effectively depolymerised to stable phenolic compounds (Figure 2-7),[7a, 63a, 63b, 63d, 65] which are

recovered in an aromatic-rich depolymerised lignin stream together with a highly-delignified holocellulosic pulp. These catalytic reductive processes also passivate the reactive functionalities on the released lignin fragments (e.g. reduction of aldehydic groups into alcohols, hydrodeoxygenation of ketones to methylene groups, see Figure 2-6),[5b, 29] and inhibit the formation of reactive intermediates in lignin depolymerisation (e.g. benzyl carbocations)[41a] which are typically prone to repolymerisation reactions.

The lignin product thus isolated from lignin-first starkly contrasts with the technical lignins obtained from conventional fractionation processes (e.g. Kraft, Organosolv, Section 2.2.1). Indeed, in these processes lignin depolymerisation yields reactive phenolic compounds, which can undergo repolymerisation, to form new cross-linked polymeric species to be found in both the reaction medium and the lignocellulosic matrix.[22a, 41a] For this reason, the upgrading of technical lignins thus released requires harsh conditions (e.g. high temperatures, elevated H_2 pressures), due to the high recalcitrance of the newly formed bonds (Figure 2-7).[18a, 27, 66] In turn, the extensively depolymerised lignin products isolated from Lignin-first approach are more prone to catalytic upgrading for the production of drop-in fuels and chemicals,[7a, 67] as well as the holocellulosic pulp, which presents a lower content of residual lignin compared to that obtained from Organosolv pulps, hence limiting the enzymatic inhibition due to the presence of lignin.[7a, 68]

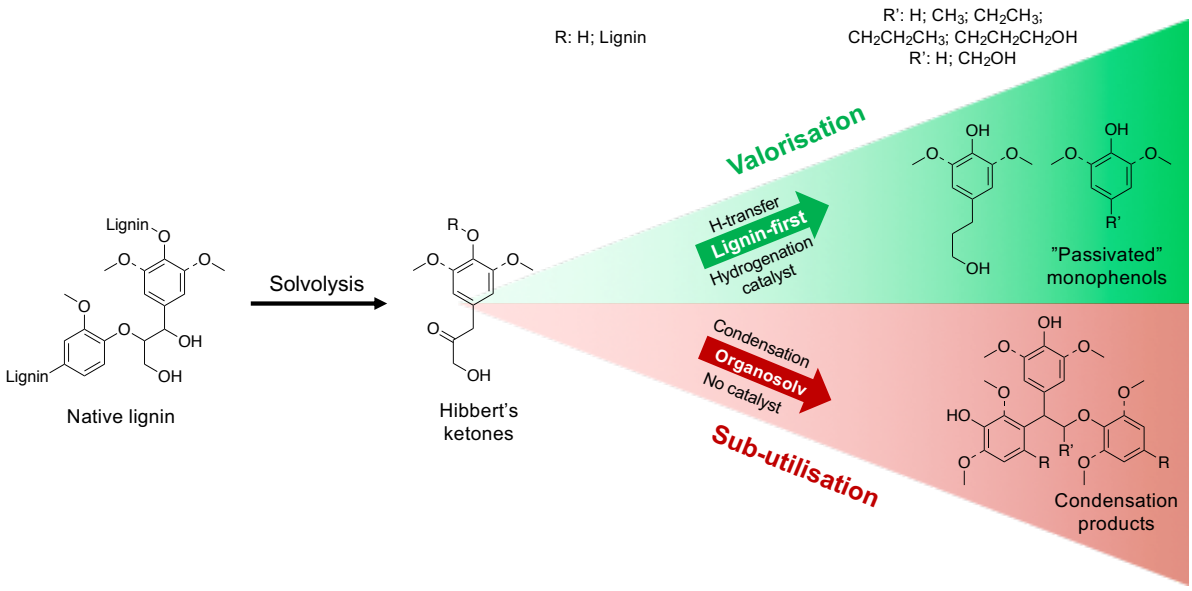


Figure 2-6 – Schematic representation of the chemical reactions occurring in occurring in conventional (e.g. Organosolv) and emerging fractionation approaches (lignin-first) for lignin valorisation.

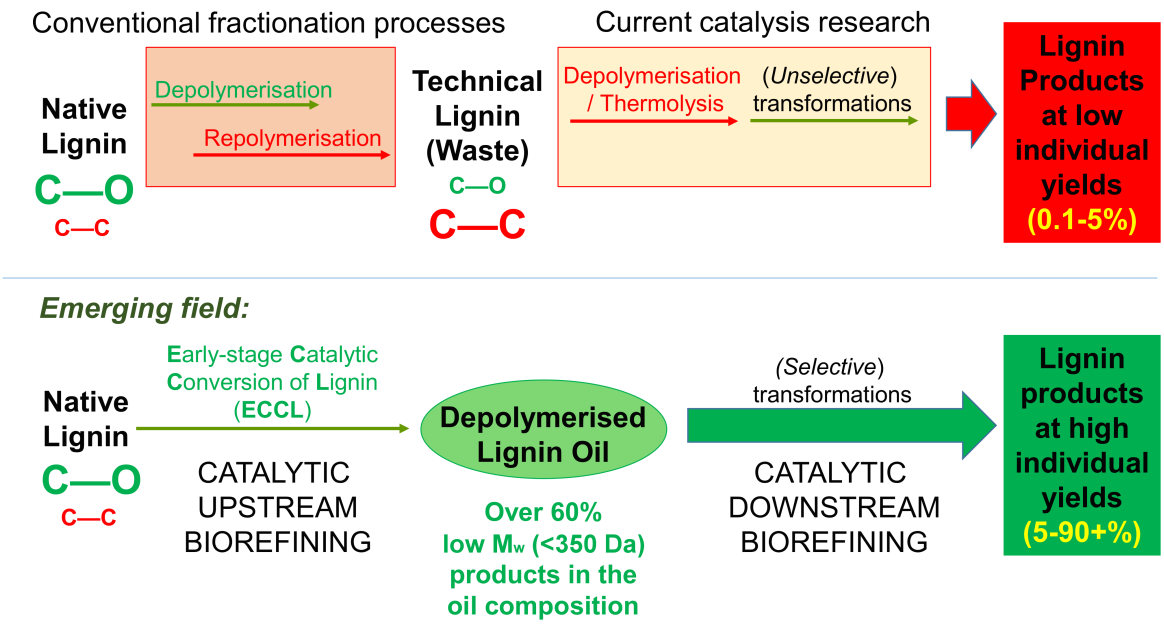


Figure 2-7 – Schematic representation of the processes occurring in conventional (e.g. Organosolv) and emerging fractionation approaches (lignin-first, based on ECCL) for lignin valorisation. Reproduced from Ref. [5b].

The lignin-first process was firstly introduced by Rinaldi et al.,[7a] who demonstrated a fractionation process, namely “Catalytic Upstream Biorefining” (CUB), which combined a hydrogenation catalyst (i.e. Raney Ni) with a hydrogen-donor solvent (i.e. aqueous solution of 2-PrOH) to establish H-transfer reactions on the solvolytically released lignin under autogenous pressure.[7a, 68] In this approach, the lignin fraction is isolated as a mixture of compounds of MW in the range 100–600 Dalton (Da), with a high individual yield of monophenols (*ortho*-(di)methoxy-4-hydroxypropylphenols as main products, *para*-alkyl-phenols in lower amounts), and a complex mixture of low- to high-MW lignin biopolymers showing high molecular diversity.[7a, 68] This lignin oil was demonstrated to be easily upgraded through hydrogeoxygenation into cyclohexanols and cycloalkanes[7a] or to kerosene/diesel fuels that can be readily integrated in existing refineries.[67] The holocellulose fraction comprising cellulose and hemicellulose is isolated as a highly delignified pulp, which can be converted by enzymatic hydrolysis into C5 and C6 sugars at high yields.[7a, 68]

In recent years, the lignin-first approach for the fractionation of biomass was tested with a broad array of experimental conditions, involving different types of lignocellulose, lignin-extracting media, and catalyst. Table 2-2 provides an extensive overview of the different lignin-first methods developed thus far, and the characteristics of the derived lignin products.

As the composition of native lignin depends largely on the feedstock employed,[18a, 19] a different set and/or amount of lignin components can be expected during the biorefining on different wood types.[63c, 69] Typically, lignins with higher syringyl content (i.e. hardwoods) result in a more efficient extraction from the lignocellulosic matrix and higher yields of monomers compared to those with higher contents of guaiacyl- or hydroxyphenyl-units (i.e. softwoods and grasses), whose

unsubstituted C_6 (and C_1 for H-units) is highly reactive towards the formation of new C-C bonds through recondensation reactions.[26a, 70] Indeed, there is a direct correlation between the frequency of β-O-4' moiety, dictated by the syringyl content in the native lignin, and the yield of monophenols recovered in the lignin fraction obtained from lignin-first biorefining.[63c] Different catalysts have been tested for lignin-first biorefining i.e. noble metal-supported catalysts[63a, 63b, 65a-f] or inexpensive Ni catalysts,[7a, 63d, 65g] which lead to different selectivity in the conversion of lignin fragments released upon fractionation, affecting in detail the degree of functionalisation of the phenolic side chain of the lignin monomers (see Figure 2-8). Indeed, lignin-first biorefining typically renders monophenols with (a) 1-hydroxypropyl-,[6a, 7a, 71] (b) propyl,[63a, 63b, 72] (c) 1-hydroxypropenyl,[73] or (d) 2-propenyl- [63c, 63d, 69a, 73] side chains depending on the employed catalytic system, defined by the specific combination of catalyst and H-donor, with the type of wood defining the functionalisation on the phenolic ring.[29, 65d, 71]

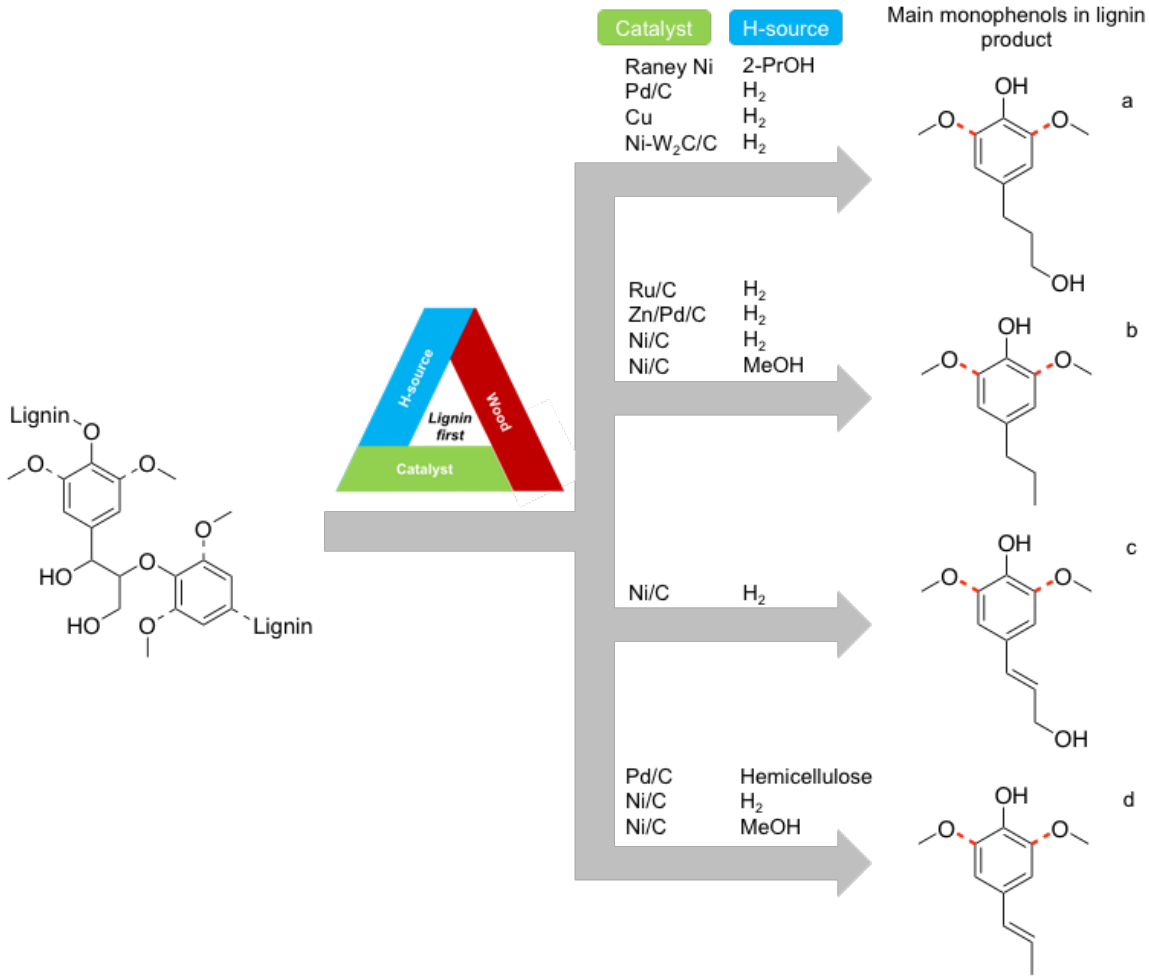


Figure 2-8 – Main lignin-derived products obtained from lignin-first biorefining in different conditions (listed in Table 2-2). Adapted from Ref. [29].

As the use of noble metals such as Pd or Ru is associated with high costs (respectively 5714$/mol and 796$/mol),[74] an efficient recovery and recycle of the catalyst is indispensable for the process. However, the catalyst is often dispersed in the solid particles of the holocellulosic pulp at the end of the process,[63d, 65b, 69a, 72b, 75] hence posing difficulties for its recovery as well as for further upgrading of the carbohydrate fraction. In this perspective, using the catalyst particles already mixed with the holocellulosic pulp for its downstream upgrading represents a clever approach to circumvent the catalyst separation step.[6a] Alternatively, microporous cages can be used to prevent the catalyst particles from mixing with the solid lignocellulose

feedstock while still getting in contact with the lignin fragments dissolved in the lignin-extracting medium.[63a, 73, 76] Notably, using Raney Ni as catalyst for lignin-first biorefining provides a solution for both issues related with the high costs of the catalyst, and its recovery. Indeed, thanks to its magnetic feature, this inexpensive catalyst (0.8$ per mol of Ni)[36] can easily and effectively be recovered from the solid fraction obtained after lignin-first by means of a magnet, and recycled for further catalytic cycles.[7a, 28a, 71]

Patented in 1926,[77] Raney Ni is a catalyst commonly employed in hydrogenation, hydrogenolysis, hydrodeoxygenation and desulfurisation.[78] and is produced by dealuminisation of a Ni:Al 1:1 wt.% alloy in high alkaline conditions (i.e. NaOH 20 wt.%) to yield a material composed of Ni 90-98 wt.% and 2-10 wt.% Al following the reactions:[79]

$$2\ Al_s + 2\ OH^-_{(aq)} + 6\ H_2O_{(l)} \rightarrow 2\ Al(OH)^-_{4\ (aq)} + 3\ H_{2\ (g)}$$

$$2\ Al(OH)^-_{4\ (aq)} \rightarrow Al_2O_{3\ (s)} + 2\ OH^-_{(aq)} + 3\ H_2O_{(l)}$$

The resulting material presents a bulk composition mainly made of Ni and a surface composition containing ca. 50 wt.% Al.[79c] The overall structure exhibits a high surface area and is rich in small pores and defect sites in the lattice which host the hydrogen produced during dealuminisation.[79c] The composition, structure and reactivity of Raney Ni are dictated by the experimental conditions for synthesis (e.g. temperature, concentration of base, composition of the starting alloy, reaction time)[79a, 79c]

The high activity towards H-transfer reactions, combined with its low cost and magnetic properties, makes Raney Ni a desirable catalyst for lignin chemistry. However, due to its large surface area and amount of absorbed hydrogen, this catalyst Is highly pyrophoric and prone to oxidation when exposed to air, which relevantly alters

its structure, therefore requiring appropriate storage to preserve its activity and extra care during handling.

The solvent plays a crucial role in lignin-first biorefining,[80] with the polarity[7a, 28a, 75a] and the pH[72a, 75b, 75c, 81] of the solvent mixture being critical parameters for delignification and lignin depolymerisation. In this context, short-chain alcohols (i.e. MeOH, EtOH, 2-PrOH) and their aqueous solutions were found to be highly effective in wood fractionation, with an accurate choice of the proportion alcohol/water being conducive to effective removal and dissolution lignin.[82] Indeed, these species can behave as H-donors, hence playing a crucial role in H-transfer systems.[7a, 63c, 63d, 71] However, primary alcohols such as MeOH and EtOH present lower H-donating capacity compared to a secondary alcohol such as 2-PrOH, and usually require an additional source of hydrogen in order to achieve high performances in lignin conversion through H-transfer reactions. For this purpose, molecular hydrogen (H_2) is widely employed as supplementary H-donor in ECCL and is associated with extensive depolymerisation of lignin (monomer yields up to 68%). However, the use H_2 in a biorefinery entails additional safety protocols for the process, along with reactors built in order to be able to withstand hydrogen pressures.[83] Furthermore, the economic feasibility of the process would require onsite hydrogen production,[67] as a distance between biorefinery and hydrogen production sites would entail additional costs for H_2 transportation.[83]

Table 2-2 – Experimental conditions and performances of current approaches for lignin-first biorefining. HW: hardwood; SW: softwood; G: grass; AR: agricultural residue. Main monophenols in lignin products are referred to Figure 2-8. a: 1-hydroxypropylphenols; b: propylphenols; c: 1-hydroxypropenylphenols; d: 2-propenylphenols.

Lignocellulosic feedstock	Wood type	Catalyst (wt% relative to weight of feedstock)	Solvent	Pressure (bar)	Additive	Temperature (°C)	Time (h)	Main monophenols in lignin products	Total yield monophenols (%)	Ref.
Poplar	HW	Raney Ni (59 wt%)	2-PrOH/H_2O 7:3 v/v	-	-	160	3	d	24	[7a]
Poplar	HW	Raney Ni (59 wt%)	2-PrOH/H_2O 7:3 v/v	-	-	180	3	d	18	[7a]
Poplar	HW	Raney Ni (59 wt%)	2-PrOH/H_2O 7:3 v/v	-	-	200	3	d	22	[7a]
Poplar	HW	Raney Ni (59 wt%)	2-PrOH/H_2O 7:3 v/v	-	-	220	3	d	35	[7a]
Poplar	HW	Raney Ni (59 wt%)	2-PrOH	-	-	180	3	d	19	[7a]
Poplar	HW	Raney Ni (59 wt%)	2-PrOH/MeOH 10:1 v/v	-	-	180	3	d	14	[7a]
Birch	HW	Ni/C (5 wt%)	MeOH	-	-	200	6	a	9	[71]
Birch	HW	Ni/C (5 wt%)	2-PrOH	-	-	200	6	a	10	[71]
Birch	HW	Raney Ni (50 wt%)	MeOH	-	-	180	3	b	13	[71]
Birch (Sweden)	HW	Pd/C (10 wt%)	EtOH/H_2O 1:1 v/v	-	-	195	15	c	35	[63c]
Birch (Finland)	HW	Pd/C (10 wt%)	EtOH/H_2O 1:1 v/v	-	-	210	15	c	36	[63c]
Poplar	HW	Pd/C (10 wt%)	EtOH/H_2O 1:1 v/v	-	-	210	15	c	22	[63c]
Spruce	SW	Pd/C (10 wt%)	EtOH/H_2O 1:1 v/v	-	-	210	15	c	12	[63c]
Pine	SW	Pd/C (10 wt%)	EtOH/H_2O 1:1 v/v	-	-	210	15	c	7	[63c]
Apple wood	HW	Ru/C (15 wt%)	MeOH	110 (H_2)	-	250	3	b	47	[72b]
Apple wood	HW	Ru/SiC (15 wt%)	MeOH	110 (H_2)	-	250	3	b	48	[72b]
Scotch pine	SW	Pd/C (5 wt%)	MeOH	30 (H_2)	Yb(III)-triflate (0.8mM)	200	2	a	24	[84]
Poplar	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	Yb(III)-triflate (0.8mM)	200	2	a	36	[84]
Oak	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	Yb(III)-triflate (0.8mM)	200	2	a	47	[84]
Birch	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	Yb(III)-triflate (0.8mM)	200	2	a	45	[84]
Oak	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	Al(OTf)3 (0.8mM)	180	2	a	46	[84]
Oak	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	HCl (0.8mM)	180	2	a	14	[84]
Oak	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	H_2SO_4 (0.8mM)	180	2	a	10	[84]
Oak	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	H_3PO_4 (0.8mM)	180	2	a	13	[84]
Oak	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	CH_3COOH (0.8mM)	180	2	a	15	[84]
Birch	HW	Ni/C (5 wt%)	MeOH	2 (N_2)	-	200	6	d	20	[69a]
Poplar	HW	Ni/C (5 wt%)	MeOH	2 (N_2)	-	200	6	d	6	[69a]
Eucalyptus	HW	Ni/C (5 wt%)	MeOH	2 (N_2)	-	200	6	d	16	[69a]
Birch	HW	Ni/C (10 wt%)	MeOH	2 (N_2)	-	200	6	b	32	[69a]
Poplar	HW	Ni/C (10 wt%)	MeOH	2 (N_2)	-	200	6	d	26	[69a]

Table 2-2 (continued) – Experimental conditions and performances of current approaches for lignin-first biorefining. HW: hardwood; SW: softwood; G: grass; AR: agricultural residue. Main monophenols in lignin products are referred to Figure 2-8. a: 1-hydroxypropylphenols; b: propylphenols; c: 1-hydroxypropenylphenols; d: 2-propenylphenols.

Lignocellulosic feedstock	Wood type	Catalyst (wt% relative to weight of feedstock)	Solvent	Pressure (bar)	Additive	Temperature (°C)	Time (h)	Main monophenols in lignin products	Total yield monophenols (%)	Ref.
Eucalyptus	HW	Ni/C (10 wt%)	MeOH	2 (N_2)	-	200	6	b	28	[73]
Mischantus	G	Ni/C (10 wt%)	MeOH	35 (N_2)	-	225	12	c	27	[73]
Mischantus	G	Ni/C (10 wt%)	MeOH	5 (H_2)	-	225	12	c	30	[73]
Mischantus	G	Ni/C (10 wt%)	MeOH	10 (H_2)	-	225	12	c	29	[73]
Mischantus	G	Ni/C (5 wt%)	MeOH	35 (H_2)	-	225	12	c	56	[73]
Mischantus	G	Ni/C (10 wt%)	MeOH	35 (H_2)	-	225	12	c	65	[73]
Mischantus	G	Ni/C (15 wt%)	MeOH	35 (H_2)	-	225	12	c	68	[73]
Poplar (WT-717)	HW	Pd/C (5 wt%)	MeOH	34 (H_2)	$ZnCl_2$ (25mM)	225	12	b	40	[63a]
Poplar (717-F5H)	HW	Pd/C (5 wt%)	MeOH	34 (H_2)	$ZnCl_2$ (25mM)	225	12	b	36	[63a]
Poplar (WT-NM-6)	HW	Pd/C (5 wt%)	MeOH	34 (H_2)	$ZnCl_2$ (25mM)	225	12	b	44	[63a]
Poplar (WT-LORRE)	HW	Pd/C (5 wt%)	MeOH	34 (H_2)	$ZnCl_2$ (25mM)	225	12	b	54	[63a]
Birch	HW	Pd/C (5 wt%)	MeOH	34 (H_2)	$ZnCl_2$ (25mM)	225	12	b	52	[63a]
Eucalyptus	HW	Pd/C (5 wt%)	MeOH	34 (H_2)	$ZnCl_2$ (25mM)	225	12	b	49	[63a]
Pine	SW	Pd/C (5 wt%)	MeOH	34 (H_2)	$ZnCl_2$ (25mM)	225	12	b	19	[63a]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	-	200	3	a	28	[75a]
Poplar	HW	Pd/C (10 wt%)	MeOH/H_2O 90:10 v/v	20 (H_2)	-	200	3	a	36	[75a]
Poplar	HW	Pd/C (10 wt%)	MeOH/H_2O 90:10 v/v	20 (H_2)	-	200	3	a	43	[75a]
Poplar	HW	Pd/C (10 wt%)	MeOH/H_2O 90:10 v/v	20 (H_2)	-	200	3	a	42	[75a]
Poplar	HW	Pd/C (10 wt%)	MeOH/H_2O 90:10 v/v	20 (H_2)	-	200	3	a	43	[75a]
Poplar	HW	Pd/C (10 wt%)	H_2O	20 (H_2)	-	200	3	a	34	[75a]
Poplar	HW	Pd/C (10 wt%)	EtOH/H_2O 10:90 v/v	20 (H_2)	-	200	3	a	38	[75a]
Poplar	HW	Pd/C (10 wt%)	EtOH/H_2O 10:90 v/v	20 (H_2)	-	200	3	a	42	[75a]
Poplar	HW	Pd/C (10 wt%)	EtOH/H_2O 10:90 v/v	20 (H_2)	-	200	3	a	43	[75a]
Poplar	HW	Pd/C (10 wt%)	EtOH/H_2O 10:90 v/v	20 (H_2)	-	200	3	a	39	[75a]
Poplar	HW	Pd/C (10 wt%)	EtOH/H_2O 10:90 v/v	20 (H_2)	-	200	3	a	26	[75a]
Poplar	HW	Pd/C (10 wt%)	EtOH	20 (H_2)	-	200	3	a	19	[75a]
Birch	HW	Pd/C (5 wt%)	H_2O	30 (H_2)	-	200	3	a	44	[65b]
Birch	HW	Pd/C (5 wt%)	MeOH	30 (H_2)	-	200	3	a	28	[65b]
Birch	HW	Pd/C (5 wt%)	Ethylene glycol	30 (H_2)	-	200	3	a	27	[65b]

Table 2-2 (continued) – Experimental conditions and performances of current approaches for lignin-first biorefining. HW: hardwood; SW: softwood; G: grass; AR: agricultural residue. Main monophenols in lignin products are referred to Figure 2-8. a: 1-hydroxypropylphenols; b: propylphenols; c: 1-hydroxypropenylphenols; d: 2-propenylphenols.

Lignocellulosic feedstock	Wood type	Catalyst (wt% relative to weight of feedstock)	Solvent	Pressure (bar)	Additive	Temperature (°C)	Time (h)	Main monophenols in lignin products	Total yield monophenols (%)	Ref.
Birch	HW	Pd/C (5 wt%)	EtOH	30 (H_2)	-	200	3	a	17	[65b]
Birch	HW	Pd/C (5 wt%)	2-PrOH	30 (H_2)	-	200	3	a	12	[65b]
Birch	HW	Pd/C (5 wt%)	1-BuOH	30 (H_2)	-	200	3	a	11	[65b]
Birch	HW	Pd/C (5 wt%)	THF	30 (H_2)	-	200	3	a	6	[65b]
Birch	HW	Pd/C (5 wt%)	Dioxane	30 (H_2)	-	200	3	a	5	[65b]
Birch	HW	Pd/C (5 wt%)	Hexane	30 (H_2)	-	200	3	a	2	[65b]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	-	200	3	a	27	[75c]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	-	250	3	a	44	[75c]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	H_3PO_4 (13mM)	200	3	a	36	[75c]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	H_3PO_4 (25mM)	200	3	a	40	[75c]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	H_3PO_4 (50mM)	200	3	a	42	[75c]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	NaOH (31mM)	200	3	a	26	[75c]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	NaOH (62mM)	200	3	a	25	[75c]
Poplar	HW	Pd/C (10 wt%)	MeOH	20 (H_2)	NaOH (125mM)	200	3	a	23	[75c]
Pine	SW	Pd/C (10 wt%)	MeOH	20 (H_2)	-	200	3	a	10	[75c]
Pine	SW	Pd/C (10 wt%)	MeOH	20 (H_2)	-	250	3	a	19	[75c]
Pine	SW	Pd/C (10 wt%)	MeOH	20 (H_2)	H_3PO_4 (25mM)	200	3	a	16	[75c]
Pine	SW	Pd/C (10 wt%)	MeOH	20 (H_2)	H_3PO_4 (50mM)	200	3	a	17	[75c]
Pine	SW	Pd/C (10 wt%)	MeOH	20 (H_2)	NaOH (62mM)	200	3	a	2	[75c]
Pine	SW	Pd/C (10 wt%)	MeOH	20 (H_2)	NaOH (125mM)	200	3	a	4	[75c]
Birch	HW	Ni/C (5 wt%)	MeOH	1 (Ar)	-	160	6	b	7	[63d]
Birch	HW	Ni/C (5 wt%)	MeOH	1 (Ar)	-	180	6	b	28	[63d]
Birch	HW	Ni/C (5 wt%)	MeOH	1 (Ar)	-	200	6	b	49	[63d]
Birch	HW	Ni/C (5 wt%)	MeOH	1 (Ar)	-	220	6	b	61	[63d]
Birch	HW	Ni/C (5 wt%)	MeOH	1 (Ar)	-	240	6	b	59	[63d]
Birch	HW	Ni/C (5 wt%)	EtOH	1 (Ar)	-	200	6	b	43	[63d]
Birch	HW	Ni/C (5 wt%)	Ethylene glycol	1 (Ar)	-	200	6	b	49	[63d]
Birch	HW	Ni/C (5 wt%)	2-PrOH	1 (Ar)	-	200	6	b	26	[63d]
Birch	HW	Ni/C (5 wt%)	Glycerol/H_2O 1:4 v/v	1 (Ar)	-	200	6	b	12	[63d]
Birch	HW	Ni/C (5 wt%)	1,4-dioxane	1 (Ar)	-	200	6	d	12	[63d]
Birch	HW	Ni/C (5 wt%)	MeOH/H_2O 1:99 v/v	1 (Ar)	-	200	6	b	9	[63d]

Table 2-2 (continued) – Experimental conditions and performances of current approaches for lignin-first biorefining. HW: hardwood; SW: softwood; G: grass; AR: agricultural residue. Main monophenols in lignin products are referred to Figure 2-8. a: 1-hydroxypropylphenols; b: propylphenols; c: 1-hydroxypropenylphenols; d: 2-propenylphenols.

Lignocellulosic feedstock	Wood type	Catalyst (wt% relative to weight of feedstock)	Solvent	Pressure (bar)	Additive	Temperature (°C)	Time (h)	Main monophenols in lignin products	Total yield monophenols (%)	Ref.
Birch	HW	Ni/C (5 wt%)	MeOH/H_2O 1:4 v/v	1 (Ar)	-	200	6	b	21	[63d]
Birch	HW	Ni/C (5 wt%)	Cyclohexane	1 (Ar)	-	200	6	b	<1	[63d]
Birch	HW	Ni/C (5 wt%)	MeOH	50 (H_2)	-	200	6	b	41	[63d]
Birch	HW	Ni/SBA-15 (5 wt%)	MeOH	1 (Ar)	-	200	6	d	27	[63d]
Birch	HW	Ni/Al_2O_3 (5 wt%)	MeOH	1 (Ar)	-	200	6	d	19	[63d]
Birch	HW	Cu/C (5 wt%)	MeOH	1 (Ar)	-	200	6	d	9	[63d]
Birch	HW	Cu-Cr oxide (5 wt%)	MeOH	1 (Ar)	-	200	6	d	2	[63d]
Pine	SW	Cu20-PMO (20 wt%)	MeOH	40 (H_2)	-	140	18	a	1	[6a]
Pine	SW	Cu20-PMO (20 wt%)	MeOH	40 (H_2)	-	180	18	a	10	[6a]
Pine	SW	Cu20-PMO (20 wt%)	MeOH	40 (H_2)	-	220	18	a	13	[6a]
Birch	HW	Ru/C (10 wt..%)	MeOH	30 (H_2)	-	250	3	b	48	[65d]
Birch	HW	Pd/C (10 wt%)	MeOH	30 (H_2)	-	250	3	a	49	[65d]
Birch	HW	Ru/C (5 wt%)	MeOH	30 (H_2)	-	200	6	b	33	[63b]
Birch	HW	Ru/C (5 wt%)	MeOH	30 (H_2)	-	250	6	b	52	[63b]
Birch	HW	Ru/C (5 wt%)	MeOH	1 (N_2)	-	250	3	b	35	[63b]
Birch	HW	Ru/C (5 wt%)	MeOH	30 (H_2)	-	250	3	b	42	[63b]
Birch	HW	Ru/C (5 wt%)	MeOH	30 (H_2)	-	250	0.5	b	33	[63b]
Birch	HW	Ru/C (5 wt%)	MeOH	10 (H_2)	-	250	6	b	47	[63b]
Birch	HW	Ru/C (5 wt%)	H_2O	30 (H_2)	-	250	6	b	25	[63b]
Birch	HW	Ni-Al_2O_3 (10 wt%)	MeOH	30 (H_2)	-	250	3	a	36	[76]
Fir	SW	Pd/C (10 wt%)	Dioxane/H_2O 1:1 v/v	35 (H_2)	-	195	5	a	15	[81]
Bamboo	HW	Pd/C (10 wt%)	Dioxane/H_2O 1:1 v/v	35 (H_2)	-	195	10	a	17	[81]
Pine	SW	Pd/C (10 wt%)	Dioxane/H_2O 1:1 v/v	35 (H_2)	HCl (10 mM)	195	5	a	10	[81]
Corncob	AR	Pd/C (10 wt%)	Dioxane/H_2O 1:1 v/v	35 (H_2)	NaOH (50 mM)	195	5	a	7	[81]
Spruce	SW	Raney Ni (100 wt%)	Dioxane/H_2O 1:1 v/v	35 (H_2)	-	195	5	a	9	[81]
Spruce	SW	Raney Ni (100 wt%)	Dioxane/H_2O 1:1 v/v	35 (H_2)	HCl (10 mM)	195	5	a	15	[81]
Spruce	SW	Raney Ni (100 wt%)	Dioxane/H_2O 1:1 v/v	35 (H_2)	NaOH (50 mM)	195	5	a	9	[81]
Spruce	SW	Rh/C (10 wt%)	Dioxane/H_2O 1:1 v/v	35 (H_2)	-	195	5	a	50	[85]
Spruce	SW	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	37	[69c]
Spruce	SW	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	21	[69c]
Spruce	SW	Ni-W_2C/C (40 wt%)	MeOH	60 (H_2)	-	235	4	a	42	[69c]

Table 2-2 (continued) – Experimental conditions and performances of current approaches for lignin-first biorefining. HW: hardwood; SW: softwood; G: grass; AR: agricultural residue. Main monophenols in lignin products are referred to Figure 2-8. a: 1-hydroxypropylphenols; b: propylphenols; c: 1-hydroxypropenylphenols; d: 2-propenylphenols.

Lignocellulosic feedstock	Wood type	Catalyst (wt% relative to weight of feedstock)	Solvent	Pressure (bar)	Additive	Temperature (°C)	Time (h)	Main monophenols in lignin products	Total yield monophenols (%)	Ref.
Aspen	HW	Ni-W_2C/C (40 wt%)	Ethylene glycol	60 (H_2)	-	235	4	a	46	[69c]
Birch	HW	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	32	[69c]
Corn stalk	G	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	37	[69c]
Birch	HW	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	40	[69c]
Birch	HW	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	26	[69c]
Poplar	HW	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	29	[69c]
Basswood	HW	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	23	[69c]
Ashtree	HW	Ni-W_2C/C (40 wt%)	H_2O	60 (H_2)	-	235	4	a	10	[69c]
Beech	HW	Ni/C (5 wt%)	MeOH	30 (H_2)	-	250	3	c	29	[72a]
Willow	HW	Ni/C (5 wt%)	MeOH	30 (H_2)	-	200	24	c	29	[72a]
Bagasse	G	Ru/C (5 wt%)	MeOH	30 (H_2)	-	250	3	c	32	[72a]
Pine	SW	Ru/C (5 wt%)	MeOH	30 (H_2)	-	200	24	c	28	[72a]
Corn stover	AR	Ni/C (H^+) (5 wt. %)	MeOH	30 (H_2)	-	200	6	c	32	[72a]
Corn stover	AR	Ni/C (5 wt%)	MeOH	30 (H_2)	H_3PO_4 (100 mM)	200	6	c	38	[72a]

2.3 Outlook

Thanks to its high degree of functionalisation, lignin in plant biomass represents an attractive feedstock for the production of bio-based fuels, chemicals and materials. However, lignin's potential in biorefinery is still under exploited, as its isolation from the lignocellulosic matrix often entails the formation of recalcitrant cross-links which drastically increase the severity of conditions needed for its downstream upgrading. Among the emerging fractionation methods for lignin valorisation, CUB constitutes a powerful approach for funnelling the conversion of lignin towards the formation of a small class of products, which offer a broad array of opportunities for the production of new value-added materials. In this context, CUB provides a way to directly synthesise chemicals with desired functionalities, which provides a clever alternative to the classic approach of fossil refineries entailing full defunctionalisation of aromatics to BTX (i.e. benzene, toluene and xylenes) followed by refunctionalisation to the required chemicals.[5b]

Due to the complex nature of the lignocellulosic matrix, there is an intricate network of processes occurring upon the catalytic fractionation of biomass, which were unveiled only to a small extent by the recent research efforts in this field. Importantly, an increased knowledge of the fundamental processes underpinning biomass fractionation could provide the tools for engineering the catalytic upstream biorefining into the selective production of compounds with the desired moieties. In this context, CUB would greatly benefit from further investigation on the physical and chemical processes responsible for the deconstruction of the lignocellulose matrix, leading to the conversion of the carbohydrate fibres as well as the release of lignin fragments, which undergo further depolymerisation and condensation reactions. The assessment

of these processes occurring in CUB represents the main objective of this dissertation. The pinnacle of this study is the definition of optimum conditions for the valorisation of both the carbohydrate and lignin fraction. In detail, the following chapters aim to investigate:

- The role of hemicellulose and the pH of the liquor on the catalytic activity of Raney Ni and the product distribution of the lignin fraction isolated from catalytic upstream biorefining (Chapter 3);
- The influence of acidic condition and the accessibility of the lignocellulosic matrix on the deconstruction of the biomass (Chapter 4);
- The time evolution of the physicochemical processes occurring upon catalytic fractionation (Chapter 5);
- The interplay of wood, catalyst and lignin-extracting medium in defining the extent of biomass deconstruction, lignin depolymerisation and condensation (Chapter 6).

3. Influence of Hemicellulose Sugars on Product Distribution from CUB of lignocellulose

The results discussed in this chapter were published in ChemCatChem and are available as reference: Chesi, C.; De Castro, I. B. D.; Clough, M. T.; Ferrini, P.; Rinaldi, R., The Influence of Hemicellulose Sugars on Product Distribution of Early-Stage Conversion of Lignin Oligomers Catalysed by Raney Nickel. *ChemCatChem* **2016,** *8* (12), 2079-2088.

3.1 Introduction

Solvolysis of the native lignin is a key step in lignocellulose fractionation,[7a, 86] and is affected by the experimental conditions (i.e. temperature) and solvent employed for extraction (i.e. pH, polarity, composition).[7a, 27, 75a, 75c, 80] Indeed, the combination of an organic solvent with water (i.e. in Organosolv fractionation) proved to be an effective extraction medium for pulp delignification, as fractionation performed in such mixtures achieved high-quality pulps amenable for paper production with little residual lignin content.[7a, 28a] Upon cooking lignocellulose in such mixtures at temperatures from 160 to 220°C, the hemicellulose fraction undergoes deacetylation, detaching from the lignin fraction. During this process, the extraction medium becomes acidified by the release of acetic and formic acid,[41a-g] which behaves as an *in-situ* catalyst for both solvolysis of β-ether lignin linkages[41f, 42] and hemicellulose hydrolysis.[41g, 42c, 43]

Despite the extensive research activity devoted to Organosolv processes thus far, the mechanistic aspects of the release of lignin from the lignocellulose matrix to the extraction medium are still poorly understood. Aiming for the optimisation of the

pulping process to achieve highly-delignified pulps, further investigation of the chemical processes involved in the solvolysis of lignin and hemicellulose upon fractionation becomes crucial. In this perspective, an interesting approach consists in 'perturbing' the acid-catalysed reactions responsible for the release of both lignin oligomers and hemicellulose sugars by neutralisation of the acids in solution. Under nearly neutral to weakly basic conditions, the acid hydrolysis of hemicellulose is suppressed, hence leading to higher preservation of the carbohydrate fraction. In turn, β-ether lignin linkages can still undergo solvolysis through radical reactions.[5b, 87] Through neutralisation of the released organic acids, it is then possible to shed light on the role that hemicellulose sugars can play on fractionation, and in detail on the activity of Raney Ni in H-transfer reactions during CUB process.

This chapter investigates the role of hemicellulose-derived sugars on the solvolytic processes occurring concurrently to the H-transfer reactions catalysed by Raney Ni upon the CUB process performed on Poplar wood, focusing on their influence on the characteristics of the lignin product and the deconstruction of lignocellulose. The chapter is divided into three main sections. The first section presents the rationale involved in the choice of the process parameters used in this study. The second part discusses the effect of neutral to weakly alkaline conditions on biomass deconstruction (i.e. delignification degree and extent of hemicellulose retention) obtained by employing inorganic bases in the Organosolv process (reference experiments) and the catalytic biorefining process with Raney Ni. For comparison, control experiments of both processes were also carried out without added bases. The final section discusses the influence of hemicellulose sugars on the activity of Raney Ni in CUB.

3.2 Results and discussion

3.2.1 Definition of experimental conditions for perturbation of acid-catalysis in CUB

The fractionation of lignocellulose results in the release of two classes of acidic species: (1) carboxylic acids i.e. acetic acid released from hemicellulose deacetylation, and formic acid produced from decomposition of hemicellulose-derived sugars;[41g] and (2) phenolic compounds i.e. phenolic monomers and oligomers derived from lignin depolymerisation.[7a, 71] Due to the difference in acidity between the carboxylic acids (pK_a~ 3.5–5) and phenols (pK_a~6.1–11.3 for guaiacyl-phenols, pK_a~7.3–10.5 for syringyl-phenols),[88] these species can be selectively neutralised and quantified through acid-base titration by careful choice of the bases to be employed. Hence, to determine the quantity of base needed to neutralise the acids released upon fractionation, the liquor obtained from the Organosolv process (control experiment) was titrated against 0.01 mol/L solutions of $NaHCO_3$, Na_2CO_3 and NaOH in 2-PrOH/ H_2O (7:3, v/v). The titration curves of Organosolv liquor against each base solution are shown in Figure 3-1, and the quantities of acid measured in the Organosolv liquor are listed in Table 3-1.

Due to its high basicity, NaOH neutralises both carboxylic acids and phenols, therefore the titre value corresponds to the total content of acidic species in the liquor (1.1 mmol/g_{wood}). Conversely, Na_2CO_3 is able to neutralise the full content of carboxylic acid, and only part of the phenolic species, owing to the similar base strength of the carbonate anion ($CO_3^{2-} + H_3O^+ \rightleftarrows HCO_3^- + H_2O$, p$K_b$=3.7) and phenoxide ion ($C_6H_5O^- + H_3O^+ \rightleftarrows C_6H_5OH + H_2O$, p$K_b$ = 4.0). Consequently, carbonate and phenolate ions will be in equilibrium, leading to a buffer system. Finally, $NaHCO_3$ neutralises only the

carboxylic acids (0.5 mmol/g_{wood}), as its basicity is insufficient for the neutralisation of the phenolic species ($HCO_3^+ + H_3O^+ \rightleftarrows H_2CO_3 + H_2O$, p$K_b$ = 7.7).

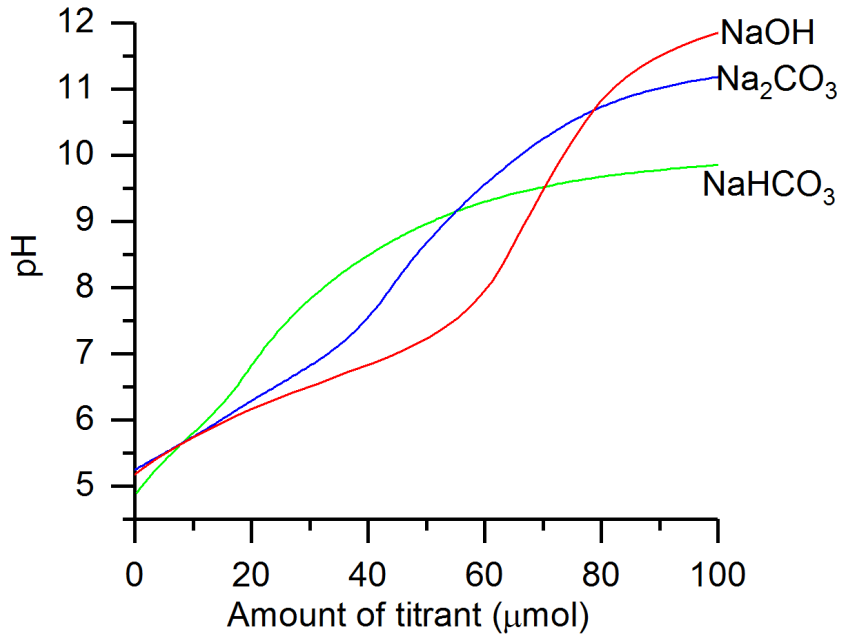


Figure 3-1 - Titration curves of Organosolv liquor against 0.01 mol/L solutions of $NaHCO_3$, Na_2CO_3 and NaOH in 2-PrOH/H_2O, 7:3 v/v.

Table 3-1 - Titre of acidic compounds determined in the Organosolv liquor.

Titrant	Titrated acids	Acid titre (mmol/g_{wood})
NaOH	Carboxylic acids and phenols	1.1
Na_2CO_3	Carboxylic acids and phenols (partially)	0.7
$NaHCO_3$	Only carboxylic acids	0.5

Based on the results of Table 3-1, three sets of experiments were designed to investigate the effect of neutral to weakly alkaline conditions on the fractionation of Poplar wood. The first set (control experiments) consists in the Organosolv and CUB process performed without added base. The second set (reference experiments) includes the Organosolv process performed with added bases, which will be

considered as reference experiments for the third set of experiments, comprising CUB process performed with added base. The fractionation experiments performed are summarised in Table 3-2. The temperature employed in fractionation (200 °C) was chosen in order to promote a sufficiently high degradation of hemicellulose,[7a] which enables a better appreciation of the preserving effect on the carbohydrate fraction produced by the neutral to weakly alkaline conditions in fractionation experiments.

Table 3-2 - Results obtained from the Organosolv process (entries 1-6) and catalytic upstream biorefining (CUB) (entries 7-12) of Poplar wood. General conditions: [a] Organosolv process: Poplar wood (17 g, chips), base (as listed), 2-PrOH:H_2O (140 mL, 7:3 v/v), 200°C for 3h;[7a] [b] CUB: Poplar wood (17 g, chips), base (as listed), Raney Ni (10 g), 2-PrOH:H_2O (140 mL, 7:3 v/v), 200°C for 3h; [c] Composition of unprocessed Poplar wood: 53% glucans; 17% xylans; 30% lignin; 1% others (daf: dry and ash-free values). [d] Klason method for determination of residual lignin seems to overestimate the quantity of lignin in highly delignified pulps. Despite performing repetitions of the analysis, in some cases sum of components in these pulps was slightly higher than 100%. [e] Calculations reported in the Methods section.

Entry	Base (experiment type)	Base amount (mmol/g_{wood})	Carbon yields of the isolated fractions (%)			Pulp composition (wt%, daf)[c]				Overall performance (%)	
			Pulp	Soluble products	Liquor pH (final)	Glucans	Xylans	Lignin	Others	Delignification[d,e]	Hemicellulose retention[e]
Organosolv process[a]											
1	None (control)	-	46	44	4.3	80	3	9	8	85	10
2	$NaHCO_3$ (reference)	0.5	45	46	5.7	77	5	8	11	88	13
3	Na_2CO_3 (reference)	0.7	56	46	6.5	67	12	11	10	78	43
4	$NaHCO_3$ (reference)	1.1	61	38	6.2	71	10	10	9	80	34
5	Na_2CO_3 (reference)	1.1	55	46	7.6	64	13	10	13	81	45
6	NaOH (reference)	1.1	54	42	6.5	69	9	11	11	79	30
CUB[b]				Lignin oil stream							
7	None (control)	-	54	43	5.5	85	12	7[d]	-	87	34
8	$NaHCO_3$	0.5	69	30	7.1	81	14	6[d]	-	88	60
9	Na_2CO_3	0.7	60	33	8.2	77	17	5[d]	1	91	49
10	$NaHCO_3$	1.1	62	31	9.5	79	17	6[d]	-	87	55
11	Na_2CO_3	1.1	58	38	9.6	84	17	4[d]	-	91	47
12	NaOH	1.1	58	36	8.2	75	15	5[d]	4	90	56

3.2.2 Effect of neutral to weakly alkaline conditions on biomass deconstruction

Table 3-2 displays the results obtained from the control, reference and CUB experiments. On average, the carbon balance was found to be 96±4% (i.e. sum of the C-yields of pulp and lignin stream) and does not include the small fraction of gaseous products obtained during fractionation (ca. 1.5% of the C content of Poplar wood), which includes products derived from demethylation and demethoxylation occurring on the lignin-derived phenols.[68] The gaseous products (i.e. methane, carbon dioxide, propane) and volatile products (i.e. methanol) thus released are inevitably lost during the workup of the products, performed through rotary evaporation.

The pH of the liquor, reached a final value of 4.3 in the control experiment (Organosolv process without added base, entry 1 in Table 3-2). Clearly, the addition of a base results in an increasing pH, as the reference Organosolv experiments (entry 2-6 in Table 3-2), reached a final liquor pH value ranging between 5.7 and 7.6. In the same way, the addition of bases to catalytic biorefining experiments resulted in a pH increase from 5.5 (entry 7) to values between 7.1 and 9.6 (entries 8–12). By comparing the final pH values of the liquor obtained from Organosolv experiments with their catalytic counterparts, it is apparent that the concurrent use of an added base with Raney Ni results in an increase in pH of 1-2 units, as a result of the prevention of formic acid formation, owing to the hydrogenation of hemicellulose sugars in the liquor, which suppresses the reaction sequence: hemicellulose sugars → furfurals → formic acid and levulinic acid.[41g, 41h, 89] Notably, the alkaline conditions are sustained during the whole duration of the CUB process (pH=7.1-9.6), hence highlighting the existence of an effect of neutral to weakly alkaline conditions on fractionation, which will be discussed further in the following sections.

The extent of pulp delignification and the retention of their original content of hemicellulose are fundamental parameters to assess the performances of the CUB process, as they provide useful information regarding the applicability of the holocellulose fibres isolated. Indeed, highly delignified pulps are desired for their better reactivity towards chemical[90] or enzymatic conversion of the carbohydrate fraction.[7a, 33, 91] The compositional analysis of the holocellulosic pulps indicates that in neutral to weakly alkaline conditions the hemicellulose is retained to a greater extent in comparison with the classic acidic conditions resulting from control experiments. Indeed, a five- to six-fold increase in hemicellulose retention was observed (from 10-34 % to 47–60 %) when bases were added in fractionation. As highlighted in Figure 3-2a, displaying the extent of hemicellulose retention against the final pH value of the liquor, the hemicellulose retention steadily increases with the alkalinity of the extraction medium up to pH=7.5, where the retention plateaus at ~50%. For comparison, previous studies discussing Organosolv fractionation in aqueous solution of EtOH reported enhanced delignification when NaOH was employed (5-35% relative to wood weight).[92] However, under such conditions, increased degradation of the cellulosic fraction takes place, resulting in an overall decrease of the pulp yield (~70-35%).[93] Clearly, this system significantly differs from the one discussed in this chapter, which employs a base loading of 1-2% relative to wood weight.

Regarding delignification, both control experiments achieved high delignification of pulps (85 and 87 %, Table 2, entries 1 and 7, respectively). Unexpectedly, the delignification extent was not relevantly affected by the increase in pH of the extraction media observed for experiments with added bases. Indeed, the plot of delignification vs. pH of the liquor displayed in Figure 3-2b shows that the extraction of lignin from lignocellulose remains constant throughout the pH window

investigated in this study. Conversely, the presence of Raney Ni does play a key role in the delignification. In fact, for the Organosolv experiments performed without added Raney Ni (entries 3–6, final pH 6.5–7.6), an average delignification of 80±1 % was achieved. In turn, the use of Raney Ni in CUB fractionation promoted an average delignification degree of 90±2 % (entries 9–12, final pH 8.2–9.6). This enhancement in delignification is attributed to the H-transfer reactions catalysed by Raney Ni occurring on the lignin fragments released from the plant structure.[7a] Indeed, H-transfer reactions occurring on the lignin linkages result in the production of stable low-MW monophenols, as they promote the reduction of carbonyl groups on the side chains of the phenolic units, typically prone to recondensation reactions.[41a] Consequently, the reprecipitation of lignin globules on the holocellulosic fibres is abated, therefore leading to higher degrees of pulp delignification.

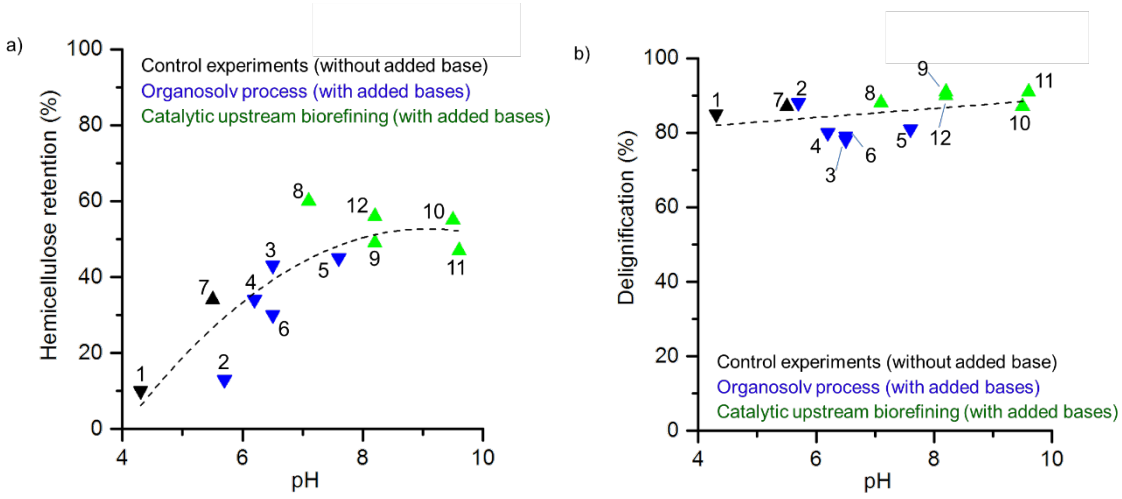


Figure 3-2 – Hemicellulose retained in pulp (a) and delignification degree (b) vs. pH of liquor. For experimental conditions, the numbers alongside the symbol refer to the entries of Table 3-2.

3.2.3 Effect of neutral to weakly alkaline conditions on the lignin stream

A qualitative assessment on the extent of depolymerisation can be obtained by gel permeation chromatography (GPC), which provides information about the apparent MW distribution of the species composing the lignin products. The GPC chromatograms of Organosolv lignin streams displayed in Figure 3-3a only show a marginal impact of the addition of bases on the apparent MW distribution to be found in the section MW>10kDa, in which the chromatogram onsets are shifted from 90kDa for lignin from the Organosolv process performed without added base (entry 1 in Table 3-2) to 50kDa for the polymer isolated from the Organosolv process carried out with added NaOH (entry 6 in Table 3-2). Hence, no relevant effect seems to be exerted by the neutralisation of organic acids released upon Organosolv fractionation of lignocellulose, or by a potential direct intervention of the alkaline additives in lignin depolymerisation reactions.

Conversely, CUB experiments resulted in the isolation of lignin as oils of reduced apparent MW, with the addition of bases further promoting lignin depolymerisation. Indeed, the fraction of products in the range 4.7–26 kDa obtained by the CUB process performed with no added base becomes significantly less populated when the pH of the extraction medium is increased. Regardless of the addition of bases in CUB (7.1–9.6, entries 8–12 in Table 3-2), all lignin oils are mainly composed of products with apparent MW lower than 200 Da.

In order to obtain semi-quantitative information on the extent of depolymerisation in lignin, the ratio "*area below low-MW region of GPC chromatogram/ area below high-MW region of GPC chromatogram*", defined in this dissertation as L_M/H_M, was calculated. Its correlation with the final pH value of the

liquor is reported in Figure 3-4. In this analysis a refractive index detection was employed, whose response is non-universal, hence providing a semi-quantitative assessment of depolymerisation. For Organosolv experiments, the ratio L_M/H_M shows no dependence on the pH of the liquor, hence suggesting that neutral to alkaline conditions do not significantly affect the extent of depolymerisation.

Conversely, the addition of bases in CUB experiments promotes a slight increase in the amount of low MW species found in the lignin oil products, at the expenses of polymeric species. In fact, the ratio L_M/H_M increases from 2.1 at pH=5.5 to 3.0 at pH=8.2. As before mentioned, acetic acid released in catalytic fractionation experiments is hypothesised to be an acid catalyst for the cleavage of β-ether lignin linkages,[41f, 42] therefore its neutralisation might suggest hampered depolymerisation of lignin. However, in alkaline conditions depolymerisation seems unaltered in Organosolv fractionation, and even enhanced in CUB fractionation. This can be explained by the fact that the cleavage of β-ether lignin linkages can also be catalysed by alkaline species,[41a] which then provide alternative routes for lignin depolymerisation. However, as further explained in the following paragraphs, the effect of alkaline additives on the extent of lignin depolymerisation is hypothesised not to related to a *direct* intervention on the reaction mechanism.

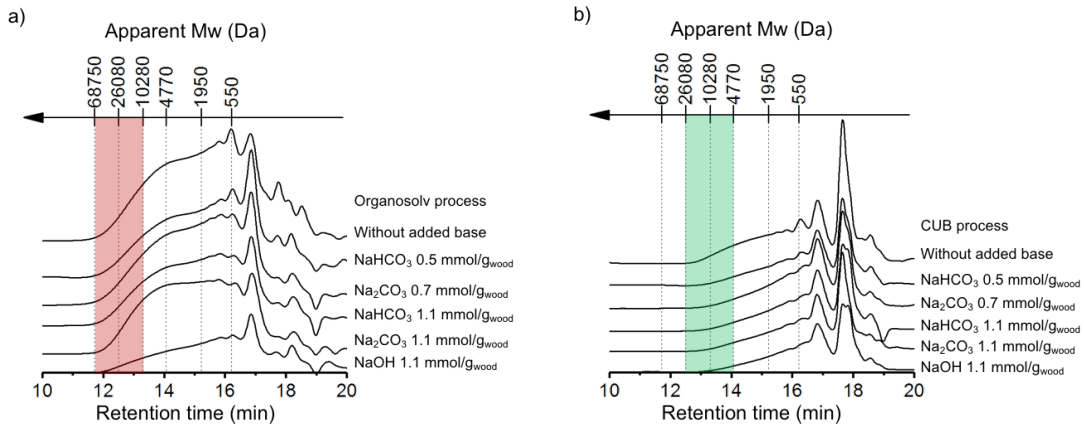


Figure 3-3 - GPC chromatograms for a) Organosolv lignin and b) CUB lignin streams obtained under various conditions (as described in Table 3-2).

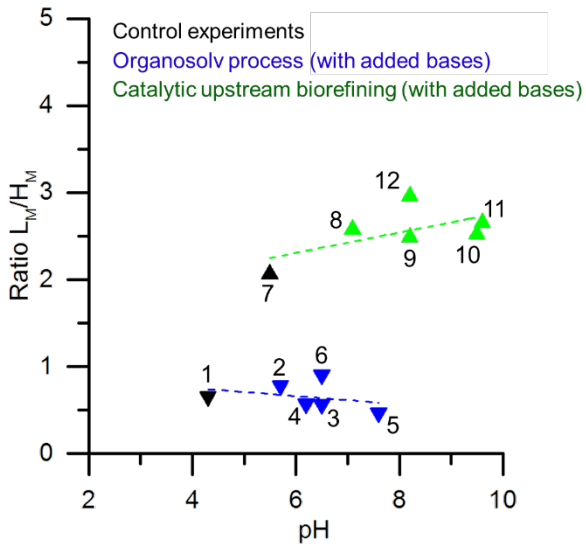


Figure 3-4 - Ratio L_M/H_M vs. pH of liquor for lignin streams obtained under various conditions (as described in Table 3-2).

Elemental analysis (CHNS) was performed on lignin products to provide insights into the effect of neutral to alkaline conditions on their elemental composition. A Van Krevelen plot comparing the organic fractions resulting from Organosolv lignins and CUB lignin oils regarding molar O/C and H/C ratios is shown in Figure 3-5. Organosolv lignins and CUB lignin oils are clustered into two distinct groups, according

to their H/C ratio. Indeed, the lignin products isolated from catalytic experiments possess higher values of H/C ratio than their Organosolv counterparts, as a consequence of the incorporation of hydrogen promoted by the Raney Ni-catalysed H-transfer processes (hydrogenation and hydrogenolysis) occurring on the released lignin fragments. The addition of a base in CUB fractionation promoted a slight increase in the H/C ratio of the lignin oil products, following a sequence not related to the pK_b of the base additive: no base (1.55±0.05, entry 7 in Table 3-2) < Na_2CO_3 (1.65±0.06 and 1.72±0.02, entries 9 and 11) < $NaHCO_3$ (1.80±0.01 and 1.76±0.02, entries 8 and 10) < NaOH (1.81±0.02, entry 12). The absence of a clear correlation between the basic strength of the added base and the increase of H/C ratio of lignin oil suggests that the relation between the type of base and the activity of Raney Ni might be due to an *indirect* effect. The H-content of Organosolv lignins was not affected by the addition of bases, as the H/C ratio was found to be 1.30±0.03 for all tests (entry 1-6). In turn, the O-content of lignins obtained with added bases was found to be lower than their counterpart obtained with no added base (decrease from O/C=0.40±0.01, entry 1, to 0.30±0.02, entry 2-6). A similar trend was observed for lignin oils obtained from CUB experiments, whose O/C ratio decreased from 0.33±0.01 to 0.27±0.01. Notably, the increased retention of hemicellulose in pulp achieved in neutral to weakly alkaline conditions limited the release of O-rich species derived from hemicellulose (i.e. sugars, polyols) in the soluble fraction, hence decreasing their overall O/C.

Previous studies on the activity of Raney Ni in presence of oxygenated compounds in 2-PrOH have highlighted the deactivation effects exerted by polyols[94] and primary alcohols ,[27, 89d] which inhibited the activity of the catalyst towards H-transfer reactions by competitive adsorption with 2-PrOH on its active sites. Alongside, sugars and sugar-derived molecules were found to decrease the activity of a Ru/C

catalyst towards the hydrodeoxygenation of lignin-derived phenolic compounds.[95] These pieces of evidence suggest the possibility that hampering the release of sugars released from hemicellulose degradation to the liquor favours the activity of Raney Ni towards H-transfer reactions. Indeed, the presence of Raney Ni promoted higher incorporation of hydrogen in lignin oils when bases were added to the CUB process. To better explore this possibility, the H/C ratio values obtained for lignin products were plotted against the extent of hemicellulose retention in the holocellulosic pulp (Figure 3-6). Notably, the H/C values for lignin oils produced in CUB experiments steadily increased together with the extent of hemicellulose retention in pulps. In comparison, the H/C values for Organosolv lignins are independent from the amount of hemicellulose released in the liquor. This evidence confirms the hypothesis of a correlation between the preservation of hemicellulose in the pulp and the activity of Raney Ni, thus related to an *indirect* effect that alkaline conditions exert on lignin depolymerisation reactions.

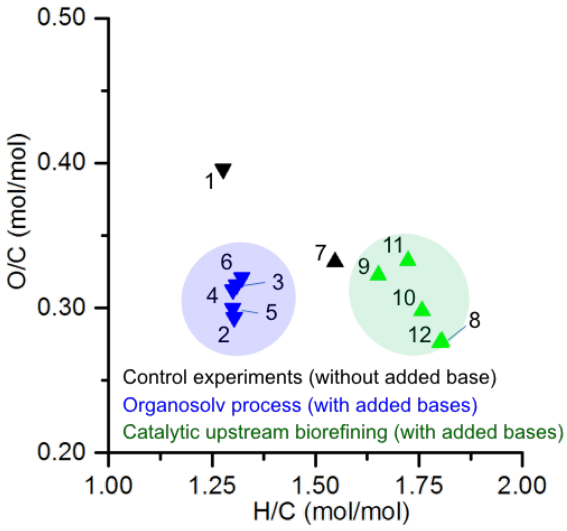


Figure 3-5 - Van Krevelen plot for Organosolv lignins (blue, numbers correspond to entries 1–6 in Table 3-2) and lignin oils from CUB (green, numbers correspond to entries 7–12 in Table 3-2).

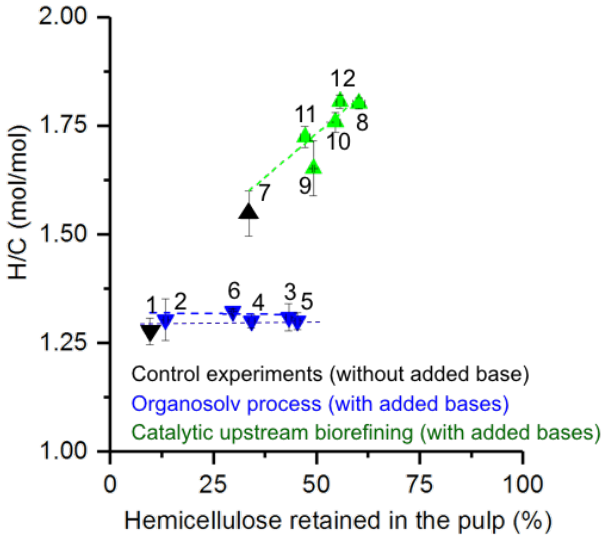


Figure 3-6 - H/C ratio versus quantity of hemicellulose retained in the pulp. For experimental conditions, the numbers alongside the symbol refer to the entries of Table 3-2.

To assess the variation of chemical composition, the lignin oil samples obtained from CUB with and without added NaOH were subjected to 2D-GCxGC-FID/MS. This technique was preferred over the 1D-GC techniques, which may oversimplify the characterisation of complex mixtures, such as the lignin oils produced by CUB.[7a] Indeed, 2D-GC techniques provide higher resolution and sensitivity than its monodimensional counterpart,[71, 96] as highlighted by the comparison between the 1D chromatogram of the lignin oil obtained from the CUB process (Figure 3-7a) and its 2D counterpart (Figure 3-7b). Whereas the 1D-chromatogram shows 4-propylphenols and 4-(3-hydroxypropyl)phenols as the only relevant components, resulting from the depolymerisation of lignin, the 2D-chromatogram also shows the presence of several minor components such as polyols and cyclohexanols and cyclohexane-1,2-diols, produced from hydrogenation of phenolic products and hydrodeoxygenation of their alkyl sidechains. These reactions contribute to the higher H-content observed for CUB

lignin oils (H/C=1.7) in comparison with that observed for Organosolv lignins (H/C=1.3).

An assessment of the differences in the composition of lignin oils was provided by quantification of target key compounds by 2D-GCxGC-FID, shown in Table 3-3. As previously discussed, the use of added bases in CUB led to increased activity of Raney Ni towards H-transfer reactions. Therefore, a comparison between hydroxyphenyl-, guaiacyl- and syringyl-units (compounds 1 and 2 in Table 3-3), and their hydrogenated counterparts (compounds 3) offers an effective assessment of the extent of HDO processes. The lignin oil from CUB without added base yielded a content of monophenols higher than that obtained from the process with added NaOH (12.9 and 7.2wt%, respectively). Conversely, the fraction of hydrogenated compounds was found to be relevantly increased when bases were used in the process (from 0.8 to 4.4 wt%), hence demonstrating more extensive hydrogenation occurring in presence of bases i.e. when a lower amount of sugars was present in the liquor.

From the contents of 4-(3-hydroxypropyl)phenols (compounds 2 in Table 3-3), the molar ratios of S/G/H phenolic units are estimated at 53.2:42.3:4.5 (CUB without added base) and 64.5:30.9:4.6 (CUB with added NaOH), both resembling the ratio obtained for native lignin of Poplar wood (S/G/H = 61.9:37.8:0.3).[97] Previous studies demonstrated high activity of Raney Ni towards demethoxylation of lignin model compounds.[89d] However, this reaction seems to be suppressed in CUB, as the fraction of H-subunits is still considerably inferior in comparison with the S- and G-counterparts.

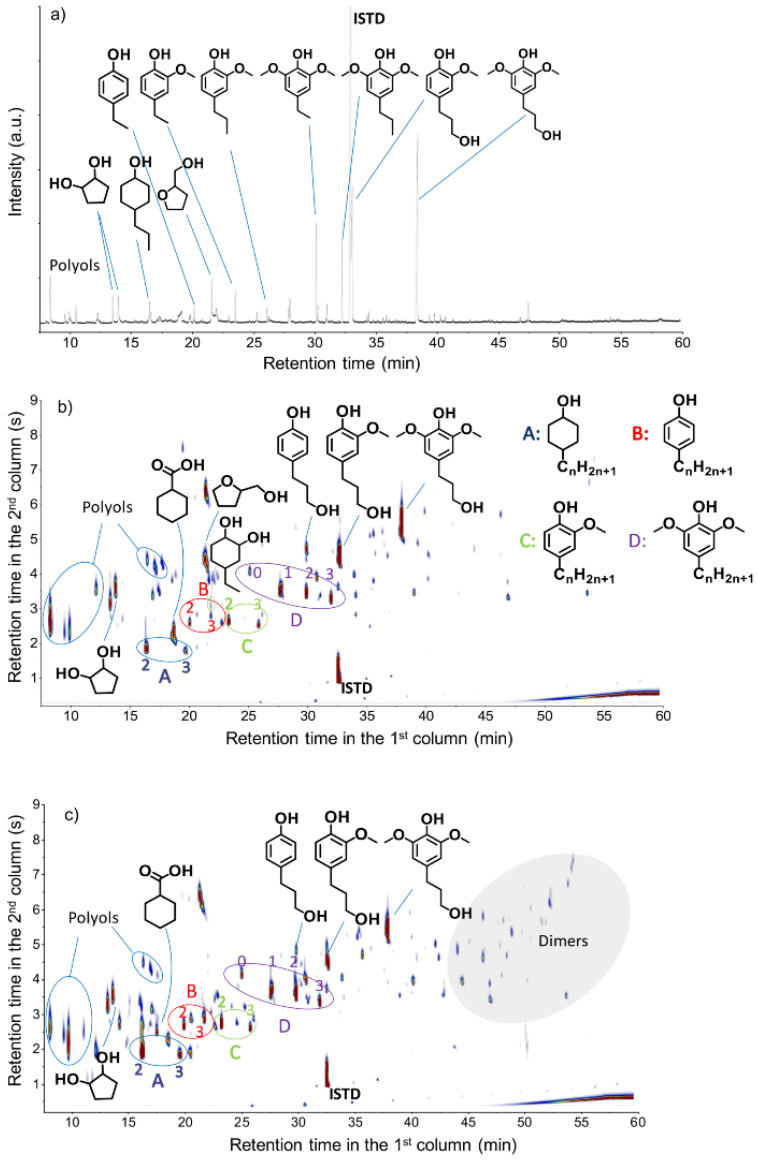
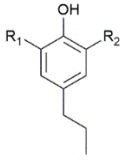
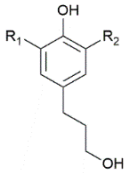
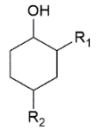


Figure 3-7 - (a) 1D-GC-FID and (b) 2D-GCxGC-FID images of lignin oil obtained from catalytic upstream biorefining process without added NaOH (entry 7 in Table 3-2), and (c) 2D-GCxGC-MS image of lignin oil obtained from the process with added NaOH (entry 12 in Table 3-2). The numbers next the spots correspond to the number of C atoms ('n') present in side-chain of the generic structure.

Table 3-3 - Content of selected components occurring in the lignin oil obtained from CUB with and without added NaOH.

Selected component		Content (wt%)	
		CUB without added NaOH	CUB with added NaOH
	R_1: H R_2: H	0.1	0.1
	R_1: H R_2: OCH_3	0.2	0.4
	R_1: OCH_3 R_2: OCH_3	1.7	1.0
	R_1: H R_2: H	0.4	0.2
	R_1: H; R_2: OCH_3	4.3	1.6
	R_1: OCH_3 R_2: OCH_3	6.2	3.9
	R_1: H R_2: CH_2CH_3	0.5	3.5
	R_1: OH R_2: CH_2CH_3	0.1	0.4
	R_1: H R_2: $CH_2CH_2CH_3$	0.2	0.5
Sum of selected compounds		13.7	11.6

3.3 Conclusions

The perturbation of the acid catalysis in the lignocellulose fractionation provided new insights into the role that the pH of the extraction media plays on the process. Indeed, the neutralisation of the acidic species released upon cooking Poplar wood in an aqueous mixture of 2-PrOH resulted in a drastic increase of the hemicellulose retention, which was enhanced five to six times in comparison to the process performed without added bases and exhibited a direct correlation with the pH of the liquor up to values of pH=7.5, after which the retention of hemicellulose plateaued. Due to the suppressed degradation of sugars from hemicellulose observed under these conditions, an increase in the activity of Raney Ni occurred, leading to a higher extent of hydrogenation on the phenolic rings deriving from lignin. Contrarily to what observed for the retention of hemicellulose, the delignification degree was found to be independent from the pH of the extraction medium, due to the existence of different pathways alternative to acid-catalysed solvolysis of β-ether lignin linkages.

In summary, this study has shown the potential of bases to indirectly improve the catalytic activity of Raney Ni through higher retention of hemicellulose in the pulps. Hence, new light is shed on the effect that monosaccharides exert on the catalytic selectivity in ECCL, which can be shifted from phenols to cyclohexanols. However, the full saturation of the aromatic ring is undesired for economical and practical reasons, as the hydrogenation of arenes requires an increase in hydrogen consumption, either as a hydrogen-donor or molecular hydrogen, naturally entailing an increase in the associated costs.

4. The role of the lignocellulose type in the lignin-first biorefining via H-transfer catalysed by Raney Ni

4.1 Introduction

Upon cooking lignocellulose in solvent mixtures containing water, the hemicellulose fraction undergoes deacetylation to release acetic acid in the liquor.[41b, 41g] Such acid catalyses the solvolysis of β-ether lignin linkages,[41f, 42a, 49c] to yield depolymerised lignin products, and the hydrolysis of hemicellulose, resulting in the release of hemicellulose-derived sugars, furfurals and formic acid,[41a, 41g, 42c, 43] with the latter further decreasing the pH of the medium. Importantly, the use of Raney Ni in lignin-first experiments for lignocellulose fractionation decreases the concentration of formic acid released in the process, hence decreasing the acidity of the medium and therefore limiting the degradation of the hemicellulose fraction.[41g, 89a, 89d]

As the acetylation levels depend on the intrinsic composition of the lignocellulosic matrix, the amount of acid released upon cooking depends on the type of wood. In fact, the quantity of acetyl groups in hardwoods (3-5 wt%) is higher than in softwoods (1-2 wt%).[98] In this perspective, higher performances in pulp delignification upon fractionation are associated with types of wood presenting higher acetylation levels, such as hardwoods (e.g. Poplar). In turn, softwoods (e.g. Spruce) and grasses (e.g. Sugar Cane Bagasse, SCB) typically show much lower performances towards lignin extraction.[28a]

Thus, a viable possibility to enhance the extraction of lignin from the lignocellulosic feedstocks could be represented by the promotion of their deconstruction through the use of acid additives in fractionation. Indeed, the addition

of acids in lignocellulose fractionation is known to improve lignin extraction and depolymerisation,[42d, 49a, 49b, 65c, 75b, 75c, 99] whose extent is directly correlated to the dissociation constant of the acid employed.[100] On the other hand, increasing the acidity of the liquor naturally leads to an increase of the degradation of the carbohydrate fraction, decreasing the quality of the produced pulps.[72a, 75b]

Despite these general notions, the impact of adding carboxylic acids additives during fractionation of softwoods still remains poorly understood. Herein, the influence of the acetylation level of the lignocellulosic feedstock, and therefore the acidity of the lignin-extracting liquor, on the performances of the CUB process was investigated. In the first section, the amount of acidic species released from Poplar wood, Spruce wood and SCB during fractionation is quantified through acid-base titration of the obtained liquor. The second section discusses the results obtained from Organosolv and CUB experiments at 180°C on Spruce and SCB, performed with acid additives in such a concentration to mimic the amount of acidic species released from Poplar wood fractionation, in an attempt to replicate its performances towards lignocellulose deconstruction. In the third section, the influence of the peculiar structure of SCB on its fractionation performances is investigated by treating this feedstock at temperatures in the range 180-240°C, in an attempt to replicate the enhancement of delignification obtained by increasing the process temperature for Poplar fractionation.[68] The acids employed in this study are: acetic acid (pK_a=4.76), naturally released during fractionation;[41b, 41g] formic acid (pK_a=3.77), which can act as source of hydrogen in a H-transfer based system;[41g, 65c, 101] or oxalic acid (pK_{a1}=1.25, pK_{a2}=4.14). For comparison, control experiments were also carried out with no added acid.

4.2 Results and discussion

4.2.1. Defining the acidic conditions for the simulation of acetylation levels in Poplar

To explore the working hypothesis that the higher acetylation level (i.e. higher content of acetic acid released during fractionation) of Poplar wood would be causing the higher delignification observed for this type of lignocellulose, compared to softwood and grasses, the acid content in the liquors obtained from Organosolv and CUB experiments performed on these feedstocks was determined by acid-base titration against 0.1 M solution of NaOH in 2-PrOH/H_2O (7:3 v/v). The quantities of acid measured by titration are listed in Table 4-1. As expected, the highest amount of acid formed in fractionation is observed for the Organosolv experiment carried out on Poplar wood (0.95 $mmol_{acid}/g_{wood}$), whereas fractionation of SCB and Spruce resulted in the formation of a smaller quantity of acid (i.e. respectively 30% and 70% lower).

The total amount of acids produced from Poplar wood during the Organosolv experiment was chosen as the target value for Spruce and SCB fractionation experiments in this study, as this value is truly representative of the acetylation level in Poplar. Indeed, the amount of acids found in the corresponding CUB liquor is altered by processes catalysed by Raney Ni.[41g] To simulate the same formation of acid obtained from Poplar wood during Organosolv, all Organosolv and CUB experiments of Spruce and SCB were performed with an amount of added acid sufficient to ensure that 0.95 $mmol_{acid}/g_{wood}$ were available in the liquor during fractionation.

Table 4-1 - Acid content in liquors produced from fractionation experiments of Poplar, Spruce wood and Sugar Cane Bagasse, and amount of acid added in fractionation experiments performed in this work. [a] Equivalent to 2.1 ± 0.3 mM; [b] Equivalent to 3.1 ± 0.4 mM; [c] Equivalent to 4.6 ± 0.3 mM.

	Acid Content released ($mmol/g_{wood}$)		**Added acid required ($mmol/g_{wood}$)**	
Wood	**Organosolv**	**CUB**	**Organosolv**	**CUB**
Poplar	0.95 ± 0.03	0.50 ± 0.01	-	-
SCB	0.66 ± 0.03	0.66 ± 0.02	0.29 ± 0.04[a]	0.29 ± 0.04[a]
Spruce	0.50 ± 0.03	0.30 ± 0.01	0.44 ± 0.05[b]	0.65 ± 0.04[c]

4.2.2 Effect of acidic conditions on lignocellulose deconstruction

Based on the results outlined in Table 4-1, three sets of experiments for Spruce wood and SCB were designed: the first set (control experiments) comprises Organosolv and CUB experiments performed without added acids. The second set (reference experiments) involves Organosolv experiments performed with added acetic acid, formic acid or oxalic acid. Finally, the third set consists of CUB experiments performed with added acids. The results obtained for all three sets of experiments are summarised in Table 4-2. In assessing the performances of the fractionation process, the carbon balance is a more accurate indicator than the mass balance.[28a, 68] However, the latter also provides useful insight into the acid-assisted fractionation, as the weight yield of the soluble products increases with the acidity of the liquor at the expense of the weight yield of pulp. Indeed, lowering the liquor pH enhances degradation of the carbohydrate matrix, leading to increased dissolution of holocellulose-derived products (i.e. oligo- and monosaccharides, lignin fragments) in the soluble fraction.[72a, 75c]

Both the degree of delignification and the removal of hemicellulose are fundamental parameters when evaluating the performances of fractionation. Indeed, the applicability of the pulps obtained from fractionation is intrinsically linked to their residual lignin content, which can hinder further downstream treatments (e.g. enzymatic hydrolysis) and, therefore, requires extensive removal.[7a, 33, 91, 102] Conversely, the removal of hemicellulose depends on the application required for the solid fraction, as it is sought-after in the perspective of biorefinery (i.e. full recovery of the whole content of biomass), while being undesired when highly pure cellulose fibres are preferred (i.e. production of paper). The performances in delignification and hemicellulose removal for the experiments performed in this work are summarised in Table 4-2. For a better visualisation of the effect of the liquor acidity on pulp delignification and hemicellulose removal, these parameters are plotted with the total amount of total H^+ available in the liquor (i.e. sum of H^+ obtained from the dissolution of acid additive in the liquor, and from the formation of acetic acid upon fractionation) in Figure 4-1.

The degradation of hemicellulose observed in fractionation experiments overall increased steadily with the acidity of the liquor, hence highlighting a positive contribution of the added acids to lignocellulose deconstruction. In turn, a direct correlation between the amount of total H^+ in the liquor and pulp delignification could not be found, suggesting that the extraction of lignin from the lignocellulosic matrix is a more complex process depending on other process variables.

Importantly, the increasing effects obtained from catalytic experiments for hemicellulose removal (11% and 3% increase respectively for SCB and Spruce) were less steep than those observed for Organosolv experiments (17% and 26% increase respectively for SCB and Spruce). Indeed, the effect that the added acids exerts on

this parameter is hampered in CUB experiments, as Raney Ni lowered the overall concentration of acids in the liquor by stopping the degradation channel of holocellulose-C6 sugars to 5-hydroxymethylfurfural (which may decompose to formic acid and levulinic acid) via sugar hydrogenation,[41g] or through direct reductive conversion of the added formic acid.[89a, 89d] The resulting decrease in the overall acid content affects the deacetylation equilibrium, leading to a reduced formation of acetic acid during fractionation.[41g]

Clearly, the low concentrations of acids employed in the catalytic experiments presented in this chapter exhibited a lower influence on the deconstruction performances, compared to those reported in past works using higher concentrations of phosphoric acid (ranging from 12 to 50mM[75c] or 100mM[72a]).

Table 4-2 - Results obtained from the Organosolv process and Catalytic Upstream Biorefining of Sugar Cane Bagasse and Spruce wood. General conditions: [a] Organosolv process: lignocellulose (17 g, chips or fibres), acid (as listed), 2-PrOH:H_2O (140 mL, 7:3 v/v), 180 °C for 3 h; [b] CUB: lignocellulose (17 g, chips or fibres), acid (as listed), Raney Ni (10 g), 2-PrOH:H_2O (140 mL, 7:3 v/v), 180 °C for 3 h; [c] The amount of oxalic acid in mmol to be employed in fractionated experiments was halved, as oxalic acid is a diprotic acid. [d] Composition of unprocessed SCB: 41% cellulose; 18% hemicellulose; 36% lignin. Spruce wood: 44% glucans; 24% xylans; 34% lignin. [e] Calculations reported in the Methods section.

Entry	Lignocellulose	Acid	Acid amount (mmol/g_{wood})	Weight yields of the isolated fractions (%,±1)		Pulp composition (wt%, daf, ±2 wt%)[d]			Overall performance (±2%)	
				Pulp	Soluble products	Glucans	Xylans	Lignin	Delignification (%)[e]	Hemicellulose removal (%)[e]
Organosolv process[a]										
1	SCB (control)	-	-	71	24	48	15	35	31	43
2	SCB (reference 1)	Acetic	0.29	69	23	49	16	34	34	41
3	SCB (reference 2)	Formic	0.29	69	26	47	14	32	39	48
4	SCB (reference 3)	Oxalic	0.14[c]	63	33	55	12	32	43	60
5	Spruce (control)	-	-	80	15	49	20	31	28	31
6	Spruce (reference)	Acetic	0.44	76	17	49	21	29	35	33
7	Spruce (reference)	Formic	0.44	68	24	49	18	26	48	48
8	Spruce (reference)	Oxalic	0.22[c]	65	27	57	16	27	49	57

Table 4-3 (continued) - Results obtained from the Organosolv process and Catalytic Upstream Biorefining of Sugar Cane Bagasse and Spruce wood. General conditions: [a] Organosolv process: lignocellulose (17 g, chips or fibres), acid (as listed), 2-PrOH:H_2O (140 mL, 7:3 v/v), 180 °C for 3 h; [b] CUB: lignocellulose (17 g, chips or fibres), acid (as listed), Raney Ni (10 g), 2-PrOH:H_2O (140 mL, 7:3 v/v), 180 °C for 3 h; [c] The amount of oxalic acid in mmol to be employed in fractionated experiments was halved, as oxalic acid is a diprotic acid. [d] Composition of unprocessed SCB: 41% cellulose; 18% hemicellulose; 36% lignin. Spruce wood: 44% glucans; 24% xylans; 34% lignin. [e] Calculations reported in the Methods section.

Entry	Lignocellulose	Acid	Acid amount (mmol/g_{wood})	Weight yields of the isolated fractions (%,±1)		Pulp composition (wt%, daf, ±2 wt%)[d]			Overall performance (±2%)	
				Pulp	Soluble products	Glucans	Xylans	Lignin	Delignification (%)[e]	Hemicellulose removal (%)[e]
CUB[b]										
9	SCB (control)	-	-	69	24	53	15	29	44	42
10	SCB	Acetic	0.29	65	28	55	16	29	48	43
11	SCB	Formic	0.29	63	22	49	15	31	44	47
12	SCB	Oxalic	0.14[c]	61	28	50	15	28	51	51
13	Spruce (control)	-	-	86	13	54	17	27	32	37
14	Spruce	Acetic	0.65	75	21	45	19	28	37	38
15	Spruce	Formic	0.65	78	17	51	19	30	32	38
16	Spruce	Oxalic	0.32[c]	75	21	50	19	25	46	40

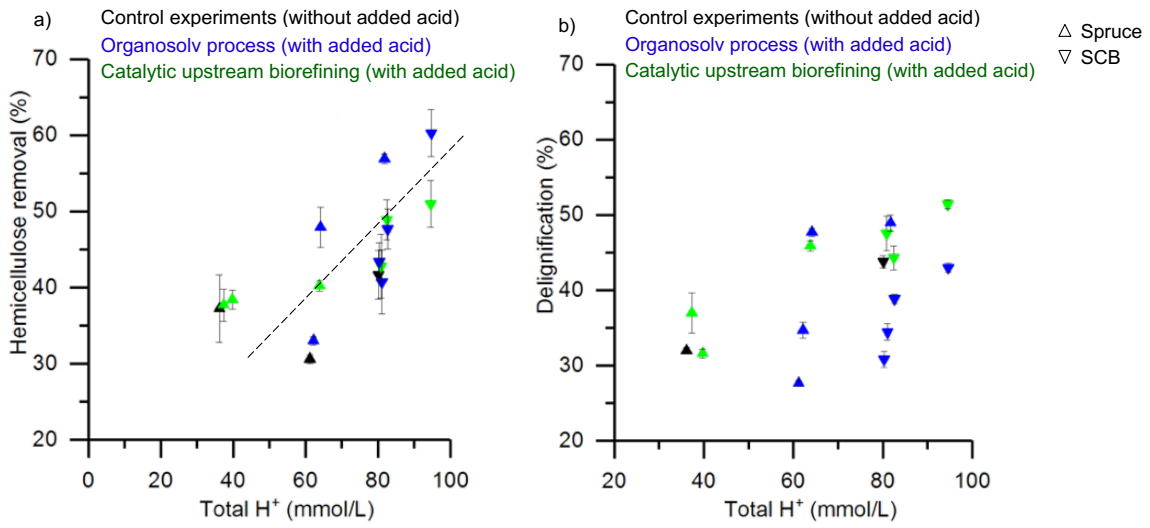


Figure 4-1. Total H^+ in reaction solution vs. (a) hemicellulose removal and (b) delignification obtained from the Organosolv and CUB process carried out under varied conditions (as described in Table 4-2). The content of total H^+ in the liquor was calculated as the sum of H^+ obtained from the dissolution of acid additive in the liquor, and from the formation of acetic acid upon fractionation.

To assess the morphological modifications occurring on the wood structure after fractionation experiments, digital microscopy was performed on untreated SCB fibres and Spruce chips, and the corresponding pulps obtained from Organosolv and CUB experiments. In the set of experiments with added acids, only the micrographs of pulps produced from fractionation experiments with added oxalic acid were presented, as they exhibited structural modifications similar to those observed on pulps produced with added formic or acetic acid.

Untreated SCB showed a highly compact and smooth surface with thick walls, covered in dark brown stains attributable to residual extractives[103] (Figure 4-3a-c). Expectedly, Organosolv and CUB processes degraded the lignocellulosic matrix as a result of the high temperature applied and the mechanical stress due to stirring. Consequently, the pulp structures appeared looser than in the untreated SCB, with

thin fibres detached from the main body of the structure, grooves and cracks generated after fibre disruption, and swelling attributable to solvolytic processes during fractionation. Organosolv test with added oxalic acid showed loosening and detachment of the fibre structure due to enhanced hydrolysis of the carbohydrate and lignin structures in acidic conditions (Table 4-2).

Spruce wood showed a compact structure of fibres crossing the chip both length-wise and perpendicularly, covered with dark particulate material likely deposited on the surface after knife-milling[104] (Figure 4-2b). Higher magnification showed the presence of pits (highlighted by arrows in Figure 4-2d) acting as channels for fluid transportation between cells, which can behave as access points for the lignin-extracting liquor during fractionation.[104] Similarly to what observed for SCB, the structure of Spruce loses cohesion after Organosolv and CUB treatment, with grooves appearing between consecutive fibres (Figure 4-4a-c). Dark particles were also found on the surface of CUB pulps, likely a residual content of Raney Ni that could not be removed during the catalyst recovery step. However, the amount of inorganics only accounted for less than 0.5wt% of the pulp composition. Organosolv test with added oxalic acid showed the presence of small fibrous residues throughout the whole chip (Figure 4-4b), which were not found in tests performed without added acid, suggesting a homogeneous “peeling” of the fibres. Importantly, Organosolv pulps show a more intense brown coloration than those obtained from CUB, which in turn presented a grey-white shade resulting from the higher degree of delignification. Despite superficial degradation observed for Spruce wood, the core structure of the chip, obtained by splitting the processed chip along its longitudinal axis and imaging the inner surface, still displayed a compact structure closely resembling untreated Spruce, evidencing

limited penetration of the solvent inside the lignocellulosic matrix regardless of its acidity (Figure 4-5).

Overall, these results show that the morphology of the lignocellulose plays a crucial role in fractionation, as the higher delignification observed for Spruce wood was related to the fact that its structure is generally more accessible for solvolytic processes than that of SCB, which in turn presented a more rigid and compact structure harder to penetrate.

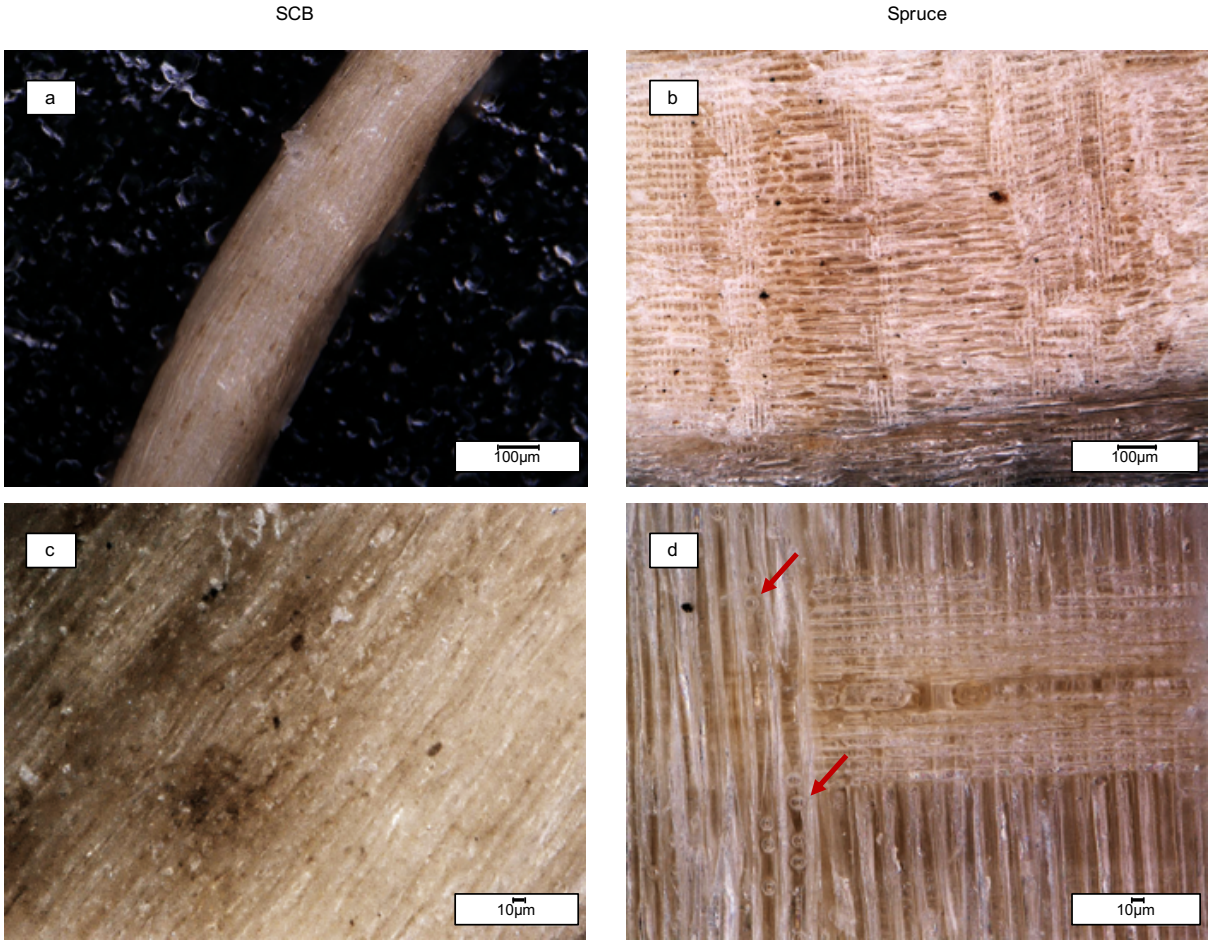


Figure 4-2. Digital microscopy images of SCB (a-c) and Spruce wood (b-d). Pits highlighted with red arrows.

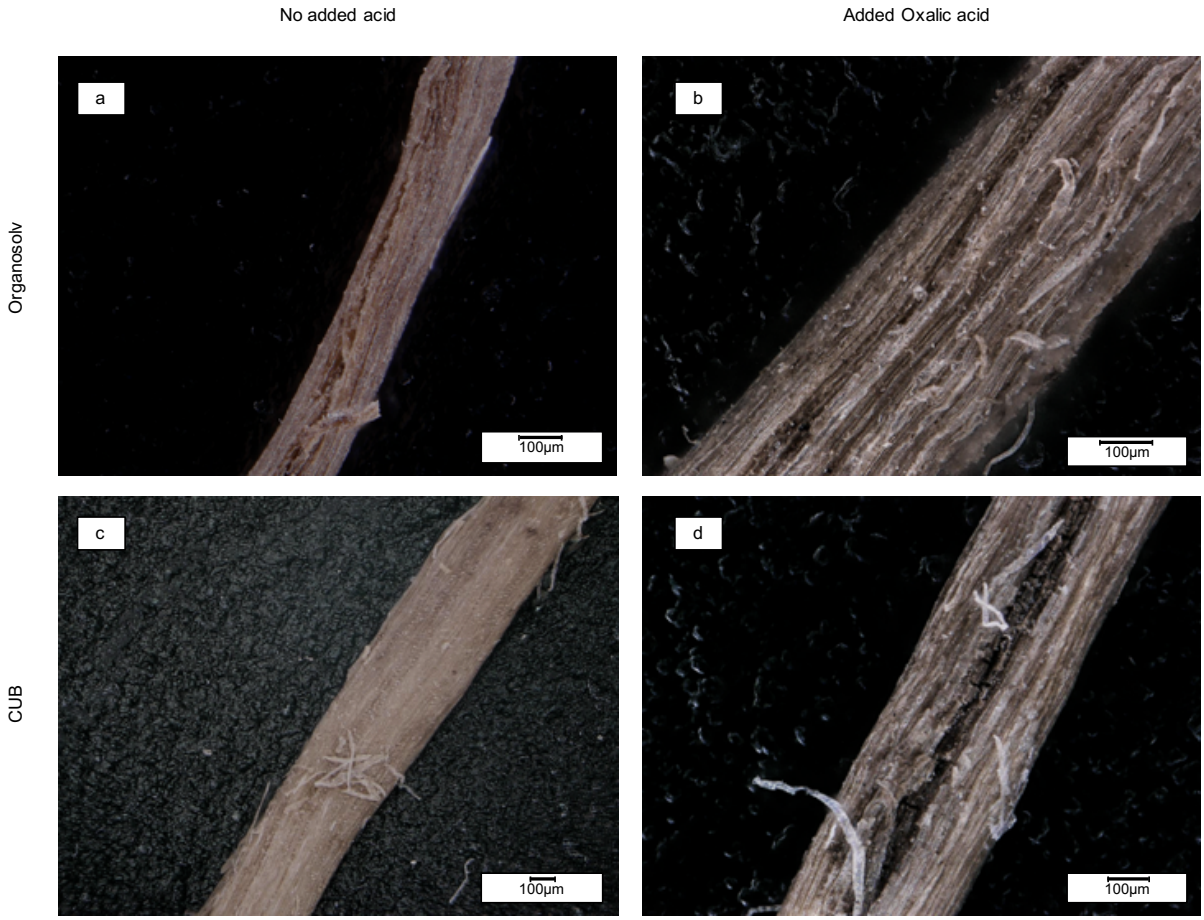


Figure 4-3. Digital microscopy images of Organosolv (top) and CUB pulps (bottom) obtained from Sugar Cane Bagasse. a-c) no added acid; b-d) oxalic acid.

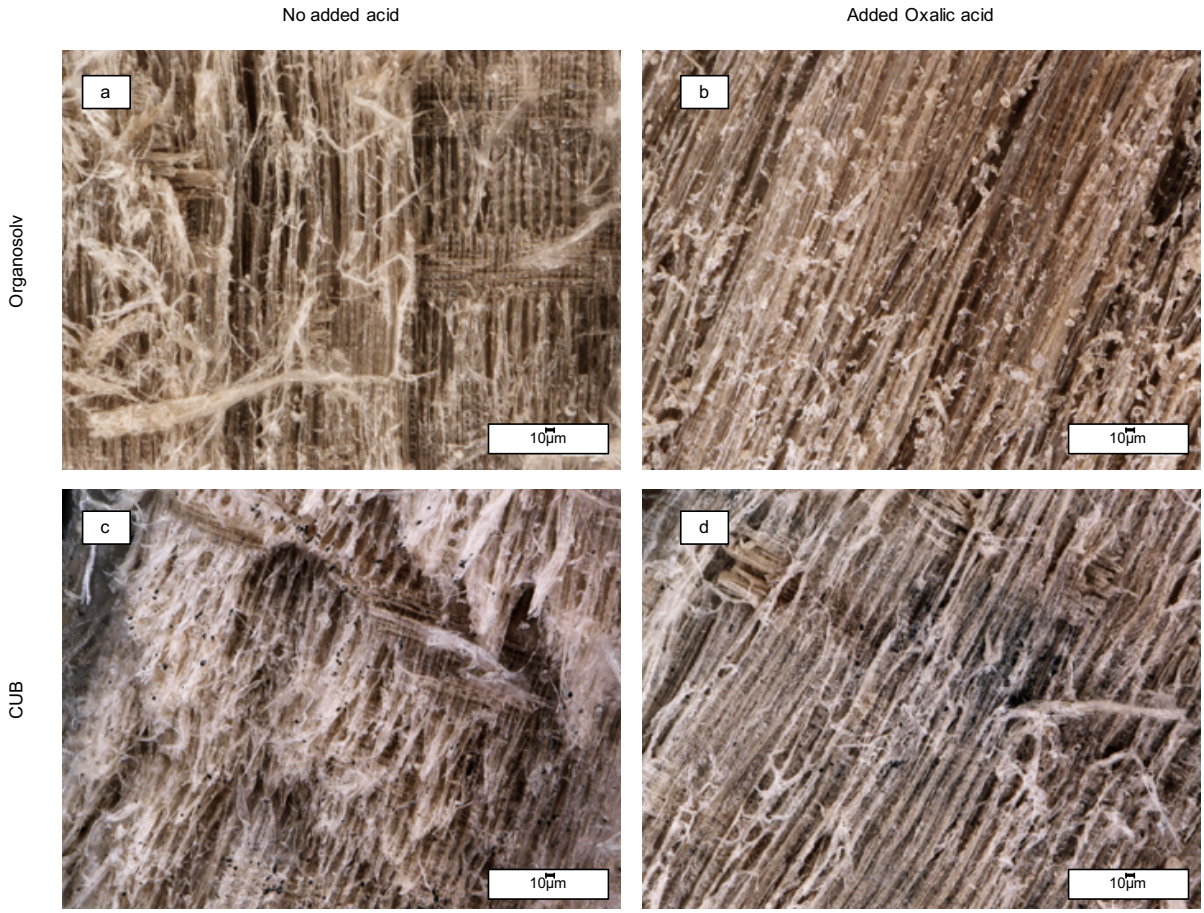


Figure 4-4. Digital microscopy images of Organosolv (top) and CUB pulps (bottom) obtained from Spruce wood. a-c) no added acid; b-d) oxalic acid.

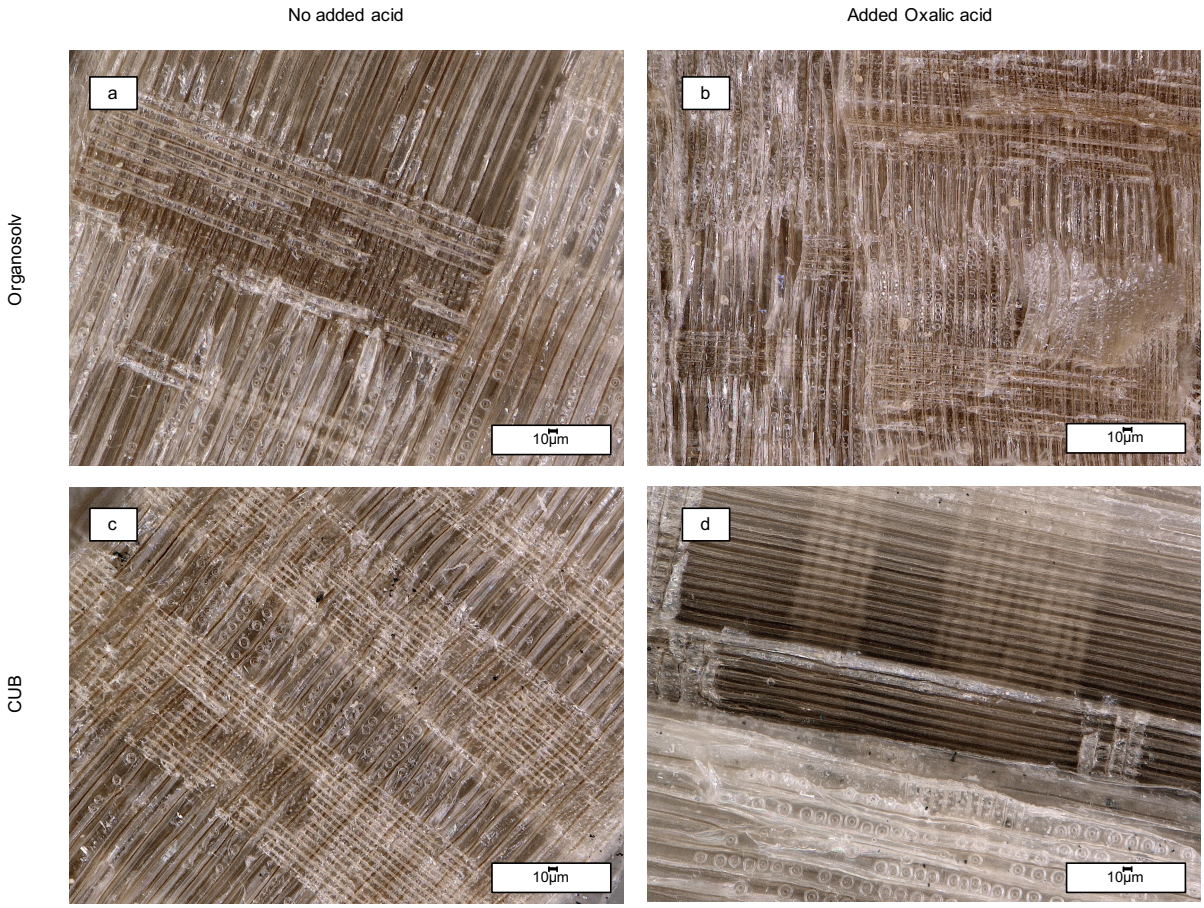


Figure 4-5. Digital microscopy images of the inner structure of Organosolv (top) and CUB pulps (bottom) obtained from Spruce wood. a-c) no added acid; b-d) oxalic acid.

4.2.3 Effect of acidic conditions on the properties of lignin streams

To assess the depolymerisation extent of lignin during fractionation, the lignin products from Organosolv and CUB processes were analysed by gel permeation chromatography (Figure 4-6). The addition of organic acids in Organosolv experiments led to a less intense signal of oligomers (i.e. peak at 17.8 min) and high-MW species from lignin (MW > 550 Da), as observed in previous work.[75c] However, the onset of the chromatograms remained almost unaltered regardless of the presence of acid additives, suggesting that recondensation occurring on lignin reactive fragments were still prevalent. In turn, Raney Ni-catalysed H-transfer reactions relevantly shifted the

apparent MW distribution of lignins towards lower values, as an indication of the lower recondensation owing to the passivation of reactive lignin fragments. In addition, Raney Ni also catalyses the reductive depolymerisation of lignin via hydrogenolysis of lignin's β-ethers.[7a, 28a] Importantly, the signal for high-MW fraction of CUB lignin streams (MW > 550 Da) became less intense when increasing the acid strength of the additive. Despite its ability to work as an H-donor,[65c] formic acid appeared to be the least effective acid in enhancing lignin depolymerisation, as its low starting concentration compared to the main H-donor in the system (i.e. 2-PrOH), and its partial decomposition on Raney Ni's surface [41g] mitigated its effects on the process.

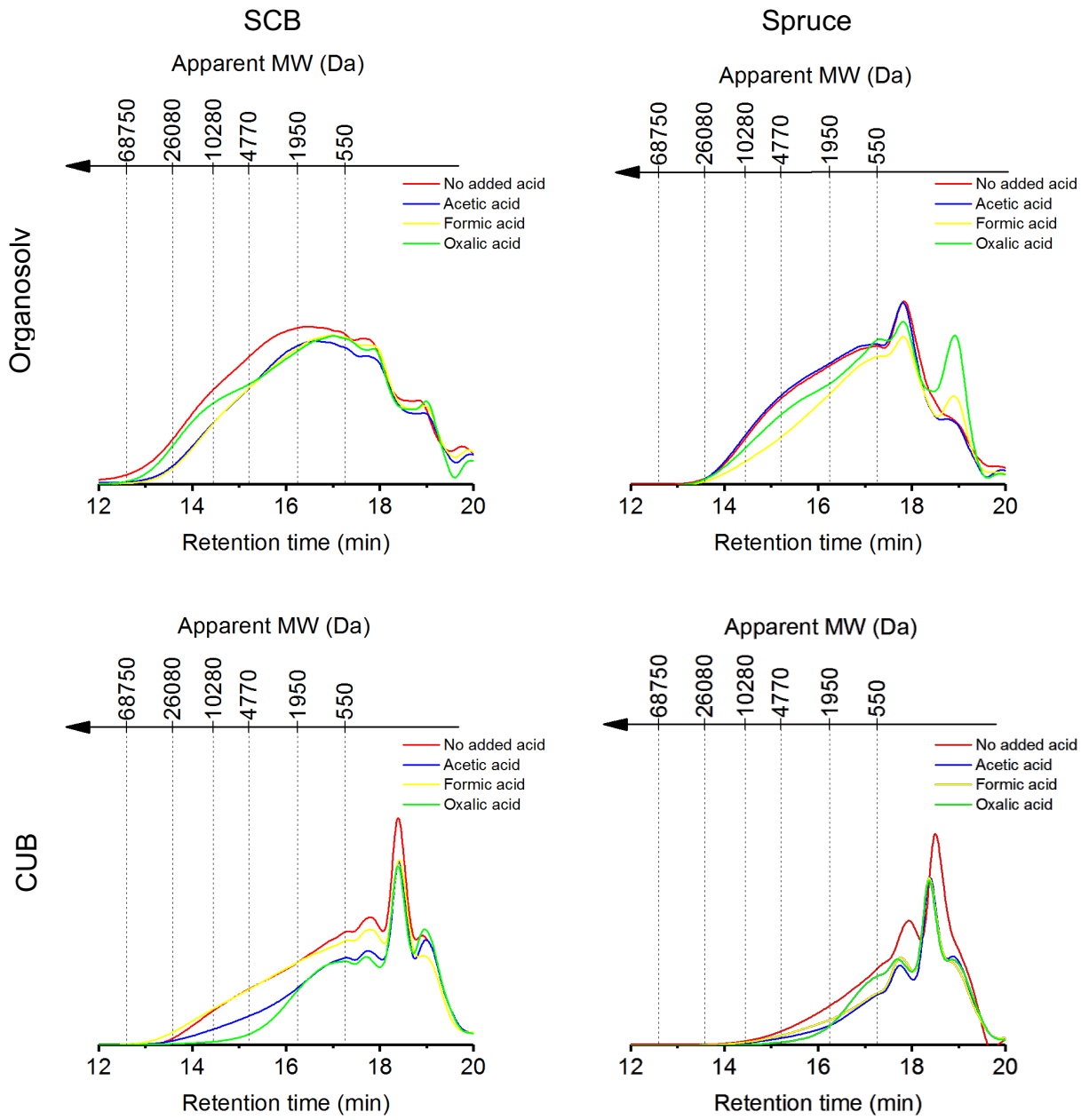


Figure 4-6. GPC chromatograms of lignin streams obtained from Organosolv and CUB experiments on Sugar Cane Bagasse and Spruce wood, carried out under varied conditions (listed in Table 4-2). The apparent MW is calculated relative to calibration with polystyrene standards.

The correlation between the acidic strength of the reaction medium and the extent of depolymerisation is better displayed in Figure 4-7, which reports the ratio low-MW species/high-MW species (L_M/H_M) against the total amount of H^+ in the liquor.

The production of lignin-derived low-MW species in Organosolv seemed not to be affected by the added acids, as L_M/H_M remained unaltered irrespectively of the lignocellulosic feedstock employed. Conversely, the ratio underwent a two-fold increase with increasing the total amount of H^+ in the liquor in CUB experiments. Although acids catalyse the cleavage of lignin's β-ether linkages,[72a, 75c, 105] their effect became apparent only in CUB experiments, where the products of acid-catalysed lignin depolymerisation are passivated through H-transfer reaction to stable species. Thereby, the recondensation process, which would otherwise counterbalance the depolymerisation, is thus hindered.

Importantly, the lignin products isolated from SCB contained a larger fraction of high-MW products in comparison to those from Spruce. This is due to the higher fraction of hydroxyphenyl units in SCB native lignin, which is more active towards recondensation reactions compared to guaiacyl-rich Spruce lignin.[4b, 18a]

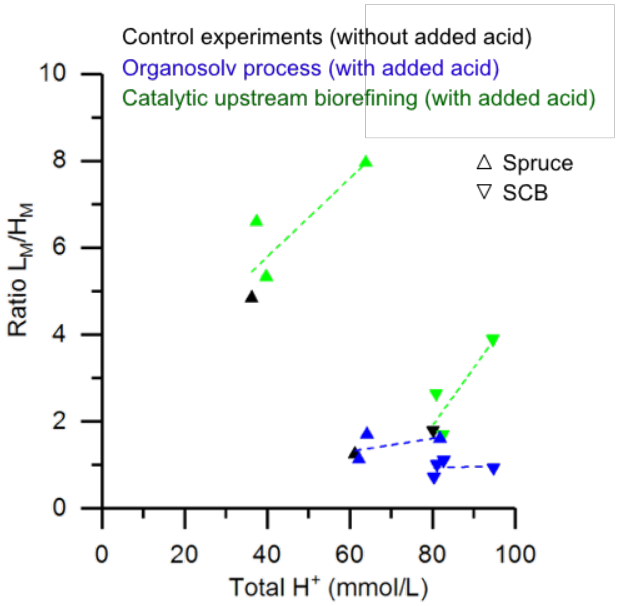


Figure 4-7 - Ratio L_M/H_M vs. total H^+ in the liquor, for lignin streams obtained under various conditions (as described in Table 4-2). The total content of H^+ in the liquor was calculated as the sum of H^+ obtained from the dissolution of acid additive in the liquor, and from the formation of acid upon fractionation (as reported in Table 4-1).

Notably, the activity of Raney Ni towards hydrodeoxygenation reactions was not affected by the acid additives, as the hydrogen content (measured by elemental analysis) and the product distribution (determined by GC-MS analysis) of the lignin streams obtained from CUB experiments did not produce any significant change when acidic conditions were employed in fractionation. Clearly, the activity of Raney Ni towards H-transfer reactions does not experience any *direct* inhibition by the acidic species in the liquor, as their total concentration (i.e. total H^+) is still too low to exert any significant effect.[41g, 106] Alongside, the minor increase in hemicellulose degradation (9% for SCB, 3% for Spruce, Table 4-2) leading to an increased release of sugars in the liquor did not produce any inhibiting effect on the activity of the catalyst, demonstrating the absence of any *indirect* deactivating effect of the added acids on Raney Ni.

4.2.4 Assessing the effect of reaction temperature on Sugar Cane Bagasse fractionation

In spite of the similar extent of deconstruction of the lignocellulosic matrix (Table 4-2), SCB showed poorer performances in lignin depolymerisation compared to Spruce, even after the addition of carboxylic acids. This observation may be related to a more extensive structure of lignin-carbohydrate-complexes (LCC), which covalently bound lignin to the polysaccharide matrix.[107] Therefore, to enhance the deconstruction of SCB, and consequently facilitate the transport of lignin fragments from the lignocellulosic matrix to the liquor, SCB was subjected to fractionation experiments at temperatures of 200-240°C. The results obtained from the fractionation experiments

performed under these conditions are reported in Table 4-4. As previously observed for Poplar,[68] increasing the process temperature from 180°C to 240°C led to a two-fold increase in the weight yield of soluble products from SCB at the expenses of the solid pulp, which in turn was extensively degraded independently of the presence of Raney Ni. At T>220 °C, the degradation of the lignocellulosic matrix to produce gaseous products (i.e. methane, carbon dioxide and propane, released at the end of the fractionation experiment)[28a, 68, 89d] and other volatile species (e.g. methanol, lost in the workup procedure)[28a] not recovered in this study, relevantly contribute to the mass balance, as they can account for ca. 6-12% of the initial weight of SCB in Organosolv experiments, and 9-26% in SCB.

The higher percentage of soluble products was correlated with a greater delignification in both the Organosolv and CUB pulps i.e. from 28-31% at 180°C to 72-90% at 240°C. Importantly, the pulps obtained from the CUB process at 200-240°C consistently experienced a higher delignification degree than that of Organosolv (Δdelignification=15-18%). Despite an extensive extraction of the absolute content of lignin from the lignocellulosic matrix, the pulps obtained at 240°C from both Organosolv and CUB were found to be rich in residual lignin. The more effective extraction of lignin was accompanied by the almost complete dissolution of hemicellulose in the liquor, as evidenced by a hemicellulose removal as high as 92-97%. The extensive disruption of the SCB fibres at high process temperatures was visible in the digital microscopy images of the pulps obtained after fractionation at 240°C (Figure 4-8). Indeed, under these conditions, the structure of the pulp fibres, whose length decreased from ca. 3cm to 0.2cm, appeared extensively ruptured and covered with microfractures. Under the investigated temperature range, it is apparent that the higher severity of experimental conditions enhanced the deconstruction of

SCB, hence promoting a better extraction of lignin from the lignocellulosic matrix. However, as this also led to extensive degradation of the solid pulp, defining a trade-off between lignin extraction and pulp preservation becomes crucial when defining the temperature of the process.

Table 4-4 - Results obtained from the Organosolv process and Catalytic Upstream Biorefining of Sugar Cane Bagasse with increased temperature. General conditions: [a] Organosolv process: Sugar Cane Bagasse (17 g, fibres), 2-PrOH:H_2O (140 mL, 7:3 v/v), 3 h, temperature as listed; [b] CUB: Sugar Cane Bagasse (17 g, fibres), Raney Ni (10 g), 2-PrOH:H_2O (140 mL, 7:3 v/v), 3 h, temperature as listed; [c] Composition of unprocessed SCB: 41% cellulose; 18% hemicellulose; 36% lignin. [d] Calculations reported in the Methods section.

Entry	Temperature (°C)	Weight yields of the isolated fractions (%)		Pulp composition (wt%, daf, ±2 wt%)[c]			Overall performance (±2%)	
		Pulp	Soluble products	Glucans	Xylans	Lignin	Delignification[d]	Hemicellulose removal[d]
Organosolv process[a]								
1	180	71	24	48	15	35	31	43
2	200	56	34	55	16	28	57	49
3	220	46	39	53	12	29	62	74
4	240	13	55	13	4	77	72	97
CUB[b]								
5	180	69	24	53	15	29	28	31
6	200	50	38	56	15	20	72	53
7	220	40	38	58	16	18	79	71
8	240	12	48	51	9	32	90	92

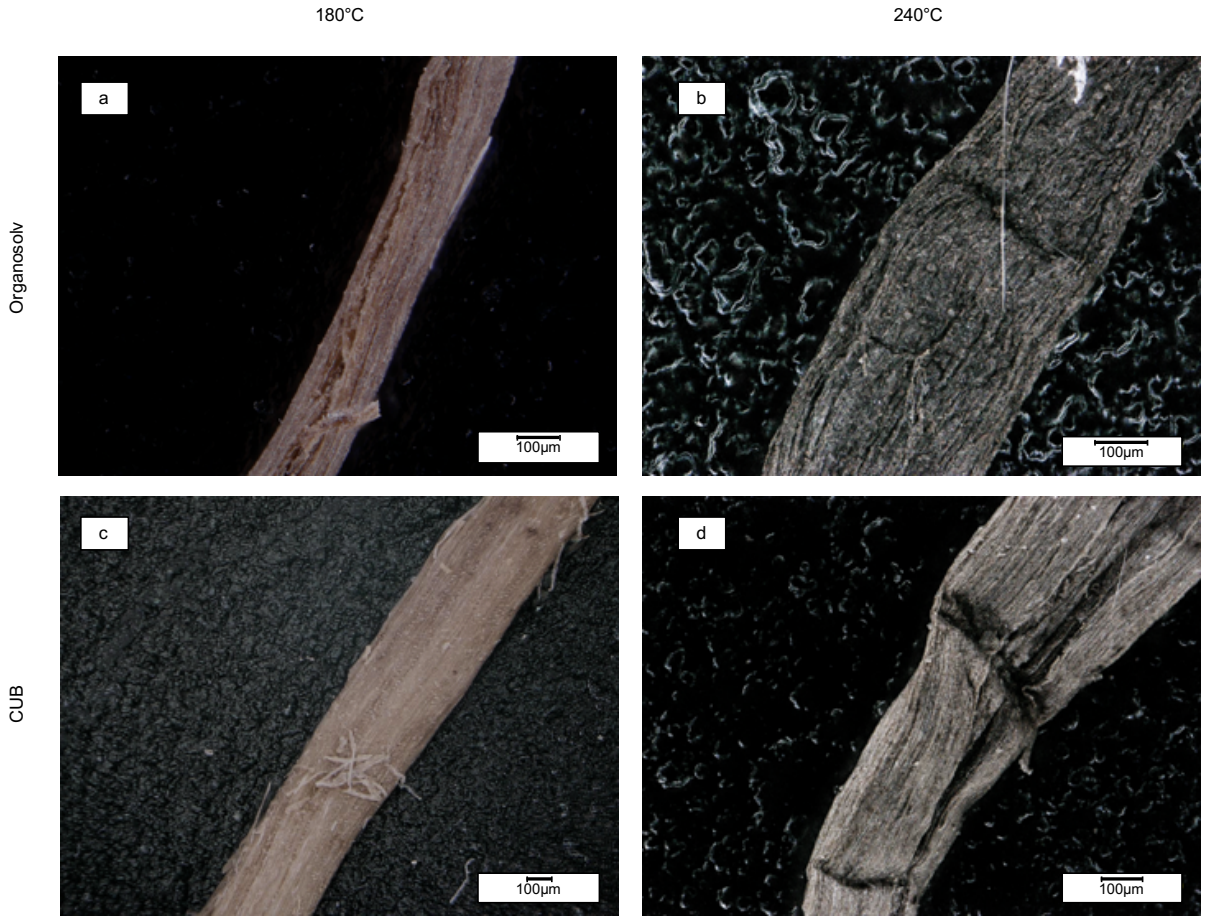


Figure 4-8 - Digital microscopy images of Organosolv (top) and CUB pulps (bottom) obtained from fractionation experiments on sugar bagasse performed at a-c) 180°C and b-d) 240°C.

Elemental analysis of the lignin products isolated from CUB experiments at 180-240°C (Figure 4-9) showed that the O/C ratio increased with the process temperature, due to the increased concentration of O-rich sugars and polyols in the liquor as a result of the increased degradation of hemicellulose (Table 4-2). Moreover, an increased amount of hydrogen was incorporated in the lignin stream with increasing temperature, suggesting that H-transfer reactions in CUB experiments occurred to a greater extent under these conditions. However, the increasing content of hemicellulose sugars and polyols found in the CUB liquor stream with increasing process temperature also contributes to elevated values of H/C ratio.

For the Organosolv lignin stream, depolymerisation of lignin was also found to be enhanced with increasing the process temperature, as the MW distribution was shifted to lower values (Figure 4-10), and the ratio L_M/H_M underwent a two-fold increase from 180 to 240°C (Figure 4-11). Interestingly, the increasing trend of L_M/H_M observed in Organosolv experiments from 180 to 220°C, due to a more extensive cleavage of the lignin linkages, was interrupted when further increasing the process temperature to 240°C, suggesting that at this temperature the recondensation reactions begin to outbalance the depolymerisation of lignin.

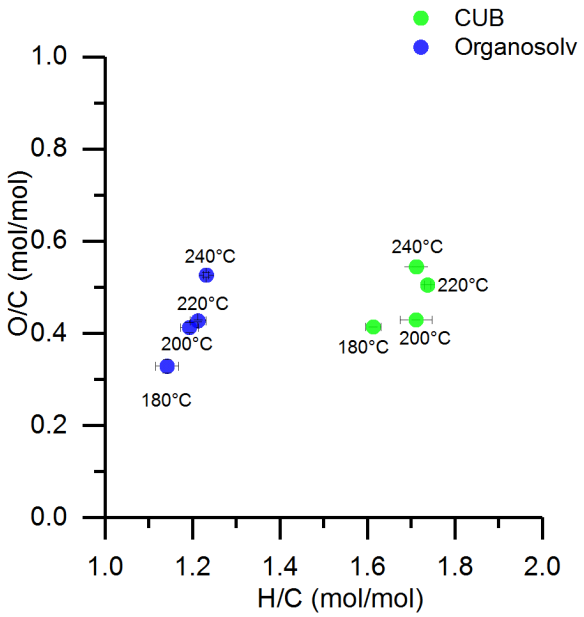


Figure 4-9 – Van Krevelen plots for lignin products obtained from the Organosolv and CUB process carried out on SCB under varied temperatures (as described in Table 4-4).

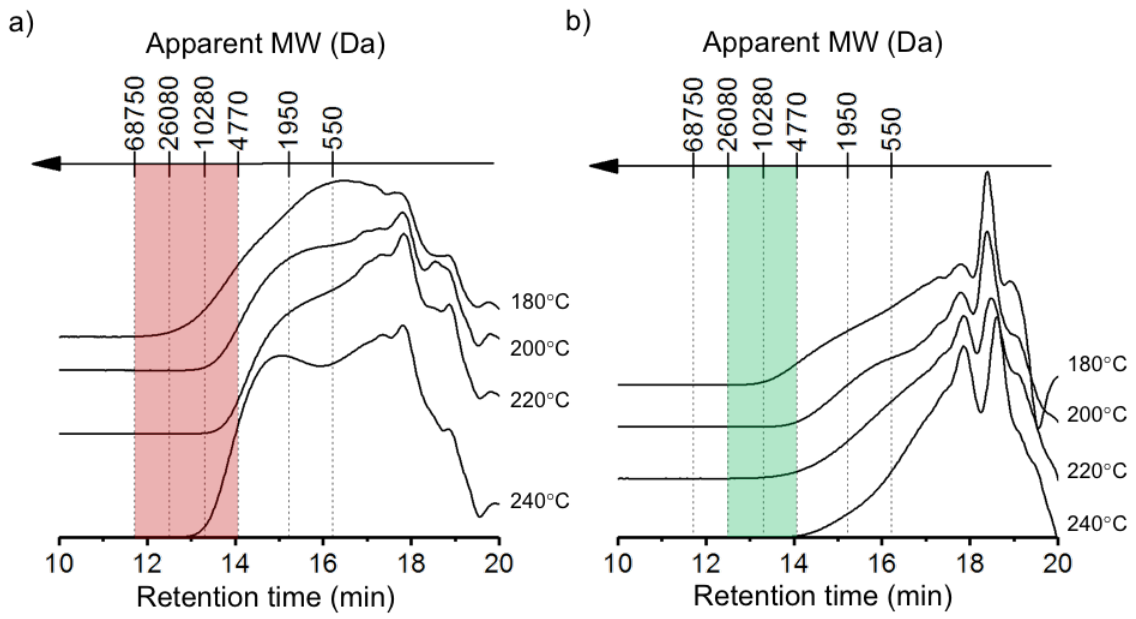


Figure 4-10 - GPC chromatograms of lignin streams obtained from the Organosolv and CUB process carried out on SCB under varied temperatures (as described in Table 4-4). The apparent MW is calculated relative to calibration with polystyrene standards.

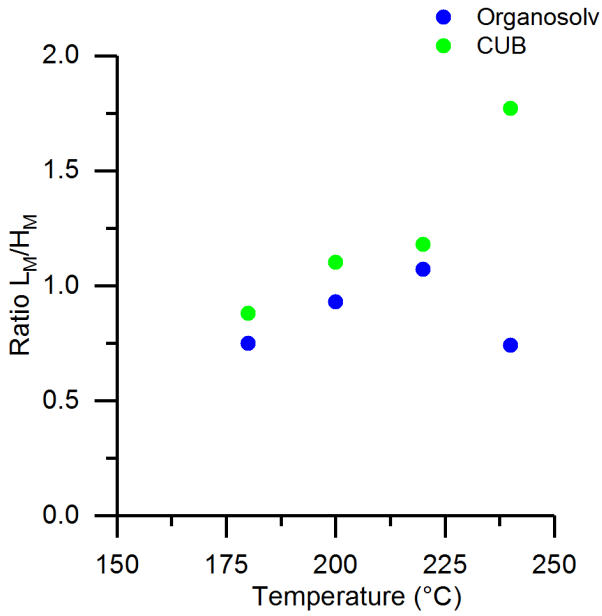


Figure 4-11 - Ratio L_M/H_M vs. temperature, for lignin streams obtained under various conditions (as described in Table 4-4).

4.3 Conclusions

Based on the hypothesis that the acetylation levels of the lignocellulosic feedstock, and consequently the acidity of the lignin-extracting liquor, are directly correlated to the extent of lignocellulose deconstruction, the lignin-first process on Sugar Cane Bagasse and Spruce wood was investigated by adding organic acids to the liquor, in such an amount to mimic the acetylation levels of Poplar wood, which typically grants high performance in pulp delignification.

Catalytic experiments allowed the isolation of extensively depolymerised lignins from both SCB and Spruce. This demonstrates that Raney Ni effectively catalyses lignin depolymerisation through H-transfer reactions on both the H-rich (i.e. SCB) and G-rich (e.g. Spruce) lignins, which are more typically prone to recondensation than S-rich lignins from hardwood.[7a, 28a] Overall, increasing the acidity of the reaction medium was conducive to enhanced deconstruction of the lignocellulosic material, as it improved extraction of lignin from the lignocellulosic matrix and subsequent depolymerisation. However, SCB showed poorer performances in lignin depolymerisation compared to Spruce wood. This singular behaviour is known to be related to the structure of native SCB lignin.[4b, 18a, 107]

To promote biomass deconstruction and increase lignin's accessibility, SCB fractionation was tested at higher process temperatures in order. Temperatures as high as 240°C enhanced the removal of lignin fragments from the SCB structure in a non-selective manner, as the delignification extent of pulps increased regardless of the presence of Raney Ni. Indeed, the higher severity conditions promoted by the increased reaction temperature promoted an extensive disruption of the SCB fibres.

In conclusion, these pieces of evidence highlight the crucial role that the structure of biomass plays in fractionation.

5. Evolution of lignin depolymerisation over time in lignin-first biorefining via H-transfer catalysed by Raney Ni

5.1 Introduction

In biorefining processes based on solvent extraction of lignin, the extent of lignin recondensation depends significantly on the residence time of the lignin fragments in the liquor. Indeed, in Organosolv fractionation (without added catalyst), lignin products isolated after only 10 min showed a narrow MW distribution, with species of MW<4 kDa. [7a] However, increasing the fractionation time to 3 h shifted the onset of the MW distribution to 15 kDa, which further increased when an 8 h reaction time was employed.[7a] A similar trend was reported for a lignin-first approach employing Ru/C and Ni/C with 30 bar H_2,[72a] wherein increased fractionation times from 3 h to 24 h shifted the MW of the resulting lignin stream towards higher values.

Overall, in lignin-first approaches, the evolution of fractionation over time has been studied primarily from the perspective of the holocellulosic pulps.[63b, 63c, 72a] In a catalytic fractionation employing a Ru/C catalyst and a H-source of molecular H_2 (30 bar),[63b] increasing the fractionation time from 0.5 h to 3 h enhanced the delignification degree from 68% to 80%. However, further prolonging the process time to 5 h did not bring about any improvement in either delignification or lignin depolymerisation. Additionally, this system required high temperatures (up to 250 °C) to achieve desirable levels of delignification (i.e. >70 %). Another lignin-first system using a Pd/C catalyst and hemicellulose as an internal H-donor at a process temperature of 210 °C achieved 75% delignification after 2h, which further increased to 92% when the reaction time was prolonged to 15 h.[63c]

In spite of the crucial role that reaction time plays in defining the properties of the lignin streams and holocellulosic pulps obtained from lignocellulose fractionation, limited information is available on the evolution of lignin depolymerisation and recondensation reactions occurring during lignin-first fractionation. A better understanding of the influence of reaction time upon these processes would, therefore, allow for a more complete understanding of lignin-first biorefining, paving the way for further optimisation. In this chapter, the evolution of the processes occurring during a Raney-Ni catalysed CUB fractionation were studied with time. CUB experiments on Poplar wood substrates were monitored across the time frame 0 – 300 min, with a specific focus on the degree of biomass deconstruction (as inferred from the extent of delignification and retention of hemicellulose in the pulp) and lignin depolymerisation (as obtained from GPC and UV-Vis analysis on the lignin streams). To allow for a more comprehensive understanding of processes unique to CUB extractions, comparative Organosolv experiments (i.e. without the addition of Raney Ni catalyst) were also performed.

5.2 Results and discussion

Organosolv and CUB experiments were performed by cooking a suspension of Poplar wood, Raney Ni (only in CUB experiments) and a mixture of 2-PrOH:H_2O (7:3 v/v) at 180 °C in a 2 mL reactor vessel under autogenic pressure.[7a] The suspension was reacted for a selected time, ranging from 5 min to 300 min. The process temperature was selected to be 180 °C, as a less extensive degradation of the lignocellulosic matrix takes place than at 200 °C,[7a, 28a, 68] allowing a better understanding of the evolution of the lignocellulose degradation and lignin depolymerisation.

To provide an initial assessment of the extent of lignocellulose deconstruction upon fractionation, the evolution of the weight yields of the holocellulosic pulps and soluble products obtained from Organosolv and CUB experiments were analysed (Figure 5-1). In both Organosolv and CUB processes, a gradual decrease in the mass of the solid residue and increase in the yield of soluble products was observed with time due to the extraction of lignin and, to a lesser extent, hemicellulose, from the lignocellulosic matrix. The release of soluble species began in the first few minutes of fractionation, with a 5 wt% decrease in the solid residue measured after just 5 min, mirrored by an equivalent increase in the weight yield of soluble products due to deacetylation of hemicellulose. The release of acetic acid is known to reduce the pH of liquors,[41b-d, 108] leading to some decomposition of hemicellulose sugars, which ultimately forms formic acid and levulinic acid.[41g] These acids behave as *in-situ* catalysts for the solvolytic cleavage of lignin linkages, leading to the dissolution of lignin fragments in the liquor.[7a, 42b, 42c] The release of soluble products from the lignocellulose matrix proceeds steadily until a *plateau* is reached after ca. 225 min in both processes. By comparing the Organosolv and CUB processes, it is apparent that CUB experiments led to larger weight reductions in the solid fraction of lignocellulose substrates. This observation is associated with the Raney Ni catalysed passivation of lignin fragments released during the CUB process, which hinders condensation of lignin fragments in the liquor with lignin species on the cellulosic fibres. Thereby, the passivation of lignin results in less redeposition of lignin on the pulps.[29, 68]

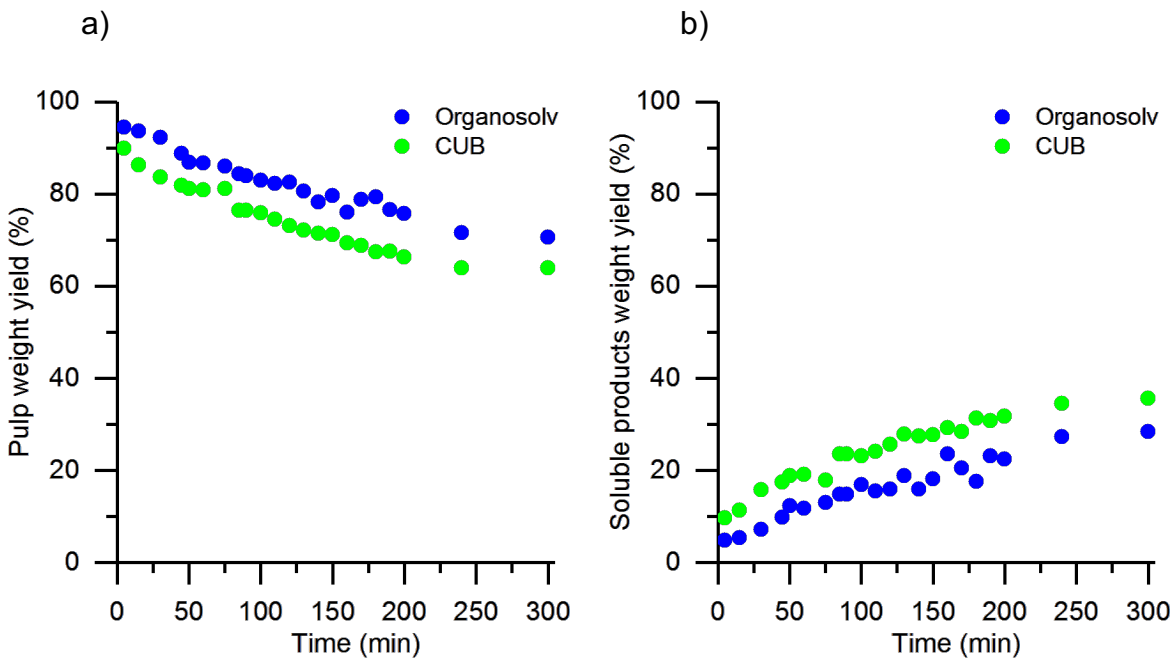


Figure 5-1 – Weight yields of (a) pulp and (b) soluble products vs. time for products obtained from the Organosolv process and Catalytic Upstream Biorefining of Poplar wood. General experimental conditions CUB: Poplar wood (0.12 g chips), Raney Ni (0.07 g), 2-PrOH/H_2O 7:3 v/v (1mL), 180 ºC for 5 – 300 min, autogenic pressure.

To determine the extent of both lignin and hemicellulose removal from the substrate over time, compositional analysis of the pulps obtained from both CUB and Organosolv processes was performed (Figure 5-2). In the first 50 min of fractionation the rate of lignin extraction is higher than hemicellulose in both processes, with delignification reaching 49% and 53% for Organosolv and CUB respectively (Figure 5-2a), corresponding to 71% and 72% of the total lignin removed after 300 min. Between 50 – 300 min, delignification slowed for both Organosolv and CUB. However, after 300 min a higher delignification degree was reached for CUB pulps (73% compared to 68% in the Organosolv process). Additionally, the delignification curves *plateau* at longer cooking times, indicating that a prolonged treatment does not lead to further extraction of lignin from the lignocellulose matrix.

The removal of hemicellulose increased with reaction time in both Organosolv and CUB processes. However, after the first 50 min of the fractionation only 7% hemicellulose removal was observed in the CUB process in comparison to 35% in the Organosolv (Figure 5-2b). By catalysing the hydrogenation of released sugars into their corresponding sugar alcohols, Raney Ni prevents further conversion of sugars into furfurals and consequently to formic acid, as typically observed in Organosolv.[41g] Therefore, the presence of the catalyst leads to less acidic liquors in CUB processes, reducing the rate of hemicellulose hydrolysis. With prolonged fractionation times (>100 min), hemicellulose removal in CUB experiments was only slightly lower than that of Organosolv, finally converging to 59% after 300 min.

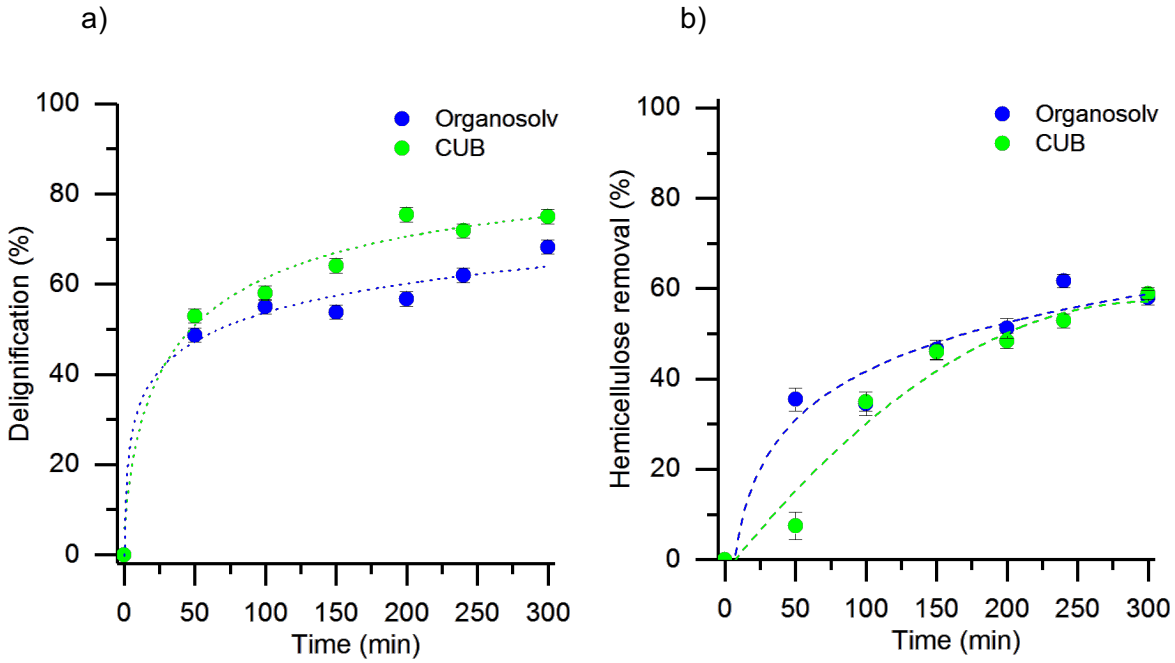


Figure 5-2 - (a) Delignification and (b) hemicellulose removal vs. time for products obtained from the Organosolv process and Catalytic Upstream Biorefining of Poplar wood in the time range 0 – 300 min. General experimental conditions CUB: Poplar wood (0.12 g chips), Raney Ni (0.07 g), 2-PrOH/H_2O 7:3 v/v (1mL), 180 ºC for 5 – 300 min, autogenic pressure.

To study the influence of cooking time on the MW distribution of lignin products, GPC analysis was performed on the lignin streams. In the explored time range, the concentration of high-MW species (MW > 550 Da, 12 – 17.3 min) in Organosolv lignin streams significantly increased, resulting in a broad MW-distribution (1 - 70k Da) including polymeric species and lignin-derived oligomers (peaks at 17.5 and 18.1 min), with lignin-derived monophenols (18.5 min) as only minor components.

Under Organosolv conditions, the low-MW fragments released from solvolysis of lignin linkages (i.e. Hibbert's ketones, benzyl carbocations) are consumed in repolymerisation reactions, resulting in the production of polymeric fractions.[29] Clearly, a prolonged residence time can further promote such reactions, resulting in an increased apparent MW of the lignin streams with reaction time.[7a] Contrarily, the introduction of Raney Ni in the fractionation system promotes lignin depolymerisation, while preventing extensive repolymerisation reactions *via* passivation of solvolytically released lignin species. As a result, the intensity of the peak attributed to low-MW species (MW < 550 Da), together with those assigned to lignin-derived oligomers, steadily increased with time during CUB fractionation (Figure 5-3b). These low-MW species are the main components of the lignin product isolated from CUB experiments after 300 min, with only a small fraction of high-MW material present. However, with increased time the onset of the chromatogram in Figure 5-3b continuously shifted towards higher values, from 10 kDa (5 min) to 41 kDa (300 min), demonstrating that despite the effective depolymerisation occurring during CUB fractionation, some repolymerisation of lignin fragments still takes place. These results allow us to conclude that long residence times are detrimental for depolymerisation, even in catalytic conditions.

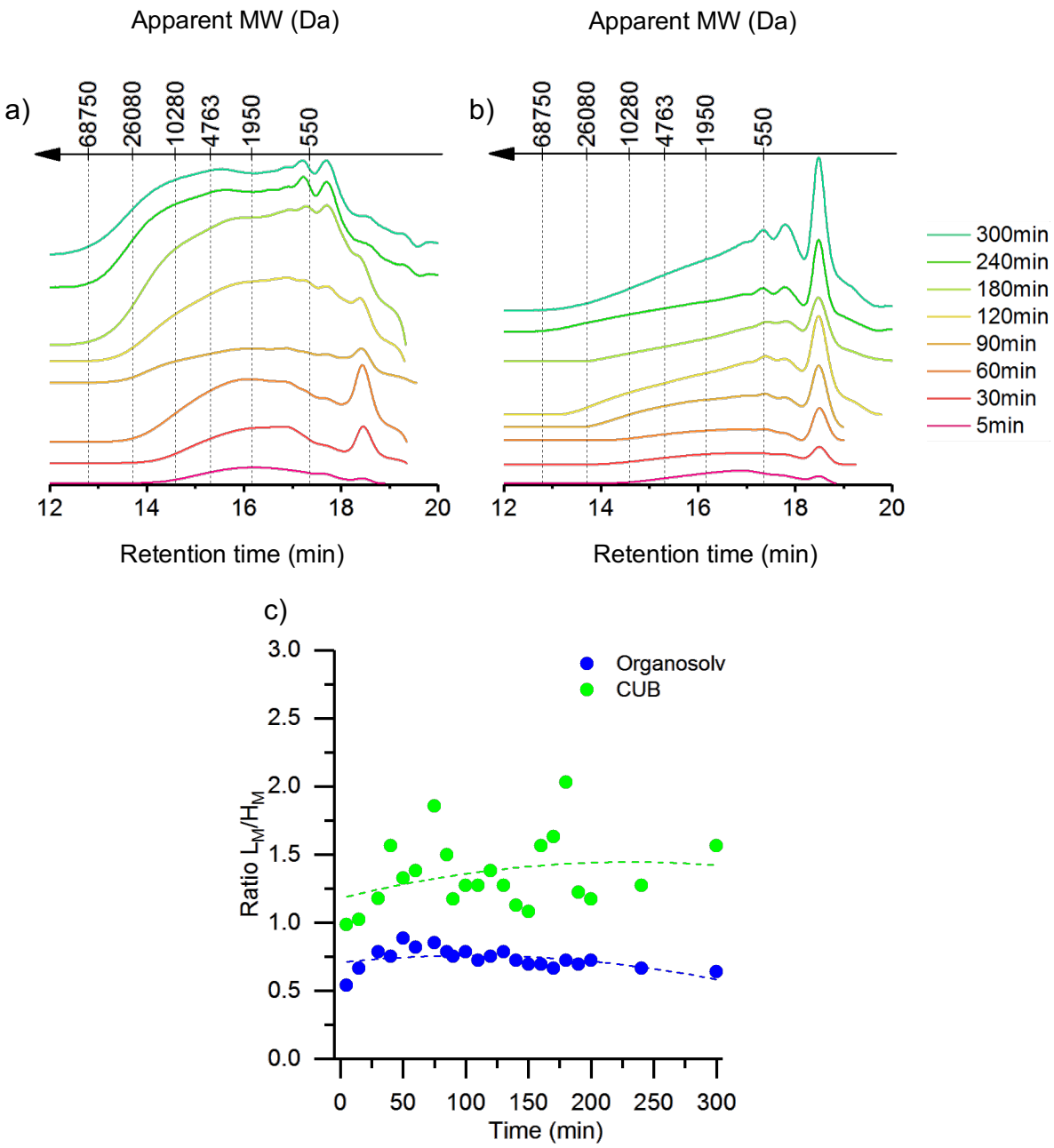


Figure 5-3 – GPC chromatograms of soluble products obtained from (a) Organosolv process and (b) CUB of Poplar wood in the time range 0 – 300 min. (c) Plot of ratio L_M/H_M vs. time for products obtained from the Organosolv process and Catalytic Upstream Biorefining of Poplar wood in the time range 0 – 300 min. General experimental conditions CUB: Poplar wood (0.12 g chips), Raney Ni (0.07 g), 2-PrOH/H_2O 7:3 v/v (1 mL), 180 ºC for 5 – 300 min, autogenic pressure.

To estimate the extent of depolymerisation occurring on lignin, the areas of the GPC chromatograms under the high-MW (H_M, MW>550 Da) and low-MW (L_M, MW<550 Da) regions were measured to give the ratio L_M/H_M. It must be noted that in this analysis a refractive index detection was employed. Figure 5-3c shows the L_M/H_M ratios for CUB and Organosolv as a function of time. In the first phase of fractionation (0 – 60 min), the release of H_M fragments was favoured over L_M species in Organosolv experiments, while CUB promoted a higher production of L_M compounds ($L_M/H_M>1$). After 60 min, the L_M/H_M ratio steadily decreased in non-catalytic experiments, whereas in presence of Raney Ni the ratio increased up to ca. 1.5.

Further insight into the evolution lignin depolymerisation can be provided by the analysis of the average molecular weight of the lignin stream. Thus, three parameters were considered: the number average molecular weight (M_n, Figure 5-4a), which is the statistical average molecular weight of the species found in the lignin stream; the weight average molecular weight (M_w, Figure 5-4b), which takes into account the molecular weight of the species in determining the molecular weight average, therefore the contribution of a specific molecule increases with its molecular weight; the Z-average molecular weight (M_z, Figure 5-4c), which is increasingly more sensitive to polymeric species.

The decreasing trend of M_n for both Organosolv and CUB lignin streams mirrored the increasing L_M/H_M ratio observed in the time range 0 – 60 min, derived by increasing depolymerisation extent in the first phase of fractionation. However, further prolonging the reaction time of Organosolv process resulted in an increase of the M_n, due to the growing size of the polymeric chains produced by recondensation reactions. In turn, as recondensation was prevented in CUB conditions, the average length of lignin-derived products from CUB was stable in the time range 60 – 300 min, resulting

in a stable Mn at these reaction times. The decreased L_M/H_M ratio in Organosolv was coupled with a linear increase of both M_w and M_z, respectively from 2 kDa to 6 kDa and from 5 kDa to 30 kDa over 300 min, suggesting that recondensation reactions are favoured with a prolonged residence time.[109] Conversely, both M_w and M_z of CUB lignin streams were consistently lower than those of Organosolv, and followed a similar increasing trend in the first 150 min. After this time, M_w and M_z began to steadily decrease to 2 kDa at 300 min. As Raney Ni is unable to catalyse the cleavage of C-C cross-links in recalcitrant polymeric species,[7a] the enhanced depolymerisation in the final phase of CUB fractionation is likely due to the lower rate of lignin extraction from the substrate (only 16% of the total lignin release occurred in the range 150 – 300 min, Figure 5-2a), leading to a lower concentration of fragments in the liquor. As a result of this low concentration, Raney Ni is able to come into contact with a larger portion of the fragments and is able to catalyse lignin depolymerisation to a greater extent than in the first half of the fractionation, more effectively preventing recondensation reactions.

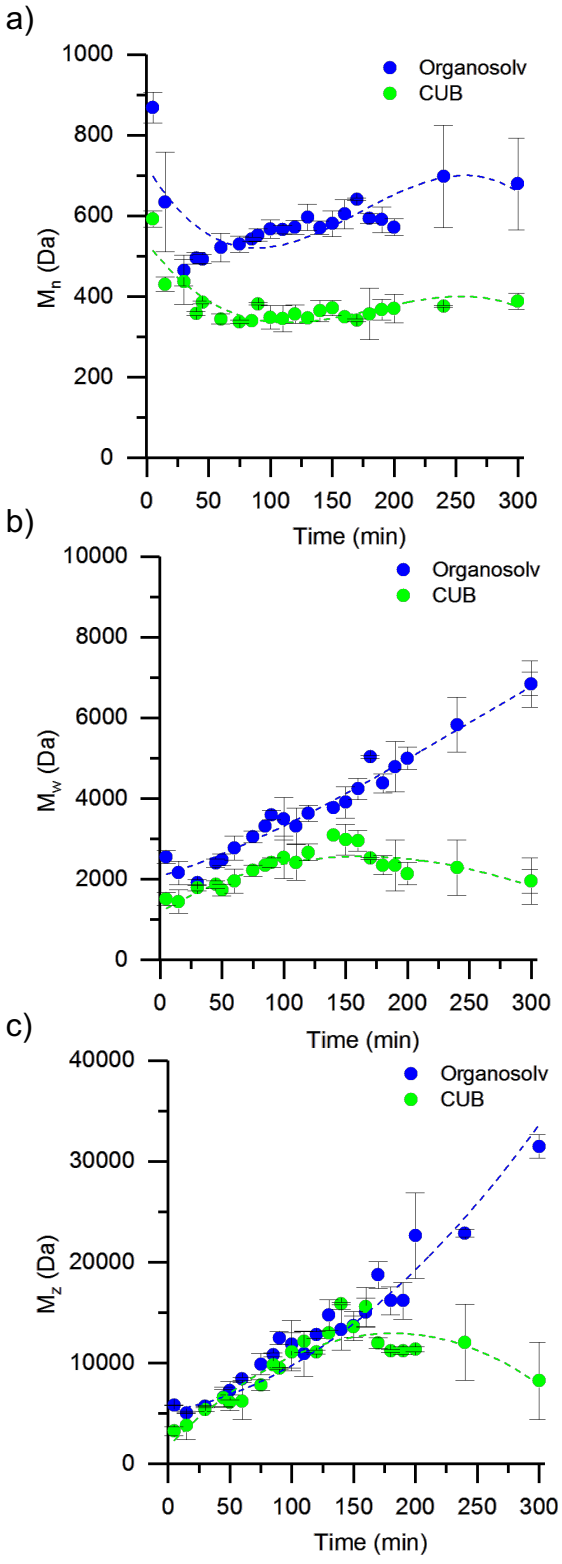


Figure 5-4 - Plot of (a) Mn, (b) Mw and (c) Mz vs. time for products obtained from the Organosolv process and Catalytic Upstream Biorefining of Poplar wood in the time range 0 – 300 min. General experimental conditions CUB: Poplar wood (0.12 g chips), Raney Ni (0.07 g), 2-PrOH/H_2O 7:3 v/v (1 mL), 180 ºC for 5 – 300 min, autogenic pressure.

The evolution of the product distribution of the main monophenols in lignin streams obtained from CUB experiment was analysed by GC-FID/MS analysis. In this work, only the compounds consistently showing a content higher than 0.1 wt% were selected for quantification. The weight content of the obtained monophenols, accounting for up to 30 wt% of the lignin streams produced from CUB experiments on Poplar, is reported in Figure 5-5 and is divided into three classes of compounds: hydroxypropyl- (H), guaiacyl- (G-) and syringyl- (S-) units. Overall, the content of monophenols in the lignin stream steadily increased across the explored time range, as a result of the constant release (Figure 5-2a) and depolymerisation (Figure 5-3) of lignin fragments from the lignocellulosic matrix. The main products found in the lignin streams were 4-(3-hydroxypropyl)-2,6-dimethoxyphenol and 4-(3-hydroxypropyl)-2-methoxyphenol, whose sum accounted for 73% of the total amount of monophenols quantified in this study. Prolonging the reaction time resulted in a higher extent of Raney Ni-catalysed hydrodeoxygenation and demethylation reactions, as an increasing fraction of these compounds was converted to the corresponding propyl-, ethyl- and methyl- derivatives. However, these compounds were isolated with low individual yields (< 2 wt%), demonstrating an overall low extent of decomposition of 4-(3-hydroxypropyl)-2,6-dimethoxyphenol and 4-(3-hydroxypropyl)-2-methoxyphenol. Due to the low abundance of H-units in native lignin from Poplar,[97] only a small fraction of hydroxyphenyl-derived compounds was found in the lignin streams, accounting for <5 wt% of the total amount of quantified monophenols.

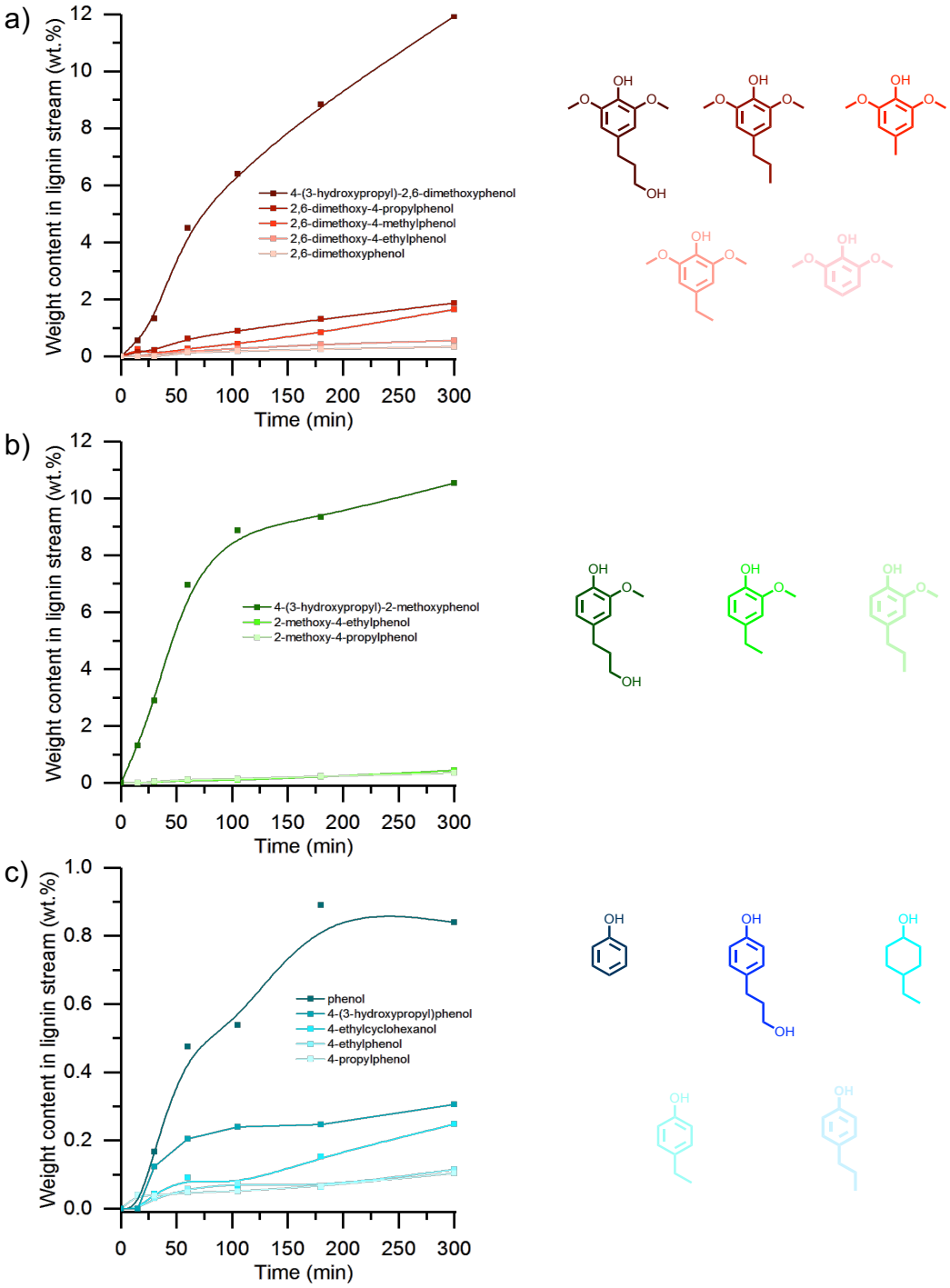


Figure 5-5 – Weight content of selected main products vs. time for lignin streams obtained from the Catalytic Upstream Biorefining of Poplar wood. a) Syringyl-units (S); b) guaiacyl-units (G); c) hydroxyphenyl-units (H). Please note that c) has a y-axis ranging from 0.0 to 1.0 General experimental conditions CUB: Poplar wood (17 g chips), Raney Ni (10.0 g), 2-PrOH/H_2O 7:3 v/v (140 mL), 180 ºC for 15 – 300 min, autogenic pressure.

To investigate the evolution of the structural composition of lignin products with time, the liquors obtained from both fractionation processes were analysed by UV-Vis spectroscopy. The UV-Vis spectra of lignin streams from both Organosolv and CUB processes are presented in Figure 5-6. Two main absorption bands at 203 nm and 275 nm were detected in the spectra. The intense peak at 203 nm is a non-selective UV absorbance by organic compounds containing unsaturated functionalities, and the less intense peak at 275 nm was ascribed to the presence of furfurals and phenols. Therefore, to some extent both peaks account for a combination of these chemical species. Nevertheless, monitoring of the absorbance at each of these wavelengths showed different trends for Organosolv and CUB process (Figure 5-7). The absorption at 203 nm appears to follow a similar trend for both Organosolv and CUB liquors with time, with Organosolv liquors consistently showing higher absorbance after 90 min. However, this is not associated to a higher population of lignin products in Organosolv liquor, as the extraction of lignin through CUB was found to be higher in the explored time frame (Figure 5-2a). In both cases, absorption at 203 nm reaches a plateau after 225 min as lignin release is not improved with prolonged fractionation times (Figure 5-2a).

A stronger absorption at 275 nm was observed in the Organosolv lignin streams when compared to the CUB. As outlined earlier, this is likely due to hydrogenation of monosaccharides to sugar alcohols in CUB systems, preventing the formation of significant quantities of furfurals and derivatives *via* further sugar degradation.[41g] Thereby, the contribution of furfurals to this absorption band is decreased in the spectrum for the CUB sample. Finally, a crucial difference in the UV-Vis spectra for Organosolv and CUB is the presence of a tailing UV-Vis band at ca. 340 nm, which is only observed in Organosolv liquors. This is related to the presence of more complex

structures generated by recondensation, oxidation of Cα position or formation of stilbene linkages.[110] Revealingly, the response at 340 nm steadily increases with fractionation time in Organosolv processes (Figure 5-7c), in good agreement with prolonged exposure times leading to increased repolymerisation and subsequent lignin recalcitrance. On the other hand, absorbance at 340 nm is negligible for CUB liquors over the entire 300 min, further corroborating the prevention of extensive recondensation reactions.

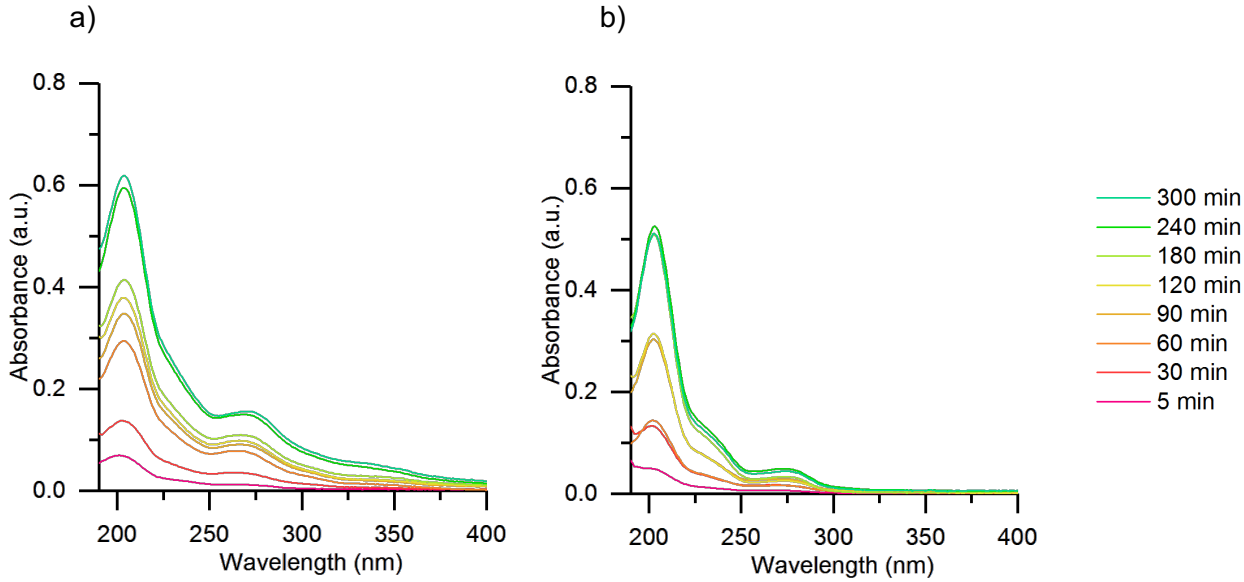


Figure 5-6 - UV-Vis absorption spectra of liquors obtained from (a) Organosolv process and (b) CUB of Poplar wood in the time range 0 – 300 min. UV curves are normalized to the weight of the sample. General experimental conditions CUB: Poplar wood (0.12 g chips), Raney Ni (0.07 g), 2-PrOH/H_2O 7:3 v/v (1 mL), 180 ºC for 5 – 300 min, autogenic pressure.

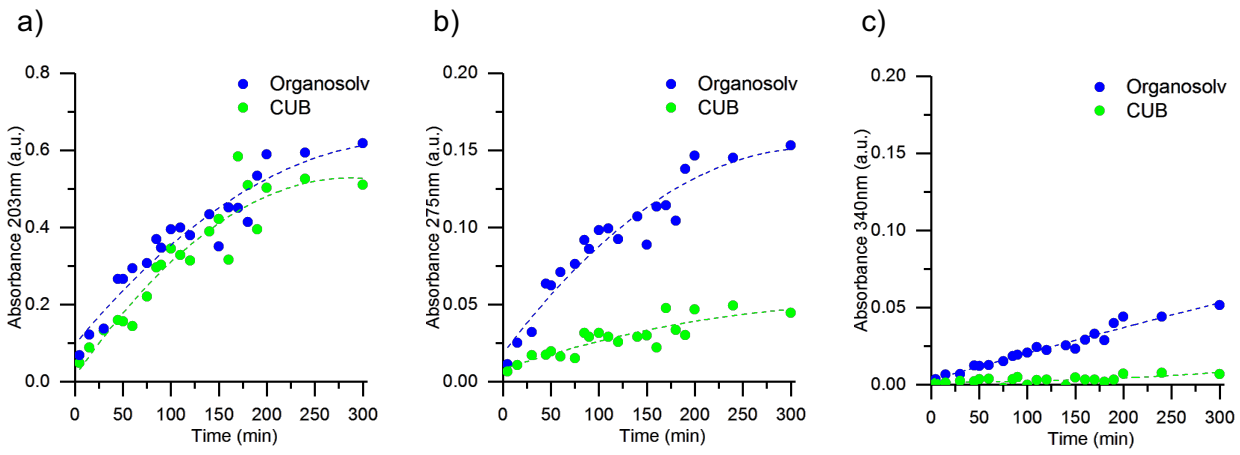


Figure 5-7 - UV-Vis absorption of liquor vs. time for products obtained from the Organosolv process and Catalytic Upstream Biorefining of Poplar wood. a) 203 nm; b) 275 nm; c) 340 nm. Please note plots b) and c) have y-axis ranging 0.0 - 0.2 a.u. General experimental conditions CUB: Poplar wood (0.12 g chips), Raney Ni (0.07 g), 2-PrOH/H_2O 7:3 v/v (1 mL), 180 ºC for 5 – 300 min, autogenic pressure.

5.3 Conclusions

Understanding the evolution of lignocellulose fractionation over time represents a crucial step in the perspective of process optimisation to obtain extensively depolymerised lignin streams and highly delignified holocellulosic pulps. Regardless of the use of Raney Ni, the initial stage (0 - 50 min) was found to be crucial for delignification with ca. 70% of all lignin recovered extracted. As the release of lignin fragments from lignocellulose decreases after 150 min, the effect of Raney Ni on lignin depolymerisation extent becomes clearer in the second part of fractionation (150 – 300 min). On the contrary, prolonged fractionation times in Organosolv experiments (i.e. without added Raney Ni) led to more extensive recondensation, producing lignins with steadily increasing apparent MW over time and leading to the reprecipitation of lignin globules on the lignocellulosic fibres.

Overall, the results demonstrate that a CUB fractionation system performed with Raney Ni as catalyst and 2-PrOH as H-donor at 180°C, requires at least 150 min for effective lignin extraction and depolymerisation. Importantly, the CUB approach required milder conditions than other lignin-first approaches for achieving good performances in terms of biomass deconstruction and lignin valorisation, needed shorter reaction times, lower temperature, and not requiring the use of molecular H_2 as H-donor.[63b, 63c, 72a]

6. The interplay of process parameters in the performance of the deconstruction of lignocellulose by a lignin-first biorefining process

6.1 Introduction

Considering that CUB involves processes in both the lignocellulosic matrix-liquor domain (i.e. lignin solvolysis and release of lignin fragments into the liquor) and the liquor-catalyst domain (i.e. Raney Ni),[7a, 28a, 29, 68] this chapter explores the working hypothesis that the deconstruction of lignocellulose and the molecular diversity of the isolated lignin oil will depend not only on the catalyst employed in the lignin-first biorefining, but also on other variables defining the quantity of H_2-equivalent species available for reductive processes catalysed by Raney Ni, and the concentration of lignin species released from the lignocellulosic matrix, namely the amount of processed wood and the volume of lignin-extracting liquor. Logically, the extent of HDO reactions for the passivation of lignin fragments should directly be related to the amount of reactant (i.e. hydrogen) and substrate (i.e. lignin-derived species themselves) available in the liquor. Building on this hypothesis, it seems more suitable for the purpose of addressing the effects and possible interactions of several factors (i.e. process parameters, or independent variables) to employ a Design of Experiment (DoE) by using factorial experiments instead of a “one-factor-at-a-time” method.

In biorefining research, the DoE approach was employed to optimise pretreatments for lignocellulose deconstruction (i.e. Organosolv[111], acid-alkali[111b], pretreatments with ionic liquids[112]) in addition to biological downstream process to

produce platform chemicals.[113] Despite its great efficacy for optimisation of complex processes, the DoE method has, however, not been applied yet in the context of lignin-first biorefining process. In this report, the CUB process was examined by using the DoE tools to assess the interaction of three independent variables: amount of wood processed ("W"), volume of lignin-extracting liquor ("L"), and amount of Raney Ni ("C"). A full 3x3 factorial design was carried out, exploring three levels for each independent variable. The dependent variables investigated in this study were chosen to gain detailed insight into the extent of formation of low MW lignin-derived species (as obtained from the ratio of the amount of low-MW to high-MW species found in the isolated lignin product; content of the most abundant monophenols), as well as the lignocellulose deconstruction (as inferred from delignification degree and hemicellulose retention). This chapter is organised into two main sections: the first section presents the results obtained from the DoE study, discussing the interaction among CUB parameters and their effect on lignin depolymerisation extent, yield of monophenolic products and biomass deconstruction performance, hence providing crucial information for process optimisation. Building on these findings, the second section addresses an experiment scaling up the optimised conditions found for the 0.25L reactor to a 10L pressure vessel, and a second experiment aiming to further improve the performances of CUB by extrapolating the DoE results. Markedly, this study provides solid evidence that the process parameters (wood loading, liquor volume and catalyst amount) strongly interact, defining the CUB performances, so that process optimisation and scaling-up cannot effectively be achieved by "one-factor-at-a-time" method, as often reported in the current literature.

6.2 Results and Discussion

6.2.1 Assessing the interaction of the experimental parameters

In the full 3x3 factorial design, the independent variables were selected as (1) quantity of processed wood (denoted by “W”), (2) volume of lignin-extracting liquor (2-PrOH:H_2O, 7:3 v/v, represented by “L”), and (3) amount of catalyst (Raney Ni , “C”). For each variable, a low (-1), central (0) and high (+1) level were defined. The values of independent variables were chosen around a typical set of experimental conditions explored by us in previous studies (i.e. W= 17g, L= 140mL, C= 10g).[7a, 28a, 68] For comparison, four experiments of Organosolv pulping of Poplar wood with no added Raney Ni were performed as control experiments. Table 6-1 lists the 27 test conditions for the complete 3x3 factorial design of CUB, with seven additional replicates and four control experiments. These experiments were performed in a 0.25L reactor vessel, at 200°C for 3h under autogenic pressure.[7a, 28a, 68] Table 6-1 allows us to address the interaction of the independent variables and their effect on the CUB dependent variables, describing the molecular uniformity of the lignin stream and the deconstruction performance (Figure 6-1).

Table 6-1 – Experimental conditions of Catalytic Upstream Biorefining process (CUB) screened in the Design of Experiments (DoE), and corresponding DoE nomenclature. The experiments highlighted with ' * ' indicate the replicates performed for the estimation of the standard deviation needed for the statistical treatment of the DoE data.

	Experimental conditions			DoE nomenclature		
Entry	Wood loading (W, g)	Solvent volume (S, mL)	Catalyst loading (C, g)	W	S	C
1*	17	60	1	-1	-1	-1
2	25	60	1	0	-1	-1
3	35	60	1	1	-1	-1
4*	17	100	1	-1	0	-1
5*	25	100	1	0	0	-1
6*	35	100	1	1	0	-1
7	17	140	1	-1	1	-1
8	25	140	1	0	1	-1
9	35	140	1	1	1	-1
10	17	60	5	-1	-1	0
11	25	60	5	0	-1	0
12	35	60	5	1	-1	0
13	17	100	5	-1	0	0
14*	25	100	5	0	0	0
15	35	100	5	1	0	0
16*	17	140	5	-1	1	0
17	25	140	5	0	1	0
18	35	140	5	1	1	0
19	17	60	10	-1	-1	1
20	25	60	10	0	-1	1
21	35	60	10	1	-1	1
22	17	100	10	-1	0	1
23	25	100	10	0	0	1
24*	35	100	10	1	0	1
25	17	140	10	-1	1	1
26	25	140	10	0	1	1
27	35	140	10	1	1	1
Organosolv						
28	17	140	-	-1	1	-
29	17	100	-	-1	0	-
30	25	100	-	0	0	-
31	35	100	-	1	0	-

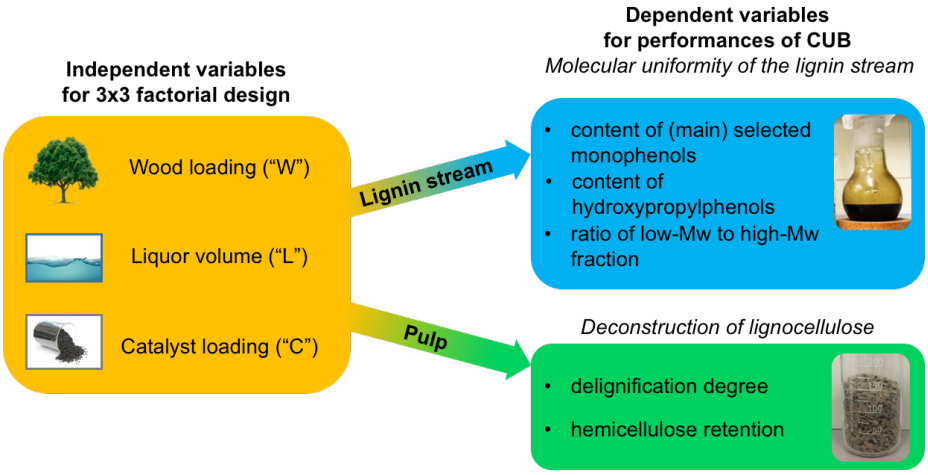


Figure 6-1 - Schematic representation of the rationale of the DoE approach performed in this study.

With regards to the lignin stream, the molecular uniformity can be described by: the ratio of low-MW to high-MW lignin fractions, estimated by the area below the gel permeation chromatogram curve in the region of 0 - 550 Da (low-MW region) and > 550 Da (high-MW region); the total content of (main) selected monophenols; the content of 4-(3-hydroxypropyl)phenols, which typically represent the most abundant products in lignin products from CUB.[7a, 28a] The deconstruction performance can be defined by the pulp properties, namely delignification degree (i.e. the remnant content of lignin in the pulp relative to the initial lignin content in the wood substrate) and hemicellulose retention (i.e. the remnant xylan content in the pulp relative to the initial xylan content in the wood substrate). Regression analysis on the results obtained from the DoE studies allowed the determination of the statistical significance of independent variables for each dependent variable, which is listed in Table 6-2. The mathematical models are presented in Table 6-4.

Table 6-2 – Summary of significant independent variables and statistics values obtained from statistical analysis. An independent variable is significant for the selected dependent variable if its p-value exceeds 0.05 (0.1 for Delignification), above which the probability that the null hypothesis (i.e. no relation exists between variables) occurs falls below 5% (10% for Delignification).

Dependent variable	Minimum value	Average	Maximum value	Statistically significant independent variable	p-value (relative)
Ratio L_M/H_M	0.5	1.2	2.0	C	0.00004
				C^2	0.0001
Total content of selected (main) phenols in lignin product (wt%)	7.4	28.9	50.4	C	<0.0000001
				W	0.0003
				C^2	0.0003
				$W*C^2$	0.0006
				L^2*C	0.007
				$L*C^2$	0.008
				L^2	0.04
				W^2*C^2	0.04
Content of 4-(3-hydroxypropyl)phenols in lignin product (wt. %)	0.8	12.7	24.6	C	0.000001
				C^2	0.00007
				$W*C^2$	0.0003
				L	0.0004
				W*C	0.01
				L^2*C^2	0.02
				W	0.02

Table 6-3 (continued) – Summary of significant independent variables and statistics values obtained from statistical analysis. An independent variable is significant for the selected dependent variable if its p-value exceeds 0.05 (0.1 for Delignification), above which the probability that the null hypothesis (i.e. no relation exists between variables) occurs falls below 5% (10% for Delignification).

Dependent variable	Minimum value	Average	Maximum value	Statistically significant independent variable	p-value (relative)
Delignification (%)	70.6	79.3	88.0	W^2*C^2	0.04
				W	0.06
				W*L	0.09
Hemicellulose retention (%)	12.1	25.7	39.3	L	0.0001
				C	0.0001
				$W*L^2$	0.01
				W	0.03

Table 6-4 – Regression equations obtained from statistical analysis. W = wood loading; L = liquor volume; C = catalyst loading.

Model equations	R^2
Ratio $L_M/H_M = 0.343059 + 0.304621\ C - 0.02134\ C^2$	0.693
Content phenols in lignin products (wt%) = $14.61841 - 0.00807\ W^2 - 0.00044\ L^2 + 2.58485\ C + 0.22179\ C^2 + 0.01157\ WC^2$ $- 0.00019\ W^2C^2 - 0.00711\ LC^2 + 0.00038\ L^2C$	0.912
Content 4-(3-hydroxypropyl)phenols in lignin product (wt%) = $-6.90435 + 0.00166\ W^2 + 0.05196\ L + 11.9645\ C - 1.13026\ C^2$ $- 0.29993\ WC + 0.03149\ WC^2$	0.843
Delignification (%) = $82.9583 - 0.27749\ W + 0.0013\ W*L - 0.00002\ W^2*C^2$	0.194
Hemicellulose retention (%) = $3.833525 - 0.005005\ W + 0.249755\ L + 0.940554\ C - 0.000024\ W*L^2$	0.657

It is clear that Raney Ni plays a crucial role on the properties of the lignin product. Indeed, the amount of catalyst employed ("C") in the CUB experiments is the independent variable with highest statistical significance for all three dependent variables connected to the molecular uniformity of the lignin stream, with p-values lower than 0.00041. Alongside, wood loading and liquor volume were also found to be statistically significant, as some of their single or binary terms exhibited p-values below 0.05. In turn, the influence of experimental conditions on lignocellulose deconstruction (i.e. delignification and hemicellulose retention) accounts for all three independent variables. In the next sections, a detailed description and discussion of the relevance of the results presented in Table 6-2 and Table 6-4 will be provided.

6.2.2 Lignin stream properties

Ratio L_M/H_M

The evaluation of the extent of lignin depolymerisation is important for processes aiming at the valorisation of lignin as platform chemicals. To assess the extent of depolymerisation, the lignin streams were analysed by gel permeation chromatography (GPC). The high-MW region included species with MW>550Da, while the low-MW region comprised only species below this threshold. Then, the ratio "*area below GPC chromatogram*$_{low\text{-MW region}}$*/ area below GPC chromatogram*$_{high\text{-MW region}}$", namely L_M/H_M, was calculated to provide direct quantitative information on the extent of lignin depolymerisation. For this analysis, a refractive index detection was employed. As indicated in Table 6-2, catalyst loading is the most significant variable affecting L_M/H_M (C, p=.0004; C^2, p=.0001), while wood loading and liquor volume exert no relevant effect on the extent of depolymerisation. For this purpose, the

representation of the dataset as a boxplot is the best way to display the dependence of L_M/H_M of lignin products obtained from the CUB process from the catalyst loading (Figure 6-2). All CUB tests performed with the lowest amount of catalyst (C=-1) are prevalently made of high-MW species, as they exhibited a L_M/H_M of ca. 0.7. This ratio increased when higher amounts of catalyst were used. Overall, the correlation between a high amount of Raney Ni employed in CUB and a low ratio L_M/H_M of lignin streams is related to the increase in catalytic sites available for H-transfer reactions, which promote depolymerisation of lignin fragments that results in a two- to three-fold increase in the L_M/H_M values. Importantly, the values obtained from experiments with C=1g were found to be similar to those for lignin products obtained from Organosolv experiments (L_M/H_M=0.6-0.7), suggesting that 1g of catalyst is insufficient to guarantee effective depolymerisation of lignin released in these conditions from Poplar wood. However, as it will be discussed in more detail next, even in the presence of low loadings of Raney Ni, the recondensation of lignin is largely suppressed.

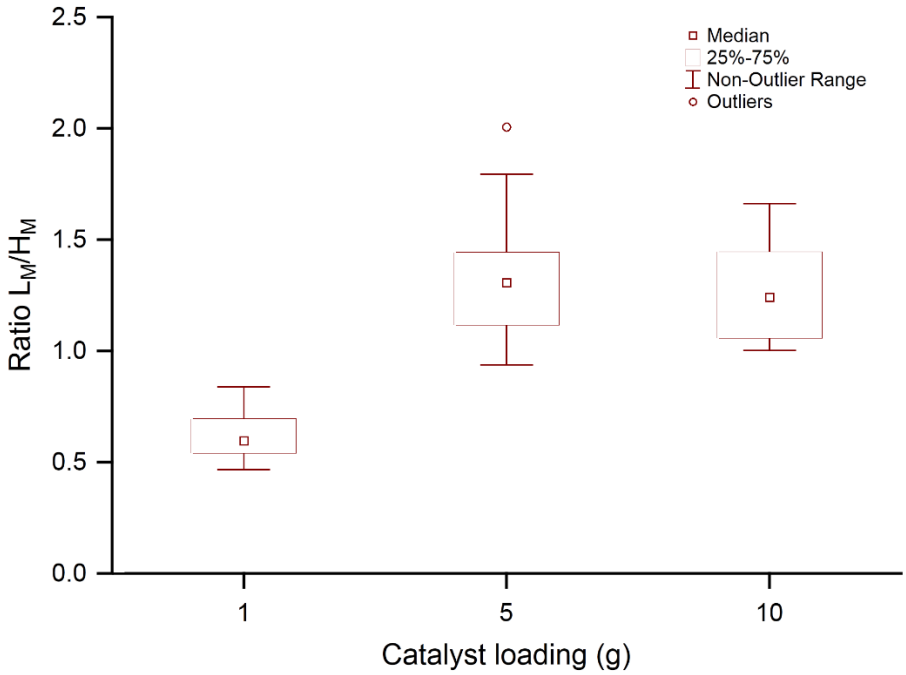


Figure 6-2 – Catalyst-loading dependence on the variability of ratio L_M/H_M of lignin products obtained from the CUB process carried out under varied conditions (as described in Table 6-1). General experimental conditions CUB: Poplar wood (17, 25 or 35g chips), Raney Ni (1, 5 or 10 g), 2-PrOH/H_2O 7:3 v/v (60, 100 or 140mL), 200 ºC for 3 h, autogenic pressure.

Content of selected (main) monophenols in lignin product

The fraction of monophenolic units in lignin products is another relevant parameter for assessing the extent of lignin depolymerisation in CUB, as well as the molecular uniformity of the lignin stream. To assess the variation in the molecular diversity and chemical composition of the low-MW fraction, the released lignin products were subjected to GC-FID/MS analysis. In this work, only the compounds consistently showing a content higher than 0.1 wt% were selected for quantification. In a similar manner to what already observed for L_M/H_M, the total content of monophenols in lignin products mainly depends on the amount of catalyst employed in CUB (C, p=<0.0000001, Table 6-2). Indeed, increasing the catalyst loading from 1g to 10g

promoted a three- to four-fold increase in the amount of monophenols recovered after fractionation (Figure 6-3) as a consequence of enhanced depolymerisation discussed previously, confirming the findings of previous reports.[69a, 114] As observed in previous studies,[7a, 28a, 71] the main compounds detected in the lignin products are substituted monophenols resulting from the coumaryl (hydroxyphenyl-units, H), coniferyl (guaiacyl-units, G), and synapyl (syringyl-units, S) subunits composing native Poplar lignin.[18a] Full saturation of the aromatic ring was not significant under the screened conditions, as no cyclohexanols and cyclohexanediols were detected above 0.1wt%. Lignin products from C=-1 (i.e. using 1g of catalyst) experiments are composed of amounts of H-, G- and S-units below ca. 5wt% (Figure 6-4). Conversely, increasing the amount of Raney Ni (C=0, 1) resulted in a drastic increase of the amount of S-units (from ca. 4wt% up to 35wt%), and a milder increase of the amount of G-units (from ca. 4wt% up to 13wt%), accounting for a more effective depolymerisation of lignin previously observed in GPC results. In turn, the amount of H-units remained constantly below 5wt% regardless of the experimental conditions employed. As H-units are only present in traces in Poplar lignin (ca. 0.3mol%),[115] their production in the CUB process can only be due to demethoxylation of G- and S-units. However, although effective on single model compounds, this reaction pathway is largely suppressed when a mixture of different monophenols is processed.[28a, 116] Hence, the presence of a relatively high fraction of H-units in the lignin products from C=-1 tests can be attributed to a lower overall concentration of monophenols in the liquor, which allowed for a more effective demethoxylation of their G- and S-counterparts.

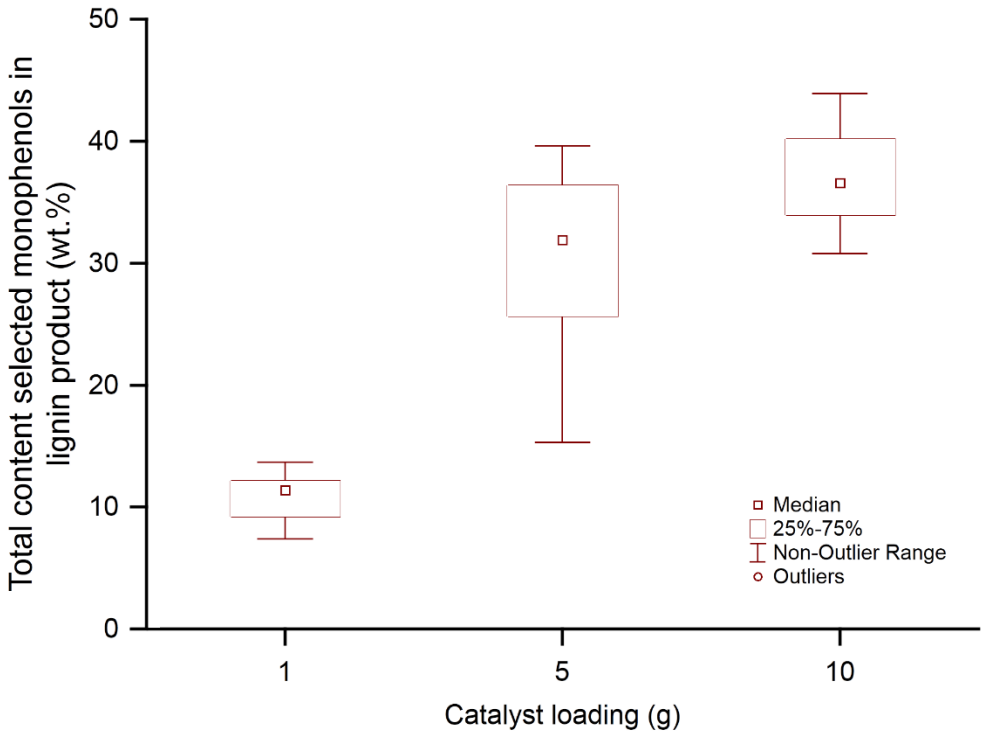


Figure 6-3 - Catalyst-loading dependence on the variability of total content of selected monophenols in lignin products obtained from the CUB process carried out under varied conditions (as described in Table 6-1). General experimental conditions CUB: Poplar wood (17, 25 or 35g chips), Raney Ni (1, 5 or 10 g), 2-PrOH/H_2O 7:3 v/v (60, 100 or 140mL), 200 ºC for 3 h, autogenic pressure.

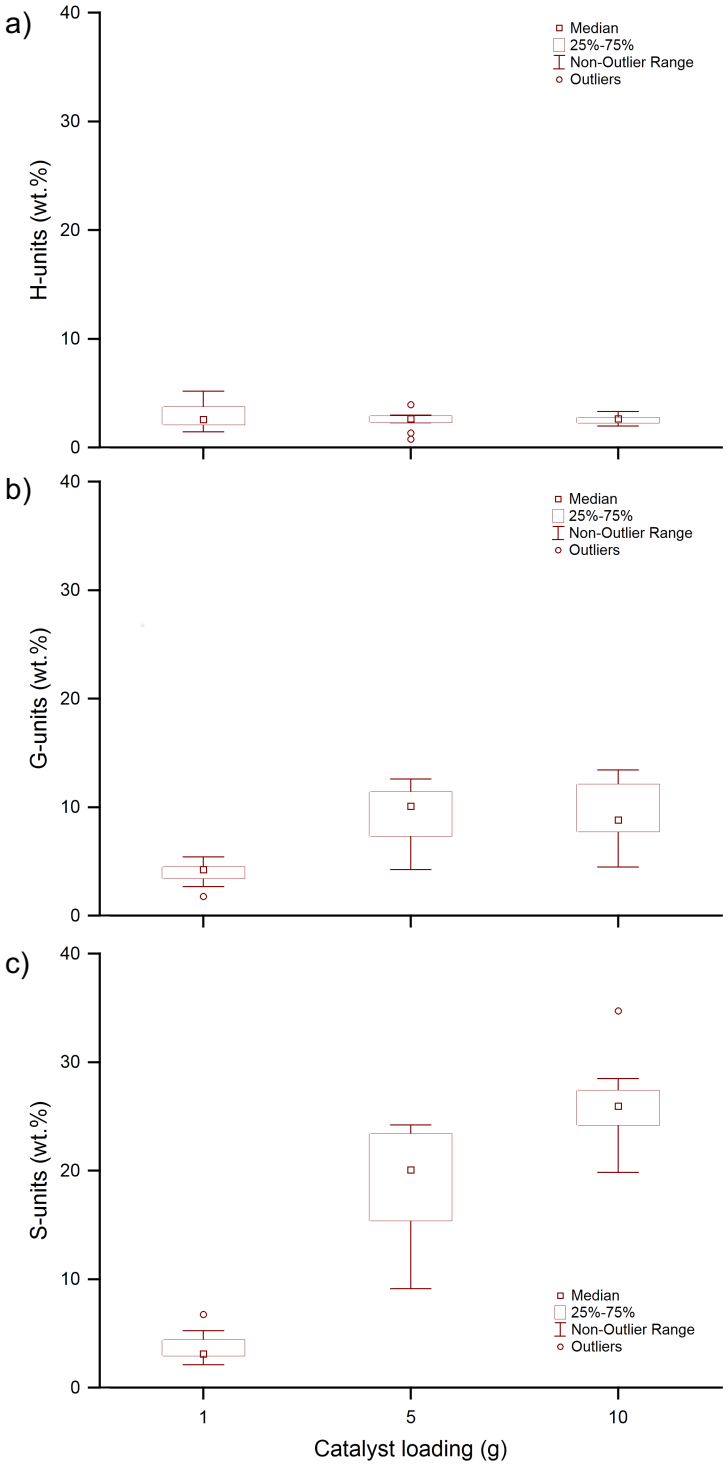


Figure 6-4 - Catalyst-loading dependence on the variability of monophenolic subunits in lignin products obtained from the CUB process carried out under varied conditions (as described in Table 6-1). a) H-units; b) G-units; c) S-units. General experimental conditions CUB: Poplar wood (17, 25 or 35g chips), Raney Ni (1, 5 or 10 g), 2-PrOH/H_2O 7:3 v/v (60, 100 or 140mL), 200 ºC for 3 h, autogenic pressure.

Content of 4-(3-hydroxypropyl)phenols in lignin product

With the aim of generating lignin streams containing monophenols with high individual yields, assessing the content of 4-(3-hydroxypropyl)phenols in the lignin products (Figure 6-5l-n), typically the most abundant monophenols produced from CUB of Poplar wood,[7a, 28a] can provide key information in evaluating the performance of the process. In CUB experiments involving a high ratio of wood to catalyst loading (i.e. W/C≥17g/g), the resulting lignin products exhibit high chemical heterogeneity, with a content of 4-(3-hydroxypropyl)phenols typically below 6wt%. Importantly, decreasing the W/C ratio to W/C≤7g/g leads to an increase in their production (values in the range 6-23wt%), as a result of higher depolymerisation extent, as discussed in the previous paragraph (Figure 6-2). Employing a liquor volume of 60mL in CUB was typically associated to the lowest content of 4-(3-hydroxypropyl)phenols in the DoE set, as these conditions favoured a shift in selectivity from 4-(3-hydroxypropyl)phenols to propyl- and ethylphenols. Indeed, the lower volume of liquor implies an increased concentration of substrate (i.e. lignin-derived species). As a result, 4-(3-hydroxypropyl)phenols produced from hydrogenolysis of lignin linkages are prone to further conversion through HDO and demethylation reactions on the surface of Raney Ni (Figure 6-6).

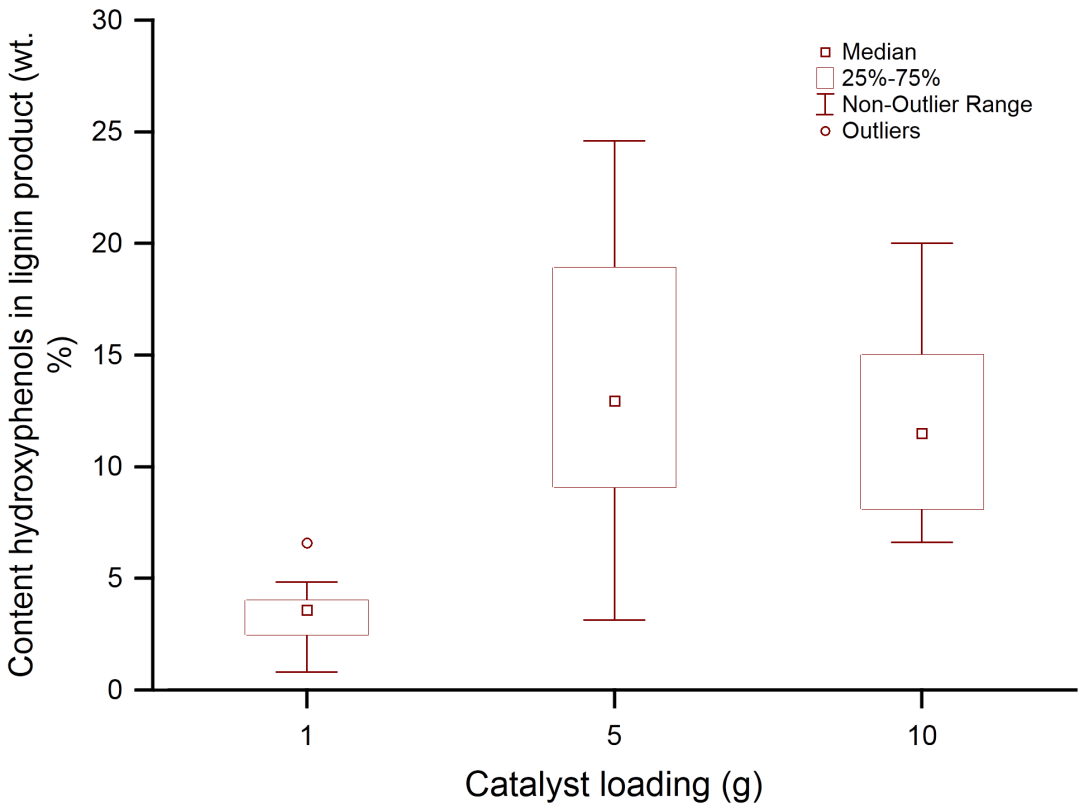


Figure 6-5 – Catalyst-loading dependence on the variability of the weight content of 4-(3-hydroxypropyl)phenols in lignin products obtained from the CUB process carried out under varied conditions (listed in Table 6-1). General experimental conditions CUB: Poplar wood (17, 25 or 35g chips), Raney Ni (1, 5 or 10 g), 2-PrOH/H_2O 7:3 v/v (60, 100 or 140mL), 200 ºC for 3 h, autogenic pressure.

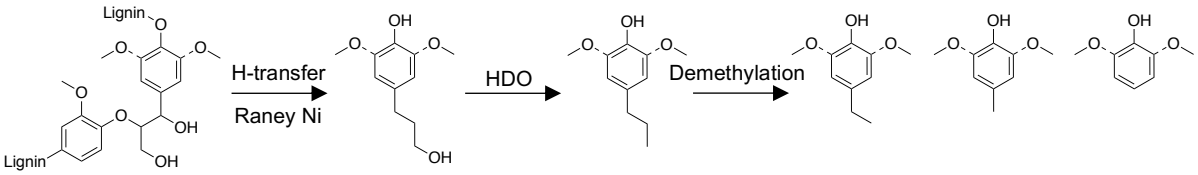


Figure 6-6 – Pathway of catalytic conversion of 4-(3-hydroxypropyl)phenols in the conditions of CUB.

Elemental analysis (CHNS) was performed on lignin products to provide insights into the extent of HDO catalysed by Raney Ni (e.g. hydrogenolysis,

hydro(deoxy)genation etc.) occurring on the lignin fragments. The resulting H/C and O/C ratios were plotted in a Van Krevelen diagram (Figure 6-7). Incorporation of hydrogen in the sets of experiments involving 5g or 10g of catalyst reproduced the one observed in previous reports (H/C of ca.1.6[7a, 28a]) and is higher than that observed for lignin products obtained in experiments with 1g catalyst (H/C<1.3). This value is closer to that obtained from Organosolv lignin (solvolytic fractionation experiment performed in the absence of Raney Ni) extracted from Poplar wood, which showed H/C=1.25 and O/C=0.4.[28a] Similarly to the H-content, the O-content increased when the catalyst loading was increased from 1g to 5-10g, as a result of the increase in the overall content of S-units from C=-1 to C=+1 experiments (Figure 6-5).

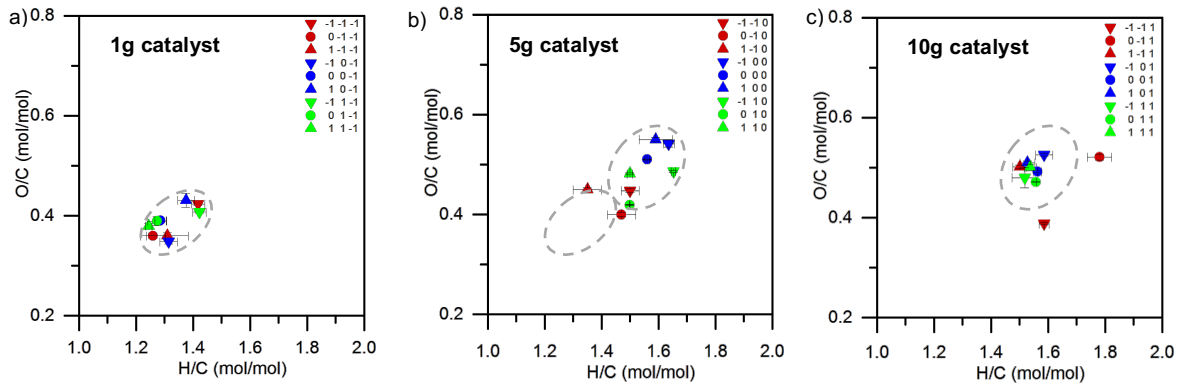


Figure 6-7 – Van Krevelen plots for lignin products obtained from the CUB process carried out under varied conditions (listed in Table 6-1). Values of H/C vs. O/C obtained from experiments with a) 1g catalyst; b) 5g catalyst; c) 10g catalyst. The chromatograms are labelled according to their DoE tag “WLC”.

6.2.3 Holocellulosic pulp properties

The extent of delignification and the retention of the original content in hemicellulose in pulp are key overall parameters to assess the performances of the CUB process in terms of biomass deconstruction, as they provide useful information regarding the

applicability of the holocellulose fibres isolated. To determine these parameters, compositional analysis of the pulps obtained from CUB experiments was performed. Differently from the parameters related to molecular uniformity of lignin stream, which are mainly affected by the catalyst loading, the two parameters connected to biomass deconstruction depend on a more complex combination of independent variables (Table 6-2), which is better discussed in the following paragraphs.

Delignification extent

All tests produce highly delignified pulps ranging from 71% to 88% (average=79%). However, CUB produced delignification values with high variability within the conditions studied, which resulted in a regression model with low R^2 (R^2=0.194, Table 6-4). As highlighted by the box plot in Figure 6-8a, increasing the amount of wood processed in CUB produced an overall decrease in the delignification extent achieved in fractionation. In turn, differently from the dependent variables relative to the molecular uniformity of lignin streams, varying the amount of catalyst alone did not seem to produce any relevant effect (Figure 6-8b). In these conditions, catalytic experiments showed delignification degrees similar to Organosolv experiments, which ranged from 73-85%. Interestingly, the combination of wood and catalyst loading showed the highest statistical significance for delignification (W^2*C^2 in Table 6-2), as tests performed with 35g wood and 10g catalyst resulted in low delignification (71-76%). However, the experiments performed in these conditions showed good performances in H-transfer reactions for the isolation of depolymerised lignin products, as discussed in the previous paragraphs. As the suspension made of 35g of wood and 10g of catalyst occupies the full volume available in the 0.25L reactor used in this study

for CUB experiments, the lower extent of delignification observed suggests poorer lignin extraction probably due to an over concentrated suspension. In these conditions, access to lignin in the lignocellulosic matrix is further hindered, therefore leading to ineffective dissolution of lignin fragments to the liquor.

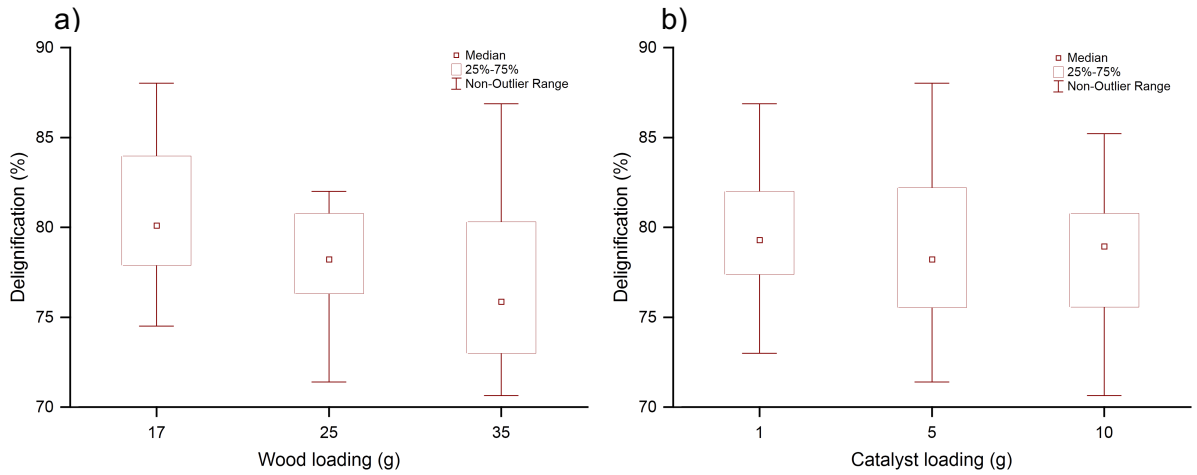


Figure 6-8 – Wood-loading (a) and catalyst-loading (b) dependence on the variability of delignification degree in pulps obtained from the CUB process carried out under varied conditions (as described in Table 6-1). General experimental conditions CUB: Poplar wood (17, 25 or 35g chips), Raney Ni (1, 5 or 10 g), 2-PrOH/H_2O 7:3 v/v (60, 100 or 140mL), 200 ºC for 3 h, autogenic pressure.

Hemicellulose retention

A wider spectrum of values is obtained for hemicellulose retention, ranging from 12% to 39%. Employing higher levels of liquor and catalyst loading in CUB is overall associated with the highest retention of hemicellulose among the screened conditions (Figure 6-9). Regarding the effect of the amount of Raney Ni, an increase from 1 to 10g resulted in a better preservation of sugars in the pulps (average of 22% and 31%, respectively), as the production of organic acids released upon fractionation[41b, 41d]

and responsible for the acid hydrolysis of the hemicellulose fibres[42c, 43] is hampered in presence of Raney Ni,[41g, 116] hence conducing to a better preservation of hemicellulose. Indeed, the correlation with the pH of the liquor well reproduces the one described in a previous report,[28a] as a hemicellulose retention of 28% was coupled with a liquor pH of 5.4 in DoE -1 0 -1, which increased to 32-37% in DoE tests "-1 0 0" and "-1 0 1" producing a liquor pH of 5.7. Moreover, tests performed without added catalyst (Organosolv, entries 28-31 in Table 6-1) showed a low extent in retention of hemicellulose (15-21%), highlighting the crucial role of Raney Ni in the preservation of hemicellulose through catalytic decomposition of organic acids. Employing 140mL of liquor in CUB is overall associated with the highest retention of hemicellulose among the screened conditions (average=31%), suggesting the presence of a concentration effect that minimised sugar release in this specific condition. Indeed, a liquor volume as little as 60mL might dissolve the organic acids released from lignocellulose while still keeping their local concentration (i.e. close to the holocellulose fibres) high, as the low volume prevents effective diffusion of these molecules in the liquid phase (i.e. away from the polysaccharide matrix). As a result, acid hydrolysis of hemicellulose is maximized in this condition, while it is abated with larger liquor volumes (i.e. L=100, 140 mL).

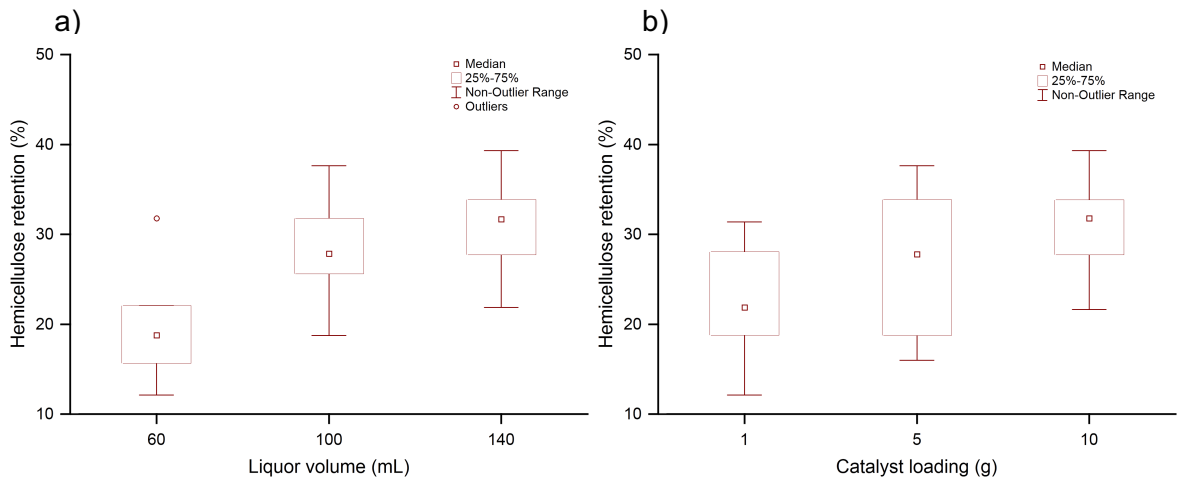


Figure 6-9 – Liquor-volume (a) and catalyst-loading (b) dependence on the variability of retention of hemicellulose in pulps obtained from the CUB process carried out under varied conditions (as described in Table 6-1). General experimental conditions CUB: Poplar wood (17, 25 or 35g chips), Raney Ni (1, 5 or 10 g), 2-PrOH/H_2O 7:3 v/v (60, 100 or 140mL), 200 ºC for 3 h, autogenic pressure.

Finally, to better visualise the non-linear effects that wood loading, liquor volume and catalyst loading exert on the performances of the CUB process, 3D surface plots were generated for each dependent variable, and reported in Figure 6-10. In these plots, the two independent variables with highest statistical significance for the selected dependent variable are included (Table 6-2). Expectedly, both L_M/H_M (Figure 6-10a) and the total content of monophenols in lignin products (Figure 6-10b) are similarly affected by the presence of Raney Ni, being catalyst loading the most crucial variable for depolymerisation through H-transfer reactions. In fact, an increase from C=1g to C=5-10g resulted in high values of L_M/H_M and phenolic contents regardless of the other variables used. However, in the latter case a weak decrease was associated to the increase of the amount of processed wood. This evidence suggests that effective depolymerisation of lignin fragments to MW species below 550Da can be achieved in these conditions. However, further depolymerisation to monophenols is mildly

hampered by the release of a larger amount of lignin fragments related to higher wood loading. In terms of molecular homogeneity, lignin depolymerisation can be funnelled towards the production of 4-(3-hydroxypropyl)phenols by combining a high liquor volume (L=100, 140mL) with catalyst loading in the range 5-10g, as a result of the better control on Raney Ni's selectivity in H-transfer reactions promoted by a low concentration of lignin fragments in the liquor and an adequate amount of catalyst (Figure 6-10c). It is apparent that effective delignification can be achieved in the conditions investigated in this study with low to medium values of catalyst loading and wood loading, to allow effective dissolution of the lignin fragments in the liquor (Figure 6-10d). In turn, the retention of hemicellulose (Figure 6-10e) can be maximized up to 39% by combining the high values of L (140mL) and C (10g), as a combination of a concentration effect linked to the liquor volume and the prevention of the production of organic acids, catalysed by Raney Ni.

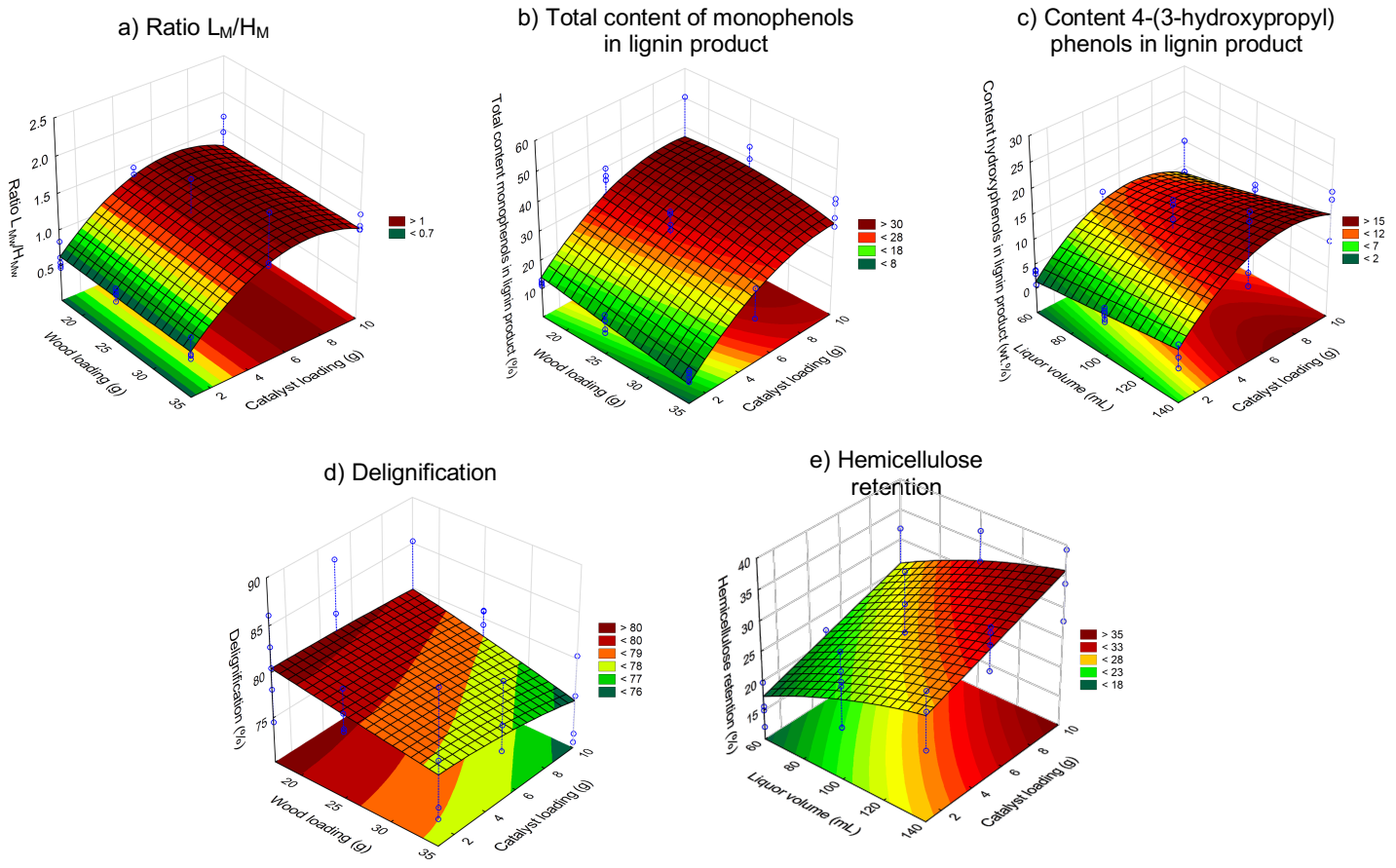


Figure 6-10 – 3D surface plots for performance responses obtained from the CUB process carried out under varied conditions (listed in Table 6-1). The values obtained for each dependent variable are displayed as blue dots. a) ratio L_M/H_M in lignin product; b) total content of monophenols in lignin product; c) content of 4-(3-hydroxypropyl)phenols in lignin product; d) pulp delignification; e) hemicellulose retention in pulp.

6.2.4 Defining the optimum conditions for Catalytic Upstream Biorefining

The surface plots in Figure 6-10 offer key information for the optimisation of the experimental conditions employed in the CUB process, as they provide the W, L and C values required to maximize the performances of the process. In the perspective of biorefinery, the final goal is to conduct lignocellulose fractionation to generate a highly delignified pulp and an extensively depolymerised lignin product with high individual yield of monophenols. Hence, to optimise the CUB process the experimental conditions allowing to maximize pulp delignification, L_M/H_M, total content of monophenols and content of 4-(3-hydroxypropyl)phenols in the lignin products were considered. The retention of hemicellulose was not included in this evaluation, as a different extent of retention might be preferred according to the chosen application for the pulp fraction. In fact, a high retention of hemicellulose in the pulp is desired in the perspective of utilisation of the whole content of biomass, however it also promotes saturation of the phenolic units, entailing an undesired increase in hydrogen consumption.[28a] Conversely, hemicellulose removal from the wood structure is preferred when highly pure cellulose fibres are required, as in the case of the paper industry.[117] Based on the experimental conditions explored in this work, optimisation of the performances in the CUB process can be achieved by processing 17g of wood in 140mL of liquor in presence of 7g of catalyst. These conditions, whose effects on process performance are discussed in the following section, are close to the test "-1 1 0", which resulted in an depolymerised lignin product (L_M/H_M=1.3±0.1) with a high amount of monophenols (38.3±1.2wt%, 4-(3-hydroxypropyl)phenols=23.7±0.9wt%), and a delignified pulp (delignification=80±2%) with relatively high amount of retained hemicellulose (36±2%). Additionally, a set of values of wood loading, liquor volume

and catalyst loading outside the ranges explored in the DoE study was defined in an attempt to further increase the performances of the process (W=1.5g, L=150mL, C=12g). This set of "extrapolated" conditions, together with the set of "optimised" conditions, was tested on the CUB process. The resulting performances in lignocellulose deconstruction and molecular uniformity of the lignin stream are discussed in the following section.

6.2.5 Testing CUB on optimised experimental conditions

To assess the suitability of the optimised conditions to a larger scale, a CUB experiment was performed in a 10L reactor using the set of "optimised conditions" (i.e. W=17g; S=140mL; C=7g), accordingly up-scaled. Additionally, a second set of conditions, namely "extrapolated conditions" (i.e. W=1.5g; S=150mL; C=12g), was tested in the 0.25L reactor used in the DoE approach. Surprisingly, the soluble products isolated from the latter CUB experiment comprised two distinct fractions, which were analysed separately: a reddish-brown lignin oil, resembling the product typically recovered from CUB;[7a, 28a] and an uncoloured, low-viscosity liquid product.
An overall assessment of the process performances obtained from CUB experiments performed in the selected conditions is summarised in Table 6-5. Importantly, the CUB experiment performed with "extrapolated conditions" produced enhanced performances towards lignin valorisation compared to DoE "-1 1 0". Indeed, these conditions coupled a high degree of delignification (89%) with the isolation of an extensively depolymerised lignin fraction (L_M/H_M=18.0). A hemicellulose retention of 60% confirmed the enhancing effect of the combination of a low W with a high L. Notably, the fractionation experiment performed in a large-scale reactor with optimised

conditions exhibited promising performances in CUB closely resembling those obtained from DoE “-1 1 0”. Indeed, this test achieved effective depolymerisation of lignin to yield a low-MW product (L_M/H_M=1.6) rich in monophenols (41.4 wt% of lignin product, 4-(3-hydroxypropyl)phenols=30.5wt%). However, lower delignification (63% compared to 80%) and higher retention of hemicellulose (51% compared to 36%) obtained in these conditions suggest a lower extent of biomass deconstruction in comparison to what observed for CUB performed in a small scale in similar conditions (DoE “-1 1 0”). Such low degree of delignification cannot be explained by extensive condensation on lignin as a result of low activity of Raney Ni in H-transfer reactions, which in turn exhibited good performances towards lignin depolymerisation. Furthermore, fractionation experiments performed in similar conditions without added Raney Ni (Organosolv experiments, 0.25L reactor scale) resulted in a delignification degree above 70% (Table 6-2). One possible explanation for this result is the limited heat transfer typically experienced in large-scale batch experiments, compared to that of smaller scale experiments.[118] Indeed, improving mixing inside the reactor by optimising the stirring rate and/or modifying the reactor design can lead to better heat transfer alongside the reaction vessel i.e. smaller temperature gradients, leading to enhanced biomass deconstruction.

The GPC analysis of the lignin fractions obtained from the new investigated conditions provided striking information regarding the extent of depolymerisation achieved (Figure 6-11). Importantly, the experiment performed with “extrapolated conditions” exhibited unprecedented depolymerisation of lignin in CUB, with the oil fraction only containing species with MW<5kDa, and the transparent fraction comprising a small amount of monophenols and light compounds below 550 Da. In

turn, the optimised conditions for CUB tested on a large-scale reactor produced a lignin product with MW distribution similar to that obtained from DoE "-1 1 0".

Notably, these similarities also extended to the chemical composition of the lignin products (obtained by GC-FID/MS analysis, Table 6-6), which presented a comparable amount of monophenols (37.1% for DoE "-1 1 0", 41.4% for optimised conditions) with a molecular distribution prevalently made of 4-(3-hydroxypropyl)phenols (30.5 wt%). The lignin fractions obtained from CUB performed with "extrapolated" conditions also presented a monophenol-rich portion, accompanied by a minor content of hemicellulose-derived polyols. Surprisingly, both fractions isolated in these conditions presented a much simpler composition compared to the DoE lignin products, with two main compounds dominating the distribution of monophenols: 2,6-dimethoxy-4-methylphenol and 2,6-dimethoxy-4-ethylphenol (compound 3 and 6 in Table 6-6), obtained from the removal of the hydroxyl group on the propyl chain of 4-(3-hydroxypropyl)-2,6-dimethoxyphenol (compound 10), and further shortening of the alkyl chain. The evidence obtained from this test, employing W/C=0.125 $g_{wood}/g_{catalyst}$ and W/L=0.01 g_{wood}/mL_{liquor} (compared to W/C=3.4 $g_{wood}/g_{catalyst}$ and W/L=0.12 g_{wood}/mL_{liquor} in DoE "-1 1 0'), contrasts with that observed in DoE tests, for which a decrease in concentration of lignin-derived species and high catalyst loading resulted in higher contents of 4-(3-hydroxypropyl)phenols (Figure 6-10c). It is apparent that such extrapolated conditions enhance the activity of Raney Ni towards deoxygenation and demethylation of lignin-derived species, favouring the production of ethyl- and methylphenols over 4-(3-hydroxypropyl)phenols, therefore providing a way to shift the selectivity from hydroxypropyl- to alkylphenols.

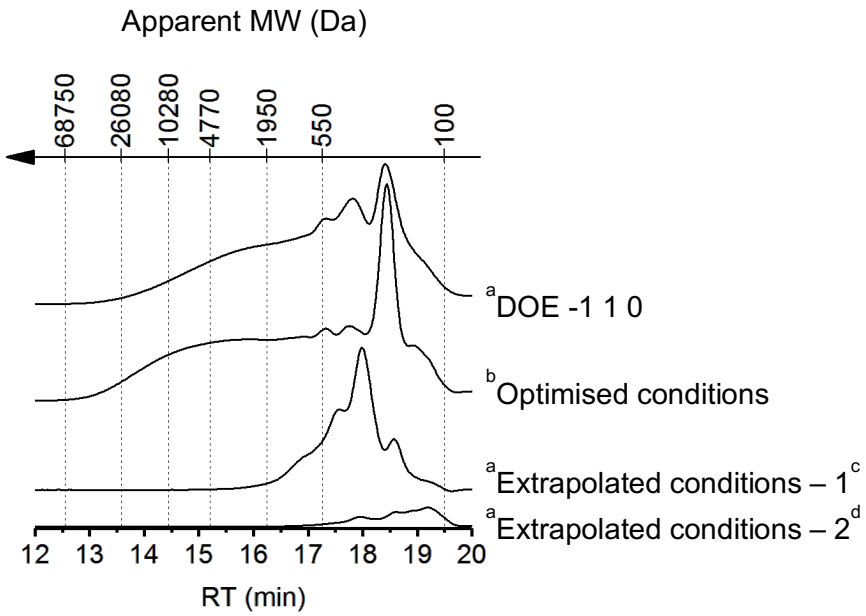
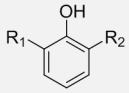
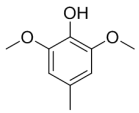
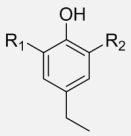
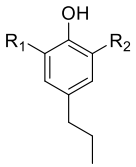
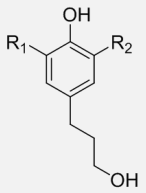


Figure 6-11 - Gel permeation chromatograms of lignin products obtained under various conditions (listed in Table 6-5). The apparent MW is calculated relative to calibration with polystyrene standards. CUB experiments were performed in [a] 0.25L or [b] 10L scale reactor. [c] Brown fraction and [d] uncoloured fraction obtained from the CUB experiment with "extrapolated conditions".

Table 6-5 - Results obtained from catalytic upstream biorefining (CUB) of Poplar wood under “optimised” and “extrapolated” conditions. [a] General experimental conditions CUB: Poplar wood (chips), Raney Ni, 2-PrOH/H_2O 7:3 v/v, 200 ºC for 3 h, autogenic pressure. [b] Average values of two tests as reported in Table 6-1. [c] Value based on the GPC analysis of the brown fraction (fraction 1 in Figure 6-11). [d] Calculations for delignification extent and hemicellulose retention are reported in the Methods section.

		Experimental conditions					Overall performance			
Entry[a]	Test	Reactor scale (L)	W (g)	L (mL)	C (g)	Ratio L_M/H_M	Content of phenols in lignin product (wt%)	Content hydroxypropyl phenols in lignin product (wt%)	Delignification (%)[d]	Hemicellulose retention (%)[d]
1	DoE 16 “-1 1 0” [b]	0.25	17	140	10	1.3	38.3	23.7	80	36
2	Optimised conditions	10	476	3920	195.5	1.6	41.4	30.5	63	51
3	Extrapolated conditions	0.25	1.5	150	12	18.0[c]	23.6	0.6	89	60

Table 6-6 - Weight content of selected monophenols occurring in the lignin products obtained from the CUB process carried out under varied conditions (listed in Table 6-5), as determined by GC-FID/MS analysis. Selected compounds: phenol (1), 2,6-dimethoxyphenol (2), 2,6-dimethoxy-4-methylphenol (3), 4-ethylphenol (4), 2-methoxy-4-ethylphenol (5), 2,6-dimethoxy-4-ethylphenol (6), 2-methoxy-4-propylphenol (7), 2,6-dimethoxy-4-propylphenol (8), 4-(3-hydroxypropyl)-2-methoxyphenol (9), 4-(3-hydroxypropyl)-2,6-dimethoxyphenol (10). [a] General experimental conditions CUB: Poplar wood (chips), Raney Ni, 2-PrOH/H_2O 7:3 v/v (amounts listed in Table 6-5), 200ºC for 3 h, autogenic pressure. [b] Brown oil fraction. [c] Uncoloured fraction.

					Content of phenols in lignin product (wt%)										
															
					1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
Entry[a]		W (g)	L (mL)	C (g)	$R_1=R_2$: H	$R_1=R_2$: OCH_3		$R_1=R_2$: H	R_1: H; R_2: OCH_3	$R_1=R_2$: OCH_3	R_1: H; R_2: OCH_3	$R_1=R_2$: OCH_3	R_1: H; R_2: OCH_3	$R_1=R_2$: OCH_3	Sum
1	DoE 16 "-1 1 0"	17	140	5	2.7	0.3	0.6	0.3	0.8	3.9	0.6	5.1	9.3	13.5	37.1
2	Optimised conditions	476	3920	195.5	2.4	0.5	0.2	0.4	0.5	1.5	0.8	4.7	15.3	15.2	41.4
3	Extrapolated conditions – 1[b]	1.5	150	12	0.9	<0.1	9.9	<0.1	0.1	11.8	0.1	0.3	0.4	0.2	23.6
4	Extrapolated conditions – 2[c]	1.5	150	12	1.1	<0.1	1.5	<0.1	<0.1	5.7	0.1	0.1	0.1	<0.1	8.7

6.3 Conclusions

The understanding and control over the fractionation of plant biomass producing high-quality pulps together with lignin streams of low molecular weight (MW) with controlled molecular diversity constitutes the overarching objective of CUB. The potential of the released lignin products for the direct synthesis of value-added materials in the existing industrial value-chain, or even in new industrial value-chains, depends on the quality, functionalisation and molecular diversity of the lignin fragments present in those streams. In this perspective, the DoE approach on the CUB here reported has provided crucial insights on the role that the amount of processed Poplar wood (W), volume of lignin-extracting liquor (L) and catalyst loading (C) exerted on biomass deconstruction and the molecular uniformity of lignin. The statistical analysis showed the catalyst loading to be the main significant variable for depolymerisation and the prevention of condensation reactions occurring on lignin, as they are directly related to the amount of catalytic sites available. While lignin depolymerisation heavily relies on the catalyst loading, the deconstruction of biomass is a more complex process requiring an appropriate combination of all three variables screened. Indeed, the wood loading and liquor volume seem not to exert any significant effect on the extent of low-MW fraction and the polymeric fraction in lignin product, differently from delignification, which in turn strongly relies on the combination of W and C values. Indeed, processing the highest amount of wood (35g) with the highest catalyst loading (10g) considered in this study resulted in ineffective extraction of lignin from the lignocellulose matrix, probably due to an overconcentration of the suspension made of wood, catalyst and liquor. Regardless of the liquor volume employed, the experiments performed with W/C≥17g/g (C=1g in this work) resembled an Organosolv-like fractionation, showing

a less effective depolymerisation. In turn, the better incorporation of hydrogen in lignin products observed in tests with W/C≤7g/g (C=5g, 10g in this work) reflected a more efficient depolymerisation of the lignin fragments, leading to the production of a higher fraction of monophenols. Notably, the selectivity of Raney Ni in H-transfer reactions can be tweaked by appropriate choice of the liquor volume and catalyst loading, which can control the extent of deoxygenation and demethylation reactions to yield 4-(3-hydroxypropyl)phenols or the corresponding alkylphenols. Through statistical analysis of the DoE results, the optimal conditions to maximize the performances of fractionation were defined. A CUB experiment performed in a 10L batch reactor with these optimised conditions exhibited promising performances in terms of lignin depolymerisation to monophenols, yielding 4-(3-hydroxypropyl)phenols with high individual yields. Importantly, these compounds can be used for the synthesis of bio-derived surfactant and detergents[119] and hold the potential for the production of new-value added materials that may replace those obtained from fossil-derived chemicals. However, the process will require further optimisation in order to improve biomass deconstruction on a larger scale. An extrapolation of the optimisation results provided a set of experimental conditions, namely “extrapolated conditions”, with the potential to further increase the performances of the process. Importantly, these conditions, employing a relevantly lower ratio of wood to catalyst amount (0.125 $g_{wood}/g_{catalyst}$), exhibited enhanced performances in fractionation, as they resulted in the isolation of a highly delignified pulp along with two extensively depolymerised lignin fractions: a first fraction comprising monophenols and lignin-derived oligomers with MW<5kDa; and a second fraction made of monophenols and hemicellulose-derived polyols of MW < 550 Da. Strikingly, both fractions exhibited a simpler chemical composition compared to the lignin oils typically isolated from CUB, with only two compounds

dominating the distribution of monophenols. Both sets of “optimised” and “extrapolated” conditions for CUB provided a strategy for funnelling lignin depolymerisation to a limited set of compounds with high individual yields. The deeper knowledge obtained in this study regarding biomass deconstruction and lignin processing in CUB provides useful information for tweaking the fractionation process to obtain a holocellulose pulp with different degrees of hemicellulose removal, and a lignin stream comprising hydroxypropyl- or alkyl-phenols that can be easily integrated into convergent approaches for the production of lignocellulose-derived chemicals and fuels.[5b, 67] The optimisation of the laboratory-scale process here achieved sets the ground for the upscaling of the CUB process, which showed promising evidence for the adaptability to a larger scale.

7. Summary and concluding remarks

This dissertation investigated the several aspects of the Catalytic Upstream Biorefining process based on the Early-stage Catalytic Conversion of Lignin concept, aiming to better understand how to engineer the fractionation of biomass, in order to obtain a carbohydrate pulp and a lignin product with the desired properties i.e. highly delignified pulps and extensively depolymerised lignin products. In detail, this dissertation targeted the processes involved in:

- the deconstruction of the lignocellulosic matrix, to release lignin fragments as well as hemicellulose-derived poly- and oligo-saccharides;
- the depolymerisation on lignin fragments released from the lignocellulosic matrix;

The role of the pH of the extraction medium in fractionation was firstly studied by performing CUB in neutral to weakly alkaline conditions. The neutralisation of the organic acids released upon fractionation of biomass through the addition of inorganic bases (i.e. $NaHCO_3$, Na_2CO_3 and NaOH) significantly lessened the extent of hemicellulose hydrolysis. Indeed, increasing the pH of the liquor from 4.3 (i.e. without added base) to 7.5 produced a five- to six-fold increase in the amount of hemicellulose retained in the carbohydrate pulp. However, the retention of hemicellulose remained constant when further increasing the pH at values higher than 7.5. The higher retention of hemicellulose in the carbohydrate pulp resulted in a lower content of monosaccharides in the liquor, which promoted an increase in the activity of Raney Ni towards hydrogenation reactions. By tuning the pH of liquor, it was possible to shift the selectivity of the catalytic reaction from aromatic monomers (at pH=4.3) to cyclohexanols (at pH=8.2). However, this possibility might be undesirable as it entails

extensive consumption of hydrogen. The delignification degree of lignocellulose was found to be pH-independent, proving that the solvolytic release of the lignin fragments from the lignocellulose matrix, typically catalysed by the acid catalysts generated *in-situ*, was effectively occurring despite the neutralisation of such acids. Notably, Raney Ni played a crucial role in promoting effective delignification upon fractionation, by drastically suppressing the condensation reactions typically occurring on the released lignin fragments, which were favoured in alkaline conditions, to yield polymeric lignin species that can reprecipitate on the carbohydrate pulps, hence increasing their residual lignin content.

The CUB process was also tested at other end of the pH spectrum. Spruce (softwood) and Sugar Cane Bagasse (SCB, grass) were subjected to CUB experiments with acid added in order to simulate the acetylation level of Poplar (hardwood), which showed better performances towards biomass deconstruction compared to the former feedstocks. The acidic conditions promoted a mild increase in the extent of hemicellulose hydrolysis, therefore the associated increase of the concentration of sugars in the liquor did not significantly affect the activity of Raney Ni, which allowed extensive depolymerisation of the lignin fragments extracted from both lignocellulosic feedstocks. However, SCB showed lower performances in lignin depolymerisation compared to Poplar and Spruce. As hypothesised by observing the compact structure of SCB by digital microscopy, the different behaviour of this feedstock in lignin depolymerisation might be related to a less effective transport of the released lignin fragments from the lignocellulosic matrix to the liquor. This hypothesis was verified through CUB experiments on SCB performed at higher temperatures (from 180°C to 240°C). Indeed, the higher deconstruction of the lignocellulosic biomass promoted under these conditions allowed for a higher extent in lignin depolymerisation, as the

lignin fragments effectively diffused from the lignocellulosic matrix to the liquor, where Raney Ni is suspended. Overall, these pieces of evidence highlighted the crucial role that the three-dimensional structure of the lignocellulosic feedstock plays in its fractionation.

In order to evaluate the time evolution of the physicochemical processes occurring upon lignocellulose fractionation, CUB was studied over 300min. This study highlighted the relevance of the first 50min of the process, which allowed the extraction of 70% of the total amount of lignin recovered after 300min. The delignification degree kept increasing up to 150min, after which no significant amount of lignin was extracted from biomass. Regarding lignin depolymerisation, Raney Ni effectively catalyses hydrogen-transfer reactions for the production of low-MW lignin products. The decrease in the rate of lignin solvolysis observed after the first 50min, which therefore released a lower amount of lignin fragments into the liquor, coincided with a mild increase in the catalytic activity, as an increase of the ratio L_M/H_M was accompanied by a decrease of the weight average molecular weight when increasing the processing time to 300min. Overall, CUB fractionation of Poplar seem to fulfil its potential with a processing time of 150min, after which no relevant increase in the process performances was observed.

Finally, a DoE approach was applied on the CUB process to study the interplay of the amount of processed Poplar wood (W), volume of the solvent mixture (S) and catalyst loading (C) in defining the performances of fractionation (i.e. biomass deconstruction and lignin depolymerisation). The DoE study highlighted the crucial role that the concentration of the lignin fragments in the liquor, defined by the employed amount of wood and solvent volume, played on both the delignification degree, while it did not seem to significantly affect the extent of depolymerisation on lignin. In turn, these

reactions were mainly depending on the amount of catalyst employed i.e. amount of catalytic sites available for H-transfer reactions. In detail, effective depolymerisation required an appropriate combination of wood and catalyst loading (W/C≤17g/g). A statistical analysis of the DoE results allowed the definition of W, S and C values for the maximisation of process performances. In the perspective of process upscaling, these “optimised” conditions were tested in a CUB experiment performed 10L batch reactor, which reproduced the high performances expected for lignin depolymerisation to monophenols. However, lower delignification was achieved in comparison to the 0.25L-scale reactor used for the DoE study, suggesting the need for further studies aimed at the optimisation of the process on a larger scale. Through extrapolation of the DoE results, another of conditions was defined aiming to further increase the process performances. A CUB experiment performed with these “extrapolated” conditions markedly decreased the extent of condensation reactions, as no species of MW>5kDa were found in the lignin product. Importantly, the production of monophenols in these conditions was funnelled towards the production of two main compounds (i.e. 2,6-dimethoxymethyl phenol and 2,6-dimethoxyethyl phenol).

Overall, this dissertation provided a better understanding of the phenomenological aspects of the Catalytic Upstream Biorefining process. In an attempt to improve the clarity on the interaction of the numerous physicochemical processes occurring upon fractionation, Figure 7-1 summarises all the findings reported in this dissertation concerning biomass deconstruction and lignin depolymerisation. From the complexity of this scheme, it is apparent that tweaking the experimental conditions of CUB in order to tune the properties of the final products is not a trivial task, due to the intricate interdependence of the factors involved in fractionation. However, the deeper knowledge obtained in this study aims to provide the right tools for this goal. The

optimisation of CUB herein achieved and the preliminary results on process upscaling represent an important step towards the application of this fractionation approach for a biorefinery scheme aimed at the production of highly-functionalised aromatic compounds and high-quality carbohydrate pulps.

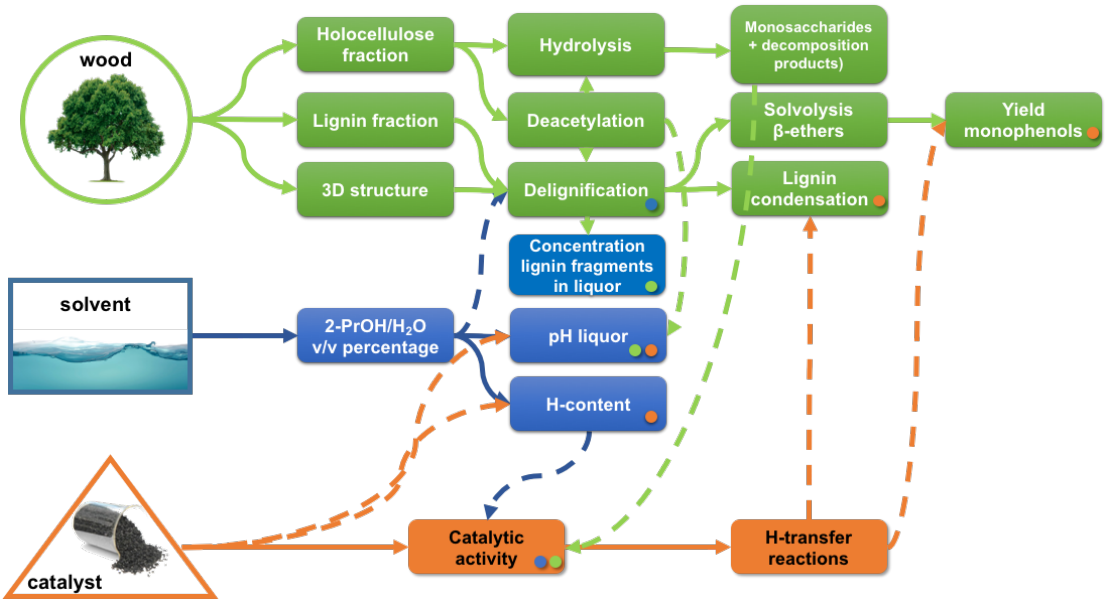


Figure 7-1 - Schematic description of the interplay of process parameters in CUB, and their effect on the physicochemical processes underpinning fractionation. Coloured dots highlight the multiple variables influencing a specific aspect of the process. Solid lines indicate the direct effects that a variable exerts on the processes mainly depending from the same variable; dash lines indicate the interplay effects that a variable exerts on the processes mainly depending from a different variable.

7.1 Future works

The morphology of lignocellulose played a crucial role in the diffusion of the lignin-extracting medium inside the lignocellulosic matrix, and subsequently the transport of the released lignin fragments from the matrix to the liquor, where the catalytic reactions can occur. An improved understanding of these processes can be provided by the in-depth analysis of the three-dimensional structure of wood through X-ray tomography. Indeed, mapping the internal structure of the lignocellulosic feedstocks (e.g. channels, pits, perforations in the plant structure) might shed light into the lower performances of softwoods and grasses towards biomass deconstruction compared to hardwoods. In this context, a simple comminution of the lignocellulosic substrate through knife- or ball-milling might increase the accessibility of lignin for its solvolytic release occurring in CUB, hence favouring biomass deconstruction.

The location of the native lignin in the lignocellulosic feedstock is equally relevant for fractionation.[120] Through fluorescence microscopy studies on untreated Poplar wood (hardwood), it was found that syringyl- (S-) and guaiacyl- (G-) and not uniformly distributed inside the plant cell structure. Indeed, the S-lignin was mainly found in the cell lumen, while the G-lignin was located in the middle lamella and vessel secondary wall regions. In turn, untreated Pine and Spruce (softwoods) showed a uniform distribution of G-lignin in the interstices between different cells, and covered with a non-fluorescent material.[120] This arrangement makes lignin less accessible in softwoods compared to hardwoods. Hence, fluorescence microscopy analysis on the pulp derived by Organosolv and CUB experiments performed on different types of lignocellulosic feedstocks could provide additional insights into the extraction of lignin occurring in fractionation. By correlating the extent of removal of the different types

with their location in the lignocellulosic matrix, it might be possible to tune the conditions of CUB to target a specific type of lignin for extraction, hence achieving a "selective" extraction of aromatic compounds. This possibility would increase the desirability of the lignin product thus isolated, which would only contain one class of monophenols.

The CUB experiment performed in a 10L reactor discussed in Section 6.2.5 provided some preliminary results for the process upscaling. These conditions produced a delignification degree lower than that achieved from experiments in a 0.25L reactor, probably as a result of the limited heat transfer typically experienced in large-scale batch experiments, compared to that of smaller scale experiments.[121] Indeed, further studies aimed at the optimisation of the process on a large scale are required. In this perspective, increasing the stirring rate might result in enhanced biomass deconstruction as a consequence of better heat transfer alongside the reaction vessel i.e. smaller temperature gradients.

8. Experimental

8.1 Chemicals and materials

Acetic acid (Sigma-Aldrich, 100%), acetone (VWR, 100%), acetonitrile (ACN, VWR, ≥99.99%), ammonium hydroxide (NH_4OH, Fisher Scientific, 35%), N,N-dimethylformammide (DMF, VWR, ≥99.90%), di-n-butyl ether (Acros, >99%), formic acid (Sigma-Aldrich, ≥98%) lithium bromide (LiBr, Sigma-Aldrich, ≥99%), methanol (MeOH, Sigma-Aldrich, ≥99.9%), oxalic acid (Sigma Aldrich, 98%), 2-propanol (2-PrOH, Sigma-Aldrich, 99.9 %), sodium bicarbonate ($NaHCO_3$, Aldrich, 99%), sodium carbonate (Na_2CO_3, Aldrich, 99%), sodium hydroxide (NaOH, Baker, 97%) sulphuric acid (H_2SO_4, Fischer Scientific, 95-97%), Raney Ni 2800 slurry (Aldrich), Poplar wood (2 mm chips, J. Rettenmaier&Sohne), Sugar Cane Bagasse (Campinas, SP, Brazil), Spruce wood (mm pellets, J. Rettenmaier & Sohne) were used as received without further purification.

8.2 Methods

8.2.1 Reactions

8.2.1.1 Organosolv process

Typically, Poplar wood (17g chips) and solvent (2-PrOH/H_2O, 7:3 v/v, 140mL) were placed in a 300 mL stainless steel pressure vessel.

In the experiments reported in Chapter 5, Poplar wood (0.12g, chips) and solvent (2-PrOH/H_2O, 7:3 v/v, 1mL) were placed in a 2 mL stainless steel pressure vessel.

In the experiments reported in Chapter 6, the amounts of Poplar wood and solvent used in the CUB experiments are reported in Table 6-1.

In the experiments reported in Chapter 3, $NaHCO_3$, Na_2CO_3 and NaOH were added to the suspension in amounts reported in Table 3-2. In the experiments reported in Chapter 4, acetic, formic and oxalic acid were added to the suspension in amounts reported in Table 4-2.

The pressure vessel was heated to a temperature of 180°C over the course of 1 h under mechanical stirring. The suspension was processed at 180°C for 3 h.

In the experiments reported in Chapter 5, the suspension was processed at different times (min): 5, 15, 30, 45, 50, 60, 75, 85, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 240, 300.

In sequence, the mixture was allowed to cool to room temperature. The liquor was separated from the pulp (holocellulose) by filtration through a filter paper. Organosolv lignin was isolated from the liquor by removing the solvent mixture at 60 °C under reduced pressure by using a rotary evaporator. Organosolv lignin was obtained as a reddish-brown solid residue.[7a, 28a, 68]

8.2.1.2 Catalytic Upstream Biorefining (CUB) process

Typically, Poplar wood (17g chips), Raney Ni (10g, wet) and solvent (2-PrOH/H_2O, 7:3 v/v, 140mL) were placed in a 300 mL stainless steel pressure vessel.

In the experiments reported in Chapter 5, Poplar wood (0.12g, chips), Raney Ni (0.07g, wet) and solvent (2-PrOH/H_2O, 7:3 v/v, 1mL) were placed in a 2 mL stainless steel pressure vessel.

In the experiments reported in Chapter 6, the amounts of Poplar wood, Raney Ni and solvent used in the CUB experiments are reported in Table 6-1.

In the experiments reported in Chapter 3, $NaHCO_3$, Na_2CO_3 and NaOH were added to the suspension in amounts reported in Table 3-2. In the experiments reported in Chapter 4, acetic, formic and oxalic acid were added to the suspension in amounts reported in Table 4-2.

The pressure vessel was heated to 200 °C in 1 h under mechanical stirring. The reaction proceeded under autogenic pressure for 3 h.

In the experiments reported in Chapter 5, the suspension was processed at different times (min): 5, 15, 30, 45, 50, 60, 75, 85, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 240, 300.

In sequence, the mixture was allowed to cool to room temperature. The liquor was separated from the solids (Raney Ni plus pulp) by filtration through a glass fibre filter (GF6, Ø 90 mm, Whatman). In sequence, the solids were re-suspended in 2-PrOH under mechanical stirring. Raney Ni was attracted to the flask bottom by a magnet externally placed at the bottom of the round flask. This procedure was repeated another four times to remove the entire catalyst content from the pulp fibres. The pulp remained in suspension and was recovered by filtration and consequently dried at 60°C for 24h. The washing aliquots of 2-PrOH were added to the liquor. Lignin products were isolated by removing the solvent at 60°C under reduced pressure by using a rotary evaporator. The lignin product was obtained as a reddish-brown oil.[7a, 28a, 68]

8.2.2 Analysis of the lignin stream

8.2.2.1 Elemental analysis

The elemental composition (CHNS/O) of holocellulose and lignin streams (ca. 2 mg) was determined by using an Elementar VarioMICRO Cube. Each analysis was repeated at least four times. The oxygen content in the sample was determined by subtraction (%O=100-%C-%H-%N-%S-%ash). From the carbon content (in gram, that is, considering the weight of substrate and isolated products), the carbon yields of pulp and lignin products were calculated by using Equations 1 and 2, respectively:[28a]

$$Carbon\ yield_{pulp} = \frac{C - content_{isolated\ pulp}\ (g)}{C - content_{poplar\ wood}\ (g)} \times 100 \quad (1)$$

$$Carbon\ yield_{soluble\ products} = \frac{C - content_{isolated\ pulp}\ (g)}{C - content_{poplar\ wood}\ (g)} \times 100 \quad (2)$$

8.2.2.2 GC-FID/MS analysis

An aliquot of lignin oil (50.0 mg) and the external standard (di-n-butyl ether, ISTD, 10.0 mg) were dissolved in 2 mL MeOH. The sample was filtered (membrane filter 0.45 µm). The sample solutions were analysed by GC-MS 2010 Plus (Shimadzu) equipped with a column ZB-1HT Inferno (30 m, 0.25 mm ID, d_f 0.25 mm). The injector temperature was 300°C. The temperature program started with an isothermal step at 40°C for 5 min. Next, the temperature was increased from 40 to 300°C by 5.2 °C min^{-1}. The program finished with an isothermal step at 300°C for 5 min.[28a] The products were identified by comparison with commercial standards (for phenol, 4-ethylphenol, 2-methoxy-4-ethylphenol, 2-methoxy-4-propylphenol, 2,6-dimethoxyphenol, 2,6-

dimethox-4-methylphenol, 4-(3-hydroxypropyl)-2-methoxyphenol, 4-(3-hydroxypropyl)-2,6-dimethoxyphenol), or with a search of the MS spectrum with the MS libraries NIST 08, NIST 08s and Wiley 9 (for 2,6-dimethoxy-4-ethylphenol, 2,6-dimethoxy-4-propylphenol). In some cases, the structure was proposed by the analysis of the EI fragmentation pattern and by comparison of retention times with authentic samples. Quantification of selected components was performed by using the FID response.[71] The response factor of the products was determined by injection of commercial standards (for phenol, 4-ethylphenol, 2-methoxy-4-ethylphenol, 2-methoxy-4-propylphenol, 2,6-dimethoxyphenol, 2,6-dimethox-4-methylphenol, 4-(3-hydroxypropyl)-2-methoxyphenol, 4-(3-hydroxypropyl)-2,6-dimethoxyphenol) or by using the Effective Carbon Number method (ECN) basing on the commercial standards injected (for 2,6-dimethoxy-4-ethylphenol, 2,6-dimethoxy-4-propylphenol, a commercial standard of 2,6-dimethox-4-methylphenol was used).[122]

8.2.2.3 GCxGC analysis

An aliquot of lignin oil (50.0 mg) and the external standard (hexadecane, ISTD, 10.0 mg) were dissolved in 2 mL MeOH. The sample was filtered (membrane filter 0.45 µm). The sample solutions were analysed by 2D-GC X GC-MS (first column : ZB-1HT Inferno 30 m, 0.25 mm ID, df 0.25 mm; second column: BPX50, 1 m, 0.15 mm ID, df 0.15 mm) on a GC-MS 2010 Plus (Shimadzu) equipped with a ZX1 thermal modulation system (Zoex). The injector temperature was 300°C. The temperature program started with an isothermal step at 40°C for 5 min. Next, the temperature was increased from 40 to 300°C by 5.2°C min^{-1}. The program finished with an isothermal step at 300°C for 5 min. The modulation applied for the comprehensive GC X GC analysis was a hot jet

pulse (400 ms) every 9000 ms. The 2D chromatograms were processed with GC Image software (Zoex). The products were identified by a search of the MS spectrum with the MS libraries NIST 08, NIST 08s and Wiley 9. In some cases, the structure was proposed by the analysis of the EI fragmentation pattern and by comparison of retention times with authentic samples. Quantification of selected components was performed by using the FID response.[7a]

8.2.2.4 Gel permeation chromatography analysis – GPC-RID/VSD

To analyse the apparent molecular weight distribution in lignin products, an aliquot of the product (50 mg) was dissolved in DMF + LiBr 0.1 wt% (1 mL) and filtered (membrane filter 0.45 µm) prior to injection. The GPC analyses were performed at 80°C with an Agilent 1260 Infinity HPLC equipped with three columns (Polargel-L guard 50x4.6mm, Polargel-M 300x7.5mm, Polargel-L 300x7.5mm, Agilent), and by using DMF + LiBr 0.1 wt% as the eluent (1.0 mL min^{-1}). For detection, a refractive index detector (RID) and a viscometer detector (VSD) were used. All responses were normalised by the sample weight. The apparent molecular weight is given relative to polystyrene standards (Agilent), and thus is only for a relative assessment of the overall changes in the apparent molecular weight distribution. The area below the GPC-RID chromatogram for MW < 550 Da was assigned to the low-MW region, while the area below the GPC-RID chromatogram for MW > 550 Da was assigned to the high-MW region. The ratio L_M/H_M was calculated by using Equation 3:

$$\frac{L_M}{H_M} = \frac{area\ below\ GPC-RID\ chromatogram_{low-Mw\ region}}{area\ below\ GPC-RID\ chromatogram_{high-Mw\ region}} \quad (3)$$

8.2.2.5 *Acid–base titration*

The titrations were carried out with a Titrino Plus 848 (Metrohm). To evaluate the content of acids in the Organosolv lignin, acid– base titrations of the liquor obtained from the Organosolv control experiment were performed. An aliquot of the liquor (0.5 mL) was diluted in a solution of 2-PrOH/H_2O (20 mL, 7:3 v/v) and placed in a beaker. The solution was titrated under magnetic stirring with a solution of $NaHCO_3$, Na_2CO_3 or NaOH (0.01 mol L 1 in 2-PrOH/ H_2O, 7:3 v/v). The equivalent point was obtained from the derivative of the titration curve. Each determination was repeated at least three times.[28a]

8.2.2.6 *UV-Vis spectroscopy analysis*

To analyse the absorption of lignin products in the UV-Vis region, 1 mL of the liquor obtained from Organosolv and CUB process was added to a solution of (2-PrOH/H_2O (7:3 v/v, 99 mL). All UV-Visible spectra were measured at 25 °C using a UV-Vis spectrometer (Agilent Technologies, UV-Vis 01) over a 200 – 900 nm spectral range, using a cuvette with a path length of 0.1 mm and a solution of 2-PrOH/H_2O (7:3 v/v) as blank.

8.2.3 Analysis of the holocellulosic pulp

8.2.3.1 *Moisture and ash determination*

The moisture in the substrate and pulp (sample weight: 1 g) was determined on a thermal balance (Ohaus MB25) as the weight loss at 105°C after 10 min. For each sample, this analysis was repeated at least three times. For the determination of the ash content, ca. 100 mg of pulp or starting material were placed in a quartz crucible.

The samples were then burned in a ventilated muffle oven under a controlled temperature program: 25 to 450°C at 7°C min^{-1}; 450 to 750°C at 2.5°C min^{-1}; and 750°C for 2 h. In sequence, the crucibles were quenched to room temperature in a desiccator. The ash was weighed. For each sample, this analysis was repeated at least three times.[7a]

8.2.3.2 Determination of pulp composition

The content of glucans, xylans and Klason lignin in the pulps were determined by acid saccharification. Typically, 50 mg of Soxhlet-extracted, ground and sieved sample (500 mm) were mixed with a 72% sulfuric acid solution (0.5 mL) under manual stirring with a spatula at 30°C for 25 min. In sequence, distilled water (10 mL) was added to the mixture and the spatula was washed to transfer any residue back to the container. The saccharification was then conducted under stirring at 130°C for 2 h. The suspension was left to cool down to room temperature. The suspension was filtered on a 0.45 µm membrane filter. The filtrate (1 mL) was neutralised with 120 µL NH_4OH 35% and analysed by HPLC. The determination of the sugar content was performed with a Perkin Elmer Series 200 HPLC equipped with a Grace Alltech Prevail carbohydrate ES 5U column (250mm length, 4.6mm ID and an Alltech 3300 ELSD detector (T: 60°C, gas flow: 2L/min, gain: 1). The analyses were carried out at 30°C by using a solution of 1 mm NH_4OH in ACN 70% in H_2O as the eluent (1 $mLmin^{-1}$). The identification and quantification of sugars was performed by comparison with authentic samples.

For the determination of Klason lignin content, the same saccharification procedure was used. After the saccharification at 130°C for 2 h, the reaction mixture was filtered

on a 0.7μm membrane filter previously weighed. The solid was washed with distilled water until neutral pH. The solid was dried in an oven at 60°C for 24h. The weight of this dried solid was considered as the residual lignin in the carbohydrate fraction.

The determination of the glucans, xylans and lignin contents was performed at least in three replicates for each sample. The average values (±2%) for the isolated pulp and its composition are 'dry, ash- and extractive- free'.

The extent of hemicellulose retained in the holocellulosic pulp produced from Organosolv and catalytic biorefining experiments was calculated by using Equation 8:[28a]

$$Hemicellulose\ retained\ (\%) = \frac{xylans_{pulp}\ (g)}{xylans_{wood}\ (g)} \times 100 \quad (8)$$

The extent of hemicellulose removed from the holocellulosic pulp produced from Organosolv and catalytic biorefining experiments was calculated by using Equation 8:

$$Hemicellulose\ removal\ (\%) = \frac{xylans_{wood}\ (g) - xylans_{pulp}\ (g)}{xylans_{wood}\ (g)} \times 100 \quad (8)$$

Delignification extent of the pulp was calculated by using Equation 10:[28a]

$$Delignification\ extent\ (\%) = \frac{lignin_{wood}\ (g) - lignin_{pulp}\ (g)}{lignin_{wood}\ (g)} \times 100 \quad (10)$$

The content of glucans, xylans, Klason lignin, acid soluble lignin and ash in the pulps discussed in Chapter 6 were determined by compositional analysis performed by

Celignis Limited (Limerick, Ireland) according to the published procedure available in Ref.[123].

8.2.3.3 Digital microscopy

Spruce wood, SCB and their Organosolv and CUB pulps were imaged with a Keyence VHX-500 digital microscope. A large set of images were obtained from different areas of the samples (at least 20 images per sample) and from different chips/fibres to guarantee the reproducibility of the observed features.

8.2.4 Statistical analysis of CUB results

The statistical analysis of the results obtained from CUB results was performed with the data software TIBCO Statistica. A Response Surface Regression analysis was performed using: wood loading (W), solvent volume (S) and catalyst loading (C) as independent variables; delignification, hemicellulose retention, L_M/H_M, Bn_{10kDa}, total content of phenols as dependent variables.

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