Lignin as a renewable aromatic resource for the chemical industry

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Thesis

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Voor Ellis, Kay, Ryan, mijn ouders en mijn schoonouders

Chapter 1

Introduction: Lignin valorization for wood adhesives and aromatic chemicals
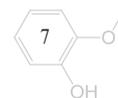
This chapter describes the background, context and topics of this thesis. Options for lignin valorization makes sense especially when this issue is positioned within the wider context of biorefinery and the biobased economy. These terms and definitions will be subsequently described followed by an introduction of lignin as a biopolymer and its versatile and intriguing properties will be discussed. This leads to the choices made in this thesis research, which is outlined at the end of this introduction.

1.1 General introduction

Today, we use and rely on many commodity consumer products like energy, materials, plastics, chemicals and transportation fuels. These consumer products largely originate from fossil resources which will be depleted sooner or later and contribute to CO_2 emissions and climate change. Therefore, alternatives are sought with low carbon emissions and these are inexhaustible resources like wind, solar energy and plant derived biomass. While energy can be produced by wind, solar systems and biomass, the other mentioned consumer products can only be made from biomass. Also to secure the energy supply, which is now unreliable due to unstable fossil oil supply chains in politically unstable countries and the expected increased demand for oil from emerging economies, plant biomass can be a suitable alternative source.

This sustainable resource is to be used within the biobased economy which is expected in the years to come to gradually take a larger share compared to the fossil-based economy. The biobased economy is not just the implementation of innovative technologies using renewable resources, but it will be a real transition with a broad and high impact on society at different levels (Langeveld and Sanders 2010). To promote the implementation of the biobased economy the governments of many countries have set ambitious goals for replacing fossil derived fuel and chemical commodities by biomass (Table 1.1).
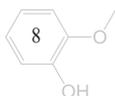
Table 1.1 Indicative goals (%) for fossil replacement by biomass.

Region	Transportation fuels			Chemical commodities			Reference
	2020	2030	2040	2020	2030	2040	
NL	10	30	30		30	20-45	Dutch ministry of Economic Affairs and Platform Biobased Raw Materials (Ree van & Annevelink, 2007)
EU	10	25					EC (2009); ERTRAC (2010)
US	10	20		18	25		Perlack et al. (2005)

Lignocellulosic biomass offers many possibilities as feedstock for the energy sector but also for the chemical industry due to its chemical composition, abundant availability and relative low costs when the conversion to products can be carried out in an economic and sustainable manner. This abundant availability is supported by the large numbers of world-wide annual lignocellulosic biomass production of about 200 billion tons (Zhang 2008) compared to the 0.3 billion tons of organic chemicals yearly produced by the chemical industry (Haveren et al. 2008). Other advantages of biomass as a feedstock are the lowered demand for crude oil supplies and less dependence on politically unstable oil exporting countries. Furthermore, sustainability criteria and fixation of atmospheric CO_2 are important drivers in using biomass resources. Disadvantages (or challenges) in using biomass are the need for fertile arable land and more complicated collection and logistic systems to mobilize this relatively low density organic material compared to crude oil. As biomass is commonly heterogeneous and has a different composition to fossil resources different processing conditions are needed. New opportunities in the production of functionalized chemicals and materials can be found due to the carbohydrate, protein and phenolic building blocks contained in biomass. In contrast to petrochemical resources that need to be cracked, decomposed and functionalized, biomass often needs to be partially defunctionalized.

The key to the most efficient use of biomass is to design a suitable and sustainable integral biorefinery to separate biomass in its major compounds in order to generate the highest value added for all fractions. According to the International Energy Agency (IEA) Bioenergy Task 42 Biorefinery: "A biorefinery is the sustainable processing of biomass into a spectrum of marketable products ranging from energy, food, feed, chemicals and materials applications" (Figure 1.1).
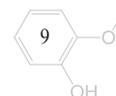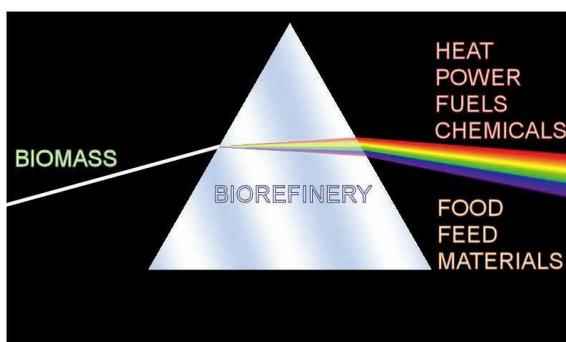
Figure 1.1 Biorefinery and its role in the transformation of biomass (IEA Task 42 Biorefineries 2010).

Schematically, a fully integrated agro-biofuel-biomaterial-biopower biorefinery using sustainable technologies is given in Figure 1.2. Ragauskas et al. (2006) stated that for a widely applicable lignocellulosic biorefinery not only the carbohydrates are of interest but the value added application of the lignin component should also be addressed.
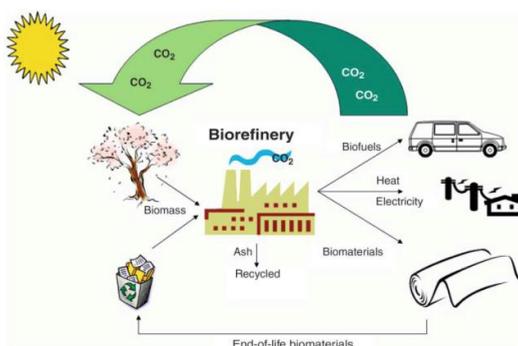
Figure 1.2 The fully integrated agro-biofuel-biomaterial-biopower cycle for sustainable technologies (Ragauskas et al. 2006).

An example of such an industrial biorefinery is the well established sustainable biorefinery operated by Borregaard in Norway as depicted in Figure 1.3. This biorefinery separates woody biomass into cellulose specialty fibres (dissolving cellulose) and lignosulfonates. Additionally, part of the dissolved lignin is converted via catalytic oxidation to vanillin and dissolved carbohydrates are fermented into the second generation (2G) biofuel bioethanol. In this way more than 90% of the wood input is used as marketable products.
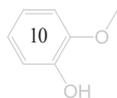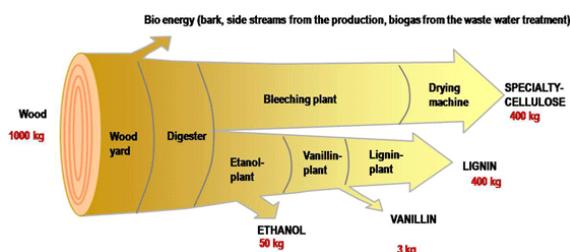
Figure 1.3 Sustainable industrial wood biorefinery operated by Borregaard, Norway (2010).

Various technologies are under development for lignocellulosic biorefineries in which the lignin fraction is mainly considered as an energy source. However, more and more biorefinery technologies make use of the possibility to separate the biomass in a carbohydrate-rich and lignin-rich fraction. Examples are organosolv and alkaline pretreatments which will be discussed in Section 1.4.

1.2 Lignin

The term lignin is derived from the Latin word for wood *lignum.* Lignin is a major constituent in structural cell walls of all higher vascular land plants. Its polyphenolic structure is well known for its role in woody biomass to give resistance to biological and chemical degradation. This is due to its hydrophobic nature and insolubility in aqueous systems preventing access of degrading chemicals and organisms. The monomeric units of phenylpropane in lignin polymers are linked in a complex network through different types of ether and ester bonds as well as carbon-carbon bonds. The lignin occurring in plant cell walls is commonly closely associated with polysaccharide structures of cellulose and hemicellulose (Figure 1.4). Wood and other lignocellulosic resources are used to extract the cellulose fibres for paper or composite applications or for the production of dissolving cellulose. To remove and dissolve the hydrophobic lignin it is chemically degraded or modified under harsh (alkaline) conditions. The residual black liquor containing the lignin fraction is mostly used as fuel feedstock for plant operation. In this way a large part (up to 40%) of the photosynthetic carbon fixed by the plants is inefficiently utilized and released in the ecosystem as CO_2.

The (bio/ecological) life cycle of lignin carbon is a major element in the closing of complete CO_2 cycle and the mineralization process of carbon from plant biomass. Natural mechanisms of lignin decomposition include the bio-degradation by microbial
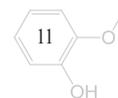
enzymes and irradiation by sun light, but also the fragmentation at elevated
temperatures or under pressure of mechanical shear. Lignin is known for its complex
chemical structure which is even more complicated by the breaking and uncontrolled
rearrangement of bonds due to radical initiated reactions. Lignin (bio)degradation is an
aerobic process and under anaerobic conditions, such as in peat soils and compost, it is
found to be stable for long periods. In soil sciences these lignin residues are referred to
as insoluble humus or humic acids.
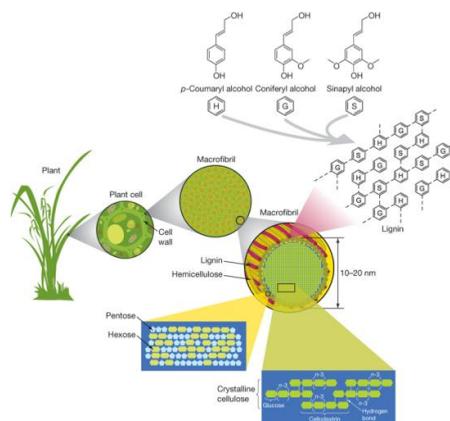
Figure 1.4 Structure of lignocellulosic biomass (Rubin 2008).

Next to the production of lignin residue (black liquor) in the pulp and paper industry,
more recently a new type of lignin residue is emerging. With the development of
biorefinery processing of lignocellulosic biomass to monomeric sugars for the
production of second generation (2G) biofuels and other desired biobased products, e.g.
'green' chemicals and biopolymers (Haveren et al. 2008), a non-digested fraction in the
spent fermentation broth will be generated. This fraction consists for a large part of
lignin. As it is known that phenolic lignin degradation products may inhibit the ethanol
fermentation process (Klinke et al. 2004) it is suggested to remove lignin by (bio)-
chemical means before the saccharification processes for an efficient production of
biofuels from lignocellulosics (Weng et al. 2008).

  In the degradation processes of lignocellulosics the recalcitrance of the lignin
polymeric structure is well known. Acid and alkaline depolymerization of lignin will
result in breaking of the ester bonds and some of the ether bonds, but the reactivity of
the liberated fragments may result in a rearranged and even more condensed polymeric
structure (Figure 1.5). Therefore the extraction conditions that are applied to

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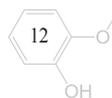
lignocellulosic biomass substantially affects the structure and properties of the resulting (technical) lignin.
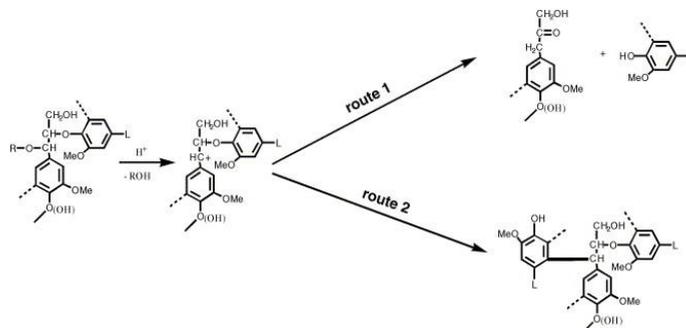
Figure 1.5 Reaction scheme showing the competition between depolymerization of a β-O-4 structure (Route 1) and repolymerization involving a lignin structure (Route 2) (Li et al. 2007).

1.3 Lignin structure

Lignin occurs widely in the middle lamellae and secondary cell walls of higher plants and plays a key role in constructive tissues as a building material, giving it its strength and rigidity and resistance to environmental stresses (Ralph et al. 2007). Lignin contents may vary in softwoods from 24-33%, in temperate zone hardwoods from 19-28%, and in tropical hardwoods from 26-35% (Dence and Lin 1992). In non-wood fibre crops the lignin content is generally lower and ranges from below 3%, in cotton and in extracted flax or hemp bast fibres, to around 11-15% for sisal and jute (Van Dam et al. 1994). In grasses such as cereal straws, bamboo or bagasse the lignin content ranges from 15-25% (Bagby et al. 1971). Compared to wood lignin, lignins from annual crops such as from grasses are reported to be less condensed (Billa et al. 2000). Some examples of these grass lignins, e.g. wheat straw and sarkanda grass, were studied in this thesis.

Besides other important properties of lignin in the cell wall, as previously described, its major functional role in woody tissues can be regarded as being a structural component. The bio-composite is composed of a stiff three dimensional crosslinked matrix (very similar to thermosetting resins like phenol-formaldehyde resins) reinforced with cellulosic fibrils, connecting the individual cells. Despite its rigidity, the lignin matrix needs a large flexibility when exterior physico-mechanical

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forces act upon it leading to shear stresses and deformation. Rearrangements of bonds within the lignin network under external stress conditions, leads to more condensed polymers (like is observed in compression wood). Self-repairing mechanisms in the cell walls by means of a radical type of reaction easily can lead to demethylation and demethoxylation, as well as the formation of novel C-C bonds and ring structures. This phenomenon is also observed when extracted lignin fragments are being handled or analyzed. The molecules tend to coagulate under certain conditions, which complicates working with lignin substantially.

From a chemical point of view, lignins are considered as complex polyphenols and despite many research efforts, its chemistry, biosynthesis and molecular biology is up till now not fully understood (Boerjan et al. 2003; Ralph et al. 2007). As a result, the lignin structure is not exactly defined, but several researchers published representations of the prominent substructures of lignin. One example is depicted in Figure 1.6 (Brunow 2001). In this figure the various functional groups are highlighted by different colours.
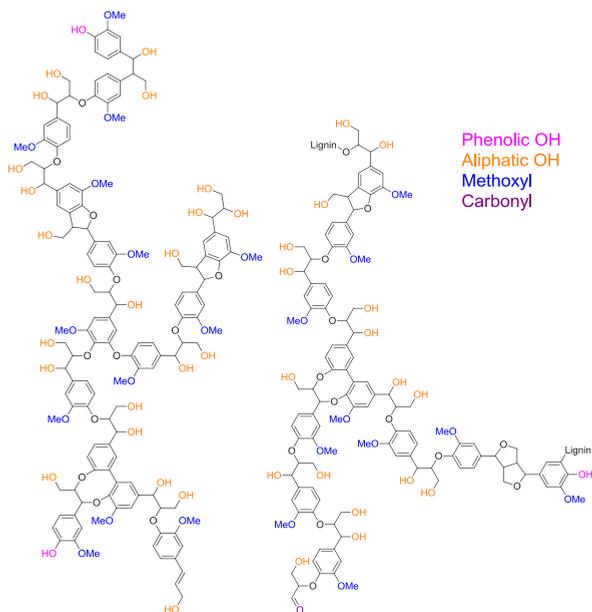
Figure 1.6 Softwood lignin structure as proposed by Brunow (2001).

Lignins are built in plants starting from three basic monolignols via oxidative phenolic coupling reactions to generate the polymer (Ralph et al. 2007). The heterogeneity of

unrevealed. Because of involvement of radical reactions during the dehydrogenative polymerization, the chemical bonding patterns appear to be randomly and display no stereo-specificity, which is exceptional for biopolymers. However, some regio-specificity and preference for the formation of the β-O-4 bond has been reported and it is suggested, but also strongly debated, that dirigent proteins play a role as template in lignin assembly (Ralph et al. 2007). The chemistry of lignin is complicated compared to other biopolymers like proteins or carbohydrates, that are linear chains or at the most branched polymers. Lignin is composed of a three dimensional network, lacking the regular and ordered repeating units of other biopolymers such as cellulose. Also restricted information is available about the crosslinking between lignin and cell wall carbohydrates. Ester and ether linkages have been reported for ferulic acid and saccharide molecules. Novel analytical and genetic tools may lead to a more complete understanding of the bio-controlled formation of this polymer in its native form (Ralph et al. 2007).

The majority (approximately two-third) of chemical bonds in the native lignin polymeric network are of the C-O-C ether linkage type between the phenylpropane units, predominantly β-O-4, while about one-third consists of C-C bonds between these units (Table 1.2). Table 1.2 also shows considerable differences in linkage occurrence between softwood and hardwood lignin. Furthermore lignin includes also branched and crosslinked structures. The more the lignin is condensed the more difficult it is to degrade and to get it dissolved in the pulping or fractionation processes.

Lignin is separated from the other lignocellulosic parts of plants by physical and/or chemical means. Not only the botanical source, but also the delignification (pulping) process and extraction procedures will highly influence the lignin structure, purity and properties. During the delignification of biomass ester and ether linkages will be largely disrupted and lignin fragments will be dissolved in the pulping liquor. The resulting technical lignin will differ significantly compared to the original lignin in the biomass. The differences in native lignin compared to technical lignins were studied by thioacidolysis, by which ether linkages in the lignin structure will be cleaved selectively, as presented in **Chapter 3**. Milled wood lignin was used as it represents the structural average of the total native lignin in wood. The structure of lignin isolated by any pulping or fractionation method is without any regular repeating unit, and lignin can thus be considered as an amorphous biopolymer.
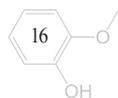
Table 1.2 Frequencies of different linkage types in native softwood and hardwood lignin per 100 C_9 units as proposed by (Henriksson et al. 2010).

Name	Structure	Softwood	Hardwood
β-O-4		40-50	50-60
β-5		10-12	3
5-5		13	3
4-O-5		3	3
β–β		3	3
Bonds to 1-position		1-3	3
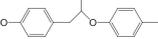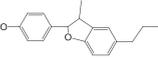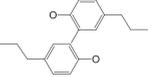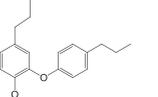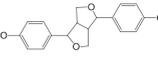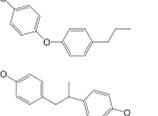
1.4 Technical lignin types and their availability

The variation in technical lignin structure is partly caused by the botanical origin of the polyphenol but equally important is the method of extraction (Lora et al. 2008; Lora and Glasser 2002). Common pulping processes used for the extraction of lignin from lignocellulosic raw materials for the production of paper are listed below. The worldwide availability of technical lignins is presented in Table 1.3.

Kraft pulping process

The most common chemical pulping process of wood today is the sulfate or kraft pulping process, a process using sodium sulfide under alkaline conditions. The lignin is partly cleaved and thiol groups were introduced at the β-position of the propane side chain (Figure 1.8) resulting in a solubilized lignin. Kraft pulp mills have evolved in large efficient integrated facilities in which the recovery of pulping chemicals and
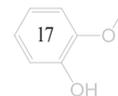
energy via combustion of the black liquor, with the solubilized lignin, is necessary. Therefore only small quantities of sulfur-containing kraft lignin for chemical use can be recovered from the black liquor. Currently only one company is producing kraft lignin commercially at a scale of about 60 kt/year (Table 1.3). As a modern kraft pulp mill nowadays may generate an energy excess relative to its needs, extraction of lignin may be allowed to give a marketable product (Lora et al. 2008). With this approach, which may be applied to most kraft mills, debottlenecking of the recovery boiler takes place while the pulp capacity could be increased (Öhman et al. 2006).
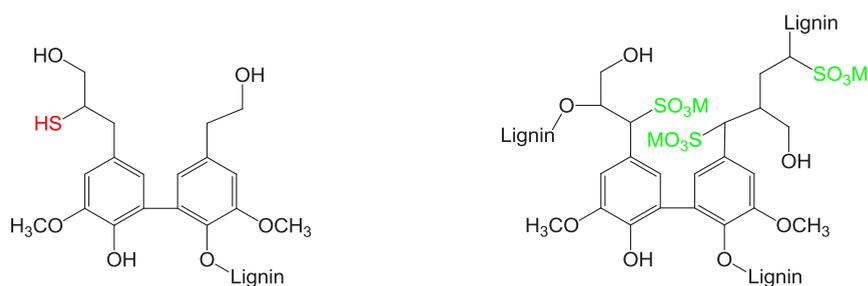
Figure 1.8 Simplified structures of kraft lignin (left) with introduced thiol group (-SH) and lignosulfonate (right) with introduced sulfonate group and counter ion (-SO$_3$M) (Holladay et al. 2007).

Sulfite pulping process

Besides the kraft process, the sulfite process is widely applied. In this process, an aqueous solution of sulfur dioxide is used at different pH's. Sulfonate groups are introduced in the lignin structure at the α-position of the propane side chain and the so-called lignosulfonates are formed (Figure 1.8). Due to the sulfonate groups most lignosulfonates are water-soluble and make these lignins different from other lignin types. Sulfite pulping does not selectively remove lignin and carbohydrates appear to be chemically attached to the lignosulfonate fragments. In some cases purified lignins are obtained by removal of the carbohydrate impurities by fermentation, chemical removal, ultrafiltration or selective precipitation (Lora et al. 2008). Currently about 1 Mt/year lignosulfonates are produced by several companies as displayed in Table 1.3.

Soda pulping process

This process uses sodium hydroxide instead of sulfide to dissolve the lignin from lignocellulosic material, such as annual fibre crops like flax and straw, and wood. Soda

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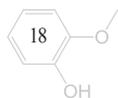
lignin is recovered by an alternative recovery process by acid precipitation, maturation and filtration giving novel types of sulfur-free lignin (Abächerli and Doppenberg 1998; Abächerli and Doppenberg 2000; Lora and Glasser 2002).

Organosolv pulping and/or fractionation processes
The use of organic solvents, e.g. ethanol, allows avoiding the formation of sulfated by-products. Organosolv pulping or fractionation enables the production of high quality cellulose AND high quality lignin. The water insoluble organosolv lignins are more pure containing a higher percentage of lignin. The major organosolv processes are the following:

- Lignol process, based on the Alcell ethanol/water pulping process,
- ASAM, Alkaline Sulfite Anthraquinone Methanol pulping,
- Organocell, Methanol pulping followed by anthraquinone/NaOH pulping,
- Acetosolv, an acetic acid/HCl pulping,
- Milox, formic acid/hydrogen peroxide delignification,
- Avidel, formic/acetic acid pulping.

These processes are not commercial yet, but have been demonstrated at pilot and demonstration scale. Organosolv pulping or fractionation of lignocellulosic biomass is nowadays one of the selected pretreatments to produce high quality cellulose for pulp and/or biofuel production together with a high purity lignin for materials and chemicals. Both the Canadian company Lignol (former Alcell process; Hallberg et al. 2010) and the French company CIMV (Avidel process; Delmas 2008) are using an organosolv fractionation technology (Table 1.3).

In this thesis, high purity organosolv lignins obtained from ethanol/water fractionations of mixed hardwoods (Alcell[tm] lignin) and wheat straw are studied for the production of aromatic chemicals described in **Chapter 5**.

Biomass pretreatment and conversion (biorefinery)
Examples of these biomass pretreatment and conversion processes are strong or dilute acid pretreated lignocellulosic biomass followed by enzymatic hydrolysis of the carbohydrates. The resulting lignin fraction contains a considerable amount of residual carbohydrates (Vishtal and Kraslawski 2011).

Steam explosion process

Woody biomass is pretreated with steam at high temperature and high pressure, followed by a rapid pressure release. The fibrous network is disrupted and liberated fibres and bundles are formed. In this process the (autocatalytic) acid hydrolysed lignin can be extracted from the cellulose, to large extent, by alkali or organic solvents (Gellerstedt and Henriksson 2008). The resulting steam explosion lignin contains a low content of carbohydrates and wood extractive impurities. It resembles the native lignin more than the other produced technical lignins as the chemical structural changes are rather limited at the process conditions applied.

In Table 1.3 an overview is given of available lignin resources, status of process development, production capacity, and lignin purity. Compared to the technical lignin production situation in 2004 as evaluated in the EUROLIGNIN network (Gosselink et al. 2004b), the major changes in 2011 are summarized hereafter:

1. soda sulfur-free lignins are produced commercially
2. organosolv sulfur-free lignins are produced at pilot scale. Up-scaling is expected in the near future.
3. initiatives for increased extraction of kraft lignins (eg. via LignoBoost technology)
4. several lignocellulosic biomass fractionation technologies are operated at pilot scale generating biorefinery lignins

Production of technical lignin is expected to increase in the coming years due to debottlenecking of the recovery boiler in pulp and paper processes, mainly in kraft processes. By extracting part (10-20%) of the lignin from the black liquor, the recovery boiler can handle more black liquor leading to an increase in the pulp capacity of the mill. The extracted lignin can be used for replacement of fossil fuel for the lime kiln in the existing kraft process or for value added applications outside the mill. Rough calculations indicated that worldwide about 40 Mt of kraft lignin per annum is extracted from wood and in Europe half of this amount (Lindgren et al. 2011). If 10-20% of this amount will be recovered by using the LignoBoost system, 2-4 Mt/year of extra kraft lignin in Europe will become available. The remaining part is still needed to generate
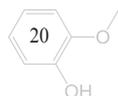
energy for the current process.

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         In a modern lignocellulosic biorefinery plant about 40% of the dried lignin-rich stream is necessary to meet the thermal requirements of 2G bioethanol production in particular for the biomass pretreatment step and the ethanol distillation part. The remaining 60% excess of lignin could be utilized as a feedstock for green chemicals and materials giving additional revenues to the biorefinery plant (Sannigrahi et al., 2010). The directives of the EC in 2020 to replace 10% of transportation fuels by biofuels (Table 1.1) will likely result in the generation of large amounts of lignin in the biofuel production from lignocellulosic biomass. In 2020 10% of the annual use of about 300 Mtonnes of transportation fuels must be generated from biomass in the EU-25. If 50% will consist of bioethanol and the other half of biodiesel for both 15 Mt will be needed. To produce 15 Mt of bioethanol, approximately double the amount 2x15=30 Mt of carbohydrates (fermentable sugars) are necessary. Assuming that half of this amount will be produced from lignocellulosic biomass as so-called 2G bioethanol, together with 15 Mt of carbohydrates (C6 and C5) from lignocellulose 5 Mt of (pure) lignin will be generated per annum. In practise, this potentially enormous lignin stream will not be highly pure but associated with other biomass components such as undigested carbohydrates, proteins and minerals. Therefore this lignin-rich stream will be even higher in amount up to 7.5 Mt/annum. In 2030 the production of 2G biofuels will further increase by a factor 2.5 to substitute 25% of the fossil-based transportation fuels. This will lead to the generation of slightly less than 20Mt/annum of biorefinery lignin. 40% of this amount needs to be used for the energy requirements of the biorefinery, which means that about 60% = 12 Mt can be potentially produced as a lignin product. Together with the additional lignin from the pulp and paper industry (2-4 Mt/year) potentially about 14-16 Mt/annum lignin will become available in the coming years in Europe. In this section it is shown that a variety of technical lignins are available or will become available in the future. As these lignins differ in purity, properties and costs these materials will be used for different applications as described in the next section. In this thesis, in particular the high purity lignins such as kraft, soda and organosolv lignin from different raw materials (wood, grass and agro residues) were studied for development of applications. These lignins were selected to minimize the influence of impurities on the behavior of the lignin in the chosen applications. However, for analytical purposes the other less pure lignins (steam explosion, hydrolysis lignin, and lignosulfonates) were used in the characterization work to show the broad applicability

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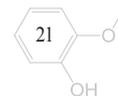
and robustness of the SEC method and to show some of the challenges in lignin characterization.

One of the challenges is the determination of the (absolute) molar mass distribution of technical lignins. Molar mass is an important parameter governing the reactivity and physico-chemical properties, such as the rheological behavior, of lignins for development of applications. This molar mass is also important for monitoring delignification, lignin oxidation and lignin depolymerization processes. As the currently used SEC methods result in large variations in molar mass, there is a strong need to develop a universal method which allow a quantifiable comparison of the (absolute) molar mass of different lignins. Therefore special emphasis was given in this thesis to develop reliable standard methods for determination of the molar mass distribution of a wide range of biorefinery lignins (**Chapter 2**).
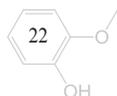
Table 1.3 Lignin resources availability per 2011.

Lignin type	Scale of operation	Volume (kt/year)	Suppliers	Sulphur presence	Purity[1]	References
Lignosulfonates (soft/hardwood)	Commercial	~1000	a) Borregaard LignoTech (NO, worldwide), b) TEMBEC (FR, US), c) Domsjö Fabriker (SE), d) La Rochette Venizel (FR), e) Nippon Paper Chemicals (JPN)	Yes	Low-medium	a) www.borregaard.com c) Christoffersson et al. (2011)
Kraft softwood	Commercial	60	Meadwestvaco (US)	Yes	High	www.meadwestvaco.com
Kraft softwood	Pilot	0.5-4	LignoBoost/Metso (SE)	Yes	High	Öhman et al. (2009)
Soda non-wood	Commercial	5-10	Greenvalue (CH, IND)	No	High	Abächerli & Doppenberg (1998)
Soda wood	Pilot/RTD	<0.5	Northway Lignin Chemical (US)	No	Medium-High	Northway Lignin Chemicals, 2010
Organosolv straw (acids)	Pilot	0.5	CIMV (FR)	No	High	Kham et al. (2005); Delmas (2008)
Organosolv hardwood (EtOH/H$_2$O)	Pilot	0.5-3	f) Lignol Innovations (CAN)[2], g) DECHEMA/Fraunhofer (DE), h) Dedini (BR)	No	High	f) Goyal et al. (1992); Hallberg et al. (2010); Lora et al. (1989); Winner et al. (1991) g) Michels & Wagemann (2011)
Hydrolysis non-wood/wood	Pilot	0.5	i) SEKAB (SE)	No	Low-medium	i) www.sekab.com, Gnansounou (2010)
Hydrolysis crop residues	Pilot	0.5	j) Inbicon (DK, US) k) Chemtex (IT, US, CHN)	No	Low-medium	j) www.inbicon.com k) www.chemtex.com
Hydrolysis LC Biomass (HCl)	Pilot/RTD	<0.5	HCl Cleantech (US, ISR)	No	Medium-high	www.hclcleantech.com
Steam explosion straw/softwood	RTD	<0.5	l) Abengoa Bioenergy (ES), m) ENEA (IT)	No	Medium	l) Gnansounou (2010) m) Zimbardi et al. (1999)

1) Impurities are generally residual carbohydrates, ash and proteins and largely depends on feedstock and process
2) Former technology of Repap. Technologies, Canada (Alcell™)

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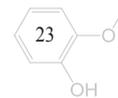
1.5 Potential applications for lignin

Lignin seems to be a versatile raw material for many applications as reviewed by (Pye 2006). The opportunities and challenges for biorefinery lignins were described in an extensive study (Holladay et al. 2007). This report demonstrates the versatility of lignin for multiple applications. Potential uses of lignin were classified in different groups as listed hereafter:

1. power-fuel-syngas
2. macromolecules
3. aromatics

These groups can also be distinguished according to the time-to-market with group 1 as current or near term applications, group 2 for medium term applications and group 3 for the longer term applications.

In the first group, lignin is used as a carbon source for energy production or is converted in energy carriers such as syngas. The second group make use of lignin's macromolecular nature and will be used in high molecular mass applications like wood adhesives (binders), carbon fibres, and for polymers like polyurethane foams (Gandini and Belgacem 2008; Abe et al. 2010). The third group uses technologies to cleave the lignin structure into monomers without sacrificing the aromatic rings for production of polymer building blocks, aromatic monomers such as benzene, toluene, and xylene (BTX), phenol, and vanillin.

In this thesis two potential value added lignin applications have been selected. One from group 2 (Lignin for binder application; phenolic resins) and one from group 3 (Production of fine chemicals from lignin; phenol derivatives). These 2 selected applications represents not the bulk applications, such as energy and (bio-)bitumen, but the more value added applications with a lower volume market as shown in the top part of the pyramid (Figure 1.9). The introduction to these applications and the results of this study will be discussed in the following sections and in **Chapters 3, 4 and 5**.
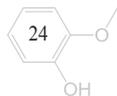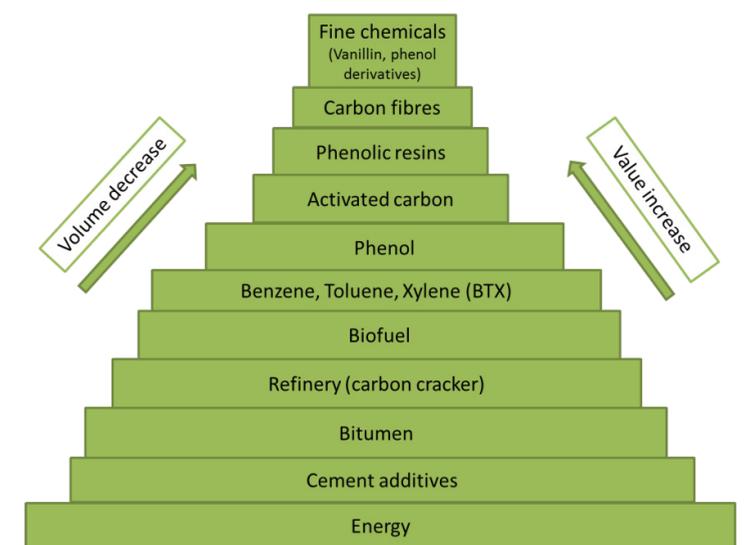
Figure 1.9 Potential lignin applications.

Another representation of the large variety of potential lignin applications is given in Figure 1.10. The selected applications in this thesis belong to the phenols and the macromolecules groups.
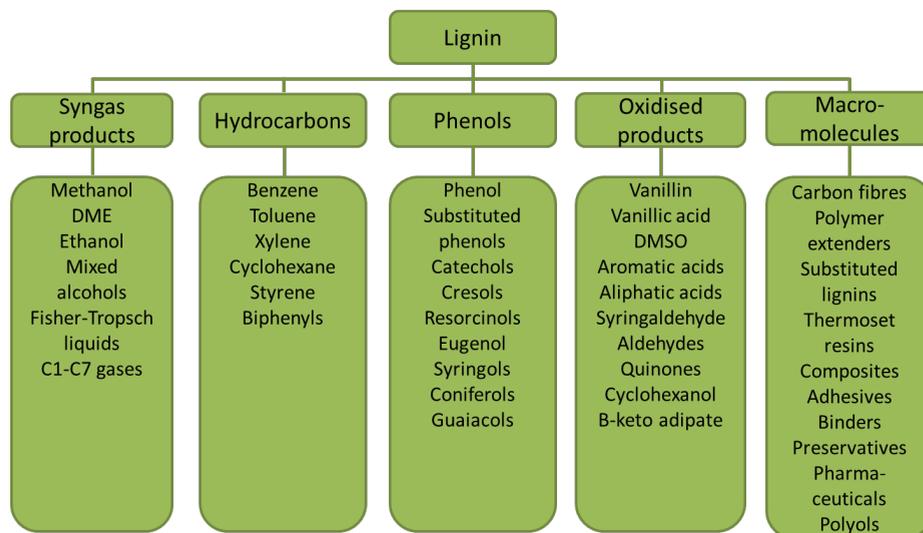
Figure 1.10 Potential lignin applications (Holladay et al. 2007; Higson 2011).

1.5.1 Lignin for binder application

Lignin's structure has a certain similarity to that of traditional fossil-based binders such as phenol-formaldehyde resins (PF) which are used for varnishes, circuit boards, billiard / pool balls and as wood adhesives for gluing fibre boards. Also in nature one of the important characteristics of lignin is its ability to act as a binder gluing cell walls together. Therefore lignin has a high potential for applications as binder.

According to the European Federation of the Plywood Industry, in 2007 European plywood production reached 3.4 million m^3 while, in line with previous years, the demand increased at about 10%/annum. Intra-European trade intensified though extra-European imports increased by 13.8% to reach 4.8 million m^3 plywood (UNECE/FAO 2008). The economic crises in 2009 lead to a decreased demand, but it is expected that this will be overcome in the coming years.

Phenol-formaldehyde resins represents about 1 million tons market on dry basis with a growth rate of circa 3%/annum (Dunky 2004; European Chemical Market reporter 2004). Phenol and phenol derivatives have received growing interest from emerging economies, not only due to the soaring cost of petroleum-derived phenol (1250 €/ton, see Figure 1.14), but also due to the increase in demand for PF resins (Tymchyshyn and Xu 2010). PF resins are formed by polycondensation of phenols in the presence of formaldehyde either under acidic (novolac resins) or basic (resol resins) conditions. The wood adhesives commonly are resol type of PF resins as shown in Figure 1.11.
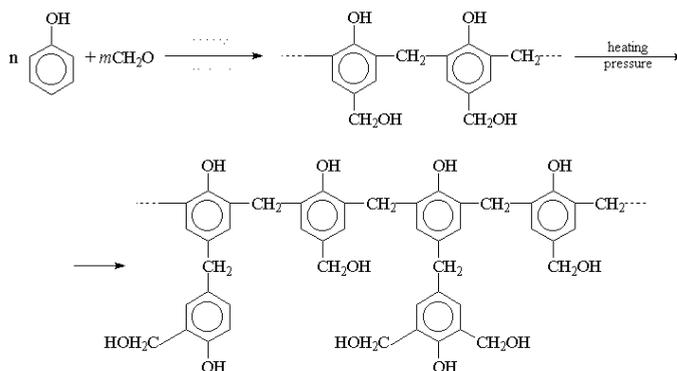
Figure 1.11 Synthesis of phenol-formaldehyde resins by polycondensation of phenols. in excess of formaldehyde developed by Baekeland (1909).

A classic example of this PF-resin was successfully used in the early 1900 years called Bakelite by reaction of phenol, formaldehyde and wood flour (Baekeland 1909). Thermosetting formaldehyde-based resins are used primarily as adhesives (binders) in the production of wood-based panels. The main wood-based panels are particleboards, medium density fibre boards (MDF), plywood and oriented strand boards (OSB). Next to PF also ureum formaldehyde (UF) and melamine ureum formaldehyde (MUF) resins are used. These formaldehyde based resins are under pressure because of formaldehyde emissions. The use of lignin in these resins is therefore two-fold:

1. Substitution of the (expensive) phenol part
2. (Emission) reduction of the carcinogenic formaldehyde by using an already crosslinked resin component.

Furthermore, PF resins seem to be better candidates for replacement by lignin than UF resins as these PF resins are dark coloured, crosslinked under alkaline conditions and represent a higher market value. PF resin glued panels are used for structural applications and can be applied in exterior environments. Most research activities on lignin based binders concentrate on substituting the phenol part with lignin in the synthesis of lignin modified phenol-formaldehyde (PF) resins (Mansouri and Salvado 2006; Tejado et al. 2007; Cavdar Donmez et al. 2008). Currently one of the main commercial applications for soda non-wood lignin is the use as partial replacement (20-30%) for phenol in the manufacture of PF resins used as binders in plywood panels (Khan et al. 2004; Khan and Lora 2006).

The conclusion of the previously described research is that lignin needs to be modified to enhance its reactivity to an acceptable level suited for the strict requirements of press rate of the panels in an industrial manufacturing process (Pizzi 2006). Methylolation with formaldehyde is a well-known modification process of lignin, analogous to the synthesis of phenol-formaldehyde resins (Figure 1.11). The major drawback is that undesired emissions of formaldehyde during processing and application may occur and the end-product is not emission free (Senyo et al. 1996). In contrast, a complete formaldehyde-free system was studied by Nimz and Hitze (1980) based on oxidative radical coupling of spent sulfite liquor by hydrogen peroxide. The resin product is suited as adhesive in particle boards. However, this approach is restricted to the spent sulphite liquor as the presence of sulfur dioxide is necessary to
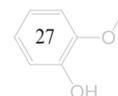
stimulate the exothermal coupling reaction. Recent papers show the development of interior wood fibre boards and natural fibre reinforced biocomposites. These are glued with organosolv straw lignin and tannin adhesive formulation in which lignin is present in considerable amounts of up to 50% (Pizzi et al. 2009; Bertaud et al. 2011; Mansouri et al. 2011). Glyoxal, a non-toxic and non-volatile aldehyde, was used as crosslinking agent.

In this thesis, another alternative formaldehyde-free modification route has been followed. To avoid formaldehyde, metaperiodate was selected as modification agent to improve the lignin reactivity for both kraft and soda lignins as described in **Chapter 4**. Periodate oxidation of lignin could result in the formation of additional carbonyl and carboxyl groups, but also in demethoxylation via the Malaprade reaction releasing methanol and *ortho*- and *para*-quinones formation (Adler and Hernestam 1955). Figure 1.12 shows a proposed mechanism representing the Malaprade reaction for a lignin model compound guaiacol.
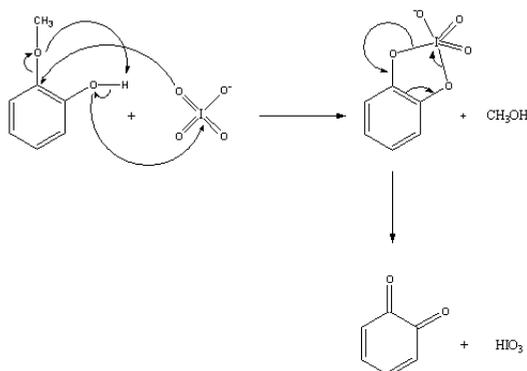
Figure 1.12 Proposed mechanism of periodate oxidation of guaiacol via the Malaprade reaction.

These lignin quinones have the ability to react with furfuryl alcohol (furan derivatives) via a *Diels-Alder* reaction. Trindade et al. (2004) used this approach for selective *in situ* oxidation of lignin in sugar cane bagasse fibres resulting in an improved reactivity towards furfuryl alcohol. They did not describe the mechanism behind this crosslinking reaction. These results lead to the choice in this thesis to study periodate as oxidation agent for development of a formaldehyde-free route to improve the lignin reactivity. Additionally, by this pathway a novel fully biobased resin based on oxidised lignin via
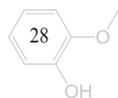
periodate and furfuryl alcohol, which is produced from lignocellulosic biomass, could be developed. The properties of binders prepared by lignin and poly-furfuryl alcohol were compared to binders prepared by PF resins partly substituted by lignin. The results are described in **Chapter 4**.

1.5.2 Lignin for production of aromatic chemicals

Lignin is up till now the only renewable resource, potentially available in enough quantities, for the industrial production of aromatics. Alternative routes to produce aromatics from other renewable feedstocks such as tannins and carbohydrates are discussed in **Chapter 6**. It seems obvious that direct and efficient conversion of lignin into discrete molecules or defined classes of high-volume, low molecular weight aromatic compounds is a very attractive goal. As petroleum resources diminish and prices increase, on one hand this goal is very attractive, but on the other hand it is a very challenging goal to achieve. Efficient production of high volume aromatics from a material as structurally complex and diverse as lignin is a big challenge but seems to be a viable long-term opportunity (Holladay et al. 2007).

      Aromatic chemicals are used in many applications. Aromatic chemical building blocks include benzene, toluene and xylene (BTX) obtained from fossil resources in a global production volume of about 36, 10 and 35 Mt/annum respectively (Cherubini and Strømman 2011). Potentially, these aromatics can be obtained from lignin, but therefore the oxygen containing functional groups need to be completely removed by dehydroxylation, decarboxylation, decarbonylation, and demethoxylation. As about 60% of all aromatics are produced starting from BTX, the conversion of biomass and lignin to these chemicals seems to be most interesting (Haveren et al. 2008). However, by focussing on phenol and phenol derivatives, the aromatic ring plus the phenolic hydroxyl needs to be maintained intact and in theory less energy will be needed to produce these compounds from the polyphenolic ligneous complex.

Phenol and some of its commercial important derivatives are shown in Figure 1.13 which are used in many applications. The production level, costs and main applications for phenol and its derivatives are given in Table 1.4. The majority of phenol is used for the production of Bisphenol-A as ingredient for polycarbonate (48%), for phenolic resins (25%) and via cyclohexanone for caprolactam synthesis (11%). Caprolactam is used to produce nylon fibres.
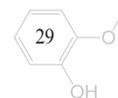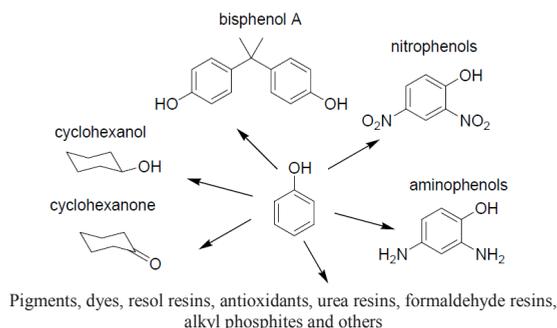
Figure 1.13 Phenol derivatives using current technology (Holladay et al. 2007).

Table 1.4 Phenol and derivatives production, market price and applications.

Product	World production (M t/y)	Market value (€/ton)[a]	Applications	Reference(s)
Phenol	8	1200	Bisphenol-A (48%) Phenolic resins (25%) Caprolactam (11%) Alkyl phenols (4%) Xylenols (4%) Aniline (2%) Various (6 %; o.a. Adipic + salicylic acid)	Stewart (2008)
Bisphenol-A	2 (projected 6 in 2015)	1600	Polycarbonate Bisphenol-A-glycidyl resins (epoxy)	Global Industry Analysts (2010)
PF-resins	1.2	1600 (range 1000 - 2500)	Wood adhesives Paints, coatings, thermosets	
Caprolactam	0.5 (from phenol) 3.5	1500	Nylon-6 - Fibres (73%) - Resins and films (27%)	
Alkyl phenolics o.a. Cresol	0.18	1100 -1500	Drilling oils additives, antioxidants, plastic processing aids, herbicides, antioxidants	
Xylenols, Cresylic acid	0.5	1100 – 1500	Polyphenylene oxide (PPO) Polyphenylene ether (PPE)	
Aniline	0.09 (from phenol) 1.3	680	Isocyanate MDI (80%) Rubber Colouring agents, pigments (10%) hydroquinone (10%)	

[a] www.icispricing.com (accessed December 2010)
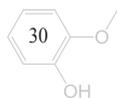
Figure 1.14 European phenol prices in 2008-2010 (ICIS, 2010).

Figure 1.14 shows that the European phenol prices can fluctuate substantially, as in 2009 when all prices of chemical dropped due to the economic crises. After that, the price of phenol has returned to an average level of about 1,250 Euro/ton. With this price level of phenol, lignin can be a very attractive cheaper raw material to substitute the phenolic part in a PF-resin, if isolation and processing can be carried out costs effectively, as discussed in the previous Section 1.5.1.

1.6 Cleavage of bonds in lignin

For the production of 'green" chemicals from lignin the different depolymerization processes have been reviewed in this section. Production of platform aromatic chemicals, that commonly are produced from refined petroleum, may be achieved along various biorefinery processing routes from the lignin enriched fractions. The controlled breaking of different linkage types in lignin needs detailed information on the stability of the bonds under different conditions and knowledge of the mechanisms of lignin decomposition. The most easily hydrolyzable bonds in lignin are the ester and ether bonds. Lignin can be degraded by biological routes with micro-organisms, by sun light (UV), and also by chemical routes at different conditions. These latter depolymerization processes for lignin will be discussed in the following sections.

1.6.1 Cleavage of carbohydrate impurities

Depending on biomass type, pulping or pretreatment/fractionation technology the lignin fraction will be contaminated with different levels of residual carbohydrates.
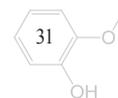
Carbohydrate fractions are often persistent in lignins when the pretreatment processes do not fully cleave all carbohydrate-lignin bonds. Covalent bonds between lignin and the cell wall carbohydrates have been studied for different plant species. The lignin-carbohydrate complexes (LCCs) are of different bonding types. The residual lignin from pine kraft pulping are predominantly linked with the hemicellulose and pectic cell wall polysaccharides (Minor 1986). LCCs linkages demonstrated for example in *Ginkgo bilboa* L. to be of ether, ester or ketal type and most commonly attached at the Cα of the lignin structure (Xie et al. 2000).

In grasses phenolic acids are present such as ferulic and p-coumaric acids that often are esterified to hemicelluloses and lignin. The ferulate-polysaccharide esters are involved in the radical initiated coupling to lignin (Ralph et al. 1995).

Organosolv fractionation, for example by ethanol-water, leads to high purity lignin with a residual carbohydrate content of <1% by weight (Lora et al. 1989b). Table 1.5 shows that organosolv lignins are the most pure technical lignins and these lignins were selected in this thesis to study the conversion to aromatic chemicals (**Chapter 5**). Together with kraft and soda lignins, organosolv lignins are suitable candidates for this application in contrast to the impure lignosulfonates and hydrolysis lignins. The latter two lignins will most likely lead to a substantial formation of non-aromatic carbohydrate derived compounds and more complicated processing.

Table 1.5 Carbohydrate content of different technical lignins.

Lignin	Feedstock	Process	Residual carbohydrates (%)	Reference
Lignosulfonate	wood	sulfite	10-25	Baumberger et al. (2007); Mulder et al. (2011)
Kraft	wood	sulfate	1-3	Baumberger et al. (2007); Mulder et al. (2011); Boeriu et al. (2004)
Soda	Non-wood	soda	2-4	Gosselink et al. (2004a); Baumberger et al. (2007); Gosselink et al. (2011)
Organosolv	Hardwood/straw	EtOH/water	0.3-1	Baumberger et al. (2007); Gosselink et al. (2004a); **Chapter 5**
Steam explosion	hardwood	Steam	2	Baumberger et al. (2007)
Hydrolysis	Wood	Acid/enzymatic	10-20	Vishtal & Kraslawski (2011)
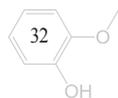
1.6.2 Biological depolymerization of lignin

Biological degradation of lignocellulosic biomass is essential for the closure of the ecological carbon cycle. The microbial degradation of biomass results in the formation of humus (humic acids), derived from incompletely decomposed lignin residues. White rot fungi are specialized to decompose the lignin in wood to obtain access to the carbohydrates in the cell walls. Fungal decay of wood results in breaking of bonds in lignin by enzymes assisted by other environmental influences (light, fluctuating temperatures, eroding water) occurring in decaying woods or bacterial composting media (Hammel 1997). The microorganisms are not using lignin carbon as energy source, but depend on the nutritional value of carbohydrates. The common lignolytic enzymes (laccases, peroxidases) operate by generation of free radicals, that initiate cleavage of linkages in lignin. As the depolymerization of lignin studied in this thesis is entirely based on chemical depolymerization of lignin (see **Chapter 5**), no further review on biological lignin depolymerization has been included.

1.6.3 Chemical depolymerization of lignin

Base-catalyzed depolymerization (BCD)

Most work related to BCD originates from the pulp and paper industry where these alkaline processes are used to depolymerise (hydrolyse) and extract lignin from lignocellulosic matrix to produce so-called wood-free cellulose fibres. Besides extensive cleavage of the β-O-4 linkages under BCD conditions the methoxyl contents in lignin decrease with the severity of alkaline conditions. However, repolymerization of lignin fragments to condensation products may occur that are formed with new bonds of methine, methylene, methyl and carboxyl functionalities as found by ^{13}C NMR (Thring et al. 1990). Kinetic studies with lignin model compounds indicate that the substitution pattern in the aromatic ring strongly affects the alkaline hydrolysis rate of the β-O-4 bonds. Electron-withdrawing groups such as in the phenoxy rings are reported to promote the alkaline cleavage (Hubbard et al. 1992).

Alcell organosolv lignin depolymerization in alkali (0-4%) yielded 7-30% liquid products. The maximum concentration of identifiable phenols was 4.4%, mostly syringol (2.4%) and limited amount of guaiacol when less severe conditions were
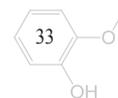
applied. Catechol was found at higher pH and temperature (Thring 1994). In Kraft lignin it was shown that the dissociation of phenolic groups at elevated temperatures in alkaline aqueous solution decreased. The apparent pKa shifts to higher values with increasing molecular weight of the lignin (Norgren and Lindström 2000). Recently, Yuan et al. (2010) studied the based catalyzed degradation of alkaline kraft lignin in water-ethanol at 220 – 300°C, with phenol as the capping agent into oligomers with a negligible char and gas production. Under the conditions applied lignin could not be degraded completely into lignin monomers.

Acid-catalyzed depolymerization

Hydrolysis under acidic conditions of lignin model compounds show α-ether elimination reactions resulting in benzylic carbonium intermediate products, that quickly rearrange into different ketones, and condensation products (Gierer 1985). Depolymerization of Alcell lignin using Lewis Acid catalysts $NiCl_2$ or $FeCl_3$ yielded gas, solid and liquid products including the formation of ether soluble monomers under different reaction conditions. Both catalysts favour condensation reactions leading to insoluble residues. The low yields of organic monomers were dominated by phenolics over ketones and aldehydes (Hepditch and Thring 2000).

Oxidative depolymerization

In general oxidative depolymerization of lignin is carried out to produce aromatics with an increase of oxygen containing groups, mostly aldehydes. The production of vanillin (3-methoxy-4-hydroxybenzaldehyde) by oxidative depolymerization of lignin, mainly from black liquor of sulfite pulping, is well known and typically is performed at 160-175°C under alkaline conditions using a copper catalyst. Borregaard is the only industrial producer of lignin derived vanillin. Especially softwood lignin is yielding relatively higher amounts of vanillin as compared to hardwood lignin where syringaldehyde may prevail (Evju 1979). The use of an alkaline wet oxidation process for wheat straw at high temperature (195°C) and pressure (12 bar oxygen) resulted in high lignin removal from the cellulose, but only low yields of monomeric phenols. Mainly low molecular weight organic acids were recovered (Klinke et al. 2002).

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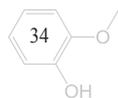
Other researchers used hydrogen peroxide for oxidative depolymerization. Kraft lignin was treated at 90°C by a biomimetic system, using hemin as a catalyst and hydrogen peroxide as an oxidising agent, which mimics the catalytic mechanism of lignin peroxidase. Relatively high yields of vanillin 19%, vanillic acid 9%, 2-methoxyphenol 2% and 4-hydroxybenzaldehyde 2% were obtained (Suparno et al. 2005). Xiang and Lee (2000) found that alkaline peroxide treatment of lignin at 80-160°C yield mainly low molecular weight organic acids (up to 50%) with only traces of aromatics which are rapidly degraded by hydrogen peroxide.

Sales et al. (2004, 2007) studied the alkaline oxidation of sugarcane soda lignin with a continuous fluid bed with a palladium chloride $PdCl_3.3H_2O/$ γ-Al_2O_3 catalyst at 100-250°C and 2-10 bar partial oxygen pressure. Total aldehyde yield on lignin was 12%. Zakzekski et al. (2010) reported other predominantly catalytic lignin oxidation processes yielding aromatic aldehydes and acids which do not exceed 10% on lignin basis. However, lignin model compounds show in some catalytic processes good conversions which are promising to further develop catalytic strategies for lignin depolymerization in a biorefinery concept.

Thermal depolymerization

Pyrolysis

Thermal degradation of lignins has been studied by thermogravimetric analysis (TGA) under different conditions with or without oxygen (pyrolysis). Study of the pyrolysis kinetics for lignocellulosics reveals that the lignin component starts decomposing at lower temperatures than the carbohydrates, but covers the whole temperature range up to 900°C. Lignin is the main biomass component responsible for the char formation. However, in oxidising atmosphere the char yields are lower. Carbonisation and solidification with maximum surface area of the char is obtained at 350-400°C (Sharma et al. 2004). Below 300°C no significant lignin degradation occurs, but volatile products are released due to dehydration, dehydrogenation, deoxygenation and decarboxylation reactions resulting from the breaking of weaker bonds and condensation reactions (Órfão et al. 1999). At higher temperatures rearrangements take place producing volatiles (syngas: CO and H_2) and reactive free radicals reactions occur when also stronger bonds are broken (Ferdous et al. 2002). Phenolic components are the main volatile products that are released during the pyrolysis stage between 250-400 along
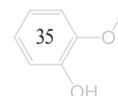
with syngas (Liu et al. 2008). TGA experiments of different lignins show that the amount of C-C bonds in the lignin enhances the char residue formation (Li et al. 2002). Further study on the molecular mechanisms behind char formation revealed that the methoxyl groups in lignin were involved and that the resulting *o*-quinone methide groups were proposed as key intermediates (Hosoya et al. 2009).

Based on TGA results pyrolysis of lignin was studied in different pyrolysis reactors. One recent study using fast fluidized bed pyrolysis of high purity soda and organosolv lignins at 400°C yielded 13-20% of condensed phenolic oil together with 30-35% char formation (de Wild et al. 2009). Up to 9% of low molecular weight phenolic compounds were quantified calculated on dry lignin. This lignin pyrolysis oil was attempted to be upgraded further by hydrodeoxygenation (HDO) to obtain phenolics, but the catalyst ruthenium on carbon (Ru/C) was too active and ring hydrogenation occurred. Further HDO treatments are discussed in the next session.

Analytical pyrolysis combined with gas chromatography and mass spectrometry (Py-GC/MS) is often used to study the thermal degradation products from whole biomass or biomass components like lignin. A softwood lignin was analyzed by Py-GC/MS revealing clear trends in the release of different products dependent on the temperature applied. In addition to volatiles like carbon monoxide, carbon dioxide, methane and C2-C3 gases aromatic monomers were produced as shown in Table 1.6.

Table 1.6 Lignin pyrolysis products at different temperature (Alén et al. 1996).

Temperature (°C)	Pyrolysis products
400 -	Vanillins, guaiacols
600 -	Vanillins, guaiacols, catechols, phenols
800 -	Aromatic hydrocarbons, other phenols
1000 -	Aromatic hydrocarbons, other phenols

Pyrolysis in combination with GC/MS has also been used to investigate the substitution patterns in lignin and its degradation products although quantification requires the addition of internal standards (Bocchini et al. 1997). Recently, the temperature dependence of the pyrolysis products of Alcell lignin and a soda non-wood lignin was investigated using Py-GC/MS. About 50 compounds were identified and quantified for each type of lignin over a temperature range of 400−800°C. The maximum yield of phenolic compounds was obtained at 600°C for both lignins, which was 17.2% for
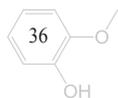
Alcell lignin and 15.5% for soda non-wood lignin. Most of the phenolic compounds had an individual yield of less than 1%. However, Alcell lignin yielded 4.29 wt % 5-hydroxyvanillin, and for soda non-wood lignin, 2-methoxy-4-vinylphenol had the highest yield at 4.15 wt % (Jiang et al. 2010). Results of these analytical studies forms the basis for the development and optimization of pyrolysis processes for whole biomass and lignin.

The production of chemicals by wood pyrolysis has been known for centuries. Acetone, methanol and acetic acid have been side products in the thermal production of charcoal and tar. The tar is a complex mixture that is composed of many different phenolics and carbohydrate derived decomposition products. Recently, pyrolysis of biomass is studied in more detail to produce liquid fuels, syngas and for the production of value added chemicals. However, the conditions for controlled depolymerization and extraction of pure chemical monomeric substances are technically not simple. Pyrolysis of lignin under non-oxidative conditions is mostly investigated for this purpose (Dorrestijn et al. 2000; Amen-Chen et al. 2001). The highly reactive aromatic radicals that are formed during the decomposition reactions at high temperature quickly rearrange to form condensed tar and char like polymers. In general, it is advantageous to use short pyrolysis times at higher temperatures, as is the case in fast pyrolysis processes, to obtain a higher liquid product yield and decreased char formation (Bridgwater et al. 1999).

Recently, an international study of fast pyrolysis of lignin was undertaken with contribution from 14 laboratories. Based on the results it was concluded that an impure lignin containing up to 50% carbohydrates behaves like whole biomass, while a purified lignin was difficult to process in the fast pyrolysis reactors and produced a much lower amount of a more enriched aromatic bio-oil. It was concluded that for highly pure lignin feedstocks new reactor designs will be required other than the typical fluidized bed fast pyrolysis systems (Nowakowski et al. 2010). Several researchers showed that inorganic alkaline catalysts such as NaOH can facilitate depolymerization of lignin by pyrolysis and influence the product composition (Amen-Chen et al. 2001).

Hydrodeoxygenation (HDO)

In the early 80's kraft lignin was converted in a two-step "Lignol" process including hydrocracking and hydrodealkylation over an catalyst bed in hydrogen into 20% phenol and 14% benzene (Huibers and Parkhurst 1982). This process was highly selective but
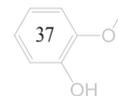
requires a high temperature stage (340-450°C). At the end of the 80's a process for liquefaction of lignin was developed using a 2-step catalytic hydrogenation with metal sulfides to yield relatively high yields of monophenols including cresols. For continuous processing phenol, methanol, and lignin tar were used as liquefying solvent (Urban and Engel, 1988). Somewhat later, kraft lignin was liquefied under (catalytic) HDO cracking conditions ranging from 300 - 550°C in a predominantly low molar mass mixture of phenolic compounds at a yield of more than 50wt% on lignin feed. The lignin phenol product was prepared to be converted to a sulfonated surfactant for oil well drilling purposes (Naae et al. 2001). Hydrocracking of organosolv (Alcell) lignin at 370-410°C in the presence of tetralin, a hydrogen donor solvent, yielded low amounts of liquid and gaseous products. Only less than 50% of the lignin is converted into a wide range of low molecular weight products (syringols, guaiacols) or demethoxylated components (phenols, catechols and their methyl or ethyl derivatives). When using a Ni catalyst higher gas yields were observed (Thring and Breau 1996).

Organocell lignin was subjected to catalytic hydrocracking in a semicontinuous reactor system using a lignin-derived slurry oil. The most complete conversion (char formation only 0.3%) was obtained at 375°C and 180 bar hydrogen pressure. Up to 12.8wt% (based on lignin) of a mixture of mono-phenols was obtained when using a sulfided NiMo catalyst. The yields of monomeric products (in wt% based on lignin) are as follows: phenol 2.3%, cresols 5.0%, xylenols 4.2%, guaiacol 1.3% (Meier et al. 1994). Conversion of lignin at temperatures between 500 and 650°C using HZSM-5 catalyst yielded both liquid and gaseous hydrocarbons. At higher temperatures the gaseous fraction increased at the cost of the liquid fraction. The major identified components in the liquid fraction were toluene (31-44%), 15% benzene, and 33% xylenes. The gas phase contains propane, ethylene, propylene, CO_2 and CO, in different ratios, depending on the applied conditions (Thring et al. 2000).

Another way to convert lignin to fuels or chemicals is by base-catalyzed depolymerization (BCD) followed by HDO. A strong base is needed to partially cleave the lignin structure. Shabtai et al. (1999; 2000) converted kraft softwood lignin (Indulin AT) and organosolv mixed hardwoods lignin (Alcell) via a 2-step process in a reformulated hydrocarbon gasoline like product. First step is BCD at 260-310°C followed by a HDO with a sulfided $CoMo/Al_2O_3$ catalyst at 350-385°C.

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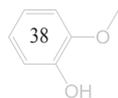
One major problem by using BCD is the high consumption of strong base such as NaOH which makes it not very attractive for economic reasons. To overcome this, Chen and Koch (2010) converted lignin with a hydrogenation catalyst (Pt or Pd on several supports) under a hydrogen atmosphere at 250-450°C into a lignin slurry with reduced oxygen content and lower acidity. This slurry was further treated with a dehydrogenation and deoxygenation catalyst at 400-900°C to form aromatic compounds, mainly alkylbenzenes.

HDO seems to be an appropriate upgrading technology for lignin and lignin derived pyrolysis oils. To minimize hydrogen consumption only partial deoxygenation must be emphasized, without ring hydrogenolysis. Both optimal catalyst performance as process conditions for lignin hydrocracking still needs to be developed. In addition, for commercial application of this process a high capital investment seems to be required which can only be justified when the resulting lignin derived products gain sufficient return on this investment.

Solvolysis

Alternatively, instead of the use of metal catalysts and hydrogen for hydrogenation, solvolytic depolymerization reactions were performed in the presence of hydrogen donors such as tetralin or anthracene derivatives (Dorrestijn et al. 1999). However the high costs of these solvents that are consumed during the process prevent practical implementation. A solution to this problem could be the use of formic acid or 2-propanol as hydrogen donors (Kleinert 2008; Kleinert and Barth 2008; Kleinert et al. 2009). In the presence of relatively large amounts of formic acid and an alcohol the resulting pyrolysis oil contains substantial amounts of aliphatic hydrocarbons, indicating that extensive hydrogenation of the resulting depolymerization products occurs (Gellerstedt et al. 2008). Another advantage of this process is the negligible formation of char.

Supercritical depolymerization

Biorefinery processes aimed at liquefaction of lignocellulosic biomass and the extraction of valuable components for fermentation or recovery of chemicals are currently studied extensively. Different fluids were used to solubilize biomass and lignin for conversion and extraction of valuable compounds.
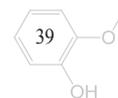
Some of these fluids and their supercritical properties are displayed in Table 1.7. The main supercritical solvent used in industry is carbon dioxide for extraction purposes, for example in decaffeination of coffee beans (Zosel 1974) and dyeing of textile fibres (Smith et al. 2000). In general, CO_2 extracted products are of higher quality and therefore representing a higher market value (Marr and Gamse 2000). Supercritical fluid extraction (SFE) has been increasingly used for example to extract essential oils, fatty acids, lipids, and bioactive compounds from biological resources as reviewed by (Herrero et al. 2010). The choice for using CO_2 as solvent is obvious as CO_2 is cheap, environmentally friendly and generally recognized as safe by the FDA (Food and Drug Administration). Supercritical CO_2 (scCO_2) has other advantages because of its high diffusivity combined with its easily tuneable solvent strength. To use CO_2 under supercritical conditions, the temperature needed is low (>31°C) and the pressure needed relatively low (>7.4MPa) in comparison to other supercritical solvents (Table 1.7). Additionally, CO_2 is a gas at room temperature and pressure, which leads to a solvent-free product after pressure expansion. A drawback of scCO_2 is its low polarity, which is comparable to hexane, but this problem can be overcome by using co-solvents to change the polarity of the supercritical fluid (Herrero et al. 2010). Furthermore, supercritical fluid processing based on CO_2, enables the easy recycling of CO_2 which is advantageous for the development of a sustainable process. Research performed on supercritical processing of lignin to produce aromatic compounds has been summarized hereafter.

Table 1.7 Supercritical fluid parameters (Reid et al. 1987).

Solvent	Critical temperature T_c (°C)	Critical pressure P_c (MPa)	Critical density (g/cm^3)
Carbon dioxide	31	7.4	0.469
Water	374	22.1	0.348
Acetone	235	4.7	0.278
Methanol	239	8.1	0.272
Ethanol	241	6.2	0.276

Depolymerization of lignin model compounds and organosolv lignin have been studied in supercritical alcohols like methanol and ethanol in a temperature range of >239°C and a pressure of >8.1 MPa. By using bases such as KOH and NaOH a high

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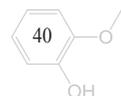
depolymerization conversion was obtained. The dominant depolymerization route is the solvolysis of ether linkages in the lignin structure while the carbon-carbon linkages are mostly stable (Miller et al. 1999; Minami et al. 2003).

Organosolv lignin was completely decomposed in supercritical water with addition of phenol to a phenolic liquid at temperatures between 400-600°C. The presence of phenol prevented repolymerization and char formation at high pressures up to 1 GPa (Fang et al. 2008). Low molecular weight fraction yields increased with longer reaction times in supercritical water without catalysts at 350-400°C and 25-40 MPa. The water soluble fraction consists of catechol (28.4%), phenol (7.5%), m-p-cresol (7.8%), o-cresol (3.8%), suggesting the cleavage of both ether and carbon-carbon (Wahyudiono et al. 2008). Okuda et al. (2004a; 2004b; 2008) used also phenol and p-cresol in supercritical water at conditions above 374°C and 22.1 MPa (Table 1.7) for complete conversion of lignin into a dimer without char formation. Phenol and p-cresol depressed crosslinking reactions due to entrapment of reactive fragments, like formaldehyde, and capping of active sites like C_α in the lignin structure. Yuan et al. (2010) used a combination of both approaches, however at milder temperatures (220 – 300°C), leading to the base-catalyzed depolymerization of kraft lignin in water-ethanol into oligomers with a negligible char and gas production. However, under the conditions applied lignin could not be completely degraded into monomers.

Oxidation of lignin and lignin model compounds with peroxide was studied under supercritical CO_2 conditions in the absence of alkali. The 5-5 biphenols were shown to be degraded and in this process mostly the formation of carboxylic acids from kraft lignin was observed (Argyropoulos et al. 2006).

For this thesis carbon dioxide was selected as supercritical solvent because of the advantages as described before. The main supercritical solvent is carbon dioxide, but acetone and water were used as co-solvent to feed lignin into the reactor. The supercritical fluid consisted of CO_2/acetone/water in a molratio of 2.7/1/1. Lignin is most likely not soluble in $scCO_2$, but it is expected to be soluble in the mentioned mixture of CO_2/acetone/water. The goal of the supercritical depolymerization of lignin is the production of monomeric phenolic compounds and these depolymerized aromatic chemicals are soluble in CO_2. By selection of a CO_2 based fluid separation of the residual lignin and the produced mono- and oligomeric aromatic compounds can be accomplished. In this novel process the aromatic products were separated from insoluble residual lignin fragments and char by adiabatic pressure release.
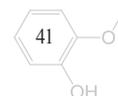
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Carbon dioxide consequently will lower the temperature in the solvent stream facilitating condensation of aromatics formed and leaving no solvent in the product mixture obtained. Thereby, the downstream processing will be substantially simplified. In this chapter it is shown that for depolymerization of lignin into aromatic monomers elevated temperatures in the range of 300-600°C are needed. In this thesis lignin depolymerization under supercritical process conditions in a carbon dioxide/acetone/water fluid was performed in a temperature range of 300-370°C (**Chapter 5**). Literature showed that this selected temperature range is high enough to depolymerize lignin and not too high to further convert lignin aromatics into gases and char. During depolymerization of lignin the formation of radical fragments is substantially occurring. To limit recondenzation of these reactive radical species, hydrogen can be used to stabilise these aromatic radicals. A renewable hydrogen source such as formic acid can be used to generate *in situ* hydrogen under hydrothermal conditions (≥300°C) as found by Yu and Savage (1998). The authors found that under these conditions formic acid is mainly decomposed by decarboxylation to CO_2 and H_2 with a typical CO_2/H_2 ratio between 0.9 and 1.2 as shown in Figure 1.15. In this thesis the effect of formic acid and the *in situ* generation of hydrogen was tested with the goal to obtain higher yields of stabilized aromatic monomers resulting from the depolymerization of lignin.
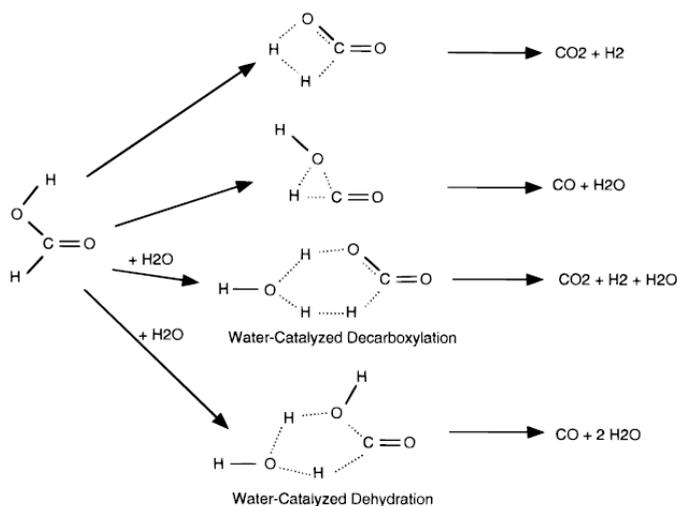
Figure 1.15 Illustration of the molecular elimination mechanisms for formic acid decomposition (Yu and Savage 1998).

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Despite all research applied on lignin conversion to monomeric aromatic chemicals, the optimal conditions for the industrial production of these compounds from lignin is still to be discovered. In general, oxidative depolymerization of lignin will result in fine chemicals with increased functional groups (eg. vanillin or syringaldehyde), whereas reductive depolymerization of lignin will produce bulk chemicals with decreased functional groups (eg. BTX). The search for suitable catalysts and process conditions for lignin conversion is still on-going as mentioned by Zakzeski et al. (2010) as:

- detailed information on the catalyst performance is lacking,
- this information is associated with the analytical challenges of lignin analysis
- presence of impurities in technical lignins complicates catalysis
- several catalysts employed were developed for petroleum refining and need to be adjusted to biorefining processes
- new catalyst materials for biorefineries needs to be developed
- biorefinery processes are still in their infancy as compared to petroleum refinery and need to become an efficient and highly integrated system

1.7 Aims and outline of this thesis

To become a renewable aromatic resource for the chemical industry lignin needs to be further characterized for its basic characteristics to elucidate its structure dependent properties. Successful introduction of lignin derived products into new markets is highly dependent on these structural related properties. In-depth knowledge about these structure – property relationships has been attained in this thesis for development of a process to improve the lignin reactivity for competition with fossil-based wood adhesives and for the production of value added green aromatic chemicals out of lignin.

First aim of this thesis is the development of a universal method for the determination of the molar mass of lignin. The method of choice is Size Exclusion Chromatography (SEC) and several methods used by different laboratories have been thoroughly evaluated. One of the recommended methods, using an aqueous solvent, is further optimized to be able to determine the molar mass of various highly dispersed technical lignins. The determination of the absolute molar mass of lignins has been an extremely challenging research task during the last decades. For this thesis, SEC in combination with Matrix Assisted Laser Desorption Ionization TimeOf Flight Mass

Spectroscopy (MALDI-TOF-MS) has been used to analyze the absolute molar mass of technical lignins. This work is described in **Chapter 2**.

Lignins potentially can be used for multiple applications and for each of them the application property demands are strongly related to analytical properties of the lignins, which needs to be established prior to application development. Specifically, correlations are demonstrated between functional properties of technical lignins and their fractions based on the principle component analysis (PCA). In **Chapter 3** it is demonstrated that the PCA model developed is able to predict the suitability of a lignin or its fraction for wood adhesive application based upon quantifiable analytical chemical data.

For enhancement of the commercial lignin utilisation in the near future the lignin reactivity, for example towards modification and crosslinking in binder and coatings applications, has to be adapted to the process and product requirements. In this thesis the goal was to improve the lignin reactivity by controlled oxidation with periodate and reaction with renewable chemicals, like highly reactive furfuryl alcohol, as described in **Chapter 4**. Development of 100% renewable and emission-free binders for wood based panels is the ultimate goal.

Final objective is to develop a sustainable process for the production of aromatic green chemicals out of lignin. In this research emphasis is given to the production of a small group of interesting phenolic chemicals by a process performed under supercritical conditions based on carbon dioxide. Results of this process development are discussed in **Chapter 5**.

In **Chapter 6** the potential of lignin to become a resource for biobased materials (wood adhesives) and biobased aromatic chemicals for the future chemical industry is described and this chapter presents the general outcome of the thesis and future perspectives within the biobased economy.
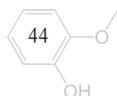
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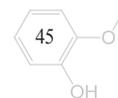
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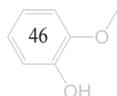
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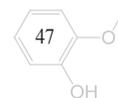
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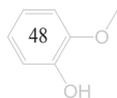
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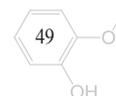
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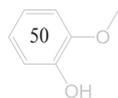
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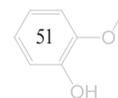
Chapter 2

Development of a universal method for the molar mass determination of lignin

Unpublished work, described in this chapter, comprises the results of the development of an improved alkaline SEC method for the molar mass analysis of highly dispersed lignins. Furthermore results of the use of MALDI-TOF-MS for the determination of the molar mass of lignin are presented and discussed.
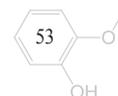
Abstract

The reactivity and physico-chemical properties of lignins are partly governed by their molar mass distribution. The development of reliable standard methods for determination of the molar mass distribution is not only relevant for designing technical lignins for specific applications, but also for monitoring and elucidating delignification and pulping processes. Size-Exclusion chromatography (SEC) offers many advantages, such as wide availability, short analysis time, low sample demand, and determination of molar mass distribution over a wide range. A collaborative study has been undertaken within the "EUROLIGNIN" European thematic network to standardise SEC analysis of technical lignins. The high-molar-mass fraction of polydisperse lignins was shown to be the main source of intra- and interlaboratory variations, depending on the gel type, elution solvent, detection mode, and calculation strategy. The reliability of two widespread systems have been tested: one based on alkali and a hydrophilic gel (e.g., TSK Toyopearl gel) and the other based on THF as solvent and polystyrene-based gels (e.g., Styragel). A set of practical recommendations has been deduced.

The recommended alkaline SEC method by the "EUROLIGNIN" network has been further improved to analyse not only technical lignins with relatively low molar mass and low polydispersity, but also highly dispersed lignins. For the analysis of the latter group the use of two hydrophilic gels with pore sizes of 500Å and more than 1000Å is recommended. With this set-up significantly improved molar mass distribution results were obtained.

The search for a suitable method to determine the absolute molar mass of lignin is ongoing for several decades. Despite the research performed on this topic only limited success has been achieved. In order to find such a method MALDI-TOF-MS (in short MALDI) and prior fractionation of polydisperse lignin have been further studied. Fractionation of organosolv lignin by organic SEC into narrow dispersed fractions resulted in appropriate MALDI spectra and an accurate correlation between SEC data and MALDI data was found.

In contrast, alkaline SEC did not result in suitable narrow dispersed lignin fractions for accurate MALDI analysis. Furthermore, organic solvent fractionation did result in increasing lignin molar mass fractions, but the highest mass fractions could not be accurately measured by MALDI.
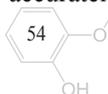
Based on the results obtained, the search for a suitable method for determination of the absolute molar mass of lignin needs to be continued. Fractionation of technical lignin into narrow dispersed fractions seems to be a prerequisite for accurate MALDI analysis.

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2.1 Introduction

Molar mass is one of the key parameter governing the reactivity and physicochemical properties of lignins, either in solution or embedded in polymer materials (Luner and Kempf 1970; Herrick et al. 1979; Yoshida et al. 1990; Constantino et al. 1996; Kubo et al.1996; Li and Sarkanen 2000; Baumberger 2002; Feldman 2002; Pouteau et al. 2003). Among the methods for molar mass determination, high-performance size exclusion chromatography (HPSEC) has several advantages: it is widely available and yields information for a wide range of molar masses (200-3x10^6 g mol^{-1}) in a single analysis step (Pellinen and Salkinoja-Salonen 1985; Chum et al. 1987; Faix and Beinhoff 1992; Gellerstedt 1992; Glasser et al. 1993). The other techniques allow determination of either M_w (light scattering, ultracentrifugation) or M_n (vapour pressure osmometry, ultrafiltration), unless previous fractionation is carried out or several techniques are combined (Marton and Marton 1964; Dolk et al. 1986; Pla 1992b). Recently applied techniques, such as mass spectrometry (Evtuguin et al. 1999; Jacobs and Dahlman 2000; Evtuguin and Amado 2003) and pulsed-field gradient NMR spectroscopy (Norgren and Lindström 2000) are still not suitable for routine experiments.

Comparative lignin SEC was found to be useful in deciphering the molecular mechanisms involved in delignification processes (Robert et al. 1984 ; Gellerstedt and Lindfors 1987; Hortling et al. 1990), in investigating cell-wall supramolecular organisation (Lawoko et al. 2005), and in assessing lignin potential for new uses (Feldman 2002). For industrial use, quantitative determination of molar masses is required. For external calibration, polystyrene standards, lignin model compounds or purified lignin fractions can be used (Pellinen and Salkinoja-Salonen 1985; Johnson et al. 1989). These results are seldom reliable. Viscosimetric detection yields the possibility to perform a universal calibration based on the hydrodynamic volume, which is proportional to the product of the intrinsic and the molar mass. In theory, this parameter is closely related to the elution volume. In the case of application of a set of standard substances of known molar masses combined with viscosity detection, the calibration curves are independent of the degree of branching and conformation. Thus, it is also possible to determine the constants K and α of the Mark-Houwink relation: $[\eta]=KM^{\alpha}$ (Glasser et al. 1993; Himmel et al. 1995; Cathala et al. 2003).

On-line multi- or low-angle laser light scattering (MALLS / LALLS) overcomes possible errors in external calibration. It provides molar mass data, as well as root-

mean-square radii (Faix and Beinhoff 1992; Pla 1992a; Glasser et al. 1993; Fredheim et al., 2002; Cathala et al. 2003). The accuracy of the determination is improved by selected filters that limit the fluorescence contribution (Froment and Pla 1989). Determination of the concentration of substances eluted from the column is commonly achieved by UV or refractive index (RI) detection. Here, it is assumed that the response coefficient is independent of the molar mass. The error caused by this simplification does not exceed 5% (Lange et al. 1983). For light-scattering, the RI increment must also be determined. This parameter is influenced by the molar mass, functional groups, and the solvent. Accordingly, the accuracy of the absolute molar mass determination can be improved if lignin fractions recovered by ultrafiltration or preparative chromatography are used for calibration (Fredheim et al. 2002; Ringena et al. 2005b).

SEC has also benefited from techniques that help to avoid association, ionic exclusion, and conformational changes in the molecules being separated. SEC systems can be aqueous or organic, based on the eluent (Table 2.1). Aqueous-based SEC is the method of choice for lignosulfonates, black liquors or alkali-extracted residual lignins and permits simultaneous monitoring of carbohydrate and lignin elution (Herrrick et al. 1979; Bikova et al.1988, 2000; Forss et al. 1989; Hortling et al. 1990; Wong and de Jong 1996; Chen and Li 2000). Organic solvent SEC is, however, more frequently applied (Mörck et al. 1986; Kelley et al. 1988; Gilardi and Cass 1993; Glasser et al. 1993; Oliveira et al. 1994; Constantino et al. 1996; Kubo et al. 1996; Baumberger et al. 1998; Norgren and Lindström 2000; Pouteau et al. 2003; Gosselink et al. 2004a; Kadla and Kubo 2004). Systems with tetrahydrofuran (THF) as organic eluent and styrene-divinylbenzene (SDVB) copolymers gels are widely applied and considered as reliable because:

1) the system is stable towards organic solvents;
2) the linear range is wide (200-3×10^6 g mol^{-1}) and the efficiency, $>50,000$ plates m^{-1} for mixed-bed gels, is high; and
3) adsorption phenomena in the case of derivatised lignins are supposed to be low (Chum et al. 1987; Johnson et al. 1989).

Traditional derivatisation techniques used are acetylation, silylation, or methylation (Gellerstedt 1992). An alternative is a pre-extraction procedure to recover lignins in their quaternary-amine complexed form for ion pair SEC (Milstein et al. 1990,
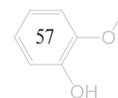
Ben-Ghedalia and Yosef 1994, Majcherczyk and Hüttermann 1997). Provided that the extraction is quantitative, this method is also suitable for lignosulfonates, which otherwise cannot be analyzed in THF. Organic SEC in polar organic solvents such as dimethylsulfoxide (DMSO), dimethylformamide (DMF) and dimethylacetamide (DMAc) does not require any derivatisation step and associative phenomena can be reduced by addition of lithium salts (Chum et al. 1987; Johnson et al. 1989). In aqueous media, pH and ionic strength strongly influence the elution of lignosulfonates and alkali lignins exhibiting polyelectrolytic behaviors (Chen and Li 2000). The use of high-molarity NaOH solutions (0.5 M) and alkali-resistant ethylene glycol-methacrylate copolymers (TSK) minimises ionic interactions (Forss et al. 1989, Himmel et al. 1995). In contrast to dextrane-based gels (Sephadex, Superdex gels), pre-packed TSK gels are not commercially available, which is a limitation.

Round Robin activities for lignin analytical methods were organized by NREL (USA) (Chum et al. 1992) in the 1990s, involving approximately 20 international laboratories. One of the conclusions was that polystyrene-calibrated SEC was not reliable, despite the apparent accuracy and reproducibility of the results for one analytical system. In particular, extremely large interlaboratory variations were obtained for a steam explosion aspen sample (M_w 1000-46,000 g mol^{-1}, M_n 500-2000 g mol^{-1}, polydispersity 7-37). Separation of the same acetylated samples on SDVB copolymer gels with THF as solvent was also not reliable. A Round Robin among four European laboratories also revealed significant variability for molar mass determinations by DMF/LiCl and THF SEC of non-wood lignins, again highlighting the need for standard procedures (Gosselink et al. 2004a).

A collaborative methodological study has subsequently been undertaken within the Eurolignin network (Abächerli et al. 2004; Gosselink et al. 2005). The major differences compared to the previous Round Robins are:

1)   a wider array of lignin samples (wood and non-wood lignin samples, various processes),

2)   increased standardisation of SEC conditions, and

3)   involvement of aqueous SEC in addition to organic SEC.

The objective was not only to test the reliability of two widespread SEC methods, but also to gain better understanding of the variation factors and to formulate

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practical recommendations. The present paper focuses on differences in the molar mass determinations and tries to assess the contribution of lignin structure and chromatographic parameters to the deviations observed. Also the recovery rate of lignins from the solubilisation and derivatisation steps is addressed.

The presented SEC results obtained within the Eurolignin Thematic network clearly shows that the aqueous SEC protocol can be accurately applied to most of the technical lignin samples. These include kraft, soda, organosolv, and other biorefinery lignins and the method can additionally be applied to modified lignins, depolymerised lignin fragments, and lignosulfonates. One of the most important advantages of this aqueous SEC methodology using an alkaline solvent is that there is no need to prior derivatise the lignins to dissolve these in the solvent. Furthermore, the use of a hydrophilic TSK gel (Toyopearl HW-55(F)) and 0.5M NaOH as eluent was recommended, because it minimise ionic interactions and adsorption to the column gel. However, when a high-molar-mass fraction is present in polydisperse lignin the established protocol is not sufficient enough due to the limited size of the gel pores present in the used column gel. As a consequence, the high-molar-mass fraction will elute in the void volume of the column and cannot be used for calculation of the molar mass of the whole lignin sample. By expanding the range of gel pores the analysis of a high-molar-mass lignin fraction will become possible. To achieve this, two similar types of gels with different and larger pore sizes were used in serial connected columns and run under identical conditions. Several technical lignin samples were analyzed by alkaline SEC using one gel and compared to SEC using two gels.

Determination of the absolute molecular weight of technical lignins has been an extremely challenging research task during the last decades. Most observed problems are limited solubility, presence of impurities, interaction between lignin molecules, interaction of lignin with stationary phases in chromatographic systems and the lack of similar and well defined lignin references. In general, SEC can only be applied for relative estimation of the molecular mass of the same class of lignins. Most commonly, an UV detector is used for determination of the molecular mass although the detector response varies with changing molecular size. In these SEC systems calibration is performed by using (sulfonated) polystyrene standards with narrow distributions.

Using a multi-detection system including UV, RI, two-angle LALLS and viscosity detectors the molar mass of lignin can be determined without the need for external calibration (Cathala et al., 2003). For the determination of the absolute molar

mass distribution of lignin several studies were performed using mass spectrometry. Evtuguin et al. (1999) used Electrospray Ionization Mass Spectrometry (ESI-MS) to analyse the absolute molar mass of lignin. Gellerstedt et al. (2008) found ESI-MS results in excellent agreement with organic SEC for bio-oils derived from depolymerised lignin. Banoub and Delmas (2003) found with Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry (APCI-MS-MS) organosolv wheat straw lignin structures up to trimers. Banoub et al. (2007) used Atmospheric Pressure Photoionization quadrupole Time-Of-Flight tandem mass spectrometry (APPI-MS-MS) for analysis of wheat straw lignin resulting in the identification of oligomeric structures. MALDI-TOF-MS is nowadays a well-established technique for elucidation of the structural composition and molecular mass of proteins, peptides, glycoproteins, and oligosaccharides. The main advantages of the MALDI technique are that it is a highly sensitive soft ionization technique predominantly resulting in the generation of single charged ions and it is applicable for a wide molar mass range (Hillenkamp et al., 1991). This technique seems to be an excellent tool for determination of the absolute molecular mass of higher molar mass lignin. Jacobs and Dahlman (2000) showed that for MALDI applications technical organosolv lignin needs to be fractionated to get well defined, narrow dispersed, fractions. Organic SEC was used to perform this lignin fractionation in THF. An accurate calibration of SEC by absolute molar mass determined by MALDI was the result. The MALDI spectrum obtained on the whole kraft lignin sample (Indulin AT) shows a broad signal with the maximum intensity of around 700 mass units. The heterogeneity of the kraft lignin macromolecule and the lack of a precise repeating unit makes it impossible to detect separate MALDI-MS peaks for the different components in the lignin polymer distribution. MALDI-TOF analysis compared to regular SEC analysis (with THF as eluent) resulted in comparable results with a maximum of about 20% deviation. To accurately determine the average molar mass a separate calibration with MALDI results for each lignin type was needed. Also Bayerbach et al. (2006) found a 20% difference in the molar mass of pyrolytic lignin when comparing SEC and MALDI-TOF-MS analysis. They found that the latter technique gave more detailed molar mass information (local mass maxima) than SEC analysis. Banoub and Delmas (2003) used MALDI-TOF-MS with delayed extraction technology and α-cyano-4-hydroxycinnamic acid as matrix and found pentameric fragments in the organosolv wheat straw lignin. Potthast et al. (1999) studied the laccase mediated polymerization of monomeric phenolic compounds by MALDI with masses up to about 2000 m/z.
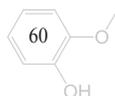
To improve the MALDI results several matrices have been used such as 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), all-trans-retinoic acid (RA), 1,8-dihydroxy-9(10H)anthracenone (dithranol), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid), and 2-aminobenzoic acid. For the analysis of lignin sinapic acid gave better results than DHB or 2-aminobenzoic acid, but the reason behind was not discussed (Bocchini et al., 1996).

Wetzel et al. (2003) found for MALDI analysis of PEG more intense fragmentation when using DHB compared to RA and dithranol. With dithranol as matrix compound with increasing laser energy more high-mass polymer goes into the gas phase. However, Mn for PEG found after subtraction of fragmentation peaks remain similar for all matrices tested. Wetzel et al. (2004) found for analysis of polystyrene (PS) that longer delay times, higher detector voltages, lower laser energy, and higher polymer concentration cause an increased signal-to-noise ratio (s/n). RA yielded higher s/n values than dithranol. Factorial design is recommended as a promising technique for understanding and optimizing MALDI analysis.

Addition of Ag-complexes to the lignin sample before measurement improves the MALDI results as found by Jacobs and Dahlman (2000) and Wetzel et al. (2004). Ag lead to an improved ionization of lignin. For accurate molar mass analysis correction for the mass of the Ag+ adduct should be performed.

Another interesting possibility for using MALDI is the use of a linear positive MALDI-TOF-MS method that could be a promising new technique for analysis of high molecular mass lignins (Mattinen et al., 2008). However, ionization of high molecular weight lignin needs to be remarkably improved to enable accurate mass analysis. One way to overcome this, is to fractionate the lignin prior to MALDI analysis by using SEC. A major drawback of the organic SEC system used by Jacobs and Dahlman (2000) is the need for prior derivatization of most of the lignin samples before SEC fractionation. Therefore, the use of the alkaline SEC, which is applicable for underivatized lignin samples, was explored for lignin fractionation. Additionally, organic solvent fractionation as described in detail in **Chapter 3** was applied to fractionate kraft and soda lignins.

Fractions resulted from alkaline SEC fractionation need to be purified to remove the majority of the salts which is necessary for an accurate MALDI analysis. For desalting several strategies can be used ranging from off-line ion exchange resins, which was used in this thesis, and solid phase extraction (SPE), to on-line electrochemical

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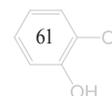
neutralisation developed for analysis of carbohydrates by HPAEC and MS (Guignard et al., 2005). Desalting will be a critical step as the solubility of the different lignin fractions is highly dependent on solvent type, ionic strength of the solvent and additives.

Table 2.1 SEC conditions used for analysis of isolated industrial or model lignins.

SEC type	Gel type	Detection	Lignin sample	
Organic SEC THF systems				
Faix et al. 1981	Microgel	UV 254 nm	MWL	ac
Robert et al. 1984	µ-Spherogel	UV 280 nm	KL	ac
Pellinen and Salkinoja-Salonen 1985	Ultrastyragel	UV 280 nm	DHP, SEL, KL	ac; sil
Mörck et al. 1986	Ultrastyragel	UV 280 nm	KL	ac
Gellersted and Lindfors 1987	µ-Spherogel	UV 280 nm	KL	ac
Kelley et al. 1988	µ-Spherogel	UV 280 nm	OSL	hp
Faix and Beinhoff 1992	Microgel	UV 253 nm	DHP, MWL	ac
Glasser et al. 1993	Ultrastyragel	Visco + RI	KL, OSL, SEL	ac
Oliveria et al. 1994; Constantino et al. 1996	PL-Gel	UV 254 nm	OSL	ac
Kubo et al. 1996	Shodex KF	UV	KL, SEL	ac
Thring et al. 1996	Ultrastyragel	UV 277 nm	OSL	-
Baumberger et al. 1998; Pouteau et al. 2003	PL-Gel	UV 280 nm	KL	ac
Norgren and Lindström 2000	-	RI	KL	ac
Cathala et al. 2003	Microgel	MALLS+RI+UV+ visco	MWL, DHP	ac
Gosselink et al. 2004a	PL Gel	UV 254 nm + RI	AL, OSL	-
Kadla and Kubo 2004	Styragel	UV 280 nm + RI	KL	ac
Majcherczyk and Hüttermann1997	TSK	UV 280 nm	KL, LS, OSL	QAM
Ben-Ghedalia and Yoseph 1994	PSM Zorbax	Diode array	AL, DL	QAM
Polar solvent systems				
Chum et al. 1987 THF/DMF, 0.1 M LiBr	µ-Spherogel	Diode array	SEL, OSL, MWL	ac
Johnson et al. 1989 DMF/THF, 0.1 M LiBr	SDVB	UV + RI	DHP, OSL	ac
Gilardi and Cass 1993, DMF	PSM Zorbax	Diode array	OSL	-
Cathala et al. 2003, DMF	Styragel	MALLS+RI+UV+ visco	MWL, DHP	-
Gosselink et al. 2004a DMF/0.2 M LiCl	Cosmosil Si-Gel 5SL/ Styragel	UV 280 nm	AL, OSL	-
Ringena et al. 2005a DMSO/water/0.05 M LiBr	PSS Gram	Diode array + RI+ visco	LS, KL, AL, SEL	-
DMAc/0.11 M LiCl	PSS PFG-PRO	+ two-angle LS		
Aqueous SEC				
Herrick et al. 1979 NaOH, Na₂SO₄, NaNO₃	Polyacrylamide Bio-gel	UV 254 nm	LS	
Callec et al. 1984, 0.5 M NaOH	Fractogel TSK	Visco + RI	Polystyrene sulfonates; Dextrans; PEGs	
Forss et al. 1989, 0.5 M NaOH	Sephadex	UV 280 nm	KL	
Hortling et al. 1990, 0.5 M NaOH	Fractogel TSK HW-55	UV 280 nm	Enz. L	
Himmel et al. 1995, 0.5 M NaOH	EG-M TSK	Visco + RI	SEL; OSL; acid hydrolysis lignins; MWL	
Wong and de Jong 1996, 0.3 M NaOH	E-M Toyopearl	UV + PAD	KL; Enz. L	
Bikova et al., 1998, 2000 DMF/0.03 M H₃PO₄/2.5 mM NaOH	Polyacrylate-methacrylate	UV + RI	Residual KL, spent liquor	
Chen and Li 2000 0.1 M Na NO₃, pH 7 or 12	Ultrahydrogel/Ultrastyragel	RI	LS; AL	
Freidheim et al. 2002, 0.01 M EDTA/ 0.05 M-Na₂SO₄ /acetonitrile	TSK	MALLS + RI	LS	
Freidheim et al. 2002 Phosphate-SDS-DMSO	Jordi Glucose-DVB	MALLS + RI	LS	

ac, acetylation ; AL, alkali lignins ; DHP, dehydrogenation polymer ; DL, dioxan lignins; DVB, divinylbenzene ; E-M, ethylene-methacrylate ; EG-M, ethylene glycol-methacrylate ; Enz. L., enzyme lignin ; hp, hydroxypropylation ; styrene-divinylbenzene; KL, kraft lignin; LS, lignosulfonates; MALLS, multi-angle laser light scattering ; MWL, milled-wood lignin; OSL, organosolv lignins; PAD, pulsed amperometric detection ; PL, Polymer Laboratories; PSM, porous silica microsphere; PSS, polystyrene sulfonates; QAM, quaternary amine; RI, refractive index ; SDVB, styrene-divinylbenzene copolymer; SEL, steam explosion lignin; sil, silylation; TSK, ethylene glycol-methacrylate ; UV, ultraviolet ; visco, viscosimetry.
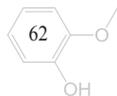
2.2 Experimental

Materials

The origin of the lignin samples is given in Table 2.2. The hardwood ion-exchanged Na-lignosulfonate was derived from an ammonium sulfite process.

Table 2.2 Origin, chemical characteristics and solubility of the industrial lignin samples.

Sample designation	Raw material	Process (supplier)	Contaminents (% dry wt.)		Solubility (%)	
			Total sugars	Ash	In the acetylation reagent	In THF after acetylation
Round Robin samples						
Kraft lignin (Curan 100)	Softwood	Kraft (Lignotech, Sweden)	1.7	7.6	92	90
Soda bagasse lignin	Bagasse	Soda (Granit, Switzerland)	1.4	1.7	98	94
Lignosulfonates	Mixed hardwood	Sulfite (LRV, France)	10.1	29.4	4.5	-
Steam explosion lignin	Aspen	Steam explosion (ENEA, Italy)	1.9	11.6	56	45
Alcell lignin	Mixed hardwood	Organosolv (Repap, Canada)	0.3	0.1	>99	>99
Additional samples for SEC and MALDI-TOF-MS						
Indulin AT	Softwood	Kraft (Meadwestvaco, U.S.)				
Soda lignin	Sarkanda grass	Soda (Granit, Switzerland)				
Sarkanda grass F1	Sarkanda Grass	Fractionated (WUR-FBR)[a]				
Sarkanda grass F2	Sarkanda Grass	Fractionated (WUR-FBR)[a]				
Sarkanda grass F3	Sarkanda Grass	Fractionated (WUR-FBR)[a]				
Sarkanda grass F4	Sarkanda Grass	Fractionated (WUR-FBR)[a]				
Sarkanda grass F5	Sarkanda Grass	Fractionated (WUR-FBR)[a]				
Residual Kraft	Eucalyptus	Kraft (NCSU)[b]				
Enzymatic Acidolysis Lignin (EAL)	Norway spruce	Enzymatic Acidolysis (NCSU)[b]				
Enzymatic Mild acidolysis Lignin (EMAL)	Eucalyptus	Enzymatic Mild Acidolysis (NCSU)[b]				

[a]Solvent fractionated Sarkanda grass soda lignin as described in **Chapter 3**.
[b]North Carolina State University, US (COST E41 lignin samples) (Argyropoulos et al., 2002; Wu and Argyropoulos, 2003)

Chemical analysis

The sugar content of the initial lignin samples was determined by high-performance anion exchange chromatography with pulsed amperometric detection (4 mm Øx 250 mm Carbopac PA1 column, Dionex, 4 mM NaOH, 1 mL min^{-1}) after a two-step acidic hydrolysis performed according to a published protocol (Gosselink et al. 2004a). Fucose was used as the internal standard. The ash content was gravimetrically determined on 1-g samples after incineration at 800°C for 8-12 h untill black carbon particles had disappeared.
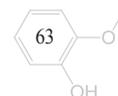
Acetylation

Acetylation was performed for each lignin sample according to a protocol modified from Gellerstedt (1992). Lignins (300 mg) were suspended in 15 ml of a 2:1 (v/v) acetic anhydride/pyridine mixture. The reaction was performed at room temperature for 6 days and was followed by methanol addition and azeotropic distillation. Aliquots of the freeze-dried residues were dispatched to laboratories for dissolution in THF (10 mg ml^{-1}) and filtration (0.45-µm GHP filters, Gelman).

Solubility

Solubility of the lignin samples in the acetylation reagent was determined gravimetrically by filtration (GFA filter, Whatman) of the suspension (100 mg of dry lignin in 9 mL of pyridine/acetic anhydride, 2:1 v/v) after 6 days stirring at room temperature. The solubility of the acetylated lignins in THF was determined by filtration (0.45-µm GHP filters, Gelman) of the suspension (10 mg ml^{-1} acetylated lignin in THF) after 1 h stirring at room temperature.

Round Robin procedure and SEC analysis

Seven laboratories in The Netherlands, France, Switzerland, Finland, Austria, Sweden, and Germany were involved in the Round Robin. Each laboratory performed the analysis on its routine system with its own column set. Furthermore:
1) Participants working with organic solvents for elution obtained samples originating from the same acetylation batch and the same commercial polystyrene standards.
2) The use of partially standardised chromatographic conditions was recommended in terms of solvents, gels and detectors.
3) The raw data were compiled in a single Excel file to ensure identical calculation procedures.

SEC was performed according to the chromatographic systems described in Table 2.3. Two of the three systems employed for aqueous SEC were similar in terms of eluent (0.5 M NaOH), calibration standards (commercial sodium-polystyrene sulfonates, Na-PSS), column material (manually packed column with ethylene glycol-methacrylate copolymer TSK gel) and UV detection (280 nm). The two systems
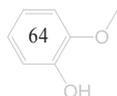
differed only in the flow rate (1 vs. 0.8 ml min^{-1}) and in the resulting column pressure. The peculiarity of the third system was a lower NaOH molarity (0.1 M) and a different stationary phase (PSS MCX 1000, 10 µm, 8x300 mm, polymeric sulfonic acid ion exchange gel based on silica). Four laboratories working with organic SEC systems applied the same eluent (stabilized or non-stabilized THF), the same type of stationary phases (SDVB gels) and UV-RI co-detection. The SDVB gels were purchased from manufacturers and exhibited various geometries and bed-types. One laboratory working with an organic system used a crosslinked polystyrene stationary phase. Substance amounts injected were approximately 50 µg for alkaline SEC and varied from 50 to 400 µg for organic SEC. In addition to aqueous and THF SEC, DMSO-water and DMAc SEC were performed according to Ringena et al. (2005a) (systems 9 and 10).

In a first step, each partner calculated the results using software that was integral part of their chromatographic system. The calculation strategy regarding integration borders and baselines was also individual. In a second step, all the raw data were compiled in a single Excel file. The retention times were converted into the corresponding molar mass using the calibration equation specific of each system and average molar masses were calculated according to Faix and Beinhoff (1992).

Table 2.3 Experimental conditions for aqueous and organic SEC analyses performed within the Eurolignin Round Robin using UV-RI co-detection and calibration with PSS (aqueous system 1-3), PS (THF system 4-8), pullulan (system 9) and PEG (system 10) standards.

System	Column		Eluent	Flow rate (ml min^{-1})
	Gel type	*Geometry*		
1	Ethylene glycol-methacrylate (manually packed TSK gel Toyopearl HW-55, TOSOH)	10/30	0.5 M NaOH	1
2	Ethylene glycol-methacrylate (manually packed TSK gel Toyopearl HW-55, TOSOH)	10/30	0.5 M NaOH	0.8
3	Sulfonic acid ion exchange gel based on silica (PSS MCX 1000, mixed bed, 10 µm)	8 mm×300 mm	0.1 M NaOH	1
4	SDVB (ViscoGel, Viscotek, mixed bed)	2×GMHHR-M (7.8 mm×300mm) and guard (6 mm×40 mm)	THF	1
5	SDVB (PL Gel, Polymer Laboratories, mixed bed, 5 µm)	7.5 mm×600 mm and guard (7.5 mm×50 mm)	THF	1
6	SDVB (Phenomenex Phenogel, linear, 5 µm)	7.8 mm×600 mm	THF	1
7	SDVB (PSS, 5 µm)	50, 100, 1000, 100000 Å (8 mm×300 mm) and guard	THF	1
8	Crosslinked PS (Styragel, Waters, 5 µm)	HR4 + HR2 + HRHR0.5 (7.8 mm×300 mm)	THF	0.8
9	Polyacrylate-methacrylate copolymer (GRAM, PSS, 10 µm)	30, 1000, 3000 Å (8 mm×300 mm)	DMSO/water LiBr	0.4
10	Silica (PFG-PRO, PSS, 5 µm)	100, 300 Å (8 mm×300 mm)	DMAc/LiCl	0.5

Alkaline SEC analysis for high-molar-mass lignin

Lignin samples of 1 mg ml^{-1} dissolved in 0.5 M NaOH were injected into one manually packed column (4.6 mm x 300 mm) with ethylene glycol-methacrylate copolymer TSK gel Toyopearl HW-55F (Tosoh bioscience GmbH, Germany; average pore size 500Å) and eluted with the same solvent. Conditions: flow 1 ml min^{-1}, column temperature 30°C, and detection at 280 nm. Second analysis was performed by SEC using two serial connected columns, each packed with respectively Toyopearl HW-75F (average pore size >1000Å) and HW-55F, under identical conditions. Standards for calibration of the molar mass distribution: sodium-polystyrene sulfonates (M_w range 891 to 976,000 Dalton, PSS Polymer Standards Service GmbH, Germany) and phenol (94 Dalton).

Lignin fractionation

Alcell lignin was fractionated by organic SEC in THF according to the method published by (Jacobs & Dahlman, 2000). 12 SEC fractions (F1-F12), evenly distributed over the SEC curve, were analyzed by MALDI-TOF-MS. Alcell lignin was fractionated by alkaline SEC using one column packed with 500Å TSK Toyopearl HW-55 (F) gel and using 0.05M NaOH as eluent at a flow rate of 1 ml min^{-1} at 30°C. Eight representative lignin fractions were collected after the UV detector at regular intervals. Kraft lignin (Indulin AT) was fractionated into 5 fractions by successive organic solvent extraction as described in **Chapter 3** (Gosselink et al., 2010).

MALDI-TOF-MS analysis

MALDI-TOF-MS spectra were acquired on a Bruker Ultraflex II instrument (Bremen, Germany). The mass spectrometer was equipped with a N2-laser (337 nm / 100 µJ). Matrices used were 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), all-trans-retinoic acid (RA), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid), and 1,8-dihydroxy-9(10H)anthracenone (dithranol).

Lignins were dissolved in the solvent of choice with a concentration of 1 g l^{-1}. For the MALDI-TOF-MS analyses solubilised lignins were mixed with the matrix solution 1:1 (v/v) to achieve different sample-matrix ratios. 1 µL of the mixture was placed on the golden MALDI target plate and dried under air flow. The positive ion
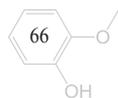
MALDI-TOF-MS spectra were collected in the reflective and linear mode. Spectra were optimized by changing matrix type, sample-matrix ratio (1:1; 1:10; 1:100; 1:1000), solvent type (0.05M NaOH, DMF, Acetone/H2O 7:3 v/v, Acetonitrile, Acetonitrile/H2O 7:3 v/v, Dioxane/H2O 9:1 v/v, NH3/H2O 3:7 v/v, and MeOH/NaOH 1:1 v/v), laser intensity, delay time, one layer or sandwich method, salts removal, or addition of $AgNO_3$.

Maltoheptaose and maltodextrin MD20 (Sigma–Aldrich GmbH, Schnelldorf, Germany) solubilised in DMF were used for the molecular mass calibration of the MALDI instrument.

Alkaline lignin fractions were treated by acid washed ion-exchange resin Dowex 50W-X8 (Sigma-Aldrich) prior to MALDI analysis.

2.3 Results and discussion

The selected lignins, produced in pilot plants or on an industrial scale, have a certain potential regarding industrial use (Avellar and Glasser 1998; Lora 2001, 2002; Lora and Glasser 2002; van Dam et al. 2005) and cover a wide range of physicochemical properties and chemical characteristics (Table 2.2). The Alcell and soda bagasse lignins exhibited high purity (>95%, as calculated from the ash and sugar contents) compared to kraft lignins (91%), steam explosion lignins (87%) and lignosulfonates (61%).

2.3.1 Representativity of the acetylated samples

Incomplete solubility is an important source of error. Different acetylation protocols are available, differing mainly in the method used to recover acetylated lignins. The Gellerstedt (1992) protocol has the advantage of limiting the possibility of fractionation, as the reagents are eliminated by successive evaporation and acetylated lignin is left in the reaction vessel. Incomplete solubility of acetylated lignins in THF is still a problem. The acetylation mixture (pyridine/acetic anhydride, 2:1 v/v) dissolved bagasse soda lignin up to 98% and kraft lignin up to 92%. In contrast, the steam explosion sample was soluble only to 56% in the acetylation reagent, while the lignosulfonate was almost insoluble (4.5%). The solubility of acetylated lignins in THF was very similar (Table 2.2). Elimination of the insoluble portion of bagasse lignin (2%) led to a lower proportion of higher-molar-mass fragments (Figure 2.1). Fourier-transform IR spectra
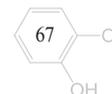
showed that the insoluble steam explosion lignin consisted of non-lignin contaminants. Indeed, this fraction exhibited a spectrum without aromatic vibrations in the fingerprint region. For lignosulfonates, only low-molar-mass compounds were soluble in THF after acetylation. These results confirm that acetylation is not suitable for lignosulfonates or contaminated samples. Lignosulfonates dissolved completely in 0.5 M NaOH, whereas steam explosion lignin also underwent fractionation in this solvent.
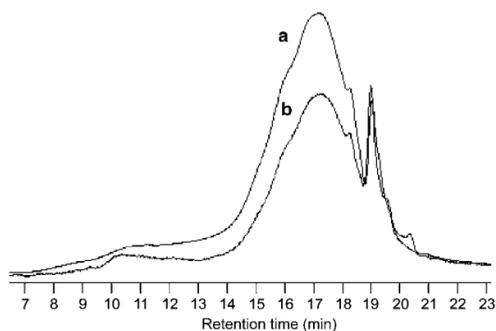
Figure 2.1 Size exclusion chromatography of an acetylated bagasse soda lignin sample without (a) and with (b) elimination of the fraction insoluble in the acetylation mixture (1:2 v/v pyridine/acetic anhydride). Chromatography in THF on a styrene-divinyl benzene column (PL Gel, Polymer Laboratories, 7.5 mm×600 mm, mixed C, 5 μm) at a flow rate of 1 ml min^{-1} with UV detection at 280 nm (system 5).

The acetylation time had no effect on the molar mass distribution (MMD) of the bagasse soda and kraft lignins. On average, 10% of average molar mass variations are due to the acetylation time. However, acetylation time dramatically modifies the elution profile of steam explosion lignin. After 10 days of acetylation time, the tailing due to low-molar masses is prolonged. It is proposed that polysaccharides are partly hydrolysed during long acetylation periods by the acetic acid liberated, which leads to better solubility.

2.3.2 SEC reproducibility

Regardless of the system, intralaboratory variations for two to four successive injections of the same sample did not exceed 15%, depending of the chromatographic system and lignin sample (average 5%, standard deviation 4%). In all cases, M_n determinations were more reproducible than M_w determinations (1.4-3.6-fold lower variation). The highest variations were observed for Mw determinations of lignosulfonates on the
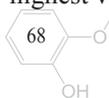
aqueous systems (9-11%). Non-sulfonated lignin samples exhibited very good intralaboratory reproducibility (1-6%) on the aqueous systems. Within the 0.3-1 g l^{-1} range, the sample concentration had no effect on the calculated molar masses. Linear calibration curves ($R^2>0.99$) were systematically obtained for all systems within the molar mass ranges investigated (580-210,500 g mol^{-1} for THF systems and 4800-142,500 g mol^{-1} for the aqueous systems). Weight-average molar masses obtained by two identical aqueous systems indicated good interlaboratory reproducibility, with a difference of 0.9-14% observed, depending on the lignin type. This was not the case, however, for number-average molar masses. Deviations of three to four were obtained between (Table 2.4).

Table 2.4 Average molar mass data (M_w, M_n) and polydispersity (M_w/M_n) of technical lignins analyzed on alkaline SEC systems.

Sample	M_w (g mol^{-1})	M_n (g mol^{-1})	M_w/M_n	System
Curan 100	11119	799	13.9	1
	9735	2755	3.5	2
	8900	200	30	3
Soda bagasse	8402	692	12.1	1
lignin	8481	2684	3.2	2
	5900	200	44.5	3
	3927	786	5.0	1
Lignosulfonates	4317	2173	2.0	2
	2200	100	22	3
	14179	1028	13.8	1
Steam explosion	22876	2977	7.7	2
lignin	11000	100	> 100	3
	3959	511	7.7	1
Alcell lignin				

Data were calculated with respect to Na polystyrene sulfonates standards.

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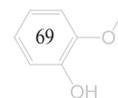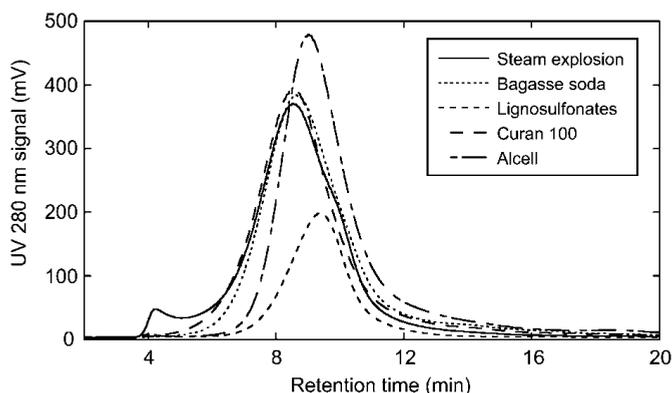
Figure 2.2 Size exclusion chromatopgraphy of technical lignins in alkaline medium. Chromatography in 0.5 M NaOH on a TSK gel Toyopearl HW 55 (manually filled HR column, 7.8 mm×300 mm) at a flow rate of 1 ml min^{-1} with UV detection at 280 nm (system 1).

Aqueous system 3 yielded elution profiles in which part of the lignins eluted with the void volume of the column. Accordingly, the molar masses exceeded the separation range of the columns. An adsorption effect was also systematically observed with system 3, as revealed by the signal after the total volume. Signals in the void volume were also observed for systems 1 and 2, but only in the case of the steam explosion lignins (Figure 2.2). Moreover, adsorption phenomena were not pronounced in these two systems. Small variations due to adsorption effects are likely to interfere with the low-molar-mass portion. The differences observed in M_n are probably due to this effect.

In contrast to aqueous SEC, the highest variations observed with organic elution solvents concerned M_w (Table 2.5). Bagasse soda lignin differed by a factor of up to 40. Differences in the high-molar-mass front tailing were partly responsible for these variations (Figure 2.3). However, variations in the main peak of the MMD (Figure 2.4) were also involved in these deviations. Lignin aggregation and/or the presence of high-molar- mass lignin-polysaccharide complexes (LCCs) may be responsible for this.
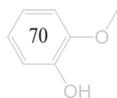
Table 2.5 Average molar mass data (M_w, M_n) and polydispersity (M_w/M_n) of acetylated technical lignins analyzed on THF-SEC systems.

Sample	M_w (g mol⁻¹)	M_n (g mol⁻¹)	M_w/M_n	System
Curan 100	39052[a]	1006[a]	38.8[a]	4
	7643[b]	1097[b]	7.0[b]	4
	50856[a]	456[a]	> 100[a]	5
	5621[b]	1285[b]	4.4[b]	5
	2700[a]	1500[a]	1.8[a]	6
	2739[b]	1311[b]	2.1[b]	6
	2630[a]	590[a]	4.5[a]	7
	2802[b]	768[b]	3.6[b]	7
	16572[a]	889[a]	18.6[a]	8
Soda bagasse lignin	43697[a]	755[a]	57.9[a]	4
	5218[b]	780[b]	6.7[b]	4
	78840[a]	249[a]	> 100[a]	5
	4841[b]	941[b]	5.1[b]	5
	1600[a]	700[a]	2.3[a]	6
	1557[b]	594[b]	2.6[b]	6
	2090[a]	510[a]	4.1[a]	7
	2244[b]	652[b]	3.4[b]	7
	17407[a]	2056[a]	8.5[a]	8
Alcell lignin	15567[a]	624[a]	25.0[a]	4
	1582[b]	642[b]	2.5[b]	4
	21849[a]	436[a]	50[a]	5
	1940[b]	771[b]	2.5[b]	5
	2400[a]	1500[a]	1.6[a]	6
	2275[b]	1228[b]	1.9[b]	6
	1440[a]	440[a]	3.3[a]	7
	1588[b]	599[b]	2.7[b]	7
	3659[a]	541[a]	6.8[a]	8

Calculation according to polystyrene standard by: [a]integration of the whole SEC profile or [b]integration of the curve domain excluding of the front tailing and late eluting portions of the profile according to Figure 2.6.
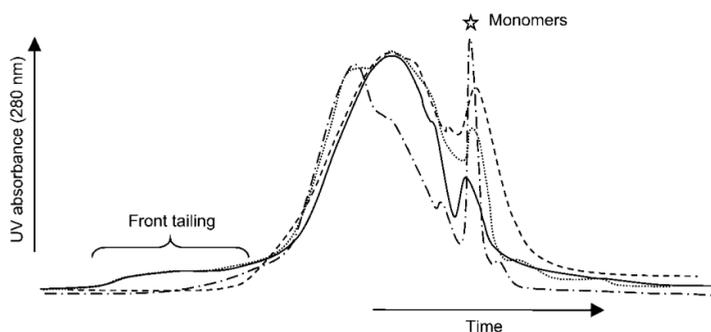
Figure 2.3 SEC profiles (arbitrary absorbance and time units) of an acetylated bagasse soda lignin sample analyzed on various THF systems. (····) ViscoGel (system 4); (—) PL-Gel (system 5); (---) Phenomenex Phenogel (system 6); (·–·) Styragel (system 8). For comparison, the abscissa scale has been adjusted by various factors depending on the system used.

It is unlikely that there are differences in the aggregation behaviour in quite similar systems. The differences in detection sensitivity, which was supported by UV/RI comparison, could better explain the deviations. Whereas good agreement between UV and RI detection was obtained with almost all systems, system 4 showed a five-fold
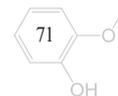
higher Mw value with UV compared to RI detection. Close examination of the elution profile indicates that a high-molar-mass fraction was detected by UV and not by RI (Figure 2.5). This is due to the well-known higher sensitivity of the UV detection for lignin. The detection of such a fraction is thus highly dependent on the detector and is a great source of Mw variations.
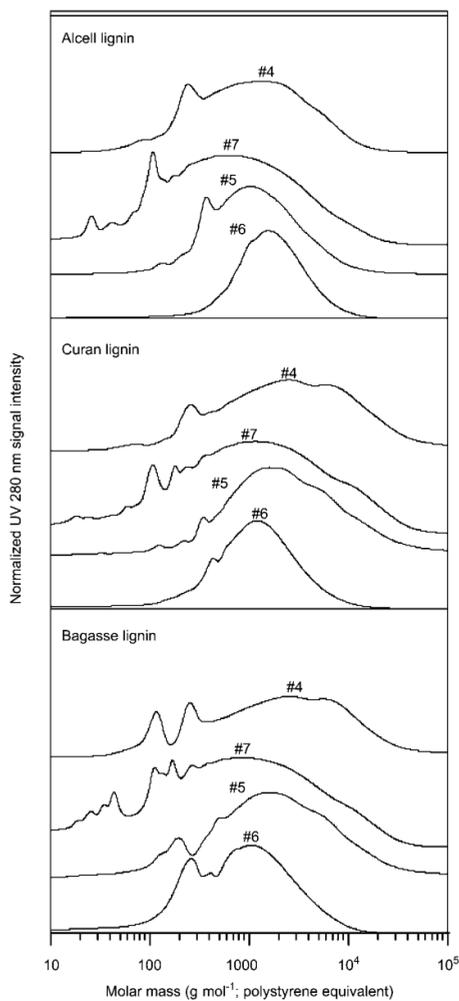
Figure 2.4 Apparent molar mass distributions (polystyrene standard equivalent) of acetylated technical lignins analyzed on the THF systems 4-7. All the curves correspond to the main peak of the elution profiles.
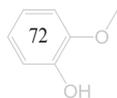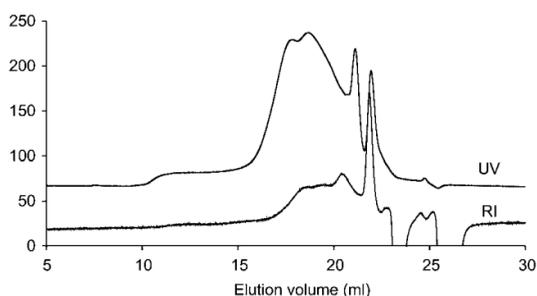
Figure 2.5 SEC elution profiles (arbitrary absorbance and time units) of a bagasse soda lignin sample analyzed with dual UV (280 nm) and RI detection. Chromatography in THF on a series of two ViscoGel columns (Viscotek, GMHHR-M 7.8 mm×300 mm and guard column, 6 mm×40 mm) at flow rate of 1 ml min^{-1} (system 4).

2.3.3 Influence of the calculation strategy

Two calculation strategies were compared: one was based on integration of the whole elution curve and the other one excluding the contribution of the high-molar-mass fraction to the chromatogram beyond the retention time of a low-molar-mass internal standard (Figure 2.6). Including the variable high-molar -mass fraction, probably overestimated by sensitive UV detectors, leads to high M_w values and large polydispersity. On the other hand, integrating the curve beyond the total volume of the column is responsible for underestimation of M_n. This method did not affect M_n significantly if applied to aqueous system 2. In this case, the difference was less than the standard deviation, but gave slightly lower M_w compared to integration of the whole curve (Table 2.6). The effect was more pronounced for the steam explosion lignin, with a two-fold decrease in M_w. The same strategy led to a three- to five-fold in M_w for analyses on DMAc/LiCl and DMSO/water/LiBr systems.

As demonstrated by the SEC results (Table 2.6), interlaboratory variation can essentially be reduced by application of calculation strategy 2. However, in this case, information concerning the high-molar-mass fractions, possibly present in the tailing, is not taken into account, so that the calculated data do not reflect the whole sample. It is thus mandatory when such a calculation is performed to conduct additional experiments suited to the molar mass range excluded from the calculation. MALDI-TOF-MS is such a tool. Corresponding investigation is now under way to check the reliability of the different SEC systems for the lignins tested in the Round Robin.
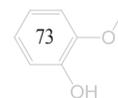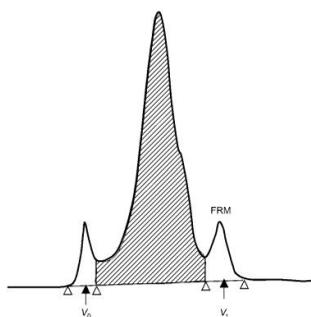
Figure 2.6 Strategy for calculation of the average molar mass data using an internal flow rate marker (FRM). The integration zone (hatching) is defined with respect to the total and void volumes (V_t and V_0, respectively). The proportion of the high- and low-molar-mass fractions is assessed by integration of the whole chromatogram, assuming that the extinction coefficient is independent of the molar mass.

Table 2.6 Influence of the calculation strategy on the average molar mass data determined from SEC analyses in 0.5 M NaOH (system 2) and polar organic solvents (systems 9 and 10).

Lignin sample, eluent (system #)	Integration of the whole curve		Integration of the main peak	
	M_w (g mol^{-1})	M_n (g mol^{-1})	M_w (g mol^{-1})	M_n (g mol^{-1})
Steam explosion, 0.5 M NaOH (2)[c]	22876	2977	12312	3079
Bagasse alkali, 0.5 M NaOH (2)[c]	8481	2684	6934	2490
Curan 100, 0.5 M NaOH (2)[c]	9735	2755	9293	2923
Lignosulfonates, 0.5 M NaOH (2)[c]	4317	2173	3833	2041
Steam explosion, DMSO/water/LiBr (9)[a]	42580	1520	9830	1430
Steam explosion, DMAc/LiCl (10)[b]	34590	2180	11580	2010

Data were calculated with respect to: [a] pullulan and glucose; [b] polyethylene glycol and oxide standards; and [c] Na-polystyrene sulfonates.

2.3.4 Improved SEC analysis

Alkaline SEC analysis of the 15 technical lignin samples on one column packed with the 500Å TSK gel Toyopearl HW-55 (F) showed reproducible results. The Eurolignin Round Robin lignins gave comparable results to the values as published by (Baumberger et al., 2007). Most lignins were effectively separated, but the chromatograms highlighted the presence of higher-molecular-mass fractions in four lignin samples: F5 (a fractionated sample of Sarkanda grass soda lignin), Steam explosion lignin, EAL and EMAL. This high-molar-mass fraction, most likely containing LCCs, elutes in the void volume V0 of the column showing a peak at about 4 min. The presence of these high molecular fractions showed that the separation range of the column packed with one 500Å gel was not sufficient to separate the whole lignin sample (Figure 2.7).

For comparative reasons two lignins, Alcell and Curan 100, without the presence of a high-molar-mass fraction are shown in Figure 2.7. Both lignins resulted in a nicely
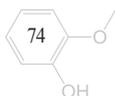
distributed macromolecule. Additionally the chromatograms of some lignins highlighted not only size-exclusion behaviour but also limited adsorption phenomena at the column stationary phase level (signal after 14 min for EMAL in Figure 2.7). Despite this limited adsorption the selected column gel is suitable for a wide range of different technical lignin types.

Except for the four samples (Sarkanda grass F5, Steam explosion, EAL and EMAL) the chromatograms obtained by both SEC methods are almost identical to each other and the M_w and M_n values found are similar. For lignins with a relatively low-molecular weight, one column filled with Toyopearl HW-55 (F) is suitable.

Lignin	SEC elution profiles	Molar mass (g mol^{-1})
Alcell		Mw = 3291 Mn = 700 Mw/Mn = 4.7
Sarkanda grass F5		Mw = 14107 Mn = 2059 Mw/Mn = 6.9
Steam explosion		Mw = 8351 Mn = 1335 Mw/Mn = 6.3
Curan 100		Mw = 7546 Mn = 1026 Mw/Mn = 7.4
EAL		Mw = 17439 Mn = 1398 Mw/Mn = 12.5
EMAL		Mw = 10814 Mn = 684 Mw/Mn = 15.8
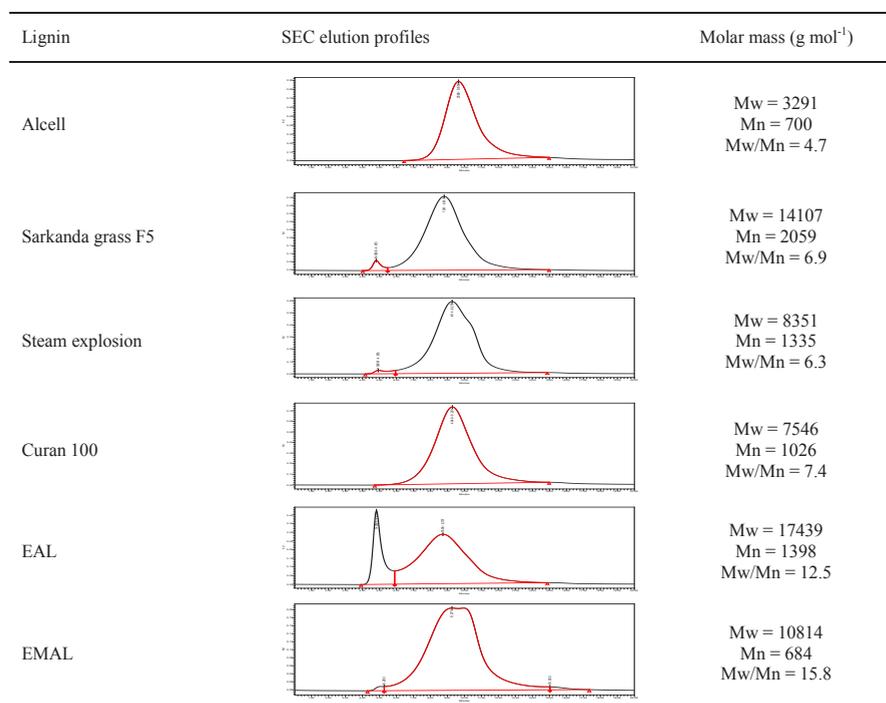
Figure 2.7 Size exclusion chromatography of technical lignins in alkaline medium using a 500Å TSK gel.

Lignin	Overlay one gel and two gels
Alcell	
Sarkanda grass F5	
Steam explosion	
Curan 100	
EAL	
EMAL	
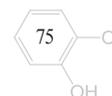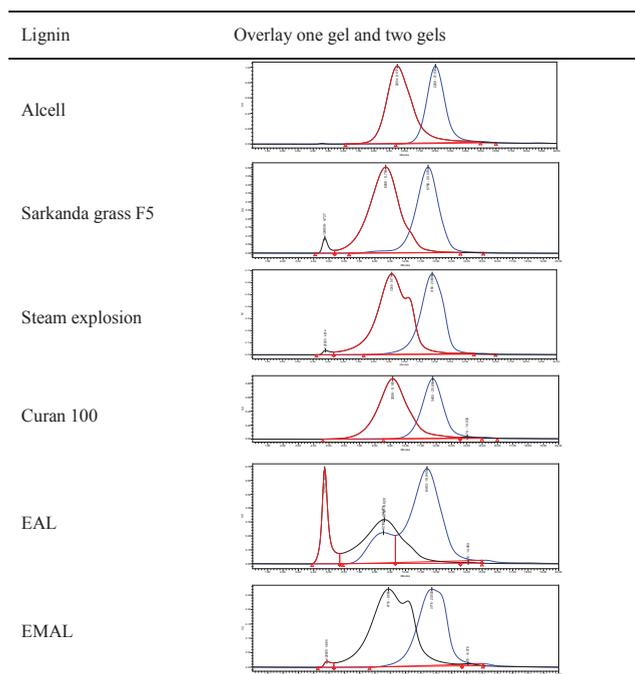
Figure 2.8 Size exclusion chromatography of technical lignins in alkaline medium using one gel (500Å; left chromatogram) and two gels (500Å and >1000Å; right chromatogram) TSK.

On one column gel the high-molar-mass fraction of S5 Sarkanda grass soda lignin was excluded from the calculation of the MMD (Figure 2.8). By using two gels this fraction was included in the separation of the whole lignin sample and contributes to the molar mass distribution of this lignin. This resulted in a significant increase of the M_w from 14 kg mol^{-1} to 39 kg mol^{-1} (Table 2.8). Figure 2.8 showed that except for EAL, the high-molar-mass fractions disappeared resulting in well-defined almost Gaussian distributions. However, the chromatogram of EAL resulted from the analysis using two gels showed still a bi-modal distribution in the molar mass, but both fractions are nicely separated. By using the two gel approach the average values for M_w considerably increase for these four lignin samples (Table 2.8), but the polydispersity did not change in the same manner depending on the different structures. All other lignins gave comparable molar mass results for both methods.
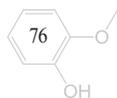
Table 2.8 Comparison of molar mass distribution of technical lignins on 1 gel and 2 gels aqueous SEC analysis.

Lignin	M_w (g mol^{-1})	M_n (g mol^{-1})	M_w/M_n	1: 500Å 2: 500Å+1000Å
Alcell	3291	700	4.7	1
	3392	706	4.8	2
Indulin AT	5198	906	5.7	1
	5341	1070	5.0	2
Sarkanda grass	5477	1024	5.3	1
	5767	1010	5.7	2
Sarkanda grass F1	1429	400	3.6	1
	1547	402	3.8	2
Sarkanda grass F2	2757	724	3.8	1
	2768	734	3.8	2
Sarkanda grass F3	3928	724	5.3	1
	3893	734	5.4	2
Sarkanda grass F4	7406	1376	5.4	1
	7370	1360	5.4	2
Sarkanda grass F5	14107	2059	6.9	1
	38999	2807	13.9	2
Steam explosion	8351	1335	6.3	1
	12869	1170	11.0	2
Curan 100	7470	894	8.4	1
	7546	1026	7.4	2
Bagasse	5854	985	6.0	1
	5882	1158	5.1	2
Lignosulfonate	3217	827	3.9	1
	3108	1103	2.8	2
Residual kraft	8086	1456	5.6	1
	8753	1452	6.0	2
EAL	17439	1398	12.5	1
	22591	2412	9.4	2
EMAL	10814	684	15.8	1
	14892	1625	9.2	2

2.3.5 MALDI-TOF-MS

In Figure 2.9 the positive reflective MALDI-TOF-MS spectra of the narrow dispersed Alcell lignin fractions, as obtained by organic SEC, are depicted. The molar mass fractions resulted in a strong linear relationship between the molar mass at the highest peak in the MALDI m/z spectrum and the retention time in the organic SEC analysis (Figure 2.10). Only, the highest molar mass fraction (F12, not shown in Figure 2.9) could not be analyzed by MALDI. These results showed that narrow dispersed lignin fractions could be measured by MALDI for their absolute molar mass. These results are in good agreement with the results previously published (Jacobs and Dahlman 2000).
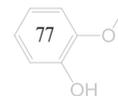
These results also showed that if the peak molar mass fraction goes beyond about 5 kg mol^{-1} the MALDI signals could be decreased due to limited ionization and flying behaviour. These narrow dispersed lignin fractions could be used for calibration of SEC.
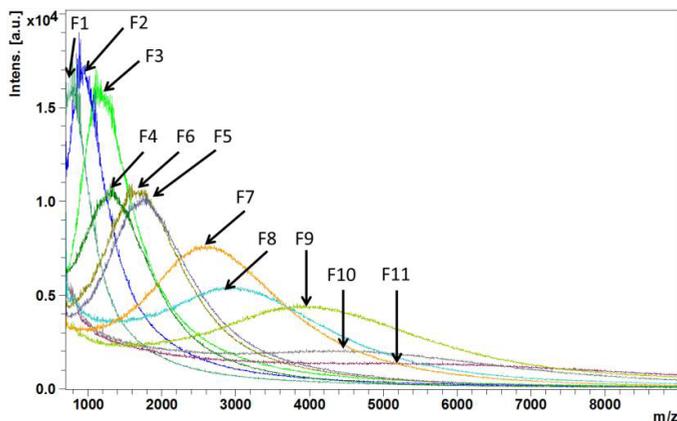
Figure 2.9 Positive reflective MALDI-TOF-MS spectra of fractionated Alcell lignin in THF (DHB used as matrix).
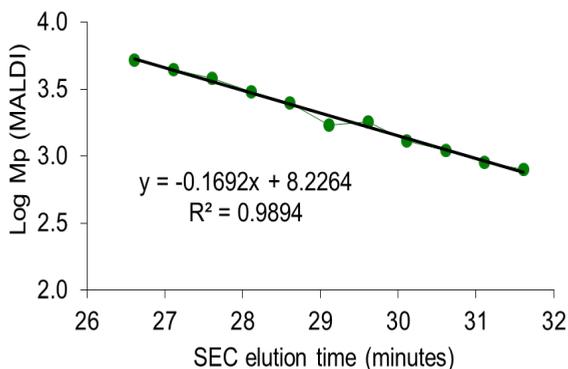
Figure 2.10 Correlation log M_p found by MALDI versus SEC elution time for fractionated Alcell lignin.

It can be concluded that fractionated organosolv lignin result in appropriate MALDI spectra, however, it would be of much higher interest for utilisation of lignin if the complete lignin sample could be measured to determine its absolute molar mass distribution. This was studied for Alcell lignin by using different solvents and

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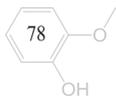
optimisation of instrument parameters. The best results were obtained when applying
DMF, 0.05M NaOH, and acetonitrile as shown in Table 2.9. When using methanol and
NaOH no clear spectra were obtained. The molar mass value (M_p) of about 1600 g mol^{-1}
was found when using DMF and 0.05M NaOH. This equals the average M_w value
measured by organic SEC in THF of Alcell lignin (Hergert et al. 2000). Although
acetonitrile resulted in the highest molar mass value, the use of this solvent is
cumbersome because of the difficulty to bring one consistent tiny droplet on the
MALDI target plate. This is due to the relatively low viscosity and relatively high
volatility of acetonitrile. DMF and 0.05M NaOH were further used for MALDI
measurements.

Table 2.9 Molar mass Alcell lignin (M_p) as function of solvent type measured by MALDI-TOF-MS
(DHB used as matrix)

Solvent	M_p (g mol^{-1})
Acetone/H_2O (7:3 v/v)	1350
DMF	1600
Dioxane/H_2O (9:1 v/v)	1300
NaOH 0.05M	1600
Acetonitrile	1950
Acetonitrile/H_2O (7:3 v/v)	1450
MeOH/NaOH (1:1)	Not measurable

Figures 2.11 and 2.12 show that an alkaline solvent is applicable for MALDI
measurements of whole lignin samples. Part of the alkaline salts present in the solvent
were removed by using an anion exchange resin. For most lignins DHB as matrix is
sufficient, but for Indulin AT an improved s/n ratio could be obtained using retinoic
acid (RA). Using CHCA, sinapic acid, or dithranol as matrix the molar masses of the
lignins studied were substantially lower than measured with DHB. Also the addition of
Ag-salts did not improve the MALDI results. This indicates that for each lignin type
optimal conditions should be found by selecting matrix type and instrument parameters.
Another important observation is that the spectra are rather broad showing the
polydispersity of these technical lignins (Figure 2.11).
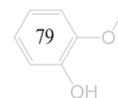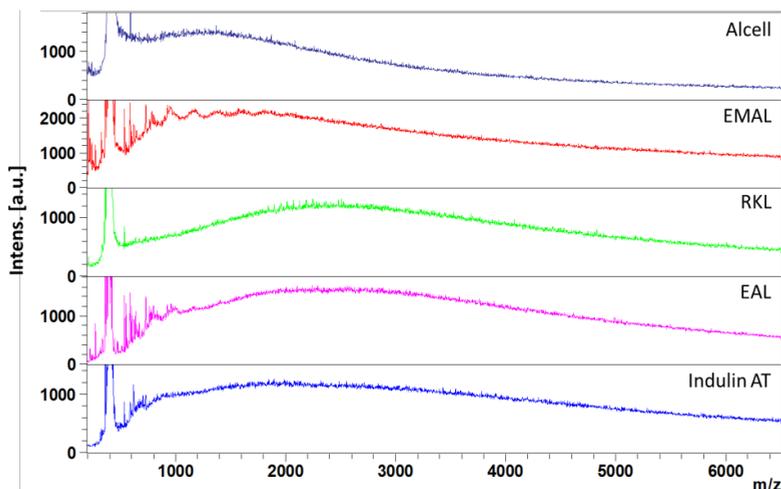
Figure 2.11 MALDI of unfractionated technical lignins in alkaline solvent (DHB used as matrix).
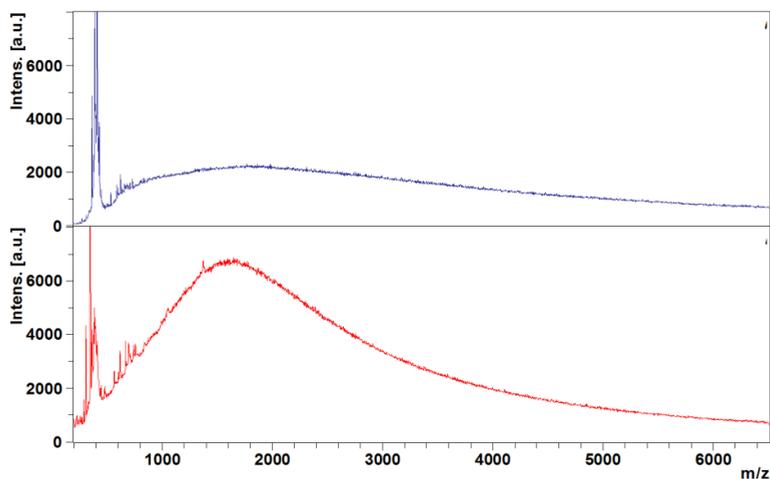
Figure 2.12 Effect of matrix type on MALDI spectrum of unfractioned Indulin AT
in 0.05 M NaOH: DHB (upper); RA (lower).

To further improve the MALDI results Alcell lignin was fractionated by SEC using an alkaline solvent. After using the ion-exchange resin, MALDI measurements were performed. This resulted in MALDI spectra of the Alcell lignin fractions with a very poor s/n (not shown). This was most likely caused by the low resolution SEC column used resulting in a limited lignin concentration in each fraction and combined with inferior ionization no clear spectra were obtained. This is an unexpected result as it is possible to obtain MALDI spectra of unfractionated Alcell lignin in the same alkaline

solvent (Figure 2.11). Further increment of the lignin concentration in each fraction did not result in higher s/n. Using a high-resolution alkaline SEC system, as used for organic SEC fractionation in THF, or ultrafiltration in an alkaline solvent may result in more narrow fractionated lignin samples.

Alternatively, kraft lignin (Indulin AT) was fractionated by organic solvent fractionation according to its molar mass (see **Chapter 3**). F1 has the lowest average molar mass and F5 the highest. Impurities such as carbohydrates and ash accumulate in the highest molar mass fractions. MALDI analysis showed that the molar mass obtained is almost comparable to the SEC analysis for fractions F1, F2 and F3 (Figure 2.13). However, MALDI analysis of unfractionated lignin, F4 and F5 give a much lower M_p than expected from the SEC analysis. These samples contain more impurities (carbohydrates, ash) which might lead to incomplete crystallization and inferior ionization of the higher molar mass lignin fractions. Additionally, the organic solvent fractionated kraft lignin resulted in more polydisperse mass fractions compared to the narrow mass fractions obtained after organic SEC fractionation of Alcell lignin. These more dispersed samples could lead to poorer spectra with a reduced s/n.
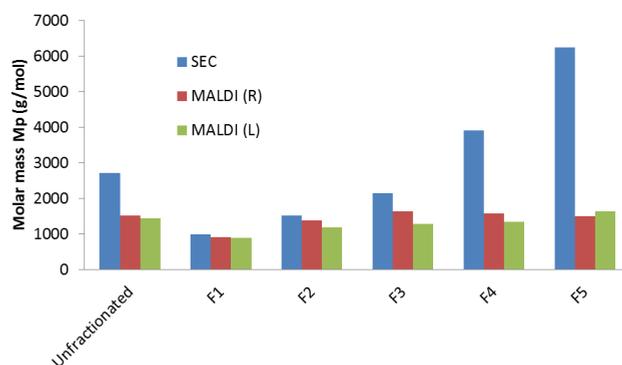
Figure 2.13 Alkaline SEC and MALDI results of unfractionated and fractionated kraft lignin. (R) is reflective and (L) linear MALDI detection method.

2.4 Conclusions

This work confirms the importance of adsorption phenomena in the course of molar mass determination by SEC. In agreement with the NREL Round Robin (1991), the steam explosion lignin sample exhibited the greatest interlaboratory and intersystem variations owing to its highest polydispersity and incomplete solubility in conventional solvents. It should be recommended that secondary effects are dependent on the structure and composition of the lignins investigated. Thus, lignins with low average molar masses, low polydispersity, and a low degree of branching (e.g., eucalyptus lignins, Evtuguin and Amado 2003) can deliver reliable results, even with a polystyrene calibration. The most important point is to systematically determine the lignin solubility in the elution medium and to perform recovery tests for lignins not yet analyzed.

It is recommended to use the 0.5 M NaOH/TSK gel Toyopearl HW-55 (F) system (1 ml min^{-1}, 25°C) and calibration with sodium sulfonated polystyrene (1,370-142,500 g mol^{-1}) for aqueous SEC. The gel is stable, adsorption of lignin onto the gel is low, interlaboratory reproducibility is high (for identical columns), and the results are similar to those obtained by DMAc- and DMSO-based systems (except for steam explosion). Good agreement between apparent and absolute MMD (preliminary MALDI-TOF investigations) also leads to the recommendation of the THF/SDVB PL Gel (1 ml min^{-1}, 25°C) system, and calibration polystyrene standards. In this case, however, the results are strongly influenced by the geometry and origin of the column and by the sensitivity of the detector.

The calculation strategy is a determining factor regarding interlaboratory reproducibility. An internal flow-rate marker may help to re-scale the time axis and delimit the integration borders. This measurement improves the reproducibility. In this case, the proportion of the front tailing (high-molar-mass fraction) should be calculated by separate integration. The results can be presented separately. Better knowledge of this high-molar-mass fraction is necessary for better SEC analytical performance.

The alkaline aqueous SEC method provides an accurate and reproducible determination of the lignin molar mass distribution in 0.5M NaOH. This method is applicable for a wide range of different technical lignins which do not need to be derivatised prior to analysis. Lignins with low average molar masses and low polydispersity give reliable results when using one column packed with a 500Å TSK gel Toyopearl HW-55(F). However, the addition of a serial connected column filled with

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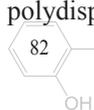
larger pores TSK gel Toyopearl HW-75(F) is recommended for the analysis of the high molecular fractions of lignin.

MALDI-TOF-MS show promising results for analysis of the absolute molar mass of lignin. Unfractionated lignin gives broad distributions while narrow fractionated Alcell lignin fractions gives good spectra. The MALDI results from these narrow distributed fractions resulted in a good correlation between SEC and MALDI data and can be used for calibration of the SEC method.

In contrast, alkaline SEC did not result in good MALDI spectra of lignin fractions. Solvent fractionation did result in increasing molar mass fractions, but the highest lignin mass fractions could not be accurately measured by MALDI. This is caused by the polydispersity of these fractions resulting in inferior ionization and poorer spectra with a reduced s/n.

Optimization of the instrument parameters, matrix type and other parameters did not result in an accurate MALDI method for whole lignin analysis probably due to the relatively high polydispersity. These results show that only narrow distributed molar mass lignin fractions can be used for accurate MALDI-TOF-MS analysis.

Acknowledgements

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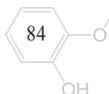
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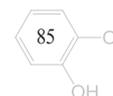
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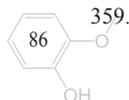
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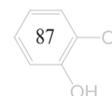
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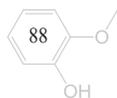
Chapter 3

Fractionation, analysis, and PCA modeling of properties of four technical lignins for prediction of their application potential in binders
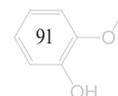
Abstract

Functional properties of technical lignins need to be characterized in more detail to become a higher added value renewable raw material for the chemical industry. The suitability of a lignin from different plants or trees obtained by different technical processes can only be predicted for selected applications, such as binders, if reliable analytical data are available. In the present paper, structure dependent properties of four industrial lignins were analyzed before and after successive organic solvent extractions. The lignins have been fractionated according to their molar mass by these solvents extractions. Kraft and soda lignins were shown to have different molar mass distributions and chemical compositions. Lignin carbohydrate complexes are most recalcitrant for extraction with organic solvents. These poorly soluble complexes can consist of up to 34% of carbohydrates in soda lignins. Modeling by principle component analysis (PCA) was performed aiming at prediction of the application potential of different lignins for binder production. The lignins and their fractions could be classified in different clusters based on their properties, which are structure dependent. Kraft softwood lignins show the highest potential for plywood binder application followed by hardwood soda lignin and the fractions of Sarkanda grass soda lignin with medium molar mass. Expectedly, the softwood lignins contain the highest number of reactive sites in *ortho* positions to the phenolic OH group. Moreover, these lignins have a low level of impurities and medium molar mass.

Keywords: binder application; degree of condensation; fractionation by successive extraction; molar mass distribution; principle component analysis (PCA); size exclusion chromatography (SEC); structural characterization; technical lignins; thioacidolysis; ^{31}P-NMR spectroscopy.
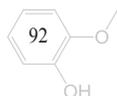
3.1 Introduction

Lignin is the most abundant renewable raw material available on earth containing aromatic ring structures. Despite its unique characteristics as a natural product with a high degree of chemical and biophysical functionalities, it is largely underexploited because of its limited commercially established applications and image as a low quality waste material. Currently, approximately 98% of the lignin extracted on industrial scales from lignocellulosic materials is burned to generate energy for pulp mills.

However, the interest in lignin as a resource for renewable raw material has been growing in the pulp and paper industry in case of increasing pulp production owing to bottleneck problems in the chemicals recovery boiler of the kraft pulp mill (Gosselink et al. 2004a). Additionally, current increased demand for fossil fuel alternatives – such as the production of transportation biofuels – intensifies the interest and need to valorize the unconverted lignin fraction. Although burning lignin is a valuable contribution for saving fossil energy sources, processing lignin into added value applications is a key factor for creating biorefinery processes, based on lignocellulosic materials, which are economically feasible.

Lignin has a high potential for applications as binder and as a source for base aromatic chemicals. The structure of lignin has a certain similarity to that of traditional fossil-based binders. Currently, lignin is processed into surfactants and adhesives and it is the source of the food additive vanillin (Gargulak and Lebo 2000). However, the main drawback of lignin utilization is its heterogeneity, leading to unpredictable and uncontrollable reactions, odor, and color.

Most research activities on binders were hitherto concentrated on substituting phenol with lignin in the synthesis of lignin modified phenol-formaldehyde (PF) resins. The final resin properties are strongly related to analytical properties of the lignins, which needs to be known prior to application development (Tejado et al. 2007). A relevant requirement is, for example, a high amount of free phenolic hydroxyl groups with numerous free reactive *ortho* ring positions. A high content of coumaryl (H-unit) and guaiacyl (G-unit) units in the lignin is more favorable than high syringyl (S-unit) content. Lignin should contain less impurities than 4% – such as carbohydrates and ash – to diminish the water sensitivity of the binder. Molar mass of lignin should not be too high to keep the binder viscosity in the desired range, and not too low to favor its contribution to the resin polymerization.
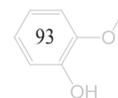
Different lignins were successfully used for up to 70% incorporation in PF binders for plywood, wafer boards, and oriented strandboards (Ash et al. 1992; Gosselink et al. 2004b; Khan et al. 2004; El Mansouri and Salvadó 2006; Tejado et al. 2007; Cavdar et al. 2008).

An important feature of lignin is its molar mass distribution, which influences its reactivity and physico-chemical properties (Baumberger et al. 2007). Mörck et al. (1986) showed that kraft lignin can be fractionated by molar mass by successive organic solvent extractions. We applied this extraction sequence to fractionate both kraft lignins and soda lignins. In-depth characterization was performed on the lignin structure, before and after fractionation, and on the presence of lignin carbohydrate complexes (LCCs).

As pointed out above, functional properties of lignin need to be characterized before application. In this paper, technical lignins from different sources (softwood, hardwood, grass), which were obtained by three technical pulping processes (kraft, soda, organosolv), were studied. The objective was to measure the properties of these lignins, which are structure dependent, aiming at the production of a binder (resin) for wood panels.

Specifically, correlations should be established between functional properties of technical lignins and their fractions based on principle component analysis (PCA). Results were compared to data of application tests, in which PF resins were mixed with unmodified lignins, as wood adhesive for plywood production. It was demonstrated that the model is able to predict the suitability of a lignin or its fractions for plywood applications based upon quantifiable analytical chemical data.

3.2 Materials and methods

Selected technical lignins

- Indulin AT, softwood kraft lignin, MeadWestvaco, USA.
- Curan 100, softwood kraft lignin, Lignotech Borregaard, Norway. Abbreviation in text for softwood kraft lignins: SW KLs.
- Sarkanda grass soda lignin, Granit, Switzerland. Abbreviation in text: grass SoL.
- P1000, mixed Sarkanda grass and wheat straw soda lignin, Granit, Switzerland.
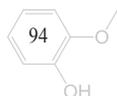
- Hornbeam (*Carpinus betulus*) hardwood soda lignin precipitated from black liquor of a Slowakian mill by Kiram, Sweden and Granit, Switzerland. (Abaecherli and Doppenberg 1998). Abbreviation in text: HW SoL.
- Organosolv lignin from mixed hardwoods (Alcell[TM]), Repap Technologies, Canada.
- Milled wood lignin (MWL) from aspen, KTH, Sweden.

Solvent fractionation

Four technical lignins (50 g) were fractionated by successive extraction with water and organic solvents (Figure 3.1). Lignin was suspended in 250 ml of the respective solvent and the suspension was continuously stirred at room temperature for 30 min. The undissolved material was filtered over a 6-µm filter paper and resuspended for a second identical extraction. The fractions from both steps were combined. Collected dissolved material was filtered over 0.45-µm filter and was vacuum dried.
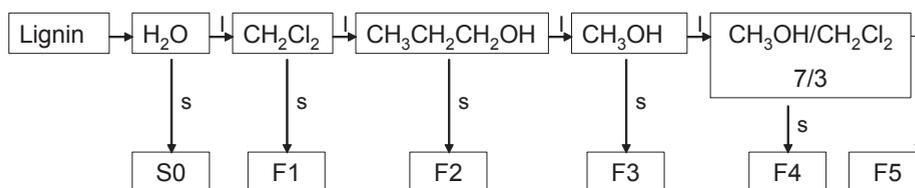
Figure 3.1 Scheme for lignin fractionation by successive extraction with organic solvents (I, insoluble material; S, soluble material).

Acetylation and organic size exclusion chromatography (SEC) analysis

Lignin (10 mg) was acetylated and purified as described by Gellerstedt (1992). Acetylated lignin was dissolved in 2 ml tetrahydrofuran (THF), filtered over a 0.2-µm filter and 20 µl was injected in three serial connected polystyrene divinylbenzene columns HR 0.5, 2, and 4 (Waters Corporation). The flow was regulated to 0.8 ml min^{-1} at room temperature. Detection: UV 280 nm. Molar mass calibration by polystyrenes with M_w ranging from 580 to 915 000 Da.
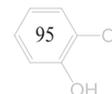
Alkaline SEC analysis

Lignin samples of 1 mg/ml dissolved in 0.5 M NaOH were injected into a manually packed column (4.6=30 cm) with ethylene glycolmethacrylate copolymer TSK gel Toyopearl HW-55F and eluted with the same solvent. Conditions: flow 1 ml min^{-1}, column temperature 25°C, and detection at 280 nm. The fractionated Sarkanda grass soda lignin was analyzed by SEC (two serial connected columns, each packed with HW-75F and HW-55F, respectively). Standards for calibration of the molar mass distribution: sodiumpolystyrene sulfonates (M_w range: 891 to 976 000 Da) and phenol.

Thioacidolysis

Lignin (10 mg) was treated with 20 ml thioacidolysis reagent containing boron trifluoride etherate and ethanethiol (Rolando et al. 1992). A part of this thioacidolysis (20 µl) product was trimethylsilylated overnight at room temperature by adding 10 ml pyridine and 80 ml of N,O-bis-(trimethylsilyl) trifluoroacetamide with 1% trimethyl chlorosilane. This product was injected on a Rtx 5 column from Restec Corporation (45 m, 0.32 µm I.D., 0.25 mm film thickness) with He as carrier gas. Temperature program started at 180°C with a heating rate of 4°C min^{-1} to 270°C followed by an isothermal step of 15 min, continued with the same heating rate to 300°C and a second isothermal step of 15 min. Injector temperature was 250°C and detector temperature was 280°C.

Another part of the thioacidolysis product was acetylated overnight at room temperature by adding pyridine and acetic anhydride 1:1 (v/v) and was subsequently purified and analyzed by organic SEC.

^{31}P NMR

In a 1-ml vial, 30 mg of lignin was mixed with 100 µl N,N-Dimethylformamide (DMF)/pyridine (1:1 v/v) and 100 µl internal standard solution containing 15 mg ml^{-1} cyclohexanol (internal standard) and 2.5 mg ml^{-1} chromium(III) acetylacetonate in pyridine. This suspension was stirred for 4 – 16 h at room temperature. Derivatization (100 µl) reagent (2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphopholane) was mixed with 400 µl of CDCl$_3$ prior to addition to the lignin suspension. After mixing, the mixture was analyzed by NMR (Bruker 300 MHz), with 30° pulse angle, inverse gated proton
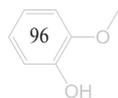
decoupling, a delay time of 5 s and 256 scans. Signal assignment was performed as described by Granata and Argyropoulos (1995).

Carbohydrates and ash

Lignin was hydrolyzed by a two-step sulfuric acid hydrolysis starting with 12 M H_2SO_4 at 30°C for 1 h followed by 1 M H_2SO_4 at 100°C for 3 h. The hydrolysate was neutralized by calcium carbonate until acidic pH as indicated by bromophenol blue. Resulting monosaccharides were separated and quantified by HPAEC-PAD on a Dionex CarboPac PA1 column and precolumn under the following conditions: sodium hydroxide/water gradient at 35 °C; flow rate 1 ml min^{-1}. Postcolumn addition of 500 mM NaOH at a flow rate of 0.2 ml min^{-1} was used for detection.

Ash in lignin was determined after complete combustion at 800°C during 4-8h.

Plywood application test

PF (47%) alkaline setting resin (pH 11) was supplied by Chimar Hellas SA, Greece. This resin was diluted to 37% PF resin. Then, 10 and 30% of this PF was replaced by lignin and the pH was adjusted to 11. Viscosity of the formulation was adjusted between 300 – 800 mPa s by adding wood powder if necessary. Birch 3-ply plywoods of 10 x 20 cm in duplicate were prepared after cold pressing and hot pressing at 140°C and 5 ton for 15 min. Subsequently, the plywood was kept 15 min in the press to cool down. The 3-ply plywood samples were sawn into seven usable test samples and tested according to EN 314-1 (Gosselink et al. 2004b). Soda grass lignin fractions F1 and F5 were not tested and for fraction F2, only the 10% substitution of PF resin was tested.

Principle Component Analysis (PCA)

PCA was performed by XLSTAT 7.1 with datasets of quantified lignin characteristics from six different lignins and a SoL of grass, which were solvent fractionated (F1 – F5). Normalization was performed by the following parameters: Log (1/carbohydrates), log (1/ash content), and log (molar mass). In addition to these, the log (1/degree of condensation) expressed by the ratio of condensed OH groups and uncondensed OH
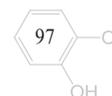
groups, log (phenolic OH), and the chemical reactivity expressed by the free *ortho* ring positions by calculation of coumaryl OH twice and guaiacyl OH once.

3.3 Results and discussion

3.3.1 Organic solvent fractionated lignin

The four selected technical lignins showed different solubility in the sequentially solvents used (Table 3.1). Industrial kraft lignins (e.g., Indulin AT and Curan 100, SW KLs) have efficiently been purified during the recovery process removing low molecular mass components as indicated by low yields of water solubles, F1 and F2 (Table 3.1). In contrast, the soda lignins (SoL) contained substantial amounts of components with low molar mass in fraction F1 and F2 as found by SEC with a low amount of attached carbohydrates (Table 3.2). Both F1 and F2 fractions consist of lignin having low molecular weights possibly contaminated with extractives such as fatty acids and resinous plant material (Mörck et al. 1986).

Table 3.1 Yields of five fractions (F1-F5) obtained from different lignins by successively applied solvent extractions (% data are wt% based on dry unextracted lignin).

Lignin	H_2O (%)	F1 CH_2Cl_2 (%)	F2 n-propanol (%)	F3 MeOH (%)	F4 $MeOH/CH_2Cl_2$ (%)	F5 Residue (%)
Indulin AT	1.4	2.5	0.9	43.0	27.3	25.0
Curan 100	4.5	3.1	1.0	29.8	20.0	41.7
Sarkanda grass soda lignin	1.3	11.6	10.0	25.3	36.7	15.1
Hardwood soda lignin	8.1	10.7	1.9	58.1	2.3	19.0
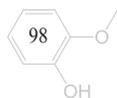
Table 3.2 Carbohydrates in solvent fractioned lignins (wt% on dry lignin).

Lignin Lignin fractions	Average sugar contents (%) obtained by total hydrolysis						Uronic acids (%)	Total carbohydrates (%)
	Ara	Xyl	Man	Gal	Glu	Rham		
Indulin AT								
unfractionated	0.25	0.55	0.05	0.44	0.33	0.01	0.00	1.62
F1	nd	nd	nd	nd	nd	nd	nd	nd
F2	0.04	0.01	0.01	0.07	0.17	0.01	0.00	0.32
F3	0.07	0.00	0.02	0.08	0.14	0.00	0.00	0.32
F4	0.07	0.02	0.03	0.06	0.44	0.01	0.11	0.73
F5	0.62	1.54	0.12	0.94	0.93	0.01	0.18	4.34
Curan 100								
unfractionated	0.29	0.43	0.04	0.76	0.26	0.00	0.00	1.78
F1	nd	nd	nd	nd	nd	nd	nd	nd
F2	0.00	0.13	0.02	0.06	0.16	0.00	0.00	0.37
F3	0.06	0.04	0.03	0.09	0.45	0.01	0.14	0.83
F4	0.05	0.02	0.03	0.06	0.46	0.01	0.12	0.75
F5	0.46	0.60	0.08	0.93	0.63	0.02	0.17	2.89
Sarkanda grass soda								
unfractionated	0.11	0.81	0.22	0.22	0.97	0.04	0.00	2.37
F1	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.19
F2	0.03	0.05	0.02	0.04	0.38	0.02	0.00	0.55
F3	0.07	0.18	0.03	0.07	0.41	0.02	0.00	0.79
F4	0.07	0.27	0.02	0.05	0.65	0.02	0.14	1.22
F5	0.27	3.61	0.99	0.73	4.00	0.04	0.37	10.01
Hardwood soda								
unfractioned	0.26	4.93	0.05	0.32	1.68	0.08	0.72	8.04
F1	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.16
F2	0.04	0.04	0.02	0.07	0.33	0.04	0.00	0.54
F3	0.12	0.09	0.03	0.12	0.58	0.05	0.18	1.17
F4	0.39	0.39	0.08	0.25	1.16	0.05	0.50	2.83
F5	0.70	22.76	0.11	0.62	6.78	0.06	3.30	34.34

Nd, not determined due to limited availability.

All lignins studied have predominantly molar masses in the range of medium to high, except for HW SoL. Figures 3.2 and 3.3 show that fractionation for both KLs and SoL occurs according to their molar mass. The F4 fractions of both SoL yielded a substantially higher M_w than the KLs. Remarkably, fraction F5 of HW SoL apparently contained moieties with medium M_w as found by SEC with organic solvent, but this result reflects the poorly soluble part in THF. This was caused by its high carbohydrate content of 34% (Table 3.2) and the poor solubility of LCCs is well known. Accordingly, soda pulping of HW was less complete in separation of carbohydrates and lignin than the other processes.

As expected, SEC in organic and alkaline medium resulted in substantial quantitative differences for the M_w of the lignins and their fractions as reported by Baumberger et al. (2007).
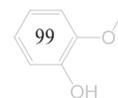
The dissolved LCCs yielded different hydrodynamic volumes for the lignin part and for the carbohydrate part in both solvents, and this strongly influences SEC results. An additional reason for differences is that different calibration standards were applied in the two systems. However, the same trend was observed during solvent fractionation (Figure 3.3). Table 3.2 shows that enrichment of LCCs occurred after successive organic solvent extraction for all lignins. Total carbohydrate content in F5 was substantially lower for the KLs than for the SoLs. Concerning KLs, these findings are in agreement with those of Mörck et al. (1986). Furthermore, selective extraction of carbohydrates occurred during fractionation. The xylan content of HW SoL in fraction F5 was approximately four times higher compared with unfractionated lignin, whereas for galactan a factor 2 was found (Table 3.2).

In conclusion, technical lignins can be easily fractionated to the application desired molar mass range by organic solvent extraction under mild conditions. The economic feasibility will be dependent on the added value of the resulting fractions and needs to be evaluated further.
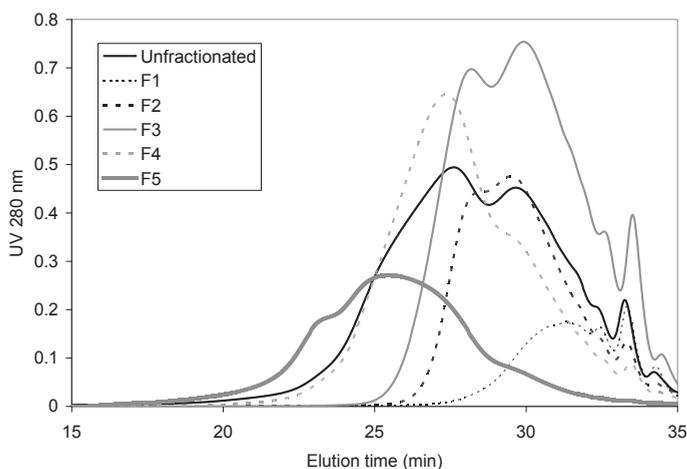
Figure 3.2 Molecular mass distribution for fractions F1-F5 and
unfractionated Indulin AT in organic SEC.
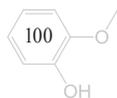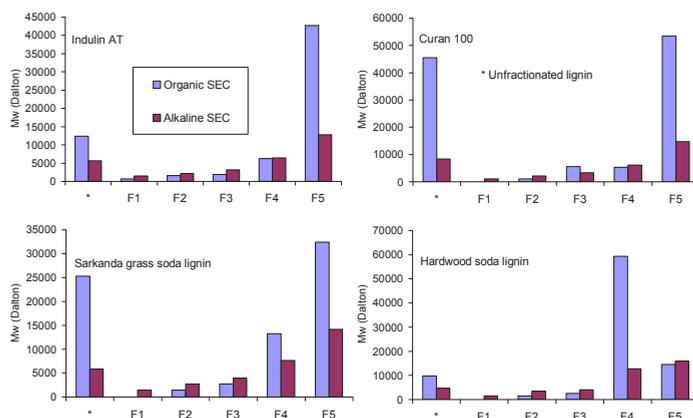
Figure 3.3 Molecular mass distribution for solvent fractionated and unfractionated technical lignins in organic and alkaline SEC.

3.3.2 Thioacidolysis and degree of condensation

Table 3.3 shows that the monomer yields obtained by thioacidolysis of technical lignins were substantially lower than that of MWL from aspen. This can easily be explained by the fact that the technical lignins are more condensed than MWL because of the secondary reactions taking place during pulping. The higher degree of condensation might influence the applicability of technical lignins as reactive part of a binder.

As well known, softwood KLs are built up mostly by G- and to a minor part by H-units. The presence of S-units (without the reactive aromatic sites in *ortho* positions to the phenolic OH) is typical for HW and grass lignins. Sarkanda grass SoL contains relatively more G-units than HW SoL (Table 3.3). Additionally, in Sarkanda grass SoL slightly more H-units are present than in HW SoL as shown in Table 3.3 and this is more pronounced in Table 3.4 by the p-hydroxyl OH content.

SEC analysis of acetylated degradation products of thioacidolysis shows a higher concentration of components with lower molecular mass obtained from MWL than from industrial lignins (Figure 3.4). This finding is in agreement with that of Christiernin et al. (2006), who observed predominantly mono- and dimeric fragments among the thioacidolysis products of MWL together with a small amount of tri-, oligo-, and polymeric fractions. In contrast, a substantial higher contribution of polymeric

fraction was observed in industrial lignins. This result is also a manifestation of the high condensation degree of industrial lignins.

Table 3.3 Thioacidolysis products from different technical lignins.

Lignin	Yield[a] (μmol/g)	H unit (μmol/g)	S/G ratio
MWL aspen	2242	nd	1.34
Indulin AT	323	nd	0
Curan 100	188	nd	0
Hardwood soda	180	nd	2.73
Sarkanda grass soda	456	8	0.57

[a]Yield of major lignin monomer products (S + G units) after thioacidolysis as quantified by GC. Nd, not detected by GC.
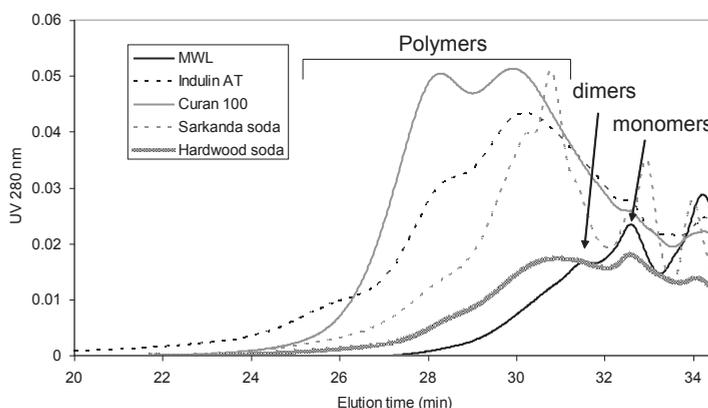
Figure 3.4 Organic SEC analysis of acetylated thioacidolysis products for different industrial lignins and milled wood lignin (MWL) from aspen.

3.3.3 Functional group distribution

^{31}P NMR revealed differences in functional groups of the lignins (Table 3.4). The results found for Indulin AT and Alcell lignin are in agreement with those reported by Granata and Argyropoulos (1995). Both SW KLs (G-types) yield comparable functional group contents. For HW SoL (SG-type), hydroxyl groups attached to S-units are predominantly present in addition those attached to G- and H-units. Expectedly, in grass SoL all three types of OH groups were found (H_{OH}, G_{OH}, and S_{OH}). Obviously, the carboxyl group content of SoLs was substantially higher than that for the KLs, indicating incorporated acidic extractives or formation of oxidized functional groups during processing.

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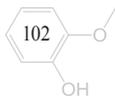
Table 3.4 Contents of functional groups (mmol/g) as determined by ^{31}P NMR.

Lignin	Functional groups (mmol/g)							Ratio
	Aliphatic OH	Cond. phen OH	Syringyl OH	Guaiacyl OH	p-Hydroxyl OH	COOH	Phenolic OH total	phenOH/ aliph OH
Indulin AT	2.08	1.30	0	1.62	0.23	0.44	3.15	1.51
Curan 100	1.78	1.55	0	1.84	0	0.43	2.39	1.90
Sarkanda grass soda	1.59	0.69	0.54	0.72	0.46	0.99	2.41	1.52
Hardwood soda	1.34	0.70	0.92	0.51	0.34	1.06	2.48	1.85
Organosolv mixed hardwoods	1.08	0.76	1.05	0.70	0.20	0.30	2.71	2.51

3.3.4 Modeling structure dependent properties

PCA was used to model structure dependent lignin properties (Table 3.5). The first two components PC1 and PC2 explained 80% of the total variance, as depicted in Figure 3.5. This biplot shows groups of lignins with distinguished properties. In particular, HW SoL and grass SoL fraction F5 are structurally different to the other lignins. These lignins contain the highest level of impurities such as carbohydrates and ash. Additionally, the molar mass of the F5 fraction of grass SoL is substantially higher which could only be analyzed by SEC adapted to a higher molar mass range.

By solvent fractionation, lignin can be classified to different groups representing substantially different structure related functional properties as shown in Figure 3.5. This classification is strongly driven by the molar mass of the different lignin fractions. Furthermore, Figure 3.5 shows for each lignin which parameters influence its position in the graph and by adjusting these parameters a lignin can be moved to another cluster. For example, HW SoL will move along the log(1/condensation) vector if the degree of condensation becomes lower owing to changing pulping and recovery conditions.

SW KLs have similar functionalities (Table 3.4) and are classified in the same cluster (Figure 3.5). Low molar mass fractions of grass SoL (F1–F3) are structurally comparable to highly pure organosolv HW lignin. The last cluster was represented by the non-wood SoL and the medium molar mass F4 fraction of grass SoL.

Table 3.5 Normalized lignin characteristics used for PCA.

Lignin	Log (1/CH)[a] (%)	Log (1/Ash) (%)	Log (M_W) (Dalton)	Log (1/DC)[b]	Log(OH$_{phen}$) (mmol/g)	Free *ortho* position[c] (mmol/g)
Indulin AT	-0.21	0.96	3.75	0.15	0.50	2.08
Curan 100	-0.25	0.00	3.92	0.08	0.38	1.84
Hardwood soda	-0.91	-0.75	3.66	1.00	0.36	1.19
Organosolv mixed HW	0.52	1.00	3.54	0.40	0.43	1.10
Sarkanda grass / wheat straw soda	-0.58	-0.04	3.68	0.38	0.39	1.68
Sarkanda grass soda	-0.37	-0.61	3.76	0.40	0.38	1.64
S. grass soda (F1)	0.73	1.00	3.16	0.50	0.40	1.57
S. grass soda (F2)	0.26	0.27	3.44	0.54	0.40	1.91
S. grass soda (F3)	0.10	0.49	3.60	0.42	0.41	1.75
S. grass soda (F4)	-0.09	0.55	3.88	0.35	0.38	1.48
S. grass soda (F5)	-1.00	-0.80	4.59[d]	0.25	0.12	0.81

[a]CH carbohydrates.
[b]DC degree of condensation = ratio of "condensed aromatic hydroxyl groups/uncondensed aromatic hydroxyl groups".
[c]In position *ortho* related to phenolic OH = 2×coumaryl hydroxyl+1×guaiacyl hydroxyl.
[d]Molar mass determined by alkaline SEC with two columns.

Biplot (axes PC1 and PC2: 80.15 %)
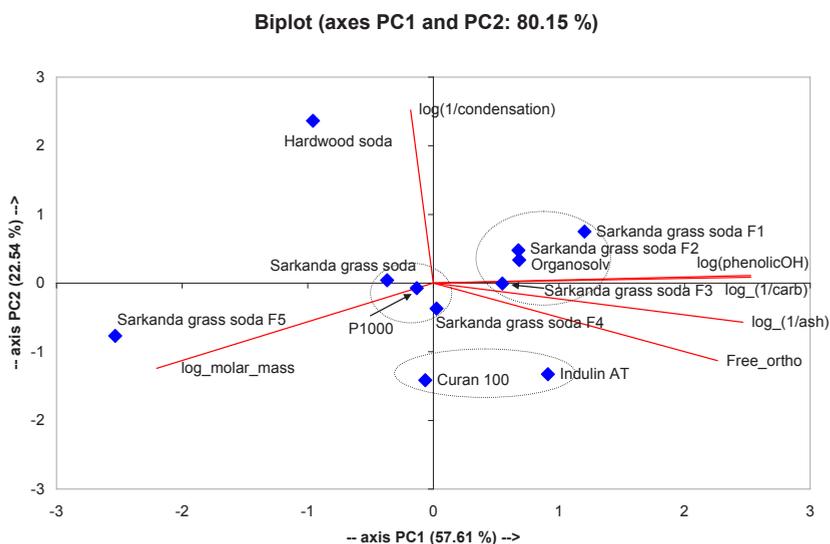
Figure 3.5 PCA biplot graph modeling lignin structural properties.

3.3.5 Lignin based wood adhesive for plywood application

Unmodified lignin based wood adhesives result in comparable glue strength as for the PF resins (Figure 3.6). Only the grass SoL fraction F2 shows a lower strength which could be caused by the low molar mass. For some lignins (grass SoL and its fractions F3 and F4) the 30% substitution result in a lower strength compared with the 10% substitution. This result indicates that these lignins act as a less reactive filler which
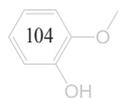
could be caused by their condensed nature (Table 3.3) and the partial removal of formaldehyde from the resin which has not been compensated. The wood failure graph (Figure 3.6) shows that Indulin AT is the best performing lignin followed by Curan 100, HW SoL, and grass SoL F4.

Organosolv lignin, non-wood SoL (P1000), and the grass SoL and its fraction F2 yield almost no wood failure. SW KLs are clustered together in the PCA (Figure 3.5) and show good performance in the plywood application. These lignins contain a high level of free *ortho* ring positions for crosslinking of the glue (Table 3.5). Surprisingly, these SW lignins contain a relatively high level of condensed hydroxyl groups (Tables 3.4 and 3.5), but this does not lead to lower adhesive performance. Therefore, the more condensed structures in the SW lignins could be more compatible with the rigid phenol-formaldehyde structure compared with the other lignins studied. Results of the PCA indicate that lignins with similar structural properties to SW KLs have high potential for use in a wood adhesive.

Although the HW SoL was classified as an outlier, its performance as wood adhesive is promising (Figure 3.6). Also, the F3 fraction of grass SoL in 10% substitution yielded good strength and wood failure. All other lignins perform less satisfactorily than the SW KLs, HW SoL, and the grass SoL in F3 and F4 fractions.
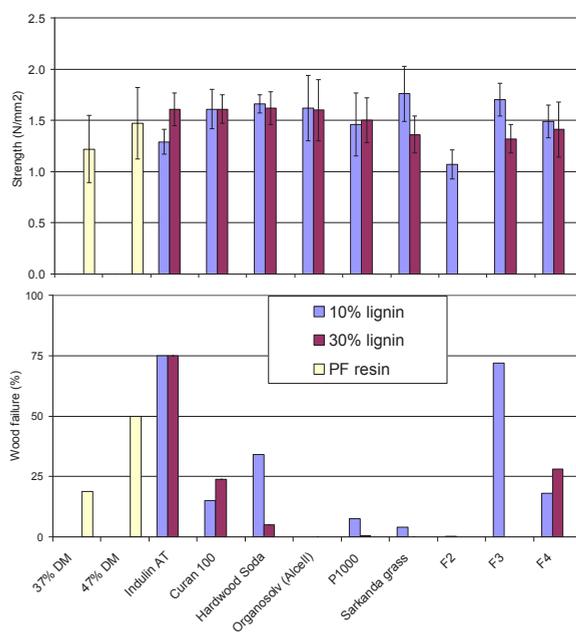
Figure 3.6 Breaking strength (top) and wood failure (bottom) for lignin based wood adhesives.

3.4 Conclusions

Successive extractions with organic solvents are suited for fractionation of different industrial lignins according to their molar mass. Kraft and soda lignins display different molar mass distributions. LCCs are less soluble in organic solvents. In soda lignins, LCCs consist of up to 34% of carbohydrates.

PCA showed that industrial lignins can be classified into different clusters. Softwood kraft lignins showed the highest potential for use as wood adhesive. These lignins contain the highest level of free *ortho* ring positions related to the phenolic OH group, low level of impurities, and medium molar mass. Additionally, the more condensed structures in the softwood lignins could be more compatible with the rigid phenol-formaldehyde structure compared with the other lignins studied.

Hardwood soda lignin does not belong to a specific cluster but is a promising component of a plywood adhesive together with medium molar mass fractions of Sarkanda grass soda lignin.

Fractionation of lignin will result in purified fractions of distinguished structure dependent functional properties and demonstrate different application potential.

Acknowledgements

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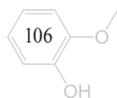
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Chapter 4

Effect of periodate on lignin for wood adhesive application
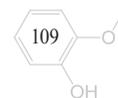
Abstract

Development of eco-friendly binders with no harmful emission during its complete life cycle is of high interest for the wood-based industry. In this paper, a fully renewable binder based on activated lignin and poly-furfuryl alcohol and a partly renewable lignin based phenol-formaldehyde (PF) binder were evaluated. Activation of kraft and soda lignins, isolated respectively from softwood and non-woods, by periodate oxidation was performed to improve lignin reactivity and application in wood adhesives. Periodate oxidation of lignin leads to higher lignin acidity, formation of quinonoid groups under more severe conditions, higher molar mass and higher reactivity towards the curing of furfuryl alcohol within a temperature range currently used in industry. Comparison of a 100% furan-based glue with a furan-based glue substituted by 10% lignin yields comparable product properties. However, periodate-activated lignin leads to lower wood failure, which might be caused by incompletely solubilised lignin particles in the acidic formulation disturbing crosslinking of the furan resin. Unmodified softwood kraft lignin performs well in a PF resin formulation at substitution levels up to 30% (w/w). Periodate oxidation of soda lignins enhances the glue performance because higher wood failure is attained. The higher molar mass after periodate treatment could be an important parameter for development of a stronger wood binder.

Keywords: lignin activation; periodate oxidation; quinone formation; renewable binders; wood adhesives.
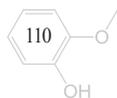
4.1 Introduction

The commonly applied synthetic resins as adhesives and coatings affect the image of wood as renewable and sustainable building and construction material. In 2005, a European R&D project called Ecobinders was initiated for the development of renewable resins based on furans and lignin that can be produced from lignocellulosic waste streams (Ecobinders 2005).

The utilization of lignin as the second abundant and underutilised renewable terrestrial biomass is challenging in the frame of the emerging biobased economy because of its complexity (Gosselink et al. 2004a; van Dam et al. 2005). Commercial applications of lignin, such as binders, are possible only if its multifunctional role in nature and its behaviour in the course of technical delignification processes are understood. Its utilisation as a macromolecule in industrial binders is expected to increase on the medium term (Holladay et al. 2007).

Furfural is produced from C5 sugars from sugar cane bagasse and corn cobs. The most important commercially available furan compound produced via catalytic reduction is furfuryl alcohol. The latter is a raw material for the production of renewable eco-friendly thermoset resins by acid-catalyzed polycondensation that are non-toxic and emission-free at all stages of the life cycle (Belgacem and Gandini 2008).

Different technical lignins can successfully be incorporated up to 70% into phenol-formaldehyde (PF) binders for particle boards, plywood, wafer boards and oriented strandboards (Ash et al. 1992; Khan et al. 2004; Gosselink et al. 2004b; El Mansouri and Salvadó 2006; Tejado et al. 2007; Cavdar et al. 2008). Based on this research, it is concluded that lignin needs to be modified to enhance its reactivity to an acceptable level suitable for the requirements of press rate of the panels in an industrial manufacturing process (Pizzi 2006). Methylolation with formaldehyde is a well known modification process of lignin, in the course of which undesired emission of formaldehyde can occur; the end product is not free from emissions either (Senyo et al. 1996). In contrast, a complete formaldehyde-free system was studied by Nimz and Hitze (1980) based on oxidative radical coupling of spent sulphite liquor by hydrogen peroxide. The product is suitable for adhesive in particle boards. However, this approach is limited to the spent sulphite liquor as the presence of sulphur dioxide is necessary to stimulate the exothermal coupling reaction.
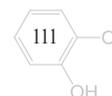
To avoid formaldehyde, periodate was selected as a modification agent to improve the lignin reactivity for both kraft and soda lignins. Periodate oxidation is a method in carbohydrate chemistry for selective oxidation of vicinal diols of anhydroglucose units (Hay et al. 1965). Additionally, periodate can selectively oxidise guaiacyl and syringyl units with free phenolic hydroxyl groups of lignin into *ortho-* and *para*-quinones via the Malaprade reaction releasing methanol (Adler and Hernestam 1955). Although sodium periodate is relatively expensive, its industrial application is described (Narayan et al. 1988). Quinones have the ability to react with furfuryl alcohol (furan derivatives) via a *Diels-Alder* reaction. Trindade et al. (2004) used this approach for selective *in situ* oxidation of lignin in sugar cane bagasse fibres as a novel fibre surface modification. These oxidised fibres exhibit an improved reactivity towards furfuryl alcohol (Trindade et al. 2004, 2005). Nordstierna et al. (2008) found more evidence for the covalent bonding of furfuryl alcohol to lignin model compounds by 2D-NMR. It is probable that the furan (pre-)polymer is similarly grafted to lignin.

In this study, periodate oxidation was applied for modification of both kraft and soda lignins isolated from softwood and non-woods, respectively, to improve lignin reactivity for use as adhesives in wood panels. The gluing performances were compared of binders prepared by 100% lignin and poly-furfuryl alcohol and binders prepared by partly lignin substituted PF binders.

4.2 Materials and methods

Materials

Data in %, if not otherwise indicated, are on w/w basis. Softwood kraft lignin (SKL) (Indulin AT): MeadWestvaco, USA. Sarkanda grass soda lignin (100-S-A): Granit SA, Switzerland. Mixed sarkanda grass/wheat straw soda lignin (P1000): Granit SA, Switzerland. Furfuryl alcohol (FA) p.a. and a prepolymerised furan resin Biorez 91ME: TransFurans Chemicals, Belgium. Alkaline 47% PF resin: Chimar Hellas SA, Greece.

Lignin oxidation

For lignin oxidation, 3 g of air-dried lignin was dissolved in 30 ml 0.05 M NaOH during 24 h under gently stirring at room temperature. The solution was adjusted to pH 5 by
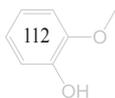
adding small quantities of 10% (v/v) aqueous HCl and this solution was preheated to the desired temperature. Lignin was treated with 1%, 5%, 10% and 50% sodium periodate (NaIO$_4$) (b.o. dry lignin) at differe nt combinations of temperature (55–95°C) and time (10–120 min). The oxidised lignins were isolated by precipitation at pH 2.5 by adding 10% (v/v) HCl and were purified by repeated water washing and centrifugation until neutral pH for further analysis.

Lignin oxidation with improved yield

In total, 100 g of air-dried lignin was dissolved in 1 l of 0.1 M aqueous ammonia, and the adjustment to pH 5 was performed with 10% (v/v) formic acid in water. The solution was preheated to 55°C. After adding 10% or 50% NaIO$_4$, the oxidation was performed during 10 min at 55°C. The reaction was stopped by 10% (v/v) formic acid in water and the lignin was precipitated at pH 3. Three more washing steps with 10% (v/v) formic acid in water were performed to remove impurities. Formic acid was removed by repeated water washing and the resulting product was freeze-dried before further testing.

Lignin characterization

The pH of lignin was determined in 1% solution in demineralised water after 4 h mixing. Residual formic acid content was quantified in this solution. After centrifugation at 3000 rpm during 15 min, 1 ml of supernatant was mixed with 1 ml of an internal standard solution of propionic acid. Then, 10 ml was injected on a Shodex Ionpak KC-811 (300 mm 8 x mm I.D.; Shodex, Tokyo, Japan) fitted with a precolumn. The eluent was 0.1% (v/v) phosphoric acid in water, He degassed, and an isocratic elution was performed with a flow of 1 ml min^{-1} during 30 min. The column temperature was 40°C and detection was carried out at 210 nm.

FT-IR spectra were recorded of 1% lignin in a KBr pellet with 64 scans in the range from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} on a Bruker Vector 22 FT-IR spectrophotometer. Spectra were baseline corrected and normalised to the C-C stretching and to C-O band in ethers and phenolic structures at 1218 cm^{-1}.
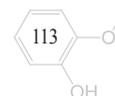
For direct determination of quinones in lignins before and after periodate treatment, the semiquantitative method with trimethyl phosphate was applied (Argyropoulos and Zhang 1998). *p*-Benzoquinone (model compound) was used for verification of the method.

Carbohydrate composition, ash and molar mass distribution of the lignins were determined as described by Gosselink et al. (2010). Elemental analysis (C, H, N and O) of untreated and periodate treated lignins was also performed.

Details of differential scanning calorimetry (DSC, Perkin Elmer): lignin and furfuryl alcohol were mixed 1:1 or 30% lignin was mixed with 37% PF resin with adjusted pH at 11. Then, 20−30 mg was transferred to a stainless steel DSC cup and hermetically closed. Temperature programme: $10°C\ min^{-1}$ from 0°C to 200°C in a nitrogen atmosphere. After annealing to 0°C, a second heating curve was taken.

Plywood application test

Unmodified and periodate oxidised lignins were tested in a formulation consisting of 10% lignin in 90% prepolymerised furan resin containing 35% water (Biorez 91ME). Then, 3% maleic anhydride (b.o. dry resin content) was added as acidic catalyst. Viscosity measurement of the formulation: Brookfield Viscometer model DVII (at room temperature). Duplicates of 3-ply birch plywoods of 10 cm x 20 cm were prepared after cold pressing for 15 min and hot pressing at 140°C (10 t for 10 min). Subsequently, the plywood was kept 15 min in the press for cooling down. Then, 47% PF alkaline setting resin (pH 11) was diluted with demineralised water to 37% PF resin; 10% and 30% of this PF resin formulation was replaced by unmodified or periodate oxidised lignin and the pH was adjusted to 11. Viscosity of the formulation was adjusted between 300 and 800 mPa× s by adding wood powder if necessary. Duplicates of 3-ply birch plywoods of 10 cm 20 cm were prepared after cold pressing and hot pressing at 140°C (5 t for 15 min). In the cooling down period, the plywood was kept for 15 min in the press. The 3-ply plywood specimens were sawn into seven test samples; they were subjected to a boiling test and mechanically tested by previously reported methods (Gosselink et al. 2004b).
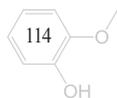
4.3 Results and discussion

Lignin oxidation by periodate ($NaIO_4$) leads to a higher lignin acidity as a result of the formation of free acidic groups. A positive correlation was found between the lignin acidity (expressed as pH in water) and the curing temperature of FA as measured by DSC (Figure 4.1). It is well known that crosslinking of FA is catalyzed by acids: the stronger the acid the lower the curing temperature of FA (Schmitt 1974). For curing of FA in a temperature range applicable for wood adhesives (120–140°C), the lignin acidity should be lower than pH 3.3. To reach sufficient lignin reactivity, the minimum amount of $NaIO_4$ on lignin is 10% as shown in Figure 4.2. Lignin treatment with 50% $NaIO_4$ leads to comparable curing temperatures of FA.
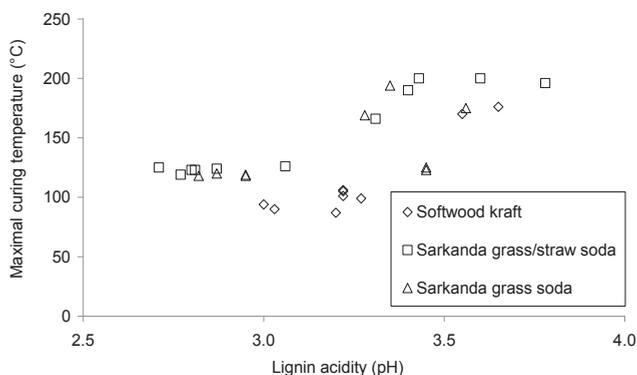
Figure 4.1 Influence of lignin acidity on maximal curing temperature of furfuryl alcohol.
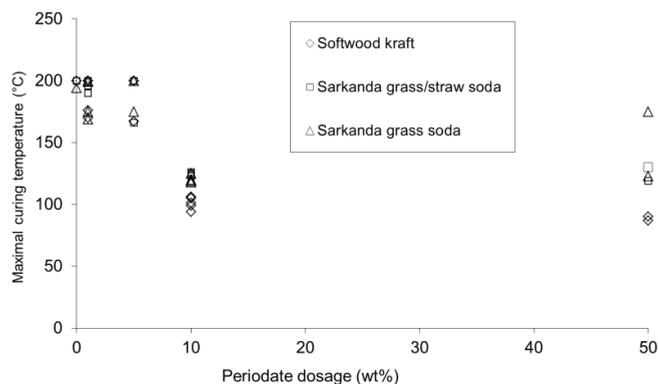
Figure4.2 Influence of sodium periodate dosage on lignin reactivity towards maximal curing temperature of furfuryl alcohol.

Periodate treatment of lignin results in a relatively high quinoid band at 1660 cm^{-1} for both soda and softwood lignins, when the dosage is higher than 1% NaIO$_4$ (Figure 4.3 and Table 4.1). The formation of the quinoid groups is more pronounced for oxidation with 50% NaIO$_4$ than that with10% NaIO$_4$. Furthermore, this treatment seems to have a negligible effect on the carboxylic acid band in lignin at 1700 cm^{-1}. There is a clear pH drop observed by periodate treatment, but the resulting pH values of the treated lignins at different dosages under mild conditions are comparable (Table 4.2). Probably, the total amount of free acidic groups released is similar at various oxidation severities. At more severe lignin oxidation (50% NaIO$_4$), the ratio of the aromatic skeletal vibrations at 1598 cm^{-1} and 1510 cm^{-1} increases, which is most probably caused by ring opening predominantly between C-3 and C-4 leading to muconic acid type structures. These newly formed acidic structures contribute to the carbonyl stretching band at 1700 cm^{-1} and increase the lignin acidity. However, this effect has its upper limits as the resulting acidities, expressed as pH in water, of 10% and 50% NaIO$_4$ treated lignin are similar (Table 4.2).
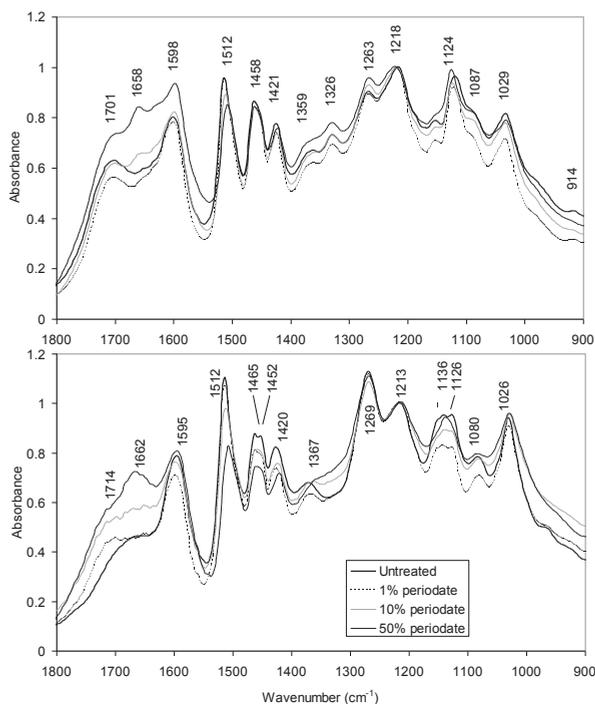
Figure 4.3 Formation of quinoid structures in mixed sarkanda grass/wheat straw soda lignin (top) and in softwood kraft lignin (bottom) by periodate treatment at 55°C during 10 min at pH 5. Conjugated C=O stretching vibration at 1658 cm^{-1} corresponds to quinoid structures.
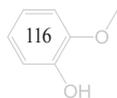
Table 4.1 Peak assignments FT-IR (Faix 1992; Trindade et al. 2004).

Band (cm^{-1})	Assignment
1709-1701	C=O str. (non-conj.)
1662-1657	C=O str. (conj., quinoid groups)
1601-1595	C-H str. arom. skeleton
1514-1512	C-H str. arom. skeleton
1464-1462	C-H asym. deform. -CH$_2$- and -CH$_3$
1427-1423	C-H in-plane deform. + arom. ring vibr.
1373-1358	O-H in-plane deform. in phen. groups
1329	C-O in S ring
1269-1265	C-O in G ring
1221-1215	C-C str. + C-O(H) and C-O(Ar) in ether and phen. structures
1153-1150	C=O str. in conj. ester (HGS lignin)
1140	Arom. C-H in-plane deform. (G)
1126-1121	Arom. C-H in-plane deform. (S)
1087-1084	C-O(H) deform. in sec. alcohols and aliph. ethers
1034-1030	C-H in-plane deform. in arom. groups + C=O str. (unconj.)
950-918	C-H out-of-plane deform. in arom. groups
856-854	C-H out-of-plane deform. (G)
835	C-H out-of-plane deform. (S + H)
818-816	C-H out-of-plane deform. (G)

Table 4.2 Composition and properties of untreated and periodate oxidised lignins (100 g scale, 55°C, 10 min, pH 5).

Experimental	Sarkanda grass soda		Mixed sarkanda grass/ wheat straw soda			Softwood kraft	
Periodate dosage (% on dry lignin)	0	10	0	10	50	0	10
Yield (%)		96		97	100		95
Composition							
Residual FA (%)	0	1.6	0	0.3	0.02	0	0.2
pH	3.3	3.0	3.5	3.1	3.1	6.3	3.1
Elem. composition:							
C (%)	59.7	60.9	64.1	62.3	58.4	62.5	63.5
H (%)	5.0	5.2	5.9	5.4	4.9	5.3	4.8
N (%)	1.2	1.4	1.5	1.3	1.4	0.9	0.4
O (%)	30.8	29.5	30.2	31.1	30.2	30.2	28.8
Total CHNO (%)	96.7	97.0	101.7	100.1	94.9	98.9	97.5
O/C	0.52	0.48	0.47	0.49	0.52	0.48	0.45
Carbohydrates (%)	2.4	2.1	4.1	3.7	2.5	1.4	1.1
Ash (%)	1.4	0.7	3.4	0.6	0.8	1.1	0.1
Quinone (mmol/g)	0	0	0	0	0.2	0	0
M_w (Dalton)	6000	8800	6800	12300	18600	5400	8700
Properties[a]							
Reactivity to FA[b] (°C)	>170[c]	116	>170[c]	122	114	>180[c]	115
30% Lignin in PF resin[d]	146	142	145	146	143	146	143

[a]Measured by DSC.
[b]Expressed as maximum peak temperature of cured FA.
[c]Curing of FA initiated, but far from completed.
[d]Maximum peak temperature of cured resin.

To maintain the aromatic structures in the activated lignin, a relatively mild treatment was selected: 10 min, 55°C, 10% NaIO$_4$ per 100 g lignin. During periodate treatment, soluble lignin fractions were removed by repeated washing with demineralized water
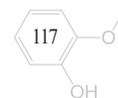
and centrifugation, resulting in yields of 60−80% for sarkanda grass soda lignin, mixed sarkanda grass/wheat straw soda and SKL. To improve the yield, the isolation procedure was changed by washing with formic acid: yields of 94−100% were obtained and the residual formic acid content in the activated lignins was lower (Table 4.2). In the worst case, 1.6% of formic acid was left in the oxidised lignin. As this concentration is much lower than that of the curing catalyst maleic anhydride (3% b.o. FA and lignin) used for the plywood test, the influence of residual formic acid can be considered as negligible on the curing of FA. This assumption was confirmed by DSC measurements.

Table 4.2 shows that a substantial part of the carbohydrates and ash were removed after periodate treatment, which can be considered as a type of purification. Part of the carbohydrates will have been oxidised by $NaIO_4$ as reported by Hay et al. (1965) and solubilised in the liquid. Ash is partly extracted under the acidic conditions applied. FT-IR spectra show that the quinoid band increases only for 50% periodate treated lignin. Lignin quinone structures of 0.2 mmol g^{-1} could be detected by the more sensitive ^{31}P NMR (Figure 4.4 and Table 4.2).

Table 4.2 shows that the O/C ratio slightly increases for mixed wheat straw sarkanda grass soda lignin and slightly decreases for the other lignins. Oxidation of lignin is not confirmed by elemental analysis because part of the lignin and impurities with a higher oxidation level, such as carbohydrates, are removed during the process.

Activated lignins have an improved reactivity towards FA with a maximal temperature around 120°C. Guigo (2008) observed similar results in a study of two periodate treated mixed sarkanda grass/wheat straw soda lignins (conditions: 10% $NaIO_4$, 55°C, pH 5, 10 min and 70 min o xidation time) in 20% concentration in furfuryl alcohol. Guigo demonstrated that periodate oxidised lignin substantially lowers the curing temperature of FA in an acceptable temperature range (137°C) for industrial processing. Furthermore, periodate oxidised lignin enables the second crosslinking stage of FA oligomers including the formation of branched structures to be performed at lower energy levels. This might be facilitated by promoted cycloaddition of quinone groups to furan structures. Compared with unmodified lignin, $NaIO_4$ lignin showed improved interactions with the furanic resin network, which is reflected by a lower activation energy barrier (Eα) at the later branching stages of polymerization. In other words, the lignin macromolecules are better integrated into the polymeric network (Guigo 2008).
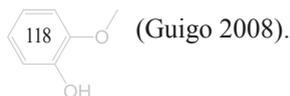
After periodate modification, the apparent molar mass of lignin substantially increases for all lignins studied (Table 4.2). On the one hand, low molar mass lignin fractions could have been removed during processing and purification, but, on the other hand, additional crosslinking of these lignins could also occur during this modification process.

Curing of PF resin, substituted by 30% lignin, occurs for both untreated and NaIO$_4$ lignins in a similar temperature range as in the case of 100% PF resin (Table 4.2). Plywood production with the substituted PF binders can be done at a pressing temperature of 140°C.
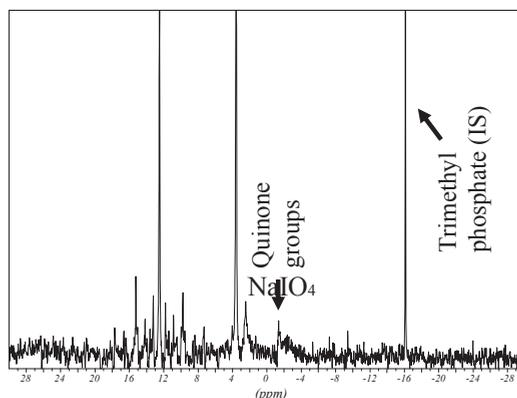
Figure 4.4 Quinone groups detected in 50% (by wt.) sodium periodate treated mixed sarkanda grass/wheat straw soda lignin by ^{31}P NMR.

Performance of lignin in plywood adhesive

Prepolymerized furan resin (Biorez 91ME) containing 35% furan in water has a viscosity of 460 mPa×s. Addition of lignin into furan resin formulations is limited to 10% because formulations at higher substitution levels are highly viscous. Furan resin containing 10% unmodified or periodate treated lignin yielded comparable or slightly inferior strength performances (Figure 4.5). However, the wood failure is substantially lower for periodate treated lignins. Both results showed that periodate treatment of lignin does not lead to better glue performance of the furan resins. Lignin yielded inferior glue properties probably owing to incomplete solubilisation of lignin particles in the acidic formulation; the acid catalysed polycondensation and network formation (crosslinking) of the furan resin is disturbed.

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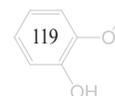
Partial substitution (10–30%) of PF resin by unmodified lignin yielded similar strength properties as 100% PF resin and the wood failure for SKL was also good (Figure 4.6). Gosselink et al. (2010) also found a higher potential of SKL for PF-based binder applications. Figure 4.6 shows that wood failure increases for periodate treated soda lignins. Periodate oxidised (10% NaIO$_4$) SKL resulted in a higher glue strength compared to unmodified lignin, but a lower wood failure. The application tests revealed that a better glue performance was obtained when periodate treated lignins were used in a PF resin. An explanation could be the increased molar mass, which is an important parameter for development of a stronger binder for wood-based panels (Gosselink et al. 2010).

The difference found for the wood adhesive performance of lignin in a furan resin and in a PF resin is mainly due to the difference in pH at which the condensation reactions of both resins take place. The furan resin is acid-catalyzed which limits the solubility of lignin and the PF resin is base-catalyzed resulting in a completely solubilized lignin.
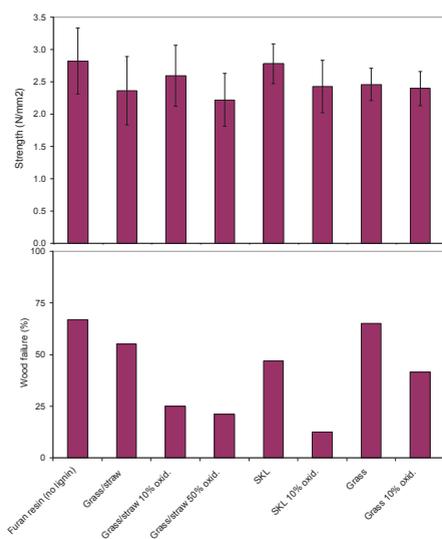
Figure 4.5 Breaking strength (top) and wood failure (bottom) of 10% (by wt.) lignin in furan wood adhesives.
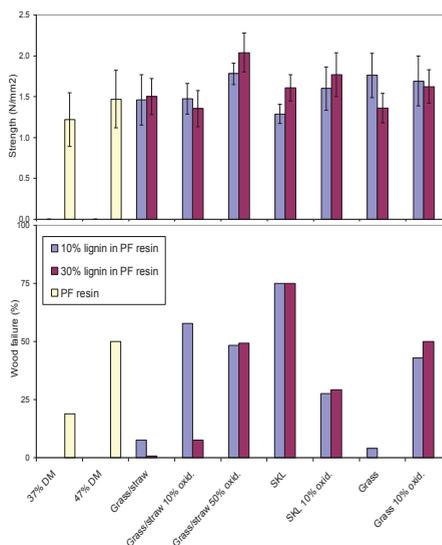
Figure 4.6 Breaking strength (top) and wood failure (bottom) of phenol-formaldehyde wood adhesives substituted by 10% (by wt.) or 30% (by wt.) lignin. DM means dry matter content of PF resin.
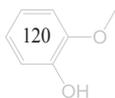
4.4 Conclusions

Periodate oxidation of lignin leads to higher lignin acidity, formation of quinoid groups at more severe treatment levels, higher molar masses and a higher reactivity towards furfuryl alcohol. In a furan-based glue, substitution with 10% lignin yields comparable plywood properties. In contrast, periodate activated lignin leads to lower wood failure. This might be caused by an incomplete solubilisation of the lignin particles in the acidic formulation which disturbs the crosslinking of the furan resin.

Unmodified SKL performs well in a PF resin at substitution levels up to 30%. Periodate oxidation of soda lignins result in better glue performance with PF resins as expressed by higher wood failure. This could be the result of the higher molar mass after periodate treatment which is an important parameter for development of a stronger binder for wood panels. It can be concluded that activated lignin by periodate oxidation can be used as a substitution component of plywood resins. The relevance of periodate oxidation is very much dependent on the resin formulation. Further research at a larger scale should show whether the achieved benefits with PF resins outweigh the extra costs generated by the periodate oxidation step.

Acknowledgements

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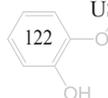
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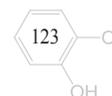
Chapter 5

Lignin depolymerization in supercritical carbon dioxide/acetone/water fluid for the production of aromatic chemicals
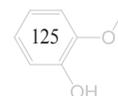
Abstract

Valorization of lignin plays a key role in further development of lignocellulosic biorefinery processes for biofuels and biobased materials production. Today's increased demand for alternatives to fossil carbon-based products expands the interest and the need to create added value to the unconverted lignin fraction. In this work organosolv hardwood and wheat straw lignins were converted in a supercritical fluid consisting of carbon dioxide, acetone and water (300-370°C, 100 bar) to a phenolic oil consisting of oligomeric fragments and monomeric aromatic compounds with a total yield of 10%-12% based on lignin. Addition of formic acid increases the yield of monomeric aromatic species by stabilizing aromatic radicals. Supercritical depolymerization of wheat straw and hardwood lignin yielded monomeric compounds in different compositions with a maximum yield of 2.0% for syringic acid and 3.6% for syringol respectively. Under these conditions competition occurs between lignin depolymerization and recondensation of fragments.

Keywords: Biorefinery, lignin valorization, phenolic chemicals, lignin supercritical depolymerization, phenols.
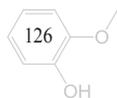
5.1 Introduction

Today's increased demand for alternatives to fossil carbon based products, such as the production of transportation biofuels and bulk "green" chemicals, expands the interest and the need to create added value to the unconverted lignin fraction. By far the most highly available and accessible biobased feedstock for the preparation of aromatic (bulk) chemicals such as phenol is lignin. Lignin is found in trees and other lignocellulosic plant-based materials representing 15-25% of their weight and about 40% of the biomass energy content (Holladay et al. 2007). The emphasis on the production of second generation biofuels using lignocellulosic biorefinery processes (Cherubini et al. 2009), will result in the production of large quantities of lignin, additionally to the lignin produced by the pulp and paper industry. Lignin is the obvious candidate to serve as a future aromatic resource for the production of liquid biofuels, biomaterials and green chemicals (Van Dam et al. 2005, Ragauskas et al. 2006, Holladay et al. 2007).

Traditionally, the use of lignin has been as a combustion fuel in pulp mills, component in binders, or additive in cement. However, due to its chemical nature, and in particular the presence of large amounts of aromatic structures, lignin may be an attractive raw material for the production of basic aromatic chemicals, such as benzene, toluene, xylene and phenol, overall reducing CO_2 emissions and the need for fossil resources. Considerable markets are present for these chemicals are present indicated by the global annual production of 8 million tonnes of phenol which are mainly used for the manufacturing of bis-phenol A used in polycarbonate production and for phenol-formaldehyde resins (Stewart 2008).

Lignin conversion to phenolic monomers reported in literature starts from lignin containing biomass (e.g. lignocellulosics) or with extracted technical lignins which was reviewed by Amen-Chen (2001) and Zakseksi (2010). The use of technical lignin has the important advantage that non-lignin components, like carbohydrates, have been removed to a large extent. The production of phenolic monomers and other chemicals from lignin has been exhaustively studied over the years. However, this has resulted in only limited industrial success including the production of vanillin from softwood lignosulfonate as a food additive (Evju 1979). Most lignin conversion processes have been studied at elevated temperatures of 250-600°C, with and without catalysts, as reviewed by Zakzeski et al. (2010). These high-temperature processes for "cracking"
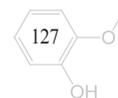
lignin led to the formation of a complex phenolic mixture of polyhydroxylated and alkylated phenol compounds as well as char and volatile components. This provides challenges for further upgrading of these complex mixtures to more homogeneous mixtures with a higher phenol content coupled with downstream processing in order to separate phenolic-like compounds. The highest yields reported for the conversion of kraft lignin in a two-step process including hydrocracking and hydrodealkylation over a catalyst bed in hydrogen are 20% phenol and 14% benzene (based on lignin) as reported by Huibers and Parkhurst (1982). This process was highly selective but needed two high temperature stages. However, in most processes the average total yield of aromatic monomers formed are in the range of 5–10%. Here it is necessary that in addition to the production of phenols, added value outlets for all products formed should be developed (Van Haveren et al. 2007).

Liquefaction of biomass for the production of fuels and chemicals by solvolysis in acetone, ethanol or water has some advantages as liberated products are diluted preventing crosslinking reactions. Furthermore it is operated at substantial lower temperature as compared to pyrolysis and gasification (Liu and Zhang 2008). Additionally, in supercritical ethanol reduction of oxygen atoms can be promoted by the hydrogen donor capacity of ethanol which is significantly induced by iron-based catalysts (Li et al. 2010, Xu and Etcheverry 2008). Depolymerization of lignin and lignin model compounds can be performed in supercritical alcohols like methanol or ethanol at high conversion rates which needs bases in combination with a temperature range of >239°C and >8 MPa. The dominant depolymerization route is the solvolysis of ether linkages in the lignin structure while the carbon-carbon linkages are mostly unaffected (Miller et al. 1999; Minami et al. 2003). Okuda et al. (2004a, 2004b) used phenol and p-cresol in water at supercritical conditions above 374°C and 22.1 MPa for complete conversion of lignin without char formation. Phenol and p-cresol did not show crosslinking reactions due to entrapment of reactive fragments, like formaldehyde, and capping of active sites like Cα in the lignin structure. Yuan et al. (2010) used a combination of both approaches, however at milder temperatures (220 – 300°C), leading to the base-catalyzed depolymerization of kraft lignin in a water-ethanol mixture into oligomers with a negligible char and gas production. Under the conditions applied, lignin could not be degraded completely into lignin monomers.
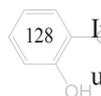
In this work, development of a novel process for the depolymerization of lignin under supercritical conditions for production of aromatic chemicals is described.

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Carbon dioxide was chosen because of its non-toxic character, its ability to form a supercritical fluid (scCO$_2$) at relatively low temperature and pressure (>31°C, >7.4 MPa) and its established industrial use, for example in decaffeination of coffee beans (Zosel 1974) and dying of textile fibres (Smith et al. 1999). In this novel process, the aromatic products were separated from residual lignin fragments and char by adiabatic pressure release. Carbon dioxide consequently will lower the temperature in the solvent stream facilitating condensation of aromatics formed and leaving no solvent in the product mixture obtained. Thereby simplifying downstream processing.

In the conversion of lignin into monomeric phenolics two main reactions compete. These are depolymerization and recondensation yielding a residual lignin char fraction. As this char represents a low value as a soil amendment (Lehmann and Joseph 2009) the main focus in this work is to minimize the formation of this carbon residue. The use of H-donating solvents, such as formic acid, and other stabilizing compounds, such as alcohols, have proven, previously, to reduce char formation (Kleinert et al. 2008, 2009).

In this work an acetone/water mixture was employed to completely dissolve the lignins which enables feeding of these lignin solutions into a pre-heated reactor at elevated temperatures of 300°C and 370°C. To bring the solvent mixture of scCO$_2$/acetone/water under supercritical conditions the pressure was adjusted to 100 bar by adding CO$_2$. Yu and Savage (1998) found that under comparable hydrothermal conditions formic acid is mainly decomposed by decarboxylation to CO$_2$ and H$_2$ with a typical CO$_2$/H$_2$ ratio between 0.9 and 1.2. Therefore formic acid was added to produce in situ hydrogen to stimulate the stabilization of aromatic radicals.

5.2 Materials and methods

Selected technical lignins and characterization

Two organosolv lignins from ethanol-water fractionation were studied: Organosolv lignin from mixed hardwoods (Alcell[tm], Repap Technologies, Canada) and organosolv wheat straw lignin (Energy research Centre of the Netherlands, ECN, The Netherlands).
Both lignins were characterized for their purity, molar mass distribution and functional groups by ^{31}P NMR (Gosselink et al. 2010).
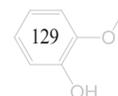
Elemental analysis of organosolv hardwood and wheat straw lignins was performed by using an EuroVector 3400 CHN-S analyzer. Oxygen content in the lignins was calculated by difference.

Supercritical depolymerization of lignin

Supercritical process conditions were investigated by using organosolv hardwood lignin (Alcell[tm]). Both organosolv hardwood and wheat straw lignins were used for a comparative study. Lignin was dissolved in acetone/water 8:2 (v/v) at 0.35 g/ml. A 100 ml hastelloy reactor (PARR Instruments Co., model 4590) was flushed with carbon dioxide to remove oxygen. 0.7 gram of lignin in solution was pumped with acetone/water 8:2 (v/v) via a 2 ml sample injection loop (Rheodyne) at a flow of 5 ml/min by a HPLC pump (Waters Corporation, model 515) into the reactor, pre-heated at 300°C or 370°C. Formic acid was introduced in the process via the same sample injection loop using the same process. A total of 30 ml of solvent was used to pump lignin and formic acid into the pre-heated reactor, followed by an adjustment of the total pressure to 100 bar by introducing carbon dioxide by using another pump (Isco, model 260D). The supercritical fluid consisted of CO_2/acetone/water in a molratio of 2.7/1/1.

Product sampling was performed by pressure release from 100 to 50 bar and collection of the compounds in 2 serial connected gas washing flasks each filled with about 200 ml acetone at room temperature. After 90 min, complete pressure release from 100 to 0 bar was performed. At each following sampling time 30 ml fresh solvent acetone/water 8:2 (v/v) and CO_2 was added to readjust the pressure to 100 bar at 300°C or 370°C. Final processing time was 3.5 h. Phenol was used to test the stability and recovery of similar compounds under the applied process conditions.

To determine the mass balance, 10 gram organosolv hardwood lignin was dissolved in 30 ml acetone/water 8:2 (v/v) and heated in the 100 ml reactor from room temperature to 300°C. CO_2 was added to adjust the pressure to 100 bar at 300°C. After 3h reaction, the gas phase was collected during pressure release in 1 liter gasbags (8 x 7" TEDLAR® Grace/Alltech). Gas phase composition was determined by GC as described in section 2.3. Water content in the lignin oil was quantified by Karl-Fisher titration. To study the Depolymerization of lignin into lower molar mass fragments all compounds collected in acetone were gently concentrated till complete dryness under a

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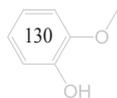
nitrogen flow at room temperature. The obtained phenolic lignin oil and dried char at 60°C were gravimetrically assessed and further analyzed as described hereafter.

Characterization of products derived from lignin

Monomeric and dimeric aromatic products were identified and quantified by respectively GC-MS and GC-FID (both Interscience) on a Restek RXI-5ms column (30m x 0.25mm x 0.25μm) in He with a temperature program starting for 2 min at 50°C and a heating rate of 10°C/min to 350°C followed by an isothermal step of 3 min. Identification of compounds was performed by comparison of MS data to the NIST library. For quantification of compounds by GC-FID calibration with pure compounds was performed using 1-methylnaphtalene as internal standard.

The molar mass distribution of the dried phenolic lignin oil was analyzed by alkaline SEC as previously described (Gosselink et al. 2010).

Gas phase composition was determined by GC-TCD analyses using a Hewlett Packard 5890 Series II GC equipped with a PoraplotQ Al_2O_3/Na_2SO_4 column and a Molecular Sieve (5A) column. The injector temperature was set at 90°C, the detector temperature at 130°C. The oven temperature was kept at 40°C for 2 minutes then heated up to 90°C at 20°C/min and kept at this temperature for 2 minutes. A reference gas (Air products) containing CH_4, CO, CO_2, ethylene, ethane, propylene and propane with known composition was used for peak identification and quantification.

Characterization of lignin char

1 mg of Alcell lignin and lignin chars were heated at 600°C for 2 minutes in a Py-GC/MS HP5890 series II with a PTV Optic 2. Evolved products were separated on an Agilent HP 5MS column (20m x 0.18mm ID) with He as carrier gas and identified by MS using the NIST library. Temperature program started at 50°C with a heating rate of 10°C/min to 300°C followed by an isothermal step of 5 min. Detector temperature was set at 280°C.
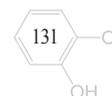
5.3 Results and discussion

Two feedstocks were used to produce organosolv lignin with a high purity, less than 1% of polymeric carbohydrates and negligible ash, relatively low molar mass and polydispersity (see Table 5.1). The carbohydrate impurities, mainly xylan, were converted to limited amounts of furfural under the process conditions applied. In contrast to hardwood lignin, in wheat straw lignin 1% nitrogen is present representing about 6% of proteins attached to the lignin structure and included in the lignin content in Table 5.1. During supercritical depolymerization of wheat straw lignin no release of nitrogen containing compounds was observed, indicating that these proteins predominantly are incorporated in the resulting char as confirmed by elemental analysis (data not shown).

Table 1 Compositional data organosolv wheat straw lignin and organosolv hardwood lignin (Alcell[tm]).

	Wheat straw	Hardwood
Lignin (%)	99	97
Carbohydrates (%)	0.8	0.3
Ash (%)	0	0
Mw (D)	2650	3400
Polydispersity	4.5	4.6
SyringylOH (mmol/g)	0.6	1.3
GuaiacylOH (mmol/g)	1.0	0.8
HydroxyphenylOH (mmol/g)	0.5	0.2
COOH (mmol/g)	0.6	0.3
N (%)	1.0	0.2
C (%)	65.7	66.1
H (%)	6.1	5.8
O (%)	27.2	27.9

To assess the stability of monomeric phenols and the recovery efficiency of the system experiments were performed with phenol as a model compound in order to study the recovery of aromatic molecules. Phenol was introduced in the preheated reactor at 300°C in a mixture of carbon dioxide/acetone/water and each 30 minutes phenol was recovered by pressure expansion in the washing flasks in acetone. In this process phenol was recovered at 98.1% together with a minor phenolic char amount of 1.7% both based on weight. Now that it was shown that phenol is almost completely stable under the process conditions applied and that the recovery system works quantitatively lignin depolymerization studies could be started.
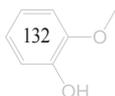
After introduction of organosolv hardwood lignin into the pre-heated reactor at 300°C in a carbon dioxide/acetone/water fluid some monomeric aromatic products have been recovered as shown in Figure 5.1 (1.8wt% on dry lignin). Without addition of formic acid treatment of lignin at 300°C yielded about 7wt% identified monomeric phenolic compounds based on dry lignin. The main products are 2,6-dimethoxy-phenol (syringol), 2-methoxy-phenol (guaiacol), and 2-methoxy-4-methyl-phenol (see Figure 5.2). These can be expected from a hardwood lignin, containing syringyl (S) and guaiacyl (G) type of structures, and is in agreement with results as has been reported by Liu et al. (2008). In this novel process the depolymerized products were separated from lignin char by adiabatic expansion of $scCO_2$. Addition of 14wt% formic acid based on lignin leads to a higher production of monomeric phenolics to a level of 10wt% on dry lignin. For the initial 60 minutes of the experiment a lag phase can be observed (Figure 5.1), which may be due to a reduction in solubility of the lignin in the acidic solvent mixture. After that, an increase in the production of monomeric phenols was observed resulting from the depolymerization of lignin. Repeated addition of formic acid to a total of 70wt% based on dry lignin starting from 30 minutes does not lead to higher yields compared to supercritical treatment of lignin without formic acid addition. Most notably from this experiment is that after 60 minutes the production of monomeric phenols ceases due to the strong acidic nature of the mixture in which the lignin and char are mostly insoluble. Furthermore formic acid leads to a significant increase for some aromatic compounds like guaiacol (1.6%), 2-methoxy-4-methyl-phenol (1.6%), 4-ethyl-2-methoxyphenol (0.6%), and syringol (3.6%). The presence of formic acid could lead to a higher degree of acid-catalyzed cleavage of ether linkages and could stabilize some of the resulting chemicals by the donation of hydrogen as reported by Yu and Savage (1998).

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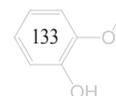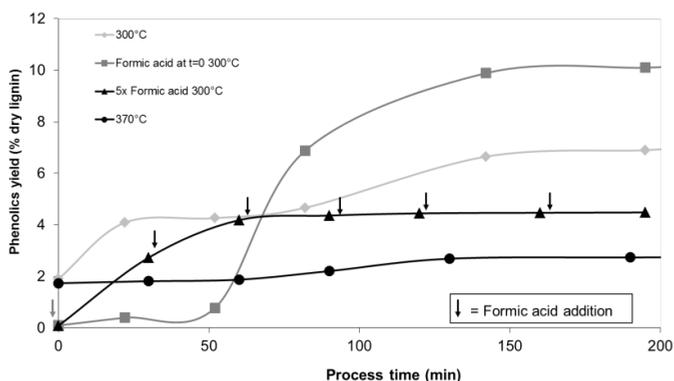
Figure 5.1 Cumulative formation of identified monomeric phenolics during supercritical depolymerization of hardwood lignin at 300 and 370°C and 100 bar in a carbon/dioxide/acetone/water mixture. 14% (based on lignin) Formic acid was added at the indicated times.
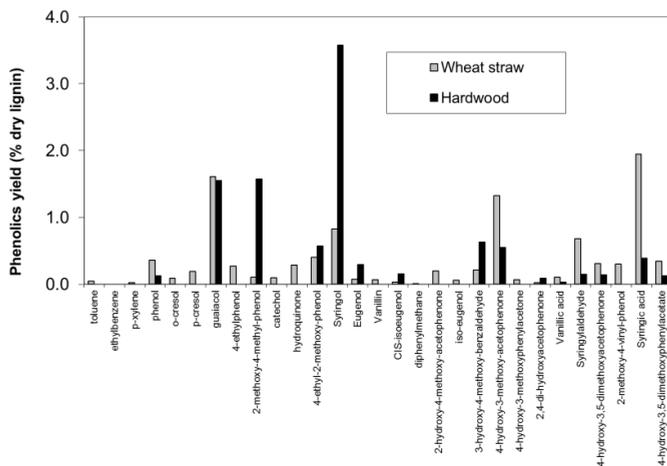
Figure 5.2 Identified phenolics produced during depolymerization of organosolv wheat straw and hardwood lignin in supercritical carbon dioxide/acetone/water fluid at 300°C, 100 bar, 3.5 h, and 14wt% formic acid (based on lignin).

Varying the formic acid dosage on hardwood lignin firstly resulted in a substantial decrease in the yield of low molar mass phenolics compared to the process where no formic acid was added. After that, a higher concentration of formic acid resulted in a higher amount of phenolics up to a maximum of 10% (Figure 5.3). At low formic acid concentration the amount of phenolics produced is decreased which might be caused by the increased acidity reducing solubilization of lignin and preventing depolymerization.
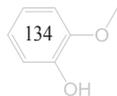
At higher formic acid concentration the hydrogen donation effect becomes more dominant to the acidic effect resulting in a higher yield of stabilized phenolics. During supercritical treatment organosolv lignin was converted into a phenolic oil of 36-45wt% based on dry lignin. This oil consists of a mixture of oligomeric lignin fragments and monomeric compounds (Figure 5.4). The average M_w is lowered from 3400 to 1200 Dalton and the polydispersity from 4.6 to 4.1. This result shows that under the conditions applied lignin is depolymerised into a lower mass phenolic oil.
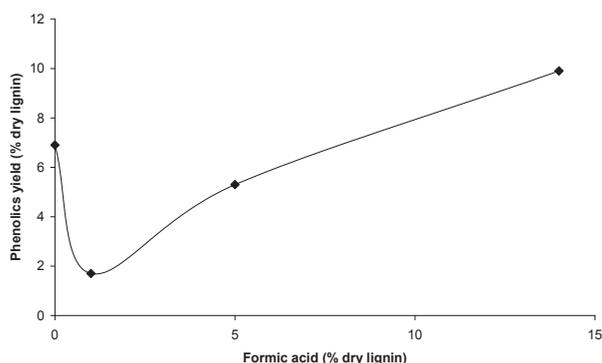
Figure 5.3 Influence of formic acid on the yield of identified phenolic compounds derived from hardwood lignin after supercritical treatment in a carbon dioxide/acetone/water fluid at 300°C, 100 bar and 3.5 h.
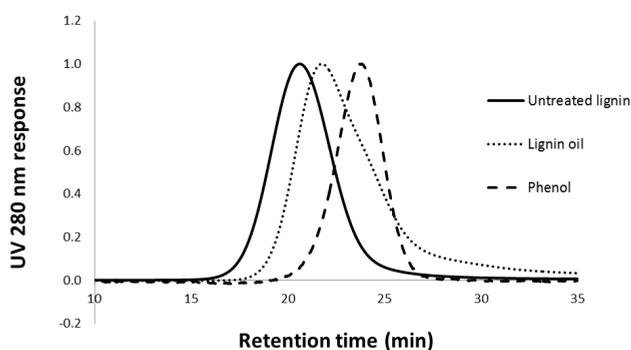
Figure 5.4 Lignin depolymerization in a carbon dioxide/acetone/water fluid at 300°C to lower mass phenolic fragments as analyzed by alkaline SEC.

Depolymerization of lignin at 370°C resulted in a lower production of monomeric phenols and the char formation is higher compared to the treatment at 300°C
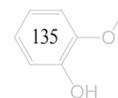
(Figure 5.1, Figure 5.5). Recondensation reactions dominates the formation of stable monomeric phenols at this temperature.

The mass balance found for depolymerization of hardwood lignin in a carbon dioxide/acetone/water supercritical fluid was closed at 94wt% (Table 5.2). Main products are gases, lignin phenolic oil, water, and char. The gas phase composition is given in Table 5.2. Formation of CO_2 from lignin could not be analyzed as CO_2 was added to the reactor as a solvent component and present in a large amount in the gas phase. The permanent gases were formed by cleavage of the propane chain on the aromatic ring and by removal of ring substituents. As lignin was heated from room temperature till 300°C and kept at this temperature for 3 hours the char (51.5%) formation is relatively large due to the long residence time. Additionally, the formed aromatic monomers were not removed during the treatment which leads to a low yield of monomeric compounds (1.7%) and a higher formation of recondensation products in the form of oligomeric structures and char. This clearly shows the beneficial effect of depolymerized product removal during the treatment yielding a much higher amount of monomeric aromatic compounds to a level of about 10%. Additionally, 16% of water was formed by dehydration reactions of lignin and the found water amount was corrected for the known water content of the supercritical fluid. After supercritical treatment the oxygen content of the residual lignin char was lowered from 27wt% in the starting lignin to about 20wt% indicating that deoxygenation took place resulting in the formation of water and oxygen containing gases like carbon monoxide.
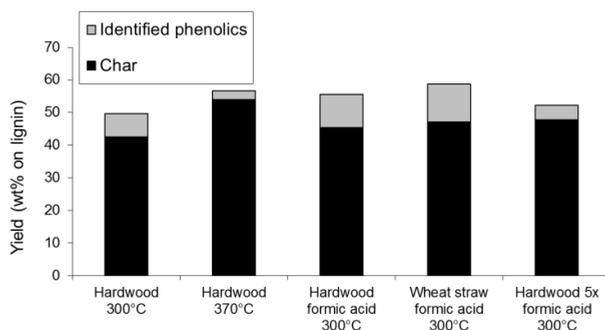
Figure 5.5 Char and identified phenolics formed during depolymerization of hardwood and wheat straw lignin in supercritical carbon dioxide/acetone/water fluid at 300-370°C, 100 bar, 3.5 h. Each dosage of formic acid is 14wt% (based on lignin).
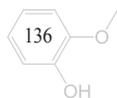
Table 5.2 Products formed during supercritical treatment of hardwood lignin in a
carbon dioxide/acetone/water fluid at 300°C and 100 bar during 3.5 h.

Product	% on dry lignin
Gases total	6.0
Methane	3.0
Carbon monoxide	1.0
Ethylene	0.01
Ethane	0.9
Propylene	0.3
Propane	0.9
Phenolic oil total	36.2
Identified monomers	1.7
Oligomers	18.6
Water	15.9
Char	51.5
Total	93.7

In the depolymerization process without formic acid addition 42wt% of char was
formed from organosolv hardwood lignin (Alcell[tm]) as depicted in Figure 5.5. A single
addition of formic acid (14wt% based on dry lignin) leads to a larger production of
monomeric phenols together with a slightly higher amount of char (45%). Repeated
formic acid addition leads to the formation of a higher amount of char (48%) and a
substantially lower production of monomeric phenols.

Both organosolv lignins from wheat straw and hardwood resulted in a similar
conversion to about 10%-12% identified aromatics and 45%-47% char (Figure 5.5).
However, the yields of the individual compounds are different for both raw materials
(Figure 5.2). In wheat straw lignin a higher amount of p-hydroxyphenyl units (H) is
present (Table 5.1) which resulted in a substantial higher amount of H-derived
phenolics (Figure 5.2). The difference in S-hydroxyl for straw and hardwood did not
result in a difference of the S-derived phenolics. The slightly higher presence of G-
hydroxyl groups in straw lignin compared to hardwood lignin (Table 5.1) resulted in a
slightly lower guaiacyl type of aromatics yield. These results indicated that for H-units
there is a positive correlation, but for S- and G-units no clear correlation with analogous
phenolic compounds could be found. Further decomposition of S- and G-derived
phenolics might be the reason for this observation. Main products derived from wheat
straw lignin are guaiacol (1.6%), syringol (0.8%), 4-hydroxy-3-methoxy-acetophenone
(1.3%), syringylaldehyde (0.7%), and syringic acid (2.0%) as shown in Figure 5.2.
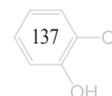
Supercritical depolymerization of lignin at 300°C in a carbon dioxide/ acetone/water mixture is not complete as found by Py-GC/MS (Figure 5.6), but only minor amounts of remaining thermally labile aromatics in the hardwood lignin char were liberated at a substantial higher temperature of 600°C during pyrolysis. The char obtained after supercritical depolymerization of lignin at 370°C contains less thermally labile aromatics as compared to the char obtained at 300°C (data not shown).
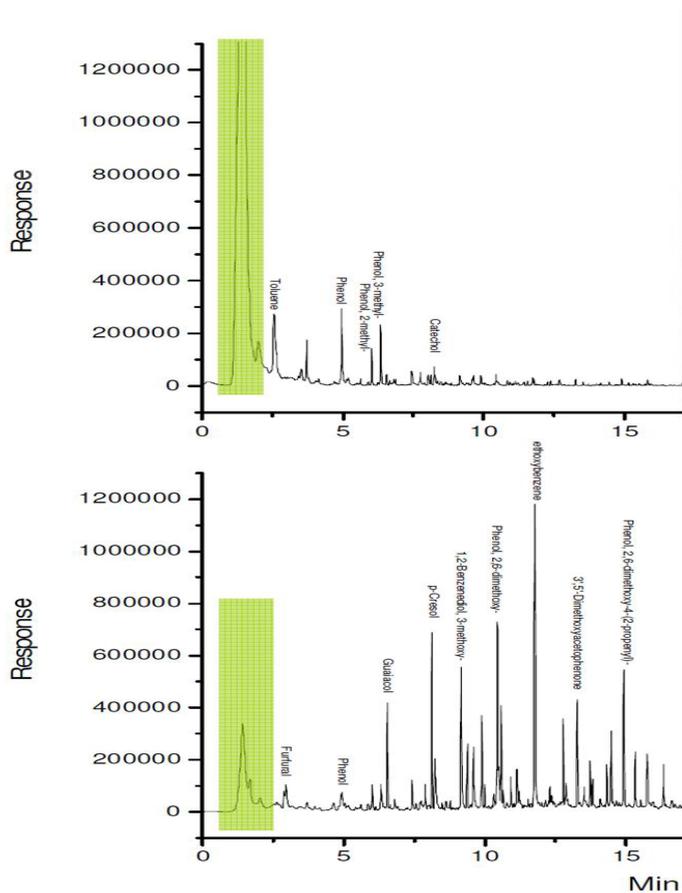
Figure 5.6 Py-GC/MS fragmentation pattern (mainly phenolic compounds) of the lignin char formed in supercritical depolymerization of organosolv hardwood lignin at 300°C (above) and of the untreated organosolv hardwood lignin (below).

Detailed MS identification of all peaks present in the chromatogram of a lignin depolymerization extract showed that 3 peaks originated from the autocondensation of acetone in the presence of formic acid under the process conditions applied. The dimeric
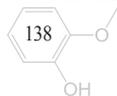
product of acetone did not interfere with the liberated aromatic compounds. However 2 other peaks, identified as 3,3,5-trimethylcyclohexanone and 3,3,6,8-tetramethyl-3,4-dihydro-1(2H)-naphthalenone, co-elute with respectively benzylalcohol and 2,6-dimethoxy-4-(2-propenyl)-phenol. These compounds were excluded from the quantification of liberated aromatic compounds from lignin.

In this novel supercritical process high purity organosolv lignin is depolymerized into a lignin oil (up to 45wt%), consisting of identified monomeric (up to 12wt%) next to oligomeric aromatics. During the process, the lignin oil is separated by pressure expansion from the remaining char. As the char represents a substantial amount, further work is needed to improve the overall conversion of lignin into valuable products.

5.4 Conclusions

Hardwood and wheat straw organosolv lignins were depolymerized in a supercritical carbon dioxide/acetone/water fluid at 300°C and 100 bar into 10%-12% monomeric aromatic compounds by using small amounts of formic acid as hydrogen donor. Furthermore, lignin is converted into a phenolic oil consisting of both monomeric and oligomeric aromatic compounds. Hardwood and straw lignin yielded a different mixture of aromatic compounds with a maximum individual yield of 3.6% for syringol and 2.0% for syringic acid based on lignin respectively. Depolymerized phenolic products and char were separated during this process by pressure expansion. As during this process competition occurs between lignin depolymerization and recondensation of fragments a substantial amount of char is formed.

Acknowledgements
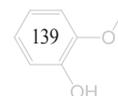
www.biosynergy.eu), which were financially supported respectively by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and the European Commission.
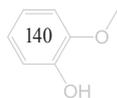
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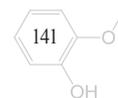
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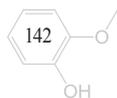
Chapter 6

Discussion and perspectives
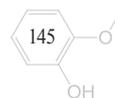
6.1 Introduction

The main aim of this thesis is to study the potential of lignin to become the renewable aromatic resource for chemical industry in the future. When carbon resources are becoming scarce and more expensive there is an urgent need to use these resources as efficient and effective as possible. This is applicable for fossil resources, which are expected to be depleted over time, but also for biobased resources. Good examples of underutilised biobased resources are the lignin containing side streams of the established pulp and paper industry and the fast upcoming lignocellulosic biorefinery industry. Most of the lignin is now used as an energy source to feed these processes. However, (part of) this lignin can be used alternatively for technical applications, most likely resulting in more value addition than the calorific fuel value and also save on the consumption of fossil resources. In order to show these benefits, a detailed life cycle assessment (LCA) is needed to support these assumptions. As far as I know this type of LCA including the utilisation of lignin for value added applications has not been published.

In **Chapter 1** of this thesis a broad picture is given on lignin sources, availability, different properties and potential applications. In a modern biorefinery process, including pulp and paper processes, the sustainable production of cellulose, hemicellulose en lignin will create the highest value for this multiproduct system. These streams can be converted into a spectrum of biobased products fitting perfectly within the biobased economy as depicted in Figure 6.1.
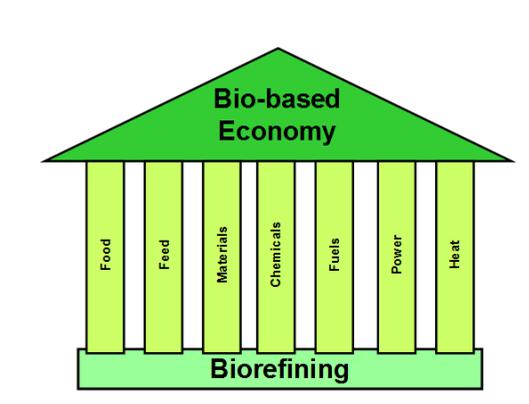
Figure 6.1 Biorefinery – the foundation to build the future Bio-based Economy
(adapted from www.iea-bioenergy.task42-biorefineries.com)

In the biobased economy, energy, fuel, food, feed, chemicals, and materials will be derived from biobased raw materials (Figure 6.1). When using lignocellulosic (LC) biomass, about 20-30% consists of lignin. As one of the major components of LC biomass, economic utilisation of lignin is necessary to optimize biomass use.

Lignin has a relatively high energy content due to its higher C/O ratio (21 MJ/kg HHV; USDA 2011) as compared to the other main, carbohydrate based, biomass components (eg. cellulose 17 MJ/kg HHV; USDA 2011), and is therefore a good source for generation of energy. Since most processes need energy input, part of the liberated lignin can serve as the energy source. However, due to its intriguing molecular structure and intrinsic natural properties, lignin is considered as a versatile raw material for many potential applications, beyond the base case conversion to energy (Doherty et al. 2011).

In lignin, the phenylpropane derived building block composition, molecular mass and the different linkages between the phenylpropane units are dependent both on biomass source and the isolation process used. Therefore, lignin represents not a single well defined biomaterial but more a cluster of different biomaterials, for each application the most suitable lignin type has to be selected. Unfortunately, there is not "one type fits all" lignin which can be used for multiple applications.
Industrial application of lignin so far has been rather limited, mainly due to complications in the multi-step recovery of lignin from product waste streams, the presence of various impurities, a non-uniform heterogeneous structure, and the unique chemical reactivity. Although many methods have been developed to overcome the first two obstacles, the economic feasibility of lignin recovery was not always justified in the past. For the two other difficulties, the heterogeneous structure and unique chemical reactivity, promising approaches are emerging (Vishtal and Kraslawski 2011). The results of this thesis are related to these latter two topics, the non-uniform structure and unique chemical reactivity. Nevertheless, there is an increasing interest of the (chemical) industry and the research community to develop breakthrough technologies for lignin conversion and novel applications. This is confirmed by the number of publications, patents and announcements of lignin related developments during the last 5 years (Table 6.1 and see **Chapter 1**).

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In Table 6.1 (and Table 1.3) the leading contributors to these lignin developments are:

- Dömsjo Fabriker, who will double their lignosulfonate production capacity to 120 kton/y by using an extra spray dryer.
- CIMV plan to start a straw biorefinery in France to produce cellulose, lignin and a sugar syrup as their main products.
- Abengoa Bioenergy, will produce steam explosion lignin at their demonstration facility.
- Lignol, who is producing on pilot scale organosolv lignin, started industrial scale testing of lignin based products with several industries active in the foundry and binder application area. This step is essential for the further development of this organosolv biorefinery on industrial scale.
- The Lignoboost concept has been successfully tested for several years and this concept is ready to be used for other industrial kraft mills.

Table 6.1 Lignin developments per 2011.

Lignin type	Scale of operation	Volume (kt/y)	Suppliers	Scale up announce-ments	Expected volume (kt/y)	Comments
Lignosulfonates (soft/hardwood)	Commer-cial	~700	a) Borregaard (NO, worldwide)	2011		a) Bali process, building pilot plant for various feedstocks. www.borregaard.no (Sjöde et al., 2010)
		60	b) Domsjö Fabriker (SE)	2011	120	b) Increased capacity, installed spray dryer
Kraft softwood	Pilot	0.5-4	LignoBoost/Metso (SE)	2011		Large Kraft pulp mills as Södra (S) are interested. 10-25% lignin can be extracted from black liquor (potentially 2Mt/y)
Kraft softwood	Pilot		FPInnovations (CAN)	2011	0.03	Lignin and furfural production from existing dissolving pulp mill under evaluation. Plans for building pilot plant.
Soda non-wood	Commer-cial	5-10	Greenvalue (CH, IND)			Current capacity of soda lignin is used in a variety of markets including resins and feed binder. Expansion seeking in Europe[1].
Waste wood, non-wood	Portable Pilot	0.5	Pure Lignin Environmental Technology (CAN)	>2009	1-2	Water soluble lignin
Organosolv straw (acids)	Pilot	1-2	CIMV (FR)	>2011	35	160 kton/y straw biorefinery
Organosolv hardwood (EtOH/H$_2$O)	Pilot	0.5-3	c) Lignol Innovations (CAN)	6/2011		c) Industrial lignin application trials conducted
			d) DECHEMA/ Fraunhofer (DE)	2010-2013		d) Building pilot plant
Steam explosion straw/softwood	RTD+ Demo	< 0.5	Abengoa Bioenergy (ES)	2009	10	30 kton/y straw biorefinery

[1]personal communication with Dr. Jairo Lora, Vice President, Greenvalue Enterprises LLC (June 2011)

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As soon as these developments have been scaled up an additional 100kt/year of lignin will be produced. This means a foreseeable increase of at least 10% more technical lignin production in the coming years. This will stimulate the further step-wise development of lignin applications.

Together with the relatively high average oil barrel price of more than $85 in 2011 it is justified to develop value added applications for lignin as the amount of lignin generated is more than sufficient to cover all heat and power demands. This leads ultimately to fully integrated lignin valorization in the pulp and paper and lignocellulosic biorefinery industries. It is expected that both in the pulp and paper industry and biorefinery industry, mostly driven by the second generation biofuel developments, more and more lignin-rich side streams will be produced. This lignin will bring extra revenues to the industry when suitable value added applications, such as wood adhesives, bio-bitumen, carbon fibres, and aromatic building blocks for polymers, have been developed. The famous industrial quote: "*You can make anything out of lignin, except money*" will than become history. Tom Browne of FPInnovations claimed that in a world where oil costs $20/barrel, the old quote is true, but things change when a barrel sells above $85 (Shaun L. Turriff 2011). Of course, extraction and processing of lignin into lignin based value added products still needs to be economically viable compared to traditional consumer products.

Besides the increased industrial activities with regard to lignin production and the industrial scale trials on lignin derived products (Lignol, CAN), several lignin related scientific networks between universities / institutes and industry were established. The International Lignin Institute (ILI) was founded in 1991 and is still promoting R&D activities in the lignin field (www.ili-lignin.com). In 2010 a dedicated lignin network was founded in The Netherlands (www.ligninplatform.wur.nl) to promote interdisciplinary research and to create a support group for the valorization of lignin in industrial production of lignin-derived chemicals and compounds. In 2010 also an extensive technology platform was created in Canada for novel materials and chemicals based on lignin to replace fossil-fuel based chemicals and products (www.lignoworks.ca). Together with the establishment of such networks the number of lignin related projects is expected to increase substantially.
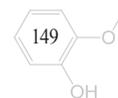
6.2 Molar mass distribution of lignin

Successful introduction of lignin into new markets is highly dependent on its structure related functional properties. An important structural property of lignin is its molecular size as the molar mass distribution partly governs its reactivity and physico-chemical properties. Development of a universally applicable molar mass distribution protocol for a wide range of technical lignins is of high importance for quality control, application development and utilisation by industrial end users. The results of this research are described in **Chapter 2**. In this chapter it is shown that an alkaline size exclusion chromatography (SEC) protocol is applicable for a wide range of different lignins which can be measured without prior derivatization. These lignins include pulp and paper lignins (lignosulfonates, kraft, soda), biorefinery lignins (organosolv, steam explosion, enzymatic hydrolysis), fractionated lignins (kraft, soda, organosolv, **Chapter 3**), modified lignins (kraft, soda, **Chapter 4**) and depolymerized lignins (organosolv lignin oil, **Chapter 5**).

Relative comparison is made between the molar mass distribution of these lignins and narrow dispersed sulfonated polystyrenes. For process development this methodology is accurate and reproducible and therefore very powerful. However, from this research and from previously reported work (Jacobs and Dahlman 2000; Mattinen et al. 2008) it becomes clear that for absolute molar mass determination most technical lignins are too polydispersed biopolymers. These technical lignins need to be fractionated to obtain narrow dispersed lignin fractions. Only narrow dispersed lignin fractions can be used for accurate absolute molar mass measurement by applying mass spectrometry like for example MALDI-TOF-MS. My research shows that optimization of the MALDI protocol leads to better signal-to-noise ratios, but does not overcome the limited signal originating from the broad molar mass distribution of the lignin molecules and the lack of regular repeating units. Only fractionation of organosolv hardwood lignin with three analytical high resolution SEC columns leads to better defined lignin fractions. These purified fractions resulted in accurate MALDI molar mass quantification, although the detection of the higher molar mass fractions shows complications. In **Chapter 2** it is shown that fractionation of lignin by alkaline SEC with one column gel does not lead to narrow fractionated lignin samples. The column gel used was manually packed and this resulted in a low resolution SEC performance.

150 Improvement of the fractionation can be achieved by using high resolution SEC

columns. Also fractionation of lignin by successive organic solvent extraction, described in **Chapter 3**, leads to fractions which are still too polydisperse for accurate MALDI analysis.

Preliminary experiments to apply ultrafiltration for alkaline fractionation of a commercially produced soda non-wood lignin show a similar trend (Abaecherli et al. 2009). The authors conclude that MALDI clearly underestimates the molecular mass compared to the values obtained by alkaline and organic SEC. This could be explained by the inferior detector response to higher molar mass lignin structures, due to limited ionisation and poor ability to fly which might be caused by strong intramolecular interaction. Here narrowing the size exclusion limits of the membranes used may lead to more suitable lignin fractions for MALDI-TOF-MS analysis. For future work it is recommended to use high resolution SEC or ultrafiltration, using narrow mass range membranes, in order to obtain more narrow-dispersed lignin fractions. These fractions can be used for absolute molar mass determination by mass spectrometry (eg. MALDI-TOF-MS or ESI-MS). When accurately characterized for its molar mass distribution these lignin fractions can be applied for absolute calibration of SEC methodologies. Moreover, well defined and characterized lignin fractions will be interesting sources for application development as described in this thesis.

6.3 Selection of suitable lignins for binder application using PCA modeling

Potentially lignin derived products can be used for multiple applications (Holladay et al. 2007) and for each application the property demands are strongly related to the analytical lignin properties, which needs to be known prior to application development. In **Chapter 3** it is demonstrated that a principle component analysis (PCA) model based upon quantifiable analytical chemical data predicts the suitability of a technical lignin or its fraction in wood adhesive applications. The lignins and their fractions are classified in different clusters based on their structure dependent properties. Kraft softwood lignins show the highest potential for plywood binder application followed by hardwood soda lignin and the fractions of Sarkanda grass soda lignin with medium molar mass. As expectedly, the softwood lignins contain the highest number of reactive sites in ortho positions to the phenolic-OH group for crosslinking reactions with formaldehyde.
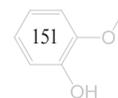
Moreover, these lignins have a low level of impurities and medium molar mass which are beneficial for gluing applications.

Results in **Chapter 3** show that fractionation of lignin by using different organic solvents results in purified fractions of distinguished structure dependent functional properties and demonstrating different application potential. In addition these results show that technical lignins consist of mixtures of lignin fragments with different molar mass distributions and chemical functionalities. These fragments can be rather easily separated and purified by organic solvent extraction at mild conditions, e.g. at room temperature. In particular the highest molar mass fractions (F5) of both kraft and soda lignins contain the so-called lignin carbohydrate complexes (LCCs) which are poorly soluble in organic solvents. These F5 fractions could also be detrimental for wood adhesive application as the non-lignin constituents can disturb resin network formation and can be more sensitive to moisture uptake leading to a higher swelling of the wood based panels. In the other fractions (F1-F4) only minor amounts of carbohydrates and ash are present and these fractions have a positive effect on the glue properties in the wood panel application. Also for other applications such a multi-criteria approach, using analytical chemical data and PCA modeling, could become an important tool to identify the most suitable lignins for further evaluation in the selected application. The work described in this thesis may serve as the basis for an application oriented tool to predict the proper lignin for the right application.

6.4 Lignin activation by periodate for wood adhesive application

Eco-friendly binders with no harmful emissions during its complete life cycle are of high interest for the wood and panel industry. The aim of **Chapter 4** was the development of a fully renewable and emission-free binder based on activated lignin and poly-furfuryl alcohol. Activation of kraft and soda lignins, isolated respectively from softwood and non-woods, by periodate oxidation was performed to improve lignin reactivity and application in wood adhesives. Periodate oxidation of lignin leads to higher lignin acidity, formation of quinonoid groups, higher molar mass and higher reactivity towards the curing of furfuryl alcohol within the temperature range currently applied in the wood panel industry. Comparison of a 100% furan based glue with a furan glue substituted by 10wt% lignin gives comparable product properties. However,

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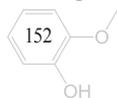
periodate activated lignin leads to lower wood failure. This may be caused by incomplete solubilised lignin particles in the acidic glue formulation that interferes with the acid catalysed polycondensation of the furan resin. In contrast, unmodified softwood kraft lignin performs well in a PF resin formulation at substitution levels up to 30% (w/w). Periodate oxidation of soda lignins enhances the glue performance since a higher wood failure is observed. The higher molar mass after periodate treatment may be an important parameter for development of a stronger wood binder. The performance of lignin in these PF resins is better than in the furanic resin because the polycondensation of the PF resin is base-catalyzed at about pH 11 and lignin is completely solubilized at that pH.

In **Chapter 4**, it is shown that only partial substitution of a furan resin or phenol-formaldehyde resin by lignin is possible. One important limitation, next to reactivity, is the viscosity increment due to the higher molar mass of technical lignins. For industrial application of a wood adhesive strict viscosity regimes are tolerated. Here lignin fractions with lower average molar mass and less impurities might go beyond the substitution levels obtained so far. Additionally, the technical potential of these lignin fractions with medium molar mass is higher according to the PCA model results described in **Chapter 3**. These medium molar mass lignin fractions can be obtained after fractionation by organic solvents (**Chapter 3**) or after depolymerization in a supercritical fluid (**Chapter 5**). The obtained lignin phenolic oil, which is a mixture of highly reactive monomers and oligomers, could be directly applied in a wood resin or after more refining.

For further improvement of lignin based binders the lignin needs to be activated during the synthesis of the prepolymerised resin formulation. Instead of using formaldehyde, recognized as a human carcinogen (US Department of Health and Human Services 2011), the search for non-toxic, emission-free alternatives is on-going. Sooner or later formaldehyde emissions will not be tolerated anymore and alternatives will become obligatory. In this thesis it is shown that a higher level of lignin reactivity can be obtained by lignin oxidation using metaperiodate ($NaIO_4$). Although interesting results were obtained with this formaldehyde-free system, in future work the effect of periodate on the lignin reactivity needs to be optimised. As periodate is a relatively expensive chemical effective recycling is needed and a fully integrated system needs to be developed.

The industrial use of lignin in wood adhesives is currently rather limited, but the use of soda non-wood lignin is a good example (Khan and Lora 2006). In this application formaldehyde is still used as crosslinking agent, but emission-free alternatives (e.g. glyoxal) are under evaluation as reported by El Mansouri et al. (2007) and Mansouri et al. (2011).

6.5 Lignin depolymerization into aromatic chemicals

Final objective of this thesis is to develop an economically feasible and sustainable process for the production of aromatic green chemicals from lignin. In this research emphasis is given to the production of a small group of interesting phenolic chemicals by a process in carbon dioxide under supercritical conditions targeting on an economically attractive yield. This work is part of an integral lignocellulosic biorefinery concept and the major steps are given in figure 6.2. In this project, called LignoValue, the main objective is the valorization of organosolv lignin into aromatic chemicals, wood adhesives and fuel additives (Gosselink et al. 2011). In the first step, called primary biorefinery step, high purity organosolv lignins were obtained from the ethanol-water fractionation of hardwood and wheat straw. These lignins were used for the depolymerization studies which are part of the secondary biorefinery step (Figure 6.2) as described in **Chapter 5**.
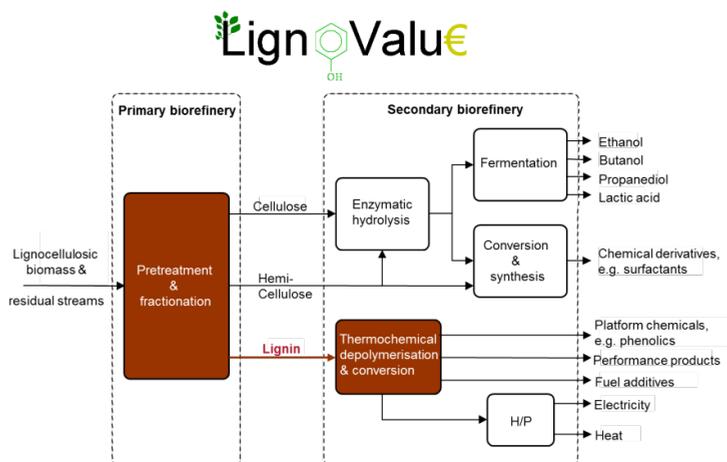
Figure 6.2 Lignocellulosic biorefinery concept with integrated lignin valorization (www.lignovalue.nl).

The results described in **Chapter 5** showed that in supercritical carbon dioxide/ acetone/water about half of the lignin was converted into depolymerised structures including oligomeric fragments and monomeric compounds. This lignin oil can be applied as substitute for the phenol part in a wood adhesive as discussed in **Chapters 3 and 4**. The average molar mass of this lignin oil is about 1200 g×mol^{-1} with a polydispersity of approximately 4. This lignin oil with a medium molar mass is according to the PCA model, described in **Chapter 2**, favourable for use in a wood adhesive. A similar lignin oil, but obtained after catalytic hydrodeoxygenation (HDO) treatment, was successfully tested in a screening plywood adhesive test. At least 75% of the fossil-based phenol could be substituted by lignin HDO oil in a lignin based PF wood adhesive while maintaining its strength properties above the standard requirements (Gosselink et al. 2011). This result indicates a good potential for further development of biobased wood adhesives derived from lignin.

Interestingly, the supercritical process developed in this research gave for some monomeric phenolic compounds a relatively high yield after depolymerization from both hardwood and wheat straw lignin (Table 6.2). These compounds represented a substantially higher value than the fuel value of about 50€/ton. Syringol, for example, is commonly used in the flavour and fragrance industry and sold for about 25-30 €/kg. Syringaldehyde has been patented for use as hair and fibre dye and as pharmaceutical precursor for obesity and breast cancer treatments and represents an even higher market value (Eckert et al. 2007). The obtained yields of the individual compounds (Table 6.2) seem promising for further downstream processing to produce the individual compounds in a purified form. Some strategies for these kind of purification routes are published by Vignealt et al. (2007) and combines liquid-liquid-extraction, followed by vacuum distillation, liquid chromatography and crystallization. These multi-step routes have to be proven to be economically interesting, but considering the values of the resulting phenolics these routes seem to be worth trying. Besides the distinct monomer streams, the residual streams may be used for applications as a wood adhesive, as bio-bitumen or as feed for a chemical cracker refinery. Another example is the use of a CO_2 expanded organic liquid to extract syringol, vanillin and syringaldehyde from lignin (Eckert et al. 2007). In future work, this extraction might be integrated to the CO_2 based supercritical depolymerization process of lignin (as described in **Chapter 5**).

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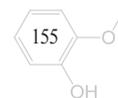
Table 6.2 Major phenolics produced by supercritical depolymerization of lignin (wt% on dry lignin).

Compound	Structure	Hardwood	Wheat straw
Guaiacol		1.6	1.6
Syringol		3.6	0.8
2-methoxy-4-methyl-phenol		1.6	0.1
2-methoxy-4-ethyl-phenol		0.6	0.4
4-hydroxy-3-methoxy-acetophenone		0.6	1.3
Syringylaldehyde		0.1	0.7
syringic acid		0.4	2.0
3-hydroxy-4-methoxy-benzaldehyde		0.6	0.2

In the supercritical depolymerization study it became clear that a large part of the lignin is converted into lignin char. Although significant depolymerization occurred, the resulting fragments tend to crosslink forming the recalcitrant char. A substantial removal of oxygen and resulting increment of carbon was observed in the residual lignin char. The oxygen content was decreased from 27% in lignin to 20% and 12.5% in the char after supercritical treatment at 300°C and 370°C, respectively. Oxygen was removed by lignin conversion into water and volatile products CO, CO_2 and methanol. The lignin char, with up to 85% carbon, could be used as resource for large volume applications such as bio-bitumen (bio-asphalt), as feed for a chemical carbon cracker refinery, activated carbon, or as carbon fibres. However, minimizing the formation of this char will be beneficial for continuous operation of this process and the economically viability is expected to increase substantially. To further depress the formation of char, more hydrogen is needed to stabilise the aromatic radicals. This can be achieved by adding a higher amount of a hydrogen donor, such as formic acid (Kleinert and Barth 2009), or by operating this process under hydrogen pressure like in HDO processes as described in **Chapter 1**. Additionally, the continuous removal of aromatic monomers from the lignin feed, which is possible in CO_2 expanded liquids, will lead to less formation of char.
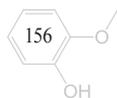
Finally, to develop an economically viable lignin depolymerization process there will be a trade-off adding more expensive chemicals, for example to stabilize the aromatic radicals, and the revenues of the lignin derived products.

6.6 Alternatives for the production of aromatic chemicals from biomass

Lignin is by far the most abundant aromatic renewable resource on earth. Therefore, researchers have always focussed on lignin as primary source for replacement of the fossil fuel derived aromatic compounds. The main aromatic compounds used in industry are phenol, BTX, and terephthalic acid (Haveren et al. 2008). Currently there is a strong desire from major brand owners (e.g. Coca Cola, Pepsi, Heinze) to "green" their product portfolio. However, results in this thesis show that there are quite some challenges to overcome for the development of an economically viable process for the production of aromatic chemicals from lignin. Therefore, alternative routes using crude biomass, tannins or (lignocellulosic) carbohydrates for the production of aromatics are under development, which will be shortly presented in this section.

A potential natural source for aromatics are polyflavonoid tannins which are industrially extracted from for example wood bark (Quebracho and Minosa) for leather tanning chemicals, wood thermosetting resins, and red wine additives (Richards 2000). Tannins are mostly composed of flavan-3-ols repeating units combined with glycosidic linkages. Tannins are less complex than lignin and therefore could serve as a raw material for the production of aromatic chemicals, although its occurrence is limited compared to the potential availability of lignin.

At the moment there is a major drive to replace terephthalic acid by biobased terephthalic acid. There are currently several routes claimed to produce this compound. Purified terephthalic acid (PTA) can be produced starting from muconic acid, which is fermentatively produced from lignocellulosic carbohydrates as developed by Draths (2011). In this process, muconic acid and bio-ethylene will form via a *Diels-Alder* reaction cyclohexene,1,4-dicarboxylic acid which will be dehydrogenated and purified to yield bio-PTA. PTA can be used together with biobased ethylene glycol to produce 100% biobased polyester (polyethylene terephthalate: PET) polymers. Applications for polyester polymers include flexible and rigid packaging materials, fibres, textiles, and

films. The world PTA demand is approximately 30 million ton/year (Cherubini and Strømman 2011).

Gevo (2011) is working on the production of biobased isobutanol from plant carbohydrates. Commercial-scale production is expected to commence in the first half of 2012. Isobutanol can be dehydrated with well-known processes to produce butenes which are building blocks for the production of materials such as lubricants, synthetic rubber, and polymers like poly(methyl methacrylate) (PMMA) and PET. For the latter purpose, isobutanol can be converted to isobutylene, iso-octene, the aromatic p-xylene, and finally PTA. Recently, Virent (2011) successfully produced p-xylene from 100% plant carbohydrates in a 37,000 L/year demonstration plant. Because the conversion of p-xylene to PTA is a well-known commercial chemical process, the biobased p-xylene can be used for the production of 100% plant-based PET. The BioForming® process is based on the novel combination of Virent's core technology with conventional catalytic processing technologies such as catalytic hydrogenation and catalytic condensation processes, including ZSM-5 acid condensation, base-catalyzed condensation, acid-catalyzed dehydration, and alkylation. Virent claims that each step can be optimized to produce the desired end-product.

Anellotech (2010) has developed a technology platform using catalytic pyrolysis for the claimed inexpensive production of chemicals and transportation fuels from non-food biomass. Vispute et al. (2010) claim that all chemical conversions can be performed in one reactor, using an inexpensive catalyst. Target green chemicals are benzene, toluene, and xylenes (BTX) which represents an existing $100 billion market.

It can be concluded that the race to produce aromatics from renewable feedstocks is wide open. It should also be emphasized that many of the above discussed alternatives are very early stage which makes it at present unclear if those routes can become costs competitive as well as sustainable.

6.7 Economic considerations

Lignin is regarded as a low value side stream from the pulp and paper industry and future biorefinery industry. Isolation, purification and drying add up to the production costs of lignin. Lignin sales values vary from low grade lignin to high grade lignin from about 50 – 750 €/ton (Figure 6.3). Lignin side streams with the lowest purity,

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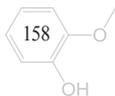
representing the lowest value, will be obtained from lignocellulosic biorefineries for production of 2nd generation biofuels. An example is the non-fermentable fraction resulting after hydrolysis and fermentation representing a value of between 50€/ton (fuel value) and >100€/ton (anticipated animal feed value for Distiller's Dried Grains and Solubles, DDGS, resulting from a first generation bio-ethanol plant). Also lignin dissolved in black liquor, which is currently used mainly as energy source for the pulp mill, can be assumed as a low purity lignin stream. These low purity lignin streams are followed by lignosulfonates with a current industrial production of 1M ton/year (Gosselink et al. 2004) at values ranging from 250 – 350 €/ton (Figure 6.3). Kraft, soda and organosolv lignins, which could be produced with a substantially higher purity, represent a value between 350 – 500 €/ton. More purified high grade lignins will be available to the market for higher prices up to 750 €/ton or even beyond this level as the costs for upgrading are included.

Figure 6.3 shows that for higher value added applications the market volume will decrease. In this thesis emphasis has been given to the development of a wood adhesive partly derived from lignin. This wood adhesive will compete with the phenolic resin market which is currently about 1 Mton/y with an average value of 1200 €/ton. The other application studied in this thesis was the production of aromatic monomers from lignin via a supercritical conversion technology. From the obtained products the monomeric phenolics fall into the phenol derivatives market, the lignin oil including mono- and oligomeric phenolics may be used for phenolic resin application, and the char may be used for bio-bitumen, for a chemical carbon cracker refinery or for the production of activated carbon. The fine chemicals market (vanillin, phenol derivatives) represents a much lower volume, typically 10-20 kton/year, with higher values as discussed previously. Bio-bitumen and a carbon cracker refiner represent large volume markets. The activated carbon market is a 1 Mton/y volume market.

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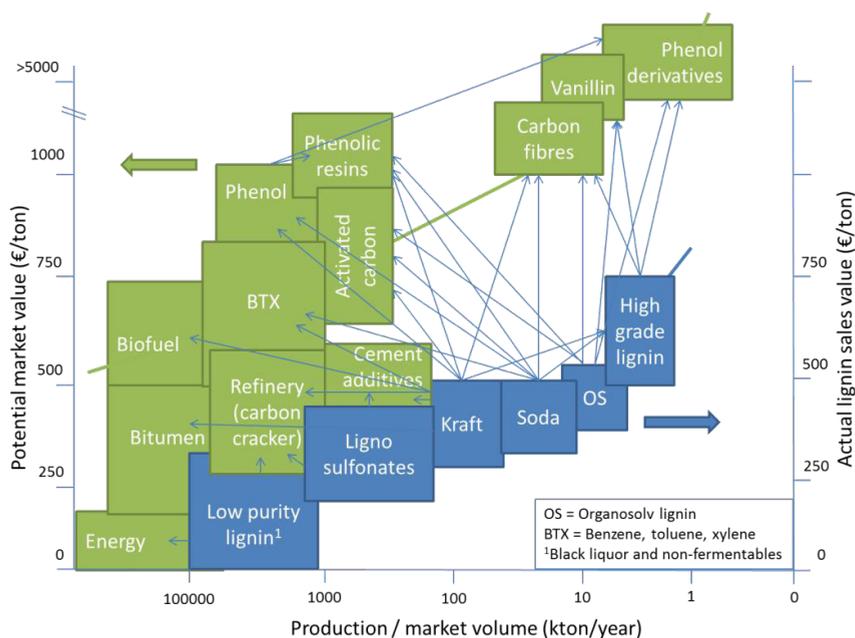
Figure 6.3 Lignin production and potential lignin derived product market and value.

In Figure 6.3 current and potential lignin-product combinations are given by the arrows. For near horizontal arrows the value increment is limited, but for oblique arrows a substantial value increment is anticipated. However, in particular for these latter applications the desired technologies need to be optimized and become costs effective to gain as much profit as foreseen. Costs for modification or conversion of lignin need to be deducted from the indicative value increments (value market minus lignin production costs) given in Figure 6.3.

Considering the value increment for these combinations it can be envisaged that various opportunities are present to create value out of lignin. A prerequisite is that both lignin production and applications should be both expanded and established while fitting the right lignin to the right application.

6.8 General conclusions

The aim of the research described in this thesis was to study the potential of lignin to become a renewable aromatic resource for the chemical industry in the future. Lignin
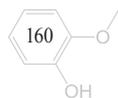
can be expected to become a widely available raw material at relatively low costs for the production of an array of products. These products range from low value and large volume to high value and low volume applications. Development of lignin based applications needs to go hand in hand with the anticipated increased production of technical lignins derived from the pulp and paper industry and the emerging lignocellulosic biorefinery industry. Breakthrough technologies need to be further developed for the development of suitable applications. Two promising value added lignin applications are described in this thesis aiming at:

1) the use of lignin in wood adhesives
2) the use of lignin for the production of aromatic chemicals

In this research a reliable SEC methodology was developed for the analysis of the molar mass distribution of a wide range of different technical lignins. This method is used for the development of both selected applications. The results showed that this method is not only applicable to unmodified technical lignins, but also for fractionated lignins, oxidised lignins, and depolymerised lignin fractions as studied in this thesis. However, the major drawback of this method is that the molar masses are calculated on a relative basis to sulfonated polystyrenes. Using MALDI-TOF-MS and prior fractionation of lignin did not solve all problems associated with the determination of the absolute molar mass of lignin. The search for a routine analysis for the absolute molar mass of lignin will be continued.

Then it is demonstrated that a principle component analysis (PCA) model based upon quantifiable analytical chemical data predicts the suitability of a technical lignin or its fraction in wood adhesive application. The lignins and their fractions are classified in different clusters based on their structure dependent properties.

Furthermore, the results presented in this thesis showed that lignins exhibiting sufficient reactive sites, medium molar mass and low level of impurities are promising candidates for the development of lignin based wood adhesives. Both lignin reactivity and formaldehyde-free crosslinking agents are needed to produce emission-free adhesives. Periodate oxidation is an interesting route to increase the lignin reactivity, but the results in this thesis showed that the lignin reactivity must be further enhanced and better understood. Alternatives to formaldehyde are being investigated and a
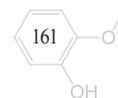
combination of activated lignin and furan compounds displays an interesting wood binder to be further developed.

Supercritical depolymerization of lignin in a carbon dioxide/acetone/water fluid resulted in depolymerised lignin oil. In this oil some monomeric aromatic compounds are present in relatively high amounts up to 3.6% (based on dry lignin). These products could be further isolated by downstream processing to obtain purified fine chemicals. The total lignin oil could be utilised for example as a wood adhesive. For continuous operation of this supercritical process, the formation of char should be further depressed by using more hydrogen or specific catalysts in the process.
Advantages of this supercritical process are:

- use of a non-toxic "green" solvent (CO_2/acetone/water)
- pressure expansion of CO_2 expanded solvent will lower the temperature facilitating the condensation of products. There is no need for an additional condenser in the process.
- temperature is relatively low compared to other thermochemical processes such as pyrolysis
- some aromatic monomers are produced in substantially higher amounts compared to the bulk products
- continuous removal of product possible
- continuous processing possible when char formation is limited or if char can be removed on-line

The results presented in this thesis contribute to a better understanding of the lignin structure, possibilities for lignin chemistry and application development. Based on these results, it is most likely that a commercial wood adhesive or resin based on lignin can be expected sooner than aromatic chemicals derived from lignin. For a wood adhesive several technical lignins can be used as directly obtained from a pulping or biorefinery process, but these lignins needs to be activated and efficiently crosslinked to obtain an emission-free adhesive. My research showed that this may be possible with periodate or by using an alternative as glyoxal being under investigation as well. In both cases further process optimization is needed to find optimal technical, economic and sustainable conditions.
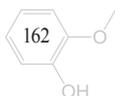
For the development of an economically viable lignin valorization route for the production of aromatic chemicals, much more research is needed to find the optimal process conditions, suitable hydrogen donors and/or biorefinery catalysts. Another important issue for this process is the use of high purity lignin, which can be produced by organosolv technology as shown in this thesis, but this technology needs a high capital investment and so far this has not been realized on industrial scale.

Finally, the progress presented in this thesis on lignin valorization will together with all the lignin research worldwide ultimately lead to the expected increased commercial utilization of lignin in the future. Although competitive routes starting from carbohydrates are under development, it seems to be justified that lignin will become a future renewable aromatic resource for the chemical industry.

6

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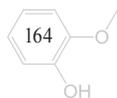
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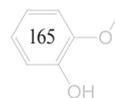
Summary

The main aim of this thesis was to study the potential of lignin to become a renewable aromatic resource for the chemical industry. As fossil resources are becoming scarce, more expensive, and exhibit negative effects on our environment, there is an urgent need for alternatives such as lignocellulosic biomass. This biomass can be used, after efficient biorefinery into its main components, for a spectrum of products and materials. However, not all biomass fractions are optimally used so far. Good examples of underutilised biobased resources are the lignin containing side streams of the established pulp and paper industry and the emerging lignocellulosic biorefinery industry. Most of the lignin is now used to fulfil the energy requirements of these processes. However, (part of) this lignin can be used alternatively for technical applications, most likely resulting in more value addition than the fuel value and also save on the consumption of fossil resources as described in **Chapter 1**.

Lignin can be expected to become a widely available raw material at relatively low costs for the production of an array of products. First calculations showed that about 15 Mt/y of dry lignin will become available in the near future (**Chapter 1**). Development of lignin based applications needs to go hand in hand with the anticipated increased production of technical lignins derived from the pulp and paper industry and the future lignocellulosic biorefinery industry. For the development of suitable applications breakthrough technologies need to be further developed. Two promising value added lignin applications are described in this thesis aiming at:

1)   the use of lignin in wood adhesives
2)   the use of lignin for the production of aromatic chemicals

In **Chapter 2** a reliable size exclusion chromatography (SEC) methodology was developed for the analysis of the molar mass distribution of a wide range of technical lignins obtained from different processes. This analytical method is used to support the development of both selected applications. The results showed that this SEC method is not only applicable to unmodified technical lignins, but also for fractionated lignins, oxidised lignins, and depolymerized lignin fractions as studied in this thesis (**Chapters 3-5**). However, the major drawback of this method is that the molar masses are calculated on a relative basis to the molar masses of sulfonated polystyrenes.
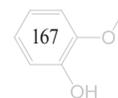
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Using MALDI-TOF-MS and prior fractionation of lignin did not solve all problems associated with the determination of the absolute molar mass of lignin. The search for a routine analysis for the absolute molar mass of lignin will be continued.

Then it is demonstrated in **Chapter 3** that a principle component analysis (PCA) model based upon quantifiable analytical chemical data predicts the suitability of a technical lignin or its fraction in a wood adhesive application (**Chapters 3 and 4**). As a result, the lignins and their fractions were classified in different clusters based on their structure dependent properties.

Furthermore, the results presented in **Chapter 4** of this thesis showed that lignins exhibiting sufficient reactive sites, medium molar mass and low level of impurities, such as carbohydrates and ash, are promising candidates for the development of lignin based wood adhesives. Both sufficient lignin reactivity and formaldehyde-free crosslinking agents are needed to produce emission-free adhesives. Periodate oxidation is an interesting route to increase the lignin reactivity, but the results in this thesis showed that the lignin reactivity must be further optimised. Alternatives to formaldehyde are being investigated and a combination of activated lignin and furan compounds displays an interesting wood binder to be further developed.

In **Chapter 5** the results of a novel process for the depolymerization of lignin in a supercritical solvent into aromatic chemicals are described. In a non-toxic "green" solvent based on carbon dioxide/acetone/water lignin was converted into a depolymerized lignin oil. In this oil some monomeric aromatic compounds are present in relatively high amounts up to 3.6% (based on dry lignin) together with oligomeric lignin structures. During the process the depolymerized aromatics were separated from the residual char by pressure expansion without the need for an additional condenser to collect these products. The compounds could be further isolated by downstream processing to obtain purified value added fine chemicals. The total lignin oil may be utilised for example as a wood adhesive. For future process optimization special emphasis should be given to lower the formation of char and in **Chapter 6** some possible routes are discussed.

The results presented in this thesis contribute to a better understanding of the lignin structure, possibilities for lignin chemistry and application development. Based on these results, it is most likely that a commercial wood adhesive or resin based on
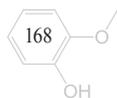
lignin can be expected sooner than aromatic chemicals derived from lignin. For a wood adhesive several technical lignins can be used as directly obtained from a pulping or biorefinery process, but these lignins needs to be activated and efficiently crosslinked to obtain an emission-free adhesive. My research showed that this may be possible with periodate and the use of furfuryl alcohol.

For the development of an economical viable lignin valorization route for the production of aromatic chemicals, much more research is needed to find the optimal process conditions, suitable hydrogen donors and/or biorefinery catalysts. Also the commercial production of high purity lignins will be necessary to further develop this process.

Finally, the results presented in this thesis together with the on-going activities worldwide will contribute to the expected increased commercial utilisation of lignin in the future. Although competitive routes starting from carbohydrates are under development, it seems to be justified that lignin will become a renewable aromatic resource for the chemical industry.
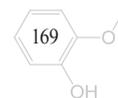
Samenvatting

De belangrijkste doelstelling van dit proefschrift was om te bestuderen welke potentie lignine heeft om een hernieuwbare aromatische grondstof voor de chemische industrie te worden. Omdat fossiele grondstoffen schaarser en duurder worden en negatieve effecten hebben op ons milieu, is er een sterke behoefte aan CO_2-neutrale alternatieven zoals bijvoorbeeld lignocellulose biomassa. Deze biomassa kan worden gebruikt, na efficiente bioraffinage in zijn belangrijkste componenten, voor een breed spectrum aan producten en materialen. Tot nu toe echter werden niet alle biomassa fracties optimaal gebruikt. Goede voorbeelden van sub-optimaal gebruikte biogrondstoffen zijn de lignine-rijke reststromen van de gevestigde pulp- en papierindustrie en van de sterk opkomende lignocellulose bioraffinage-industrie. Tot nu toe wordt het merendeel van de lignine gebruikt om aan de energie behoeftes van deze processen te voldoen. Echter, een deel van de lignine zou als alternatief kunnen worden gebruikt voor technische applicaties, welke zullen moeten resulteren in een hogere toegevoegde waarde dan de energiewaarde en tevens ook een besparing geeft op het gebruik van fossiele grondstoffen, zoals beschreven is in **Hoofdstuk 1**.

Van lignine wordt verwacht dat het op grote schaal beschikbaar komt voor relatief lage kosten en zal worden ingezet voor diverse producten. Globale berekeningen geven aan dat ongeveer 15 miljoen ton/jaar droge lignine beschikbaar zal komen in de nabije toekomst (**Hoofdstuk 1**). De ontwikkeling van lignine applicaties moet echter hand in hand gaan met de voorziene productie toename van technische lignines. Voor de ontwikkeling van geschikte applicaties zullen doorbraak technologiën en geschikte analytische methoden verder moeten worden ontwikkeld. Twee veelbelovende hoogwaardige lignine toepassingen worden beschreven in dit proefschrift met de volgende doelstellingen:

1)   het gebruik van lignine in houtlijmen
2)   het gebruik van lignine voor de productie van aromatische chemicaliën

In **Hoofstuk 2** werd een betrouwbare chromatografische methode ontwikkeld voor de analyse van de molmassa verdeling van een breed scala aan technische lignines die verkregen werden van verschillende processen. Deze analytische methode wordt gebruikt om de ontwikkeling van beide geselecteerde applicaties te ondersteunen. De
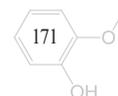
resultaten laten zien dat de ontwikkelde SEC methode niet alleen toepasbaar is voor niet-gemodificeerde technische lignines, maar ook voor gefractioneerde lignines, geöxideerde lignines en gedepolymeriseerde lignine fracties die zijn bestudeerd in dit proefschrift (**Hoofdstukken 3-5**). Een belangrijk nadeel van deze methode is echter dat de moleculmassa's worden berekend relatief ten opzichte van de molmassa's van gesulfoneerde polystyrenen. Door gebruik te maken van MALDI-TOF-MS, voorafgegaan door de fractionering van lignine, werden echter niet alle problemen opgelost die meespelen met de bepaling van de absolute molmassa van lignine. Daarom zal de zoektocht naar een routinematige analyse van de absolute molmassa van lignine worden gecontinueerd.

Vervolgens wordt in **Hoofdstuk 3** gedemonstreerd dat een hoofdcomponentenanalyse (PCA) model, dat gebaseerd is op kwantificeerbare analytische chemische data, de geschiktheid van een technische lignine of zijn fractie in een houtlijm applicatie voorspelt (**Hoofdstukken 3 en 4**). Dit resulteerde in een classificatie van de bestudeerde lignines en hun fracties in verschillende clusters, gebaseerd op de structuur-afhankelijke eigenschappen. Verder laten de resultaten in **Hoofdstuk 4** zien dat lignines die voldoende reactive groepen, een gemiddelde molmassa en een laag niveau aan onzuiverheden, zoals koolhydraten en as, bevatten veelbelovende kandidaten zijn voor de ontwikkeling van lignine gebaseerde houtlijmen. Zowel voldoende lignine reactiviteit als formaldehyde-vrije crosslinkers zijn nodig om emissie-vrije lijmen te produceren. Periodate oxidatie is een interessante route om de lignine reactiviteit te vergroten, maar de resultaten in dit proefschrift lieten zien dat de lignine reactiviteit verder geoptimaliseerd moet worden. Alternatieven voor formaldehyde-houdende lijm worden momenteel onderzocht. Een combinatie van geactiveerde lignine en furaan componenten geeft een interessante houtlijm die nog verder ontwikkeld moet worden.

In **Hoofdstuk 5** worden de resultaten van een nieuw proces in een supercritisch oplosmiddel voor de depolymerisatie van lignine in aromatische chemicaliën beschreven. In een niet-toxisch "groen" oplosmiddel, welke bestaat uit kooldioxide/aceton/water, werd lignine omgezet in een gedepolymeriseerde lignine olie. In deze olie komen een aantal aromatische monomere componenten voor in relatief hoge concentraties tot maximaal 3.6% (berekend op droge lignine) tesamen met
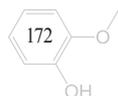
oligomere lignine structuren. Gedurende het proces werden de gedepolymeriseerde aromaten gescheiden van de gevormde "char" door drukverlaging zonder dat er een additionele condensor nodig is om de producten te verzamelen. De componenten kunnen verder worden geïsoleerd met behulp van scheidingstechnologieën om gezuiverde hoogwaardige fijnchemicaliën te verkrijgen. De lignine olie zou bijvoorbeeld kunnen worden toegepast als een houtlijm. Voor procesoptimalisatie moet vooral speciale aandacht gegeven worden aan het verder reduceren van de vorming van "char". In **Hoofdstuk 6** worden een aantal mogelijke routes hiervoor besproken.

De resultaten die in dit proefschrift worden beschreven, leveren een bijdrage aan een beter begrip van de ligninestructuur, mogelijkheden voor ligninechemie en applicatie ontwikkeling. Gebaseerd op deze resultaten is het zeer waarschijnlijk dat een commerciële houtlijm of hars bestaande uit lignine sneller kan worden verwacht dan productie van aromatische chemicaliën uit lignine. Voor een houtlijm kunnen een aantal technische lignines worden gebruikt, die direct worden verkregen uit een pulp- of bioraffinage-proces, maar deze lignines moeten wel geactiveerd worden en efficiënt worden vernet om een emissie-vrije lijm te verkrijgen. Mijn onderzoek laat zien dat dit mogelijk is met periodaat en het gebruik van furfuryl alcohol.

Voor de ontwikkeling van een economisch levensvatbare lignine valorisatieroute voor de productie van aromatische chemicaliën, is nog meer onderzoek nodig om de optimale procescondities, geschikte waterstofdonors en/of bioraffinage-katalysatoren te vinden. Ook zal de commerciële productie van lignines met een hoge zuiverheid nodig zijn om dit proces verder te ontwikkelen.

De resultaten die in dit proefschrift worden gepresenteerd zullen tesamen met de reeds gestarte wereldwijde activiteiten bijdragen aan de verwachte toenemende commerciële toepassing van lignine. Hoewel er concurrerende routes voor de productie van aromaten, die beginnen met koolhydraten, worden onderzocht, lijkt het goed onderbouwd dat lignine een hernieuwbare aromatische grondstof voor de chemische industrie zal worden.

Acknowledgements / dankwoord

Ruim 6 jaar geleden besprak ik met Ed de mogelijkheid om van mijn lignine onderzoek
een promotieonderzoek te maken. Hiervoor moesten nog wel een aantal geschikte
projecten worden geacquireerd om het onderzoek te financieren en dat is gelukt.
Daarnaast moesten we op zoek naar een promotor en een begeleidingsteam. Deze waren
snel gevonden met Johan als promotor en Göran, als lignine professor, Ed, Elinor en Jan
als persoonlijke begeleiders vanuit WUR. Johan, ik ben je heel dankbaar dat ik je toen
als promotor heb kunnen enthousiasmeren, hoewel lignine geen hoge prioriteit had in
het Wageningse onderzoek. Gedurende de jaren heb ik gemerkt dat ik wel degelijk mijn
enthousiasme kon overbrengen op jou, maar ook op anderen binnen WU-VPP. En het
doet me deugd om ook in jouw presentaties en bedrijfscontacten het lignine onderwerp
regelmatig voorbij te zien komen. Johan, ik heb veel van je geleerd, met name om ook
"out of the box" te denken, en je hield met altijd scherp bij het onderzoek. Dear Göran,
your contribution to this lignin valorisation project was essential to make it successful.
Although we did not met on a regular basis during the years, our meetings and
communications were always fruitful to keep on track. I still remember my stay in
Stockholm in 2006 at your lab as one of the necessary steps to make this PhD trip a
success. Your colleagues, in particular Jiebing Li and Liming Zhang, gave me a warm
welcome, teached me how to characterize lignin in a more detailed manner, rent me a
bike to discover Stockholm and learn me that Swedish people love sweet "fika". For
this occasion I brought the famous "stroopwafels" to Sweden. We will keep in contact!
Jan, samen met jou ben ik bij ATO op dezelfde dag begonnen en ik vind het een eer om
jou als co-promotor te hebben. Ik heb me er altijd over verbaasd hoe snel jij artikelen
kunt bekijken en kunt voorzien van een hoop kritische noten, die (helaas voor mij)
meestal zeer relevant waren. Daarnaast was je altijd een prettige collega, mentor en
inspirator voor dit onderzoek. Ed, zoals gezegd, je stond aan het begin van dit
promotietraject en daar ben ik je "eeuwig" dankbaar voor. We hadden al jaren
samengewerkt in het lignine veld en ik heb daar veel van kunnen leren en ik denk dat
we een goede tandem vormden met vele gezellige momenten. Totdat je besloot om er
tussenuit te knijpen naar Avantium, maar gelukkig ben je altijd betrokken gebleven bij
mijn onderzoek. Daarvoor ook mijn oprechte dank, want ik weet dat je nieuwe baan
veel tijd vergt. Daarnaast ben ik heel blij dat je mijn paranimf wilt zijn.

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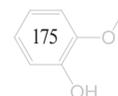
Elinor, naast Johan mijn begeleider vanuit WU-VPP, en ook iemand die me altijd kritisch heeft gehouden ten aanzien van de chemie in het lignine onderzoek. Daarnaast kon je me altijd enorm motiveren met je optimische view en heb je als English-native speaker een belangrijke bijdrage gehad in het succesvol afronden van de artikelen.Voor dit promotieonderzoek waren een aantal projecten belangrijk om te beginnen Ecobinders waarin ik samen met Wim van Rhijn en Philippe Willems de coordinatie verzorgde. Wim, ik ben je heel erkentelijk dat je dit project hebt willen coordineren en we hebben in deze jaren een goede vriendschappelijke band opgebouwd. Ik hoop dat we elkaar in de toekomst nog vaker tegenkomen en wellicht in gezamenlijke projecten. Philippe, ik denk nog vaak terug aan de gezellige bourgondische momenten in België en projectmeetings op leuke Europese locaties. Ik heb je leren kennen als een goede business developer en inspirator voor bedrijven om biobased ontwikkelingen daadwerkelijk uit te voeren. In Rotterdam zeggen ze: "Geen woorden, maar daden" (dit zal mijn familie leuk vinden!) en die slogan past bij jou. Daarnaast wil ik ook Bôke Tjeerdsma bedanken voor zijn bijdrage aan dit project en de gezamenlijke organisatie van een meeting in Holland zal me altijd bij blijven. We kennen elkaar al geruime tijd en ik hoop je nog vaak te ontmoeten.

Een ander belangrijk project was LignoValue waarvoor ik als eerste de grondlegger Hans Reith van ECN wil bedanken. Door jou ben ik destijds gevraagd om samen LignoValue op te tuigen en daarna te coordineren. Vanaf het eerste moment heb ik je leren kennen als een geweldige inspirator met een enorme betrokkenheid om een dergelijke activiteit tot een goed einde te brengen. Hiervoor ben ik je zeer erkentelijk. Na het binnenhalen van LignoValue verdween je naar de achtergrond, omdat je Biosynergy ging leiden, maar ik heb je in dat project ook nog veel ontmoet. Hans, bedankt en ik denk dat we af ten toe nog wel een Amsterdam's biertje zullen drinken. Gelukkig blijven we de komende jaren samenwerken in Biocore.

Verder wil ik de andere collega's van ECN bedanken, met name Wouter en Paul, waarmee ik gedurende LignoValue ook een hele goede vriendschappelijke band heb opgebouwd. Het was altijd erg prettig om samen op reis te gaan en naast werk ook tijd te hebben om prive gesprekken te voeren. Ook met jullie zal ik de komende jaren nog blijven samenwerken. Daarnaast wil ik de andere projectdeelnemers bedanken te beginnen met Erik Heeres, Arjan Kloekhorst, Tony Bridgwater en Daniël Nowakowski. 176 Verder hadden we binnen LignoValue een prima samenwerking tussen ECN, RUG,
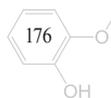
Aston University, WU-VPP, Avantium, Bayer, BASF en WUR-FBR. Daarnaast is Peter Berben van BASF mijn opponent vanuit de industrie. Hiervoor wil ik hem hartelijk danken. Gentlemen, we had a succesful project and I would like to thank you all for your valuable contribution during the projectmeetings, visits and cooperation. In het bijzonder wil ik Arjan bedanken voor zijn bijdrage aan Hoofdstuk 5, waarin hij de pyrolyse GC-MS analyses heeft uitgevoerd en ik "zijn" GC mocht gebruiken voor gasanalyses. Paul was de eerste die promoveerde, met resultaten vanuit LignoValue, en ik hoop dat jij de laatste wordt. Succes! Hoewel mijn bijdrage aan het Biosynergy project beperkt was vergeleken met Lignovalue wil ik wel alle onderzoekers van dit project bedanken. Uiteraard gaat mijn grootste dank uit naar coordinator Hans Reith.

Ik heb nu al veel mensen bedankt, maar heel veel dank gaat natuurlijk uit naar mijn Wageningse collega's. Dit zijn er veel geweest in de loop der jaren en zonder iemand te kort te willen doen wil ik er een aantal bedanken. Ten eerste Wouter met wie ik al jaren samenwerk in verschillende projecten en ook in dit lignine promotietraject heb je een essentiële rol gespeeld. Je wist altijd oplossingen te vinden om een analyse uit te voeren of te verbeteren, een proces te optimaliseren of om een geintje uit te halen. Bedankt voor je support en ik ben heel blij dat je mijn paranimf wilt zijn. Daarnaast wil ik Hans v.d.K., Jacinta, Edwin, Willem S., Jan-Gerard, Paulien, Wim, Nicole, Richard O., Steef, Jeroen, Jocco, Gülden, Martien, René, Harriette, Rob, Ronald, Wolter, Bert, Arie, Daan, Jacco, Willem V., Frits, Jan S., Hans M., Jacqueline, Herman, Guus, Eric (statistiek) en Barend (NMR) bedanken voor de geweldige sfeer en collegialiteit. Natuurlijk wil ik ook mijn collega's van de Food BU Arnoud, Kees, Rian en Sander bedanken voor hun belangrijke bijdrage aan de supercritische lignine experimenten en analyses. Bedankt! Hoewel ik geen "echte" AIO was binnen de leerstoel WU-VPP, heb ik me wel thuis gevoeld tussen de veelal jonge collega's. Bedankt voor de gezellige tijd, het bijzondere kerstdiner bij Ruud thuis en succes met jullie (promotie)onderzoek. Verder wil ik Gerda enorm bedanken voor het beantwoorden van mijn vragen, regelen van vergaderingen en het opsturen van documenten. Je stond altijd klaar om iets te doen.

Bert en Wim (Preduxion) wil ik erg bedanken voor het cover ontwerp en het drukwerk.

Tot slot wil ik Ellis bedanken voor de enorme steun en liefde die zij heeft gegeven om dit gerealiseerd te krijgen. Ook Kay en Ryan worden bedankt; papa krijgt nu meer tijd voor jullie! Ook mijn ouders en mijn schoonouders hebben een belangrijke bijdrage geleverd door veelvuldig als oppas te fungeren. Super bedankt!   Richard

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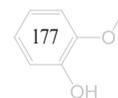
Curriculum vitae

Richard Gosselink was born on September 27, 1967 in Hengelo (Gld), The Netherlands. After graduation at the secondary school "Het Ludgercollege" in Doetinchem, he started in 1985 the study analytical chemistry at the University of Applied Science "Hogeschool Arnhem Nijmegen". In the fourth year he did his internship at the KEMA in Arnhem on the subject "Recovery and analysis of polycyclic aromatic compounds in coal gasification residues". After graduation in 1989 he obtained his BSc and he served the army for about one year. After that, he started his career in Wageningen in 1990 at the research institute called "Agrotechnological Research Institute (ATO-DLO)" and is still working there. The institute is nowadays called "Food & Biobased Research" and is part of Wageningen University and Research Centre. He started as a research assistant and became researcher and project manager after about seven years. His field of expertise is biomass characterisation, biomass fractionation and valorization of cellulose and lignin. The last ten years he focuses on the theme lignin valorization. He co-ordinated the EUROLIGNIN network (2002-2005), a FP6 EU project called ECOBINDERS (2005-2008), a Dutch funded biorefinery project focussing on lignin valorisation (LIGNOVALUE, 2006-2010) and is WP leader in a recently started FP7 EU biorefinery project (BIOCORE, 2010-2014). He is also board member of the International Lignin Institute (ILI) from 2005 and initiated in 2010 the Wageningen UR Lignin Platform. He acts also as a reviewer for several international journals.

From November 2005 until December 2011 he worked on his PhD-research at the Food & Biobased Research institute (WUR-FBR) in close collaboration with Wageningen UR Valorisation of Plant Production Chains (WU-VPP). For this PhD-research, he worked for three weeks at the Royal Institute of Technology, KTH, Stockholm, Sweden in 2006. The results of this PhD-research are described in this thesis.
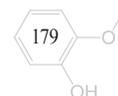
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Gosselink, R.J.A., Teunissen, W., van Dam, J.E.G., de Jong, E., Gellerstedt, G., Scott, E.L., Sanders, J.P.M. Lignin depolymerisation in supercritical carbon dioxide/acetone/water fluid for the production of aromatic chemicals. Bioresour. Technol. *(submitted)*

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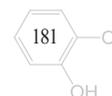
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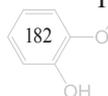
Overview of completed training activities

Discipline specific activities

Meetings and workshops ECOBINDERS, 2005-2008

Meetings and workshops COST E41, 2005-2008

Short Term Scientific Mission, COST E41, KTH, Stockholm, Sweden, 2006

Biomassa, kans of bedreiging?, Wageningen, 2007

Lignin conference, ILI Forum 8, Rome, Italy, 2007

Renewable Resources and Biorefineries 4, Rotterdam, 2008

European Workshop on Lignocellulosics and Pulp 10, Stockholm, Sweden, 2008

Course Renewable Resources for the Bulkchemical Industry, Wageningen, 2010

Renewable Resources and Biorefineries 6, Düsseldorf, Germany, 2010

European Workshop on Lignocellulosics and Pulp 11, Hamburg, Germany, 2010

Course Biorefinery, Amsterdam, 2010

International Biomass Valorisation Congress, Amsterdam, 2010

General courses

Project Management, Wageningen, 2001

Presentation Skills, Wageningen, 2001

Advanced Project Management, Wageningen, 2002

Statistics, Wageningen, 2002

Acquisition Training / Commerciële vaardigheden, Wageningen, 2003

Scientific Writing, WGS, Wageningen, 2008

Mini workshop 'How to write a world-class paper', Wageningen, 2010

Optionals

Preparation of PhD research proposal, Wageningen, 2005

Participation in theme and group meetings LSG WU-VPP, Wageningen, 2006-2010

Organization International Biomass Valorisation Congress, Amsterdam, 2010

Glossary

α-position: see Figure G.1 first position in phenylpropane unit

β−position: see Figure G.1 second position in phenylpropane unit

β−O−4 linkage: On the β−position linked with an ether bond to the 4-position on the second aromatic ring (Figure G.1)

γ-position: see Figure G.1 third position in phenylpropane unit
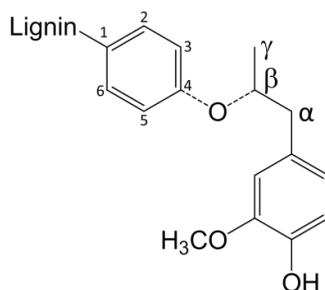
Figure G.1 Guaiacyl unit in lignin with positions in propane chain and β−O−4 linkage.

γ-Al_2O_3: alumiumoxide support for catalyst

[η]: intrinsic viscosity, it is a measure of a solute's contribution to the viscosity η of a solution

2D-NMR: two dimensional NMR

2G bioethanol: Second Generation bioethanol orginated from lignocellulosic biomass via so-called second generation technologies

Ag-complexes: silver-complexes

barrel: oil barrel, 42 US gallons, 159.0 L

BCD: Base-Catalyzed Depolymerization

BTX: Benzene, Toluene, Xylene

C_α: α-position

^{13}C NMR: Carbon Nuclear Magnetic Resonance
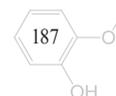
C2-C3 gases: gases with 2 or 3 carbons, e.g. ethylene or propylene

C5 sugars: hemicellulose sugars, e.g. xylose and arabinose

C-C bonds: Carbon-Carbon bonds in an organic structure

CHCA: α-cyano-4-hydroxycinnamic acid

CH_3OH: methanol

CIMV: Compagnie Industrielle de la Matière Végétale, French wheat straw biorefinery company

CO: carbon monoxide

CO_2: carbon dioxide

C-O-C: Carbon-Oxygen-Carbon bonds in an organic structure

CoMo: Cobalt molybdenum

DDGS: Distiller's Dried Grains and Solubles, residue in first generation bio-ethanol plant

DHB: dihydroxybenzoic acid

Diels-Alder reaction: The Diels–Alder reaction is an organic chemical reaction (specifically, a cycloaddition) between a conjugated diene and a substituted alkene, commonly termed the dienophile, to form a substituted cyclohexene system

DMAc: dimethylacetamide, organic solvent

DMF: dimethylformamide, organic solvent

DMSO: dimethylsulfoxide, organic solvent

DSC: Differential Scanning Calorimetry

EOS: Energy Research Strategy funding body of the Dutch Ministry of Economic Affairs, Agriculture and Innovation

ESI-MS: Electrospray Ionization Mass Spectrometry

EtOH: ethanol

F: fraction

FA: furfuryl alcohol

$FeCl_3$: Iron(III)chloride

FT-IR: Fourier Transform InfraRed spectroscopy
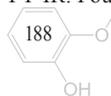
GC: Gas Chromatography

G-unit: Guaiacyl unit

H: hydrogen atom

H_2: hydrogen gas

He: helium gas

HCl: hydrochloric acid

HDO: Hydrodeoxygenation. Removal of oxygen-containing groups/compounds.

HHV: Higher heating value (HHV) is the potential combustion energy when water vapor from combustion is condensed to recover the latent heat of vaporization. Lower heating value (LHV) is the potential combustion energy when water vapour from combustion is not condensed.

HIO_3: hydrogen iodate, iodic acid

HPAEC-PAD: High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection

HPLC: High Performance Liquid Chromatography

H-unit: coumaryl-unit

HW: Hardwood

HW SoL: Hardwood Soda Lignin

HZSM-5 catalyst: zeolite catalyst in acidic form

KL: Kraft Lignin

KOH: potassium hydroxide

KTH: Royal Institute of Technology, Stockholm, Sweden

LC: lignocellulosic biomass

LCCs: Lignin-Carbohydrate Complexes

MALDI(-TOF-MS): Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry

Mark-Houwink relation: $[\eta]=KM^{\alpha}$, $[\eta]$: intrinsic viscosity, α and K: Mark-Houwink parameters depend on the particular polymer-solvent system, M: molecular weight

MDI: methylene diphenyl diisocyanate, crosslinking agent in polyurethane

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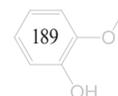
M_n: number average molar mass

M_p: molar mass at peak maximum

M_w: weight average molar mass

MWL: Milled Wood Lignin, lignin obtained via dioxane/water extraction of wood

$NaIO_4$: sodium (meta)periodate

NaOH: sodium hydroxide

$NiCl_2$: nickel(II)chloride

NiMo: nickelmolybdenum

NIST: The National Institute of Standards and Technology, US

NREL: National Renewable Energy Laboratory, US

OH groups: hydroxyl groups

PCA: Principle Component Analysis, is a mathematical procedure for exploratory data analysis and for making predictive models (this thesis)

$PdCl_3.3H_2O$: palladium(III)chloride.hydrate

Polydispersity: M_w/M_n, reflects the broad range of molecular size of a polymer

Pd: palladium

PEG: polyethylene glycol

PET: polyethylene terephtalate

PF: phenol-formaldehyde resin

Pt: platinum

PTA: purified terephtalic acid

Pyrolysis: thermal treatment in absence of oxygen

RA: retinoic acid

R&D: Research & Development

RI: Reflective Index (detection)

Round Robin: any activity in which a group of researchers is interacting for example on the evaluation and validation of an analytical methodology
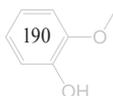
RTD: Research and Technology Development

ScCO$_2$: carbon dioxide under supercritical conditions, temperature >31°C and pressure >7.4MPa

SEC: Size Exclusion Chromatography

SoL: Soda Lignin

S-unit: Syringyl unit

SVDB: styrene-divinylbenzene copolymer

SW: Softwood

SW KL (SKL): Softwood Kraft Lignin

TCD: Thermal Conductivity Detector

TGA: Thermogravimetric Analysis

THF: Tetrahydrofuran

TSK gel: ethylene glycol-methacrylate copolymers gel

UV: Ultraviolet light

ZSM-5: Zeolite catalyst

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