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Dissertation

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Impact of type and pretreatment of lignocellulosics on lignin and pulp properties

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Übereinstimmungserklärung:

Die Übereinstimmung dieses Exemplars mit dem Original der Dissertation zum Thema: „Impact of type and pretreatment of lignocellulosics on lignin and pulp properties“ wird hiermit bestätigt.

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Abstract

The depletion of fossil fuels and the need to deal with climate change lead to an increasing interest in renewable resources. Lignocellulosic biomass in general, and agricultural residues in particular, could serve as an excellent starting material for the production of cellulose, basic chemicals, lignin and bioethanol in a biorefinery framework as they are abundant, do not compete with food production and are distributed worldwide. Two factors considerably influence the composition and properties of biorefinery products: biomass feedstock and pretreatment process. Their influence on the separability of raw material into a carbohydrate and lignin fraction as well as the composition and properties of these products are objectives of this study. Hereby, the focus is on lignin, as its structure is particularly dependent on the aforementioned factors complicating its further utilisation.

Different agricultural biomass namely barley straw, coconut shell powder, hemp shives, horse manure, maize straw, miscanthus, oat husk, pretreated alfalfa, rape straw, sunflower stalks, tomato stalks and wheat straw were investigated regarding their suitability for lignocellulose separation using alkaline soda pulping. Best separation into a carbohydrate and lignin fraction was achieved for pretreated alfalfa, miscanthus, wheat and barley straw. The purity of the obtained lignin fractions varied in the wide range of 57% KLASON-lignin content for sunflower stalks and 81% for pretreated alfalfa prior to further purification by dialysis. Lignin fractions were characterised by means of FTIR spectroscopy, elementary analysis, thioacidolysis, size-exclusion-chromatography, thermodesorption, differential scanning calorimetry and different wet chemical methods for determination of functional groups. Lignins could be classified into groups, within which they show similar characteristics: (A) horse manure, rape straw, (B) sunflower stalks, tomato stalks, (C) barley-, maize- and wheat straw and (D) hemp shives, miscanthus. In addition, promising lignin candidates were found for several applications. Thus, by screening different agricultural residues, it is possible to choose a specific raw material, in order to produce lignin with desired properties and functionalities.

The effect of the pretreatment process was studied based on wheat straw. It was subjected to conventional alkaline pulping, microwave-assisted alkaline pulping and organosolv pulping using formic acid and hydrogen peroxide. Pulping parameters were varied in order to attain best possible separation into a carbohydrate and lignin fraction. Of the varied parameters the concentration of both sodium hydroxide and formic acid has the highest impact on yield and purity of the products for alkaline and organosolv pulping, respectively. It additionally influences the content of functional groups of the lignin fraction and is hence, an important parameter for lignin customisation for subsequent utilisation. Furthermore, the possibility of reducing pulping time by using microwave-assisted pulping instead of conventional alkaline pulping is promising, as the obtained carbohydrate fraction has a low intrinsic viscosity, which may enhance enzymatic hydrolysis.

Contents

List of abbreviations

AM	microwave-assisted alkaline pulping
AP	conventional alkaline pulping
Ar	aryl
bs	barley straw
CC	cell corner
CD	condensation degree
cf	confer
CI	condensation index
CML	compound middle lamella
cp	coconut shell powder
CrI	crystallinity
DP	degree of polymerisation
DSC	differential scanning calorimetry
eg	exempli gratia
FTIR	Fourier transformation infrared
G	guaiacyl
GC	gas chromatography
H	p-hydroxyphenyl
hm	horse manure
hs	hemp shives
IAT	Indulin AT
mi	miscanthus
MS	mass spectrometry

ms	maize straw
MWL	milled wood lignin
oh	oat husks
OP	organosolv pulping
P	primary wall
pa	pretreated alfalfa
Prec. prod.	precipitation product
PS	polysaccharide
QM	quinone methide
rs	rape straw
S	syringyl
S1, S2, S3	secondary wall layers
SEC	size exclusion chromatography
SEM	scanning electron microscopy
ss	sunflower stalks
ts	tomato stalks
VOC	volatile organic compound
ws	wheat straw
XRD	x-ray diffraction

1. Introduction

Our todays world strongly depends on fossil fuels to meet the needs of electricity, transportation and platform chemicals. However, global reserves are very limited. Already by the year 2079 coal will be the only fossil fuel remaining, as oil and gas will be depleted assuming consumption at 2006 rates [195]. In addition, the need to deal with climate change requires that large portions of these fossil reserves should not be used. This is of particular urgency, as CO_2 emission is predicted to rise by 20% until 2040 leading to a global temperature increase of 3.6 °C [108, 151]. Thus, many governments have announced new policies in order to keep the global temperature rise below 2 °C [108].

Whereas meeting electricity needs may be possible by solar, wind and water power, a sustainable transportation sector is expected to rely on biomass [147]. Ethanol, a high-octane fuel, can be obtained by conversion of biomass as illustrated in Figure 1.1. By this, CO_2 emission is reduced compared to burning fossil fuels, as no more is released than was absorbed during growth of plants. Thus, the total contribution of CO_2 to the atmosphere is close to zero assuming a sustainable supply of biomass feedstock, short transfer distances and high efficiency of the biorefinery [222]. This can be achieved by utilisation of lignocellulosic biomass such as wood and residual non-food parts of crops. Lignocellulosics consist of cellulose and hemicelluloses, making up the carbohydrate fraction, as well as lignin, a polyphenolic material. To ensure high efficiency, their complete valorisation is essential. In order to achieve this, a treatment of biomass is crucial prior to the conversion

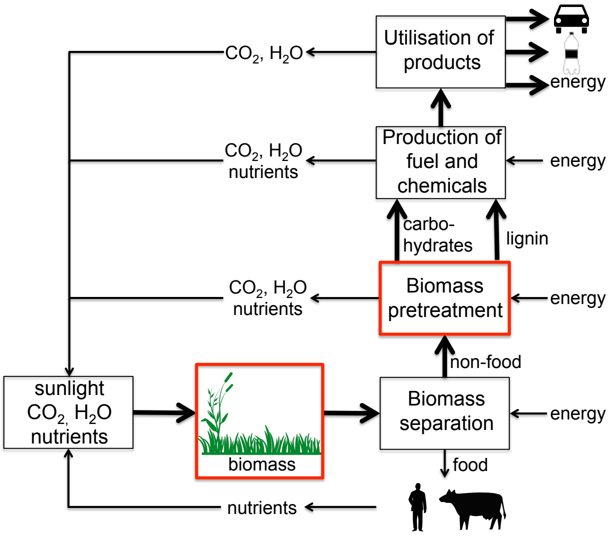


Figure 1.1.: Sustainable production of fuels and platform chemicals from biomass in an integrated biomass production-conversion system (modified from Huber, Iborra and Corma [103])

of carbohydrates to ethanol by enzymatic hydrolysis and fermentation. This pretreatment not only increases the accessibility of the carbohydrate fraction to enzymes, but it also removes potential enzyme inhibitors, such as lignin [206]. By this, separate fractions for individual usage are obtained. Whereas ethanol can be produced from the carbohydrate fraction, the utilisation of lignin nowadays is in most cases limited to an energetic use. However, as it is the only renewable source of aromatic compounds increased efforts are made to include it in several value-added applications to additionally meet the need of platform chemicals and other up to now petroleum based products.
Major challenges for an efficient biorefinery producing value-added products are:

1. Efficient separation of biomass into a carbohydrate and lignin fraction
2. Control of product composition and properties.

The objective of this study is to elucidate the influence of biorefinery parameters to cope with the mentioned tasks. Two key factors are proposed to considerably influence the outcome of the process: biomass feedstock and pretreatment process.

The biomass feedstock of a future lignocellulosic biorefinery may vary to ensure sustainable supply throughout the whole year. In addition, different biomasses may have to be processed simultaneously resulting in products with varying qualities and properties. Though extensive studies have been conducted on different lignocellulosic raw materials, the results reported are not comparable due to differences in pulping procedures and/or parameters. Furthermore, the interest in lignin outside the context of the pulp and paper industry is comparatively new (published items on this topic have doubled from 2010 to 2014 [210]). Thus, there is still a general lack of data on the composition of the lignin fraction with regard to different biomass feedstocks. The focus of this study is on agricultural residues, such as straws and stalks, and hence on herbaceous biomass. Their use is advantageous as they are abundant and do not compete with food production.

The second key parameter influencing the outcome of the biorefinery process is the pretreatment of biomass. One of the advantages of using herbaceous biomass is that traditional pretreatment methods used in pulp mills, like sulphate (Kraft) and sulphite pulping, are not necessary. Thus, sulphur-free and more environment-friendly procedures can be applied. In addition, this exhibits properties of the lignin fraction different to the common Kraft-lignin enabling new possibilities for its utilisation.

Both key parameters biomass feedstock and pretreatment process set the approach of this study as follows:

1. Variation of biomass feedstock using the same pretreatment process (Chapter 3)
   - Separation of twelve agricultural herbaceous biomasses
   - Separation by alkaline soda pulping as pretreatment process based on its high technological readiness and the possibility for integrating a biorefinery concept in pulp mills
   - Investigation of pulp composition
   - Investigation of lignin composition and detailed study on structural features to reveal lignins which are either similar and hence, substitutable among one another, or show unique features, which are promising for certain applications

2. Variation of pretreatment process using the same biomass feedstock (Chapter 4)
    - Separation of wheat straw, as it is the most abundant agricultural residue in Europe
    - Separation by alkaline soda pulping, organosolv (formic acid and hydrogen peroxide) and microwave-assisted alkaline pulping
    - Investigation of pulp composition
    - Investigation of lignin composition and detailed study on structural features to reveal lignins, which are promising for certain applications

By this, extended knowledge is acquired on the influence of raw material and pretreatments on product properties, especially with regard to lignin, providing the basis for a highly efficient lignocellulosic biorefinery.

2. Lignocellulosic biomass: feedstock, composition, separation and products

The following section will elucidate the need for utilising renewable biomass as well as its global availability with regard to agricultural lignocellulosic waste. The composition of relevant biomass constituents and their behaviour during different pretreatment procedures will be discussed. Finally, utilisation possibilities for products obtained from lignocellulosic biomass will be outlined.

2.1. Lignocellulosic biomass feedstock

Lignocellulosic biorefineries utilise biomass consisting of the residual non-edible parts of crops or other non-food sources. Some challenges, hindering commercialisation are related to feedstock availability and processing [222]. This work focusses on herbaceous agricultural residue, though other lignocellulosic feedstock, such as woody biomass, will also play a role in future biorefinery concepts [222]. Table 2.1 lists important crops and their residues. Crop production data is based on information collected and published by the Food and Agriculture Organization of the United Nations (FAO) [77] for the year 2013. It becomes clear that agricultural feedstock varies strongly, depending on the investigated region. Whereas in Europe and North America different kinds of straw predominate, bagasse and stalks account for the major part in South America and Africa. Thus, a future biorefinery concept has to be very flexible towards biomass feedstock or different concepts need to be developed depending on the surroundings. Globally, sugar cane bagasse and maize straw can produce the largest amounts of ethanol from a single biomass feedstock.

By using typical residue to crop ratios, it is possible to estimate the global quantity of agricultural by-products, such as straw, stalks or hulls. However, only 50 to 80% of accruing straw is collectable without risking humus reduction [237]. Thus, for calculation of the amount of straw and stalks available, their total amount was multiplied by a factor of 0.5 to account for sustainable land management. By this, approximately 4040 Mt of agricultural residues are available worldwide per year. Assuming an average lignin content of 20%, this results in 808 Mt lignin.

Table 2.1.: Quantity of major agricultural by-products based on crop production data published by FAO [77] (if not indicated otherwise) and their potential ethanol production in 2013

Crop	Residue	Ratio	Production $[\mathrm{Mt\cdot a^{-1}}]$		Potential bioethanol production [2]			
		residue/crop	Crop	Residue [1]	from C6 $[\mathrm{L\cdot t^{-1}}]$	from C5 $[\mathrm{L\cdot t^{-1}}]$	Sum $[\mathrm{L\cdot t^{-1}}]$	$[\mathrm{GL\cdot a^{-1}}]$
Europe								
Wheat	Straw	2.1[119]	225.7	237.0	240	149	389	92
Sugar beet	Pulp	0.3	167.5	50.3	156	65	221	11
Maize	Straw	2.9[119]	118.9	172.4	253	127	380	65
Potato	Peel	0.04[157]	114.3	4.6	358	76	434	2
Barley	Straw	2.1[119]	85.9	90.2	271	107	378	34
Rye	Straw	2.1[119]	14.9	15.6	216	142	358	6
Oat	Straw	2.1[119]	14.5	15.2	268	142	410	6
Sunflower	Stalk	3.7[157]	31.8	58.8	239	111	350	21
Rape	Straw	3.7[157]	25.6	47.4	238	107	344	16
Asia								
Sugar cane	Bagasse	0.6	747.0	448.2	258	157	415	186
Rice	Straw	1.8[119]	671.0	603.9	280	125	405	245
Wheat	Straw	2.1[119]	320.0	336.0	240	149	389	131
Maize	Straw	2.9[119]	305.1	442.4	253	127	380	168
Coconut	Shell	0.1[119]	51.9	5.2	173	169	342	2
North America								
Maize	Straw	2.9[119]	367.9	533.5	253	127	380	203
Wheat	Straw	2.1[119]	95.5	100.3	240	149	389	39
Soybean	Straw	2.9	94.7	137.3	244	81	325	45
	Pulp	0.34	94.7	32.2	227	236	463	15
Sugar beet	Pulp	0.3	30.4	9.1	156	65	220	2
Sugar cane	Bagasse	0.6[119]	27.9	16.7	258	157	415	7
Sorghum	Straw	1.5[119]	9.9	7.4	227	156	383	3

[1] For straw or stalk, the amount available is considered to be only 50% of the accruing residue in order to ensure sustainable soil management.

[2] A conversion rate of 100% is assumed.

Continued: Quantity of major agricultural by-products based on crop production data published by FAO [77] (if not indicated otherwise) and their potential ethanol production in 2013

Crop	Residue	Ratio residue/crop	Production [Mt·a^{-1}] Crop	Production [Mt·a^{-1}] Residue [1]	Potential bioethanol production [2] from C6 [L·t^{-1}]	from C5 [L·t^{-1}]	Sum [L·t^{-1}]	[GL·a^{-1}]
South America								
Sugar cane	Bagasse	0.6[119]	868.7	521.2	258	156	414	216
Soybean	Straw	2.9	145.9	211.6	244	81	325	69
	Pulp	0.34	145.9	49.6	227	236	463	23
Maize	Straw	2.9[119]	127.0	184.2	253	127	380	70
Cassava	Stalk	0.2[119]	28.8	2.9	222	92	314	1
	Peel	0.02[119]	28.8	0.6	426	8	435	0.3
Africa								
Cassava	Stalk	0.2[119]	157.7	15.8	222	92	314	5
	Peel	0.02[119]	157.7	3.2	426	8	435	1
Sugar cane	Bagasse	0.6[119]	97.4	58.4	258	156	414	24
Maize	Straw	2.9	71.0	103.0	253	127	380	39
Yam	Peel	0.02	60.6	1.2	491	4	495	1
Oceania								
Sugar cane	Bagasse	0.6[119]	29.1	17.5	258	156	414	7
Wheat	Straw	2.1[119]	23.3	24.5	240	149	389	10
Barley	Straw	2.1[119]	7.9	8.3	271	107	378	3
Rape	Straw	3.7	4.1	7.6	238	106	344	3
World								
Sugar cane	Bagasse	0.6[119]	1911.1	1146.7	258	156	414	475
Maize	Straw	2.9[119]	1018.1	1476.2	253	127	380	560
Rice	Straw	1.8[119]	740.9	666.8	280	125	405	270
Wheat	Straw	2.1[119]	715.9	751.7	240	149	389	293
Sum			4386.0	4041.4				1598

[1] For straw or stalk, the amount available is considered to be only 50% of the accruing residue in order to ensure sustainable soil management.

[2] A conversion rate of 100% is assumed.

Furthermore the content of C6 sugars (sum of glucan, mannan and galactan) as well as C5 sugars (sum of xylan and arabinan) of the agricultural by-products allows the calculation of potential ethanol production based on the following conversions:

$$\mathrm{C_6H_{12}O_6} \longrightarrow 2\,\mathrm{C_2H_5OH} + 2\,\mathrm{CO_2}$$
$$3\,\mathrm{C_5H_{10}O_5} \longrightarrow 5\,\mathrm{C_2H_5OH} + 5\,\mathrm{CO_2}$$

This, coupled with the amount of agricultural residues produced, gives a value for potential annual bioethanol production. Surely, this is only a rough estimate, as it assumes a conversion rate of 100%. Conversion from sugar to ethanol is, however, strongly dependent on biomass pretreatment, hydrolysis and fermentation procedure. Hydrolysis by concentrated sulphuric acid, for example, typically converts approximately 76% of cellulose and 90% of hemicellulose into monomeric sugars [47]. If, as described by Demirbaş [47], the additional loss by fermentation is assumed to be 25% and 50% for cellulose and hemicellulose, respectively, only 57% of original cellulose and 45% of hemicellulose can be converted to ethanol. Although, there are numerous attempts to increase hydrolysis and fermentation efficiency, only half of the in this study (cf. Table 2.1) estimated global potential second generation ethanol production would be realistic at this point, being 800 $\mathrm{GL{\cdot}a^{-1}}$. According to BPs "Statistical Review of World Energy 2014", the worldwide consumption of light distillates consisting of aviation and motor gasoline is 1280 $\mathrm{GL{\cdot}a^{-1}}$ [29]. As 1 L of ethanol could replace 0.72 L of gasoline, approximately 1700 GL ethanol would be needed annually for complete replacement [116].

Thus, feedstock enlargement is needed. This is possible by including biomass from wood, energy crops, algae as well as industrial and municipal waste. In addition, a general increase in agricultural production has to be taken into account. For instance, a study by Kim and Dale [116] estimated the potential bioethanol production similar to this compilation. Their results indicate a potential global ethanol production from lignocellulosic biomass of 442 GL per year. In their study they investigated data sets provided by the Food and Agriculture Organization of the United Nations (FAO) from 1997 to 2001. However, the agricultural production has changed strongly over the past years. The world production of rice, for example, has increased by approximately 20% when compared to data from 2013 [77]. The same trend applies to other major crops such as sugar cane, wheat and maize. On the other hand, additional uses of agricultural residue have to be taken into account. China, for example, produces more than 9 million tons of straw pulp for paper production annually [175]. Other uses are as bedding material and as livestock feed, though straw is known to be a poor-quality feed due to low digestibility and low voluntary intake [234].

Besides biomass availability, the logistic of its supply constitutes another principal challenge. Here, a system needs to be established in a way that maintains the economic and ecological viability while providing the needed quantities of resources [222]. Additional biorefinery challenges and perspectives are discussed in publications from, for instance, Wertz and Bédué [222], Menon and Rao [153] and the International Energy Agency [106].

2.2. Composition and structure of lignocellulosics in herbaceous biomass

The composition and structure of lignocellulosics in herbaceous, hence non-woody, biomass differs from the one in wood. As this study focusses on the utilisation of agricultural residues from herbaceous plants, the following section will present their characteristics in addition to fundamentals on plants and their constituents in general.

2.2.1. Composition

The composition of plants is known to vary depending strongly on species, tissue, age, climate and soil. This is illustrated by the standard deviation of the content of cellulose, hemicellulose, lignin and ash within one type of agricultural residue as shown in Table 2.2. Accordingly, cellulose is the most abundant polymer in plants, followed by hemicelluloses and lignin.

Cellulose

Cellulose accounts for approximately 35-45% of the dry matter of cereal straw (Table 2.2) and is of great importance for the mechanical strength of cell walls and acts as their framework. In addition, it is the most abundant polymer in the world, with an estimated production of $1.5 \cdot 10^{12}$ tons [140].

Table 2.2.: Composition of different agricultural residues in % according to the Energy research Centre of the Netherlands [58], unless otherwise indicated. The number of used datasets is given in round brackets

Raw material	Cellulose	Hemicellulose	Lignin	Extractives	Ash
Barley straw	41.2	23.6	18.0	4.6[a]	6.0
	± 7.5 (3)	± 1.9 (3)	± 4.4 (3)	(1)	± 2.7 (9)
Maize straw	38.3	25.2	14.8	-	6.1
	± 8.4 (5)	± 4.5 (5)	± 2.4 (5)	(0)	± 2.1 (7)
Rape straw	40.5	23.5	20.0	1.9 [a] [113]	6.0
	± 6.4 (2)	± 5.0 (2)	± 0.0 (2)	(1)	± 1.9 (9)
Rice straw	35.7	23.7	14.0	3.4[b] [175]	18.5
	± 5.5 (5)	± 2.1 (4)	± 4.5 (5)	(1)	± 2.6 (13)
Sunflower stalks	36.8	33.5	17.5	6.9[a] [187]	4.6
	± 2.5 (2)	(1)	(1)	(1)	(1)
Wheat straw	37.9	26.8	18.3	5.4[b]	6.4
	± 6.6 (18)	± 5.5 (18)	± 5.1 (18)	(1)	± 2.7 (48)

[a] ethanol extractives

[b] ethanol/toluene extractives

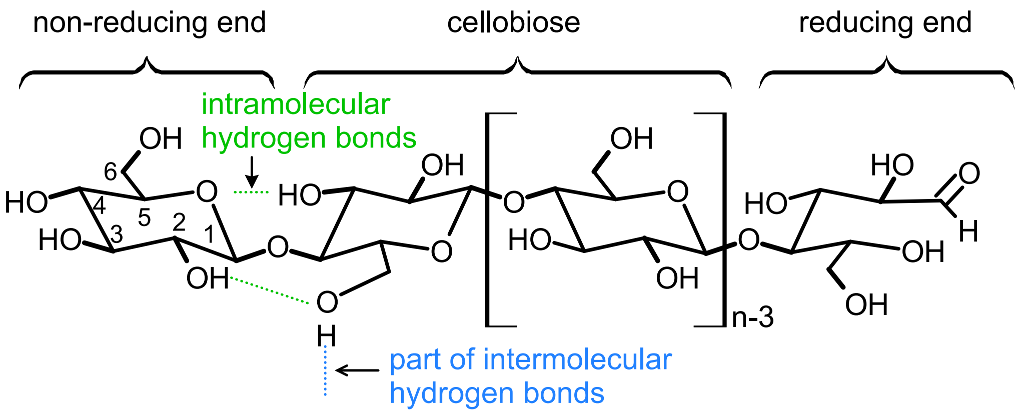
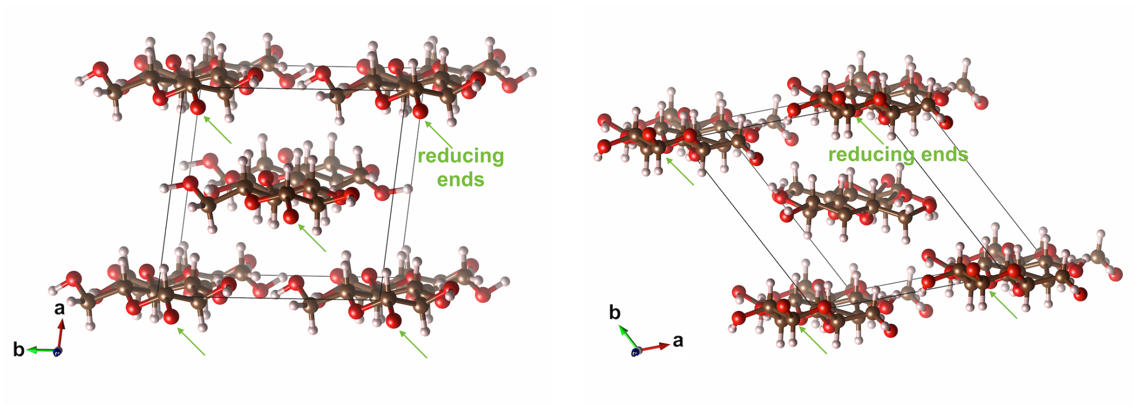


Figure 2.1.: Structure of cellulose (n=DP) (modified from Wagenführ and Scholz [214])

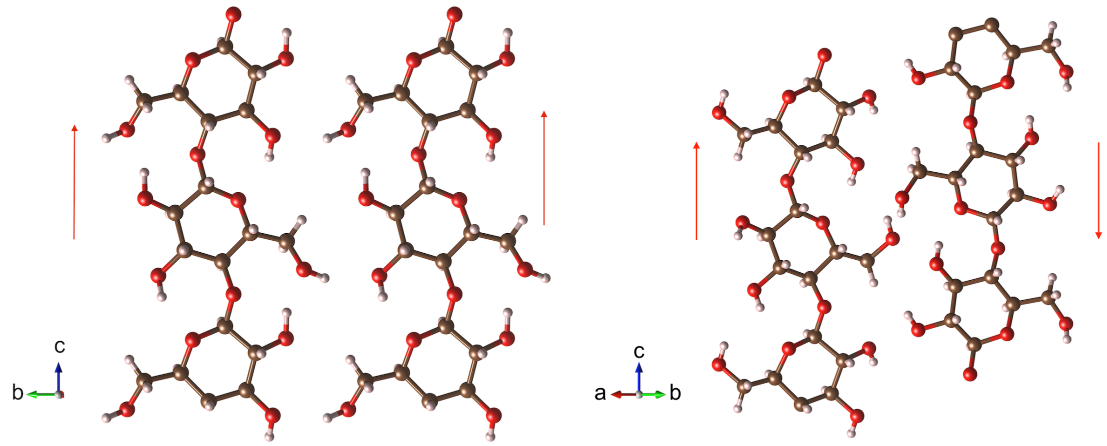
Cellulose is a linear homopolymer composed of D-glucopyranose units linked by β-1,4-glycosidic bonds with anhydro-cellobiose as repeating unit as shown in Figure 2.1. In the cell wall, cellulose consists of 6,000 (primary wall) to 16,000 glucose units (secondary wall). Its degree of polymerisation (DP) as isolated polymer depends on the origin and isolation procedure. In wood pulp, the values are typically between 300 and 1700. Cotton and other plant fibres have DP values in the range of 800–10,000 [117].

The presence of three hydroxyl groups per unit, namely at C2, C3 (both secondary) and C6 (primary), result in the formation of various strong hydrogen bonds. Intramolecular hydrogen bonds between C6 hydroxyl and C2 hydroxyl as well as between C5 oxygen and C3 hydroxyl are responsible for stabilising the glycosidic bond and for adding stiffness to the native cellulose chain. Intermolecular hydrogen bonds between the hydroxyls of C6 and C3, result in the formation of bundles called fibrils and hence, in the building of supramolecular structures. These fibrils are either highly or less ordered, forming crystalline or amorphous regions, respectively. The areas alternate every 10 to 20 nm in direction of the fibre axis as observed by Bhattacharya, Germinario and Winter [22] using atomic force microscopy imaging.

The degree of crystallinity of cellulose has been discussed to be a key factor for enzymatic hydrolysis in line with a biorefinery framework. In general, an increase in hydrolysis rates is observed with decreasing cellulose crystallinity [36, 127]. Thus, the measurement of a crystallinity index (CrI) has attracted increasing interest. Park et al. [172] have compared X-ray diffraction (XRD) and solid-state ^{13}C NMR for determination of CrI. Both have shown to be suitable, provided that the complete XRD or NMR spectrum is taken into account. Typical values are 60-70% for wood pulp and about 45% for regenerated cellulose [65].



(a) Cellulose I_β (b) Cellulose II



(c) Cellulose I_β (d) Cellulose II

Figure 2.2.: Crystal structures of cellulose I_β and II: (a-b) projection with their unit cells (black frame) along the fibre axis **c** with arrows indicating the reducing end group (c-d) orientation of cellulose chains indicated by arrows (based on unit cell data published by French [78], created with VESTA)

Cellulose is known to crystallise in several different polymorphs (I-IV). As types of III and IV are only obtainable by treatment of cellulose I or II with liquid ammonia and/or heating to at least 200 °C, they are outside the scope of this work. Cellulose I is the native, and most abundant, crystalline form, consisting of counter-running cellulose chains. There are two different types of cellulose I, cellulose I_α (triclinic) and I_β (monoclinic), the latter being the one dominating in plant-based cellulose fibrils [91]. Cellulose in wheat straw, for instance, comprises only the cellulose I_β crystal structure [142]. Various conventions have been used over the years to designate the unit cell dimensions. French [78] has just recently suggested a standardised convention for indexing the dimensions of cellulose units with c as the fibre axis, an obtuse monoclinic angle γ, and the a-axis shorter than the b-axis. Therefore, the unit cell dimensions for cellulose I_β are: $a = 7.784$ Å, $b = 8.201$ Å, $c = 10.380$ Å and $\gamma = 96.55°$ [78]. The unit cell for cellulose I_β, based on this crystal structure data, is shown in Figure 2.2a. Its chains are arranged in parallel with the reducing ends being located at the same end of the chain assembly (cf. Figure 2.2c).

Mercerisation in strong (≥ 18%) sodium hydroxide solution leads to a widening of the lattice, as Na^+ cations penetrate its intracrystalline spaces [97]. This results in a new lattice, called Na-cellulose I, with large distances between the cellulose molecules. Furthermore, it is, unlike cellulose I, most likely made of antiparallel chains [166]. When removing the linked Na^+ cations, the allomorph cellulose II with the unit cell dimension $a = 8.10$ Å, $b = 9.03$ Å, $c = 10.31$ Å and $\gamma = 117.10°$ is formed [78]. The corresponding unit cell is shown in Figure 2.2b. Apart from the treatment with sodium hydroxide, the dissolution and precipitation of cellulose I also leads to a transformation to cellulose II, as shown for all kinds of regenerated cellulose. Cellulose II is the stable thermodynamic form and the transformation from type I to II is therefore irreversible [222]. In contrast to cellulose I, its crystal structure consists of anti-parallel chains as shown in Figure 2.2d. Both types of cellulose can be identified by their characteristic X-ray diffraction pattern [78].

The strong hydrogen bonding also effects the physico-chemical properties of cellulose. While many reagents have the ability to cause swelling of cellulose, only few can overcome these forces and cause cellulose dissolution. Examples for non-derivatising solvents are the aqueous solution of cupriethylenediamine hydroxide, N-Methylmorpholine N-oxide (NMMO) and a system composed of lithium chloride and dimethylacetamide [223].

Hemicellulose

The term "hemicellulose" describes a group of various non-cellulosic polysaccharides. In contrast to cellulose, they are branched polymers of lower molecular weight and consist of various sugar units apart from β-glucose. These units can be divided into pentoses, such as β-D-xylose and α-L-arabinose, hexoses, including β-D-mannose and α-D-galactose, as well as hexuronic acids and deoxy-hexoses [69]. The main chain (backbone) of hemicelluloses consists of either one unit, for instance xylose in xylan, or of two or more units as mannose and glucose in glucomannans, which are linked β-(1→4). Possible branch points are (1→2), (1→3) and/or (1→6) [186]. Table 2.3 summarises typical hemicellulose types in hardwoods, softwoods and grasses. Hemicelluloses in the latter are xylans containing glucuronic acid and/or its 4-O-methyl-ether as well as arabinose attached to C2 or C3 of the xylose units. In addition, they can be partly acetylated. In grasses, arabinose from arabinoglucuronoxylan is found to be covalently linked to the acid group of ferulic acid via its primary hydroxyl at the C5 position as shown in Figure 2.3 [94, 199]. This plays a significant role in cell wall development, biomass separation and subsequent polysaccharide utilisation as they may also enter lignification reactions [94].

The recovery of hemicelluloses from biomass is desirable and has great potential, as they have been demonstrated to be applicable as gels, films, coatings and adhesives in the food as well as non-food field. Furthermore, hemicelluloses have been proved to be a potential fermentation feedstock for production of ethanol, acetone, butanol and xylitol [181].

Table 2.3.: Hemicelluloses with regard to the type of biomass (according to Fengel and Wegener [69] and Rowell [186])

Hemicellulose type	Units	% in biomass	DP
Hardwood			
Glucuronoxylan	**β-D-Xyl**, 4-O-Me-α-D-GluA, Acetyl	15-30	200
Glucomannan	**β-D-Man, β-D-Glu**	2-5	200
Softwood			
Glucomannan	**β-D-Man, β-D-Glu**, α-D-Gal, Acetyl	20-25	100
Arabinoglucuronoxylan	**β-D-Xyl**, 4-O-Me-α-D-GluA, α-L-Ara	7-10	100
Grasses			
Glucuronoxylan	**β-D-Xyl**, 4-O-Me-α-D-GluA	20-30	<100
Arabinoglucuronoxylan	**β-D-Xyl**, 4-O-Me-α-D-GluA, α-L-Ara		

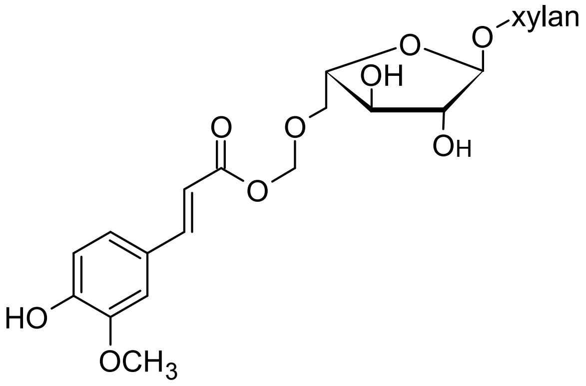


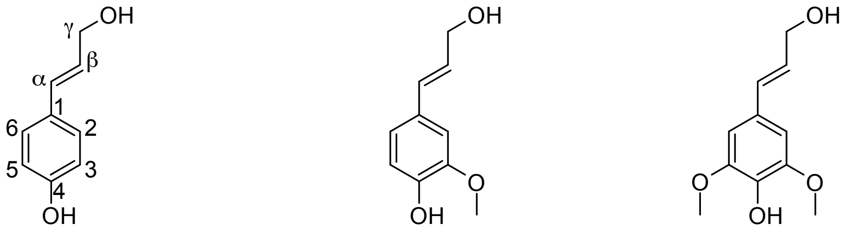
Figure 2.3.: Ferulate attachment to polysaccharides (according to Hatfield, Ralph and Grabber [94])

Lignin

Lignin is an amorphous polyphenolic material, which conferres mechanical strength as well as the ability of conducting water to the plant. It is primarily derived from the three monolignols p-coumaryl, coniferyl and sinapyl alcohol, which are termed p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) if involved in linkages. They are shown along with the designation of atoms in the phenyl propane units in Figure 2.4a. The proportion of these monolignols differs depending on botanical origin, cell type and cell wall layer. With some exceptions lignin from angiosperms (hardwood) mainly consists of guaiacyl and syringyl, whereas lignin from gymnosperms (softwood) mainly consists of guaiacyl units. In contrast, all three structural units are included in considerable amounts in lignin from graminoids [31]. Furthermore, less abundant units than the three main monolignols have been identified and may be incorporated into the polymer at varying levels. These include ferulates (which form crosslinks between lignin and hemicelluloses), coniferaldehyde and acylated monolignols containing acetate, p-coumarate or p-hydroxybenzoate moieties [222].

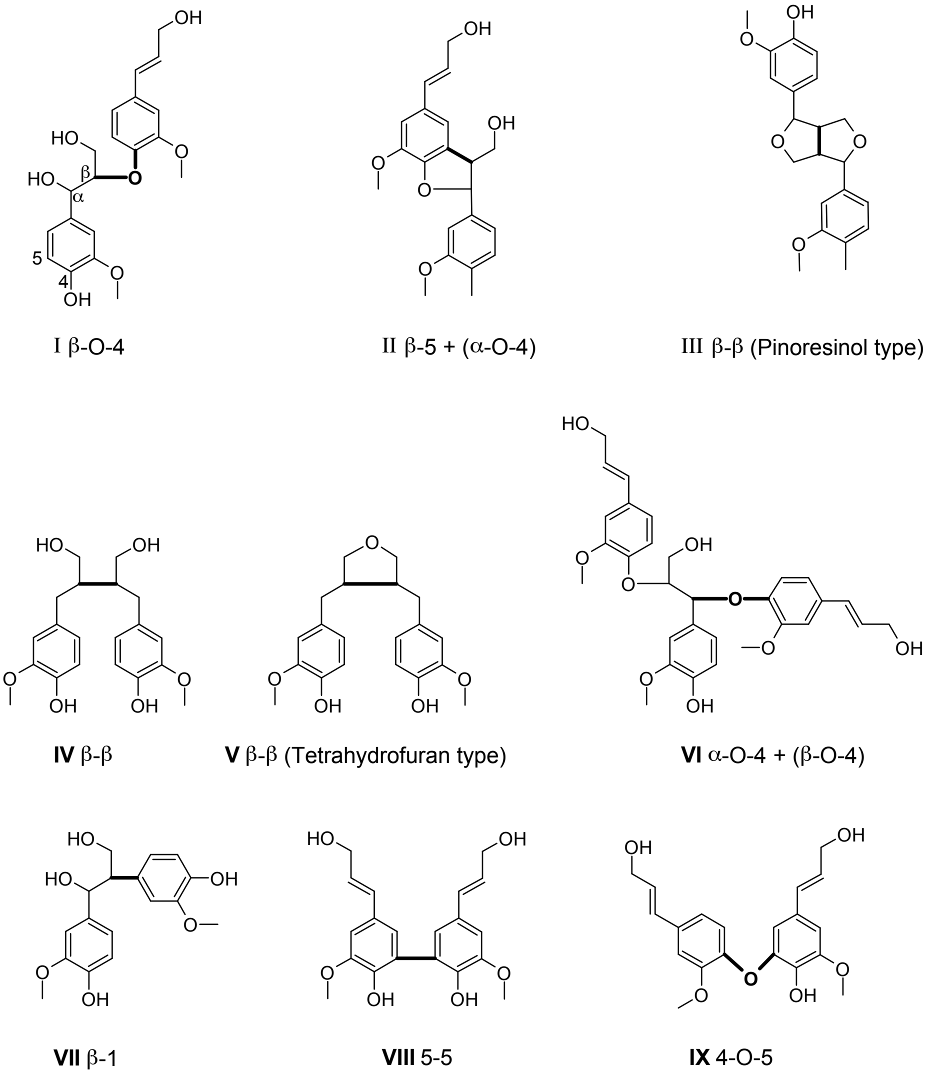
In addition, other functional groups can be present besides hydroxyl and methoxyl groups already included in the main monolignols. According to Zakis [235], there are four types of carbonyl groups naturally occurring in lignin, namely at the C_α-, C_β-, C_γ- as well as C4-position. Other types may be present in highly modified lignins, for instance ortho-quinones after demthylation and oxidation. Brunow and Lundquist [31] further describe the presence of carboxyl groups at the C_α- and C_γ-position.

To better understand the structure and type of linkages of lignin, its biosynthesis is outlined in the following. For biosynthesis of lignin, phenyl propane units are synthesised via the shikimate pathway in the cytoplasm followed by transportation to the cell wall. The first step for building up the lignin macromolecule is enzymatic dehydrogenation of monolignols yielding resonance-stabilised phenoxy radicals. Thereafter, polymerisation proceeds via two routes: dimerisation and lignification. The first leads to coupling of two monolignols, preferably involving the β-position, and is presumably the first step in lignin biosynthesis [31]. The second includes incorporation of mono- or dilignols in an existing lignin molecule as new end group as well as coupling of two oligomers [27, 146]. This results in different types of linkages, which are illustrated in Figure 2.4b.



Monolignol	p-Coumaryl alcohol	Coniferyl alcohol	Sinapyl alcohol
Structural unit	p-Hydroxy phenyl unit (H)	Guaiacyl unit (G)	Syringyl unit (S)
Occurence	Grasses	Hardwoods, softwoods, grasses	Hardwoods, grasses

(a)



(b)

Figure 2.4.: Structural features of lignin: (a) Building units of lignin depending on type of biomass (b) Different types of linkages: structures I-V arise from dimerisation, whereas the linkages in structure VI-IX are typical for lignification (modified from Dimmel [51])

Table 2.4.: Type of lignin linkages and their frequency in %

	No. in Fig. 2.4b	Spruce [2]	Beech [164]	Wheat straw [46]
β-O-4	I	48	} 65	77
α-O-4	VI	6-8		-
β-5	II	9-12	6	11
β-β	III	-	-	4
β-β	IV	2	5.5	-
β-β	V	-	2	-
β-1	VI	7	15	-
5-5	VIII	9.5	2.3	3
4-O-5	IX	3.5	1.5	-
others		7	2.5	5

During dimerisation, at least one of the phenyl propane units is coupled at its β-position giving rise to structures I-V. In contrast, 5-5 and 5-O-4 linkages, which are responsible for branching, are only formed by coupling between preformed oligomers [146]. In general, the formation of aryl ether linkages is strongly favoured due to the high electron density at the phenolic oxygen. Thus, this type of interunit linkage accounts for over 50% of linkages as shown in Table 2.4 for lignin from spruce, beech and wheat straw [68]. Del Río et al. [46] determined a high proportion of β-ether linkages in wheat straw lignin as determined by 2D-NMR. This is advantageous, as this bond is easily cleaved chemically, providing a basis for industrial and analytical processes. However, the finding of del Río et al. [46] is inconsistent with results from Sun [202] as well as Banoub [16], proposing the α-ether and β-5 bond to be the dominant linkage type, respectively.

Besides linkages between lignin units, lignin-carbohydrate linkages play a significant role for biomass separation processes, as the obtained lignins tend to be associated with carbohydrate impurities. Covalent bonds via glycosidic, ester and benzyl ether linkages between lignin and carbohydrates have been proposed and demonstrated using model compounds [146]. However, it has been difficult to obtain evidence on nature and frequency of such linkages in plants, as they are subjected to change during isolation [146]. An early review was authored by Merewether in 1957 [154] with many following (e.g. Koshijima and Watanabe [120], Hatfield, Ralph and Grabber [94]), especially due to advanced possibilities by the application of new techniques. Balakshin et al. [14], for instance, developed a quantitative approach to characterise linkages in lignin–carbohydrate complexes (LCC) using a combination of ^{13}C NMR and HSQC 2D NMR techniques. Both pine and birch preparations showed the presence of different amounts of benzyl ether, γ-ester and phenyl glycoside bonds between lignin and carbohydrates. Iiyama et al. [105] further demonstrated that in wheat internodes ferulic acid is etherified to lignin as well as esterified to arabinoxylans and hence, cross-links lignin and polysaccharides. In contrast, p-coumarates, which are also found as constituent of grass lignins, are ester-linked to the γ-position of phenylpropanoid sidechains [191]. According to studies of Iiyama et al. [105] they are, however, not acting as cross-linking agents.

The type and frequency of lignin-carbohydrate bonds plays a significant role for biomass separation. Whereas ester bonds are easily hydrolysed during alkaline pulping, this does not apply to ether bonds. Studies using model compounds indicate that benzyl ether bonds between lignin and hemicelluloses are generally resistant to alkaline hydrolysis, but cleave by acid hydrolysis [121]. Thus, a more detailed knowledge on the nature and frequency of lignin-carbohydrate bonds is required in order to improve biomass separation. This implies the development of an applicable method that can be regarded as ideal for the isolation of representative native lignin with unaltered structures.

The physico-chemical properties of lignin depend strongly on origin, impurities and separation method. This is reflected by their behaviour in various solvents. Although all of them, with the exception of lignosulfonic acids, are water-insoluble, alkali lignins can be dissolved in aqueous alkali solutions. In addition, organic solvents such as dimethyl sulphoxide, dimethyl formamide and pyridine can be good solvents, though some lignins are only dissolved partially owing to a relatively high-molecular-weight fraction. Lignins isolated by hydrolysis of carbohydrates using mineral acids, such as KLASON-lignin are generally considered to be insoluble in various common solvents. However, they are soluble in alkali solutions to a minor extend [235].

With lignin being the only biobased aromatic feedstock, it plays a key role in biorefinery concepts. A range of lignins with varying properties are available depending on type of biomass and separation process resulting in diverse application possibilities as outlined in Section 2.4.2.

Extractives

The term extractives summarises a heterogeneous group of substances, which can be extracted from biomass using solvents such as acetone, ethanol, toluene or dichloromethane. The main extractives are fatty acids, di- and triglycerides, sterols and steryl esters. In most hard- and softwoods, extractives are only a minor component accounting for 2 to 4% of the total dry matter [67]. In contrast, their content in non-wood biomass is generally higher. In wheat straw, for instance, a proportion of 5.4% of ethanol-toluene extractives is reported [58]. Both, culm and leaf, are covered with semicrystalline wax particles, mainly consisting of long-chain alkanes, alkyl alcohols and fatty acids. Their function is to protect the plant from water loss, from the effects of high or low temperature as well as from insect predation [230].

Though being a minor compound, extractives play a role in industrial alkaline pulping processes, as they are partly dissolved in the black liquor and accumulated in the pulp, causing technical and economic problems. Especially the trend towards completely closed water circuits, within the scope of working environmentally friendly, results in a higher deposition of extractives.

Ash

In contrast to soft- and hardwoods, ash in cereal straws can account for up to 20% of the total dry matter. Apart from impurities due to inherent soil, which is of special importance for agricultural residue, straws and other fibrous biomass are rich in mineral matters. About two-thirds of the total mineral content is silicon dioxide, which is deposited in form of biomineralised silica within epidermal cells [230]. These silica bodies have been imaged in grass samples by Dietrich et al. [50]. Whereas silica has been reported to have a positive effect on plant growth and on plant resistance, its occurrence is rather problematic for chemical pulping. In this regard, it causes difficulties during recovery of alkaline black liquor and results in excessive scaling and fouling on boiler units [158].

Some desilication processes have been proposed by removal of silica in the black or green liquor. Lei et al. [135] showed that silica can be effectively lowered by a hot-water pretreatment of the raw material. Thus, there are possibilities to overcome the problem of silica accumulation during pulping of non-wood biomass.

2.2.2. Classification, structure and ultrastructure of herbaceous biomass

The agricultural residue used in this study is obtained from herbaceous, hence non-woody, plants. Their anatomical structure differs from the one in wood. Whereas the latter is enabled to secondary diameter growth due to the existence of cambium, herbaceous plants by definition lack this type of tissue and are only enabled to primary growth [230]. Even the coconut palm, though large, perennial and hard, does not consist of woody tissue and is hence, herbaceous [150]. Table 2.5 lists the herbaceous biomass relevant to this study and the botanical classification of the original plant.

Table 2.5.: Used herbaceous biomass and botanical classification (Order, Family, monocotyledon (M) and dicotyledon (D)) of the original plant

Biomass	Order	Family	M/D	By-product from...
Barley straw (ba)	Poales	Poaceae	M	grain production
Coconut shell powder (cp)	Arecales	Arecaceae	M	fibre production
Hemp shives (hs)	Rosales	Cannabaceae	D	fibre production
Horse manure (hm)	-	-	-	livestock farming
Maize straw (ms)	Poales	Poaceae	M	grain production
Miscanthus (mi)	Poales	Poaceae	M	none: energy crop
Oat husk (oh)	Poales	Poaceae	M	grain production
Pretreated alfalfa (pa)	Fabales	Fabaceae	D	protein production
Rape straw (rs)	Brassicales	Brassicaceae	D	seed production
Sunflower stalks (ss)	Asterales	Asteraceae	D	seed production
Tomato stalks (ts)	Solanales	Solanaceae	D	vegetable production
Wheat straw (ws)	Poales	Poaceae	M	grain production

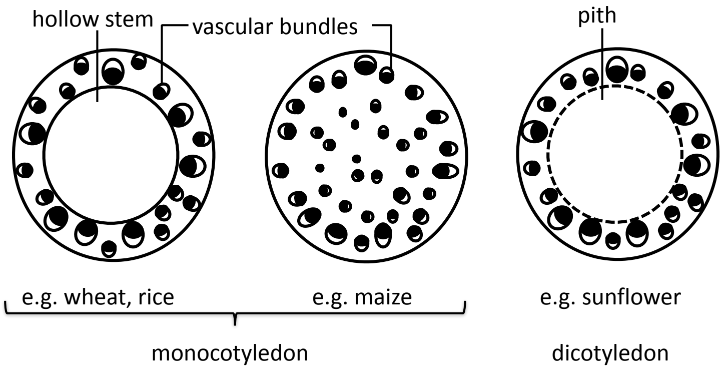


Figure 2.5.: Cross section of stems of mono- and dicotyledons (modified from Xu [230] and Stern [200])

As a large portion of the used agricultural residues are made of stems and stalks, they are described in more detail in the following. In contrast to woody stems, herbaceous stems include numerous vascular bundles embedded in a ground tissue of parenchyma storing cells. Figure 2.5 illustrates the arrangement of these bundles in mono- and dicotyledons. Each is orientated so that its xylem (black) is closer to the centre and its phloem (white) is closer to the epidermis. In monocotyledons these vascular bundles appear scattered throughout the stem. In some monocotyledonous grasses, such as wheat, they are arranged in a circle near the periphery of the stem due to its hollow centre [200]. Herbaceous dicotyledons, for instance those of the families *Asteraceae* (sunflower) and *Solanaceae* (tomato plants), have their vascular bundles arranged circular separating the cortex from the pith. Nonetheless, they do not make complete rings as in woody dicotyledons [200, 230]. Common for all herbaceous plants is that the vascular bundles branch off to set leaves at regular intervals, leading to the formation of nodes and internodes [230].

Whereas there are differences concerning the anatomical structure of wood and herbaceous plants, their cell structure is similar, consisting of middle lamella (ML), primary wall (P) and secondary walls (S1, S2, S3). If the S3 layer forms the last fibrillar layer at the luminal border, it is often also referred to as tertiary wall (T). Figure 2.6 illustrates the differences between cell wall layers based on their content of lignin, hemicelluloses and cellulose as well as on the arrangement of cellulose microfibrils, the latter being crucial for the mechanical behaviour of the plant fibre [72, 230].

It is worth to be noted, that besides the differences between cell wall layers, there are also differences between cell types. For instance, in parenchyma cells cellulose fibrils are arranged more randomly, whereas a rather longitudinal arrangement to the fibre axis has been reported for epidermal cells [233]. Furthermore, Meyer et al. [155] report, that the occurrence of syringyl lignin is restricted to the parenchyma cells flanking the vascular bundles, while guaiacyl is deposited only in cells of the vascular bundle.

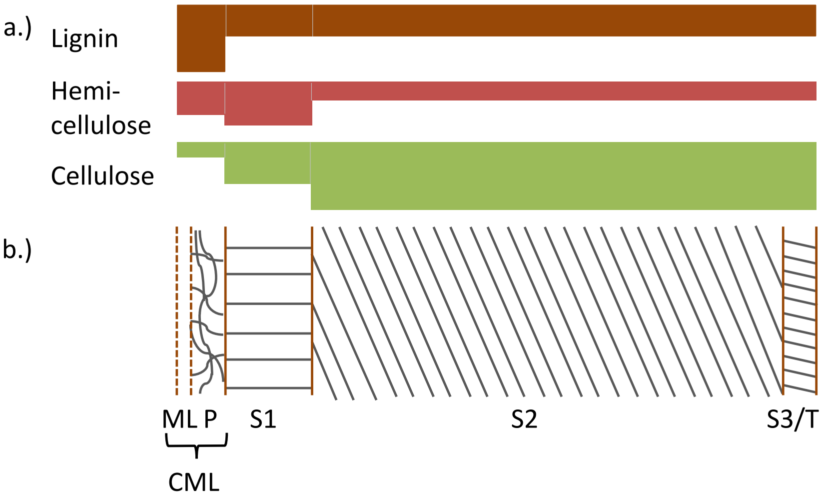


Figure 2.6.: Cell wall structure a.): Distribution of lignin, hemicellulose and cellulose in the cell wall of spruce according to Fengel and Wegener [66] b.): Arrangement of cellulose microfibrils in various wall lamellae in wheat straw (according to Xu [230] and Zhai and Lee [238])

Middle lamella

The middle lamella, which is the layer between walls of adjacent cells, is basically free of cellulose. In contrast, the highest concentration of lignin is found in the middle lamella being 41.2% in wheat straw ML [238]. As the transition from middle lamella to the adjacent primary cell walls (P) is not very clear, the term compound middle lamella (CML) is used to include ML and P [72]. In addition, there is a common area between three or four cells called cell corner (CC). It is known to consist mainly of lignin, with up to 66.4% in wheat straw, and to be the initial point for lignin deposition [230]. Lignin, deposited at the cell corners and in the compound middle lamella during the early stages, is enriched in p-hydroxyphenyl (H) and guaiacyl (G) units [184]. Thus, due to the lack of syringyl units, which are associated to β-O-4 linkages with high frequency, it is proposed to have a more cross-linked structure with a higher content of carbon-carbon bonds [97].

Primary cell wall

The primary cell wall is a thin layer consisting of cellulose, hemicellulose, pectin and protein, embedded in lignin. In this layer, cellulose microfibrils are usually oriented in a random manner resulting in a unstructured network. For wheat straw, however, also a netlike structure has been described [238]. Furthermore, it is assumed to be a layer rich in hemicelluloses, though their quantification in the different layers has been reported to be difficult [230].

Secondary cell wall

With some exceptions, the secondary wall consists of three layers (S1, S2, S3), with S2 forming the main portion with 70–90% of the total cell wall [230]. As the cellulose content increases from outer to inner cell wall layers, it is highest in the secondary wall, especially in S2 and S3. Within the layers of the secondary wall, cellulose fibrils are aligned in parallel with their orientation changing between S1, S2 and S3 as shown in Figure 2.6. Furthermore, they are more densely packed in S2 and S3 compared to S1.

Although the concentration of lignin and hemicelluloses is the lowest in S2, a major proportion is located in this layer due to its thickness. Thus, up to 67.5% of the overall lignin content in wheat straw is located in the secondary wall [238]. This lignin contains a mixture of guaiacyl and syringyl units [184].

2.3. Chemical separation of lignocellulosics

Lignocellulosic biomass inherently resists degradation of its carbohydrate fraction by enzymes and microorganisms as used for hydrolysis and fermentation for ethanol production. This recalcitrance is caused by several factors such as the partially crystalline nature of cellulose, the epidermal tissue (especially its content of waxes), the composite nature of the plant cell wall and the structural heterogeneity of its constituents [218]. Thus, a pretreatment step leading to a reactive cellulosic intermediate while minimising the formation of compounds that interfere with the downstream process is crucial.

A number of pretreatment procedures are currently under investigation, which can be divided into biological, physical, physico-chemical, and chemical methods [206]. Biological pretreatments use wood-attacking microorganisms, which have various effects. Depending on the species used, white rot fungi, for instance, is capable of an extensive lignin degradation or of uniform depletion of cellulose, hemicellulose and lignin [163]. This offers some advantages such as low use of chemicals and energy [227].

Physical pretreatments only include mechanical size reduction by chopping and grinding primarly aiming at increasing the surface area and decreasing cellulose crystallinity as well as its degree of polymerisation. Refining additionally results in disruption of the middle lamella and increased accessibility of lignin.

Physico-chemical pretreatments furthermore cover the addition of chemicals as for instance water in autohydrolysis and steam explosion, or ammonia in ammonia fibre expansion, also known as AFEX. This additionally leads to degradation and deesterification of hemicellulose and changes in lignin morphology [33, 40].

Chemical pretreatments employ a range of different chemicals and usually include raising the temperature to the range of 140–210 °C [227]. Established procedures for using woody biomass are the Kraft process, which is carried out using sodium hydroxide and sodium sulphide, as well as the sulphite process, which uses sulphites or bisulphites [75]. If herbaceous plants are used as raw material instead of wood, sulphur-free and hence more environment-friendly procedures can be applied. These include acid hydrolysis, alkaline pulping using sodium hydroxide, also known as soda pulping, and organosolv pulping using alcohols or carboxylic acids with or without the addition of oxidising agents. The following sections will give a more detailed description of the alkaline soda process and the organosolv process. The latter will focus on the specific case of using formic acid instead of methanol or ethanol. In addition, the effect of microwave-assisted pulping will be discussed.

2.3.1. Alkaline pulping using sodium hydroxide

Alkaline soda pulping, which uses sodium hydroxide as pulping and sodium carbonate as make-up chemical, is one of the most important processes for non-wood fibre pulping. It is conventionally performed in digesters at temperatures between 160 and 180 °C. The amount of sodium hydroxide is adjusted to compensate for its consumption by acidic biomass compounds. Furthermore, the pH needs to stay above 11 during pulping to prevent precipitation of dissolved lignin [51]. A major disadvantage of alkaline pulping of non-wood plants is that due to their high silica content, irrecoverable salts are formed and incorporated into the biomass [126]. However, Lei et al. [135] showed that the silica content can be effectively lowered by a hot-water pretreatment. Thus, the process and recovery of chemicals could still be improved.

As soda pulping was already used commercially in 1855 for pulp production from poplar, it is well-studied under the aspect of subsequent paper making [197]. However, the process needs to be re-evaluated as there are important differences whether the focus is on subsequent paper or ethanol production. For the latter complete or nearly complete lignin removal is not necessary, as it was shown that only 30—50% of the initial lignin needs to be removed in order to significantly increase biomass digestibility [180]. Other results suggest, that it is enhanced by altering, not removing, lignin [54, 185]. Pulp strength properties, bleachability and susceptibility to yellowing are further objectives of the paper industry, which do not apply to ethanol production [13].

The pretreatment with sodium hydroxide has several effects on lignocellulosic material. However, not all of them are advantageous for subsequent hydrolysis and fermentation. The dominant effects are: [180]

- Swelling of fibres and increase of internal surface area
- Degradation of carbohydrates by alkaline hydrolysis and peeling reaction
- Partial conversion of cellulose I to II
- Removal of acetyl groups from xylan
- Cleavage of ester bonds between lignin and polysaccharides
- Lignin fragmentation reactions and lignin removal
- Lignin condensation reactions
- Removal of waxes, silica and cutins that coat plant tissue

Reactions of carbohydrates and lignins, as being the major constituents of lignocellulosic biomass, will be discussed in detail in the following.

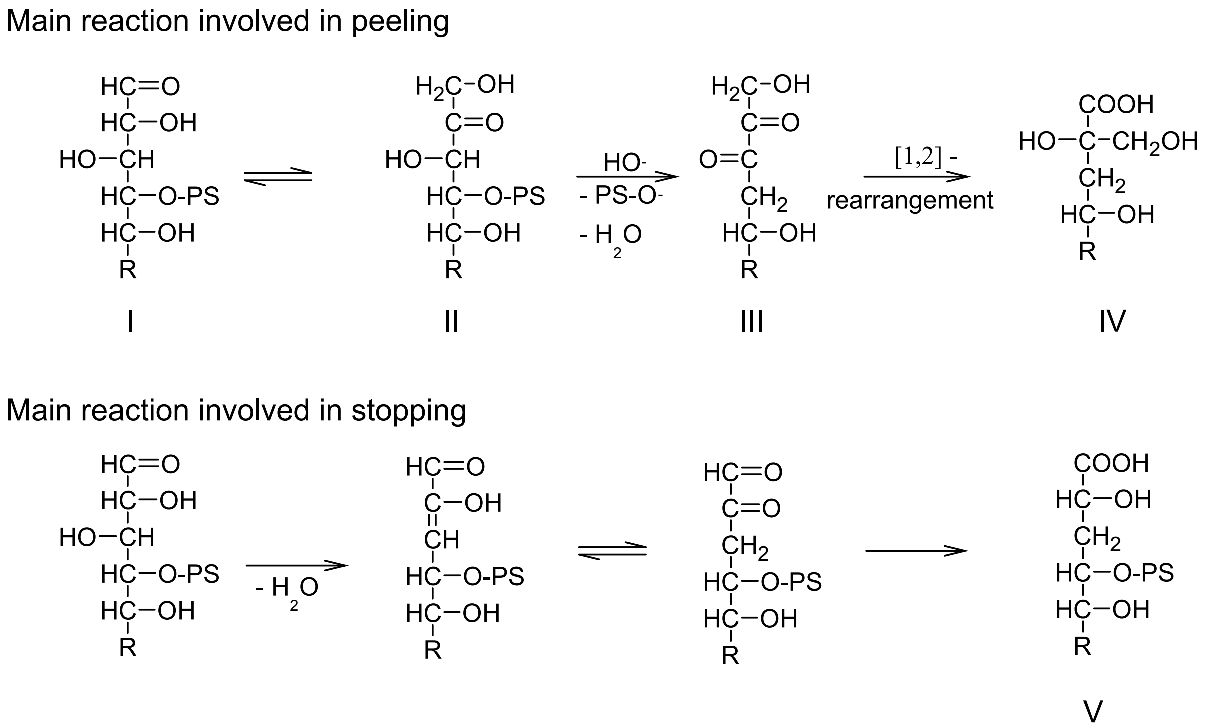


Figure 2.7.: Main reactions involved in peeling and stopping reaction with PS = polysaccharide chain and R = CH_2OH (cellulose, mannan) or H (xylan) according to Fengel and Wegener [70]

Reaction of carbohydrates

During alkaline pulping, carbohydrates undergo endwise degradation (peeling) as well as alkaline hydrolysis of glycosidic bonds and acetyl groups [70].

As hemicelluloses are of much lower molecular weight than cellulose and as they are not crystalline, they do not present the same barriers to accessibility as does most of cellulose [118]. Thus, the loss, based on dissolution and degradation, is particularly high for glucomannans and xylans. However, cellulose losses occur as well [180].

Carbohydrate degradation starts at temperatures above 100 °C from the reducing end of the polysaccharide chain, known as primary peeling. The main reaction pathway is shown in Figure 2.7. The initial step proceeds via the open-chain form of the reducing end (I), which is present in an equilibrium state with its corresponding ketose (II). At high pH the polysaccharide chain at C4 is cleaved and the residual monomer (III) is transformed to gluco-isosaccharinic acid in case of cellulose and mannan, as well as to xylo-isosaccharinic acid in case of xylan (IV) via an 1,2-rearrangement. Other possible pathways result in formation of lactic acid and, after elimination of formic acid, of 2,5-dihydroxypentanoic acid. In order to stabilise polysaccharides against alkaline peeling, the use of anthraquinone or related compounds are beneficial [70, 75].

In contrast, the so-called stopping reaction, which competes with alkaline peeling, leads to the stabilisation of polysaccharide chains by the formation of an alkali-stable metasaccharinic acid end group (V), as shown in Figure 2.7.

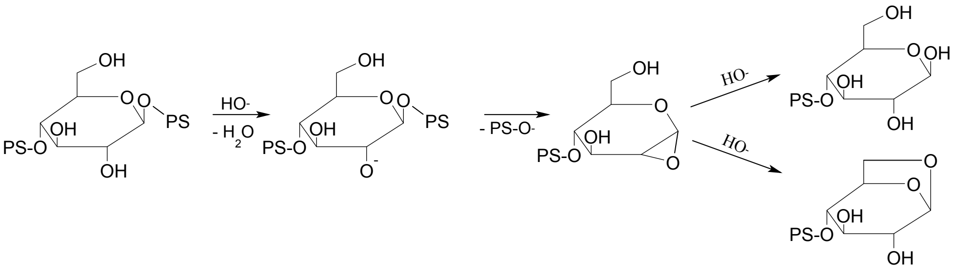


Figure 2.8.: Hydrolysis of glycosidic bonds at high pH with PS = polysaccharide chain leading to a new reducing end group (upper part) or the formation of an 1,6-anhydroglucose end (lower part) (according to Fengel and Wegener [70])

Besides the 3-deoxyaldonic acid units, 16 other stabilising terminal acidic units have been detected in alkali-boiled hydrocellulose [118]. In addition, physical stopping occurs when a cellulose molecule is peeled back to a crystalline region due to inaccessibility of the reducing end with regard to alkali [7].

At temperatures above 150 °C alkaline hydrolysis of the glycosidic bond sets in as shown in Figure 2.8. This random alkaline scission of glycosidic linkages results in considerable weight loss and hence, in decrease of the degree of polymerisation. Possible products are a new reducing end as well as an 1,6-anhydroglucose unit (levoglucosan). By the formation of new reducing ends, degradation proceeds via secondary peeling.

Reaction of lignin

The main concepts of alkaline delignification, besides the deprotonation of phenolic hydroxyl groups, are 1. the fragmentation of lignin by cleavage of interunit linkages and 2. the introduction of hydrophilic groups, both proceeding exclusively by nucleophilic reactions during pulping. Their impact is strongly dependent on the type of lignin unit (phenolic or non-phenolic) and on the stage of pulping. The aspects of alkaline delignification have been reported in detail, most notably by Gierer [82–84]. There are three stages in the process of alkaline delignification [82, 180].

- Initial stage: activation energy approx. 61 kJ·mol^{-1}, temperature < 150°C
- Bulk stage: activation energy approx. 150 kJ·mol^{-1}, includes heating from 150 to 170 °C and pulping at approx. 170°C
- Residual stage: activation energy approx. 120 kJ·mol^{-1}, temperature approx. 170°C

The reactions during alkaline pulping are diverse. The most important ones are illustrated in Figure 2.9. As the initial stage of pulping is characterised by its low activation energy it mainly involves phenolic units, which are easily converted to quinone methide intermediates (1) (Figure 2.9 part **a**). This conversion is accompanied by the elimination of an existing α-aroxy substitutent. The obtained quinone methide is relatively unstable [212]. Subsequent cleavage of β-aryl ether linkages is rapidly induced in the presence of hydrogen sulfide ions. However, it does not proceed during sulphur-free alkaline pulping, as HO^- is a weaker nucleophile than HS^-. Thus, the formation of alkali-stable structures takes place via enolisation, including two possibilities for rearomatisation. One involves deprotonation of the β-carbon (1.1), the other a retrograde aldol condensation with loss of C_γ as formaldehyde (1.2). Retrograde aldol condensation can also lead to cleavage of the carbon-carbon bond between C_α and aromatic nucleus as shown in reaction (1.3). It is further relevant for the cleavage of linkages between C_α and C_β as well as between C_β and C_γ in both phenolic and non-phenolic units as shown in reaction scheme (4) (Figure 2.9 part **c**). However, only a minor portion of lignin fragmentation can be ascribed to this reaction pathway [83]. This also applies to cleavage of methyl-aryl ether bonds in methoxyl groups resulting in the formation of minor amounts of methanol.

Condensation reactions including the addition of an internal nucleophil compete with the discussed fragmentation reactions. Though, being only comparatively weak nucleophils, carbanions can be incorporated as the equilibrium is shifted due to an irreversible re-aromatisation step (1.4). Furthermore, a condensation of phenolic units with formaldehyde, which may be liberated from reaction (1.1), results in the formation of diaryl methane structures (2). Lignin condensation reactions have been reported to be the most important reaction type for decreasing or even preventing complete delignification [51].

In contrast to phenolic units, fragmentation through cleavage of the β-ether linkage does take place in non-phenolic units. As it requires relatively drastic conditions, it is reserved to the bulk phase of pulping [83]. However, in this case it is the dominant delignification process. The pathway is outlined in Figure 2.9 part **b** as reaction (3). It involves the participation of an adjacent ionised hydroxyl group and proceeds via formation of an oxirane intermediate. Despite this adverse transition state, this reaction is strongly favoured due to the vicinity of HO^- to the reaction centre as well as the change in entropy after passing the oxirane intermediate to give 1,2-glycols [83].

The topochemical effect of alkaline delignification of wheat straw has been reported by Zhai and Lee [238]. According to their studies, there is only little difference in delignification when comparing the three morphological regions cell corner, middle lamella and secondary wall. All three undergo a rapid initial phase of lignin removal accounting for nearly 70% of delignification, followed by a slower supplementary phase. However, Whiting and Goring [224] describe a more rapid delignification of the secondary wall tissue compared to the middle lamella during Kraft pulping of spruce. They propose this difference to be due to preferential hemicellulose removal from the secondary wall as well as different lignin composition and hence, reactivity. Chiang and Funaoka [38] report that during the initial phase of delignification, about 15% of guaiacyl units were removed, whereas the removal of syringyl was insignificant at this stage. The latter are, however, preferentially removed during the bulk phase of alkaline pulping. They further emphasise that the involvement of syringyl units facilitate delignification in general, as they are associated with high frequency with β-O-4 linkages and condensation is reduced due to the lack of reactive sites on syringyl nuclei [39].

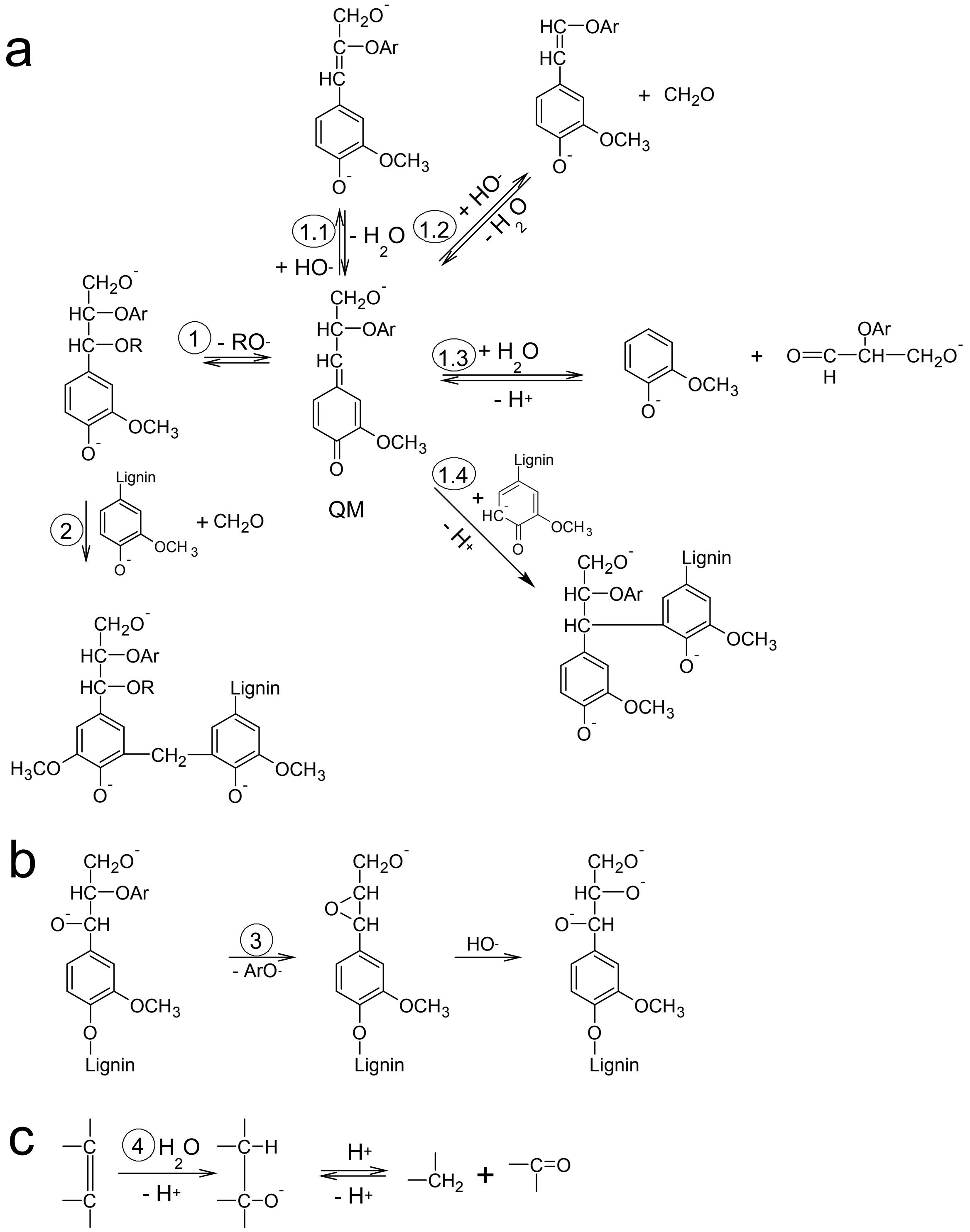


Figure 2.9.: Main reaction pathways of a) phenolic units via quinone methide (QM) b) non-phenolic units c) both units, during pulping with HO^{-} (summarised from Gierer [82, 83])

2.3.2. Organosolv pulping using peroxyformic acid

The use of organic solvents for pulping has the advantage of the possible recovery of initial chemicals by distillation. Lignin of high purity can be obtained having a high potential for further utilisation. The use of peroxyformic acid in particular is advantageous with regard to its high selectivity. In addition, the pulping process can be operated at low temperatures and at atmospheric pressure. The silica of the biomass, a particular issue for alkaline pulping of straws, remains in the obtained pulp facilitating an efficient recovery of cooking chemicals [137].

Nonetheless, the use of organic solvents in general carries a fire and explosion risk due to their volatility and hence, increases engineering costs. In case of formic acid, the corrosive character makes it necessary to work with glass or enamelled steel. Among the organic solvents two main groups have been in the focus of research: alcohols (mainly methanol and ethanol) and organic acids (formic and acetic acid).

The milox process (Milieu Pure Oxidative), is a three-stage process developed by the Finnish Pulp and Paper Research Institute and Kemira, with a pilot plant built in 1991 at Kemira Chemicals' Oulu plant site [232]. It uses formic acid and hydrogen peroxide to generate peroxyformic acid *in situ.* The "natural pulping" process, originally patented by the Natural Pulping AG and used in this study, is very similar, though based on slow addition of hydrogen peroxide at a constant rate [196]. Peroxyformic acid is formed as follows:

$$\mathrm{HCOOH} + \mathrm{H_2O_2} \rightleftarrows \mathrm{HCOOOH} + \mathrm{H_2O}$$

The delignifying character of peroxyformic acid is based on its generation of hydroxonium ions by heterolytic cleavage of the peroxidic bond [204]:

$$\mathrm{HCOOOH} + \mathrm{H^+} \rightleftarrows \mathrm{HCOOH} + \mathrm{HO^+}$$

However, the stability of peroxyformic acid is poor due to several competing decomposition reactions. Filippis, Scarsella and Verdone [74] report the irreversible decomposition to water and carbon dioxide to be the predominant pathway:

$$\mathrm{HCOOOH} \longrightarrow \mathrm{CO_2} + \mathrm{H_2O}$$

Thus, major effects during peroxyformic acid pulping are caused by the acidic media, the use of an organic solvent as well as the generation of hydroxonium ions as delignifying agents. The dominant effects of peroxyformic acid pulping are [88, 139, 211]:

- Increase in pore volume
- Hydrolysis and oxidation of polysaccharides
- Degradation and oxidation of monosaccharides
- Lignin oxidation, fragmentation, condensation and dissolution
- Changes in lignin morphology and distribution

Reactions of carbohydrates and lignins, being the major constituents of lignocellulosic biomass, will be discussed in the following.

Reaction of carbohydrates

In acidic medium, as present during acid-catalysed organosolv pulping, the main reactions of carbohydrates are hydrolysis of the polysaccharide chain as well as dehydration of glucose and xylose forming 5-(hydroxymethyl)furfural (HMF) and furfural, respectively. Especially due to the use of peroxyformic acid, oxidation reactions are additionally relevant.

Figure 2.10 illustrates the dominant pathway of acidic hydrolysis of carbohydrates at low pH. In the initial step the glycosidic oxygen is protonated. This is followed by a cleavage of C-O yielding an intermediate cyclic carbocation, which is stabilised by addition of water. Another pathway proceeds to a minor extend via protonation of the ring oxygen and an intermediate non-cyclic carbocation. Chang, Ma and Cen [34] showed, that increasing the temperature and acid concentration promote the hydrolysis of wheat straw.

In addition, dehydration reactions of monosaccharides are relevant for pulping in acidic medium. Figure 2.11 illustrates the formation of hydroxymethylfurfural and furfural, being the major products derived from pentoses and hexoses, respectively. These furan aldehydes are known to directly affect glycolytic and fermentative enzymes resulting in reduced cell mass, growth rate and hence, ethanol productivity [171].

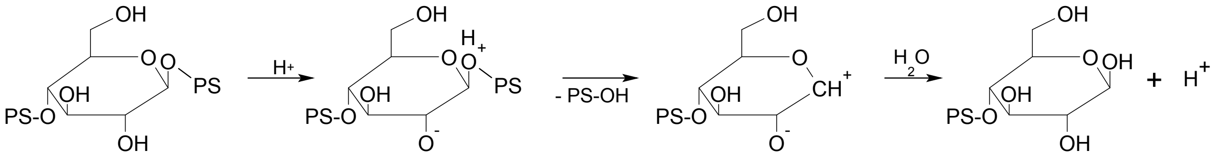


Figure 2.10.: Acid hydrolysis of carbohydrates with PS = polysaccharide chain (adapted from Fischer and Bäurich [75])

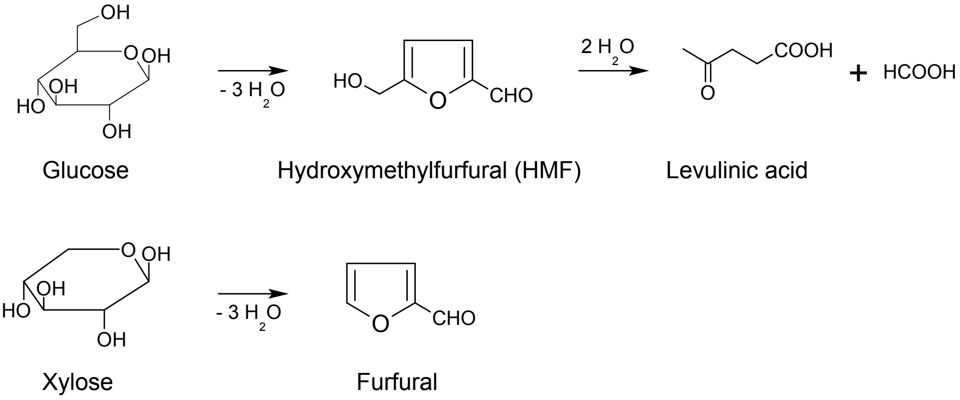


Figure 2.11.: Formation of hydroxymethylfurfural, levulinic acid, formic acid and furfural from monosaccharides in acidic medium (according to Fengel and Wegener [71])

They are, however, versatile platform chemicals and extended research has been conducted for their production from biomass [131, 148]. HMF can further undergo decomposition reactions, yielding formic acid and levulinic acid, with the latter being identified as high-value biobased chemical by the US Department of Energy [221]. Though furfural has also been described to decomposite to formic acid [171], Dunlop [55] reports that relatively long exposure at elevated temperatures is required to bring about extensive destruction by acids.

At conditions present during pulping using peroxyformic acid, also oxidation reactions involving poly- and monosaccharides are relevant. Both, hydroxyl groups and reducing end-groups may be subject to oxidative attack, resulting in aldehyde, keto and carboxyl groups. The ring structure can be preserved, as in the case of uronic acid formation, or destroyed by cleavage of C-O or C-C linkages. Products, other than uronic acids, are aldonic acids from hexoses as well as aldaric and pentosic acids from hexuronic acids. Furthermore, the use of formic acid leads to formylation as described by Gonzaléz et al. [88]. According to their study, the incorporation of formyl groups occurs with parallel deacetylation of approximately 82-97% of original acetyl groups [88].

Reaction of lignin

Delignification with organic acids is based on their function as acid as well as organic solvent enabling both, degradation and dissolution of lignin [134]. In contrast to alkaline pulping, both phenolic and non-phenolic arylpropane units can be converted into quinone methide intermediates during acidic pulping. This conversion is accompanied by elimination of the α- or, in the case of conjugated structures, γ-substituent. If this substituent is an adjacent unit, the conversion therefore results in lignin fragmentation [83].

Additionally, reactions with HO^+ based on electrophilic addition to aromatic and olefinic structures have to be taken into account in the case of peroxyformic acid pulping. Typical reactions involving hydroxonium ions are summarised in Figure 2.12 [84]. Ring hydroxylation, displacement of side chains as well as the cleavage of β-aryl ether bonds result in an increase in phenolic hydroxyl content. Furthermore, carbonyl and ester groups are formed during oxidative demethylation and oxidative ring cleavage, respectively. Overall fragmentation as well as increased hydrophilicity due to demethylation and formation of additional phenolic hydroxyl groups is achieved resulting in increased lignin solubility. Li et al. [137] confirm enhanced fragmentation by the addition of hydrogen peroxide, as the lignin obtained was of significantly lower molecular weight compared to solely using formic acid and contained less β-O-4 linkages than the corresponding milled wood lignin. Furthermore, Sun et al. [201] report a higher reactivity of p-hydroxyphenyl units towards peroxyformic acid compared to syringyl and guaiacyl structures.

In addition to fragmentation, condensation reactions occur in acidic media. As in alkaline media, external and internal nucleophiles compete for quinone methide intermediates. Ede and Brunow [56] report the major reaction pathway of β-aryl-ether model compounds in 99% formic acid to be condensation between the α-carbon and available aromatic groups, rather than cleavage of β-aryl linkages. Thus, the degree of lignin polymerisation is increased during pulping using solely formic acid, indicating the advantage of adding hydrogen peroxide.

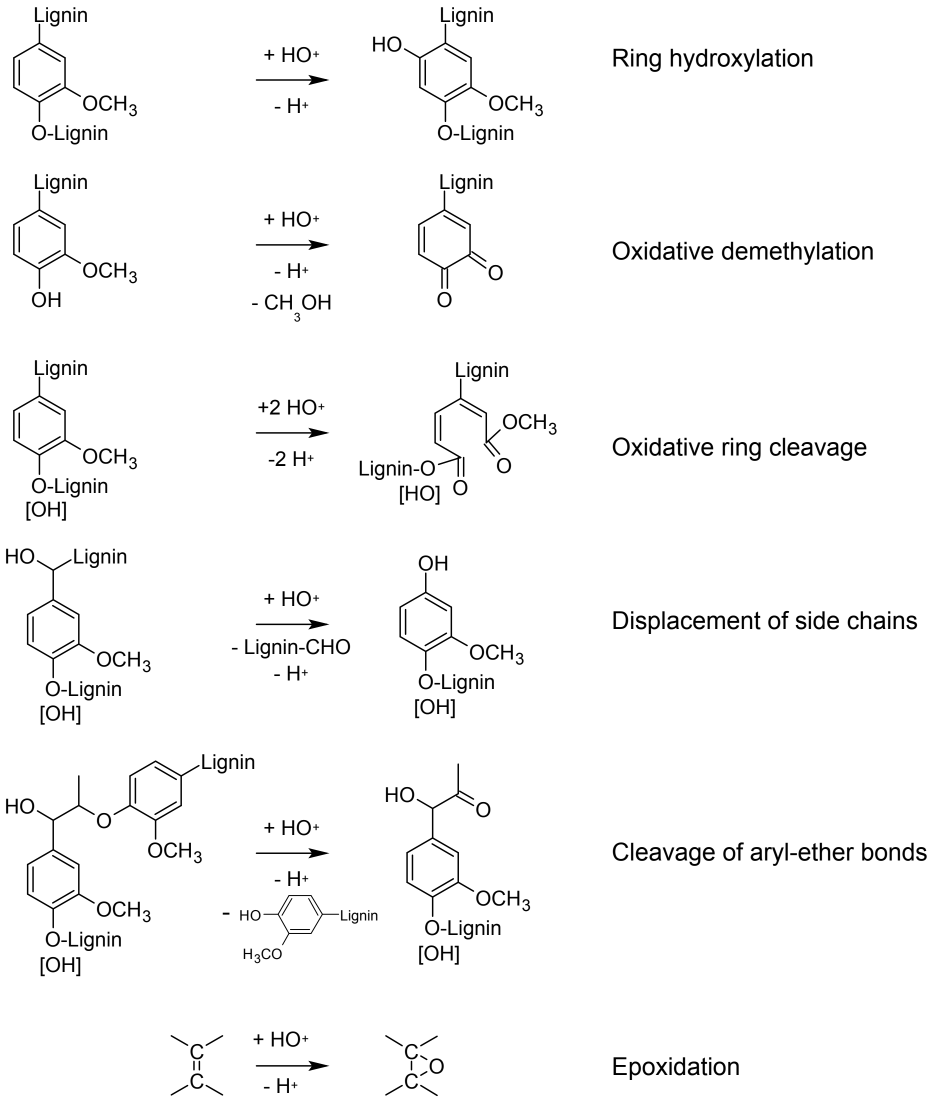


Figure 2.12.: Reactions of aromatic and olefinic structures with hydroxonium ions (according to Gierer [84])

Li et al. [137] report strong signals in FTIR spectra of lignins obtained by both formic acid as well as peroxyformic acid pulping, which correspond to their formate esters. Thus, they propose esterification of phenolic and aliphatic hydroxyl groups in the delignification process. Formylation of lignin during peroxyformic acid pulping was also documented by Hortling, Poppius and Sundquist [101] using ^{1}H-NMR.

Besides chemical changes, lignin is modified with regard to morphology and distribution. Donohoe et al. [54] describe the formation of spherical droplets on and within cell walls. This can be advantageous for subsequent enzymatic hydrolysis of pulp, as the accessibility of cellulose microfibrils is improved. Indeed, this relocation phenomenon might explain the mechanism for enhanced digestibility of dilute acid and hot water pretreated biomass [185].

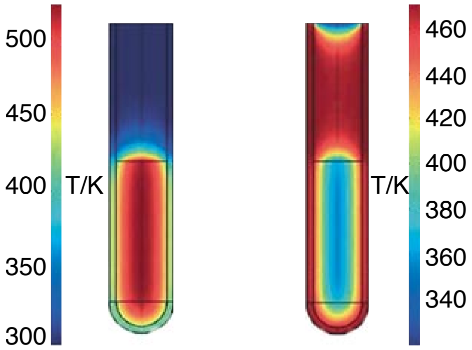


Figure 2.13.: Difference in the temperature profiles after 1 min of microwave irradiation (left) and treatment in an oil-bath (right). Microwave irradiation raises the temperature of the whole volume simultaneously, whereas in the oil-heated tube the reaction mixture in contact with the vessel wall is heated first (modified from Schanche [192]).

2.3.3. Microwave-assisted pulping

As the aim of biomass pretreatment is to disrupt their recalcitrant structure, microwave heating could be advantageous over conventional heating. Whereas the latter is based on superficial heat transfer, microwave-assisted pulping uses the ability of direct interaction between a heated object and an applied electromagnetic field to create heat. This results in simultaneous rapid heating as shown in Figure 2.13. Besides, so-called "hot spots" are created, as polar parts are selectively heated [102]. This explosion effect among the particles can improve the disruption of recalcitrant structures of lignocellulose. Besides the thermal effects, microwave irradiation also includes non-thermal effects, such as an increased mobility in solids as well as stabilisation of polar transition states and intermediates due to the electromagnetic field [44]. Microwave-assisted pulping of non-wood biomass was studied in water [159], sodium hydroxide [102, 185, 238], ethanol [159], sulphuric acid [159], acetic and propionic acid [87]. Hu and Wen [102] showed that microwave-assisted alkaline pulping of switchgrass leads to a high sugar yield of 99%. Zhu et al. [240] further state, that enzymatic hydrolysis of wheat straw, which was treated by microwave-assisted alkaline pretreatment had a higher hydrolysis rate.

While there is proof for an enhancing effect on enzymatic hydrolysis of pulp obtained by microwave-assisted pulping, there is a lack of data regarding the recovery and composition of the lignin fraction [43, 141, 217].

2.4. Products of the separation process

Recent advances in biotechnology enable production of several traditional chemicals from renewable biomass instead of petroleum. However, their commercialisation is hindered in many cases, as they have to meet many end-use requirements at a competitive cost. Further, it has to be shown, that their complete life cycle is environmentally friendly.

The European Commission addresses these difficulties by funding of specified research (e.g. Horizon2020), developing clear and unambiguous standards as well as encouraging Member States to give preference to biobased products. By this, the biobased share of all chemical sales is expected to rise with a compounded annual growth rate of close to 20% [59, 60].

2.4.1. Products derived from carbohydrates

In 2004, a report from the U.S. Department of Energy identified twelve building block chemicals that can be produced from sugars via biological or chemical conversion and that can be additionally converted to a number of high-value biobased chemicals or materials [221]. The list, updated 2010 by Bozell and Petersen [28], is shown in Table 2.6.

Biochemical transformation of biomass into fuel is represented almost entirely by ethanol. Many reviews are available on the potential and technology of bioethanol production from lignocellulosic feedstock, for instance by Alvira et al. [5], Balat [15], Demirbaş [47], Kim and Dale [116] as well as Talebnia, Karakashev and Angelidaki [206]. Nonetheless, the commercial availability was until recently reserved to bioethanol of the first generation. The first commercial plant worldwide for the production of cellulosic ethanol has just started its production in the first half of 2013 (Beta-Renewables in Crescentino, Italy) and has the potential of producing 40,000 tons of bioethanol per year [21]. More will follow in near future, for example DuPont with its commercial facility expected to be online this year. For further information the International Energy Agency [107] has collected data on current pilot and demonstration projects, for instance by Inbicon, and additionally released a corresponding report [11]. Besides of the application as fuel, ethanol is of interest as precursor of ethylene, which serves as raw material for polyethylene, ethylene oxide and polyvinylchloride. This pathway offers an alternative to fossil-based commodity plastics and provides a direct interface between biorefinery and petrochemical industry [28]. Though being the main driver, other products from carbohydrates besides ethanol will be introduced in the following.

As described in Section 2.3.2, acid-catalysed dehydration of pentoses yields furfural. Though unfavourable during biomass pretreatment, as it acts as inhibitor of subsequent enzymatic hydrolysis, it has a wide range of industrial applications as a solvent and for the production of polytetramethylene ether glycol, a precursor for elastic fibre production [6]. It can furthermore serve as replacement for petrochemicals in Nylon manufacture [190]. Additionally, furfural can be used for production of levulinic acid.

Table 2.6.: Top chemical opportunities from biorefinery carbohydrates and the technical state of their biobased production: + not established ++ large/pilot scale +++ commercial scale (according to Bozell and Petersen [28], and the U.S. Department of Energy [221])

Compound	Conversion	Utilisation	State
Ethanol	Biological (yeast)	Fuel Synthesis of ethylene and acetic acid	+++
Furfural	Chemical (dehydration)	Platform chemical Solvent	+++
HMF	Chemical (dehydration)	Synthesis of liquid alkanes and FDCA	+
FDCA	Chemical (oxidation of HMF)	Replacement for terephthalic acid → PET analog	+
Glycerol	Biological (yeast)	Gasoline fuel blend Synthesis of derivatives	+++
Isoprene	Biological (bacteria)	Rubber	++
Hydrocarbons	Biological (microalga or bacteria)	Platform chemicals Solvents	++
Lactic acid	Biological (bacteria)	Polylactic acid prod. Platform chemical Fibre prod.	+
Succinic acid	Biological (bacteria)	Specialty chemical Synthesis of e.g. maleic anhydride, succimide	++
Hydroxypropionic acid / aldehyde	Biological (bacteria)	Antimicrobial properties Prod. of acrylates Prod. of 1,3 propane diol	+
Levulinic acid	Chemical (hydration of HMF)	Platform chemical	+
Sorbitol	Chemical (reduction of glucose) Biological (bacteria)	Prod. of hydrocarbons Food & medical industry	+++
Xylitol	Chemical (catalytic hydrogenation of xylose)	Food & medical industry	++

Levulinic acid has drawn much attention, as it may serve as platform chemical for the synthesis of various organic compounds and hence, offers one of the larger families of potential industrial derivatives among the listed compounds. For instance, various levulinate esters may be used in the flavouring and fragrance industry or as blending components in biodiesel [34]. Furthermore, δ-aminolevulinate as well as a bisphenol derivative are obtainable, which can be used as herbicide and substitute for bisphenol A, respectively [85]. Thus, its diverse application possibilities, low cost and ready availability from both 5-carbon and 6-carbon sugars suggests that levulinic acid could be a building block of central importance within the biorefinery [221].

The biobased production of isoprene from bacterial conversion of carbohydrates has been pushed by a collaborative research agreement of Genencor and Goodyear in 2008 as a domestic approach to rubber production. The pilot and commercial development of BioIsoprene monomer is planned for 2015 [23]. This is a major step towards independence on petroleum-based feedstock with isoprene being a high value hydrocarbon with a world market of 1–2 billion US$ [28].

In contrast, the production of sorbitol based on hydrogenation of glucose is already practised commercially by several companies and has a current production volume in the order of approximately 312 million US$ annually [221].

To conclude, modern biotechnology allows the industry to target new and previously abandoned products derived from carbohydrates. This, coupled with increasing fossil feedstock costs, will improve the economics of future biorefineries.

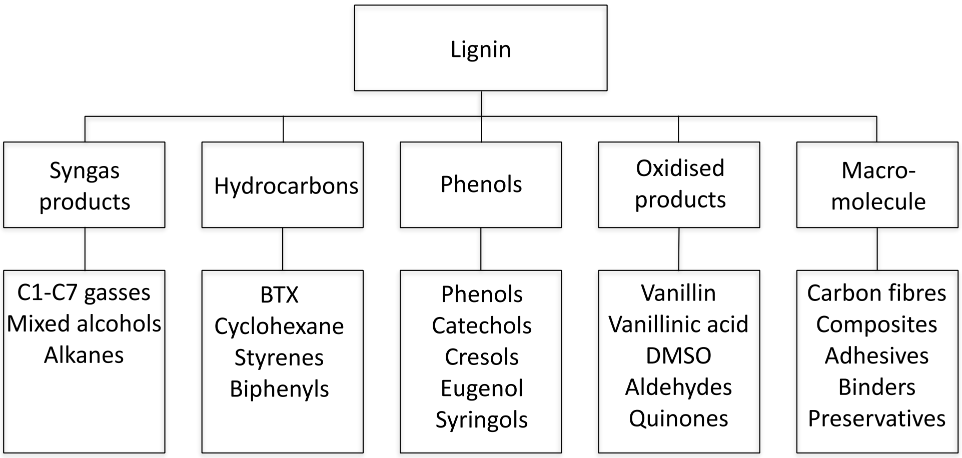


Figure 2.14.: Potential products from lignin (modified from IEA [104])

2.4.2. Products derived from lignin

The majority of accruing lignin is burnt in order to drive the process of pulp production. Whereas the energetic use of Kraft lignin requires increased care in order to avoid emission of SO_2, burning of sulphur-free lignins is considered to be less problematic [134]. Aside from the energetic use, there are various possibilities for a material use of lignin as macromolecule as well as after degradation, with an overview given in Figure 2.14.

Utilisation of the macromolecule

Lignin as macromolecule possesses properties suitable for many material applications and can hence, play a role in replacing petroleum-based components and/or components causing health or environmental hazards. As lignin can be isolated using various methods and raw materials, a broad product range is available. The type of application may vary depending on molecular weight, content of inorganics and sugar, content of functional groups (e.g. methoxyl, phenolic hydroxyl), solubility, miscibility with other components, as well as thermal properties, especially glass transition and decomposition temperature of the lignin. Lignosulphonates, for instance, are mainly used based on their surface-active properties. Table 2.7 shows some possibilities tested for sulphur-free lignins, as they will be produced by biorefineries. It lists the direct use of black-liquor or isolated lignin, as well as the utilisation after blending with other polymers or chemical modification. Also the combination of chemical modification and blending is advantageous in some cases.

The use of black liquor has clearly the advantage of omitting lignin recovery. It can directly be added to cement replacing 15% water improving concrete workability and compaction by up to 85% [57]. Another application of black liquor is the production of gels, which can be used in oil wells as drilling mud. Nonetheless, the non energetic usage of black liquor is only a niche market and the use of isolated lignins offers a more versatile utilisation as macromolecule.

Table 2.7.: Utilisation of lignin as macromolecule

Utilisation of...	after...	as...	Reference
black liquor	addition to cement	concrete	[57]
	reaction with phenol, formaldehyde and cross-linking agent	gel	[145]
isolated lignin	no treatment	filler in rubber	[122]
	no treatment	filler in paints	[19]
	addition of cross-linking agent	gel, absorbent	[174]
	addition of fibre, additives	biocomposite	[161]
	thermal pretreatment and purification	carbon fibre	[110] [99]
	chemical modification		
	• acetylation	carbon fibre	[239]
	• oxidative ammonolysis	fertiliser	[76]
	• sulphonation	dispersant	[20]
	blending with natural polymers		
	• protein	plastic, adhesive	[53]
	• starch	film	[18, 32]
	blending with synthetic polymers		
	• epoxy resin	adhesive, foam	[53, 64]
	• phenol formaldehyde	adhesive, foam	[53, 64]
	• poly(ethylene oxide)	carbon fibre	[111]
	• asphalt	asphalt concrete	[205]

As shown in Table 2.7, the utilisation of isolated lignin covers a broad range of application possibilities, especially after blending with other polymers and/or chemical modification. Nevertheless, its direct use is limited due to its poor thermal processability and poor spinnability. Nonetheless, processes have been developed to improve direct lignin application. The company Tecnaro, for instance, patented a procedure for thermoplastic processing of lignin after addition of fibres and water to give moulds [208]. Isolated lignin could furthermore be used to replace polyacrylonitrile, which is almost exclusively used to produce high strength carbon fibres leading to a generation of toxic by-products such as hydrogen cyanide [239]. A replacement would be preferable to overcome environmental issues and to decrease production costs. Carbon fibres, lightweight and fatigue resistant materials, are produced by fibre spinning followed by fibre thermostabilisation and carbonisation at approximately 1000 °C. Though spinning of fibres is possible directly from isolated lignin, Kadla et al. [110, 111] showed that blending with polyvinyl alcohol or polyacrylonitrile enhances the spinnability and tensile strength of fibres. However, typical tensile strengths reached with lignin are still low in comparison to commercial polyacrylonitrile based carbon fibres [173, S.15].

By mixing lignin with other compounds, blends with lower cost than those of a single component or with more desirable properties, altered to meet specific needs, are obtainable [64]. For instance, up to 35% of phenol could be replaced by lignin in phenol-formaldehyde resins without alteration of properties [53]. However, the rate of reaction is determined by the number of reactive sites in the used lignin [64]. Thus, a preceding chemical modification by demethylation, hydroxyalkylation or phenolation has been used to improve lignin blends [4, 146]. Furthermore, the ability to control the hydrophilicity of lignin by derivatisation results in miscibility with any of the natural and synthetic polymers listed in Table 2.7. This further leads to an improvement of tensile strength and bulk modulus as well as to protection of the product against oxidative degradation.

Chemical modification of lignin is not only advantageous for improving its miscibilities with other polymers, but it is also used to introduce new functionalities. Oxidative ammonolysis of lignin, for instance, involves the reaction with ammonia and oxygen under pressure and results in accumulation of nitrogen, demethoxylation, demethylation as well as oxidation reactions [165]. Due to this artificial humification, lignin can be converted to a fertiliser providing nitrogen with immediate as well as long-term availability. A successful realisation for sulphur-free lignin was shown by Fischer and Schiene [76].

Clearly, the utilisation of lignin as macromonomer is a broad field. Several reviews are available, for instance by Doherty, Mousavioun and Fellows [53], Gandini and Belgacem [80] as well as Lora [145].

Utilisation after depolymerisation

The valorisation of lignin for the production of platform, bulk and fine chemicals is crucial, as it represents the only source to produce renewable aromatic compounds on which society currently depends [236]. Nonetheless, lignin depolymerisation is challenging because of 1. variation in C-C and C-O bond strengths in lignin, 2. recondensation of reaction products, 3. heterogeneity of products in the product stream and hence, 4. difficulty in product separation and purification. Thus, down-streaming processes of lignin have still not reached commercial plant scale in 2014 [229].

Table 2.8 summarises methods currently studied for lignin depolymerisation resulting in a variety of products. Liquid oil, as obtained by pyrolysis, constitutes of a complex mixture of many aromatic and non-aromatic compounds with a wide molecular weight distribution from light hydrocarbons to high-molecular weight polymers [10]. Hydrogenolysis, pyrolysis in the presence of hydrogen, leads to higher yields of monophenols as well as less char formation and is hence, a promising method for lignin depolymerisation [170].

Compared to pyrolysis, higher temperatures are used for gasification, which leads to cracking of the aromatic ring, carbonyl, carboxyl and methoxyl functional groups. As a consequence, hydrogen, carbon dioxide, carbon monoxide and methan are released. This gas mixture can be used for the synthesis of methanol and FISCHER–TROPSCH fuels as well as for power generation by combustion in gas turbines [10]. The usage of sulphur-free lignin is advantageous in this case as no H_2S is formed [144]. Cracking of lignin can also be achieved by catalytic cracking allowing the use of lower temperatures (300-500 °C). The products formed are found to be strongly linked to catalyst structure.

Table 2.8.: Lignin depolymerisation: methods and products (based on Lora [144], Zakzeski et al. [236] and Azadi et al. [10])

Method	Conditions	Products
Pyrolysis	thermal treatment (300-600 °C) oxygen-free, partly with catalyst	liquid oil, char, gas
Gasification	thermal treatment (700-1000 °C) partly with catalyst	synthesis gas (H_2, CO, CO_2, CH_4)
Catalytic cracking	acid + zeolite/silica-alumina	low molecular weight aromatic compounds
Hydrolysis	catalyst + base	phenolic compounds
Reduction	H_2 + catalyst	phenol, BTX[1]
Oxidation	metal oxide hydrogen peroxide	aromatic aldehydes and carboxylic acids

[1] benzene, toluene, xylene

Lignin reduction allows the direct production of platform chemicals such as phenol, benzene, toluene and xylene. These can be further processed to fine chemicals using technology already developed by the petroleum industry [236]. By oxidation, on the contrary, lignin is converted to aromatic aldehydes as well as carboxylic acids and hence, to chemicals with high functionalities. Furthermore, the direct production of fine chemicals, such as vanillin, is possible.

Despite the increasing interest in using lignin as source for platform chemicals, there is still a lack of profound understanding of catalyst-lignin interactions and of techniques for product separation and purification. This complicates the valorisation of actual lignin streams. However, Zakzeski et al. [236] point out that there are analogies between the 20th century petroleum refinery and the 21st century biorefinery. Thus, it will still take considerable effort for biorefinery procedures to become as efficient and integrated systems as are petroleum refineries in the present.

3. Alkaline pulping of agricultural products and by-products

Feedstock supply is one of the major challenges of a lignocellulosic biorefinery and efforts are made to enlarge the basis of suitable biomasses (eg. Horizon 2020, call for competitive low-carbon energy). Pulping of many different agricultural residues has been reported in the literature [37, 143, 188]. However, they mainly focus on the carbohydrate fraction for ethanol production. Nonetheless, value-added valorisation of lignin is inevitable for a biorefinery concept.

This chapter is dedicated to the separation of different lignocellulosic raw materials into a pulp and lignin fraction using conventional alkaline pulping. Besides the separability into these fractions, this study focusses on the characterisation of the lignin fractions in order to reveal similar and unique structural features and to explore utilisation possibilities accordingly.

3.1. Composition of raw material

In the framework of a lignocellulosic biorefinery the raw material can origin from various sources, such as agricultural by-products, energy crops or wood chips and may therefore be very heterogeneous. They do not only differ in plant species, but also in the part of plant (stalk, husk, leaf, cob) that is used.

Table 3.1 shows the composition of the considered raw materials (determined according to Section 6.5) and the abbreviations used for following figures and discussions. The determined contents of holocellulose, lignin, extractives and ash generally sums up to approximately 100%. High ash contents, however, have shown to be problematic for the used wet-chemical determination, though high care was taken by ash-correcting initial and resulting weight. One major problem when working with agricultural products is their contamination with soil apparent as high contents of ash. This varies strongly depending on the harvesting method, soil and plant as can be seen in Table 3.1. Further information about their origin and, if applicable, pretreatment is given in Section 6.1.

Wheat (ws), barley (bs) and maize straw (ms) show a similar composition as they are obtained from plants belonging to the same family *Poaceae* at similar harvest times. Though belonging to the same class, the composition of oat differs, as husks were studied in this case. They are rich in hemicellulose and contain less lignin compared to the aforementioned straws, as described in the literature [220].

High contents of hemicelluloses are also found in rape straw (rs), sunflower stalks (ss) and tomato stalks (ts), with the latter even exceeding the content of cellulose. As hemicelluloses are already studied for different applications other than fermentation, for instance as additives in paper or films, the listed raw materials could be promising candidates in

Table 3.1.: Composition of raw materials used for conventional alkaline pulping (%, on dry matter basis)

Raw material	Cellulose	Hemicellulose	Lignin	Extractives	Ash	Sum
Barley straw (ba)	42.2	23.9	19.8	5.7	7.5	99.1
Coconut shell powder (cp)	24.1	19.6	47.9	2.3	3.5	97.4
Hemp shives (hs)	49.6	26.1	17.2	4.9	3.5	101.3
Horse manure (hm)	37.3	12.1	31.5	5.8	10.6	97.3
Maize straw (ms)	38.5	26.1	21.4	5.7	12.0	103.7
Miscanthus (mi)	57.3	21.3	24.5	2.5	1.5	107.1
Oat husk (oh)	38.8	38.9	16.4	1.4	2.9	98.4
Pretreated alfalfa (pa)	39.2	23.7	12.6	10.9	7.5	93.9
Rape straw (rs)	36.2	36.4	16.8	2.5	15.9	107.8
Sunflower stalks (ss)	31.2	31.6	15.1	10.6	6.0	94.5
Tomato stalks (ts)	19.2	26.4	15.8	2.4	25.9	89.7
Wheat straw (ws)	44.2	30.7	19.3	3.9	2.6	100.7

this area [156]. Here, further studies on their exact composition of hemicelluloses is necessary. Furthermore, a high content of cellulose was found in miscanthus (mi), which makes it an interesting starting material for dissolving pulp production. In contrast, coconut shell powder (cp), which is obtained after defibration of coconut shell and subsequent removal of coir fibre, distinguishes itself by its high content of lignin. It makes up almost half of the material.

Sunflower stalks and pretreated alfalfa (pa) show comparatively high contents of ethanol-toluene extractives and hence high contents of free fatty acids as well as other acids such as azelaic and maleic acid, as these are typical compounds extracted by this solvent mixture [175]. The high ash moieties found in rape straw and tomato stalks are problematic, as it causes difficulties during recovery of alkaline black liquor and results in excessive scaling and fouling on boiler units [158]. Thus, removal of attached soil prior to pulping is important, but has shown to be difficult, as sand strongly adheres especially to tomato stalks.

3.2. Separation into a carbohydrate and lignin fraction

The separation of lignocellulose into a carbohydrate and a lignin fraction is desirable in order to fully exploit the potential of each component. For alkaline pulping, sodium hydroxide was used and the parameters were as follows: liquid/solid ratio = 6.2 mL/g, $\omega(\mathrm{NaOH}) = 3\,\mathrm{wt\%}$, $\theta = 160\,^{\circ}\mathrm{C}$, time at maximum temperature = 30 min, H_{32}-factor = 320. The pulp was separated from the black liquor, washed and dried. The lignin fraction was precipitated from the black liquor at pH=1 and the precipitation product was separated, washed and dried. A high separability is characterised by high yield and purity of pulp and precipitation product. The studied raw materials showed a very different behaviour towards alkaline pulping regarding their separability into these fractions, which will further

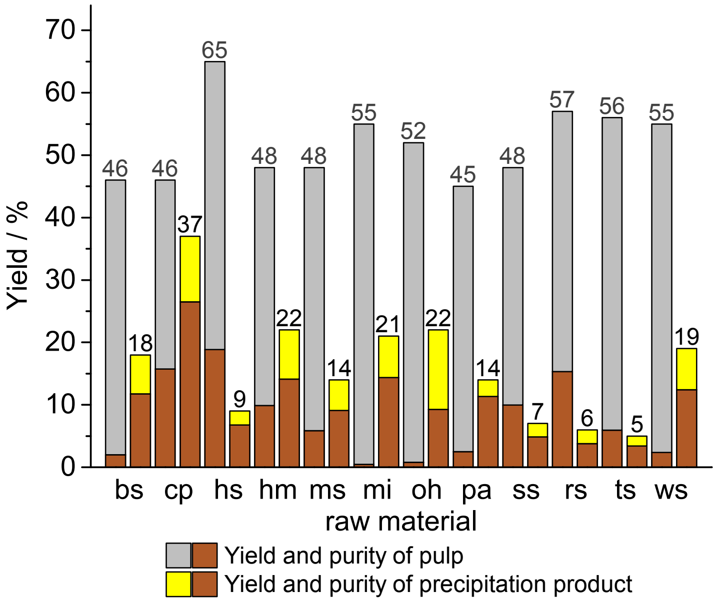


Figure 3.1.: Yield and purity, given as respective KLASON-lignin content, of pulp and precipitation product with regard to raw material

be referred to as pulp and precipitation product for carbohydrate and lignin fraction, respectively.

Figure 3.1 illustrates the yield and purity of pulp and precipitation product obtained from the studied materials. Values are given as respective KLASON-lignin content with regard to the raw material. Generally, alkaline pulping using the parameters specified in Section 6.4.1 showed to be suitable for the separation of most raw materials. Pulps obtained from barley straw (bs), pretreated alfalfa (pa), miscanthus (mi) and wheat straw (ws), for instance, contain only minor lignin impurities of less than 6% KLASON-lignin. This delignification is also reflected in considerable yields of precipitation products. For pretreated alfalfa, 89% of the original lignin is recoverable.

On the contrary, the tested alkaline pulping procedure is not suitable for separation of hemp shives (hs), rape straw (rs), sunflower stalks (ss) and tomato stalks (ts). In these cases, the percentage of lignin in pulp is higher compared to the raw material. Thus, alkaline pulping of these materials leads to a higher solubilisation of hemicelluloses than of lignin, resulting in a low recoverable amount of the latter.

Nonetheless, for some materials at least one fraction could be obtained with high purity. Pulp obtained from coconut shell powder, for instance, still contains a high amount of lignin impurities. However, the yield of precipitation product is high with regard to the raw material due to its high original lignin content. It is worth noting that this lignin is also of comparatively high purity having a KLASON-lignin content of 71.6%. This could be further increased up to 86.5% after purification using dialysis. In contrast, the obtained precipitation product from oat husks (oh) is of very low purity even after purification by dialysis having a KLASON-lignin content of 55.6%. Nonetheless, it is an excellent material for production of pulp, as pulp yield was as high as 52% and it consisted of 96% of holocellulose. It is noteworthy that the used pulping parameters, which were optimised with regard to wheat straw, are not the optimum parameters for all of the studied materials. When using oat husks, for instance, studies carried out by Gläser [86] have shown that a sodium hydroxide concentration of 2 wt% instead of 3 wt% significantly improves the yield of pulp by 14%.

In conclusion, the decision whether one of these materials is suitable for alkaline pulping strongly depends on whether the aim is the separation into a carbohydrate and lignin fraction or the production of either of them.

3.3. Characterisation of pulp

Several factors have been reported to influence enzymatic hydrolysis of pulp into monosaccharides. Among them are the content of lignin and hemicelluloses as well as the degree of polymerisation. On these key indicators lies the focus in the following section [36].

3.3.1. Composition

A successful separation of lignocellulosics is also reflected in the composition of the obtained pulp, shown in Table 3.2. As for the composition of raw materials, high ash contents have shown to cause disturbances in the gravimetrical analysis of constituents, despite using ash-correction for initial and resulting weights, leading to some sums exceeding 100%.

Lignin content in pulp is not found to correlate with its content in the corresponding raw material. It is lowest in pulp from barley straw (bs), miscanthus (mi), oat husks (oh) and wheat straw (ws). In addition, the content of cellulose exceeds 70%, making these pulps promising starting materials for bioethanol production. As cellulose content of miscanthus pulp is highest with approximately 80%, it could, after further hemicellulose

Table 3.2.: Composition of pulp obtained from different raw materials by conventional alkaline pulping (%, on dry matter basis)

Raw material	Cellulose	Hemicellulose	Lignin	Extractives	Ash	Sum
Barley straw (ba)	70.4	11.9	4.3	2.3	8.7	97.6
Coconut shell powder (cp)	38.5	12.1	34.2	2.8	10.4	98.0
Hemp shives (hs)	59.4	17.5	19.0	2.1	1.9	99.9
Horse manure (hm)	62.3	11.5	20.6	2.8	10.5	107.7
Maize straw (ms)	69.7	12.0	12.1	1.0	14.9	109.7
Miscanthus (mi)	79.0	12.5	0.8	0.8	1.6	94.7
Oat husk (oh)	70.9	24.6	1.5	0.6	1.3	98.9
Pretreated alfalfa (pa)	73.7	15.8	5.5	4.3	9.4	108.7
Rape straw (rs)	49.1	21.1	26.9	2.0	5.3	104.4
Sunflower stalks (ss)	59.4	10.9	20.8	1.2	5.0	97.3
Tomato stalks (ts)	50.1	0.0	10.6	2.0	25.0	85.7
Wheat straw (ws)	76.5	12.3	4.3	1.7	2.6	97.4

removal by variation of pulping parameters or additional hot water pretreatment, serve as dissolving pulp.

Besides the removal of lignin, hemicellulose removal increases cellulose accessibility and hence, digestibility by enzymes [160, 168]. With some exceptions, the content of residual hemicellulose is in a quite narrow range between 10.9% for sunflower stalks and 17.5% for hemp shives. For rape straw and oat husks its content in pulp exceeds 20%, being the raw materials with the highest original hemicellulose content (cf. Table 3.1). However, no relationship was found between hemicellulose content in raw material and pulp. In case of tomato stalks, for instance, complete hemicellulose removal is achievable despite its initial content of 26.4%. Though hemicelluloses are removed completely, tomato stalks are not suitable for bioethanol production in the harvested form, as the extremely high ash content of 25.0% found in pulp may not only cause technological problems, but may also act as enzyme inhibitor [138]. In addition, washing is made difficult as the soil strongly adheres to the stalks.

3.3.2. Intrinsic viscosity

Besides the composition of pulp obtained by different raw materials, the intrinsic viscosity was determined to obtain information about the degree of polymerisation, which can be estimated as "true viscosity average degree of polymerization" (DP_v) according to Kes and Christensen [114]. This value is used with considerable reservation, especially as pulp samples contain hemicelluloses and lignin moieties besides cellulose. Results are shown in Figure 3.2. The intrinsic viscosity is not determinable for all pulp preparations, as some contain high amounts of lignin, which interferes with the method established for cellulose samples.

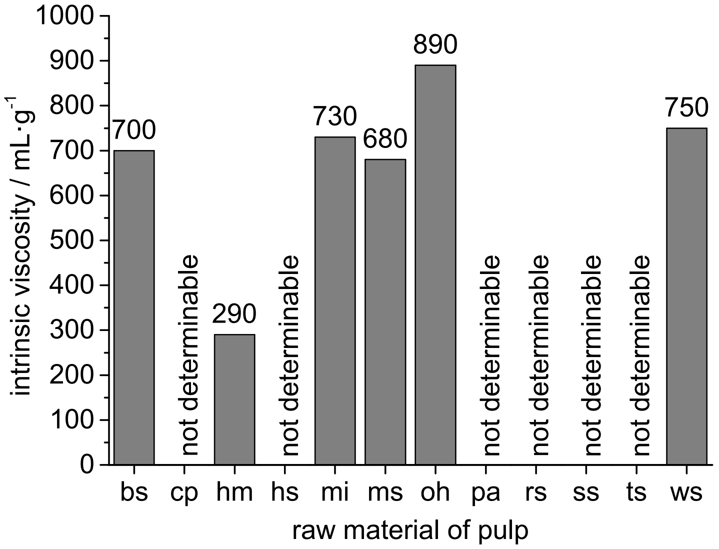


Figure 3.2.: Intrinsic viscosities of pulp obtained from different raw materials by conventional alkaline pulping

For pulp obtained from straws intrinsic viscosities in the comparatively narrow range of 680 to 750 $\mathrm{mL \cdot g^{-1}}$ were determined. Thus, it can be concluded that these pulps have similar degrees of polymerisation of approximately $\mathrm{DP_v} = 3\,100 - 3\,600$. Thus, degradation during pulping is evident based on DP range of 7 000 to 15 000 for plant cellulose reported by Fengel and Wegener [65]. Hallac and Ragauskas [92], however, report a cellulose DP range of $1800 - 4000$ in agricultural residues using the same viscosimetry measurement technique. Based on this, the degradation of cellulose is not as apparent under the conditions used for alkaline pulping. Furthermore, intrinsic viscosity of pulp obtained from oat husks (oh) is even higher resulting in a $\mathrm{DP_v}$ of approximately 4500.

On the contrary to the studied herbaceous residues, intrinsic viscosity for pulp obtained from horse manure (hm) is very low being 290 $\mathrm{mL \cdot g^{-1}}$ ($\mathrm{DP_v} \approx 900$). This clearly illustrates the effect of previous digestion of gramineous raw material. The low degree of polymerisation may significantly enhance enzymatic digestibility by the increasing number of cellulose chain reducing ends allowing more exoglucanase effective activity. Thus, pulp obtained from horse manure is proposed as starting material for further studies [92].

A mild pulp delignification is proposed for samples excluded in this section using acetic acid and sodium chlorite in order to enable determination of intrinsic viscosities. This procedure was used, for instance, by Kumar et al. [128]. Though well established, this method has been in discussion to significantly reduce the chain length of cellulose [128].

3.4. Characterisation of precipitation product

As utilisation of the lignin-rich precipitation product is inevitable for an economic biorefinery concept and as it provides the only source of renewable aromatic compounds, the following section focusses on its characterisation with regard to the different raw materials used. Elucidating structural details of lignin is of importance for finding future applications. To achieve this, Fourier transform infrared (FTIR) spectroscopy and elementary analysis are used to provide information on non-modified sample structure. These methods are supported by thioacidolysis coupled with gas chromatography and mass spectrometer (GC/MS), size-exclusion-chromatography as well as different wet-chemical methods. Thermal desorption coupled with GC/MS and differential scanning calorimetry are used to provide information particular for applications based on thermal processing.

3.4.1. Composition

High purity of the precipitation products, expressed as their KLASON-lignin content, is crucial for most potential applications. Figure 3.3 shows the KLASON-lignin of all precipitation products after washing and after further purification using dialysis, by which impurities of low molecular weight were removed. Dialysis was successful to a certain extend for all precipitation products, leading to higher lignin contents. For some, however, the purification effect was only minor. Thus, the impurities could involve carbohydrates linked to lignin as well as high molecular weight carbohydrates.

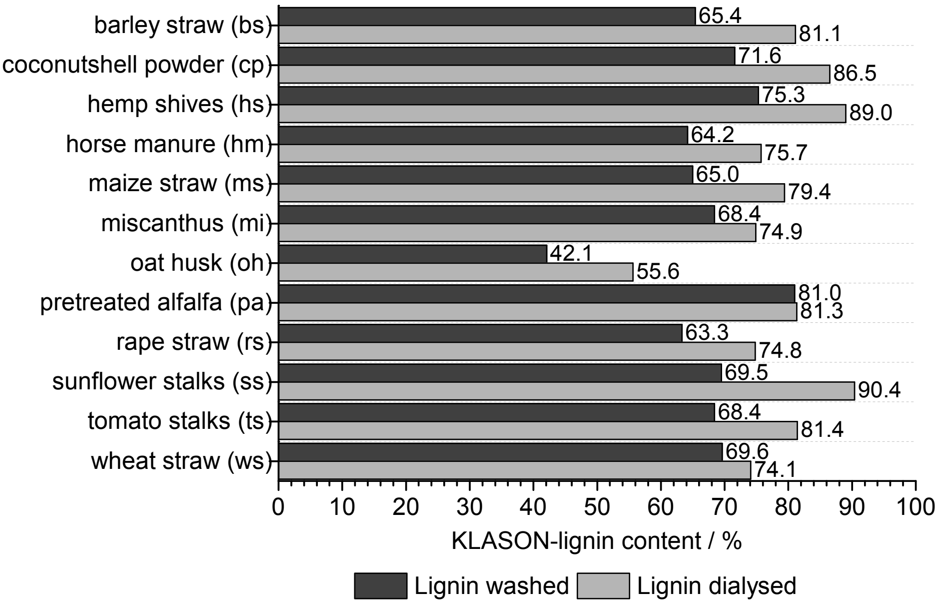


Figure 3.3.: Lignin content of precipitation products obtained from different raw materials by conventional alkaline pulping after washing and dialysing

For a more detailed characterisation, the precipitation product of wheat straw was delignified using sodium chlorite and acetic acid. Whereas ash was only responsible for 1.0% of the found impurities, the separated holocellulose was found to be responsible for 76.5%. It consisted of 57.28% xylose, 2.39% arabinose, 2.33% glucose and 0.05% galactose. As a mild hydrolysis procedure was used in order to preserve xylose, hydrolysis into monomers was not complete. This is also revealed by the sum of monomers being only 62.05%. The relative composition of the separated holocellulose is similar to the composition of hemicelluloses in wheat straw with xylose being the main component followed by arabinose, glucose and galactose [181]. By this, hemicelluloses were found to be the predominant impurity for alkaline wheat straw lignin as described by Lu and Ralph [146].

As it was not possible to obtain lignin from oat husks with a KLASON-lignin content of at least 60% it shall not be considered in the following sections. Kraftlignin (Indulin AT) is used as well-known lignin for comparison.

3.4.2. Fourier transform infrared spectroscopy

FTIR spectra of dialysed precipitation products from selected raw materials are given in Figure 3.4. All bands were assigned according to Faix [62]. To enable comparison of spectra, they were normalised to the aromatic skeletal vibration at 1510 cm^{-1}.

The aliphatic groups are represented by the C-H stretching vibrations at 2920 cm^{-1} and 2850 cm^{-1} in methyl and methylene groups [62]. The Aliphatic Index, given in Table 3.3, combines the intensities of these peaks and expresses the ratio of aliphatic to aromatic contributions. It varies depending on the used raw material. With a value of 2.5 it is highest for lignin from pretreated alfalfa (pa). However, the aliphatic contribution of lignin from pa originates not only from aliphatic lignin side chains, but also from fatty acids present in the lignin preparation [25].

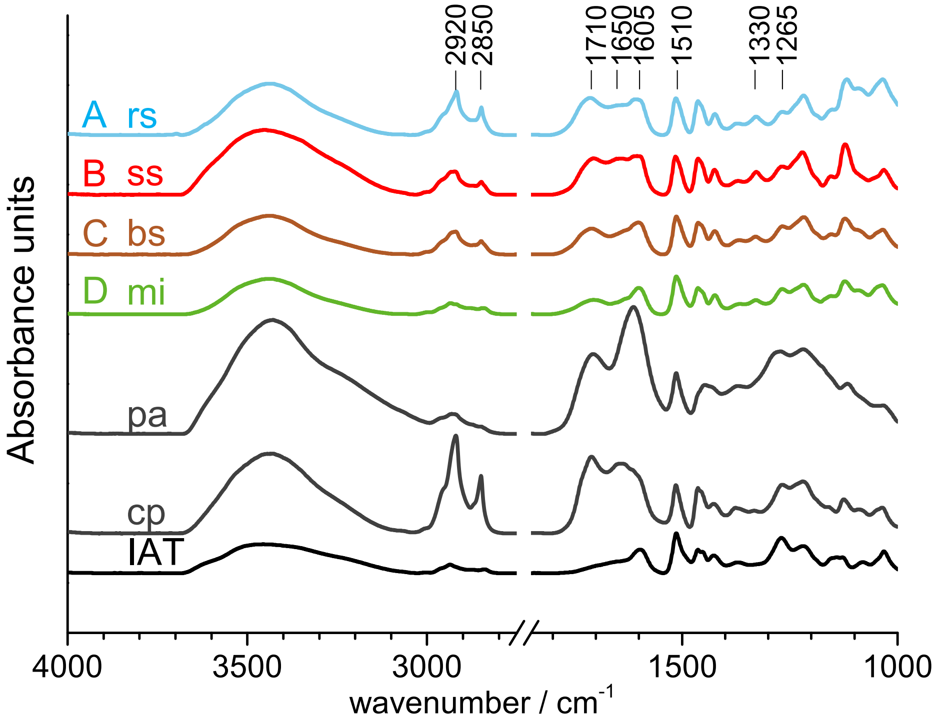


Figure 3.4.: FTIR spectra of dialysed precipitation products obtained from different raw materials by conventional alkaline pulping and Indulin AT

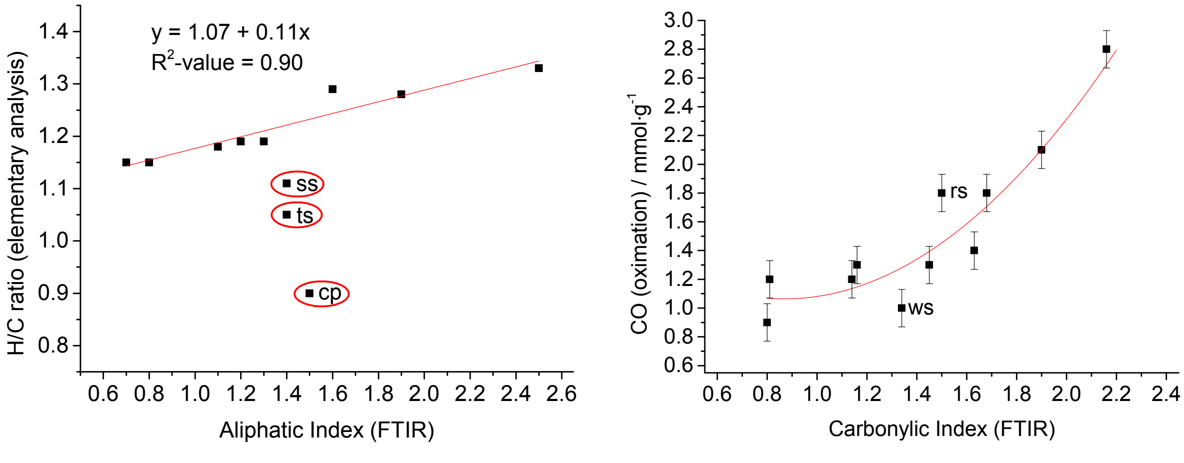
Table 3.3.: Results from FTIR spectroscopy of dialysed precipitation products obtained from different raw materials by conventional alkaline pulping and Indulin AT

Prec. prod.	Aliphatic Index	Carbonylic Index	S/G	Condensation Index
	$I_{(2920+2850)}$	$I_{(1710+1650)}$	$I_{(1330:1265)}$	$\frac{\sum Min_{(1550-1050)}}{\sum Max_{(1600-1030)}}$
hm	1.9	1.7	0.8	0.8
rs	1.6	1.5	0.9	0.7
ss	1.4	1.6	0.9	0.5
ts	1.4	1.5	0.9	0.5
bs	1.0	1.1	0.8	0.6
ms	1.2	1.1	0.8	0.7
ws	1.3	1.3	0.8	0.6
hs	0.7	0.8	0.8	0.6
mi	0.8	0.8	0.7	0.6
cp	1.5	1.9	0.8	0.7
pa	2.5	2.2	0.8	0.7
IAT	0.8	0.6	0.5	0.5

This assumption is supported by GC/MS results after thioacidolysis of alfalfa lignin, where palmitic acid, oleic acid and stearic acid were identified, as will be discussed in Section 3.4.5. The lowest Aliphatic Indices have precipitation products obtained from hemp shives (hs) and miscanthus (mi) as well as Indulin AT (IAT), indicating high aromatic proportions in these lignins.

In order to further elucidate the informative value of the Aliphatic Index, its relationship to the hydrogen to carbon ratio (H/C ratio) obtained by elementary analysis is illustrated in Figure 3.5a. When omitting lignin from sunflower stalks (ss), tomato stalks (ts) and coconut shell powder (cp), a correlation between these variables was found according to Fishers test [52] (error probability of 1% (P=0.99), degree of freedom f=n-2=6). The H/C ratio has been in discussion as expression for the degree of aromaticity [225]. The correlation found with the Aliphatic Index supports its use, at least with regard to most lignins. As mentioned, the joint presentation of the values reveals, that the correlation is not applicable to precipitation products obtained from sunflower stalks, tomato stalks and coconut shell powder as all of them have a high Aliphatic Index with a comparatively low value for the H/C ratio. This indicates a high proportion of aromatic linkages such as 5-5 or 4-O-5 linkages, as thereby the H/C ratio is decreased without affecting the intensity of C-H stretching vibration in methyl and methylene groups.

The absorbances at 1650 and 1710 cm^{-1}, originate from carbonylic stretching vibrations in unconjugated ketones, carbonyl and ester groups as well as in conjugated p-substituted aryl ketones, respectively [62]. Both peaks are likely to superimpose contributions of carboxyl and aldehyde groups, which absorb around 1700 cm^{-1} [62]. The Carbonylic Index, given in Table 3.3, combines the intensities of peaks at 1650 and 1710 cm^{-1} with regard to the aromatic skeletal vibration. It is highest for precipitation products from coconut shell powder (cp) as well as pretreated alfalfa (pa), which accordingly have high proportions of ketones, such as α-carbonyl groups. The lowest values have precipitation products from hemp shives (hs) and miscanthus (mi) as well as Indulin AT (IAT).



(a) (b)

Figure 3.5.: Relationship between (a) Aliphatic Index and H/C ratio determined by elementary analysis and (b) Carbonylic Index and carbonyl content determined wet-chemically by oximation

In order to further elucidate the informative value of the Carbonylic Index, its relationship to the wet-chemical determination of carbonyl groups is shown in Figure 3.5b. Whereas the FTIR method also includes esters and contributions of carboxyl groups, these are not covered by the wet-chemical oximation method. Despite these differences, both methods give similar trends for the studied lignin samples. Thus, in most of the studied precipitation products a major proportion of C=O bonds is present as ketones. Precipitation product obtained from wheat straw, however, has a high Carbonylic Index with a comparatively low value for wet-chemical carbonyl content. Here, a higher proportion of ester groups may account for this variance.

The syringyl to guaiacyl ratio (S/G ratio) was estimated as ratio of the intensity at 1330 $\mathrm{cm^{-1}}$, assignable to mainly syringyl, and the intensity at 1265 $\mathrm{cm^{-1}}$, assignable to guaiacyl but also including carbonyl contributions. Though obviously superimposed by interfering vibrations, the ratio of those peaks has shown to be useful to give a rough estimate of the syringyl to guaiacyl ratio, which is given in Table 3.3. It varies only slightly between 0.7 for precipitation product obtained from miscanthus (mi) and 0.9 for precipitation product obtained from rape straw (rs), sunflower stalks (ss) and tomato stalks (ts). For more reliable results for the S/G ratio by FTIR spectroscopy the influence of carbonyl could be reduced, for instance by reaction with $NaBH_4$.

As condensation occurs parallel to fragmentation reactions during pulping, the formation of new C-C linkages indicates the severity of isolation. Faix [61] suggests, that this is reflected by condensation indices (CI), which are further determinable by FTIR spectroscopy. A major drawback of this method is that differences are not as distinct, as CIs are within a small range between approximately 0.4 and 0.6 for milled wood lignin and KLASON-lignin, respectively [61]. Table 3.3 gives CIs calculated by data obtained from FTIR spectra of the studied precipitation products. As the CIs of all lignin preparations are higher than or equal 0.5, it can be concluded, that they are highly condensated. This is not surprising, giving the harsh reaction conditions used during alkaline pulping compared to isolation as milled wood lignin. Values exceeding the range studied by Faix [61] are determined for precipitation products from pretreated alfalfa, coconut shell powder, maize straw, rape straw and horse manure. The latter has the highest CI being 0.8. This is plausible considering that not only alkaline pulping contributes to the severity of its isolation, but also chemical and enzymatic procedures during digestion of the primary grass.

To conclude, the Aliphatic and Carbonylic Indices determined by FTIR spectroscopy allow the classification into groups, within which lignins are similar in this regard. Based on these contributions the following groups are proposed:

A horse manure (hm), rape straw (rs)

B sunflower stalks (ss), tomato stalks (ts)

C barley- (bs), maize- (ms) and wheat straw (ws)

D hemp shives (hs), miscanthus (mi)

No substitutes could be found for lignin obtained from pretreated alfalfa (pa) and coconut shell powder (cp). As FTIR spectroscopy allows prediction of structural and chemical information on lignin, a chemometric approach using Principal Component Analysis (PCA) is proposed for further studies.

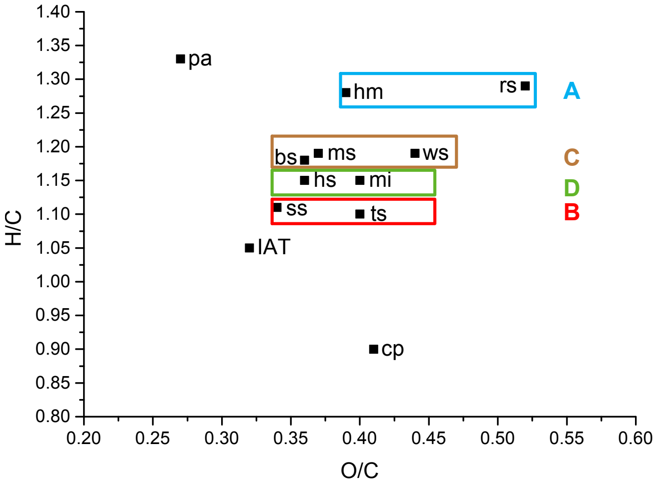


Figure 3.6.: H/C and O/C ratios derived by elementary analysis of dialysed precipitation products obtained from different raw materials by conventional alkaline pulping and Indulin AT

3.4.3. Elementary analysis

Figure 3.6 shows results derived by elementary analysis of the studied precipitation products based on the H/C versus O/C diagram suggested by Krevelen and Schuyer [124]. Typical values of the H/C and O/C ratio of lignin varies in the range of 0.80 to 1.35 and 0.30 to 0.50, respectively, and may overlap with brown coal [193]. These values can be shifted by impurities included in the lignin fraction, such as long-chain fatty acids (H/C ratio ↑, O/C ratio ↓) and carbohydrates (H/C ratio ↑, O/C ratio ↑). In addition, they are influenced by the lignin structure itself: by the degree of condensation (H/C ratio ↓) and by the content of functional groups, for instance -OCH_3 (H/C ratio ↑, O/C ratio ↑).

Nonetheless, most of the studied precipitation products are within a typical range of H/C and O/C ratios for lignin. Lignins with similar H/C ratios are grouped, leading to the same classification as was found by FTIR spectroscopy. The H/C ratio decreases from approximately 1.29 for group A, to 1.20 for group C, to 1.15 for group D and finally to 1.10 for group B. As discussed in Section 3.4.2, the H/C ratio correlates with the Aliphatic Index determined by FTIR spectroscopy, when omitting group B (precipitation products from tomato and sunflower stalks). The O/C ratio is within a similar range between 0.34 and 0.45 for all four groups, though it is higher for the precipitation product from rape straw, being 0.52. However, no further distinction between the groups can be made by O/C ratio.

Compared to the proposed groups, precipitation products from pretreated alfalfa and from coconut shell powder show different characteristics. The H/C ratio of precipitation product from pretreated alfalfa (pa) is 1.33 and hence, comparatively high. A plausible explanation is its content of fatty acids, also found by GC/MS of thioacidolysis products (cf. Section 3.4.5). These fatty acids shift the H/C ratio to higher and the O/C ratio to lower values. Precipitation product from coconut shell powder (cp) also shows a unique H/C ratio, being 0.90 and hence, lower than that of benzene. By this, it shifts closer to values typical for brown coal (H/C: 0.70 to 1.10 and O/C: 0.10 to 0.40) and hence, to increased effective heating values [17, 193].

3.4.4. Functional groups determined wet-chemically

The content of functional groups of the precipitation products depends on the used raw material, as shown in Figure 3.7. Their overall content varies between 9.9 mmol·g^{-1} for pretreated alfalfa and 13.2 mmol·g^{-1} for hemp shives.

The content of aliphatic hydroxyl is not only dependent on the content of aliphatic side chains, but may also arise from carbohydrate impurities. However, the content of these impurities varies strongly. This results in a wide range of aliphatic hydroxyl content from 2.1 to 5.4 mmol·g^{-1} for lignin from sunflower stalks and wheat straw, respectively. Carboxyl groups also arise from carbohydrate impurities, as hemicelluloses in grasses contain considerable amounts of glucuronic acid (cf. Section 2.2.1). The content is between 1.2 mmol·g^{-1} for group D and 2.5 mmol·g^{-1} for precipitation product obtained from coconut shell powder and herewith in the same range as determined for soda wheat straw, hemp and flax lignins using the same type of method [89] .

As described in Section 3.4.2, the carbonyl content is in agreement with the Carbonylic Index determined by FTIR spectroscopy. Furthermore, the groups, classified by FTIR spectroscopy and elementary analysis, can also be found by wet-chemical functional group determination. However, as most of these methods are based on derivatisation reactions as well as several process steps, the classification is not as clear as obtained by other analytical tools based on non-modified samples.

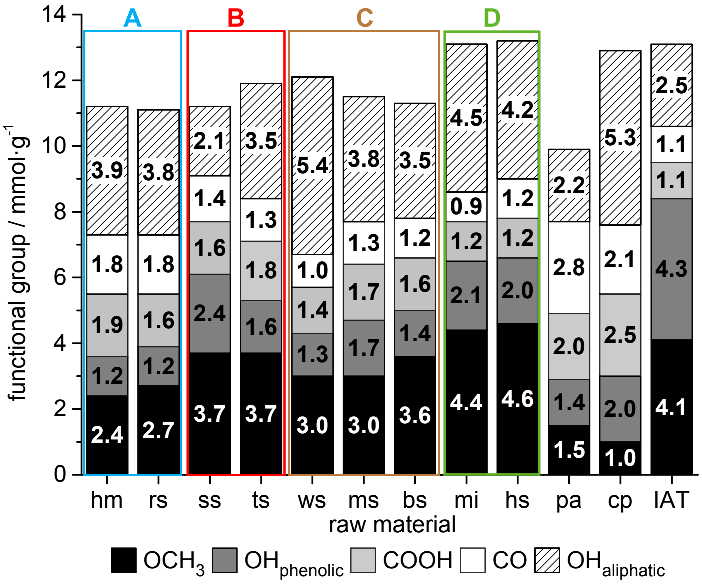


Figure 3.7.: Content of different functional groups of dialysed precipitation products obtained from different raw materials by conventional alkaline pulping and Indulin AT

As illustrated in Figure 3.7, group A lignin is characterised by low methoxyl- and phenolic hydroxyl but high carboxyl and carbonyl content. In opposition, high methoxyl- and phenolic hydroxyl, but low carboxyl and carbonyl content were determined for group D lignin. Precipitation products of group C were found to have medium levels of these functional groups. Sunflower and tomato stalk precipitation products (group B) have similar levels of methoxyl, carboxyl and carbonyl groups. The content of phenolic hydroxyl, however, differs significantly being 2.4 and 1.6 mmol $\cdot g^{-1}$ for precipitation products from sunflower and tomato stalks, respectively. This functional group was not included in classification into groups by FTIR spectroscopy or elementary analysis.

Precipitation products from coconut shell powder and pretreated alfalfa, which already showed unique characteristics revealed by FTIR spectroscopy as well as by elementary analysis, stand out from other lignin preparations due to their low methoxyl and high carbonyl content. It is noteworthy, that both dialysed precipitation products are of high purity exceeding 85% KLASON-lignin content. They are hence examples for the unsuitability of using methoxy group content as indicator for lignin purity as suggested in the literature [31].

The content of phenolic hydroxyl groups of precipitation products from all studied raw materials is low compared to a softwood Kraft-lignin as shown for Indulin AT (IAT), which contains two to three times as much of this functional group.

3.4.5. Thioacidolysis-GC/MS

Chemical degradation techniques, such as permanganate oxidation, hydrogenolysis or thioacidolysis, provide valuable information about lignin building units. Thioacidolysis, which is used in this study, is a solvolysis of dialysed precipitation product in dioxane-ethanethiol with boron trifluoride etherate resulting in cleavage of the β-O-4 bonding. Thus, it allows the determination of type and amount of units involved in this linkage [132]. The results of thioacidolysis are given in Table 3.4.

According to thioacidolysis, the majority of studied precipitation products are of the HGS-type, which is typical for grass lignin. Exceptions are precipitation products obtained from coconut shell powder (cp), sunflower stalks (ss) and rape straw (rs). As no H-derived degradation products were detected, they are assignable to the GS-type. Precipitation product obtained from hemp shives further contains only traces of H-derived degradation products and can hence also be considered as GS-type. Typical for plants of the family *Poaceae* is their high proportion of guaiacyl units [42]. This is supported by the low S/G-ratio of precipitation products obtained from wheat, barley and maize straw as well as from miscanthus, which all belong to this category. In contrast, syringyl is the major unit in precipitation products obtained from sunflower stalks (family *Asteraceae*) and hemp shives (family *Cannabaceae*).

The S/G ratio determined by thioacidolysis differs from the one determined by FTIR spectroscopy, by which all precipitation products were found to have an S/G ratio below one. This clearly illustrates major drawbacks for both methods. On the one hand, results from FTIR spectroscopy are influenced by C=O stretching vibration, which may distort results obtained for precipitation products containing high amounts of this functional group. On the other hand, only units originally bond via ether linkages are included in the calculation of S/G ratio in the case of thioacidolysis.

Table 3.4.: Ratio of H, G and S units, yield and condensation degree (CD) from thioacidolysis of dialysed precipitation products obtained from different raw materials by conventional alkaline pulping based on their KLASON-lignin content and Indulin AT (IAT)

Group	Prec. prod.	H	G	S	Yield [μmol·g^{-1}]	CD [%]
A	hm	0.50	1.00	0.62	186	96
	rs	0.00	1.00	0.71	484	89
B	ss	0.00	1.00	1.71	588	87
	ts	0.00	1.00	0.54	540	88
C	bs	0.08	1.00	0.96	382	91
	ms	0.09	1.00	0.94	182	96
	ws	0.03	1.00	0.68	381	91
D	hs	0.01	1.00	1.11	673	85
	mi	0.14	1.00	0.74	724	84
	cp	0.00	1.00	0.59	42	99
	pa	0.05	1.00	0.63	76	98
	IAT [1]	0.00	1.00	0.00	323	93

[1] Data reported by Gosselink et al. [90]

Furthermore, syringyl units are more likely to be released than guaiacyl and p-hydroxyphenyl units, as the latter two may be also involved in aromatic C-C and diaryl ether linkages due to their vacant C3 or/and C5 position. Thus, lignins with a high content of C-C linkages expressed as high degree of condensation are poorly represented by this method.

The condensation degree (CD) can be calculated from the yield of thioacidolysis degradation products as suggested by Lapierre [132]. In contrast to the condensation index calculated from FTIR spectra, which expresses the relative amount of C-C linkages, the condensation degree obtained from thioacidolysis expresses the amount of lignin units participating in C-C as well as in diaryl ether linkages often referred to as "condensed bonds" [45]. Despite these differences, the results from both methods are in excellent agreement, revealing the same lignins to be highly condensated. The calculation of CD is based on the assumption that thioacidolysis yield is 80% for the β-O-4 linked structures and that the average molecular weight of phenylpropane units is 180 g·mol^{-1}. The condensation degrees, which range between 84 and 99%, are given in Table 3.4. Except for group A, the condensation degrees of the lignin preparations are similar within one group. It is highest for precipitation product obtained from coconut shell powder, followed by pretreated alfalfa. Thus, these precipitation products are likely to be poorly represented by thioacidolysis. This is supported by preliminary results from pyrolysis-GC/MS, which reveal, that precipitation product obtained from coconut shell powder has a H/G ratio of seven times as much as miscanthus, though none H-derived products are found by thioacidolysis (cf. Annex Figure A.5). As mentioned before, H-units are more likely to be involved in C-C and diaryl ether linkages due to their vacant C3 and C5 position. Thus, the precipitation product of coconut shell powder is an example for their underestimation by thioacidolysis.

Furthermore, some special features can be found by GC/MS of thioacidolysis degradation products, as can be seen in Figure 3.8. Lignin obtained from pretreated alfalfa (pa), for instance, contains large proportions of palmitic, oleic as well as stearic acid. This content of fatty acids is also responsible for its high H/C ratio found by elementary analysis as discussed in Section 3.4.3. In addition, a degradation product obtained from xylan can be identified in wheat straw lignin, further supporting the presence of carbohydrates in this precipitation product as discussed in Section 3.4.1. The chromatogram of lignin obtained from horse manure is shown as an example for lignin containing an especially high amount of H-units, the degradation products of which could be identified at retention times of 7.99 and 16.50 min. As they are released by thioacidolysis, it can be concluded, that horse manure lignin contains large amounts of uncondensed H-units, despite their vacancies at C3 and C5.

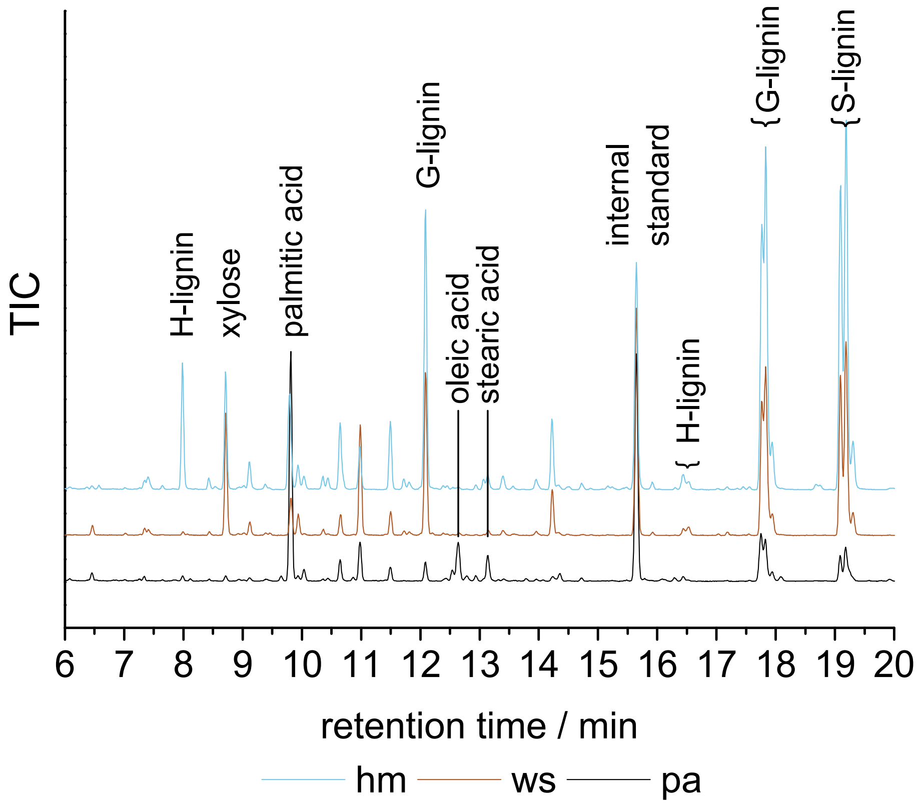


Figure 3.8.: Chromatogramm of lignin obtained from horse manure, wheat straw and pretreated alfalfa after thioacidolysis

Table 3.5.: Results from size-exclusion-chromatography of dialysed precipitation products obtained from different raw materials by conventional alkaline pulping and Indulin AT

Group	Prec. prod.	M_n [g·mol^{-1}]	M_w [g·mol^{-1}]	M_w/M_n (PDI)
A	hm	900	5500	5.8
	rs	800	4400	5.8
B	ss	1200	5900	5.1
	ts^1	800	4900	6.4
C	bs	800	6200	7.5
	ms	900	5300	5.7
	ws	1100	6700	6.3
	ws^1	600	3100	4.9
D	hs	700	5700	7.8
	mi	600	5100	8.2
	cp	1800	7700	4.2
	pa	1200	6700	5.5
	IAT	700	5300	8.2
	IAT^1	500	4100	7.5

^{1} Usage of a ready-prepared column, leading to lower values for M_n, M_w and PDI

3.4.6. Size exclusion chromatography

The molecular weight of lignin is of particular interest for its application as it affects its solubility in solvents, miscibility in blends and the mechanical properties of products. Size exclusion chromatography (SEC) was used in order to determine the molecular mass of the lignin preparations relative to sodium-polystyrene sulphonates. The results are presented as number average (M_n) and weight average (M_w) molecular mass in Table 3.5. Furthermore, the polydispersity index (PDI) is given, calculated as the ratio of M_w and M_n.

The M_n ranges between 600 g·mol^{-1} and 1800 g·mol^{-1} for lignin from rs and cp, respectively. They are within a typical range for soda lignins as values are reported between 600 and 1400 g·mol^{-1} for soda wheat straw and soda hemp lignin, respectively [145]. Two different columns were used for analysis: a non-commercial column, which was used for the majority of samples and a commercial ready-prepared column. Interestingly, the results differ significantly with regard to both the molecular weight and the PDI. The commercial ready-prepared column resulted in lower values for M_n, M_w and PDI compared to the non-commercial column, as shown for wheat straw lignin (ws) and IAT in Table 3.5. For lignin obtained from tomato stalks only the result obtained by the commercial ready-prepared column is available. However, it can be assumed that its values for M_n, M_w and PDI would have been higher, if measured with the first non-commercial column based on the findings from ws and IAT.

Considering this, similar values for M_n are obtained within the proposed groups. Classification into groups, however, is not possible as differences between the groups are not as distinct. The highest values for M_n and M_w are found for precipitation product obtained from coconut shell powder. This underlines its high degree of condensation as found by thioacidolysis GC/MS and FTIR spectroscopy (cf. Sections 3.4.5 and 3.4.2).

A high PDI, as found for group D, indicates a high content of impurities and/or a very heterogeneous distribution of molar masses of the lignin molecules. As one of the main challenges in industrial utilisation of lignin is its lack of uniformity, most of the studied precipitation products would benefit from solvent fractionation. For instance, Gosselink et al. [90] proved the suitability of successive extraction with organic solvents for fractionation of different industrial lignins according to their molar mass. In addition, the fractionation of wheat straw lignin obtained during this study using acetone/water mixtures and ethyl acetate was shown by Boeriu et al. [26].

3.4.7. Differential scanning calorimetry

Providing information on the thermal behaviour of lignin is important in order to choose suitable applications for its subsequent valorisation. Differential scanning calorimetry (DSC) measures the energy input required to keep the lignin sample and a reference at the same temperature as a function of temperature. By this, direct information on enthalpy changes associated with phase transitions is provided. During glass transition, which occurs in amorphous polymers, intermolcecular hydrogen bonds break and molecular motion is enhanced resulting in a change of the polymer from a hard brittle condition into a viscous or rubbery one and vice versa [109].

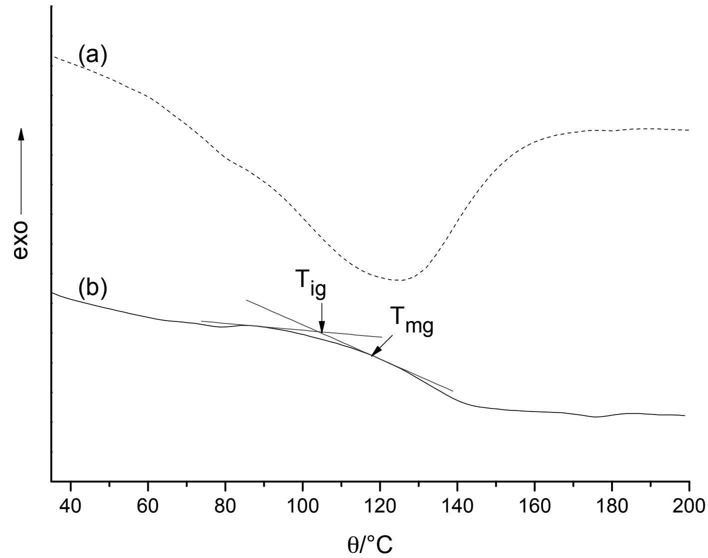


Figure 3.9.: DSC of lignin obtained from wheat straw (a) without and (b) with annealing at 90 °C, illustrating the determination of glass transition temperature as extrapolated onset temperature T_{ig} and midpoint temperature T_{mg}

This change, which is indicated by an endothermic shift in the DSC curve, makes the glass transition temperature T_g an important key indicator for thermal processing of lignin. The determination of glass transition temperature is strongly effected by the thermal history of the sample as well as by impurities, which can result in a broad endothermic peak covering and extending the glassy state [41]. This effect is shown in Figure 3.9 (a) for wheat straw lignin. In order to minimise this effect, annealing of polymer samples at 15 to 20 K above the glass transition temperature prior to DSC measurements has reported to be advantageous [98]. For lignin, however, radical coupling reactions may be activated at temperatures around the glass transition state introducing irreversible changes in thermal behaviour and hence, T_g values [41]. Thus, annealing was carried out at 90 °C for 30 minutes, as suggested by Li and McDonald [136]. The resulting curve is shown in Figure 3.9 (b), which also illustrates the determination of T_g. Two values can be used for its designation: the extrapolated onset temperature T_{ig} as well as the midpoint temperature T_{mg}. Both were shown to be the most reproducible values for glass transition temperature [162]. Values for T_{ig} as well T_{mg} of lignins from different raw materials are listed in Table 3.6.

For commercial Kraft lignin Indulin AT (IAT), Li and McDonald [136] report a T_{mg} of 147 °C, when annealing at 90 °C, which correspond exactly to the result in this work, though there is another, but weaker, signal at 112.9 °C.

Table 3.6.: Results from DSC of dialysed precipitation products obtained from different raw materials by conventional alkaline pulping and Indulin AT: T_{ig}: extrapolated onset temperature, T_{mg}: midpoint temperature, ΔT: T_{mg} - T_{ig}

Group	Prec. prod.	First signal			Second signal		
		T_{ig} [°C]	T_{mg} [°C]	ΔT [°C]	T_{ig} [°C]	T_{mg} [°C]	ΔT [°C]
A	hm	94.1	118.1	24.0			
	rs	92.6	116.2	23.6			
B	ss	93.1	121.6	28.5			
	ts	97.6	128.4	30.8	173.2	182.3	9.1
C	bs	99.8	125.0	25.2	149.2	161.0	11.8
	bs[1]	109.5	135.3	25.8	158.7	183.0	24.3
	ms	111.8	126.2	14.4			
	ws	103.0	120.6	17.6			
D	hs	95.4	110.4	15.0	143.6	153.2	9.6
	mi	107.9	121.6	13.7			
	cp	115.2	135.7	20.5			
	cp[1]	116.8	144.7	27.9			
	pa	88.8	106.4	17.6			
	IAT	102.8	112.9	10.1	134.7	147.2	12.5
	IAT[1]	116.2	121.3	5.1	143.5	154.5	11.0

[1] DSC was carried out after annealing at 105 °C, instead of 90 °C

It is further worth noting that annealing at 105 °C is found to be necessary in order to prevent an endothermic relaxation peak for IAT as well as for lignin from barley straw and coconut shell powder. The resulting glass transition temperature T_{mg} for IAT is 154 °C being in excellent agreement with the result of Cui et al. [41] reporting a T_{mg} of 153 °C when annealing at 105 °C. This shows, once again, how important the detailed description of method is for a reproducible determination of glass transition temperature. Nevertheless, difficulties have been reported for the measurement of glass transition temperature of soda wheat straw lignin in a round robin. The determined T_{mg}, if detected at all, covered a broad range between 146 – 180 °C, though using a common method [198]. A smooth transition between two signals, as found for lignin from maize straw in this study, further complicated the analysis. Despite these difficulties, several results can be stated in the following.

For the majority of precipitation products a distinct glass transition below 130 °C is detected. Thus, they are below glass transition temperatures reported for wheat straw soda lignin within the scope of a round robin [198] with a mean value of 155 °C. Since thermoplastic materials are always processed above their glass transition temperature, these comparatively low values for T_{mg} are advantageous. In contrast, a high T_{mg} of 144.7 °C is observed for lignin obtained from coconut shell powder (cp). This may result from its high molecular weight of $M_w = 7700$ g·mol^{-1}. However, no relation was found between T_g and molecular weight, though described elsewhere [136, 144]. A special feature is noted for lignin obtained from barley straw (bs), maize straw (ms) and hemp shives (hs), as they show two signals for T_g. The transition between those signals is moreover very smooth. This indicates a high degree of heterogeneity, which is also suggested by the high polydispersity index (PDI) of beyond 7.5 for lignins from barley straw and hemp shives. Other lignin preparations in this study generally have a PDI between 5 and 6 (cf. Section 3.4.6).

3.4.8. Thermal desorption

Thermal desorption at elevated temperatures with subsequent GC/MS analysis informs about the type of volatile moieties of lignin samples. These are of particular interest for their application, as volatile organic compounds (VOCs) are either present in the isolated lignin or are formed as lignins are processed at high temperature. Their emission plays a significant role in causing health hazards and is therefore subject to increasing restrictions [215]. Especially monoterpenes, sesquiterpenes, phenols (particularly guaiacol), sulfides as well as medium long aliphatic alcohols and aldehydes (particularly hexanal and heptanal) are seen as problematic [215]. In addition, the odour threshold values of these VOCs are extremely low. For example, the odour threshold value for guaiacol in water solutions is reported to be 3 to 21 ppb and for reduced sulphur compounds such as dimethyl disulphide and dimethyl trisulphide to be 5 and 0.2 ppb, respectively [112]. This drawback reduces the applicability of lignin-based composites and supports the need of their determination in lignin emissions. There are various methods for VOC measurement, for instance gravimetric determination (ASTM D2369) [9], static headspace analysis (ASTM D6886) [8] or TD GC/MS, the latter being particular suitable for simulation of VOC formation in the temperature range of thermoplastic processing [112, 115].

Table 3.7.: Thermal desorption of lignin obtained from different raw materials by conventional alkaline pulping and Indulin AT with colour indicating peak intensity: 🔴 not detected, 🟡 detected, 🟢 considerable amounts present

Label	Substance	hm	rs	ss	ts	bs	ms	ws	hs	mi	cp	pa	IAT
3	Dimethyl disulphide	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟡
4	Hexanal	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟡	🔴
5	Furfural	🟢	🟢	🟡	🟡	🟡	🟢	🟢	🟡	🟢	🔴	🟡	🔴
6	Guaiacol	🔴	🔴	🔴	🟡	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢
7	Benzofurane, 2,3-dihydro-	🟢	🟡	🟡	🟡	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🔴
8	Guaiacol, 4-vinyl-	🟢	🟡	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🟢
9	Syringol	🟢	🟡	🟡	🟡	🟡	🟡	🟡	🟡	🟡	🔴	🔴	🔴
10	Vanillin	🟢	🟢	🟢	🟡	🟡	🟢	🟢	🟢	🟢	🔴	🟢	🟢
11	Isoeugenol	🟡	🟡	🟡	🟡	🟡	🟡	🟡	🟡	🟡	🔴	🟡	🟢
12	Guaiacol, 4-propyl-	🔴	🟡	🟡	🟡	🟡	🟡	🟡	🟢	🟢	🔴	🔴	🟢
13	Acetovanillone	🔴	🟡	🔴	🟡	🟡	🟡	🔴	🟡	🔴	🔴	🟡	🟢
14	Guaiacylacetone	🟡	🟡	🟡	🟡	🟡	🟡	🟡	🟡	🟢	🔴	🟡	🟢
15	Dodecanoic acid	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟡	🔴
16	Acetophenon, 3,5-dimethoxy-	🟢	🟢	🟢	🟢	🟢	🟢	🟡	🟢	🟢	🔴	🟡	🔴
17	Homovanillic acid	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢
18	Syringaldehyde	🟡	🟢	🟢	🟡	🟡	🟡	🟢	🟢	🟢	🔴	🟡	🔴
19	Syringol, 4-propenyl-	🟡	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟡	🔴
20	Acetosyringone	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🔴	🟢	🔴
22	Coniferylalcohol	🔴	🔴	🟡	🟡	🔴	🔴	🔴	🟢	🟢	🔴	🟡	🔴
23	Tetradecanoic acid	🔴	🔴	🔴	🔴	🟡	🟡	🔴	🔴	🔴	🔴	🔴	🔴
24	Homosyringaldehyde	} 🟡	🟢	🟡	🟡	🟡	🟡	🟢	🟢	🟡	🔴	🔴	🔴
25	Desaspidinol												
26	Neophytadiene	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🔴	🟢	🔴

Lignin samples were subjected to thermal desorption with subsequent qualitative GC/MS analysis. Gas chromatograms of selected lignins as well as further details e.g. retention time are listed in the Annex (cf. Figure A.3 and Table A.1). Table 3.7 summarises the peak intensities of substances and classifies them as: 🔴 substance not detected, 🟡 substance detected (peak height > 13.000 absorption units = limit of detection), 🟢 considerable amounts present (peak height > 400.000 absorption units). This enables an easy and fast comparison between numerous samples.

Kraft lignin (IAT) releases various compounds, such as isoeugenol (11), acetovanillone (13), guaiacylacetone (14) and odour-intensive guaiacol (6), which are not, or only in traces, present in emissions from the studied precipitation products from herbaceous plants. Furthermore, dimethyl disulphide is detected in IAT, which is also known for its low odour threshold, as already described. In contrast, other compounds are formed by thermal desorption of the precipitation products from herbaceous plants. For instance, furfural (5) and 2,3-dihydro-benzofurane (7) are released due to the presence of carbohydrate impurities. Precipitation product from coconut shell powder, however, distinguishes itself by its inertness. Here, no substances are released during heat treatment, which could make it an excellent starting material for biocomposites. Unique features are also found for lignin from pretreated alfalfa, as it releases traces of hexanal. This compound, together with other saturated and unsaturated C5 to C11 aldehydes, is highly odorous and hence, undesirable in indoor air, limiting its further application possibilities [189].

3.5. Lignin applications

Depending on the application, required lignin properties may vary. In the following, lignin obtained from different raw materials will be discussed with regard to selected application possibilities.

For application in **biocomposites**, a low content of carbohydrate impurities in the used lignin preparations is advantageous, as they easily burn when processed thermally. In addition, low values for the glass transition temperature allow a low processing temperature. The absence of volatile organic compounds with low odour threshold further ensures a broad applicability of produced biocomposites. A higher molecular weight of the lignin improves its affinity to the matrix and hence, the mechanical properties [209]. Based on this, lignin from coconut shell powder is a promising candidate for use in biocomposites. Aside from its comparatively high glass transition temperature, it fulfils all of the mentioned requirements. Furthermore, it is noteworthy that the glass transition temperature is still low compared to Kraft-lignin. In addition, lignins of group A and B are of interest, as they have the lowest values for glass transition temperature and are separable in considerable purity.

If used as **binder** a high amount of free phenolic hydroxyl groups with numerous free reactive ortho ring positions is a relevant requirement [90]. Thus, a low proportion of methoxyl groups and syringyl units as well as a low degree of condensation is favourable. In addition, lignin should contain less than 4% impurities in order 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 favour its contribution to the resin polymerisation [90]. Lignin obtained from sunflower stalks has the highest content of phenolic groups in this study. Furthermore, it is of high purity (90.4% KLASON-lignin content) and the detailed elucidation of its impurities is therefore proposed for further studies. In addition, the degree of condensation is low. Besides its favourable properties, it has a high content of syringyl with an S/G ratio of 1.71 (determined by thioacidolysis GC/MS) as well as a high content of methoxyl groups.

For application in **thermosetting resins**, phenolation of lignin has shown to be advantageous [176, 177]. Here, a low content of ash and a high number of aliphatic hydroxyl groups increase the degree of derivatisation [177]. The ash content of all precipitation products could be eliminated by dialysis. Furthermore, precipitation products obtained from coconut shell powder and group D are promising due to their high content of aliphatic hydroxyl groups. Though, this also applies to lignin from wheat straw, it is not proposed for this application, as its aliphatic hydroxyl groups most likely originate from its carbohydrate moieties.

For production of **carbon fibre**, lignin of high purity and high carbon content is desirable. Low content of methoxyl group is favourable, as fibres from softwood lignin precursor have been reported to manifest more rapid oxidative cross-linking than hardwood lignin [179]. Therefore the use of lignin from herbaceous plants may generally have high potential. In addition, C-O bonds in interunit lignin linkages, such as β-O-aryl ether, are to be minimised in order to yield a more favourable lignin for structural carbon-fibre production [179]. Based on these requirements, lignin obtained from coconut shell powder is suggested as a promising candidate, due to its low H/C ratio and its low content of methoxyl groups.

For production of **hydrogels**, lignin purity is not as crucial as carbohydrate impurities may contribute to gel formation. Whereas the content of carbonyl and carboxyl groups does not influence the swelling capacity, a high content of hydroxyl is beneficial [219]. Furthermore, molar masses of between 4000 and 6000 $g{\cdot}mol^{-1}$ may be favourable, though further studies are needed for confirmation [219]. Based on their high content of hydroxyl groups lignins of group D are proposed as well as lignin obtained from wheat straw.

For the **catalytic valorisation** of lignin, high purity is inevitable as proteins, inorganic salts and other potential poisons generally complicate catalysis [236]. Though this applies to precipitation products obtained from sunflower stalks, hemp shives and coconut shell powder, further purification of this product stream may open up this application to precipitation products of all studied raw materials. Additional advantageous properties of lignin regarding the performance of catalysts remain unclear, as most studies use model compounds instead of actual lignin streams [236].

4. Pulping of wheat straw using different procedures

Two factors considerably influence the composition and properties of biorefinery products: biomass feedstock and pretreatment process. Whereas different raw materials were discussed in the previous chapter, the focus of this section is on the effect of different pretreatments (alkaline using sodium hydroxide and organosolv using formic acid and hydrogen peroxide) and the influence of process parameters. This is followed by direct comparison of conventional alkaline, microwave-assisted alkaline and organosolv pretreatment. As wheat straw is the most abundant agricultural residue in Europe, it was selected as raw material for the experiments [234].

4.1. Conventional alkaline pulping

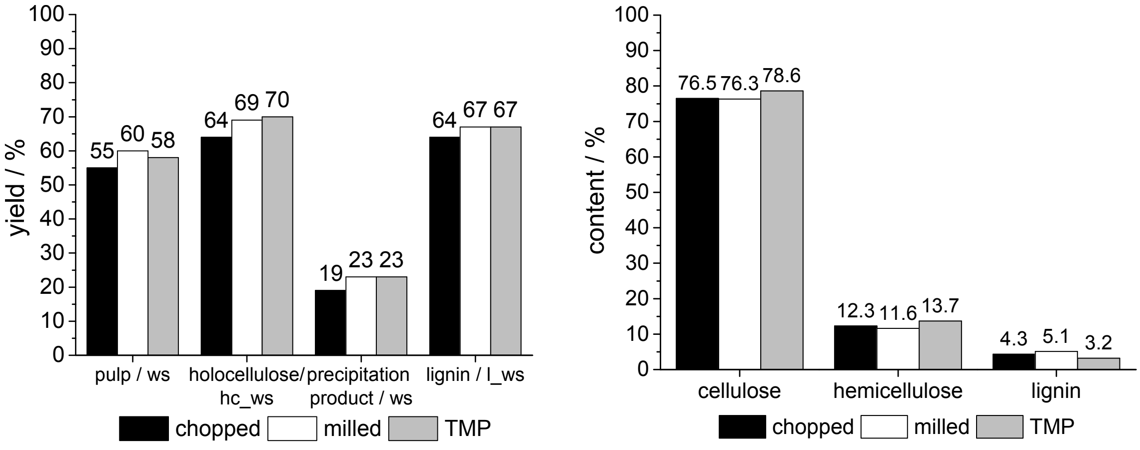
This section deals with alkaline pulping carried out in an autoclave using aqueous sodium hydroxide. The standard parameters were as follows: liquid/solid ratio = 6.2 mL/g, $\omega(\mathrm{NaOH}) = 3\,\mathrm{wt\%}$, $\theta = 160\,°\mathrm{C}$, time at maximum temperature = 180 min. The pulp was separated from the black liquor, washed and dried. The lignin fraction was precipitated from the black liquor at pH=1 and the precipitation product was separated, washed and dried.

First, the effect of comminuting wheat straw is discussed, followed by variation of pulping and precipitation parameters. Finally, data obtained from a scale-up experiment from laboratory to pilot scale are presented.

4.1.1. Effect of physical/mechanical pretreatment

In order to study the effect of comminution of wheat straw on its separability by alkaline pulping, three different methods were used to prepare the raw material:

- chopping: fibre length = 40 − 100 mm
- milling: fibre length < 4 mm
- thermo-mechanical pulping (TMP): fibre length = 0.1 − 2.0 mm



(a) (b)

Figure 4.1.: Effect of comminution on (a) Yield of pulp and precipitation product with regard to wheat straw (ws) and yield of holocellulose and lignin with regard to their contents in wheat straw (hc-ws, l-ws) and (b) Composition of pulp (on dry matter basis)

These different physico-mechanical pretreatments do not lead to a significant difference in composition of wheat straw. Furthermore, they only slightly influence product yield and composition after alkaline pulping (standard parameters as defined in Section 6.4.1), as can be seen from Figures 4.1a and 4.1b, respectively. For product yield, there is no significant difference whether the wheat straw was milled or refined by thermo-mechanical pulping beforehand. If chopped wheat straw was used, the yield of pulp as well as precipitation product are slightly lower. For the composition of pulp, no significant difference was found between pulp obtained from chopped or milled wheat straw. Pulp obtained from wheat straw pretreated by TMP, has the lowest lignin content, as TMP improves the accessibility and removal of lignin due to disruption of the middle lamella and thermal softening [96]. However, this effect is not relevant in this case, as the content of lignin in pulp was already low for the other grinding methods. Furthermore, TMP requires a high energy input of 677 $\mathrm{kWh \cdot t^{-1}}$ compared to chopping, which uses an energy input of approximately 10 to 50 $\mathrm{kWh \cdot t^{-1}}$ [24]. Thus, chopping of wheat straw prior to pulping is sufficient for the pulping parameters used. However, TMP could be advantageous when using milder pulping conditions.

4.1.2. Variation of pulping parameters

Pulping is one of the most expensive steps of bioethanol production and it furthermore affects costs of prior and subsequent operation steps [218]. Understanding the influence of pulping parameters such as temperature, time and sodium hydroxide concentration on product yield and composition is therefore crucial for identifying saving potentials. Even such a well-known process like alkaline pulping has to be reviewed under the aspect of bioethanol production as the objectives differ from the ones of the pulp and paper industry. For example, short cellulosic chains and hence low values for intrinsic viscosity are desirable for conversion into bioethanol. Furthermore, the recovery of lignin for the preparation of value-added products is preferable rather than burning as done in pulp mills. With these considerations in mind, the optimisation experiments were carried out based on the following aims:

1. High yield of both, pulp and precipitation product
2. Low residual lignin content in pulp, as it has been discussed as a potential inhibitor in subsequent enzymatic hydrolysis [36]
3. Low to medium intrinsic viscosity of pulp
4. High content of lignin in precipitation product (evaluated based on washed precipitation product)

In addition, control of phenolic hydroxyl groups in lignin is relevant, as it strongly influences its further application. Whereas a high content is favourable for crosslinking and derivatisation reactions, a low content improves its compatibility with polypropylene [26].

For the experiments the liquor ratio was kept constant at 6.2 $\mathrm{mL \cdot g^{-1}}$. TMP-refined wheat straw was used for experiments in this section. The parameters maximum temperature, time at maximum temperature and concentration of sodium hydroxide were varied as discussed in the following.

Variation of maximum temperature

The maximum temperature θ_{max} of the process was varied as follows: 100, 120, 140 and 160 °C, which correspond to a gauge pressure of approximately 1.4, 2.0, 3.1 and 5.1 bar, respectively. Time at maximum temperature as well as concentration of sodium hydroxide were kept at standard conditions as defined in Section 6.4.1. As the autoclave is heated by wall and floor heating periodically, the temperature varied with a value of about ± 5°C along the set temperature value. Figure 4.2 shows typical values for temperature and the dependent gauge pressure for a pulping process carried out in a 2-L-autoclave.

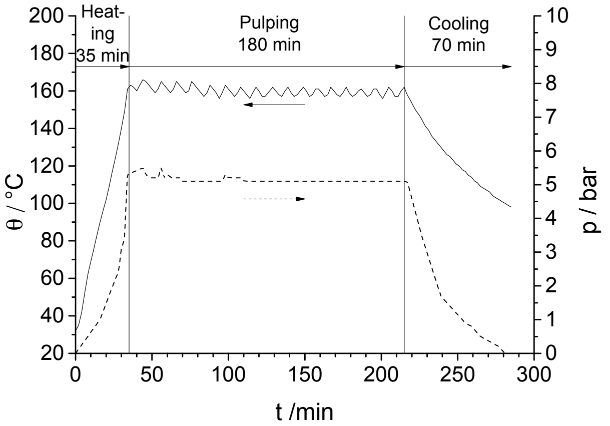


Figure 4.2.: Process data obtained from a typical alkaline pulping procedure carried out in an autoclave of 2-L capacity

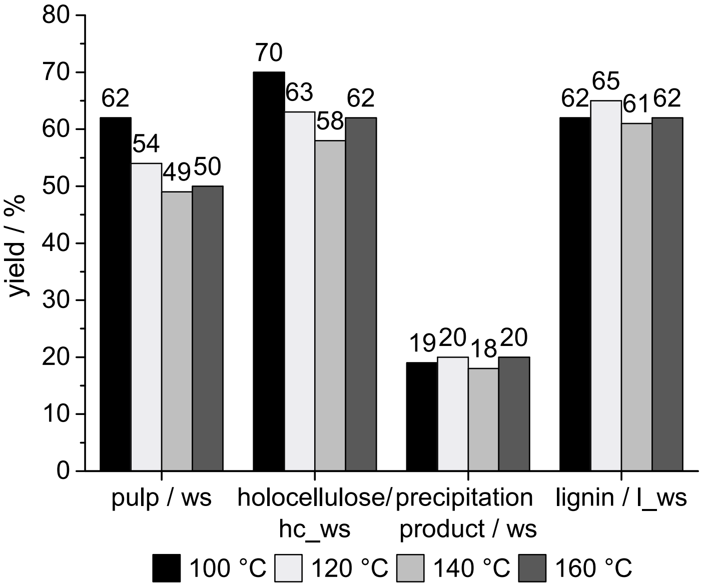
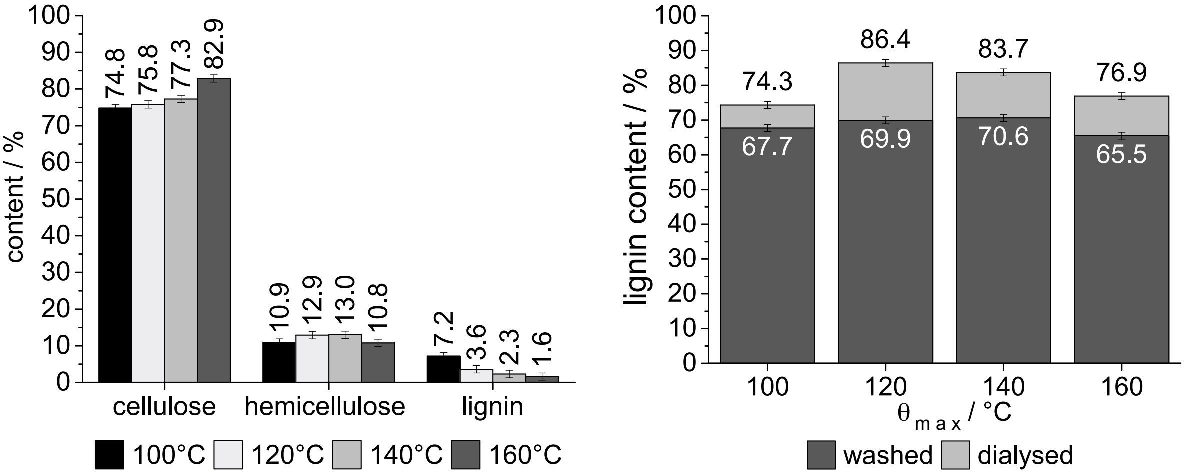


Figure 4.3.: Yield of pulp and precipitation product with regard to wheat straw (ws) and yield of holocellulose and lignin with regard to their contents in wheat straw (hc-ws, l-ws) as a function of maximum temperature

The set maximum temperature has a large impact on pulp and holocellulose **yield**, as both decrease when increasing the pulping temperature from 100 to 140 °C (Figure 4.3). A further increase of θ_{max} to 160 °C does not significantly influence pulp yield. The yield of precipitation product is not influenced by pulping temperature as it ranges between 18 to 20% in relation to wheat straw for all studied pulping temperatures. Thus, a low value for θ_{max} (100 °C) already results in high yields for both, holocellulose and lignin.

In order to obtain **pulp** containing only a low amount of residual lignin, a pulping temperature higher than 100 °C is proposed as lignin content decreases from 7.2 to 3.6 % when the temperature is elevated from 100 to 120 °C (Figure 4.4a). For temperatures between 120 and 140 °C the composition of pulp remains unchanged. If the temperature is further increased to reach 160 °C, the content of cellulose increases from 77.3 to 82.9 %, as the content of lignin and hemicelluloses slightly decreases. Thus, the main step of delignification occurs between 100 to 120 °C in contrast to results from conventional Kraft-pulping of wood where it occurs during the "bulk phase" at 150 to 170 °C [82].

The degree of polymerisation (DP) of cellulose can be obtained by determination of intrinsic viscosity. However, for calculation of DP from intrinsic viscosity a model based on pure cellulose is used [114]. As pulp obtained by alkaline pulping is rich in hemicellulose, this might influence intrinsic viscosity in a manner which cannot be considered when converting to DP. Thus, conversion to DP is omitted at this point and the results are discussed in terms of intrinsic viscosity.



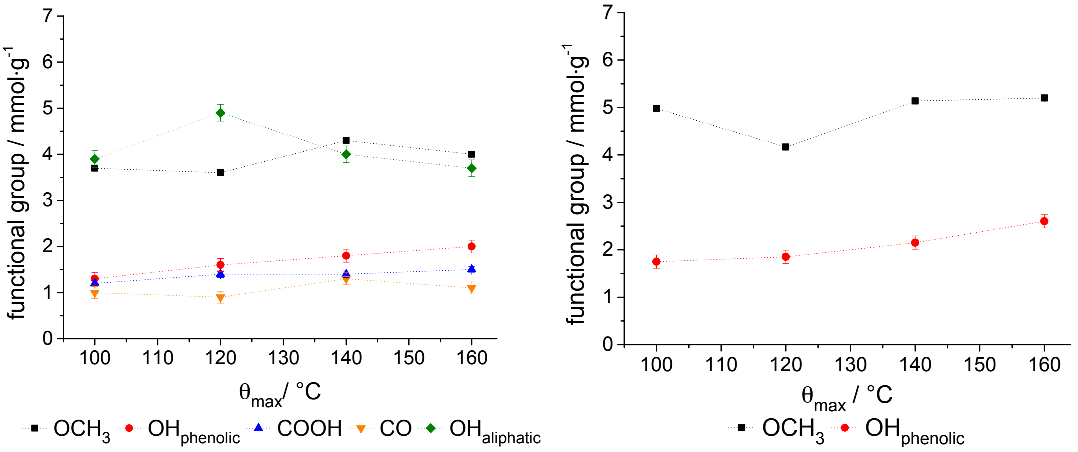
(a) (b)

Figure 4.4.: Composition of products depending on maximum temperature of alkaline pulping (a) Composition of pulp (b) Lignin content of precipitation product

If the maximum temperature was raised from 100 to 120 °C, the intrinsic viscosity of the pulp increased from 540 to 690 mL·g^{-1}. A possible explanation is that short-chained carbohydrates are dissolved from the pulp at higher temperatures. This assumption is supported by the decrease in pulp and holocellulose yield in the considered temperature range as discussed before. However, further increase in temperature does not affect intrinsic viscosity as it remains constant at approximately 680 mL·g^{-1} for temperatures between 120 and 160 °C.

The purity of **precipitation product** is not as dependent on pulping temperature as the purity of pulp. More particularly, the lignin content of the washed precipitation products varies within a narrow range of 5.1% for the studied temperatures. It should be noted, that the largest difference occurs between 140 and 160 °C, as the lignin content decreases from 70.6 to 65.5%, suggesting a further decrease if the temperature would be elevated further. Thus, pulping temperatures of about 120 to 140 °C are proposed as optimum conditions, especially as lignin contents exceed 80% for the dialysed precipitation products.

The functional group content is shown in Figure 4.5a for the dialysed precipitation products. Furthermore, Figure 4.5b shows functional groups that are characteristic for lignin (methoxyl and phenolic hydroxyl) in relation to the KLASON-lignin content of the precipitation product. By this, the impact of differing lignin contents can be excluded. The pulping temperature does not influence the content of carbonyl as it fluctuates around 1.0 mmol·g^{-1} within the error margin. The effect on carboxyl groups was also found to be modest, as they increase marginally from 1.2 to 1.5 mmol·g^{-1} in the temperature range studied.



(a) (b)

Figure 4.5.: Functional group content of (a) dialysed precipitation product and (b) dialysed precipitation product per KLASON-lignin, as a function of maximum temperature of alkaline pulping

The influence of pulping temperature on the content of aliphatic hydroxyl groups is not clear. No trend is observable as the content is 3.9 and 3.7 mmol·g^{-1} for 100 and 160 °C, respectively. Nevertheless, it has to be noted that its content is significant higher for the precipitation product obtained at 120 °C being 4.9 mmol·g^{-1}. This additionally coincides with the lower methoxyl group content in relation to the KLASON-lignin content as shown in Figure 4.5b. This would be plausible, if this precipitation product contains comparatively high amounts of carbohydrate impurities. However, this is unlikely as it has the highest content of KLASON-lignin in this test series.

The content of phenolic hydroxyl groups is clearly affected by an increase in temperature as it increases from 1.3 to 2.0 mmol·g^{-1} in relation to the precipitation product and from 1.8 to 2.6 mmol·g^{-1} in relation to the KLASON-lignin content. This increase in phenolic hydroxyl is due to fragmentation reactions of lignin which occur during alkaline pulping (cf. Section 2.3.1). However, as the pulping experiments at 100, 120 and 140 °C were stopped during the initial phase of pulping, which includes the heating period of up to 150 °C, lignin fragmentation involves mainly α-ether linkages in phenolic units [82]. In contrast, β-ether linkages of non-phenolic units also undergo fragmentation via neighbouring group participation during the bulk phase ($\theta = 150$–170 °C) [82, 83]. This leads to a sharp increase of phenolic hydroxyl content between 140 and 160 °C. Thus, to obtain lignin with a high content of phenolic hydroxyl groups, a pulping temperature higher than 150 °C is applicable.

To conclude, high yields of both pulp and precipitation product are obtained choosing a low pulping temperature of approximately 100 to 120 °C. The highest purities of both products were achieved for a temperature between 120 and 140 °C. However, lower rates of enzymatic hydrolysis are achieved if pulp, which was obtained at 120 °C was used, compared to pulp obtained at 160 °C [185]. Thus, this might justify the application of higher pulping temperatures. This is also favourable, if lignin with a high content of phenolic hydroxyl is desired for the purpose of derivatisation or cross-linking.

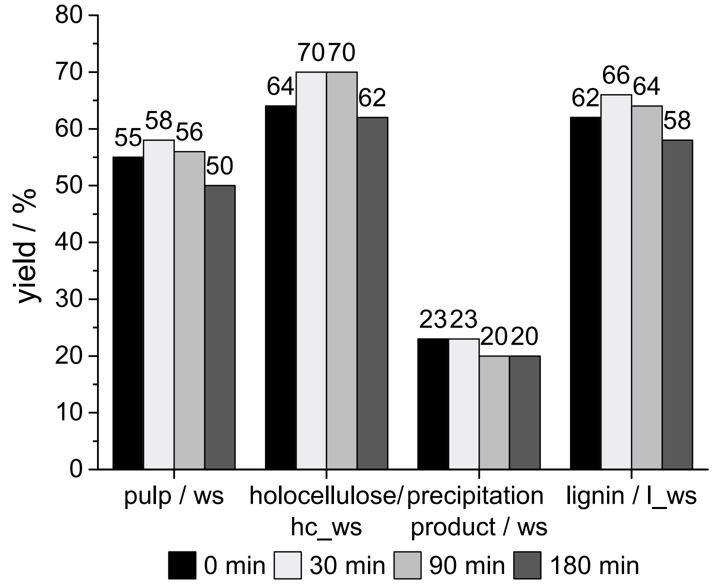


Figure 4.6.: Yield of pulp and precipitation product with regard to wheat straw (ws) and yield of holocellulose and lignin with regard to their contents in wheat straw (hc-ws, l-ws) as a function of time at maximum temperature

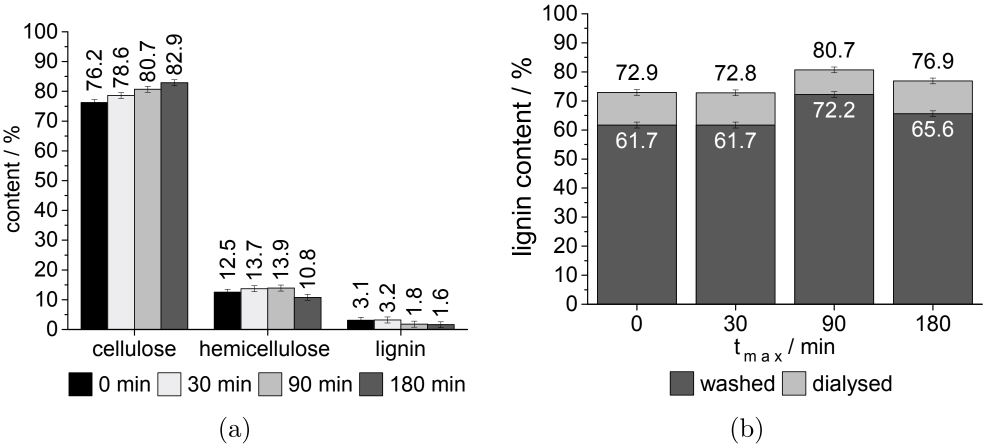
Variation of time at maximum temperature

The time at maximum temperature t_{max} was varied as follows: 0, 30, 90 and 180 minutes. The time was taken when the maximum temperature was reached as shown in Figure 4.2. Maximum temperature as well as concentration of sodium hydroxide were kept at standard conditions as defined in Section 6.4.1.

The time at maximum temperature influences both **yield** of holocellulose and lignin. The yield of holocellulose increases with increasing t_{max} from 0 to 30 minutes. Nevertheless, it decreases when t_{max} is set to 180 min due to a decrease in pulp yield which drops from 56% for $t_{max} = 90$ min to 50%. Thus, prolongation of pulping duration leads to degradation of holocellulose. This effect, also known as alkaline peeling reaction of carbohydrates, is well described for alkaline pulping processes and is outlined in Section 2.3.1. The yield of precipitation product decreases slightly from 23 to 20% if the pulping time is 90 minutes or longer, resulting in a decrease in lignin yield. Thus, a maximum yield of holocellulose and lignin can be proposed for $t_{max} = 30$ min.

When looking at **pulp** composition, the time at maximum temperature has only little impact, as the content of cellulose increases from 76.2 to 82.9% when extending pulping from 0 to 180 minutes (Figure 4.7a). The lignin content of pulp is already very low for $t_{max} = 0$ min being 3.1%. If the time is extended to $t_{max} = 180$ min, it decreases only slightly to reach 1.6% .

The intrinsic viscosity of pulp and hence, its degree of polymerisation, is only little influenced by pulping time, as it remains constant at approximately 800 mL·g^{-1} for 0 and 30 minutes. If increased further, a slight decrease to 700 mL·g^{-1} is noted. This might be due to peeling reaction, a relatively slow process leading to cleavage of glucose from cellulose (cf. Section 2.3.1).



(a) (b)

Figure 4.7.: Composition of products depending on time at maximum temperature of alkaline pulping (a) Composition of pulp (b) Lignin content of precipitation product

The time at maximum temperature does not influence the purity of **precipitation product** between 0 and 30 minutes (Figure 4.7b). If a longer time is chosen, lignin content increases from 61.7 to 72.2% for 90 minutes and decreases again to 65.5%. Thus, in order to obtain a precipitation product with a high lignin content a time at maximum temperature of 90 minutes is proposed.

The influence of time at maximum temperature on functional group content, shown in Figure 4.8a is most obvious between 0 and 30 min. In this range the content of aliphatic hydroxyl decreases rapidly from 5.6 to 4.8 mmol·g^{-1} with an ongoing downward trend. As this effect occurs despite similar lignin contents in the studied precipitation products, it is unlikely to be caused by carbohydrate impurities. Thus, this is rather due to cleavage of C-C linkages of the propanoid side chain by retrograde aldol condensation, which has been described for alkaline pulping by Gierer [83].

Furthermore, the content of carbonyl groups decreases from 1.8 to 1.1 mmol·g^{-1}. High contents of carbonyl groups are reported to be typical for protolignins [241]. Whereas the cleavage of C-C-linkages between C_α and C_β as well as between C_α and aromatic nucleus results in formation of carbonyl, a cleavage of the bond between C_β and C_γ with release of formaldehyde would decrease the carbonyl group content of lignin during alkaline pulping [83]. The content of carboxyl groups increases from 1.3 to 1.6 mmol·g^{-1} and remains at this level for prolonged pulping times. Carboxylic groups are formed by cleavage of ester groups in alkaline media, which are present in form of acetyl groups in carbohydrates, ester linkages between lignin and carbohydrates or fats. Figure 4.8b shows the content of methoxyl and phenolic hydroxyl in relation to the KLASON-lignin content of the precipitation product. Thereby, it is clearly demonstrated that the slight increase in methoxyl content observed in Figure 4.8a is only caused by little differences in lignin contents of the precipitation products and not by changes in composition of lignin units. In the contrary, an increase of phenolic hydroxyl content is considerable.

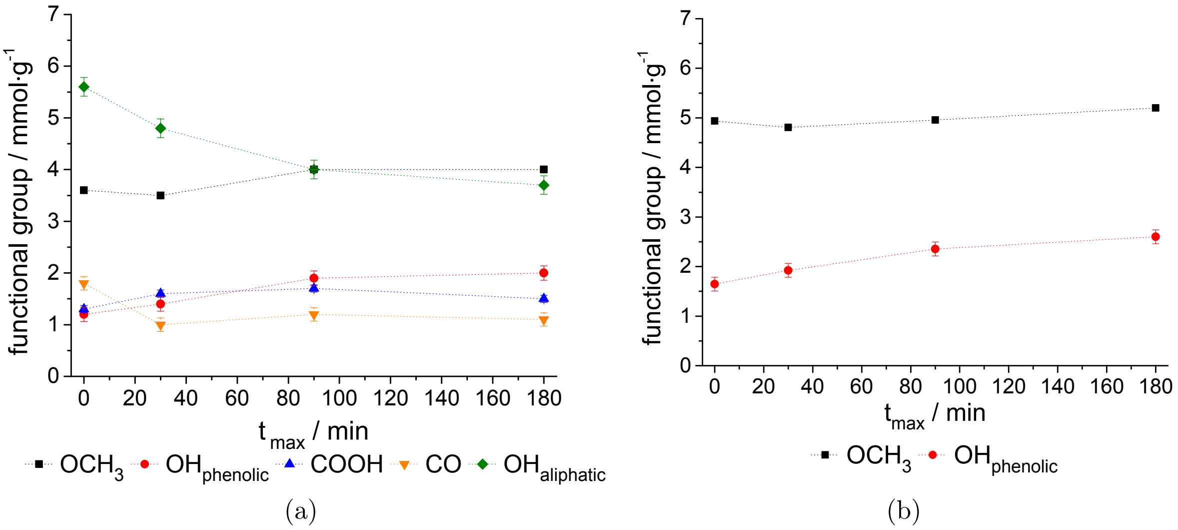


Figure 4.8.: Functional group content of (a) dialysed precipitation product and (b) dialysed precipitation product per KLASON-lignin, as a function of time at maximum temperature of alkaline pulping

In summary, to achieve the goals proposed for the pulping process with regard to time at maximum temperature the following considerations have to be taken into account. Times of 0 and 180 minutes are not advisable, as they lead to low product yields, whereas a time of 30 minutes is optimal in this terms. On the other hand, the purity of precipitation product and its content of phenolic hydroxyl is higher, if a time of 90 minutes was applied. Thus, times of 30 to 90 minutes are proposed, depending on lignin utilisation and energy consumption of the process.

Variation of concentration of sodium hydroxide

The concentration of the used aqueous sodium hydroxide solution ω(NaOH) was varied as follows: 1, 3, 6, and 9 wt%. Maximum temperature as well as time at maximum temperature were kept at standard conditions as defined in Section 6.4.1.

Out of all varied parameters, the concentration of sodium hydroxide has the largest impact on yield of pulp and precipitation product (Figure 4.9). If it was set at ω(NaOH) = 1 wt%, the maximal **yield** of pulp being 56% is obtained, though still containing considerable amounts of lignin. This is also reflected by the low yield of both holocellulose and of precipitation product. If a concentration of sodium hydroxide of 3 wt% was used, delignification proceeds leading to a decrease in pulp yield and an increase in yield of holocellulose, precipitation product and lignin. Nonetheless, a further increase of ω(NaOH) promotes lignin and holocellulose degradation, resulting in reduction of their yields.

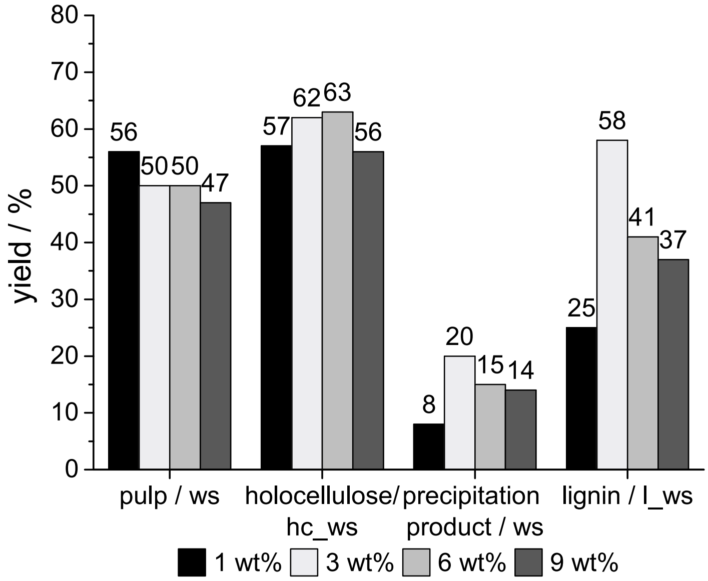
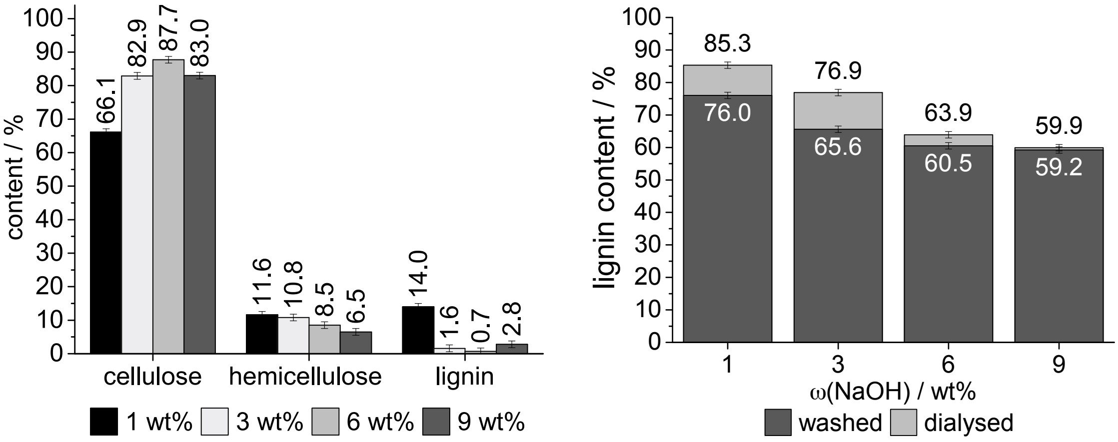


Figure 4.9.: Yield of pulp and precipitation product with regard to wheat straw (ws) and yield of holocellulose and lignin with regard to their contents in wheat straw (hc-ws, l-ws) as a function of concentration of sodium hydroxide used for alkaline pulping

The composition of **pulp** is also strongly influenced by the concentration of sodium hydroxide solution. The content of lignin sharply decreases from 14.0 to 1.6% if $\omega(\mathrm{NaOH})$ is increased from 1 to 3 wt%. The removal of lignin is coupled with an increase in cellulose content from 66.1% to a maximum value of 87.7%. Nevertheless, if a concentration of $\omega(\mathrm{NaOH}) = 9$ wt% was used, cellulose content decreases due to promotion of alkaline peeling reaction, as described in Section 2.3.1. The content of hemicellulose, however, decreases continuously with increasing $\omega(\mathrm{NaOH})$ due to their excellent solubility in alkali [12].

Intrinsic viscosity of pulp slightly increases from 610 to 700 $\mathrm{mL \cdot g^{-1}}$ for $\omega(\mathrm{NaOH}) = 1$ wt% and 3 wt%, respectively. However, if increased further, the higher concentration of sodium hydroxide leads to alkaline peeling reaction and hence, to short-chained cellulose. This is obvious due to the drop in intrinsic viscosity from 700 to 400 $\mathrm{mL \cdot g^{-1}}$ if $\omega(\mathrm{NaOH}) = 3$ wt% and 9 wt%, respectively. Kubes et al. [125] also report that it is alkali charge that has a profound effect on pulp viscosity, as pulp viscosity declines rapidly when raising the effective alkali charge at constant temperature and cooking time.

As sodium hydroxide concentration is known to influence crystallinity and crystalline structure of cellulose, x-ray diffraction is used to show the influence of sodium hydroxide concentration on pulp obtained by conventional alkaline pulping. Figure 4.11 shows x-ray diffraction diagrams of pulp obtained using $\omega(\mathrm{NaOH}) = 3$ and 9 wt%. In the first case, only peaks typical for cellulose I_β are found, whereas peaks for cellulose II are absent (cf. Annex Figure A.2 for peak deconvolution). This indicates, that the obtained cellulose is still in its natural, non-regenerated form. In the latter case, peaks for cellulose I_β as well as for cellulose II were found. Furthermore, the crystallinity slightly decreases from 69 % to 61 % for $\omega(\mathrm{NaOH}) = 3$ and 9 wt%, respectively.



(a) (b)

Figure 4.10.: Composition of products depending on concentration of sodium hydroxide used for alkaline pulping (a) Composition of pulp (b) Lignin content of precipitation product

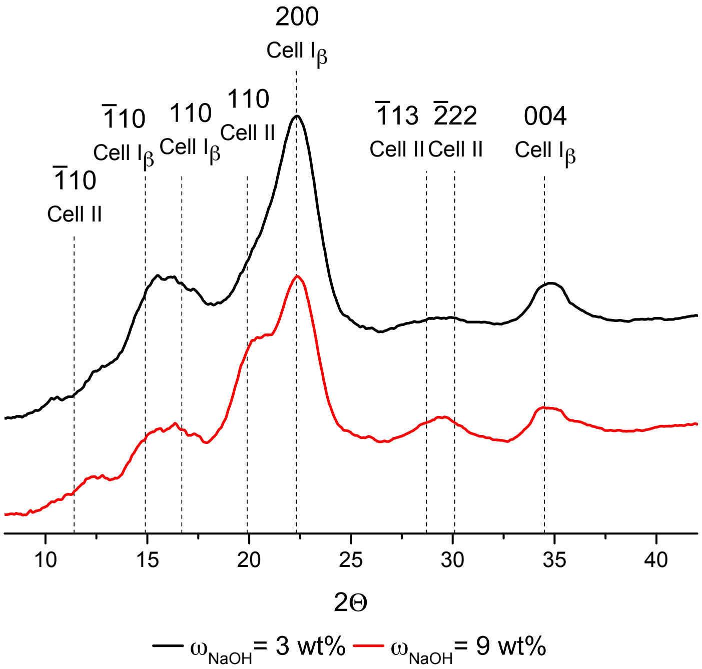
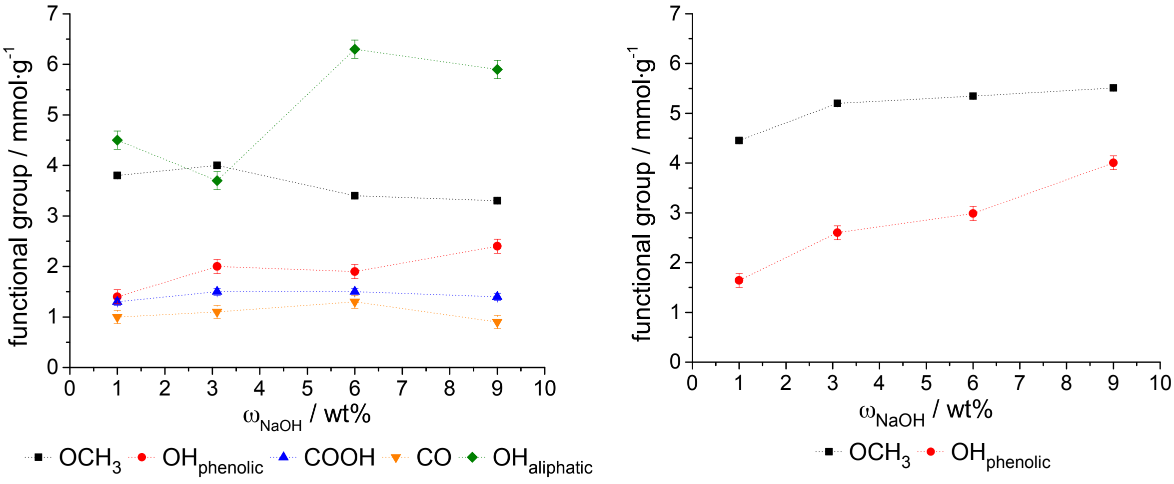


Figure 4.11.: X-ray diffractograms of pulp obtained using $\omega(\mathrm{NaOH}) = 3$ and $9\,\mathrm{wt\%}$

For purity of **precipitation product**, the concentration of sodium hydroxide was also found to be the most influential parameter, as the lignin content decreases continuously from 76.0% to 59.2% if ω(NaOH) is increased from 1 wt% to 9 wt%. Interestingly, the purification by dialysis, which usually amounts to about 10%, decreases with increasing concentration of sodium hydroxide. This finding supports the assumption that low lignin content is caused by high molecular weight impurities, such as long-chained carbohydrates. As more hemicelluloses are solved with increasing ω(NaOH), the increase in impurities in the precipitation product could be due to the formation of new lignin-carbohydrate linkages. These could be formed, for instance, during lignin precipitation in the black liquor at low pH. This is supported by studies from Xiang et al. [228], who describe a reaction between glucose and acid-soluble lignin in acidic media.

The influence of sodium hydroxide concentration on the content of functional groups is shown in Figure 4.12. Whereas no significant impact on carbonyl and carboxyl groups can be observed, methoxyl as well as aliphatic and phenolic hydroxyl groups are strongly influenced. Interestingly, the content of aliphatic hydroxyl groups decreases between ω(NaOH) = 1 and 3 wt%, though the content of impurities, which mainly consist of carbohydrates (cf. Section 3.4.1), increases. A possible explanation is the cleavage of C-C linkages of the propanoid side chain by retrograde aldol condensation as described by Gierer [83].

The content of methoxyl groups is particular interesting being characteristic for lignin. As can be seen from Figure 4.12a its content decreases in the precipitation product due to the decreasing lignin content. However, in relation to the KLASON-lignin content of the precipitation product it increases between ω(NaOH) = 1 and 3 wt% (Figure 4.12b). As this goes hand in hand with pulp delignification, it can be concluded that units, which are rich in methoxyl groups, are preferable dissolved at sodium hydroxide concentrations of 3 wt% or higher. This is in contrast to results obtained by Rencoret et al. [182], which imply



(a) (b)

Figure 4.12.: Functional group content of (a) dialysed precipitation product and (b) dialysed precipitation product per KLASON-lignin, as a function of concentration of sodium hydroxide used for alkaline pulping

preferential removal of S-lignin. As soon as the delignification process is completed at $\omega(\text{NaOH}) \geq 3\,\text{wt\%}$ (as proven by low lignin content in pulp, see Figure 4.10a), the content of methoxyl groups per KLASON-lignin remains at approximately 5.4 mmol·g^{-1}.

Furthermore, the content of phenolic hydroxyl increases with increasing concentration of sodium hydroxide. This is due to cleavage of α-ether linkages in phenolic as well as of β-ether linkages in non-phenolic units. Subsequently, new phenolic units are separated which may undergo further fragmentation leading to alkaline lignin peeling [83]. This is further supported by low lignin yields for high sodium hydroxide concentrations as discussed before.

To conclude, a sodium hydroxide concentration of higher than 1 wt% is preferable to meet the aims of separation procedure. High sodium hydroxide concentrations also result in a decrease of cellulose crystallinity, which may enhance enzymatic hydrolysis. However, this conversion to amourphous cellulose proceeded only to a minor extend. In addition, high values of $\omega(\text{NaOH})$ lead to lignin fragmentation in a way, which makes its recovery difficult or impossible. Though it also results in high contents of phenolic hydroxyl groups, this effect is outweighed by the low yield and purity of the precipitation product. Thus, a sodium hydroxide concentration of 3 wt% is suggested for the alkaline separation of wheat straw.

4.1.3. Variation of precipitation procedure

The usage of chemicals, especially of those that cannot be recovered, is to be minimised for economic and environmental reasons. Therefore, the precipitation procedure was studied in dependence on precipitation method, precipitant and pH. An overview is given in Table 4.1. Apart from the varied parameters the standard procedure described in Section 6.4.1 was followed. The influence of the parameters is studied in terms of yield and purity of precipitation products, content of functional groups as well as particle sizes, with the latter being of major importance for separation of precipitation product from the acidified black liquor.

No influence on the yield of precipitation product was found for the different precipitation parameters as shown in Figure 4.13. With regard to pH, however, García et al. [81] report an increase in yield by approximately 14% when decreasing pH from 2.57 to 0.72. As lignin structure varies depending on the pulping parameters used, pH for precipitation of maximum possible precipitable lignin might vary accordingly.

Table 4.1.: Parameters varied for precipitation procedure after alkaline pulping

Sample	Method	Precipitant	pH
1/HCl/1	1: precipitant is added to black liquor	HCl	1
1/HCl/3	1: precipitant is added to black liquor	HCl	3
2/HCl/1	2: black liquor is added to precipitant	HCl	1
1/H_2SO_4/1	1: precipitant is added to black liquor	H_2SO_4	1
1/H_2SO_4/3	1: precipitant is added to black liquor	H_2SO_4	3
2/H_2SO_4/1	2: black liquor is added to precipitant	H_2SO_4	1

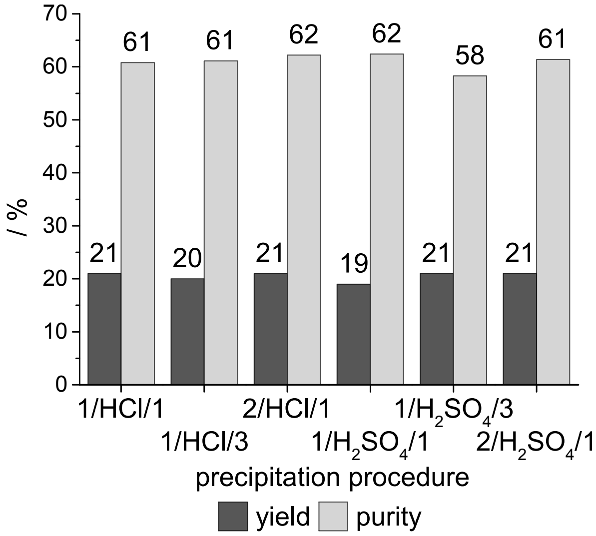


Figure 4.13.: Yield in relation to wheat straw and purity given as content of KLASON-lignin depending on precipitation procedure

This is further suggested by another pulping experiment carried out at 185 °C for 45 min. Here, the yield of precipitation product was 17 and 24% for pH = 3 and 1, respectively, supporting the findings of García et al. [81]. Thus, further investigations in this area can help to decrease consumption of chemicals.

For the study of purity of precipitation product, given as KLASON-lignin content in Figure 4.13, no dependence on precipitation method, precipitant or pH was found.

Figure 4.14 shows the content of functional groups depending on the precipitation procedure. Some variations are observable, especially with regard to the content of aliphatic hydroxyl groups. Nonetheless, these are still within the error margin and, in addition, no clear dependency on one specific parameter could be identified. This goes hand in hand with results from García et al. [81], who did not find significant differences between lignin fractions precipitated at pH = 2.57 and 0.72 by FTIR spectroscopy.

Particle size of the precipitation product before separation from the black liquor turned out to be of special interest, as it substantially influences the separation of precipitation product from the black liquor. A large particle size of preferably higher than 150 µm was aimed at in order to facilitate separation by techniques operating at large scale as for instance chamber filter press. In addition, a particle size of more than 5 µm is inevitable even if separation is performed by centrifuge. Thus, the particle size of the precipitation product in the acidified black liquor was studied by laser diffraction.

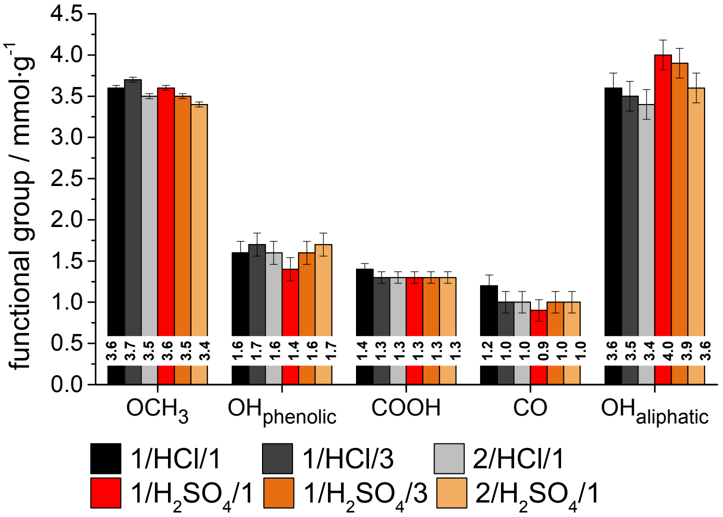


Figure 4.14.: Functional group content of precipitation product depending on precipitation procedure

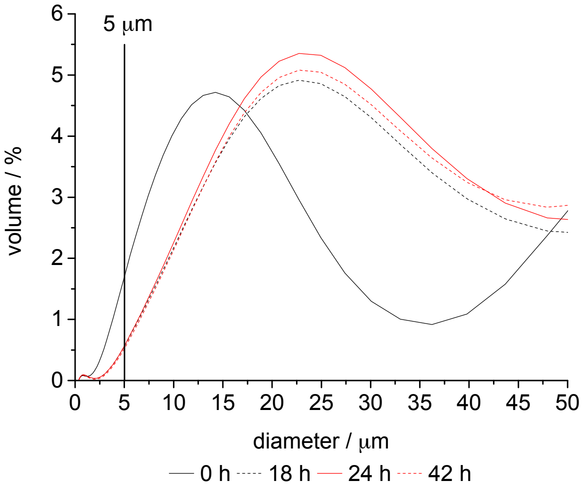
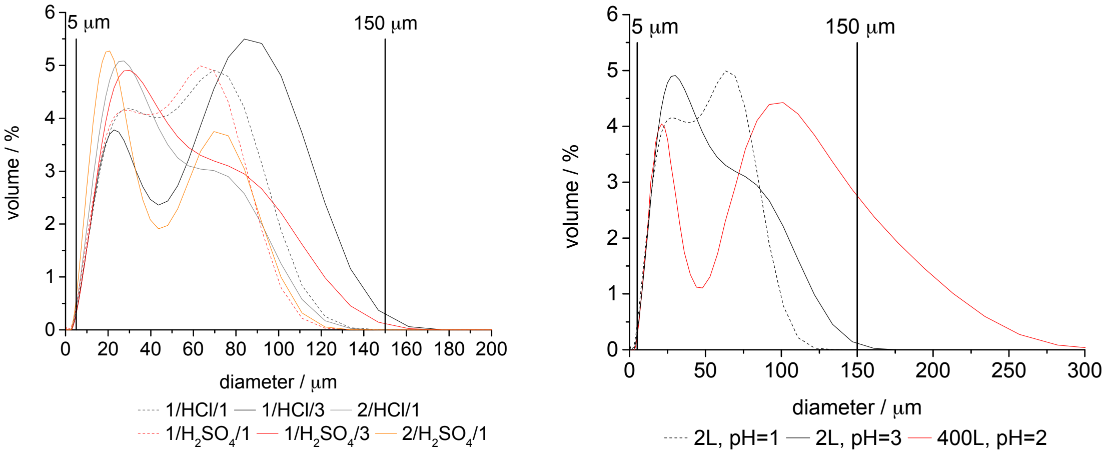


Figure 4.15.: Particle size of precipitation product in dependence of residence time in acidic media

The influence of residence time of precipitation product in the acidified black liquor on the particle size is studied between 0 and 42 hours (Figure 4.15). For a residence time of 0 hours, hence immediately after acid precipitation, the peak maxima was at 14.2 µm. Prolongation of residence time to 18 hours (standard setting) or longer leads to a shift to approximately 23 µm. Furthermore, the volume of particles having a diameter of less than 5 µm could be significantly reduced. Thus, the precipitation product should not be separated immediately after addition of acid in order to simplify the separation process.

The influence of precipitation method, precipitant and pH on particle size distribution are shown in Figure 4.16a. In most cases, two peaks were observed at approximately 20 – 30 µm and 65 – 95 µm, the latter supposably being an agglomerate of particles. Furthermore, the particle size ranges between 5 and 160 µm.



(a) (b)

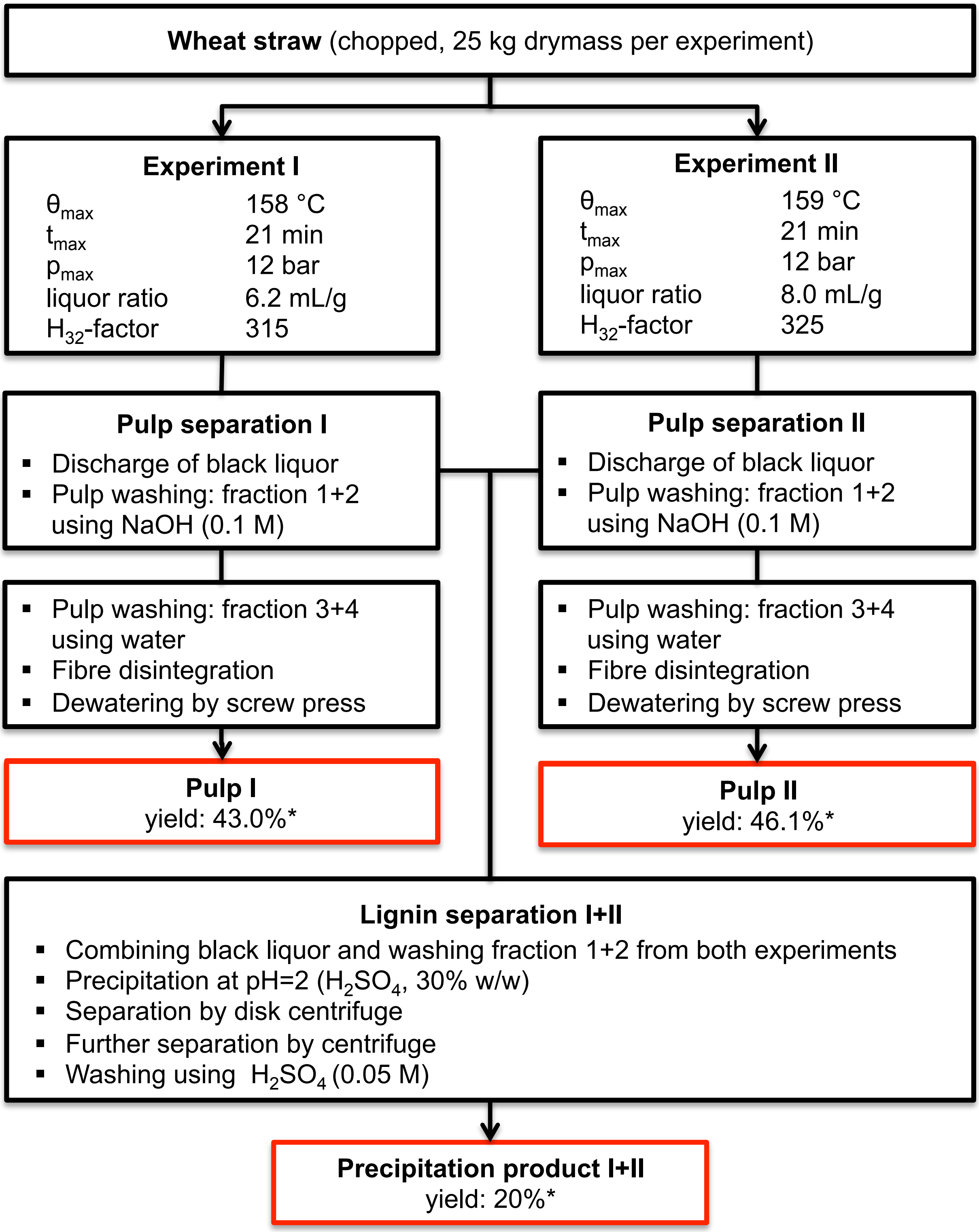
Figure 4.16.: Particle size of precipitation product: influence of (a) precipitation method/precipitant/pH (b) precipitation reactor

Whereas there is no dependence between particle size and neither precipitation method nor precipitant, a precipitation at pH = 3 leads to slightly higher particle sizes in comparison to precipitation at pH = 1.

In addition, Figure 4.16b shows the particle size of precipitation product obtained in a stirred precipitation reactor at a temperature of 9 °C. H_2SO_4 was used as precipitant, which was added to the polymer solution (according to method 1), to set pH to 2. For comparison, the particle size distribution of two precipitation products obtained from the non-stirred precipitation at room temperature at pH = 1 and 3 are shown. Here, the second peak maxima and hence the particle size of the agglomerate is shifted to a particle size of approximately 100 µm. Furthermore, the diameter of a considerable percentage of the particles exceeds the 150 µm mark. Hence, this is an significant improvement compared to the other studied precipitation procedures and provides opportunities for further studies.

4.1.4. Scale-up experiments

For a successful implementation of a process, experiments in pilot scale are crucial for closing the gap between lab and industrial scale. Every step to a higher technical readiness level (TRL), raises new kinds of problems that need to be addressed properly before proceeding further [167]. This section deals with scale-up experiments from 2-L to 10-L (TRL 4) to 400-L (TRL 5-6). The 2-L and 10-L scale were both conducted in similar laboratory autoclaves (unstirred, wall and floor heating) using the same procedure for product separation as described in Section 6.4.1. Set parameters were: $\theta_{max} = 160\,°C$, $t_{max} = 30\,min$, $\omega_{NaOH} = 3\,wt\%$, liquor ratio = $6.2\,mL{\cdot}g^{-1}$. However, this method cannot be directly transferred to pilot scale. The process scheme used for 400-L scale is shown in Figure 4.17. Two pulping experiments were carried out with separate preparation of pulp. In contrast, lignin was precipitated from combined black liquors and washing fractions from both pulping experiments. Major changes made in comparison to lab scale operation are listed in Table 4.2.



* in relation to wheat straw

Figure 4.17.: Procedure for scale-up experiments of alkaline pulping carried out in a fixed-bed reactor of 400-L capacity at Fraunhofer CBP

Table 4.2.: Major adjustments made for pilot scale (400-L) compared to lab scale (2-L and 10-L)

Type	Lab scale	Pilot scale
Heating	Wall and floor heating	Recirculation of pulping liquor in fixed-bed reactor
	No preheating of liquor	Preheating of liquor in separate vessel
Process control	By time and temperature	By H_{32}-factor
Gauge pressure	5-6 bar	12 bar
Liquor/solid ratio	$6.2\,mL{\cdot}g^{-1}$	I: $6.2\,mL{\cdot}g^{-1}$; II: $8.0\,mL{\cdot}g^{-1}$ Addition of pipe volume
Pulp preparation	No fibre disintegration Dewatering by Büchner funnel	Fibre disintegration Dewatering by screw press
Lignin precipitation	By HCl at pH=1 Unstirred at approx. 20 °C	By H_2SO_4 at pH=2 Stirred at 9 °C
Lignin separation	By lab centrifuge	By disk centrifuge followed by lab centrifuge
Lignin residence time	Approx. 15 h	Up to several weeks

For instance, recirculation of pulping liquor enables fast heating of the process in large scale and simplifies lignin dissolution. In addition, lignin precipitation had to be carried out using H_2SO_4 instead of HCl as it is less aggressive towards the used equipment.

These adjustments and the larger scale, lead to differences in **product yields** as shown in Figure 4.18. As for the first scale-up experiment the liquor to solid ratio did not fully compensate transfer losses for liquor circulation, the following discussion of results regarding the pulp fraction refers to the second experiment. Whereas pulp yield is not significantly influenced by a scale-up from 2-L to 10-L, it decreases by 10% for the 400-L scale. Though, both reactor and disintegrator were rinsed to completely discharge pulp, transfer losses cannot be excluded. Losses due to extensive carbohydrate fragmentation is excluded, as this would have resulted in lower hemicelluloses moieties in the pulp.

In contrast, the same yield of precipitation product is attainable compared to lab-scale. It must be noted that in case of the 400-L scale experiment the yield of precipitation product can only be given as an approximation as its separation was difficult and time-consuming given the low particle size as discussed in the previous section, despite the improved precipitation in a cooled stirred vessel. Thus, a fixed volume of stirred slurry was concentrated using a disk centrifuge. Afterwards, the precipitation product was separated and washed using a laboratory centrifuge as was used for the lab-scale experiments. This resulted in a prolonged residence time in the acidified black liquor which also contains solved hemicellulose. This may have promoted the reaction of lignin with dissolved sugar monomers and oligomers leading to a lower purity of the precipitation product compared to smaller scales, as shown in Figure 4.19b, and hence to a low lignin yield. This is supported by studies from Xiang et al. [228], who describe a reaction between glucose and lignin in acidic media by consumption of glucose in the presence of lignin.

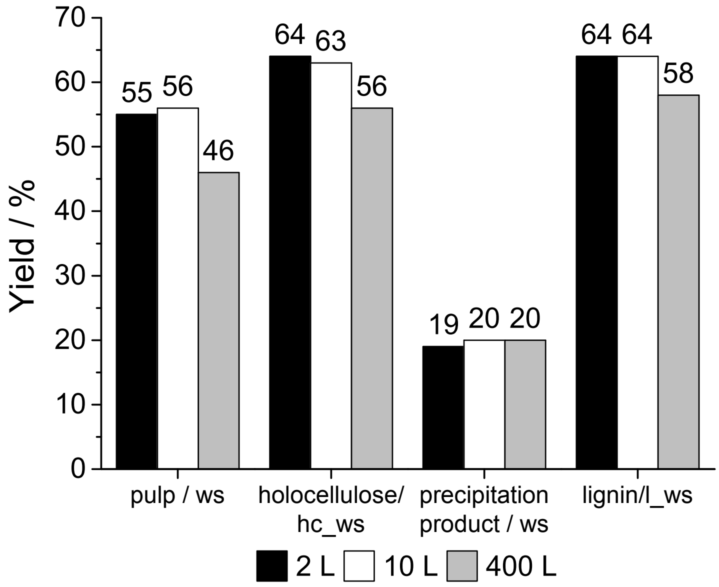
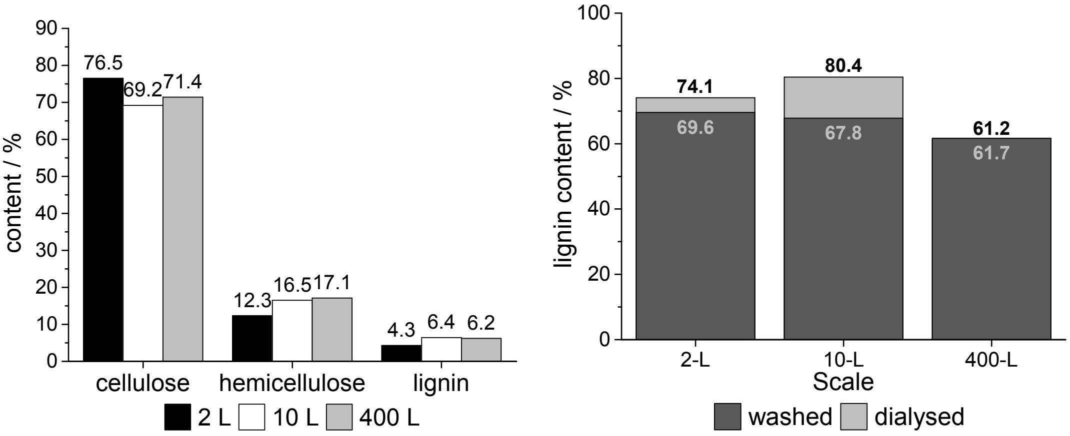


Figure 4.18.: Yield of pulp and precipitation product with regard to wheat straw (ws) and yield of holocellulose and lignin with regard to their contents in wheat straw (hc-ws, l-ws) depending on scale of alkaline pulping



(a)

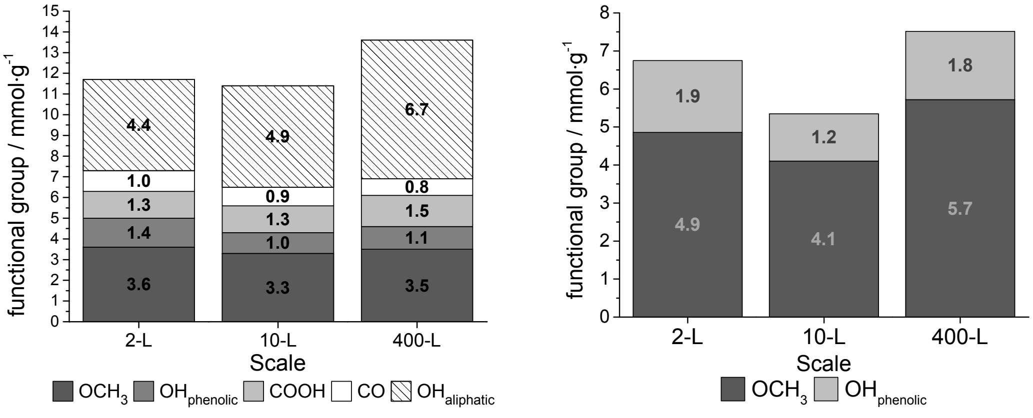
(b)

Figure 4.19.: Composition of products depending on scale of pulping (a) Composition of pulp (b) Lignin content of precipitation product

The composition of **pulp** obtained at pilot-scale did not significantly differ from pulp obtained at 10-L scale (see Figure 4.19a). However, both contain less cellulose and more lignin as well as hemicellulose compared to pulp obtained at 2-L-scale. This indicates that there are differences in severity of pulping between the 2-L-scale and the others. Though the maximum temperature was reached in less than 40 minutes for all scales, the pulping procedure at 2-L-scale might have been more severe, as the set temperature was likely to be reached in the centre of the digester. Thus, temperature control, which is usually fixed at the top edge of a digester, was shown to be one major issue when transferring pulping procedures to different digester and hence, to higher TRL.

The low purity of the **precipitation product** is reflected in the content of functional groups as the aliphatic hydroxyl content, strongly influenced by carbohydrate moieties, is approximately 2 mmol·g^{-1} higher compared to preparations obtained at lab scale (Figure 4.20a). Similar contents of carbonyl and carboxyl groups are obtained for all scales.

As discussed for pulp, the process scale resulted in different pulping severity. This is also reflected in the content of functional groups of the precipitation product. The precipitation product obtained from pulping at 10-L-scale contains less phenolic hydroxyl groups compared to 2-L-scale with regard to KLASON-lignin content as shown in Figure 4.20b). Thus, less ether linkages are cleaved and the procedure is not as severe. Furthermore, it contains less methoxyl groups and, as the lignin content in pulp is higher for pulp obtained at 10-L-scale, this supports the thesis that lignin, which contains less methoxyl groups is dissolved first [38]. For 400-L-scale, it has to be noted that the precipitation product was subjected to a long residence time in acidified black liquor due to difficulties in the separation process (cf. Table 4.2). This might have caused further structural changes, besides the ones caused by the pulping procedure. The precipitation product is characterised by a high content of methoxyl and phenolic hydroxyl groups. This effect is generally accompanied by advanced lignin dissolution, as shown for variation of sodium hydroxide concentration (cf. Section 4.1.2). However, this is not the case as the content of residual lignin in pulp is similar to the 10-L-scale.



(a) (b)

Figure 4.20.: Functional group content of (a) dialysed precipitation product and (b) dialysed precipitation product per KLASON-lignin, depending on scale of alkaline pulping

To conclude, the scale-up of the pulping procedure up to pilot scale was successful with regard to delignification and separation of pulp as well as lignin precipitation. Higher pulp yields might be possible after further optimisation of the process. With regard to lignin the problem of separation and washing should be addressed.

4.2. Organosolv pulping

As pulping is one of the most expensive steps of bioethanol production, the influence of pulping parameters on product yield and composition is advantageous for identifying saving potentials. In the following section, the time at maximum temperature as well as the concentration of formic acid is varied for organosolv pulping based on formic acid and hydrogen peroxide. Organosolv pulping was conducted using the following parameters as standard setting: liquid/solid ratio = 27 mL·g^{-1}, addition of 46 mL of hydrogen peroxide solution (30 %, w/v), addition of HCOOH (98 wt%) and water to adjust to ω(HCOOH) = 60 wt%, θ = 103 °C, time at maximum temperature = 60 min. The pulp was separated from the liquor, washed and dried. The lignin fraction was precipitated in water, centrifuged and lyophilised.

4.2.1. Variation of time at maximum temperature

The time at maximum temperature t_{max} was varied as follows: 40, 60 and 90 minutes.

Figure 4.21 shows the product **yields** with regard to the time at maximum temperature. The yield of pulp is not significantly influenced, as it remains at approximately 50% for the studied time range. The same applies to yields of holocellulose and precipitation product. The yield of lignin was lower for t_{max} = 60 min compared to 40 as well as 90 minutes due to a slightly lower yield of precipitation product combined with a likewise slightly decreased purity as shown in Figure 4.22b.

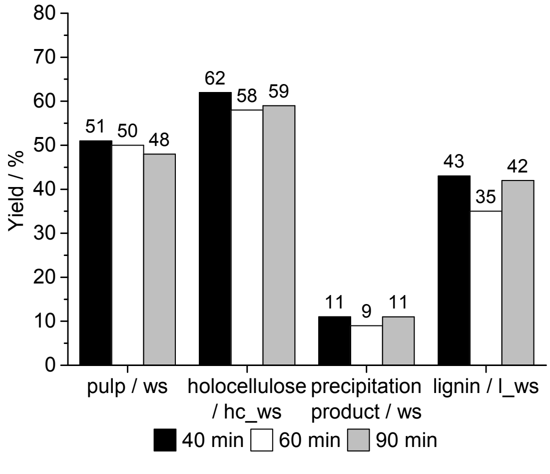
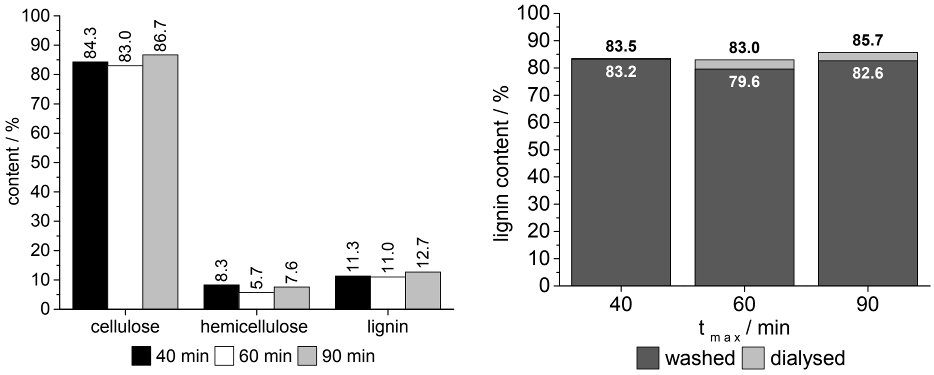


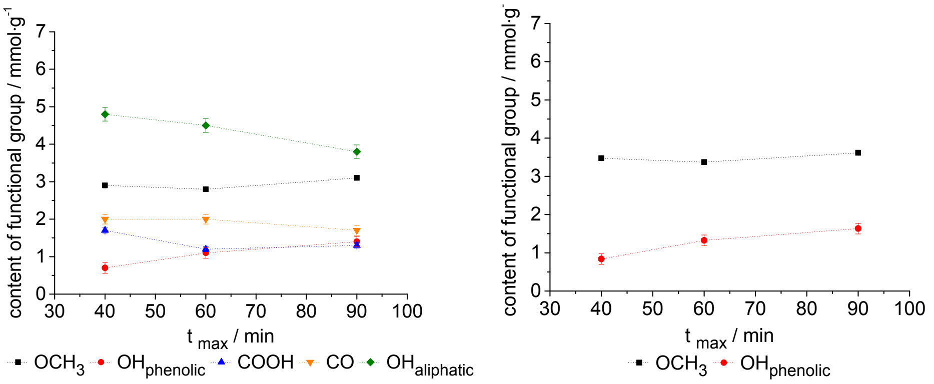
Figure 4.21.: Yield of pulp and precipitation product with regard to wheat straw (ws) and yield of holocellulose and lignin with regard to their contents in wheat straw (hc-ws, l-ws) as a function of time at maximum temperature of organosolv pulping



(a)

(b)

Figure 4.22.: Composition of products depending on time at maximum temperature of organosolv pulping (a) Composition of pulp (b) Lignin content of precipitation product



(a)

(b)

Figure 4.23.: Functional group content of (a) dialysed precipitation product and (b) dialysed precipitation product per KLASON-lignin, as a function of time at maximum temperature of organosolv pulping

Pulp composition is not influenced by time at maximum temperature as shown in Figure 4.22. Thus, even shorter pulping times are proposed for further studies.

The same applies to the purity of the **precipitation product**, which is also not influenced by the time at maximum temperature. In contrast, the content of functional groups in the precipitation product is strongly influenced by the time at maximum temperature. For instance, the content of aliphatic hydroxyl decreases from 4.8 to 3.8 mmol·g^{-1} for 40 and 90 minutes, respectively (Figure 4.23a). As the lignin content of precipitation product remains nearly constant, carbohydrates are unlikely to cause this effect. Thus, the decrease of aliphatic hydroxyl content is due to displacements of side chains in lignin (cf. Section 2.3.2). As this is caused by attack of HO$^+$ ions, this reaction is accompanied by an hydroxylation at C1 [84]. Indeed, the content of phenolic hydroxyl groups increases from 0.8 to 1.6 mmol·g^{-1} with regard to KLASON-lignin as shown in Figure 4.23b. Other functional groups are not, or only to a minor extend, influenced by the time at maximum temperature.

It can be concluded, that pulping time has only a minor influence on the outcome of the pulping procedure with regard to both product yield and composition. Thus, short times at maximum temperature are suggested for peroxyformic acid pulping, unless the focus is on lignin production for further derivatisation or cross-linking reactions.

4.2.2. Variation of concentration of formic acid

The concentration of aqueous formic acid solution ω(HCOOH) was varied as follows: 30, 50, 60, and 80 wt%. Maximum temperature as well as time at maximum temperature were kept at standard conditions as defined in Section 6.4.3.

In contrary to the time at maximum temperature, the concentration of formic acid has a high impact on product yield (Figure 4.24) as well as product composition (Figure 4.25). The **yields** of holocellulose and lignin show two opposing trends, which have to be taken into account when choosing the optimal concentration of formic acid. Whereas the yield of pulp and holocellulose decreases with increasing ω(HCOOH), yield of precipitation product and lignin is increased. This is caused by hemicellulose degradation going hand in hand with the delignification process [75].

As delignification and hemicellulose degradation proceed and the content of hemicellulose and lignin in **pulp** decreases, the relative content of cellulose in pulp increases steadily as shown in Figure 4.25a. Hemicellulose degradation is also relevant for the purity of precipitation product, which increases with increasing concentration of formic acid. This is due to depolymerisation of xylan caused by formic acid [101].

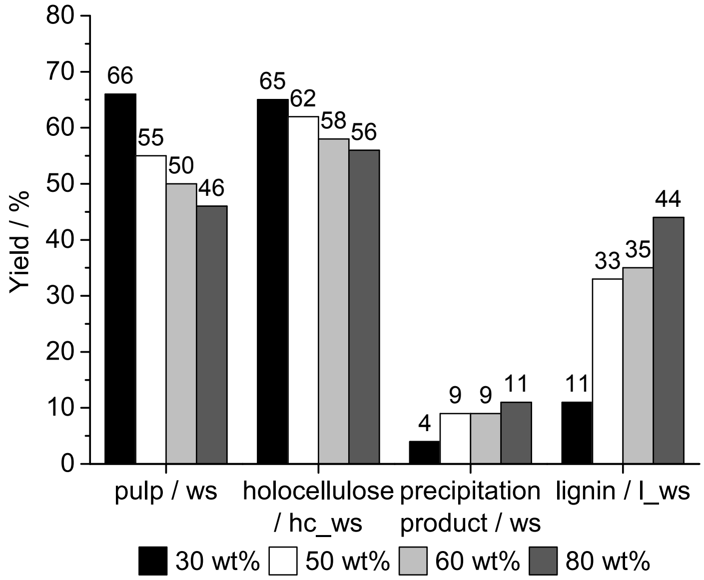
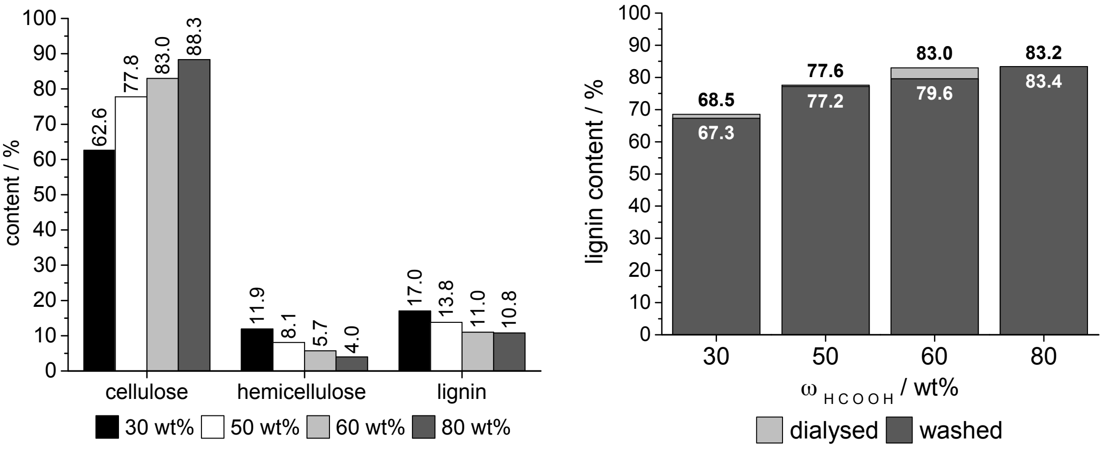


Figure 4.24.: Yield of pulp and precipitation product with regard to wheat straw (ws) and yield of holocellulose and lignin with regard to their contents in wheat straw (hc-ws, l-ws) as a function of concentration of formic acid used for organosolv pulping

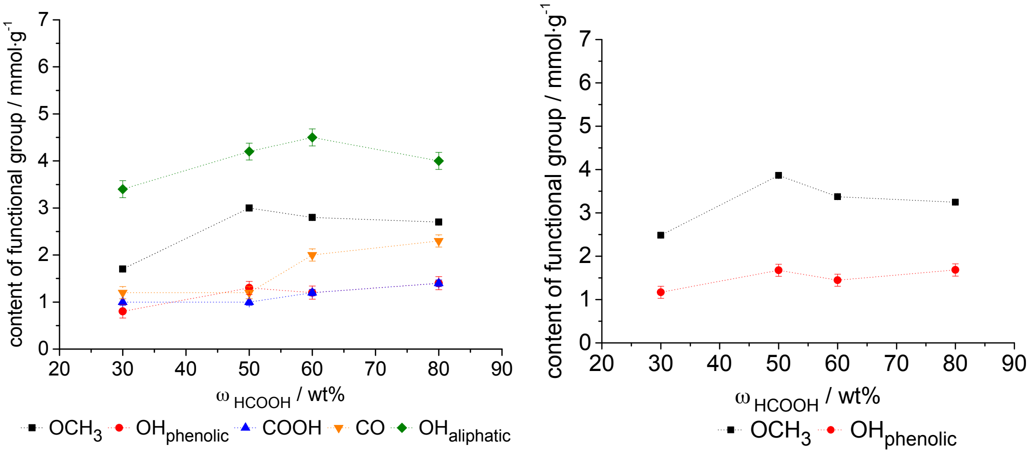


(a) (b)

Figure 4.25.: Composition of products depending on concentration of formic acid used for organosolv pulping (a) Composition of pulp (b) Lignin content of precipitation product

The content of functional groups in the **precipitation product** is also affected by formic acid concentration (Figure 4.26). The content of aliphatic hydroxyl, for instance, is increased from 3.4 to to 4.5 mmol·g^{-1} when increasing formic acid concentration from 30 to 60 wt%. As the purity of lignin also increases, carbohydrate impurities are unlikely to account for this effect. However, solvolytic cleavage of α-ether linkages is known to occur in acidic media resulting in the corresponding alcohol in the aliphatic side chain [49]. A further increase of ω(HCOOH) up to 80 wt% leads to a decrease in aliphatic hydroxyl content, as found for prolonged pulping times. One explanation is the displacement of side chains in lignin (cf. Section 2.3.2). As this is caused by attack of HO$^+$ ions, this reaction would be accompanied by an hydroxylation at C1 [84], as was the case for the parameter time at maximum temperature. However, the content of phenolic hydroxyl groups in not significantly increased. Thus, another reaction pathway may account for the decrease in aliphatic hydroxyl content, which also goes hand in hand with an increase in carbonyl content from 1.2 to 2.3 mmol·g^{-1}. Under strongly acidic conditions the hydroxyl-substituted side chain undergoes dehydration and rearrangement reactions leading to the introduction of carbonyl groups in form of Hibbert's ketones [216]. Furthermore, the reactive species HO$^+$ leads to oxidative demethylation as well as oxidative ring opening reactions, which both result in carbonyl formation [35, 84]. In addition, hydrogen peroxide itself is an oxidising agent. However, its concentration was kept constant for the pulping experiments.

The content of methoxyl groups was reported to decrease during formic and peroxyformic pulping as demethylation reactions take place [84]. This can be confirmed as the methoxyl content in milled wood lignin obtained from wheat straw was reported to be 5.1 mmol·g^{-1} [169] and hence significantly higher than the contents found in this study with a maximum content of 3.0 mmol·g^{-1}. Furthermore, a decrease in methoxyl is observed for the range between ω(HCOOH) = 50 and 80 wt%, especially with regard to KLASON-lignin (see Figure 4.26b). In contrast, a significant increase of methoxyl content from 1.7 to 2.8 mmol·g^{-1} is noticeable if the formic acid concentration is raised from 30 to 50 wt%.



(a) (b)

Figure 4.26.: Functional group content of (a) dialysed precipitation product and (b) dialysed precipitation product per KLASON-lignin, as a function of concentration of formic acid used for organosolv pulping

As in the case of carbonyl groups, there are two possible explanations with one depending on peroxyformic acid formation. In this case, the irreversible decomposition of peroxyformic acid into CO_2 and H_2O promoted by increased concentration of H^+ has to be taken into account as it results in a lower formation of active HO^+ species [74]. Thus, the demethylation reaction could be promoted by a lower concentration of formic acid. A second explanation for the increase in methoxyl group content within the discussed range of ω(HCOOH) could be that lignin which is dissolved first and at lower concentration of formic acid contains less methoxyl groups compared to lignin, which is dissolved at a formic acid concentration of 50 wt% and above. This is also supported by the increase of lignin yield by 33 % (see Figure 4.24) reflecting the step-wise delignification process. In addition, Sun et al. [201] report a higher reactivity of p-hydroxyphenyl units towards peroxyformic acid compared to syringyl and guaiacyl structures. This effect was also observed for alkaline pulping (cf. Section 4.1.2).

To conclude, a formic acid concentration of 50 to 60 wt% should be used in order to achieve best delignification results accompanied by high lignin yields on the one hand and a high pulp yield on the other hand.

4.3. Comparison of conventional alkaline, microwave-assisted alkaline and organosolv pulping

As pulp and lignin are known to be strongly influenced by the pulping procedure used for preparation, this section is dedicated to comparison of conventional alkaline (AP) and organosolv pulping (OP), with their optimisation of parameters already being discussed in Section 4.1 and Section 4.2. In addition, microwave-assisted alkaline pulping (AM) is taken into account as a fast alternative to conventional pulping. Furthermore, it has shown to be advantageous in terms of subsequent enzymatic hydrolysis [102, 240]. However, there is a lack of data concerning the lignin fraction. Thus, this will be the focus of this section. It has to be noted, that optimisation regarding power or pulping duration of AM is not subject of this study and the same sodium hydroxide concentration was used as for AP. Chopped wheat straw was used for experiments of this section with the pulping parameters specified in Section 6.4.

4.3.1. Separation into a carbohydrate and lignin fraction

The yield of pulp with regard to the pulping procedure is shown in Figure 4.27a. There is no significant difference between conventional and microwave-assisted alkaline pulping. This result is unexpected in so far as pulp obtained by the latter has a relative high content of both, hemicellulose and lignin, as shown in Figure 4.27b. As less has been solved of these substances, compared to both conventional alkaline pulping and organosolv pulping, the pulp yield was expected to be higher. Thus, pulp yield is additionally decreased by loss of cellulose, for instance due to cleavage of chains by generation of hot spots [102]. Organosolv pulping leads to a slightly lower yield of pulp compared to both alkaline procedures. Delignification is poor as it is for microwave-assisted alkaline pulping. However, the favoured depolymerisation of xylan results in a pulp that is rich in cellulose compared to both alkaline procedures.

For the yield of precipitation product, conventional and microwave-assisted alkaline pulping lead to similar results (see Figure 4.28a). This is interesting in view of the fact that pulp obtained by the latter was not as delignified. Thus, the alkali-soluble lignin in the black liquor obtained by microwave-assisted pulping is easier to precipitate and separate. For organosolv pulping the yield of precipitation product was low due to low delignification of pulp. Although, the purity of washed precipitation product is with a value of 79.6 % the highest compared to both alkaline methods, this cannot compensate for the low yield of precipitation product and results therefore in a low lignin yield.

Whereas the purity of the precipitation product is within a similar range for AP and AM, it is considerably higher for OP. This is presumably due to its lower content of carbohydrate impurities, as acidic organosolv pulping leads to intensive hydrolysis of lignin-hemicellulose bonds [231]. Furthermore, peroxyformic acid is known to have a depolymerising effect on xylan [101].

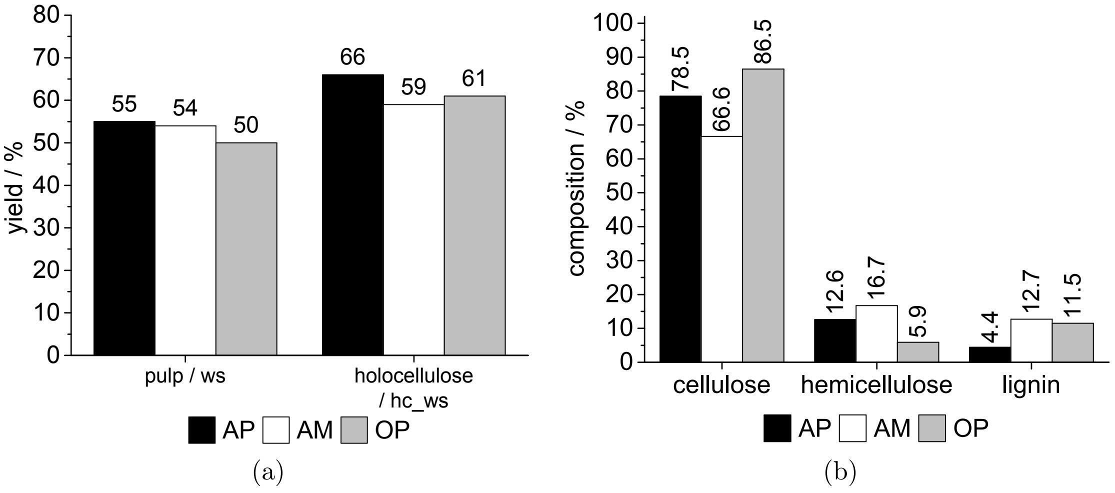


Figure 4.27.: (a) Yield of pulp with regard to wheat straw (ws) and yield of holocellulose with regard to its content in wheat straw (hc-ws) and (b) Composition of pulp depending on pulping procedure (AP: conventional alkaline, AM: microwave-assisted alkaline, OP: organosolv)

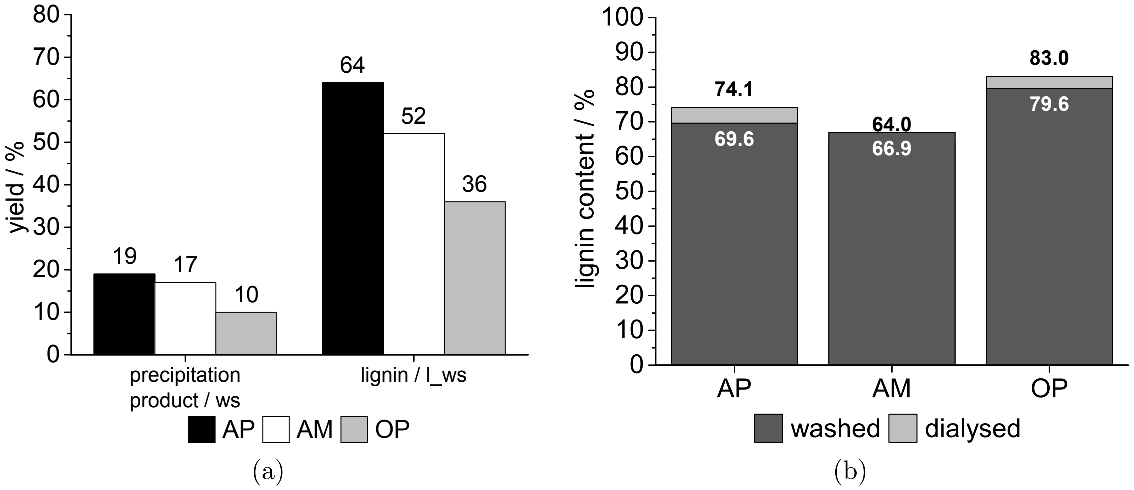


Figure 4.28.: (a) Yield of precipitation product with regard to wheat straw (ws) and yield of lignin with regard to its content in wheat straw (l-ws) and (b) Composition of precipitation product depending on pulping procedure(AP: conventional alkaline AM: microwave-assisted alkaline OP: organosolv)

4.3.2. Pulp

Several factors have been reported to influence enzymatic hydrolysis of pulp into monosaccharides. Among them are the degree of polymerisation and crystallinity. Thus, these are the key indicators focussed on in the following section [36].

Intrinsic viscosity

The intrinsic viscosity was determined to obtain information about the degree of polymerisation, which can be estimated as "true viscosity average degree of polymerization" (DP_v) according to Kes and Christensen [114]. This value is used with considerable reservation, especially as pulp samples contain hemicelluloses and lignin moieties besides cellulose.

The measured intrinsic viscosities of the obtained pulps were 810, 400 and 440 $mL \cdot g^{-1}$ ($DP_v = 4000$, 1450, 1700) for conventional alkaline pulping, microwave-assisted alkaline pulping and organosolv pulping, respectively. Thus, using a microwave system results in significant cleavage of cellulosic chains supposably due to generation of hot spots. Despite the fact, that this results in low pulp yield, it could be advantageous for subsequent enzymatic hydrolysis, despite the higher lignin content in pulp. The same applies to the intrinsic viscosity of pulp obtained by OP. It is similar to that reported for pulp obtained by pulping using acetic acid being 465 $mL \cdot g^{-1}$ [203] and hence, low compared to pulp obtained by AP.

X-ray diffraction

X-ray diffractograms reveal that cellulose is of type I_β for all pulps and no conversion into cellulose II has taken place (cf. Annex Figure A.2 for example of peak deconvolution).

The influence of crystallinity of pulp on enzymatic hydrolysis rate has been in discussion [36]. It is evident that absolute values for crystallinity index (CrI) obtained by the deconvolution method are likely to be too high, as pulp obtained by conventional alkaline pulping (AP) has a CrI of 99% though still containing amorphous hemicellulose moieties. However, relative evaluation of values is possible. The CrI of wheat straw is 56% using this method. Alemdar and Sain [3] report a CrI of 58% using a similar XRD method. When using XRD peak height method as proposed by Segal et al. [194], CrI is reported to be 35% [178]. Due to removal of lignin by alkaline pulping (AP and AM) the CrI of the obtained pulps is significantly increased. When comparing CrI of pulps obtained by AP and AM, it is lower for AM. This goes hand in hand with its higher content of amorphous hemicelluloses and lignin.

In contrast, OP pulp has the lowest CrI being 58%, similar to the one of wheat straw, despite having the highest cellulose content. This is in line with results reported by Haverty et al. [95], who describe only small variations in CrI between the raw material miscanthus and the performic acid treated pulp, being 69.6% and 62.9%, respectively. Thus, the acidic pretreatment leads to an increase in cellulosic amorphous moieties due to disruption of hydrogen bonding within cellulose. In contrast, swelling in sodium hydroxide used for AP and AM results in conversion from cellulose I to II at high concentrations [123, S.104], but not necessarily in disruption of the crystal lattice.

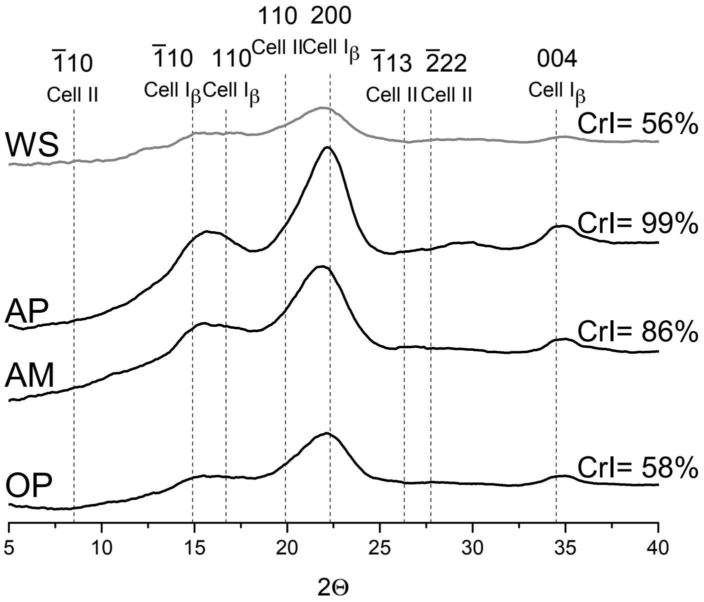


Figure 4.29.: X-ray diffractograms of wheat straw (WS), pulp obtained by conventional alkaline pulping (AP), microwave-assisted alkaline pulping (AM) and organosolv pulping (OP)

For instance, Kunze and Fink [129] describe the effect of an aqueous solution with 6% sodium hydroxide on cellulose as negligible. They further report that the lattice of cellulose I can be completely destroyed by addition of urea to sodium hydroxide. Thus, this is suggested as a pretreatment of wheat straw prior to enzymatic saccharification, as it may enhance accessibility and hence, rate of hydrolysis.

Scanning electron microscopy

Changes in fibre morphology by different pretreatments can be visualised using scanning electron microscopy (SEM) as shown in Figure 4.30. A magnitude of 350x was used for wheat straw and 3500x for pulp.

Defibration is evident for all three pulping processes. Comparatively long and straight fibres are obtained from AP of approximately 5 to 10 µm thickness. In addition, some larger parts of 50 to 100 µm thickness are present. Their surface is mostly smooth with some furrows along the fibre, probably due to drying effects. Pulp obtained from AM is extremely irregular containing parts as large as the original wheat straw stalk as well as fibres of approximately five µm thickness. In contrast to fibres from AP, fibre ends appear frayed. Compared to AP and AM, pulp from OP is more homogeneous, as larger parts are nearly absent. The fibre surface is rougher and scaled. This may be advantageous for enzymatic hydrolysis due to increased accessibility.

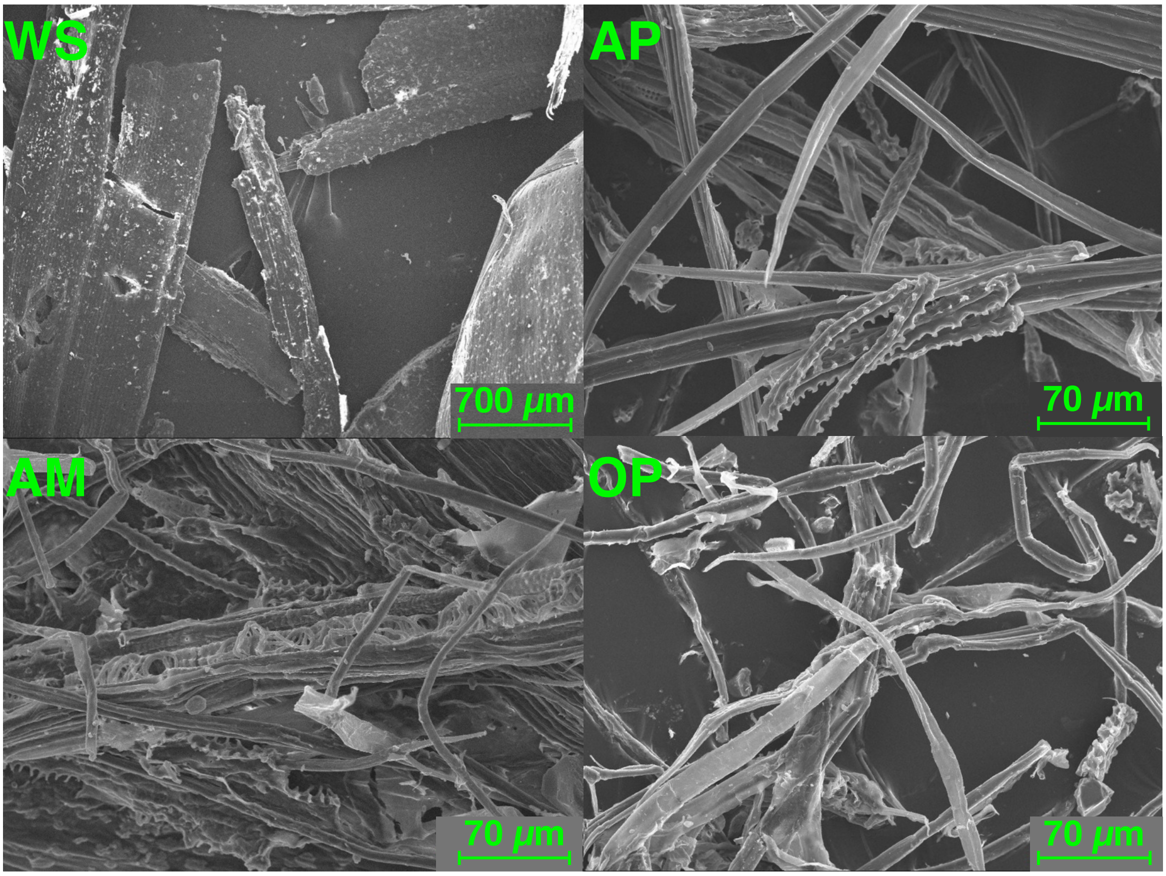


Figure 4.30.: Images obtained by scanning electron microscopy of wheat straw (WS), pulp obtained by conventional alkaline pulping (AP), microwave-assisted alkaline pulping (AM) and organosolv pulping (OP)

4.3.3. Precipitation product

The following section focusses on the characterisation of the lignin fraction with regard to the different pretreatment processes. This is done by means of FTIR spectroscopy, elementary analysis, thioacidolysis GC/MS, size-exclusion-chromatography as well as different wet-chemical methods. In addition, thermal desorption GC/MS and differential scanning calorimetry are used to provide information particular for applications based on thermal processing.

FTIR spectroscopy

FTIR spectra of the studied lignin preparations are shown in Figure 4.31. To simplify the discussion, Table 4.3 sums up the most important peaks and results.

The region between 2920 cm^{-1} and 2850 cm^{-1} is assigned to C-H stretching vibrations in methyl and methylene groups. As the spectra were normalised to the aromatic skeletal vibration at about 1510 cm^{-1}, the intensity of these bands represents the aliphatic contributions, including carbohydrates, fatty acids and aliphatic side chains in lignin, in relation to the aromatic part. As done for lignins from different raw materials in Chapter 3, the intensities of these peaks are combined as Aliphatic Index and given in Table 4.3.

Precipitation products obtained by AP and AM do not show considerable differences in this area. In contrast, the Aliphatic Index is approximately twice as high for the precipitation product obtained by OP. Carbohydrate impurities are unlikely to account for this effect, as the purity of precipitation product is higher when obtained by OP compared to both alkaline procedures.

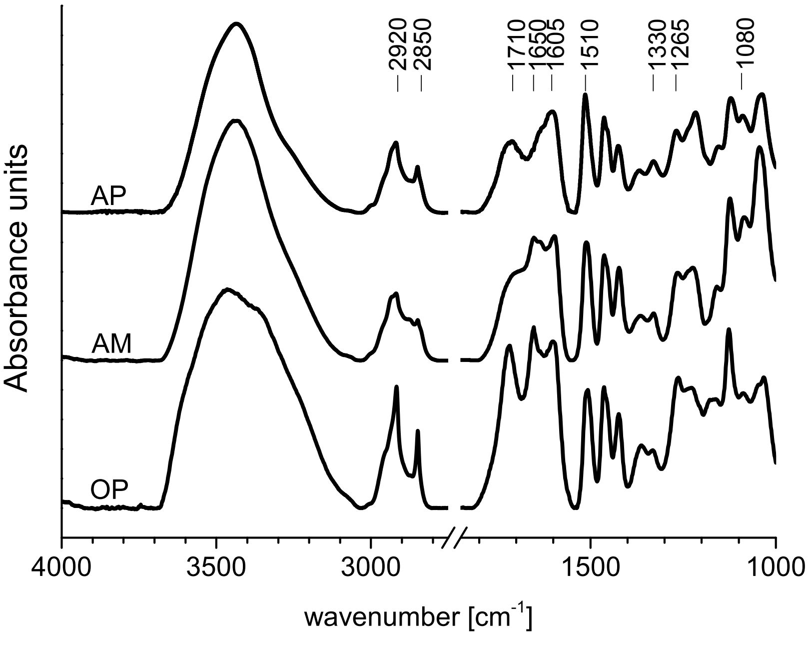


Figure 4.31.: FTIR spectra of lignin obtained by conventional alkaline (AP), microwave-assisted alkaline (AM) and organosolv pulping (OP)

Table 4.3.: Results from FTIR spectroscopy of dialysed precipitation products obtained from wheat straw by conventional alkaline (AP), microwave-assisted alkaline (AM) and organosolv pulping (OP)

Prec. prod.	Aliphatic Index $I_{(2920+2850)}$	Carbonylic Index $I_{(1710+1650)}$	S/G $I_{(1330:1265)}$	Condensation Index $\frac{\sum Min_{(1500-1050)}}{\sum Max_{(1600-1030)}}$
AP	1.3	1.3	0.8	0.6
AM	1.0	1.4	0.8	0.7
OP	2.3	2.1	0.8	0.7

A plausible explanation is the scission of carbon-carbon linkages between C_α and C_β or between C_β and aromatic nuclei in lignin due to retrograde aldol reactions during alkaline pulping [83]. Though only being a minor reaction pathway, this could be responsible for the differences between lignin obtained by AP and OP in this spectral region. In addition, precipitation product obtained by OP using a formic acid concentration of 60 wt%, as done for this section, has the highest content of aliphatic hydroxyl groups compared to other concentrations. Thus, displacement of sidechains or formation of Hibbert's ketones, which would both results in a decrease in the Aliphatic Index of OP, proceed only to a minor extend as discussed in detail in Section 4.2.

The Carbonylic Index (cf. Table 4.3) is also comparatively high for precipitation product from organosolv pulping as it shows bands with high intensities at 1710 cm^{-1} and at about 1650 cm^{-1}. As the band at 1710 cm^{-1} can be assigned to C=O originating from unconjugated ketones but also from ester groups, its strong intensity supports the process of esterification during formic acid pulping, as proposed by Ede and Brunow [56].

Furthermore, this region reveals small differences between AP and AM lignin, as the Carbonylic Index is slightly higher for the latter. According to the band at about 1650 cm^{-1}, the higher amount of carbonyl groups found wet-chemically in AM lignin (cf. Figure 4.33a) originates from C=O in conjugated p-substituted aryl ketones.

Condensation indices (CI) calculated by data obtained from FTIR spectra, are shown in Table 4.3. As stated in Chapter 3, the CI estimates the severity of isolation as condensation occurs parallel to fragmentation reactions during pulping [61]. The CIs of all three precipitation products are higher than 0.5, which are hence, highly condensated. This is plausible, giving the harsh reaction conditions used in this study compared to isolation as milled wood lignin (CI: 0.37 – 0.42) [61]. The condensation indices of the studied lignin preparations are furthermore similar being 0.6 for AP and 0.7 for AM as well as OP.

The S/G ratio, calculated by FTIR data, was 0.8 for all wheat straw lignin preparations, independent of the pulping method used. This is also supported by similar contents of methoxyl groups determined wet-chemically. Furthermore, Dalimova and Abduazimov [42] report a similar S/G ratio of 0.72 for milled wheat straw lignin. This implies that syringyl and guaiacyl units are dissolved at the same rates regardless of the pulping procedure, at least under the applied parameters. However, different results are obtained when determined by thioacidolysis, as will be discussed in the corresponding section.

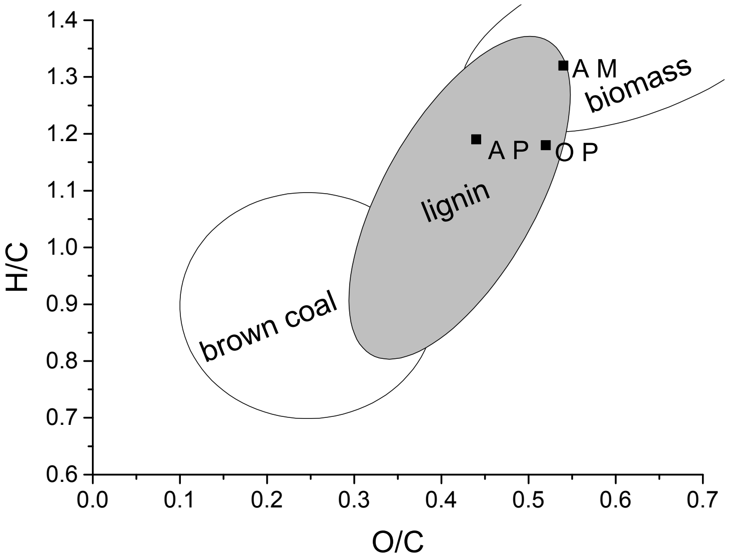
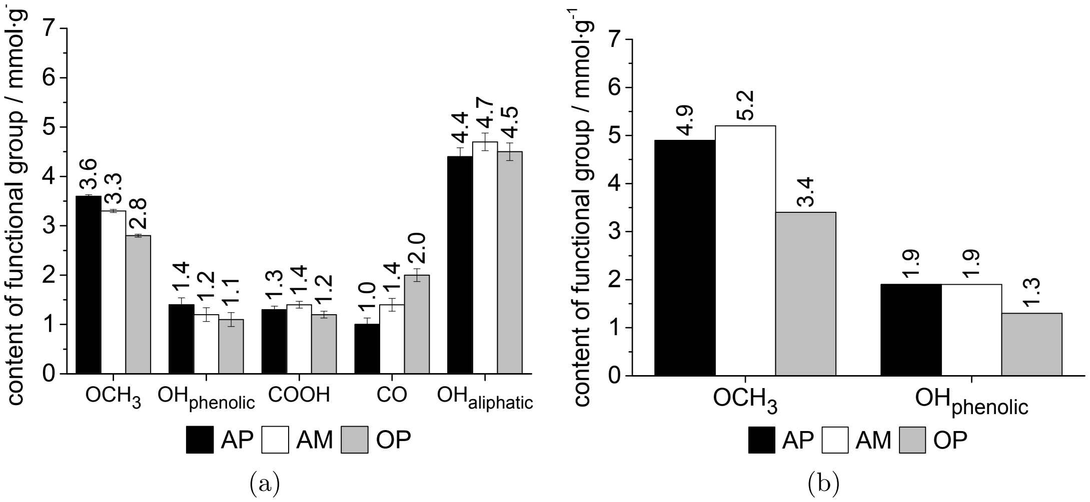


Figure 4.32.: Van-Krevelen diagramm on basis of elementary analysis of lignin obtained by conventional alkaline (AP), microwave-assisted alkaline (AM) and organosolv pulping (OP)

Elementary analysis

The elementary analysis revealed that lignin preparations obtained by AP and OP have H/C and O/C ratios, which are within a range typical for lignin (H/C ratio: 0.80 – 1.35 and O/C ratio: 0.30 – 0.55 [193]). However, the precipitation product obtained by AM is on the boundary between lignin and biomass for both H/C ratio (1.32) and O/C ratio (0.54). This reflects its high content of impurities, which could not be reduced by purification via dialysis.

As illustrated in Figure 4.32, the O/C ratio of precipitation product obtained by OP is also slightly higher, compared to AP. The high O/C-ratio of OP lignin can be caused by the reactive species OH^+ formed during peroxyformic acid pulping leading to ring hydroxylation as well as oxidative ring opening reactions [84]. Furthermore, it can be caused by lignin esterification [56]. Elementary analysis reveals similar H/C ratio for AP and OP lignin being 1.19 and 1.18, respectively. However, lignin obtained by AM has a significant higher H/C ratio of 1.32. Both, precipitation product obtained by AM and OP do not fit in the correlation found for H/C ratio and Aliphatic Index in Section 3.4.2 for precipitation products obtained by AP. Thus, their H/C ratio is not only influenced by their content of aliphatic contributions (carbohydrates, fatty acids, aliphatic side chains in lignin), but also by other factors such as the content of carbonyl groups, the degree of condensation or the S/G ratio. Therefore, the low degree of condensation determined by thioacidolysis could be responsible for the high H/C ratio found for the precipitation product from AM compared to AP.



(a) (b)

Figure 4.33.: Functional group content of (a) dialysed precipitation product and (b) dialysed precipitation product per KLASON-lignin, obtained by conventional alkaline (AP), microwave-assisted alkaline (AM) and organosolv pulping (OP)

Functional groups determined wet-chemically

The content of functional groups is not only influenced by the pulping procedure, but also strongly by pulping parameters, as discussed in Section 4.1 for conventional alkaline (AP) and in Section 4.2 for organosolv pulping (OP). Thus, it is noteworthy that this Section focusses on differences between conventional alkaline, microwave-assisted alkaline and organosolv pulping using standard parameters.

Figure 4.33 shows the content of functional groups in the three different preparations. When comparing AP and AM, similar contents of functional groups are observed for their precipitation products apart from the content of carbonyl groups, which is higher for AM compared to AP. High contents of carbonyl groups are reported to be typical for protolignins [241] and can be obtained by short pulping times when using alkaline pretreatments (cf. Section 4.1.2). This was the case for AM, where heating of the vessel occurred rapidly and pulping time was shortened to 10 minutes.

Differences in the content of methoxyl groups are due to different lignin contents of the precipitation products as revealed in Figure 4.33b. This is noteworthy, as pulp obtained by AM contained more residual lignin compared to AP. Experiments in Section 4.1.2 showed, that lignin with a low content of methoxyl groups is dissolved first during conventional alkaline pulping. If this would also apply to microwave-assisted alkaline pulping, the content of methoxyl groups would have been significantly lower. Thus, the topochemistry of delignification is different for AM as polar parts are selectively heated [102].

Differences of both alkaline pulping procedures to OP are basically the lower content of methoxyl groups as well as the higher content of carbonyl groups of the latter. This goes hand in hand with typical reaction pathways of lignin degradation during peroxyformic acid pulping, such as oxidative demethylation and oxidative ring opening reactions, which were thoroughly discussed in Sections 2.3.2 and 4.2.2.

Table 4.4.: Results from thioacidolysis-GC/MS of dialysed precipitation products obtained by conventional alkaline pulping (AP), microwave-assisted alkaline (AM) pulping and organosolv pulping (OP) based on their KLASON-lignin content

Prec. prod.	H [µmol·g^{-1}]	G [µmol·g^{-1}]	S [µmol·g^{-1}]	H/G/S	S/G	Yield [µmol·g^{-1}]	CD [%]
AP	7.4	226.2	154.9	2:58:40	0.7	389	91
AM	53.4	406.7	476.3	6:43:51	1.2	936	79
OP	28.7	168.3	103.4	10:56:34	0.6	300	93

Thioacidolysis-GC/MS

Thioacidolysis is used as chemical degradation method to provide information about lignin building units allowing the determination of type and amount of units involved in β-O-4 linkages [132].

Results from thioacidolysis of the three precipitation products are shown in Table 4.4. The hereby found S/G ratios differ from those found by FTIR spectroscopy, where all three samples have an S/G ratio of 0.8. If determined by thioacidolysis, precipitation product obtained by AM has a significant higher S/G ratio compared to AP and OP. Furthermore, precipitation product obtained by OP has a high content H- and low content of S-units. This is in accordance with its low content of methoxyl groups determined wet-chemically.

The yield of degradation products is more than twice as much for AM, suggesting higher amounts of uncondensed aryl-ether structures compared to AP and OP lignin. As microwave-assisted alkaline pulping was controlled by pressure instead of temperature, delignification might have remained in its initial stage ($\theta =$ approx. 140 °C) [82]. However, in the case of OH$^-$ as active species, the cleavage of β-aryl ether linkages is reserved to non-phenolic units in the bulk stage of delignification at higher temperature (cf. Chapter 4.1). Thus, when comparing with other pulping procedures, a higher proportion of β-aryl ethers remained uncleaved during microwave-assisted alkaline pulping, resulting in the higher yield of degradation products by thioacidolysis.

This assumption is also supported by the condensation index CD, which expresses the amount of lignin units participating in C-C as well as in diaryl ether linkages. As the CD of milled wood lignin from wheat straw is reported to be 82% [183], the additional cleavage of ether linkages is evident for conventional alkaline pulping at bulk stage as well as for organosolv pulping, particularly due to reactions involving HO$^+$, as both their CDs exceed 90%. This is different in the case of lignin obtained by microwave-assisted alkaline pulping, which has a similar CD compared to MWL, which is a mild isolation procedure for lignin resulting in a CD of 79%. Nevertheless, the effect of lignin isolation is evident in all cases as the CD of native wheat straw in relation to its lignin content is only 73% [132].

Table 4.5.: Results from SEC of dialysed precipitation products obtained from conventional alkaline (AP), microwave-assisted alkaline (AM) and organosolv pulping (OP)

Pre. prod.	M_n [g·mol^{-1}]	M_w [g·mol^{-1}]	M_w/M_n (PDI)
AP	1100	6700	6.3
AM	600	7600	12.4
OP	1200	7600	6.5

Size-exclusion-chromatography

The molecular weight of lignin is of particular interest for its application as it affects its solubility in solvents, miscibility in blends and the mechanical properties of products.

The molecular weights of the dialysed precipitation products relative to sodium-polystyrene sulfonates have been studied by size-exclusion-chromatography (SEC) and the results are presented in Table 4.5. Both precipitation products obtained by alkaline pulping (AP and AM) are within a typical range for wheat straw soda lignins as values are reported between 600 [145] and 1770 g·mol^{-1} [169]. Interestingly, the number-average molecular weight of AM is lower and its weight-average molecuar weight higher compared to AP, resulting in a high polydispersity index. By this, precipitation product obtained by microwave-assisted pulping is very heterogeneous containing high moieties of higher as well as much lower molecular weight as the one from AP. The high molecular weight fraction goes hand in hand with low lignin fragmentation as found by thioacidolysis GC/MS. Furthermore, the high PDI reflects the high content of impurities, as the lignin content of the precipitation product is only 64.0% and hence, 10.1% lower compared to AP. Sugar oligomers, for instance, could account for the low molecular weight fraction. In addition, the microwave-treatment proceeds very heterogeneous as shown by SEM (cf. Section 4.3.2). Thus, this may also result in non-uniform lignin fragmentation and dissolution. Both, the number- and weight-average molecular weight of OP is higher compared to AP. Since lignin degradation is mainly based on cleavage of ether-linkages, this is also reflected by their lower content of phenolic hydroxyl groups determined wet-chemically.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) is used to study the thermal behaviour of the lignin preparations. In order to evaluate a further valorisation including those involving thermal processing, their glass transition is of particular interest, being a temperature range where a polymer changes from a hard brittle condition into a viscous or rubbery one [109]. For details on its determination from DSC graph see Section 3.4.7.

Table 4.6 lists values for the glass transition temperature of the studied precipitation products determined as extrapolated onset temperature T_{ig} as well as midpoint temperature T_{mg}. Their difference, given as ΔT, provides information about the heterogeneity of the sample. Though no distinct relation could be found between ΔT and the polydispersity index, determined by SEC, lignin obtained by AM is interesting in this regard. Its glass transition is comparatively broad, indicating a high heterogeneity of its composition, which coincides with its high polydispersity index of 12.4.

Table 4.6.: Results from DSC of dialysed precipitation products obtained from conventional alkaline pulping (AP), microwave-assisted alkaline (AM) and organosolv pulping (OP): T_{ig}: extrapolated onset temperature, T_{mg}: midpoint temperature, ΔT: T_{mg} - T_{ig}

Prec. prod.	T_{ig} [°C]	T_{mg} [°C]	ΔT [°C]
AP	103.0	120.6	17.6
AM	97.2	143.7	46.5
OP	84.6	115.1	30.5

The lowest value for T_{mg} has the precipitation product obtained by organosolv pulping, which is hence proposed to be the favourable pulping procedure with regard to utilisation as composites.

Thermal desorption

The determination of volatile substances released from lignin by thermal desorption with subsequent GC/MS analysis permits the detection of compounds causing health hazards and/or being odour-intensive, which would make them unsuitable for utilisation in composites as discussed in Section 3.4.8.

Lignin samples were subjected to thermal desorption with subsequent qualitative GC/MS analysis. Table 4.3.3 summarises the peak intensities of substances and classifies them as: ● substance not detected, ● substance detected (peak height > 13.000 absorption units = limit of detection), ● considerable amounts present (peak height > 400.000 absorption units). This enables an easy and fast comparison between numerous samples. Further details e.g. retention time and gas chromatograms of selected lignins are listed in the Annex (Figure A.4 and Table A.1).

Numerous compounds released by Kraft lignin (IAT), including the odour-intensive guaiacol (6), are not, or only in traces, present in emissions from the studied wheat straw lignin preparations. In contrast other compounds, are formed by thermal desorption of lignin from herbaceous plants. For instance, furfural (5) and 2,3-dihydro-benzofurane (7) are released due to the presence of carbohydrate impurities. When comparing different pulping methods, it becomes clear, that lignin obtained by organosolv pulping (OP) emits the least substances. In addition, the compounds that are released differ from the alkaline preparations (AP, AM). This includes the emission of 4-hydroxy-2-methoxy-cinnamaldehyde (21) as well as acetic acid (1) and/or methyl formate (2). Precipitation products obtained by AP and AM release the same substances, though in varying amounts. An exception is coniferyl alcohol, which is only present in emissions from AM.

To conclude, organosolv pulping results in lignin, which emits fewer volatile compounds compared to those obtained by alkaline pulping methods, making it more suitable for applications involving thermal pretreatments.

Table 4.7.: Thermal desorption of dialysed precipitation products obtained from conventional alkaline pulping (AP), microwave-assisted alkaline (AM) and organosolv pulping (OP) with colour indicating peak intensity: 🔴 not detected, 🟡 detected, 🟢 considerable amounts present

Label	Substance	AP	AM	OP	IAT
1	Acetic acid	🔴	🔴	🟡	🔴
2	Methyl formate				
4	Dimethyl disulphide	🔴	🔴	🔴	🟡
5	Furfural	🟢	🟢	🟡	🔴
6	Guaiacol	🔴	🔴	🔴	🟢
7	Benzofurane, 2,3-dihydro-	🟢	🟢	🟢	🔴
8	Guaiacol, 4-vinyl-	🟢	🟢	🟢	🟢
9	Syringol	🟡	🟡	🟡	🔴
10	Vanillin	🟢	🟢	🟡	🟢
11	Isoeugenol	🟡	🟡	🟡	🟢
12	Guaiacol, 4-propyl-	🟡	🟡	🟡	🟢
13	Acetovanillone	🔴	🔴	🔴	🟢
14	Guaiacylacetone	🟡	🟡	🟡	🟢
16	Acetophenon, 3,5-dimethoxy-	🟡	🟡	🔴	🔴
17	Homovanillic acid	🔴	🔴	🔴	🟢
18	Syringaldehyde	🟢	🟡	🟡	🔴
19	Syringol, 4-propenyl-	🟢	🟢	🔴	🔴
20	Acetosyringone	🟢	🟢	🔴	🔴
21	Cinnamaldehyde, 4-hydroxy-2-methoxy-	🔴	🔴	🟢	🔴
22	Coniferyl alcohol	🔴	🟢	🔴	🔴
24	Homosyringaldehyde	🟢	🟡	🔴	🔴
25	Desaspidinol				

4.3.4. Lignin applications

In the following section, lignin obtained by the studied pulping procedures will be discussed with regard to selected application possibilities. More details on advantageous properties are presented in Section 3.5.

For production of **biocomposites**, lignin obtained by organosolv pulping is favourable, as it has the lowest glass transition temperature and highest purity facilitating thermal processing. Furthermore, it releases comparatively low contents of volatile organic compounds, which is particularly desirable for composites for indoor-use.

If lignin is to be used as **binder**, relevant requirements are high purity, high content of free phenolic hydroxyl groups, a low proportion of methoxyl groups and syringyl units as well as a low degree of condensation [90]. Lignin obtained by organosolv pulping is of high purity and contains less methoxyl groups compared to lignin obtained by alkaline pulping. However, the condensation degree determined by thioacidolysis is significantly lower for lignin obtained by microwave-assisted pulping, which additionally has a higher content of phenolic hydroxyl groups. Further optimisation of lignin separation and purification after this pulping process may hence, also result in a promising candidate for application as binder.

For application in **thermosetting resins**, phenolation of lignin has shown to be advantageous, which is enhanced by a low content of ash and a high number of aliphatic hydroxyl groups [177]. The ash content of all precipitation products could be eliminated by purification using dialysis. The content of aliphatic hydroxyl groups is similar for precipitation products obtained by AP, AM and OP. However, the precipitation products obtained by both alkaline procedures have a lower content of KLASON-lignin compared to OP and aliphatic hydroxyl groups are hence, partly caused by carbohydrate moieties. These in turn are not suitable for thermal applications. Thus, precipitation product obtained by OP is considered to be favourable, which additionally releases the least amount of VOCs.

For production of **carbon fibre**, lignin of high purity, high carbon content, but low content of methoxyl groups and β-O-aryl ether linkages is favourable [179]. Lignin obtained by organosolv pulping meets these requirements best out of the studied pulping procedures.

In contrast, lignin purity is not as crucial for production of lignin **hydrogels**, as carbohydrate impurities may also contribute to gel formation. However, a high content of hydroxyl has reported to be beneficial [219]. Thus, in this case, lignin obtained by conventional or microwave-assisted alkaline pulping is favourable over organosolv pulping.

For the **catalytic valorisation** of lignin, high purity is inevitable as proteins, inorganic salts and other potential poisons generally complicate catalysis [236]. Thus, precipitation product obtained by organosolv pulping is favourable. Additional advantageous properties of lignin regarding the performance of catalysts remain unclear, as most studies use model compounds instead of actual lignin streams [236].

To conclude, lignin application is strongly dependent on its composition, which in turn is determined by the pulping procedure. However, amendment of pulping parameters and lignin precipitation (two-step precipitation [133], reheating [1]) as well as the use of lignin fractionation will allow versatile applications.

5. Summary and links to future studies

Lignocellulosic biomass is an attractive raw material for bioethanol production, as it is abundant and does not compete with food production. However, lignocellulosic feedstock, even if limited to herbaceous plants, is very heterogeneous. Therefore, the relationship between raw material and the properties of the carbohydrate and lignin fraction, separated by a pretreatment step, is not entirely understood. This applies particularly to lignin due to its various types of units and linkages. However, as it is the only renewable source of aromatic compounds, its utilisation is of particular interest. In addition, its conversion into value-added products will considerably contribute to the feasibility of future biorefineries.

With biomass being a key factor to influence product properties, the first part of this study was aiming at:

A) Investigation of separability of various agricultural herbaceous biomasses by alkaline soda pulping to enlarge biomass feedstock

B) Elucidation of lignin composition with regard to the used raw material

- Revealing lignins with similar structural features, which are substitutable among one another
- Revealing lignins with unique structural features, which are promising for certain applications

A) Alkaline soda pulping was carried out using the following herbaceous biomasses: barley straw, coconut shell powder, hemp shives, horse manure, maize straw, miscanthus, oat husk, pretreated alfalfa, rape straw, sunflower stalks, tomato stalks and wheat straw. Suitability for lignocellulose separation by alkaline pulping was found to differ, strongly depending on the agricultural residue used. Best separation into a carbohydrate and lignin fraction was achieved for pretreated alfalfa, miscanthus, wheat and barley straw. In contrast, lignin content in pulp obtained from hemp shives, sunflower stalks and rape straw is higher compared to the raw material and hence, delignification via alkaline pulping unsuccessful. If the focus is only on pulp production, oat husks are an excellent raw material as pulp yield exceeds 50% and it consisted of 96% of holocellulose. If, on the other hand, the focus is on lignin production, coconut shell powder is favourable, as large amounts of lignin (37% of raw material) are separable with a purity of up to 86.5%.

B) FTIR spectroscopy was used to reveal lignins with similar structural characteristics. These could be further elucidated especially by elementary analysis and functional group analysis resulting in the following classification into groups, within which lignins are similar: (A) horse manure, rape straw, (B) sunflower stalks, tomato stalks, (C) barley-, maize- and wheat straw and (D) hemp shives, miscanthus. By this, a step towards standardisation of lignin properties is made.

By elucidating structural features of the lignins promising candidates were in addition successfully revealed for several applications, which are to be investigated in further studies.

Lignins of group A and B are generally of interest for applications implying thermal processing, as they have the lowest values for glass transition temperature and are separable in considerable purity.

Lignins obtained from sunflower stalks and from group D are promising for the application as binder as well as for derivatisation and cross-linking reactions (phenolation, hydrogel production), as they have high contents of phenolic hydroxyl groups and are separable in comparatively high purity. Lignins from group D have additionally a high content of aliphatic hydroxyl groups.

An enhanced knowledge on advantageous lignin properties is additionally needed for specific applications. This particularly applies to its catalytic valorisation. As a majority of the reported catalytic systems involve the use of pure lignin model compounds, next steps imply the use of actual lignin streams to obtain performance information with regard to lignins obtained from different biomasses and pretreatments. For the production of hydrogels, the Institute of Plant and Wood Chemistry (TU Dresden) will continue its research on the elucidation of structure-function relationships based on findings of this study.

Lignin from coconut shell powder is of particular interest due to its unique structural features. It distinguishes itself by its high purity, the complete absence of volatile compounds up to 250 °C, a high molecular weight and a lower glass transition temperature compared to conventional Indulin AT. By this, it is ideal for the application in biocomposites. Due to its low H/C ratio and its low content of methoxyl groups it is furthermore interesting for carbon fibre production. In addition, the high content of hydroxyl groups is favourable for cross-linking and derivatisation reactions. Further studies are needed by thermogravimetric analysis for determination of the decomposition temperature as well as by 2D NMR to elucidate the type of linkages in detail. The unique and promising properties revealed for lignin from coconut shell powder may justify its use, though delignification of the carbohydrate fraction was poor. Thus, there are three possible ways for its application and further studies have to proceed accordingly:

1. Use of its lignin fraction as by-product of ethanol production

   ⟶ Examination of enzymatic hydrolysis of its carbohydrate fraction: the high lignin content in the pulp may be tolerated if an enzyme complex obtained from *Penicillium verruculosum* is used

2. Use of its lignin fraction, but not as part of ethanol production

   ⟶ Test series to optimise extraction parameters: due to its low methoxyl content, a similar degree of delignification may be achievable using milder parameters and omitting the cost intensive pulping procedure used as pretreatment for ethanol production

3. Direct use of the raw material, as it already has a lignin content of 47.9%, omitting the complete pretreatment step and ethanol production

   ⟶ Investigation of the applicability as biocomposite

These results present another step towards understanding the influence of biomass on product properties, especially with regard to lignin, and hence towards enlarging the biomass feedstock for biorefineries.

Not only the raw material, but also the process used for lignocellulose separation significantly influences product yield and composition. Such a pretreatment is inevitable for subsequent ethanol production, as the carbohydrate moiety of lignocellulosic biomass is, compared to starch and sucrose, more difficult to access and convert. Thus, this recalcitrance of cellulose results in increased conversion costs. Extended research on pulping procedures and parameters and their influence on product properties will help to exploit saving potentials and to open the way to customised products.

This leads to the objectives of the second part of this study:

A) Investigation of influence of pulping parameters on biomass separability as well as on pulp and lignin composition based on wheat straw

B) Comparison of different pulping procedures with regard to wheat straw separability, pulp and lignin composition with particular focus on structural features of lignin

A) The parameters maximum temperature, time at maximum temperature and concentration of sodium hydroxide were varied for alkaline soda pulping. A pulping temperature of 120 °C combines high yields and purities of both pulp and precipitation product. However, higher rates of enzymatic hydrolysis may justify the application of elevated pulping temperatures. For the residence time at maximum temperature 30 minutes are optimal in terms of product yields. On the other hand, the purity of precipitation product and its content of phenolic hydroxyl is higher, if a time of 90 minutes was applied. The concentration of sodium hydroxide has the highest impact on yield and purity of the products compared to other pulping parameters. A sodium hydroxide concentration of 3 wt% is suggested for the alkaline separation of wheat straw. By this, nearly complete delignification of pulp as well as a maximum yield of precipitation product was achieved. In addition, the content of hydroxyl groups of the lignin fraction is strongly influenced, making the concentration of sodium hydroxide an important parameter for lignin customisation. Alkaline pulping was successfully transferred to pilot scale, as both pulp and lignin were obtained in similar yields and purities compared to lab scale. Lignin recovery was identified as bottle neck of the pretreatment process, as it is complicated due to its low particle size.

For organosolv pulping based on peroxyformic acid the time at maximum temperature and concentration of formic acid were varied. Short pulping times of up to 40 minutes are suggested, as its influence on both product yield and composition is negligible. If the focus is, however, on lignin for further derivatisation or cross-linking reactions, pulping time can be prolonged in order to enhance fragmentation and hence, the formation of phenolic hydroxyl groups. A formic acid concentration of 50 wt% or higher should be used in order to achieve best delignification results accompanied by high lignin yields. On the other hand, pulp yield decreases strongly if formic acid concentration is increased much further. Thus, a formic acid concentration of 50 to 60 wt% is recommended. Formic acid concentration was further found to strongly influence the content of methoxyl, carbonyl and aliphatic hydroxyl groups in the lignin fraction.

B) Conventional alkaline pulping, microwave-assisted alkaline pulping and organosolv pulping on basis of peroxyformic acid were compared with regard to wheat straw separability, pulp and lignin composition as well as structural features of lignin. Conventional alkaline pulping resulted in highest yield and purity of pulp as well as highest yield of lignin-rich precipitation product. The effect of microwave-assisted alkaline pulping was most evident by low intrinsic viscosity and hence, low degree of polymerisation, of the obtained pulp. It could be shown, that reduction of pulping time by using microwave-assisted pulping instead of conventional alkaline pulping is possible, though pulping parameters need further optimisation to improve pulp delignification and purity of lignin fraction. In addition, a study should be carried out on the topochemistry of delignification during microwave-assisted pulping, which may differ from conventional pulping as indicated by findings of this study.

The purity of lignin fraction was highest for organosolv pulping. The lignin fraction obtained by organosolv pulping further emitted fewer volatile compounds compared to those obtained by alkaline pulping methods, making it more suitable for applications involving thermal pretreatments. In addition, it contains the least amount of methoxyl groups and may be particularly suited for carbon fibre production.

The presented study provides a basis for a multiple species approach within a biorefinery framework. This is advantageous over using only one raw material as it reduces the dependency on seasonal and regional availability of biomass, lowers the amount of needed storage capacities and allows short transport distances. In addition, fast-growing undemanding plants, such as prairie cordgrass, should be taken into account besides agricultural residues, as such species can grow on marginal lands, which are not suited for crop production. Next, the influence of using mixtures of agricultural residues, which are harvested at similar times, on product properties should be studied in detail. A process needs to be selected as pretreatment, which is flexible towards different raw materials, delivers high quality products, is environmentally friendly, cheap, safe and allows decentralised production of bioethanol. Though findings of this study favour alkaline pulping for biomass separation and organosolv pulping with regard to most lignin applications, there are several other alternatives, for instance hydrothermal pretreatment. Joint efforts in research and politics are needed to make progress. Based on this, the biorefinery can deliver customised products from renewable resources to become an efficient, highly integrated system and meet the chemical and fuel requirements of the 21st century.

6. Experimental

This chapter lists the used materials and their supplier followed by an overview and description of methods.

6.1. Raw materials

Table 6.1 lists the used raw materials for pulping experiments. Table 6.2 lists all chemicals used in this study.

Table 6.1.: Used herbaceous biomass

Biomass	By-product from	Obtained from
Barley straw (ba)	grain production	Agrargenossenschaft Rossau e.G
Coconut shell powder (cp)	fibre production	University of Nong Lam after removal of coconut fibre
Hemp shives (hs)	fibre production	ATB Agrartechnik Bornim
Horse manure (hm)	livestock farming	local stable in Freital horses were fed on pastures
Maize straw (ms)	grain production	Agrargenossenschaft Rossau e.G
Miscanthus (mi)	none: energy crop	Sieverdingbeck in Velen-Ramsdorf
Oat husk (oh)	grain production	Peter Kölln KGaA
Pre-treated alfalfa (pa)	protein production	Joanneum Research Graz after protein extraction using water
Rape straw (rs)	seed production	Agrargenossenschaft Rossau e.G
Sunflower stalks (ss)	seed production	Agrargenossenschaft Rossau e.G
Tomato stalks (ts)	vegetable production	Israel
Wheat straw (ws)	grain production	Agrargenossenschaft Rossau e.G

Table 6.2.: List of chemicals

Chemical	Supplier
Acetic acid, 100%, p.a.	Carl Roth GmbH+Co KG
Acetic acid, potassium salt $\geq$99 %	Acros Organics
Acetic anhydride $\geq$99 %, for synthesis	Carl Roth GmbH+Co KG
Aniline, for synthesis	Merck KGaA
L-Arabinose, $\geq$99 %	Sigma-Aldrich Chemie GmbH
Barium chloride, p.a.	VEB Laborchemie Apolda
Benzoic acid, p.a.	inventory
Bromine	inventory
Cupriethylenediamine, 1 mol·L^{-1} (aq.)	VWR Chemicals
N,N-dimethylformamide $\geq$99.8 %, p.a.	Carl Roth GmbH+Co KG
Dimethyl sulfoxide $\geq$99.5 %, for synthesis	Carl Roth GmbH+Co KG
Ethanol $\geq$96 %, denatured	inventory
Ethanol $\geq$96 %	inventory
Formic acid $\geq$99 %	Fluka
D-Galactose $\geq$99 %	Sigma-Aldrich Chemie GmbH
D-Glucose $>$99.5 %	Sigma-Aldrich Chemie GmbH
p-Hydroxy-benzoic acid	Sigma-Aldrich Chemie GmbH
Hydrochloric acid 32 %, technical grade	Carl Roth GmbH+Co KG
Hydrochloric acid 32 %, p.a.	Carl Roth GmbH+Co KG
Hydrochloric acid 0.1 mol·L^{-1} $\pm$ 0.2%	Carl Roth GmbH+Co KG
Hydroiodic acid 57%, p.a.	Acros Organics
Hydroxylammonium chloride $\geq$99,5 %, p.a.	Carl Roth GmbH+Co KG
Isopropyl alcohol	inventory
D-Maltose, p.a.	inventory
D-Mannose, p.a.	inventory
Methyl red	inventory
Nitric acid, 65%, p.a.	Carl Roth GmbH+Co KG
Phenol $\geq$99.5 %, p.a.	Carl Roth GmbH+Co KG
Phenolphthalein	inventory
o-Phosphoric acid $\geq$85 %, p.a.	Carl Roth GmbH+Co KG
Phosphorous (red) (stabilised) for synthesis	Merck KGaA
Phosphorous pentoxide $\geq$99 %	Carl Roth GmbH+Co KG
Potassium bromide for spectroscopy	Merck KGaA
Potassium iodide $\geq$99.5 % p.a.	Carl Roth GmbH+Co KG
Pyridine $\geq$99 %, for synthesis	Carl Roth GmbH+Co KG
L-Rhamnose $\geq$99 %	Sigma-Aldrich Chemie GmbH
Sodium acetate $\cdot$ 3 H_2O $\geq$99 %	Carl Roth GmbH+Co KG
Sodium acetate $\geq$99 %, p.a.	Carl Roth GmbH+Co KG
Sodium chlorite $\geq$79 %	Carl Roth GmbH+Co KG
Sodium hydroxide $\geq$99 %, granulate	Carl Roth GmbH+Co KG
Sodium hydroxide 0.1 mol·L^{-1}	Carl Roth GmbH+Co KG
Sodium thiosulphate 0.1 mol·L^{-1}	Carl Roth GmbH+Co KG
Starch (soluble) p.a.	Merck KGaA
Sulphuric acid, $\geq$95 %	VWR Chemicals
tetra-*n*-butylammonium chloride, for synthesis	Merck KGaA
tetra-*n*-butylammonium hydroxide, 40% in methanol	Alfa Aesar
Toluene, 99 % p.a.	inventory
Triethanolamine $\geq$99 % p.a.	Merck KGaA
Trifluoroacetic acid, $\geq$99.8	Merck KGaA
Vanillin $\geq$99 % p.a.	Fluka
D-Xylose $\geq$99 %	Sigma-Aldrich Chemie GmbH

6.2. Overview of methods

Three different pretreatments (conventional alkaline pulping, microwave-assisted alkaline pulping and organosolv pulping) were used in order to obtain pulp and a precipitation product that is rich in lignin. Methods used for characterisation of these products are shown schematically in Figure 6.1.

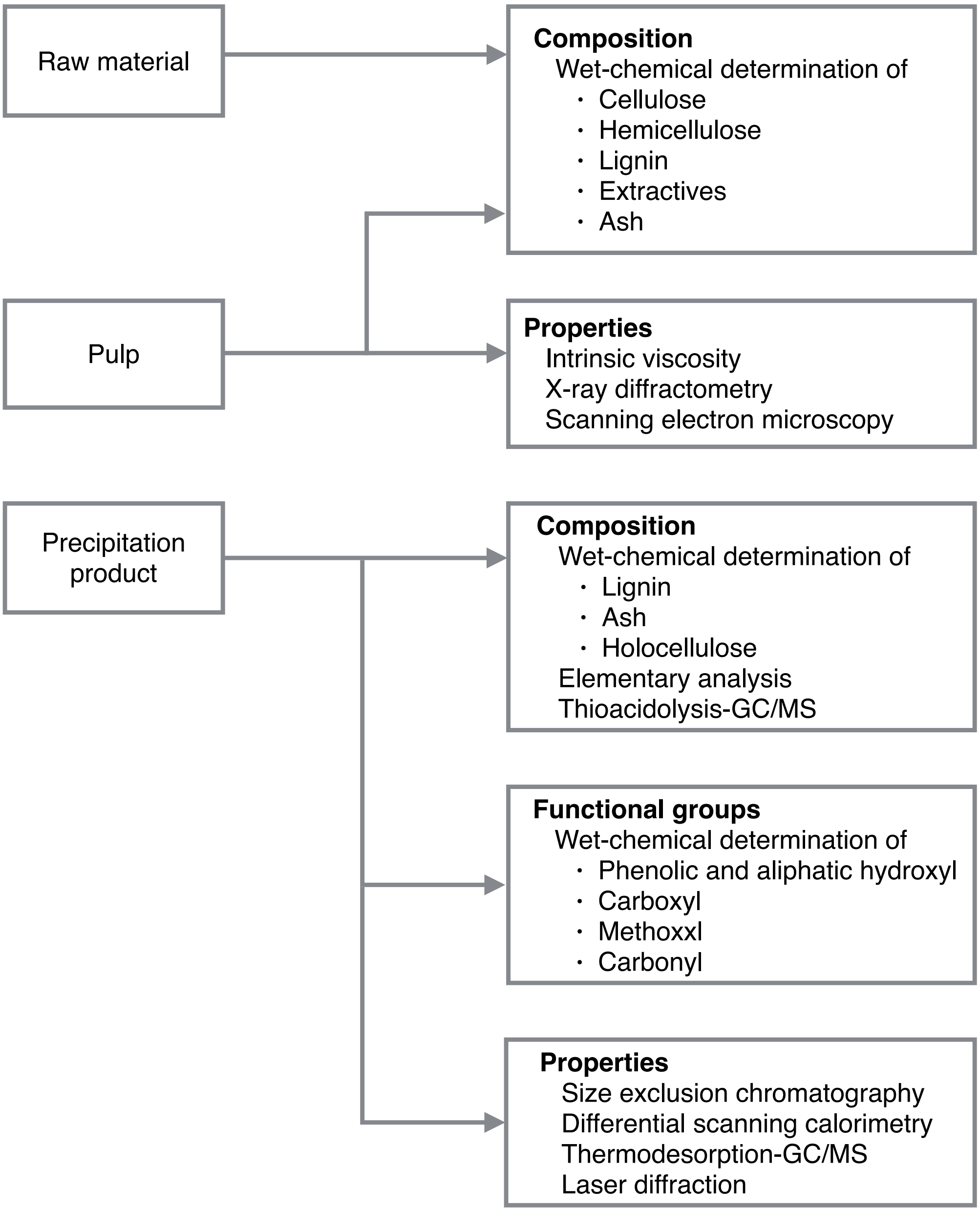


Figure 6.1.: Overview of methods

6.3. Physical/mechanical pretreatment

In order to study the effect of comminution on alkaline pulping, wheat straw was prepared in three different ways prior to pulping:

- chopped to a length of circa four to ten centimetre using a shredder (Hege)
- milled to pass a 4 mm mesh using a laboratory mill (Retsch SM100)
- as thermo-mechanical pulp using a 12-zoll-refiner (Andritz) and a disk gap of 0.15 mm without preliminary steaming leading to a fibre length of 0.1 to 2.0 mm, was carried out at and by staff of Technische Universität Dresden (Chair for Wood and Fibre Materials Technology)

6.4. Pulping procedures

6.4.1. Conventional alkaline pulping

Alkaline pulping was performed as described by Rossberg et al. [185] and Gläser [86] using an aqueous solution of sodium hydroxide. Experiments were partly carried out by B. Gläser.

Alkaline pulping was carried out using an autoclave (unstirred, wall and floor heating) of 2-L capacity. The parameter used were as follows: liquid/solid ratio = 6.2 mL·g^{-1}, ω(NaOH) = 1, **3**, 6 or 9 wt%, θ_{max} = 100, 120, 140 or **160** °C, time at maximum temperature = 0, 30, 90 or **180** min, H^{32}-factor = 3, 30, 100, 230, 320, 900 or **1270**. For alkaline pulping of different raw materials in Chapter 3 and for comparison of pulping methods in Section 4.3 the following parameters were used: liquid/solid ratio = 6.2 mL/g, ω(NaOH) = 3 wt%, θ = 160 °C, time at maximum temperature = 30 min, H^{32}-factor = 320.

Pulp was separated from the black liquor using a Büchner funnel, washed twice with an aqueous solution of NaOH (0.1 mol·L^{-1}) (washing fraction one and two) and afterwards with deionised water (washing fraction three). The black liquor and washing fraction one and two were combined. The lignin fraction was precipitated at pH = **1** or 3 using **HCl (ca. 6 mol·L^{-1})** or H_2SO_4 (ca. 3 mol·L^{-1}). It was left for a residence time of approximately 18 hours at room temperature. Afterwards, it was separated by centrifugation (4100 g, 10 min), followed by washing with **HCl (0.1 mol·L^{-1})** or H_2SO_4 (0.05 mol·L^{-1}) and drying at room temperature (lignin I). For further purification, the precipitation product was dialysed using Spectra/Por®3 membranes (regenerated cellulose, molecular weight cut off: 3500) until the conductivity was below 10 µS·cm^{-1} and lyophilised (lignin II). If not specified further, the standard procedure (printed bold) was used.

6.4.2. Microwave-assisted alkaline pulping

Microwave-assisted alkaline pulping was performed as described by Rossberg et al. [185], Gläser [86] and Gähler [79] using an aqueous solution of sodium hydroxide. Experiments were carried out by B. Gläser and K. Gähler.

The pulping procedure was carried out using a microwave system (type: MDS 2000 from CEM) with following parameters: liquid/solid ratio: $6.2\,\mathrm{mL{\cdot}g^{-1}}$, $\omega(\mathrm{NaOH}) = 3\,\mathrm{wt\%}$, power = 609 Watt, maximum gauge pressure: 6.2 bar, time at maximum pressure = 10 min (determination as described by instruction manual). Pulp and lignin were prepared as described in section 6.4.1.

6.4.3. Organosolv pulping

Organosolv pulping was performed using formic acid and hydrogen peroxide following the procedure described by Siegle [196]. Experiments were carried out by M. Hörnicke [100] at the former Sächsisches Institut für Angewandte Biotechnologie e.V. (now Universität Leipzig).

The pretreatment was performed in a 3-L round bottom flask with reflux condenser with constant stirring using an anchor stirrer (90 rpm) at the following parameters: liquid/solid ratio = $27\,\mathrm{mL{\cdot}g^{-1}}$, addition of 46 mL of hydrogen peroxide solution (30 %, w/v), addition of HCOOH (98 wt%) and water to adjust to $\omega(\mathrm{HCOOH}) = 30$, 50, **60** or 80 wt%, $\theta_{max} = 103\,°\mathrm{C}$, time at maximum temperature = 40, **60** or 90 min. Pulp was separated using a Büchner funnel, washed with deionised water and dried at 105 °C. Lignin was precipitated in water in a ratio of 1:5 (pulping liquor:water, v/v). Thereafter, the suspension was centrifuged directly for 10 minutes with 11 000 g and lyophilised. For further purification, the precipitation product was dialysed as described in Section 6.4.1. If not specified further, the standard procedure (printed bold) was used.

6.5. Characterisation of raw material

Dry weight and ash

Dry weight and ash were determined as described by Matissek [149].

To determine the dry weight of the sample, it was weighed into a dry crucible and dried at 105 °C until constant mass is reached. The dry weight was calculated as shown in Equation (6.1).

$$\text{dry weight}\,(\%) = \frac{m_2 - m_1}{m_3 - m_1} \cdot 100\% \tag{6.1}$$

m_1	Weight of dry crucible [g]
m_2	Weight of sample in dry crucible before 105 °C [g]
m_3	Weight of sample in dry crucible after 105 °C [g]

The dried samples were further used to determine ash content. Before incineration, the samples were carefully heated above the flame of a Bunsen burner. This is necessary, as uncontrolled burning of the sample could lead to loss of ash. Afterwards, the crucible was placed into a furnace at 525 °C for approximately five hours. The crucible was then placed into a desiccator and allowed to cool down. Black particles were crushed using a glass rod, which was rinsed with deionised water afterwards to quantitatively rinse all particles in the crucible. The sample was dried at 105 °C and then placed into the furnace at 525 °C again until constant mass was reached. The content of ash was calculated as shown in Equation (6.2).

$$\mathrm{ash}\,(\%) = \frac{m_2 - m_1}{m_3 - m_1} \cdot 100\% \tag{6.2}$$

m_1	Weight of dry crucible [g]
m_2	Weight of sample (dry) in dry crucible before 525 °C [g]
m_3	Weight of sample (dry) in dry crucible after 525 °C [g]

Extractives

The content of extractives was determined as ethanol-toluene extractives as described by Bremer et al. [30].

A portion of 5 g of sample was weighed into an extraction thimble, which was placed in a Soxhlet apparatus. 200 mL of a mixture of ethanol and toluene (1:1, v/v) was placed into a 500 mL round bottom flask, which was connected to the Soxhlet apparatus. The mixture was boiled for six hours. Afterwards, it was partly poured into a dry weighed 100 mL round bottom flask and evaporated using a rotary evaporator. This procedure was repeated until all of the solvent was evaporated. The extractive-containing round bottom flask was dried at 40 °C until constant mass was reached. The content of extractives was calculated as shown in Equation (6.3). The extractive-free material was used for further analysis (content of cellulose, holocellulose and lignin).

$$\mathrm{extractives}\,(\%) = \frac{m_2 - m_1}{m_3} \cdot 100\% \tag{6.3}$$

m_1	Weight of dry round bottom flask [g]
m_2	Weight of extractives and round bottom flask after 40 °C [g]
m_3	Weight of sample (dry) [g]

Cellulose

Determination of cellulose content was carried out based on the procedure developed by Kürschner and Hoffer [130] and as described in detail by Bremer et al. [30].

A portion of 1 g of the extractive-free sample was weighed into an Erlenmeyer flask. 25 mL of a mixture of nitric acid in ethanol (1:4, v/v) was added followed by one-hour boiling under reflux. The solid was separated from the solution using a dry weighed glass filter (40 µm). This procedure was repeated twice. Afterwards the solid was again separated and washed with ethanol and hot deionised water. The sample was boiled again using 100 mL of deionised water for 30 min. The solid was again separated using the glass filter and washed with deionised water. The glass filter was then dried at 105 °C. The content of cellulose was calculated as shown in Equation (6.4).

$$\text{cellulose}\,(\%) = \frac{m_2 - m_1}{m_3 \cdot (100\% - E)/100\%} \cdot 100\% \tag{6.4}$$

m_1	Weight of dry glass filter [g]
m_2	Weight of cellulose in glass filter after 105 °C [g]
m_3	Weight of sample (dry, ethanol-toluene extracted) [g]
E	Extractives as calculated by Eq. 6.3 [%]

Furthermore, ash correction of the initial and resulting weights is recommended for samples with high ash contents.

Holocellulose

The content of holocellulose was determined based on Wise [226] and described in detail by Bremer et al. [30].

A portion of 0.3 g of extractive-free sample was dispersed in 45 mL water, followed by addition of 60 µL glacial acetic acid and 0.3 g sodium chlorite. The mixture was kept at 75 °C in a shaking water bath for five hours. Glacial acetic acid and sodium chlorite were added after every hour. Afterwards, the holocellulose was separated by filtration using a dry weighed glass filter (40 µm), washed with deionised water and dried at 105 °C. The content of holocellulose was calculated as shown in Equation 6.5.

$$\text{holocellulose}\,[\%] = \frac{m_2 - m_1}{m_3 \cdot (100\% - E)/100\%} \cdot 100\% \tag{6.5}$$

m_1	Weight of dry glass filter [g]
m_2	Weight of holocellulose in glass filter after 105 °C [g]
m_3	Weight of sample (dry, ethanol-toluene extracted) [g]
E	Extractives as calculated by Eq. (6.3) [%]

Furthermore, ash correction of the initial and resulting weights is recommended for samples with high ash contents.

Hemicellulose

The content of hemicellulose was calculated by subtracting the content of cellulose from the content of holocellulose as shown in Equation 6.6.

$$\text{hemicellulose}\,[\%] = \text{holocellulose} - \text{cellulose} \tag{6.6}$$

holocellulose	Content of holocellulose as calculated by Eq. 6.5 [%]
cellulose	Content of cellulose as calculated by Eq. 6.4 [%]

Lignin

Lignin content was determined as acid-insoluble (KLASON) lignin in accordance with TAPPI method T222 om-83 [207]. Acid-soluble lignin was not determined for the reasons stated by Hatfield and Fukushima [93].

A portion of 0.5 g of the extracted sample was mixed with 15 mL of 72% sulphuric acid. This mixture was left at room temperature for 2 h with occasional stirring and macerating the material using a glass rod. Afterwards, the mixture was transferred to a 1-L round bottom flask containing 300 mL of deionised water. Additional water is used to rinse the beaker and to adjust the total volume of the solution to 575 mL. This solution was boiled under reflux for 4 h. After cooling down to room temperature the insoluble lignin was separated by filtration using a glass filter of fine porosity (16 µm). The lignin was washed with deionised water and dried at 105 °C. The content of lignin was calculated as shown in Equation 6.7.

$$\text{lignin}\,(\%) = \frac{m_2 - m_1}{m_3 \cdot (100\% - E)/100\%} \cdot 100\% \tag{6.7}$$

m_1	Weight of dry glass filter [g]
m_2	Weight of lignin in glass filter after 105 °C [g]
m_3	Weight of sample (dry, ethanol-toluene extracted) [g]
E	Extractives as calculated by Eq. 6.3 [%]

Furthermore, ash correction of the initial and resulting weights is recommended for samples with high ash contents.

6.6. Characterisation of pulp

6.6.1. Chemical composition

The composition of pulp samples was determined analogous to the composition of raw material (see Section 6.5).

6.6.2. Properties

Intrinsic viscosity

The intrinsic viscosity was determined according to ISO 5351/1.

A portion of about 100 mg of pulp was weighed into a dissolving flask with rubber gasket. The quantity of sample used was dependent on the expected limiting viscosity number and could vary approximately 10 mg. Afterwards, 25 mL of deionised water was added, followed by 25 mL of cupri-ethylene-diamine solution (1 mol·L^{-1} Cu and 2 mol·L^{-1} of ethylene-diamine) and 10 pieces of copper. The flask was placed in a shaking device for two hours and in a water bath (25 °C) for at least 30 minutes. For measuring the intrinsic viscosity an Ubbelohde viscometer was used. The intrinsic viscosity was calculated based on Martin's formula shown in Equation 6.8.

$$lg[\eta] = lg\frac{\eta - \eta_0}{\eta_0 \cdot c} - k[\eta] \cdot c \tag{6.8}$$

$[\eta]$	intrinsic viscosity [mL/g]
$\frac{\eta - \eta_0}{\eta_0 \cdot c}$	viscosity number [mL·g^{-1}]
k	empirical constant (for cellulose in CED = 0.13)
c	concentration of cellulose in solvent [g·mL^{-1}]

The relationship between intrinsic viscosity η and DP_v has been formulated according to Kes and Christensen [114] as shown in Formula 6.9.

$$\eta = 2.45 \cdot DP^{0.70} \tag{6.9}$$

X-ray diffraction

X-ray diffraction was carried out at and by staff of Technische Universität Dresden (Chair for Inorganic Chemistry II) as described by Rossberg et al. [185].

Measurements were carried out in transmission mode by a STOE Stadi P system using Cu-radiation (λ=1.54 Angstroms), Ge (111)-monochromator and an Image Plate Position Sensitive Detector (IP-PSD) in Debye-Scherrer geometry. The data was collected for one hour at a resolution of 0.01° 2Θ, from 3 to 50° 2Θ. Crystallinity was determined by peak deconvolution as described by Park et al. [172]. Eight Gaussian crystalline peaks were separated at 12.2°, 14.9°, 16.7°, 19.9°, 22.9°, 28.8°, 30.1° and 34.5° 2Θ and one broad at

21.5° 2Θ accounting for the amorphous contribution using the software Origin. CrI is calculated from the ratio of the area of all crystalline peaks to the total area.

Scanning electron microscopy

Scanning electron microscopy was carried out as described by Rossberg et al. [185] and was performed by Ernst Bäucker at the Institute of Forest Utilization and Forest Technology (Technische Universität Dresden) using a Jeol JSM-T330 A, operated at 15 kV.

6.7. Characterisation of precipitation product

6.7.1. Chemical composition

Dry weight and the content of ash and KLASON-lignin in the precipitation product were analysed as described in Section 6.5, without removing extractives prior to analysis.

Holocellulose

For determination of holocellulose in the precipitation product, the sample was delignified using the procedure described in Section 6.5. Afterwards, the obtained holocellulose was hydrolysed using the mild hydrolysis technique described by Fengel et al. [73], which aims at preserving xylose.

In contrast to plant or pulp samples, the delignification treatment needed to be repeated for three times until complete delignification was achieved. Delignification was proved by FT Raman spectroscopy due to absence of the band at 1605 cm^{-1}. For hydrolysis of holocellulose, a portion of 20 mg holocellulose was weighed into a round bottom flask followed by addition of 3 mL of trifluoroacetic acid (TFAA) (100 %). After swelling overnight, 1.108 mL deionised water was added. The mixture was heated under reflux at 105 °C for 35 min. Afterwards, 9.233 mL of deionised water was added and the mixture was again heated for two hours. The sample was washed with deionised water for 20 times until neutral, followed by complete evaporation of the water. The content of xylose, glucose, arabinose and galactose in the hydrolysed holocellulose sample was determined by HPLC-MS using a ReproGelPb-column and water with a flow of 0.6 mL·min^{-1} as eluent.

6.7.2. FTIR spectroscopy

FTIR spectroscopy was carried out at Technische Universität Dresden (Chair for Bioanalytical Chemistry).

FTIR spectra were obtained on a Nicolet 210 using KBr pellet technique. Spectra were measured between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1} with 100 scans and normalised to 1510 cm^{-1}.

Elementary analysis

Elementary analysis was carried out at and by staff of Technische Universität Dresden (Chair for Physical Organic Chemistry) on a Hekatech EA 3000 Euro Vector CHNSO.

6.7.3. Functional groups

Phenolic hydroxyl and carboxyl groups

The method described is an adapted method according to Dence [48]. It is only suitable for lignin without acidic groups having a lower pKs value as RCOOH, such as RSO_3H. In such a case the original method with addition of HCl has to be used.

1. Preparation of the titration device
A titration device (Titroline α by Schott) with a Pt 6280 electrode by Schott was used. Tetra-*n*-butylammonium chloride ($1\,mol\cdot L^{-1}$) was used as electrolyte for the electrode. For preparation of the standard solution a portion of 62.5 g of tetra-*n*-butylammonium hydroxide (TnBAH) was weighed into a 3-L chemical flask, followed by addition of 2 L of isopropyl alcohol.

2. Concentration of TnBAH standard solution
A portion of 0.15 g benzoic acid was weighed into a titration vessel, followed by addition of 30 mL of dimethylformamide. The mixture was stirred for 15 to 30 min before titration. The concentration of the TnBAH-solution is calculated as shown in Equation 6.10

3. Sample preparation
A portion of 0.35 g of lignin was weighed into a titration vessel, followed by addition of 0.07 g of 4-hydroxybenzoic acid and 30 mL of dimethylformamide. The mixture was stirred for 15 to 30 min before titration. A blank is run on a solution containing 0.07 g of 4-hydroxybenzoic acid dissolved in 30 mL of dimethylformamide. The content of carboxyl and phenolic hydroxyl is calculated as shown in Equation 6.12 and 6.13, respectively.

4. Calculation
Concentration of TnBAH-solution in $mol\cdot L^{-1}$

$$c(TnBAH) = \frac{m}{V \cdot 0.12212} \tag{6.10}$$

m	Weight of benzoic acid [g]
V	Volume of TnBAH [mL]
0.12212	Molar mass of benzoic acid [$g\cdot mol^{-1}$]/1000

Theoretical consumption a, assignable to the internal standard (4-hydroxybenzoic acid) in mL

$$a = \frac{m}{0.13812 \cdot c(TnBAH)} \tag{6.11}$$

m	Weight of 4-hydroxybenzoic acid [g]
0.13812	Molar mass of 4-hydroxybenzoic acid [g·mol^{-1}]/1000
$c(TnBAH)$	Concentration of TnBAH-solution as calculated in Eq. 6.10 [mol·L^{-1}]

Calculation of carboxyl groups in mmol·g^{-1}

$$COOH = \frac{(V_x - a) \cdot c(TnBAH)}{m} \tag{6.12}$$

V_x	Titration volume up to the first inflection point [mL]
a	Theoretical consumption as calculated in Eq. 6.11 [mL]
$c(TnBAH)$	Concentration of TnBAH-solution as calculated in Eq. 6.10 [mol·L^{-1}]
m	Weight of lignin sample [g]

Calculation of phenolic hydroxyl groups in mmol·g^{-1}

$$OH_{phenolic} = \frac{(V_y - V_x - a) \cdot c(TnBAH)}{m} \tag{6.13}$$

V_y	Titration volume up to the second inflection point [mL]
V_x	Titration volume up to the first inflection point [mL]
a	Theoretical consumption as calculated in Eq. 6.11 [mL]
$c(TnBAH)$	Concentration of TnBAH-solution as calculated in Eq. 6.10 [mol·L^{-1}]
m	Weight of lignin sample [g]

Total hydroxyl groups

The total content of hydroxyl groups (aliphatic and phenolic) was determined after acetylation of lignin using a mixture of acetic anhydride and pyridine according to an in-house method.

$$\mathrm{R{-}OH + (CH_3CO)_2O \xrightarrow{\textit{pyridine}} R{-}OCOCH_3 + CH_3COOH}$$

1. Acetylation of lignin
A portion of 500 mg of lignin, which was dried over phosphorous pentoxide, was placed into an Erlenmeyer flask with ground joint. 10 mL of pyridine (purified by distillation) was added, followed by 10 mL of acetic anhydride (purified by distillation). Air in the flask was expelled by nitrogen and the flask was sealed with a stopper. Afterwards, the flasks was placed in a shaking water bath at a temperature of 40 °C for about eight hours. The lignin was precipitated by pouring the acetylation mixture into 100 mL of HCl (0.1 M). It was then separated by filtration using a glass filter of fine porosity (16 μm), washed with HCl (0.1 M) in order to remove excess pyridine and finally with deionised water. The sample was then dried over phosphorous pentoxide.

2. Determination of total hydroxyl groups
A portion of 60 mg of acetylated and dried lignin was weighed into a 50 mL two necked flask, a magnetic stir bar was added, followed by 4 mL of H_2SO_4 (50% (v/v)). The flask was connected to a reflux condenser and the second neck of the flask was closed with a stopper. All joints were sealed with joint grease, which was tested as a blank value to have no influence on the subsequent distillate. The mixture was heated to its boiling point using an oil bath at 120 °C and boiled for one hour with stirring. Afterwards, it was lifted out of the oil bath and circa 10 mL of deionised water was added through the reflux condenser. The mixture was then again boiled for further 10 min, lifted out of the oil bath and left to cool down for 10 min. After having rinsed the reflux condenser with deionised water, the flask was connected to a short path distillation apparatus with a thermometer and a 100 mL round bottom flask as receiver flask. The stopper of the second neck of the flask was rinsed carefully and a dropping funnel without pressure compensator was connected instead. The mixture was heated and deionised water was added through the dropping funnel every time the temperature dropped below 100 °C. The distillation was stopped, when circa 70 mL of distillate was received. The distillate was then quantitatively transferred into a wide neck Erlenmeyer flask and 3-5 drops of phenolphthalein were added before titration with NaOH (0.05 M). Addition of barium chloride is useful as a test for possible contamination with H_2SO_4. If barium sulphate is precipitated, the distillate has to be discarded as it contains H_2SO_4. A blank is performed with unacetylated lignin.

3. Calculation
The total hydroxyl content is calculated as shown in Equation 6.14.

$$\mathrm{OH_{total}}\ [\mathrm{mmol{\cdot}g^{-1}}] = \frac{c \cdot V \cdot \tau}{m - (42.0 \cdot c \cdot V \cdot \tau)} \cdot 1000 \tag{6.14}$$

c	Concentration of HCl [mol·L^{-1}]
V	Volume of HCl required [mL]
τ	Factor of HCl
m	Weight of the sample [g]
42.0	Molar mass of -CO-CH_3 subtracted by molar mass of H [g·mol^{-1}]
1000	Unit conversion mol to mmol

Aliphatic hydroxyl groups

The content of aliphatic hydroxyl was calculated after determination of total and phenolic hydroxyl groups as shown in Equation 6.15.

$$\mathrm{OH_{\ aliphatic}}\ [\mathrm{mmol{\cdot}g^{-1}}] = \mathrm{OH_{\ total}} - \mathrm{OH_{phenolic}} \tag{6.15}$$

$\mathrm{OH_{\ total}}$	Content of total hydroxyl groups as calculated in Eq. 6.14 [mmol·g^{-1}]
$\mathrm{OH_{\ phenolic}}$	Content of phenolic hydroxyl groups as calculated in Eq. 6.13 [mmol·g^{-1}]

Methoxyl groups

The determination of methoxyl groups was based on the procedure proposed by Vieböck and Schwappach [213], described in detail by Zakis [235] and was partly carried out by A. Rudolf. Reactions proceed according the following scheme:

$$\begin{aligned}
\mathrm{R{-}OCH_3} + \mathrm{HI} &\longrightarrow \mathrm{R{-}OH} + \mathrm{CH_3I}\\
\mathrm{CH_3I} + \mathrm{Br_2} &\longrightarrow \mathrm{CH_3Br} + \mathrm{IBr}\\
\mathrm{IBr} + 2\,\mathrm{Br_2} + 3\,\mathrm{H_2O} &\longrightarrow \mathrm{HIO_3} + 5\,\mathrm{HBr}\\
\mathrm{HIO_3} + 5\,\mathrm{HI} &\longrightarrow 3\,\mathrm{I_2} + 3\,\mathrm{H_2O}\\
3\,\mathrm{I_2} + 6\,\mathrm{Na_2S_2O_2} &\longrightarrow 6\,\mathrm{NaI} + 3\,\mathrm{Na_2S_4O_6}
\end{aligned}$$

After reaction of lignin with hydriodic acid, methyl iodide is released and quantitatively transported from the reaction medium into the absorber using CO_2. After reaction with bromine, the released iodine is determined by titration with sodium thiosulphate solution. As reference material vanillin was used.

1. Preparation of the Vieböck Schwappach apparatus

An apparatus as shown by Vieböck and Schwappach [213] was used. The scrubber was filled with a suspension of red phosphorus in water (1%). The trapping unit was filled with 15 mL of a potassium acetate solution (20 g in 180 mL glacial acetic acid) and 0.225 mL of bromine. The solution was distributed evenly among the two parts of the trapping unit. A test tube containing a solution of sodium acetate in formic acid was connected to the end of the trapping unit.

2. Sample preparation

A portion of 20-25 mg of dry lignin was weighed into a reaction flask, followed by addition of 0.2 g red phosphorus, 0.5 g phenol, 6 drops of acetic anhydride and 5 mL of hydroiodic acid. The reaction flask was then connected to the Vieböck Schwappach apparatus.

3. Procedure

Carbon dioxide gas was passed slowly through the gas inlet into the reaction flask with a speed of approximately one bubble every two seconds. The oil bath was heated to 140 °C and the mixture was boiled for one hour (time taken from boiling point). Afterwards, the trapping tube was disconnected and its content was poured into an Erlenmeyer flask containing 1.5 g of sodium acetate dissolved in little deionised water. The trapping tube was washed several times with approximately 150 mL of deionised water. Formic acid was added dropwise until discolouration of the solution was achieved. After 5 min, 3 drops of methyl red were added, followed by 10 mL of a solution of potassium iodide (10 g in 100 mL deionised water) and 5 mL of sulphuric acid (diluted with deionised water 1:1, v/v).

4. Titration

The solution was titrated with a standard solution of sodium thiosulphate ($0.1\,\mathrm{mol{\cdot}L^{-1}}$) using a starch solution (1%) as indicator.

5. Calculation

The methoxyl content is calculated as shown in Equation 6.16.

$$\mathrm{OCH_3}\ [\mathrm{mmol{\cdot}g^{-1}}] = \frac{c \cdot {}^{V}\!/_{6} \cdot \tau}{m} \tag{6.16}$$

c	Concentration of $Na_2S_2O_3$ [$\mathrm{mol{\cdot}L^{-1}}$]
V	Volume of $Na_2S_2O_3$ required [mL]
6	Ratio of $n(OCH_3)/n(Na_2S_2O_3)$
τ	Factor of $Na_2S_2O_3$
m	Weight of the sample [g]

Carbonyl groups

The determination of carbonyl groups was carried out according to Zakis [235] and Faix, Andersons and Zakis [63]. This procedure is based on oximation of carbonyl groups using hydroxylamine hydrochloride. The released hydrochloric acid reacts with triethanolamine, which was added in order to shift the equilibrium of the reaction to the oxime formed. Excess base is titrated using standard solution of hydrochloric acid.

$$R_2C{=}O + NH_2OH \cdot HCl \rightleftharpoons R_2C{=}NOH + HCl + H_2O$$
$$HCl + (CH_2CH_2OH)_3N \longrightarrow (CH_2CH_2OH)_3NH^+ + Cl^-$$

1. Preparation of solution one
Triethanolamine (1.2 g) was weighed into a 50-mL volumetric flask. Ethanol (96% (v/v), undenatured) was added to the mark.

2. Preparation of solution two
Hydroxylammonium chloride (0.7 g) was weighed into a 50-mL volumetric flask and dissolved in 5 mL of deionised water. A portion of 25 mL of solution one was added followed by ethanol (96% (v/v), undenatured) to the mark.

3. Procedure
A portion of 80 mg of dry lignin was placed into a test tube. Dimethyl sulfoxide (2 mL) was added and mixed thoroughly using a test tube mixer until the lignin was dissolved. Afterwards, 5 mL of solution two was added and the solution was again mixed. The tube was sealed with a rubber stopper and placed in a water bath at a temperature of 80 °C for two hours. The solution is transferred quantitatively into a titration vessel and titrated using HCl (0.05 mol·L^{-1}) to pH 3.3.

4. Calculation
The carbonyl content is calculated as shown in Equation 6.17.

$$\text{CO [mmol·g}^{-1}\text{]} = \frac{c \cdot V \cdot \tau}{m} \tag{6.17}$$

c	Concentration of HCl [mol·L^{-1}]
V	Volume of HCl required [mL]
τ	Factor of HCl
m	Weight of the sample [g]

6.7.4. Thioacidolysis-GC/MS

Thioacidolysis was carried out at and by staff of Holzforschung München (Technische Universität München).

The procedure was performed as described by Rolando, Monties and Lapierre [183] with the following amendments: a portion of 15–30 mg lignin was added to 8 mL thioacidolysis reagent and thioacidolysis products were quantified by GC/MS. For calculation of S/G ratio the following degradation products were taken into account (with R being G and S): $\mathrm{R\text{-}CHSEt\text{-}CHSEt\text{-}CH_2SEt}$ (erythro and threo), $\mathrm{R\text{-}CH_2\text{-}CHSEt\text{-}CHSEt_2}$ and additionally $\mathrm{G\text{-}CH_2\text{-}CHR_2}$, as it is found in noteworthy concentrations in Kraft and soda lignins. Peak identification was done as suggested by Rolando, Monties and Lapierre [183].

Condensation degree (CD) calculation is based on the assumption that thioacidolysis yield is 80% for the β-O-4 linked structures and that the average molecular weight of C_3C_6 units is $180\,\mathrm{g{\cdot}mol^{-1}}$. These considerations result in the following equation for its calculation:

$$\mathrm{CD} = 100\% - Y/M \cdot 100\% \cdot 1.25$$

CD	Condensation degree in %
Y	Amount of degradation products based on 1 g KLASON-lignin in µmol
M	Amount of 1 g C_3C_6 based on a molecular weight of $180\,\mathrm{g{\cdot}mol^{-1}} = 5555.56$ µmol
1.25	Factor to account for a thioacidolysis yield of 80% for β-O-4 linked units

6.7.5. Size exclusion chromatography

All measurements were carried out at and by staff of Wageningen University and Research Centre (WUR).

Molecular mass was determined by size exclusion chromatography (SEC) equipped with UV and RI-detection. A solution of NaOH (0.1 M) with a flow of 1 mL/min was used as eluent. Polystyrene standards were used for calibration in the molecular weight range of 891 to 1.02 x 106 $\mathrm{g{\cdot}mol^{-1}}$.

6.7.6. Differential scanning calorimetry

Differential scanning calorimetry was carried out at and by staff of Technische Universität Dresden (Chair for Inorganic Chemistry II).

For determination of glass transition temperature, 20-40 mg of lignin were weighed into aluminium crucibles with perforated lids. Samples were annealed at 90 or 105 °C for 10 min, then cooled to 0 °C, prior to heating to 200 °C. For both cycles the temperature rate was 10 K/min under an argon stream (30 mL/min). Both, the extrapolated onset temperature (T_{ig}) as well as the midpoint temperature (T_{mg}) are given as glass transition temperatures.

6.7.7. Thermodesorption-GC/MS

Thermodesorption-GC/MS was carried out by M. Bremer and J. Kaliner.

TD measurements were carried out using the pyrolyzer unit EGA/PY 3030 D Frontier Lab, which was connected to a gas chromatograph coupled with a mass spectrometer 5977 MS 7890B Agilent. Approximately 150 µg of purified precipitation product was weighed into a sample vessel, heated at 30 K·min^{-1} from 50 to 250 °C and kept for 5 minutes in a pyrolysis chamber. Volatiles were led to a cold trap, and thereafter, to the gas chromatography column (HP 5-ms, 30 m x 250 µm, film: 0.25 µm) for separation. The oven was programmed as follows: initial temperature 45 °C, rate of increase 4 K·min^{-1} to 280°C. A constant carrier gas flow of 1.2 mL·min^{-1} was used. The scan range of the mass spectrometer was $m/z = 50-600$. Compounds were identified using mass spectra library ChemStation and mass spectra published by Meier and Faix [152].

6.7.8. Laser diffraction

Laser diffraction on a LS 200 (Beckman Coulter GmbH) was used to determine particle size. To avoid dissolution of lignin particles, the pH inside the measuring cell was adjusted to $pH = 4$ using hydrochloric acid. By this, the particle size was stable for at least one hour as shown by multiple measurements. Particle size was calculated based on the Fraunhofer-approximation.

A. Annex

A.1. Pulping

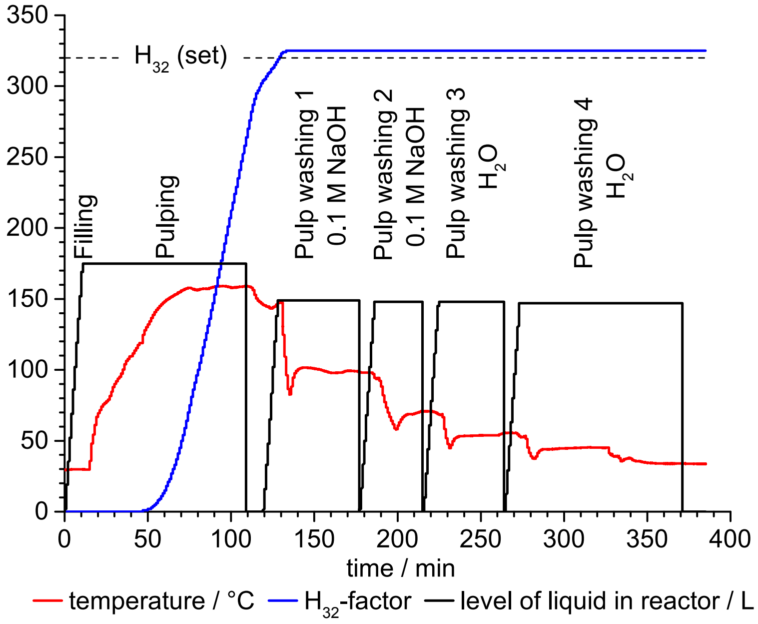


Figure A.1.: Process scheme of alkaline soda pulping in 400-L scale showing the level of liquid in the reactor, temperature and H_{32}-factor

A.2. Pulp

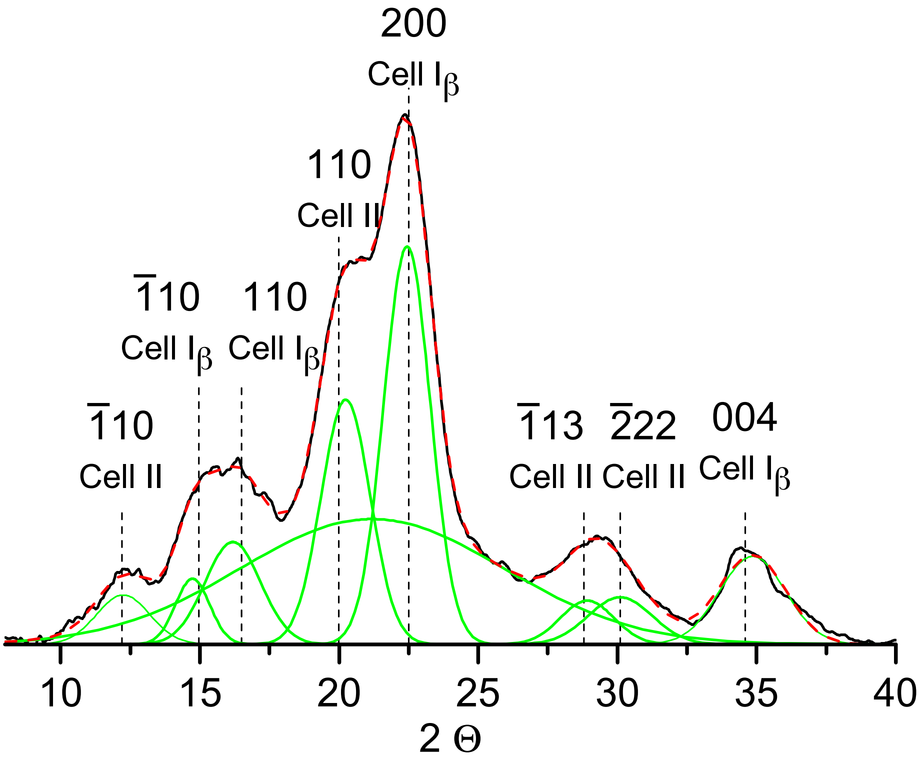


Figure A.2.: Deconvolution of x-ray diffractogram of pulp obtained by conventional alkaline pulping using $\omega(\mathrm{NaOH}) = 9\,\mathrm{wt\%}$

A.3. Precipitation product

Table A.1.: Thermodesorption of lignin obtained from different raw materials by conventional alkaline pulping and Indulin AT

Label	Substance	MW	Ret.time
1	Acetic acid	60	2.03
2	Methyl formate	60	2.03
3	Dimethyl disulfide	94	3.01
4	Hexanal	100	3.75
5	Furfural	96	4.35
6	Guaiacol	124	11.57
7	Benzofurane, 2,3-dihydro-	120	15.93
8	Guaiacol, 4-vinyl-	150	19.07
9	Syringol	154	19.74
10	Vanillin	152	20.28
11	Isoeugenol	164	20.72
12	Guaiacol, 4-propyl-	166	20.79
13	Acetovanillone	166	20.98
14	Guaiacylacetone	180	21.26
15	Dodecanoic acid	200	21.38
16	Acetophenon, 3,5-dimethoxy-	180	21.43
17	Homovanillic acid	182	21.82
18	Syringaldehyde	182	21.86
19	Syringol, 4-propenyl-	194	22.01
20	Acetosyringone	196	22.14
21	Cinnamaldehyde, 4-hydroxy-2-methoxy-	178	22.15
22	Coniferyl alcohol	180	22.15
23	Tetradecanoic acid	228	22.18
24	Homosyringaldehyde	196	22.26
25	Desaspidinol	210	22.26
26	Neophytadiene	278	22.43

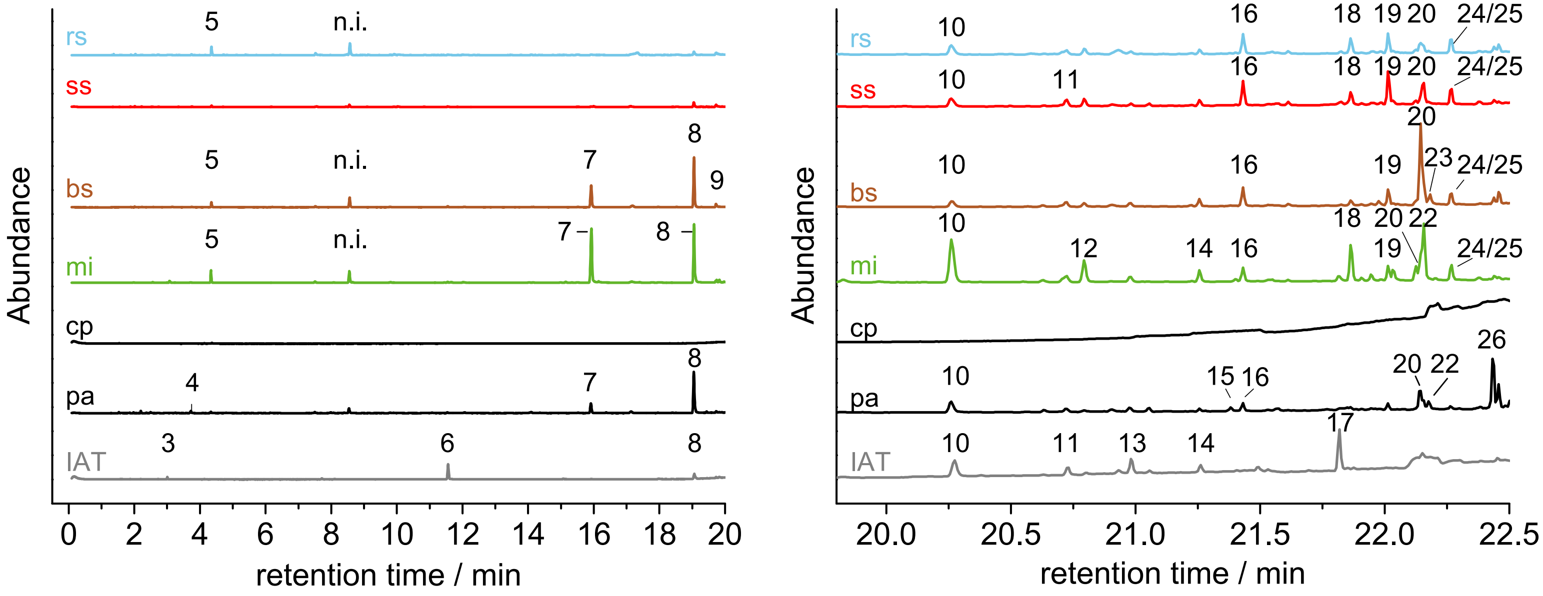


Figure A.3.: Chromatograms after thermal desorption of selected lignins obtained from different raw materials by conventional alkaline pulping and Indulin AT

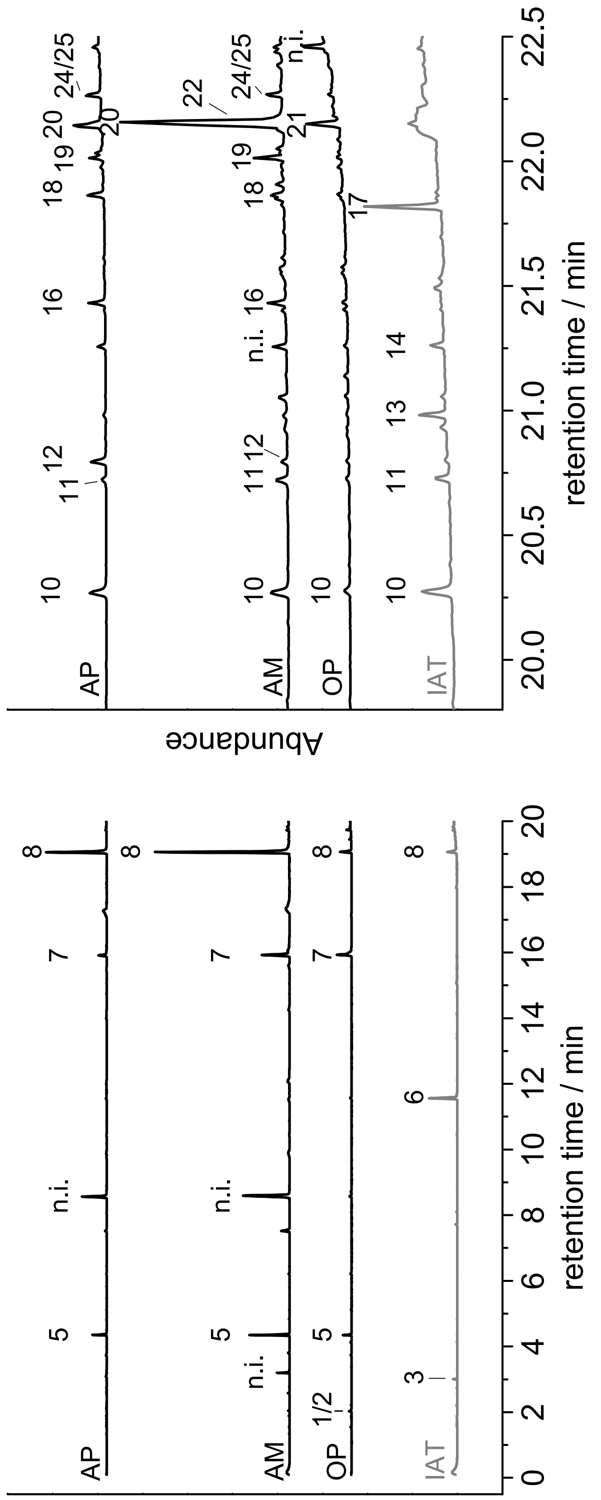


Figure A.4.: Chromatograms after thermal desorption of dialysed precipitation products obtained by conventional alkaline (AP), microwave-assisted alkaline (AM) and organosolv pulping (OP)

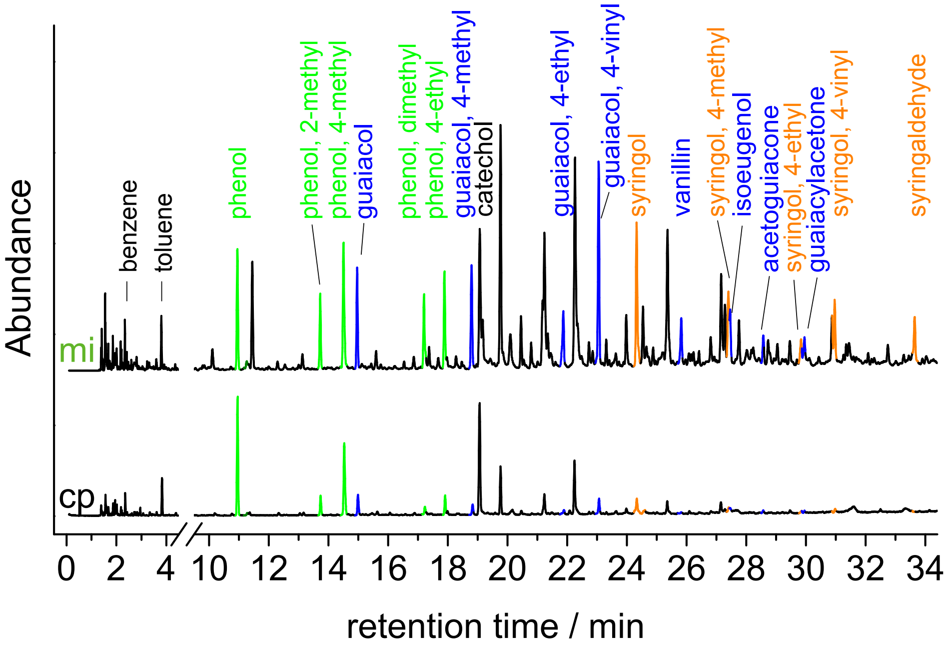


Figure A.5.: Chromatogramm of pyrolysis degradation products of dialysed precipitation products obtained from miscanthus and coconut shell powder ($T_{\text{pyrolysis}} = 650\,^{\circ}\text{C}$)

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Art is I; science is we.

(Claude Bernard)

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